Time-of-day Effects in Arousal:

Disrupted Diurnal Cortisol Profiles in Children with ADHD

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Abstract

Background. Fluctuations in attention-deficit hyperactivity disorder (ADHD) symptoms related to regulatory deficits in arousal states are themselves characterized by circadian rhythms. Though cortisol is an important circadian arousal-related marker, studies focusing on across-the-day cortisol variations in ADHD are scarce. There is no study with multiple measurements to take into account interday and intraday variability.

Methods. Salivary cortisol was sampled five times a day (awakening, 30 min after awakening, noon, 4PM, 8PM) across five consecutive days in 33 children with ADHD (22 with and 11 without oppositional defiant disorder; ODD) and 33 class- and sex-matched controls (aged 6-12). The cortisol awakening response (increase from awakening to 30 min after awakening) and the diurnal cortisol profile (across-the-day variations) were compared for ADHD with ODD (ADHD+ODD) and without ODD (ADHD) subgroups and the control group.

Results. The cortisol awakening response was not significantly different between groups. However, longitudinal analyses to evaluate cortisol profiles across the day revealed a significant group x time effect ($p<0.001$). More specifically, compared to each other, the ADHD subgroup showed a flatter slope with relative morning hypo-arousal and evening hyperarousal, whereas the ADHD+ODD subgroup showed a steeper slope with relative morning hyperarousal and evening hypo-arousal ($p<0.001$).

Conclusions. Findings support time-related arousal disruptions in children with ADHD associated with the presence or absence of ODD comorbidity. We recommend research on cortisol in larger samples for a better understanding of arousal mechanisms involved in ADHD not only with and without ODD but also with other comorbities which may have implications for timing of arousal-based treatments.

Keywords: ADHD; ODD; circadian; HPA axis; cortisol; arousal

Attention-deficit hyperactivity disorder (ADHD) is one of the most prevalent psychiatric disorders in children, characterized by pervasive symptoms of inattention, hyperactivity and impulsivity in home and school settings (Taylor, et al., 2004). These behavioural symptoms have been explained by deviations from an optimal arousal level (hypo- or hyperarousal) (Sonuga-Barke, Wiersema, van der Meere, & Roeyers, 2010). Such deficits in arousal modulation in ADHD have been studied by examining cortisol level in saliva, which is a reliable peripheral measure to evaluate hypothalamic-pituitary-adrenal (HPA) axis functioning (Kirschbaum & Hellhammer, 1989). Cortisol influences regulation of attention, behaviour, and emotion by affecting processes within the prefrontal cortex (PFC) and hippocampus (HC) (Erickson, Drevets, & Schulkin, 2003). Anatomical and functional abnormalities in these brain regions have been observed in ADHD (Himelstein, Newcorn, & Halperin, 2000; Plessen, et al., 2006), and altered cortisol concentrations in these regions may be associated with maladaptive control of attentional, behavioural, and emotional processes in ADHD (Hastings, Fortier, Utendale, Simard, & Robaey, 2009).

It is well-established that behavioural symptoms and physiological functions fluctuate across the day (Antrop, Roeyers, & De Baecke, 2005) in circadian rhythms thought to be driven by the suprachiasmatic nucleus (SCN) in the ventral hypothalamus. Arousal is characterized as a physiological and psychological state of being awake, aware and alert that also varies across the day (for a review see: Silver & LeSauter, 2008). Evidence for disrupted circadian regulation of arousal patterns in ADHD has been reported for eveningness preference (Susman, et al., 2007), sleep-wake disturbances (Cortese, Faraone, Konofal, & Lecendreux, 2009) and excessive daytime sleepiness (Lecendreux, Konofal, Bouvard, Falissard, & Mouren-Simeoni, 2000), higher heart rate levels particularly expressed during specific times of day (Imeraj, et al., 2011), and afternoon hyperactivity problems (Antrop, et al., 2005). Results are mixed (Cohen-Zion & Ancoli-Israel, 2004), so further clarification of time-of-day effects in arousal is important. From a theoretical perspective, disrupted circadian arousal-related effects in ADHD (i.e., hypo- and hyperarousal at different times of day) may reflect critical mechanisms underlying behavioural fluctuations in ADHD. From a practical point of view, knowledge of such effects could potentially guide more appropriate timing of diagnostic and research assessments as well as delivery of treatments for ADHD.

Only minor attempts have been made to evaluate time-of-day effects in cortisol levels as a proxy for arousal patterns in ADHD. Variations in cortisol levels in ADHD have been investigated mostly with regard to stress tasks (Hong, Shin, Lee, Oh, & Noh, 2003). Time information reaches the HPA axis through connections

between the circadian pacemaker and the paraventricular nucleus of the hypothalamus which contains corticotropin releasing hormone neurons. This circadian input is reflected in a typical diurnal secretion pattern of cortisol -the end product of the HPA axis- with a trough around midnight, a large trend upward toward morning and a peak 30 to 45 min after awakening, called the cortisol awakening response (Haus, 2007).

The available evidence on across-the-day fluctuations in cortisol levels in ADHD points towards distinctive diurnal patterns (Kaneko, Hoshino, Hashimoto, Okano, & Kumashiro, 1993). However, findings are inconsistent across studies and suggest both hypo- and hyperarousal patterns at different times of day. Blomqvist et al. (2007) reported awakening cortisol level in 13-year old children with ADHD was reported to be lower than in age-matched controls, but Freitag et al. (2009) found the awakening level and the awakening response (change after awakening) to be normal in 6-to-13 year old children with ADHD without comorbid disorders. In the general population, attention problems in 8-13 year old boys have been related to a lower morning-to-afternoon cortisol ratio, but whether this was due to lowered awakening or elevated afternoon cortisol level or both was not specified (Susman, et al., 2007). In a subgroup (10-12y), elevated evening levels were found in both boys and girls (Sondeijker, et al., 2007), but a study of preschool children reported higher awakening levels (Hatzinger, et al., 2007). Some studies have investigated the effects of comorbidity of ADHD with oppositional defiant disorder (ODD), which is the most frequent comorbidity in clinical samples and is associated with poor prognosis. For example, cortisol levels were lower than normal in children with comorbid ADHD and ODD/conduct disorder (CD) (King, Barkley, & Barrett, 1998) and in clinic-referred disruptive boys (McBurnett, Lahey, Rathouz, & Loeber, 2000), but this deficit may be normalized by treatment with stimulant medication (Kariyawasam, Zaw, & Handley, 2002). In some studies of ADHD children, lowered awakening response (Freitag, et al., 2009) or lower basal values (Kariyawasam, et al., 2002) were associated with comorbid ODD, but this pattern was not confirmed by others (Hastings, et al., 2009). Such discrepancies suggest that ADHD with comorbid ODD/CD may differ from ODD and CD and other aggressive behaviour without ADHD. According to the hypo-arousal theory (Raine, 1996), the latter have typically been linked to both lower basal cortisol levels (McBurnett, et al., 2000) and lower morning values (Pajer, Gardner, Rubin, Perel, & Neal, 2001).

A major limitation of previous research is restricted sampling of cortisol, which is highly variable within individuals and across time of day. Some studies have assessed only awakening cortisol during one (Blomqvist, et al., 2007; Hatzinger, et al., 2007) or two (Freitag, et al., 2009) study days, while others included multiple daytime measurements (Kaneko, et al., 1993; Sondeijker, et al., 2007; Susman, et al., 2007) across a single study day. There is a consensus that intra- and interday variability in cortisol patterns call for full circadian

evaluation with multiple time points sampled on a given day across several days (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003; Houtveen & de Geus, 2009), but this methodological standard has not been applied in ADHD research.

Cortisol levels were obtained over multiple days (three weekdays and two weekend days) with multiple measurements across each day (two waking and three consecutive samples) in ADHD children compared to age-matched controls. This allows for evaluation of across-the-day fluctuations in cortisol based on average levels of the individual participants. Though some authors reported cortisol awakening response to be a reliable predictor for consecutive daytime levels (Edwards, Clow, Evans, & Hucklebridge, 2001), others questioned this relationship (Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009). Therefore, we assessed the cortisol awakening response (increase in cortisol levels from awakening to 30 min after awakening), and diurnal profile (variations in cortisol levels across the day). Since the literature suggests that ODD comorbidity may influence these findings, two subgroups were taken into account -ADHD with ODD (ADHD+ODD) and without ODD (ADHD). Based on our hypothesis of disrupted arousal-related circadian rhythm in ADHD (i.e., hypo- and hyperarousal at different times of day), we expected (i) a significant difference in cortisol increase after awakening among the three groups (ADHD+ODD, ADHD, and controls), and (ii) a significant interaction of group x time, reflecting that group differences in cortisol level depend on the time of day when samples were collected for these measurements. The influence of other factors that may modulate levels of arousal such as age, sex, pubertal stage, body mass index (BMI) (Adam, 2006; Oskis, et al., 2009), and internalizing problems (such as depressive and anxiety symptoms) (Van den Bergh, Van Calster, Pinna Puissant, & Van Huffel, 2008) was evaluated, also.

Methods

Subjects

Participants were 33 children (ages 6-12 years) diagnosed with ADHD-Combined Type and 33 sex- and age-matched normal developing control children selected within the same class as the child with ADHD. Control children had no formal psychiatric or medical diagnosis and were medication-free. Children with ADHD were recruited from a child psychiatric outpatient unit where they were diagnosed based on a child psychiatric evaluation (parents' history of the child's symptoms as well as information from teachers, and if necessary a school observation). Prior to participation, a structured interview for parents (Diagnostic Interview Schedule for Children, PDISC-IV) was used to confirm the clinical diagnosis of ADHD-Combined Type and to establish

comorbid conditions (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000). An ODD diagnosis was obtained in 22 children (of which two were also diagnosed with CD). This defined two ADHD subgroups (ADHD+ODD; n=22 and ADHD; n=11). Exclusion criteria were an IQ<80 (Wechsler Intelligence Scale for Children, WISC-III-R; Wechsler, 1991), autism spectrum disorder, chronic medical conditions, and any medication except for methylphenidate. Twenty-eight children with ADHD took methylphenidate, but all were medication-free at least 72 hours prior to participation to avoid confounding with an acute effect of methylphenidate that increases circulating cortisol (Volkow, et al., 1995).

The Child Behaviour Checklist (CBCL; Achenbach, 2001) was used to screen for current behavioural and emotional problems; including internalizing problems that have been related to alterations in cortisol levels (Van den Bergh, et al., 2008). This questionnaire, completed by parents, has good reliability and validity (Achenbach, 2001). It contains 112 items rated on a 3-point Likert scale (not at all to often) yielding broadband scales for internalizing (combines scales of withdrawn, somatic complaints and anxious/depressive behaviour) and externalizing (combines scales of delinquent and aggressive behaviour) problems. Pubertal stage and BMI were also assessed because of their impact on circadian expression of cortisol (Adam, 2006; Oskis, et al., 2009).

Procedure

This study was approved by the Ethical Committee of the Ghent University Hospital, Belgium. After parents of children with ADHD provided written informed consent, the school and teacher were asked for their cooperation. Teachers selected three sex- and age-matched normally developing classmates without formal psychiatric of medical diagnosis, and if all parents provided written consent, one control was selected randomly to participate in the study.

We measured diurnal cortisol levels of the ADHD and control children in the naturalistic home and school environment during five consecutive days (three weekdays/two weekend days). The participants were randomly assigned to start with weekdays or weekend days, and this resulted in 18 children being evaluated from Tuesday evening to Sunday evening and 15 children from Friday evening until Wednesday evening. On the first data collection day, the cortisol sampling protocol was explained to parents and teacher, and children were trained in the saliva collection procedure. After five days, children received a reward (small toy selected by themselves plus an entrance ticket for a leisure park) for their participation.

Salivary Cortisol Sampling and Analysis

Saliva cortisol was collected using the "Salivette" device (Sarstedt®), which provides a non-invasive measure of the unbound -bioactive- cortisol fraction that is highly correlated with circulating plasma levels. Compared to venipuncture or saliva collection in a laboratory setting, saliva sampling in a naturalistic setting does not induce stress (Kirschbaum & Hellhammer, 1989).

Participants were instructed to collect five saliva samples at specific times across the day: directly after awakening (Cort1), 30 min post-wake time (Cort2), noon (Cort3), 4PM (Cort4), and 8PM (Cort5). Differences were expected in bed- and awakening times during weekdays and weekend days, so parents noted these times on each of the study days.

The "Salivette" consists of a cotton swab that participants chew gently for 1 min. To avoid known problems, participants were asked not to drink or eat sour products (low pH) or not to brush their teeth (blood contamination) 30 min before sampling. After saliva collection, the swab was transferred into a plastic tube and stored in the refrigerator at home until the end of the five days, when all tubes were collected. Parents or teachers wrote the sampling time on Cort1 and Cort2 tubes and, when deviating from the expected time, on Cort3, Cort4 and Cort5 tubes. Parents and teachers were asked to set an alarm to remind children of the cortisol collection during the day (Cort3/Cort4). To further enhance compliance and accurate reportage of sampling times, participants were told that accuracy of sampling times could be monitored from the samples in the lab. Samples were excluded when sampling time deviated >15 min from waking and expected hours. Of a total of 1650 cortisol samples, 90 samples (5%) were randomly missing due to forgetting, deviating sampling times or insufficient saliva collection.

Samples were centrifuged at 4°C (2000 g for 10 min) and stored at -26°C to maintain stability until assay. Samples were analyzed in two batches by the Brussels University Hospital, Department of Hormonology by radioimmuno assay (RIA, Diasorin, Italy), using a modification of an unextracted RIA method for serum cortisol. Briefly, 200 µL saliva was pipetted into the coat tube and incubated with ^{125}I cortisol for 45 min at 37°C. The modified cortisol assay had a range from 0.5-30 µg/L and within- and between-run coefficients of variation of <5% and <10%, respectively.

Statistical Analyses

Demographic variables (age, sex, BMI, pubertal stage), CBCL scores, and sleep-wake hours were compared between ADHD+ODD, ADHD, and control group using analysis of variance (ANOVA) or chi-square analysis as appropriate. Weekday and weekend sleep-wake hours were also compared using ANOVA.

To compare the awakening response between ADHD subgroups and control group, the area under curve (AUC=(Cort2–Cort1)x4) was calculated to estimate the average increase. To investigate the diurnal cortisol decline in ADHD+ODD, ADHD, and control groups, mixed model analysis was conducted with SAS PROC MIXED. Mixed models are preferred over repeated measures ANOVA for the study of longitudinal data as they have greater flexibility to model time effects and correlation patterns between repeated measurements and handle missing data more appropriately (Gueorguieva & Krystal, 2004). The diurnal profile was based on four approximately equally spaced time points: awakening (Cort1), noon (Cort3), 4PM (Cort4), and 8PM (Cort5). For each of the four post-awakening time points, samples across days (up to 5 when there were no missing data) were averaged by the SAS program. Averaging data cancels out random day-to-day variation and increases precision. This can lead to an increase in effect size (ES), the standardized mean difference between groups (Cohen $d=(mean1–mean2)/SD_{pooled}$), which is independent of sample size but depends on precision of measurement (Swanson, et al., 2001). ES values are defined as small (0.2), moderate (0.5), and large (0.8) to help interpret clinical relevance of statistically significant effects (Cohen, 1992). The distribution of cortisol concentrations showed positive kurtosis and skewness, so analyses were performed with the log-transformed values. The SAS Proc Mixed procedure requires the specification of models: Plausible covariance-pattern models (heterogeneous compound symmetry, heterogeneous autoregressive, and unstructured covariance patterns) were fitted, with or without inclusion of random intercept effects for subject and for day of evaluation (within subject). The best fitting mixed model was selected by likelihood ratio comparison tests. Analysis was performed with fixed effects of group (ADHD+ODD vs ADHD vs control), time (four time points per day), and group x time in a model controlling for day order (day1 to day5) and weekday vs weekend day. Possible confounders (age, sex, BMI, pubertal stage, and internalizing problems) were entered one by one, and those that did not significantly contribute to variations in cortisol levels were excluded from the model. To decompose the group x time interaction effect, two orthogonal contrasts were defined. The first was a contrast of the control group vs ADHD group. The second was a contrast of the ADHD subgroups (ADHD+ODD vs ADHD). The mathematical relationship of time and cortisol level was additionally assessed by evaluating the linear, quadratic, and cubic effects for the main effect of time and for the group x time interaction.

Results

Descriptive Statistics

The ADHD+ODD, ADHD, and control group were not significantly different with respect to age, sex, pubertal stage, BMI, awakening and sleeping times. Weekday and weekend days were not significantly different for sleeping time (paired $t(65)=-1.63$; ns) but were for waking times (paired $t(65)=-13.649$; $p<0.001$). The average CBCL ratings for the ADHD subgroups were significantly higher than for the control group (see Table 1).

TABLE1

Cortisol Awakening Increase

The cortisol awakening response (increase from awakening to 30 min after awakening) was not significant between groups ($F(2,61)=0.62$; $p=0.54$). Means, standard deviations, and effect sizes at Cort1/Cort2 time points are provided in Table 2. To evaluate whether the awakening response may be different for weekdays or weekend days, analyses were repeated for weekdays ($F(2,61)=0.01$; $p=0.99$) and weekend days ($F(2,61)=1.87$; $p=0.16$) separately, yielding similar results.

Cortisol Daytime Decline

Variations in cortisol levels at the 4 post-awakening times across the day were best modeled using a heterogeneous first-order autoregressive covariance structure and a random intercept effect for subject. Age, sex, BMI, pubertal stage, and internalizing problems were excluded from the final model as these factors did not significantly contribute to variations in cortisol levels. For a model controlling for day of evaluation ($F(4,1166)=0.74$; $p=0.57$) and weekday vs weekend day ($F(1,1166)=3.87$; $p=0.05$), there was a significant effect of time ($F(3,1166)=767.73$; $p<0.0001$) and group x time ($F(6,1166)=4.05$; $p<0.001$), but no main effect of group ($F(2,1166)=0.69$; $p=0.50$). Polynomial contrasts of the time factor showed a significant linear effect ($p<0.0001$) reflecting a linear decline in cortisol levels from awakening to evening, a quadratic effect ($p<0.001$) reflecting a steeper decline in the morning than in the afternoon, and a cubic effect ($p<0.0001$). The orthogonal contrast of the overall ADHD group and the control group revealed that the linear ($p=0.75$), quadratic ($p=0.26$), or cubic ($p=0.07$) interaction components were not significantly different. The second orthogonal contrast -comparing the ADHD+ODD and ADHD subgroups- revealed a significant difference between groups in the linear component

($p<0.001$). The quadratic ($p=0.20$) or cubic ($p=0.19$) components of the interaction were not significant which may however be due to a lack of power to detect such differences. Despite the small number of participants in ADHD subgroups, the ADHD+ODD subgroup showed significantly higher morning and lower evening levels, resulting in a steeper linear decrease in cortisol levels throughout the day as compared to the ADHD subgroup. Comparable results were obtained in additional analyses where ODD symptoms were considered as a continuous dimension (published as online supplementary material). A visual representation of group differences in cortisol levels (µg/L), averaged across 5 days, is presented in Figure 1. Means, standard deviations, and effect sizes at each time point are provided in Table 2.

Confirmatory analyses of weekdays and weekend days separately were performed and revealed similar findings: the linear component of the group x time interaction for the ADHD+ODD and ADHD subgroups differed significantly during weekdays ($p<0.01$) and weekend days ($p=0.01$).

TABLE2

FIGURE1

Discussion

To the best of our knowledge, this is the first study of cortisol and ADHD with such a large number (25) of observations for each individual, which were obtained over five days with five measurements across each day in ADHD children compared to age-matched controls. This study aimed to investigate whether variation in cortisol levels across the day would be consistent with the theory of an abnormal diurnal arousal pattern associated with ADHD (i.e., time-specific hypo- vs hyperarousal). We assessed (i) the cortisol awakening response (increase from awakening to 30 min after awakening), and (ii) the daytime decline (variations in intra-day cortisol levels based on four time points across the day: awakening, noon, 4PM, 8PM).

With respect to the awakening response, we found no evidence for group differences, which is partly in line with Freitag et al. (2009) who reported no different awakening response in children with ADHD but a significantly lower awakening response in children with ADHD+ODD. This discrepancy may be explained by methodological differences in timing of cortisol assessments; Freitag et al. (2009) assessed awakening response at four time points (0, 30, 45, and 60 min after awakening), while we had only two time points (0 and 30 min after awakening), and they did not assess the post-awakening pattern, while we assessed it across the entire day.

With respect to the daytime decline, we found a significant interaction of time and group; orthogonal contrasts showed this effect was related to across-the-day differences between the ADHD subgroups and not to

an overall difference between the ADHD group (average of the two subgroups) and the control group. Most studies in children with ADHD reported time-specific differences in cortisol levels (Blomqvist, et al., 2007; Kaneko, et al., 1993). This discrepancy may be explained by methodological differences in diagnosis: these studies did not subgroup ADHD cases based on the presence or absence of ODD, which is very common on most clinical samples. Since the two subgroups (ADHD+ODD and ADHD) showed differential patterns, subgroup differences may have existed but cancelled each other. Studies in general population samples have shown a lower morning-to-afternoon ratio associated with the presence of attention problems (Susman, et al., 2007), which partly support our findings of a flattened decline in the ADHD subgroup.

Our results support the hypothesis that arousal-related circadian rhythms may be altered in children with ADHD. Since we found that alterations in the diurnal cortisol pattern in our ADHD sample are based on the presence or absence of comorbid ODD, we can extend the hypothesis by suggesting differences in the pathophysiology of ADHD+ODD and ADHD. In the regulation of attention, behaviour, and emotion, differential neurophysiologic effects of cortisol have been described in the HC, amygdala, and PFC as these brain regions contain different cortisol receptors (Hastings, et al., 2009). Whereas the HC and amygdala contain both mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), the PFC only contains GR (Sanchez, Young, Plotsky, & Insel, 2000). Binding on MR occurs for most of the circadian cycle as these receptors have a high affinity for cortisol. GR on the contrary have a lower affinity for cortisol resulting in increased cortisol binding only when circulating cortisol rises: for example, during peak moments of circadian activity, i.e. the ‘wake-up’ period (Reul & Kloet, 1985). Therefore, we propose that the action of cortisol in these brain regions is highly time-dependent and differentially altered in children with ADHD+ODD and ADHD.

This contrasts with theoretical frameworks that have defined ADHD and ODD in terms of hypo-arousal deficits (Raine, 1996; Sonuga-Barke, et al., 2010), largely ignoring circadian effects on arousal. Rather than being chronically hypo-aroused, our findings suggest the subgroups of children with ADHD+ODD and ADHD may be hypo- or hyperaroused at different times of day. If these findings could be replicated in larger samples, this knowledge on time-of-day effects in cortisol level may guide timing of interventions with arousal level as a presumed target. Though time-of-day effects on different modalities of interventions for ADHD have not been assessed systematically (Swanson, et al., 2004), we hypothesize that the ADHD+ODD and ADHD subgroups may benefit most from different treatment modalities at different times of day. For example, the mechanism of action of stimulant medication has predominantly been associated with an increase in arousal, and some speculate that its therapeutic effects are due to the correction of a state of underarousal. This leads to the

hypothesis that morning treatment with medication may be more beneficial for children with ADHD than with ADHD+ODD. In contrast, the mechanism of action of non-pharmaceutical treatments such as relaxation or mindfulness have predominately been associated with a decrease in arousal, suggesting that children with ADHD+ODD may benefit most from this intervention in the morning rather than in the afternoon/evening.

Although this study has some clear strengths associated with the evaluation of intra- and interday cortisol variability, some limitations must be taken into account. First, salivary cortisol samples were collected in the naturalistic environment of children, which resulted in some non-compliance. Due to financial constraints, it was not possible to use electronic monitoring devices to assess time of saliva collection, which would be a good addition to future studies. Second, the overall sample size in this study was limited leading to small sample sizes for the evaluation of the clinical subgroups (ADHD+ODD and ADHD). Third, this study did not include subjects with ODD without ADHD. It is not clear whether differences found in ADHD+ODD and ADHD subgroups are specific for ADHD, ODD, or the comorbid condition. Fourth, we did not control for activity levels which may have influenced cortisol concentrations and which may have differed for the ADHD and control groups in similar school settings (same classrooms) during the weekdays or in different home settings during the weekend days. Fifth, the study lacked of an objective test of arousal (i.e., multiple sleep latency test) that could have been coupled to measure of cortisol. Sixth, we did not assess current nor previously experiences of stress, which have been related to higher cortisol levels. In future studies, it may be valuable to document stressful experiences (e.g., prenatal exposure to teratogens and stress, separation, and abuse) that may cause long-lasting or even permanent alterations of the HPA axis function by affecting steroid receptors in the PFC and HC (McBurnett, et al., 2000; Meaney, et al., 1985). Finally, we did not adjust for effects of age, sex, BMI, and pubertal stage, which were not significantly related to cortisol levels in our study but may be present and operate to confound our results.

Conclusion

In summary, in our analyses of cortisol levels within- and across-days, we observed a significant ADHD subgroup x time interaction reflecting a reversal of a group difference in cortisol levels from the morning to evening. This suggests that the ADHD subgroup may be relatively hypo-aroused in the morning but hyperaroused in the evening, while the ADHD+ODD subgroup may be relatively hyperaroused in the morning and hypo-aroused in the evening. Though further research is recommended to validate our findings in larger samples and to explore the association of ADHD with other comorbidities, knowledge on time-specific arousal deviations in ADHD subgroups may guide the timing of arousal-based treatments.

Key points:

- Evidence in literature for time-specific arousal problems in ADHD
- Limited and inconsistent results on across-the-day cortisol variations in ADHD
- This study supports time-specific cortisol deviations in ADHD with and without comorbid ODD
- Clinical implications for the timing of arousal-based treatments in ADHD with or without ODD

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Table 1

Test Statistics of Demographic Variables, Awakening and Sleep Times, and CBCL Ratings

	Control M(SD)/n(%)	ADHD+ODD M(SD)/n(%)	ADHD M(SD)/n(%)	$F(2,64)/\chi^2(2)$
Age	8.88(1.62)	9.09(1.54)	8.82(1.54)	<1
Sex (n)	26m (79), 7f (21)	17m (77), 5f (23)	9m (82), 2f (18)	<1
Pre-pubertal stage				
- Pubic hair stage (n)	26m (100), 6f (86)	14m (82), 5f (100)	9m (100), 2f (100)	4.55(m); 1,08(f)
- Genital stage (n)	26m (100)	16m (94)	9m (100)	<1
- Breast stage (n)	6f (86)	4f(80)	2f (100)	<1
BMI	15.82(1.63)	17.12(2.13)	15.04(1.33)	3.06
Weekday sleep time	20:38(0:30)	20:16(1:10)	20:35(0:24)	1.48
Weekday awakening time	6:59(0:21)	6:52(0:24)	6:59(0:19)	<1
Weekend sleep time	21:04(2:43)	20:49(3:11)	21:37(0:36)	<1
Weekend awakening time	8:02(0:38)	8:05(0:43)	8:01(0:43)	<1
CBCL internalizing	46.33(9.28)	63.10(9.15)	62.09(7.74)	27.03***
CBCL externalizing	44.64(8.53)	86.95(7.70)	62.09(5.43)	66.51***

Note. ADHD+ODD=attention-deficit hyperactivity disorder with oppositional defiant disorder; ADHD=ADHD without ODD; M=mean; SD=standard deviation; n=number in sample; %=percentage of sample; m=males; f=females; CBCL=child behaviour checklist

***p<0.001

Table 2

Summary Statistics for Cortisol Concentrations (µg/L) on Five Time Points Across the Day, Averaged Across Five Days

Time	Control M(SD)	ADHD+ODD M(SD)	ADHD M(SD)	Control vs ADHD ES	ADHD+ODD vs ADHD ES
Awakening	8.11(3.18)	8.53(5.10)	7.06(1.38)	0.02	0.39
30 min after awakening	9.01(2.34)	9.99(4.70)	8.61(1.88)	0.16	0.39
Noon	3.89(1.21)	3.32(0.73)	3.33(0.59)	0.83	0.02
4PM	2.97(1.01)	2.88(0.50)	3.04(0.77)	0.05	0.25
8PM	1.61(0.59)	1.29(0.50)	2.14(1.23)	0.04	0.91

Note. M=mean; SD=standard deviation; ES=effect size (Cohen *d*); ADHD+ODD=attention-deficit hyperactivity disorder with oppositional deviant disorder; ADHD=ADHD without ODD

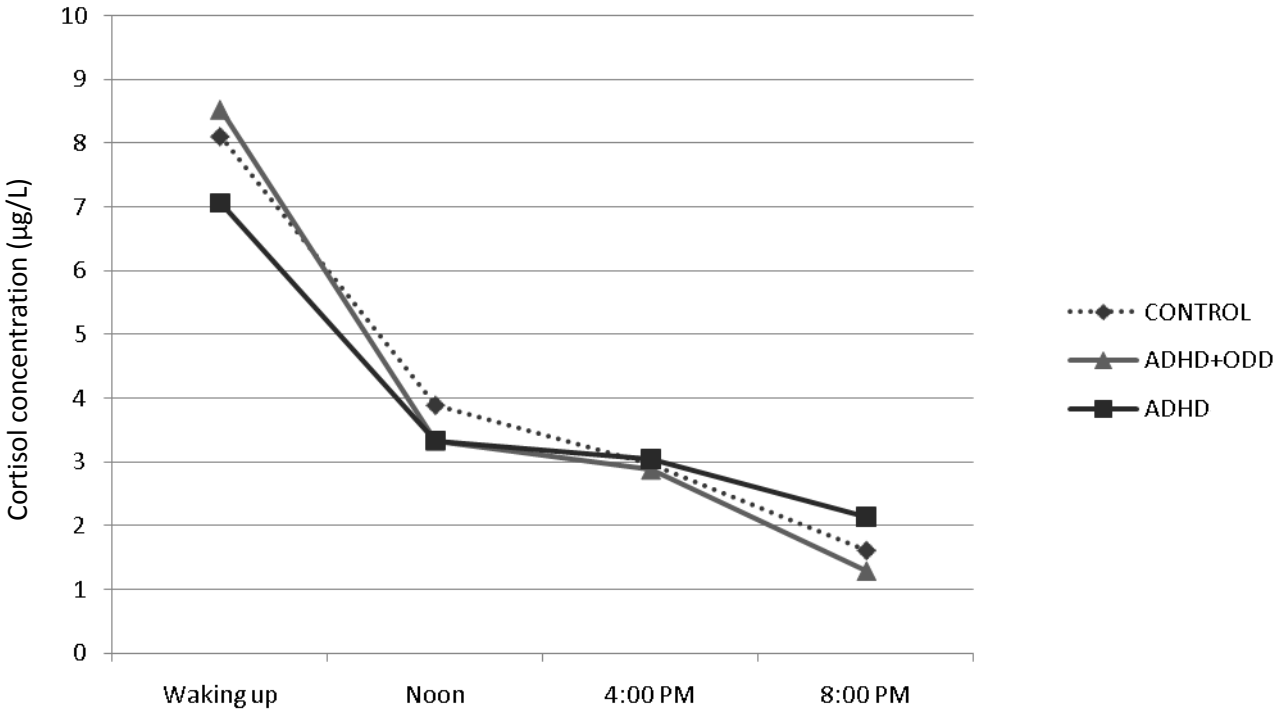

Figure 1. Group differences in cortisol concentrations across the day, averaged across five days.

Additional analyses with ODD symptoms considered as a continuous dimension

Next to the analyses reported in the manuscript text where ODD was considered as a categorical measure (clinical subgroups of ADHD with and without ODD), additional analyses where ODD symptoms were considered as a continuous dimension were performed. First, we included diagnostic group (ADHD vs control), time (four time points), ODD (continuous measure), and the two- and three-way interactions of these terms in a mixed model. Though there was a significant main effect of time ($p < 0.0001$) and a significant interaction effect of time*diagnostic group ($p < 0.01$) and time*ODD ($p < 0.05$), the 3-way interaction time*diagnostic group*ODD was not significant ($p = 0.39$). However, this analysis seemed rather problematic as the distribution of ODD symptoms was very different in the ADHD in the control group (rarity of these symptoms in the latter group). Therefore, in a second set of analyses, we explored the effect of ODD problems as a continuous measure in the ADHD group and the control group separately. In these models, we included time (4 time points), ODD (continuous measure), and the interaction term. With respect to the control group, there was a significant effect for time ($p < 0.0001$), but not for ODD ($p = 0.80$) or the interaction term ($p = 0.53$). In contrast, for the ADHD group, a significant effect of time ($p < 0.0001$), ODD ($p < 0.01$), and time*ODD ($p < 0.01$) was found. These results seem to reflect similar disruptions as found in clinical ADHD subsamples (ADHD with and without ODD): a 1-point increase on the ODD problem scale was associated with (i) a 3.4% decrease in evening cortisol; and (ii) a 2.3% increase in morning cortisol concentration.