Universitat de les Illes Balears

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THE EFFECT OF PROGRESSIVE MUSCLE RELAXATION IN THE BASAL CORTISOL RESPONSE OF HIGH AND LOW NEUROTICISM STUDENTS

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EvoCog

IFISC

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Doctoral Programme of Cognition and Human Evolution

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Karin Chellew Gálvez

Director: Gloria García de la Banda

Co-director: Phil Evans

Ponent: Enric Munar Roca

Doctor by the Universitat de les Illes Balears

To my parents, Tito and Lily

and to my godparents, Olivia and Roberto

“We have negative mental habits that come up over and over again. One of the most significant negative habits we should be aware of is that of constantly allowing our mind to run off into the future. Perhaps we got this from our parents. Carried away by our worries, we're unable to live fully and happily in the present. Deep down, we believe we can't really be happy just yet—that we still have a few more boxes to be checked off before we can really enjoy life. We speculate, dream, strategize, and plan for these "conditions of happiness" we want to have in the future; and we continually chase after that future, even while we sleep. We may have fears about the future because we don't know how it's going to turn out and these worries and anxieties keep us from enjoying being here now.”

Thich Nhat Hanh

Peace is every breath: A practice for our busy lives (2012, p. 23)

Acknowledgements

Infinitive thanks:

To my whole family who is always there to support me and gives me the wings to pursue my goals and help me to carry on all these years. I know that it has not always been easy. To my girls, Vicky, Malen, Julia F. C., Natalia, Julia U., and Emnita, for always being there when I needed it the most! To my very good friends from IFISC, specially to: Pedrito, Enrico, Miguel, and Przemek for all those great times that we shared this last two years at lunch, *rutas martianas*, "fiestas *multiculturales"*, outings, paintballs, etc., that gave me the strength and motivated me to carry on. Guys, YOU ROCK! To María Saletas who always was there to help me. To Diego, who always made me laugh and made my day a little bit less stressful at the bar. To my colleagues, good friends, and my second family: Vicky, Jacobo, Pep, Malen, and Selene for all these years of hard work at the office, travels, breakfasts, etc. Guys you are the best, thank you for all the good experiences! To Esperança, thank you for all those moments that we shared, at breakfast, travelling by car, conversations, etc., they were very special to me. To all the extraordinary people and professors that I had the pleasure to meet in so many places all these years, thank you for all that I learned from you! To you Przemek, you have made my last years a very special time. To Phil Evans, thank you to be part of this process and thank you to share all your knowledge and time with me. Finally, to Gloria, since the moment I met you, you have been an inspiration and a role model for me. I have no words to express my gratitude to you for always being there for me, to share all your knowledge, time and dedication.

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PREFACE

The aim of the present PhD dissertation is to assess how personality traits influence the cortisol response of undergraduate students in three different conditions; stressful, baseline, and relaxation. This work starts evaluating the cortisol response facing a stressful situation (public speaking) of psychology undergraduate students. Then continues with the study of daily cortisol (baseline cortisol) in students with extreme scores in Neuroticism (N). Finally, this dissertation ends with the evaluation of the efficacy of abbreviate progressive muscle relaxation (APMR), to reduce overall levels of both psychological and physiological stress in undergraduate students scoring high and low in N. These three studies have resulted in two publications:

- García-Banda, G., Servera, M., **Chellew, K.**, Meisel, V., Fornes, J., Cardo, E., Perez, G., Riesco, M., & Doctor, R. M. (2011). Prosocial personality traits and adaptation to stress. *Social Behavior & Personality, 39*(10), 1337-1348. doi: 10.2224/sbp.2011.39.10.1337.
- García-Banda, G., **Chellew, K.**, Fornes, J., Perez, G., Servera, M., & Evans, P. (2014). Neuroticism and cortisol: Pinning down an expected effect. *International Journal of Psychopathology, 91*, 132-138. doi: 10.1016/j.ijpsycho.2013.12.05

And, a future publication, which manuscript has been recently submitted:

- **Chellew, K.**, Evans, P., Fornes, J., Perez, G., & García-Banda, G. (Submitted). The effect of progressive muscle relaxation on daily cortisol secretion. *International Journal of Stress Management.*

RESUM

Els trets de personalitat juguen un paper rellevant en les diferències individuals en la secreció del cortisol. No obstant això, la naturalesa i els mecanismes subjacents d'aquesta relació tot i romanen poc clars. El cortisol, producte final de l'eix Hipotàlem-pituïtari-Adrenal (HPA), és un glucocorticoide que el nostre cos secreta naturalment seguint un pronunciat cicle diürn, amb nivells elevats davant de situacions particularment estressants (reactivitat del cortisol). La present tesi doctoral té com a objectiu elucidar com els trets de personalitat influeixen en la resposta del cortisol d'estudiants universitaris en tres condicions diferents: estressant, basal i de relaxació. Aquest treball comença avaluant la resposta del cortisol davant d'una situació estressant (parlar en públic) en estudiants de psicologia. Esperàvem que la reactivitat del cortisol estigués positivament relacionada amb Obertura, Amabilitat i Responsabilitat, i negativament amb Extraversió, Neuroticisme i Psicoticisme. En el nostre segon estudi, avaluem el perfil de secreció de cortisol basal en estudiants universitaris amb puntuacions extremes en Neuroticisme (N) tractant de demostrar una associació teòrica esperat entre N i la secreció de cortisol diürn. Pensàvem que participants amb puntuacions altes en N exhibirien constantment nivells elevats de cortisol diürn basal comparat amb aquells amb puntuacions baixes en N. Finalment, volíem examinar si una setmana de Relaxació Muscular Progressiva Abreujada (APMR) era eficaç per reduir nivells totals d'estrès psicològic i fisiològic de participants amb puntuacions extremes en N. Els nostres resultats confirmen, en primer lloc, que parlar en públic augmenta significativament la secreció de cortisol en comparació amb una activitat acadèmica

no estressant. A més a més, Responsabilitat ha estat associada amb un augment significatiu dels nivells de cortisol, i Psicoticisme amb una a la baixa. En segon lloc, trobem que Neuroticisme ha estat associat amb una elevada secreció de cortisol davant de situacions d'estrès diari, encara que només després dels primers 45 min. després de despertar (CAR). Aquesta associació ha estat independent del gènere i edat dels participants, si fumaven o no, l'hora de despertar, o del dia de l'estudi. Finalment, en tercer lloc, APMR és una eina eficaç per disminuir tant l'estrès psicològic com fisiològic en tots els participants, independentment de puntuacions altes o baixes en Neuroticisme, el gènere, o l'edat dels participants.

RESUMEN

Los rasgos de personalidad juegan un papel relevante en las diferencias individuales en la secreción del cortisol. Sin embargo, la naturaleza y los mecanismos subyacentes a esta relación aún permanecen poco claros. El cortisol, producto final del eje Hipotálamo-Pituitario-Adrenal (HPA), es un glucocorticoide que nuestro cuerpo secreta naturalmente de acuerdo a un ciclo diurno pronunciado, con niveles elevados ante situaciones estresantes (reactividad del cortisol). El objetivo de la presente tesis doctoral ha sido elucidar cómo los rasgos de personalidad influyen en la respuesta del cortisol de estudiantes universitarios en tres condiciones distintas: estresante, basal y de relajación. Este trabajo comienza evaluando la respuesta del cortisol ante una situación estresante (hablar en público) en estudiantes de psicología. Esperábamos que la reactividad del cortisol estuviera positivamente relacionada con Apertura, Amabilidad y Responsabilidad, y negativamente con Extraversión, Neuroticismo y Psicoticismo. En nuestro segundo estudio, evaluamos el perfil de secreción de cortisol basal en estudiantes universitarios con puntuaciones extremas en Neuroticismo (N). Con ello pretendíamos demostrar de forma experimental una asociación planteada a nivel teórico entre N y secreción de cortisol diurno. Así esperábamos que los participantes con puntuaciones altas en N exhibieran niveles elevados de cortisol diurno basal comparado con participantes con puntuaciones bajas en este rasgo. Por último, queríamos examinar si una semana de Relajación Muscular Progresiva Abreviada (APMR) era efectiva en reducir los niveles totales de estrés psicológico y fisiológico de participantes con puntuaciones extremas en N. Nuestros resultados confirman,

en primer lugar, que hablar en público aumenta significativamente la secreción de cortisol en comparación con una actividad académica no estresante. Además, Responsabilidad se asoció con un aumento significativo de la respuesta de cortisol, y Psicoticismo con una respuesta a la baja. En segundo lugar, encontramos que altos niveles de Neuroticismo se asociaron con una secreción elevada de cortisol en situaciones de estrés diario, aunque solo después de los primeros 45 min después de despertar (CAR). Esta asociación fue independiente del género y edad de los participantes, si fumaban o no, de la hora de despertar, o del día del estudio. Por último, en tercer lugar, APMR fue eficaz en disminuir tanto el estrés psicológico como fisiológico en todos los participantes, independientemente del género, la edad o de la puntuación de Neuroticismo de los participantes.

ABSTRACT

Personality traits play a significant role in individual differences in cortisol response (LeBlanc, Ducharme, & Thompson, 2004). However, the nature and underlying mechanisms of the relationship between cortisol secretion and personality traits still remain unclear. Cortisol, an end product of the Hypothalamic-Pituitary-Adrenal axis (HPA), is a glucocorticoid that our body naturally secretes according to a pronounced diurnal cycle with increased values under stressful situations (cortisol reactivity). The aim of the present PhD dissertation was to elucidate how personality traits influence the cortisol secretion of undergraduate students in three different conditions; stressful, baseline, and relaxation. This work began by evaluating the cortisol response facing a stressful situation (public speaking) of psychology students. We believed that cortisol reactivity would be positively related to Openness, Agreeableness, and Conscientiousness, and negatively to Extraversion, Neuroticism and Psychoticism. In our second study, we assessed the baseline cortisol in students with extreme scores in Neuroticism (N) attempting to prove a theoretical expected association between N and diurnal cortisol secretion. We postulated that high N participants would display elevated diurnal background levels of cortisol compared to low N participants. Finally, we examined whether one week of Abbreviated Progressive Muscle Relaxation (APMR) was effective in reducing overall levels of psychological and physiological stress of high- and low-N participants. Our results confirmed, firstly, that public speaking significantly increased cortisol secretion when compared to a non-stressful academic activity. In addition, Conscientiousness was associated with an enhanced cortisol response to public

speaking, and Psychoticism with a blunted one. Secondly, we found that high levels of Neuroticism were associated with elevated cortisol secretion on daily stress, but only after the first 45 min following awakening (CAR). This association was independent of sex and age, smoking status, awakening time, and day of study. Finally, in third place, APMR was effective in decreasing both psychological and physiological stress in all participants independently of their N-score, gender, or age.

Chapter 1

Introduction and theoretical framework

1. Introduction

Traditionally, aberrant cortisol dynamics and personality traits have been closely linked to mood and anxiety disorders (Oswald et al., 2006). Evidence suggests that personality traits may play a significant role in individual differences in cortisol response (LeBlanc, Ducharme, & Thompson, 2004). However, the nature and the underlying mechanisms of the relation between cortisol secretion and personality traits still remain unclear. Cortisol, the HPA end product, is a glucocorticoid that our body naturally secretes according to a pronounced diurnal cycle with increased values under particularly stressful conditions (cortisol reactivity). In this thesis, cortisol responses to public speaking were examined to test the hypotheses that reactivity would be positively related with openness, agreeableness, and conscientiousness, and negatively to extraversion, neuroticism and psychoticism. Moreover, there are strong theoretical arguments that those high on neuroticism (N) should normally exhibit higher prevailing levels of the stress-linked hormone cortisol. Thus, in the second study presented in this thesis we tried to prove expected associations between N and diurnal cortisol secretion. We had one simple but clear theoretically derived formal hypothesis, that high N participants would constantly display elevated diurnal background levels of cortisol compared to low N participants. Finally, we wanted to examine whether an intervention consisting of one-week of Abbreviate Progressive Muscle Relaxation (APMR) was effective in reducing overall levels of both psychological and physiological stress of high- and low-N participants.

In this chapter we present a description of the main concepts used along this thesis that include: personality, neuroticism, stress, cortisol, daytime cortisol

circadian cycle (CAR and AUC), cortisol reactivity, and Abbreviated Progressive Muscle Relaxation (APMR). In the second chapter, we will introduce the three studies that form part of this thesis, and finally, in the last chapter we discuss and highlight the main results and limitations of these studies, reach conclusions, and suggest future lines of research that arise from this work.

2. Personality

Eysenck (1970) defined personality as: *"A more or less stable and enduring organization of a person's character, temperament, intellect, and physique, which determines his unique adjustment to the environment. Where character denotes a person's more or less stable and enduring system of conative behaviour (will); temperament, his more or less stable and enduring system of affective behaviour (emotion); intellect, his more or less stable and enduring system of cognitive behaviour (intelligence); physique, his more or less stable and enduring system bodily configuration and neuroendocrine endowment"* (p. 243).

In this thesis we focused on the Eysenck's temperament aspect of personality that refers to intrapersonal processes (cognitive, emotional, and motivational) that determine our individual behaviour. More specifically, personality can be described as consistent behaviour patterns, stable across time and consistent across situations and can be quantitatively assessed (Burger, 2011).

Paunonen (1998) pointed out that there is some intuitive appeal to the conceptualization that factors of personality are organized hierarchically, arranged according to the breadth of the behaviour domains represented (see Figure 1). There are several approaches that have a hierarchical structure to describe, explain, and assess personality traits. In the present thesis we are going to present two of the most used and well-known models: the Eysenck's biosocial approach (Eysenck & Eysenck, 1969; Eysenck & Eysenck, 1985a) and the psycholexical approach of the Five-Factor Model (FFM; Costa & McCrae, 1985).

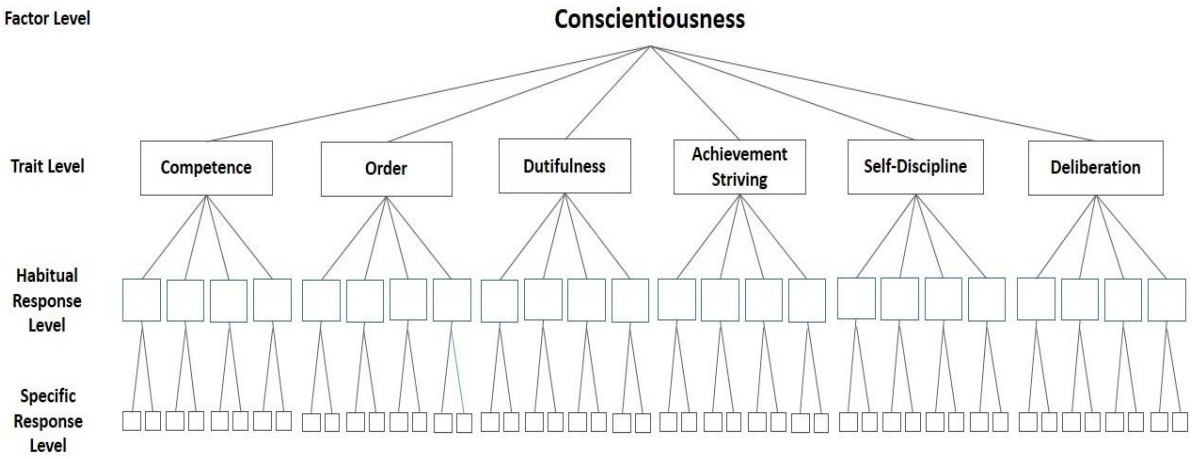


Figure 1. Representation of a hierarchical model of personality organization. Adapted from *"Hierarchical organization of personality and prediction of behavior,"* by S. V. Paunonen, 1998, *Journal of Personality and Social Psychology, 74*(2), p. 539. Copyright 1998 by the American Psychological Association, Inc.

In both approaches two basic personality traits are acknowledged, neuroticism and extraversion. People with high scores in emotional instability or 'neuroticism' dimension are moody, touchy and anxious, whereas those with low scores are relaxed, even tempered and calm. People with high scores in extraversion are enthusiastic, sociable, talkative and optimistic. On the contrary, introverts are reserved, pessimistic and keep themselves to themselves. Eysenck proposed a third dimension called psychoticism, reflecting impulsive, antisocial, aggressive, cold, egocentric, lack of empathy, creative, and tough-minded behaviours (Eysenck, 1991, 1992a, 1992b). In the FFM of Costa and McCrae (1985) three more fundamental traits were included: openness, agreeableness, and conscientiousness. Openness involves active imagination, aesthetic sensitivity, attentiveness to inner feelings, preference for variety, and intellectual curiosity. Characteristics such as trust, straightforwardness, altruism, compliance, modesty,

and tender-mindedness are components of agreeableness. Qualities of order, dutifulness, achievement striving, self-discipline, and deliberation are characteristics of conscientiousness (Costa, McCrae, & Dye, 1991; Costa and McCrae, 1992b).

2.1. Neuroticism

The personality trait of neuroticism (N) refers to relatively stable tendencies to respond with negative emotions to threat, frustration, or loss (Costa & McCrae, 1992a). Individuals in the general population vary noticeably on this trait, ranging from frequent and intense emotional reactions to minor challenges to almost non-emotional response even if they are facing significant difficulties (Lahey, 2009). N is operationally defined by items referring to irritability, anger, sadness, anxiety, worry, hostility, self-consciousness, and vulnerability, which have been found to be substantially correlated with one another in factor analyses (Costa & McCrae, 1992a). People who score low in N, contrary to people high in N, tend to be more calmed and confident, and appear to cope better with stress.

N is believed to reflect a stable disposition involving specific biological and psychological mechanisms that produce its robust association with psychopathology. In fact, N has been shown prospectively to predict the development of emotional disorders including major depression, posttraumatic-stress disorder (PTSD), phobias, and panic attacks (e.g., Breslau, Davis, & Andreski, 1995; Clark, Watson, & Mineka, 1994; Hayward, Killen, Kraemer, & Taylor, 2000; Krueger, Caspi, Moffitt, Silva, & McGee, 1996). In addition, females N scores have been shown to be slightly but significantly higher than in males (Costa, Terracciano, & McCrae, 2001),

which could explain why we found more women developing these type of disorders than men.

One essential aspect of neuroticism is that it involves individual differences in cognition and information processing, producing biases, specifically, under stressful situations. In fact, high-N individuals, compared with low-N ones, show heightened attention to negative or threatening information (rather than neutral information), as evidenced by a faster response to and a slower detachment from negative or threatening stimuli (Ormel et al., 2013). Such negative responses to challenges are both frequent and out of proportion to the circumstances for individuals who are high in this trait (McCrae & Costa, 2003). Therefore in our last two studies we included extreme N-scores participants (high vs. low) in order to observe better differences between these two groups.

In the next section, we will introduce the concept of stress and its definition, the main stress sources (stressors) that could affect us. This include the description of major life events and hassles, and finally how we can measure them.

3. Stress

Stress occurs when environmental demands overwhelm one's abilities to meet those demands (Lazarus & Folkman, 1984) and is an influential construct in health research (Keller et al., 2012). The interactionist model of stress (Lazarus & Folkman, 1984; Mischel, 2009) suggests a bidirectional relationship between an individual's response and a stressful situation. The recent development of this model (Conger & Donnellan, 2007) differentiates between stress causation (stress, such as life events, may lead to a change in an individual's personality characteristics that in turn affects their response to stressful events) and stress selection (one or more individual personality characteristics may increase the degree to which a life event is experienced as stressful). In this study we focused in the latest one.

There are several sources of stress that can interfere in our lives: chronic stress, acute stress and daily stress (APA, 2014). Chronic stress refers to the constant and persisting stress stimuli over an extended period of time that can lead to psychological and physical debilitation and it can result in serious health conditions. Acute stress is the most common form of stress among humans and, contrary to chronic stress, it refers to particular short-term stressful situations (e.g., an exam, an interview) that produces an acute stress response, but that decreases or disappears after the stressor is no longer available. Finally, another common source of stress is daily stress, which refers to minor stressful events such as, daily annoyances and hassles (e.g., making decisions, meeting deadline at work or university, traffic jams, etc.) that are present in everybody's life. What is relevant about this type of stress is that continue exposure to them can generate as much

damage in the long-term than chronic stress. In this thesis we focus on acute and daily stress because we considered that both stressors are part of the student's life. We did not include chronic stress because it would needs a longitudinal study design and what was out of our study scope.

In relation to acute stress, we believe that undergraduate students have frequently to face several academic stressors (e.g., academic exams, written assays, public speaking, etc.), that generate an acute stress response. Public speaking has been proved to be a potent social stimuli widely used in stress research (Schoofs, Hartmann, & Wolf, 2008). Specifically, this task involves social evaluation by peers (Andrews et al., 2007) that triggers intense emotional responses provoking changes in HPA-axis activity and, consequently, eliciting strong cortisol responses (Dickerson, Mycek, & Zaldivar, 2008). Therefore, in our first study we use 10-minute-long public speaking presentation to assess individual's stress responses.

Minor stressors are another source of stress that people have to face frequently and reflect the daily stress load. However, not every person will interpret them as stressful or respond in the same way than other person may do. These differences may be explained by some personality traits, in particular, neuroticism (N). As we mention previously, people high in N are characterized by the tendency to perceive more stressors and to respond negatively to these situations than people low in this trait (Lahey, 2009). Thus, in our last two studies presented here, we studied only participants with extreme N-scores to denote clearly differences between them in relation to daily stress.

Psychological or perceived stress can be assessed by using self-reports that collect information about the number, frequency and intensity of the stressors experienced during a period of time (e.g., hassles, recent life experiences, etc.).

However these instruments present some limitations, mostly due to a high intra- and between-subjects variability in their ratings. For instance, a person who has to describe its level of stress in several time points could use the same value but denoting something different (intra-subjects) or, two people using the same score may signify something completely different from one participant to another (between-subject). Finally, the instruments used to evaluate stress may be not sensitive enough to denote changes over time. Therefore, in order to obtain a reliable measure of perceived stress we included in our last study the SRLE scale. This scale has been shown to be a decontaminated hassles measure to determine accurately how much everyday stressors affect physical and mental health (Kohn & Macdonald, 1992), and sensitive enough to detect individual differences and changes over time (de Jong, Timmerman, & Emmelkamp, 1996).

To complement this subjective measure (SRLE), we also include an "objective" measure to increase the reliability of our results and, to demonstrate that stress is not only affecting us psychologically, but also physiologically. Indeed, when we are experiencing stress, immediately our brain generates a signal that provokes a physiological response activating the HPA-axis (e.g., Gaab, Sonderegger, Scherrer, & Ehlert, 2006). As a result, our organism gets ready to deal with daily stress. A good and reliable biomeasure of stress is the hormone, cortisol – end product of HPA-axis activity.

4. Cortisol

Cortisol is the main glucocorticoid hormone responsible for mobilizing energy to deal with daily activities and contributes fundamentally to the maintenance of basal and stress-related homeostasis (McEwen, 2003). However, Selye (1936) recognized the paradox that the physiologic systems activated by stress can not only protect and restore but also damage the body. The primary biological mechanism underlying stress regulation and adaptation is the hypothalamic-pituitary-adrenal (HPA) axis (see Figure 2).

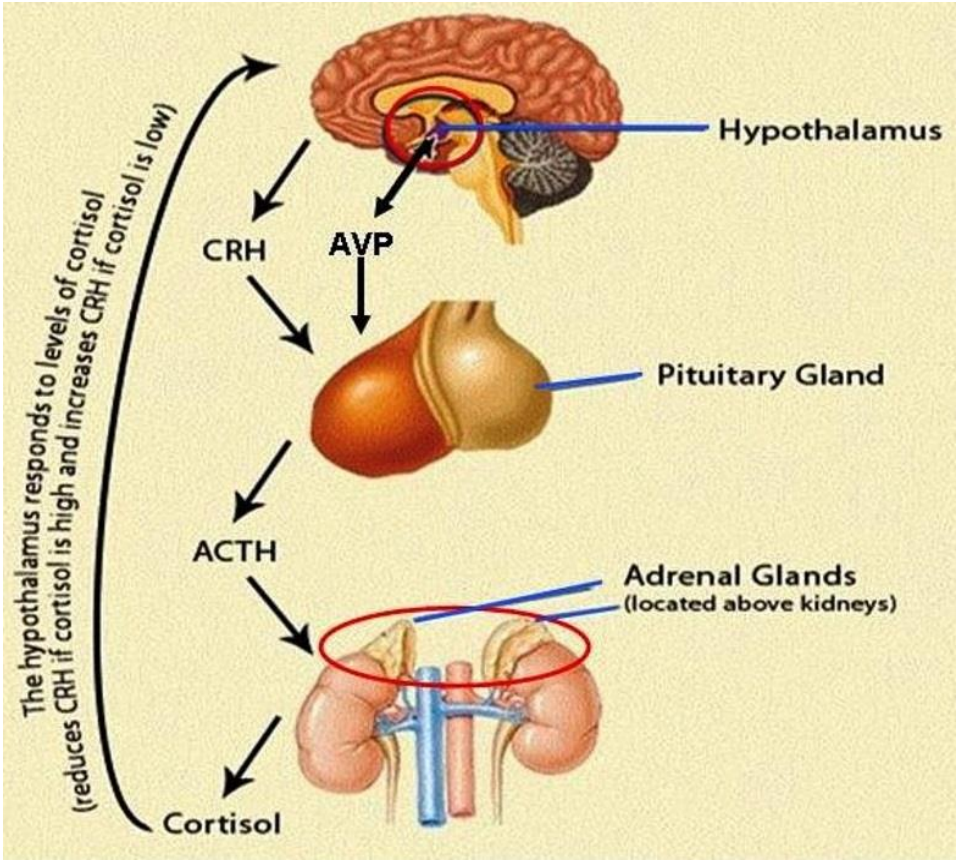


Figure 2. Illustrates the physiological cascade involved in the hypothalamic-pituitary-adrenal (HPA) axis and the regulation feedback after the cortisol secretion. Adapted from "http://total-body-psychology.com.au/stress-response-hpa-axis/".

The HPA axis activity is governed by the secretion of corticotrophin-releasing hormone (CRH) and vasopressin (AVP) from the hypothalamus, which in turn activates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, which finally stimulates the secretion of the glucocorticoids from the adrenal cortex, such as cortisol. Once the glucocorticoids are secreted into the blood stream, they interact with their receptors in multiple target tissues including the HPA axis. The activated glucocorticoid receptor (GR), in turn, induces a feedback inhibition signal on both CRH and AVP from the hypothalamus and directly on the secretion of ACTH from pituitary that leads the reduction of HPA axis activity.

Glucocorticoids not only influence the activity of pituitary but also many other functions of the central nervous system such as, arousal, cognition, mood and sleep, also the activity and direction of intermediary metabolism, the maintenance of a normal cardiovascular tone, the activity and quality of the immune and inflammatory reaction, including the manifestations of the sickness syndrome, as well as growth and reproduction (Chrousos & Kino, 2007). Glucocorticoids influence the brain, regulating the neuronal survival, neurogenesis, and sizes of complex anatomical structures such as the hippocampus, the acquisition of new memories and the emotional appraisal of events (Herbert et al., 2006). In that way, any generalized change in the glucocorticoid signalling system would be followed by corrective, compensatory changes in the activity of the HPA axis.

However, sometimes this “compensatory” activity does not occur. In fact, whereas cortisol generally helps the organism face daily life activities, disturbed patterns of cortisol secretion are potentially detrimental in long-term, for instance, the deregulation of daytime cortisol activity has indeed been associated with stress-

related pathologies, including depression, post-traumatic stress disorder, anxiety, externalizing behaviours, and cognitive deficits, as occur with chronic stress.

4.1. Daytime cortisol circadian cycle

Daytime cortisol cycle refers to the cortisol secreted along the day. The cortisol secretion presents a circadian rhythm of 24 hours that follows a characteristic diurnal rhythm with several secretory episodes of short duration and high amplitude (Fries, Dettenborn, & Kirschbaum, 2009). Under normal conditions, the typical diurnal cortisol profile shows a sharp rise upon awakening, called cortisol awakening response (CAR), where thereafter there is a steady decline over the rest of the day with lower levels in the evening and night (Tsigos & Chrousos, 2002). The CAR appears to be a distinct feature of the hypothalamus-pituitary-adrenal (HPA) axis, superimposing the circadian rhythmicity of cortisol secretion (Fries, Dettenborn, & Kirschbaum, 2009) as it adds a substantial incremental effect to the linear trend of increasing cortisol concentrations in the early morning hours (Wilhelm, Born, Kudielka, Scholtz & Wüst, 2007). More importantly, the awakening itself is a consistent, recurring, and strong stimulus for HPA activity (Wilhelm et al., 2007).

4.1.1. Cortisol Awakening Response (CAR)

The CAR is a discrete and distinctive part of the cortisol circadian cycle. In healthy adults salivary free cortisol concentrations increase by between 50 and

160% in the first 30-45 min immediately post-awakening (approximate average increase of 9 nmol/l, range 4–15 nmol/l, estimated to be equivalent, to about three secretory episodes; Clow, Thorn, Evans, & Hucklebridge, 2004). This response was coined the "Cortisol Awakening Response" (CAR) by Federenko et al. in 2004 (see Figure 3).

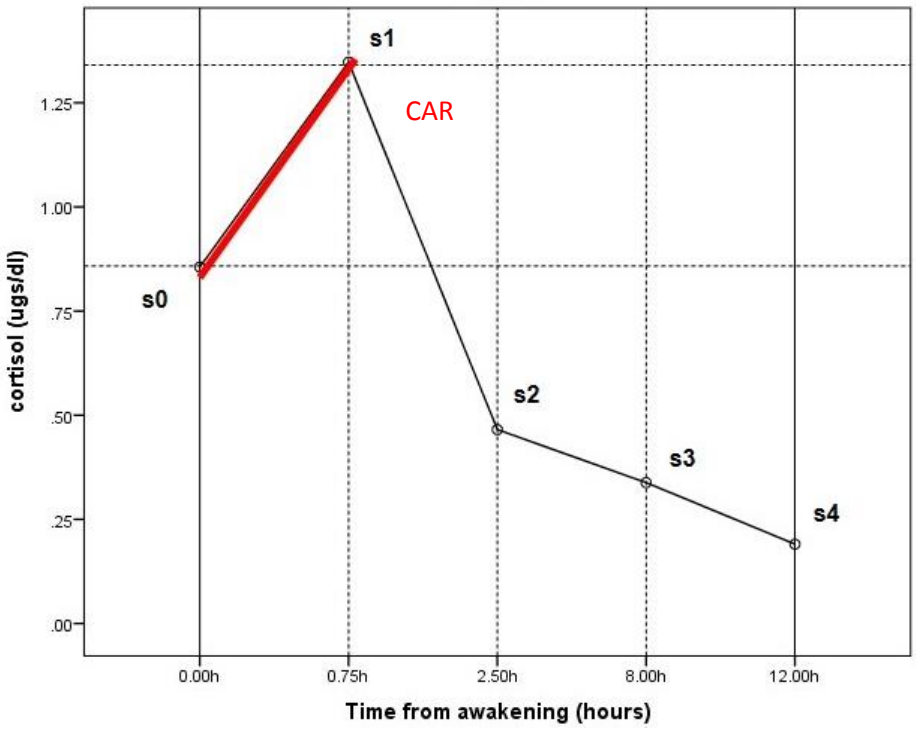


Figure 3. Characteristic of the cortisol diurnal profile during 12hs period where the Cortisol Awakening Response (CAR) is represented, between 0h (wake-up) and first 45 minutes (0.75h) after awakening.

Pruessner et al. (1997) were the first who proposed that the repeated assessment of this cortisol increase after awakening in saliva might represent a useful and easy index of cortisol regulation. Indeed, the CAR has been attracting

attention from researchers for a number of reasons. First, the CAR is one of the most important and easy parameter of HPA axis function to measure. Second, the CAR is under somewhat independent control from cortisol output during the remainder of the day, and associations between the CAR and cortisol sampled later in the day are quite low (Edwards, Evans, Hucklebridge, & Clow, 2001). Third, twin studies have documented a genetic influence on the CAR that is distinct from the heritability of daytime cortisol levels (Kupper et al., 2005). Finally and very important, the magnitude of the CAR appears to be associated with psychosocial factors and health in potentially significant ways. In fact, Chida and Steptoe (2009) suggest that the magnitude of the CAR may be a distinctive indicator of HPA function and dysfunction.

Early psychological studies of the CAR indicated that the response was heightened among individuals experiencing job stress, overload, and low self-esteem (Schulz, Kirschbaum, Pruessner, & Hellhammer, 1998; Pruessner, Hellhammer, & Kirschbaum, 1999; Wüst, Federenko, Hellhammer, & Kirschbaum, 2000). Others suggest that the CAR might be an indicator of chronic psychosocial stress (e.g., Bhagwagar, Hafizi, & Cowen, 2005). Furthermore, larger CARs were suggested to be a marker of neuroendocrine activation as the individual contemplated the challenges of the day ahead (Chida and Steptoe, 2009). By contrast, a diminished CAR appeared to be present in people suffering from post-traumatic stress disorder (de Kloet et al., 2006; Wahbeh & Oken, 2013), men with systemic hypertension (Wirtz et al., 2007), and women with mild or moderate depression (Stetler & Miller, 2005).

Nonetheless, the CAR is particularly volatile and variable intra- and between-subjects. This variability has been reported at the awakening time but not later during the day (Kupper et al., 2005) and may be affected by biological mechanisms of control. These mechanisms are the suprachiasmatic nucleus circadian clock (Postnova, Fulcher, Braun, & Robinson, 2013), the hippocampal regulation (Fries et al., 2009), and biological processes associated with sleep-wake cycles (Smyth, Clow, Thorn, Huckebridge, & Evans, 2013). Therefore, in our second and third study, due to the fact that cortisol secretion during the first 45 min is too volatile, we also evaluated the area under the curve (AUC), which represents the total cortisol secreted during a predefined period of time, which seems to be more stable than the CAR to evaluate changes over time.

4.1.2. Area under the curve (AUC)

The area under the curve (AUC) is another cortisol secretion parameter that provides relevant information about the functioning of HPA. The AUC is often used to estimate total cortisol secretion during a predefined time period (Hansen, Garde, & Persson, 2008). In research and clinical settings, the salivary cortisol measures are used as a physiology indicator of responsiveness of the HPA axis to determine the health consequences of stress.

In research involving repeated measurements of a response variable, there is a need to derive parameters that summarize the information contained in the multivariate data. The AUC is a good parameter to get this information and it is computed following a trapezoidal formula separated into triangles and rectangles.

Pruessner, Kirschbaum, Meinlschmid, & Hellhammer (2003) provide a simple formula for the computation of two types of AUC that reveal different information (see Figure 4): AUCg (area under the curve to the ground) and AUCi (area under the curve to the increase).

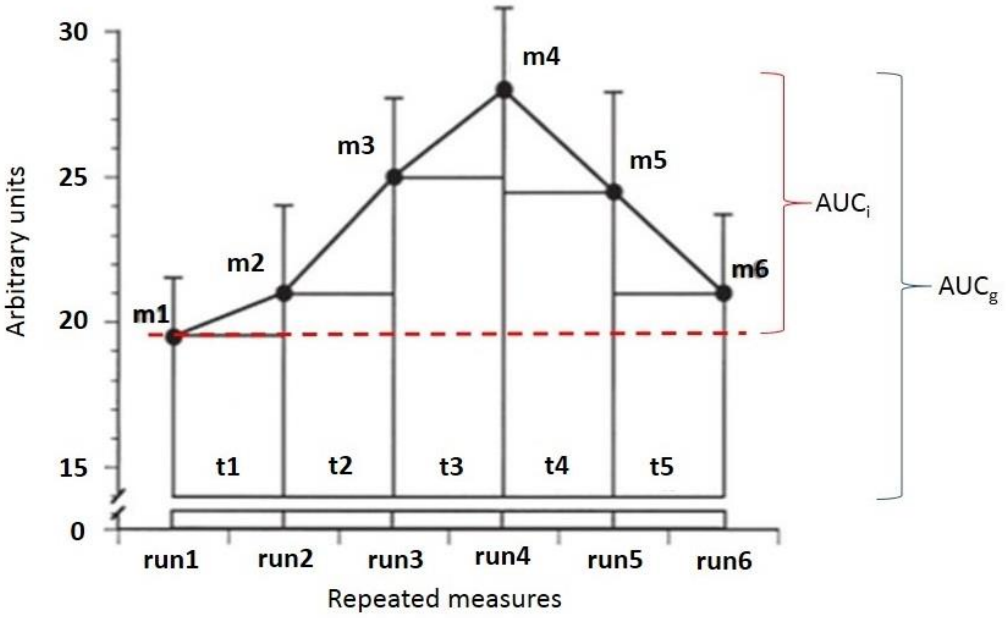


Figure 4. Time course of an artificial dataset with six measurements; the triangles and rectangles illustrate the composition of the area under the curve (AUC). '*m1' to 'm6'* denote the single measurements, and '*t1'* to '*t5'* denote the time interval between the measurements. Adapted from *"Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change"* by J. C. Pruessner, C. Kirschbaum, G. Meinlschmid, and D. H. Hellhammer, 2003, *Psychoneuroendocrinology, 28*, p. 918. Copyright 2003 by Elsevier Science Ltd.

The AUCi is calculated with reference to the baseline measurement and it ignores the distance from zero for all measurements and emphasizes the changes over time. AUCg is the total area under the curve, and it takes into account both *sensitivity* (the difference between the single measurements from each other) and *intensity* (the distance from these measures from ground), and it is assumed to be a measure more related to total hormone output.

Both formulas are basically simple additions of areas consisting of triangles and rectangles:

$$AUC_g = \sum_{i=1}^{n-1} \frac{(m_{(i+1)}+m_i)\cdot t_i}{2}$$

$$AUC_i = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2}\right) - \left(m_1 \cdot \sum_{i=1}^{n-1} t_i\right)$$

In this study we focused on the AUCg parameter and we divided it in two different parameters: AUC0_45min (the area under the curve the first 45 min after awakening) and AUC075h_12h (also called “total cortisol secretion” – TCS in our last study), which represent the cortisol secreted during the remainder of the 12h period after CAR (see Figure 5).

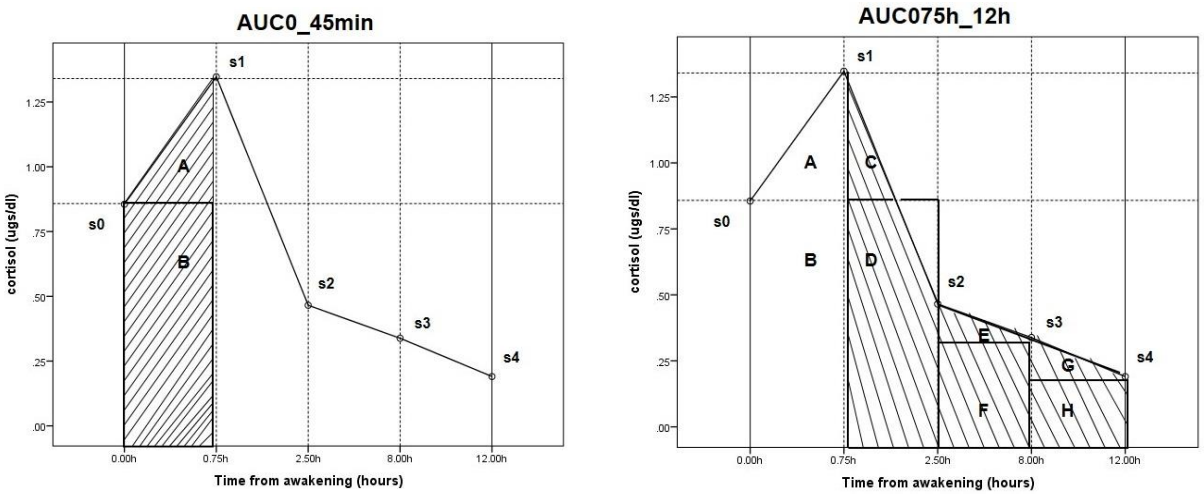


Figure 5. Plots showing two forms of AUCg: AUC0_45min and AUC075h_12h *or* TCS.

We have described how the cortisol diurnal profile normally behaves under daily situations and several ways to assess the functioning of HPA-axis obtained from saliva samples. However, this daytime cortisol profile can be supplemented by episodic *cortisol reactivity* to unpredictable, uncertain, and novel psychological challenges (Bosch et al, 2009).

4.2. Cortisol Reactivity

The HPA-axis is a major endocrine system adapting the organism to bodily and environmental challenges by inducing behavioral and physiological changes, improving the organism's ability to adjust homeostasis (Tsigos & Chrousos, 2002). Cortisol plays a crucial role in the organism's efforts to adjust to these challenges. In fact, under unpredictable, uncertain, and novel challenges an increase in cortisol secretion is considered an adaptive response to a mismatch between the individual

needs and factors in the environment (de Kloet, 2003; McEwen, 2000). This reactivity has widely been considered an endocrine index of the stress response (Miller, Chen, & Cole, 2009). However, some studies have been found that not always there is an increase in the cortisol response during the acute stressful situation, but also a flat or blunted cortisol response (e.g., Loft et al., 2007; Roy, 2004; Saxbe, Repetti, & Nishina, 2008), which have been associated with a poor or less adaptive response.

An excessive demand over time on energy mobilization due to frequent stress, failed shutdown of the stress reactivity system or inadequate response to challenges, generates an allostatic load (McEwen, 1998). McEwen (2000) define allostatic load as a result of the effort that our body as to tolerate trying to keep the allostasis (homeostasis) during stressful demands. This load at some point might affect not only the HPA axis function (McEwen, 2008; Oswald et al., 2006), but also the autonomic nervous system function (Appelhans & Luecken, 2006; Chida & Hamer, 2008; Thayer, Åhs, Fredrikson, Sollers, & Wager, 2011), and produce changes in the structure of the brain with a decline of cognitive functions (Herbert et al., 2006; McEwen, 2012; Lupien, McEwen, Gunnar, & Heim, 2009). Concretely, the accumulation of this load (called overload by McEwen) sometimes lead to target tissue pathology, as occurs in chronically stressed or depressed individuals (Chrousos 2000; Gold & Chrousos, 2002).

Although all human beings encounter stressful events, they do not respond identically to such experiences. Whereas some individuals adapt flexibly to the changing demands of stressful experiences, others cope far less effectively. Thus, in relation to these differences in cortisol responses, a large number of studies have found a substantial inter- and intra-individual variability to similar challenges (Chida &

Steptoe, 2009; Kudielka, Hellhammer, & Wüst, 2009; Thorn, Hucklebridge, Evans, & Clow, 2009) and it has been suggested that personality traits may play a significant role in their explanation (LeBlanc, Ducharme, & Thompson, 2004; Oswald et al., 2006; Pruessner et al., 1997).

5. Personality and Cortisol: relation between concepts

Whenever the brain needs energy, the HPA-axis will help to allocate glucose to the brain, increasing this dynamic (higher cortisol peaks) under stressful conditions (Peters et al., 2004). As we mentioned above, there is some inter- and intra-individual variability in cortisol secretion, and part of this variability may be explained by personality traits. Associations between cortisol and personality have been found under stressful situations (acute stress) and under daily activities (daily stressors).

In relation to acute stress, associations between neuroticism and elevated urinary cortisol have been documented in academic exams (García de la Banda, Martínez-Abascal, Riesco, & Pérez, 2004) and mental arithmetic tasks (Habra, Linden, Anderson, & Weinberg, 2003). On the contrary, neuroticism was also linked to blunted salivary cortisol responses to public speaking (Garcia de la Banda, Martinez-Abascal, Pastor, et al., 2004), mental arithmetic tasks (Phillips, Carroll, Burns, & Drayson, 2005), and cold exposure (LeBlanc & Ducharme, 2005). There are also other personality traits that have been related with cortisol. A diminished cortisol response to stress has been linked to extraversion (Garcia de la Banda, Martínez-Abascal, Pastor, et al., 2004; Kirschbaum et al., 1995; Oswald et al., 2006), but directionally opposite results have been reported by LeBlanc and Ducharme (2005). Openness has been linked to higher cortisol levels evaluated by the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), while participants low in agreeableness (who tend to be mistrusting and hostile) secreted more cortisol compared to people that have higher scores in agreeableness (in males; Suarez, Kuhn, Schanberg, Williams, & Zimmerman, 1998).

On the contrary, conscientiousness, considered a buffer for stress (Korotkov, 2008) and a good predictor of health and longevity (Friedman, 2008), has not been associated with cortisol secretion (Nater, Hoppman, and Klumb, 2010; Oswald et al., 2006; Schoofs, Hartmann, & Wolf, 2008). Finally, no association has been found in previous studies between psychoticism and cortisol (Kirschbaum, Bartussek, & Strasburger, 1992; Schommer, Kudielka, Hellhammer, & Kirschbaum, 1999).

In relation to daily stress, a small body of research has tried to relate basal cortisol and personality. In particular, the trait of neuroticism. In fact, neuroticism has been not only associated to psychological stress but also with excessive cortisol secretion that predisposes individuals to psychopathology (Lahey, 2009; Ormel et al., 2013). However, the direction of this association remained inconclusive, since some studies found increased, showing individuals with high N scores to have greater cortisol levels (Nater, Hoppmann, & Klumb, 2010; Portella, Harmer, Flint, Cowen, & Goodwin, 2005) and others decreased cortisol concentrations (Hauner et al., 2008; LeBlanc & Ducharme, 2005). In addition, Mikolajczak et al. (2010) found that high N was associated with significantly less flexibility of the CAR, when weekend and weekday profiles are compared. Also a flatter diurnal cortisol slope has been linked to higher N, but only in male participants (Hauner et al., 2008). Nonetheless, in a majority of studies, the predicted link between high N and high basal cortisol it has not been found (Adler, Wedekind, Pilz, Weniger, & Huether, 1997; Chan, Goodwin, & Harmer, 2007; Ferguson, 2008; Gerritsen et al., 2009; Hill, Billington, & Krägelog, 2013; Riese, Rijsdijk, Rosmalen, Snieder, & Ormel, 2009; Schommer, Kudielka, Hellhammer, & Kirschbaum, 1999; Van Santen et al., 2011).

This generally inconsistent picture in regard to personality and cortisol might partly reflect methodological differences. For example, early researchers used the

Eysenck Personality Questionnaire-Revised (EPQ-R; Eysenck & Eysenck, 1985b) to assess personality measures (Schommer et al., 1999) while later researchers relied on the NEO Personality Inventory (Costa & McCrae, 1985) or selected other personality questionnaires such as the Freiburg Personality Inventory (Fahrenberg, Hampel, & Selg, 1989) used by Brandtstädter, Baltes-Götz, Kirschbaum, and Hellhammer (1991). In our three studies we include both questionnaires to solve this problem and to guarantee the reliability of our results. Another problem is that in some studies laboratory stressors have been used with a lack of independent validation of their stress-eliciting properties. Speak in front of an audience, is a proven social stressor and has become commonly used in the study of stress (Schoofs, Hartmann, & Wolf, 2008). Therefore, in our first study we used 10-minutes public presentation as an acute stressor to assess the stress response.

A rigorous assessment of cortisol is critical in this type of studies as well. However, when cortisol reactivity has been assessed, only a pretest and posttest measure has been typically used. Many studies used the first saliva sample, before the stressful situation, as a pretest condition, without taking into consideration that this cortisol level value may be influenced by anticipation. Therefore, in both stressful and basal cortisol studies we include baseline measures of cortisol a week before to the specific situation (acute or daily stress), to control the effect of anticipation or novelty respectively.

Additionally, many researchers exploring personality and cortisol activity have relied on samples consisting of either adults or very young children (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). We consider that late adolescence represents the ideal time for the evaluation of cortisol, because is the period before personality and cortisol patterns have been further affected by a long history of major life events

or psychopathology, permitting a relatively “clean” observation of the inter-correlation pattern between cortisol and personality traits (Hauner et al., 2008). For that reason this population has been our target sample in the three studies.

In sum, we have used both personality questionnaires, naturalistic academic settings to assess acute stress, including baseline cortisol measures in all studies, and focused on young population. In addition, in our second and third study, we used both electronic monitoring devices and manual registration of the exactly time when each saliva sample was collected to increase compliance. We included as well several control features that could be affecting cortisol secretion, such as stress level, food or drinks intakes, smoking status, medication, etc. Finally, sample characteristics were also controlled: similar number of participants in relation to sex, participant’s homogeneity in age and education, and careful selection of extreme high versus low scores in neuroticism.

6. APMR as an intervention to reduce psychological and physiological stress

Relaxation is a behavioural intervention used to release bodily tensions and promote positive feelings to deal with stressors. Relaxation is one of the primary components of all kinds of meditation, which induce a pleasant and deep relaxed state of body and mind (Hussain & Bhushan, 2010). More importantly, relaxation techniques are a central component in most comprehensive programs for the treatment of stress-related problems (Benson, Beary & Carol, 1974), which is the focus of our study.

Jacobson (1938) discovered that by systematically tensing and relaxing different muscle groups and by learning to focus on and discriminate between the resulting sensations of tension and relaxation, a person may almost alleviate muscle contractions and experience a feeling of deep relaxation (Bernstein & Borkovec, 1973). Progressive Muscle Relaxation (PMR) training produces extraordinarily low levels of muscle tension, and that patients suffering from a variety of psychological and somatic disorders experience significant relief when they practice this technique (Lehrer, 1978). However, even when PMR was originally conceived by Jacobson, requiring more than 40 individual sessions, was not until 1973 when Bernstein and Borkovec streamlined Jacobson's training approach and specified in a step-by-step manner the procedures for administering relaxation training to clients in 8 to 12 sessions. Bernstein and Borkovec provided a protocol called Abbreviated Progressive Muscle Relaxation (APMR), that involves shorter sessions and only 16 muscle groups rather than the nearly 30 indicated by Jacobson.

APMR is one the most techniques used with a long history of studies showing positive results based on clinical population with several diseases and sub-clinical population. Certainly, APMR has shown to produce effective psychological changes which include, reduced anxiety levels (Rausch, Gramling, & Auerbach, 2006), decreased perceived stress (Emery, France, Harris, Norman, & Van Arsdalen, 2008) and increased feelings of relaxation (Pawlow & Jones, 2002), and physiological benefits, such as decreased cortisol levels (Krajewski, Saverland, & Wieland, 2011; Pawlow & Jones, 2002, 2005).

Due to its historical pedigree, its well-defined and easily taught procedures, its comparatively low cost delivery, and some evidence of efficacy, APMR has become justly popular as a promising stress-reduction intervention. Given such promise, it is an ideal candidate for more detailed scrutiny of its efficacy. However, mostly of the existing studies that assess changes in cortisol present some limitations in their designs. For instance, using only a single session of APMR for 20 min and just one cortisol measure taken immediately before and after (Dolbier & Rush, 2012; Pawlow & Jones, 2005), or applying two APMR sessions spaced seven days apart, but again assessing cortisol immediately before and after each session (Pawlow & Jones, 2002), or using good cortisol measures but very small sample size (n=7) in both control and experimental groups (Krajewski et al., 2011). So, even when in essence all these studies are positive in suggesting that cortisol decreases immediately before and after APMR sessions (Dolbier & Rush, 2012; Pawlow & Jones, 2002, 2005) or during a six-month period of daily practice (Krajewski et al., 2011), none of these studies examine changes in measures designed to be stable estimates of prevailing cortisol levels, such as AUC, in a meaningful period one-week before and after a well-controlled intervention.

Therefore, in our last study, a carefully constructed cortisol measure (TCS) and an appropriate self-report (SRLE) was used in order to provide a robust physiological and psychological markers of stress. We investigated whether 5 consecutive days of APMR training impacts on 'prevailing' levels of stress comparing one-week before and one-week after the intervention, rather than just its efficacy in reducing spot measures of stress from start to finish a single session of APMR. We also examine whether the efficacy of this intervention may be modulated by neuroticism.

Chapter 2

Cortisol response under a stressful situation: Public speaking

Introduction

Cortisol, end product of the HPA-axis, follows a circadian rhythm (24 hours) of secretion with a significant peak in the first 0.75h after awakening allowing us to face daily activities. However, this basal rhythm is supplemented by episodic cortisol reactivity under stressful situations. Current research shows that university is a very stressful time for at least 50% of the student body (Regehr, Glancy, & Pitts, 2013). Potential stressors derive from the need to adjust to heavy academic demands and the need for students to immerse themselves in a novel social network (Dolbier & Rush, 2012). Public speaking is one of the most frequent stressors students experience during their academic training and is a proven social stressor used to assess cortisol reactivity. Finally, we have described previously that personality plays a significant role in the way people react under similar stressful conditions, and therefore some of these traits are associated with differences in cortisol response to challenge. However, in prior research several inconsistencies have been found in the relationship between personality and cortisol reactivity that might partly reflect some methodological issues.

Therefore in our study we wanted to control several factors that in our opinion might help to clarify some of these inconsistencies. First, in order to increase the validity of personality assessment, we used both EPQ-R and NEO-FFI questionnaires. Second, we used a 10-minute-long class public presentation to evaluate cortisol response, as a students' real life stressor (opposite to laboratory stressors). Third, in order to increase methodological rigor we include additional baseline cortisol sampling taken on a previous day to the stressful situation but at the

same time that the public speaking samples were taken. Finally, because late adolescent represents the period before personality and cortisol patterns have been further affected by a long history of major life events or psychopathology, will permit us a "clean" observation of the relationship between cortisol and personality, we focused on this group population.

Summing up, the aim of this first study was to compare salivary cortisol secreted by a group of university students in two different conditions: non-stressful and stressful. Differences in cortisol reactivity will be correlated with personality traits. Our first hypothesis was: public speaking (stressful condition) will increase cortisol secretion compared with a daily academic activity (baseline condition). We anticipated also that cortisol reactivity would be positively related to Openness, Agreeableness and Conscientiousness, and negatively related to Extraversion, Neuroticism and Psychoticism.

Method

Participants

Seventy-five students from University of Balearic Islands (UIB) participated in our study, 56 were woman and 19 were men (mean age = 20.9 years). All were volunteers enrolled in a Psychology of Personality class at UIB across three academic intakes with no exclusion criteria except the provision of both complete questionnaire and complete cortisol data.

Instruments

- Participants completed the 83-items of the Spanish version of the Eysenck Personality Questionnaire-Revised (EPQ-R; Eysenck & Eysenck, 1997), using a dichotomous yes/no response. This questionnaire provides three major personality dimensions: Extraversion (E), Neuroticism (N) and Psychoticism (P).
- Participants also completed the revised short-form version of the Spanish NEO Five Factor Inventory (NEO-FFI; Costa & McCrae, 1999). This inventory consists in 60-items of which measure the five major personality dimensions: Neuroticism (N), Extraversion (E), Openness (O), Agreeableness (A) and Conscientiousness (C). Participants responded on a 5-point Likert scale from 0 (*totally disagree*) to 4 (*totally agree*).

Procedure

At the start of the semester, students completed the EPQ-R and the NEO-FFI scales, and after providing written informed consent, four salivary samples were taken to assess cortisol levels during the late afternoon (between 4 and 6 pm). Two baseline samples were taken on a day during a class period where the student had no paper, examination, or class participation at approximately the same time when the samples were taken during the stressful day. Two stress samples were taken for each participant during a class performance, where the student providing the sample had to give a 10-minutes presentation to the class and answer classmates' questions after the presentation. The first sample was taken just prior the presentation and the second 30-minutes after (see Figure 1).

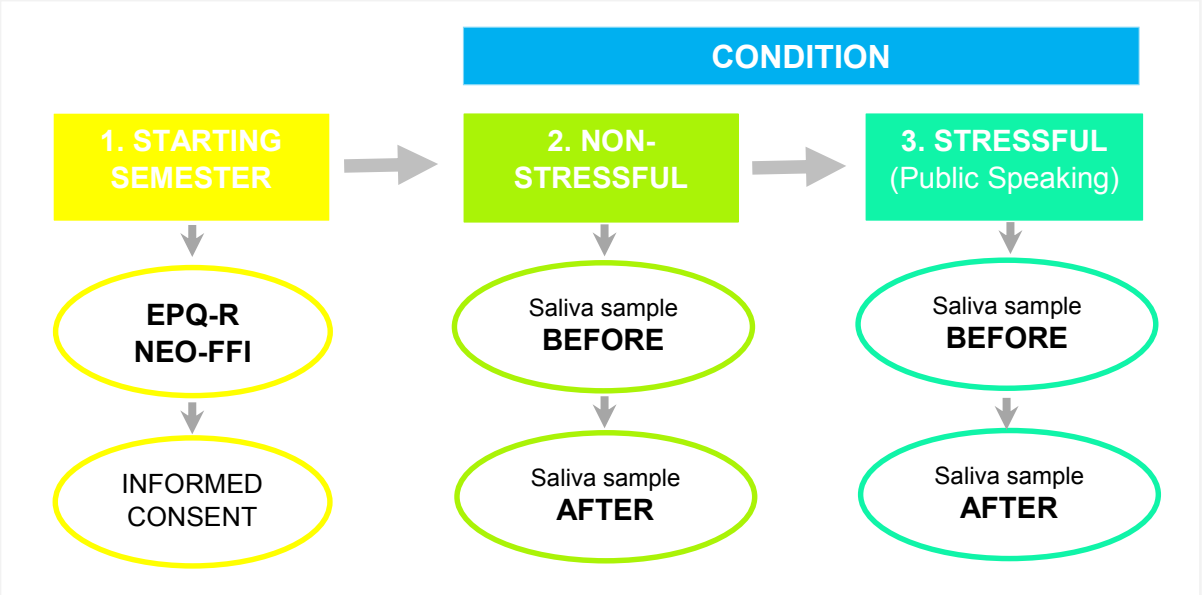


Figure 1. Procedure and saliva sample collection.

Salivary cortisol samples were collected with a cotton swab chewed for one minute, stored in a capped plastic vial ("Salivette" Sarstdet Inc.). These samples were centrifuged at 3000g for 3 minutes, and then the filtrates were stored frozen at -80ºC until analysis. Before analysis, the samples were thawed, mixed, centrifuged and analyzed without pre-treatment. To reduce error, all samples of each participant were analyzed in one assay. Salivary cortisol was measured using a modification of the Bayer ADVIA Centaur cortisol assay, a competitive direct chemiluminescence's immunoassay that uses a rabbit polyclonal antibody. Endogenous cortisol contained in the samples competes with a cortisol labeled with acridinium-ester for the binding sites of the anti-cortisol rabbit polyclonal antibody-coated paramagnetic particles. The intra- and inter-assay coefficients of variation were less than 10% for 0.30 µg/dL of cortisol.

Statistical analysis

A two-way repeated measures analysis of variance (ANOVA) was used to test the effects of stress/baseline and first/second sample of cortisol, and Pearson correlations were carried out to examine associations between cortisol and personality measures.

Results

Table 1 provides a summary of descriptive statistics for personality dimensions and cortisol levels at the two conditions. Distributions of cortisol data were substantially positively skewed, so values were analyzed as logarithmically transformed with one extreme outlier removed.

Table 1

Descriptive statistics for personality traits and cortisol measures

	Minimum	Maximum	*M*	*SD*
NEO-FFI Neuroticism	1.00	44.00	23.36	8.63
NEO-FFI Extraversion	6.00	46.00	31.37	8.37
NEO-FFI Openness	11.00	48.00	31.91	7.43
NEO-FFI Agreeableness	12.00	43.00	28.59	6.21
NEO-FFI Conscientiousness	6.00	40.00	27.56	7.30
EPQ-R Extraversion	6.00	19.00	13.13	3.30
EPQ-R Neuroticism	2.00	23.00	12.09	5.01
EPQ-R Psychoticism	0.00	19.00	5.03	3.27
CortBL1	0.02	2.19	0.55	0.38
CortBL2	0.09	2.00	0.44	0.28
CortPS1	0.24	3.05	0.82	0.60
CortPS2	0.10	2.40	0.71	0.47

Note: Cortisol levels are presented in µg/dL. CortBL1: Baseline cortisol at time 1 taken at the beginning of an ordinary class. CortBL2: Baseline cortisol at time 2 taken at the end

of that class. CortPS1: Stress cortisol at time 1 taken at the beginning of a class where participants had to speak in public. CortPS2: Stress cortisol at time 2 taken at the end of the class.

Two-way repeated-measures ANOVA was performed with the first and second cortical assessments on the same day and non-stressful (baseline) vs. stressful (stress) condition (see Figure 2). A significant main effect was obtained for the non-stressful /stressful factor ($F(1, 73) = 34.99$, $p = .000$, partial $\eta^2 = .32$, non-transformed $M_{\text{no-stress}} = .50$; $M_{\text{stress}} = .77$), also for first (before) vs. second (after) sample ($F(1, 73) = 21.20$, $p = .000$, partial $\eta^2 = .22$, non-transformed $M_{\text{first}} = .69$; $M_{\text{second}} = .58$). There was no interaction effect ($F(1, 73) = 1.15$, $p = .29$, partial $\eta^2 = .02$).

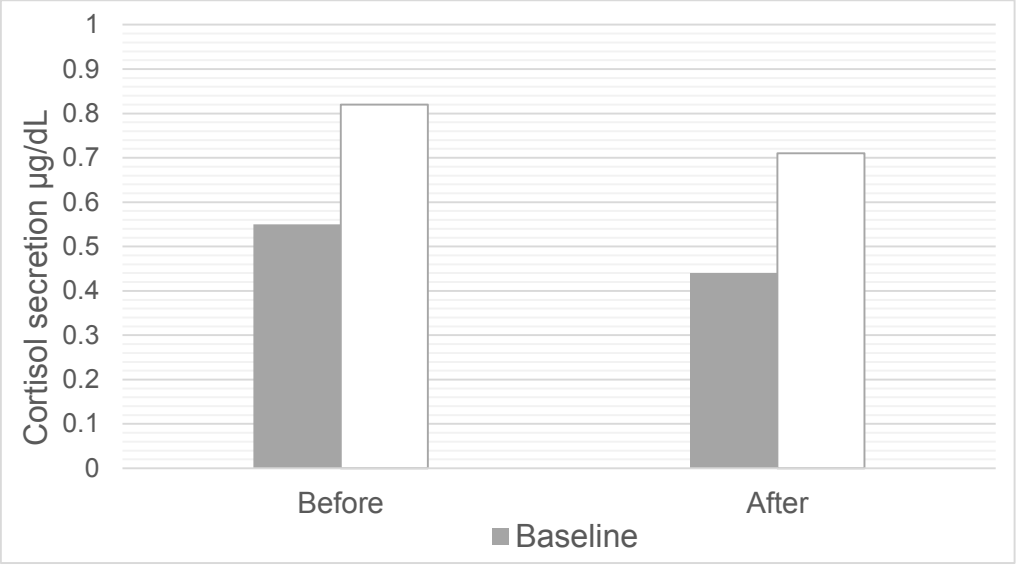


Figure 2. Cortisol secretion before and after the presentation in baseline and stressful condition.

We examined next correlations between personality and cortisol measures. All personality measure distributions were plausibly normal except for

Conscientiousness and for Psychoticism, which were negatively and positively skewed respectively. Both, therefore, underwent a square root transformation (with prior reflection for negative skew). Pearson correlation coefficients were run between cortisol scores and the personality variables. The two cortisol measures taken at the beginning and at the end of both baseline (CortBL) and stress (CortPS) conditions were averaged and examined to reflect mean cortisol on each day; both had significant positive skewness and were log transformed. The difference score, reflecting the cortisol response to the stressor, was calculated by subtracting the baseline average from the stress day average. The distribution of this variable was plausibly normal without transformation. The correlations between personality variables and the three cortisol scores appear in Table 2. Neuroticism scores on the EPQ-R and NEO-FFI questionnaires were positive and highly correlated (.73), as it was Extraversion (.61). Psychoticism (EPQ-R) and Conscientiousness (NEO-FFI) were negatively correlated (-.48). Cortisol response to stress was related to Conscientiousness (.31) and inversely to Psychoticism (-.31). No significant correlations were found between personality and the average scores at baseline and stressful condition, except a small negative relationship between baseline cortisol and the personality trait of Conscientiousness (-.23).

Finally, although the sample was primarily females, and therefore there were not enough males to make a powerful analysis, it is possible that being male or female may have some influence on the cortisol response. However, multiple regression analyses controlling for gender and also for year of recruitment into the study did not significantly alter the univariate effect sizes reported above.

Table 2

Pearson product moment correlations for cortisol and personality dimensions

	NEO Neuroticism	NEO Extraversion	NEO Openness	NEO Agreeableness	sqrNEO Conscientiouness	EPQ Extraversion	EPQ Neuroticism	Log CortLB	Log CortPS	Cort PS-BL
NEO Neuroticism								-.06	-.09	-.14
NEO Extraversion	-.08							-.16	-.14	-.02
NEO Openness	.12	.23*						-.01	.08	.01
NEO Agreeableness	-.06	.20	.02					-.01	.06	.08
Sqr(reflex)NEO Conscientiousness	-.39***	-.03	-.11	.16				-.23*	.02	.31**
EPQ Extraversion	-.22	.61***	-.01	.01	-.02			-.07	-.13	-.07
EPQ Neuroticism	.73***	-.11	-.01	-.04	-.14	-.34**		-.03	.13	.15
EPQ sqr Psychoticism	.14	.14	.38***	-.30**	-.48***	.04	.02	.02	-.19	-.31**

Note: logCortBL = log Baseline cortisol. LogCortPS = log Public Speaking cortisol. CortPS-BL = cortisol changes between baseline and public speaking scores. sqrNEO Conscientiousness = square root of reflex of NEO Conscientiousness scores. sqrEPQ Psychoticism = square root transformation of EPQ Psychoticism scores.

* $p < .05$, ** $p < .01$, *** $p < .001$.

Discussion

Our results confirm that public speaking increased significantly cortisol secretion when compared with a non-stressful academic activity, validating our task as an effective stressor to trigger the HPA-axis activity. Our second hypothesis is partly confirmed: Conscientiousness and Psychoticism appear to be the only personality traits related with cortisol secretion levels, both playing a relevant role in the response to stress. Particularly, conscientiousness was associated with an enhanced cortisol response to stress, which it has been associated with a better adaptation behavioural style to stress conditions (Roy, 2004). On the contrary, psychoticism was related with a blunted (plain) cortisol response. Blunted cortisol reactivity may reflect poorer response to the stressful demands, could be associated with certain underlying deregulation of the HPA system (Phillips, Carroll, Burns, & Drayson, 2005), and persistent aggression (McBurnett, Lahey, Rathouz, & Loebeer, 2000). Indeed, blunted cortisol reactivity has been implicated in the development of psychopathic personality traits (O'Leary, Loney, & Eckel, 2006).

Chapter 3

Cortisol response under daily situations in participants with extreme scores on neuroticism

Introduction

So far, our results have shown that Conscientiousness is directly and Psychoticism is inversely associated with cortisol reactivity produced by an acute stressor as public speaking. But, what happens with cortisol when we are not facing a particular stressful situation? Are personality traits also associated to daily cortisol secretion?

The "wear and tear" model of stress (McEwen & Wingfield, 2010) introduced the concept of allostasis, maintaining stability through changes, to refer to the neural, endocrine and immune activation with which our body reacts when facing short-term stress. On the contrary, they used allostatic load to allude to the price the body pays for this repetitive effort over time resulting in chemical imbalances and perturbation in (e.g. cortisol) diurnal rhythms (McEwen, 2000). According to this model some people have to make more effort than others to maintain homeostasis.

Neuroticism (N) is a relatively stable trait that can be defined as the predisposition to respond with intense emotional reactions to psychological stressors (Lahey, 2009). In fact, individuals high on N perceive and have more stressors, respond exaggeratedly to them (called hyperractivity; Suls, 2001), and require significantly more time to recover (Suls & Martin, 2005). Therefore, we would expect that those high on Neuroticism (N) would normally exhibit higher levels of the stress-linked hormone cortisol, reflecting greater frequency and intensity of HPA stimulation from the psychological domain.

Several studies have tried to explain the relationship between daily cortisol secretion and neuroticism with surprisingly inconsistent results. Some founded high-

N associated with higher levels of daily cortisol (AUC; Nater, Hoppmann, & Klumb, 2010) and elevated CAR (Portella, Harmer, Flint, Cowen, & Goodwin, 2005); with less CAR flexibility, when week days and weekends are compared (Mikolajczak et al., 2010), and with flatter diurnal cortisol slope, but only in high-N males (Hauner et al., 2008). Nonetheless, other studies have not found a significant relationship between N and the CAR (Chan et al., 2007; Hill et al., 2013; Riese et al., 2009; Van Santen et al., 2011), cortisol slope (Ferguson, 2008), or awakening/evening cortisol (Gerritsen et al., 2009).

Some methodological issues might explain these inconsistent results. First, differences in demographic characteristics of sample populations such as sex and age. Second, diversity in the scales used to measure N. Third, differing statistical power reflecting different samples sizes. However, crucial for cortisol measurement are factors related to the adequacy of timing and frequency of salivary cortisol samples along the day, synchronization (or not) of cortisol sampling times in relation to awakening time, number of sampling days over which daily levels are averaged to obtain valid “typical” values over time, and finally, adequacy of procedures to ensure participants strict adherence to protocol in relation to time sampling, which is essential if saliva collection is carried out by participants.

The data presented here were obtained from a large research project studying individual differences and various interventions on diurnal cortisol profiles of students. In this study, we have applied sufficient methodological rigor to achieve valid results regarding expected associations between N and diurnal cortisol secretion. We wanted 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 four year cohorts, we recruited extreme N score

participants, we collected the diurnal cortisol profile over two days rather than a single day, and we controlled timings of saliva samples using an electronic monitoring device (MEMS track-cap, see Appendix).

Our hypothesis is that high-N participants would have elevated diurnal levels of cortisol compared to low-N individuals. We also wanted to look at the associations between N and two dynamic parameters of the diurnal cortisol profile, the cortisol awakening response (CAR) and the subsequent fall over the day (AUC0.75-12h). In respect of diurnal fall, we expected that high N might be associated with a flatter fall. In relation to the CAR, due to the high inconsistency in the literature, we did not consider any effect a priori.

Method

Participants

NEO-FFI personality inventory data was collected over four academic years from 3.843 first-year students of University of Balearic Islands (mean age = 20.87, 62% female). Fifty-eight percent (N = 2202) wanted to continue collaborating. From those, 883 participants were selected by their extreme high and extreme low scores on Neuroticism, based on NEO-FFI 15th and 85th female and male percentiles (Costa & McCrae, 1999), and invited to participate in the next study stage consisting in a demanding two-day protocol involving careful assessment of the diurnal cortisol cycle. Of these, 185 initially agreed to participate, but 67 could not continue (e.g., time constraint, illness, lack of commitment to whole study design, 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 included 113 participants (see Figure 1 below).

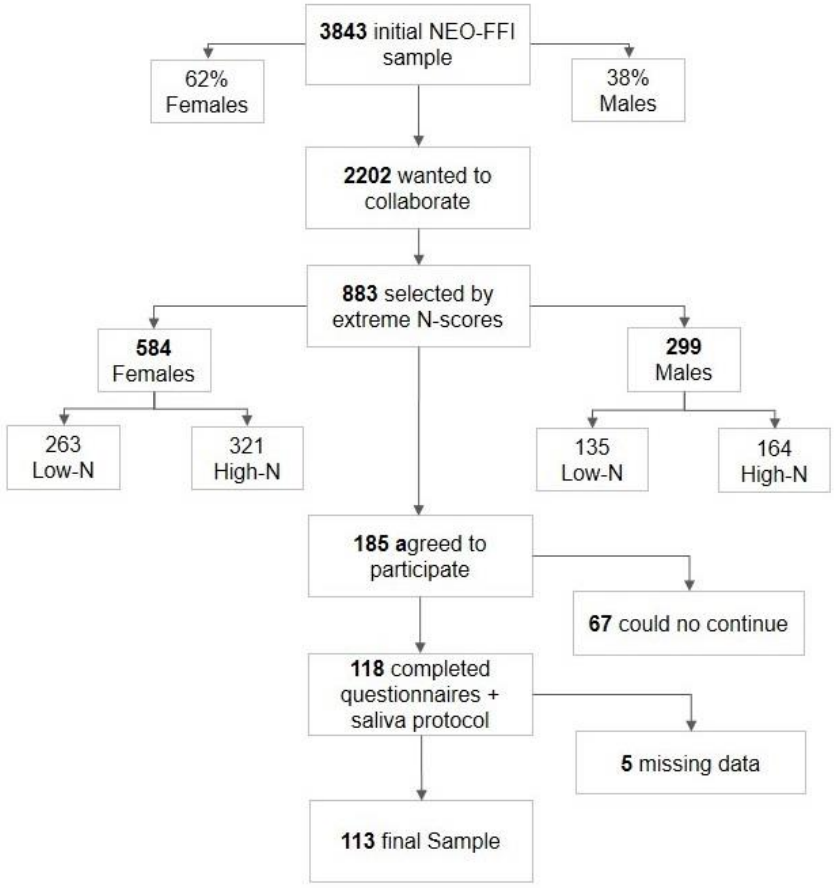


Figure 1. Diagram showing the participants flow through each stage of our study.

Instruments

Personality measures

Neuroticism: NEO-FFI (Costa & McCrae, 1999) personality inventory was used to assess the trait of neuroticism with the 12-item subscale (score range: 0 to 48).

Participants responded on a 5-point Likert scale from 0 (strongly disagree) to 4 (strongly agree). Internal consistency values for our sample were .83 (original values from .74 to .89; Costa & McCrae, 1992, 1995). Participants also completed the 24-item Neuroticism subscale of the Eysenck Personality Questionnaire-Revised (EPQ-R; Eysenck, Eysenck, & Barret, 1985) using a dichotomous yes/no response. Original internal reliability coefficient for the Neuroticism scale was .85 for females and .88 for males. in our subsample, higher internal Neuroticism consistency values were founded for females (.936) and males (.943).

Cortisol assay procedures

Student saliva samples were collected with a cotton swab chewed for 1 minute, stored in a capped plastic vial ("Salivette" Sarstedt Inc.), centrifuged at 3000g for 3 minutes, and then the filtrates frozen at -80°C until analysis. Samples were thawed, mixed, centrifuged and analysed without pre-treatment. Salivary cortisol was measured using a modification of the Bayer ADVIA Centaur cortisol assay, a competitive direct chemiluninescence immunoassay that uses a rabbit polyclonal antibody. Endogenous cortisol contained in the samples competes with cortisol labelled with acridinium-ester for the binding sites of the anti-cortisol rabbit polyclonal antibody-coated paramagnetic particles. The intra- and interassay coefficients of variation were less than 10% for 0.30μg/dL of cortisol.

Adherence electronic monitoring

Following Kudielka, Hawkley, Adam and Cacioppo (2007) protocol, timing cortisol adherence was measured by MEMS Track Caps device (AARDEX, Ltd.,

Zug, Switzerland). Participants took an absorbent cotton swab at each assigned sampling time from a plastic bottle with a microchip lid that recorded the time of each opening (see Appendix). After collecting a saliva sample, participants stored the swab in a pre-labeled plastic tub (Saliventte, Sarstedt, Barcelona, Spain). Participants completed the information protocol each time the plastic bottle was opened to compare with the MEMS time (Broderick, Arnold, Kudielka, & Kirschbaum, 2004). In addition, at the briefing session participants programmed their mobile phones to beep at the established times in order to enhance compliance.

Procedure

As shown in Figure 2, first year UIB students completed the NEO-FFI personality inventory assessing Neuroticism and gave informed consent in their classes. Those interested in continuing the collaboration provided their e-mail address and mobile phone number.

People selected by their extreme scores in N were called and invited to continue in further steps of the study. During the first meeting was explained the research aims and tasks, and participants were instructed how to collect saliva samples, to use the MEMS track and to fill accurately the protocol sheet. In addition were asked to set their mobile phone alarms in order to increase the adherence to protocol required sampling times. Students were also requested to complete the EPQ-R personality questionnaire to validate the scores obtained by the NEO-FFI N-subscale used to select our extreme groups.

Five samples were collected synchronized to awakening: at awakening, and 0.75h, 2.5h, 8h, 12h after awakening, on two days of a specific week (Tuesday and

Thursday) following the McArthur Network protocol on salivary cortisol measurement (Stewart & Seeman, 2000). In addition, 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. following Adam and Kumari (2009) recommendations to control for covariates (see Appendix). This protocol provided us with relevant information to check for confounding effects and was used as well to assess cortisol sampling time compliance.

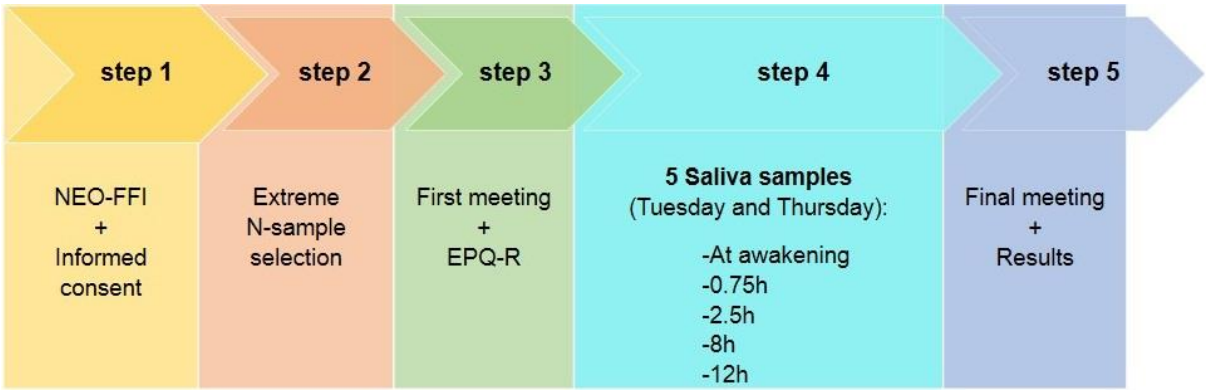


Figure 2. Study design and procedure of saliva sample collection.

Treatment of data

In line with previous studies, analyses were conducted on composite cortisol measures encapsulating those key elements, which make up the diurnal cortisol cycle. Raw data for all participants is plotted against time in Figure 3, and shows the typical brief and dramatic CAR in the period immediately following awakening, followed by an equally dramatic but slower diurnal fall to values 12h after awakening.

The nature of the composite measures can also be gleaned in Figure 3 from the enclosed areas denoted by capitalized letters “A” to “F”.

Individual differences in levels of cortisol were assessed as areas under the curve (AUC) with respect to time. AUC0h-12h was computed comprising the areas A+B+C+D to estimate total cortisol secretion over the 12h period from awakening (see Figure 3). In addition two components of this total secretion were separately examined: 1) total cortisol secreted during the 0.75h period following awakening (AUC0h-0.75h) comprising areas A+B, when the CAR typically occurs, and 2) cortisol secreted during the remainder of the 12h period (AUC0.75h-12h) comprising areas C+D in Figure 3.

In terms of dynamic movement rather than levels, the CAR itself can be defined as the AUC of increase from the first awakening sample during the CAR period (area “A” in Figure 3) and is commonly abbreviated AUCi. Since we had only two measures: 0 (awakening) and +0.75h post awakening, AUCi in this case is synonymous with the simple difference between 0 and +0.75h samples. While the fall in cortisol from 0.75h to 12h (areas E+F in Figure 3) gives a measure of decline from typical CAR peak, it is generally considered that the CAR is independent of underlying diurnal fall, and a preferred measure of diurnal fall (used here) is slope coefficient from 0-12h excluding CAR period values (in this case at 0.75h). All three AUC cortisol composite measures were significantly positively skewed and for all inferential analyses were logarithmically transformed so as to normalize distributions and windsorized to 3sd to minimize outlier effects. Slope coefficients were so extremely positively skewed that the measure was transformed to an ordered quartile scale.

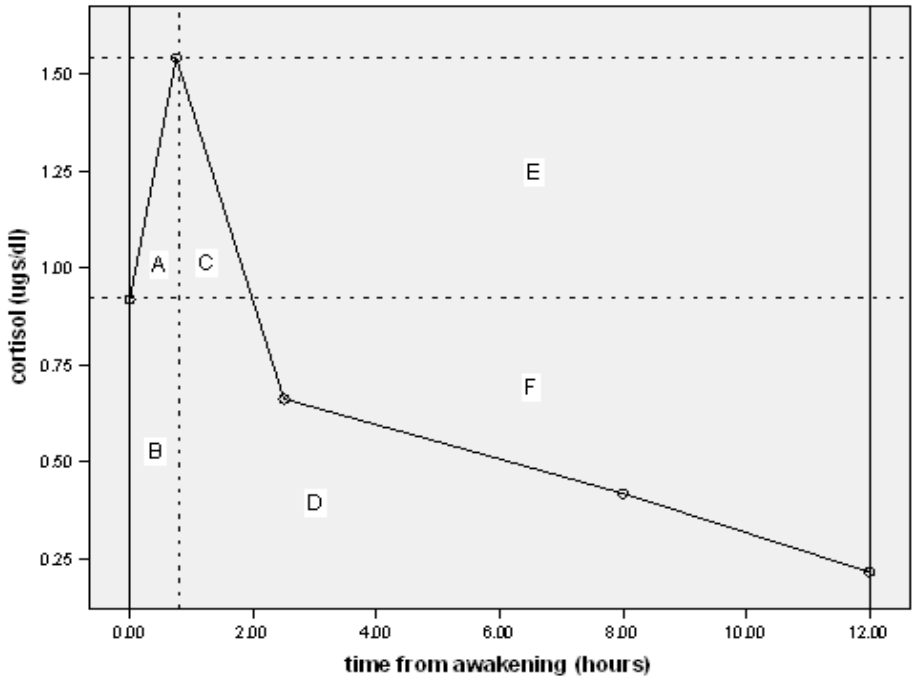


Figure 3. Mean cortisol of all participants plotted against time of day (relative to individual awakening times). For explanation of areas denoted by capitalized letters, see text above.

Effects on outcome measures were examined using mixed regression modelling, an approach considered most appropriate for multilevel designs incorporating multiple repeated measures over time with fixed and random parameters (Blackwell et al., 2006). The approach has been used in particular specifically to model dynamic aspects of the diurnal cortisol cycle (Ranjit et al., 2005; Adam, 2006; Smyth, Clow, Thorn, Hucklebridge, & Evans, 2013), and in this case enabled us to examine over the two study days the effects of earlier versus later awakening time on cortisol secretion. All model dimensions specified participant identity ("subject variable") as a random variable and the following fixed effects for all analyses: intercept, awakening time (covariate), and high- versus low-N, study group, sex, age, smoking status and study day (repeated measure) as factors.

Since study days were limited to two, models assumed a compound symmetry covariance structure. In analyses of dynamic "change" measures (CAR and slope of diurnal fall) initial awakening level of cortisol was included as an additional covariate. Just over half of participants were aged 17-19, while the remainder constituted a long tail of older students in their 20s and 30s. Age was therefore best modelled as a dichotomous variable reflecting this binary distributional characteristic. Awakening times were grand-mean centred in the primary analyses, thus examining the effect of absolute "clock-time" of awakening on cortisol measures. However we also performed analyses of participant-centred awakening times to examine specifically the purely within-participant effect of whether changes in awakening time from day 1 to day 2 were related to respective cortisol changes.

It is known that use of MEMS when participants are informed that sampling time is being recorded reduces overall timing error considerably. Accordingly the data from 226 cases (113 participants x 2 days) was first modelled without recourse to checks on sample timing and these results are the ones presented in the results section. However MEMS information was used in re-runs of these analyses to control statistically for real sampling time being on average earlier or later than protocol instructions. In the case of AUC0h-0.75h and the CAR itself, where even greater timing accuracy is essential, analyses were re-run including a factor which indicated whether a CAR time interval was accurate +/- 10 min, or earlier, or later by 10 min that the required 45 minutes. In all these analyses, effects were checked for comparability of magnitude and significance with effects found to be significant in the primary analyses.

Results

Table 1 compares Neuroticism groups on age, sex, smoking status, and mean awakening time over the two days of the study. The low neuroticism group was slightly but significantly older. No other differences were significant.

Table 1

Socio-demographic variables in low and high neuroticism groups

	Neuroticism	
Variable	Low (*n* = 49)	High (*n* = 69)
Age (*M, SD)*	22.78 (6.67)	20.42 (3.69)
Sex (F/M)	30 / 19	47 / 22
Smokers (%)	18.40	20.30
Awakening time (hh:mm) (*M, SD*)	8:08 (1:33)	8:00 (1:18)

Neuroticism and cortisol secretion

Effects and associated significances of all mixed regression modelling are presented fully in Table 2. Our expectations that those high on N would tend to have higher secretion of cortisol were confirmed. The effect was significant ($p < .008$) for the analysis of total secretion over 12h from awakening (AUC0h-12h). However separate analysis of the very high secretion present in the brief 45 minute CAR period (AUC0h-0.75h) immediately following awakening indicated no secretion difference whatsoever ($p < .846$) between high and low N groups. Analysis of the rest of the 12h period (AUC0.75-12h), omitting the CAR period, re-confirmed the

finding of significant difference (p < .007). Clearly the overall 12h effect was exclusively driven by secretion differences in the overwhelmingly greater part (11.25h) of the total study period after the CAR.

Table 2

Main effects for neuroticism and potential confounding / extraneous variables on cortisol measures. Cohort group has numerator df = 5, all other factors and covariates of awakening time and level have df = 1

Effect	df	F	*p*		Df	F	*p*
AUC0h-12h				**AUC0.75h-12h**			
Intercept	208.354	188.914	.000	Intercept	208.736	172.433	.000
Neuroticism	103.579	7.427	.008	Neuroticism	103.606	7.614	.007
Cohort group	103.399	9.410	.000	Cohort group	103.425	8.473	.000
Sex	103.768	8.973	.003	Sex	103.797	9.585	.003
Smoker	103.003	.109	.742	Smoker	103.032	.116	.734
Study day	111.800	1.241	.268	Study day	111.862	1.519	.220
Age	103.796	1.496	.224	Age	103.825	1.238	.268
Awakening time	207.662	13.034	.000	Awakening time	208.104	13.540	.000
AUC0h-0.75h				**Awakening level**			
Intercept	209.641	124.756	.000	Intercept	210.501	27.640	.000
Neuroticism	104.099	.038	.846	Neuroticism	102.889	.001	.973
Cohort group	103.915	11.711	.000	Cohort group	102.703	3.993	.002
Sex	104.297	.523	.471	Sex	103.100	.057	.812
Smoker	103.531	.006	.936	Smoker	102.334	.430	.513
Study day	112.449	2.414	.123	Study day	111.384	1.585	.211
Age	104.327	3.869	.052	Age	103.129	1.660	.200
Awakening time	209.168	.248	.619	Awakening time	210.220	4.031	.046
CAR				**Slope**			
Intercept	209.133	116.087	.000	Intercept	201.344	13.848	.000
Neuroticism	103.928	.149	.700	Neuroticism	102.990	2.464	.120
Cohort group	105.469	9.228	.000	Cohort group	100.707	2.547	.033
Sex	104.118	.822	.367	Sex	103.205	5.793	.018
Smoker	103.474	.551	.460	Smoker	102.557	.029	.864
Study day	113.519	.706	.403	Study day	113.265	.179	.673
Age	105.220	2.668	.105	Age	104.357	.367	.546
Awakening time	208.927	3.032	.083	Awakening time	208.831	.671	.414
Awakening level	209.206	279.206	.000		204.201	133.849	.000

Neuroticism groups did not differ in initial cortisol levels recorded at awakening time itself ($p < .973$), nor in the magnitude of the CAR rise ($p < .700$), nor in the slope of diurnal fall ($p < .120$), although the trend in these data were in line with expectation of steeper diurnal fall for the low- N groups. Mean predicted diurnal profiles are plotted in Figure 4a for high and low N groups. The plot clearly shows the difference between the groups in cortisol secretion only emerges after the CAR period, and also shows the trend towards less steep diurnal decline in the high N group.

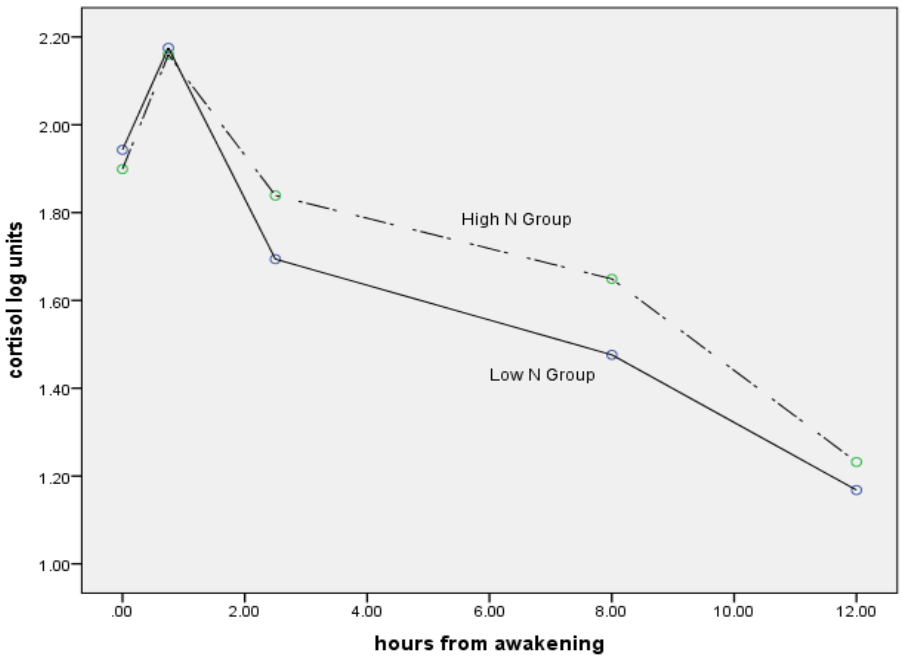


*Figure 4*a. Diurnal cortisol profiles for high- and low-N groups.

Other variables in the analysis

Potential confounding variables including demographics of age and sex were included in the analyses for control purposes. Effects an associated significances are given in Table 2. There were significant effects for sex of participant. Higher

secretion in males was evident for total diurnal secretion ($p < .003$) outside the CAR period (AUC0.75h-12h) but not in the CAR period itself. Male participants also had significantly less negative slopes indicating flatter diurnal fall ($p < .018$). Both these effects are evident in the mean predicted diurnal profiles plotted in Figure 4b.

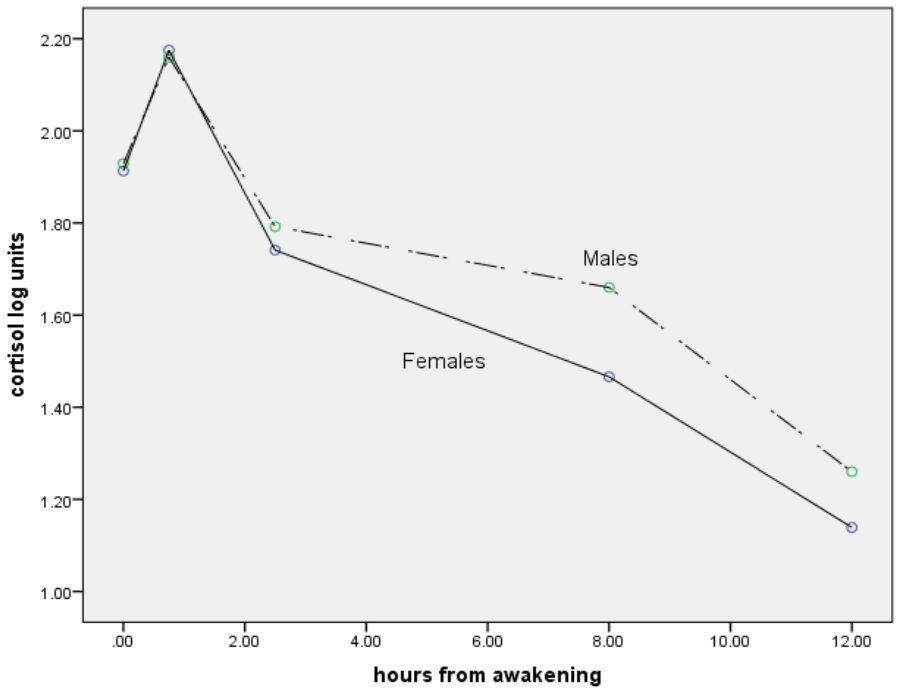


Figure 4b. Diurnal cortisol profiles for male and female participants.

Age effects were not found, but there were trends for the younger participants to have higher levels of cortisol during the CAR period ($p < .052$) and a greater CAR ($p < .105$). No differences were apparent between the two days for any cortisol measure. Covariates were however significant in a number of analyses (see Table 2). Earlier awakening time was associated with greater cortisol secretion ($p < .001$) over the 12h period excluding the CAR period (AUC0.75h-12h). Earlier awakening was not however associated with greater cortisol during the CAR period itself

(AUC0h-0.75h); indeed it was associated with lower cortisol values for the first (awakening) sample ($p < .046$).

When between and within participant awakening time effects were separated out in the analysis of AUC0h-0.75h both were significant, i.e. participants who tended on average to wake earlier in terms of absolute clock-time had higher average cortisol values than those who woke later ($p < .033$), and participants' tended to have relatively higher AUC0.75h-12h values on their own earlier rather than later awakening day ($p < .004$). Both effects are illustrated in Figure 5a and 5b, by plotting slope lines from regression predicted values of cortisol for both participants' mean awakening times (5a) and for participant-centred times (5b). The association between awakening time and initial awakening level of cortisol reported above was entirely a between-participants effect ($p < .008$).

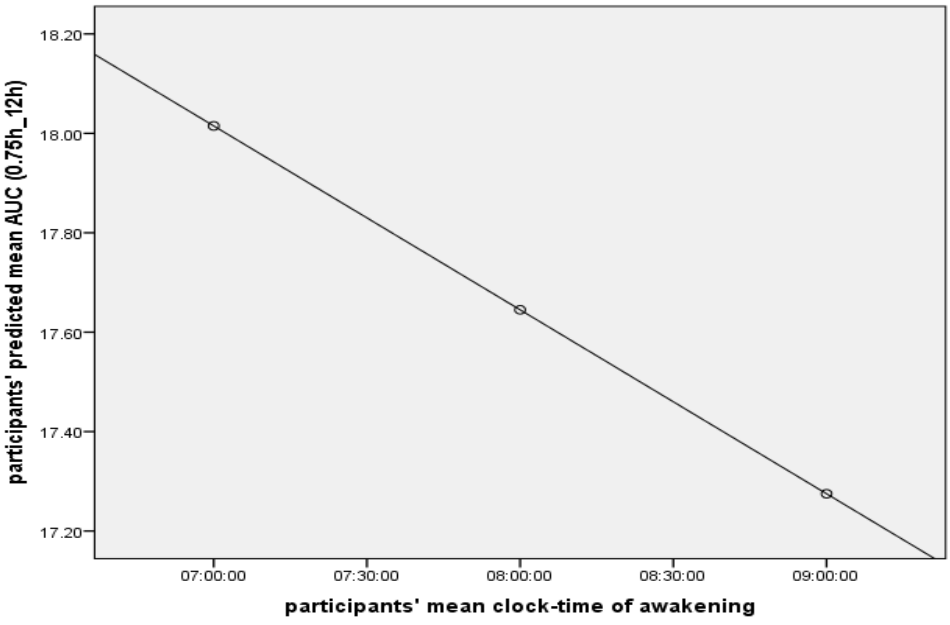


*Figure 5*a. Between participants: early "wakers" tend to have higher $AUC_{0.75h\text{-}12h}$ (b slope = -.40; s.e. = .17).

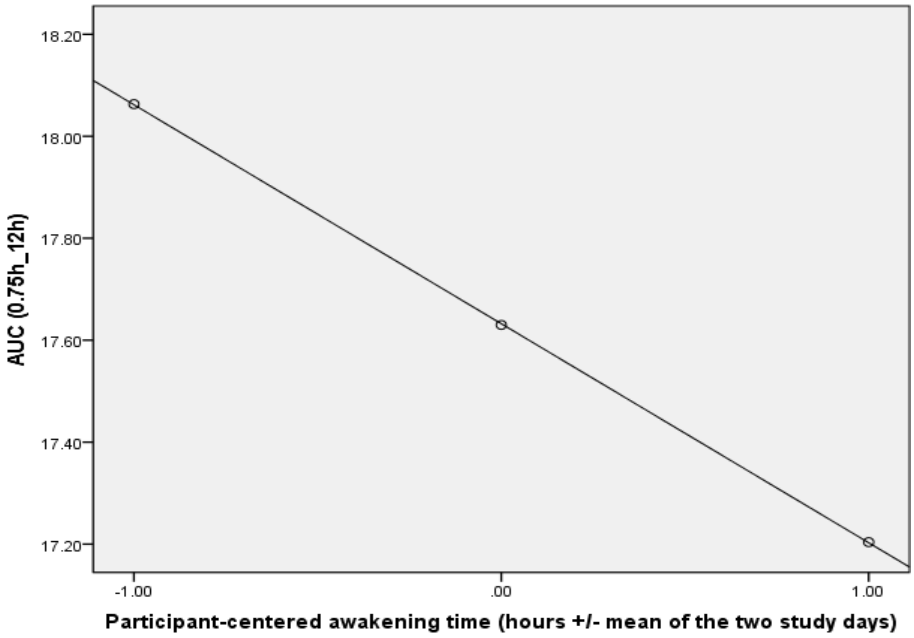


Figure 5b. Whithin participants: earlier than average awakening predicts higher $AUC_{0.75h\text{-}12h}$ (b slope = -.43; s.e. = .15)

Higher initial awakening values of cortisol were, as expected, associated with smaller CAR rises and greater diurnal fall. As expected there were highly significant mean differences in all cortisol measures except slope across different cohort groups tested in different months and years, underlining the absence of normative absolutes in salivary cortisol measurement either across different laboratories or assay batches across time, and the necessity of coding for cohort in this kind of study design (Smith et al., 2013).

Timing Accuracy and MEMs data

MEMs data were available for the vast majority (91%) of study days, and indicated that participants were generally adhering well to protocol requirements in regard to timing of saliva samples. Thus, on 88% of days the average time of collecting saliva was within 30 min of the protocol derived time. When the modelling summarized in Table 2 was repeated with the addition of a covariance reflecting time of sampling earlier or later than protocol requirement, effects reported in Table 2 were similar in terms of magnitude, and all significant effects remained significant. While control for timing did not alter principal findings, MEMs data did illuminate the potential sensitivity of cortisol measures to timing error. Lower total secretion of cortisol, for both AUC0.75h-12h and AUC0h-12h measures, was significantly associated with days where sampling had been on average later than protocol requirements (F = 5.31; df = 1,172.83; p < .022). In regard to the CAR, on 87% of days participants were accurate to within 10 min of the required protocol interval of 45 min. When a factor denoting early, on time, or late saliva sampling was added to the analysis of CAR magnitude, it was not significant and the analysis yielded similar effect sizes and led to identical significance decisions as in the Table 2 analysis.

Discussion

With respect to our hypothesis, we have demonstrated with a degree of methodological rigor, that high levels of neuroticism are linked to elevated cortisol secretion during the daytime. This predicted association between N and cortisol secretion was independent of sex and age of participant, smoking status, awakening time, and the particular day of the study. This effect was consistent throughout all

cohorts. While this association was apparent for most of the diurnal profile, it was not evident during the CAR period. These findings support several lines of evidence that the period of massive rise in the brief 0h-0.75h CAR period should be seen separate from the rest of the diurnal cycle (Clow et al., 2010), probably due to different control mechanisms and with potentially different correlates (Postnova et al., 2013; Fries et al., 2009; Smyth et al., 2013; Van Santen et al., 2011; Mangold et al., 2012; Evans et al., 2012; Evans et al., 2011).

Control variables were significantly independent predictors of cortisol secretion. Sex of participant was associated with diurnal cortisol secretion (outside CAR period), showing higher levels and flatter slopes in males. These results are in line with previous findings (Larsson et al., 2009; Hauner et al., 2008; Kudielka & Kirschbaum, 2005). A within- and between-participant significant effect was found between awakening time and cortisol where earlier waking hours was associated with greater total cortisol secretion outside CAR period. Inside the CAR, earlier awakening was associated with lower starting values of cortisol and a tendency to higher CARs, in line with previous literature (see Clow et al., 2010 for a review).

Summing up, high levels of N are associated with elevated cortisol secretion, but only after the CAR, independently of sex and age, smoking status, awakening time, and day. These results are consistent throughout four-year cohorts. In addition, the CAR period is independent of the rest of the diurnal fall supporting previous literature. Finally, sex and awakening time are significantly independent predictors of cortisol secretion: men present higher levels of cortisol and flatter slopes and; earlier wakening hours generate higher levels of total cortisol and, inside the CAR, produce lower starting cortisol values and bigger CARs.

Chapter 4

Abbreviated Progressive Muscle Relaxation to reduce psychological and physiological stress

Introduction

As we have already presented, personality traits play a significant role in individual differences in cortisol secretion. In Chapter 2, we have found that prosocial personality traits may be good predictors of an adaptive cortisol response to acute stress. On the contrary, antisocial personality traits are associated to a blunted cortisol response to public speaking and therefore could be a good indicator of impairment in stress reactivity. Chapter 3, was dedicated to demonstrate that Neuroticism is not only associated with a negative bias in attention and appraisal, increased emotional reactivity and ineffective coping, but also with elevated cortisol secretion during the daytime. Indeed, our results showed that total cortisol secretion (after CAR period) was approximately 20% higher in high N than low N participants. Reduced feedback inhibition of the HPA-axis increases cortisol levels in our system. When cortisol is constantly elevated depress our immune system, and in long-term, have been associated with several psychosomatic and psychological diseases (Wilhelm, Born, Kudielka, Scholtz, & Wüst, 2007). Therefore, in this last study we considered that Abbreviated Progressive Muscle Relaxation (APMR) could be a suitable stress-management technique to decrease psychological and physiological stress and to produce states of relaxation.

APMR it is a popular stress-reduction intervention due to its well-defined, easily taught procedures and low cost delivery, however, the findings related to its efficacy are somehow limited. In this study, an appropriate self-report measure and the same carefully constructed cortisol parameter (used in Chapter 3) were selected to provide a more detailed and intensive examination of APMR efficacy. We

investigate whether APMR impacts on prevailing levels of stress from one week before the intervention to a week following the intervention, rather than just examining its efficacy in reducing stress levels from start to finish APMR session. We also examine whether the efficacy of this intervention may be modulated by the personality trait of neuroticism.

It is known that stress occurs when external demands overload our abilities to manage them in an adaptive way. The transactional model of stress (Lazarus & Folkman, 1984; Mischel, 2009) suggests a bidirectional relationship between an individual's response and the stressful situation. We believe that some personality traits, as neuroticism, increase the degree to which a life event could be appraised as stressful.

Most of the studies that investigate stress use self-report measures. However, self-reports can lead to low reliability due to high intra- and inter-participant variability. In effect, what is stressful for one person may not be for another and two people using exactly the same score may be signifying something very different. The Survey of Recent Life Experiences (SRLE) is a hassles survey to determine accurately how much everyday stressors are affecting our psychological health (Kohn & McDonald, 1992) and has been shown to be sensitive enough in detecting individual differences and changes over time (de Jong, Timmerman, & Emmelkamp, 1996). Therefore, in this study we used the SRLE as an accurate and reliable measure of stress.

A common used objective measure in this area of research is the so-called stress hormone cortisol. However, as we have seen earlier, cortisol awakening response (CAR) is a particularly volatile and variable period within and between

participants (Smyth, Clow, Thorn, Hucklebridge, & Evans, 2013). For this reason, in this study, using methodological techniques to ensure accurate timing, we wanted to provide more reliable estimates of daily cortisol secretion by excluding the first 45 minutes post-awakening (the CAR) and averaging daily cortisol measures of two non-consecutive week days.

Use of cortisol measures as "biomarkers" of changes in stress has been often used in APMR intervention studies, however, with limited conclusions (e.g., Dolbier & Rush, 2012; Krajewski, Saverland, & Wieland, 2011; Pawlow & Jones, 2002, 2005). When larger samples sizes have been used, effects have often been restricted to immediate stress reduction, even across a single APMR session. Limited reliability and validity of cortisol measures due to the use of very few or even single "spot" measures of cortisol. In addition, where such limitations are less evident and detailed cortisol profile measurement has been taken over a more extensive APMR trial period, it cannot be generalized due to very small samples. Therefore, while studies, incorporating cortisol measures, have generally been positive and supportive in the efficacy of APMR, there is a need for studies to examine changes in measures chosen to provide stable estimates of prevailing cortisol, using adequate cortisol sampling over a meaningful period of one week before and after a well-controlled intervention. These are the aims of the study reported here.

In order to increase the reliability, we used a single fully-trained professional and member of the research team to conduct all interventions, minimizing any possible variation from APMR protocol across groups. We also incorporated for half of the participants an additional baseline measure of cortisol a week before the pre-treatment common to all participants. This permitted us to assess the degree to which any cortisol reduction following intervention might simply reflect temporal

"habituation" to the novelty rather than being a real marker of intervention efficacy. We also incorporated a measure of Neuroticism (N) in this study as a potential moderator of any intervention effects. N is characterized by vulnerability to high prevailing levels of chronic distress (Bolger & Zuckerman, 1995), has been associated with stress and is a risk factor for the development of psychopathology (Lahey, 2009; Ormel et al., 2013). In fact, as we have reported in chapter 3, we have found a clear association between high-N scores and high levels of basal cortisol secretion (Garcia-Banda et al., 2014). Finally sex, gender and smoking status were controlled for in all analyses.

Thence in this final study we wanted to explore the effect of one-week of APMR training on perceived stress and diurnal cortisol secretion in high- and low-N participants. We hypothesized that one week of intensive APMR delivered by a trained professional would be effective in reducing stress, evidenced by both self-report and cortisol measures. Furthermore we also examine whether individual difference variables, notably, neuroticism might modulate any stress reduction.

Method

Participants

First year students from the University of Balearic Islands were recruited annually into this APMR intervention study from a larger sample of students who had already provided psychometric data for research purposes and had been pre-selected according to their high (> 85th percentile) or low (< 15th percentile) NEO-FFI-Neuroticism score (Costa & McCrae, 1999). Over the course of the four years, six groups attended a week's course of APMR training. 101 student volunteers

provided complete data, including saliva samples and attendance at one-week course APMR relaxation training (see Figure 1). From this sample 66 were female (mean age = 21.18; *SD* = 5.141). Due to the fact that age was extremely skewed, for analysis purposes, it was best viewed as dichotomous variable: a younger group aged 18-20 years, and a long tail students older than 20. In total, 63 were high-N and 38 were low-N (see Table 1).

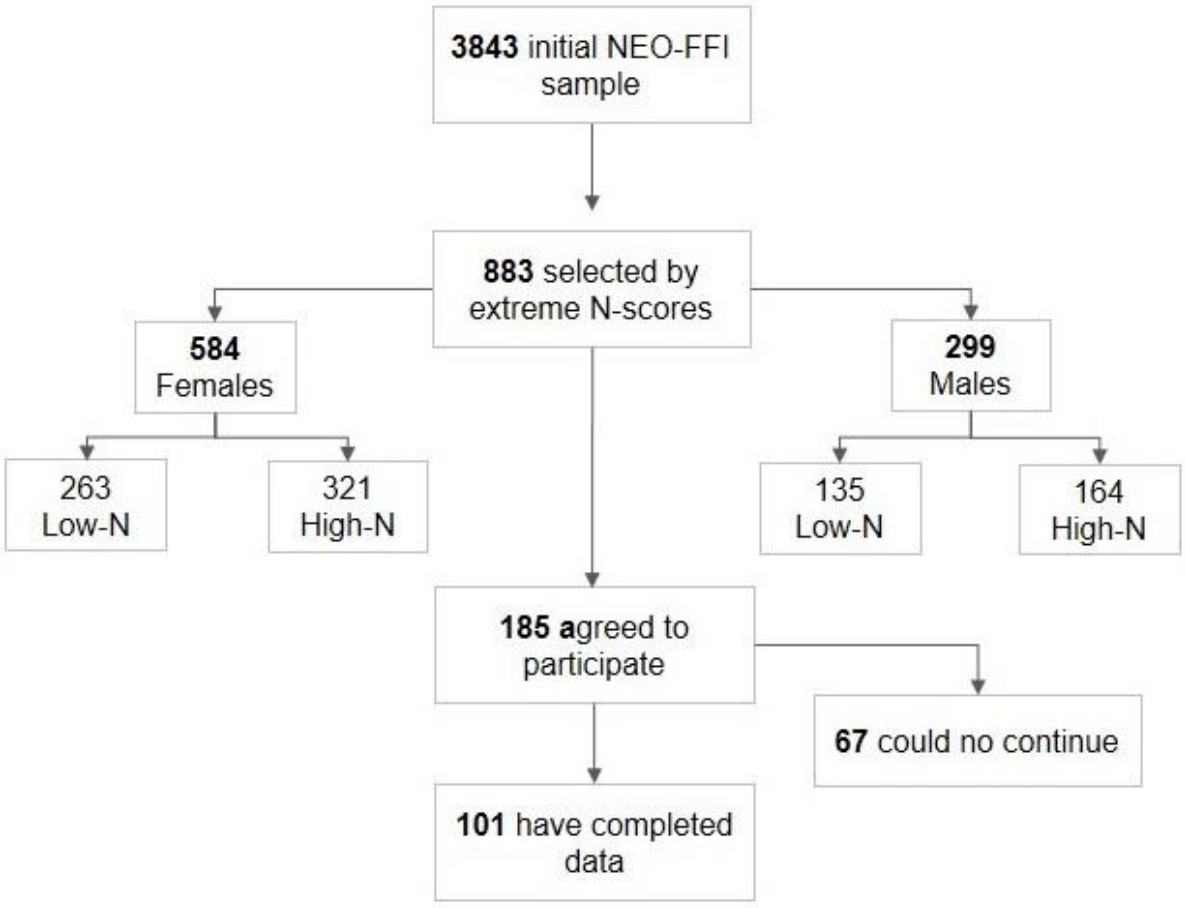


Figure 1. Sample collection process and final sample.

Table 1

Sample characteristics in relation to baseline cortisol assessment

	Groups			
	Additional baseline		Single baseline	
Characteristics	n	%	n	%
Neuroticism				
Low-N	19	41.3%	19	34.5%
High-N	27	58.7%	36	65.5%
Age				
Younger	26	56.5%	41	74.5%
Older	20	43.5%	14	25.5%
Gender				
Female	32	69.6%	34	61.8%
Male	14	30.4%	21	38.2%
Smoking status				
Non-smoker	32	69.6%	44	80%
Smoker	14	30.4%	11	20%
Total	46	45.5%	55	54.5%

Intervention

APMR consists of five 45 minutes session of tensing and releasing 16 muscle groups (dominant and non-dominant hand and forearm, dominant and non-dominant biceps, forehead, upper cheeks and nose, lower cheeks and jaws, neck and throat, chest, shoulders and upper back, abdominal or stomach region, dominant and non-dominant thigh, calf and foot) designed to produce both cognitive and physiological relaxation. Instructions encouraged participants to focus on sensations associated with release of muscle tension and feelings of comfort. They were advised not to tense muscle groups that felt strained or that aggravated pain.

Measures

Neuroticism: NEO-FFI (Costa & McCrae, 1999) was used to evaluate neuroticism using 12-item subscale (score range = 0 to 48). Participants responded on a 5-point Likert scale from 0 (*totally disagree*) to 4 (*totally agree*). The internal consistency value for our sample was .83 (original N value .74-.89; Costa & McCrae, 1992b, 1995). As mentioned above, participants were recruited for this APMR intervention study from a large pool of students already pre-selected as very high or very low on Neuroticism according to percentile norms. Thus neuroticism in this study was investigated as a dichotomous variable.

Survey of Recent Life Experiences (SRLE): The SRLE was developed by Kohn and McDonald (1992) and covers the following areas: mundane annoyances, domestic responsibilities, work, romance, friends, family, other social relationships, finances, environment, time pressure, competitive standing (in terms of abilities, attractiveness, etc.), and future security. Participants indicated the extent of their recent life experiences over the past month on the following 4-point scale: 1 = not at all part of my life; 2 = only slightly part of my life; 3 = distinctly part of my life; and 4 = very much part of my life. Total SRLE score is computing by adding all the values given (1 to 4) to each question (range: 41-164). The internal consistency value for our sample was .93 (original value .90; Khon & McDonald, 1992).

Cortisol: Salivary cortisol measures were collected with a cotton swab chewed for one minute, stored in a capped plastic vial (“Salivette” Sarstdet Inc.). These samples were centrifuged at 3000g for 3 minutes, and then the filtrates were stored frozen at -

80ºC until analysis. Before analysis, the samples were thawed, mixed, centrifuged and analyzed without pre-treatment. To reduce error, all samples of each participant were analyzed in one assay. Salivary cortisol was measured using a modification of the Bayer ADVIA Centaur cortisol assay, a competitive direct chemiluminescence's immunoassay that uses a rabbit polyclonal antibody. Endogenous cortisol contained in the samples competes with a cortisol labeled with acridinium-ester for the binding sites of the anti-cortisol rabbit polyclonal antibody-coated paramagnetic particles. The intra- and inter-assay coefficients of variation were less than 10% for 0.30 µg/dL of cortisol.

Adherence Electronic Monitoring: Timing cortisol adherence was measured by MEMS Track Caps device (AARDEX, Ltd., Zug, Switzerland). Participants took an absorbent cotton swab at each assigned sampling time from a plastic bottle with a microchip lid that recorded the time of each opening. After collecting a saliva sample, participants stored the swab in a pre-labeled plastic tube (Salivette, Sarstedt, Barcelona, Spain). Participants completed the information protocol each time the plastic bottle was opened to compare with the MEMS time (Broderick, Arnold, Kudielka, & Kirschbaum, 2004). In addition, participants programmed their mobiles to beep at the established times in order to further enhance compliance. An AARDEX interface and software were used to transfer time collection from the MEMS to PC. Discrepancy between MEMS and protocol-required timing of saliva samples could thus be used to check the sensitivity of any hypothesised effects to degree of timing errors.

Procedure

First year UIB students gave informed consent in their classes. Those who wanted to participate in the study provided their e-mail address and mobile phone number (see Figure 2).

In order to evaluate the effect of APMR on perceived stress participants completed the SRLE scale the week before and after the training. Cortisol secretion was assessed by collecting five measures of cortisol across the day (awakening, 45 min, 2.5h, 8h and 12h), on two days (Tuesday and Thursday), one-week before (pre) and one-week after (post) the intervention. In half of the six cohort groups we included an additional baseline cortisol measure taken two-weeks prior to the pre-intervention. Additionally, participants used the MEMS Caps to register each time they took a saliva sample. Moreover, students 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, or they did sport, etc. (see Adam & Kumari, 2009).

Participants attended five APMR sessions on five consecutive days (Monday to Friday) during the morning (8:30-9:30h). The sessions were conducted by an expert and university trainer (third author) in this technique who remained blind to collected data until the completion of this study. The APMR was performed following strictly the standard procedures set forth by Bernstein and Borkovec (1973).

Participants were not given reimbursement for their participation, although at the end of the study they received detailed information about their personality and cortisol profiles.

Figure 2. Study design and procedure of saliva and SRLE sample collection and APMR training week.

Treatment of Data and Statistical Analysis

Outcome measures were examined for normality of distribution. Extreme outlying scores (+/- three-standard deviation) were winsorized and in the case of cortisol was root transformed to reduce skewness statistics to approximately twice standard error or less.

TCS parameters were calculated as areas under the curve (AUC) of cortisol measures collected at 0.75h, 2.5h, 8h, and 12h after awakening on Tuesday and Thursday for each time period (pre- and post-intervention), using the standard trapezoid formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Outcome effects were examined using mixed regression modeling (MRM), an approach considered most appropriate for multilevel designs incorporating repeated measures over time with fixed and random parameters (Blackwell, Mendes de Leon, & Miller, 2006). This approach has been used in particular to model dynamic aspects of the diurnal cortisol cycle (Smyth et al., 2013), and in this case enabled us to examine over two time periods (pre and post) the effect of APMR on SRLE and

TCS. Similar two-level models were constructed for both dependent variables. In each case we assumed random intercepts and random slopes at the first level (Model A), which were modelled as outcomes at level 2 (Model B) when between-persons covariates were introduced. The goal in Model B was to determine which person-level characteristics might modulate differences at the within-person level.

Model A represents solely within-person effects and included the fixed covariates of pre- and post- intervention weeks, wake-time, and, for TCS only, sampling day within weeks (Tuesday vs. Thursday). The dichotomous variable of sampling day was effect coded such that zero represented cortisol secretion effects for other variables averaged across both sampling days. For similar reasons and following convention (Blackwell et al., 2006), wake-time was participant-centred such that scores represented the purely within-participant effect of changes in wake-time across occasions, with each score computed as a person's raw wake-time minus their own mean wake-time over all four study days. Thus again a value of zero in the model equations would represent conditions in which wake-time was assumed to be average for each participant. Finally the key covariate of the intervention (pre vs. post) was dummy coded (0/1) such that 0 (and therefore the intercept in the model) represented SRLE or TCS pre-intervention.

In Model B, the following level 2 (between-persons) fixed covariates were included: sex, age category, smoking status, neuroticism group, and allocation (or not) to an additional baseline (pre-intervention) assessment of outcome measures. All these dichotomous variables were effect coded such that -1 represented the category values of female, younger age, non-smoker, low neuroticism, and absence of additional baseline assessment, and +1 represented binary opposites. A preliminary full Model B was run including all covariates to examine their statistically

independent effects on baseline (intercept coefficient) levels of dependent variables, and all two-way interactions involving intervention (pre-post slope coefficient). The latter test within the model for possible modulation of within-person intervention effects by between-persons covariates. The final Model B presented here involved backward elimination of covariates with no significant effects on intercept or intervention slope coefficients.

Results

Full details of all analyses including coefficients for estimating all Model A and final Model B effects on both stress measures are given in Table 2.

Table 2

Effects of the intervention on the two outcome variables

	Model A			*Final Model B*		
SRLE	Coefficient	(SE)	*p*<	Coefficient	(SE)	*p*<
Fixed effects						
Intercept	72.03	(1.91)	.001	69.21	(1.60)	.001
Intervention	-6.90	(1.12)	.001	-6.84	(1.1)	.001
Wake-time	1.22	(3.39)	.719			
Intervention*Wake-time	-2.00	(6.16)	.746			
Neuroticism				10.74	(1.52)	.001
	Variance	(SD)	*p*<	Variance	(SD)	*p*<
Random effects						
Level 1 residual	58.86	(8.6)	.001	52.71	(18.01)	.003
Intercept	289.9	(46.08)	.001	183.7	(30.93)	.001
Linear slope	0.00			10.52	(32.55)	.747
TCS	Coefficient	(SE)	*p*<	Coefficient	(SE)	*p*<
Fixed effects						
Intercept	1.050	(.001)	.001	1.055	(.001)	.001
Intervention	-.003	(.001)	.012	-.003	(.001)	.009

Day	-.001	(.001)	.444			
Intervention *Day	-.001	(.001)	.532			
Wake-time	-.004	(.001)	.001	-.004	(.001)	.001
Intervention*Wake-time	-.000	(.002)	.855			
Additional baseline				-.007	(.001)	.001
Gender				.003	(.001)	.029
Age				-.003	(.001)	.003
	Variance	(SD)	*p*<	Variance	(SD)	*p*<
Random effects						
Level 1 residual	.000107	(.000)	.001	.000107	(.000)	.001
Intercept	.000138	(.000)	.001	.000083	(.000)	.001
Linear slope	.000001	(.000)	.523	.000014	(.000)	.432

Note. SE = standard error; SD = standard deviation; TCS = total cortisol secretion.

Effect of APMR on SRLE

The results of Model A indicate that the intercept (denoting SRLE baseline) before APMR training was 72.03. The slope coefficient for the intervention effect was statistically significant (-6.90, $p < .001$) and is an estimate of the reduction in SRLE measured stress in the week following the intervention, with wake-time held at its zero (mean) value. Wake-time was not associated with overall SRLE scores nor with changes in SRLE post-intervention. The final model B yielded similar intercept (69.21) and intervention slope (-6.84) values, the latter remaining highly significant ($p < .001$) and, expressed in percentage terms, the intervention was followed by an approximately 10% reduction in SRLE measured stress (see Figure 3).

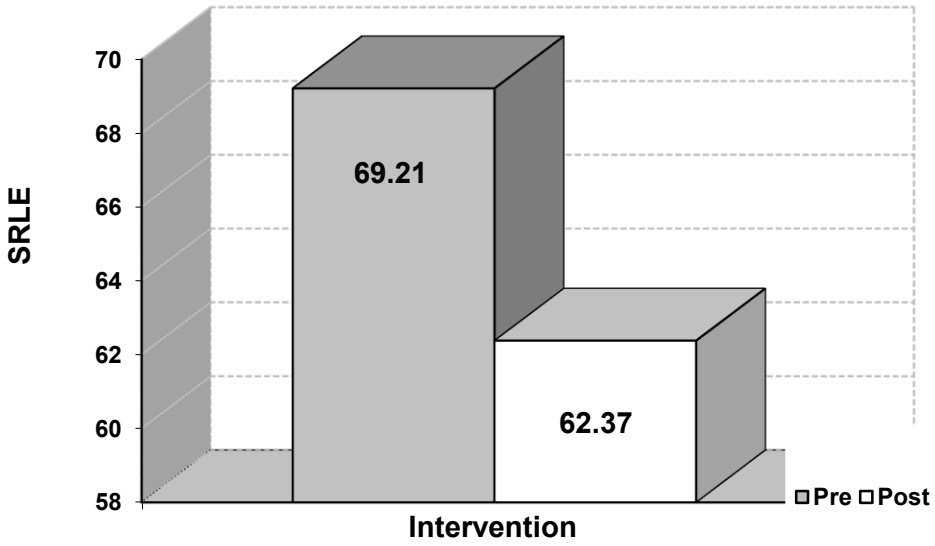


Figure 3. Effect of APMR intervention on SRLE following the final Model B.

Model B yielded a significant main effect coefficient (10.74, $p < .001$) for neuroticism. This coefficient estimates that high neuroticism participants tended to report 10.74 (approximately 16%) more SRLE stress units than the study average, and low neuroticism participants equivalently less. The Neuroticism x Intervention interaction was not significant, so there was no suggestion that Neuroticism modulated the main finding of a reduction in SRLE stress following the intervention. Equally there were no other significant main effects on overall level of SRLE stress reporting and no evidence of modulation of the significant intervention effect, with all other terms being excluded from the final model in the process of process of backward elimination (see Table 2).

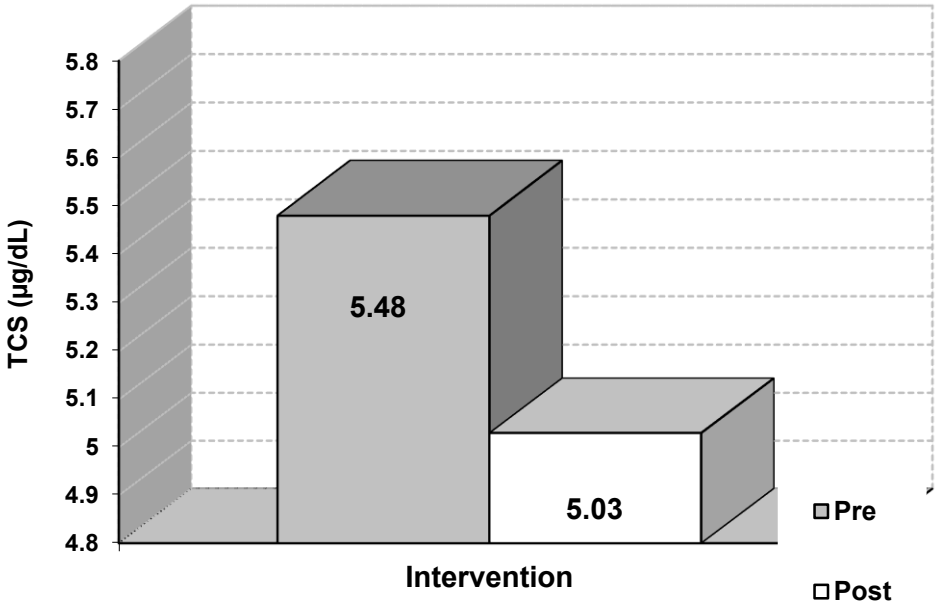
Effect of APMR on Cortisol Secretion

The results of Model A using MRM analysis indicate that average total cortisol secretion (TCS) before APMR training (intercept) was 1.055 root units (equivalent to 5.48 µg/dl). The estimate of slope coefficient for intervention was -.003 root units (-0.45 µg/dl), $p < .012$. In this case slope was equal to differences between pre and post intervention means, with sampling day and wake-time held at zero (mean) values. Thus APMR was followed by a significant decrease in cortisol secretion a week after the training. Later wake-time was significantly associated ($p < .001$) with lower cortisol regardless of time point (pre- or post-intervention) with a significantly negative slope coefficient of -.004 in the equation based on root units. This would translate into a reduction of 0.56 µg/dl for every hour that participants might wake up later than their own typical average time. The intervention*wake-time interaction was not significant indicating that the efficacy of the intervention in reducing cortisol was not associated with any pre-post changes in wake-time.

Model B yielded closely similar estimates of intercept and intervention slope to Model A indicating a similar degree of significant ($p < .009$) cortisol reduction of approximately 8% following the intervention (see Figure 4). None of the level 2 covariates interacted significantly with the intervention covariate, suggesting that the degree of cortisol reduction did not depend on gender, age, neuroticism, smoking status or whether participants' baseline cortisol was assessed once or twice before the intervention. Regardless of pre or post occasions, males had overall higher levels of cortisol than females ($p < .029$), younger students (<21 years) had higher levels of cortisol than older ones ($p < .003$), and participants whose baseline cortisol was assessed twice before the APMR intervention had significantly lower levels of cortisol than participants who had a single week pre-intervention baseline ($p < .001$).

In contrast to the SRLE analysis and against expectation, neuroticism was not significantly associated with overall greater cortisol secretion and did not enter the final Model B, although there was a quite strong trend for those higher in neuroticism to secrete more cortisol.

Figure 4. Effect of APMR intervention on cortisol secretion



following the final Model B.

Discussion

These findings contribute to the understanding of the effect that abbreviated progressive muscle relaxation has on psychological and physiological stress, in a study with several strengths. At the same time, this study confirm that SRLE is a cleaned hassles measure to determine accurately how much stress participants have over one-week. Also that TCS provides a stable estimation of prevailing

cortisol levels after a meaningful period of one-week, which represents a robust and reliable marker to assess changes in cortisol secretion. Hence, using a carefully constructed cortisol measure (TCS) and an appropriate self-report (SRLE) we can confirm that one-week of APMR decrease significantly prevailing levels of psychological and physiological stress one-week after the intervention. And this later is our major finding.

Our results also shown that people in the extra baseline condition had significantly lower levels of cortisol on average. No evidence was found that baseline condition interacted in any way with the intervention effect and, therefore, influenced the efficacy of APMR in reducing cortisol secretion. Later wake-time was significantly associated with lower cortisol secretion. Indeed, those individuals who woke-up later present lower cortisol secretion, in comparison with people who woke-up earlier. However, in terms of the focus of this study, the wake-time effect was independent of intervention effects, and there was no evidence that the efficacy of the APMR in reducing TCS was mediated by wake-time differences between the pre- and post-intervention.

Neuroticism appears to be a potential moderator of self-reported psychological stress but not of cortisol measures. Unsurprisingly, high-N participants present higher levels of psychological stress than low-N ones. Finally, sex, age category and smoking status were not related to psychological stress. On the contrary, sex and age effects were apparent on cortisol measures. Male and young people present higher levels of TCS. Nonetheless, sex, age category and smoking status were not related either to psychological or physiological stress changes produced by APMR training. Therefore, APMR was equally effective to decrease

perceived stress and cortisol secretion independently if they were female or male, younger or older or if they were smokers or not.

In sum, APMR was effective to decrease both psychological and physiological stress in all participants independently of their N-score, sex or age. As expected, people high in neuroticism experienced more stress than people low in this trait, however, no differences were found between these two groups on cortisol.

Chapter 5

General discussion and conclusion

1. General discussion

The present PhD work aimed to explore the activity of the hypothalamic-pituitary-adrenal (HPA) axis as a neuroendocrine regulator of the stress response. Its end product, cortisol, was examined under three different conditions (i.e., acute stressful, daily stress, and relaxation). Its relationship with personality traits was also explored.

Our first study focused on the effects of an academic acute stressor on cortisol in undergraduate psychology students. We hypothesized that the common stressor of public speaking will increase cortisol secretion compared with the control condition. As expected, public speaking was associated with elevated cortisol compared to baseline levels taken at equivalent times on a regular academic day. These results were in line with those found by Andrews et al. (2007) using social evaluation, and Schoofs, Hartmann, Wolf (2008) using oral examination. It appears that social evaluative tasks such as public speaking do elicit robust and reliable cortisol responses, as found in previous studies (e.g., Dickerson, Mycek, & Zaldivar, 2008).

Our second hypothesis was partially confirmed. Some personality traits did correlate with cortisol secretion levels, but not as much we had expected. Conscientiousness (C) and Psychoticism (P) were the only personality traits related with changes in cortisol secretion under public speaking.

Conscientiousness was linked to heightened cortisol secretion during public speaking that may be associated with a better coping under a stressful situation. Het and Wolf (2007) showed that female participants with higher cortisol levels coped better with the emotional load of public speaking than did participants with regular or even blunted cortisol secretion, concluding that a more pronounced cortisol response to acute stress may be adaptive in reducing negative effects of stress on mood.

Conscientiousness (C) is a personality dimension strongly related to health, and may exert part of its influence by modifying the appraisal of the negative effects of daily stressors (Gartland, O'Connor, Lawton, & Ferguson, 2014). High conscientious individuals display good self-control and present a high level of responsibility, which may help them to anticipate, cope, and respond better to expected stressful events (Nater, Hoppmann, & Klumb, 2010). Our results offer a sight of one biological mechanism that could explain why people high in conscientiousness enjoy better health and live longer respect to people with lower levels in this trait (Friedman, 2008).

Contrary, we found relatively smaller cortisol responses during public speaking in participant with high scores in psychoticism. This dimension has found to be highly correlated with psychopathy and impairment in stress reactivity in previous studies (O'Leary, Loney, & Eckel, 2007). In the literature there is a consistent account of antisocial traits, impulsive, defiant, and aggressive behaviour to low cortisol reactivity in children and adolescents (Shirtcliff et al., 2009). Our results are consistent with a view that blunted cortisol reactivity under stress may sometimes form part of what is likely to be a wider biological profile associated with antisocial behaviour.

In our second study, we have demonstrated that participants that score high in Neuroticism (N) tend to exhibit higher prevailing levels of cortisol after the CAR period in daily life situations. This result was replicated in different cohorts over different years using careful control features. We know that high-N scores are associated with negative biases in information processing (Chan, Goodwin, & Harmer, 2007), difficulties in regulating emotions (Mikolajczak, Roy, Luminet, Fillée, & de Timary, 2007), impaired self-control (Uziel & Baumeister, 2012), and now we have demonstrated that they are also linked to elevated cortisol secretion during the daytime under non-stressful circumstances. Interestingly, the predicted associations between N and cortisol secretion were shown to be independent of sex and age of participant, smoking status, awakening time, and the particular day of the study.

Nevertheless, sex of participant was associated with diurnal secretion (outside the CAR), with higher levels and flatter slopes in males. A greater diurnal decline (slope) was observed in females, in line with Larsson, Gullberg, Råstam and Lindblad (2009). Regarding the slope, our results are similar to the findings for adolescents reported by Hauner et al. (2008), and the conclusions of Kudielka and Kirschbaum (2005). Moreover, Hauner et al. (2008) also reported a significant interaction between sex and neuroticism, where males with high N defined a sub-group with particularly limited slope.

Associations between awakening time and cortisol secretion were also significant. This finding suggests an entirely within-participant effect where the total cortisol secretion (outside the CAR) for a single participant tended to be greater if they had awakened earlier, regardless of the absolute clock-times involved. Directionally identical between-participant effect of awakening time was also evident, suggesting that those who habitually woke earlier tended to have higher secretion

totals. Finally inside the CAR period, earlier awakening was associated with lower starting values of cortisol and a tendency to higher CARs.

However, even though we found an association between N and during most of the diurnal period, it was not evident in the period following awakening (CAR). This gives strong support to several studies (e.g., Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010) that show that the period of massive rise in the brief 0-0.75h should be seen separately from the rest of the diurnal cycle, underpinned by different control mechanisms, e.g. the suprachiasmatic nucleus circadian clock (Postnova, Fulcher, Braun, Robinson, 2013), hippocampal regulation (Fries, Dettenborn, & Kirschbaum, 2009), and biological processes associated with sleep-wake cycles (Smyth, Clow, Thorn. Hucklebridge, & Evans, 2013). In addition, the CAR period measures may also be more reflective of cognitive functioning (Evans, Hucklebridge, Loveday, & Clow, 2012; Evans et al., 2011) in anticipation of the demands of the upcoming day (Fries et al., 2009).

Our last study analyzed the effect of Abbreviated Progressive Muscle Relaxation (APMR) using reliable measures to assess changes in perceived stress and cortisol secretion. This was a pioneer study using both psychological assessment and cortisol daily profiles pre- and post-one-week APMR intervention in people with extreme scores on neuroticism.

These findings demonstrated that APMR can significantly reduce both psychological and physiological stress in a study which has several strengths. SRLE was used as a cleaned hassles measure (Kohn & McDonald, 1992) suitable for determining accurately how much stress participants have experienced over one-week. The cortisol measure used in this study was also chosen and constructed

carefully. To do that, we paid special attention to timing accuracy of saliva sample collection, we excluded the samples that represent the CAR period, and adequate multiple sampling during the course of four days, two days each period. Under these conditions, construction of a TCS measure was able to provide a stable estimation of a participant's mean cortisol level over the period around the sampling days. We make a particular emphasis to the fact that in averaging (over two days), the TCS measure attenuates the influence of within-day acute cortisol responses to daily stressful demands and excludes entirely the influence of within-participant fluctuations in the most volatile period of the diurnal cycle (CAR).

Thus, using two robust outcome measures (SRLE and TCS), we can confirm that a one-week of APMR decreased significantly prevailing levels of psychological and physiological stress one-week after the intervention. These results are in line with an existing literature which suggest that APMR reduces perceived stress (Broadbent et al., 2012; Dolbier & Rush, 2012; Kaspereen, 2012; Scheufele, 2000) and cortisol secretion (Krajewski, Sauerland, & Wieland, 2011; Pawlow & Jones, 2002, 2005) immediately after intervention. However, our study goes further, confirming that efficacy is evidenced not only by immediate effects, but also by longer-term reduction of prevailing levels of psychological and physiological stress indices.

In terms of control features of the design, approximately half the participants provided stress outcome measures in an additional baseline trial one week before the pre-intervention week common to all participants. If cortisol reduction between pre- and post- intervention found for all participants were due to a simple temporal effect, reflecting perhaps "habituation" in response to novelty and challenge of the saliva collection protocol itself, then the efficacy of the intervention might appear to

be significantly less in the group whom the post-intervention represented their third week of saliva sample collection. Results showed no significant influence of an additional baseline exposure to saliva collection on the efficacy of the APMR intervention. Despite this result, the groups which were selected for additional baseline cortisol assessment did have in general significantly lower cortisol levels (i.e., both pre and post intervention). These differences between cohorts are not unexpected. As is the case when differing average values pertain for equivalent studies from different laboratories, such differences in absolute cortisol values cannot readily be interpreted.

Another variable which was examined and statistically controlled in this study was wake-time. People who woke up later had lower cortisol secretion compared with people who woke up earlier. Wake-time effects on cortisol secretion levels have been reported before in the psychophysiology literature (Edwards, Evans, Hucklebridge, & Clow, 2001; Kudielka & Kirschbaum, 2003; Okun et al., 2010). However the crucial finding, in terms of the focus of this paper, was that the efficacy of the APMR in reducing TCS was independent of wake-time.

Finally, in regard to neuroticism, high-N participants compared to low-N ones were found to have significantly higher levels of self-reported psychological stress but not cortisol secretion, although there was a trend towards the latter. Besides these findings, no modulation effect was found for neuroticism on the efficacy of APMR. This confirm that APMR was equally effective in reducing psychological and physiological stress in both high- and low-N groups. Sex, age, and smoking status were not related to psychological stress. However, sex and age effects were apparent for cortisol measures. Male and younger people presented higher levels of TCS, which is in line with the results found by Seeman, Singer, Willkinson and

McEwen (2001). However none of these variables in any way modulated the pattern of stress reduction apparent over the trial period for all participants.

2. Limitations

The three studies compiled in this dissertation presents several limitations. In our first study we included a limited sample formed by psychology students. Thus, our sample may not be representative of the entire student community, and we cannot generalize the results obtained. Gender was another limitation (male being unrepresented). It seems that gender interactions can offer a better understanding of the functioning of cortisol response to psychosocial exposure. Finally, our range of questionnaire scores was somewhat restricted, due to the fact that most of the participants had middle scores, so a wider sample would be desirable in the future.

In the second and third study we have tried to cover most of our first study limitations (different study degrees, more male and extreme score participants were included). Nevertheless, one of the limitations of our second study was the use of the broad domain of neuroticism scale without including lower order facets of the N-NEO-FFI inventory. This approach may yield to more robust facet specific associations with cortisol parameters because they focus on a more homogeneous range of behaviour than higher-order traits approaches (Ormel et al., 2013).

Finally in our third study, the main limitation was the reliance on student sample. There must be caution in generalizing from a basically healthy and young adult population to more “distressed” populations across the fuller adult age range. Such populations may be more difficult ones to investigate with the same degree of experimental control but they may also be ones where the need for efficacious intervention is more necessary. Longer follow-up would also be desirable to demonstrate if enduring gains might indicate the extent to which a “life-skill” has

been acquired from this relatively short and cost-effective intervention. Moreover, there is still a need to include in the future a control group activity (e.g., remain seated) in order to confirm the effectiveness of this intervention in decreasing both psychological and physiological stress. Finally, in order to generalize these results would be interesting to include young adult populations with middle N-scores.

3. Conclusion

The main objective of this work was to examine associations between cortisol, personality and relaxation. From the results of this first study, we can conclude that conscientiousness, a trait associated with resiliency to psychological disorders and health, was associated with an enhanced cortisol response to stress. Psychoticism, a trait consistently linked to more pronounce externalizing disorders, was associated with blunted cortisol responses. To our knowledge, this is the first study to show that pro-social and antisocial personality traits predict opposite cortisol responses to acute social stressors.

The present work also contributes by confirming a highly plausible theoretical prediction (but surprisingly inconsistent in the literature). Individuals who score high in neuroticism will show elevated cortisol secretion in their diurnal profile. This study also gives strong support to several lines of evidence that show that the CAR period should be seen separate from the rest of the diurnal cycle. Importantly, the predicted associations were shown to be independent of sex and age of participant, smoking status, awakening time, and the particular day of the study. And it was consistent over different cohorts recruited in different years.

Finally, we can conclude that APMR is an effective intervention able to reduce perceived stress and cortisol secretion in university students. And what is more important, maintaining these reductions a week after the training. Efficacy effects were independent of individual differences in wake-time, neuroticism, sex, age and smoking status. Thus, given the high rates of stress and stress-related mental health problems reported by students (Regehr, Glancy, & Pitts, 2013), university health services may consider the benefits of making this type of intervention widely

available as a way of reducing student stress and more serious anxiety and depression stress-related symptoms (Bewick, Koutsopoulou, Miles, Slaa, & Barkham, 2010). Relaxation training might offer real benefits to students as they seek to cope with the challenges of their degree journey.

In future studies, due to the fact that high-N individuals present a highly levels of perceived stress, it would be interesting to include a cognitive intervention approach (e.g., mindfulness) addressed to manage dysfunctional cognitions, emotions, and maladaptive behaviours to reduce stress perception in this population.

3.1. Future lines of research

Two opposite lines of research arise from this PhD dissertation. The first focuses on understanding the biological nature of Neuroticism (N) focusing in the study of the relationships among N-personality trait, internalizing symptomatology, and genetic composition. This line of research will require the development of explanatory hypotheses related to N's heterogeneity of behaviour, where the facets of N are of main importance (as suggested by Ormel et al., 2013). Most studies aiming to find genetic susceptibility factors have focused on the serotonin hypothesis. According to this hypothesis, serotonin would be the neurotransmitter at the centre of the chemical imbalances behind internalizing disorders. The serotonin transporter gene SCL6A4, also known as 5-HTT, is involved in the reuptake of serotonin at brain synapses. The promoter of the SCL6A4 gene has two common allelic variants, short (S) and long (L). The presence of the short version translates into more serotonin in the synapse space (Holden, 2008), and this characteristic has been related with neuroticism. However, other polymorphisms have been described for this gene. Therefore, in order to take into account all possible genetic variance of this locus, sequencing the whole gene in each one of the individuals involved in the study, would be advisable. Thus, the aim of this future study would be to explore the genetic basis of neuroticism and internalizing symptomatology by sequencing the whole genomic regions of serotonin gene, and to study the role of stressors in this relationship.

The second will focus on implementing mindfulness interventions in schools and prove through longitudinal studies that children who have developed mindfulness skills are better equipped to deal with stress in their young and adult lives (e.g., adapting to university demands). Mindfulness is a meditative practice of bringing mindful awareness to moment-to-moment experience that similarly to APMR aims to improve the psychological and physiological wellbeing of individuals. Numerous studies have documented the benefits of teaching mindfulness to adults within the context of Mindfulness-Based Stress Reduction (MBSR; Kabat-Zinn, 1990) programs. Additionally to this problem is that in the literature we can find that there is an increase of children perceiving and experiencing more stress, and thus developing more negative psychological symptoms (e.g., anxiety, depression) than several decades ago.

There are already several research groups around the world applying mindfulness to help children to recognize and manage stress, but most of them are based on clinical population (i.e. ADHD children Susan Bögels and her team from the Netherlands). We consider that developing school-based interventions will be a preventive tool to reduce stress, stress-related mental health and behavioural problems in children. Moreover, it will be a proactive one helping children to achieve more positive personal and academic outcomes by enhancing attention, self-regulation, social competence, and well-being. As van de Weijer-Bergsma, Langenberg, Brandsma, Oort & Bögels, (2012) suggested these interventions can be complemented by MBSR programs for teachers in order to help them to cope with their own stress and as a result, being able to improve their interactions with their students. Moreover, involving parents in MBSR training will be of great help for children to expand their mindfulness skills from school to home context.

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Appendix

1. **Personality questionnaires** (EPQ-R, NEO-FFI)

EPQ-R

Nombre: Sexo: Hombre ☐ Mujer ☐
Edad: Fecha:
Centro:
Estudios:
Profesión:

Por favor, conteste cada pregunta poniendo un aspa (X) sobre el SI o NO que le siguen. No hay respuestas correctas o incorrectas, ni preguntas con trampa. Trabaje rápidamente y no piense demasiado en el significado exacto de las mismas.

	SI	NO
1. ¿Se para a pensar las cosas antes de hacerlas?		
2. ¿Su estado de ánimo sufre altibajos con frecuencia?		
3. ¿Es una persona conservadora?		
4. ¿Se siente a veces desdichado sin motivo?		
5. ¿Alguna vez ha querido llevarse más de lo que le correspondía en un reparto?		
6. ¿Es usted una persona más bien animada o vital?		
7. Si usted asegura que hará una cosa, ¿siempre mantiene su promesa, sin importarle las molestias que ello le pueda ocasionar?		
8. ¿Es una persona irritable?		
9. ¿Le tiene sin cuidado lo que piensan los demás?		
10. ¿Alguna vez ha culpado a alguien por algo que había hecho usted?		
11. ¿Son todos sus hábitos buenos y deseables?		
12. ¿Tiende a mantenerse apartado/a en las situaciones sociales?		
13. A menudo, ¿se siente harto/a?		
14. ¿A cogido alguna vez alguna cosa (aunque no fuese más que un alfiler o un botón) que perteneciese a otra persona?		
15. Para usted, ¿los límites entre lo que está bien y lo que está mal son menos claros que para la mayoría de la gente?		
16. ¿Le gusta salir a menudo?		
17. ¿Es mejor actuar como uno quiera que seguir las normas sociales?		
18. ¿Tiene a menudo sentimientos de culpabilidad?		
19. ¿Diría de sí mismo que es una persona nerviosa?		
20. ¿Es usted una persona sufridora?		
21. ¿Alguna vez ha roto o perdido algo que perteneciese a otra persona?		
22. ¿Generalmente toma la iniciativa al hacer nuevas amistades?		
23. ¿Los deseos personales están por encima de las normas sociales?		
24. ¿Diría de si mimo que es una persona tensa o muy nerviosa?		
25. Por lo general, ¿suele estar callado/a cuando esta con otras personas?		
26. ¿Cree que el matrimonio esta anticuado y debería abolirse?		
27. ¿Puede animar fácilmente una fiesta aburrida?		
28. ¿Le gusta contar chistes e historias divertidas a sus amigos?		
29. ¿La mayoría de las cosas le son indiferentes?		
30. ¿De niño, fue alguna vez descarado con sus padres?		
31. ¿Le gusta mezclarse con la gente?		
32. ¿Se siente a menudo apático/a y cansado/a sin motivo?		
33. ¿Ha hecho alguna vez trampas en el juego?		
34. ¿A menudo toma decisiones sin pararse a reflexionar?		
35. ¿A menudo siente que la vida es muy monótona?		
36. ¿Alguna vez se ha aprovechado de alguien?		
37. ¿Cree que la gente pierde el tiempo al proteger su futuro con ahorros y seguros?		
38. ¿Evadiría impuestos si estuviera seguro de que nunca seria descubierto?		
39. ¿Puede organizar y conducir una fiesta?		
40. ¿Generalmente, reflexiona antes de actuar?		

41. ¿Sufre de los “nervios”?		
42. ¿A menudo de siente solo?		
43. ¿Hace siempre lo que predica?		
44. ¿Es mejor seguir las normas de la sociedad que ir a su aire?		
45. ¿Alguna vez ha llegado tarde a una cita o trabajo?		
46. ¿Le gusta el bullicio y la agitación a su alrededor?		
47. ¿La gente piensa que usted es una persona animada?		
48. ¿Cree que los planes de seguros son una buena idea?		
49. ¿Realiza muchas actividades de tiempo libre?		
50. ¿Daria dinero para fines caritativos?		
51. ¿Le afectaría mucho ver sufrir a un niño o animal?		
52. ¿Se preocupa a menudo por cosas que no debería haber dicho o hecho?		
53. ¿Habitualmente, es capaz de liberarse y disfrutar en una fiesta animada?		
54. ¿Se siente fácilmente herido en sus sentimientos?		
55. ¿Disfruta hiriendo a las personas que ama?		
56. ¿Habla a veces de cosas de las que no sabe nada?		
57. ¿Prefiere leer a conocer gente?		
58. ¿Tiene muchos amigos?		
59. ¿Se ha enfrentado constantemente a sus padres?		
60. ¿Cuándo era niño, hacia enseguida las cosas que le pedían sin refunfuñar?		
61. ¿Se ha opuesto frecuentemente a los deseos de sus padres?		
62. ¿Se inquieta por cosas terribles que podrían sucederle?		
63. ¿Es usted más indulgente que la mayoría de las personas acerca del bien y del mal?		
64. ¿Se siente intranquilo por su salud?		
65. ¿Alguna vez ha dicho algo malo o desagradable acerca de otra persona?		
66. ¿Le gusta cooperar con los demás?		
67. ¿Se preocupa si sabe que hay errores en su trabajo?		
68. ¿Se lava siempre las manos antes de comer?		
69. ¿Casi siempre tiene una respuesta “a punto” cuando le hablan?		
70. ¿Le gusta hacer cosas en las que tiene que actuar rápidamente?		
71. ¿Es (o era) su madre una buena mujer?		
72. ¿Le preocupa mucho su aspecto?		
73. ¿Alguna vez ha deseado morirse?		
74. ¿Trata de no ser grosero con la gente?		
75. ¿Después de una experiencia embarazosa, se siente preocupado durante mucho tiempo?		
76. ¿Se siente fácilmente herido cuando la gente encuentra defectos en usted o en su trabajo?		
77. ¿Frecuentemente improvisa decisiones en función de la situación?		
78. ¿Se siente a veces desbordante de energía y otras, muy decaído?		
79. ¿A veces deja para mañana lo que debería hacer hoy?		
80. ¿La gente le cuenta muchas mentiras?		
81. ¿Se afecta fácilmente por según qué cosas?		
82. Cuando ha cometido una equivocación, ¿está siempre dispuesto a admitirlo?		
83. Cuando tiene mal humor, ¿le cuesta controlarse?		

NEO-FFI

Nombre: Sexo: Hombre ☐ Mujer ☐
Edad: Fecha:

Instrucciones: este cuestionario consta de 60 frases. Lea cada frase con atención y marque la alternativa (A a E) que refleje mejor su acuerdo o desacuerdo con ella.

A= En total desacuerdo; B= En desacuerdo; C= Neutral
D= De acuerdo; E= Totalmente de acuerdo

1. A menudo me siento inferior a los demás	A	B	C	D	E
2. Soy una persona alegre y animosa	A	B	C	D	E
3. A veces, cuando leo poesía o contemplo una obra de arte, siento una profunda emoción o excitación	A	B	C	D	E
4. Tiendo a pensar lo mejor de la gente	A	B	C	D	E
5. Parece que nunca soy capaz de organizarme	A	B	C	D	E
6. Rara vez me siento con miedo o ansioso	A	B	C	D	E
7. Disfruto mucho hablando con la gente	A	B	C	D	E
8. La poesía tiene poco o ningún efecto sobre mí	A	B	C	D	E
9. A veces intimido o adulo a la gente para que haga lo que yo quiero	A	B	C	D	E
10. Tengo unos objetivos claros y me esfuerzo por alcanzarlos de forma ordenada	A	B	C	D	E
11. A veces me vienen a la mente pensamientos aterradores	A	B	C	D	E
12. Disfruto en las fiestas en las que hay mucha gente	A	B	C	D	E
13. Tengo una gran variedad de intereses intelectuales	A	B	C	D	E
14. A veces consigo con artimañas que la gente haga lo que yo quiero	A	B	C	D	E
15. Trabajo mucho para conseguir mis metas	A	B	C	D	E
16. A veces me parece que no valgo absolutamente nada	A	B	C	D	E
17. No me considero especialmente alegre	A	B	C	D	E
18. Me despiertan la curiosidad las formas que encuentro en el arte y en la naturaleza	A	B	C	D	E
19. Si alguien empieza a pelearse conmigo, yo también estoy dispuesto a pelear	A	B	C	D	E
20. Tengo mucha auto-disciplina	A	B	C	D	E
21. A veces las cosas me parecen demasiado sombrías y sin esperanza	A	B	C	D	E
22. Me gusta tener mucha gente alrededor	A	B	C	D	E
23. Encuentro aburridas las discusiones filosóficas	A	B	C	D	E
24. Cuando me han ofendido, lo que intento es perdonar y olvidar	A	B	C	D	E
25. Antes de emprender una acción, siempre considero sus consecuencias	A	B	C	D	E
26. Cuando estoy bajo un fuerte estrés, a veces siento que me voy a desmoronar	A	B	C	D	E

27. No soy ni tan vivo ni tan animado como otras personas	A	B	C	D	E
28. Tengo mucha fantasía	A	B	C	D	E
29. Mi primera reacción es confiar en la gente	A	B	C	D	E
30. Trato de hacer mis tareas con cuidado, para que no haya que hacerlas otra vez.	A	B	C	D	E
31. A menudo me siento tenso e inquieto	A	B	C	D	E
32. Soy una persona muy activa	A	B	C	D	E
33. Me gusta concentrarme en un ensueño o fantasía y, dejándolo crecer y desarrollarse, explorar todas sus posibilidades	A	B	C	D	E
34. Algunas personas piensan de mi que soy frío y calculador	A	B	C	D	E
35. Me esfuerzo por llegar a la perfección en todo lo que hago	A	B	C	D	E
36. A veces me he sentido amargado y resentido	A	B	C	D	E
37. En reuniones, por lo general prefiero que hablen otros	A	B	C	D	E
38. Tengo poco interés en andar pensando sobre la naturaleza del universo o de la condición humana	A	B	C	D	E
39. Tengo mucha fe en la naturaleza humana	A	B	C	D	E
40. Soy eficiente y eficaz en mi trabajo	A	B	C	D	E
41. Soy bastante estable emocionalmente	A	B	C	D	E
42. Huyo de las multitudes	A	B	C	D	E
43. A veces pierdo el interés cuando la gente habla de cuestiones muy abstractas y teóricas	A	B	C	D	E
44. Trato de ser humilde	A	B	C	D	E
45. Soy una persona productiva, que siempre termina su trabajo	A	B	C	D	E
46. Rara vez estoy triste o deprimido	A	B	C	D	E
47. A veces reboso felicidad	A	B	C	D	E
48. Experimento una gran variedad de emociones y sentimientos	A	B	C	D	E
49. Creo que la mayoría de la gente con la que trato es honrada y fidedigna	A	B	C	D	E
50. En ocasiones primero actúo y luego pienso	A	B	C	D	E
51. A veces hago las cosas impulsivamente y luego me arrepiento	A	B	C	D	E
52. Me gusta estar donde está la acción	A	B	C	D	E
53. Con frecuencia pruebo comidas nuevas o de otros países	A	B	C	D	E
54. Puedo ser sarcástico y mordaz si es necesario	A	B	C	D	E
55. Hay tantas pequeñas cosas que hacer que a veces lo que hago es no atender a ninguna	A	B	C	D	E
56. Es difícil que yo pierda los estribos	A	B	C	D	E
57. No me gusta mucho charlar con la gente	A	B	C	D	E
58. Rara vez experimento emociones fuertes	A	B	C	D	E
59. Los mendigos no me inspiran simpatía	A	B	C	D	E
60. Muchas veces no preparo de antemano lo que tengo que hacer	A	B	C	D	E

2. Rating scales

SRLE (41)
Escala de Experiencias Vitales Recientes

NOMBRE:	EDAD:	SEXO:
TRABAJAS:	ESTUDIOS:	

Instrucciones. A continuación te presentamos una lista de experiencias que la gente tiene en un momento u otro de su vida. Por favor, indica en qué medida ha formado parte de tu vida cada una de dichas experiencias en el ***ultimo mes***. Para contestar debes poner una **X** en el espacio reservado para la valoración de cada experiencia (de 1 a 4) en el margen derecho de la pagina. El criterio que debe utilizar es el siguiente:

1	2	3	4
No ha formado parte de mi vida en absoluto	Solo debilmente ha formado parte de mi vida	Ha formado parte de mi vida de forma marcada	Ha formado parte de mi vida de forma muy intensa

	1	2	3	4
1. Desagrado por sus actividades diarias				
2. Desagrado por su trabajo y/o estudios				
3. Conflictos etnicos o raciales				
4. Conflictos con su familia politica o la de su novio o novia				
5. Haber sido defraudado o decepcionado por los amigos				
6. Conflictos con su superior en el trabajo				
7. Rechazo social				
8. Tener demasiadas cosas que hacer al mismo tiempo				
9. No ser valorado suficientemente				
10. Conflictos economicos con miembros de la familia				
11. Un amigo traiciona su confianza				
12. Que no valoren o aprecien sus aportaciones				
13. Tener problemas para rendir de acuerdo con sus propias metas				
14. La gente se aprovecha de usted				
15. No disponer de tiempo libre				
16. Problemas para disponer de dinero en efectivo				
17. Tener muchas responsabilidades				
18. Insatisfaccion con el trabajo y/o estudios				
19. Tomar decisiones sobre relaciones intimas				
20. No disponer de tiempo suficiente para atender las obligaciones				
21. Tener cargas economicas				
22. Evaluacion de su trabajo inferior a la que ud. Piensa que merece				
23. Experimentar altos niveles de ruido				
24. Evaluacion de su trabajo inferior a lo esperado por usted				
25. Conflictos con miembros de la familia				
26. Encontrar su trabajo agotador				

(POR FAVOR CONTINUA EN LA PAGINA SIGUIENTE)

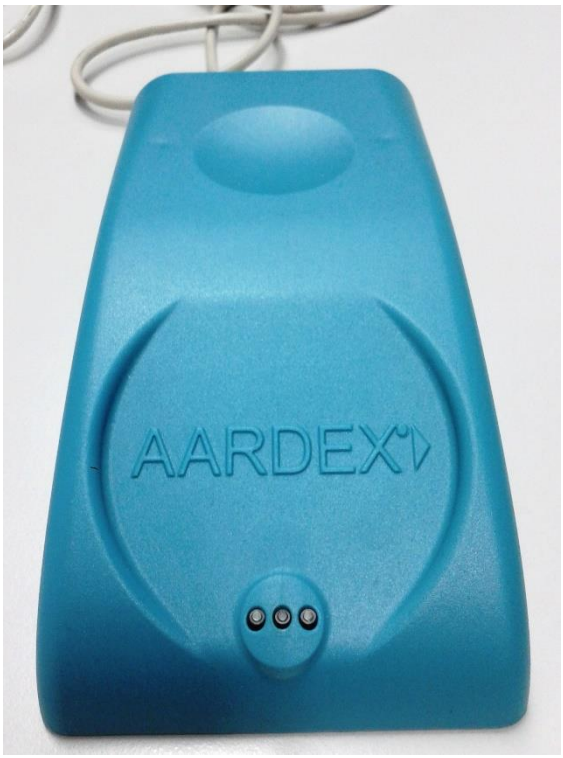
	1	2	3	4
27. Conflicto con los amigos				
28. Intentar conseguir un prestamo				
29. Ser timado o estafado en la compra de bienes				
30. Interrupciones indeseadas en su trabajo/estudio				
31. Estar aislado socialmente				
32. Ser ignorado				
33. Insatisfaccion con su apariencia fisica				
34. Insatisfactorias condiciones de la vivienda				
35. Encontrar el trabajo/estudios aburrido				
36. Fracaso para conseguir el dinero que esperaba				
37. Chismorreos sobre una persona querida				
38. Insatisfaccion con su bienestar fisico				
39. Chismorreos sobre usted				
40. Dificultad para manejar la moderna tecnologia				
41. Trabajo duro para cuidar y mantener la casa				

Por último, señala con una X de 0 a 10 cuánto de estresado/a te sientes en tu vida cotidiana

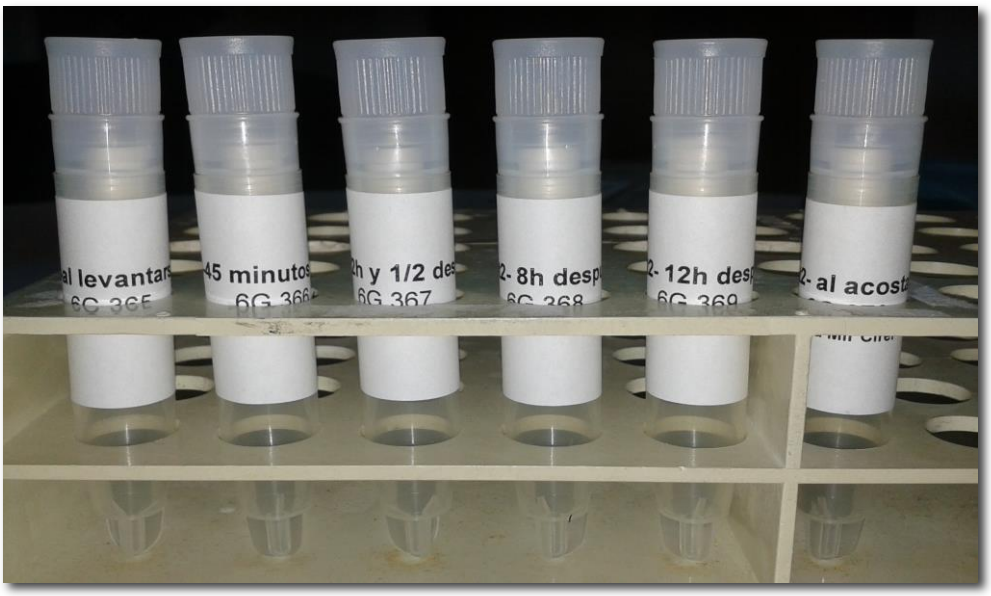
0	1	2	3	4	5	6	7	8	9	10
NADA										MUY

3. Mems Caps

4. AARDEX



5. Saliva collection device



6. Centrifuge for saliva samples machine (Macrotronic – Selecta)

PROTOCOLO DE RECOGIDA DE MUESTRAS DE SALIVA

NOMBRE Y APELLIDOS:

MOMENTOS DE RECOGIDA DE MUESTRAS	EJEMPLO	HORAS RECOGIDA DE SALIVA	Nº DE CIGARRILLOS	Nº de CAFÉS	Nº de Bebidas con cafeína	HORAS DE EJERCICIO	SIESTA SI	SIESTA NO	Nº de bebidas ALCOHÓLICAS	Hora de las COMIDAS	¿cómo te sientes de estresado/a? Valora (nada) 0-10 (muy)
Hora de levantarse	7.15h										
45 minutos después	8h										
2 horas y 1/2 después de levantarse	9.45h										
8 horas después de levantarse	15.15h										
12 horas después de levantarse	19.15h										
A la hora de acostarse	11h										

Traer las muestras al Despacho B-208 el 20 de marzo de 8.30-9.30h

IMPORTANTE: Evitar comer, lavarse los dientes, beber o fumar, 20 minutos antes de recoger la muestra de saliva

¿Estás tomando medicación? SI ☐ NO ☐ ¿Qué medicación estás tomando?

¿Señala cuántas horas has dormido esta noche?	Menos 5 h	5	6	7	8	9	10	Más10h

¿Cómo has dormido?	Muy mal	Mal	Regular	Bien	Muy Bien

MUJERES:

¿Cuándo fue el último día de tu regla?

¿Estás tomando anticonceptivos? SI ☐ NO ☐

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