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Cognitive Performance and Morning Levels of Salivary Cortisol and α-Amylase in Children Reporting High vs. Low Daily Stress Perception

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The aim of the present study was to assess the effects of daily stress perception on cognitive performance and morning basal salivary cortisol and alpha-amylase levels in healthy children aged 9-12. Participants were classified by whether they had low daily perceived stress (LPS, n = 27) or a high daily perceived stress (HPS, n = 26) using the Children Daily Stress Inventory (CDSI). Salivary cortisol and alpha-amylase were measured at awakening and 30 minutes later. Cognitive performance was assessed using the Cognitive Drug Research assessment system. The HPS group exhibited significantly poorer scores on speed of memory ($p < .05$) and continuity of attention ($p < .05$) relative to the LPS group. The HPS group also showed significantly lower morning cortisol levels at awakening and at +30 minutes measures in comparison with the LPS group ($p < .05$), and mean morning cortisol levels were negatively correlated with speed of memory ($p < .05$) in the 53 participants. No significant differences were observed between both groups in alpha-amylase levels. These findings suggest that daily perceived stress in children may impoverish cognitive performance via its modulating effects on the HPA axis activity.
Keywords: children, self-perceived stress, salivary cortisol, salivary alpha-amylase, cognition

El objetivo del presente estudio fue evaluar los efectos de la percepción de estrés diario sobre el rendimiento cognitivo y los niveles matutinos basales de cortisol y alfa-amilasa salivar en niños sanos de edades entre los 9 y los 12 años. Los participantes fueron clasificados en función de si su nivel de percepción de estrés diario era bajo (LPS, n = 27) o alto (HPS, n = 26), empleando el Children Daily Stress Inventory (CDSI). Se midió el cortisol y la alfa-amilasa salivar al despertar y 30 minutos más tarde. El rendimiento cognitivo se evaluó mediante el sistema de evaluación Cognitive Drug Research. El grupo HPS obtuvo puntuaciones significativamente más bajas en velocidad de memoria ($p < .05$) y continuidad de la atención ($p < .05$) con respecto al grupo LPS. El grupo HPS también mostró niveles significativamente más bajos de cortisol matutino al despertar y a los 30 minutos en comparación con el grupo LPS ($p < .05$), y sus niveles medios de cortisol matutino correlacionaron negativamente con la velocidad de la memoria ($p < .05$) en los 53 participantes. No se observaron diferencias significativas entre los grupos en los niveles de alfa-amilasa. Estos resultados sugieren que la percepción de estrés diario en niños puede disminuir su ejecución cognitiva a través de sus efectos moduladores en la actividad del eje HPA.
Palabras clave: niños, estrés auto-percibido, cortisol salivar, alfa-amilasa salivar, cognición

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The primary aim of the present study was to assess the effects of high daily stress perception on early morning activity of the hypothalamic-pituitary-adrenocortical (HPA) and symphatetic-adrenal-medullary (SAM) systems and on cognitive performance in healthy children. Although it has been hypothesized that a high level of stress or hassles during childhood may have both short- and long-term effects on physical and psychological health (Tennes & Kreye, 1985), until recently the impact of everyday stressors has received little attention (Turner-Cobb, 2005) while there has been significant attention to the impact of major life events and stressors (e.g., maltreatment or loss). The lack of systematic research on the effects of "non traumatic" daily stressors during childhood is surprising, as chronic and continuous stressful events experienced early in life are known to play a specially important modulating role on physiological stress system as well as on learning and memory processes (Bremner & Narayan, 1998; Heffelfinger & Newcomer, 2001; Heim & Nemeroff, 2001).

Stress has been generally defined as a state occurring when an individual perceives that the demands of the environment exceeds his or her ability to cope with those demands (Adler et al., 1994). According to the transactional model of stress and coping, a good knowledge of stressors and their management by the child is key in understanding how stress is experienced by children. Lazarus & Folkman (1984) proposed a two-step approach to evaluate this process. The potential harm or benefit of a given event needs first to be assessed, followed by an evaluation of the resources and skills of the individual to cope with the situation. All these variables are interdependent on one another and directly affect the type of emotion experienced by the individual (Folkman & Lazarus, 1985). In our bibliographic review, we have not found any psychoendocrinological study in the child literature since this theoretical perspective. On the contrary, the majority of the reviewed papers, which it will be commented below, examine the effects of objective stressors (e.g., the exposition to environmental noise) or the effects of psychosocial risk factors (e.g., the membership to a specific socioeconomic status group or to get an immigrant status) on stress hormones and/or on cognitive performance.

In children, daily or everyday mild stress may be caused by moderately unpleasant events occurring in their family, school, and/or deriving from health changes (Birch, 1998; Del Barrio, 1997; Milgram, 1992). Marital conflict between parents (Cummings, Davies, & Simpson, 1994), as well as financial difficulties in the family (Flinn & England, 1997; Langrock, Compas, Keller, Merchant, & Copeland, 2002; Seifer et al., 1996), fear of poor health (Parmelee, 1997), being overweight (Birch, 1998; Stefanello, 2004), perceived or actual discrimination by teachers (Piekarska, 2000), problems and difficulties in the study of subjects (Roeser & Eccles, 1998; Torsheim & Wold, 2001; Torsheim, Aaroe y Wold, 2003) or bullying by other children at school (Raviv et al., 2001; Richters & Martinez, 1993) have all been shown to negatively impact the health and psychological development of children. The perception of these stressors can induce in children changes in the neuroendocrine stress axis, namely: the symphatetic-adrenal-medullary (SAM) system (also termed the LC-NE system) and the hypothalamic-pituitary-adrenal (HPA) axis (Aston-Jones & Cohen, 2005; Chrousos & Gold, 1992; Turner-Cobb, 2005).

Adrenaline (A) and noradrenaline (NA) are the main adrenergic hormones of the SAM system. This system has its cephalic region in the locus coeruleus. From this nucleus, the main source of NA afferents to the rest of the brain, a dense network of noradrenergic axons extend through-out the cerebral cortex to multiple cortical and subcortical areas, including hippocampus, amygdala, thalamus, hypothalamus, bed nucleus of stria terminalis, nucleus accumbens, as well as descending projections which synapse at the level of thoracic spinal cord to inervate the adrenal medulla. The SAM system acts rapidly (in just seconds) after the onset of the perception of a physical or psychological stressor. The sympathetic branch of the autonomic nervous system represents the neural effectors of this system. It supports the fight/flight response releasing noradrenaline since the postganglionar cells in numerous body's organs and structures. This neural response is complemented by an also rapid humoral response, the releasing of NA and, especially, A to the bloodstream from adrenal medulla. Both actions induce a series of physiologic and metabolic changes vital for adaptation. Chronic stress has been associated with potentiated release of noradrenaline with exposure to subsequent stressors, and early stress has been associated with lifelong increases in sensitivity of the noradrenergic system (Bremner & Vermetten, 2001). The precise role of catecholamines in cognition is an issue that remains unclear in the literature. The SAM system function has been classically related to arousal, memory-enhancing effects of emotional information, and more recently to the optimization of reward seeking behaviors (Aston-Jones & Cohen, 2005). Nevertheless, contradictory findings have been obtained from both human and animal studies of attentional processes where both over- and under-arousal are associated with poor performance (Skosnik, Chatterton, Swisher, & Sohee, 2000).

On the other hand, cortisol is considered the primary glucocorticoid hormone produced by the HPA axis in humans. After stress perception, corticotropin releasing factor (CRF) is secreted from hypothalamus and acts in the anterior lobe of the pituitary gland to promote the release of adrenocorticotropin hormone (ACTH) into the bloodstream. ACTH acts then on adrenal cortex where it induces the releasing of glucocorticoids (GCs; corticosterone in some animals and cortisol in humans). Under basal conditions, cortisol follows a circadian rhythm with elevations in the morning and decreases over the day. After exposure to stressor, cortisol levels rise less rapidly than catecholamines (10-15 minutes), acting in numerous areas of the body and brain. In the brain it modulates neurophysiological, emotional and cognitive functioning. To produce these effects,

cortisol binds to two receptor subtypes: the mineralcorticoid (MR or type-I) and glucocorticoid (GR or type-II) intracellular receptors with differential affinity. MRs bind GCs with an affinity that is about 6-10 times higher than of GRs (Reul & DeKloet, 1985). MRs and GRs receptors present also a different distribution in the brain. MRs are present in the hippocampus, parahippocampal gyrus, entorhinal and insular cortices. GRs are present in paraventricular nucleus and other hypothalamic nuclei, the hippocampus and parahippocampal gyrus and cortical structures (preferentially in the prefrontal cortex) (Diorio, Viau, & Meany, 1993; McEwen, DeKloet, & Rostene, 1986; McEwen, Weiss, & Schwartz, 1968; Meany & Aitken, 1985).

In contrast to the unclear adrenergic hormones functions on cognition, GCs are more salient modulators of perception, arousal, attention and memory (Erickson, Drevets, & Schulkin, 2003). The hippocampus, a sensitive structure to GCs, is a key cerebral structure for declarative or explicit memory. Animal and human data had shown that the cumulative exposure of the hippocampus to high levels of GCs contributes to hippocampal atrophy and memory impairments (Landfield, Baskin, & Pitler, 1981; Landfield, Waymire, & Lynch, 1978; Lupien et al., 2005; McEwen, 2000). These results have given rise to the "glucocorticoid-cascade hypothesis" which suggests that there exist a significant relationship between cumulative exposure to high levels of GCs, impaired memory function and atrophy of the hippocampus (Sapolsky, Krey, & McEwen, 1986). Prefrontal cortex is another key-structure of the brain essential for cognitive functions as attention, executive functions and/or working memory. In the primate's brain, GRs are present with higher density in frontal lobes and show a lower density in the hippocampus in contrast with the levels originally described in rodent literature (Lupien et al., 2005; Patel et al., 2000; Sanchez, Young, Plotsky, & Insel, 2000). Some works have shown that cognitive functions sensitive to frontal deficits (e.g., working memory) are more sensitive to GCs administration than cognitive function sensitive to hippocampal damage (Lupien, Gillin, & Hauger, 1999; Lupien et al., 2005; Young, Sahakian, Robbins, & Cowen, 1999). In general, cognitive performance shows a well-documented inverted-U (curvilinear) shape relationship with glucocorticoids concentrations in animal and human studies (Lupien et al., 2005) being the ratio of MR/GR occupation a major proposed determinant of the direction of GC-induced cognitive changes (DeKloet, Oitzl, & Joels, 1999). Moderate levels are optimal while extremely low or high concentrations have adverse cognitive outcomes (Beckwith, Petros, Scaglione, & Nelson, 1986; Lupien & McEwen, 1997).

In the few studies on stress, hormones and cognitive function in children, GCs have been by far the most studied stress hormones in comparison to catecholamines. As indicated above, we have not found any studies which have assessed the effects of subjective perception of stress in cognitive performance of children. However, several studies have examined GCs-cognition relation for high risk groups. Lupien, King, Meaney, & McEwen (2000; 2001) examined cognitive performance in children coming from different socioeconomic status (SES) groups. They reported non-significant differences in selective attention, declarative memory, non-declarative memory and language despite evidence that lower SES children exhibit higher levels of cortisol than higher SES children. In other studies, children living in noisy environments have been found to exhibit cognitive deficits in some but not all areas (e.g., complex reading, selective attention and concentration tasks). These deficits were accompanied by elevated 8-h overnight urinary and morning salivary cortisol as well as increased blood pressure relative to children living in less noisy areas (Evans, Lercher, Meis, Ising, & Kofler, 2001; Ising & Ising, 2002). Nevertheless, in some of these studies cognitive deficits have not been accompanied by differences in basal levels of cortisol (Haines et al., 2001a; 2001b).

Non-invasive methods for the determination of urinary and salivary cortisol and urinary catecholamines permit the study of endocrine activity in child populations. These methods allow jointstudy of both arms of the stress axis (Charmandari, Kino, Souvatzoglou, & Chrousos, 2003; Chrousos & Gold, 1992). Salivary alpha-amylase (sAA) has been proposed as a possible non-invasive marker of adrenergic activity (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Chatterton, Vogelsong, Lu, & Hudgens, 1997; Gilman, Thornton, Miller, & Biersner, 1979; Nater et al., 2005; 2006; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004; Rohleder, Wolf, Maldonado, & Kirschbaum, 2006; Stegeren, Rohleder, Everaerd, & Wolf, 2006). In 2004, Rohleder et al. reported that while the well-known circadian pattern of salivary cortisol is characterized by a morning peak observed approximately 30 minutes after waking and a slow decline during the day, salivary alpha-amylase levels (also sAA awakening response) appears to exhibit the opposite circadian pattern. SAA basal levels appear to be also sensitive to the influence chronic stress levels (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). Chatterton et al. (1997), in line with this results, also reported a significant statistically increase in morning sAA activity between a control-day and the jump-day in subjects exposed to the first skydiving jump. Hence, in the present study, we will measure free salivary cortisol as a marker of morning HPA axis activity and sAA as a possible marker of morning adrenergic activity.

In sum, the primary goal of the present study has been to investigate possible hormonal differences associated with accumulated daily perceptions of mild stressors in healthy children and the effects that these hormonal differences have on cognitive performance. The specific objectives of this study were to assess the effects of the high or low daily stress perception on cognitive performance (specifically, attention, working memory and episodic memory) and on morning salivary cortisol and salivary alpha-amylase levels. Finally, we examined whether these hormones mediated the effect of daily stress perception on cognitive performance.

Method

Participants

This study is part of an ongoing epidemiological study on the effects of perceived stress in 9- to 12- years-old children. From an initial pool of 265 boys and girls attending five public schools located in the metropolitan area of Malaga (Spain), 116 were selected exclusively according to its extreme Children Daily Stress Inventory score's (criteria for inclusion; CDSI score ≤ or ≥ [mean ± 1 SD]). The groups were defined as Low Perceived Stress (LPS, CDSI scores ≤ [mean = 12.49 – SD = 5.44]) and High Perceived Stress (HPS, CDSI scores ≥ [mean = 12.49 + SD = 5.44]) groups. Children with middle scores in CDSI were deliberately excluded in this study. Consent to participate was solicited from the families of these children and 62 (53.44 %) families were agreed to take part in our study. All the participants were in good general health according to parent report except nine children who were excluded because they were taking prescription medication. The sample thus consisted of 53 children, 27 children in the LPS group (13 girls and 14 boys) and 26 children in the HPS group (10 girls and 16 boys). The study was conducted according to the principles laid down in the Declaration of Helsinki (1996).

Measures

Assessment of Child Daily Stress. Children Daily Stress Inventory (CDSI) consists of 48 items that assess common problems for children. The inventory was developed according to criteria set by a panel of experts at the University of Malaga and subsequently refined to adjust to the understanding of children. The items of the CDSI describe daily life events documented in the literature to trigger stress and linked to adjustment problems on emotional, social, and school domains. They are organised in three areas, Health, School and Family (e.g., "I have been sick several times this school year", "My teachers are very demanding", "I find my homework difficult", "I spend too much time alone at home", "A close relative has recently died") and are introduced by the question: "Does this happen to you in the last year?" which has to be answered with either YES or NO. Test-retest reliability of the total score of CDSI was .81 and its convergent validity with composite scales of the Behavior Assessment System for Children (BASC, Reynolds & Kamphaus, 1992) were .62, .48, .58, –.51 for emotional symptoms index, school maladjustment, personal adjustment and clinical maladjustment respectively (Fernandez, Hierrezuelo, Blanca, Morales, & Muñoz, 2005; Trianes, Blanca, Fernandez, Escobar, & Maldonado, 2007).

Cognitive Assessment. The Cognitive Drug Research (CDR) computerised assessment system has been specifically developed to measure changes in cognitive function over time. It has been shown to be sensitive in previous studies with child samples (Wesnes, Pincock, Richardson, Helm, & Hails, 2003). To assess attention, working memory and episodic memory functions, a selection of tasks from the CDR (see the specific tasks below) were administered at school on one single occasion during the morning. All 53 children were presented with exactly the same battery of tests. All tasks were computer-controlled, the information being presented on VGA colour monitors, and the responses recorded via response modules containing two buttons, one marked «NO» and the other «YES». All tests and task instructions were presented in Spanish. In order to instruct the children in the use of the button box and overcome initial anxiety, three tasks (Simple Reaction Time, Choice Reaction Time and Digit Vigilance) were completed in a shortened form before the beginning of the assessment. Completion of the entire battery took around one hour. The following tasks from the CDR system were chosen and administered in the following order: word presentation, immediate word recall, picture presentation, simple reaction time, digit vigilance task, choice reaction time, spatial working memory, numeric working memory, delayed word recall, word recognition, picture recognition. Detailed descriptions of the tests used are provided by Wesnes, Ward, McGinty, & Petrini (2000).

The factor structure of the CDR test system has been established using principal components analysis (see Wesnes et al., 2000, for details). This analysis confirmed the construct validity of the battery, by demonstrating that the task measures within theorized cognitive domains of attention, working memory and episodic secondary memory, loaded together on common factors. The five composite factor scores comprise Power of Attention, Continuity of Attention, Quality of Working Memory, Quality of Episodic Secondary Memory, and Speed of Memory. These composite measures were used in the analyses.

Morning Salivary Cortisol and Salivary Alpha-Amylase Determinations. To determine cortisol and alpha-amylase levels, saliva samples were obtained using the Salivette sampling device (Sarstedt). Subjects were instructed to abstain from breakfast and not to brush their teeth until the end of the sampling period. Children were trained to chew slightly the Salivette device for exactly one minute. Two samples were obtained in the morning at home to assess the cortisol awakening response and early morning salivary alpha-amylase levels, the first immediately after awakening and the second 30 minutes later (Meinlschmidt & Heim, 2005). The net increase in cortisol between awakening and 30 minutes after waking has been found highly sensitive to group differences (Meinlschmidt & Heim, 2005; Pruessner, Hellhammer, & Kirschbaum, 1999). The sAA response to awakening was originally described by Rohleder et al. in 2004 although was examined in adult men. Rohleder et al. (2006) have also showed recently that valid measures of salivary alpha-amylase can be obtained by using salivettes without assessing flow rate. The collection of saliva samples and its conservation was supervised by parents. Samples were immediately frozen at home at -20º C and collected later during the day by an investigator.

For analysis, the samples were thawed and spun at 3,000 rpm for 5 minutes to obtain a clear, watery supernatant with low viscosity. For free cortisol determination 20 µl saliva were removed for analysis using a time-resolved fluorescence immunoassay (DELFIA; Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). The lower detection limit of this assay is 0.43 nmol/l with interassay and intraassay coefficients of variance of <10% across the expected range of cortisol levels. For the alpha-amylase assay an enzymatic colorimetric assessment was employed (Kirschbaum, Wolf, & Rohleder, 2003; Rohleder et al., 2004). Briefly, 20 µl of diluted saliva (1:625) were pipetted into a 96-well microplate, to which 80 µl of the substrate (ethyliden-G_7PNP; Roche Diagnostics, Mannheim, Germany) was added. The microplate was then placed into a water bath (37 ºC) for 90 s. Two readings were obtained at 405 nm and 37 ºC at an interval of 5 minutes. Alpha-amylase activity was calculated as the change in OD_{405nm} between the two readings against a standard of 8 alpha-amylase concentrations (0 to 785 U/mL; LinTrol, Sigma, St. Louis, USA). The most common index of salivary alpha-amylase activity is the determination of enzyme level per volume of saliva or enzyme units per milliliter (Nater et al., 2004). Inter- and intra-assay variation was below 10%.

Procedure

Before starting data collection, mothers of the selected children were invited to an information session in which a general explanation (oral and written) of the investigation was given. Special attention was put in two points: (a) the instruction of mothers (orally and written information) and children on the timing of saliva sampling, (b) appropriate conservation of samples in the home freezer (–20º C) and (c) mothers have to fill a record sheet with observations on any violation of protocol. Two trained examiners for cognitive testing were introduced to all children and mothers during this meeting. Mothers filled a questionnaire about family demographics, parent education and parent occupation from which SES data were derived. Home saliva samples were collected two to three days before cognitive testing on a typical school day in the middle of the week (tuesday, wednesday or thuesday). To avoid anticipatory stress responses, home saliva sampling was not conducted on the day of testing. All the children reported awakening time, sleep duration and sleep quality during the night before. On the day of cognitive testing, the CDR-battery was administered once to each participant between 9:30 and 11:30 AM. Cognitive assessment was conducted in quiet and suitable rooms in each school.

Statistical Analyses

Sex, age and anthropometric data of the two groups [height, weight, body mass index (BMI), waist-hip Ratio (WHR)] were compared using either a χ^2 test (for categorical data) or a Student's *t* test (for continuous data). Cognitive variables were compared between both groups (LPS and HPS) using a two-tailed *t*-test. Sex effect on morning cortisol and alpha-amylase levels were assessed through two-way (group by time) within-subject ANOVAs with repeated measurements on time. For salivary cortisol and alpha-amylase, 2×2 ANOVAs with the level of perception of stressors (HPS and LPS groups) as a grouping factor and time (awakening and +30-min samples) as a repeated factor were conducted. Adjustment of degrees of freedom was conducted according to Greenhouse-Geisser, where appropriate. Relationship between mean cortisol levels and cognitive variables were examined through bivariate and partial Pearson-moment product correlations. All analyses were performed using SPSS 11 for Mac OS X. Unless indicated, all results shown are mean ± standard deviation (*SD*). A criterion of $p < .05$ was set for significance.

Results

Anthropometric and Sociodemographic Indicators of LPS and HPS Groups

No statistically significant differences were observed in anthropometric data (height, weight, body mass index and waist-hip ratio), gender or age between LPS and HPS groups (see Table 1). Mothers of children of both groups did not differ in indicators of SES (all *p*s > .1).

Table 1
Comparison of Anthropometrics Indicators, Sex and Age of the Low Perceived Stress and High Perceived Stress Groups (Mean ± SD)

Parameters	LPS Group (*n* = 27)	HPS Group (*n* = 26)	*p*
Height (cm)	140.37±8.43	142.35±9.88	*ns*
Weight (kg)	38.33±10.35	42.27±13.64	*ns*
Body Mass Index	13.53±2.99	14.69±4.08	*ns*
Waist-Hip Ratio	0.84±0.05	0.86±0.08	*ns*
Age (years)	10.78±1.18	11.19±0.89	*ns*
Male/Female Ratio	14/13	16/10	*ns*
Awakening Time	7:49±0:19	7:38±0:30	*ns*

Differences in Cognitive Performance between the LPS and HPS Groups

The results of the analyses on cognitive performance are presented in Table 2. Compared to the LPS group, the HPS group showed significantly lower continuity of attention, $t = 2.18$, $p = .034$, Cohen´s $d = 0.60$, effect size $r = .28$, and slower speed of memory, $t = –2.56$; $p = .013$, Cohen's $d = –0.70$, effect size $r = –.33$ (speed memory scores mean time spent in the memory task). They did not differ significantly on either of the two attention subscales (power of attention) or on two of the three memory subscales (quality of working memory and quality of episodic secondary memory), thus their performance was not poorer across the board.

Morning Basal Salivary Cortisol and Salivary Alpha-Amylase Levels in LPS and HPS Groups

Cortisol levels at awakening and 30 minutes later for the 53 participants are presented in Table 3 and Figure 1 (panel A). The repeated measures ANOVA revealed a significant main effect of time of measurement, $F(1, 51) = 28.78$, $p < .001$, with no main effect of sex of child, $F(1, 51) = 1.24$; *ns*. The effect size for the time effect was $\eta^2 = 0.361$ with an observed power of 1. A 2 (Group) × 2 (Time) ANOVA with repeated measures in the last factor including six covariates (waking time, sleep quality, age, sex, BMI and WHR) was conducted. The results yield no effect of the covariates thus they were removed and the analysis recomputed without covariates. Cortisol showed an absolute mean increase of 8.5 nmol/l (55.55%) from waking to 30 minutes later. Using criteria proposed by Wüst, Wolf, et al. (2000) participants were grouped as exhibiting or not exhibiting a cortisol awakening response. We found that most exhibited this response (76 vs. 24%). Distribution of responders and non-responders was not different by groups, $\chi^2(1) = 2.533$, $p = .11$. A significant group effect in morning cortisol levels was observed, with the HPS group showing lower cortisol levels at both time points in comparison with LPS, $F(1, 51) = 4.42$, $p = .04$. The effect size for the group effect in the cortisol awakening response was $\eta^2 = 0.080$ with an observed power of 0.542. The time by group interaction was not significant, $F(1, 51) = 0.16$, $p = .68$. Thus, the groups did not differ in cortisol awakening response (CAR).

Because the CAR did not differ by group while cortisol levels at both time points did, we examined whether the two measures could be averaged to provide a morning cortisol level measure for use in subsequent analyses. Therefore the values were averaged. To assess whether mean morning cortisol levels were associated with the group difference in cognitive performance observed for speed of memory and continuity of attention, we computed bivariate correlation coefficients. Morning cortisol levels were negatively correlated with speed of memory ($r = –.26$, $p = .03$). Thus children with lower morning cortisol levels performed more poorly on this cognitive task. When LPS/HPS group was partialled out of the association between

Table 2
Cognitive Scores of Low Perceived Stress and High Perceived Stress Groups on Subscales of the Cognitive Drug Research Computerized Assessment System (Mean ± SD)

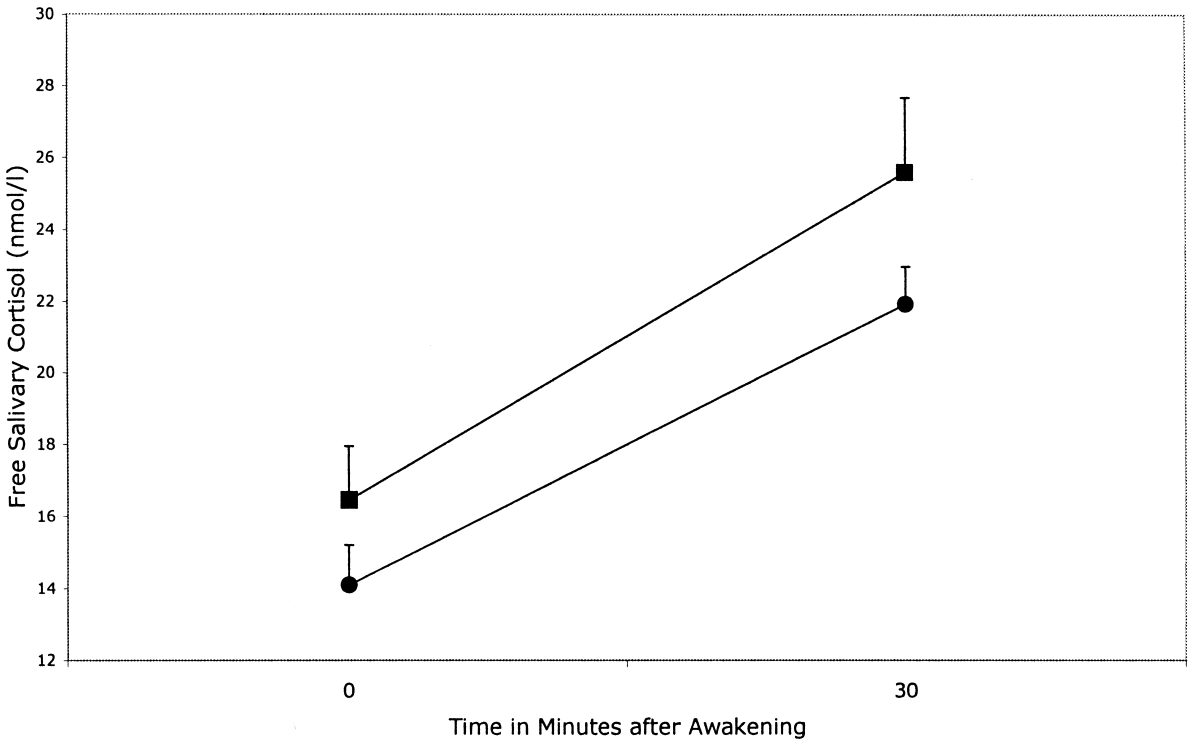
Cognitive Factors (ranges)	*N*	LPS (*n* = 27)	HPS (*n* = 26)	*p*
Power of Attention (900-5000)	53	1468.32±218.76	1484.32±239.93	*ns*
Continuity of Attention (0-95)	53	84.96±8.49	80.46±6.32	< .05
Speed of Memory (2000-10000)	53	4165.77±1037.86	4943.04±1166.22	< .05
Quality of Working Memory (0-2)	53	1.60±0.20	1.52±0.28	*ns*
Quality of Episodic Memory (0-400)	53	157.87±25.87	143.61±44.66	*ns*

Table 3
Comparison of Cortisol (Nmol/L) and Salivary Alpha-Amylase (U/Ml) of the Low Perceived Stress and High Perceived Stress Groups (Mean ± SEM)

Parameters	LPS Group (*n* = 27)	HPS Group (*n* = 26)
Awakening Cortisol	16.45±1.50	14.10±1.11
Cortisol + 30 min	25.60±2.08	21.93±1.04
Cortisol Increase*	9.13±2.85	7.83±1.27
Awakening sAA	42.86±6.23	44.23±4.85
sAA + 30 min	47.06±5.62	54.05±6.97
sAA Increase*	4.19±7.27	9.82±6.07

Note. *Increase = 30-min measure – awakening measure. sAA = salivary Alpha-Amylase

(A)



(B)

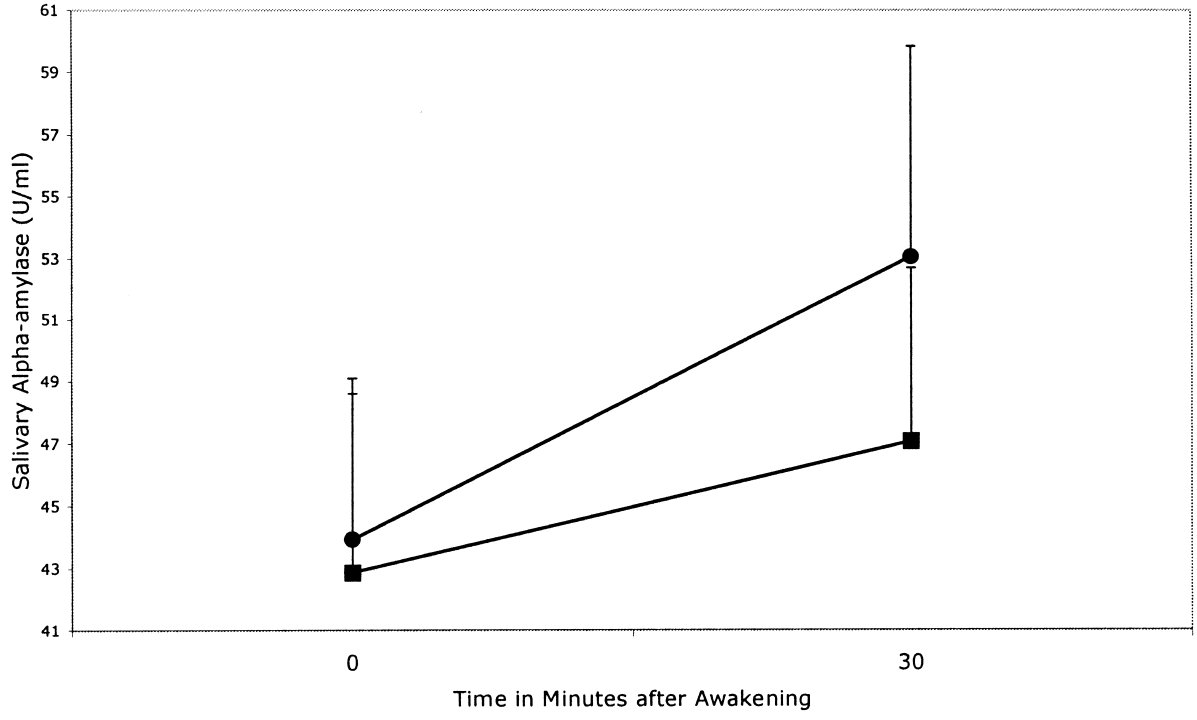


Figure 1. Panel A. Mean cortisol awakening response (± SEM) in the Low Perceived Stress group (■; $n = 27$) and in the High Perceived Stress group (●; $n = 26$). Panel B. Mean awakening alpha-amylase response (± SEM) in the Low Perceived Stress group (■; $n = 27$) and in the High Perceived Stress group (●; $n = 26$).

morning cortisol levels and speed of memory, the correlation decreased to r = –.18 (p = .09). This result suggests that although group of stress has an impact on the association between mean morning cortisol levels and performance in speed of memory, it is not the only factor explaining this relationship. No significant correlations were observed between mean cortisol levels and continuity of attention, suggesting that the impairment of HPS group in sustained attention were not associated with basal levels of glucocorticoids.

Regarding morning salivary alpha-amylase levels (see Table 3 and Figure 1, panel B), repeated-measures ANOVA yielded neither a significant group effect, $F(1, 51) = 0.26$, p = .61, nor a time effect, $F(1, 51) = 2.17$, p = .14. The interaction time by group was not significant either, $F(1, 51) = 0.35$, p = .55. No differences were observed between both sexes in this parameter.

Discussion

The results of the present study show that children with high self-reported perception of mild daily stressors in several domains of their lives score more poorly on several test of cognitive performance than children with low self-reported perception of stressors. Their profile of lower cognitive performance comprises a poorer ability to sustain concentration and longer time taken to retrieve information from working and/or episodic memory. In addition, children reporting higher perceived stress had lower early morning cortisol levels than did children reporting lower perceived stress. Finally, early morning cortisol was significantly and negatively correlated with the speed of memory. This correlation was present even after controlling for perceived stress group, suggesting some degree of association between lower HPA axis functioning and poorer memory performance.

The effects of high self-perceived stress on the speed of memory, a factor which reflects the ability to store, hold and retrieve information of an episodic nature, reported in the present study are consistent with the view that the effects of corticosteroids on cognition are selective (Lupien et al., 1999). One possible explanation of the pattern of results observed here may result from an extension of Lupien and McEwen´s (1997) proposal that an inverted U-shaped relationship exists between GCs and the nature and magnitude of cognitive function. Lupien et al., (2002) showed in two elegant studies that GCs have modulatory effects on learning and memory. A marked reduction in morning circulating levels of GCs decreased the performance on a 20 min. delayed memory task when compared to a placebo condition, while the administration of high dose of GCs at the time of the circadian trough had a positive impact on cognitive efficiency. These findings are consistent with the results reported here with the notable difference that in our study we examined long term effects of chronic life stressors in contrast of these studies using laboratory stressors and exogenous administration of synthetic GCs (Sauro, Jorgensen, & Pedlow, 2003). Is well known that regions of the amygdala and hippocampus, which contains GRs and MRs, participate in a memory system specific to autobiographical (or episodic) events (Fink et al., 1996) in which moderate concentrations of cortisol has a facilitator effect on memory while high or low levels are associated to cognitive impairments (Akirav & Richter-Levin, 2002; Roozendaal, 1999). The HPS group in our study might be representing this last case.

The execution in continuity of attention, a factor which reflects de ability of the volunteer to sustain attention and which is dependent of the activity of the anterior attentional system (Posner & Rothbart, 1994), were also poorer in the HPS group although this factor was not significantly correlated with the lower cortisol levels of this group. Nevertheless, Davis, Bruce, & Gunnar (2002) have reported also in healthy children (5-6 years-old) that poorer accuracy in neuropsychological tasks which assessing brain structures involved in the anterior attentional system are associated with lower circadian cortisol concentration at home and in the lab in response to a neuropsychological assessment. This last result is also coherent with the recent study conducted by Blair, Granger, & Razza (2005) on children (4-5 years-old) in which moderate increase in cortisol followed by down-regulation of this increase was positively associated with measures of executive function. In sum, the literature suggests a facilitatory role for moderate levels of cortisol in studies on arousal, attention and concentration (Erickson et al., 2003). In our study, lower cortisol levels as observed in the HPS group might be related to specific impairments in limited areas of children's cognition (speed of memory) principally regulated by the hippocampus but not to other cognitive deficits observed like continuity of attention (or at least the attention task was not sensitive enough to detect the effect). In any case, further research is necessary to improve our knowledge of the relationship between morning salivary cortisol levels and cognitive performance in children as well as the potential indirect mechanisms underlying this association.

The lower levels of cortisol in the HPS group represent an interesting but unexpected finding that must be discussed thoroughly. The studies examined in the introduction suggest that low SES (which is usually associated to higher exposure to unspecific stressors) in childhood is accompanied by an elevation in the secretion of glucocorticoids. In our study, however, a high frequency of self-reported perception of stressors was not associated with increased morning levels of cortisol. On the contrary, the HPS group exhibited lower levels of cortisol after waking up than the LPS group. This finding apparently violates the central dogma that stress should be associated with elevated cortisol concentrations (Gunnar & Vazquez, 2001). However, early experiences of

prolonged chronic stress during childhood have been associated in the scientific literature with low basal levels of cortisol and a flattening of expected daytime rhythm (Gunnar & Vazquez, 2001). A distant relationship with parents, living with less sensitive parents, less responsive and low quality parental care or living with parental conflicts are experiences that have been consistently associated in the literature with lower basal cortisol levels in the morning hours in low-risk populations of children (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Spangler & Grossman, 1993; Spangler & Schieche, 1998). This corpus of research suggest that hypocortisolism may be a feature associated with a number of stress-related states across the lifespan which may possibly be conferring risks for certain psychosomatic disorders. However, as we used a cross-sectional experimental design in our study we may only detect relationships and speculate on the hypothetical causes of such cortisol down-regulation (for comprehensive reviews see Fries, Hesse, Hellhammer, & Hellhammer, 2005; Gunnar & Vazquez, 2001; Heim, Ehlert, & Hellhammer, 2000).

One possible and tempting explanation is that the decreased response in the HPS group might be the result of down-regulation at some level in the HPA axis after chronic, repeated and continued impact of minor physical and psychosocial stressors (Fries et al., 2005). In adult populations, low SES or burnout syndrome, conditions clearly associated with chronic stress, have also been associated with low morning cortisol and decreased well-being (Brandtstadter, Baltes-Götz, Kirschbaum, & Hellhammer, 1991; Pruessner et al., 1999). Alternative explanations, such as a minor adrenocortical dysfunction, cannot be ruled out. The use of the low-dose dexamethasone test in a future study with these groups may help in the knowledge of the possible mechanism of this down-regulation of cortisol.

The difference in methods used and the criterion for children's classification employed in the present study are factors that may also account for this discrepancy. First, we measured the effects of the frequency of self-perceived stressors on morning salivary cortisol levels with strict respect to awakening time. Although the physiological role of the CAR has not been clearly defined, evidence suggests that it is under a distinct regulatory influence, different from the rest of the diurnal cortisol secretory cycle (Clow, Thorn, Evans, & Hucklebridge, 2004). This approach has several relevant advantages compared with other traditional determinations of cortisol regulation (Pruessner, Hellhammer, Pruessner, & Lupien, 2003). Early morning cortisol response to awakening has been shown to be a reliable biological marker for adrenocortical activity (Edwards, Evans, Hucklebridge, & Clow, 2001; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Wüst, Federenko, Hellhammer, & Kirschbaum, 2000). This response, partly genetically determined, is measured with strict reference to the time of waking. It shows good intra-individual stability across days and weeks and allows one to differentiate between subgroups of healthy individuals (Pruessner et al., 1997; Wüst, Wolf, et al., 2000). The studies conducted on child daily stress and reviewed in the introduction can be broadly divided in two groups according to the methods used for cortisol determination. For instance, Evans & English (2002) employed 12-h overnight (8 PM-8 AM) urine samples for cortisol determination while Lupien et al. (2000; 2001) and Flinn & England (1997) examined levels of morning salivary cortisol without strict reference to awakening and its circadian pattern. Obviously, the values of cortisol levels in overnight urine samples or measured in saliva without strict reference to awakening are not direct comparable markers of adrenocortical activity. Second, and more importantly, in the present study we selected children according to their subjective and private perception of a wide range of stressors in major domains such as health, family and school and not according to objective exposition to environmental stressors like noise or the membership to a specific familiar SES. If personality and/or coping resources or coping strategies are underlying variables to this association is a question that must be response in future research (Korte, Koolhaas, Wingfield, & McEwen, 2005).

Finally, our study is also the first from our knowledge exploring awakening salivary alpha-amylase levels in child populations with different degrees of stress perception. The research on salivary alpha-amylase as a SAM activity marker has just started and has been so far exclusively conducted in adult populations (Chatterton et al., 1996; 1997; Nater et al., 2005; 2006; 2007; Rohleder et al., 2006; Stegeren et al., 2006). The first and only reports of circadian variations and awakening response of this salivary enzyme (Kirschbaum et al., 2003; Nater et al., 2007; Rohleder et al., 2004) have shown that alpha-amylase is possibly secreted in a circadian fashion in a pattern that seems to mirror the rhythmic changes in cortisol levels. Hence, the non-statistically significant difference in alpha-amylase levels between HPS and LPS groups observed in the present study might be interpreted as non-differential adrenergic activity, at least, in the morning hours. Further research is necessary to improve our knowledge on the utility of sAA as a stress marker, especially, in child samples, where its future may be especially promising.

In spite of the several important limitations of the present study like the sample size, the levels of both cortisol and alpha-amylase collected on a single day and the non use of electronic monitoring devices to control adherence, this study provides sufficient evidence that greater accumulated self-perception of non-traumatic stressors during childhood is associated with a poorer performance of sustain concentration and speed memory being impairs in speed of memory associated to low cortisol levels observed in the HPS group. These interesting initial results warrant a larger follow up study.

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