PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
Doctorado en Neurociencias

Tesis Doctoral

"PSYCHOSOCIAL STRESS EFFECTS ON ATTENTIONAL CONTROL MEDIATED BY BRAIN OSCILLATORY ACTIVITY."

Por

Ismael José Palacios García

Septiembre 2017

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Santiago, Septiembre 2017

Creating a world in which the healthy qualities of mind are investigated and valued

Dedicated to Andres Fuentealba Grant (1987-2016)

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Abstract

Attention is a crucial cognitive function. It's being constantly reoriented by means of either top-down or bottom-up factors. Some studies have suggested that stress-related states can disturb the balance between those factors. However, the neural mechanisms underlying those changes, have not been completely elucidated. Here we explore the neural correlates of psychosocial stress and the differential impact of its physiological and psychological components over an attentional task. Forty-two healthy participants were subject to EEG while exposed to either the Trier Social Stress Test or a comparable control protocol. Immediately before and after those protocols participants performed an attentional task. The induced stress response was verified by measuring changes on heart rate, salivary cortisol concentration and the score in the STAI scale. Psychosocial stress induced an increase of salivary cortisol, heart rate and self-reported anxiety. Stressed subjects had worse accuracy in the attentional task than controls. This effect directly correlated with the self-reported anxiety level. Anxiety was also correlated with both the heart rate and salivary cortisol response. Interestingly, correct trials in the control group were associated with a gamma increase (30-65 Hz). Conversely, correct trials of the stress group correlated with an increase of beta activity (12-30 Hz). The beta activity correlated positively with anxiety and negatively with attentional accuracy, while gamma band was uncorrelated with any of the measures. Finally, we report a frequency specific coupling between heart signal and brain oscillatory activity. In particular, we found that heart R peak was phase locked to beta band activity. Surprisingly, the coupling decreased after psychosocial stress induction. Altogether the results highlight: 1) the relevance of the immediate stress-dependent anxiety to understand the effects of social stressful situations over attentional control. Subjective experience of anxiety is apparently more relevant at this temporality than the physiological stress response, which could be playing only an indirect role by affecting the perception of the current body state, 2) the differential cognitive strategies and neural mechanism via which stressed subjects can achieve

the task. Such observation might have important implications for designing therapeutic interventions to deal with social stress and stress in general.

Resumen

La atención es una función cognitiva crucial. Se está reorientando constantemente mediante factores 'Top-Down' y 'Bottom-Up'. Algunos estudios han sugerido que los estados relacionados con el estrés pueden alterar el equilibrio entre dichos factores. Sin embargo, los mecanismos neuronales subyacentes a esos cambios, no han sido bien estudiados hasta la fecha. Aquí exploramos los correlatos neurales del estrés psicosocial y el impacto diferencial de sus componentes fisiológicos y psicológicos sobre una tarea de atención. Cuarenta y dos participantes sanos fueron sometidos a EEG, mientras fueron expuestos a la prueba de estrés social TSST o a un protocolo Control comparable. Inmediatamente antes y después de dichos protocolos los participantes realizaron una tarea de atención. La respuesta al estrés se verificó midiendo los cambios en la frecuencia cardíaca, la concentración de cortisol salival y la puntuación en la escala de ansiedad STAI. Como era esperado, el estrés psicosocial indujo un aumento del cortisol salival, la frecuencia cardíaca y la ansiedad auto-reportada. Los sujetos estresados tuvieron peor precisión en la tarea atencional que los controles. Este efecto se correlacionó directamente con el nivel de ansiedad auto-reportado. La ansiedad también se correlacionó con la frecuencia cardíaca y la respuesta del cortisol salival. Curiosamente, los ensayos correctos en el grupo de control se asociaron con un aumento en la actividad oscilatoria cerebral de tipo gamma (30-65 Hz). Por el contrario, los ensayos correctos del grupo de estrés se correlacionaron con un aumento de la actividad beta (12-30 Hz). La actividad beta se correlacionó positivamente con la ansiedad y negativamente con la precisión atencional, mientras que la banda gamma no se correlacionó con ninguna de las medidas. Finalmente, se reportó un acoplamiento de frecuencia entre la señal cardíaca y la actividad oscilatoria cerebral. En particular, se encontró que los intervalos R de la respuesta cardíaca estaban se acoplaron específicamente con la fase de la actividad cerebral en frecuencias del rango beta beta. Sorprendentemente, el acoplamiento disminuyó después de la inducción del estrés psicosocial.

En conjunto, los resultados destacaron: 1) la relevancia de la ansiedad dependiente del estrés para comprender los efectos del estrés social sobre el control atencional. La experiencia subjetiva de la ansiedad es aparentemente más relevante en esta temporalidad que la respuesta fisiológica, que estaría jugando un papel indirecto al afectar la percepción del estado corporal actual, 2) las estrategias cognitivas diferenciales y los mecanismos neurales a través del cual los sujetos estresados pueden lograr la tarea. Tales observaciones podrían tener importantes implicaciones en el diseño de intervenciones terapéuticas para tratar el estrés social y el estrés en general.

Outline

The current thesis consists of 5 chapters. The first chapter presents a general introduction and background regarding the field of study. At the end, the goals and hypothesis are explicitly exposed. The second chapter includes the part of the thesis associated with the goal and hypothesis 1, and contains information that is expected to be published as the paper 1. The third chapter includes the part of the thesis associated with the goal and hypothesis 2, and contains information that is expected to be published as the paper 2. The chapter 4 is a general discussion about the chapters 1, 2 and 3. Finally, chapter 5 reports on recent findings that are yet in a preliminary form and do not directly link with the preceding findings in chapter 2 and 3, and therefore has been termed ‘Coda’, to indicate that it is related but do not precisely follows the general idea of the thesis.

Chapter 1: General Introduction

The stress response has been present probably accompanying the evolution of the nervous system from the very beginning, however, it was not until the middle of the last century (1936) when the Austro-Hungarian physician Hans Selye defined it as “The non-specific response of the body to any demand made upon it”[1]. Selye showed that the exposure to different types of noxious

physical or emotional stimuli (as deafening noise, extremes of heat or cold or even to perpetual frustrating situations), induced an inter-related adaptive response termed ‘General adaptation syndrome’. It develops in three stages; the alarm stage, in which all the system turns on deciding between ‘Fight or flight’; the resistance stage, given by the fading of the response as a sign of adaptation and the exhaustion stage, in which the finite adaptation energy runs out and the body becomes the victim of constant wear and tear as a product of a stronger and longer presence of the noxious stimuli [2].

As was exposed by Hans Seyle, living organisms maintain a dynamic internal equilibrium (homeostasis), which is constantly challenged by physical or psychological factors (stressors)[3,4]. Thereby, *Stress* can be defined as the state in which the homeostasis is threatened or perceived to be so [5]. Two types of stress can be recognized in humans, the “good stress” generally refers to those experiences that a person can master and which leave a sense of exhilaration and accomplishment, and the “bad stress” refers to experiences where the sense of control is lacking, they are physically dangerous and exhausting, and involve emotional draining [6].

Whether stress is ‘good’ or ‘bad’ it will be strongly determined by the individual’s perception of stress. This in turn should be affected by major life events as trauma or abuse, generally produced by the environmental circumstances of home, work or neighborhood, and by individual differences given by genetic, epigenetic and developmental features. Once a particular experience is perceived as stressful, both a behavioral and physiological response is triggered as a mechanism of adaptation (Allostasis). If those mechanisms are de-regulated, the body may suffer the tear and wear of it (Allostatic load), leading to disease if the stress persist over time (Figure 1) [7]

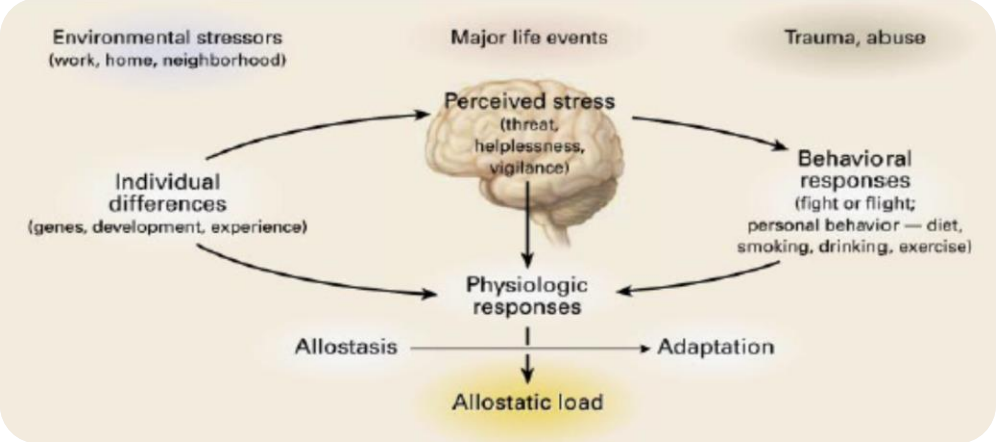

Figure 1 l Schematic representation of the different elements which affect and are related with stress perception. Taken from McEwen [7]

Nowadays stress numbers are disturbing. This year, the statistic brain research institute from New York showed that the 77% of U.S population regularly experiences physical symptoms (fatigue, 51%) caused by stress, the 76% experiences psychological symptoms (irritability or anger, 50%) caused by it and the 48% of the population feels that stress has increased in the last 5 years. In addition, employers reported an annual cost of 300 billion dollars in stress-related health care and missed work (Statistic Brain Research Institute, American Institute of Stress, NY, 2017). In Chile the numbers are not more encouraging. A survey performed in 2014 showed that 39% of the population feels acutely stressed, 26% feels chronically stressed and only 35% feels relaxed (Chile 3D-2013, GFK Adimark. 2014).

Certainly, stress is a response that has allowed the evolution and adaptation of most living beings. However, there is a thin line between adaptation and the de-regulation of the system, leading to disease [4] or cognitive impairments [8]. Moreover, it has been well demonstrated how stress affects the new generations either via direct transfer of the stress hormones and molecules from the stressed mother to the fetus [9] or through lifestyle-related epigenetic modifications on the gametes [10,11].

1.1 Stress induces a highly coordinated physiological response

Stressful experiences induce a body state characterized by changes at the physiological and psychological levels. In humans, the brain is the responsible for the orchestration of this response [12] because it: (1) determines what is threatening or not, and therefore, what is stressful to the subject; (2) regulates the physiological, cognitive, emotional and behavioral response from the individual against a specific experience; (3) can change structurally and physiologically in an adaptive or maladaptive manner as a result of coping with the stressful experience; and (4) processes external sensory inputs from the environment and internal inputs from the body[13]. Notably, the limbic system, including the amygdala, hippocampus and the prefrontal cortex (PFC), has been considered the specific modulator of those processes[14].

The activation of the Hypothalamic-Pituitary-Adrenal-Axis (HPAA) and the Autonomic Nervous System (ANS) are the principal means via which the brain influences all the organs in response to stress [15]. The response has a specific temporality that begins with the rapid activation and release of norepinephrine (NE) from the ANS (within seconds), followed by the release of cortisol, the main effector of the HPAA (within few minutes)[16,17]

The HPAA response begins with the activation of the paraventricular nucleus of the hypothalamus, which releases the corticotrophin releasing factor (CRF). The CRF travels through the portal system and reaches the anterior pituitary promoting the release of corticotrophin hormone (ACTH). Finally, the ACTH enters the bloodstream and acts over the cortex of the adrenal gland, which secretes glucocorticoids (GC)[15]. GCs are the principal effectors of the axis, regulating the homeostasis of the whole body. Interestingly, GCs can enter the cell through diffusion, binding to the glucorticoids (GR) and mineralocorticoids (MR) receptors. Attached to any of the receptors, the GCs translocate into the nucleus inducing changes in the gene expression. Given that the GR and MR have different affinity to cortisol (MR has higher affinity), each cell can sense and respond differently to a broad spectrum of GC concentrations [4].

The HPAA has a feedback system at each level. Both the hypothalamus and anterior pituitary have receptors for CRH, ACTH and GC. Depending on the concentration of each factor, the system can down-regulate itself turning off the response [18]. Additionally, it has been shown that the amygdala can release an amygdaloid CRF which can also promote the stress response and regulate the HPAA [19].

The ANS responds rapidly to stressors controlling cardiovascular, respiratory and endocrine function among other things, via sympathetic and parasympathetic systems [15]. The sympathetic innervation of peripheral organs comes from efferent preganglionic fibers (cholinergic), whose cell bodies lie in the spinal cord. Heart, smooth muscle and other peripheral organs are innervated by post ganglionic neurons (noradrenergic), which are synapsed by preganglionic neurons in the sympathetic ganglia. The ANS activation is given primarily by noradrenergic cell groups from the Locus Ceruleus (LC) and other regions of the pons and medulla. There is also humoral sympathetic contribution through the activation of the adrenal medulla which contributes with the circulating NE[20].

Interestingly, there are reciprocal connections between CRF and NE neurons allowing the stimulation between each other, mostly via alpha noradrenergic receptors. In addition, it has been shown that CRF and NE neurons from the LC, interact with the hippocampus, amygdala and mesocorticolimbic system[20].

1.2 From stress in animals to social stress in humans

The physiological stress response described above is one of the principal survival responses that have allowed us to face, for instance, a dangerous situation, a predator or natural disasters[6]. However, there are situations in which the physical integrity and survival are not threatened. Nonetheless they: (1) are perceived as novel, uncontrollable, unpredictable, or ambivalent, and in which the individual anticipates psychological consequences, and (2) are produced by social stressors, like a social evaluative threat, an economic reward situation or even by thoughts of the

personal life. This type of situations or experiences are responsible for the psychosocial stress (PSS)[21].

In 2004 Dickerson and Kemeny [22] did a meta-analysis reviewing 208 different PSS laboratory protocols in order to study which were the necessary elements for eliciting the activation of the response. They found that a mixture of a public speech and a demanding cognitive task lead to a maximal HPAA activation. They also showed that a social evaluative threat and uncontrollability were key concepts at the moment of a suitable social stress induction. The social stress protocol that better fits with the previously exposed requirements is the Trier Social Stress Test (TSST).

The TSST was designed to induce PSS and consists of 4 stages: (1) Anticipation, in which the participants are asked to prepare a five minutes speech about their personal skills for a sham work; (2) Speech, here the participants have to face an expressionless committee of experts and give the speech during 5 minutes. If the speech is shorter, the committee can either ask the participant to continue or make some questions; (3) Mathematical task, the participants have to subtract 13 to 1000 consecutively until 0 during 5 minutes. Every time the participant fails, they have to start again from the beginning; (4) Rest, finally the participants can rest and the committee adopts a friendly attitude [23].

It has been shown that the TSST induces an increase of plasma and salivary cortisol[24] and of the ACTH [17] (it is worth to mention that salivary cortisol peak has a delay of approximately 10 minutes). Both changes reveal an activation of the HPAA. On the other hand, the TSST also induces the activation of the ANS, observed through an increase of the heart rate, the skin conductance, α-amylase[25] and the release of epinephrine and nor-epinephrine[17]. Notably, the mentioned parameter does not change in the same way in subjects with PFC damage[26], highlighting the relevance of the psychological perception of stress for the physiological activation (Figure 1).

At the psychological level, the TSST induces an increase of the negative affect measured through the Positive and Negative Affect Schedule (PANAS) [27], a decrease of calmness and increase of bad mood, measured through the Multidimensional Mood State Questionnaire (MDBF)[28], an increase of the perceived stress measured through analogues scales [25] and an increase of the state of anxiety, using the State and Trait Anxiety Inventory (STAI)[29].

It is worth to mention that there are some gender differences associated to the TSST reactivity. For instance, younger men have higher release of ACTH just after the TSST than women[24], however no differences between gender were found on cortisol and heart rate reactivity[30]. Psychologically, it was shown that the perceived stressfulness was higher in women than in men[24]. In the same line, women reported more irritability, fear, confusion and less happiness than men just after the TSST[30]. Finally, it was also shown that the menstrual cycle phase and the use of oral contraceptive can also affect the reactivity of the HPAA in women. Women in luteal phase had a higher cortisol reactivity than those in follicular phase or those using oral contraceptives[31].

1.3 Social stress induces behavioral/cognitive changes

The TSST has been one of the main protocols to evaluate the effects of PSS over cognition. Also, some research uses students with high academic pressure as study models (Academic social stress (AS)). The next section will be focused on PSS and cognition using both the TSST and AS study models.

Research of PSS and cognition have focused mainly on attention and memory. Olver, et al. [32] developed a three phase protocol including a baseline, stress (just after stress induction) and post stress (after 30 minutes of debriefing) cognitive measurements using the TSST as stressor. They found that spatial memory (spatial 2-back paradigm) was negatively affected after the stress induction; however, it recovers after the debriefing period. Attention (choice reaction time) on contrary was affected after the TSST but remained impaired after the debriefing period. The authors highlighted that the impaired attention after stress and the debriefing period may be due

to an increased stress-related noradrenalin release (remains high after 30 minutes) in the PFA, resulting in a saturation of the alpha-1 receptor and leading to attentional impairments. However, in this study no correlation analysis between the cognitive impairments and the HPAA or ANS reactivity, or with any psychological response was performed.

In accordance, Schoofs, et al. [27] demonstrated that working memory performance is negatively affected after the TSST compared to a control protocol. The stressed group was consistently impaired in the 2-back and 3-back levels but only in the first of the four blocks of task. Interestingly, the effects were correlated with the increase of cortisol, but not with ANS response nor with the positive or negative mood. They argued that stress-related cortisol release may affect working memory by its own. In contrast, it was shown that the effects of cortisol over memory are only present when individuals are psychologically stressed[33]. Interestingly, the effects of stress over memory do not only require the HPAA activation, but also an activation of the ANS. Using an n-back paradigm, Elzinga and Roelofs [34], showed that the negative effects of the TSST over working memory were only present at the moment of maximal HPAA and ANS activation. Here the results were also discussed in terms of PFC impairments, in which the interaction between NE and the basolateral complex of the amygdala (BLA) affects how the PFC regulates the effects of glucocorticoids over memory.

Concerning attention, an fMRI study on students with academic stress showed that attentional control was negatively correlated with the perceived stress, and with the functional connectivity between PFC and other brain regions as amygdala and posterior parietal cortex [35]. The authors highlight as a caveat the lack of physiological measurements as cortisol or any ANS indicator. In accordance, Sänger, et al. [36], used a change detection task in which the subjects had to indicate which of two presented bars changed its orientation respect the previously shown slide. They showed that there was a negative correlation between cortisol reactivity and the fronto-central P300 ERP component amplitude only in the stressed participants. Interestingly, they related the

fronto-central P300 component with the integrity of the frontal lobe functioning and focal attention. Unfortunately, no psychological measurements was performed.

In stroke patients it was shown that cortisol reactivity after the TSST was lower as the mPFC damage was more extended. Additionally, participants showed heightened self-reported stress and altered autonomic control of the heart rate in response to the TSST [26]. The latter findings support the idea that the mPFC is involved in the regulation of both the physiological and psychological response against the TSST.

Using attentional tasks with emotional stimuli, it was shown that cortisol reactivity to the TSST directly modulates the attentional bias to the negative stimuli. Subjects with high cortisol reactivity take shorter to name the color of an image with negative emotional content compared to an image with neutral content. Interestingly, after the stress induction the avoidant attitude turns into a vigilant one, in which the participants take more time in color-naming negative emotional content images compared with neutral. Despite the fact that they also found an increase of anxiety after the TSST, there was no correlation between it and cortisol, heart rate or attentional bias [37]. Similar attentional bias was found in highly anxious individuals [38]. All together this raise the idea that anxiety by itself might alter or redirect the attention to threatening or emotional stimuli.

Precisely, the idea of anxiety affecting attention was developed by Eysenck, et al. [39] with the 'Attentional Control Theory'. Firstly, they highlighted the definition of anxiety as 'A state in which an individual is unable to instigate a clear pattern of behavior to remove or alter the event/ object/interpretation that is threatening an existing goal'[40]. Thereby, behavior will be affected by the anxiety causing experience, independent of the particular task. The Attentional Control Theory then proposed that: 'anxiety increases the allocation of attention to threat-related events. More specifically, an anxious subject allocates attentional resources preferentially to threat-related stimuli, whether internal (e.g., worrisome thoughts) or external (e.g., threatening task-irrelevant distractors)'. In the case of PSS-related anxiety, the 'threatening' stimulus can be the PSS

experience itself, favoring the disruptions of goal-directed tasks temporally close to the PSS experience.

Despite strong evidence showing that acute *psychosocial stress* alters attention, it is still unclear whether those effects are more related to the psychological experience, the physiological response or a mixture of both. The first research question to be addressed in this thesis will try to disentangle the relative influence of these factors.

1.4 From physiology and behavior to the brain activity

With the emerging of Neuroscience, many behavioral studies are incorporating a measure of brain activity on their analysis, using either imaging techniques as fMRI or through the study of the electric or magnetic activity in the scalp with the electroencephalography (EEG) or magnetoencephalography (MEG), respectively. Due to the high temporal resolution of both EEG and MEG, they allow the study of brain activity associated to a specific and fast event, as for instance a fast attentional task. Two main type of analysis can be implemented, the Event Related Potentials (ERP) and the Oscillatory Activity. During the last 20 years an exponentially increasing number of works have related oscillatory activity at different frequencies with almost the complete spectrum of cognitive processes, including memory, attention and consciousness[41-43].

In general, neurons can fire action potentials in a regular manner inducing rhythmic activation of output synapses and generating oscillatory activity in all postsynaptic target cells. If several neurons fire action potentials regularly, the oscillatory output signal will be amplified at such level to be measured with EEG [44].

The oscillatory activity can be studied at very low frequencies, including delta (1-4 Hz) and theta (4-8 Hz) range, low frequencies, including alpha (8-12 Hz) range and high frequencies, including beta (12-30 Hz) and gamma (>30 Hz) range. Different frequencies have been related with different cognitive processes or at least with part of them. It is likely that cognition emerges as a

coordination between different brain regions, involving different frequencies[41,45]. According with the research question 1, the current section will be focused on the evidences relating oscillatory activity with attention.

Attention is constantly being balanced through a continuous interplay between a top-down network (goal-directed attention) and bottom-up network (stimulus-driven attention)[46]. The first one is supposed to be more dependent of endogenous components, while the second is limited by exogenous ones [47]. It has been shown that tasks involving mostly top-down processing are commanded by communication between fronto-parietal regions at beta frequency (13-30 Hz) meanwhile, tasks involving unexpected bottom-up changes of the stimulus are related with increased gamma oscillatory activity between the same regions [48]. The authors suggested that top-down communication via lower frequency oscillations results in a 'Broadcast' of top-down signals on a larger anatomical scale. They also added that there is a more robust mechanism for longer range coupling because it is less sensitive to spike timing delays. On the other hand, higher frequency coupling might enhance the local representations of the stimulus which then is passed forward from parietal to frontal cortices.

Following the same idea, Bastos, et al. [49] did a hierarchical categorization of different areas of the visual system (primary cortex, dorsal and ventral stream, frontal areas) according with the procedures described in Markov, et al. [50]. They studied the feed-back and feed-forward influences between different pairs of areas while the monkey was performing a visual attentional task. Similarly, they found that feed-back influences were carried out by beta frequency synchronization and feed-forward influences by gamma frequency.

Beyond visual attentional tasks, increased beta activity has also been related with top-down control deployment to maintain an internal cognitive state. Thus, it should be expected an increase in beta activity if the system has to actively maintain the current cognitive set and a beta band decrease if the setting is disrupted by an unexpected event[47]. Moreover, the same authors

proposed that beta band activity could also increase during events of sensory mental imagery, however the idea has not been experimentally tested.

1.5 Oscillatory activity in stress related states

There are few works designed to study the oscillatory activity during states of PSS and most of them are focused on alpha band activity since it has been related with cognitive inactivity, suppression of sensory incoming information [51] and increased inward attention [52].

Students with high academic pressure showed an asymmetrical decrease of alpha activity in the right PFC, which based on the mentioned inhibitory role of alpha band, can be interpreted as an increased right prefrontal activity [53]. This results are in good agreement with the idea that left PFC is associated with positive moods, while right PFC with negative ones [54].

Other study has shown that a higher baseline anxiety state correlates with an increase of alpha oscillatory activity (8-12 Hz) and a decrease of delta activity (less than 4 Hz)[55]. Alpha power is stronger when attention is directed internally, towards mental imagery or thoughts[56]. These results suggest that an elevated state of anxiety may induce an attentional change from the current goal-directed tasks to task-irrelevant intrusive threat-related contents.

In studies involving situations with anxious apprehension, it was shown that after a negative monetary feedback there was an increase of beta-delta coupling[57]. Similar results were also observed in frontal regions during speech anticipation [58] and in a paradigm of exogenous cortisol intake [59]. An interesting idea discussed by Miskovic, et al. [58] is the possibility that the beta-delta frequency coupling was related with an increased communication between cortical-subcortical regions.

Beta band activity has been related with increased top-down task monitoring and with higher anxiety levels, also it has been proposed to control the maintaining of internal cognitive states. However, the beta band activity has never been assessed (neither has gamma band) while a

previously stressed subject is performing an attentional task. Moreover, the relationship between the psychological, physiological and cognitive effects of PSS and the oscillatory bran activity has yet to be explored. The second research issue to be addressed in this thesis will try to characterize the oscillatory response associated to PSS.

1.7 Summary and proposal

PSS situations are characterized by the induction of an increased response of the HPAA and the ANS, together with changes in the psychological perception of the current state. At the behavioral/cognitive level, it is well described that PSS entails failures to perform working memory and attentional tasks. Interestingly, the state of anxiety, which is one of the PSS psychological outcomes, was proposed as a key element affecting attention. Thereby, high levels of anxiety may redirect attention to threatening stimuli or situations which are often out of the current task, affecting the goal-directed behavior. One way to measure the relationship between top-down v/s bottom-up influences during a particular task is through the study of oscillatory brain activity, where beta band activity (13-30 Hz) is strongly related with top-down feedback processing while activity in the gamma range (>30 Hz) relates with bottom-up feed forward. As a consequence, an individual requiring higher top-down control to perform a task, should have increased beta band activity, while, individuals performing a task, exclusively driven by the intrinsic features of the task, should exhibit higher activity in the gamma range.

In the field of PSS, it is still unclear how the physiological response (HPAA, ANS) and the stress dependent state of anxiety might interact in order to affect each other and behavior. Moreover, the neural correlates of PSS have been poorly understood until date. Particularly, the oscillatory activity has never been assessed in the context of an attentional task.

We propose a stress induction (TSST) procedure flanked by two attentional task and resting state periods (PRE-POST). Electrical brain activity (EEG) and heart rate (EKG) will be measured during the complete session. In addition, five saliva samples will be taken and the anxiety level will be

assessed with the STAI questionnaire before and after the stress induction. Half of the participants will be part of the experimental group (stress induction, TSST). The other half will be subjected to a Control protocol including exactly the same measurements with a modified version of the TSST, consisting of the same mental and physical effort but without the social evaluative component.

After the experiment we will have: (1) the state of anxiety, reactivity to cortisol and heart rate response associated to the PSS induction, in addition to the performance in the attentional task after the TSST compared to baseline; (2) the continuous brain activity during the attentional task.

Our experimental design may provide new and valuable insight to understand how PSS is perceived and affects goal-directed behavior. Given the high prevalence of PSS in modern society and its role as a main etiologic factors for psychiatric disease (as depression and bipolar disorder), our research has an important medical and therapeutic value since it could increases our tools to deal with and treat stress and stress related states.

1.8 General goal

To study the physiological, psychological and neurophysiological mechanisms via which PSS affects goal-directed attention.

1.9 Specific goals

a. To study the interaction between heart rate, salivary cortisol and the self-reported state of anxiety with performance over an attentional task.
b. To explore the neural correlates of the physiological and psychological state induced by PSS during an attentional task.

1.10 General hypothesis

The PSS induces a state characterized by a physiological and psychological response. In particular, the psychological perception of the PSS experience as stressful (in a process that can be affected by the physiological response (HPAA, ANS)), provokes the increase of the self-reported state of anxiety. This PSS-dependent psychological state triggers an attentional shift away from the current task, resulting in worse accuracy in an attentional task. Despite those effects, stressed subjects can re-allocate attention to the current task, specifically by increasing the top-down influences, reflected in the increase of fronto-parietal Beta band oscillatory activity.

1.11 Specific hypothesis

a. The PSS-dependent increase of anxiety modulates behavioral performance by allocating attention out of the current task the subject is doing. In turn, the physiological stress-related response given by the activation of the ANS and the HPAA favors the increase of the self-reported anxiety, but only if the situation was already perceived psychologically as stressful.
b. The PSS protocol triggers an attentional shift away from the current task, which is observed by the increase of alpha oscillation during the resting state previous to the task. However, stressed subjects redirect attention to the task by increasing top-down monitoring mechanisms, which are observed as an increase of oscillatory activity at Beta frequencies.

Chapter 2: Relationship between heart rate, self-reported state of anxiety, salivary cortisol and attentional performance

Immediate effects of psychosocial stress over attention depend on subjective experience and not directly on stress-related physiological changes

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2.1 Abstract

Acute psychosocial stress is associated with physiological, subjective and cognitive changes. In particular, attention, considered one of the main processes on driving cognition, has been separately related with different stress outcomes such as anxiety, cortisol levels and autonomic response. Nonetheless, the specific contribution and association between those outcomes and attention is still not fully understood. In order to study this association, 42 male participants performed an attentional task just before and immediately after being exposed to an experimental treatment, designed to induce psychosocial stress (Trier Social Stress Test; TSST), or to a

matched no-stress control condition. Salivary cortisol concentrations, heart rate, and the self-reported anxiety were measured to assess the physiological response to stress and the subjective experience during the protocol respectively. As expected, psychosocial stress induced an increase in heart rate, salivary cortisol and a state of anxiety. The behavioral analysis revealed that the control group improved their performance in the attentional task after the protocol, while the TSST group showed no changes. Moreover, after dividing the stress group into those with high and low anxiety, we observed that participants of the high anxiety group not only failed to improve their performance but rather worsened. Finally, after the testing of different single-level mediation models, we found that anxiety state is enough to explain the changes on attention, but also can acts as a mediator between the effects of heart rate and cortisol and attention performance. Our results suggest that the immediate effects of acute psychosocial stress over attention are highly dependent on the participant's subjective experience, which in turn is reciprocally affected and can mediate the stress-related physiological changes.

Psychosocial stress, Attentional control, Single-level model, Salivary cortisol, Heart rate, anxiety, Trier Social Stress Test -TSST.

2.2 Introduction

Many daily life experiences are characterized by unpredictable and uncontrollable situations in which the individual anticipates psychological consequences, generally produced by social factors. Those situations are responsible for psychosocial stress [21]. The psychosocial stress response includes a physiological component characterized by the activation of the hypothalamic pituitary adrenal axis (HPAA) and the autonomic nervous system (ANS), resulting among other things in an increased release of cortisol and elevated heart rate, respectively. Changes at the

physiological level have been strongly related with cognitive performance, among which highlights, working memory [60], memory retrieval [61] and selective attention [62].

Besides the physiological response, social stress is also associated with a psychological component such as, an emotional experience/perception of stress or the increase of the self-reported state of anxiety[25]. In particular, the state of anxiety can be conceptualized as "the state in which an individual is unable to instigate a clear pattern of behavior to remove or alter the event/object/interpretation that is threatening an existing goal''[40]. Interestingly, as individuals select the suitable information to carry out a specific task according to their goals, process referred as attentional control [63] [64,65], higher levels of anxiety may affect the attentional control process. Concordantly, Eysenck, et al. [39] developed "The Attentional Control Theory", where they discussed how anxiety may affects attentional control and cognitive performance. In line with this, several works have addressed the effects of anxiety over cognition, for instance, Harris and Cumming [66] demonstrated that individuals with high levels of anxiety performed worse in a task involving prospective memory, a type of task that involves the recalling of a formed planned intention at some future point in time. Even more, it has been suggested that elevated levels of anxiety induces impairments on task switching, reflected as more errors and longer time to complete the task [67].

As shown before, both the physiological and psychological response are separately implicated on cognitive/behavioral changes. However, during a psychosocial stress situations both responses interact exerting a mixture of outcomes that can vary depending on the type, timing and severity of the stressor and task [68]. One of the most common experimental settings to induce stress is the "Trier Social Stress Test" (TSST), which includes a job interview and a mathematical task in front of an expressionless committee [23]. Studies using the TSST have revealed some of the impairments that social stress may produce at cognitive levels, including working memory [27], attention [32], dual-task [28] and over second order processes as metacognition [69].

Despite most of the studies using the TSST report a physiological and psychological response associated with it, the relationship between both responses in the context of a cognitive task have not been fully understood yet. Interestingly, a recent study [70] proposed a pharmacological designed to study the effects of the TSST over the psychological response in the absent of the physiological one. The results showed that suppressing the physiological response did not impact the emotional response to the stressor, giving rise to the question if the physiological stress response has a real contribution on the emotional experience. The latter study however, did not include a cognitive task. Similar caveats can be found in other studies in which there is a relationship between psychological perception of stress and cognition (Task-switching), but without considering a physiological component [35] or works showing a strong dependency between the physiological response (Cortisol reactivity) and the cognitive performance (Selective attention), but without assessing the role of the psychological stress response on it [37].

There are strong evidences linking both the physiological and psychological response related to social stress with cognition. However, the relationship between those variables in relation with a specific behavioral/cognitive outcome is still not understood. Moreover, in the field of attention, there are evidences that posit the anxiety as a relevant factor, however, few studies relate this psychological state (anxiety) with stress dependent cognitive changes.

Regarding the previous ideas, we aimed to study -under the same experimental context- the relationship between physiological response, the psychological experience and the cognitive performance associated to Psychosocial Stress. Specifically, we studied the relation between cortisol reactivity, heart rate, self-report state of anxiety and performance over an attentional task in individuals that were exposed to a Stress protocol (TSST) or a control one.

We hypothesize that (1) psychosocial stress will impairs the individual's performance over an attentional task, (2) the physiological response – measured through salivary cortisol concentration and heart rate - will be positively correlated with the self-reported state of anxiety and (3) In

accordance to the Attentional Control Theory, the psychosocial-stress-dependent increase of the state of anxiety -and not the physiological response- will mediates the participant's performance impairments in the attentional task.

2.3 Methods

2.3.1 Participants

Forty-nine (49) male participants were recruited and assigned to the stress (n = 24) or control (n = 25) group. Seven of these participants were excluded (4 controls) due to failure to follow the instructions during the experiment or problems with the data acquisition. The resulting 42 healthy non-medicated volunteers (mean age ± SD = 25 ± 3.8 years) were recorded between 12.00-14.30 hours and were instructed to avoid smoking cigarettes, drinking coffee or tea and eating food including bubble gum 2 hours before the experiment. All participants gave their written informed consent prior to the study in accordance with the guidelines of the Bioethics Committee of the Faculty of Medicine at Pontificia Universidad Católica de Chile, which approved the research protocol.

2.3.2 Procedures

Participants underwent electroencephalogram (EEG), electrocardiogram (EKG) and electrooculogram (EOG) electrodes placement (EEG and EOG data are not shown).The experiment began with a first application of an attentional task (pre-condition) flanked by 90 seconds of resting state recording. After this, the TSST or the control protocol was conducted, finishing with a second application of the same flanked attentional task (post-condition) (Figure 1). In addition to attentional performance, the heart response and salivary cortisol were monitored to assess the activation of both the ANS and the HPAA, respectively. The electrocardiogram was registered as an external electrode in the EEG recording setup, using a BioSemi ActiveTwo ® system, constantly throughout the experiment. Salivary samples for cortisol measurement were taken in 5 different moments (Section 2.5). Finally, participants were asked to fill the state of

anxiety scale just before and after the TSST/Control protocol. The number of interruptions (Cortisol sampling – anxiety scale) during the procedure were kept to a minimum in order to maintain the participant's involvement and the natural physiological and subjective states as induced by the protocol.

2.3.3 Stress induction and Control protocol

Psychosocial stress was induced with a EEG-adapted version of the Trier Social Stress Test [23]. The protocol consisted of an interview simulation in which the participants must expose their personal attributes for applying to a fictional job (5 minutes) in front of three people acting as referees (serious and in an expressionless attitude) and a video camera, followed by an arithmetic task (5 minutes), consisting on subtracting 1000 minus 13 consecutively until 0, every time that the participant failed, he was told by one of the referees to start again. The protocol follows the Kirschbaum, et al. [23] guidelines, with the exception that the referees were the ones who entered the room with the participant prepared for the evaluation inside.

The control protocol included the same procedures but in front of the experimenter (good mood and friendly attitude) instead of people acting as referees. The same physical and mental effort was induced but without the psychosocial stress component. After the protocol, participants were informed that no judgments were made about their presentation and that the camera was turned off.

2.3.4 Attentional task

An adaptation of a task-switching paradigm by Liston, et al. [71] was used as the attentional task. Two circles, each subtending 4,6° of the visual space and equidistant of the monitor center, were presented for 700 ms. Each circle was red or green and moved upward or downward. In between both there was a letter "M" for movement or "C" for colour. The subject was instructed to choose the green circle when the letter was the "C" and the upward circle when the letter was the "M". Each trial began with a central white fixation cross of variable duration (600-1000 ms). The

complete trial involved the central fixation followed by 700 ms of the colored and mobile circles. Accuracy and reaction times were recorded on a trial by trial basis by using the Psychopy software [72]. Participants were trained with three blocks of 12 trials, which corresponded to color, movement and color/movement discrimination. The experiment involved four blocks of 64 trials separated by 1 minute rest between blocks.

We modified the task-switching paradigm [71] by increasing the task speed on approximately 2 times. This change was made in order to reach a mean of accuracy of 70% of correct answers, by turning the task substantially more difficult than previously reported.

2.3.5 Physiological measurements

EKG activity was monitored during the sessions using 2 external electrodes (BioSemi ActiveTwo ®) positioned 2 fingers under the left collarbone and over the left hip in order to recognize the R peaks on a specific period of time and then to obtain the heart rate of this period. Seven different periods of 90 seconds were used to calculate heart rate; during the four resting state periods (see 2.2) and at the beginning of different Control/TSST tasks (Anticipation-Speech-Math) (Figure 1). We decided to consider only the first 90 seconds of the Control/TSST tasks in order to equate the variability produced by comparing periods with different length. Heart rate was obtained and calculated using custom-made Matlab scripts and Kubios software [73].

In addition to heart response, 1 ml of saliva samples were collected on salivary tubes at five different time points (Figure 1): just before the Control/TSST treatment (Baseline) and after 0 (+ 0), 10 (+ 10), 25 (+ 25) and 40 minutes (+40) of Control/TSST treatment. Immediately after the saliva collection the samples were preserved at -20°C until the analysis. Saliva samples were sent to the Molecular Biology Laboratory of the Universidad de La Frontera, for quantitative determination of cortisol concentration. Salivary concentrations of cortisol were obtained using an enzyme immunoassay commercial kit following the manufacturer's instructions (DRG Salivary Control ELISA Kit, DRG Instruments GmbH, Germany)[69].

2.3.6 Psychological response

The perceived stress scale [74] and trait anxiety scale [75] were applied before any procedure, in order to assess the daily and baseline subjective stress state and the trait of anxiety, respectively. Regarding the psychological experience of our experimental design, participant were asked to complete the state of anxiety scale [75] just after and before the control/TSST protocol (Figure 1). The psychological response associated to our experiment was focused exclusively on the stress dependent self-reported anxiety acquisition.

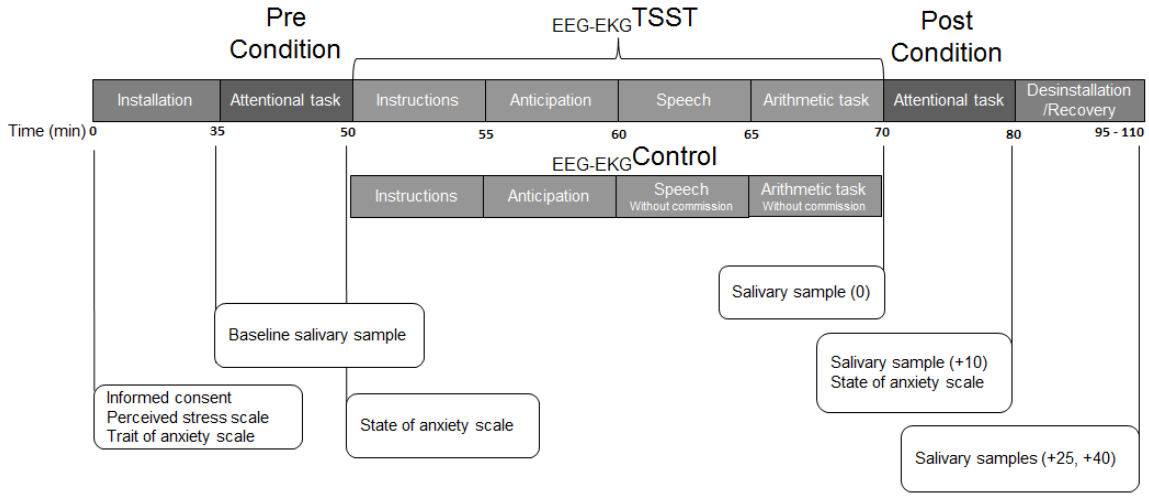

Figure 1l: General experimental protocol. The diagram considers all of the measures collected over time. Numbers indicate time in minutes.

2.3.7 Single-level model

We studied the relationship between salivary cortisol, heart rate, self-reported state of anxiety and attentional performance by testing different single-level models. The model implementation involves the presence of initial (X), outcome (Y) and a mediator (M) variables. In other words, there is an initial independent variable X that can better explains the dependent outcome variable Y when the mediator variable M is present [76]. The mediated effect was studied through the estimation of the regression equation that predicts the outcome Y from the initial variable X

$$Y_i = \beta_0 + \beta_c X_i + r_i \quad (1)$$

and through the estimation of the regression equation predicting the outcome Y from the initial variable X and the mediated variable M

$$Y_i = \beta_0 + \beta_{c'} X_i + \beta_b M_i + r_i \quad (2)$$

The difference between the coefficients associated to the initial variable X, (c – c´) reflects the mediation effects of the variable M over the predicting effects of the variable X over the outcome Y.

Additionally, the mediation effects can also be estimated through the estimation of the regression equation, which predicts the mediator M from the initial variable X

$$M_i = \beta_0 + \beta_a X_i + r_i \quad (3)$$

Taking the product between the regression coefficient of the mediator M explaining the outcome Y (*b*) from equation (2) and the regression coefficient of the initial variable X explaining the mediator M (*a*) from the equation (3) we obtain a second estimate of the mediation effects (*a*b),* [77].

Finally, the mediation estimation can be calculated using

$$ab = c - c' \quad (4)$$

Where *a* is the coefficient of the initial variable X predicting the mediator M, *b* is the coefficient of the mediator M predicting the outcome Y, *c* is the coefficient of the initial variable X predicting the outcome Y and c´ is the coefficient of the initial variable X predicting the outcome Y in presence of the mediator M[1] [76].

[1] The classical mediation requires the fulfillment of 4 conditions: (1) an initial relation between X and Y variables, (2) a relation between X and M variables, (3) a relation between M and Y variables, and (4) a decrease of the relation between X and Y variables in the presence of M.

We used the single-level functions implemented on the Matlab Multilevel Mediation/Moderation Toolbox (M3) v.0.9 (T.D.W. and M.A.L). The toolbox calculates the first-level path coefficients (PC) using standard ordinary least squares multiple regression and determines p values through the bootstrapping approximation (See supplementary method of Wager, et al. [78] for more detail).

This study was designed to find the relation between stress levels -measured through the salivary cortisol, heart rate and the state of anxiety- and the changes on the attentional performance in control and experimental protocol. Given that there is not a clear and defined relationship between those variables and attention, six different models using attentional performance as outcome Y and changing the initial variable X and the mediator M within salivary cortisol, heart rate and the state of anxiety were tested. Therefore, each variable was considered the initial variable X using the other two variables as the mediator. For each model the a, b, c, c´ and ab coefficients were obtained. The significance of each coefficient was tested using bootstrapping (n=10000)

A unique value of each variable was calculated for the analysis, the delta of change (value after each protocol minus baseline) for salivary cortisol, state of anxiety and attentional performance, and the area under the curve for the heart rate response, considering the 42 participants for each model estimation.

2.3.8 Statistical analysis

Differences between groups in their daily perceived stress and trait of anxiety were evaluated through a two-tailed unpaired t test. The effects of the psychosocial stress (TSST) on attentional performance, physiology and the state of anxiety were evaluated with a two-way repeated measures analysis of variance (ANOVA) together with the Bonferroni post-test.

The relation between the delta attentional performance and anxiety was evaluated using Pearson´s correlation. All analyses were performed using GraphPad Prism software (GraphPad Software, San Diego CA, USA). Data in the graphs are presented as the mean ± S.E.M.

2.4 Results

2.4.1 Physiological and psychological stress response.

We measured the heart rate, salivary cortisol and self-reported state of anxiety score in order to corroborate the success of our experimental and control design. Participants reported an increase in their state of anxiety only after the stress protocol (Figure 2A; Group x Time interaction; $F(1,41) = 42.03$; $p<0.001$. Bonferroni post hoc test; Pre-Treatment, $t = 2.122$, $p>0.05$. Post-Treatment, $t = 8.493$, $p<0.001$), while after the control protocol the anxiety was almost unaffected. In addition, we observed a higher heart rate response in the stress group compared to control (Figure 2B; Group x Time interaction; $F(6,240) = 9.77$; $p<0.001$). Bonferroni post hoc test revealed that these differences were significant at the anticipation ($t = 3.014$, $p<0.05$), speech ($t = 5.736$, $p<0.001$) and math ($t = 7.00$, $p<0.001$) time points (Figure 2b). Salivary cortisol also increased significantly after the stress protocol in comparison with the control (Figure 2C; Group x Time interaction; $F(4,160) = 8.793$; $p<0.001$), using Bonferroni post hoc test we found that this increase was specifically significant at the sampling time +10 ($t = 2.909$, $p<0.05$) and +25 ($t = 2.740$, $p<0.05$). Altogether, these results showed that both the stress (TSST) and control protocols were implemented properly.

Lastly, participants reported no differences in relation to their trait of anxiety (Figure S1a, $p>0.05$) or daily levels of subjective perceived stress (Figure S1b, $p>0.05$). Thus, we are able to assume that the results of this report are due to the experimental protocol instead of external daily stress or intrinsic personality features of the participants.

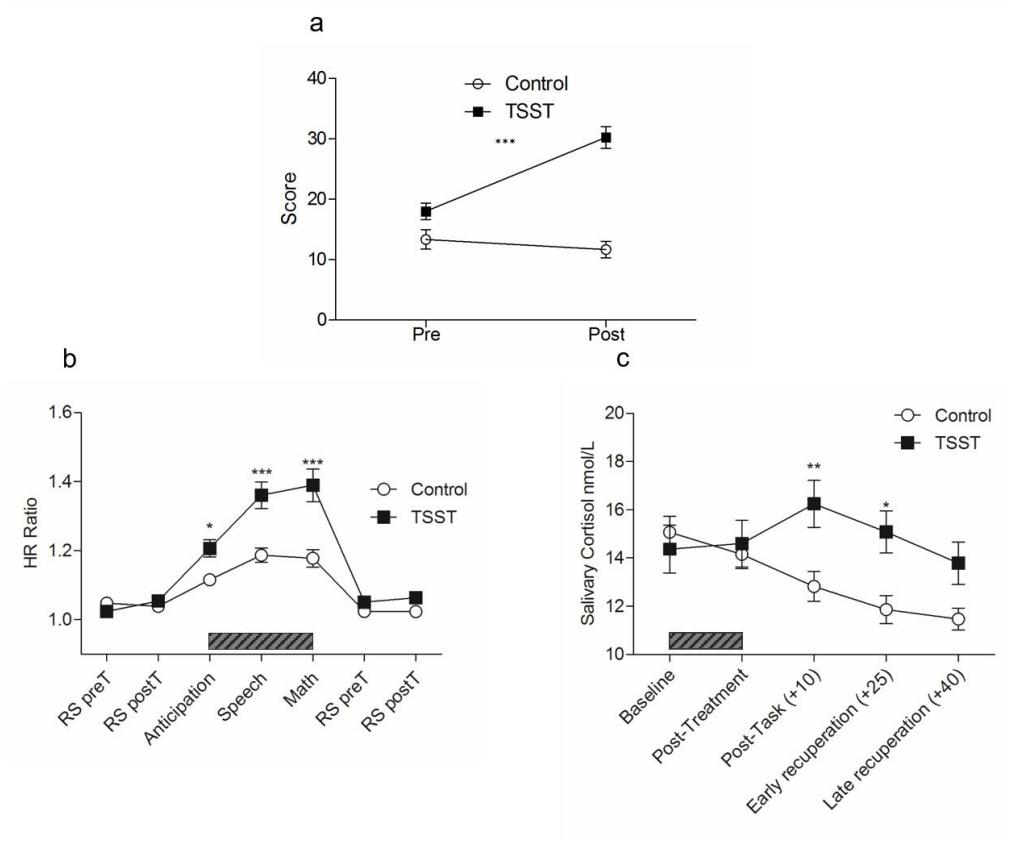

Figure 2| Psychological and physiological stress response against the Trier Social Stress Test (TSST) or Control protocol. a) Self-reported state of anxiety score was calculated by subtracting the baseline measure from the score in the state of anxiety scale after the TSST or control protocol. b) Mean heart rate during two 90 seconds of resting state flanking the baseline attentional task, during 90 seconds of anticipation, speech and math moments of the TSST or control protocol, and during another 90 seconds of resting state flanking the attentional task after treatment (RS preT: Resting state pre-task, RS postT: Resting state post-task). c) Salivary cortisol was measured at five points during the protocol, including the baseline period just after the setup installation, just after the treatment (Time=0), and after 10, 25 and 40 minutes of it. Grey grating bars in b and c indicate either TSST or control protocol. Error bars represent standard errors of the means (SEM). n= 21 participants per group. *P<0.05, **P<0.01, ***P<0.001

2.4.2 Attentional performance improvement is affected by psychosocial stress

We measured accuracy as the number of correct trials and reaction time after and before both protocols. Psychosocial stress impairs the improvement of performance, which could be observed after the control protocol (Figure 3A; Group x Time interaction; $F(1,40) = 5.870$; $p<0.05$). However, a similar reduction in reaction time for correct answers was observed after both

protocols (Figure 3B; Time x Group interaction; $F(1,40) = 0.002$; $p>0.05$; Time main effects; $F(1,40) = 35.87$; $p<0.001$). Suggesting that the effects of stress over performance were not due to fatigue or a bad disposition for performing the task. Moreover, it can also be suggested that at the moment of a correct answer participants of both groups were equally attentive.

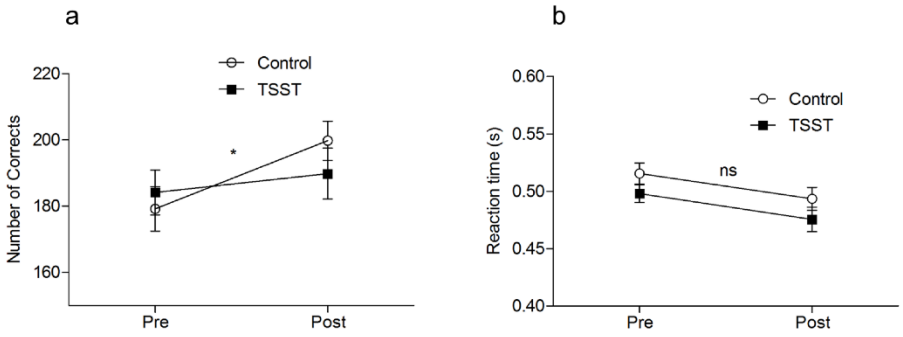

Figure 3l Effects of psychosocial stress on attentional performance. Attentional accuracy as the number of corrects trials (a) and reaction times of those trials (b) were measured during the attentional task before (pre) and after (post) treatment. Error bars represent standard errors of the means (SEM). N=21 per group **P<0.05, ns=non-significant*

2.4.3 Attentional performance differs between individuals with low or high anxiety acquisition

After carry out a Pearson correlation analysis between the change in attentional performance and both the physiological activation and the self-reported state of anxiety, we observed that the only stress outcome which correlates with attentional performance was anxiety, in which as far as anxiety acquisition increased the attentional performance diminished (Table S1, Figure 3a). In order to study deeply the negative relation between attentional performance and anxiety, we separated the TSST group in those with low and high anxiety acquisition levels using the median as a cut point (Figure 3) and then we compared the attentional performance for the 3 groups (Control, Low anxiety TSST and High anxiety TSST). Interestingly, participants with low anxiety

(mean, 5.9 ± 2.002; n=10) acquisition behaved similarly to controls (mean, -0.6 ± 0.89; n =21) (Figure 3b; Group x Time interaction; $F(1,29) = 0.03$; $p>0.05$). On the other hand, in those with high anxiety (mean, 17.91 ± 0.9091; n = 11) acquisition, the attentional performance not only failed to improve, but it was worse than before, as it differed significantly from participants with low anxiety and controls (Figure 3b; Group x Time interaction; $F(1,19) = 9.081$; $p<0.01$). The same procedure was done for high and low cortisol and heart rate responders, however, there were no differences in performance between low and high responders in none of the cases (Figure S2a-b).

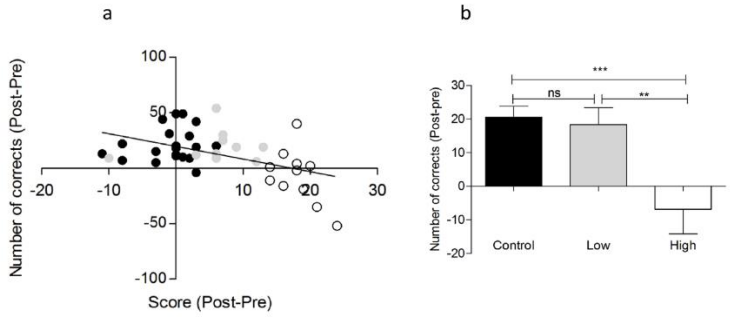

Figure 4l Dependence between attentional performance and the state of anxiety. a) Scatter plot of the whole sample divided between control (black circles), low anxiety acquisition (grey circles) and high anxiety acquisition (white circles) groups. b) Attentional accuracy as the number of correct responses was measured after treatment and corrected using the baseline of controls, low and high anxiety groups (n: Control=21, Low=10, High=11). Error bars represent the standard errors of the mean (SEM). ***P<0.01, ***P<0.001*

2.4.4 Relation between attentional performance and stress response.

We used the Matlab toolbox (M3) to study the relation of different stress variables and attentional performance for the 42 participants. Table 1 shows the path coefficient, standard error and the significance for each different single-level model. We found that anxiety was negatively related with the attentional performance independent of the changes on cortisol (Table 1A, c´, ab) and heart rate (Table 1B, c´, ab), nevertheless, changes on cortisol (Table 1C, a) and in heart rate

(Table 1E, a) were reciprocally related with changes on anxiety (Table 1A, B, a). Interestingly, neither cortisol (Table 1C, c´) nor heart rate (Table 1E, c´) were related with the attentional performance by themselves, however, there was a significant effect in the presence of anxiety as mediator for both (Table 1C, E, ab). Finally, both cortisol and heart rate were mutually related (Table 1D, F, a).

Table 1: Mediation analysis values

Seed /Mediator	*a*	*b*	*c´*	*ab*
A) Anxiety/Cortisol	0.17**	0.24	-1.19**	0.04
B) Anxiety/HR	0.02***	6.76	-1.32**	0.16
C) Cortisol/Anxiety	0.77*	-1.19**	0.24	-0.93*
D) Cortisol/HR	0.04*	-1.34	-0.71	0.01
E) HR/Anxiety	8.04**	-1.31**	6.69	-10.83**
F) HR/Cortisol	3.88*	-0.69	-1.33	-2.83

Each box includes the path coefficient for each single-level model considering the 42 participants. See section 2.7

2.5 Discussion

We proposed an experiment designed to study the relation between three different landmarks of psychosocial stress, such as cortisol, heart rate and anxiety, and performance on an attentional task. Our hypothesis were confirmed and are summarized as follows; (1) acute psychosocial stress induced a failure to improve performance in the attentional task, which was clearly observed after the control protocol (Figure 3); (2) the stress-related physiological response, including both salivary cortisol and heart rate increase were positively correlated with the self-reported state of anxiety (Table S1); (3) the effect of psychosocial stress over attentional control was directly related with the self-reported state of anxiety augmentation (Table 1). In particular, participants of the stress group with high anxiety acquisition exhibited worse performance after the TSST compared to baseline, while those with low anxiety acquisition improved their

performance as much as participants of the control group did (Figure 4). Thereby, our results highlight the relevance of the stress-dependent self-reported state of anxiety over attentional control.

According with the classical proposal of mediation [76], none of the six single level models tested fulfilled the four requirements for establishing mediation (See Method, 2.7). The single-level model which better fitted those requirements was tested using the state of anxiety as the mediator variable and salivary cortisol or heart rate as initial variables (Table 1, C,E), with attentional performance as the outcome variable. Both single models failed on the first requirements due that neither cortisol nor heart rate were statistically related with the attentional performance, despite that, contemporary approaches have proposed that the first requirement is not mandatory, as long as the indirect effect carried by the relation between X and M, and M and Y was present [79]. Nevertheless, this type of relation, labeled indirect effect, is not considered a classic mediation [80]. Accordingly, our analyses suggest that both the salivary cortisol and heart rate are related indirectly with attentional performance through their relationship with the self-reported state of anxiety. Thereby, there is an indirect effect between physiological response and attentional performance through the self-reported state of anxiety. Interestingly, in our single level models (Table 1, C, E) the path coefficients c´ and ab had opposite sign. This phenomenon is referred as inconsistent mediation and it occurs when the pretended mediator variable M acts as a suppressor variable [81] [2].

The observed relationship between the self-reported state of anxiety and the attentional performance is in agreement with Abercrombie, et al. [33], who highlight the relevance of the psychological perception of stress on memory. This hypothesis is also in accordance with Liston,

[2] Both cortisol and heart rate may have a positive relation with attentional performance (Table 1 C, E, c´), however in the presence of anxiety this relationship turns negative (Table 1 C,E, c´+ab). Therefore, an acute increase of the physiological response might have beneficial effects over attention as long as the state of anxiety does not increase. Further research designs to study this relationship are necessary to unravel this issue.

et al. [35] who showed that as far as daily stress perception increased, attentional performance did not. However, other works have not found interaction between stress, psychological states and task performance [27,28].

Analogously, we did not find any direct relation between attentional performance and cortisol response. That could be explained by the fact that the second attentional task was done just after the stress protocol, moment in which salivary cortisol have not reached the peak yet (Figure 2C). The time lag since the stressor occurrence until cortisol exert its effects is about 30 minutes [16]. This issue was discussed by Het, et al. [82] in the field of memory, in which apparently the moment of the treatment application in relation to the course of the study (before learning v/s before retrieval) are substantial over the effect of stress on memory. In agreement, studies in which performance was evaluated just at the moment of the greater cortisol concentration showed a negative correlation between behavioral performance and the cortisol response[27,37,83]. Even more, the effects of psychosocial stress over cognitive functions were not present just after the stress induction but comes gradually on time[84]. In line with our results, some works have not found any correlation between cortisol and performance even though the task was performed in the peak of cortisol concentration[85]. The lack of relationship could also be explained due that at the moment of the second attentional task (Figure 1), the increase in cortisol was not accompanied by an increase in the autonomic response, measured through heart rate increase (Figure 2b,c). This has been described as necessary, at least, for working memory impairments [34]. Apparently, the autonomic stress response is as involved in stress-dependent alterations as the cortisol response [60].

Understanding the interaction between the physiological response itself with the psychological response in the context of psychosocial stress is definitely an issue that have been unraveled until know. Our analysis showed a positive and reciprocal interaction between cortisol reactivity, heart rate increase and anxiety acquisition. The results are in line with Schlotz, et al. [86] which

addressed that the relationship between cortisol and performance was explained by changes in trait anxiety, yielding stronger relationship in subjects with higher trait anxiety. In contrast, high trait anxiety was associated with lower neuroendocrine reactivity during psychosocial stress [87], concluding that higher anxiety was associated with the inability to respond to stress. A recent work, which intended to explore in more detail the relationship between physiological and psychological stress response, concluded that the emotional experience of stress and the physiological stress response were two dissociated systems [70]. This is in contrast with what we found, it is likely that the state of anxiety was a more reliable psychological stress marker than the used by them (Mood). Interestingly, they deeply discussed the possibility that the psychological stress markers used in general could be not the valid and appropriated measures especially at the moment to study their relationship with body response in the context of psychosocial stress.

Theoretically, the dynamic model of stress and sustained attention by Hancock and Warm [88] proposes that stress produces a reduction of available attentional capacity, which can be explained as a psychological adaptability. In this way, there would be a direct relationship among stress, psychological state and attention, which is in agreement with our results and others [35,89]. Interestingly, Eysenck, et al. [39] proposed that elevated states of anxiety affects attentional control, disrupting the top-down/bottom-up balance, thereby attention is strongly allocated to threat related stimulus. Following this idea, it was shown that the state of anxiety was associated with increased attention to threatening images [38,90]. Even more, other study showed that highly anxious participant reorder better images of angry faces (compared to neutral) that have been presented before and then have been put in different locations, realizing an increased emotional memory due to anxiety [91]. Our attentional task lacks in emotional or threating stimulus, however, if we consider the psychosocial stress experience as the threat or challenge, then we should expect an increased attention to this experience even if it occurs in the past. As the attentional capacity is

limited, the allocation of the attentional resources out of the task (external task-irrelevant distractors or internal stress past experience related thoughts) may diminish the possibility of performing well on it.

It is worth to mention that our study was restricted only to men, because it has been well described that the menstrual female cycle and the oral contraceptive can strongly affect the stress neuroendocrine response [31]. Moreover, it has also demonstrated that the relationship between the subjective stress response and cortisol depends on the menstrual cycle phase [92]. For this reason, the results of this work cannot be extended to female population. More works including bigger and more spread samples are needed.

Finally, some works have started to explore the neural correlates associated with stress, anxiety and attentional control. One of those works showed a negative relation between oscillatory brain activity at delta (4-6 Hz) and beta (13-29 Hz) frequencies and the anxiety driven attentional avoidance [93]. Moreover, given that beta frequency activity has been related with goal directed top-down processes [48,49], we should expect an increased beta activity in situation in which increased top down control is required (as after psychosocial stress experience). Further works to explore the relationship between beta activity, anxiety and attentional control are required to improve the understanding of how social stress affects behavior.

2.6 Conclusion

The results of the present study highlight the relevance of the immediate stress-dependent anxiety acquisition to understand the effects of social stressful situations over attentional control. Subjective experience of anxiety is apparently more relevant at this temporality than the physiological stress response, which could be playing only an indirect role by affecting the perception of the current body state. This finding is relevant because it brings back the focus to the subjective psychological experience and not to the stress-induced involuntary physiological changes. Such observation might have important implications for designing therapeutic

interventions to deal with social stress and stress in general. It could be far more beneficial to develop focused strategies to deal with the psychological self-perception in stressful environments than trying to cutback the natural stress-induced physiological reaction which could even be used to the subject's benefit.

2.7 Supplementary Information

Table S1: Correlation between stress outcomes and attentional performance

	Corrects trials	Reaction Time	Anxiety state	Heart rate	Salivary cortisol
Correct trials *R* *p*	1	-	-	-	-
Reaction time *R* *p*	-0.0099 0.9499	1	-	-	-
Anxiety state *R* *p*	-0.4946 0.0007***	-0.0384 0.8139	1	-	-
Heart rate *R* *p*	-0.0708 0.6557	-0.0079 0.9602	0.4016 0.0084**	1	-

Salivary cortisol *R* *p*	-0.1870 0.2356	-0.0272 0,8641	0,3506 0,0228*	0,4074 0,0074**	1

Correct trials: Correct trial post treatment – baseline
Reaction time: Reaction time of correct trials post treatment - baseline
Anxiety state: Self-reported state of anxiety post treatment – baseline.
Salivary cortisol increase: Salivary cortisol (+10) – baseline.
Heart rate response: Area under the curve of the heart rate response.
*N = 42 participants. *P<0.05, **P<0.01, ***P<0.001*

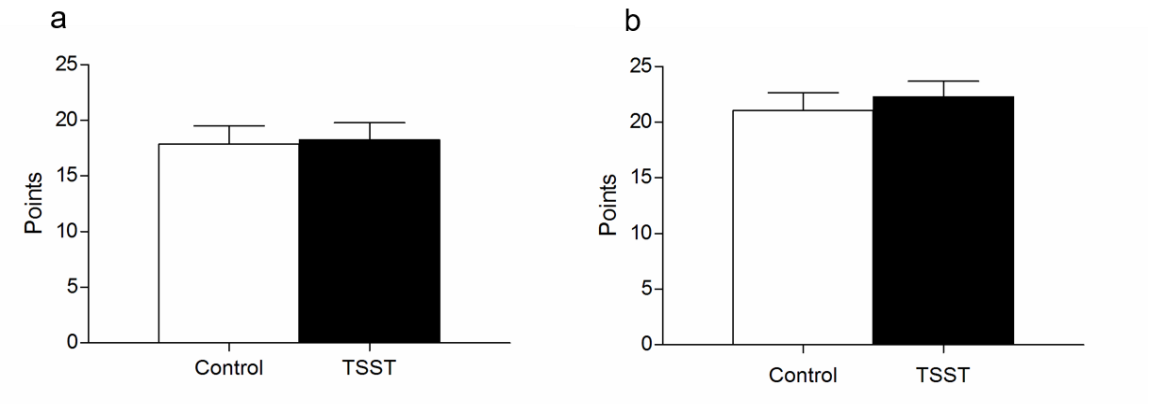

Figure S1l Levels of baseline perceived stress and trait of anxiety. Participants were asked to fill the Trait of Anxiety (a) and the Cohen Perceived Stress (b) questionnaires at the moment of the arrival and accordingly with the experiences they had the last two weeks. Error bars represent the standard errors of the mean (SEM).

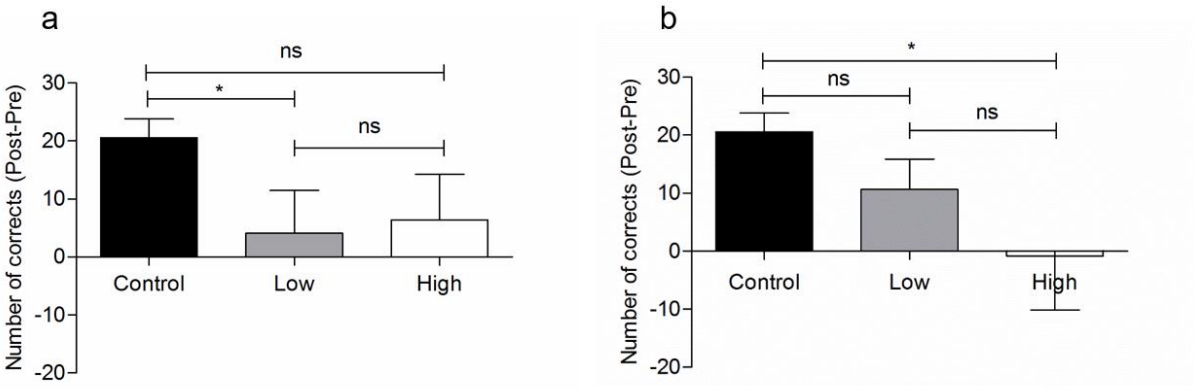

Figure S2l Dependence between attentional performance, heart rate and salivary cortisol. Attentional accuracy as the number of correct responses was measured after treatment and corrected using the baseline of controls, low and high heart rate (a) and cortisol responders (b) (n: Control=21, Low=10, High=11). Error bars represent the standard errors of the mean (SEM). ***P<0.01, ***P<0.001*

2.8 Acknowledgement

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Chapter 3: Electrophysiological mechanism associated to PSS induction during an attentional task and in resting state

Increased beta band activity as a coping mechanism during stress impaired goal-directed attention.

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3.1 Introduction

During daily life, individuals shift their attention between endogenous top-down influences, such as goals, memories and expectations; and exogenous bottom-up influences represented by sensory stimulation[46]. Neuroimaging studies have demonstrated that top-down influences originate from a wide-brain fronto-parietal network controlling endogenous attention and goal-oriented behaviors[46,94]. In addition, electrophysiological studies focused on Local Field Potential (LFP) oscillatory responses, such as power and phase synchronization, have shown that top-down modulation correlates with an increase of beta-band activity around 13-28 Hz, whereas stimulus-dependent bottom-up modulation is related with an increase of gamma band activity (>30 Hz)[48,95-99]. These oscillatory influences are asymmetrically exerted over different brain areas, configuring a functional hierarchy of top-down and bottom-up influences through the brain[49,100]. Among several other factors, psycho-social stress (PSS) can strongly impair both, the function

and structure of critical top-down controlling brain networks[101]. Socially stressed subjects perform worse than non-stressed subjects in attentional tasks. Further, this decrease in performance is associated with a lower functional connectivity between dlPFC cortex and other regions such as insula, ventral parietal posterior cortex and anterior cingulate cortex[35]. Conversely, patients with stroke-related lesions over PFC have consistently lower cortisol plasmatic values during PSS experiences[26]. Altogether, these findings point to a reciprocal interaction between critical components of the fronto-parietal attentional network and PSS.

Yet, the functional mechanisms underlying the effects of stress over cognitive performance and network modulation are still unclear, and frequently reduced to the cellular effects of glucocorticoids in the brain[4]. Moreover, we still lack an unequivocal cognitive function that can explain the interactions between PSS and brain-wide attentional networks. Some authors posit anxiety as an important attentional modulator[39]. Particularly, it has been observed that high anxious individuals have biased attention to emotional and threatening images[38,90] and increased memory to angry faces compared to neutral ones[91]. Thereby, anxiety may affect top-down control of attention, either allocating top-down attention towards an emotional ongoing stimulation, or to focusing on previous experiences interpreted as psychologically threatening.

Psycho-social stress induces a behavioral state that includes an increase of anxiety[29], together with an enhanced activation of both, the Hypothalamic-Pituitary-Adrenal-Axis (HPAA) and the Autonomic Nervous System (ANS)[24]. However, the behavioral consequences of PSS are not exclusively dependent of the physiological neuroendocrine and autonomic response[70]. Recently, it has been described that PSS-dependent self-reported increase of anxiety, but not the physiological response activation, is controlling the allocation of attentional resources, whereas the physiological response activation is directly associated with the psychological perception of stress-related experiences. Thus, autonomic and neuroendocrine responses may induce an increase of self-reported state of anxiety, allocating top-down attentional resources towards the

emotional on-going response. Yet, the relationship between those PSS landmarks and the oscillatory activity associated to the balance between top-down and bottom-up shifts during attentional tasks has not been studied.

Here, we hypothesize that after the PSS-dependent increase of anxiety, attentional resources would be redirected towards the stress-related threatening experience, instead of a sensory-driven attentional task. As a consequence of the shift in the allocation of attentional resources, we would observe an increased number of consecutives mistakes (Error or blank trials). Crucially, the process of attentional shift towards the emotional on-going response, should follow an enhancement of the endogenous top-down monitoring revealed through the increase of beta-band oscillatory activity. Finally, if the attentional allocation is affected by the increase of stress-related self-reported anxiety, we should expect a positive correlation between the increase of anxiety with both, the number of consecutives mistakes and beta-band activity enhancement.

3.2 Methods

3.2.1 Participants and procedures

Forty-two male non-medicated volunteers (mean age ± SD = 25 ± 3.8 years) were recruited between 12.00-14.30 hours. Once the electroencephalogram (EEG), electrooculogram (EOG) and electrocardiogram (EKG) electrodes (BioSemi ActiveTwo ® system) were placed, the experiment began with the baseline application of an attentional task and 90 seconds of resting state recording (pre-condition). After this, the TSST or the control protocol was conducted, finishing with a second application of the same attentional task and 90 seconds of resting state recording (post-condition). Additionally, participants were asked to fill the state of anxiety scale (STAI) [75] just before and after the TSST/Control protocol (Figure 1A).

All participants gave their written informed consent prior to the study in accordance with the guidelines of the Bioethics Committee of the Faculty of Medicine at Pontificia Universidad Católica de Chile, which approved the research protocol.

3.2.2 Stress induction and Control protocol

Psychosocial stress was induced with a EEG-adapted version of the Trier Social Stress Test [23]. The protocol consisted of an interview simulation in which the participants must expose their personal attributes for applying to a fictional job (5 minutes) in front of three people acting as referees (serious and in an expressionless attitude) and a video camera, followed by an arithmetic task (5 minutes), consisting on subtracting 1000 minus 13 consecutively until 0, every time that the participant failed, he was told by one of the referees to start again. The protocol follows the Kirschbaum, et al. [23] guidelines, with the exception that the referees were the ones who entered the room with the participant prepared for the evaluation inside.

The control protocol included the same procedures but in front of the experimenter (good mood and friendly attitude) instead of people acting as referees. The same physical and mental effort was induced but without the psychosocial stress component. After the protocol, participants were informed that no judgments were made about their presentation and that the camera was turned off.

3.2.3 Attentional task

An adaptation of a task-switching paradigm by Liston, et al. [71] was used as the attentional task. Two circles, each subtending 4,6° of the visual space and equidistant of the monitor center, were presented for 700 ms. Each circle was red or green and moved upward or downward. In between of both there was a letter “M” for movement or “C” for colour. The subject was instructed to choose the green circle when the letter was the “C” and the upward circle when the letter was the “M” (After two block the subjects were instructed to choose the red circle when the “C” appeared and the downward circle for the “M”). Each trial began with a central white fixation cross of variable duration (600-1000 ms). The complete trial involved the central fixation followed by 700 ms of the colored and mobile circles. Participants were trained with three blocks of 12 trials, corresponding

to solo color, solo movement and color/movement discrimination. The experiment involved four blocks of 64 trials separated by 1 minute rest between blocks.

Three different measurements of accuracy; the number of correct trial, the maximal number of consecutive failed trials (error or omission) and the number of episodes during the task with more than 2 consecutives failed trials (error or omission) and reaction times were recorded on a trial by trial basis by using the Psychopy software [72]. The final value for each of the measurements was calculated as the difference between the post-condition task scores minus the baseline (Pre-condition).

3.2.4 Physiological and Psychological Response

EKG activity was monitored during the sessions using 2 external electrodes (BioSemi ActiveTwo ®) positioned 2 fingers under the left collarbone and over the left hip. Five different periods of 90 seconds were used to calculate heart rate; during the 2 resting state periods (see 2.2) and at the beginning of different Control/TSST tasks (Anticipation-Speech-Math) (Figure 1). We decided to consider only the first 90 seconds of the Control/TSST tasks in order to equate the variability produced by comparing periods with different length. Heart rate was obtained and calculated using custom-made Matlab scripts and Kubios software [73].

The perceived stress scale [74] and trait anxiety scale [75] were applied before any procedure, in order to assess the daily and baseline subjective stress state and the trait of anxiety, respectively. Regarding the psychological experience of our experimental design, participant were asked to complete the state of anxiety scale [75] just after and before the control/TSST protocol (Figure 1). The psychological response associated to our experiment was focused exclusively on the stress dependent self-reported anxiety acquisition.

3.2.5 EEG Recording and Pre-processing

EEG data was obtained using 64 electrodes (Biosemi ® ActiveTwo) arranged according to the international 10/20 extended system. Four additional electrodes were used to detect eyes

movements. Two of them were placed vertically arranged to right pupil (Vertical EOG) and the other two were placed just at the outer canthus of both eyes (Horizontal EOG). Additionally, two electrodes were placed over the right and left mastoids to be used as an offline re-reference

EEG, EKG and EOG data were collected with a 2048 Hz sampling frequency and referenced online using the CMS and DRL electrodes incorporated in the recording system. The initial pre-processing, including the down-sampling to 1024 Hz and the re-reference to mastoids was performed using Matlab 7.8.0 (The Mathworks, Inc.) with EEGLAB v7.1.7.18b toolbox [102].

3.2.6 Resting State EEG Data Analysis

Each resting state period was divided in 0.5 seconds epochs. Noticeable artefactual epochs were rejected by visual inspection and subjected to a further Independent Component Analysis (ICA). Both the blink and cardiac components were rejected from the data using EEGLAB v7.1.7.18b toolbox [102].

The artefact free data was filtered and analyzed using the FieldTrip toolbox (Release date 2016-09-07)[103]. An initial band pass filter between 0.5-80 Hz was applied, followed by a discrete fourier transform filter between 48:0.01:52 Hz using 10 seconds of mirror padding for each epoch. Power spectrums were obtained after applying a hanning multi-tapering Fourier Transform over the filtered artefact free data.

The connectivity analysis was performed using the weighted phase lag index (WPLI) [104], a measure of phase-synchronization corrected for volume conduction. The analysis was performed between a frontal and parietal 9-electrodes cluster centered in the electrodes Fz and Pz, respectively.

3.2.7 Task EEG Data Analysis

Continuous EEG data was band-pass filtered using a infinite impulse response (IIR) Butterworth filter implemented in Matlab EEGLAB v7.1.7.18b toolbox [102]. Afterwards the data was epoched between -500 and 1000 millisecond time-locked to the stimulus onset and cleaned from artifact

using the same procedure described in *Resting State Data Analysis*. Data was transformed from the time domain into the frequency domain using the fast Fourier transform implemented in Matlab 7.8.0 (The Mathworks, Inc.) in overlapping windows (300 millisecond) in steps of 30 millisecond. The resulted time-frequency data was normalized into a Z-score relative to the baseline (From -500 to 0 millisecond) data, using custom-made Matlab scripts.

3.2.8 Statistical Analysis

The effects of the psychosocial stress (TSST) over heart rate and the state of anxiety were evaluated with a two-way repeated measures analysis of variance (ANOVA) together with the Bonferroni post-test. The relationship between accuracy, reaction times, heart rate, the self-reported state of anxiety and EEG activity was calculated using Pearson´s correlation. Both the graph and analysis of ANOVAs and correlation were performed using GraphPad Prism software (GraphPad Software, San Diego CA, USA). Statistical analysis of power spectrum and connectivity during resting state periods were assessed through a permutation test (1000 repetitions) corrected by multiple comparison implemented on custom made Matlab scripts. Time-frequency charts during the attentional task were compared between themselves using a bin by bin permutation test (2^{21} repetitions) corrected by multiple comparison but without assuming independency of the data. All the bins with p values lower than 0.05 were considered as significant. Data in the graphs are presented as the mean ± S.E.M.

3.3 Results

We implemented a modified version of the Trier Social Stress Test (TSST), together with a control protocol, adapted to EEG-EKG registration[23]. The EEG and EKG response was monitored throughout the complete experiment. The experiment consisted in an initial 90 seconds of resting state period, followed by an attentional task (Pre-condition). Then, the TSST or the control protocol were presented, to finish with another 90 seconds of resting state period followed by the attentional task (Post-condition) (Figure 1A). Before and after each protocol presentation,

participants self-reported their experience using the state of anxiety questionnaire[75]. This experimental setup allowed us to explore the influence of PSS induced changes on the oscillatory activity during resting state, and through all the correct answers in the attentional task. Those changes were also related with both, the autonomic nervous system (ANS) reactivity and the self-report state of anxiety.

3.3.1 Physiological and subjective stress markers

First, we measured heart rate and the state of anxiety as outcomes of the stress response. A 2 (group) X 5 (time) repeated measurements ANOVA was performed for heart rate analysis. As expected, there were no differences in heart rate between groups during resting state periods (Figure 1B, RS pre-TSST and RS post-TSST, Bonferroni post-test; $p>0.05$). Conversely, we observed an increase of the heart rate during the execution of the speech and arithmetic phases in both, TSST and control protocols (Figure 1B, grey bar. Time effect $F(4,160) = 21.93$; $p<0.001$). This overall increase in heart rate was significantly stronger during the stress condition (Figure Figure 1B, Group x Time interaction effect $F(3,160) = 3.645$; $p<0.01$). Specifically, heat rate increases were observed during the arithmetic and speech phases of the task, but not during the anticipation period (Figure 1B, Bonferroni post-test; ** $p<0.01$, ***$p<0.001$).

Second, we quantified the state of anxiety across subjects using the STAI. With this instrument at a hand, we evaluated the state of anxiety score after the presentation of both attentional tasks (Figure 1A, discontinuous black lines). We observed a strong increase of the state of anxiety score after the execution of the TSST protocol, which was absent after the execution of the control protocol (Figure 1C, Group x Time interaction effect $F(1,40) = 38.04$; $p<0.001$). This effect was elicited only after the TSST post condition attentional task (Bonferroni post-test; $p<0.001$), indicating that the TSST protocol specifically triggers a stress-related increase on state of anxiety levels.

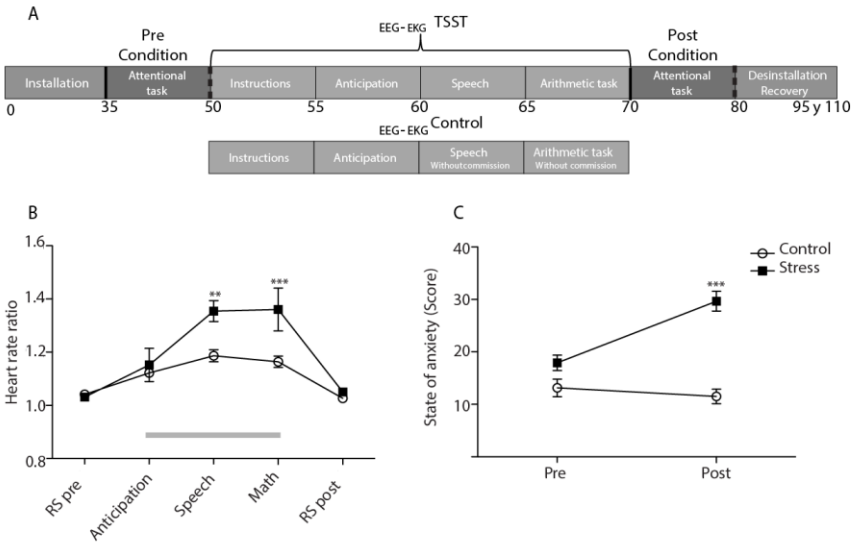

Figure 1| Schematic diagram of the experimental setup. Continuous and discontinuous black vertical lines indicate resting state periods and state of anxiety inventory request, respectively. Numbers below the diagram indicate time in minutes (A). Heart rate during the experimental procedure for the PSS and Control group. Grey bar depicts the moment of PSS induction or Control protocol, (RS pre = Resting state Pre, RS post = Resting state post), **p<0.01, ***p<0.001 (B). State of anxiety score during baseline (Discontinuous black line after pre condition attentional task) and after PSS induction or Control protocol (Discontinuous black line after post condition attentional task), ***p<0.001 (C).

3.3.2 Scalp oscillatory activity during resting state

Then, we measured the resting state scalp power spectrum during 90 seconds before each attentional task (pre and post condition). The power spectrum was obtained using a multitaper FFT analysis over 500 ms epochs. We averaged the power spectrum across all channels to then compare across conditions. This analysis revealed a slight power increase around 10 Hz during the resting state period after the execution of both, TSST and control protocols. This increase was not significantly different between groups. Moreover, we did not find high-frequency power differences throughout conditions. However, convincing absence of power differences does not necessarily preclude phase synchronization differences among signals[105,106]. Therefore, we assessed the phase-synchronization differences between fronto-parietal electrodes using the weighted phase lag index (WPLI), a reliable measure of phase consistency, even in the presence

of volume conduction[104]. With this analysis, we observed an increase of alpha frequency-band synchronization after the presentation of both protocols. Crucially, the increase of the alpha frequency-band WPLI values turned significant only after the presentation of the stress protocol (Figure 2C, D).

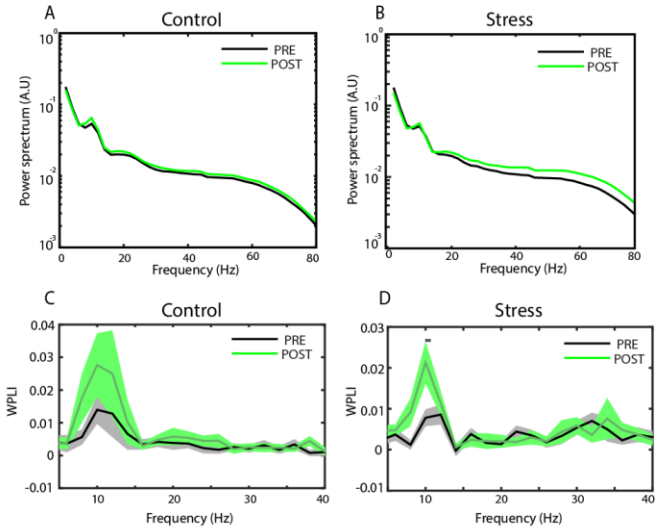

Figure 2l Power spectrogram (A-B) and phase synchrony through the WPLI (C-D) during baseline and after either the Control protocol (A-C) or the PSS induction (B-D), (Cpre = Control Baseline, Cpost = Control post, Spre = PSS baseline, Spost = PSS post). Black line in D indicates $p<0.05$, permutation test corrected by multiples comparisons. Solid lines and shaded area illustrate mean and SEM, respectively.

3.3.3 Behavioral performance on the attentional task

Before and after both the presentation of the TSST an control protocols, participants performed a version of the attentional control task used by Liston, et al. [35]. Task performance was assessed by the total number of correct trials, the maximal number of consecutive fails, the average number of two or more consecutive fails episodes and reaction time values for correct trials. We found that the self-reported state of anxiety, but not the heart rate response, was directly associated with performance in the attentional task (Table 1). Interestingly, we found an inverse relationship between PSS-dependent self-reported state of anxiety and the number of correct trials and a

positive correlation between PSS--dependent self-reported state of anxiety and the maximal number of consecutive failed trials (Table 1). Furthermore, our analyses revealed no relationship between anxiety and the average number of episodes for two or more consecutives fails, and anxiety with reaction times (Table 1). Both groups, control and TSST, had faster reaction times after the execution of the protocol despite changes on anxiety (Figure S1D), suggesting that PSS effects over performance were not due to fatigue or any other task learning disruption. Additionally, we found that the average number of episodes of two or more consecutives fails decreases similarly in both control and TSST groups (Figure S1C), but there was an increased number of maximal consecutives failed trials after the PSS induction (Figure S1B), suggesting that participants of both groups suffered a similar number of attentional lags, but stressed group exhibited longer ones.

Table 1: Correlation between stress outcomes and attentional performance

	Corrects	Max fails	Number of events	Reaction time	Anxiety state	Heart rate
Corrects						
R	1	-	-	-	-	-
p						
Max fails						
R	-0.743	1	-	-	-	-
p	<0.0001***					
Number of events						
R	-0.2812	0.297	1	-	-	-
p	0.0712	0.0563				
Reaction time						
R	0.056	-0.142	0.039	1	-	-
p	0.724	0.369	0.804			
Anxiety state						
R	-0.528	0.486	0.173	-0.073	1	-
p	0.0003***	0.001**	0.273	0.644		
Heart rate						
R	-0.1581	0.265	0.130	-0.173	0.404	1
p	0.323	0.089	0.411	0.272	0.008**	

Max fails: Maximal number of successive fails (Errors+blanks) post treatment –Baseline
Number of events: Number of episodes with more than 2 successive fail post treatment - Baseline
Reaction time: Reaction time of correct trials post treatment - Baseline

Anxiety state: State of anxiety post treatment – baseline.
Salivary cortisol: Salivary cortisol concentration (+10) – Baseline.
Heart rate: Area under the curve of the heart rate response during the complete experiment.
*N = 42 participants. *P<0.05, **P<0.01, ***P<0.001*

3.3.4 Oscillatory activity associated to the correct trials of the attentional task

Next, we wondered whether PSS exerts any impact in the oscillatory dynamic during the execution of the attentional task. Therefore, we compiled all the previously filtered correct trials in epochs of 1.5 s, lasting from 0.5 s pre-stimulus to 1 s post-stimulus onset. Then, we averaged the baseline normalized power across electrodes and subjects. Control and Stress-induced groups depict similar time-frequency dynamics during Pre and Post trials. In both groups, we observed an early increase of power at low frequencies, reflecting the evoked potential associated to the stimulus onset, followed by a beta-band activity decrease, starting at 0.2 s after stimulus onset. We also found a late increase of gamma-band activity (Figure 3A, left middle bottom up panels) after 0.4 s stimulus onset. Importantly, the difference charts revealed significant differences between both, Stress and Control groups. The control group showed an increase of gamma-band activity in the Post control condition, centered around 0.18 s post-stimulus onset. This gamma-band increase was followed by a beta-band decrease between 0.2-0.5 s (Figure 3A, top-right panel, permutation test, $p<0.05$ bins are showed in grey box). In the Stress-induced group, we observed a huge increase of beta-band power in the Post-TSST (Figure 3A, bottom-right panel, permutation test, $p<0.05$ bins are showed in grey box). Differences charts were compiled into an early (110-280 ms, Figure 3B) and late (300-470 ms, Figure 3C) window. As mentioned before, there was an increase of gamma band (35-40 Hz) for the control group during the early window. During the late window, the increase of beta band after the PSS induction was accompanied by a decrease of the power at the same frequency for the control group between 22-28 Hz. The topology of the beta increase during the second half of the trial was present through all the scalp

with higher intensity at frontal-temporal regions (Figure 3D). Altogether, these results suggest that, depending on previous exposure to PSS, different brain rhythms are elicited while participants are engaged in an attentional task.

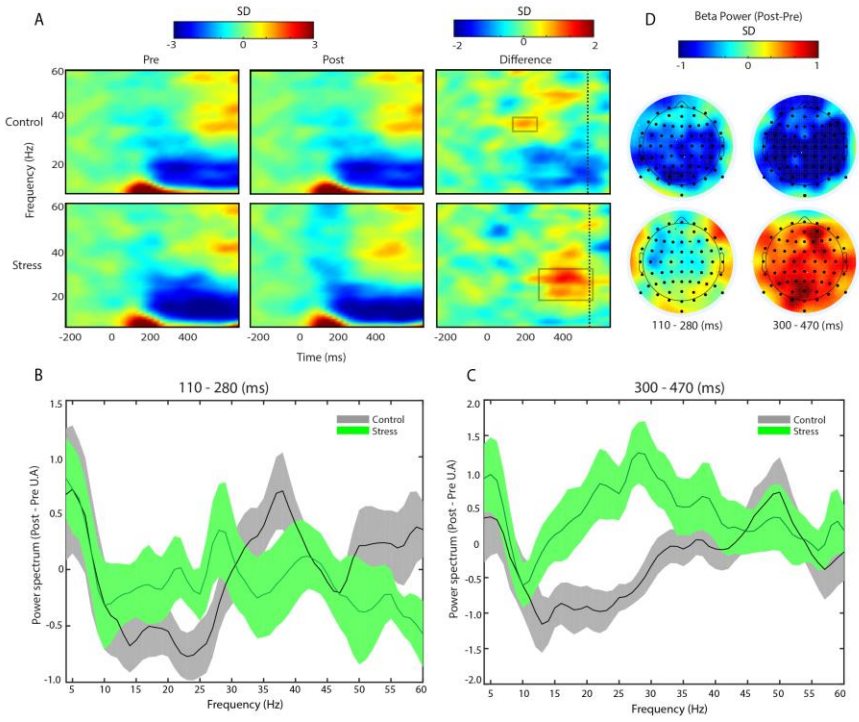

Figure 3l Time-frequency charts of correct trials for both groups during baseline and after either the Control protocol or PSS induction. Grey squares indicate regions in which one difference chart is significantly higher than the other (Permutation test). The difference chart indicate the subtraction Post minus Pre charts and the dot vertical lines in there show the mean reaction time (A). Spectrograms of the Post-Pre difference of the early (110 – 280 ms, B) and late (300 470 ms, C) window of the correct trial. Topography of the beta power (12-29 Hz) during the first and second half of the correct trial (D).

3.3.4 Correlations between spectral power, attentional performance and stress markers

Finally, we quantified the relationship between the observed beta activity with both, the attentional performance and PSS markers. For each subject, we calculated: the beta-band amplitude magnitude change during the task, the difference between correct answers across conditions, the

maximal number of consecutive fails, the average number of episodes with two or more consecutives fails, the self-reported state of anxiety and the heart rate increase (area under the curve) across conditions. Then, we performed a Pearson correlation between beta-band activity and the abovementioned parameters, across all participants. We observed that beta-band power during the task was negatively correlated with the number of correct trials (Figure 4A, $R = -0.4299$, $p<0.01$), positively correlated with the maximal number of consecutives mistakes (Figure 4B, $R = 0.4186$, $p<0.01$) but not correlated with the number of episodes with 2 or more consecutives fails (Figure 4C, $R = -0.083$, $p>0.05$). A positive correlation between beta-band activity and both, the self-reported state of anxiety (Figure 4D, $R = 0.4228$, $p<0.01$) and the heart rate reactivity was also observed (Figure 4E, $R = 0.3$, $p<0.05$). These correlations seem to suggest that oscillatory changes in the beta band are directly related with performance differences between groups. Moreover, it is likely that the self-reported state of anxiety plays a key role inducing changes at the attentional performance and the brain rhythms underlying task attentive behaviors.

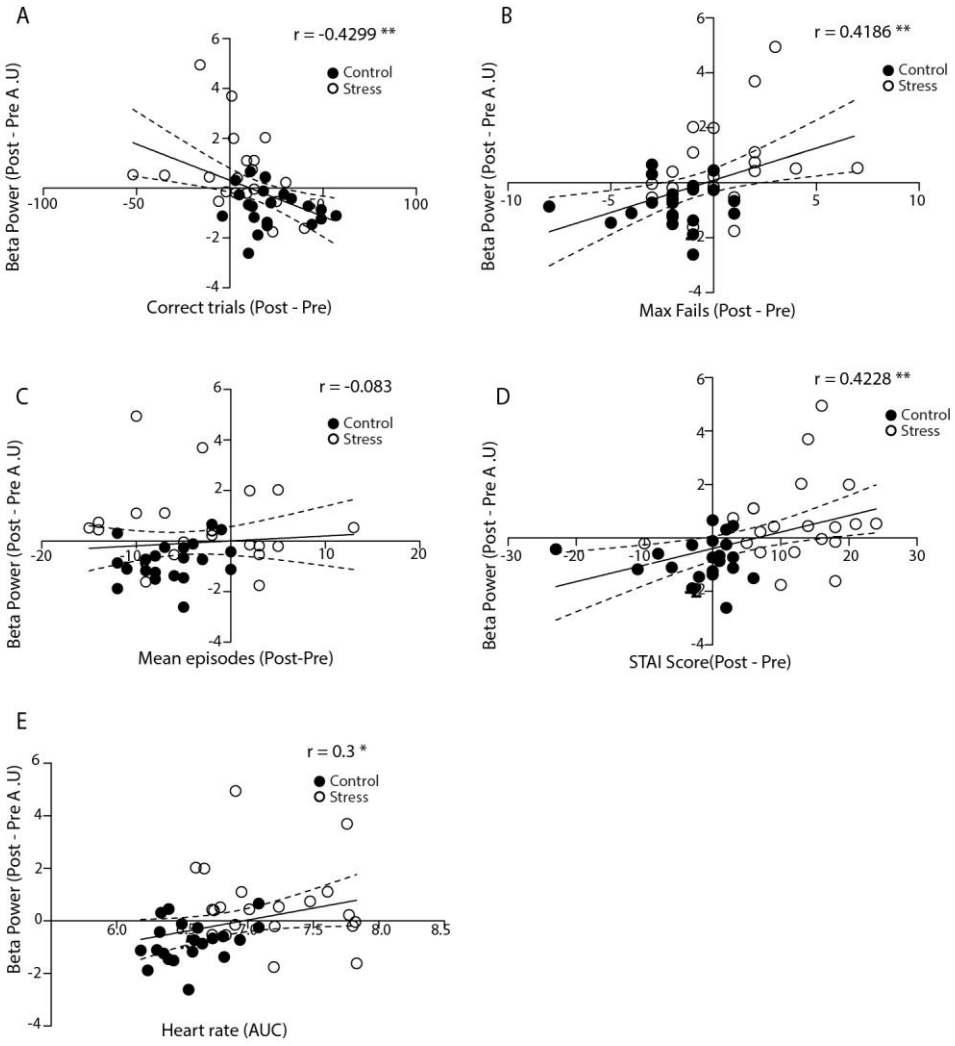

Figure 4l Person correlation between beta power difference (Post-Pre) and correct trials (A), Maximal number of consecutive fails (B), number of episodes with 2 or more consecutives fails (C), self-reported state of anxiety (D) and Heart rate (Area under the curve, E). *p<0.05, **p<0.01.

3.4 Discussion

As predicted, we found a positive correlation between self-reported state of anxiety and the number of consecutives fails (errors + blanks) in the attentional task, but not with the number of episodes in which they committed a consecutive fail. This suggest that independent of the anxiety levels, individuals tend to allocate the attention out of the task, however, as the anxiety increase the re-allocation of it back to the task is more difficult. There was no correlation between behavioral performance and the physiological activation, but the last correlate positively with the self-reported state of anxiety, bearing out the idea that (at this temporality), the physiological response plays a role on the psychological perception of PSS rather than affecting cognition directly. At an electrophysiological level, we did not found differences on power amplitude during the resting state periods. Conversely, we found an increase of fronto-temporal alpha (8-12 Hz) phase synchronization in both groups, however this increase turns significant only after the PSS induction. We then evaluated the electrophysiological activity associated with correct trials in the attentional task. Interestingly, we found an increase of early gamma band (35-45 Hz) in individuals exposed to the control protocol, unlike those exposed to the PSS induction in which there were a late increase of beta band activity (22-29 Hz). Finally, there was positive correlation between beta band activity with self-reported anxiety and the maximal number of consecutive fails.

3.4.1 PSS induces the allocation of attention out of the task

We showed that the increase of anxiety was directly correlated with attentional allocation out of the task (Table 1). This results is in line with the evidences showing that an increased state of anxiety allocates attention to emotional or threatening stimuli [37,38,107]. In our design, the attentional task lacked in emotional component, moreover, the only threatening component during the experiment was the TSST. Thereby, it should be expected an increased attention to either internal intrusive thought associated with the previous stressful experience (TSST) or to develop effective strategies to reduce the self-perceived anxiety associated to this experience [39]. Additionally, there

are also evidences showing that PSS induction is related with worse performance on non-emotional tasks [28,32,89].

We suggest that after PSS induction, attention was directed internally to the previously experienced stressful situation. It has been shown that internally-directed attention is related with increased alpha power [52,56] and synchronization[108]. Interestingly, we found an increase of alpha fronto-parietal synchronization during resting state after both protocols, however, this increase was statistically significant only after the PSS induction (Figure 2C-D). According with the behavioral results, it seems that during resting state there is an increase of inner attention after both protocols, but this increase is higher after the TSST suggesting that those individuals are more strongly engaged to endogenous elements as thoughts, inner strategies, etc.

3.4.2 The allocation of attention on the ongoing task correlates with an increase of beta band activity

Interestingly, correct trials in the control individuals were driven by a gamma band increase (Figure 3A). The observed increase of gamma band activity in control individuals is in accordance with the literature of perception [106] and attention [109,110], and reflects an increased stimulus driven bottom-up attention [48,100,101], which is expected on participants highly focused on the ongoing task. Moreover, it has been shown that during attention to external stimuli there is an increase of gamma activity which is decreased when the stimuli is unattended [111]. Conversely, corrects trials of stressed individuals associated with an increase of beta band activity (Figure 3C). According to previous works beta band activity increase is related with higher top-down modulation[48,101,112], which can be also seen as an increased goal-directed attention [46]. Thereby, our results suggest that stressed individuals required greater top-down modulation to achieve the correct response. Because stressed individuals have higher functional connectivity between prefrontal-amygdale [113], one possibility is that they exerts some compensatory mechanism to counterbalance the deregulatory influences from the amygdale. On the other hand, it would be possible that the

increased beta band activity reflects the continuous effort of the PFC in order to maintain the attention in the task and not on the elevated levels of intrusive endogenous thoughts.

3.4.3 The stress-dependent increase of self-reported anxiety is directly associated with beta band activity

We showed that as far as the beta band activity increased the self-reported anxiety, the maximal number of consecutives mistakes and heart rate increased as well. The strong relation between anxiety, performance and beta band activity is in line with the ‘’attentional control’’ hypothesis proposed by Eysenck, et al. [39]. Thereby, the increase of anxiety turns attention out of the ongoing task to previously threatening experience leading to higher amount of consecutives mistakes. The individuals are able to redirect attention to the task by increased top-down control which is reflected as increased levels of beta band activity. Interestingly, we found a positive correlation between heart rate and beta band activity, the increased heart rate reflects an increase of autonomic nervous system [114]. In this line, nor-epinephrine (NE) has been proposed as one of the main molecular effectors of PSS[115]. Moreover, it has been shown that the stress related increase of NE persists during about 30 minutes [116] and can binds α1 receptors in the PFC inducing attentional impairments[117]. The locus coeruleus NE projections can also reach regions in the amygdale and hippocampus[20], favoring emotional activation. Altogether, the evidences suggest that the rapid release of NE may leads to PFC impairments through α1 receptors binding. Those impairments might induces inappropriate management of thoughts and emotions, by the switching of control from PFC to the amygdale[101].

We believe that this study highlights the complexity of the PSS response in relation to behavior, emotional response and brain activity, thus demanding a multi-level approach. Moreover, this findings revealed the compensatory strategies allowing stressed subjects to cope with the stressful experience. However, this compensation comes at the price a stronger cognitive and physiological tear and wear. This work may open a new perspective for an integrative treatment of stress in the clinical domain.

3.5 Supplementary information

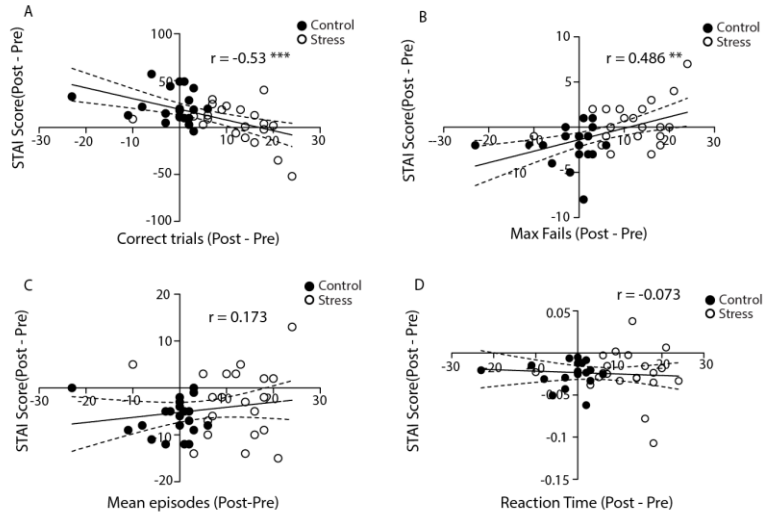

Figure S1l Person correlation between self-reported state of anxiety difference (Post-Pre) and correct trials (A), Maximal number of consecutive fails (B), number of episodes with 2 or more consecutives fails (C) and Heart rate (Area under the curve, D). **p<0.01, ***p<0.001.

3.6 Acknowledgement

This work was supported by a doctoral fellowship and internship research fellowship (CONICYT 21140884, Chile) to I.P., by FONDECYT (1130810, Chile) to J. R. S., by FONDECYT (1120752, Chile) to E.R., by a Fund for Innovation and Competitiveness (FIC) of the Chilean Ministry of Economy, Development and Tourism, through the Millennium Scientific Initiative (IS130005) to J. R. S. and E.R. ……

Chapter 4: General Discussion and Conclusion

PSS experiences induce a state characterized by a physiological activation and psychological changes involving, for instance, the increase of anxiety [24].The cross talk between those stress components in the context of an attentional task and, moreover, the neural correlates of it were still unclear.

The current thesis aimed to study how the different elements of the PSS response interacted between themselves in order to affect cognition, using the brain oscillatory activity as a new tool to further the understanding and interpretation of those effects.

The main results of the work can be summarized as: 1) The PSS dependent state of anxiety was the variable that better explained the effects of stress over the attentional task. Conversely, the physiological activation favored the increase of anxiety, however, it was not directly related with the behavioral outcomes. 2) Correct answers in the attentional task of the stressed participants were accompanied with an increase of beta band activity spread on fronto-parietal regions, unlike control participants in which correct answers were characterized by an increase of gamma band. Interestingly the increase of beta band activity was correlated with the increase of anxiety and the decrease of attentional accuracy.

4.1 Anxiety is the key mediator of the effects of PSS over attention

The improvement in the attentional task observed after the control condition was disrupted after the PSS condition. However, we noted that after splitting the stress group in those with high and low anxiety, participants with low anxiety improved as much as controls, while those with high anxiety not only failed to improve but worsen. We propose that anxiety is a key mediator of the effects of PSS over attention. In contrast to a significant amount of evidence [27,37], we did not find any direct relation between cortisol or heart rate response and cognitive behavior. It has been proposed that cognitive flexibility is only affected when cortisol concentration was in the peak [84],

however in our design, the attentional task was performed just after the stress induction, while cortisol has not reach the peak yet. Our design then evaluated the immediate effects of PSS induction. Thus, our results suggest that those immediate effects are dependent of the current psychological state of the subject more than the physiological one. Accordingly, Ali, et al. [70], showed that the immediate psychological response to the PSS did not depend on the physiological one. In particular, the pharmacological blockade of the physiological response to the TSST did not affect the psychological response to it, since participants of the dexamethasone-propranolol condition showed an almost absent physiological response but with the same emotional experience to the TSST than the placebo group. As shown in the Chapter 1, Figure 1, we propose that there is an initial PSS perception that depends mainly of the psychological perception of the experience. However, as shown by our mediational analysis (Chapter 2, Table1), this initial psychological experience can be affected in a next step by the conscious perception of the physiological changes induced by the PSS. Interestingly, if the physiological activation does not come with the anxiety acquisition, the behavioral disruption is not present. Conversely, we observed a trend to improve performance, however, the sample size of our experiment did not allow us to corroborate this issue. On the other hand, if the initial psychological PSS-dependent increase of anxiety comes with a physiological activation, attentional disturbances are likely to be observed[33].

Translating the stress response mechanism into the field of the experience of emotions we can explain our results using the classic Schachter and Singer theory. They propose that the experience of emotion requires both the physiological response and the interpretation of it, considering the particular situation the person is in at the moment [118]. Our results might be explained following the Schachter-Singer proposal since the stress response elicits a physiological response, however, this response can only alter cognition if it is interpreted

subjectively/psychologically as a negative experience. Thus, PSS would affect attention when the physiological changes produced by it, were psychologically evaluated as a negative experience[3].

4.2 Beta band activity increase reflects continuous top-down monitoring after the PSS induction

Beta band activity has been strongly related with motor and cognitive control, in a process conceptualized as the maintenance of the 'status quo'. In other words, there is a close relationship between beta band activity and the maintenance of a current state in order to prepare the body for expected or predictable changes, at cognitive and motor levels [47]. Here we showed that correct trials of stressed individuals were accompanied by an increase of the beta band activity, unlike participants of the control group which showed an increase in the gamma range (Chapter 3, Figure 3).

In the domain of the motor control, according with a more classical interpretation of the beta band, our results are congruent with the evidences positing that beta band is attenuated during voluntary movements [119]. As shown in Chapter 3, Figure 3, there was a huge decrease of beta band in both groups (Control and Stress) and in both time points (Pre and Post). Alternative hypothesis have related beta band activity with the expectancy of an upcoming event, in which enhanced beta band in the dorsal stream is related with the expectations of an upcoming event [120]. Our results suggest that when stressed participants are engaged in the task, they are more expectant for the forthcoming change. This idea is in accordance with Elling, et al. [121], who showed that stressed individuals have increased exogenous attention during a stressor anticipation. Further research with specific source localization are needed to identify with more certainty which process is really happening.

[3] Other relevant aspects are discussed in more detail in chapter 2, section 'Discussion'.

Regarding the cognitive domain, Engel and Fries [47] hypothesized that: 'tasks involving a strong endogenous top-down component should be associated with high beta band activity, whereas one should observe a decrease of beta band activity in paradigms where the behavioral response of the subject is largely determined by exogenous, bottom-up factors'. Also it has been documented that settings involving weak endogenous top-down component are related with increased power in the gamma oscillations [45]. Our results suggest that participants of both groups reach correct answers using different cognitive mechanisms. The increased beta band activity in the stressed participants suggest an enhanced endogenous top-down component at the moment of solving the task, while the increased gamma band in the control individuals suggest a behavioral mechanism based on the exogenous bottom-up component. Interestingly, the power of beta band correlated positively with the state of anxiety, meaning that when the state of anxiety increased, the beta band power increased too (Chapter 3, Figure 4). It is likely that stressed participant required more top-down influences to stay task engaged, because the increased state of anxiety induces a continuous redirection of attention to intrusive stress and threat related thoughts, and to endogenous strategies to decrease the current anxiety self-perceived state, leading to less available attentional resources to perform the task [39]. Thereby, the individuals have to be constantly in a self-monitoring process to redirect the attention to the task. It can be suggested that independent of the type of task someone is performing (dual-task, task-switching, working memory, etc) [27,28,35], if the task requires a continuous and stronger top-down attentional component to succeed in it, a stress dependent increase of anxiety might disrupt performance by allocating attentional resources to task irrelevant elements[4].

It is worth mentioning that both the state of anxiety and the power in beta band correlated positively with the maximal amount of consecutive errors or blanks trials (Chapter 3, Figure 4). This finding supports our hypothesis that the stress dependent increase of anxiety induces the

[4] Other relevant aspects are discussed in more detail in chapter 3, section 'Discussion'.

redirection of the attention out of the task, eliciting longer episodes without correct answers. Interestingly, as far as the out of task episodes are longer, participants have to increment their endogenous top-down mechanisms to come back to it (the task), which can be explicitly observed through the increase of beta band activity.

The stress dependent attentional changes can be discussed in terms of the PFC deregulation. As it was shown in the chapter 1, the PFC orchestrates the brain's activity for the regulation of behavior, emotion and thoughts [122]. Also, the PFC has direct and indirect connections with brainstem nucleus such as the LC (NE) and substantia nigra (Dopamine). Under non stressful situations, optimal levels of catecholamine enhance the PFC regulatory function, however, under stressful experiences there is an exacerbated release of both NE and dopamine, impairing PFC regulations but strengthening amygdale function, setting up a 'Vicious cycle' (Chapter 4, Figure 1) [101]. Thereby, during and after stress situations there is an increased amygdale modulatory activity which favors the redirection of attention to emotional stress related stimuli, thoughts or experiences. As mentioned above, the amygdale hyper-activity in turn, correlates with impairments of PFC regulation, leading to the mentioned goal-directed attentional disruption. Although PSS induces goal-directed attentional impairments given by the exacerbated catecholamine release, the amygdale hyper-activation and the PFC dysfunction [101], however we showed that individuals use compensatory mechanisms to counteract those effects and achieve the current task as best they can.

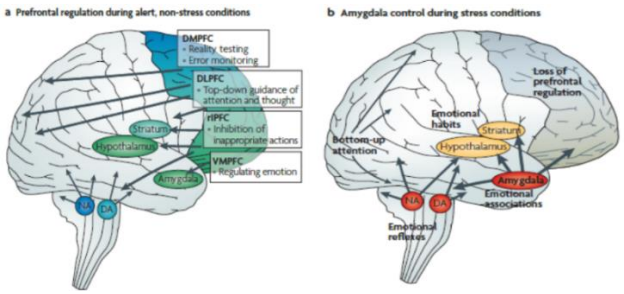

Figure 1l Model of PFC and amygdala regulation during non-stress condition and during stress. Taken from Arnsten [101]

4.3 Limitations and methodological issues of the current study

During the development of the thesis we recognized some limitations and methodological issues that should be exposed. Firstly, the study was done only with university men population. It has been shown that reactivity against stress differs between men and women [31], and also between children, young and elderly population [24], thus, it is difficult to extrapolate our results to those groups.

In relation to the task, it would have been interesting to incorporate a third attentional task during the peak of cortisol, or after the stress response had ceased. Then, the possibility of a cortisol mediated response [84] and the long term effects of the PSS over cognition could have been assessed[32]. Also, to really prove the hypothesis that the PSS-dependent increase of anxiety affects goal-directed attention independent of the current task, it would have been more adequate to incorporate several tasks of different nature, such as working memory, task switching (our task) or sustained attention, instead of only one type.

Interestingly, one of the main outcomes of the present work was the relationship between anxiety, behavioral performance and beta band activity. However, it was not clear whether the observed effects were restricted only to anxiety or other psychological variables as the perceived stress [35], positive or negative affect [33], etc. Studies including bigger batteries of psychological, physiological and cognitive variables together are needed to disentangle in more detail the effects of PSS over behavior.

Finally, our results are supported by evidences associated with stress-related deregulations of PFC [53,101]. Unfortunately, our work was methodologically restricted, because the EEG has not the appropriate spatial resolution to explore with more precision which brain areas are related with the stress-related beta band increase. Future research combining EEG with fMRI or recording the

specific position of each electrode are needed to obtain results with both the spatial and temporal resolution desired.

4.4 Implications and future directions

Stress and in particular PSS experiences are affecting more and more people around the world, inducing in many cases health problems which can vary between a simple headache to the development of a psychiatric disease such as depression or bipolar disorders[5]. Works including different analysis levels of PSS, such as a psychological, physiological, behavioral and neurobiological views, are extremely beneficial to increase an integrated understanding on how stress operates over the individuals mind, body and behavior. That valuable knowledge will contribute to the design of contemporary, holistic and integrated strategies to cope with daily stress. In this line, it is highly necessary to increase the research not only in the study of stress as such, but also in the strategies or actions to deal with it. Works assessing the role of cognitive control practices such as meditation, or lifestyle habits including dietary or physical activity over PSS reactivity might also give rise to preventive measures.

Interestingly, the development of technology associated to the physiological and electrophysiological measurements have increased to the point where the laboratory is not always necessary. This allows us to design naturalistic experiments either inside the stressful work office or in particular places where the stressful lifestyle is absent, and therefore the reactivity to stress might change or be associated to other type of stimuli like for instance, animals, food or temperature rather than social approval. Those variables and factors will now be open for exploration by a new generation of integrated and ecologically situated studies.

[5] See chapter 1.

Coda: Brain-heart coupling at beta frequency is disrupted after PSS

5.1 Introduction

There are brain mechanism destined to monitor what is happening in the body. It has been shown that the activation and connectivity of the Default-Mode Network (DMN) correlates with the ongoing modulation of skin conductance [123] and heart rate [124,125]. Ziegler, et al. [125] found that during resting state there was a positive correlation between the BOLD signal in the ventromedial PFC (vmPFC) and the length of the R-R intervals. The authors highlight the importance of the vmPFC in the modulation of efferent vagal activity, they also add that it is likely that vmPFC connections with other Central Autonomic Network (CAN) [6] structures such as the hypothalamus, midbrain and brainstem might also be involved in the mediation.

Going further, Babo-Rebelo, et al. [126], developed an experiment to study the relationship between the content of spontaneous thoughts with the Heart Evoked Potential (HEP), the cortical representation of the cardiac information. Thoughts were categorized on those in which the person was the agent of the thought ('I' category) and in those in which the participants were thinking about themselves ('Me' category). They found that vmPFC HEP covaried with the 'Me' dimension of the self while the ventral precuneus HEP with the 'I' dimension of the self. Additionally, the heart-monitoring has also behavioral implications. It was demonstrated that frontal and parietal HEP amplitude preceding a stimulus detection was predictor of the detection accuracy. Interestingly, the results could not be attributable to changes in cortical excitability, volume conduction or changes in heart rate or heart variability[127].

[6] The term 'central autonomic network' (CAN) has been coined to describe the cortical and subcortical brain structures that ensure the complex integration of sensory information from different parts of the body and accomplish the appropriate. The anterior cingulate cortex, insular and vmPFC, multiple nuclei of the diencephalon, midbrain, pons and medulla oblongata are discussed as main components of this reciprocally interconnected functional network.

Finally, a recent work showed a coupling between slow gastric phase and the alpha cortical amplitude. Moreover, they showed that the coupling directionality was from gut to brain, suggesting that brain activity is not only dependent on brain neurons and networks proprieties but also on body influences[128].

Brain-heart coupling has been considered as an important factor on processing of self and the representation of the current state. Also it was shown that brain-gut coupling can emerge at specific oscillatory frequency. However, heart activity has not been related with any specific brain frequency. Furthermore, the brain-heart coupling has not been assessed during the state induced by PSS.

Is the heart coupled with the brain at a specific frequency? If yes, Might this coupling be affected by PSS?

5.2 Method and Results

Previous reports have shown coupling between phases of cortical rhythms and spontaneous body behaviors such as microssacades [129] or slow gastric rhythms[128]. Interestingly, fluctuations of those spontaneous behaviors as microssacades and slow gastric rhythms shapes cortical rhythms at alpha and gamma frequencies[128,130], respectively. The relation between other type of physiological rhythms as heart rate and brain oscillation in resting state have been unexplored. In order to study this relationship, we assessed the phase locking between heart rate and brain fluctuations by using the Pairwise Phase Consistency indicator (PPC)[131]. The PPC is a useful method to explore the phase relationship between spikes and brain oscillations as local field potentials (LFP)[132]. In particular, a spikes channel involves ‘ones’ and ‘zeros’ throughout time, every ‘one’ indicating the occurrence of a spike. Thereby, the PPC should be suitable for any signal that could be binarized. Because the heart rate signal is perfectly binarizable, it is possible to study the relation between the ‘R-R’ complex and the phase of an EEG signal.

Here we proceeded as follows, for each 90 seconds resting state period, all the R peaks were found and ± 1 seconds epochs were obtained centered on the R peak. The phase of EEG signal was obtained by taking the Fast Fourier transform locally around every R peak. Statistics was calculated using the PPC. All the analysis were performed using the Matlab FieldTrip toolbox (Release date 2016-09-07) [103].

5.2.1 Brain-Heart beta phase coupling decreases after PSS induction

It has been shown that the heart evoked potential can be affected by the cardiac field [133,134]. In order to find the highly contaminated EEG component, Babo-Rebelo, et al. [126] used the PPC estimator over the independent components of the EEG signal and eliminated all the components with PPC over 0.2 between 12-30 Hz frequencies. The same procedure were adopted in our analysis but eliminating all the component with PPC over 0.1 at the same frequencies. Thereby, our EEG data should be clean from the cardiac field.

PPC spectrum between heart beat and EEG data showed a remarkable phase locking between heart rate and oscillatory brain activity, around 10-30 Hz, centered in 20 Hz (Figure 1A, B; Black lines). The described phase locking was unaffected after control protocol (Figure 1A). Interestingly, PSS induced a significant decoupling between 17-24 Hz (Figure 1B; $p<0.05$; Permutation test corrected by multiple comparisons), consistently through the electrodes of the scalp (Figure S2B). The mentioned effects were better appreciated taking the differences between the PPC post minus pre conditions. The difference for the controls PPCs was nearby zero for all the frequencies, analogously, it can be clearly appreciated that after the stress protocol the phase coupling was strongly decreased at beta frequencies (Figure 1C). The presented results suggest for first time that social stress disrupts the coupling between heart rate and brain oscillatory activity (Figure 2C), independently of the heart rate (Figure S1A) and heart variability (Figure S1B).

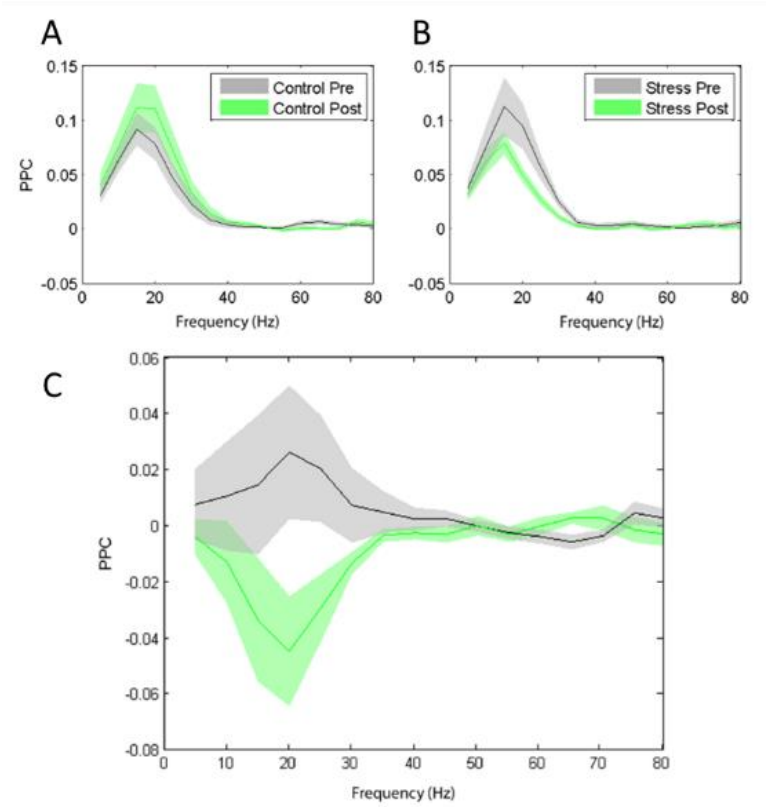

Figure 1l Mean whole brain Phase Pairwise Consistency spectrograms using free of artefact EEG data during baseline and after the control protocol (A) and the PSS induction (B). Post condition minus baseline spectrograms for control condition (Black line) and stressed condition (Green lines) (C). Shaded area indicated the SEM. Black horizontal line in B and C indicate $p<0.05$.

5.2.2 Control 1: Brain-Heart beta phase coupling decrease after PSS induction was absent in contaminated components

To ensure the differences between groups were given especifically by a brain procces and not by the contamination of the cardiac field. We compared the highly contaminated components of the EEG signal between groups. Firstly, we found that, as was predicted, PPC values for the contaminated components reach almost 0.4, which is fairly high compared with the values observed in the literature [132]. Interestingly, as shown in Figure 1 the PPC spectrum of contaminated component reached also a peak at beta frequencies (Figure 2), however there were no differeces neither for the control group (Figure 2A) nor for the stress one (Figure 2B). If the

differences observed in Figure 1 would have been by the cardiac field, then it should be expected to observe those differences in the contaminated component as well.

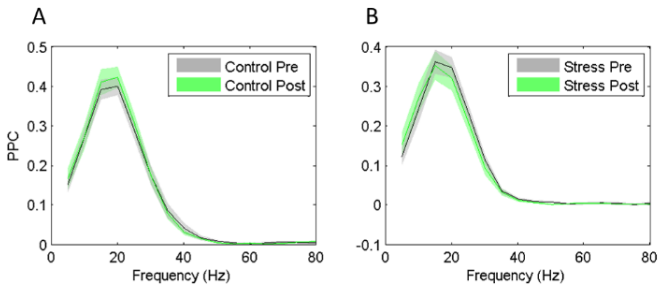

Figure 2l Phase Pairwise Consistency spectrograms using artefactual independent components during baseline and after the control protocol (A) and the PSS induction (B). Shaded area indicated the SEM.

5.2.3 Control 2: Brain-Heart beta phase coupling was time-locked to the heart beat

To test if the observed effects were truly locked to heart beat we created for each participant three surrogates heartbeat, all of them with the same rate as the original. The first one with constant interbeat interval (Heart rate variability = 0), the second one with random interbeat interval distribution (Heart rate variability = original) and the third one with Gaussian interbeat interval distribution (Heart rate variability = original). None of the three surrogate heartbeats was clearly coupled with EEG phases in a frequency range between 0-80 Hz (Permutation test, corrected by multiple comparison, $p > 0.05$)). Moreover, PPC values were almost zero for all cases in all frequencies and without differences between groups (Figure 3)

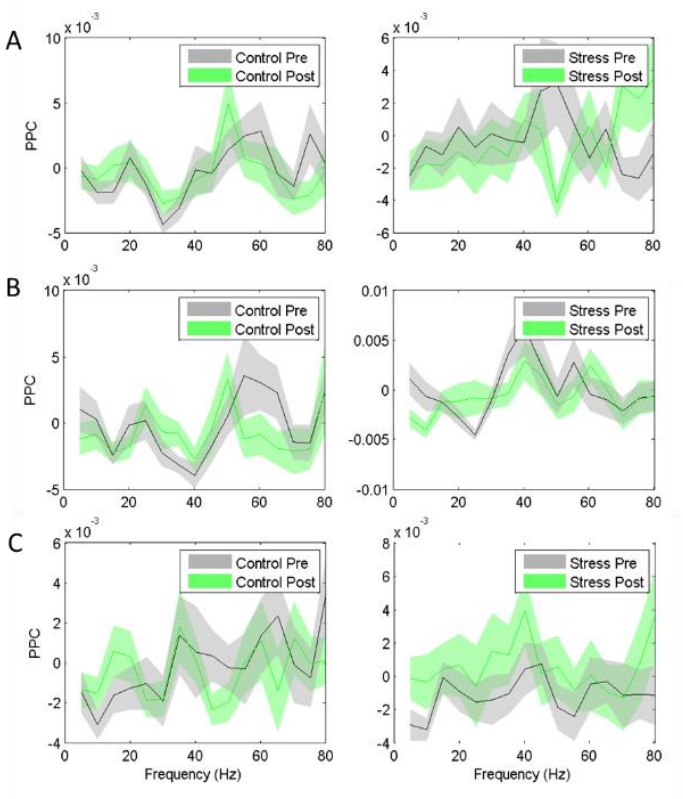

Figure 3l Mean whole brain Phase Pairwise Consistency spectrograms of surrogated heart signals with constant (A), random (B) and Gaussian variability distribution (C), during baseline and after the control protocol and the PSS induction. Shaded area indicated the SEM.

5.3.4 Control 3: Heart evoked potential (HEP) was not affected by PSS

Most of works assessing the relationship between heart and brain use the HEP as indicator [127,135]. The epoched and artifact free EEG data was used to calculate the R peak triggered average of potential for each subject [103]. As expected, there was an initial negative sharp deflection of potential locked to the R peak that was similar for both condition. Arround 200 millisecond there was a second broader negative deflection lasting until 400 millisecond. The features of this second deflection did not differed between groups (Permutation test, corrected by multiple comparison, $p > 0.05$) being similar in latency and amplitude (Figure 4).

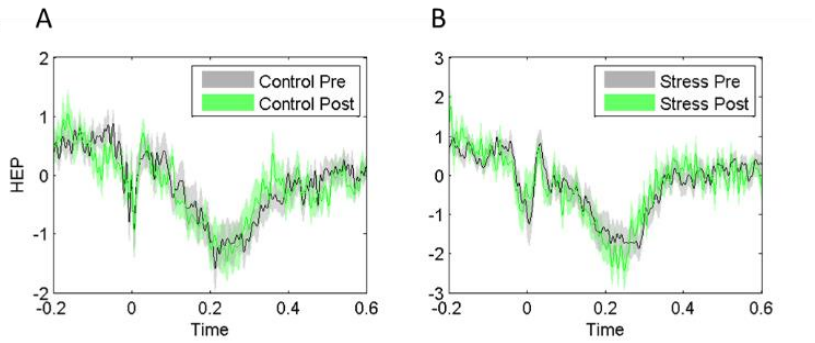

Figure 4l Central (C1, CZ, C3) Heart Evoked Potential (HEP) spectrograms using free of artefact EEG data during baseline and after the control protocol (A) and the PSS induction (B). Shaded area indicated the SEM.

5.4 Discussion

Our exploratory approach was firstly intended to assess whether the heart signal and brain oscillations were phase coupled at any specific frequency. We found a consistent phase coupling between R-peak and beta band brain oscillations (20-28 Hz) in controls and stressed participants during baseline and post-treatment resting state periods. Secondly, we wanted to analyze if the coupling was affected by PSS induction. Interestingly, we observed a significant decrease of the beta coupling after the PSS induction, which was unaffected after the control protocol (Figure 1). To our knowledge this is the first time that heart signal is associated with a specific oscillatory activity in the brain. The significance of the results and the interpretation of those was obscured by the lack of evidences in the heart-brain field. However, some controls were performed in order to make our results more reliable. In the current section, the results will be discussed in term of the controls we performed and possible interpretations will be exposed using indirect evidences as background.

While no works have associated the coupling we found with a cardiac artefact, studies assessing the heart evoke potential (HEP) have recognized the presence of a cardiac field artefact (CFA) [126,133,134,136]. In order to clean the EEG signal from the CFA, we calculated the PPC estimator over the independent component (After the ICA) and removed from the EEG data all the components

with a mean PPC in the frequency range of 12-30 Hz higher than 0.1[126]. Comparing the artefact free EEG signal (Figure 1) with the artefactual components, we observed that in contrast with the artefact-free EEG signal (values around 0.1), the artifactual components had PPC values higher than 0.4 at the peak (Around 25 Hz). The PPC values described by Vinck, et al. [131] in the original article were in the same range as artefact-free EEG data (0.1). Additionally, the groups differences observed using clean EEG data (Figure 1), were not present when the comparison was performed using the artefactual components (Figure 2), suggesting that the coupling disruption observed after the PSS induction was a reliable effect of brain activity and not of the CFA artefact. Moreover, if the brain-heart decoupling after PSS induction (Figure 1C) were due to the CFA, we would expect a similar decrease of amplitude in the HEP, however, as shown in Figure 4 there were no group differences neither at zero latency (generally associated with the CFA [136]) nor after 200 millisecond of the R peak (where the effects over the HEP are generally observed)[126]. Finally, we showed that the coupling and the groups differences were time-locked to the heart R-peak, as there were not present in surrogated data (Figure 3). Altogether the results suggest that the beta band coupling and decoupling after PSS induction is a reliable effect of brain activity associated with heart.

The locus coeruleus-dependent nor-adrenaline release seems to be a good candidate explaining the observed coupling. Anatomically, the LC sends projections and receives inputs from an extensive number of regions. In particular, LC innervates extensively the cortex in an excitatory way through the activation of α1-noradrenergic excitatory receptors present in pyramidal cells or to a lesser degree through the binding to α-2 noradrenergic inhibitory receptors presents on gabaergic interneurons. Additionally, LC affects the heart function through projections to parasympathetic vagal nuclei including the dorsal motor nucleus (DMN) of the vagus and the nucleus ambiguus (See 2.3.1.3 Samuels and Szabadi [137]), and sympathetic nucleus as the rostroventrolateral medulla (See 2.3.2 Samuels and Szabadi [137]). Interestingly, LC projections

induce in both cases an increase of heart rate via inhibition by α2-receptors, or through α1-receptors activation.

From a functional perspective, inputs from the LC are the responsible of the alerting network proposed by Petersen and Posner [65], involving regions of frontal and parietal cortices. Also, LC has been directly linked with the attentional ventral and dorsal networks proposed by Corbetta and Shulman [46]. The latter idea was extensively developed in the theory of Locus Coeruleus-Norepinephrine function [138]. They propose that LC neurons exhibit low levels of tonic activity during unaroused states facilitating sleep, moderate levels when the organism is engaged in a focused task and high levels when the organism is not committed to a task and is exploring the environment. Interestingly, the transitions between different tonic levels are enabled by cortical inputs from prefrontal regions that projects strongly to LC and are sensitive to task context. Thereby, LC tonic signals enables transitions between behavioral states depending on the environmental demands. Finally, the theory highlights that the top-down influences over ventral bottom-up networks may occurs directly or in directly via LC projections.

As was exposed by the theory of Locus Coeruleus-Norepinephrine function [138], the LC modulates the way attention shifts in relation to different tasks, environments and individual demands and also exerts it influences over the autonomic system. It could be that the LC-NE system comprises information from both the autonomic state (Heart rate) and the cortical attentional networks. Thereby, the LC-dependent coupling between the heart signal and cortical activity could be associated to a top-down monitoring of the body signals. Thus, the information from the autonomic system may be helpful at the moment of developing a specific task.

Regarding the decoupling observed after PSS induction, it is possible that as the heart and brain systems have different times to recover from PSS induction, thus explaining the uncoupling between them. The heart rate is back to baseline levels (Figure S1A), while the α1-noradrenergic receptors in the prefrontal cortex can stay activated during 30 minutes after the PSS induction [116].

Together these evidences favor the idea that increased levels of stress-related NE release induces imbalance of the brain-body communication.

At a philosophical level, Park, et al. [127] proposed that the relationship between brain and heart signals carry information of the subjective dimension of experience. In addition, they added that the ability to say 'I', requires the existence of a biological substrate for the definition of the organism as an entity. The same author developed that idea in more detail and propose the 'neural subjective frame' concept[139], as the basic biological mechanism defining the subject as a biological entity. The neural subjective frame was also described as a low-level block of subjective experience which is not explicitly experienced by itself but can underlie self-consciousness and subjective emotional feelings. In other words they proposed that an important part of perception, emotions and self-consciousness is sustained by the implicit and continuous monitoring of the state of the internal organs as heart, gut, etc. The mentioned theory is in line with the interpretation of our results in which there is a highly specific coupling between higher order cortical top-down activity and heart signal. It could be speculated that part of the top-down monitoring of attention, emotions, errors and inhibitions[101], also includes an implicit but continuous monitoring of the bodily state, this monitoring would be the basis of the neural subjective frame mentioned above. During PSS experiences it is likely that the deregulation that occurs at levels of attention, emotions etc., also includes deregulations of the bodily subjective frame.

Here we showed for first time that higher order brain mechanism includes information from the bodily changes, which was observed through the phase coupling between beta band oscillatory activity and heart R-peak. Interestingly, this coupling was disrupted after the PSS induction, suggesting that the top-down bodily continuous monitoring (Neural subjective frame) is as affected by PSS as other PFC functions as attentional control, error monitoring and emotional regulation.

The psychological perception of stress as such was demonstrated to be a limiting factor while coping with a cognitive task. The coupling between brain and viscera seems to be an innovative

and illuminating measure which might reflect in an implicit way how the brain is sensing the body changes and state. The development of studies directed to explore this coupling might contribute valuable information in the fields of stress and emotion.

5.5 Supplementary information

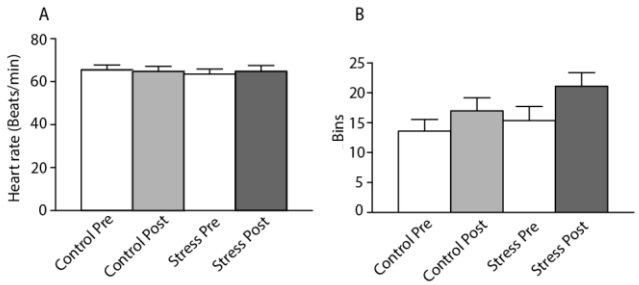

Figure S1l Heart rate (A) and Heart variability (B) during resting state periods for controls and stressed participants.

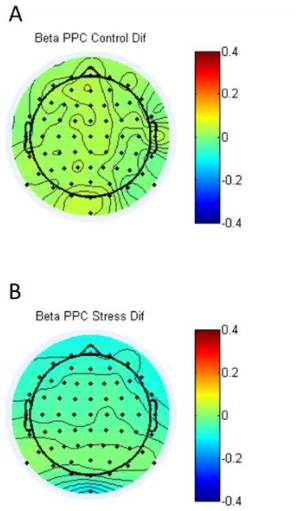

Figure S2l Topography of the differences of beta PPC for control (A) and stressed (B) participants.

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8 Publications

Palacios-Garcia I, Villena-Gonzalez M, Campos-Arteaga G, Artigas C, Jaramillo K, Silva J, Rodriguez E (2017). Immediate effects of psychosocial stress over attention depend on subjective experience and not directly on stress-related physiological changes. (To be submitted in Psychophysiology)

Palacios-Garcia I, Silva J, Bosman C, Rodriguez E (2017). Increased beta band activity as a coping mechanism during stress impaired goal-directed attention. (To be submitted in Scientific Reports)

Palacios-Garcia I, Silva J, Bosman C, Rodriguez E (2017). Brain-heart coupling at beta frequency is disrupted after psychosocial stress. (In preparation)

Palacios-Garcia I, Villena-Gonzalez M, Campos-Arteaga G, Artigas C, Jaramillo K, Silva J, Rodriguez E (2015). Psychosocial stress affects attentional control and neural oscillatory activity. Psychoneuroendocrinology. 2015 Nov;61:44-5. Epub 2015 Aug 8.

Conference presentations

Palacios-García, I., Villena-Gonzalez, M .,Campos-Arteaga, G., Artigas-Vergara, C., Silva, J., Bosman, C., Rodríguez, E. From mind to body: Assessing the relationship between stress markers and goal-directed attention. Europe Mind and Life Summer Research Institute, Germany, 2017

Palacios-García, I., Villena-Gonzalez, M ., Campos-Arteaga, G., Artigas-Vergara, C., Silva, J., Rodríguez, E. Psychosocial stress induces oscillatory activity changes during an attentional task reflecting different cognitive strategies. Annual meeting of the international society for Neuroscience. San Diego, United States, 2016

Villena-González, Mario., **Palacios-García, Ismael**., López, Vladimir., Rodríguez, Eugenio. Visual and auditory perception are differentially affected by visual imagery and inner speech. 23rd annual meeting of the cognitive neuroscience society. New York, United States, April 2-5.

Palacios-García, I., Villena-González, M., Campos-Arteaga, G., Artigas-Vergara, C., Jaramillo, K., Silva, J., Rodríguez, E. Can you control your attention when you are stressed? XI Annual meeting of the Chilean society of Neuroscience 2015

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