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Comparative analysis of acute stress responses in children: exploring behavioral and emotional differences between sexes

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ABSTRACT:

Stress is crucial for our well-being, and how we handle it relates with our vulnerability to develop mental health problems. However, its effect on preadolescents, particularly regarding sex differences, still raises unexplored questions. This final degree project compares the acute stress response in children from Sabadell, Spain (n=20, 10 girls, from 7 to 8 years), using the Trier Social Stress Test for Children (TSST-C). Stress perception is measured with the Self-assessment Manikin (SAM), stress behaviors via the OBSERVER XT, and facial expressions with the Py-Feat program. Results are correlated with the Strengths and Difficulties Questionnaire (SDQ) and the Social Phobia and Anxiety Inventory for Children-Parent report (SPAI-CP). The TSST-C effectively induced stress in children. Not many sex-differences were found except that girls exhibited a quicker subjective stress recovery than boys. Additionally, externalizing conduct problems were associated with less anger and surprise facial expression while social phobia and anxiety was associated with higher neutral and less anger expressions. This study underscores the necessity of investigating stress's impact on this age group and hints at potential disparities between sexes.

Key words: acute stress, TSST-C, children, subjective stress, emotion expression, stress behavior, sex differences.

RESUMEN

El estrés es crucial para nuestro bienestar, y la manera en que lo gestionamos está relacionado con nuestra vulnerabilidad a desarrollar problemas de salud mental. Sin embargo, su efecto en los preadolescentes, particularmente en lo que respecta a las diferencias de sexo, aún plantea incógnitas sin explorar. Este trabajo de fin de grado compara la respuesta al estrés agudo en niños de Sabadell, España (n=20, 10 niñas, de 7 a 8 años), utilizando el *Trier Social Stress Test for Children* (TSST-C). La percepción del estrés se mide con el *Self-assessment Manikin* (SAM), los comportamientos de estrés a través del OBSERVER XT y las expresiones faciales con el programa Py-Feat. Los resultados se correlacionan con el Cuestionario de Fortalezas y Dificultades (SDQ) y el Inventario de Fobia Social y Ansiedad para Niños-Informe de Padres (SPAI-CP). El TSST-C indujo efectivamente estrés en los niños. No se encontraron muchas diferencias de sexo, excepto que las niñas mostraron una recuperación subjetiva del estrés más rápida que los niños. Además, los problemas de conducta externalizantes se asociaron con menos expresiones faciales de ira y sorpresa, mientras que la fobia social y la ansiedad se asociaron con una mayor expresión neutral y menos expresiones de ira. Este estudio remarca la necesidad de investigar el impacto del estrés en este grupo de edad y sugiere posibles disparidades entre los sexos.

Palabras clave: estrés agudo, TSST-C, niños, estrés subjetivo, expresión emocional, comportamiento de estrés, diferencias de sexo.

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1. INTRODUCTION

This study aims to evaluate and compare acute stress responses in a psychological procedure by using the Trier Social Stress Test for children (TSST-C) between healthy girls and boys. We measured self-appraisal, behavioral responses and facial emotional expressions over the stress task. We also explored the association of these parameters with externalizing conduct problems and social phobia and anxiety.

1.1 Brief history, evolution of the term and paradigms

Stress is a multifaceted phenomenon examined through various perspectives and theoretical models that currently holds a key role influencing our well-being. Before coining the term, Claude Bernard (1813-1878) introduced the concept of an internal environment enveloping cells - which he termed *"milieu intérieur"* - sustained through a constant process of compensatory adjustments in bodily functions. In 1929, Walter B. Cannon (1871-1945) introduced the term "homeostasis" and postulated that upon a challenging situation the activation of the sympathoadrenal system is triggered and acts as a cohesive functional unit, meaning its components work together as a single entity to perform a specific function or achieve a common goal (Cooper, 2008). The term "stress" was later incorporated by Hans Selye (1907–1982), who described it as a "nonspecific response of the body to any demand" in his book *The stress of life* back in 1956 (Tan & Yip, 2018). Years later, Selye proposed three universal stages of coping with a stressor - the "General Adaptation Syndrome" or GAS - consisting of an initial "alarm reaction", analogous to Cannon's *"fight or flight"* response, a stage of adaptation and, eventually, a stage of exhaustion. This paradigm would suggest the necessity to differentiate "good" stress, categorizing it as "eustress" from "bad" stress, "distress" in 1975, defending that stress is not only a process for threats to health and life but also ensures adaptation to the existing environment and anticipating future challenges (Lu, Wei & Li, 2021). Then, stress is considered the process of challenge to the state of homeostasis, where the *stress response* is the compensatory mechanisms aimed at restoring homeostasis and adapting to biological, psychological, and/or social situations, and a *stressor* is the stimulus that causes this process to occur (Achotegui, 2007). As mentioned previously, if the organism's homeostasis is strongly challenged, a state of extreme discomfort, unease and anguish that can adversely affect an individual's well-being will happen. This is known as *distress,* or negative form of stress typically negative and harmful. On the opposite side, *sustress* refers to an inadequate stress, where homeostasis is not challenged due to a lack or inadequacy of stressors. Such a situation might reduce the resilience of homeostasis, diminishing its ability to adapt and posing a risk to overall health. Lastly, if homeostasis is moderately challenged, *eustress,* it can induce a mild stress response that increases the capacity of homeostasis and benefits health (see in Figure 1, Lu, Wei & Li, 2021). The type of stress and the effectiveness of the *stress response* determine whether it facilitates the restoration of health-promoting homeostasis (positive effects) or leads to bodily harm or diseases (negative effects).

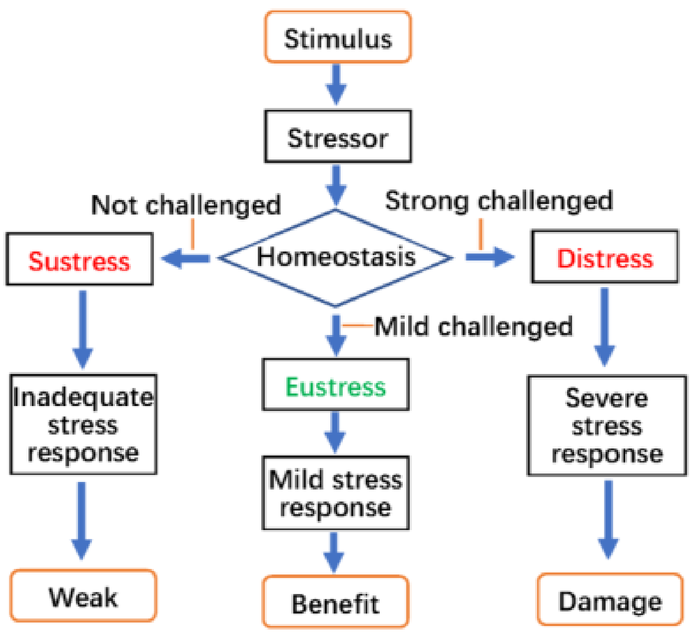


Fig. 1: *Framework of the stress system. (Lu, Wei & Li, 2021).*

It is also important to differentiate acute stress from chronic stress, for which the first will be the main focus of this study. Acute stress refers to a short-term response to an immediate stressor. It is an adaptive reaction which aims to prepare the body to survive, triggering the *"flight or figh*t" response, and subsiding once the stressor is no longer perceived or has ceased. On the other hand, chronic stress refers to a prolonged and ongoing state of stress arousal that persists over an extended period as there is, or the organism perceives, a persistent stressor over time. Like intense acute stress, this persistence is desadaptative as the organism doesn't possess the resources to adapt to the situation and may contribute to problematic health conditions due to prolonging the state of stress (Achotegui et al. 2007 & Armario et al. 2000).

1.2 What is Stress?

It is widely acknowledged to define "stress" as wide physiological and behavioral changes, which have evolved over time, in response to circumstances that pose a threat to one's physical or psychological well-being. These stress-inducing events may manifest as real, anticipated or symbolic and cannot be successfully handled under the normal homeostatic mechanisms. Human experiences of stress are diverse and can manifest in various ways such as general life stressors which include challenges like having a family member in poor health or transitioning between jobs. There are more extreme events, such as terrorist attacks or natural disasters that constitute catastrophic stressors. Childhood maltreatment is another significant source of stress, along with how some individuals may face minority stress. This spectrum of stressors highlights the complex and multifaceted nature of stress in the human experience. The World Health Organization ([WHO], 2023) defines the term as "a state of worry or mental tension caused by a difficult situation. (...) The way we respond to stress, however, makes a big difference to our overall well-being". This statement is of the utmost relevance as individual differences in acute stress responses and its relationship with mental health are central themes in this project, with emphasis on acute stress in children. Because, even if stress serves as a crucial mechanism for adapting to the demands of our environment, outcomes lie in how well this adaptive process functions. An inadequate or excessive stress response can be associated with heightened vulnerability to the development of various health and psychopathological conditions while an adequate stress response allows us to adapt successfully in face of adversity (Kumar et al., 2013, Joos et al., 2019 & Ripoll, 2011). This raises the question of: what really influences how we react to stress? And why do people handle the same stressful situations in such different ways? The answer is that stress is a multifactorial process and its response is subjected to a variety of characteristics from either the stressor, the subject who experiences it and the context involved during the process (Kumar et al., 2013). When finding ourselves in a stressful situation, in order to react we first need to evaluate the stimuli and decide if we ought to consider it a stressor and react accordingly. This cognitive assessment is known as *appraisal* and it involves the subjective interpretation of the significance and potential impact of a stressor on one's well-being (Lazarus & Folkman, 1984). The appraisal process is highly individualized and influenced by various factors such as our personal experiences, genetics, and the environment we grew up in, as they result in the individual differences that determine the diversity in stress appraisal related to diversity in physiological and behavioral response. For instance, interaction between genetic predisposition and childhood trauma can cause vulnerability to mental disorders. This means having more difficulty to cope with excessive stressful circumstances resulting in a higher probability of developing a psychopathological condition. However, this vulnerability wouldn't manifest in stress-free circumstances at adulthood (Johnson et al., 2019). As mentioned previously, stress is a process which includes affective, cognitive, behavioral and endocrine factors. On an affective level, the person experiences a variety of emotional responses that can oscillate depending on the nature of the stressor, individual coping mechanisms, and past experiences. These emotional responses are an essential part of the overall stress response, contributing to the individual's emotional subjective appraisal of the situation and influencing subsequent behavioral adaptations. On a behavioral and cognitive level, an individual response to stress is often influenced by a wide range of psychological variables such as learned behavior, cognitive appraisal, and the interaction between the subject and their environment. The individual may perform, for example, cognitive processes such as rumination or health-risk strategies oriented toward reducing the tensions by avoiding dealing directly with the problem. Avoidance coping is involved in the association between coping efforts and emotional distress, and is generally linked to more depressive symptoms (Holahan et al., 2005). On the other hand, behavioral coping strategies may be vocal, as some individuals become more talkative, or motor, such as freezing, fidgeting, involuntary movements or looking for social support as relying on others. Facial expressions can also be shaped by stress (Mayo et al., 2019).

1.3 Stress: A physiological response

On a physiological level, our body has specific responses for each type of challenge to the homeostasis. Systemic stimuli (e.g. infections, temperature extremes, toxins, allergic reactions…) involves a specific physiological response to be solved, but when a challenge is strong the specific responses are not enough to deal with it. In such cases, a general stress response is added to the specific ones involving releasing certain substances which will prepare the body for immediate and intense activity and help the body recover after the stress is over. Additionally, the stress response helps the body remember the situation, so it can better handle similar challenges in the future. Regardless of the particular nature of the stressor, the stress response involves the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis. The HPA axis primarily mediates the slower and more prolonged stress response, while the SAM axis initiates a rapid response, promoting alertness and vigilance in the face of stressors. The efficacy of these two axes as valuable biological markers has been demonstrated, exhibiting a proportional response to stress intensities (Armario et al., 2020). The duration of stress exposure, along with specific stressor characteristics, not only influences the peak response but also establishes a positive correlation with the duration of activation of the HPA axis during the post-stress period or recovery phase. Specifically, the magnitude of reactivity in the acute stress response is quantified by assessing the change from baseline to peak levels of neuroendocrine enzymes, such as alpha-amylase (SAM) or hormones, such as cortisol (HPA), as elucidated in the subsequent sections. The hypothalamus is the crucial brain structure which regulates homeostasis, specifically the paraventricular nucleus of the hypothalamus (PVN). Part of the hypothalamus connects with the autonomic nervous system (SAM) and enteric nervous system (ENS), controlling involuntary functions like heart rate and blood pressure, integrating autonomic and neuroendocrine responses to preserve homeostasis. A different part of the PVN can also activate the HPA axis. In some circumstances, both axes are co-activated while they can also function separately. The activation of the SAM axis begins with the activation of the hypothalamus. The activation signal then traverses down the sympathetic neurons within the spinal cord, reaching the postganglionic neurons, which subsequently release noradrenaline. This chain ultimately leads to the secretion of the alpha-amylase, an enzyme involved in the breakdown of carbohydrates (see Figure 2). Alpha-amylase is released not only from the salivary glands but also from other tissues, and it contributes to the mobilization of energy resources by breaking down complex carbohydrates into simpler sugars (Yamane et al., 2023). Furthermore, the activation of the hypothalamus also induces the release of catecholamines, including adrenaline and noradrenaline, from the adrenal medulla. These crucial hormones enter the bloodstream, initiating a chain reaction of physiological responses aimed at rapidly preparing the body for immediate action. Adrenaline and noradrenaline exert their effects on diverse target organs and tissues, triggering a spectrum of changes such as an increase in heart rate and blood pressure, sudoration, dilatation of pupils, redirection of blood flow to vital muscles, and a rise in respiratory rate. Moreover, the SAM axis accelerates the mobilization of energy reserves, ensuring the prompt liberation of glucose and fatty acids into the bloodstream. This quick energy release is designed to fuel the escalated metabolic demands associated with the "fight-or-flight" response. The orchestrated activation of the SAM axis plays a critical role in enhancing physical preparedness and optimizing the body's capacity to effectively confront and manage stressful situations, thereby enabling adaptive responses in challenging circumstances. Upon activation of the HPA axis, the PVN releases the neuropeptide corticotropin-releasing hormone (CRH) into the bloodstream. This CRH stimulates the anterior pituitary gland to secrete the adrenocorticotropic hormone (ACTH), which in turn prompts the adrenal cortex to release glucocorticoid hormones into circulation, which facilitate the entrance of glucose in tissues to activate a better adaptive response. One of the principal glucocorticoids released is cortisol, and it peaks between 20-30 minutes after the introduction of the stressor. This hormone exerts a widespread influence on almost every organ and tissue in the human body while performing functions such as regulating energy expenditure, suppressing inflammation, elevating blood pressure and glucose levels, etc... Consequently, cortisol is commonly used as a biomarker of low and moderate stress, as in high-stress situations the adrenal cortex becomes saturated and most of the ACTH doesn't translate to cortisol (Allen et al, 2016). Furthermore, glucocorticoids function as part of a negative feedback loop (see Figure 2). In circumstances where cortisol levels are low, it mostly binds to high-affinity mineralocorticoid receptors (MR). However, during elevated levels of cortisol such as after stress exposure, it increasingly binds to glucocorticoid receptors (GR) which exerts inhibitory control over cortisol synthesis by suppressing the activation of the HPA axis activation through this feedback loop, restoring the normal homeostatic levels. This functions habitually under resting conditions or in acute-stress situations (specially). When discussing chronic stress and the prolonged activation of the HPA axis, these mechanisms often become dysregulated, leading to a surge in cortisol levels and, consequently, significant health negative aftermaths (de Voogd et al., 2022).

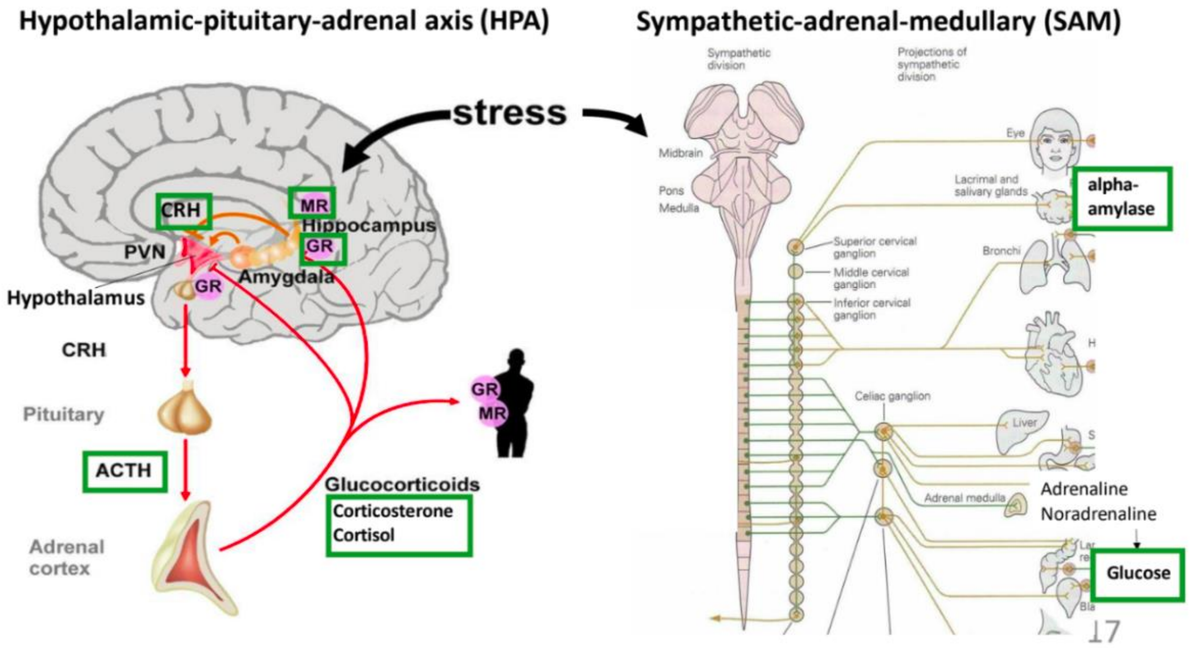


Fig. 2: *On the left side, illustration of the hypothalamic-pituitary-adrenal (HPA) axis (CRH; corticotropin-releasing hormone, ACTH; adrenocorticotropic hormone). Arrows represent secretion and/or stimulation, as CRH stimulates the pituitary gland to secrete ACTH, and ACTH stimulates the adrenal cortex to secrete cortisol. The negative feedback loop is shown, as cortisol levels stimulate the hypothalamus and decreases ACTH and CRH's secretion by binding to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). On the right side, illustration of the sympathetic-adrenal-medullary (SAM) axis. Arrows represent secretion and/or stimulation as the hypothalamus stimulates the adrenal medulla. Postganglionic neurons are activated and secrete noradrenaline, which promotes alpha-amylase secretion. Parallzelly, hypothalamus activation results in noradrenaline and adrenaline secretion, which increases glucose levels as a mechanism to obtain energy to execute the stress response (Nadal, 2023).*

All in all, this physiological response provides a coordinated reaction that allows for both an optimal strategy and homeostatic restoration by stimulating energy mobilization, metabolic alterations, immune system stimulation, and inhibition of the digestive and urinary organs on a systemic level. Not only the involvement of these axes is important for the acute stress response, but the co-activation of the HPA axis and SAM axis play a key role. These two systems have been found to be co-activated during stress response and its asymmetry or uncoordination has been associated with negative outcomes such as social adversity, antisocial or aggressive behaviors, drug addiction and eating disorders. Specifically, psychopathology manifestations associated with asymmetry in either form has been identified when Low-HPA is paired with High-SAM, and conversely. Such combinations are associated with higher scores in the Child Behavior Checklist (CBCL) for externalizing and internalizing domains. This indicates that a dissociation between the axes is correlated with a greater frequency or intensity to problems such as depression, somatic complaints, anxiety, social withdrawals, rule-breaking behavior, aggression and attention difficulties (Wadsworth et al., 2019 & Pham et al., 2023).

2. THE TSST-C

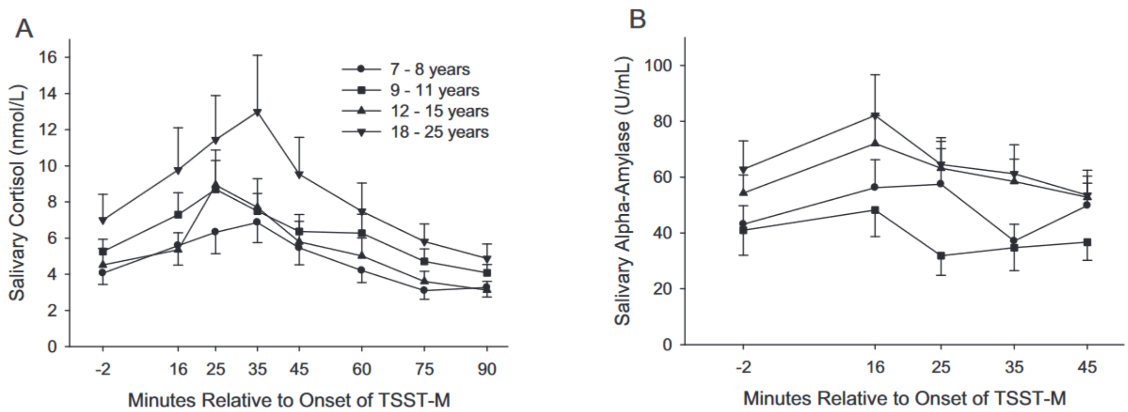
As previously stated, this project aims to investigate the stress response in children with the Trier Social Stress Test for children (TSST-C) and compare the results between sexes. Originally developed in 1993 by Kirschbaum, Pirke, and Hellhammer, the TSST was specifically designed to elicit acute stress within a controlled experimental setting. Notably, this test has demonstrated its capacity to trigger activation of the hypothalamic-pituitary-adrenal (HPA) axis, verified via saliva samples to evaluate cortisol levels and other hormones (salivary or plasmatic) as well as showing an increase in subjective stress such as the appraisal (Hellhammer & Schubert, 2012). The TSST is also recognized for its efficacy in characterizing individual differences across a broad age spectrum, while also demonstrating predictive utility. Another notable consideration is how the test could serve as a valuable tool for estimating the effectiveness of interventions, allowing for a comparison of pre- and post-intervention stress-response measurements. In the standard TSST protocol, participants are instructed to deliver a presentation in an interview-style setting, followed by a mental arithmetic challenge in front of an interview panel, introducing the element of social-evaluative threat. Other variations of the TSST have been introduced such as the TSST-G, for groups instead of a single participant, and TSST-C, specifically adapted for children and the test used for this study. The process will be explained later. The TSST-C also provides multiple parameters in order to measure stress response. Biological markers gathered through saliva samples are used to gather data about cortisol and sAA (salivary alpha-amylase) levels. Along with that, visual and behavioral data (*in situ* by the panel of experts and recorded by a video recorder and microphone) is collected. This provides information related to the behavioral response of the children during the tasks. While the TSST-C is meticulously standardized, it is crucial to consider the influence of physiological and psychological factors on the acute stress response. For instance, state covariates such as medication or drink intake can affect physiological markers and thus provide inaccurate data by contaminating the salivary samples (Allen et al., 2016).

3. STRESS IN CHILDREN

Stress is the main non-genetic factor of vulnerability to mental disorders. According to the World Health Organization ([WHO], 2023), one in seven 10 to 19 year-olds, an alarming number of children and adolescents across the globe, experience stress-related disorders, including anxiety and depression, at an early age. These data underscore the urgency to comprehend the root causes and mechanisms underlying childhood stress, enabling the implementation of strategies to lessen its adverse effects and promote resilience.

2.1. Age differences in stress

Looking at biological markers, longitudinal studies in young has shown how a blunted cortisol response in adolescents predicts at adulthood substance abuse in general, nicotine use and antisocial behavior (Evans et al., 2016), especially in boys with high testosterone levels (Susman et al., 2017). Meanwhile, higher cortisol reactivity/recovery predicts at adulthood suicidal ideation and depressive symptoms. In another study, in females only, higher peer stress predicted suicide attempt in those with blunted cortisol responses (Eisenlohr-Moul et al., 2018, and Shalev et al., 2019). More investigation regarding cortisol hyper-activity suggests early-life adversity as a possible cause in the short-term while it leads to cortisol hypo-reactivity in the long term (Hosseini-Kamkar et al., 2021, Johnson et al., 2019 & Reilly et al., 2019). Cross-sectional research suggests that high levels of exposure to uncontrollable stressors, such as community violence and less family support, are also associated with blunted cortisol activity (Joos et al., 2019). Not always a blunted response is related to externalizing problems. Hyperresponsive adolescents, who showed a hyperactivation of the HPA axis have been reported to show greater emotional (internalizing) and behavioral (externalizing) problems (Bendezú et al., 2021). In addition, children seem to show a lower response than adults during an acute stress laboratory test on a physiological (lower levels of cortisol and salivary alpha-amylase) and subjective level (see Figure 3; Yim et al., 2015) and on coping strategies. Adolescents have reported to use more voluntary engagements than children, who use more emotion suppression and voluntary disengagement. This supports the hypothesis that adolescent's cognitive development allows them to approach challenges in a more engaged way (Johnson et al., 2019).



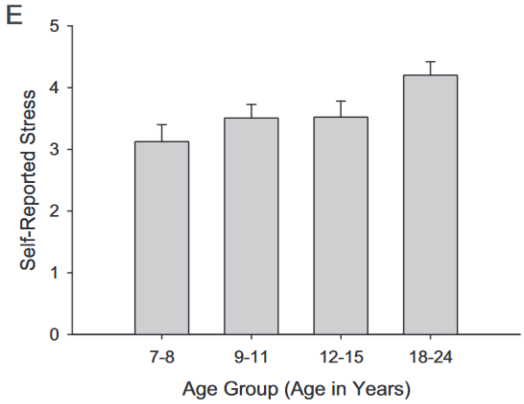


Fig. 3: *Children (n=22; 7-8 years, 9 female) show lower stress response in salivary cortisol samples (A), salivary alpha-amylase (B) and self-reported stress (E) in comparison to other age groups during the TSST-M (Trier Social Stress Test-like procedure; Yim et al., 2015).*

In light of the above, not only studying stress responses at an early age can be useful to predict the probability of having dysregulation in the future or show specific behaviors, but also as a way to identify non-adaptive stress responses and design an effective intervention. In the present day, despite the worsening conditions and problems faced by children, along with rising rates of emotional and behavioral issues, investigation on psychosocial stress and its relation to maladaptive outcomes in childhood has lagged behind similar research with adults. (McMahon et al., 2003 & Confederació SALUD MENTAL ESPAÑA, 2022)

2.2. Sex differences in stress

It is crucial to highlight the important gap of research in current research concerning sex-based differences in acute stress response during childhood, which remains despite the growing body of evidence underscoring notable distinctions in stress responses between sexes in adulthood and differences between age groups. Cortisol responses in adults are higher in men than in females and the similar results can be observed when exploring ACTH responses in adults (see Figure 4; Uhart et al., 2006 & Kudielka et al., 2004b).

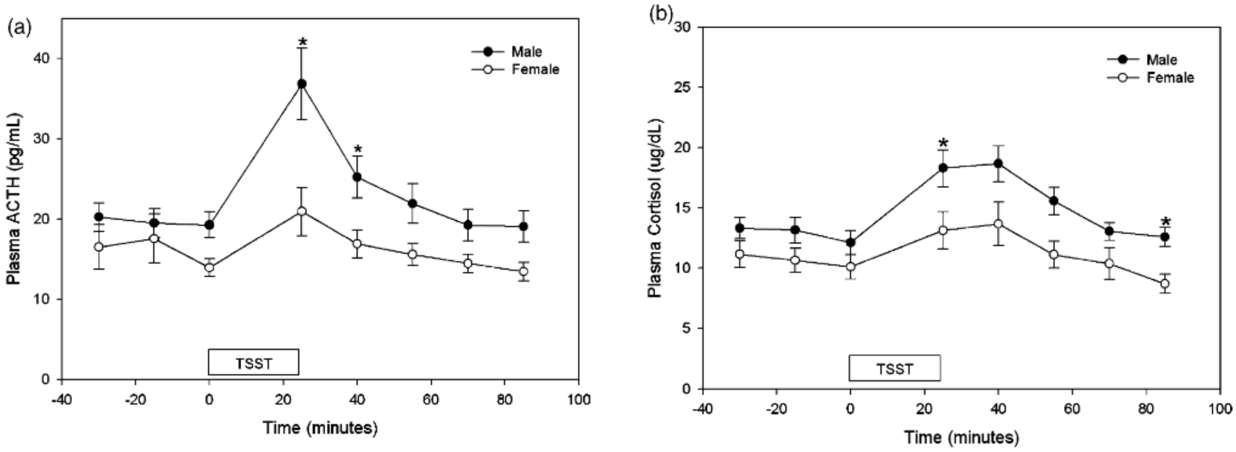


Fig. 4: *(a) Plasma ACTH (pg/mL) response to TSST (a test for acute-stress where the participants must do a public speaking and mental arithmetic exercises in front of a jury) by sex. Values reflect unadjusted means (SE). *Plasma ACTH levels (ug/dL) differed significantly by sex at the following time points: 25 min, p=0.007; 40 min, p=0.021. (b) Plasma cortisol response to TSST by sex. Values reflect unadjusted means (SE). *Plasma cortisol levels differed significantly by sex at the following time points: 25 min, p=0.027; 85 min, p=0.006 (Uhart et al., 2006).*

We can also find differences in cardiovascular regulation. Males have shown more vasoconstrictive response, followed by a less vasodilatory response, and longer time to return to baseline level than the females during a stressful task consisting of a mental arithmetic test (Chen et al., 2012). When focusing on heart-rate, elderly people reveal no sex-differences. However, adult women were found to have a larger heart rate response to the TSST than men. These studies suggest that men are more prone to be vascular reactors whereas women are more cardiac reactors maybe due to physiological and endocrine factors (Kudielka et al., 2004a). On a more behavioral level, stress responses in males tend to be “fight or flight” while, in women, it is “tend and befriend” due to evolutionary, or maybe cultural, mechanisms (Wang et al., 2007, Youseff et al., 2018 & Eschenbecket al., 2018). Regarding this aspect, Mayo and collaborators (2019) associated higher observation of angry facial expressions in men during a stressful situation whereas, in women, displays of anger weren't observed even though they self-reported greater anger than men. This adds up to the “tend and befriend” theory, perhaps increasing the likelihood of social approach under stressful conditions. Another study by Blasberg et al. (2023) found that women showed a higher tendency to smile during stressful situations in comparison to men. Regarding subjective stress, no differences between male and females have been found when using a non-psychological stressor (Goldfarb et al., 2019) or using the TSST-VR in adults (Liu & Zhang, 2020).

In children, sex differences in HPA axis reactivity appear to be present as girls show higher cortisol response to social stress tests, contrary to results in adults (DeVeld et al., 2012, Hostinar et al., 2015 & Ji et al., 2016). When looking at cardiovascular responses, another study by Kudielka et al. (2004a) showed girls had a significantly higher heart-rate during and after the stress task, but not previously (see Figure 5). So, it would be expected for girls to show higher physiological reactivity in the social stress task if this current study included a physiological measure.

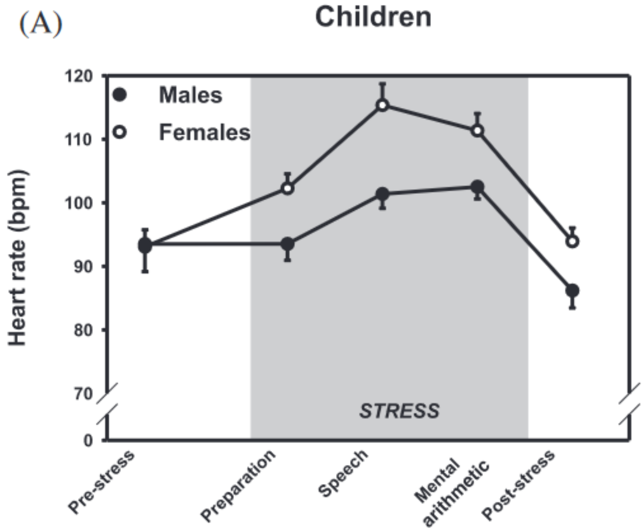


Fig. 5: *Graphic illustrating heart rate levels before, during and after the stressor (speech and mental arithmetic) between male and female children (n=31, 15 girls; age range: 9–15). Means and SEM (standard error of mean) are shown. Statistically significant differences in heart rate during the stressful task (speaking in front of an audience) were found (Kudielka et al., 2004a)*

Regarding behavioral response, similar results as the studies in adults can be observed as girls resort to seeking social support and problem solving more than boys, who tend to avoid coping, or use avoidance as a coping mechanism (Eschenbeck et al., 2018). The same can be observed at facial emotional expressions. Girls show lower displays of anger, and other externalizing emotions, along with higher displays of positive emotions in comparison to boys (Chaplin et al., 2013). That's why we expect boys to show emotions promoting avoidance such as anger, whereas girls should show more prosocial facial expressions such as happiness. However, sex-differences in emotion expression could be moderated by age, task valence and interpersonal context (Chaplin et al., 2013). Additionally, facial muscle activity, such as eyelid tightening, has been associated with greater subjective stress reactivity which leads us to wonder if there might be an association between perceived stress and expression of facial emotion (Blasberg et al., 2023). Regarding subjective response, girls show higher perceived stress after exposure in comparison to boys (Raffington et al., 2020). However, when in a silent environment, girls have been seen to recover faster than boys after performing a stressful task (Shu et al., 2020). This implies that factors, such as the soundscape, could influence subjective stress levels in children, and these could vary depending on sex. Besides, psychological differences between sexes at this age have been observed, as girls report more fear of failure and criticism than boys. Therefore, the socio-evaluative nature of the stress task could make the task more stressful for girls (Gullone et al., 2000). In this study, we expect girls to show higher subjective stress as all participants shared similar recovery conditions.

2.3. Objectives and hypothesis

The main objective of this study is to examine and compare acute stress responses, during the Trier Social Stress Test for Children (TSST-C), in participants aged 7 to 8, focusing on sex differences. Firstly, we expect the TSST-C to increase stress appraisal in comparison to baseline. Secondly, due to the fact that girls seem to have a higher HPA axis and subjective response on stressful conditions, we expect to observe higher perceived stress in girls (Kudielka et al., 2004a, Hostinar et al., 2015, Ji et al., 2016, DeVeld et al., 2012, Poole et al., 2016, Burkholder et al., 2015 & Raffington et al., 2020). However, no research has been found exploring sex differences in anxious behavior in children, such as self-touch or fidgeting (Burkholder et al., 2015). Therefore, we expect girls to show higher frequency of socially anxious behavior during the stress task based on their higher physiological and subjective responses. Additionally, given the fact that children with social phobia and anxiety have been observed to show a higher subjective anxiety, we also expect to find an association between the social phobia and anxiety and higher perceived stress during the study (Krämer et al., 2012 & Stadelmann et al., 2017). Moreover, children with social anxiety have been found to express less facial emotions, compared to non-anxious children in a non-stressful situation (Melfsen et al., 2000). No studies about facial emotional expression during acute stress response in children with social phobia and anxiety were found. Apart from that, Stadelman et al., (2017) found no differences in subjective stress between children with externalizing conduct problems and healthy children, even if externalizing problems have been associated with lower activation of the HPA axis (Marsh et al., 2008 & Benzedú et al., 2021). So we hypothesize that we will find no association between externalizing conduct problems and subjective stress. Regarding facial emotional expressions, not much literature is found in children, as studies exploring facial emotional expressions during the TSST-C are not common. However, boys with conduct problems have been seen to show no correspondence between facial expressions and autonomic reactivity while, in the control group, an association was found between sad expressions and autonomic reactivity (Marsh et al., 2008). In addition, it is of interest to study possible relations between facial emotional expression and externalizing conduct problems, as Grabell et al., (2020) found facial expression with eye constriction showed clinical utility as an indicator for irritability and externalizing behaviors in children. Moreover, boys have been found to express more externalizing conduct problems than girls during childhood (Tiet et al., 2001, Kovess-Masfety et al., 2021 & Álvarez-Voces & Romero, 2024). So, we expect to observe higher scores in the externalizing conduct problems subscale of the SDQ in boys.

4. MATERIALS AND METHODS

4.1. Participants characteristics

The participants, 20 subjects (50% females) with ages 7 to 8, were part of a longitudinal and epidemiological study still ongoing (from the Universitat Autònoma de Barcelona, UAB) about the development of behavioral and emotional problems in youth (Grup Laboratori Humà, more information available at https://webs.uab.cat/labhuma/). This general population sample was recruited voluntarily and anonymously from phase 1 of a longitudinal study at public and private schools from a city in Catalunya. The eligibility criteria was, fundamentally, age (children in their first primary school year at the first assessment). As for the exclusion criteria: Participants taking medications affecting the endocrine system, children diagnosed with intellectual disabilities, severe psychiatric disorders, severe neurodevelopmental disorders, visual or auditory impairments (subjects lacking hearing aids or corrective devices hindering normal conversation) and endocrine diseases that may interfere with salivary cortisol levels (such as Addison's and Cushing's) were excluded from the study. Those undergoing antibiotic treatment due to severe infections were rescheduled. Health-related screening interviews were also carried out through both telephone and in-person sessions with the mother, father, or caretaker, conducted by an investigator. Regarding ethical aspects of the study, all participants were informed of the implications of the study and gave their oral assent plus informed consent from the parents, and all investigators provided the certificate of negative sexual offenses.

4.2 Procedures

Participants were accompanied by their parents to the meeting point, where Researcher A guided the family to the facilities designated for the corresponding session. In session 1 the parents participants answered the Social Phobia and Anxiety Inventory for Children-Parent Report (SPAI-CP) and completed other tests for the original study. In session 2, they met with Researcher B (rB) in room 1 (R1), who conducted a brief interview addressing the participants' health and collected measurements of the child. Once completed, parents proceeded to another room (R2) to answer questionnaires with Researcher A (rA). Meanwhile, the child remained with Researcher B, engaging in a 30-min habituation period involving drawing, painting, and watching animal videos for relaxation. This habituation period ensures that there are no interferences between the stress of being in a new environment (laboratory) and the stress originated from the TSST-C. After the habituation period, Researcher B escorted the child to another room (R3) where the TSST-C would take place. Scores for the Strengths and Difficulties Questionnaire (SDQ) were obtained on the first session, approximately two weeks prior to the second session (see in Figure 6).

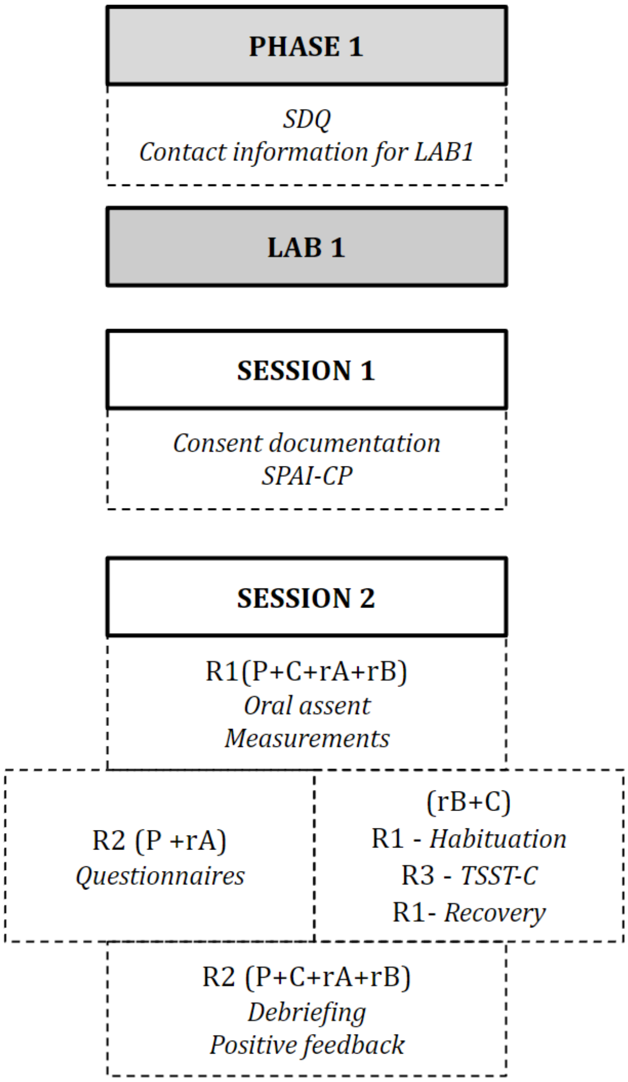


Fig. 6: *Visual representation of the longitudinal study. The SDQ scores were obtained during Phase 1 along with contact information for the rest of the study. The next phase, Lab 1, consisted in session 1 and 2. In session 1 consent documentation was filled and SPAI-CP scores obtained. In session 2, the TSST-C took place. R1=room 1, R2=room 2, R3=room 3, rA=researcher A, rB=researcher B, P=parent/s, C=child.*

The version of the TSST-C was used as a laboratory social and cognitive stressor to induce acute stress in the participants. Before beginning the test, Researcher B explained the tasks the participants would perform. Afterwards, Researcher B would leave the room and return 15-min later, once the TSST-C had finished, and return to the first room (R1) to start the recovery period of 30-min and the debriefing period of 15-min, where they were given positive feedback about their work and compensation for their time and participation in the study. Children answered the SAM at arrival (min -30), before the beginning of the TSST-C (min 0, baseline measure), during the speech task (min 7 '30), during the arithmetic task (min 12' 30) and during the recovery1 (min 30) and recovery 2 (min 45). Through the session, saliva samples were taken (see Figure 7).

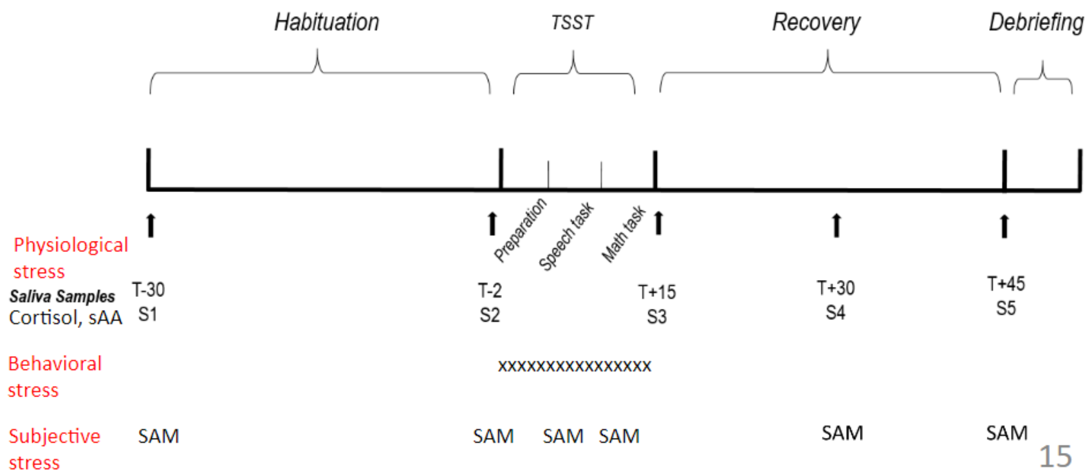


Fig. 7: *Timeline of events, Self Assessment-Manikin (SAM) responses and salivary samples (S). The time scale begins at the start of the TSST-C (T) that consists of a preparation, speech task and math task.*

The TSST-C consisted of a public speaking and arithmetic task that was performed in front of two judges (one male and one female) that remained without giving either positive or negative feedback. A video camera was placed to record their facial expression and their movements. Children were told to imagine a hypothetical situation where they were in a new class and had to talk about themselves to the panel of investigators while trying to hold eye contact with the panel. Additionally, they were told that they would compete amongst the other participants and the judges would choose the best performance. They were given 5-min to prepare the speech and they had 5-min for the speaking task. If a participant did not freely fill the whole 5-min of the task and stayed silent for 15 seconds the judges would prompt them to continue. If they still stayed silent a second time, the judges would ask them pre-prepared questions about themselves. For the arithmetic task, participants were instructed to count backwards beginning from a 100 without making any mistakes (e.g. 100, 99, 98, 97...). If the participants answered wrong, a judge would ask them to start over from the beginning. At the third mistake, the participants were told to do the same task but starting from 40. If a participant failed to execute the simplified task accurately as the protocol (e.g. weren't able to subtract correctly from either 100 or 40), the task was substituted by an alternative which consisted of adding from 0 to 100, incrementing by 1.

4.3. Measures and materials

The materials and instruments used for this study include:

4.3.1. Self-assessment Manikin (SAM)

The SAM is a pictorial scale composed of 5 points (range 1 calm or relaxed - 5 excited or stressed) useful so that the participants can rate their perceived levels of stress during the process (Bradley & Lang 1994). For this study, the arousal dimension was used (see Figure 8). Each participant was given a brief explanation about the scale and was asked to rate their perceived levels of stress a total of 6 times during the session. The first measure was taken at *arrival* (SAM1 at minute -30, as the minute 0 was the start of the TSST), the second one before the TSST-C (SAM2, *baseline* measure), measures taken during *speech task* and *math task* followed during the TSST-C (SAM3 and SAM4 at minutes 0, 7'30 and 12'30) and twice more after the test, consisting of *recovery 1* and *recovery 2* (SAM 5 and SAM6 minutes 30 and 45).

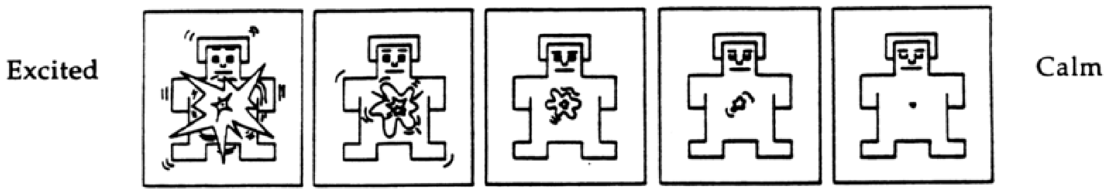


Fig. 8*: Image of the Self Assessment Manikin or SAM, by Bradley, M. M., & Lang, P. J. (1994).*

4.3.2. Strengths and Difficulties Questionnaire (SDQ)

Parent's participants answered the external conduct problems scale from the Strengths and Difficulties Questionnaire (SDQ) designed by Robert Goodman at the Institute of Psychiatry in London (1997). This study used the Spanish version first validated by Rodríguez Hernández et al. (2013); *Cuestionario de capacidades y dificultades (SDQ-Cas).* The SDQ is freely available on their webpage; www.sdqinfo.org and serves as an emotional and behavioral screening tool designed for the assessment of children and adolescents. It can be completed by parents with children from 4 to 17 years old. In this study, only the external conduct problems scale was used, composed of 5 items and responses are recorded on a 3-point Likert scale ranging from "not true" to "certainly true". Sample items include *"Roba cosas en casa, en la escuela o en otros sitios (Steals from home, school or elsewhere)"* and *"A menudo miente o* *engaña (Often lies or cheats)"*. For the data analysis, SDQ total scores are calculated in percentile.

4.3.3. Social Phobia and Anxiety Inventory for Children-Parent Report (SPAI-CP)

Parents also completed the Spanish version of the Social Phobia and Anxiety Inventory for Children-Parent Report (SPAI-C-P; Turner, Beidel, Dancu & Stanley, 1989) named *Inventario de Fobia y Ansiedad Social* (not yet published by Olivares & Olivares-Olivares, 2023), during the first session. This inventory is a brief measure of social anxiety in children, evaluated by parents, corresponding to the Spanish version of the SPAI-CP for children. The scale consists of 26 items, each scored in a 3-point scale from 0 to 2 ranging from *"never or hardly ever"* to *"most of the time or always"*. Sample items include "*My son/daughter gets scared when meeting new kids"* or *"My son/daughter is afraid of asking questions in class"* (these items are translated directly from the Spanish version). The maximum total score is 52, which indicates that the child experiences phobia with a high frequency in a broad range of social settings. And children with risky scores would be excluded from the TSST. The questionnaire has shown good reliability (α=0.810).

4.3.4. Behavioral Coding

The evaluation of the behavioral response was done through the visualization of the recordings and a coding method adapted from Burkholder et al. (2015) who used a coding scale (Children and Adolescent Stress and Emotion Scale, CASES) developed for their study in stress' impact on the regulation of anxiety expression in children and from Poole et al. (2016) collecting behavioral data on a 5-point scale which included frequency and intensity of the behavior and computed socially anxious behavior by standardizing and summing each measure (i.e. physical anxiety, speech inhibition and verbal anxiety). For this study, two different investigators registered the total time duration of the following three behavioral patterns during the speech and math task with the OBSERVER XT in 10 participants (5 girls): 1) *Self-touch,* referring to the repetitive touching or handling of one's body including clothes, accessories and external objects such as the microphone, the wall or items they bring with themselves. 2) *Swinging or Balancing*, continuous rhythmic movement on the vertical or horizontal axis of the body trunk. 3) *Freezing*, the absence of body movement along with an observable muscular tension on.

4.3.5 Facial Emotion Expression

To evaluate the behavioral response in a complementary way, Py-Feat (Python Facial Expression Analysis Toolbox, Cheong et al., 2023) was used to score facial expressions of emotion. Py-Feat is a software written in Python programming language, popular for being an open, completely free and easy to learn source. It also compiles to all major operating systems, its functionality for scientific computing in research increasing during these past years due to its features. Face detection results include a confidence score for each detected face. In other words, Py-Feat distributes the total probability of expressing an emotion among the 7 basic emotions (anger, disgust, fear, happiness, sadness, surprise, and neutral) with the normalized means. After the face is identified as an image, Py-Feat uses a standard coordinate facial landmark scheme. In addition to the basic properties of a face in an image, it also includes models for detecting deviations of specific facial muscles (i.e., action units; AUs) from a neutral face expression using the Facial Affect Coding System or FACS (Ekman & Friesen, 1978), which consists of a system that quantifies the activation of facial muscles, extracting facial expression information. Finally, Py-Feat includes models for detecting the presence of specific emotion categories by classifying the images based on how much the face resembles an emotion. It is important to note that Py-Feat is sensitive to occlusion of the image. Performance substantially drops in the detection of anger, fear, sadness and surprise by the occlusion of eyes, while disgust, happy and neutral detection drop when the mouth is blocked (Cheong et al., 2023). In this study, the 20 videos of the participants are analyzed through Py-Feat during the TSST-C and results are compared between sexes.

4.4 Data analysis

Data was analyzed using IBM SPSS Software for Windows, version 22 (IBM Corp., Spain).

Descriptives and correlations were computed for perceived stress to explore the consistency between the basal measures and the ones taken during the stress task. A repeated-measures ANOVA was included to determine if perceived stress differed across time between sexes. Homogeneity was checked by means of the BOX test and sphericity with the Mauchly test. If sphericity was not achieved, the Greenhouse-Geisser correction was performed modifying the degrees of freedom. If homogeneity was not achieved, data were log-transformed. Given the pedagogic value of the degree project, no correction of the significance was performed with post-hoc contrasts. For analyses of facial emotion expression, descriptives were calculated for each emotion and a repeated-measures ANOVA was performed to compare each emotion versus the neutral expression. Descriptives were also coded for stress-behavior after dividing the subjects by sex, and a Student t-test was performed. Correlations were performed between all the variables (perceived stress during the TSST-C, social phobia, conduct problems, facial emotion and coded stress-behavior) to study potential relationships by means of Pearson's coefficients (two-tailed).

5. RESULTS

5.1 Perceived stress

To assess the evolution of perceived stress across the session, a mixed ANOVA was performed with the log-transformed data with the between-subject factor SEX(2) and the repeated measure TIME(6). Perceived stress changed across the session [TIME: $F(2.55, 45.97)=12.51$, $p<0.001$], and this change was the same in both sexes [SEX x TIME: NS], without being global differences between sexes [SEX: NS]. Polynomial contrast analysis showed that in both sexes, a quadratic trend was observed [$F(1,18)=33.45$, $p<0.001$], indicating that the task was effectively increasing perceived stress. Perceived stress during the speech task and the math task increased in comparison to baseline [$F(1,18)=10.58$, $p<0.01$; $F(1.18)=11.47$, $p<0.01$, respectively]. Due to the pedagogic value of this final degree project, and given the inspection of Figure 10, small sex differences were observed and will be considered. In boys, perceived stress during the math task increased in comparison to the speech task, whereas in girls the opposite pattern was found [Contrast repeated, SEXxTIME: $F(1,18)=5.29$,$p<0.05$]. Additionally, girls showed a trend towards a faster decrease of perceived stress post-TSST-C (differences at recovery 1), in comparison to boys [$F(5,90)=12.51$, $p=0.054$].

Correlations for perceived stress are presented in Table 1. Perceived stress at arrival was positively correlated with the baseline, $r(18)=0.75$, $p<0.001$, and recovery 2, $r(18)=0.71$, $p<0.001$. Baseline perceived stress correlated positively with recovery 1, $r(18)=0.67$, $p<0.01$, and recovery 2, $r(18)=0.83$, $p<0.001$. Perceived stress during the speech task correlated positively with the measure taken during the math task, $r(18)=0.61$, $p<0.01$, and perceived stress during recovery 1 correlated positively with recovery 2, $r(18)=0.51$, $p<0.05$. In these last measures, a stronger correlation was observed in girls, $r(18)=0.81$, $p<0.01$, compared to boys, $r(18)=0.64$, $p<0.05$. For the other measures, the same pattern was observed in each sex. All other correlations were not statistically significant, suggesting the dissociation between perceived stress during resting conditions and during stress.

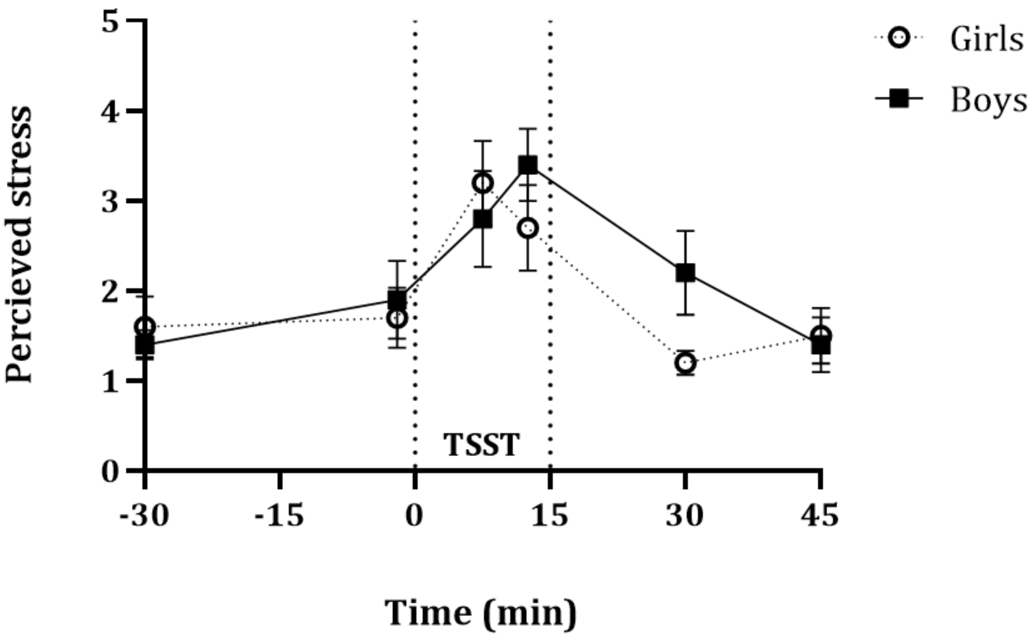


Fig. 9: *Perceived stress before, during and after the TSST-C. Scores are means and the error bars represent mean standard error (SEM, n=20, 10 girls with ages 7 to 8).*

Table 1

Two tailed Pearson's correlation between self-reported stress pre-, during and post- TSST-C (n=20).

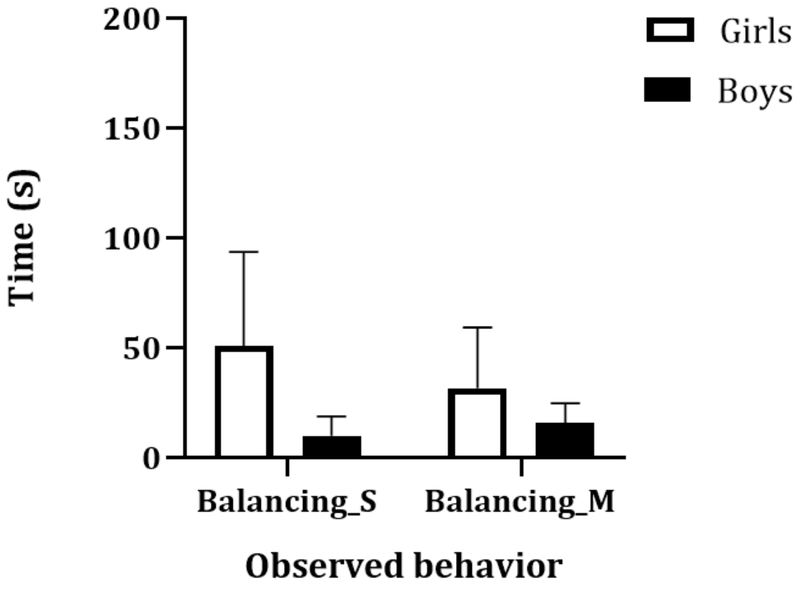
		sam1	sam2	sam3	sam4	sam5	sam6
sam1	Correlación de Pearson	1	,745**	,368	,160	,325	,707**
	Sig. (bilateral)		,000	,110	,501	,162	,000
	N	20	20	20	20	20	20
sam2	Correlación de Pearson	,745**	1	,170	,038	,667**	,829**
	Sig. (bilateral)	,000		,475	,874	,001	,000
	N	20	20	20	20	20	20
sam3	Correlación de Pearson	,368	,170	1	,606**	-,202	,251
	Sig. (bilateral)	,110	,475		,005	,394	,286
	N	20	20	20	20	20	20
sam4	Correlación de Pearson	,160	,038	,606**	1	,042	-,098
	Sig. (bilateral)	,501	,874	,005		,861	,681
	N	20	20	20	20	20	20
sam5	Correlación de Pearson	,325	,667**	-,202	,042	1	,508*
	Sig. (bilateral)	,162	,001	,394	,861		,022
	N	20	20	20	20	20	20
sam6	Correlación de Pearson	,707**	,829**	,251	-,098	,508*	1
	Sig. (bilateral)	,000	,000	,286	,681	,022	
	N	20	20	20	20	20	20

*Note: **Significant correlation $p<0.01$. *Significant correlation $p<0.05$. SAM scores indicate stress perception during the session. Sam1 taken at arrival, sam2 taken before the TSST-C (baseline), sam3 during the speech task, sam4 during the math task, sam5 after the TSST-C (recovery 1) and sam6 after sam6 (recovery 2).*

5.2 Behavioral Stress

Freezing behavior was not registered during the observations. Means between two independent observers were calculated for 10 subjects selected at random (see Figure 10). Differences between boys and girls remained statistically non-significant even after removing an identified outlier.

(A)



(B)

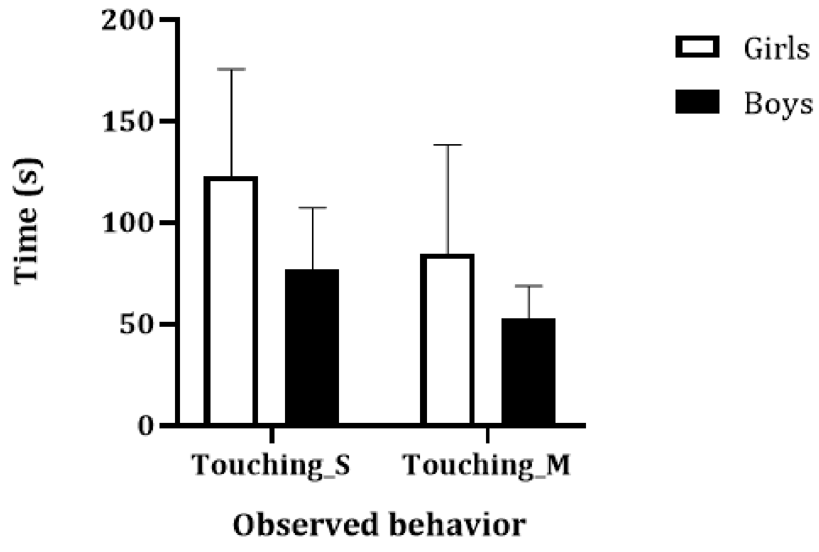


Fig. 10: *In graph A, stress behavior Balancing observed during the speech task (S) and math task (M). Error bars show standard error of the mean (SEM, n=10, 5 girls). In graph B, stress behavior Touching is observed during the speech task (S) and math task (M). Error bars show standard error of the mean (SEMn=10, 5 girls).*

5.3 Facial Emotion Expression

Facial emotion expression means are presented in Figure 13. A mixed ANOVA was performed with the between-subject factors SEX(2) and repeated measure EMOTION(7). The analysis indicated no statistically significant differences between sexes in the emotions expressed during the TSST-C [SEX and SEX x EMOTION: NS]. A contrast analysis was performed to compare each emotion versus the neutral expression. Neutral expression was more frequent during the TSST-C in comparison to anger [$F(1,18)=4.67$, $p<0.05$], disgust [$F(1,18)=38.87$, $p<0.001$], fear [$F(1,18)=33.46$, $p<0.001$] and happiness [$F(1,18)=15.03$, $p<0.001$]. The same tendency was found for surprise [$F(1,18)=3.74$,$p=0.069$].

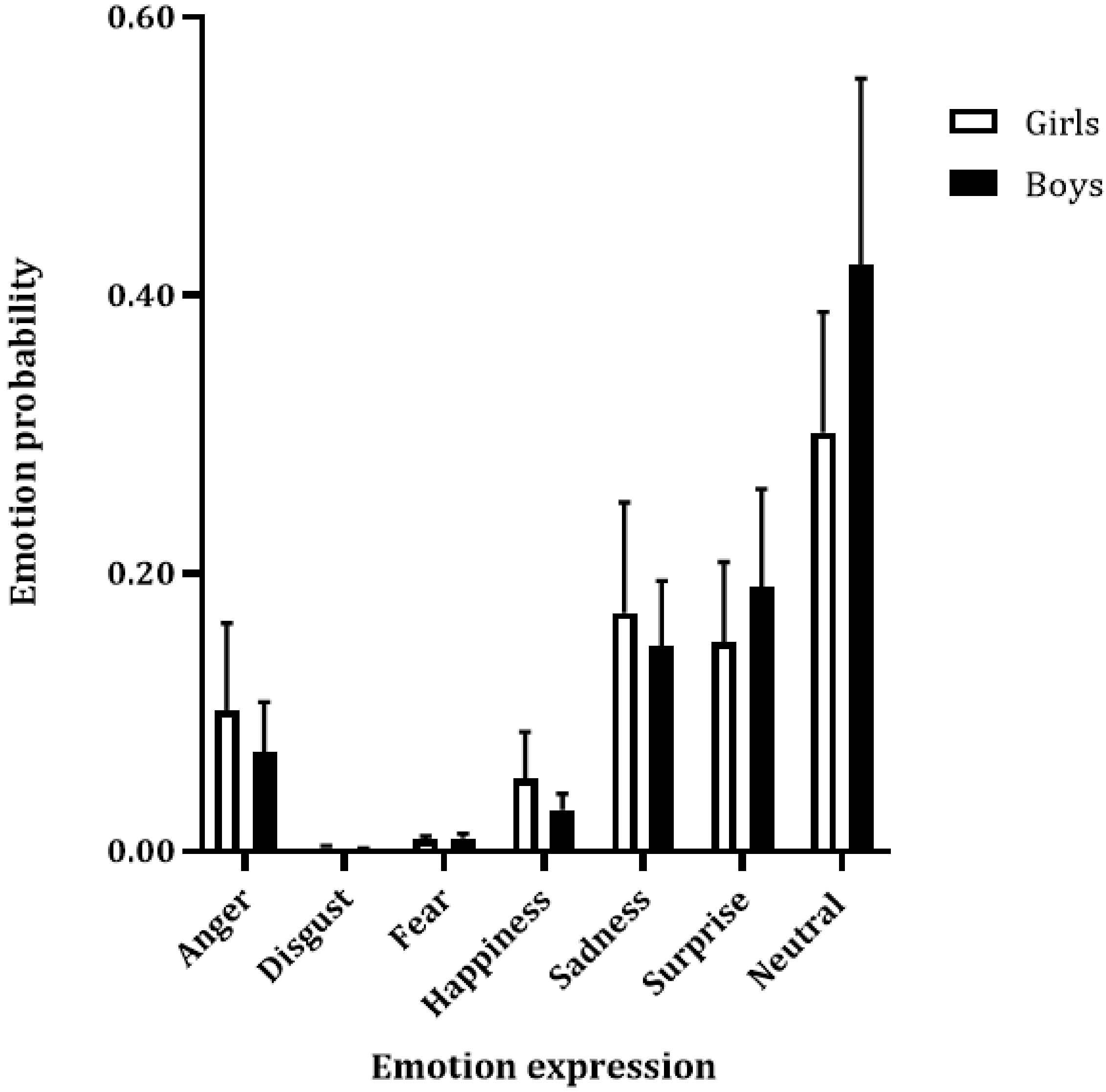


Fig. 11: *: Mean percentage of facial emotions detected with PY-FEAT using recorded videos of the participants during the TSST-C in girls and boys (n=20, 10 girls). Error bars show standard error of the mean (SEM).*

5.4 Correlational analysis

Externalizing conduct problems were found (trend level) to positive correlate with social phobia and anxiety, $r(18)=0.39, p=0.091$, whereas they were negatively correlated with the expressions of surprise, $r(18)=-0.58$, $p<0.01$, and anger, $r(18)=-0.46$, $p<0.05$, during the TSST-C. Externalizing conduct problems also were found (trend level) to positively correlate with disgust, $r(18)=0.39$, $p=0.093$, and sadness, $r(18)=0.39$, $p=0.094$. Meanwhile, social phobia and anxiety were positively correlated with neutral expression, $r(18)=0.53$, $p<0.05$, and negatively with anger, $r(18)=-0.47$, $p<0.05$. Balancing during the speech task correlated positively with anger, $r(18)=0.68$, $p<0.05$, and balancing during the math task, $r(18)=0.98$, $p<0.001$. Touching during the speech task was also found to be positively correlated with touching during the math task, $r(18)=0.79$, $p<0.01$. Happiness expression was observed to positively correlate with disgust, $r(18)=0.49$, $p<0.05$, and fear, $r(18)=0.53$, $p<0.05$, whereas surprise correlated negatively with sadness, $r(18)=-0.58$, $p<0.01$. Additionally, neutral expression was found to negatively correlate with anger, $r(18)=-0.60$, $p<0.01$, and fear was positively correlated with disgust, $r(18)=0.61$, $p<0.01$.

6. DISCUSSION

6.1 Perceived stress

On a first note, the TSST-C has successfully increased subjective stress and it returned to its baseline at the recovery period in agreement with similar studies in the same age range (DeVeld et al., 2012, Hellhammer et al., 2012 & Ji et al., 2016). We initially hypothesized that girls would exhibit higher subjective stress during the stress task as previously done studies in children (Hostinar et al., 2015 & Raffington et al., 2020) and with the TSST-C (Kudielka et al., 2004a, Ji et al., 2016 & DeVeld et al., 2012). Besides, girls have been seen to report higher fear of socio-evaluative situations than boys (Gullone et al., 2000). However, our results indicated only subtle differences between sexes. Interestingly, girls appeared to demonstrate a quicker recovery from stress despite reporting similar perceived stress levels to boys in the final measure, suggesting differences during the recovery process. These results align with Shu et al. (2020), who observed girls (from ages 8 to 12) recovered faster during a silent soundscape from subjective stress caused by a stressful task. Additionally, perceived stress increased in the math task, in comparison to the speech task whereas the opposite was observed in girls. It would be of interest to explore if there is an interaction between sex and coping mechanisms, as the use of suppression (keeping one's emotions to one-self) has been related to lower cortisol reactivity in girls, but not in boys (DeVeld et al., 2012). Besides, coping mechanisms have been found to vary between sexes, for which should also be included in future studies (Wang et al., 2007, Youseff et al., 2018 & Eschenbecket al., 2018). All in all, sex differences may be present and further research is needed.

6.2 Stress behavior

Behavioral stress was assessed by means of balancing and touching behavior. Due to the lack of studies, we expected that girls would exhibit more pronounced signs of behavioral stress, drawing from evidence indicating their higher physiological reactivity to stress (DeVeld et al., 2012; Hostinar et al., 2015; Ji et al., 2016), psychological stress responses (Gullone et al., 2000), and cardiovascular stress reactions (Kudielka et al., 2004a). However, no differences between sexes were found. Freezing was not registered, so this behavior may not be as frequent in children as we initially believed. Additionally, as a sign of consistency, balancing during the speech task was associated with balancing during the math task. Similarly, touching behavior during the speech task was associated with touching during the math task. An unexpected result was that balancing was associated with higher expression of anger during the TSST-C. No previous studies were found regarding the correlation between balancing behavior and emotion expression in stressful situations.

6.3 Facial emotion expression

Regarding facial emotion expression, we hypothesized that boys would be more likely to display emotions promoting avoidance, such as anger or disgust, while girls would exhibit more prosocial expressions like happiness (Chaplin et al., 2013) and that subjective stress could be associated with facial emotion expression (Blasberg et al., 2023). Our results did not align with these expectations, as we found no differences in the display of emotions between boys and girls nor any correlation between subjective stress and emotion expression. In both sexes, neutral expression predominated during the task, compared to the majority of the other emotions. No literature has been found regarding the effect of acute stress in emotion expression in children nor about the correlations between the expressed emotions during stressful situations.

6.4 Social phobia and anxiety and conduct problems

We hypothesized that participants with higher SPAI-CP total scores would report increased stress appraisal during the TSST-C (Krämer et al., 2012 & Stadelmann et al., 2017). No evidence was found to corroborate this hypothesis. However, social phobia and anxiety was associated with higher neutral expression and less anger expression during the stress task. These results align with Melfsen and collaborators (2000), as children were found to have reduced general facial expression in comparison to non-anxious children. Future studies are needed to explore how social phobia and anxiety interact with stress and affect emotional expression in children. In addition, no sex differences were found in social phobia and anxiety, agreeing to the study mentioned previously (Stadelmann et al., 2017). Our results align with our previous expectation, as scores on the externalizing subscale of the Strengths and Difficulties Questionnaire (SDQ) exhibited no association with subjective stress during the

TSST-C, as found by Stadelmann et al. (2017). In addition, externalizing conduct problems were associated with less surprise and anger expression, and showed a tendency towards being associated with higher disgust and sadness. No previous studies focused on these associations have been found to compare with our present results. Studies exploring facial emotional expression during the TSST-C are not common, a possible association between emotion recognition and expression should be contemplated in future research. Additionally, no sex-differences were found in externalizing conduct problems scores, contrary to the consulted studies (Tiet et al., 2001, Kovess-Masfety et al., 2021 & Álvarez-Voces & Romero, 2024). When looking at specific behaviors of externalizing conduct problems, Tiet et al. (2001) found no sex-differences on stealing, lying and relational aggression in children. Besides, lying and substance use increased with age whereas relational aggression and impulsivity peaked in early adolescence in both sexes. On the other hand, Álvarez-Voces and Romero (2024) suggest girls with conduct problems seem to desist more than boys possibly due to cultural expectations and biological factors. Further research is needed to explore sex-differences in externalizing conduct problems through age and their development.

7. CONCLUSIONS

7.1 Strengths and implications

This final degree project addresses a notable gap in the existing literature by focusing on stress in children, an area that has been understudied. Particularly, it focuses on the lack of stress-children studies that specifically examine sex differences. These results highlight the importance of including individual differences rather than relying solely on sex as a predictor of stress response in this age. Moreover, unexpected associations have been found which require further exploration. By contributing to the underexplored line of study of this final degree project, our research provides valuable insights into how girls and boys cope, experience and recover from stress.

7.2 Limitations and future directions

Our investigation faced several limitations, the most notable being the sample size and non-clinical condition of our sample may not generalize to clinical samples. Future studies would benefit from larger, more diverse samples including a range of ethnicities and mental health conditions. This would also enhance the applicability of our results to a broader demographic and clinical populations. Additionally, including physiological measures such as salivary samples to analyze cortisol levels, could provide a more comprehensive understanding of children's stress responses. In addition, longitudinal studies are also needed and this present study was mainly cross-sectional and only partially longitudinal, as the SDQ measure was taken the previous year. Moreover, exploring other relevant stress responses, such as analyzing speech content, cognitive response (such as self-rating or thoughts), quality of voice during stress tasks, or even frequency and duration of eye-contact, could offer deeper insights into how children experience and cope with stress.

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