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STRESS AND EMPATHY – A FOCUS ON CORTISOL AS AN EARLY BIOMARKER

SHISHIR BALIYAN

Doctoral Program in Psychology of Health

Thesis directors:

César Venero Nuñez

UNIVERSIDAD NACIONAL DE EDUCACIÓN A DISTANCIA

Carmen Sandi Pérez

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

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I would like to dedicate this work to acknowledging the irony and unfairness of looking for empathy in laboratory rats, to my family, my friends and to the Mayfly, there and nevermore.

Gracias a todxs

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ANOVA	analysis of variance
AUC_G	total diurnal cortisol output
AVE	Average Variance Extracted
BRCS	Brief Resilient Coping Scale
CA·	*Cornu Ammonis* layer 3
CA1	*Cornu Ammonis* layer 1
CAR	Cortisol Awakening Response
CFA	Confirmatory Factor Analysis
CFI	Comparative Fitness Index
CI	Confidence Interval
Conf	Confinement
COVID-19	Coronavirus disease 2019
CR	Construct Reliability
CRH	Corticotropin Releasing Hormone
DASS	Depression, Anxiety and Stress Scale
DCS	Diurnal Cortisol Slope
df	degrees of freedom
EC	Empathic Concern
e-Corsi	electronic Corsi block-tapping test
EFA	Exploratory Factor Analysis
EQ	Empathy Quotient
FL	Family Loneliness
GC(s)	Glucocorticoid(s)
GDS	Geriatric Depression Scale
GFI	Goodness of Fit Index
GR(s)	Glucocorticoid Receptor(s)
HP	Hippocampus
HPA	Hypothalamus-Pituitary-Adrenal
IRI	Interpersonal Reactivity Index
JASP	Just Another Statistical Program
KMO	Kaiser-Meyer-Olkin Index
M	Mean
MDD	Major Depressive Disorder

MR(s)	Mineralocorticoid Receptor(S)
mRNA	Messenger Ribonucleic Acid
NNFI	Non-Normed Fit Index
NY	New York
PD	Personal Distress
PET	Pictorial Empathy Test
PETS	Pictorial Empathy Test-Spanish Version
PSS	Perceived Stress Scale
PSS-H	Perceived Helplessness
PSS-SE	Perceived [lack-of] Self-Efficacy
PT	Perspective Taking
PVN	Paraventricular Nucleus
QRC	Quality Of Relationships With Cohabitants
RMSEA	Root Mean Square Error Of Approximation
SD	Standard Deviation
SDS	Social Desireability Scale
SELSA	Social And Emotional Loneliness Scale
SL	Social Loneliness
SPSS	Statistical Program For The Social sciences
SRMR	Standardized Root Mean Square Residual
ToM	Theory Of The Mind
T. PSS	Total Perceived Stress
TIPI	Ten-Item Personality Index
UNED	Universidad Nacional de Educación a Distancia
X^2	Chi-square goodness of fit test
Δ	Change in

ABSTRACT

Empathy, a crucial psychobiological process for adequate social functioning and bond formation, is a complex phenomenon that relates to (i) feelings elicited by witnessing another individual's experience (emotional empathy), and to (ii) being capable of knowing another's mental state (cognitive empathy). In addition, it is important to differentiate between dispositional and situational empathy in the context of the two empathy facets (i.e., emotional and cognitive). In line with the increasing interest in the fields of psychology and neuroscience -particularly social neuroscience- in understanding empathy, the goal of this work is to advance knowledge on factors and mechanisms regulating empathy in humans.

One of the key challenges in empathy research is to have psychometrically-reliable and validated instruments. The Pictorial Empathy Test (PET) is one of the few brief tests for situational affective empathy and perhaps the only one to have complete psychometric validity. A first goal of this dissertation has been the translation, validation and adaptation of the PET to the Spanish. To this end, two studies were carried out, including the description of the process (i.e., translation and confirmation; sub-study 1.1) and the confirmation of the internal structure and convergent and discriminant validity of the Spanish version of the test (sub-study 1.2). The obtained results reinforce the utility of PETs as a short, adaptable (specifically for neuroimaging research) screening tool for situational emotional empathy and put forward a validated version to use in Spanish cohorts.

Empathic behavior and abilities are influenced by several factors including age, sex, psychological status and even hormones. Apart from the well documented role of oxytocin in bond-formation and empathy, empirical evidence suggests that cortisol, the principal glucocorticoid of the hypothalamic-pituitary-adrenal (HPA) axis in humans, is also associated with empathic behavior. In this dissertation, we explored the idea that the HPA axis is implicated in the bi-directional adaptation to everyday processes, such as empathy. Using the Spanish version of the PET questionnaire developed under Study 1, we investigated the impact of stressors on empathy and analyzed the contribution of psychological factors to determine different effects. To this end, we took advantage of the situation of strict social confinement imposed by the Spanish

government during the first stages of the COVID-19 pandemic. Indeed, limitations on social interactions and involuntary social-distancing policies are potentially stressful and can exacerbate feelings of loneliness and alter emotional well-being. Thus, in Study 2 we addressed the impact impinged by the pandemic and associated confinement on a young-adult sample. To this end, we explored changes in individuals' psycho-cognitive profile from pre-pandemic to under-confinement conditions. Specifically, we focused on the characterization of state-trait empathy, perceived stress, working memory, prospective volunteering, attention capacities and trait extraversion scores obtained both, before the pandemic and during the first strict home confinement; the latter including as well measures of depression and anxiety. In addition to these analyses, the collected data was used to explore the possible role that baseline (i.e., pre-pandemic) diurnal cortisol indices may play in predicting the emotional impact of the COVID-19 pandemic, attempting to identify a potential biomarker of at-risk groups following public health crises.

The results of the present thesis revealed that participants' social and family loneliness increased during long-term strict home confinement, while prospective volunteering tendencies and extraversion decreased. Also, there was an increase in participants' self-reported perceived stress and Perspective-Taking scores, in parallel with an improvement in executive function (visuospatial working memory). Importantly, after adjusting for relevant confounders, moderation analyses revealed that in young adults with high pre-pandemic extraversion, a higher total diurnal cortisol output (AUC_G) predicted a larger increase in social loneliness during confinement, while in individuals with low extraversion, AUC_G was negatively related to change in loneliness. Furthermore, not only did resilient coping moderate the association between perceived stress scores and pre-pandemic AUC_G and cortisol awakening response (CAR), but also AUC_G and CAR had indirect effects confinement-led changes in perceived self-efficacy. These changes were parallelly mediated by the pandemic-caused increase in visuospatial working memory and Perspective-Taking abilities. In summary, our findings highlight the utility of total diurnal cortisol output in predicting the social impact of stress (COVID-19 home confinement), presenting this hormone as a potential biomarker for a priori identification of at-risk groups during public health crises.

In a final study (Study 3), we revisited the role for cortisol in social and prosocial behaviour, hypothesizing that empathy/prosocial behaviours are adjusted by ideal levels of internal emotional arousal reflected in corresponding moderately high levels of cortisol. We also postulated that this relation may be easier to encounter in an aged sample group (old age people show higher emotional empathy), via a trait measure of emotional empathy (state measures may present excessive inter-individual variation) and in females (females tend to score higher on empathy tests). To address this issue, we measured subjects' trait empathy scores using the interpersonal reactivity index (IRI) to measure empathic concern (emotional empathy) and perspective taking (cognitive empathy) in a sample of 72 community-dwelling females aged 60-84 years old. Cortisol indices were assessed by collecting five saliva samples throughout the day to estimate the cortisol awakening response (CAR), overall cortisol secretion over the post-awakening period (post-awakening AUC_G), total diurnal cortisol output AUC_G as well as the diurnal cortisol slope (DCS). We found that individuals with high empathic concern scores showed higher diurnal cortisol levels as compared to individuals with low empathic concern scores. Hierarchical regression modelling controlling for relevant confounders such as age, awakening hour, sleep duration, years of education and depressive-like symptoms scores revealed that higher post-awakening cortisol AUC_G, higher diurnal cortisol AUC_G, as well as a steeper diurnal cortisol slope were associated with higher trait emotional empathy scores. No associations were found between cortisol indices and perspective taking. Thus, these findings provide new evidence for the use of diurnal cortisol indices as possible biomarkers for emotional empathy in older females.

Altogether, this thesis provides novel and strong evidence indicating how several diurnal cortisol indices are associated with emotional empathy. In addition to providing a new empathy test for Spanish samples, our work reveals key factor and processes underlying mental and emotional constructs. Furthermore, we have pinpointed a potential novel biomarker for the *a priori* identification of populations vulnerable to develop mental health alterations during exposure to public-health emergencies. We hope that our work will contribute to the advancement of the research into the neuroendocrine basis of empathy.

RESUMEN

La empatía, un proceso psicobiológico crucial para un adecuado funcionamiento y formación del vínculo social, es un fenómeno complejo que incluye tanto los sentimientos que surgen al presenciar la experiencia de otro individuo (empatía emocional), como la capacidad de conocer el estado mental de otro sujeto (empatía cognitiva). Además, es importante diferenciar entre empatía disposicional y situacional en el contexto de las dos facetas de la empatía (es decir, emocional y cognitiva). En línea con el creciente interés en los campos de la psicología y las neurociencias -particularmente la neurociencia social- por comprender la empatía, el objetivo de este trabajo es avanzar en el conocimiento de los factores y mecanismos que regulan la empatía en humanos.

Uno de los principales retos en la investigación de la empatía es disponer de instrumentos psicométricos validados. En este sentido, el Pictorial Empathy Test (PET) es uno de los pocos test breves que evalúan la empatía afectiva situacional y quizás el único que tiene una validez psicométrica completa. Un primer objetivo de esta tesis ha sido la traducción, validación y adaptación del PET en una población española. Para ello, se llevaron a cabo dos subestudios que describen este proceso de traducción y confirmación del mantenimiento de significado en la versión española del Pictorial Empathy Test (PETs) en el subestudio 1 y la confirmación de la estructura interna y validez convergente y discriminante del test en el subestudio 2. Los resultados de esta investigación confirman la utilidad de la versión española del PET como una herramienta de detección de duración breve y adaptable (específicamente para la investigación de neuroimagen) para evaluar la empatía emocional situacional y permiten su uso en cohortes españolas.

El comportamiento empático está influenciado por varios factores que incluyen la edad, el sexo, el estado psicológico e incluso las hormonas. Además del conocido papel de la oxitocina en la formación de vínculos y la empatía, la evidencia empírica sugiere que el cortisol, el principal glucocorticoide del eje hipotálamo-hipofisario-suprarrenal (HPA) en humanos, también está asociado con el comportamiento empático.

En esta tesis hemos explorado si la actividad del eje HPA está implicada en una adaptación bidireccional de procesos cotidianos como la empatía. Usando

la versión española del PET desarrollado en el primer estudio, hemos investigado el impacto de los estresores en la empatía y analizado la contribución de los factores psicológicos para determinar distintos efectos. Para ello, hemos realizado un estudio antes y durante la situación de confinamiento social estricto impuesto por el gobierno español durante los primeros estadios de la pandemia COVID-19.

Las limitaciones en la interacción social y políticas similares de distanciamiento social involuntario son potencialmente estresantes y además, pueden exacerbar los sentimientos de soledad y alterar el bienestar emocional. Por tanto, hemos investigado el impacto de la pandemia y su correspondiente confinamiento en una muestra de adultos jóvenes. Para ello, hemos explorado los cambios en el perfil psicocognitivo de los individuos desde las condiciones previas a la pandemia hasta la situación de confinamiento. En concreto nos hemos centrado en la caracterización de la empatía estado-rasgo, el estrés percibido, la memoria de trabajo, el voluntariado prospectivo, las capacidades de atención y la extraversión antes y durante el primer confinamiento estricto de la pandemia. Además, se utilizaron los índices diurnos de cortisol previos a la pandemia para explorar el posible papel que pueden desempeñar en la predicción del impacto emocional de la pandemia de COVID-19 con el fin de identificar un biomarcador potencial de grupos de riesgo en situaciones de crisis sanitaria.

Los resultados de este estudio revelaron que la soledad social y familiar de los participantes aumentó durante el confinamiento domiciliario estricto a largo plazo, mientras que las tendencias prospectivas de voluntariado, así como la extraversión disminuyeron. Además, hubo un aumento en el estrés percibido de los participantes y en las puntuaciones de “Toma de perspectiva” de la empatía, en paralelo con una mejora en la función ejecutiva (memoria de trabajo visoespacial). Es importante destacar que, después de ajustar los factores de confusión relevantes, los análisis de moderación revelaron que, en adultos jóvenes con una alta extraversión antes de la pandemia, una mayor producción total de cortisol diurno (AUC_G) predijo un mayor aumento de la soledad social durante el confinamiento, mientras que, en individuos con baja extraversión, un mayor AUC_G se relacionó negativamente con el cambio en la soledad social. Además, el afrontamiento resiliente no solo moderó la asociación entre las puntuaciones de estrés percibido, el AUC_G pre-pandémico y la respuesta al despertar del cortisol (CAR), sino que también observamos que los índices AUC_G y CAR se relacionaron indirectamente con los cambios provocados por el confinamiento en la autoeficacia percibida. Estos cambios fueron mediados paralelamente por el aumento en la memoria de trabajo visuoespacial y las habilidades empáticas de “toma de perspectiva” observado durante la pandemia. En resumen, nuestros hallazgos destacan la utilidad de usar el índice de cortisol total liberado lo largo del día para predecir el impacto social del estrés (durante el confinamiento domiciliario por COVID-19), presentando a esta hormona como un biomarcador potencial para la identificación *a priori* de grupos de riesgo durante crisis de salud pública.

En un último estudio hemos revisado la evidencia empírica del papel del cortisol en el comportamiento social y prosocial, hipotetizando que es posible que los comportamientos de empatía/prosocial se ajusten a los niveles ideales de reacción emocional interna reflejada en unos niveles moderadamente altos de cortisol. También hemos postulado que esta relación puede ser más fácil de encontrar en una muestra de personas mayores (que muestran mayor empatía emocional), a través de una medida de rasgo de empatía emocional (las medidas de estado pueden presentar una variación interindividual excesiva) y en mujeres (dado que tienden a obtener puntuaciones más altas en pruebas de empatía que los hombres). Para abordar esta cuestión se analizaron las puntuaciones de rasgo de empatía mediante la aplicación del índice de reactividad interpersonal (IRI), que permitió medir la preocupación empática (empatía emocional) y la toma de perspectiva (empatía cognitiva) en una muestra de 72 mujeres de 60 a 84 años. Los índices de cortisol se evaluaron recogiendo cinco muestras de saliva a lo largo del día para estimar la respuesta de cortisol al despertar (CAR), la secreción total de cortisol durante el período posterior al despertar (AUC_G posterior al despertar), la producción diurna total de cortisol AUC_G, así como la pendiente diurna de cortisol (DCS). Observamos que las personas con puntuaciones altas en preocupación empática mostraron niveles más altos de cortisol diurno en comparación con las personas que tenían puntuaciones bajas. El modelo de regresión jerárquica que controla los factores de confusión relevantes como la edad, la hora de despertar, la duración del sueño, los años de educación y las puntuaciones de síntomas de tipo depresivo reveló que una mayor AUC_G de cortisol después del despertar, una mayor AUC_G de cortisol diurno, así como una pendiente más pronunciada de cortisol diurno se asociaron con puntuaciones más altas en empatía emocional de rasgo. No encontramos asociaciones entre los índices de cortisol y la toma de perspectiva. Por tanto, estos hallazgos aportan una nueva evidencia para el uso de índices diurnos de cortisol como posibles biomarcadores para la empatía emocional en mujeres mayores.

En conjunto, esta tesis proporciona una evidencia novedosa de cómo varios índices diurnos de cortisol se asocian con la empatía emocional. Además de proporcionar una versión española validada de una prueba de empatía para muestras españolas, nuestro trabajo revela diversos factores críticos y procesos que subyacen a los constructos mentales y emocionales. Más aún, hemos identificado que el cortisol diurno puede ser un nuevo biomarcador potencial para la identificación *a priori* de poblaciones vulnerables a desarrollar alteraciones en el estado de salud mental durante situaciones de emergencia de salud pública. Esperamos que nuestro trabajo contribuya al avance de la investigación sobre las bases neuroendocrinas de la empatía.

1. INTRODUCTION

1.1 Empathy

This work primarily focuses on empathy in humans, although versions of empathy are increasingly being delineated in other species (with more literature especially with rodents) as well. It is important to distinguish between empathy in human *vs* non-human species given not only the ease of confirming the rationale behind certain actions/feelings simply via inquiry but also because humans are, arguably, a more monogamous species as compared to, say, rodents. This is important given the role of pair bonding in empathy as well as the hormonal correlates of empathy (Shirtcliff et al., 2009).

1.1.1 Origin of empathy

Empathy, the capacity to sense and even share in the mental state of another is considered a highly complex phenomenon experienced and executed by humans. While the very nature of the process makes it difficult to define and delineate, often leading to claims that it is an emotion uniquely human, one needs to look back at the origins of the same to understand it thoroughly. Another obstacle to having both - an elegant and widely accepted – description of empathy is that it is a relatively recently evolved construct and is applicable to relatively few species (De Waal et al., 2006). As is reflected in the previous few lines, not only does empathy have a multitude of definitions depending on the perspective from which it is described; philosophy, psychology, neuroscience, sociology or even art but there also exists uncertainty concerning how exactly to categorize “empathy”. Is it a feeling, an ability, an emotion, a cognitive process or an amalgam of the above?

In order to address the ambiguity of empathy one has to move to the shallow waters of the Gulf of Mexico and back to a time roughly 65 million years from today, a period when the earth was dominated by dinosaurs. An asteroid 12 km wide struck the region, forming the 200 km wide Chicxulub crater and leading to the fifth mass extinction. The resulting immediate destruction by the explosion and resulting tsunamis was followed by weeks of acid rain and the resulting dust cloud led to a drastic drop in temperatures and to the extinction of around 80% of the species present. All animals larger than 25kg disappeared and with them the dinosaurs, the domineering force on earth up until then. This event was critical to the further development of empathy because it allowed for mammals to flourish both in variety and size (mammals up until then being smaller than the domestic cat). Mammals are crucial to the discussion of empathy because of the amount of time and energy they invest into the upbringing of the offspring. Mammals tend to have fewer number of pups but each has a better chance of surviving. This is due to the amount of time the offspring spend under the care of their parents, developing relatively large brains. Reptiles on the other hand have large litters of small-brained but well-developed offspring which are ready to autonomic survival right after hatching and do not need parental care. In the case of mammals, the need and *desire* to care for their offspring, necessitated by their incapacity of independent survival, has been postulated to be aided by the formation of the mother-offspring bond. The mother-offspring bond in turn has been speculated to be at the origin of empathy (MacLean, 1985; Swain, Lorberbaum et al., 2007; for review see Hastings et al., 2006). This makes sense when one considers how important it is for mammalian parents, specifically mothers, to not only provide appropriate care to their recently born offspring but to understand the when and why of the necessities. Empathy too consists of the basic understanding of the condition of another, it is also considered to be one of the sources of prosocial or helping/caring behaviour.

1.1.2 One definition of empathy?

Given that empathy is a recently developed construct, there is also a myriad of definitions associated with it. The English term *empathy* (from Greek *em* (in) *pathos* (feeling), *empatheia*) itself was coined hardly 100 years ago, when looking for a translation for the German word (*Einfühlung* (feeling-into)) coined by philosophy student Robert Vischer studying form and aesthetics (Vischer, 1873). Thus, in its original form and up until the 1940s empathy was solely used to describe the act of projecting oneself into objects and shapes and bring to life inanimate things. Early psychological experiments, where subjects were asked to empathize with objects yielded responses like “organic sensation in chest; cool object in and against it” when asked to imagine being a bowl (Perky, 1910). More than half a century later and following shifts of definitions, empathy was again found to correlate with the ability and frequency of imaginations (Rabinowitz and Heinhorn, 1985). Eventually experiments were carried out where observations were made of how subjects could remember previous sad memories when they saw photographs of sad people and thus empathize with the expressions. The above could be defined as a part of emotional empathy and it coincides with more recent results where previous experience has been shown to aid in the ability to empathize (Batson et al., 1996; Eklund et al., 2009). Following this, during the second world war, social psychologists designed experiments about empathy focused on the ability of subjects to predict the decision making of another by taking the perspective of the other without having their own internal state suffering any changes nor contaminating the predictions with personal influences/biases. More recently, empathy has been looked at from different perspectives and given how empathy is a complex process involving many subcomponents there are many possible definitions which describe part of the construct and are thus, strictly speaking, incomplete without necessarily being incorrect.

Below are described some of these definitions.

a. Cognitive empathy or empathic accuracy

The knowledge, accurate or not, about the internal state including cognitive and emotional condition of another has been considered by clinicians and academicians as empathy (Preston and de Waal, 2002; Wispe, 1986). This same ability has also been used in other terms like cognitive empathy (Eslinger, 1998; Zahn-Waxler et al., 1992) or empathic accuracy (Ickes, 1993).

b. Mimicry or imitation

The adoption of a physical posture which replicates that of another has been considered to be empathy. This physical imitation of another may also consist of matching the facial expression of another as well and such concrete cases fulfil the definition of empathy for certain philosophers (Gordon, 1995). Psychologist have termed this as motor mimicry in their studies (Dimberg et al., 2000; Hoffman, 2000) or as imitation (Lipps, 1903; Meltzoff & Moore, 1997). De Waal and Preston argue for a similar mimicry model but in place of motor mimicry they consider empathy to be represented by the mimicry of the neural representation of the observed (de Waal and Preston, 2017).

c. Emotional contagion or affective empathy

Catching the feelings that another person is experiencing is one of the most widely understood meanings of empathy. The definition here considers that the observation of another person who is exhibiting an emotion can cause the replication of the same or similar feelings in the observer although these feelings need not be of the same intensity. Thus, the contagion of emotions is considered by certain psychologists (e.g., Hatfield, Cacioppo, & Rapson, 1994) as the process of coming to feel as another person. Similar definitions exist for the terms affective empathy (Zahn-Waxler et al., 1992), and automatic emotional empathy (Hodges and Wegner, 1997). Overall, this sharing of the physiology of another is used as a definition for empathy by philosophers (e.g., Darwall, 1998; Sober & Wilson, 1998), neuroscientists (Damasio, 2003; Decety & Chaminade, 2003; Eslinger, 1998), and psychologists (Eisenberg & Strayer, 1987; Preston & de Waal, 2002).

d. Aesthetic projection or aesthetic empathy

The original definition of empathy, the act of projecting oneself into inanimate objects, although out of use in psychology remains relevant in philosophy and art (for e.g., Gernot et al., 2018: *emotion contagion and appreciation of abstract art*). To empathise meant to project our feelings and experiences into forms of art and nature. For example, one carries out the aesthetic transfer of their subjective self into an expansive landscape they may be witnessing, and thus reciprocate a feeling of vastness from oneself to the “object” and vice-versa, thus arguing that empathic processes are essential to aesthetic appreciation (Lanzoni, 2009).

e. Imagining oneself in the situation of another

Role taking (Mead, 1934), cognitive empathy (Povinelli, 1993), empathy (Mead, 1934), projective empathy or simulation (Darwall, 1998), are some terms used to describe the interpersonal imagining of self (imagine-self) in another's situation. This helps one understand how one would think, feel or act if in the place/situation of another.

f. Imagining the other in certain situations

Perspective taking (Ruby and Decety, 2004), psychological empathy (Wispé, 1968), empathy (Ruby and Decety, 2004; Adolphs, 1999), projection (Adolphs, 1999), imagine other (Batson, 1991) or imagine them (Stotland, 1968) are terms used to describe the imagining of another in a certain situation. This involves knowledge of another person and predictions about how they would feel and act under certain stimuli.

g. Observer distress for another that is suffering

Sympathy (Darwall, 1998; Eisenberg & Strayer, 1987; Preston & de Waal, 2002; Sober & Wilson, 1998; Wispé, 1986), sympathetic distress (Hoffman, 1981, 2000), pity or compassion are some terms used to describe this definition of empathy. Here empathy, or empathic concern are used to describe the *other-oriented* emotions evoked concerning the situation of another. So, one may feel sad for another person who is apparently scared, and this is termed as empathic concern. Of note is the feature that the emotions are evoked not for oneself, so one doesn't feel sad *per se*, but feels sad for another and the emotions need not be the same in intensity nor similar in content.

h. Observer distress on witnessing the suffering of another

Empathy (Krebs, 1975), empathic distress (Hoffman, 1981) and personal distress (Batson, 1991) are terms that have been used to describe the visceral state of distress aroused by witnessing the suffering of another. It is important to note that the witness is distressed by what they observe, not due to emotional contagion (definition c) nor because they feel sad for the other person (definition g).

It is important to note that this apparent vagueness concerning the *definition* of empathy is often caused by the difficulty inherent to confirming (or refuting) the presence of one but not the other phenomenon described above. To illustrate using an example, when watching a sad video clip, if the observer starts to cry it would not be erroneous to assume this as a sign of empathy. However, the basis behind the emotional response may be because the witness is simply affected by the emotions on display, a form of emotional contagion or affective empathy (definition c) but one cannot discard the fact that it may also be based in mimicry/imitation (definition b) or be borne from the observer having put oneself in the position of the demonstrator (assuming the video clip shows the cause of sadness as well) and thus the observer sets in motion the process of empathy from cognitive empathy or empathic accuracy (definition a). Since all the above phenomenon are accepted definitions of empathy, in order to parse the presence/absence of one from the other (and from others that may also be involved) in the above example, one would need further investigation which may not be the focus of the study.

As for the current affair of events, in this thesis we use empathy as a multifaceted construct, a perspective increasingly common across social neuroscience and psychology. A brief representation of this is that in this work empathy is considered to be composed of two distinct, yet related, processes (Cox et al., 2012). A) Cognitive empathy is closely related to the theory of mind (ToM) and defined from the point of view of knowing the mental state of another; and B) Affective or emotional empathy, this dimension is closely related to the feelings aroused (shared or otherwise) by witnessing another´s experience (de Vignemont & Singer, 2006). Both processes are complex and compose parts of some of the definitions described earlier. A critical addition to the two processes is the ability to regulate one's emotions (emotional regulation) (Zaki, 2020), be capable of identifying the source of the emotions and to be aware of the difference between the self and the other (self-other). This last part is important given that essentially empathy consists of a self-other overlap. The self-other overlap is defined as any process where a witness experiences a state comparable to that of the demonstrator by activation of the witness's subjective inner characterization of experiencing the demonstrators state, whether ecologically or by conscious simulation. Both motor and neural representations are involved in self-other overlap, thus there can be an activation of neural components by simply witnessing something and this activation would be similar to if the witness were to experience the observed thing themselves (Preston and Hofelich, 2012). As mentioned earlier, this process does not require conscious awareness of the overlap (e.g., Decety & Jackson, 2006; Gallese, 2001; Preston & de Waal, 2002b; Singer, 2006). However, consciously experienced resonance that the witness can identify, emote to, and ruminate over is the form generally discussed in psychology and is considered as crucial to the experience of "true empathy" (Stueber, 2010; Batson & Shaw, 1991; Eisenberg & Miller, 1987; Hoffman, 2000). This added distinction for true empathy is important as one of the effects of the self-other overlap is the risk of the witness failing to carry out emotional regulation and getting overwhelmed by the emotions (concept 3) ending in the inability to pay attention to possible needs of the demonstrator.

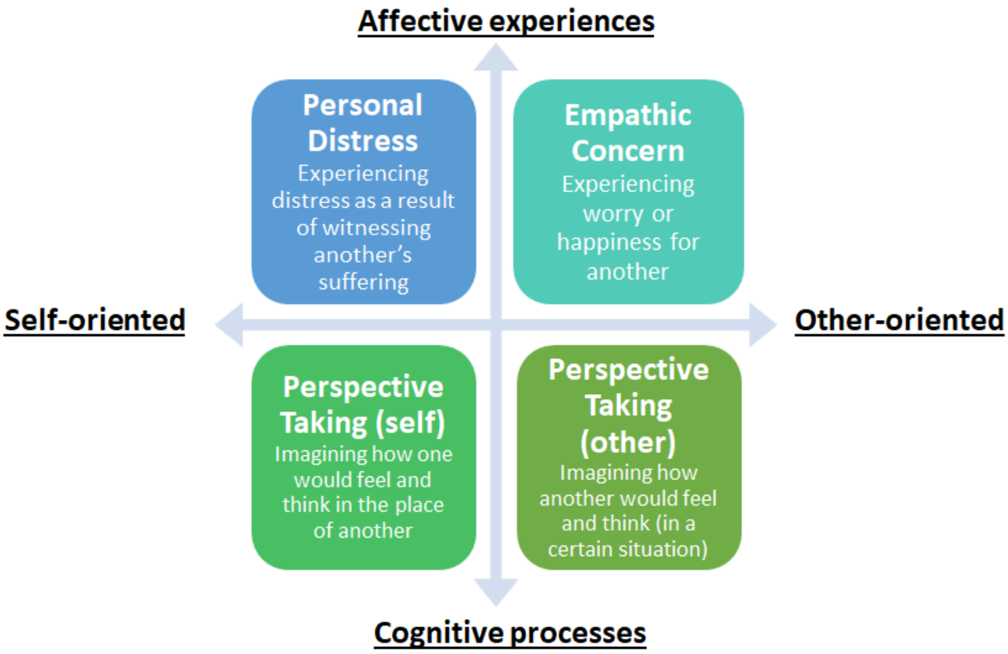

Figure 1. *Overall, empathy can be described as a process roughly in the middle of sensations that are based in the self vs the other and based on processes that are affective vs cognitive. When neither of these four directions is excessively dominant but all four of them are present, is when 'true empathy' can be said to be in play. The taking of perspective itself has two different concepts underlying the process. One is where one imagines the person in certain conditions and we use our knowledge of that person to imagine what they are feeling, while the other is where the person doing the empathizing imagines themselves in the place where the other is, imagining oneself in the conditions being suffered by the empathised and thus change our perspective.*

The self-other overlap is integral to a discussion about empathy also because it is inherent to the perception-action model of empathy described below.

The perception action model considers that in order to understand or perceive – and thus bring out the possibility to share – another, at least the activation of similar experience is needed in the observer's neural system. This is a dynamic-systems view based on the biological explanation of the distribution of discrete experiences into abstract knowledge, memories, etc. This includes representation of feelings like "butterflies in the stomach" or "uncontrollable rage". According to the perception action model, one's neural representations for those experiences get triggered when we are exposed to them, allowing for further processes of empathy to take place. The neural level overlap needed in order to empathy to be triggered has been demonstrated by functional neuroimaging studies (for review see Xiang et al., 2018). Thus, although to an extent top-down processes like memory and general knowledge can aid or even substitute neural representation but having no prior experience (i.e., no neural representation) makes empathizing not possible.

1.1.3 Measurement of Empathy

Empathy as a tool (specifically considering its cognitive aspects) has multiple uses in daily interpersonal interactions. It promotes prosocial (Lockwood et al., 2012; Decety et al., 2016) and altruistic behaviour (Marsh, 2016; FeldmanHall et al., 2017), aids in the development and maintenance of morality (Maibom, 2014; Decety and Cowell, 2014) and arguably helps regulate and reduce violence and aggressive behaviour (Pascual-Sagastizabal et al., 2019; Gantiva et al., 2021 but also see Vachon et al., 2014). Empathy helps stimulate interpersonal bonding, group cohesion (Stürmer et al., 2006; de Vignemont and Singer, 2006) while also being involved in intergroup contexts in the form of ingroup favouritism (preferring one's ingroup) and outgroup derogation (disfavouring one's outgroup) (Fuchs, 2017).

In order to properly explore the relation of empathy with other concepts and fields of study it is crucial to not only define the construct precisely but to also have the instruments and/or techniques needed to register, measure, and record the aspect of empathy of interest. A recent review by Hall and Schwatz (2019) provides evidence of how less than half of the research published (they analysed the literature from the years 2001-2013 and 2017) carried out exploring empathy is employed using an instrument adequate for the corresponding study. The section below describes the most frequently used methods to record and quantify empathy.

a. Self-report based

Measures which consist of participants being instructed to directly respond about their own emotions, perceptions, behaviours, beliefs, attitudes or intentions are known as self-report measures/instruments. In the context of empathy, generally the pattern followed is of presenting subjects with a statement and asking them to either evaluate how well that statement describes their behaviour/personality or respond with the degree of accuracy that statement has concerning their prospective decision making/life choices (Neumann et al., 2015). Some of the most widely used recent self-report instruments measuring empathy and its correlates are the:

1. Balanced Emotional Empathy Scale (Mehrabian, 1996)
2. Multidimensional Emotional Empathy Scale (Caruso & Mayer, 1998)
3. Empathy Quotient (Baron-Cohen & Wheelwright, 2004)
4. Feeling and Thinking Scale (Garton & Gringart, 2005)
5. Basic Empathy Scale (Joliffe & Farrington, 2006a)
6. Griffith Empathy Measure (Dadds et al., 2008)
7. Toronto Empathy Questionnaire (Spreng, McKinnon, Mar, & Levine, 2009)
8. Questionnaire of Cognitive and Affective Empathy (Reniers et al. 2011)
9. Interpersonal Reactivity Index (Davis, 1980; Spanish version: Pérez-Albéniz et al., 2003)

The instrument mentioned last in this list, the Interpersonal Reactivity Index (IRI), is one of the most widely used self-report measures (Hall and Shwartz, 2019). The IRI has been translated into Spanish (Fernández et al., 2011; Pérez-Albéniz et al., 2003), French (Gilet et al., 2013), German (Lauterbach & Hosser, 2007; Paulus, 2009), Chinese (Chiang et al., 2014; Dong & Wang, 2010) as well as across the Dutch, Swiss, Belgian, U.S, Italian and Swedish populations.

The IRI assesses two components each of cognitive and affective trait empathy. The cognitive components measured are Perspective-Taking (taking into account others' viewpoints; e.g., "I try to look at everybody's side of a disagreement before I make a decision") and Fantasizing (relating with fictional characters in literature and movies; e.g., "I really get involved with the feelings of the characters in a novel"). The emotional components include Empathic Concern (feeling for others in need; e.g., "I often have tender, concerned feelings for people less fortunate than I") and Personal Distress (self-oriented, negative arousal as a result of others' distress; e.g., "In emergency situations, I feel apprehensive and ill-at ease") (Davis, 1983). The four components are measured via four subscales assessed using 28-items (Pérez-Albeniz et al., 2003). In the IRI, the participants are asked on a 5-point scale (1 = it does not describe me well, to 5 = it describes me very well) how accurate situations exploring empathy were in describing them.

Self-reported measures of empathy can be complemented and compared with measures that are not dependant on subjective respondent responses. Behavioural measures of empathy involve observing/recording respondents' reactions to stimuli under controlled environments.

b. Behavioural Measures

1. Comic Strip Task (Völlm et al. 2006)
2. Picture Stories (Nummenmaa, Hirvonen, Parkkola, & Hietanen, 2008)
3. Kids Empathetic Development Scale (Reid et al. 2011)
4. Picture Viewing Paradigms (Westbury and Neumann, 2008)

The last of these measures consists of pictorial resources used as stimulant for a behavioural response, this behavioural response may be recorded via further questioning or by observation of the subject with or without psychobiological measures. Pictorial resources have been used to measure both the cognitive and emotional dimensions of empathy (Cognitive measures: Interpersonal Perception Task, Costanzo & Archer, 1989; Reading the Mind in the Eyes test, Baron-Cohen et al., 2001; Amsterdam emotion recognition test; Israelashvili, Oosterwijk, et al., 2019; Geneva emotion recognition test, Schlegel et al., 2014; Emotional measures: Lang et al., 1993; Dziobek et al., 2008; Ali et al., 2009; Derntl et al., 2009; Seara-Cardoso et al., 2012; Westbury & Neumann, 2008).

c. Psychobiological measures of empathy

There are also available a wide range of psychophysiological techniques to measure empathy and its subsets. Neuroimaging, subtle movements of facial muscles (facial electromyography), electroencephalography, blink response (pupillary feedback), electrodermal activity (galvanic skin response), cardiovascular measures etc., are some examples of techniques available.

1.1.4 A self-report/pictorial instrument for use in the Spanish population

As seen earlier, there is a wide variety of tests and distinct questionnaires developed to be able to study the range of constructs that combine together to form empathy (see previous section for details). The principal tools used to carry out research on empathy are the different types of self-report instruments mentioned above, at times combined with behavioural and/or neural assessment (Singer et al., 2006). Currently, the questionnaires employed most often [Emotional Empathy Scale, Mehrabian & Epstein, 1972; Interpersonal Reactivity Index (IRI), Davis, 1980; and Empathy Quotient scale, Baron-Cohen & Wheelwright, 2004)] boast of multiple advantages. These questionnaires are inexpensive to obtain and employ, they are simple and straightforward to understand and analyse, and they are time-friendly, with some versions which can be finished in a few minutes. Nonetheless, these self-report measures also present certain difficulties and limitations:

i) Self-report questionnaire instruments have been exclusively developed for the analysis of trait empathy, also known as dispositional empathy. Instruments to measure state (or situational) empathy are unavailable.

ii) Given that the process which these measures employ, *viz* via inquiry about the respondents' tendency to respond to scenarios and not via actual real-time reactions leaves them open to conscious or subconscious deception in responses. This is often a result of a social desirability bias and may also be a result of simply differences in responder memory, subjective interpretation of the self's actions and even how well a respondent knows oneself (Wilson and Dunn, 2004).

iii) Given that these instruments provide scenarios on which respondents are expected to base their replies, the personal assessment and interpretation of each situation also introduces an undesirable external variable to the responses. For example, responses to an item of the Empathic Concern subscale of the Interpersonal Reactivity Index, "Sometimes I don't feel very sorry for other people when they are having problems", may cause responders to understand the terms sometimes/very sorry/problem in manners very different from one another.

iv) Even if respondents successfully avoid giving socially desirable responses and other variables (self-knowledge, memory, interpretation etc.) are comparable in a sample, such self-report instruments do not strictly reflect objective interpersonal abilities of their respondents. In a recent meta-analysis study carried out by Murphy and Lilienfeld (2019) self-report measures of empathy were found to only weakly-to-moderately predict social comprehension performance. Correlations between the two were between the range of r = .1, $p<.001$ and r= .2, $p<0.001$. Similar findings were obtained in the study carried out by Israelashvili et al (2019).

v) Finally, since current measures focus on dispositional empathy, they tend to lack responses which are ecologically valid reactions (or representations of the construct).

To be able to address such drawbacks of simple self-report measures, Lindeman, Koirokivi and Lipsanen (2018) devised, in Finnish, the Pictorial Empathy Test (PET), a concise method that assesses emotional (affective) empathy responses. The PET merits specific attention because, as described above, even though picture viewing paradigms have often been used as behavioural measures of both emotional and cognitive dimensions of empathy, most of them are incomplete from a psychometric point-of-view. To my knowledge, thorough factorial psychometric evaluation and confirmation that those constructs are being measured is not available for any emotional empathy instrument except for the PET. In Lindeman et al. (2018), the authors present the PET with all testing, measurement and assessment necessary to have a robust instrument complete with exploratory and confirmatory factor analysis of emotional empathy. Additionally, PET composes of photos obtained from open-source material available in the public domain via Wikimedia Commons and has been made freely available for use. Open-source material is of great practical importance in the speed and breadth of availability and access to research material, thereby helping accelerate the scientific process (Canessa & Zennaro, 2009).

1.1.5 Importance of psychometric testing

While psychological tests help measure psychological aspects of humans (e.g., empathy, depression), psychometrics is the methodology based on evaluating the aspects and properties of the psychological tests themselves. Ideally, each psychological test must be checked for psychometric features such as validity or reliability. Each psychological test consists of different measurement items, which may be statements, questions or in the case of PET, images associated to questions. Depending on what the test has been designed to measure, in the case of PET, only one dimension of empathy, situational state empathy, statistical testing needs to be done to confirm that indeed, only one construct is being measured by the PET. Thus, overall, PET is measuring a unidimensional construct. One needs to confirm *what* that construct is (confirm it is emotional empathy being measured) and that all items of PET reliably explore this construct (display conceptual homogeneity), that they all have something in common while also not measuring the same construct in exactly the same manner.

Factor analysis is the most common way to test for and to confirm the dimensionality (number of constructs explored) of an instrument. Broadly, factor analysis can be carried out in two manners: the exploratory factor analysis (EFA) and the confirmatory factor analysis (CFA). As the name suggests, EFA is generally used at the initial stages of the development and analysis of a test and is implemented to look for the latent constructs that may be getting measured on the basis of the theory behind the test as well as on the basis of statistical analysis. In simple terms, this process is largely based on looking for inter-item correlation matrices. If all 7 items of PET strongly correlate with each other and there is no formation of two groups of items (say 3 and 4 items) which correlate strongly within each group but not with items of the other group, then it is proof that the 7 items are measuring something similar (Furr, 2021).

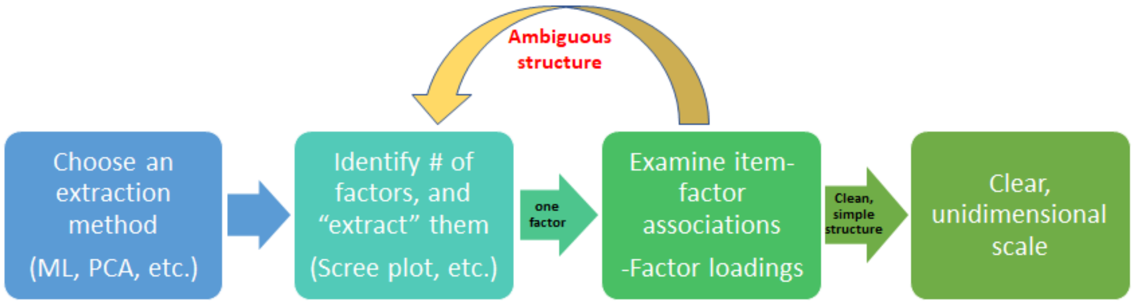

Figure 2. *Schematic flowchart for a unidimensional Exploratory Factor Analysis*

To begin EFA, one first has to select an extraction process which determines the basis of the type of relation the items will have with one another. This is followed by the comparisons of the items with one another and the identification of the number of factors (latent constructs) being measured by the items. This is done on the basis of eigenvalues, scree plots and parallel analysis. In short, values seen or graphed (as in scree plots) are used to place a cut-off which cues one to how many statistically acceptable latent constructs can be identified by the extraction method used. Finally, each item is then studied with respect to its relation with the construct that has been identified to be measured by the instrument. Thus, the Factor Loadings (usually with values from -1 to 1) allow for a rough idea about how strongly is each item related to the factor as well as the amount of "weight" each item adds to the formation of the latent construct, apart from statistics denoting how much unique weight is added by an item. Thus, factor loading values that are close to 0 are generally pointers to the possible exclusion of certain items without much loss to the instruments measuring capability (Hair et al., 2019).

In comparison to an EFA, a confirmatory factor analysis (CFA) is used at a stage where the latent constructs behind an instrument's items are well known. Hence, it is a confirmatory procedure to test if the known dimensions are in fact what the items are measuring in practice. Although there are inherent fundamental similarities between EFA and CFA, there are some differences which make the use of CFA imperative to, say, the adaptation of a test from one culture and language to another. The confirmatory factor analysis is not just another manner of exploring an instrument's internal structure but allows test developers and evaluators to understand the degree to which their theoretically driven models are consistent with the data that has been collected from respondents. This can then help in posterior changes to the hypothesis based on the internal structure obtained (Hair et al., 2019).

Psychological tests are useful only to the degree that they accurately measure true psychological aspects. That is to say that if two people carry out the PET, we would like to assume that any difference between the scores of the two subjects is caused by actual differences in dispositional emotional empathy and not due to measurement error or other variables. Put differently, measurement errors and the true score of a subject responding to PET would give one the observed score. The measurement error may be such that it increases and decreases the scores of one or the other subjects, and such that the error has nothing to do with the true score of the subjects, these two are taken as given assumptions to accurately explore a test instruments reliability (error which always changes the score in one direction is not problematic given that this would occur for every subject). That a test is reliable in the measurement scores that is produces is one of the most basic and important psychometric properties and this feature is called test reliability and is based on the standard error of measurement (i.e., the average size of the error scores) (Furr, 2021).

Arguably the most important psychometric component of all tests is the measure of validity. Validity statistics tell us that indeed the instrument is actually measuring what it is supposed to be measuring. All the items of PET may have high factor loadings to one single latent dimension and they may all be robustly measuring differences between individuals which reflects true differences (high reliability). However, it is still possible that PET does not in fact measure emotional empathy and that there is some other concept that is being measured (an associated concept or simply an unrelated concept being measured by chance). In order to confirm and really be sure that the dimension being measured is the desired one, validity analyses need to be run. A more elaborate definition of validity also tells us how exactly validity is confirmed or rejected:

validity is "*the degree to which evidence and theory support the interpretations of test scores entailed by the proposed uses*" of a test (American Education Research Association, the American Psychological Association, and the National Council on Measurement in Education, 1999, p. 9). Construct validity can be measured by checking for content validity (no construct-irrelevant content and no underrepresentation of dimensions in the scale used). It additionally includes convergent and discriminant validity checks. Convergent validity checks are tests to confirm if theoretically known relations are maintained as they should when using the instrument under examination and discriminant validity correlation checks explore if the instrument is capable of discriminating between related but distinct constructs (for example if PET can distinguish between cognitive and emotional empathy) (Furr, 2021).

1.2 Stress, the HPA axis and Cortisol

Somewhat similar to the debate surrounding the description of empathy, stress has been a source of much discussion as to what it exactly is and what should or should not be called “stress”. One of the earlier descriptions of stress was that of a non-specific response of an organism to noxious stimuli (Seyle, 1957). The scope of the phenomenon was made more precise by including the phenomenon of ‘stressor’ and ‘stress response’. The term homeostasis, coined by Cannon (1932), is also closely implicated with stress and stress response, with the maintenance of a complex dynamic internal equilibrium being known as homeostasis and stressors being stimuli which disturb homeostasis and the stress response being the response of an organism aimed at maintaining the same. Stress being considered as anything that alters the homeostatic state has been questioned by various authors, principally because most functions and actions carried out by organisms are in order to avoid the alteration of homeostasis (Levine, 2005; Romero et al., 2009), such as the search for nutrition. Hence, stress is better conceived as change in homoeostasis born from the uncontrollable and/or unpredictable nature of the stressor (for review see Koolhaas et al., 2011), the COVID-19 pandemic along with the confinement measures may be considered as a stressor that fits this definition very well.

Given the definition of stress, it is not surprising that there is a wide variety of events and phenomenon that serve as stressors. An intuitive division concerning types of stressors can be made on the lines emotional (mental/psychological) stressors and physical (external or internal) stressors. Further subdivisions of the types of stressors can be made concerning the degree of stress induced as well as their duration (Lazarus and Folkman, 1984). A stressor is only considered so when it either exceeds thresholds of the stress response system (thus activating it) due to its intensity or temporal span of action. When the stress response system is activated, compensatory responses are initiated and changes take place in the central nervous system and in peripheral organs/tissues. Activation of the central nervous system involves neural pathways implicated in adaptive functions like arousal, attention, vigilance and other neural systems which are meant to inhibit bodily functions which are not immediately necessary nor helpful (nonadaptive) for a swift return to homeostasis. The response includes diverting more resources to the brain (oxygen and energy) and to the heart and skeletal muscles, a response crucial and in preparation of survival under the most dangerous of situations (Joëls, Sarabdjitsingh, and Karst, 2012). This response is a quick-response which is followed by a slower, more complex response related to the HPA axis. In this neuroendocrine circuit, the limbic and hypothalamic systems coordinate cognitive, emotional, neuroendocrine, and autonomic nervous system responses, which dynamically determine, for each individual, their behavioural, neural, and hormonal stress response (Lucassen et al., 2014). Another manner of looking at the different stressors is the type of response they produce; eustatic or allostatic. This distinction differs on the basis of each individual given that a stress may be allostatic when the individual is not capable of positively adapting to the stress (in this case called distress) while it may be eustatic when the individual experiences a net positive effect on health, motivation, cognition and well-being, thus adapting successfully to the stress (in this case called eustress) experienced. The fact that the same stressor may be allostatic for one individual but eustatic for another is also reason for the term stress being slightly ambiguous. Added to this the fact that the subjective experience of stress is not always reflected in the increase in physiological markers of stress, cortisol and catecholamines (Kirschbaum et al., 1999). These same markers, especially cortisol (more details below), do not necessarily have similar levels across individuals, in fact, cortisol has a dynamic basal secretion level which changes intra-individually in accordance with the day-night cycle and the sleeping-awakening profile of each individual. The hypothalamus is the most relevant structure in the regulation of the autonomic nervous system and stress. It integrates the sensory and visceral information received from the cerebral cortex, the entorhinal cortex (EC), the hippocampus (HP), the amygdala, some thalamic nuclei, the basal ganglia and the cerebellum and, activates the sympathetic response directly from the paraventricular nucleus of the hypothalamus (PVN) or through the nucleus of the solitary tract (NTS) under situations of stress.

1.2.1 HPA axis activation

The Hypothalamic–Pituitary–Adrenal axis (HPA axis or Limbic-HPA axis) is a principal neuroendocrine system which governs the stress machinery and is additionally involved in various processes, among them metabolic, immune and inflammatory systems. It is composed of influence to and feedback interactions from three components: the hypothalamus, the pituitary gland and the adrenal glands (Toni, 2004).

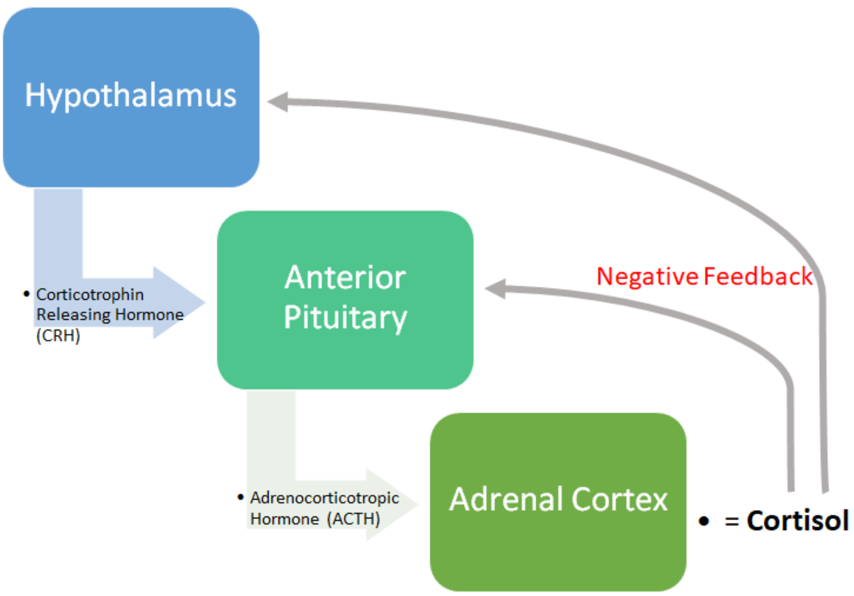

Figure 3. *Schematic gist of the HPA axis.*

The PVN neurons of the hypothalamus synthesize corticotropin-releasing hormones (CRH) and are primarily responsible for the secretion of the "stress hormone" cortisol. Through a series of blood capillaries in the median eminence, CRH reaches the adenohypophysis, stimulating corticotropic cells that, in turn, release adrenocorticotropic hormone (ACTH). In parallel, the magnocellular cells of the hypothalamus release other hormones in the neurohypophysis, such as oxytocin and vasopressin. Through blood circulation, ACTH reaches the adrenal cortex, and stimulates the release of glucocorticoids (GCs) like cortisol that have been synthesized in the fascicular layer of the adrenal cortex. Thus, GCs are the end product of HPA axis activation. Once released into the bloodstream, GCs have direct effects on the brain and the rest of the body. When GC levels are elevated due to over-activation of the HPA axis, GCs like cortisol themselves participate in the regulation of their levels through negative feedback mechanisms (Rich & Romero, 2005). This is because the GCs act directly on the adenohypophysis and the PVN; or indirectly, they influence other brain structures such as the HP, the amygdala, or the noradrenergic system, thus modulating the activity of the hypothalamus (Jankord & Herman, 2008). It should be noted that other signalling pathways act in concert with the HPA axis, thus regulating energy resources. Among these parallel signalling pathways are the Hypothalamic-Adipose-Gonadal axis and the immune system.

1.2.2 Glucocorticoids

Basal plasma levels of GCs are determined by activity based circadian variations (Liston et al., 2013; Qian, Droste, Lightman, Reul, & Linthorst, 2012). Thus, in humans, lowest plasma cortisol levels are observed during deep rest at night while during the morning (as the body prepares for the day and the system is “turning-on”) GCs reach peak concentrations. GCs have the capacity to act on the central nervous system and most peripheral organs via specific receptors found in these organs. These receptors are characterized by different affinity to GCs, as well as by their distribution, and are divided into two classes: 1) the mineralocorticoid receptor (MR; type I); and 2) the glucocorticoid receptor (GR; type II) (Russell, Kalafatakis, & Lightman, 2015).

MRs are bound by mineralocorticoids (aldosterone being the main steroid hormone of this family) and are characterized by high affinity for binding to GCs. MRs have a more limited localization than GRs; they are found mainly in cardiovascular tissues, liver and kidneys, as well as in most corticolimbic regions of the brain, including those involved in the regulation of the HPA axis (De Kloet, Vreugdenhil, Oitzl, & Joëls, 1998). Although more abundant, GRs have a six- to tenfold lower affinity than MRs for GCs and are found in high concentrations in the HP, basal ganglia neurons and in the lateral and medial septum of the amygdala (Patel et al., 2000). More specifically, human HP shows abundant expression of GRs in both *Cornu Ammonis* layer 1 (CA1) neurons and dentate gyrus neurons, with the lowest levels demonstrated in the CA3 subregion of HP. Additionally, GRs are expressed in astrocytes as well. Although GRs levels remain stable with age, hypercortisolaemia and decreased mRNA for GRs in the HP has been observed in patients with major depressive disorder (MDD) and schizophrenia (Wang et al., 2013). The research group led by Bruce McEwen first postulated the association between chronic stress and neuronal damage in the glucocorticoid cascade hypothesis (Sapolsky, Krey, & McEwen, 2002). According to this hypothesis, if exposure to high levels of GCs was prolonged over a long period of time, corresponding to situations of chronic stress or HPA axis dysfunction, it would induce progressive damage in key areas of the brain involved in HPA axis regulation, with the HP being particularly vulnerable. In the sections below we will study this hypothesis specifically in relation to different psychological states, traits and conditions.

1.2.3 Cortisol and its diurnal indices

Cortisol is synthesized from cholesterol in the *zona fasciculate*, the intermediate region of the human adrenal cortex. The cells there produce cortisol in the mitochondrion, which then gets secreted out into the extracellular space and into the bloodstream. Cortisol has a low molecular weight and is lipophilic, thus, free cortisol (when unbound from corticosteroid-binding globulin) enters other cells via passive diffusion, making it possible to measure unbound cortisol in various body fluids (sweat, tears, urine, blood). Saliva too contains free cortisol in easily detectable levels and one of the preferred sources due to sampling ease (Elias et al., 2014).

As we know, cortisol is the main adrenal glucocorticoid hormone that is under control of the Hypothalamic-Pituitary-Adrenal (HPA) neuroendocrine axis, that exerts not only a primary role in the stress response (Herman et al., 2005), but also a major circadian signal (Spencer et al., 2018). In the absence of a stressful situation, cortisol secretion shows a strong circadian rhythm, with high concentrations at awakening and low levels at night (Hucklebridge et al, 2005). Typically, in the first 30-45 min following awakening, there is a rapid increase in cortisol concentrations (the cortisol awakening response, or CAR). Subsequently, cortisol levels show a fast decrease during the following few hours, with a small rise during lunch, and later they decline progressively reaching the lowest levels around midnight (Pruessner et al., 1997; Elder et al., 2014; Kudielka and Wüst, 2010). In the last decade, research on the diurnal rhythm of cortisol is gaining attention for its utility as a biomarker of not only the stress response, but also of general HPA axis function reflecting cognitive state and emotional well-being (Adam & Kumari, 2009; Stone et al., 2001). It has been noted that this diurnal fluctuation of cortisol is integral to the development as well as degradation of synapses in the brain, which in turn helps in learning and adaptation (Liston and Gan, 2011; Liston et al., 2013). So, while too much of cortisol (or its absence when it should be released) is detrimental to health, a flat diurnal rhythm of cortisol too is pernicious to health (McEven and Karatsores, 2015; McEven, 2019). It is important to keep in mind that these fluctuations and the rhythm of cortisol is a function of the need of the organism and their activity.

As described above, the HPA-activity and stress reactivity-based changes in cortisol reveal an abundant amount of information concerning the condition that a person or a group of individuals may be in, and hence, there has also been a lot of interest in studying the same. As a result of this widespread attention, many other indices of HPA functioning, similar to but apart from CAR, have been recorded (for example; baseline output, total area under the curve with respect to ground (Diurnal cortisol AUC_G), area under the curve from baseline to peak). Khoury (2015) and her colleagues carried out a restricted review of six principal journals publishing research about HPA activity in relation to behaviour. They identified as many as 15 indices being used although as previously reported (Dickerson et al., 2004; Stalder et al., 2016) many have been used without a proper rational, or citation, for the chosen index. Although Khoury et al (2015) investigated stress reactivity, they summarised that most information from the 15 indices they had identified was concentrated in only total baseline cortisol output and in the change in cortisol levels hence this thesis focuses on the four principal indices mentioned next. The CAR has been proposed as a useful index of adrenocortical activity that provides relevant information on the activity and proper function of the HPA axis (Schmidt-Reinwald et al., 1999; Adam and Kumari 2009). Apart from CAR, other indices of interest are overall output of cortisol across the daytime hours (Diurnal cortisol AUC_G). Total cortisol output across the day has been shown to be a distinct variable independent of CAR (Edwards et al., 2001, Maina et al., 2009). It is important to keep in mind that AUC_G provides a baseline output value and is not necessarily related to the body's stress reactivity nor the anticipatory stress response (Engert et al., 2013). Total output over the period of CAR (post-awakening AUC_G) is also another index which is novel and potentially records aspects of CAR and of AUC_G in parallel, therefore being of special interest. Finally, the diurnal cortisol slope (DCS) is a much-used index concerning how sharply cortisol levels differ across night-time and post-awakening sample levels.

1.2.4 Cortisol, COVID-19 pandemic, psychological stress, and loneliness

"Pains of imprisonment" was a term coined by Sykes (2021; reprinted from 1958) and was used to first describe all the subjective and objective stresses faced by prisoners over the course of their imprisonment. The five aspects to the stress were: deprivation of freedom, of material and services, of relationships, of autonomy and of security. Boredom, unemployment, loss of contact with reality, hallucinations, attempts at self-harm (Gibbs, 1982), loss of life skills, extreme loneliness, humiliation, impaired sense of self, violation of sexual identity (Irwin & Owen, 2005), and feelings of stress and anxiety alongside other psychological difficulties (Crewe, 2011) are additional aspects of incarceration. House arrests are an alternative means to detain someone in their own house, which too has been shown to have similar, only if not as intense, impact as above (Payne and Gainey, 2004; Vanhaelemeesch, Vander Beken and Vandevelde, 2014). Now is a good moment to comment that people who experienced long-term forced home confinement due to the COVID-19 pandemic control measures implemented by their governments reported very similar feelings to their obligatory “house-arrest”. Of the five aspects to “pains of imprisonment” described by Sykes (1958), loss of freedom, relationships, autonomy and personal security were reported by nursing home residents who underwent stringent, even if unavoidable, lockdown measures (Kaelen et al., 2021). Boredom, loss of contact with reality, loss of life skills, loneliness, stress and anxiety and other psychological issues are some of the effects of COVID-19 pandemic and confinement that a wide number of studies from across the globe have reported (for reviews see Rodríguez-Fernández et al., 2021; Brooks et al., 2020; Cachón-Zagalaz et al; Serafini et al., 2020; Xiong et al., 2020; a review and meta-analysis- Prati and Mancini, 2021). The striking qualitative similarity between the subjective experiences and the objective measures of psychological suffering permits for the perspective that home confinements due to the global pandemic have been and are sources of considerable stress.

It is well known that living together and forming societies contributes to the survival and reproductive success of humans. This organizational system provides protection against environmental threats and is associated with increased life expectancy (House et al., 1988; Lillard et al., 1995). Moreover, strong social bonds help promote the proper functioning of regulatory systems and help maintain individuals’ homeostatic balance (Kiecolt et al., 2001; Waltz, et al., 1988). Many genetic, molecular and hormonal changes have helped to support this type of social organization and there are a large number of studies in which it has been described that individuals who receive social support or are more integrated within a social network have greater cognitive capacity and are less likely to experience deleterious cognitive changes over the course of their life (Berkman & Syme, 1979; Fratiglioni et al., 2004; Seeman, Lusignolo et al., 2001; Zunzunegui et al., 2003; Fratiglioni et al., 2004; Gow et al., 2013; Seeman et al., 2001; Zunzunegui et al., 2003). Other studies have suggested that people who live alone are twice as likely to develop dementia (Fratiglioni et al., 2000). Finally, it should be noted that individuals with a good social network enjoy better health and a longer life expectancy than individuals who have a small social network and/or low-quality social relationships (Gow et al., 2013).

Currently, social isolation could be divided into two types: physical isolation (Cornwell & Waite, 2009a; Hawthorne, 2008; Waite & Hughes, 1999) and isolation related to social participation (Benjamins, 2004). However, Cornwell and Wait (2009b) propose to study social isolation in two different ways; one of them aimed at understanding it as disconnection from the social world; the second, explained from the point of view of the perception of the feeling of isolation or loneliness. Some studies point out that the risks that social isolation can pose to health are comparable to those associated with cigarette smoking or obesity (House, 2001). People who lack social connections, or experience frequent feelings of loneliness, tend to suffer higher rates of morbidity and mortality (Brummett et al., 2001; Seeman, 2000; Uchino et al., 1996), as well as infections (Pressman et al., 2005), depression (Heikkinen & Kauppinen, 2004) or cognitive impairment (Barnes et al., 2004). Younger adults are more likely to be classified as socially isolated and depressive symptoms are strongly associated with perceived social isolation (Hawthorne, 2008). Therefore, social disconnection would be marked by a lack of social relationships and low levels of participation in social activities, and perceived isolation or loneliness, would be understood as the feeling of loneliness and a perceived lack of social support (Yi & Hwang, 2015).

Loneliness is a complex unpleasant feeling rooted in a state of mind in which one´s interpersonal relationships are perceived as inadequate (Peplau and Perlman, 1982). In 1973, Weiss proposed that loneliness is composed of social and emotional dimensions (Weiss, 1973). Social loneliness results from a sense of dissatisfaction with one's general social life and interactions, while emotional loneliness consists of dissatisfactory intimate emotional ties, as with a spouse, parent or sibling. Loneliness can affect all age groups, but its prevalence is higher in young (18-30 years) and elderly (>80 years) adults (Hawkley et al., 2020). As consequence of the COVID-19 pandemic, governments across the globe had enforced population confinements during early 2020. In Spain the population underwent a strict, 50-day lockdown with citizens obliged to remain inside their residence. Such extensive confinements accompanied by overhauling changes to daily social interactions demand more attention for two main reasons; firstly, because recent studies have reported sharp increments in feelings of loneliness and worsening of mental health following lockdowns (Pierce et al., 2020; Groarke et al., 2020) and; secondly, because it is well known that long-term loneliness increases the risk of detrimental health consequences, including higher blood pressure, anxiety, depression and all-cause mortality (Cacioppo and Cacioppo, 2014; Martín-María et al., 2020). Although the impact of the pandemic on loneliness has made things harder for all individuals affected, evidence suggests that younger adults (18-24 years) merit specific attention for being the group most hard hit (Groarke et al., 2020).

In the last decade, a dysregulation of the HPA axis has been proposed as a potential mechanism through which loneliness may trigger pernicious health effects (Cacioppo et al., 2002; Nowland et al., 2018). Thus, in some studies loneliness feelings were found related to higher AUC_G (Lai et al., 2018; Pressman et al., 2005). In contrast, other studies found either no relationship between loneliness and cortisol (Cacioppo et al., 2002) or even a flattening of the diurnal cortisol rhythm (Doane and Adam, 2010). Although these studies in young-adult samples show contrasting results, they suggest an important relation between cortisol and loneliness. A growing body of evidence points towards loneliness as not only a cause of, and to correlate with stress, but also a possible consequence of biological factors, like individual differences in cortisol output (Cacioppo and Hawkley, 2009; Campagne, 2019). Studies such as those done by Stone et al. (2013), and Cole et al., (2015), provide evidence of how prior stress can precede posterior emergence of symptoms of loneliness. Similarly, reduction of stress via mindful meditation, known to alter cortisol output (Brand et al., 2012), has been shown to reduce loneliness as well (Creswell et al., 2012; Lindsay et al., 2019). In a recent study in young adults, cortisol levels moderated the relation between loneliness and social connections (Kornienko et al., 2020).

Individual differences in genetics and personality are also known to play a crucial role in this interdependent relationship between loneliness and stress. In this regard, Boomsma et al., (2005) and Gao et al., (2017) reported that genetic factors significantly contribute to loneliness, and the latter also suggest that loneliness is genetically associated with personality. The role of personality in individual differences in loneliness can be expected given how the need of social connections or the impact/threat of their loss - as by current pandemic and the confinement periods - varies from person to person (Cacioppo and Patrick, 2008). Multiple cross-sectional studies in young adults have reported that loneliness relates inversely with extraversion (Atak, 2009; Buecker et al., 2020; Cacioppo et al., 2006; Cheng and Furnham, 2002; Stokes, 1985). According to Eysenck's theory of personality (Eysenck, 1963), extraversion is associated with lower levels of cortical arousal which leads to a greater need for stimulation. This need is in turn met by behaviours that increase the type and degree of interpersonal and social relations. Following results of their study and interpreting them in light of Eysenck's theory, Saklofske and Yackulic (1989) discussed how "*for the extravert, loneliness may occur when limitations are placed on the opportunity to interact with others on a regular basis*." (p. 4). Concerning the relationship between extraversion and cortisol, it has been reported that extraversion relates with more positive objective life events (Magnus et al., 1993) and aids attention to positive aspects of stressors (Hemenover & Dienstbier, 1996). Low extraversion has been associated with both, low and high basal cortisol outputs (Hauner et al., 2008; Laceulle et al., 2015; Ouanes et al., 2017). In two separate studies using young-adult samples, researchers found extraversion to predict cortisol responses (Pérez et al., 2004; Limone et al., 2021), providing findings for a relationship between extraversion and the HPA system. Extraversion has also been found to moderate the relationship between peer attachment and loneliness (Lu et al., 2014).

Thus, the strict social confinements present a salient opportunity for studying the existence of a relationship between cortisol output and the propensity to experience changes in loneliness, empathy, extraversion, working memory and perceived stress outside laboratory settings. Given that the literature suggests extraversion as a possible moderator, it would be reasonable to examine if the relationship between AUC_G and loneliness differs when extraversion varies between individuals.

1.2.5 Cortisol and its impact on behaviour

CAR has been shown to be related to maladaptive neuroendocrine patterns which then reflect in psychosocial and health related correlates (for reviews see Kudielka and Wüst, 2010, Chida and Steptoe, 2009, Fries et al., 2009, Clow et al., 2004). Similarly, previous research also indicates that a flatter DCS is related to chronic stress and worse physical and mental health, while subjects with steeper slopes are physically and mentally healthier (for meta-analysis and review see Adam et al., 2017).

Cortisol and mental health (perceived stress, anxiety and depression)

There is literature which allows the speculation of the possibility that baseline cortisol profiles and HPA axis function may influence mental health. Mean cortisol levels have been shown to be negatively related to shy/anxious or internalizing behaviour in preschool boys, but not girls (Tout et al., 1998). Similarly, basal (trait) cortisol and externalizing behaviour has also been shown to have a negative relation only for adolescent boys (Shirtcliff et al., 2005). A meta-analysis (k=72, n=5,480) of basal cortisol and externalizing behaviour (angry/aggressive) in children revealed a negative relation between the two. Furthermore, they found that the age of the children moderated the relation in a way that externalizing behaviour was related to higher basal cortisol in pre-schoolers but with lower basal cortisol values for elementary school (6-10 years) aged children. Furthermore, no relation was found between basal cortisol and externalizing behaviour in adolescents (11-16 years) (Alink et al., 2008). Booth, Granger and Shirtcliff (2008) showed that adolescent girls (and not in middle schoolers nor boys) present lower levels of stable trait-like basal cortisol levels to relate with poor quality social relationships (parents, siblings and peers). This association was independent of any internalizing or externalizing tendencies, presenting further proof of an adaptive nature to the cortisol-behaviour link. Below we consider other psychological conditions of interest and explore their possible relation with basal cortisol.

- **Perceived stress and diurnal cortisol profiles**

We as individuals generally tend to be perceptive to periods of time of events that cause us stress. This ability to be able to perceive oneself as under stress is produced by a subjective self-evaluation. Thus, perceived stress is the belief or self-analysis that one has about the amount and quality of stress they are under. Given the definition of stress itself, self-reported perceived stress interprets the uncontrollability and unpredictability of issues one is facing at the moment or over a certain period of time (Phillips, 2013). By default, it is a qualitative measurement about one’s life and ability to handle life-events (Lazarus and Folkman, 1984). Generally perceived stress is measured using the Perceived Stress Scale (PSS) (Cohen, Kamarck and Mermelstein, 1983). The PSS is the most translated and widely used tools used to measure stress perception and presents robust psychometric properties. It is a 14-item self-report questionnaire developed to assess how “unpredictable, uncontrollable, and overloading” is one’s life perceived to be (Cohen et al., 1983, p. 387). Sample items include the following: “In the last month...how often have you felt in control of everything?”, and “...how often have you felt that you were able to control the difficulties in your life?”. Half of the questions are positively stated and reverse coded and are supposed to be responded to on a 5-point Likert-type scale. Given the kind of instrument PSS is, measuring psychological stress, one may assume that physiological stress response as measured by cortisol and results from the PSS will correlate well. However, although there is an obvious link between the two, associations that have been looked for have been found to be weak and ambiguous (Cohen et al., 2000; Hjortskov, Garde, Ørbæk, & Hansen, 2004; Lackschewitz, Hüther, & Kröner-Herwig, 2008; Schlotz et al., 2008). Campbell and Ehlert (2012) reviewed almost 50 studies and concluded that less than 25% of them presented a corelation between perceived stress and cortisol reactivity. However, concerning baseline cortisol values and stress, in a neuroimaging study by Henckens (2016) and colleagues, high basal cortisol levels were related to posterior stress resilience and lower stress-induced amygdala activity. Findings by Volmann and Weekes (2006) revealed that basal cortisol levels moderated the relationship between stress and health outcome leading them to conclude that the HPA system acted on stress-related illnesses, while waking cortisol levels can predict emotional responses to potentially traumatic events (Pineles et al., 2013), post-traumatic stress disorder (PTSD) (Pervanidou et al., 2007), and even socioemotional adjustment (Smider et al., 2002). Lowell, Moss and Wetherell (2011) also encountered that cortisol mediated perceived stress related health complaints. Although cortisol stress response levels may not correlate well with perceived stress scores, there is evidence suggesting that basal cortisol levels may relate with and even predict levels of posterior self-perceived psychological stress. This is further supported by the fact that the stress response and basal HPA axis dependant basal cortisol values act on differing systems. Different profiles of corticosteroid receptor activation belong to either process thus possibly pointing to differential neural pathways (de Kloet et al., 1998).

- **Anxiety and diurnal cortisol profiles**

Anxiety may be defined as a state which is "*characterized by an unpleasant affective experience marked by a significant degree of apprehensiveness about the potential appearance of future aversive or harmful events*" (DiTomasso and Gosch, 2002). This condition can be measured using various self-report instruments like the Spanish version of the Depression, Anxiety and Stress Scale (DASS; Ruiz et al., 2017). The DASS is composed of 7 question items for each scale, thus giving a measure of depression-like symptoms, anxiety and self-perceived stress. Some researchers suggest certain children are at a higher risk to develop anxiety given that they have a lower threshold for stimuli induced HPA axis activation increasing cortisol (Kagan, Reznick and Snidman, 1987). Thus, anxiety problems should be associated with higher diurnal cortisol levels. But other researchers speculate that assuming early life stress leads to anxiety, anxiety issues would be associated with lower diurnal cortisol output (van der Vegt et al., 2010). This would occur given eventual dampening of the HPA axis caused by frequent early life stress led cortisol elevations (Gunnar and Vazquez, 2001). High basal level of cortisol has been shown to contribute to clinical anxiety (Muller, Koen and Stein, 2005; Rainnie et al., 2004). Girls with high morning cortisol and dampened CAR also showed anxious attachment traits (Oskis et al., 2011). In a large study, Greaves-Lord et al. (2007) comment how alterations in HPA axis activity may result in anxiety issues after finding that adolescents (n=1768) having higher morning cortisol levels and CAR display persistent anxiety. High basal cortisol causes psychopathologies and blunts HPA-responsiveness as happens in anxiety; hence reduction of basal cortisol may lead to a reduction in anxiety (Dunko et al., 2006). In fact, weeklong dietary supplementation with amino acids L-lysine and L-arginine have shown to concomitantly reduce anxiety symptoms as well as basal cortisol levels (Smriga et al., 2007). Hence basal cortisol levels may be related to anxiety although it is not clear if this would reflect in a general young-adult sample that have suffered stress.

- **Depression and diurnal cortisol profiles**

One of the most consistent research results in biopsychology is the presence of higher basal cortisol levels in adult patients of major depressive disorder (Heim and Nemeroff, 1999; Pariante, 2003; Yehuda et al., 2005). Additionally, CAR has been demonstrated to predict major depressive disorder (Adam et al., 2010; Vrshek-Schallhorn et al., 2013). In humans, chronic environmental stressors such as relationship problems, health problems, or overwork are associated with depressive-type disorders. It has been postulated that the damage that chronic stress can produce in the HP is a central mechanism that leads to depressive symptoms through its influence on the activity of the HPA axis (Delgado and Palacios et al., 2011). Given the widespread literature supporting the role of basal cortisol in the presence of depression one may see the same in a general population sample.

Understandably, all three psychological constructs; perceived stress, anxiety and depression are susceptible to control and change by the personality of each individual. Coping is a personal process that changes according to situational context of stress and individual appraisal of stressor is integral to coping (Lazarus and Folkman, 1984). 'Resilient coping' is defined as the ability of individuals to maintain relatively stable and functional psychological and physical conditions when faced with an extremely disruptive situation (Bonanno, 2004). Importantly, the reported decisive impact of stress on depression and anxiety (Bernice & Wilding, 2004; Melchior et al., 2007, for review see Hammen, 2005) has been shown to be moderated by resilience and coping style (Johnson & Sarason, 1978; Beasley et al., 2003; Bitsika et al., 2013). Resilient coping incorporates cognitive and behavioural strategies like committed active problem solving towards adverse and stressful circumstances (Sinclair & Wallston, 2004). Hence, predictably, this capacity (and its absence) would direct any impact a stressful situation might have on psychological conditions like self-perception of stress, anxiety and depression and may be involved with the physiological coping/response reflected in the basal cortisol values of individuals.

1.3 Cortisol and Empathy

Finally, we revisit the exploration of the idea that the HPA axis – apart from helping individuals adjust to high stress and challenges -is also implicated in the bi-directional adaptation to more everyday processes. We do this by looking at the mother-child bond. One can recall in the discussion about empathy how the ontogenetic roots of empathy are speculated to have their origins in the mother-child bond (for review see Hastings, Zahn-Waxler, McShane, 2006). Insula mediated action representation, essential functional architecture for empathy, also modulates our emotional content in respect to witnessing others (Carr et al., 2003). Hence, given that the limbic system-based mother-child bond (the likely ontogenetic root of empathy) is also at the heart of HPA axis (even termed the Limbic-Hypothalamus-Pituitary-Adrenal axis; Vazquez, 1998; Gunnar and Vazquez, 2001), cortisol may serve as a partial mechanism for the development/maintenance of the empathy processes. Based on empirical support for cortisol's role in social and prosocial behaviour it may be that empathy/prosocial behaviours are adjusted by ideal levels of internal emotional arousal reflected in corresponding moderately high levels of cortisol (Shirtcliff et al., 2009). Noteworthy are phenomenon where the mother and the child have been reported to exhibit cortisol profiles in sync with one another (Nofech-Mozes et al., 2019). Interestingly, such synchronization of the HPA axis dependent cortisol output between two individuals has also been registered in the case of old-age romantic partners, reinforced by partner presence (Pauly et al., 2020). There are studies showing how social competence positively correlated with cortisol levels in new or uncertain situations (Granger et al., 1994; Gunnar et al., 1997), while it correlated negatively with cortisol levels in stable, familiar situations (Gunnar et al., 1997). Also, a longitudinal study revealed that youth with more internalizing behaviour in childhood had lower basal cortisol levels in adolescence and youth with externalizing behaviours in childhood too displayed dampened diurnal cortisol rhythms (Ruttle et al., 2011). These data reveal the posterior impact left by negative experience-behaviour combination; however, it is open to speculation if similar biological markers may be detectable for arguably positive phenomenon like empathy. Yet, given the width of evidence available, even though direct evidence between empathy and cortisol may not be available, there is reason to believe such a relation may exist.

COVID-19, cortisol and perceived stress

We already know that stressors can exert effect on the cognitive condition, especially for executive functions [for e.g., decision making (Starcke & Brand, 2012); working memory (Luethi et al., 2009; Qin et al., 2009)]. The influence of stress on cognitive processes depends not only on the intensity and duration of the stressor, in a way that stress has a bidirectional effect on cognition (Salehi et al., 2010), but also on the cognitive process under study (Lupien et al., 2009; Sandi, 2013). While high or chronic stress can be detrimental to cognitive performance, mild to intermediate stress can even boost it (Sandi, 2013). GCs are the main players in a series of physiological changes that occur in stressful situations. However, most of this research has been executed in laboratory settings and hardly any literature exists about cognitive aspects of the pandemic´s impact or the relation between baseline cortisol indices and cognitive/emotional conditions generated by the stress. Cognitive capacities themselves are in turn related to the emotional aspects of personality (Munoz et al., 2015). Influencing either cognitive state or emotional processes leads to impact and changes in the other. For instance, Allott et al. (2015) reported the negative relationship between working memory and perceived stress. It has also has been reported that problem-solving ability is negatively associated to posterior self-reported perceived stress (D´Zurilla & Sheedy, 1991). In fact, cognitive capacity is known to buffer against the deleterious impact of stress (Riglin et al., 2016). In addition, previous literature has shown the robust existence of a relationship between CAR and working memory (Almela et al., 2012; Evans et al., 2011; Moriarty et al., 2014; but also see Franz et al., 2011). Nevertheless, to our knowledge, these is does not exist previous research inspecting whether baseline diurnal cortisol indices can serve as predictive biomarkers for changes in cognitive performance nor about how these changes may relate to stress perception in healthy subjects.

2. OBJECTIVES

- **Objective 1**

Given that the PET is a robust instrument based on generating valid reaction-based responses, it fits the criteria for use in further study of emotional state empathy, however, PET is available in Finnish and has been tested on a Finnish population. In order to use this instrument in Spain one needs to test its psychometric and construct properties and validity in Spain, hence, the Objective 1 of this dissertation was:

To translate, culturally adapt, and validate the Pictorial Empathy test for use in a Spanish population and test its internal validity, reliability and measurement invariance.

- ***Hypothesis 1***

PETs scores will correlate with participant scores on other scales of empathy.

- ***Hypothesis 2***

PETs scores will strongly correlate with the empathic concern subscale of the IRI and present low or moderate correlations with the other three sub-scales.

- ***Hypothesis 3***

Female subjects will present higher PETs scores than male subjects.

- ***Hypothesis 4***

PETs respondent age will present a positive correlation with PETs scores.

- ***Hypothesis 5***

PETs scores will be positively associated with pro-social behaviour.

➢ **Objective 2**

Given that the COVID-19 pandemic and its associated confinement have been reported to be a source of distress. And, keeping in mind that psychosocial and health correlates have been proposed to associate with maladaptive neuroendocrine patterns, the Objective 2 of this dissertation was:

To compare pre-pandemic state empathy, extraversion and social and emotional loneliness levels with during-confinement empathy, loneliness and extraversion levels in a young-adult sample. To explore if individual pre-pandemic psychobiological profile predicts impact of COVID-19 confinement on loneliness.

- ***Hypothesis 6***

Participants will exhibit an increase in self-reported loneliness and a decrease in self-reported extraversion and hypothetical volunteering.

- ***Hypothesis 7***

Pre-pandemic diurnal cortisol output will predict the propensity to changes in social loneliness and pre-pandemic extraversion would moderate this relationship.

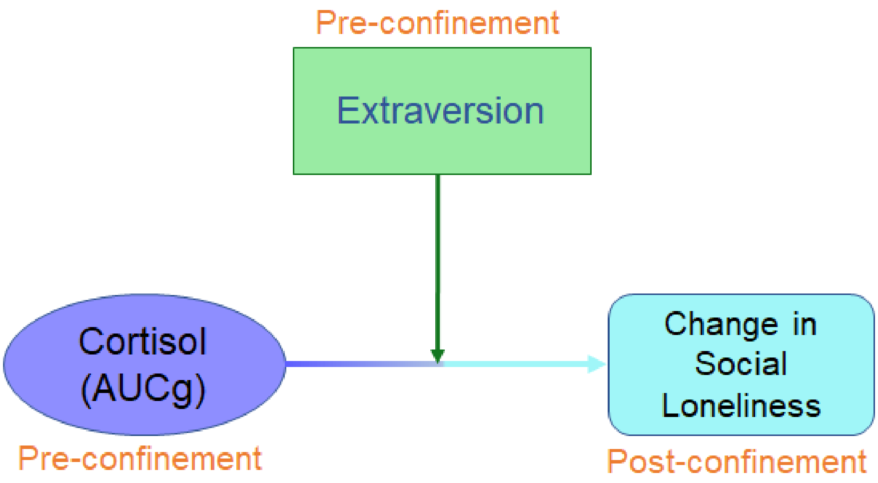

Figure 4. *Conceptual models for the theoretical framework of the hypothesis 7.*

- **Objective 3**

Given that the COVID-19 pandemic and its associated confinement have been reported to be a source of distress. And, as elaborated in the introduction, psychosocial and health correlates have been proposed to associate with maladaptive neuroendocrine patterns, the Objective 3 of this dissertation was:

To compare pre-pandemic trait empathy, working memory and perceived stress levels with during-confinement empathy, working memory and perceived stress levels in a young-adult sample. To explore individual differences in the susceptibility to the impact of the COVID-19 pandemic and test the role of cortisol and resilience in the same.

- ***Hypothesis 8***

Participants will exhibit an increase in perceived stress, empathy and working memory.

- ***Hypothesis 9***

Individual baseline cortisol indices will predict during-confinement perceived stress, anxiety and depression levels and this relation will be moderated by resilient coping.

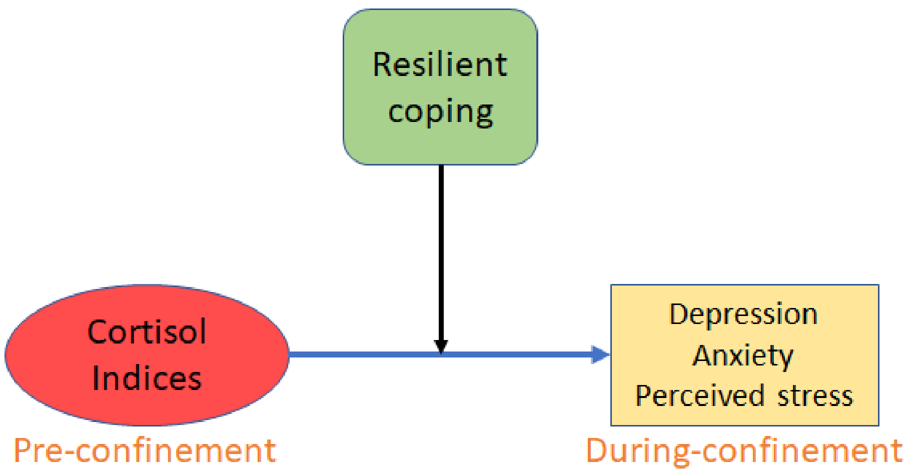

Figure 5. *Conceptual models for the theoretical framework of the hypothesis 9.*

- ***Hypothesis 10***

Individual baseline cortisol indices will predict the change in perceived stress and this relation will be mediated by the change in cognitive abilities.

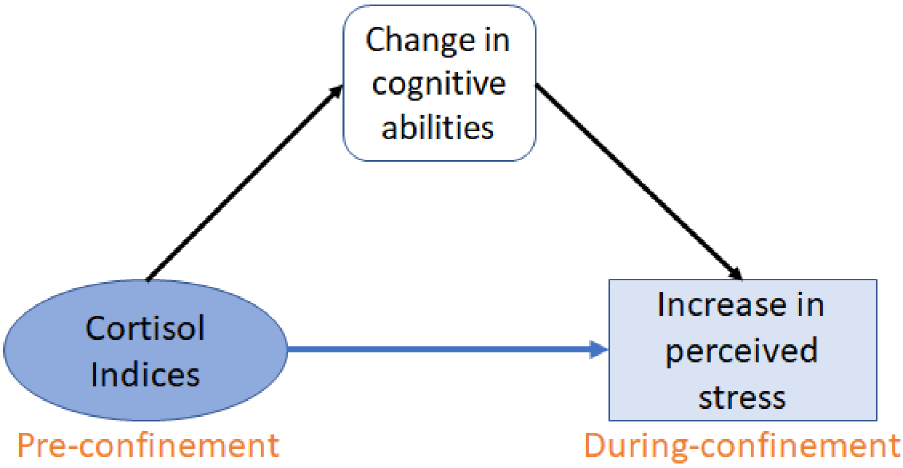

Figure 6. *Conceptual models for the theoretical framework of the hypothesis 10.*

- **Objective 4**

Given how previous literature points to the fact that cortisol may serve as a partial mechanism for the development/maintenance of the empathy processes (Shirtcliff et al., 2009), as described in the introduction, the Objective 4 of this dissertation was:

To explore the relation between baseline total diurnal cortisol output and trait emotional empathy scores in an aged female sample.

- ***Hypothesis 11***

Baseline diurnal cortisol profiles will be associated to emotional empathy of subjects.

2.1 Objective 1

2.1.1 Materials and methods: Sub-study 1

To verify PET's convergent and discriminant validity across a Spanish resident population sample, we not only examined hypotheses already part of the original PET study (Lindeman et al., 2018), but also incorporated the assessment of supplementary relationships between conceptually relevant measures. Thus, we first expected individuals' PET scores to correspond with their scores on other scales of emotional empathy (Hypothesis 1). To this end, we used the 23-item Spanish version of the Empathy Quotient scale (EQ; Redondo & Herrero-Fernández, 2018) in Sub-study 1 of Objective 1 and the Spanish Interpersonal Reactivity Index (IRI; Pérez-Albeniz et al., 2003) in Sub-study 1 and 2 of Objective 1. The Empathy Quotient consists of three dimensions integrated into the test using three sub-scales: Cognitive Empathy, Social Abilities, and Emotional Reactivity and we hypothesised that high PET scores would correlate specifically with the Emotional Reactivity sub-scale. Likewise, the IRI consists of four distinguishable constructs of empathy (Perspective Taking; Fantasy; Empathic Concern and Personal Distress). Among these constructs, the subscale Empathic Concern is the one that measures emotional empathy (feeling moved due to the condition of others) and thus we expected robust correlations of PETs with this subscale and low or moderate correlations with the other three dimensions (Hypothesis 2).

On the basis of previous longitudinal studies, empathy measuring instrument design literature and review of the biological basis of sex-based empathetic tendencies (Mestre et al., 2009; Reniers et al., 2011; for review see Christov-Moore et al., 2014), female subjects were predicted to report greater PET scores than the male subjects (Hypothesis 3). According to Sze et al. (2012), there is an effect of age on state emotional empathy in response to empathy induction. This study was carried out by using images similar to PET in that they were photographs of children, women and men which were in distress. In parallel with the images the subjects were asked how emotionally distressed they felt on observing the images. A strong effect of age on state emotional empathy in response to empathy induction was observed. Based on this and on previous literature positively associating age with emotional empathy (Sun et al., 2018; Khanjani et al., 2015; Wieck & Kunzmann, 2015), we hypothesized that age would also be positively related with PET scores (Hypothesis 4). Finally, we expected PET scores to be positively associated with pro-social behaviour (Hypothesis 5). Apart from the evidence available concerning the relation between prosocial behaviour and affective empathy (Schroeder et al., 2015), the rationale for this last hypothesis is based on observations of comforting behaviour towards those in distress as seen in humans across different ages; babies/toddlers (Davidov et al., 2013), adults (Stel et al., 2008) and across species; prairie voles (Burkett et al., 2016), rats (Bartal et al., 2011).

The principal objective of Sub-study 1 was to test and explore the effectiveness of the Spanish translation and to explore if the translated PETs reflected a reliable and robust internal consistency, and if it loaded on a single factor when employed in a face-to-face testing environment. Another major concern was to check how the test performed when administered to a Spanish population. Figure 7 displays a flow-schematic depicting the principal phases in the design and development of the Pictorial Empathy Test, Spanish version (PETs). Hypotheses 1, 2, 3 and 4 were tested. The study subjects were 79 university undergraduate psychology degree students who first gave informed consent for the research and were given course credit for taking part in the same (78% females; mean age 20.68; SD 5.19; range 18–53: 92% Caucasians). Previously, necessary ethical committee approval was received from the University of Almeria (UALBIO2020/020), and the National University of Distance Education (UNED) and all following procedures described herein were in accordance with and complied with the Helsinki Declaration for human research.

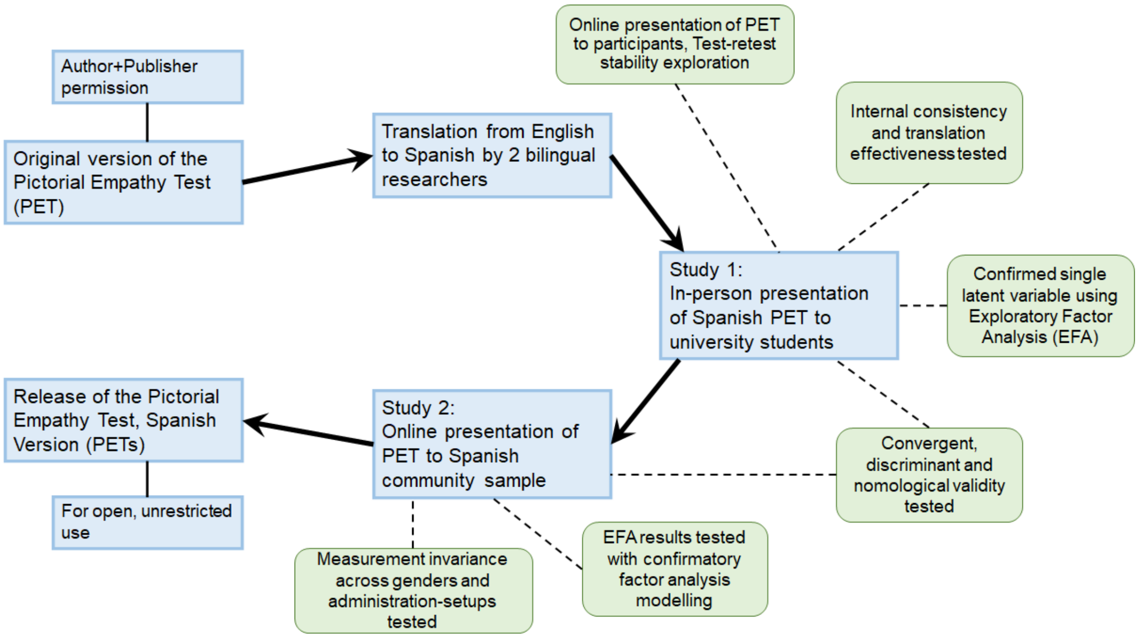

Figure 7: *Flow-schematic depicting the principal phases in the design and development of the Pictorial Empathy Test, Spanish version (PETs)*

a. Materials

Pictorial Empathy Test (PET)

The Pictorial Empathy Test is composed of seven photographs (an example image shown below, Figure 8. Please refer to the appendix for the entire PETs test), at the bottom of each image there is the presence of a recurring question prompting participants to note down (with pencil or on a computer) and register their immediate self-affective state, *viz*, how emotionally moved they felt, as a result of observing each image, by employing a five-point Likert-type scale; *"¿Cuánto considera que esta fotografía le conmueve emocionalmente?"* [1 = *Nada emotivo* (not at all), 2 = *un poco* (a little bit), 3 = *Me produce algunos sentimientos* (it arouses some feelings), 4 = *Bastante emotivo* (quite a lot), 5 = *Mucho* (very much)]. This Spanish material for the PETs made use of the same seven photographs of people in suffering, presented sequentially as was in the original study and test. To obtain the Pictorial Empathy Test score, an average score of the responses can be calculated but also the total score can be used too, given that the average score can never be more than 7 and the total score can never be lower than 7. This open-source material can be seen and sourced from the Appendix I of this thesis as well as from the supplementary material added to the research paper Baliyan et al. (2022). These photographs were obtained from Wikimedia Commons and with the greatest resolution available. These images when presented occupied thirty-three percent of the computer screen and were displayed on a white background. The participants were provided the PET content by a personal computer and were given instructions to note down their responses on paper. *Original version of PET translated with permission from *European Journal of Psychological Assessment*, 34(6), 421–431; ©2016 Hogrefe Publishing.

***Figure 8**. Photograph 2 of the PET and the PETs.*

Convergent and Divergent Scales

Self-reported questionnaire-based dispositional empathy was evaluated with the 23-item version of the Empathy Quotient self-report (EQ-Short; Redondo & Herrero-Fernández, 2018). The EQ is made up of statements describing situations or environments to which participant responses are recorded on a 4-point Likert-type scale (1 = strongly disagree, to 4 = strongly agree). This instrument was scored with respective Likert-scale choices scored as 0, 0, 1, and

2. This scoring pattern was employed in order to heighten the distinction between strong and weak empathic responses (Baron-Cohen & Wheelwright, 2004). Thus, the total EQ score (Cronbach's α = .90; McDonald´s ω = .91), emotional reactivity score (α = .75; ω = .75), cognitive empathy score (α = .91; ω = .91), and social skills score (α = .62; ω = .63) were calculated and used for further analysis.

Interpersonal Reactivity Index (IRI) was made use of in order to assess three of the four subscales, empathic concern (Cronbach's α = .70; McDonald´s ω = .71) for emotional empathy, perspective taking (α = .65; ω = .70) as an index of cognitive empathy and personal distress (α = .50; ω = .56). The three sub-scales were captured by 21 items (Pérez-Albeniz et al., 2003) which prompted subjects to respond on a five-point scale (1 = it does not describe me well, to 5 = it describes me very well) to how accurate scenarios concerning empathy were in describing them.

The tendency for prosocial behavior was evaluated employing the sixteen item Spanish version of the Adult Prosocialness Scale (Caprara et al., 2005). The study participants reported on a five-point Likert-type scale if the 16 statements were never/almost never true (coded as 1), to almost always/always true (coded as 5). Reliability (α; ω) of the scale was (.91; .91).

The Spanish version of the Marlowe and Crowne Social Desirability Scale (Ferrando & Chico, 2000) was made use of in order to explore the magnitude up to which study subjects were willing to deceive so as to control the image of themselves that they expose outwardly. Subjects reported with either true or false to 33 items and the number of statements which were responded to as true (after correcting for inverse items) was the total score. A greater score reflected a greater tendency towards social desirability and thus to a greater possibility that subjects may not respond honestly. Reliability (α; ω) of the scale was (.55; .57).

b. Statistics

The principal objective of sub-study 1 was to have access to a dataset which would serve for tentative exploration of the impact of translating the PET from Finnish to English and then again from English to Spanish. We decided to do so in order to be cautious about the possible loss of meaning given that there were two degrees of separation between the original study and the Spanish translated scale. In addition, even though the stimuli upon which PET is based is very salient and the same images were made use of in the Spanish version of PET, we were conservative in assuming excessive communalities between the Nordic culture of Finland and Spanish culture. This re-confirmation of the basal meaning of the test was done in line with earlier validation of translated instruments measuring empathy. For example, in various studies the Jefferson Empathy Scale has been first explored using an Exploratory Factor Analysis (EFA) then followed by a Confirmatory Factor Analysis (CFA) (Portugal; Magalhães et al., 2011 Italy; Montanari et al., 2015 United Kingdom; Tavakol et al., 2011 Spain; Valentín et al., 2019., Alcorta-Garza et al., 2016 Australia; Williams et al., 2013). EFA are better suited for a sample size that may be smaller than that recommended by a CFA and thus the sample size for sub-study 1 was intentionally modest (further discussion below). In summary, considering the possibility of information loss over two sessions of translations, the existence of possible cultural differences, the PET being a new measure, and the modest sample size, we decided to investigate the data for any possible, although not probable, deviation from the factor structure of the original pictorial empathy test. Hence, overall, this was kept in mind when considering to carry out an exploratory factor analysis as compared with doing a confirmatory factor analysis on the overall data obtained in sub-study 1. Henson & Roberts, (2006) commented that EFA should be done before doing a CFA on another, separate sample group and they also expressed that "*EFA may indeed be warranted during instrument development, even when theoretical expectations are present regarding the number of factors.*" (p. 407). Therefore, by design, sub-study 2 was developed keeping in mind not only a larger sample size and a broader population composition but also as the following step so as to confirm that the information extracted from the EFA was robust and replicable. All statistical tests were carried out using free-software JASP (v 0.12.2, https://jasp-stats.org/) and/or SPSS statistical package version 26 (SPSS Inc, Chicago, IL). Study sample size was estimated as acceptable based on *n:p* (sample size to number of items) ratio being stronger than 10:1 (Hair, 2009) in conjunction with the wide communality expected across factor loadings (MacCallum et al., 1999; Kyriazos, 2018). Although Confirmatory Factor Analysis (CFA) also requires a mostly agreed upon sample-size of ten participants per free parameter (Schreiber et al., 2006), other researchers have supported a number greater than 200 as acceptable for data analysis (Garver & Mentzer, 1999; Hoelter, 1983). Hence, we reserved carrying out the CFA for sub-study 2.

Construct validity was tested via EFA, and was started with the exploration of model-fit by forcing a two-factor structure with varimax rotation. However, results were more robust when using a unifactorial design which had been predicted by the use of parallel analysis (Horn, 1965) and the Scree test. Following this the new factor was derived accordingly (Cattell, 1966). The Bartlett´s sphericity test was employed in order to confirm that the data was fit to be used to carry out an EFA. Kaiser–Meyer–Olkin (KMO) index is used to confirm if sampling adequacy for the model and MSA values measure the same for individual variables, all values must be above 0.6 to proceed (Cerny & Kaiser, 1977). Further rotation was not used because of the unidimensional condition of the factorial structure. Multiple constructs and their relationships with PET were evaluated by using the Pearson´s correlation coefficient, providing convergent validity. Given that the sample sizes across the two genders were not equal, making detection of unequal variances more unlikely (Delacre et al., 2017), differences on the PET scores were calculated using Welch´s t-test. Reliability statistics used Cronbach's and McDonald's tests. Values above 0.9 were considered excellent; 0.8 = very good; 0.7 = good, 0.6 = acceptable and 0.5 = questionable.

2.1.2 Results: Sub-study 1

The reliability (Cronbach's α; McDonald´s ω) of PET´s set of seven photographs was .77 and .79 respectively. Exploratory Factor Analysis was carried out using the unweighted least squares method in order to study the Spanish version of PET´s initial internal structural patterns. Bartlett´s test confirmed significant correlations across items ($X^2 = 139.2$, $p < .001$) and measures of sampling adequacy values were greater than 0.7 for each item and KMO index was = .77 overall, reflecting adequacy to proceed with EFA. Based on the results from the original study (Lindeman et al., 2018), we expected to extract a single factor from our data. We confirmed the single factor structure via the unifactorial result obtained by carrying out parallel analysis to extract factors based on the number of items and sample size. Furthermore, the Scree test showed the formation of the ¨elbow¨ at factor 2 (Figure 9), thus confirming the presence of a single factor.

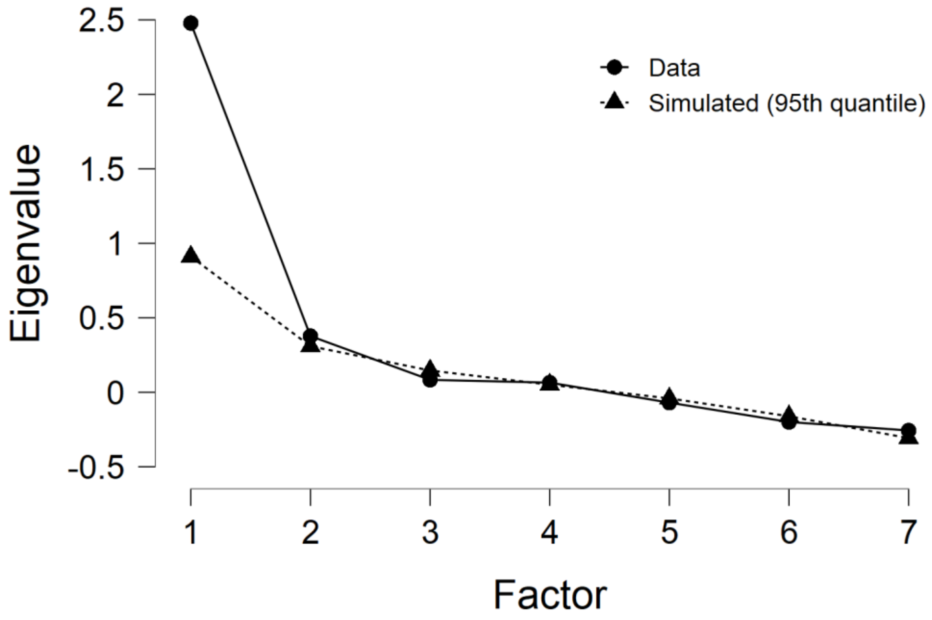

Figure 9. *Scree-plot for Pictorial Empathy Test Exploratory Factor Analysis. The above graph shows the presence of a sharp elbow at factor #2 (x axis) and the standardized Eigenvalue (y axis) of factor number 2 to 7 are all below 1, therefore implying the presence of a single factor behind the 7 items of PET.*

Therefore, our results replicate the factor structure of the PET original version and all factor loadings by the individual items were above 0.4 (Table 1). The PET scores ranged from 2.57 to 5.00 (M = 4.18, SD = 0.55). To evaluate the convergent and divergent validity of PET, correlations were calculated between the PET scores and the other variables that theoretically related to affective empathic reactions (Table 2).

Table 1. *Exploratory Factor Analysis: Pictorial Empathy Test item loadings.*

Item #	Loading
1	.626
2	.609
3	.770
4	.519
5	.635
6	.524
7	.416

Table 2. *Pearson correlation values (r) of the scores obtained in PET with respective instrument used in each study.*

Variable	**PET**	
	Sub-study 1	Sub-study 2
Empathizing Quotient Scale	.119	--
Emotional Reactivity	**.389****	--
Social Abilities	.046	--
Cognitive Empathy	-.028	--
Empathic Concern (Subscale of IRI)	**.441****	**.477****
Perspective Taking (Subscale of IRI)	**.338****	**.208****
Adult Prosocialness Scale	**.358***	--
Reported age	--	**.155****

Note. PET = Pictorial Empathy Test; IRI = Interpersonal Reactivity Index; * = $p < .01$, ** = $p < .001$.

We observed a significant positive correlation between PET and the emotional reactivity index of EQ, whereas the cognitive aspects of EQ did not correlate with PET scores (Table 2). We made use of the interactive calculator (Lee, 2013) to check if the differences between the correlations of PET scores and scores on the 2 dimensions of the Empathy Quotient (EQ) would be statistically significant as previously documented by Steiger (1980). The correlation between emotional reactivity and PET scores was significantly different than those with the cognitive subscales (z-score: 3.338, $p < .001$).

Regarding PET´s correlations with specific IRI subscales (Hypothesis 2), positive correlations between both, the empathic concern (EC) as well as perspective taking (PT) subscales were encountered. The obtained correlation index was greater between PET scores and IRI-EC ($r = .459$, $p < .001$) when compared to IRI-PT ($r = .338$, $p = .002$). PET scores did not correlate with the personal distress subscale of the IRI ($r = .088$, $p = .439$), whereas their correlation with the adult prosocialness scale was positive and highly significant ($r = .358$, $p = .004$).

On running the Pearson's Product Moment Correlation analysis none of the mean scores for each of the 7 items of PET nor the total score correlated with the Social Desirability Scale (SDS) (highest value being for total PET score (($r = .19$, $p = .13$); Table 4. Partial correlations between the IRI subscales and PET, while controlling for SDS, revealed no differences with the unadjusted correlation values (Table 2). Finally, results of Welch´s test run with PET scores supported Hypothesis 3 as females scored higher on the PET ($M = 4.29$) than men ($M = 3.83$), F (1, 21.52) = 7.115, $p = .014$.

Table 3: *Correlation table of PD and F of IRI with PET scores from Sub-study 1.*

	PET	PD
PD	0,088	
F	0,031	**,281****

*Note. PET=Pictorial Empathy Test; IRI=Interpersonal Reactivity Index; PD=Personal Distress; F=Fantasy. Pearson coefficients shown. * p<0.05*

Table 4: *Correlation table of PET and individual PET items (Pet 1 – PET 7) with SDS from Sub-study 1.*

	SDS	PET 1	PET 2	PET 3	PET 4	PET 5	PET 6	PET 7
PET 1	0,192							
PET 2	0,184	**,471****						
PET 3	0,128	**,384****	**,505****					
PET 4	0,098	**,268***	**,298****	**,424****				
PET 5	0,110	**,551****	**,362****	**,532****	**,256***			
PET 6	-0,084	**,263***	0,221	**,422****	**,344****	**,302****		
PET 7	0,168	**,230***	**,239***	**,299****	**,307****	0,123	**,383****	
PET TOTAL	0,193	**,713****	**,720****	**,753****	**,607****	**,668****	**,568****	**,569****

*Note. PET=Pictorial Empathy Test; SDS=Social Desirability Scale. Pearson coefficients shown. * p<0.05; ** p<0.01*

2.1.3 Materials and methods: Sub-study 2

The objective of the Sub-study 2 was to not only replicate the results of Sub-study 1 while employing a broader and bigger selection of the Spanish population but to also use an online questionnaire when doing so. At the same time, hypotheses 1, 2, 3 and 5 were tested in this study. Subjects were 580 Spanish residents (77% females; 22.2% males; 0.9% preferred not saying; 90.6% Caucasians) and they gave informed consent prior to participation in the study. The mean age was 34.4 years (SD = 12.90, range 15–78) and subjects were recruited to the online study by the use of social media platforms and by using student mailing lists. All necessary ethical committee permissions were obtained beforehand and all procedures were run in accordance with the Helsinki Declaration for human research.

a. Materials

Besides responding to PET, participants filled the Empathic Concern (EC; $\alpha = .76$; $\omega = .77$) and Perspective Taking (PT; $\alpha = .77$; $\omega = .79$) subscales of the Interpersonal Reactivity Index (IRI).

b. Statistics

All statistical tests were carried out using free-software JASP (v 0.12.2, https://jasp-stats.org/) and/or SPSS statistical package version 26 (SPSS Inc, Chicago, IL).

Reliability statistics used Cronbach's and McDonald's tests (for details see Sub-study 1). When studying the confirmatory factor models, reliability was also measured using composite reliability (CR), values above 0.7 are considered good. CR was calculated in Excel (Microsoft) using the standardized factor loadings obtained during the CFA done in JASP, the square of the sum of the 7 factor loadings was divided by the square of the sum of the factor loadings added to the sum of the error variances.

Confirmatory factor analysis (CFA) was run using JASP. The following indices were used: chi-square (x2) goodness of fit test which should turn out non-significant; (X2/degrees of freedom [df]), values lower than 3 indicate good fit; the comparative fit index (CFI), which ranges from 0 to 1 and with a minimum good fit value of 0.90; the root mean square error of approximation (RMSEA), which should be lower than 0.05; the goodness of fit (GFI) index, where values greater than 0.9 reflect good fit; the Non-normed fit index (NNFI), where values greater than 0.95 reflect good fit and the standardized root mean square residual (SRMR) index, with values lower than 0.08 indicating a good fit (Hu & Bentler, 1999).

Convergent validity was tested for the CFA. Model convergent validity was measured using the average variance extracted of the items. This value was calculated by using the factor loadings obtained for each item during the CFA and dividing the sum of the squares of the 7 factor loadings by the number of items (7). This calculation was carried out using the standardized factor loadings obtained in JASP and then doing the calculations described in Excel. Values greater than 0.5 are acceptable and reflect that on average, the construct explains 50% or more of the variance of its indicators (Hair et al., 2019). Multiple constructs and their relations to PET were measured using Pearson´s correlation coefficient, providing convergent validity.

Measurement invariance or group invariance was studied across genders and for administration formats. It was measured across 4 gradually restrictive steps involving multi-group CFA. This test was conducted to ascertain that PET validly measures the same constructs across groups and genders (Brown, 2006; Little, 1997). The measurement was carried out using JASP with the threshold for invariance being a change of RMSEA greater than 0.015 and of CFI greater than 0.01 (Chen, 2007). Differences of PET scores were measured using the Welch´s t-test since the sample sizes across the genders were unequal, making detection of unequal variances more unlikely (Delacre et al., 2017).

2.1.4 Results

PET´s presented good reliability (α = .86; ω = .88) and we confirmed the unifactorial structure of PET supported by the previous experiment, Sub-study 1. CFA was carried out using diagonally weighted least squares as the estimation method. This extraction method is recommended when fitting structural equation models with ordinal variables because it provides a more robust inference as compared with different estimation methods (Li, 2016). A good fit of the one factor solution was found, $X^2 = 14.732$, $p = .397$, $X^2/DF = 1.052$, $CFI = .999$, $GFI = 0.995$, $NNFI = .999$, $RMSEA = .010$ and $SRMR = .046$. Standardized estimations and the factorial structure are shown in Figure 10.

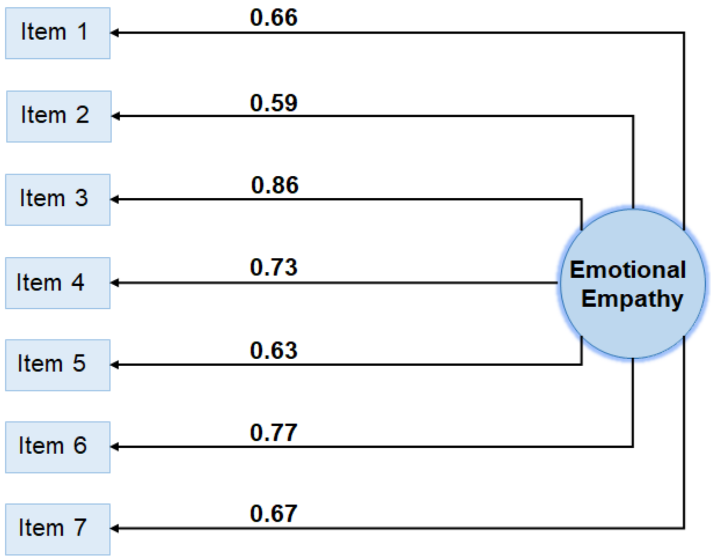

Figure 10. *Factor loadings as obtained via the confirmatory factor analysis model for the Spanish version of the Pictorial Empathy Test (PETs).*

Table 5. *Confirmatory Factor Analysis: Pictorial Empathy Test item loadings.*

Item #	**Std. Error**	**z-value**	**p**	**Std. Est. (all)**
1	0.033	20.663	< .001	**0.664**
2	0.033	21.321	< .001	**0.587**
3	0.033	22.019	< .001	**0.859**
4	0.030	20.059	< .001	**0.733**
5	0.030	19.188	< .001	**0.631**
6	0.028	18.800	< .001	**0.773**
7	0.034	19.985	< .001	**0.670**

Acceptable convergence was observed in the model with Average Variance Extracted AVE = 0.503, and good internal consistency was observed in the construct reliability index CR = 0.87 (Fornell & Larker, 1981). Additionally, as can be observed in Table 5, all factor loadings are near or greater than 0.60 which indicates acceptable to good item contribution to the latent construct.

There were no changes in CFI and RMSEA indices which reflects the absence of any measurement variance in terms of factor structure and factor loadings across both genders (Table 6). Results in table 6 also point towards equality of intercepts and thus comparisons between means of two groups can be carried out. Therefore, when comparing the means to test for gender differences (Hypothesis 3), Welch´s *t*-test showed that females (*M* = 4.29, *SD* = .64) scored significantly higher than males (*M* = 3.91, *SD* = .80) on PET, *F* (1, 178.18) = 24.61, *p* < .001. Combined, PET scores ranged from 1.71 to 5.00 (M = 4.20, SD = 0.69).

Table 6. *Goodness-of-Fit statistics for Confirmatory Factor Analysis and multigroup measurement invariance analyses.*

Models	**X^2**	**p**	**CFI**	**RMSEA**	**RMSEA 90% CI**	**SRMR**
1. Sub-study 2: Confirmatory Factor Analysis	**14.73**	**.397**	**.999**	**.010**	**-**	**.046**
2. Male/Female measurement invariance						
Equal form *(configural invariance)*	**20.32**	**.853**	**.999**	**.001**	**.000 - .026**	**-**
Equal factor loadings (*metric invariance*)	**33.94**	**.470**	**.999**	**.001**	**.000 - .043**	**-**
Equal indicator intercepts (*scalar invariance*)	**36.14**	**.645**	**.999**	**.001**	**.000 - .035**	**-**
3. In-person/Online testing measurement invariance						
Equal form *(configural invariance)*	**14.81**	**.980**	**.999**	**.010**	**.000 - .026**	**-**
Equal factor loadings (*metric invariance*)	**28.31**	**.742**	**.999**	**.010**	**.000 - .056**	**-**
Equal indicator intercepts (*scalar invariance*)	**37.96**	**.645**	**.999**	**.010**	**.000 - .066**	**-**

Note. X^2 *= Chi-square goodness of fit test; CFI = Comparative Fit Index; RMSEA = Root Mean Square Error of Approximation; CI = Confidence Intervals, GFI = Goodness of Fit Index, SRMR = Standardized Root Mean-square Residual.*

The factorial structure of Sub-study 1 (PET conducted in-person) was compared with the participants of Sub-study 2 (PET conducted online) to explore measurement invariance across administration formats. A hundred-and-ten participants from Sub-study 2, matched in sex and age, were selected so as to match Sub-study 1 participants. Following this, multigroup comparison analysis (Table 6) indicates that the questionnaire presented the same factor structure, the factor loadings were equivalent and strong factorial invariance existed between the two groups.

In order to be able to check the convergent and divergent validity of the PETs, correlations were calculated between the PETs scores and the other self-reported measures (Table 2). PETs scores displayed significant correlations with empathic concern ($r = .477$, $p < .001$) and cognitive empathy ($r = .208$, $p < .001$) as when measured by the Interpersonal Reactivity Index. We observed that the correlation between empathic concern (EC) and PETs scores was significantly stronger than that of perspective taking (PT) and PETs (z-score: 2.612, p = .005).

Finally, in order to check for the effect of age (hypothesis 4) on PETs scores, we explored the correlation between participant age and PETs scores. As predicted, a significant positive correlation between the subject age and PETs score was found (r = .155, p < .0001).

2.2 Objective 2

2.2.1 Materials and methods

a. Procedure

This research was conducted across two time points, pre-pandemic (24.11.2019 to 30.11.2019) and during-confinement (24.4.2020 to 30.4.2020) (Figure 11). The first step at both time points was obtaining relevant ethics committee approval (UALBIO2020/020) and obtaining informed consent from participants.

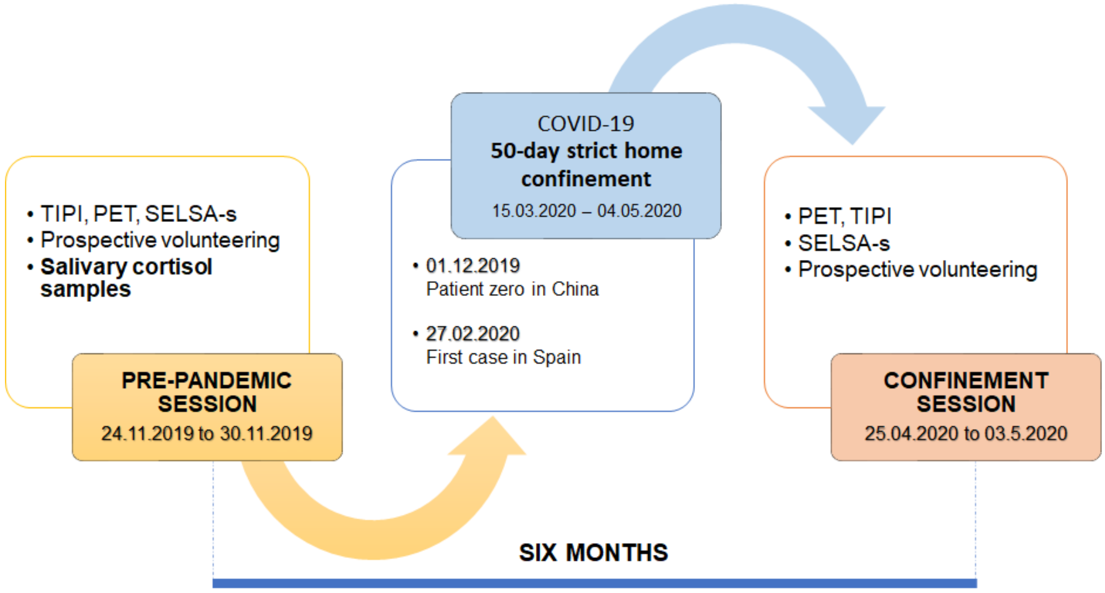

Figure 11. *Study timeline. SELSA-s = Social and Emotional Loneliness Scale-Short; PET = Pictorial Empathy Test; TIPI = Ten-Item Personality Index.*

All procedures complied with specifications outlined by the European Communities Council Directive 2001/20/EC and the Helsinki Declaration for biomedical research involving humans.

Participants

First-year university students were invited to take part in a study investigating personality. Seventy-nine students participated (78% females; mean age 20.68; *SD* 5.19). Following receival of the invitation to the second assessment, 45 of these students consented and their data were collected during home confinement.

Table 7. *Characteristics of the study sample for cohort that participated in both sessions of the study. SD = Standard Deviation;* AUC_G *(ug/dL) =Total diurnal cortisol release.*

	Mean (SD)
Sex	80% female
Age	21.09(6.42)
Ethnicity	93.6% Caucasian
AUC_G	3.880(1.492)

Cortisol

During pre-pandemic assessment, participants were given saliva sample collection vials (Salivette®, Sarstedt) along with detailed verbal and written instructions concerning sample collection. Subjects collected 5 samples, at 0, 30 and 45 minutes after awaking, 7 hours following awakening and right before sleeping. Relevant health condition, oral contraceptives and improper/ill-timed sample collection (>+/-5 minutes) were employed as exclusion criteria. Eleven subjects´ cortisol samples were eliminated from the analyses because they did not provide all the saliva samples, provided saliva samples with haemic or food/beverage contamination or saliva collection times deviated +5 min from the expected time point during the post-awakening measures. Valid samples were used to assess the area under the curve with respect to the ground (AUC_G). Samples were analysed via a commercially available enzyme-linked immunosorbent assay (Salimetrics®) having a sensitivity of <0.007 µg/dL with inter- and intra-assay precision of 4.9% and 2.9% respectively.

Questionnaire measures

Social and Emotional Loneliness Scale for Adults-s (SELSA): SELSA-S (Yárnoz-Yaben, 2008) measures three distinct facets of loneliness via its two subscales of social loneliness and emotional loneliness. The emotional loneliness subscale is further broken down into romantic loneliness and family loneliness. The items consist of descriptions of feelings and subjects are instructed to mark how accurate those descriptions are for themselves using a 7-point Likert-type scale. Scale reliability; McDonald's ω pre-pandemic = .82 and during-confinement = .84.

Ten item personality inventory (TIPI): TIPI (Romero et al., 2012) was used to quantify extraversion during both sessions of the study. The TIPI consists of items representing characteristics of personality and subjects are asked to mark how well each item describes them on a 7-point Likert-type scale. Scale reliability; McDonald's ω pre-pandemic = .81 and during confinement = .68.

Pictorial Empathy Test (PET): PET (Baliyan et al., 2022) was used to quantify situational emotional empathy during both time points of assessment. PET consists of 7 photographs of people in suffering, each image followed by inquiry of the immediate empathic reaction of the subject to those stimuli using a 5-point Likert-type scale. Scale reliability; McDonald's ω pre-pandemic = .77 and during confinement = .79.

Prospective volunteering: After completing PET, subjects were asked to answer, assuming having available 30 days of vacations, how many days would they dedicate to volunteering with a non-government organization working to aid people in suffering. During confinement subjects again underwent the PET and answered the same question about prospective volunteering.

b. Statistics

For cortisol levels, we calculated total diurnal cortisol liberation via the area under the curve with respect to the ground plotting each individual subject's cortisol samples collected; at their respective awakening, +30min, +45min, 7 hours following awakening and at their respective bedtime (Pruessner et al., 2003).

The changes in values of variables of interest were estimated by subtracting the confinement session scores from the pre-pandemic scores thus allowing us to calculate the extent of the changes which were then used as dynamic variables. Thus, Wilcoxon's signed ranks test was utilized to investigate changes in loneliness, state empathy, volunteering tendencies and extraversion from pre-pandemic to values during confinement. Correlations were used to explore the association among the quality of relationships in the house, number of cohabitants and the aforementioned variables, the significance level was set at $p \leq 0.05$, two-tailed, for all analyses.

To run our proposed moderation models, we utilized regression-based path analysis via the PROCESS plugin (version 3.5) for SPSS. PROCESS is a macro to estimate and probe interactions (Hayes, 2017). We estimated model 1 for moderation (working hypothesis) in PROCESS using 5000 bootstrap samples and 95% bias-corrected bootstrap confidence intervals. In the moderation model, we tested whether AUC_G related to changes in social loneliness as measured using SELSA-S, while being moderated by prior extraversion scores. Effect sizes are provided as standardized coefficients (β) but unstandardized coefficients are also provided for the moderation analyses to provide readers with the opportunity to interpret raw scores. No uni- or multivariate outliers were found among the variable values used in the analyses. All statistical analyses were carried out through the statistical package for social sciences (SPSS) version 25.0 (IBM, Armonk, NY).

2.2.2 Results

a. Impact of the pandemic and forced quarantine

Pre-pandemic values were compared to during-lockdown values using Wilcoxon's signed ranks tests for measures of social and family loneliness, perspective volunteering tendencies, the pictorial empathy test and extraversion. We observed a significant increase in family and social loneliness ($z = -2.031$, $p = 0.04$ & $z = -2.5089$, $p = 0.04$ respectively). Regarding measures of empathy, we observed a significant decrease in the total number of days offered volunteering during confinement, while no significant change was observed among PET scores ($z = -4.294$, $p < 0.01$; $z = -1.846$, $p = 0.07$). Trait extraversion decreased post-confinement ($z = -2.001$, $p = 0.04$).

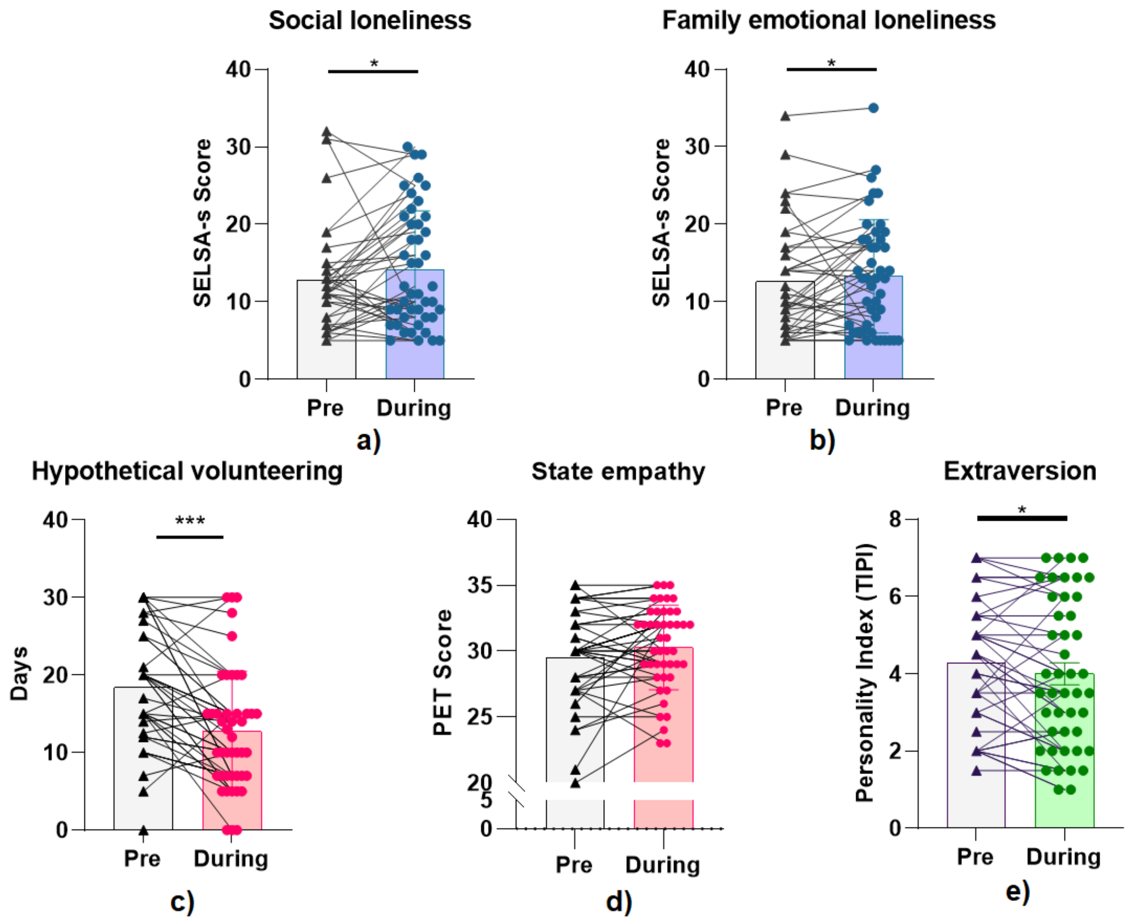

Figure 12. *Pre-confinement and during-confinement scores. Bar graphs showing (**a**) an increase in Family emotional loneliness (**b**) an increase in Social loneliness (**c**) a decrease in number of days dedicated to a hypothetical volunteering choice (**d**) no change in scores on state empathy and (**e**) a decrease in extraversion scores as measured by the TIPI. SELSA-s = Social and Emotional Loneliness Scale-Short; PET = Pictorial Empathy Test; TIPI = Ten-Item Personality Index. * p < 0.05; *** p < 0.001.*

Unadjusted correlations

Spearman correlation coefficients between study variables are displayed in Table 8. Among the most important correlations, we observed that the quality of the relations with cohabitants negatively correlated with change in family loneliness (r=-.32, p = 0.017). Interestingly, change in social loneliness correlated with change in volunteering duration, such that increase in volunteering corresponded with a higher increase in social loneliness (r=.43, p<0.001).

Moderation Analyses

Using path-analysis models, we investigated our working hypothesis (Figure 1); whether pre-pandemic diurnal cortisol output and post-confinement change in social loneliness had a relationship, which was moderated by extraversion. Sex, residence change during confinement and the number and the quality of relations with cohabitants were covariables controlled for. The pattern of results did not differ on exclusion of all covariates, underscoring the robust association between the variables of interest.

AUC_G with pre-extraversion as moderator. The overall model was significant $F(7,34) = 2.90$, $p=0.02$, showing that 44% of the variance in change in social loneliness was predicted by AUC_G, pre-extraversion and their interaction. AUC_G and pre-extraversion's interaction significantly predicted change in social loneliness (AUC_G*pre-extraversion: $\beta = 0.52$, $p = 0.01$). Simple slopes (at mean and +/-1 SD pre-extraversion score) are presented in Figure 13. Johnson-Neyman significance regions analysis revealed that when pre-extraversion is more than 5.62 ($\beta=0.83$), AUC_G and change in social loneliness are significantly positively related, $b=2.40$; $\beta=0.47$, $p = 0.05$. However, at and below low extraversion scores (<2.5) AUC_G and change in social loneliness are negatively and significantly related $b=-4.0$; $\beta=-.75$, $p = 0.05$. Moderation models with change in family loneliness were not statistically significant.

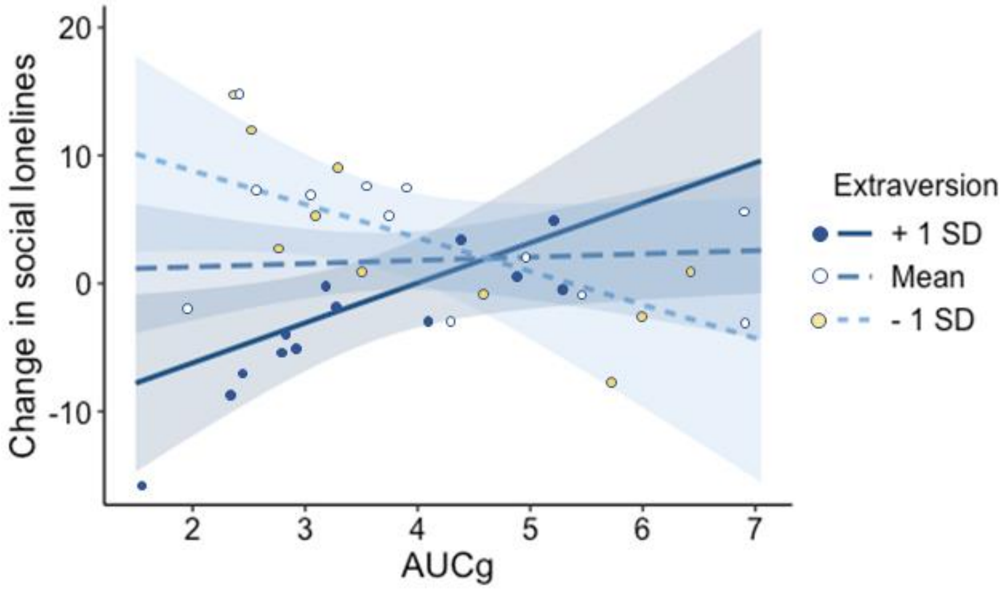

Figure 13. *Simple slopes plots of conditional effects representing the association between pre-extraversion and pre-lockdown cortisol AUC_G. AUC_G = Cortisol index for area under curve from ground; SD=Standard deviation.*

Table 8. *Unadjusted correlation matrix. AUC_G =Cortisol index for area under curve from ground; QRC=Quality of relationships with cohabitants; F. L=Family Loneliness; S. L=Social Loneliness; Δ=Change in score calculated as pre-pandemic values subtracted by during conferment scores for the respective variables; PET=Pictorial Empathy Test. Spearman coefficients reported; *p<0.05; **p<0.01*

	Residence Change	QRC	During Volunteering	Pre Extraversion	During Extraversion	Pre F.L	Pre S.L	During F.L	During S.L	Pre PET	Post PET	ΔVolunteering	ΔFamily loneliness	ΔSocial loneliness	ΔPET
QRC	0,069														
During Volunteering	**,295***	-0,204													
Pre Extraversion	**,353***	0,061	0,195												
During Extraversion	**,311***	0,104	0,115	**,813****											
Pre Family Loneliness	-0,268	-0,270	-0,296	-0,177	-0,220										
Pre Social Loneliness	-0,073	0,205	**-,326***	-0,214	-0,191	**,582****									
During Family Loneliness	-0,130	**-,510****	0,014	-0,132	-0,091	**,627****	0,160								
During Social Loneliness	**-,318***	-0,162	-0,013	**-,518****	**-,530****	**,451****	**,502****	**,537****							
Pre-PET	-0,123	-0,011	0,252	0,055	-0,071	-0,131	**-,303***	-0,138	-0,003						
During-PET	-0,044	0,234	0,169	0,001	-0,116	-0,247	-0,255	-0,243	0,086	**,592****					
ΔVolunteering	-0,004	-0,107	**,462****	-0,079	-0,132	0,065	-0,006	0,179	0,284	0,185	0,125				
ΔFamily Loneliness	0,152	**-,317***	**,418****	0,101	0,065	**-,324***	-0,215	**,478****	0,066	-0,164	0,051	0,257			
ΔSocial Loneliness	-0,244	-0,209	0,268	**-,368***	**-,513****	0,220	-0,173	**,327***	**,672****	**,343***	**,425****	**,427****	0,128		
ΔPET	0,062	0,166	-0,024	-0,114	-0,067	-0,195	-0,088	-0,132	0,003	**-,494****	**,321***	0,112	0,217	0,153	
AUCg	-0,065	0,203	0,181	-0,106	0,047	-0,288	-0,225	-0,171	-0,125	-0,131	0,165	0,075	0,273	-0,001	**,321***

2.3 Objective 3

2.3.1 Materials and methods

a. Participants

79 university first-year undergraduate psychology course students (78% females; mean age 20.68; *SD* 5.19; range 18–53) were recruited to take part in a research project investigating empathy and spatial working memory. During the home confinement session, 45 of these participants consented to also be part of this second assessment. Of the 45 participants, 36 were females, and the mean age of the total sample was 20.67 years (*SD* = 0.42, age range 18-53). Participant demographic characteristics are described in Table 9 and Table 12 (Appendix II). The students were given course credit for being part of this research. Data during-home-confinement (referred to as during-confinement) was collected when the subjects were still in confinement although this date was close to the (as of then) unannounced final date of the lifting of restrictions at the very end of the strict confinement, (relaxed <5 days from mean subject response date), this timing helped keep the participant responses free of effects of any confinement upliftment relief.

b. Characteristics of the study sample

Table 9. *Demographic characteristics of subjects that participated in both phases of the study. Scores at pre-pandemic (AUCg, CAR) and during confinement (anxiety, depression, resilient coping) sessions of the study.*

	Mean (SD)
Gender	80% female
Age	21.09(6.42)
Ethnicity	93.6% Caucasian
AUCg	3.880(1.492)
CAR	0.126(0.115)
BRCS	13.911(3.636)
DASS (Anxiety)	8.356(6.079)
DASS (Depression)	9.422(5.864)

Note. SD=Standard Deviation; AUCg (ug/dL)=Total diurnal cortisol release; CAR (ug/dL)=Cortisol Awakening Response; BRCS=Brief Resilient Coping Score; DASS=Depression, Anxiety and Stress Scale.

c. Procedure

This experiment was composed of 2 sessions, the 'pre-pandemic' session and the 'during-confinement' session. The pre-pandemic assessment was carried out in-person and participants were given an informed consent statement with data about the study and their rights (for e.g., voluntary participation, confidentiality, right-to-withdraw, research team contact). After the collection of demographic data, the subjects performed the cognitive tests and responded to the questionnaire instruments. Thereby, self-reported perceived stress, empathic proclivity, working memory capabilities and five same-day saliva samples were collected just prior to the beginning of the global pandemic (24.11.2019 to 30.11.2019). All students who took part in the pre-pandemic study received an e-mail 6 months later, inviting participation in a study concerning the COVID-19 pandemic. After their acceptance, we required these participants to respond to relevant questionnaires and undergo cognitive testing towards the end of the 50-day strict government decreed lockdown (24.4.2020 to 30.4.2020), by doing this we got access to both, pre- and during-confinement data of the same subjects.

Both sessions of data collection were approved by the ethical committees of the University of Almeria (UALBIO2020/020), Spain and the National University of Distance Education (UNED), Spain. All procedural methods used concerning participants and data handling met the conditions outlined by the European Communities Council Directive 2001/20/EC and the Helsinki Declaration for biomedical research involving humans.

d. Saliva sample collection

Towards the termination of the pre-pandemic session, all participants were given saliva collection tubes and verbal as well and printed-out instructions concerning the procedure and steps to follow so as to collect the 5 saliva samples. Participants collected their samples at zero, thirty and forty-five minutes after awakening, two hours after lunch and just before bedtime and they were asked to do this on a typical, non-weekend day. Subjects were instructed to neither eat, smoke, take any stimulants (such as coffee, caffeinated drinks, or tea), nor brush their teeth at least 1 hour before the collection of either sample.

e. Cognitive tests

Corsi block-tapping test: Subjects were informed about the methodology to carrying out the Corsi block-tapping test in the forward and backward order. The apparatus employed was made of a wooden board (23 × 28 cm) upon which 9 cubes were arranged in an irregular pattern (Corsi, 1972). For the Corsi-forward test, the instructor touched the wooden cubes in series of increasing length (from two to nine cubes) and participants were instructed to touch the cubes in the same sequential order as done in the demonstration by the instructor. Two trials with different patterns (but of the same length) were carried out for each series and if a correct response was received the number of cubes touched increased by 1 in the next series. Corsi-backward test consisted of instructing the participant to touch the wooden cubes in the order reverse to that shown by the instructor. The final result the overall total scores for Corsi-forward, Corsi-backward (each correct response was counted as 1 point to the score) and the total, i.e., the sum of series touched without error in either pattern, forward or backward. The test was ended when the participant was not able to advance to the next series after having two failed attempts at the same series length.

Change-location task: Scope and control of attention was evaluated by using the change-location visual array task (Shipstead et al., 2012). This test composes of testing for the maintenance capacity of working memory (Cowen et al., 2005) and it does not involve either distractions or higher order complex processing. This type of tasks when run with one to three circles tend to show very high accuracy across most subjects. However, four circles lead to a decrease in accuracy implying that this is where focal attention of most subjects is getting challenged. For the present change-location task a fixation point was shown for 1000 ms which was then followed by the display of four differently coloured circles, spread out on the computer screen, for 150 ms. A black screen was presented next, for 900 ms, followed again by four circles in the same location as earlier, but with one being of a different colour than earlier. Participants were required to select the circle they believed to have changed in colour. Subjects were given verbal instructions which were followed by twelve practice trails. These practice trails were then followed by 64 test trials that were divided into two equal blocks. This test was run using a personal computer and the instructor left the testing room in order to avoid possible distractions for the test subject. The test's duration was about nine minutes, and the number of correct responses and errors was registered and the *K*-index [(proportion correct × number of circles) − 1] was a reflection of the performance level at the test.

Electronic Corsi block-tapping test (e-Corsi): The e-Corsi was functionally similar to the previously described traditional/physical Corsi block-tapping task. The forward and backward sequences were presented as series of squares lit one by one in a pre-determined order. At the end of the lighting sequence, subjects were asked to reprise the series in the same sequence by clicking the squares in the order that they had flashed earlier.

The above tasks were both performed via online methods (e-Corsi was used in the place of traditional Corsi, please check the discussion for commentary about the absence of repetition and/or digitisation effects) in the during-confinement session of the study.

f. Questionnaire measures

Perceived Stress Scale (PSS): The Spanish version of the self-PSS was sent to the participants to be filled-out (Remor, 2006) at home, during the pre-pandemic, as well as during the home-confinement sessions of the study. This instrument instructs participants to report the frequency (in the last 30 days) with which they have had the thoughts, feelings and sensations as described by the 14 items. Respondents are supposed to reply on a five-point Likert scale and the total score is calculated by inverting the answers on the reversed items and adding together the scores of the 14 items. Scores with higher totals signify reflect greater self-perceived stress. As mentioned, the PSS is composed of both of positively-worded (the inverse items) and negatively-worded statements describing daily life situations. These two types of statements reflect the 2 constructs being measured, perceived self-efficacy (also known as perceived coping) and perceived helplessness (also known as perceived distress) (Hewitt et al., 1992). Perceived self-efficacy can be described as the subjective interpretation of how capable one is in dealing with prospective situations (Bandura, 1982).

Interpersonal Reactivity Index (IRI): The Spanish version of the IRI (Pérez-Albéniz et al., 2003) was employed to calculate cognitive and emotional empathy via the two sub-scales; Empathic Concern (EC) and Perspective Taking (PT), respectively (Cox et al., 2012). Either sub-scale consisted of 7 statements with participants requested to reply on a five-point Likert-type scale concerning how appropriately each statement describes them.

Brief Resilient Coping Scale (BRCS): Resilience coping abilities were evaluated by employing the Spanish adaptation of the Brief Resilient Coping Scale, BRCS (Limonero et al., 2014), this instrument was only used at the during-confinement condition.

Depression Anxiety and Stress Scales (DASS): Self-reported depression-like symptoms and anxiety levels were evaluated by making use of the validated Spanish version of the Depression Anxiety and Stress Scale (Ruiz et al., 2017). The DASS consists of 14 items, 7 items for both, the depressive and the anxiety measuring scale. To avoid overburdening the subjects and in order to reduce the total time they had to spend on the study, we choose to do without the sub-scale used to evaluate stress, given that the PSS was being responded to as well. The DASS was employed only at the during-confinement session of the study. Subjects were asked to fill-out the instrument using a 4-point Likert-type scale. The statements prompted subjects to analyse how well did the item-described situations capture their feelings, thoughts and sensations during the last 30 days (instead of the original scale where the subjects are asked only about the previous week, this was modified so as to get a broader and more consistent perspective of the impact of the pandemic and confinement in general, and not only the effects of the latter part of the confinement).

g. Data management and statistical analyses

For baseline cortisol profiles, we calculated 2 indices using the collected pre-pandemic cortisol samples. Samples provided were for the moments of awakening, +30min, +45min, +7 hours after waking and right before going to sleep. The two indices calculated were (i) the area under the curve with respect to the increase using the salivary samples collected at awakening, and 30 and 45 min after awakening, used as a measure for the Cortisol Awakening Response

(CAR) (that is, the pattern of the dynamic cortisol increase following awakening) and (ii) the area under the curve with respect to the ground for the total output of all five saliva samples of the day (AUC_G) (Pruessner et al., 2003a).

Pre-pandemic mean scores were compared with during-confinement values so as to process the impact of the COVID-19 pandemic and confinement on perceived stress, empathy, working memory and attention. Paired samples t-tests or Wilcoxon's test were calculated where applicable. The change in scores was obtained by subtracting the during-confinement session scores from the pre-pandemic phase scores thereby allowing quantification of the intensity of the changes occurred and usage of these dynamic representation as variables. Correlation analyses were performed to study the association between cortisol indices, self-perceived stress, working memory performance on the Corsi Block-Tapping task, and empathy before and during the confinement. The significance level was set at $p \leq 0.05$, two-tailed, for all analyses.

To test the postulated moderation and mediation models hypotheses, regression-based path analysis using PROCESS plugin (version 3.5) was used, a macro for estimating and probing interactions (Hayes, 2017) in SPSS. In the said plugin, Model 1 for moderation (hypothesis 9) and model 4 for parallel mediation (hypothesis 10) were run, employing 5000 bootstrap samples and 95% bias-corrected bootstrap confidence intervals (CI).

In moderation models, we tested if cortisol values (i.e., CAR and AUC_G) were associated to depression-like symptoms and anxiety (as evaluated via DASS) and perceived stress (via PSS), while being moderated by resilient coping (i.e., BRCS). In mediation models, we evaluated whether cortisol indices (i.e., CAR and AUC_G) were associated with the changes in perceived stress (i.e., PSS) through the changes in executive function performance (Corsi-block tapping task scores) and cognitive empathy (Perspective-Taking subscale of IRI scores).

No uni or multivariate outliers were observed in the variables tested across all analyses. Statistical analyses were performed using the statistical package for social sciences (SPSS) version 25.0 (IBM, Armonk, NY).

2.3.2 Results

a. Changes across pre-pandemic and during home confinement scores

To study the psychoneurological effect of COVID-19, pre-pandemic scores were compared to during home confinement values (Figure 14) for the variables of self-reported perceived stress (perceived helplessness, perceived self-efficacy and total perceived stress), empathy (perspective taking-cognitive empathy and empathic concern-emotional empathy), spatial working memory (Corsi block-tapping test forward, backward and total) and attention capacity (Change location task). Paired samples tests indicated that there was a significant increase in perspective taking (during home confinement perspective taking: M = 20.5, *SD* = 3.53; pre-pandemic Perspective Taking: M = 18.9, *SD* = 3.65) (t(44) = -3.431, p < 0.01), however no change in Empathic Concern was observed (z = -0.515, p = 0.61). Self-reported perceived helplessness and total perceived stress increased significantly (t (40) = -3.707, p = .004 & z = -2.563, p = 0.01 respectively), however there were no significant changes in perceived self-efficacy (z = -1.861, p = 0.06). Surprisingly, changes in the scores of the Corsi block-tapping test indicated a significant improvement in performance during home confinement Corsi-forward (M = 8.8, *SD* = 1.45) and total Corsi scores (M = 16.7, *SD* = 2.51) with respect to pre-pandemic Corsi-forward (M = 9.7, SD = 1.45; t(34) = -2.714, p = 0.10) and total Corsi scores (M = 17.7, *SD* = 2.60; t(34) = -2.675, p = 0.011). Corsi-backward did not display significant change across the during-confinement (M = 7.8, *SD* = 1.53) and pre-pandemic (M = 8.0, *SD* = 1.86) sessions (t (34) = -0.719, p = 0.477). Finally, no significant changes were recorded in the during-confinement attention task (Change-Location) performance in relation to the pre-pandemic scores of the subjects (z = -1.414, p = 0.157).

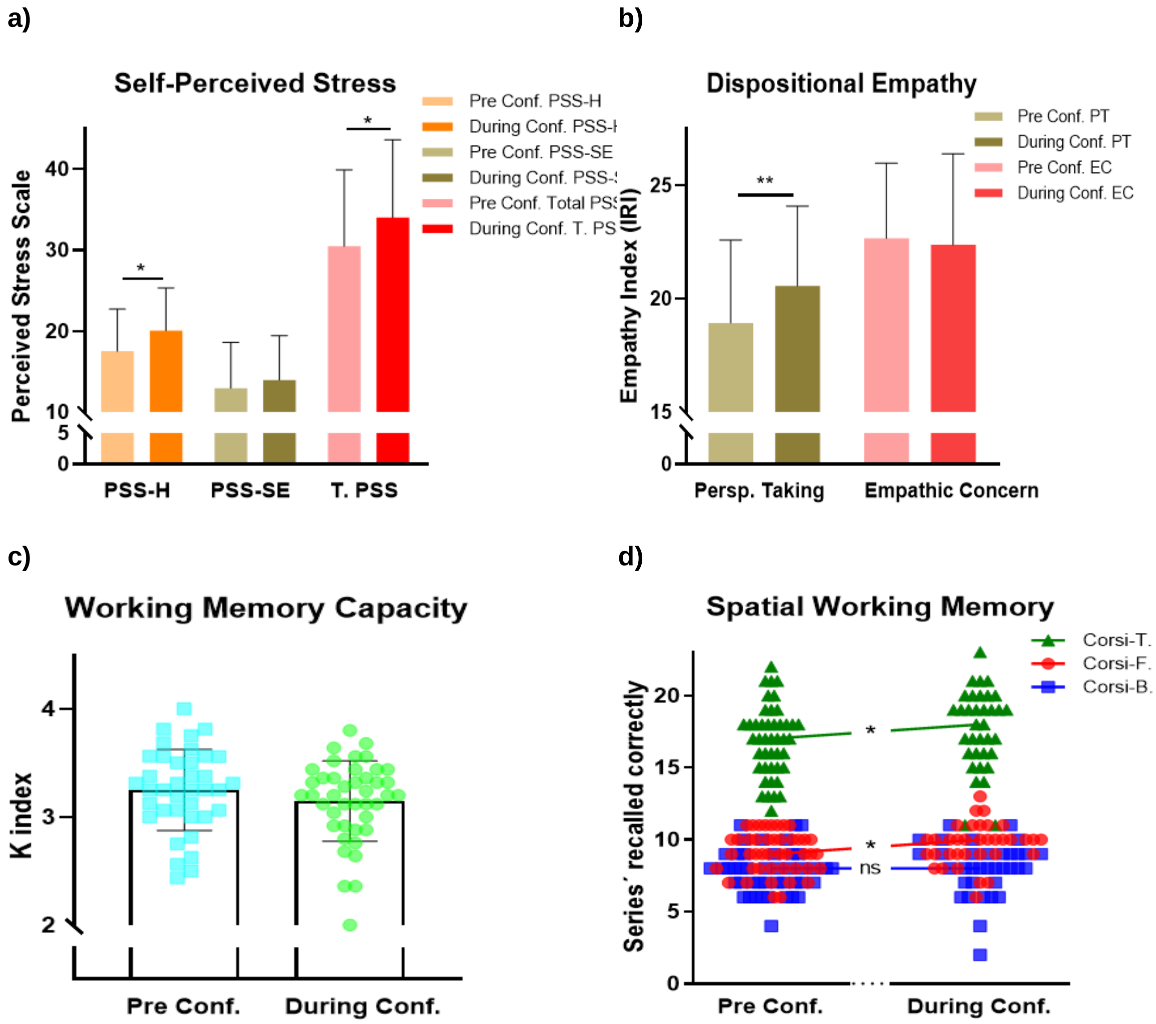

Figure 14. *Pre confinement and during confinement scores: a) Bar graphs showing an increase in perceived helplessness (PSS-H) and total perceived stress (T. PSS) b) An increase in perspective taking (PT) c) An increase in number of block-sequences correctly recalled during Corsi-Forward and Corsi-Total and d) Scores on the change-location task. Note: Conf.: Confinement; PSS-SE: Perceived [lack-of] Self-Efficacy; Perceived Stress Scale IRI: Interpersonal Reactivity Index; EC: Empathic Concern.*

b. Moderation Analyses

Using moderation models, we investigated hypothesis 9 (Figure 5); if basal cortisol indices (AUC_G, CAR) and during-confinement total self-reported perceived stress, depression-symptoms and anxiety had a relationship which was moderated by resilient coping capacity (i.e., the BRCS score). All moderation models are displayed in Table 4. Figures and graphs of simple slopes are presented only for AUC_G and CAR models in relation to total during-quarantine self-reported perceived stress (moderated by resilient coping capacity). Other significant models' (i.e., AUC_G to depression and to anxiety) tables and graphs are available in the Appendix II at the end of this document.

AUC_G. The overall model was significant (F (3,34) =7.87, p<0.01, r^2=0.41), presenting information showing 41% of the variance in during-confinement perceived stress is predicted by AUC_G, resilience score and their interaction,

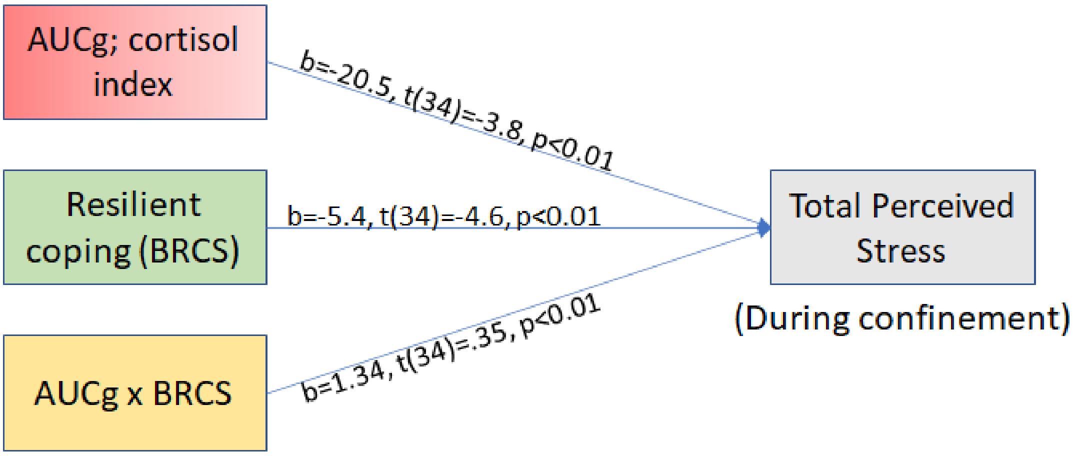

Figure 15. *AUC_G and resilience and their interaction significantly predicted total during-confinement perceived stress (AUC_G: b=-20.52, p<0.01; Resilience: b=-5.4, p<0.01; AUC_G*Resilience: b=1.34, p<0.01), where the interaction was responsible for 26% of the variance in perceived stress after the confinement.*

Simple slopes (at mean and +/-1 standard deviation of the Brief Resilient Coping Scale -BRCS- score) are presented in Figure 16. Johnson-Neyman significance regions analysis shows that when resilient coping is less than a BRCS score of 13.72, AUC_G and during confinement PSS are significantly negatively related (b=-2.10, p=0.05), and as resilience decreases, the relationship of AUC_G and PSS becomes even more negative with the relationship at the lowest resilience (BRCS score 5) at b=-13.80, p<0.01. Additionally, when BRCS scores are higher than 16.83, AUC_G and during-confinement PSS are once more significantly related, but positively (b=2.11, p=0.05), and at the highest resilience scores (20), AUC_G and during confinement PSS are strongly positively related (b=6.35, p<0.01).

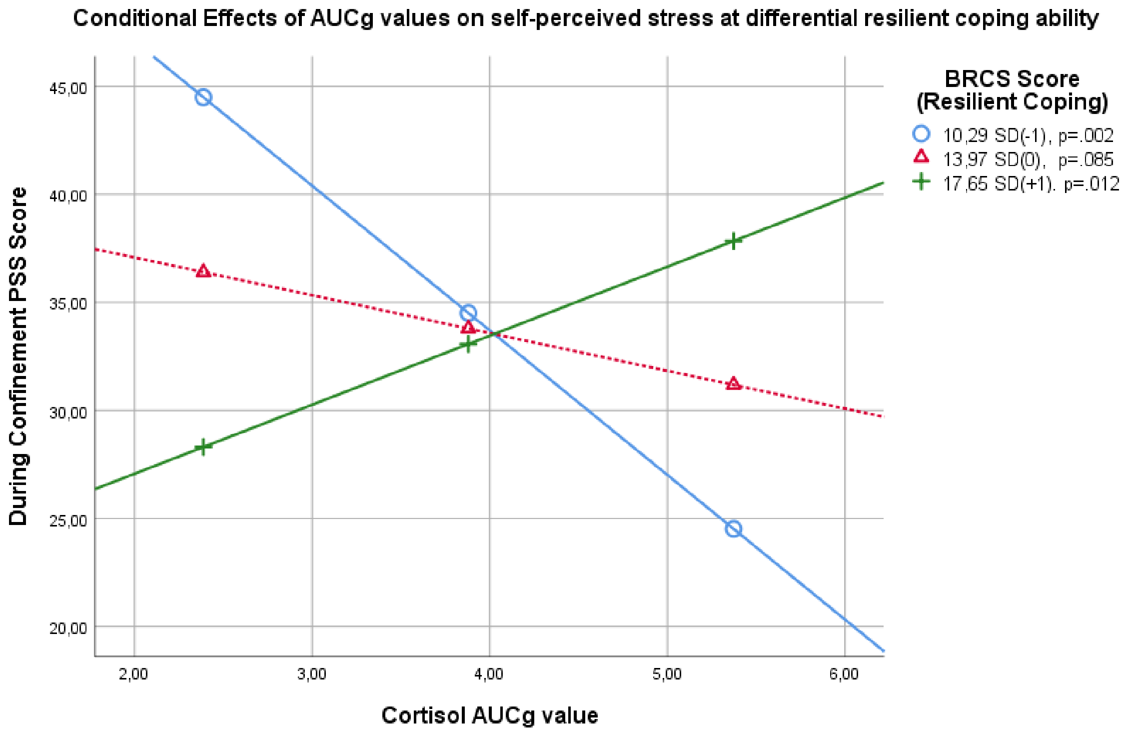

Figure 16. *Simple slopes (conditional effects) representing the association between Resilient Coping and pre-pandemic daytime cortisol AUC_G predicting home confinement total perceived stress. BRCS=Brief Resilient Coping Score, AUC_G=Cortisol index for area under curve from ground.*

CAR. The overall total model was significant (*F* (3,35) =4.10, p=0.01, r^2=.26), displaying that 26% of the variance in during-quarantine self-reported perceived stress is predicted by the Cortisol Awakening Response, resilience score and the interaction of the two (Figure 17). CAR and resilience and their interaction significantly predicted total home confinement perceived stress scores (CAR: b=-158.3, p=0.04; resilient coping: b=-2.21, p=0.02; CAR*resilient coping: b=10.74, p=0.04 respectively), where the interaction was responsible for 10% of the variance in self-reported perceived stress during-confinement. Johnson-Neyman significance regions analyses showed that when resilient coping was less than 8.25, CAR and home confinement PSS were significantly related (b=-70.62, p=0.05), as resilience decreased, the relationship of CAR and PSS became more negative with effect at lowest resilience (BRCS score 5) (b=-105.6, p=0.041).

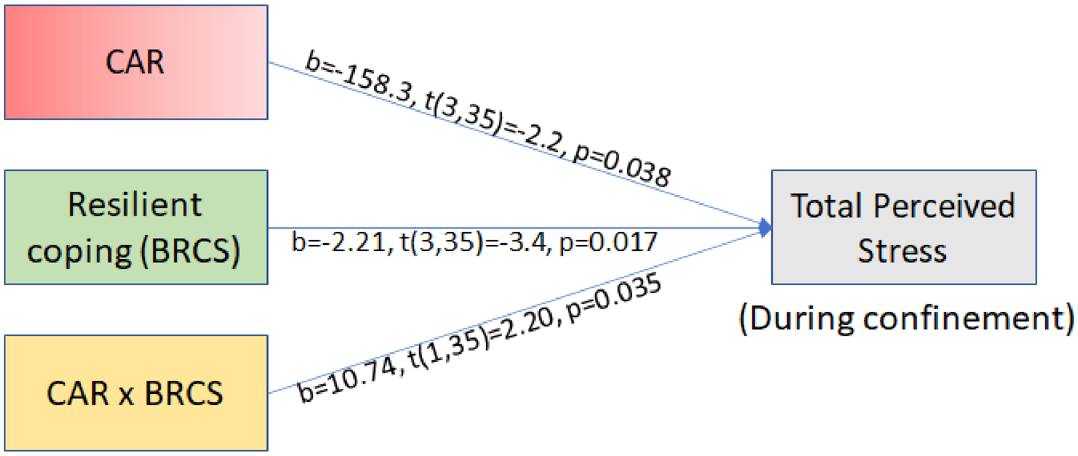

Figure 17. *Simple moderation analysis statistical model with unstandardized regression coefficients. BRCS=Brief Resilient Coping Score, CAR=Cortisol Awakening response.*

While significant moderation models were identified in the relation between AUC_G, self-reported depressive-like symptoms score and anxiety (with resilient coping as moderator; figures and data in Appendix II at the bottom of this document), CAR did not present any significant moderation models with respect to the DASS scale score of Depression or Anxiety. Moderation models were also run (as earlier, with resilient coping as moderator; figures and data in Appendix II at the end of this thesis) to explore how the two sub-scales of the Perceived Stress Scale, measured by the two latent constructs, were related to cortisol one by one. Results revealed that the models were mostly governed by the perceived self-efficacy values. Similar data was recorded for CAR, where up to 43% of perceived self-efficacy was predicted by CAR, resilient coping and their interaction as against 26% for total PSS (refer to Appendix II).

c. Mediation Analyses

Hypothesis 10 (Figure 6) was tested by the use of mediation models. We explored if CAR and AUC_G cortisol indices at pre-pandemic levels (i.e., at baseline/basal levels) were indicative of changes in the self-reported perceived stress *via* their relationship with the change in scores obtained on the Corsi Blocks task, a reflection of cognitive function (working memory), and change in Perspective-Taking scores, a reflection of cognitive empathy. While none of the cortisol indices was indicative of changes in the total self-reported perceived stress via changes in total Corsi task performance, we did encounter changes in positive perceived stress (perceived self-efficacy) being affected by cortisol variables via the changes in Corsi-forward scores and Perspective-Taking scores.

AUC_G. We encountered that the AUC_G (total cortisol output across a day) was positively related with changes in obtained scores on Corsi-forward (ES=.402, p=0.04) and Perspective Taking (ES=0.905, p=0.04). More positive change in performance on the Corsi-forward (ES=-2.262, p<0.01) and more positive change in perspective taking (ES=-0.905, p<0.01) were related to lower decrease in the self-reported perceived self-efficacy. Importantly, the mediation analyses indicated a significant indirect effect (ES=-1.7311; 95% CI = [-3.071, -0.929]) of AUC_G on changes in self-reported perceived self-efficacy via changes in Corsi-forward (ES=-0.911; 95% CI = [-1.79, -0.241) and perspective taking (ES=-0.821; 95% CI = [-1.89, -0.220). The results show that a higher AUC_G associated with better improvement of perspective taking and spatial working memory during-confinement, this in turn related to lower increase in the worsening of self-reported perceived self-efficacy during-confinement. This model explained up to 54% of the variance seen in the change in self-reported perceived self-efficacy (F (3,22) =8.47, p=<0.01). Very noteworthy was the direct effects of AUC_G on during-quarantine change in self-reported perceived self-efficacy were also statistically significant (ES=1.944, 95% CI = [.623, 3.264], p<0.01) and greater AUC_G foretold greater change in perceived stress. Tellingly, the suppressing effect of the direct and indirect effects on each other led to a statistically non-significant total-effects model (F (1,24) =0.0804, p=0.78, r^2=0.003), where, overall, AUC_G did not predict change in perceived self-efficacy following the COVID-19 pandemic and associated confinement. There was no predictor or correlational association between the two mediators and no interaction effects (between AUC_G and either of the mediators) were noted.

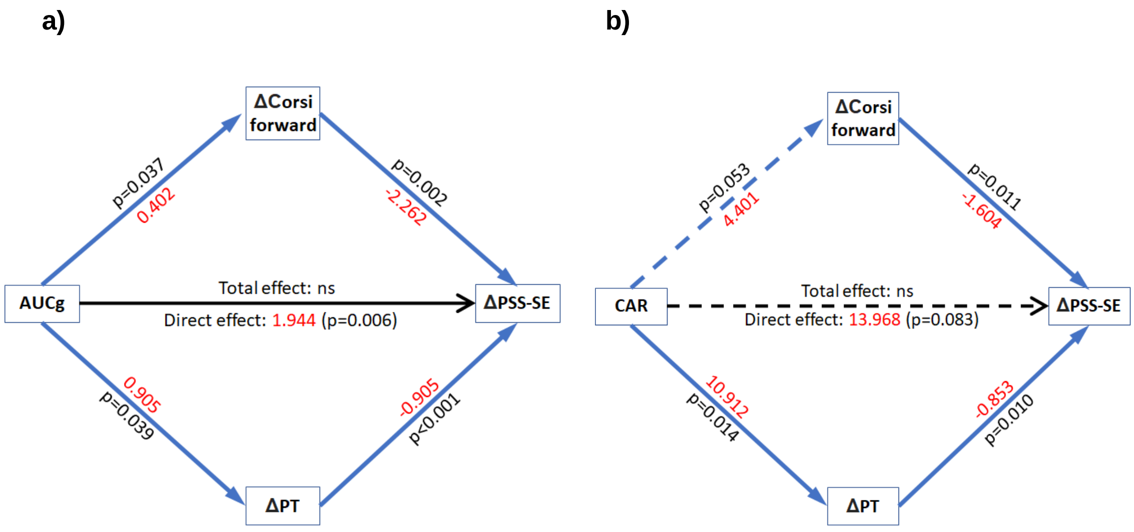

Figure 18. *Mediation model for AUC_G cortisol predicting change in perceived self-efficacy. PT=Perspective Taking, PSS-SE=Perceived stress scale Self-Efficacy, AUC_G=Area Under Curve cortisol, CAR=Cortisol Awakening Response, ns=non-significant, effect sizes in red. (right): Mediation model for CAR predicting change in perceived self-efficacy.*

CAR. CAR had a trend to being positively related with changes in performance of Corsi-forward (ES=4.401, p>0.05) and significantly predicted changes in perspective taking (ES=10.912, p=0.01). Again, higher positive change in performance on the Corsi-forward (ES=-1.604, p=0.01) and higher positive change in perspective taking (ES=-0.853, p=0.01) were related to poorer decrease in the perceived self-efficacy. Critically, mediation analyses indicated an indirect effect of CAR on changes in perceived self-efficacy via changes in Corsi-forward (ES=-7.061; 95% CI = [-16.43, -2.088) and perspective taking (ES=-9.317; 95% CI = [-20.77, -2.013), giving a total indirect effect of (ES=-16.378; 95% CI = [-32.241, -6.178]). In summary, a higher CAR was related to a higher improvement of perspective taking and spatial working memory during-confinement, this then was associated to a lower decrease of perceived self-efficacy reported by the subject during-confinement. The overall model explains up to 39% of the variance seen in the change in self-reported perceived self-efficacy (F (3,23) =4.81, p=<0.01). The direct effect of CAR on home confinement change in perceived self-efficacy was not statistically significant (ES=13.97, 95% CI = [-1.99, 29.93], p>0.08) and, although not significant, it displayed a statistical trend towards an effect contrary to that which CAR has on PSS via change in PT and Corsi-forward. Once more, the suppressing effect of the direct and indirect effects on each other led to a statistically non-significant total effects model (F (1,25) =4.815, p=0.760, r^2=0.003), where overall, CAR did not predict change in perceived self-efficacy amid COVID-19 caused, government decreed home-confinement. As before, no correlational between the two mediators and no interaction effects (between CAR and either of the mediators) were recorded.

2.4 Objective 4

2.4.1 Materials and methods

a. Participants

We initially recruited 102 older female adults to participate in this study. Thirty participants were excluded following application of specific *a priori* exclusion criteria described below. The final sample was composed of 72 Caucasian older females (age range 60-84 years; *M*=68.81). All participants provided informed consent and the study was approved by the Ethics Committee at the UNED and adhered to the tenets of the Declaration of Helsinki. Exclusion criteria were: suffering a disabling chronic disease or psychiatric disorders (e.g., psychosis), alcohol or other drug abuse, current use of medication which may interfere with cortisol sampling (corticosteroids, benzodiazepines, etc.) or moderate depression (GDS scores ranged from 0 – 15, a score of 9 and above indicated moderate and severe depression and was used as cut-off). Nineteen participants were excluded because they did not meet the inclusion criteria, and eleven were eliminated because they did not provide all the saliva samples, provided saliva samples with haemic contamination or saliva collection times deviated +6 min from the expected time point.

b. Questionnaire and cognitive measures

Participants completed two subscales of the Spanish version (Pérez-Albéniz et al., 2003) of the Interpersonal Reactivity Index (IRI); Perspective Taking (PT; cognitive empathy; scale reliability McDonald's ω=0.65) and Empathic Concern (EC; emotional empathy; scale reliability ω=0.70). Each sub-scale consisted of 7 items with subjects instructed to reply on a 5-point Likert-type scale about how adequately each item described their thoughts and feelings in a variety of situations. An item from the PT subscale, for example; *I sometimes find it difficult to see things from the "other guy's" point of view*.

c. Cortisol analyses

Detailed verbal and written instructions were provided concerning saliva sample collection protocol and compliance. Salivary samples were collected using Sarstedt Salivettes at awakening, +30 min and +45 min after waking, in the afternoon and at bedtime, and were stored at -80ºC until analysis. Cortisol levels were analyzed using an enzyme-linked immunosorbent assay (Salimetrics®) having a sensitivity of <0.007 µg/dL with inter- and intra-assay precision of 5.9% and 3.6% respectively. Four cortisol indices were calculated individually for each participant as a function of their respective sample collection times: (i) the area under the curve of the first 3 samples with respect to the increase over the first morning sample (i.e., the pattern of the Cortisol Awakening Response (CAR)); (ii) post-awakening cortisol AUC_G (as measured by calculating the area under the curve with respect to the ground for the first 3 samples); (iii) the diurnal cortisol slope (DCS), calculated as bedtime cortisol minus awakening cortisol/time interval between awakening and bedtime and; (iv) the total cortisol released throughout the waking hours (Diurnal cortisol AUC_G), as measured by calculating the area under the curve with respect to the ground for all five samples (Pruessner et al., 2003).

d. Statistical analyses

Age, awakening hour, sleep duration, years of education and depressive-like symptoms were used as confounders in all analyses. Outliers were identified and winsorized (which did not meaningfully change any results) and cortisol data logarithm transformed for normality. Empathic concern and perspective taking scores were used to categorize participants into high or low (above or below the median) groups. Repeated-measures ANOVAs were performed with time as within-subject factor and empathic concern or perspective taking as a between-subject factor. Greenhouse–Geisser values were used when the requirement of sphericity was violated. All relevant comparisons used Bonferroni adjustments for the *p* values. Hierarchical multiple regression modelling was used to examine the association of each cortisol index with older females´ empathy subscale scores. Age, depression scores, awakening hour, sleep duration and years of study were added as the first block in step 1 following stepwise analysis, and each cortisol index individually in step 2.

2.4.2 Results

a. Participant characteristics

Enrolled participants are a subset of an ongoing study about psychological characteristics in older adults. Participants reported a mean level of PT and EC of 16.5(*SD* 5.1) and 21.2(*SD* 4.7) respectively, and there was considerable variability between individuals across both scales (range 6–27; 10–28 respectively). A correlation matrix between all studied variables is presented in the supplementary material (Table 15; Appendix II).

b. Diurnal cortisol pattern differences between empathy scores

Participants' cortisol concentrations differed significantly from zero at waking, t(71) = 19.01, $p<.001$, increased significantly from waking to +30min post-awakening, t(71) = 4.94, $p<.001$, and decreased significantly from +30min to +45min post-awakening, t(71) = −2.67, $p=.009$; following established awakening cortisol release patterns.

When introducing empathic concern as a between-subject factor, significant time × empathic concern interaction was found in diurnal cortisol levels [$F(2.94, 191.35) = 3.52$, $p=0.017$, $\eta p^2 = 0.051$]. Bonferroni adjusted comparisons revealed that difference in cortisol levels between individuals with high (I) and low (J) empathic concern scores at awakening did not reach levels of conventional significance (I-J = 0.26, $p=0.069$, 95% CI = -0.02 to 0.54), but significant differences were found at +30min and +45min post-awakening (I-J = 0.32, $p = 0.008$, 95% CI = 0.09 to 0.56 and I-J = .37, $p=0.001$, 95% CI = 0.15 to 0.58 respectively) (Figure 19). No differences were seen in cortisol levels when the sample was split across the median of perspective taking scores (all $p>0.15$).

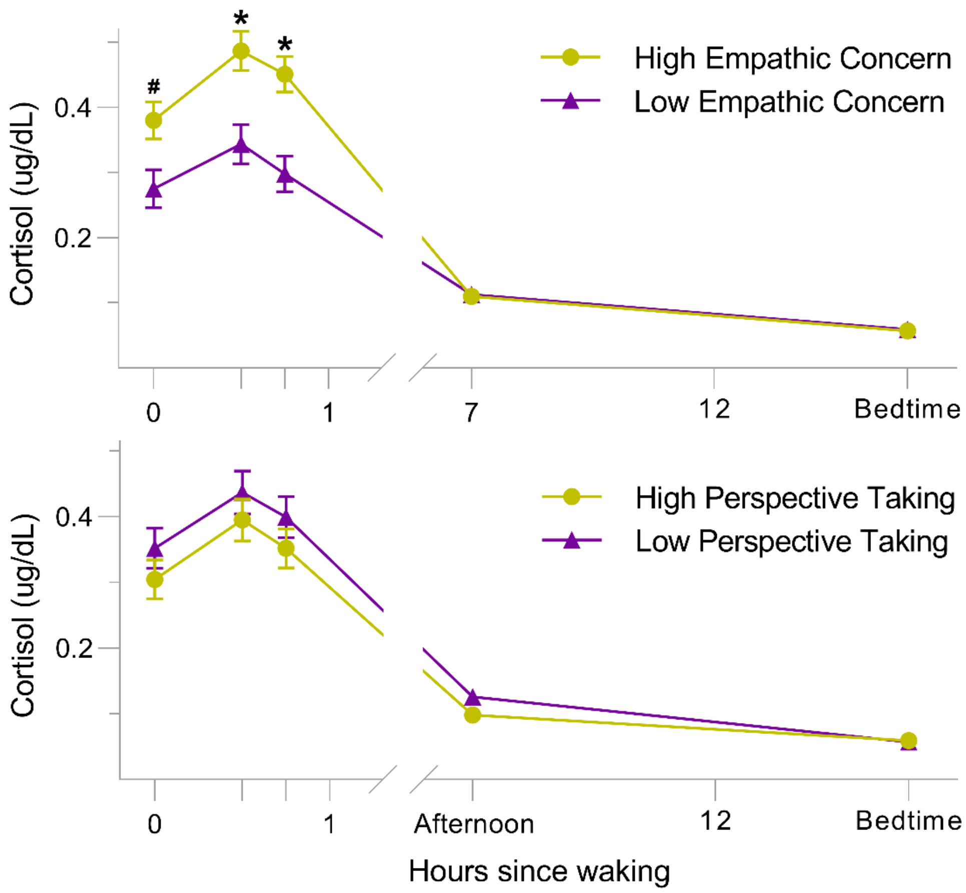

Figure 19. *Diurnal cortisol pattern in individuals with high and low empathy scores (for Empathic Concern and Perspective Taking). Individuals with high empathic concern scores showed higher cortisol levels at the awakening (#p=0.069), +30min (*p=0.008) and, +45 min (*p=0.001) timepoints than individuals with low empathic concern scores. For simplicity, plotted values are non-transformed means. Error bars represent SEM.*

c. Association between cortisol indices and empathy

Associations between cortisol and empathic concern are depicted in Figure 20 and summarized results presented in Table 10. No significant relationships were found between CAR and EC nor between any cortisol index and PT (all p>0.13).

Table 10. *Regression analyses with cortisol indices as predictors and empathy subtypes as dependent variables, adjusted for age, awakening hour, sleep duration, years of education and depression scores.*

	Empathic Concern			**Perspective Taking**		
	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*
Post-Awk. AUC_G	0.14	**0.38****	0.001	0.01	-0.12	0.28
CAR	0.01	0.08	0.491	0.00	0.02	0.82
Diurnal AUC_G	0.08	**0.29***	0.015	0.00	-0.06	0.59
DCS	0.06	**-0.25***	0.033	0.03	0.16	0.13

Note: AUC_G=Area under curve with respect to the ground, *DCS*=Diurnal cortisol slope, ΔR^2= change in R^2. *p<0.05, **p<0.01

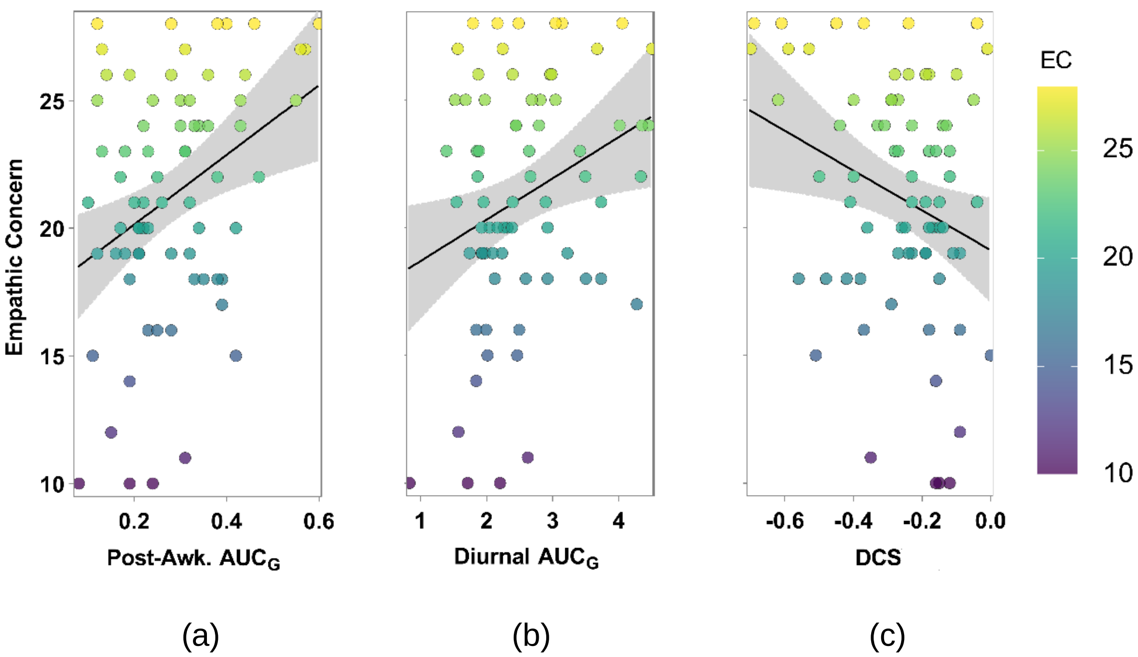

Figure 20. *Association between cortisol indices and empathic concern (EC). Post-awakening cortisol AUC_G with EC (a), Diurnal cortisol AUC_G with EC (b), and DCS with EC (c). AUC_G=Area under curve with respect to the ground, DCS=Diurnal cortisol slope.*

3. DISCUSSION

The first objective of this thesis was to translate and culturally adapting PET to the Spanish population. To this end, following a first translation and tuning of the content, we aimed at evaluating its psychometric properties in a sample of university students. The goal was also to test the construct validity of the PET Spanish version in traditional paper-and-pencil (hereafter referred to as in-person) experimental settings (as the original study was only online) and to confirm that the measurements represented ecologically valid state empathic reactions. Furthermore, we aimed at confirming the validity and factor structure, via use of CFA, of PET across a broad Spanish community sample. Finally, we aimed at testing measurement invariance and at offering this Spanish PET version without copyright restrictions. Our EFA successfully replicated the unidimensional factor structure of PET. Positive correlations between PET and established measures of emotional empathy reflect that the construct being measured by PET is similar to those measured by the emotional empathy aspects of the EQ and IRI scales. Therefore, we obtained evidence for highly satisfactory convergent validity. We observe a significant positive correlation between empathic reactivity and PET scores. Also, the difference between cognitive empathy subscale-PET vs emotional reactivity subscale-PET correlations was significant. These data provide discriminant validity results given that the Spanish PET version, a test for situational emotional empathy, correlates with the emotional empathy subscales of EQ, but not with the cognitive empathy subscales. Significant gender differences between PET scores are consistent with previous results in emotional empathy studies (Reniers et al., 2011; Christov-Moore et al., 2014). Similarly, further nomological validity of PET is reflected in the positive correlation between PET and the adult prosocialness scale (Stel et al., 2008). Therefore, hypotheses 1, 2, 3 and 4 are supported by our results. For Sub-study 2 our goals were two-fold. We wanted to replicate the results of Sub-study 1 within a larger, more representative selection of the Spanish population while using an online-questionnaire format. We also wanted to carry out the validation of the one-factor structure of the scale, convergent and discriminant validity analyses and test for multigroup invariance.

Confirmatory factor analysis displayed excellent model fit indices for the unidimensional factor structure of PET as revealed in Sub-study 1. Results from Sub-study 1 confirming hypotheses 3 -females scoring higher than males- and 5 -age dependency of PET scores- were replicated in Sub-study 2. Although the factorial structure of PET may be stable across gender and administration formats, this is not a guarantee that the instrument works equally and has the same meaning across the distinct groups (Borsboom, 2016). Measurement invariance for PET was established not only across genders, but also across two different modes of data collection, online and in-person. Convergent and divergent validity was highly satisfactory with relevant correlations between IRI and PET being confirmed. Divergent validity using the IRI scores was established by demonstrating that PETs´ correlation with IRI-empathic concern (a measure of affective empathy) is significantly stronger than that of PET and IRI-perspective taking, a measure of cognitive empathy. Therefore, hypotheses 1, 2, 3 and 5 were supported by the data.

In order to measure state affective empathy related constructs an ecologically valid, fast, easy to adapt-and-translate measure is currently required. While other similar tests are time-intensive (MET-30 minutes, Dziobek et al., 2008; SAM-Faces-40 items, Seara-Cardoso et al., 2012) and their construct validity is yet to be confirmed, PET is a brief, image-based test constructed in a straightforward-to-translate single-question format. For the aforementioned reasons, we considered PET an instrument meritorious of adaptation for the Spanish population.

In the process of adapting the test, we kept in mind not only confirming validity by replicating the tests carried out by the original authors, but also adding new dimensions of convergent, discriminant and nomological validity. Apart from retesting the convergent and divergent validity measures already tested in the original study, we decided to include 3 new dimensions. The first was using the Interpersonal Reactivity Index as a measure of empathy to compare PET against. IRI is one of the most widely translated measure of empathy still in use and we considered it an important instrument for PET´s validation. Apart from that, for nomological validation we decided to study how age correlates with PET scores. The main reason to do so was the study by Sze et al. (2012), (although see also Beadle and Vega, 2019) wherein the authors presented to subjects distressing photos of children, women and men experiencing suffering in conjunction with 5-point Likert scale type options inquiring how emotionally distressed the subjects felt right after seeing those stimuli. Their conclusion was that there is an effect of age on state emotional empathy in response to empathy induction and since the model used is very similar to PET, we hypothesized that age would positively relate with scores on PET. The other new dimension explored, supported by the literature, was testing if higher pro-sociality would correspond to higher scores in PET. All the mentioned hypotheses about convergent validity and the effects of age and sex on PET scores were met.

Apart from using additional dimensions to study the validity of PET, we also employed additional statistical tests to study PET´s psychometric properties. We tested for and confirmed measurement invariance in order to ensure suitability of employing PET across the two most common experimental paradigms (in-person and online). We also obtained satisfactory results about PET´s construct stability across females and males individually. As mentioned earlier, measurements of empathy that rely on self-reports tend to capture the subject´s self-perception and not necessarily their true empathic ability (Israelashvili et al., 2019, for review see Murphy & Lilienfield, 2019). Our results here provide a reaction-based instrument that addresses this issue and can thereby facilitate a more precise exploration of, say, bidirectional relationships like the role of stress on altruism mediated by state/situational empathy. As such, both the original and Spanish versions of PET can serve as a valuable and brief supplement to studying state empathy and its role across a wide range of clinical and preclinical conditions and behavioural tendencies. Although PET is based on the witnessing of empathy invoking situations, the score is still derived from immediate self-report. This leaves PET vulnerable to subjects who may avoid reporting their authentic reactions (or lack thereof) and respond falsely in a pattern representative of a person capable of affective empathy. Since the original version of PET lacked a check for response manipulation, in this research we included the social desirability scale to explore this possibility. Neither of the PETs 7 items individually, nor the total score presented signs of socially desirable responding.

While we consider the PET to be a generalized test of affective empathy, there are specific details which may provide clues to help identify the contribution of the distinct components of emotional empathy as a whole; emotional contagion, personal distress, and empathic concern. Previous research has established how the empathic concern (EC) subscale of IRI is moderately correlated ($r = .35$) with situational empathic responses (Zickfeld et al., 2017). Thus, Eisenberg et al., (1994) found a correlation of EC with situational empathy reactions measured by monitoring heart rate and facial reactions while viewing evocative video recordings of children in hardship. In addition, Light et al., (2015) found a similar correlation using facial electromyography. Results of the current study exhibit similar correlations between the dispositional empathy measured by empathic concern of IRI and PET scores. However, as shown in the study by Chiesa and colleagues (2015), implicit arousal of subjects, as recorded via pupillary dilation on witnessing a painful stimulus (face receiving a slap) was predicted by the empathic concern scale of a dispositional empathy instrument, the IRI, but not by the personal distress subscale. The absence of a relation between dispositional personal distress and situational empathic concern was also replicated in results of Sub-study 1 (Table 3) and may be interpreted as PET largely inducing and measuring empathic concern and not personal distress. The distinction is interesting given how personal distress is the self-focused reaction to another's emotional condition, while empathic concern is other-focused and is considered motivation for altruism in response to witnessing another's condition (Zahn-Waxler et al., 1990; Decety, 2010). Further tentative proof that it is concern for the other in distress - coupled with the motivation to alleviate this distress - that is being recorded by PETs can be derived from the robust correlation ($r = .36$, $p < 0.01$) between adult prosocialness scale scores and the PETs scores. Due to distinct causal motivation, personal distress does not coincide reliably with prosocial behaviour (Batson & Powell, 2003; Eisenberg et al., 2006), as seen in results from sub-study 1, where personal distress scores did not correlate with scores at the adult prosocialness scale.

In conclusion, our study presents a new and validated version of PET for the Spanish population. We further examine and confirm the reliability, validity and unifactorial structure of the test, in online as well as in-person contexts and offer this version for unrestricted re-use.

Moving onto objective 2, this dissertation examines the prospective association between cortisol, social and family loneliness and extraversion. It documents the impact of COVID-19 pandemic confinement in association with biological markers of stress and attends to possible psychobiological features relevant to identification of vulnerable groups. Firstly, via a longitudinal study, we examined the impact of the pandemic confinement on loneliness in young adults. Our results showed that, during long-term home confinement, most subjects reported increased feelings of social and family loneliness. The pandemic and confinement's effects were also reflected in participants reporting themselves to having lower extraversion trait-like characteristics and a substantial decrease in prosocial tendencies, as evidenced by the diminished prospective volunteering. Importantly, individual differences in pre-pandemic total output of cortisol (AUC_G) were able to predict the impact of strict social confinement on social loneliness, an association moderated by pre-pandemic extraversion scores, affecting the intensity and direction of the relation.

As the feelings of loneliness may be influenced by the students having had to change their primary residence as a result of the forced long duration confinement, or by the number and/or quality of relations with the cohabitants they shared their residence with, we considered these variables as potential confounders. Also, given sex differences concerning loneliness scores (Borys & Perlman, 1985) across young adults (Cramer & Neyedley, 1998) and specifically during COVID-19 (McQuaid et al., 2021), we added sex as another covariate in all relevant analyses. Interestingly, results of the current study complement the results obtained by another longitudinal study concerning the relation between loneliness and cortisol, also carried out during the COVID-19 pandemic. Across 52 early-adolescent youth, Jopling et al., (2021) found that the pandemic-led increase in loneliness was associated with higher awakening cortisol. The apparent impact of increased loneliness on cortisol production observed in that study when paired with our results of diurnal cortisol output together with extraversion predicting change in loneliness points towards a possible bi-directional relationship between loneliness and the HPA axis in adolescent and young adult humans. In a previous study, higher extraversion was related with lower CAR (van Santen et al., 2011), while in older adults, lower extraversion was related with elevated diurnal cortisol output (AUC_G) (Ouanes et al., 2017). In the present study we observed that most subjects with higher pre-pandemic extraversion levels showed a reduction in loneliness during the pandemic, an effect that may be related to strong social support when encountering stressors

(Swickert et al., 2002). Findings of some previous studies had indicated that, compared to introverts, extraverted individuals experienced higher stress levels (Liu et al., 2020) and larger declines in social connectedness (Folk et al., 2020). However, extraversion has also been shown to be related to lower perceived stress and better emotional regulation (Barańczuk, 2019) and to be a protective factor against anxiety during the COVID-19 pandemic (Nikčević et al., 2021). This association, where extraversion relates with perceived stress, both positive and negatively, fits with results of the current study and the moderating role of extraversion. The complex nature of loneliness not only draws attention to the fact that individuals vary in their quantity and quality of relationship needs, but also that distinct types of personality fulfil these needs dissimilarly. Thus, overall, the presented moderation model highlights how diurnal cortisol output and personality type (highly social or personalities more tolerant of isolation) shape individual differences in sensitivity to restricted social contact. Additionally, the finding that extraversion pivots the increase or decrease of perceived change in social loneliness during home-confinement at different pre-pandemic diurnal cortisol levels may help understanding the -sometimes contradictory- results reported across studies exploring the relation between cortisol and loneliness.

According to the evolutionary theory of loneliness, perceived lack in quantity and/or quality of intimacy or companionship motivates one to make new or strengthening existing social connections (Cacioppo et al., 2006). While we did not see any changes in state/situational emotional empathy, as measured by the PETs, nor in trait emotional empathy, as reported in results from objective 3, we observed a strong decrease in prospective volunteering intentions. Given that the viral pandemic and imposition of lockdown specifically prevented social contact, working with strangers via a charitable organization for helping strangers can be less appealing owing to the heightened risk of viral contagion. For most people, being quarantined is a stressful experience that increases anxiety and depressive symptoms (for rev see Brooks et al., 2020). Therefore, it may be speculated that the negative psychological impact of quarantine may affect the appeal of volunteering, which itself is associated with possible psychological dangers like exhaustion, nervousness and depression (Capner and Caltabiano, 1993; Mitchell et al., 2004) apart from the obvious psychological fear of contagion. Therefore, given the circumstances, we consider our results about emotional empathy and prosocial behaviour to not be at odds with one another. Interestingly, in the correlation results, we also noticed that those who suffered greater worsening in social loneliness were willing to spend more time volunteering. Thus, subjects who had experienced greater social loneliness also had stronger motivation to increase their social interactions and, perhaps, attend to the heightened feeling of inadequate social connections and reduce their social pain.

Change in social loneliness has a strong inverse relationship with individuals' extraversion score during confinement. Previously, Cacioppo et al., (2006) not only showed loneliness to be related to extraversion, but also found that high loneliness is associated to greater shyness and lesser sociability among young adults. Cheng and Furnham (2002) showed how extraversion had direct and indirect effects on loneliness, while Mund and Neyer (2016) carried out a 15-year longitudinal study to show loneliness predicted future development, even magnitude, of extraversion traits. Here, we also observed that strict social confinement during COVID-19 pandemic reduced extraversion scores. Extraversion is generally associated with being cheerful, optimistic, preferring social encounters and experiencing more daily positive emotions (McCrae and Costa, 2003). While personality dimensions are generally stable and withstand major life events (Specht et al., 2011), there is also research into the temporary effects of depression and anxiety disorders on personality (Karsten et al., 2012), as well as the more lasting impact of interventions (Roberts et al., 2017). In fact, Sutin and colleagues (2020) also observed changes in personality (lower neuroticism) following the COVID-19 pandemic. Our view is that the decrease in extraversion observed in our study reflects situation-caused personality states changing one's self-perception (Fleeson, 2007; 2004; 2001). Nonetheless, while trait measures could be expected to revert to their previous values when the immediate acute pandemic situation has ended, our data strongly supports the need for future longitudinal studies on the topic to include personality assessments. Finally, our pre-pandemic observations did replicate the negative relation between loneliness and empathy as reported previously (Beadle et al., 2012). However, subsequent during-confinement exploration of the same relation revealed that the association had been lost. The asynchronization of this relationship may be due to the abrupt and "forced" nature of the increase in loneliness which did not coincide with a corresponding decrease in empathetic tendencies.

Some limitations should be considered when interpreting the results of this study. Given the circumstances, the sample size of the study was constrained, and it was not possible to collect saliva samples during the confinement. Moreover, it was not feasible to have a control group not submitted to the lockdown. The recruitment procedure leaves the results open to sample selection bias. Nevertheless, we deem our results highly informative, given how few studies involving natural stressors of this kind are available and the inherent challenges to obtaining relevant data. In conclusion, loneliness among the population must be monitored closely given that although transient loneliness promotes the desire to socialize, the failure to socialize risks entering a self-reinforcing loneliness feedback loop (Cacioppo & Hawkley, 2009). Given the continuously emerging waves of COVID-19 pandemic over the past couple of years, and the concomitant re-confinement measures frequently applied in many countries, social connections are under constant strain and, at the same time, they may be needed more than ever. Results from our study point towards the need of longitudinal studies exploring the transition of the state-of-mind into loneliness and explore possible biomarkers as prodromic or causal links underlying loneliness.

When exploring the hypotheses of objective 3, we reveal that individual cortisol profiles predict how long-lasting stressful circumstances impact perspective taking, working memory and eventually, perceived self-efficacy in dealing with prospective situations. Thus, these results go beyond previous work indicating that the causal effect of stress on depression and anxiety (Andrews & Wilding, 2004; Melchior et al., 2007, for review see Hammen, 2005) are moderated by resilience and coping style (Johnson & Sarason, 1978; Beasley et al., 2003; Bitsika et al., 2013), by showing the potential predictive capacity of basal diurnal cortisol for the development of stress-related psychopathologies.

First, we explored the relationship between cortisol, resilient coping and mental health (depression, anxiety and perceived stress). Resilient coping incorporates cognitive and behavioural strategies like committed active problem solving towards adverse and stressful circumstances (Sinclair & Wallston, 2004). Thus, we expected resilient coping to moderate the direction and intensity of cortisol's prediction of mental health. We focused on CAR and AUC_G as the principal cortisol indices given their importance as risk factors predicting stress-related disorders such as depression and post-traumatic stress disorder (Lemoult et al., 2015; Pineles et al., 2013; Pernavides et al., 2007). Additionally, treatment for depression and PTSD has correlated with normalization of cortisol profiles (Vythilingam et al., 2004; Olff et al., 2007) and a decrease in perceived stress (Fava et al., 1992). Our moderation models support hypothesis 9 (Figure 5), stating that pre-pandemic total diurnal cortisol secretion (i.e., AUC_G) predicted the depressive-like symptoms, anxiety levels and total perceived stress reported. This relationship was moderated by resilient coping capacity in a manner that high reported coping corresponds to a positive relationship between the predictor AUC_G and mental health. Low resilience scores, however, invert the relationship such that at low AUC_G levels, subjects reporting low resilient coping have high depressive-like symptoms/anxiety/perceived stress during home confinement.

To our knowledge, there are no previous studies where diurnal cortisol indices have been used to predict perceived stress after a long-term stressful event in healthy young adults. Our data has tentative similarities with previous cross-sectional studies like that of Ruiz-Robledillo et al, (2014), who showed that high resilient coping was associated with low cortisol and better perceived health and social support, while our moderation model shows high resilient coping (+1SD) at low cortisol levels (-1SD) also predicts lower during confinement perceived stress (-1SD). Moreover, for low AUC_G (-1SD) at low resilience levels predicts high perceived stress (+1SD), while for CAR, at very low resilience levels (8.25 BRCS score and below), flat CAR responses correlate with high perceived stress (+1SD). This result draws attention to the study by O´Connor and colleagues (2009), who reported that high perceived stress predicted flattened CAR profiles. We found that pre-pandemic AUC_G, but not CAR, were related to depressive-like symptoms and anxiety due to the COVID-19 pandemic. Among the sparse relevant literature, Lemoult et al. (2015) reported similar results in that young girls with high AUC_G indices showed a higher susceptibility to onset of depression later in adolescence after experiencing negative life events, but this was not observed in girls with a high CAR index. Similar to results in the present study, other authors have not found a relation between CAR and depressive symptoms (Carnegie et al., 2014), while certain studies do report CAR to be predictive of depressive symptoms (Hardeveld et al., 2014; Stroud et al., 2019;

Schuler et al., 2017). Our findings are in line with the diathesis-stress models of depression postulating that prodromal vulnerability factors, such as altered function of HPA axis, interact with environmental stressors to increase risk for depression (Hammen, 2005; Monroe and Simons, 1991). Thus, it may be hypothesized that high diurnal cortisol levels occur in those individuals with greater stress-related arousal state and more susceptiblility to the impact of stressors. Although it is not well understood how a higher AUC_G can increase susceptibility to stress, in depressed patients it has been proposed that elevated diurnal cortisol levels can alter functioning in brain areas that exert HPA axis negative feedback loops (Kudielka et al, 2006; Schmidt-Reinwald et al., 1999) and play a crucial role in emotional processing, hindering the ability to cope with future stressful events (Holsboer, 2000; Schuhmacher et al., 2012). Hence, a high AUC_G during pre-pandemic might be considered an early biomarker of inefficient HPA axis negative feedback, which, in turn, can alter individuals' reactivity to stressful events.

Overall, the finding that resilient coping can increase or decrease perceived stress at different basal diurnal cortisol conditions may be relevant to understand the often-reported contradictory results obtained across studies exploring relations between psychological and physiological stress, including a negative relation (Faresjö et al., 2013; Yang et al., 2001), no relation (Hjortskov et al., 2004) or even a positive relation (Weibel et al., 2003; González-Cabrera et al., 2014; 2017).

Another objective of this thesis was to examine the relationship between individual cortisol, cognition and stress perception with the specific prediction (hypothesis 6) that the changes in cognitive capacity of the subjects will mediate the relationship between pre-pandemic cortisol and the changes in perceived stress (Figure 10). We used the dynamic values obtained via subtracting the home confinement scores from the pre-pandemic scores for a concise representation of the neuropsychological effects of COVID-19. We expected a predictive relationship between cortisol and cognition owing to the study by Moriarty et al., (2014) where they showed CAR's inverted-U association with spatial working memory, the same task explored in the current study using the Corsi-block tapping test. The prefrontal cortex is an underlying functional brain region implicated in both, working memory performance and the ability of perspective taking (Shamay-Tsoory et al., 2009; Lara et al., 2015). Additionally, the prefrontal cortex is one of the brain structures that exerts a negative feedback on the HPA axis activity (Herman et al., 2005), while CAR occurs during post-awakening reversal of sleep inertia and reactivation of the prefrontal cortex activity (Balkin et al., 2002). The mediation model presents results in-line with the above since CAR and AUC_G indices are related to change in spatial working memory and perspective taking (cognitive empathy). The model further shows that the increase in working memory and perspective taking, in turn, correlates with a decrease in the worsening of perceived self-efficacy (keeping in mind perceived self-efficacy was the driving factor behind total perceived self-efficacy being predicted in the moderation models). Previous investigators have demonstrated that both genetic and cognitive factors could be implicated in the response to stress (Gibb et al., 2013). In addition, it has been reported that after adverse circumstances/events, those subjects with higher cognitive abilities showed more positive results, like better academic performance and better social acceptance (implicating empathy) and friendship (Masten et al., 1999; Riglin et al., 2016).

Otto et al., (1997) showed that perceived improvement in problem solving was associated with lower perceived stress. Improved cognition may allow for more cognitive capacity to process and use the novel circumstances in order to more efficiently manage them (Southwick, et al., 2005), and this could in turn influence change in perceived self-efficacy. We believe this effect of higher cognition also applies with respect to improvement in perspective taking correlating with attenuated decrease in perceived self-efficacy as seen in the current study. Although there are few studies on the matter and results are inconclusive, Gambin & Sharp (2018) found an inverse relationship between cognitive empathy and social/separation anxiety in inpatient adolescents. Worth noting is a recent study also studying the impact of COVID-pandemic and confinement replicating the results we describe in their own study sample where following the pandemic they observed an increase in the cognitive empathy scores (Baiano et al., 2022). Apart from the indirect effect of cortisol indices (both AUC_G and CAR) on perceived self-efficacy, AUC_G also directly affects perceived self-efficacy albeit in the opposite direction. AUC_G is directly related to a worsening of perceived self-efficacy while indirectly, through cognitive capacities, it relates to improvement in self-efficacy after COVID-19 quarantine. This suppression by the two effects on one another leading to an overall total effect being non-significant is a known cause of missing relevant relations (Loeys et al., 2015) and may perhaps be an explanation as to why the relationship between cortisol and perceived stress has been elusive.

Apart from studying the prospective capacity of pre-pandemic diurnal cortisol indices as predictors of the individual differences concerning impact of COVID-19 on cognitive functioning and emotional well-being, we also observed that strict long-term confinement conditions during the COVID-19 pandemic led to an overall significant increase in perceived stress, visuo-spatial working memory and trait-like perspective taking in young adults compared to pre-pandemic time. An increase in perceived stress was not surprising given not only the fear of infection, but also the uncertainty caused by the preventive measures (e.g., the pre-emptive quarantine) and the ensuing change in routine (Brooks et al., 2020; Xiang et al., 2020). This increase in uncertainty is apparent when looking at the two constructs that the perceived stress scale evaluates; perceived helplessness and perceived self-efficacy. Our results indicate that the increase in total perceived stress is driven primarily by an increase in perceived helplessness. This is congruous with the condition the general public has found itself in; obliged to stay inside their homes to avoid an invisible threat the subjects, as individuals, have little control over.

It is well known that GCs, like cortisol, are key regulators of both intrinsic and extrinsic (Pavlovian and spatial conditioning) learning and memory (Sandi & Pinelo-Nava, 2007), whose neuroanatomical bases are found in corticolimbic areas, mainly in the hippocampal formation (Russell et al., 2015), prefrontal cortex and amygdala (Sandi & Pinelo-Nava, 2007). Within this system, GCs interact at multiple levels and, depending on the period of exposure, structural changes or even functional consequences may occur with an opposite or bidirectional outcome to that expected (Joëls et al., 2004). Although, if the exposure to stress is prolonged to three weeks there is generally a certain worsening of learning abilities, especially in spatial learning (Sandi, 2004). At the synaptic level, GCs modulate presynaptic activity and postsynaptic glutamatergic and GABAergic responses that are involved in the molecular phenomena of synaptic potentiation and depression (reduction of neuronal synapse efficiency) of learning processes (Russell et al., 2015). At the cellular (physiological and structural) level, GCs influence the electrical properties of neuronal activity (Joëls et al., 2012) and the volume of dendritic spines, both important features for efficient communication between neurons (Jeanneteau & Chao, 2013). Moreover, GCs interact with noradrenergic and cholinergic circuits innervating the HP and amygdala, and affect memory formation and the regulation of behavioural adaptations (Manzanares, Corchero, & Fuentes, 1999). The variable nature of these effects is determined by the phase of the GCs circadian cycle (as with cortisol), GC concentrations, timing, and type of learning analysed (Roozendaal, 2002; Schwabe, Tegenthoff, & Wolf, 2012). Sharp increases in cortisol just immediately before learning can promote processes such as memory formation, consolidation, and recall of emotionally poignant stimuli (Cahill, Gorski, & Le; McReynolds et al., 2010; Roozendaal, 2002). However, if stress occurs temporally long before learning, the genomic consequences elicited by the action of cortisol (GCs) may impede memory processes (Buchanan, Tranel, & Adolphs, 2006). In addition, chronic exposure to elevated levels of GCs may impair spatial memory establishment and retrieval (Dumas, Gillette, Ferguson, Hamilton, & Sapolsky, 2010; Roozendaal, 2002). In view of all these investigations one could conclude that the amount of circulating GCs could have a net negative or positive consequences on memory consolidation or learning; it has been suggested that the effects of these hormones (specifically cortisol) on learning and memory follow an "inverted U" curve; such that, very low or high levels would impair learning, while moderate amounts of GCs would enhance it, although this curve does not seem to be reflected in emotional memories (Finsterwald & Alberini, 2014; Sandi & Pinelo-Nava, 2007; Venero et al., 2002). Thus, in the light of the COVID-19 pandemic and associated confinement caused stress, the second hypothesis of Objective 3 was to see if the stress caused would improve or lead to deterioration of working memory of the participants. Concerning this, the significant increase in the performance of the Corsi block-tapping test, a measure of visuo-spatial working memory span, we believe this result to be an observation of the bidirectional effect of stress on cognitive abilities (Schoofs et al., 2008, Salehi et al., 2010; Weerda et al., 2010). Thus, although the pandemic has caused an increase in stress, we may speculate that the intensity of this stress has not been high enough to be detrimental to our subjects' short-term working memory. One argument against this conclusion could be that there is a learning effect caused by the repetition of the cognitive task. However, on top of the 6-month gap between the two tests, previous research has noted the absence of any learning-effect when Corsi is repeated from traditional versions to e-Corsi (Brunetti et al, 2014; Siddi et al., 2020). Nor are there any differences between Corsi administered face-to-face in laboratory settings or via the use of e-Corsi (Robinson 2016). If anything, Claessen et al. (2015) observed results where traditional Corsi-forward reproduction resulted in higher accuracy compared to e-Corsi. On the other hand, short-term working memory to maintain objects in a spatial series demands active spatial attention (Smyth & Scholey, 1992; 1994). This implies a direct role of attention scope and control in Corsi memory span (Cowen, 2004; Escamilla et al., 2020). Therefore, it may be argued that improved performance in the Corsi test is not a reflection of better working memory span *per se*, but it is an effect of an increase in working memory capacity. However, the absence of differences between pre-pandemic and during confinement change-location task scores discards the possibility that the pandemic and the associated confinement had changed focal attention capacity specifically, a result in line with previous research showing that changes in positive or negative emotion/mood had no impact on spatial attention (Bendall & Thompson, 2015).

Similar to working memory, we observed an increase in perspective taking, but not an increase in empathic concern, as measured by the interpersonal reactivity index (IRI). Perspective taking and mentalizing are empathy systems that require more complex cognitive systems (de Waal, 2007) and perspective taking has been shown to be dependent on the prefrontal cortex (Montag et al. 2008; Shamay-Tsoory et al., 2009). Thereby, our observation of an increase in perspective taking is in line with improvement of cognitive systems as seen with working memory, a cognitive process also governed by the prefrontal cortex (for rev. see Lara et al., 2015). Additionally, the absence of increase in empathic concern, a process not strongly related to the prefrontal cortex and cognitive systems (Bernhardt and Singer, 2012) is also worth noting.

Overall, while it has been shown that both cortisol and perceived stress have a distinct genetic component (Luo et al., 2017; Rietscel et al., 2017). Rietschel et al. (2017, pp 8), also make note of the absence of a biological relation between cortisol and perceived stress in their study. However, they mention how

¨*Phenotypic and genetic correlations with psychological variables may only become evident in cohorts with more pronounced or specified environmental impacts… Under the challenge of more adverse environments, stronger variance might occur in those phenotypes, partially driven by distinct genetic factors*¨. We believe the COVID-19 pandemic and the following lockdown may have created conditions triggering the emergence of a relation between cortisol and perceived stress.

Although our study provides important information not only about the relationships between cognitive capacities, psychological stress and specially the basal HPA cortisol indices, but also regarding the impact of COVID-19 epidemic, some limitations should be considered when interpreting the results of this study. Given the circumstances, the sample size of the study was constrained, and for similar reasons, biological stress markers during home confinement could not be collected. Given the participant demographic, we strongly encourage similar studies with other demographic profiles, where the effects of COVID-19 pandemic may vary and thus so could their interrelationships. Similarly, a note of caution to the generalizability of the results, we suggest these results be indicative, given how few studies there are about natural stressors of this kind and the inherent complexity as compared to laboratory settings. Finally, given how widespread COVID-19 has been, it was not possible to have a control group which did not pass through a confinement. This should be kept in mind when interpreting the differences observed between the pre-pandemic and during confinement sessions of the study. While seasonal changes tend to impact depression like symptoms in the direction contrary to our results (Soreni et al., 2019), there may be other factors involved in these changes, apart from the situation caused by COVID-19 pandemic.

In conclusion, the exploration of objective 3 presents new data concerning how people confront long-term stressors (like the pandemic-confinement) and establishes how the impact of such crises varies according to individual HPA axis set-up. Furthermore, it argues for attention to coping ability, cognitive function and overall contextual landscape of the stimuli under study for a more complete interpretation of the dynamic between physiological and psychological stress.

So far, we have seen evidence about how the abnormal secretion of the glucocorticoid hormone cortisol connects chronic psychosocial stress to worsened health (Chrousos, 2009). However, the HPA axis and cortisol are also critical for the execution of “normal” day-to-day activities (via higher affinity MRs system, Bartels et al., 2003), are indispensable in the allocation of energy and nutrients to organs and tissues that need them and thus help the body prepare for metabolic/cardiovascular tasks (Adam and Epel, 2007), immune responses and anti-inflammatory actions (El-Farhan, Rees and Evans, 2017). Thus, if an individual wants to do activities which are demanding metabolically, it is the function of the HPA axis (in conjunction with other systems) to activate itself and allow for those behaviours to be executed. It is noteworthy that the previous sentence talks about activating the HPA axis based on a ‘*want’*, which correctly implies that it is not necessarily stress responses which involve the activation of this neuroendocrine system. In fact, appetitive and rewarding stimuli, such as sexual activity too elicit HPA stimulation with similar intensities as do “stressful” stimuli (Ralph et al., 2016; Bonilla-Jaime et al., 2006). Additionally, the intensity with which the HPA axis is activated often relates to the behaviour thus the metabolic demand of the action(s). Thus, often, arousal is seen as an essential physiological necessity in preparation and execution of behaviour (Pfaff et al., 2008; Koolhaas et al., 2011). This brings us to the Objective 4.

This dissertation is the first research to examine the association between individual cortisol patterns (diurnal and post-awakening) and empathy in older females. Individuals with higher empathic concern showed higher cortisol levels post-awakening. Specifically, while we observed no relationship of perspective taking scores (cognitive empathy) with awakening or diurnal cortisol levels, higher empathic concern scores (emotional empathy) were associated with higher post-awakening cortisol output AUC_G and diurnal cortisol AUC_G, as well as a steeper DCS.

The activity of brain areas involved in emotion processing, emotional contagion and emotion priming such as the amygdala, the ventromedial prefrontal cortex as well as the rostral anterior cingulate cortex (Decety and Jackson, 2006) can be affected by cortisol (Harrewijn 2020; Wheelock et al., 2016). The fact that affective empathy, rather than perspective taking, involves emotional contagion and the visceral/emotional response to empathy (Doherty, 1997) may explain the results of the current study where we see a relation between several cortisol indices (post-awakening cortisol AUC_G, diurnal cortisol

AUC_G and DCS) and EC, but not with PT. Remarkably, Shirtcliff et al., (2009) described how children displaying callous unemotional symptoms (i.e., lack of empathy, lack of guilt, etc.) show low circulating cortisol levels and emotional hypoarousal. Consequently, they postulated that empathy may be facilitated by moderately high cortisol levels reflecting an optimal state of emotional arousal and a resultant level of internal distress that facilitates empathic behaviour. Their observation may further explain results of the current study where we show that higher output of cortisol after awakening is observed in older females with high empathic concern. Probably, within a normal range, higher cortisol levels (implicating both indices, post-awakening AUC_G and diurnal AUC_G) may enable individuals to be readily aroused vicariously by another's sadness, pain or distress. Having said that by this viewpoint, personal distress, the self-focused feels of discomfort generated by witnessing the suffering of another, might also be related to the LHPA axis and is recommended as an aspect meriting future attention. The only other research that we are aware of concerning older adults and the relation between cortisol and empathy found that individuals with greater perspective taking demonstrated higher basal cortisol on average, but no relation was found between empathic concern and cortisol (Pauly et al., 2020). Unfortunately, specific cortisol indices were not explored, nor were sex-differences taken into account despite the well-established sex-differences in empathy (Derntl et al., 2010), making it difficult to compare these earlier results with ours. Similarly, differences in sample characteristics such as age and the use of a mixed-sex sample may explain the discrepancy between results from a previous study in college students, in which CAR related to emotional empathy (Johnson et al., 2014) and results from the present study, where CAR was not related to empathy.

Among the different cortisol indices, CAR is known to be related to distinct psychological conditions and psychiatric disorders (Chida and Steptoe 2009), but the psychological significance of post-awakening cortisol AUC_G remains little explored. According to our regression modelling results, cortisol output after awakening (AUC_G) is a very robust predictor of emotional empathy. Therefore, it may be speculated that post-awakening cortisol AUC_G reflects how the emotional psychosocial factors pertinent to each individual interact with the HPA axis. However, it is likely that this baseline bottom-up impact of morning cortisol on empathy is complemented by stress-reactivity dependent top-down processes where activity of brain regions, like the prefrontal cortex, implicated in empathy and empathic behaviour negatively impacts the HPA axis output (Hoover et al., 2007; de Kloet et al., 2018). Results of the current study also provide evidence that lower empathic concern scores are related to flatter diurnal cortisol slopes. Previously, other authors have indicated that a flatter DCS is related to chronic stress and worse physical and mental health, while subjects with steeper slopes are physically and mentally healthier (for review see Adam et al., 2017). Thereby, our present results may be interpreted in this sense as individuals with steeper DCS exhibited higher capacity to experience emotional empathy, although again, it is likely that the two processes reinforce each other.

Some limitations of this study are noteworthy. Firstly, we did not corroborate self-reported saliva sample collection reports using objective measures such as electronic track caps. Secondly, the correlational nature of the results precludes drawing conclusions about causal relationships. Finally, in line with previous research self-report questionnaires were used to measure empathy, but addition of more ecological measures of empathy are desirable. Despite these limitations, results of the current study provide important new evidence for a relation between cortisol and emotional empathy in female older adults. This together with the earlier evidence points to the possibility that over the course of the lifespan the interaction between the HPA system and the general act of emotional empathy leaves such an imprint on both systems that they are predictive of the functioning of the other. That is to say, the LHPA axis influences emotional empathy-like behaviour and feelings and the general personality-behavioural profile have continuous influences on the LHPA axis such that each influence the other's development and design. This viewpoint is congruous with a bi-directional psychophysiological interaction where top-down processes like emotion processing (e.g., empathy) influence and in turn get influenced by bottom-up systems like the physiological response of the body to arousal (e.g., LHPA axis activity). The results from the empathy in old-age females point towards possible reflection of the impact and gradual imprint of diurnal cortisol release making it possible to infer affective empathy responses and vice-versa. Possibly this relationship develops and grows stronger over time, making it more likely to encounter in aged participants and thereby explaining why we did not encounter it in the sample used in objective 3. However, there may be other explanations to its absence in the young-adult university student sample, like the smaller sample size or the usage of the mixed-sex sample. Hopefully, future studies can build upon the present results and will be able to disentangle the role of cortisol on empathy and empathy-like behaviour.

4. CONCLUSIONS

- We provide a Spanish version of the Pictorial Empathy Test (PET), and its validation for the measurement of situational emotional empathy. We present evidence that confirms its unidimensional internal structure and its psychometrically correctness, including good discriminant and convergent validity and high reliability.

- In a young-adult sample, the COVID-19 pandemic and the associated 50-day social confinement led to increases in social and family loneliness and to a significant decrease in trait extraversion and willingness to help others.

- Pre-pandemic diurnal cortisol indices were related to changes in the feeling of social isolation following social confinement. This relation was moderated in intensity and direction by the pre-pandemic scores of trait extraversion.

- Subjects that had undergone home-confinement exhibited an increase in cognitive empathy, self-perceived stress and spatial working memory.

- Individual diurnal cortisol indices were able to predict posterior levels of perceived stress, anxiety and depression, and the direction and intensity of this relationship was moderated by resilient coping.

- Individual pre-pandemic diurnal cortisol levels predicted posterior post-stress change in perceived stress and this relation was mediated by the post-stress change in spatial working memory.

- Total diurnal cortisol output plots revealed that old-aged females with high empathic concern scores showed higher diurnal cortisol levels as compared to counterparts with low empathic concern scores.

- Diurnal cortisol index levels are related to trait emotional empathy. Specifically, higher post-awakening cortisol AUC_G, higher total diurnal cortisol AUC_G, as well as a steeper DCS were found to be associated with higher trait emotional empathy scores even after controlling for relevant confounders.

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6. APPENDICES

Appendix I

Please find below the complete Spanish version of PET, PETs. The PETs is composed of the question, “¿Cuánto considera que esta fotografía le conmueve emocionalmente?”, followed by the five options: [1 = nada de emotivo, 2 = un poco, 3 = me produce algunos sentimientos, 4 = bastante emotivo, 5 = mucho]. This question is placed below each image along with the response options. Please use the original open source provided to download and use full resolution copies of the photographs shown. Do not forget to cite the license and author of the images when used (for e.g., at the end of the research experiment).

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Appendix II

Table 11: Moderation models.

predictors	Model 1 - Depression (DASS)				Model 2 - Anxiety (DASS)			
	b(SE)	t	LLCI: ULCI	Δr^2	b(SE)	t	LLCI: ULCI	Δr^2
AUC_G	-10.609(3.410)	-3.111	-17.540: -3.679		-10.266(3.869)	-2.653	-18.129: -2.403	
BRCS	-2.560(.748)	-3.423	-4.080: -1.040		-2.557(.849)	-3.012	-4.281: -.832	
AUC_G*BRCS	.664(.224)	3.012	.216: 1.112	.192	.685(.25)	2.741	.177: 1.194	.173

predictors	Model 3 - Depression (DASS)				Model 4 - Anxiety (DASS)			
	b(SE)	t	LLCI: ULCI	Δr^2	b(SE)	t	LLCI: ULCI	Δr^2
CAR	-56.461(46.87)	-1.205	-151.622: 38.700		-18.41(51.49)	-.358	-122.745: 85.925	
BRCS	-.79(.413)	-1.915	-1.628: .047		-.522(.425)	-1.153	-1.440: .397	
CAR*BRCS	3.402(3.099)	1.098	-2.889: 9.694	.030	1.678(3.398)	.493	-5.223: 8.573	.007

predictors	Model 5 - PSS ([lack of] Self-Efficacy)				Model 6 - PSS (Helplessness)			
	b(SE)	T	LLCI: ULCI	Δr^2	b(SE)	T	LLCI: ULCI	Δr^2
AUC_G	-11.788(2.895)	-4.072	-17.673: -5.904		-8.734(3.325)	-2.627	-15.490: -1.977	
BRCS	-3.286(.635)	5.173	-4.576: -1.995		-2.112(.729)	-2.910	-3.604: -.640	

AUC_G*BRCS	.771(.187)	4.121	.391: 1.151	.249	.572(.215)	2.663	.136: 1.009	.166

predictors	Model 7 - PSS ([lack of] Self-Efficacy)				Model 8 - PSS (Helplessness)			
	b(SE)	t	LLCI: ULCI	Δr^2	b(SE)	t	LLCI: ULCI	Δr^2
CAR	-122.262(38.110)	-3.208	-199.631: -44.893		-37.021(43.895)	-.843	-126.176: 52.135	
BRCS	-1.679(.336)	-5.005	-2.360: -.998		-.533(.387)	-1.379	-1.318: .252	
AUC_G*BRCS	8.229(2.520)	3.266	3.114: 13.344	.174	2.508(2.903)	.864	-3.386: 8.402	.020

Note. b=Unstandardized effect size; SE=Standard Error; DASS=Depression, Anxiety and Stress Scale; LLCI=Lower Limit of Confidence Intervals 95%; ULCI=Upper Limit of Confidence Intervals 95%; AUC_G=Cortisol index for area under curve from ground; BRCS=Brief Resilient Coping Score; CAR=Cortisol Awakening response. Models 1, 2, 5, 6, 7: conditional effects (for 16th, 50th and 84th percentile) graphs below

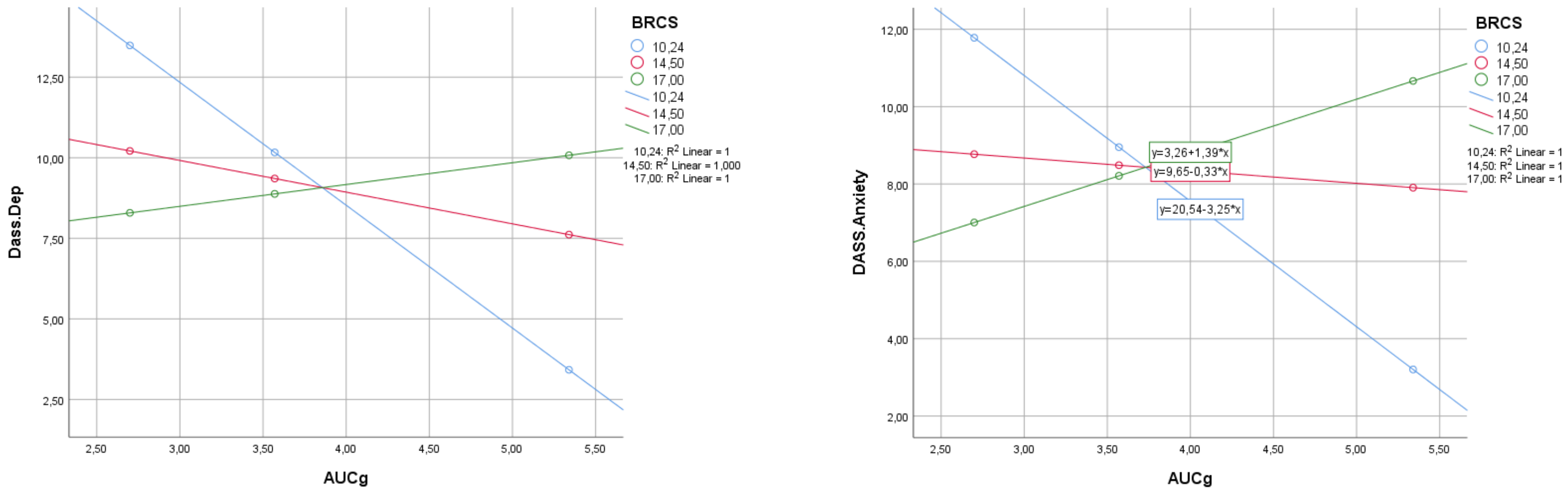
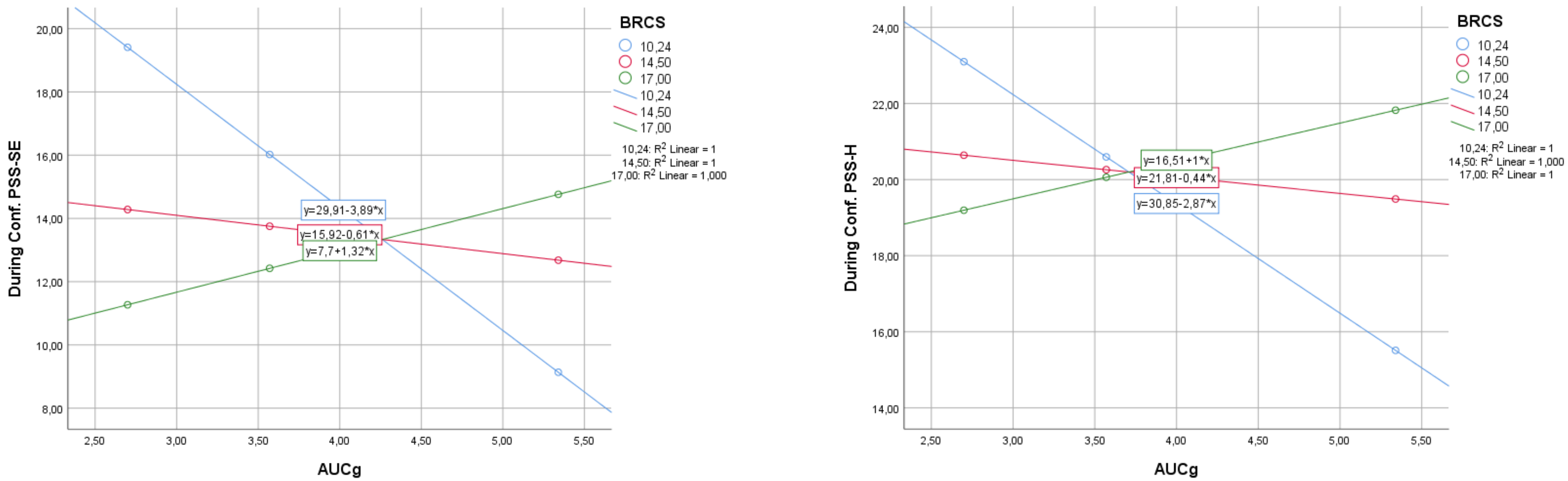


Figure 21. *(Above) Conditional effects simple slopes for model 1 (figure (a)) and 2 (figure (b)):*

Note. AUC_G=Total cortisol index for the day; BRCS=Brief Resilient Coping Score; DASS=Depression, Anxiety and Stress Scale

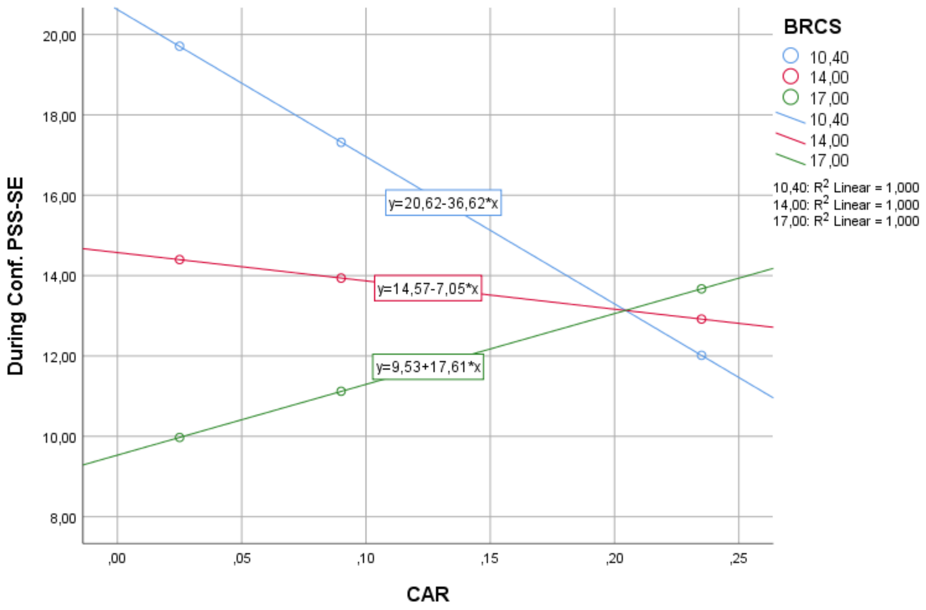
__Figure 22.__ (Below) Conditional effects simple slopes for model 3 (figure 1(c)) and 4 (figure (d)):



(c) **(d)**

Note. PSS-SE=Perceived Stress Scale-Self-Efficacy; PSS-H=Perceived Stress Scale-Helplessness; AUC_G=Total cortisol index for the day.

Figure 23. *(Below) Conditional effects simple slopes for model 7 (figure 1(e)):*



(e)

Note. PSS-SE=Perceived Stress Scale-Self-Efficacy; BRCS=Brief Resilient Coping Score; CAR=Cortisol Awakening Response.

Table 12. Scores at pre-pandemic stage for the subject pool which abstained from participating in the during confinement phase and scores for the 'during confinement' cohort.

	Non-participant cohort	During confinement cohort
AUC_G	3.61(1.02)	3.89(1.49)
CAR	0.10(0.09)	0.12(0.11)
Age	20.12(2.50)	21.09(6.42)
IRI: Perspective Taking	20.09(4.22)	18.93(3.66)
IRI: Empathic Concern	21.74(4.33)	22.67(3.30)
PSS: Helplessness	16.17(5.23)	17.51(5.21)
PSS: Self-Efficacy	11.25(4.09)	12.95(5.67)
PSS: Total	27.42(8.31)	30.46(9.45)
Corsi-Forward Score	8.66(1.56)	8.88 (1.45)
Corsi-Backward Score	8.00(1.27)	7.77(1.54)
Corsi-Total Score	16.66(2.27)	16.66(2.52)
Change-Location Score	3.08(0.39)	3.17(0.35)

Note. Mean and Standard Deviation (SD) presented; AUC_G (ug/dL)=Total diurnal cortisol release; CAR (ug/dL)=Cortisol Awakening Response; IRI=Interpersonal Reactivity Index; PSS=Perceived Stress Scale.

Table 13: Unadjusted correlation matrix.

	AUCg	CAR	ΔEC	ΔPT	ΔF.corsi	ΔB.corsi	ΔT.corsi	ΔCL	ΔPssH	ΔPssSE	ΔT.Pss	AnxietyDASS	DepDASS
CAR	**,423****												
ΔEC	-0,135	-0,195											
ΔPT	0,010	**,375***	0,228										
ΔF.corsi	0,206	**,419***	-0,140	0,271									
ΔB.corsi	0,183	0,271	0,174	-0,103	-0,030								
ΔT.corsi	0,334	**,428***	-0,010	0,086	**,634****	**,685****							
ΔCL	0,281	0,109	0,032	0,009	**,376***	-0,100	0,140						
ΔPssH	0,129	0,009	-0,107	-0,170	-0,183	-0,122	-0,282	0,062					
ΔPssSE	0,218	0,029	-0,143	-0,229	**-,393***	-0,025	-0,289	-0,129	**,420****				
ΔT.Pss	0,165	0,047	-0,085	-0,150	-0,291	-0,112	-0,339	0,145	**,831****	**,792****			
AnxietyDASS	0,118	0,040	-0,163	-0,275	0,127	0,094	0,163	0,230	0,212	**,408****	**,408****		
DepDASS	-0,021	-0,161	**-,371***	-0,277	0,028	-0,024	0,006	-0,094	0,200	**,471****	**,361***	**,685****	
BRCS	-0,064	-0,039	0,035	0,190	-0,052	-0,257	-0,170	-0,008	-0,110	-0,083	-0,124	-0,209	-0,187

Note. DASS=Depression, Anxiety and Stress Scale; AUC_G =Cortisol index for area under curve from ground; BRCS=Brief Resilient Coping Score; CAR=Cortisol Awakening response; PT=Perspective Taking; EC=Empathic concern; CL=Change Location; PssH=Perceived Helplessness; PssSE=(lack of)Perceived Self-efficacy; T.Pss=Total Perceived Stress Scale; F.corsi=Corsi Forward; B.corsi=Corsi Backward; T.corsi=Corsi Total Spearman coefficients reported; *p<0.05; **p<0.01

Table 14: Moderation models with Age and Sex as covariables.

predictors	Model A – AUC_G to Perceived stress				Model B – AUC_G to DASS-Depression			
	b(SE)	t	LLCI: ULCI	Δr^2	b(SE)	t	LLCI: ULCI	Δr^2
AUC_G	-21.036(5.612)	-3.747	-32.469: -9.602		-11.060(3.506)	-3.154	-18.203: -3.918	
BRCS	-5.435(1.216)	-4.467	-7.913: -2.957		-2.595(.760)	-3.414	-4.143: -1.047	
AUC_G*BRCS	1.374(.362)	3.792	.656: 2.112	.262	.689(.226)	3.041	.227: 1.150	.201
Sex	-1.063(3.540)	-.300	-8.273: 6.147		-.213(2.211)	-.096	-4.718: 4.294	
Age	-.165(.299)	-.551	-.774: .444		-.186(.187)	-.995	-.566: .195	

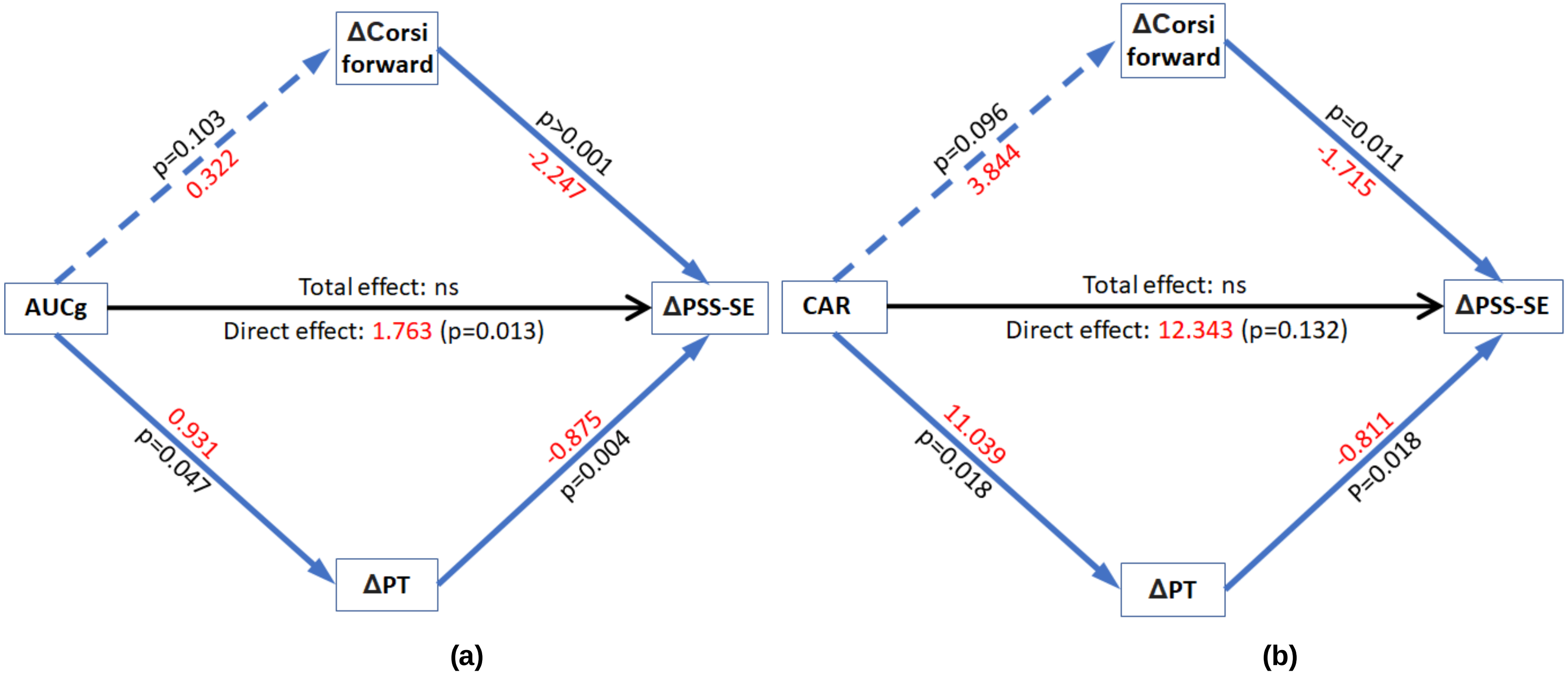
predictors	Model C – AUCg to DASS-Anxiety							
	b(SE)	t	LLCI: ULCI	Δr^2				
AUC_G	-11.1410(3.895)	-2.859	-19.076: -3.205					
BRCS	-2.6139(.8445)	-3.095	-4.334: -.893					
AUC_G*BRCS	.7354(.251)	2.923	.223: 1.247	.195				
Sex	-1.098(2.456)	-.447	-6.102: 3.906					
Age	-.321(.207)	-1.548	-.744: .101					

predictors	Model D – CAR to Perceived stress	

	b(SE)	t	LLCI: ULCI	Δr^2				
CAR	-169.988(78.240)	-2.173	-329.172: -10,804					
BRCS	-2.198(.668)	-3.291	-3.556: -.8389					
CAR*BRCS	11.493(5.205)	2.208	.904: 22.089	.108				
Sex	-2.008(4.091)	-.491	-10.332: 6.316					
Age	-.109(.334)	-.329	-.785: .587					

Note. b=Unstandardized effect size; SE=Standard Error; DASS=Depression, Anxiety and Stress Scale; LLCI=Lower Limit of Confidence Intervals 95%; ULCI=Upper Limit of Confidence Intervals 95%; AUC_G=Cortisol index for area under curve from ground; BRCS=Brief Resilient Coping Score; CAR=Cortisol Awakening response.

Figure 24. *(Below) Mediation models while controlling for sex and age: (a) AUC_G cortisol predicting change in perceived self-efficacy; (b) CAR predicting change in perceived self-efficacy. PT=Perspective Taking; PSS-SE=Perceived stress scale Self-Efficacy; AUC_G=Total diurnal cortisol release; CAR=Cortisol Awakening Response; ns=non-significant. Effect sizes in red.*



Indirect effect(s) of AUC_G on ΔPSS-SE:

	Effect	SE	LLCI	ULCI
TOTAL	-1,6083	,6761	-3,2368	-,5659
del.PT	-,8145	,4971	-2,0384	-,1148
del.d.co	-,7938	,5336	-1,9907	,1730

Indirect effect(s) of CAR on ΔPSS-SE:

	Effect	SE	LLCI	ULCI
TOTAL	-15,5422	7,2477	-31,7071	-3,2584
del.PT	-8,9501	5,2149	-21,0601	-,6471
del.d.co	-6,5921	4,6302	-17,2346	,6602

Table 15. *Pearson correlation coefficients*

	Empathic Concern	Perspective Taking	Post-Awk AUCg	CAR	Diurnal cortisol AUCg	DCS	Age	GDS score	Awakening hour	Sleep duartion
Perspective Taking	0,215									
Post-Awk AUCg	**,378****	-0,074								
CAR	0,082	0,082	0,066							
Diurnal cortisol AUCg	**,245***	0,050	**,697****	0,152						
DCS	**-,276***	0,183	**-,789****	**,468****	**-,367****					
Age	0,015	-0,080	0,087	-0,112	0,058	-0,079				
GDS score	-0,195	**-,456****	-0,090	-0,128	-0,199	-0,052	0,166			
Awakening hour	-0,079	-0,064	**,254***	-0,123	0,048	**-,330****	0,017	0,073		
Sleep duartion	0,176	-0,222	0,157	-0,087	-0,211	**-,328****	0,020	0,043	**,519****	
Years of education	-0,111	**,307****	**-,249***	-0,037	-0,164	0,192	-0,224	**-,235***	-0,065	-0,016

Note: AUCg = Area under curve with respect to ground; CAR = Cortisol Awakening Response; DCS = Diurnal Cortisol Slope; GDS = Geriatric Depression Scale. **p*<0.05; ***p*<0.01