Psychoneuroendocrinology (2015) 60, 7—17

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/psyneuen

Psychological and cortisol reactivity to experimentally induced stress in adults with ADHD

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Received 28 January 2015; received in revised form 4 May 2015; accepted 4 May 2015

KEYWORDS
ADHD;
Adults;
Test anxiety;
Psychological stress;
Cortisol reactivity;
HPA axis

Summary Individuals with ADHD suffer from increased vulnerability to environmental and mental stressors and may be at increased risk for chronic stress in everyday life. The Hypothalamic-Pituitary-Adrenal (HPA) axis is a critical physiological system that mediates responses to stress. The present study seeks to examine test performance, test anxiety, self-reported psychological stress and cortisol reactivity to mental-cognitive stress in adults with ADHD when compared with healthy controls. Stress was induced by an arithmetic ability test. Psychological stress was assessed repeatedly throughout the experimental session. Salivary cortisol, an indicator of the HPA axis function, was evaluated immediately upon arrival, as well as 1 min and 20 min post-test completion. Results revealed higher levels of test anxiety and poorer performance on the test in the ADHD group. The ADHD and control groups showed no difference in base-line levels of subjective stress and in subjective stress levels 20 min after the test. In contrast, individuals with ADHD reported significantly higher levels of stress at the test anticipation phase and 1 min post-test completion. Cortisol response to stress differed according to group: in the ADHD group, 20 min post-test cortisol levels were significantly higher than base-line cortisol levels. This was not evident in the control group. These results suggest greater activation of the HPA axis in response to stress in adults with ADHD when compared with healthy controls. Adults with ADHD do not differ from controls in basal levels of subjective

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http://dx.doi.org/10.1016/j.psyneuen.2015.05.008

stress and cortisol, but do have stronger psychophysiological reactions in response to stressful challenges. The present findings are among the first to demonstrate significant alterations in cortisol reactivity to stress in adults with ADHD.

1. Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder affecting 3—7% of children, frequently persisting into adulthood (Akinbami et al., 2011; Bell, 2011; Faraone et al., 2003; Kessler et al., 2005, 2006; Polanczyk et al., 2014; Simon et al., 2009; Willcutt, 2012; Wolraich et al., 2012). The main symptoms of ADHD include persistent inability to sustain attention, deficit in inhibition, hyperactive behavior and impulsivity (American Psychiatric Association, 2000, 2013). Consequently, ADHD may substantially affect the individual's behavioral and cognitive functioning, leading to functional impairment in academic, family and social settings (Barkley, 2006; Spencer et al., 2007). ADHD may also lead to heightened responses to various mental and social stressors and increased risk of chronic stress in everyday life (Hirvikoski et al., 2009; Lackschewitz et al., 2008). In recent years there has been growing interest in understanding the psychobiological stress response in children and adults with ADHD.

The hypothalamic-pituitary-adrenocortical (HPA) axis is considered a critical physiological system that mediates response to stress. In the face of environmental stressors, the HPA axis is activated, resulting in the secretion of cortisol from the adrenal cortex to the blood stream. The secretion of cortisol, which represents a biological indicator of arousal, is an adaptive response that alerts the individual to environmental changes and promotes the recovery of homeostasis. Studies have found the measurement of cortisol as an indicator for adrenocortical activity to be of high predictive value for psychological stress (von Dawans et al., 2011; Foley and Kirschbaum, 2010). Elevated serum cortisol immediately begins to interact with corticoid receptors to inhibit the stress response via negative feedback (Ma et al., 2011). Cortisol is capable of modulating psychological processes related to learning and memory through its action at receptors in limbic structures such as the amygdala and hippocampus. Dysregulation of the HPA axis reactivity may have detrimental effects on health and well-being (Fairchild, 2012; McEwen and Gianaros, 2010).

To date, most studies in the field of ADHD and HPA axis function and reactivity have been done on children, yielding inconsistent results (for review see Fairchild, 2012; Imeraj et al., 2012). Some studies have focused on basal cortisol secretion in naturalistic conditions. In this line of research, studies of diurnal patterns of cortisol have demonstrated a relationship between ADHD symptoms and altered circadian cortisol patterns. Results are inconsistent, showing both hypo- and hyper-arousal deviations at different time points. Studies considering cortisol awakening response (CAR) have also suggested a dysregulation of the HPA axis in ADHD, although findings were inconsistent (Imeraj et al., 2012; Ma et al., 2011). Sondeijker et al. (2007), in a large general population study, found that ADHD is not associated with significant changes in basal cortisol secretion. Freitag et al. (2009) reported a reduced CAR in children with ADHD and comorbid Oppositional Defiant Disorder (ODD) but a normal CAR in children with ADHD alone. Other studies on children with ADHD have evaluated cortisol levels in response to stress. Findings on cortisol reactivity to stress have been mixed, with some providing evidence for blunted cortisol responses to psychological stress, and others revealing normal or even elevated cortisol reactivity to stressors (Christiansen et al., 2010; Hong et al., 2003; King et al., 1998; Lee et al., 2010; Maldonado et al., 2009; Pesonen et al., 2011; Randazzo et al., 2008; Shin and Lee, 2007; Stadler et al., 2011; van West et al., 2009). Some of these inconsistencies may be partly explained by the high comorbidity rates of ADHD with other disorders (e.g., ODD and Conduct Disorder). For example, Stadler et al. (2011) found blunted cortisol reactivity to experimentally induced stress in boys with comorbid ADHD and disruptive behavior problems. Hastings et al. (2009) found greater cortisol reactivity to stress in boys with comorbid ADHD and anxiety, but diminished reactivity in ADHD boys with comorbid disruptive behavior and oppositional problems. Another aspect that may contribute to inconsistencies in results is the consideration (or lack of consideration) of the different ADHD subtypes; it appears that cortisol reactivity to stressors may be dependent on the specific ADHD subtype (Maldonado et al., 2009).

Far fewer studies on HPA axis and cortisol reactivity have been conducted on adults with ADHD than on children. Longitudinal studies suggest that childhood ADHD persists into adulthood in 30—60% of cases, either as a residual type or as a full clinical condition, accounting for problems in the management of normal daily activities and of academic and work-related tasks (deZwaan et al., 2011; Kessler et al., 2005; Simon et al., 2009). Adults with ADHD may be more vulnerable to environmental stressors and homeostasis may be frequently disturbed by internal or external stimuli that the individual perceives as threatening and which elicit a psychophysiological stress reaction. This may decrease the individual's capacity to cope with environmental demands, increasing the likelihood of encountering adversity (Garcia et al., 2012; Hirvikoski et al., 2009). Garcia et al. (2012) found an association between ADHD severity and negative life events in adults. The role of ADHD severity was significant even after controlling for the effect of comorbid disorders. Yim et al. (2010) compared cortisol responses of non-ADHD children and young adults to an identical laboratory stressor and reported that no differences were found between children and adult responses. It remains unclear, however, whether the results obtained in cortisol studies with ADHD children apply to ADHD adults, who may interpret and respond to potential stressors differently than children. Hence, evaluating the psychophysiological stress response among adults with ADHD is of great importance.

Corominas et al. (2013) examined CAR in adults with ADHD in order to assess possible differences between the combined and inattentive ADHD subtypes. Results showed no significant differences in the mean increase of CAR between the inattentive and combined subtypes. This study did not include a comparison group of healthy individuals. Hirvikoski et al. (2009) compared adults with ADHD with healthy controls regarding diurnal salivary cortisol in the everyday environment and salivary cortisol before and after cognitive stress in a laboratory setting. Results of this study showed that individuals with ADHD reported significantly more self-perceived stress than controls, and subjective stress correlated with the amount of stressors in everyday life. The two groups were comparable with respect to overall diurnal cortisol levels and rhythm, as well as in pre- and post-stress cortisol concentrations. Post-stress cortisol (but not baseline cortisol) concentration was positively correlated with impulsivity. The group with high post-stress cortisol also reported higher levels of self-perceived stress and more stressors in everyday life. The diagnosis of ADHD significantly increased the risk of belonging to the group with high post-stress cortisol levels. However, this study was limited by a high incidence of comorbid disorders, since participants were recruited from a Neuropsychiatric Unit of a University Hospital, with 57% of the ADHD group having at least one additional diagnosis (e.g., depression, panic disorder, anxiety and borderline personality). Lackschewitz et al. (2008) examined psychological and physiological stress responses in adult ADHD subjects in comparison with healthy controls under laboratory conditions. Participants with ADHD reported greater subjective stress but no group differences in cortisol reactivity were seen. The sample size in this study was relatively small ($n = 18$) and one-third of the ADHD group had comorbid psychiatric disorders.

Young adults with ADHD often demonstrate academic difficulties that result in significantly lower achievement scores and less overall academic success than peers (Barkley, 2006; Barkley et al., 2008; Frazier et al., 2007; Weyandt and DuPaul, 2006). One factor that has been strongly suggested as a contributor to lower academic performance, in the general population, is test-related anxiety (Chapell et al., 2005; Zeidner, 1998). Reaser et al. (2007) found that college students with ADHD tend to possess a negative attribution style with regard to their test performance as well as motivational deficiencies. Nevertheless, very few studies have directly assessed levels of test anxiety in adults with ADHD (Dan and Raz, 2012; Lewandowski et al., 2013; Nelson et al., 2014). Test anxiety may be associated with a more negative self-perception as well as with poorer test performance, greater self-perceived stress and alterations in cortisol reactivity to psychological stress in individuals with ADHD.

The aim of the present study is to examine test anxiety, test performance, self-reported psychological stress and cortisol reactivity to mental-cognitive stress (arithmetic ability test) in adults with ADHD (without comorbid psychiatric conditions), in comparison with healthy controls. We expected participants with ADHD to report higher basal (trait) levels of test anxiety and to demonstrate poorer performance (lower grades) on the arithmetic test. Based on the relevant literature, it was hypothesized that ADHD adults and healthy controls would show comparable baseline levels of both subjective stress and cortisol prior to exposure to the stress manipulation. However, in response to the laboratory stressor, higher levels of subjective stress accompanied by alterations in cortisol reactivity in the ADHD group compared with controls were expected.

2. Methods and materials

2.1. Participants

Forty nine undergraduate students (mean age 26.62 ± 1.88 years) participated in this study. They were divided into two groups: participants diagnosed with ADHD ($n = 24$; 16 females, mean age 27.16 ± 1.83), and healthy controls without ADHD ($n = 25$; 19 females, mean age 26.04 ± 1.80). Inclusion criteria for the ADHD group were (a) at least six symptoms either of inattention or of hyperactivity-impulsivity on the DSM-IV based ADHD Rating Scale (DuPaul et al., 1998), and (b) previous professional diagnosis: participants provided documentation of valid professional diagnosis of adult ADHD previously made by either a neurologist or psychiatrist from an established clinic in the field of psychoeducational assessment (the formal assessment must include a sound clinical interview). In many cases these documents were the ones submitted to the College's Support Center for Students with Learning Disabilities in order to get special exam accommodations. Students who could not provide such relevant documents were not included in the study.

Inclusion criteria for the control group were (a) fewer than four symptoms either of inattention or of hyperactivity-impulsivity on the ADHD Rating Scale-IV, and (b) no previous diagnosis of ADHD. The ADHD and control groups did not differ with respect to mean age, education or ethnicity distribution. Participants with a prior history or comorbidity with other neurological or mental/psychiatric disorders were excluded from the study. Participants were asked several questions to control for past or present mental/psychiatric disorders, e.g., ''Are you suffering, or have you ever been diagnosed as suffering from some kind of mental/psychiatric/personality disorder?'', ''Are you taking, or have you ever been treated with, some kind of psychiatric medication?'' Participants were recruited using departmental mailing lists and through snowball sampling. Written informed consent was obtained from all participants and they were given course credit according to their academic requirements. The study was approved by the Institutional Review Board and is in accordance with the declaration of Helsinki.

2.2. Measures

2.2.1. ADHD questionnaire

The assessment questionnaire for ADHD (DuPaul et al., 1998) included 18 items based on the symptoms listed in the DSM-IV for ADHD diagnosis. These symptoms include measurements of attentiveness (9 items), hyperactivity and impulsivity (9 items). Participants were asked to choose whether each described situation was correct or incorrect with respect to them[1]. The ADHD and control group significantly differ in mean values of attentiveness (ADHD: $M = 7.46 \pm 1.06$, control: $M = 1.33 \pm 1.17$; $p = 0.0001$), and hyperactivity-impulsivity (ADHD: $M = 4.38 \pm 2.70$, control: $M = 1.63 \pm 1.40$; $p = 0.0001$).

2.2.2. Online continuous performance test (OCPT)

This test was used in order to confirm and validate group selection and to further examine mean differences between the ADHD and control groups in terms of sustained attention and response inhibition abilities. The OCPT aligns with standard CPT design and is programmed for delivery over the Internet. A detailed description of this test can be found elsewhere (Raz et al., 2014). The total net test time of the OCPT is 19 min. The test has two conditions: low target frequency and high target frequency. The first half of the test (low target frequency) includes 224 trials (56 targets, 168 non-targets), with a target to non-target ratio of 1:3. In the second half of the test (high target frequency), the target to non-target ratio is reversed and set to 3:1 (168 targets, 56 non-targets). Manipulation of target frequency in CPTs is designed to highlight the sustained attention component in the low-target frequency condition and the response inhibition component in the high-target frequency condition. Four primary measures are extracted for analyses: errors of omission (defined as the number of targets to which a participant did not respond), errors of commission (defined as the number of times a participant incorrectly responded to a non-target), response times, and response time consistency (standard deviations in response times). These measures are extracted per condition (low and high target frequencies).

2.2.3. The Friedben test anxiety scale (FTAS; Friedman and Bendes-Ya'akov, 1997)

The FTAS is a 23-item scale consisting of the following three subscales: (a) Cognitive Obstruction: poor concentration, failure to recall, difficulties in effective problem solving before or during a test (e.g., ''On a test I feel like my head is empty, as if I have forgotten all I have learned''); (b) Tenseness: physical and emotional discomfort (e.g., ''I am very tense before a test, even if I am well prepared''); and (c) Social Derogation: concerns about being socially belittled and deprecated by significant others or peers following failure on a test (e.g., ''If I fail a test I am afraid I will be considered stupid by my friends''). Respondents were asked to answer how frequently they experienced feeling the described situations on a 6-point scale (1 = almost never, 6 = almost always). Scores were calculated by reversing raw scores on five items and averaging the mean scores of all items in each subscale. Their range, therefore, was 1—6, with lower scores reflecting a lower level of test anxiety. Internal consistencies of the whole questionnaire and of the three sub-scales were high (Total test anxiety, $\alpha = 0.953$; Cognitive Obstruction, $\alpha = 0.937$; Tenseness, $\alpha = 0.91$; Social Derogation, $\alpha = 0.934$).

[1] ADHD subtypes according to the self-report rating scale: out of the 24 participants in the ADHD group, 16 were classified as the inattentive subtype (attention deficit disorder; ADD), 7 as the combined subtype (inattentive + hyperactive-impulsive) and 1 as the hyperactive-impulsive subtype.

2.2.4. Computerized visual analog scale (VAS)

The VAS (constructed with Qualtrics software; http://www.qualtrics.com) was used to assess participants' subjective psychological stress. Consistent with common VAS presentations, the scale was a 100 mm horizontal line (Ahearn, 1997) divided into 10 equal-sized partitions (scaled from 0 to 100). The left edge of the scale was marked ''not stressed at all'' and the right edge was marked ''very stressed'' (for relevant uses, see Buhr and Dugas, 2009; Rossi and Pourtois, 2012). A sliding locator was initially positioned at the midpoint of the scale. The experimenter instructed the participants to use the computer mouse to place the locator at the scale position representing their current level of stress (''How stressed do you feel right now?''). The score (value between 0 and 100) was automatically calculated obtaining the exact value in reference to its location on the slider. The participants were not informed of this numerical value. The VAS was measured at four time points during the experimental session.

2.3. Arithmetic ability test

To assess mental load we devised a 34-question test consisting of various arithmetic problems that can be solved without the use of a calculator. The test included 20 problems taken from Raven's Progressive Matrices Test (Raven, 1938), a widely used, nonverbal test of analytic intelligence which has been found to predict performance on a wide range of reasoning tasks (Carpenter et al., 1990). We chose questions from sets C—D—E, suitable for participants above the age of 14. In addition, we devised 14 basic arithmetic problems consisting of addition, subtraction, division, multiplication and simple exponentiation operations. A sample problem is $60{:}[20{:}(9-4) + 3 \times 3 + 2]$. In accordance with the Raven test, we offered eight possible answers for the arithmetic problems. Participants had 12 min in total to complete the test. During the test, a countdown timer appeared on the computer screen. The questions were presented in a small, printed booklet and participants were asked to write their answers down on an attached answer sheet. To increase the perceived importance of the test, logos of the academic institution, the council for higher education, and the national institute for testing and evaluation were visible on top of the booklet. To simplify assessment, test scores were standardized to a mean of 0 and a standard deviation of 1. Internal consistency of the test was $\alpha = 0.796$.

2.4. Cortisol saliva assessment and analysis

Salivary cortisol levels were measured at three time points during the experimental sessions. Subjects were instructed not to eat, drink, chew gum, smoke or brush teeth for 60 min prior to coming to the lab. They rinsed their mouth thoroughly with cold water 5 min prior to collection of the first and last saliva samples; 2 ml samples of saliva were collected into polypropylene tubes. Samples were maintained at room temperature until the session was completed, then stored at 4 °C until the day following the collection, and then frozen at −20 °C until assayed. All samples remained frozen prior to assay and then were centrifuged at $2000 \times g$ for 10 min.

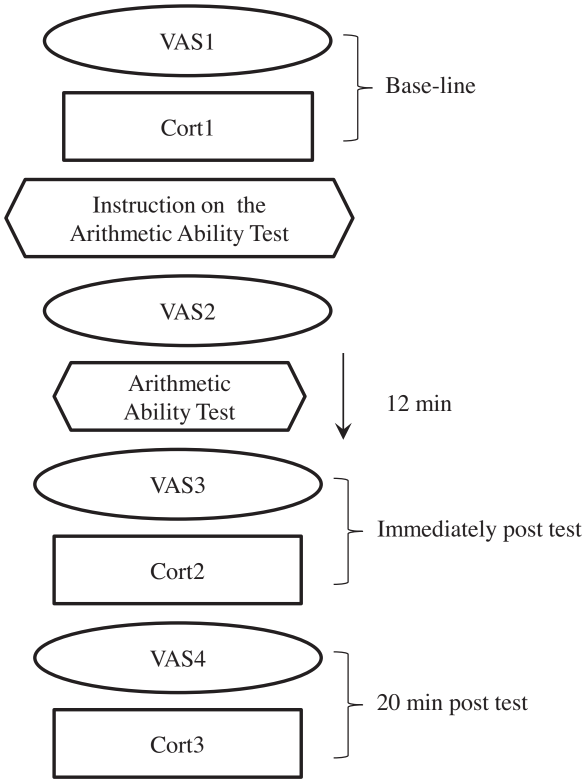

Figure 1 Schematic description of the procedures during the experimental session (second meeting).

Salivary cortisol concentrations were analyzed in duplicate (the average of the duplicates was used in all analyses) using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (IBL International, Hamburg, Germany) according to manufacturer's protocol. The intra- and inter-assay coefficients of variance were below 7.3% and 9.3%, respectively.

2.5. Procedure

Two hundred and twenty undergraduate students completed the ADHD questionnaire, and were asked to report whether or not they had been previously diagnosed with ADHD. Those who met criteria for either the ADHD or control groups were invited twice to the laboratory. On the first visit, they completed a demographic (personal details) questionnaire, test anxiety questionnaire and the OCPT. OCPT performance among the ADHD group compared with the control group is summarized in Table 1. Participants were told that during the second meeting they would be asked to provide saliva samples but were not informed about the arithmetic ability test they would have to solve. Participants from the ADHD group were instructed to abstain from using methylphenidate 24 h prior to OCPT testing as well as prior to the second experimental session.

Approximately one week after the first meeting, participants were invited for the experimental session (see Figure 1 for schematic description of the procedures). All experimental sessions took place between the hours of 13:00—17:00. Upon arrival at the lab, experimenters confirmed that participants had refrained from eating, drinking, chewing gum, smoking or brushing teeth for at least 60 min before arrival, and participants were asked to rinse their mouths thoroughly with cold water. Subsequently, they completed the first VAS (VAS1; baseline), with the first collection of saliva samples immediately following (Cort1; baseline). Participants were then introduced to the arithmetic ability test and instructed on how to complete it. Immediately following the explanation and, prior to actually starting the test, participants completed the VAS2. They then had 12 min to complete the test. Upon completion of the test, participants completed the VAS3 and supplied a second sample of saliva (Cort2). Fifteen minutes afterwards participants were asked to rinse their mouth once again and 5 min later (20 min after completion of the test) they completed the VAS4 and supplied the last sample of saliva (Cort3). The time latency of 20 min post-test was selected since many studies have shown that cortisol in saliva peaks approximately 20—30 min after the onset of mild laboratory-induced stress (von Dawans et al., 2011; Fibiger et al., 1986; Hubert and de Jong-Meyer, 1989; Kirschbaum and Hellhammer, 1989). During the 20 min interval, participants were not allowed to leave the room, talk, eat, drink or smoke; they were allowed to read the monthly college newsletter.

2.6. Data analysis

Independent-sample *t*-tests (Student's *t*-tests) were used to compare OCPT, test anxiety and arithmetic ability scores between the ADHD and control groups. The relationship between ADHD and VAS scores was analyzed by 4×2 repeated measures analysis of variance (mixed-design ANOVA). Time (VAS1/2/3/4) was the within-subject factor, and Group (ADHD/control) was the between-subject factor. To assess cortisol reactivity to the stress challenge (arithmetic ability test) we used a 3×2 repeated measures analysis of variance (mixed-design ANOVA). Time (Cort1/2/3) was the within-subject factor, and Group (ADHD/control) was the between-subject factor. Follow-up independent sample *t*-tests (Student's *t*-tests) were used to break down main effects of Group and interaction effects. In cases of multiple comparisons, the Bonferroni correction was applied; reported *p*-values appear with the correction applied.

3. Results

3.1. Test anxiety

Analysis of the self-reported test anxiety scores revealed significant differences between the ADHD and control groups in two of the three sub dimensions of the test anxiety scale —Cognitive Obstruction test anxiety [$t(47) = 4.92$, $p = 0.0001$, Cohen's $d = 1.42$] and Tenseness test anxiety [$t(47) = 3.70$, $p = 0.001$, Cohen's $d = 1.07$], as well as in Total test anxiety [$t(47) = 3.95$, $p = 0.0001$, Cohen's $d = 1.14$]. In comparison to controls, participants with ADHD had significantly higher levels of Cognitive Obstruction, Tenseness and Total test anxiety (Figure 2). Participants with ADHD also tended to have higher levels of Social Derogation test anxiety, but this difference did not reach statistical significance ($p = 0.14$).

Table 1 OCPT performance among ADHD and control groups: means and standard deviations for omission errors, commission errors, reaction times (RT) and response time consistencies (RTSD), and the corresponding between-groups t and p values. Role of funding source: This study was supported by a small scholarship provided by the College Research Authority. Money was used to purchase salivary cortisol ELISA kits and pay for research assistant.

	Control		ADHD		t	p
	sd	Mean	sd	Mean		
Low target						
Omissions	0.92	0.52	1.98	1.54	2.30	0.03
Commissions	0.94	0.84	2.58	1.79	1.68	0.10
RT (ms)	63.78	405.24	156.63	490.09	2.47	0.02
RTSD (ms)	30.03	66.66	68.26	115.80	3.24	0.003
High target						
Omissions	2.13	1.24	9.52	5.46	2.12	0.04
Commissions	3.44	4.24	6.11	8.42	2.96	0.005
RT (ms)	55.02	362.41	88.78	394.40	1.52	0.14
RTSD (ms)	21.08	66.72	68.67	133.47	4.54	0.0001

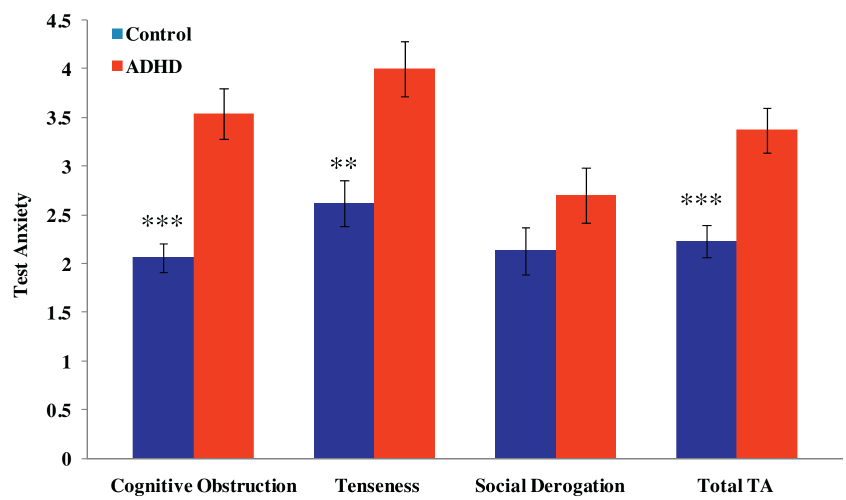

Figure 2 Differences between ADHD and control groups in levels of Cognitive Obstruction, Tenseness, Social Derogation and Total test anxiety. Error bars represent SEM. Asterisks are showing the results of a comparison between groups: $^{**}p < 0.01$, $^{***}p < 0.0001$.

3.2. Computerized visual analog scale (VAS)

A 4 × 2 repeated measures ANOVA revealed a significant Time effect (within-subject effect) [$F(3,141) = 18.82$, $p = 0.0001$, $\eta_p^2 = 0.28$], such that reported levels of stress were higher immediately before (VAS2) and immediately after (VAS3) the test when compared with base line (VAS1) and 20 min post-test (VAS4) levels of stress. There was also a significant main effect of Group [$F(1,47) = 7.78$, $p = 0.008$, $\eta_p^2 = 0.14$], such that participants with ADHD reported higher levels of stress compared with controls. These effects were subsumed under a significant Time × Group interaction effect [$F(3,141) = 2.88$, $p = 0.038$, $\eta_p^2 = 0.06$]. Post hoc comparisons showed that participants with ADHD reported higher levels of stress than controls immediately before (VAS2; $p = 0.02$) and immediately after (VAS3; $p = 0.01$) the test, but there were no differences between the groups in base line (VAS1; $p = 0.80$) and 20 min post test (VAS4; $p = 0.28$) levels of stress (Figure 3).

3.3. Arithmetic ability test

Participants with ADHD had significantly lower scores (47.86 ± 15.08), compared with controls (62.56 ± 14.88), on the arithmetic ability test [$t(47) = -3.42$, $p = 0.001$, Cohen's $d = -0.98$].

3.4. Cortisol saliva levels

A 3 × 2 repeated measures ANOVA revealed significant within-subject Time effect [$F(2,94) = 20.18$, $p = 0.0001$, $\eta_p^2 = 0.30$], such that cortisol levels 20 min post-test (Cort3) were higher than both base line cortisol levels (Cort1) and cortisol levels immediately after the test (Cort2). While there were no main effects of Group in cortisol, analysis revealed a significant Time × Group interaction effect [$F(2,94) = 5.51$, $p = 0.005$, $\eta_p^2 = 0.11$]. Follow-up comparisons showed that within the ADHD group, (but not within the control group), cortisol levels were significantly higher 20 min post-test (Cort3) than base-line levels (Cort1) and immediately post-test levels (Cort2) ($ps = 0.0001$) (Figure 4a). Another way to look at these results is to calculate the difference in cortisol levels between Cort3 and Cort1 and between Cort3 and Cort2 (Cort3—Cort1; Cort3—Cort2), and to compare those differences between the ADHD and control groups. *T*-test analysis revealed that the difference in cortisol between Cort3 and Cort1 was significantly higher in the ADHD group than in the control group [$t(47) = 2.16$, $p = 0.036$, Cohen's $d = 0.65$]. Similar results were found for the difference in cortisol between Cort3 and Cort2 [$t(47) = 3.39$, $p = 0.001$, Cohen's $d = 1.00$] (Figure 4b). These results suggest that the cortisol response to the test was significantly more pronounced in participants with ADHD than in controls.

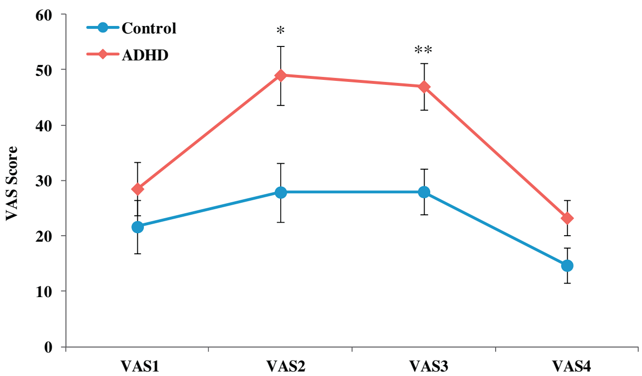

Figure 3 Differences between ADHD and control groups in levels of self-reported stress on the Visual Analog Scale (VAS). Error bars represent SEM. Asterisks are showing the results of a comparison between groups: $^{*}p < 0.05$, $^{**}p < 0.01$.

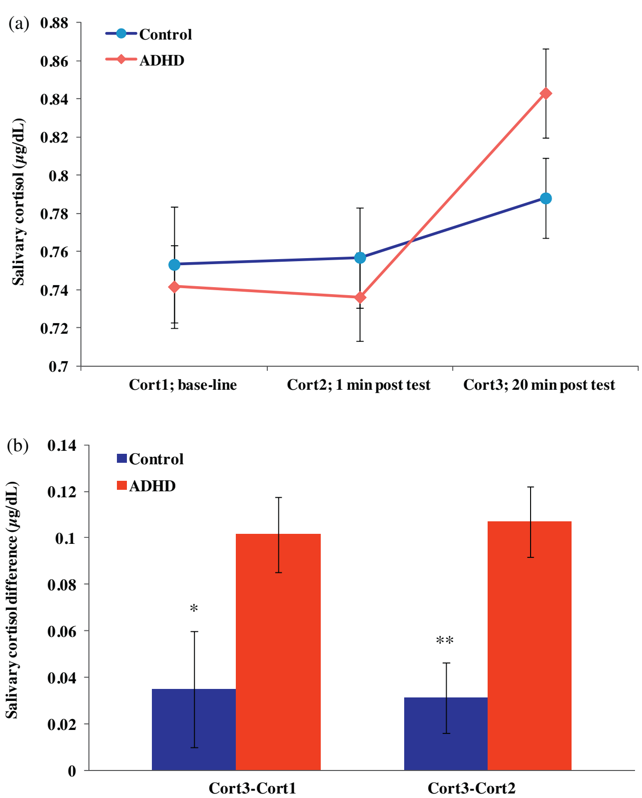

Figure 4 (a) Salivary cortisol in ADHD and control groups at base-line (Cort1), immediately after the test (Cort2) and 20 min after the test (Cort3). (b) Differences in cortisol between Cort3 and Cort1 and between Cort3 and Cort2 for the ADHD and control groups. Error bars represent SEM. Asterisks are showing the results of a comparison between groups: $^{*}p < 0.05$, $^{**}p < 0.01$.

4. Discussion

In the present study we examined test anxiety, test performance, psychological stress and cortisol reactivity to stress in adults with ADHD compared with healthy controls. We found that participants with ADHD had significantly higher base line levels of Cognitive Obstruction test anxiety, Tenseness test anxiety and Total test anxiety. It seems that individuals with ADHD tend to perceive test situations as more threatening than those without ADHD. Anxiety disorders are a common comorbid condition among individuals with ADHD, with prevalence commonly ranging from 15% to 35%, and even reaching as much as 50% (Kessler et al., 2006; Mancini et al., 1999; Schatz and Rostain, 2006). Despite the well documented comorbidity of ADHD with anxiety disorders, very few studies have directly evaluated test anxiety among adults with ADHD (Dan and Raz, 2012; Lewandowski et al., 2013; Nelson et al., 2014). The current results are in line with Nelson et al. (2014) who found that college students with ADHD reported higher total test anxiety as well as specific aspects of test anxiety, including worry (i.e., cognitive aspects of test anxiety) and emotionality (i.e., physiological aspects of test anxiety). Lewandowski et al. (2013) reported significant differences between ADHD and non-ADHD students regarding their perceptions of, and anxiety during, test taking. Those with ADHD perceived themselves as having more difficulty in reading under timed conditions and reported more test-related anxiety than their peers. Adults with ADHD often demonstrate academic difficulties that result in lower grades than peers and less academic success overall (Barkley, 2006; Barkley et al., 2008; DuPaul and Volpe, 2009; Frazier et al., 2007). Test anxiety may be a major factor contributing to such lower academic performance; indeed, the results of the present study show the performance of participants with ADHD to be significantly poorer on the arithmetic test. Worry, which is considered a fundamental cognitive component of test anxiety, has been found to be particularly related to academic under-achievement (Wine, 1971; Wong, 2008; Zeidner, 1998). The Worry component closely corresponds to the Cognitive Obstruction scale of the test anxiety questionnaire used in this study. Attentional interpretation of the Worry component that may be specifically relevant to ADHD has been suggested by several researchers (Sarason, 1984; Tobias, 1985; Wine, 1971). According to this interpretation, Worry is an attention-demanding cognitive activity involving self-preoccupying, intrusive thoughts that interfere with task-focused thinking. Thus, high test-anxious individuals may be more susceptible to cognitive interference, deficits in allocation of attention and reduction in the cognitive capacity for task solution when confronted with challenging tasks; this may prove to be even truer for individuals with ADHD. It is difficult to determine whether students with ADHD perform less successfully due to test anxiety or due to a history of poor performance on tests that exacerbates anxiety regarding future performance. Future studies may try to resolve this issue by assessing test anxiety among students with and without ADHD who achieved compatible/similar academic test scores in the past; or by assessing test anxiety among students with ADHD who have lower academic achievements compared to those with higher academic achievements.

Related, and in line with our hypothesis, the ADHD group showed a stronger subjective response to stress than controls. Upon arrival at the laboratory and before being exposed to the challenge of the test, individuals with ADHD reported similar base-line levels of stress to those reported by controls. However, immediately after exposure to the task at hand (at the test anticipation phase) those with ADHD reported significantly higher levels of stress than controls. Such difference in the level of subjective stress was also seen immediately after completion of the test. Twenty minutes thereafter, the between-group difference in subjective stress had dissipated. These results further support the notion of heightened susceptibility to mental stressors among adults with ADHD, and are in line with Lackschewitz et al. (2008) who found a large difference in subjective stress ratings between the ADHD and control groups during the stress anticipation phase and during the actual stress phase but not in the baseline condition. The researchers suggested that ADHD subjects are more intensely affected by the psychological dimension of the stress response, not only during the actual presence of the stressor but also while anticipating it.

It is plausible that greater test anxiety and elevated psychological stress while anticipating and solving a test may contribute to poorer performance beyond the deficits attributed to the inherent characteristics of ADHD. Therefore, to alleviate test anxiety and perceived stress, intervention programs for adult ADHD students are strongly advised to incorporate cognitive-behavioral stress management techniques.

In addition to subjective-psychological stress, the present study focuses on evaluation of the physiological stress response, as reflected in cortisol reactivity to mental/cognitive stress. Generally, the stress manipulation resulted in elevation in cortisol levels, suggesting that the arithmetic test was sufficient to activate the HPA axis. Interestingly however, cortisol response to stress clearly interacted with group. In the ADHD group, cortisol levels 20 min post-test were significantly higher than base-line cortisol levels, while this was not evident in the control group. In other words, the differences between cortisol levels pre- and post-test were significantly greater in the ADHD group than in the control group. These results suggest greater activation of the HPA axis in response to stress in adults with ADHD in comparison to healthy controls. Reports on HPA axis and cortisol reactivity to stress in adulthood ADHD are scarce. Our results are in partial agreement with Lackschewitz et al. (2008) and Hirvikoski et al. (2009) who found similar pre- and post-test levels of cortisol in adults with ADHD and controls. However, contrary to the findings of the present study, the above mentioned studies did not find an interaction between cortisol reactivity to stress and ADHD. Nevertheless, our findings, together with previous reports on adult ADHD, seem to contradict the notion of reduced basal cortisol secretion and cortisol hypo-reactivity to stress (a notion derived mainly from early studies on childhood ADHD) (Fairchild, 2012). The secretion of cortisol results, among other things, in increased heart and breathing rates and higher blood pressure, and is considered a biological indicator of arousal (Saplsky, 2000). The greater reactivity of cortisol to the test challenge in the ADHD group may correspond to the higher levels of subjective psychological stress reported by this group. Indeed, the measurement of cortisol as an indicator for adrenocortical activity has been found to be of high predictive value for psychological stress (von Dawans et al., 2011; Foley and Kirschbaum, 2010). Importantly, such cortisol-related physiological arousal and changes in bodily sensations may be subjectively perceived as positive (by low test-anxious persons) or negative (by high test-anxious persons) and thus may have facilitative or debilitative effects on test performance. That is, the levels of arousal per se may not have critical impact on test performance, but rather what persons say to themselves about the arousal they experience (Wong, 2008). In the present study, individuals with ADHD reported higher levels of test anxiety which may be related to the adaptation of a more negative perception of the test situation and of the sense of physiological arousal, resulting in greater subjective psychological stress as well as poorer test performance overall. Importantly however, the main objectives of this study have been tested as separate or independent objectives. Due to the relatively small sample, direct statistical analyses of the associations between test anxiety, test performance and psychophysiological stress responses were not conducted. Therefore, while our data clearly shows that an ADHD adult population exhibits poorer test performance, higher levels of test anxiety, and greater psychological and cortisol responses to stress, the possible associations between these variables should be interpreted with caution. As it stands, we can only speculate, based on the relevant literature, about possible theoretical or logical relationships between these variables. The actual statistical associations between them should be assessed in future studies with larger samples that afford adequate statistical power.

In the interpretation of our results, a few other considerations need to be taken into account: first, the samples used may be somewhat biased since we recruited college students; the current sample may represent a unique subpopulation of relatively high-functioning ADHD adults who managed to complete high-school and successfully enter college. Moreover, while community-based studies suggest that ADHD is more frequently observed in males (Gaub and Carlson, 1997), females comprised the majority of participants of both the ADHD and control groups in the current study. Replication of the results in samples of varying makeup, gender, education and ethnicity will help establish the generalizability of our results. Second, due to the relatively small sample size, in analyses of the current data, no distinction was made between the different ADHD subtypes (i.e., combined, inattentive, hyperactive-impulsive). Although the three subtypes belong to the same diagnostic entity, it is well established that they are different from each other in several important behavioral and physiological aspects (Bell, 2011). It is therefore highly possible that psychological stress and alterations in cortisol reactivity are dependent on the specific ADHD subtype (Maldonado et al., 2009). Future studies are encouraged to assess psychological and physiological responses to stress among the different ADHD subtypes. Third, in the present study, only three saliva samples were collected, with the focus on assessing base-line levels of cortisol in comparison with peak levels of cortisol (i.e., the rising phase of the cortisol response). This design did not allow for the investigation of the recovery/down regulation of the cortisol response to stress. With the present protocol, it is hard to say whether the increase in cortisol levels in the ADHD group is very short lived or prolonged and whether the non-ADHD group ultimately show a response to the arithmetic task that is somewhat delayed in time compared to the ADHD group. The collection of additional post-stress samples, in future studies, is warranted, in order to assess the recovery phase of cortisol and to verify that the apparent group difference in reactivity is not explained by differences in the latency of the response. Fourth, given the specific sample that was assessed in this study (high-functioning undergraduate students, most of them known to the authors personally from class) we used few general questions to control for present or past comorbid disorders; using a formal symptom checklist (e.g., SCL-90-R or the Brief Symptom Inventory) would have been preferable. It will also be useful to collect data on IQ as well as to control for the use of oral contraceptives among female subjects since sex steroids like estrogen may be a potential modulator of cortisol response to stressors (Boisseau et al., 2013). Finally, we used a stress manipulation in a laboratory setting in which the outcomes of the test bear no actual or significant implications on participants' real life. In addition, the arithmetic-based stressor did not appear to elicit a cortisol response in the control group and the subjective response to stress was very weak. To further investigate the relevance of the current findings, future research may evaluate subjective stress and cortisol reactivity to real life stressors that actually may threaten the individual's life goals.

In summary, to our knowledge, the current study is the first to examine psychological stress and cortisol reactivity to laboratory stressors in adults with ADHD with no comorbid psychiatric disorders, and the first to report a more pronounced cortisol response to stress in individuals with ADHD, reflected in the larger delta between base-line and post-test salivary cortisol. Alterations in cortisol reactivity to stressors in adult ADHD should be further investigated considering various samples, stressors and ADHD subtypes.

Role of funding source

This study was supported by a small scholarship provided by the College Research Authority 50621. Money was used to purchase salivary cortisol ELISA kits and pay for research assistant.Conflicts of interest

None declared.

Acknowledgment

This study was supported by a Migal-Tel Hai research authority grant.

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