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Research paper

Effects of psychosocial stress on the hormonal and affective response in children with dyslexia

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ABSTRACT

Introduction: Research on stress and dyslexia has mainly focused on chronic and contextual stress caused by the school environment. Our goal was to test individual differences in the hypothalamic-pituitary-adrenal (HPA) axis reactivity of dyslexic and non-dyslexic children and the related emotional manifestations associated with exposure to a psychosocial stressor.
Methods: Eighty-one children (11–14 years old; 38 dyslexic) were exposed to the Trier Social Stress Test adapted to children or to a control condition. The salivary cortisol response, anxiety, and mood were measured before and after the stress.
Results: Dyslexic children did not show the expected cortisol response, as the highest percentage of children who were non-reactive to stress was found in this group. Cortisol reactivity to stress was related to higher levels of anxiety and lower positive affect in the non-dyslexic children.
Conclusion: These results suggest a pattern of hypo-activation of the HPA axis to psychosocial stress in children with dyslexia.

1. Introduction

Many studies have shown that the hypothalamic-pituitary-adrenal (HPA) axis is activated when a person experiences stress. Cortisol is the primary hormone released by this axis when facing a stressor, either physical or mental, along with catecholamines (adrenaline and noradrenaline). Thus, the perception of threatening events activates the HPA system and, subsequently, the increased secretion of cortisol. The stress response also has important psychological components (emotional arousal, focus on threat vigilance, or self-defence). Cortisol is the most widely used biological marker of stress, and cortisol elevations also occur in response to novel and unpredictable situations [1,2].

Biological reactivity to psychological stressors is designed to prepare the organism for challenge or threat. Boyce and Ellis [3] and Ellis et al. [4] proposed the "biological sensibility to context" theory. This theory suggests that stress reactivity is not a unitary process; instead, it incorporates counter-regulatory circuits that serve to modify or temper physiological arousal. In addition, the effects of high reactivity phenotypes are bivalent, exerting both risk-augmenting and risk-protective effects in a context-dependent manner. This theory suggests that heightened stress reactivity may not only reflect exaggerated arousal in conditions of challenge, but also an increased biological sensitivity to context, with potential for negative health effects in situations of adversity and positive effects in situations of support and protection. From an evolutionary perspective, these authors consider that, due to the developmental plasticity of the stress response systems and their structured context-dependent effects, these systems may constitute conditional adaptations: evolved psychobiological mechanisms that monitor specific features of childhood environments as a basis for calibrating the development of stress response systems to adaptively match these environments. Taken together, these theoretical perspectives lead to a novel hypothesis: that there is a curvilinear, U-shaped relationship between early exposure to adversity and the development of stress-reactive profiles, with high reactivity phenotypes disproportionately emerging within both highly stressful and highly protected early social environments [3].

In addition, according to Thompson [5], over time, HPA functioning can disturb the neurological circuitry that underlies the body's regulation as a response to chronic stress. Furthermore, this alteration occurs, in part, due to repeated exposure to stressful events, which affects the limbic and cortical processes (motivation, memory, thinking, and emotional regulation) that regulate HPA activity.

Early exposure to harsh, dangerous, or unpredictable environments adversely affects the development of the central nervous system.

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Children exposed to stressful relationships or experiences show different patterns of HPA axis functioning, compared to children who do not experience these stressful relations or experiences [6]. It has been suggested that the effect of exposure to traumatic events is partially dependent on a child's harmful early experiences. Greater cortisol secretion has been associated with lower levels of harm, and the lowest levels of cortisol reactivity have been observed in children who experienced the most traumatic events and the highest levels of harm in the previous year. Cortisol reactivity to stress in children has been examined in laboratory settings in order to simulate an adverse environment similar to situations experienced in real life. A large number of studies have examined how children respond to an acute psychosocial stressor such as the Trier Social Stress test for children [7]. When investigating children from 10–13 years old, the results indicated significant increases in the cortisol response to the TSST [7–11]. However, children who experienced early life stress showed an attenuated cortisol rise in response to the TSST [12,13].

Recent studies suggest that childhood exposure to adversity influences later HPA axis functioning. The parenting style in childhood, a putative moderator of adversity, may be important in determining HPA reactivity [12]. Children with dyslexia have been described as having difficulty in tolerating novelty and environmental stressors [14]. In this regard, the importance of parenting in the behaviour, emotional development, and academic context of these children suggests that it may also be important in the biological development of their stress-sensitive systems. Dyslexia is a neurodevelopmental disorder characterised by slow and inaccurate word recognition [15]. According to the Diagnostic and Statistical Manual of Mental Disorders [16], developmental dyslexia (DD) is categorized as a specific learning disorder, with a pattern of learning difficulties characterized by problems with accurate or fluent word recognition, poor decoding, and poor spelling abilities. In this particular pattern, it is important to specify any additional difficulties that are present, such as difficulties with reading comprehension or math reasoning. The essential associated features include persistent difficulties in learning the essential academic skills. The individual's performance on the affected academic skills is well below average for his/her age, and learning difficulties are usually apparent in the early school years. According to the UK´s Rose report [17], and from an educational perspective, dyslexia would be a learning difficulty that primarily affects the skills involved in accurate and fluent word reading and spelling. Characteristic traits of dyslexia are difficulties in phonological awareness, verbal memory, and verbal processing speed. Dyslexia occurs across the range of intellectual abilities. It is best thought of as a continuum, rather than a distinct category, and there are no clear cut-off points. Co-occurring difficulties may be seen in aspects of language, motor co-ordination, mental calculation, concentration, and personal organisation, but these are not, by themselves, markers of dyslexia. A good indication of the severity and persistence of dyslexic difficulties can be obtained by examining how the individual responds or has responded to well-grounded intervention. The estimated prevalence in the academic domains of reading, writing, and mathematics ranges from 5% to 15% in school-age children across different languages and cultures, and it is more common in males (ratio from 2:1 to 3:1) than in females [16]. The neural basis of DD is similar across alphabetic languages, and it is commonly associated with anatomical and functional abnormalities in the left hemisphere, specifically, under-activation of the temporoparietal and occipitotemporal regions, as well as low activation of the left inferior frontal gyrus [15].

In addition to these features, as suggested above, children with dyslexia have been described as especially vulnerable to stressful environments [14]. Certain aspects of a school environment can be perceived as threatening for these children, which may induce stress or anxiety and negatively affect their health. Tests and assessments are stressful for all children; however, for the dyslexic child, it is a greater challenge to remember facts and information that most children can easily recall. In such cases, dyslexic children may perceive the stressor as excessive and choose to run away from it, either physically by avoiding the test, or emotionally by making themselves sick in class so that there is a legitimate reason for their absence. Dyslexic children who stay and try to do their best would be like a child in a fight with one hand tied behind his/her back. These children may proceed with the task, but with an unfair disadvantage that makes them likely to fail [18].

Alexander-Passe [14] investigated whether dyslexic children experience higher levels of stress at school. Three academic year groups, 3–5 (8–10 years old), 6–9 (11–13 years old), and 10–12 (14–16 years old), were selected. This study included four sources of stress (teacher interaction, academic stress, peer interaction, and academic self-concept) and three manifestations of stress (emotional, behavioural, and cardiac responses). Collectively, their results indicate important differences between dyslexics and controls, especially in peer interactions and academic self-concept. Dyslexic children feel stressed by their classmates' perceptions of them, and they have low feelings of self-worth related to their academic ability. According to Alexander-Passe [14], "when school-aged dyslexics begin to question their own self-worth, their ability to achieve in academic settings is affected". Thus, school-aged children with dyslexia have feelings of fear, shyness, and loneliness, which are also manifested in symptoms such as nausea, tremors, or rapid heartbeat. In relation to age, the children in the 3–5 year group are perceived to have higher stress than the 10–12 year group. For the author, one explanation could be that teachers know and understand their pupils' abilities and difficulties, whereas in the 3–5 year group, they put pressure on their pupils without having full knowledge of their abilities and difficulties.

In this regard, we propose that children with specific learning disabilities like dyslexia could be particularly vulnerable to stress and anxiety associated with the school environment. In children with dyslexia, there are excessive emotional and physiological reactions to stressful circumstances [14], but it is not known whether or not this is accompanied by a corresponding increase or decrease in the neuroendocrine response. In the literature, little is known about stress-related hormones in these children.

Research on the association between stress-related events and dyslexia mainly focuses on the contextual chronic stress elicited by the school environment. To our knowledge, no studies have examined the performance of both non-dyslexic and dyslexic children on the same cognitive tasks for the purpose of measuring cortisol reactivity. Discovering how non-dyslexic and dyslexic children react to stress at the biological level allows us to better understand how a given cognitive task and the way it is perceived affect cortisol secretion. The primary goal of the current study was to test whether individual differences in dyslexic and non-dyslexic children's HPA axis reactivity and emotional manifestations vary depending on exposure to a stressful situation similar to school. Furthermore, we expected a pattern of hypoactivation of acute HPA-axis functioning, considering the severity and chronicity of perceived stress in a clinical sample with dyslexia, compared to a control reference group.

2. Methods

2.1. Participants

Eighty-one children from 11 to 14 years old participated in this study and were randomly assigned to a stressful condition ($n = 39$) or a non-stressful control condition ($n = 42$). All the participants were individually tested in a single experimental session. The selection of the sample was carried out using a general questionnaire that explored characteristics of the children's physical, psychological, and daily habits. Exclusion criteria were severe visual or hearing problems, cardiovascular, endocrine, neurological or psychiatric disease, having been under general anaesthesia in the past year, experiencing a stressful event in the past year, or use of a drug related to heart, emotional, or endocrine function, or one that could affect their hormone levels. Participants were instructed not to eat, drink (except water), or exercise in the two hours preceding the laboratory session. In addition, selected subjects underwent a comprehensive neuropsychological assessment prior to the detection and diagnosis of dyslexia. This evaluation was conducted by professional neuropsychologists from the Neuropsychology Unit of the University of Murcia. The procedure followed was:

1 Screening tests (4 tests to assess learning difficulties):

The school's Educational Guidance Teams and psychologists administered four tests chosen to collectively measure the following aspects:
two reading comprehension tests, one on orthographic decision, and another on access to the lexicon. One of the reading comprehension tests was subsequently eliminated because it was not sensitive to detecting the risk of presenting dyslexia. The screening instruments are described in López-Escribano et al. [19]. In the screening, subjects who obtained scores below one standard deviation on any of the three group tests were considered at risk for dyslexia, and these children were referred for neuropsychological evaluation.

1 WISC-IV and TALE (when the screening was positive):

All the children were given the Wechsler intelligence scale for children (WISC-IV), the adapted Spanish version, and the T.A.L.E (test of reading and writing analysis) [20]. With regard to the intellectual level, children with scores below 80 on the scales of verbal comprehension and/or perceptive reasoning were eliminated. On the reading-writing test, children were selected who had scores two school years below their normative group on any of the reading parts (accuracy and/or speed in reading letters, syllables, words and texts) or on the written part.

1 Neuropsychological Assessment (subjects who tested positive on the previously described tests).

In addition, they underwent a complete neuropsychological evaluation in order to rule out other types of alterations that could interfere with reading performance.
For the control group, the screening was negative, and their values on the rest of the tests were within the limits for normal scores.

The sample was distributed as follows: 43 non-dyslexic (ND) and 38 dyslexic (DD) subjects. Of the 43 non-dyslexic participants, 22 (12 girls and 10 boys) were exposed to the stress condition and 21 (12 girls and 9 boys) to the control condition. Of the 38 dyslexic participants, 17 (6 girls and 11 boys) underwent the stress condition, and 21 (9 girls and 12 boys) were in the control condition. Table 1 provides a description of the sample. Significant age differences were found between groups

Table 1
Sociodemographic characteristics of evaluated children (n = 81). The values represent mean and standard deviations of the mean in parenthesis (SD).

Characteristics	Group Dyslexic (n = 38)	Non-dyslexic (n = 43)	p
Age mean (SD) (Years)	12.61 (0.94)	12.16 (0.37)	0.006
Range of age	12.37–12.83	11.95–12.37	
Sex			
Male	23	19	
Female	15	24	
Birth-weight (SD) (Kg)	3.21 (0.39)	3.31 (0.66)	0.44
BMI (SD) (Kg/m^2)	20.52 (3.50)	19.87 (3.51)	0.41
Full term children (%)	30 (78.9)	35 (81.4)	0.78

because, in the dyslexic group, three children had repeated a school year.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Research Committee of the University of Valencia. All the children participated voluntarily, and written informed consent was obtained from the children and from one parent.

2.2. Procedures

Sessions were held between 13.30 h and 18.00 h in order to control for possible circadian rhythm effects [21]. As Fig. 1 shows, the entire procedure lasted approximately 1.5 h. It included the administration of questionnaires on anxiety and mood scales and the collection of salivary samples (to determine cortisol). In addition, other tests were completed that were not analysed in this study.

2.2.1. Questionnaires

2.2.1.1. State anxiety. All the participants completed the Spanish adaptation [22] of the Spielberger State Anxiety Scale for Children (STAI-C) [23]. The STAI-C is a widely-used, self-administered questionnaire to measure anxiety in children. It consists of two separate 20-question rating scales, one for "state" anxiety (acute, transitory) and the other for "trait" anxiety (chronic, pervasive). On the state version, participants are asked how they feel at the time of being questioned, and on the trait version, participants are asked how they feel generally.

Items are answered "hardly ever", "sometimes", or "often", and they are given a score from 1 to 3. This instrument is age-appropriate for prepubescent children, and it has acceptable psychometric properties [24]. In this study, we used only the state anxiety scale on two occasions, before and after the assessment in both conditions.

The alpha value for the scale was 0.87.

2.2.1.2. Negative and positive affect. Participants' affect was assessed right before and after the TSST-C, using the standardized children's version of the Positive and Negative Affect Schedule [25,26]. The measure includes a list of adjectives corresponding to a range of emotions. Participants rate to what extent they feel each emotion at this moment on a 5-point scale (0 = not at all, 4 = extremely). Before the TSST-C, participants were asked to report how they felt "at this moment", and after the procedure they were asked to report how they felt during the TSST-C. Alpha values for both scales were 0.87 for positive affect and 0.89 for negative affect.

2.2.2. Experimental procedure: Trier Social Stress for Children (TSST-C) or control condition

Instructions for the tasks were given for 5 min in both conditions. The stress was induced by a children's version of the well-known Trier Social Stress Test [7]. The Trier Social Stress Test for Children (TSST-C) involves uncontrollability and high levels of social-evaluative threat. It consists of a verbal task and a mental arithmetic task in front of a camera and a committee composed of a man and a woman, who act as observers in a neutral and rather reserved fashion.

The speech task involves listening to the beginning of a story begun by the examiner in front of the committee. After listening to the introduction of the story, the children have 5 min to think about and elaborate on a continuation (preparation), and then another 5 min to tell the committee about the continuation they created. The arithmetic task (5 min), in turn, consists of serially subtracting the number 7 from other 4-digit numbers as fast and accurately as possible. In the case of an incorrect answer, the committee asks the child to return to the initial number and start again. It is of methodological importance to note that, in contrast to the TSST for adults, the members of the audience on the TSST-C were instructed to provide the children with appropriate positive feedback, either facial or verbal.

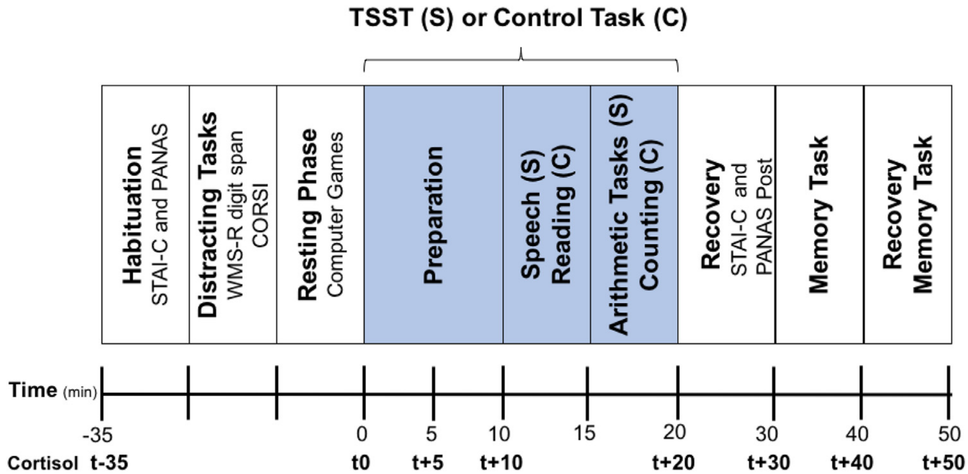

Fig. 1. Timeline of the stress (TSST, S) and control (C) conditions. Sequential salivary cortisol sampling (t − 35 to t + 40). State Anxiety Inventory-State (STAI-S), Positive and Negative Affect Schedule (PANAS).

The control condition also involves verbal and mental arithmetic tasks. However, they do not take place in front of a camera and a committee. The children are given 5 min to think about their favourite book or film, which they then tell the examiner about. Instead of the mental arithmetic task, a modified version of the well-known domino game is used. The aim of this game is to obtain the sum of 7 (number of dots on the "open" end of the tile + number of dots on the tile placed by the player = 7). For example, picture the first move of the game, when a single tile is on the table and both of the tile's ends are still free to use. If the right half of this tile has 3 dots on it and the left half has 2 dots on it, the child can either place a tile with 4 dots on the right side of this tile (4 + 3 = 7) or one with 5 dots on the left side of this tile (2 + 5 = 7). The children play this game with the examiner.

2.2.3. *Hormonal response*

HPA axis activity was evaluated by cortisol levels [27]. Saliva was sampled at eight different points in time using the Salivette sampling device (Sarstedt, Nümbrecht, Germany): baseline (t − 35), sample 0, sample +5, sample +10, sample +20, sample +30, sample +40 and sample +50. Intra- and inter-assay precision expressed as the coefficient of variation was below 10%.

2.3. *Data analysis*

For the analyses, measures of cortisol levels were log-transformed to approach a normal distribution. For easy interpretation of the figures, the values represent raw values and not transformed values; they are mean ± standard error of mean (S.E.M).

A number of analyses (ANOVAs and ANCOVAs) with repeated measures on one factor were computed to identify effects of time (cortisol: 8 samples; anxiety and positive and negative affect: 2 measures: pre-and post-assessment), group (dyslexic/non-dyslexic children), and condition (stress/control). Baseline cortisol level (first sample t − 35) was entered as a covariate to control for its possible influence when there were baseline differences in the groups. Greenhouse–Geisser corrections were applied when appropriate, and only corrected results are reported. Effect sizes (η^2) are indicated as a measure of explained variance, with 0.01 defined as a small effect size, 0.06 as a medium effect size, and 0.14 as a large effect size [28]. Differences between mean cortisol samples of the groups (dyslexic/non-dyslexic children by stress/control conditions) were assessed with mixed-effects analysis of variance.

Main effects on cortisol reactivity (calculated as the maximum increase in cortisol, compared to the pre-stress cortisol, during the TSST or control condition) were tested by univariate ANOVA. To distinguish between individuals who show cortisol reactivity (responders) and those do not (non-responders), we used a threshold classification criterion of a 2.5 nmol/L baseline-to-peak-increase [29,30]. All the reported correlations are Pearson correlations. Two-tailed tests with p-values <0.05 were considered significant. p-values were corrected with the Bonferroni correction when testing post-hoc planned comparisons. All statistical analyses were performed with SPSS (version 22).

3. Results

3.1. *Cortisol responses to stress*

Baseline cortisol levels (t − 35) revealed differences between the dyslexic and non-dyslexic groups (F (1,80) = 10.801, p = 0.002), with the dyslexic group showing lower levels. Thus, differences in cortisol levels between the DD and ND groups at t − 35 were taken into account in the following analyses to control for their possible influence on the cortisol response to the TSST-C.

A repeated-measures ANCOVA was carried out to analyse the cortisol response to the TSST. Time was entered as a within-subject factor (0, +5, +10, +20, +30, +40, +50), with group (dyslexic/non-dyslexic) and condition (stress/control) as between-subject factors, whereas cortisol levels at t − 35 were entered as a covariate. The analysis showed main effects of condition (F (1, 76) = 7.027, p = 0.010, η^2 = 0.085), time (F (6456) = 3.030, p = 0.006, η^2 = 0.038), and the condition x time interaction (F (6456) = 10.919, p = 0.000, η^2 = 0.126). There were no differences in cortisol levels between conditions on the 0 min, +5 min and +10 min samples; however, the cortisol levels were higher in the TSST-C condition than in the control condition in later samples (+20, +30, +40, +50 min; all $p \leq 0.007$). In the stress condition, cortisol levels increased, reaching their peak after the TSST-C (+30 and +40 min samples) (+0, +5, +10, +20 < +30: $p \leq 0.009$; +0, +5 < +40: $p \leq 0.02$), and then starting to decrease in the last saliva sample (+50 min sample) (+30, +40 > +50: $p \leq 0.003$) (see Fig. 2).

In the control condition, cortisol levels decreased over time, but the differences were only significant between the 0 min, +5 min, and +10 min samples and the +50 min sample (+0 vs +50, p = 0.007; +5 vs +50, p = 0.004; +10 vs +50, p = 0.018).

We investigated whether the differences found in the stress condition occurred in the same way in both groups of stressed participants (dyslexic/non-dyslexic). Cortisol responses decreased from baseline, but reached peak levels at +30 and +40. Afterwards, cortisol levels decreased in the last saliva sample in both stressed groups. However, these differences over time were significant in the stressed non-dyslexic

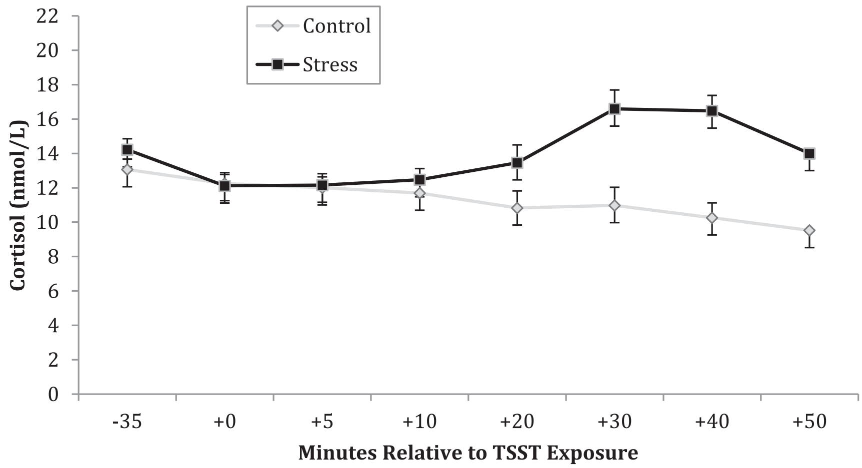

Fig. 2. Mean ± S.E.M of cortisol levels (nmol/L) at the eight sampling points in the two study conditions (stress/control). Error bars represent standard error of the mean (S.E.M).

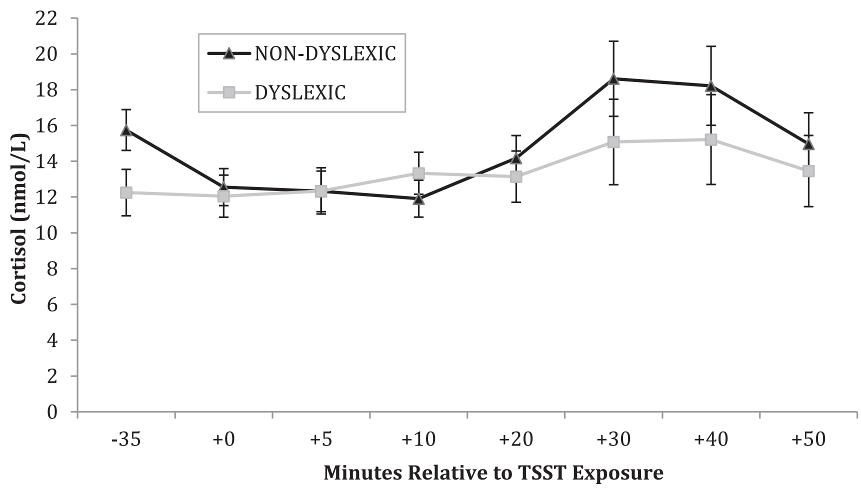

Fig. 3. Mean ± S.E.M of cortisol levels (nmol/L) at the eight sampling points in the two stressed groups (non-dyslexic: $N = 22$; dyslexic: $N = 17$). Error bars represent standard error of the mean (S.E.M).

group ($p \leq 0.02$), but not in the stressed dyslexic group (see Fig. 3).

We also analysed the cortisol reactivity to stress as the maximum increase in cortisol ($t + 30$) compared to the stress cortisol before the TSST-C ($t + 0$). In the ANCOVA adjusting for average baseline cortisol ($t - 35$), there was a significant difference between the stressed non-dyslexic group and the two control groups (F (1,76) = 5.503; $p = 0.002$, $\eta^2 = 0.178$) (stressed non-dyslexic vs dyslexic control: $p = 0.011$; stressed non-dyslexic vs non-dyslexic control: $p = 0.005$). These measures in the dyslexic stressed group did not differ significantly from the other groups (see Fig. 4).

There are no clear guidelines for determining a definitive cortisol response to distinguish between individuals who show cortisol reactivity (responders) and those do not (non-responders), but some have suggested a fixed threshold classification criterion, such as a 2.5 nmol/L baseline-to-peak-increase [29,30]. In all, 72.7% ($N = 16$) of the non-dyslexic children in the stress condition exceeded this threshold, whereas only 35.3% ($N = 6$) of the dyslexic children did so in the same condition (see Table 2).

3.2. Psychological measures

Repeated-measures ANOVAs were used to analyse the psychological response to the TSST-C. Time (pre- vs post-assessment) was entered as a within-subject factor, with group (dyslexic/non-dyslexic) and condition (stress/control) as between-subject factors. For each ANOVA, we included either state anxiety, negative affect, or positive affect as dependent variable. No differences were found between the scores on the state anxiety and negative affect scales of the dyslexic and non-dyslexic participants on the measures collected before the stress task (anxiety: DD: mean = 28.92, SD = 4.923; ND: mean = 28.67, SD = 5.727; F (1,80) = 0.043, $p = 0.837$; negative affect: DD: mean = 16.61, SD = 2.843; ND: mean = 17.35, SD = 3.323; F (1,80) = 1.155, $p = 0.286$). However, the two groups differed on positive affect before stress, with the dyslexic group reporting less positive affect (DD: mean = 22.97, SD = 2.604; ND: mean = 24.98, SD = 2.568; F (1,80) = 12.111, $p = 0.001$). The condition x time interaction was significant for state anxiety and negative affect. In the stress condition, scores on the state anxiety and negative affect scales were significantly higher at post-assessment (anxiety: mean pre-assessment = 28.005, SD = 0.867; mean post-assessment = 37.832, SD = 1.112; $p = 0.000$; negative affect: mean pre-assessment = 16.759, SD = 0.502; mean post-assessment = 18.537, SD = 0.659; $p = 0.000$). In the control condition, scores on the negative affect scales were significantly lower at post-assessment (mean pre-assessment = 17.119, SD = 0.479; mean post-assessment = 16.167, SD = 0.630; $p = 0.032$).

Comparing only the groups in the stress condition on the psychological measures, anxiety and negative affect increased from pre- to post-assessment in both stressed groups (dyslexic/non-dyslexic; $p \leq 0.004$). Positive affect decreased from pre- to post-assessment in both stressed groups, but these differences were significant in the

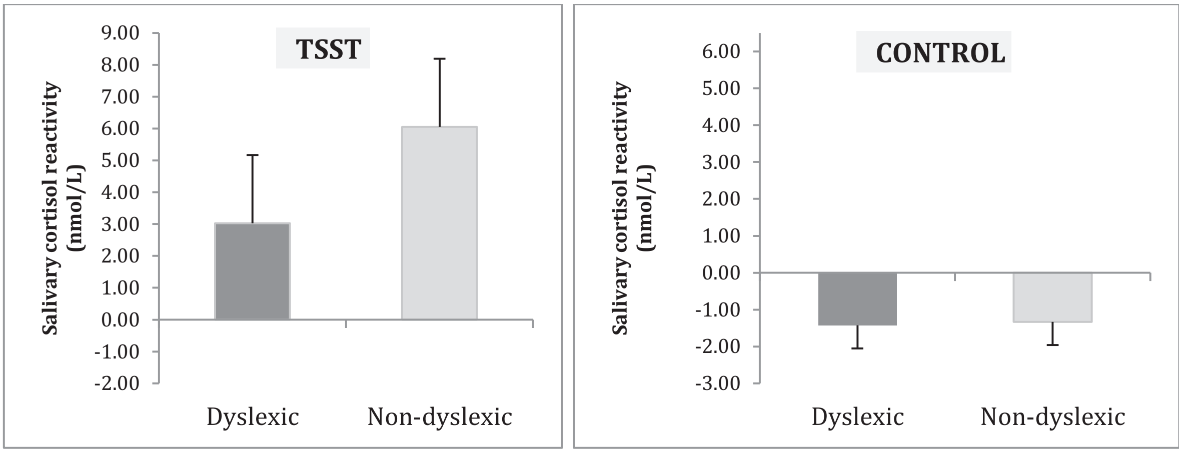

Fig. 4. Salivary cortisol reactivity by group (dyslexic and non-dyslexic) and by condition (stress/control). Depicted values are means, and error bars represent the S.E.M (standard error of the mean).

stressed dyslexic group ($p = 0.043$), but not in the stressed non-dyslexic group ($p = 0.078$) (see Fig. 5).

3.3. The relationship between the stress response and psychological measures

In order to test whether changes in cortisol reactivity to stress were associated with the psychological measures (post-treatment minus baseline on anxiety, negative or positive affect scores) in the two groups of stressed subjects, Pearson correlations were calculated. Results showed a significant correlation between cortisol reactivity and anxiety scores ($r = 0.464$, $p = 0.030$) and, as a trend, between cortisol reactivity and positive affect scores ($r = -0.421$, $p = 0.051$) in the stressed non-dyslexic group. No associations were found between these measures in the stressed dyslexic group.

4. Discussion

The present study compared cortisol and psychological reactions to acute stress exposure in dyslexic and non-dyslexic children. To our knowledge, this is the first study to examine the relationship between neuroendocrine and psychological responses to stress in dyslexic children.

The TSST-C has been used with children as young as 7 years old, and it has been found to be the most effective laboratory stressor task if the goal is to activate the HPA axis [31]. In this study, children in this age group (12–14 years old) were stressed during the TSST-C because they showed a higher cortisol response than control children, coinciding with different studies that indicated increases in the cortisol response to the TSST in children from 10–13 years old [7–11].

A first result obtained was the difference between the cortisol levels of the two groups at baseline. We think this result could be due to the fact that the initial situation might be a stressor that would act differently in each group, and so this measure ($t - 35$) was considered a covariate in the subsequent statistical analyses.

When we investigated whether the differences found in the stress condition occurred in the same way in dyslexic and non-dyslexic children, we found significant differences in the profiles of the two groups. In non-dyslexic children, we found significant differences over time in response to the TSST-C, revealing the expected curve, with a significant maximum increase in cortisol levels at $t + 30$, compared to the pre-stress cortisol level ($t + 0$). Of the 22 non-dyslexic children exposed to the stressor, 72.7% ($n = 16$) were responders, showing a change in their cortisol levels in response to the stressor, whereas 27.3% were non-responders. However, in the dyslexic group, the expected curve was not found; 64.7% of the 17 dyslexic children ($n = 11$) exposed to the stressor were non-responders, showing no change in the cortisol level in response to the stressor, whereas only 35.3% were responders.

In general, our results show that non-dyslexic children displayed a higher response to stress than dyslexic children, who exhibited an attenuated cortisol reactivity to the TSST-C. This attenuation could be explained by the context sensitivity hypothesis [3], which suggests that early life stress leads to an initial heightened stress response, but when this stress is maintained over time, this level tends to be suppressed.

Table 2
Cortisol levels (nmol/L) in the stress (TSST) and control conditions in dyslexic and non-dyslexic groups. The values represent mean and standard error of the mean in parenthesis (S.E.M.).

	TSST Dyslexic($N = 17$)	Non-dyslexic($N = 22$)	CONTROL Dyslexic ($N = 21$)	Non-dyslexic($N = 21$)
Cortisol response (nmol/L)				
$t + 0$	12,048 (3,8273)	12,555 (5,50501)	10,9748 (4,31971)	12,9933 (4,98330)
$t + 25$	15,076 (9,5095)	18,607 (10,08520)	9,5490 (2,98747)	11,6600 (4,58872)
Cortisol reactivity*	3,03 (8,82)	6,05 (9,89)	−1,43 (3,14)	−1,33 (2,87)
% Cortisol responders/ non-responders**	35,3% ($n = 6$)/ 64,7% ($n = 11$)	72,7% ($n = 16$)/ 27,3% ($n = 6$)	14,3% ($n = 3$)/ 85,7% ($n = 18$)	4,8% ($n = 1$)/ 95,2% ($n = 20$)

* Maximum increase in cortisol, compared to pre-stress cortisol ($t + 0$), during the TSST.
** % Cortisol responders using the fixed threshold classification criterion of a 2.5-nmol/L. baseline-to-peak increase.

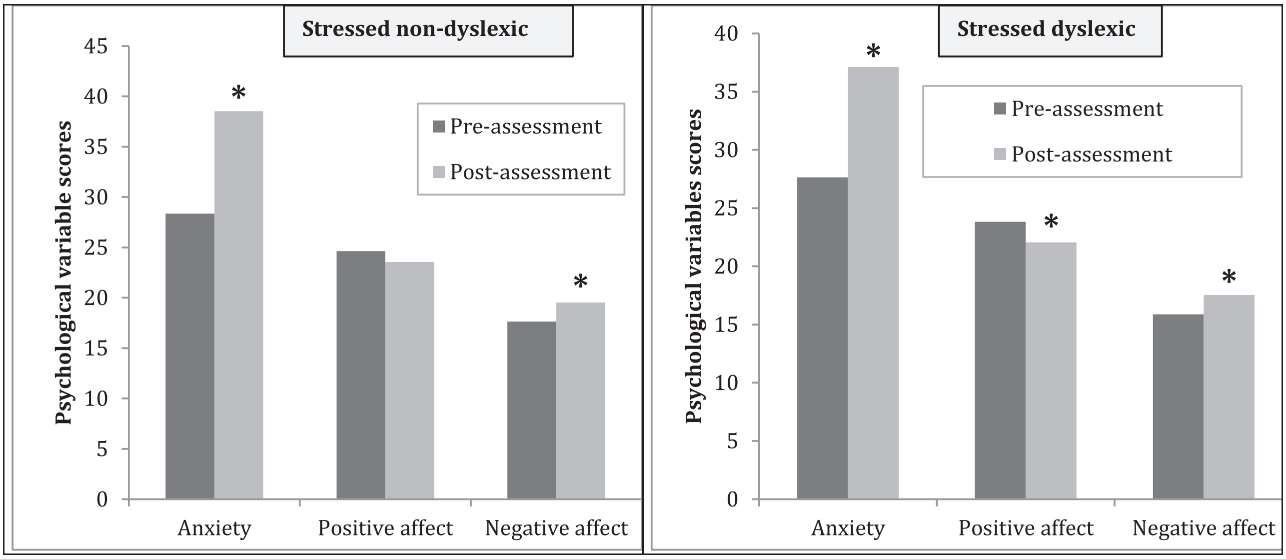

Fig. 5. Mean scores of psychological measures (anxiety, positive and negative affect) in the two stressed groups (non-dyslexic: $N = 22$; dyslexic: $N = 17$) in pre- and post-assessment. Error bars represent standard error of the mean (S.E.M). (*$p < 0.05$).

This suppression may be indicative of an adaptive response because the consequences of chronic exposure to glucocorticoids are known to include negative effects on brain structures [32,33]. At the moment, we do not know of any studies that show evidence of this relationship in dyslexic subjects, although several authors have found that children who experienced early life stress show an attenuated cortisol rise in response to the TSST-C [34,12,13]. Another possible explanation would be that dyslexic children, unable to physically flee from the stress situation, and having to continue with the task even though they know or think that they will fail, chose to hide their failure under a "couldn´t care less" attitude, whose physiological manifestation could be an attenuated cortisol reactivity to stress [35].

In previous work [36,37], pubertal development has been shown to predict increased cortisol responses to this task and other psychosocial stressor tasks. Thus, despite the literature on this question [38], we may have chosen to assess the impact of dyslexia on responsivity to stress at a developmental period that involves enhanced HPA hypo-responsiveness. However, we found no group differences in pubertal status, and few children in any of the groups were past the mid-point of the pubertal stage. Therefore, the attenuated HPA responsiveness found in dyslexic children exposed to stress not might be associated with the pubertal development stage.

There is evidence that dyslexic children are especially vulnerable to stressful environments, as suggested by Alexander-Passe [14]. Certain aspects of a school environment can be perceived as threatening for these children, which may induce stress. In this regard, the continued exposure to reading and writing during the first years of school produces stress in dyslexic children. It is possible that the attenuation of the reactivity to stress in dyslexic children can be attributed to a childhood spent in a chronically stressful school context.

Participants exposed to stress showed a higher negative psychological state, with higher scores on negative affect and state anxiety after the task, whereas in participants in the control condition, negative affect significantly decreased post-task. As expected, these results confirm previous findings showing negative reactions to acute psychological stress [39,40]. In addition, comparing only the two groups of stressed subjects (dyslexic and non-dyslexic), we found an increase in negative affect and state anxiety from pre- to post-task. However, in the case of positive affect, only the dyslexic group showed a significant decrease post-task. In addition, scores on the anxiety and negative affect scales did not differ between dyslexic and non-dyslexic participants at baseline. Thus, although the two groups differed on positive affect before the stress, only the dyslexic group reported less positive affect after it. In this regard, psychological discomfort manifested by dyslexic children after exposure to the TSST-C (decrease in positive affect from pre to post only in the dyslexic group) could be explained by the fact that these children learn to respond anxiously in situations where they have failed in the past. They learn to anticipate failure and, thus, approach new situations with fear and anxiety, which could explain dyslexic children's lower positive affect before the stress. Exposure to the TSST-C is a situation that is very similar to what these children experience every day because they are constantly frustrated and faced with failure at school. Dyslexic children are under constant stress because it takes them so much longer to master many basic skills, which increases fatigue, making them more susceptible to anxiety. Children require great resilience to resist the strain on their self-esteem. The great importance placed on literacy in school contributes to dyslexic children facing work they cannot do all day long, whereas their peers learn quickly. This situation often results in feelings of embarrassment, humiliation, anxiety, anger, frustration, and guilt [41,42,14]. Eventually, a situation of learned helplessness arises where students no longer even attempt to do something new or something they have failed at in the past. They see no point in trying because they are convinced that they will fail. Anxiety increases the impact of dyslexia, leading to a vicious cycle of increased anxiety, decreased motivation, frustration, and failure [41].

Furthermore, we found that cortisol reactivity was positively correlated with anxiety, and negatively with positive affect, in stressed non-dyslexic children. These results are consistent with studies that have shown significant correlations between biological stress markers (cortisol) and psychological stress markers in the adult population [43]. The expected response is that psychosocial stress will trigger the endocrine stress system and increase the subjective emotional experience of stress. Although considerable efforts have been made to link changes in the activity of the hormonal stress systems to changes in the subjective emotional experience of stress in adults, this association has not been studied in children. In our study, this association was not found in stressed dyslexic children. This lack of correlations between subjective and physiological measures in the stressed dyslexic children could be explained by the observed attenuated cortisol response to the TSST-C. In these children, whereas subjective experience seems to respond specifically to social-evaluative stress, their cortisol reactivity to stress could be chronically diminished, suggesting a previous generalized hyperreactivity in daily life [44]. Another possible explanation for this lack of association between the endocrine system and the subjective response to stress would be the dissociation between the two responses found in some studies, where the endocrine response is suppressed, and a subjective response to stress is found [45].

Because there is evidence that psychosocial support has the potential to modify or prevent alterations in HPA axis functioning [46], one limitation of our study is the lack of specific information about family support, school support, and measures of psychological adversity. Thus, it would be interesting to include this information in future studies. Taking these aspects into account could help us to better understand the hypo-responsiveness of the HPA axis and the associated behavioural changes in dyslexic children. Another limitation of the study is the lack of evidence in the literature to explain the differences found between the groups (dyslexic and non-dyslexic) in the basal cortisol levels. Future studies should measure cortisol throughout the day in children with dyslexia to find out whether their HPA axis dysregulation is a generalized (tonic) condition or an acute effect due to the experimental situation.

In summary, the present findings suggest that dyslexic children show reduced cortisol levels in response to a TSST-C. This observation provides new evidence that a dysregulated HPA axis might play a role in dyslexia. Further studies are needed to determine the pathogenesis of this altered response in this disorder..

Ethical statement

The manuscript meets the guidelines for ethical conduct and report of research with humans. Also, the ethics committee of the University of Valencia has approved all the experimental procedures reported on the manuscript. All participants gave informed consent for participating in the present research

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Declarations of interests

None of the authors have any conflict of interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tine.2019.03.001.

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