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Self-reported symptoms of depressed mood, trait anxiety and aggressive behavior in post-pubertal adolescents: Associations with diurnal cortisol profiles

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ABSTRACT

The association between self-reported symptoms and diurnal cortisol profiles was studied in post-puberty adolescents (29 boys and 29 girls, $M_{age} = 15.06$ years). The adolescents completed the Children's Depression Inventory, State Trait Anxiety Inventory, and an Aggressive behavior scale. The diurnal cortisol profile was derived from three saliva samples, collected at awakening, noon and evening on a week-end day. Univariate repeated measurement regressions revealed that depressed mood and trait anxiety were strongly and aggressive behavior was weakly related to the diurnal cortisol profile: greater emotional distress was associated with flatter diurnal cortisol profiles. Multivariate analysis, however, revealed that only trait anxiety made an independent contribution. Further analyses suggested that trait anxiety was related to elevated evening cortisol rather than to decreased awakening cortisol and that from a trait anxiety score of 38 onwards, high anxious adolescents show clearly higher evening cortisol than low anxious adolescents. These data suggest that anxiety disorder comorbidity might explain some of the differences in HPA-axis function among depressed patients.

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Introduction

Mood and anxiety disorders are among the most common disorders. Some subtypes of these disorders are associated with changes in the activity of the hypothalamus-pituitary-adrenocortical (HPA)-axis activity. It is suggested that the full maturation of the HPA-axis – including the full diurnal cortisol rhythm, which is reached with the attainment of Tanner stage three – may have implications for the heightened risk of psychopathology noted among post-pubertal adolescents (Forbes et al., 2006; Goodyer et al., 2001; Gunnar and Quevedo, 2007). Different cortisol measures have been studied, e.g., changes in cortisol referring to overall hypersecretion over 12 or 24 h, changes in absolute levels of cortisol secretion at different times during the day such as lowered morning cortisol or elevated evening cortisol, and changes in diurnal or circadian cortisol rhythms. It is clear that post-pubertal adolescents have been understudied with respect to cortisol rhythms, a measure that clearly is gaining in importance (Adam, 2006).

In a recent meta-analysis, Miller et al. (2007: 38) concluded that even when a person does not develop a full-blown psychiatric condition,

greater emotional distress is associated with flatter diurnal cortisol profiles. Also, to the extent that people report higher levels of distress, they showed greater daily cortisol output and elevated afternoon/evening cortisol, though morning levels may be somewhat lower. A high, flattened diurnal profile has been shown to be indicative of a chronically stressed, hyperactive HPA-axis (Deuschle et al., 1997; McEwen, 2002; McEwen and Wingfield, 2003; Rosmond et al., 1998) and is associated with depression in adult outpatients (den Hartog et al., 2003; Sachar et al., 1970; Young et al., 2006), or adolescent outpatients (Kaufman and Charney, 2001; Forbes et al., 2006). Other dysregulations imply low cortisol levels at awakening which remain constant throughout the day. Such a low, flattened diurnal profile (i.e., lacking of expected strong diurnal rhythm) reflects reduced cortisol output (hypocortisolism) and is seen in adult patients with posttraumatic stress disorders, atypical depression or chronic fatigue syndrome (Gunnar and Quevedo, 2007; Heim et al., 2000, 2004; Miller et al., 2007; Van Praag et al., 2004) and in neglected children (e.g., orphanage-reared children) (see Gunnar and Vazques, 2001; Gunnar and Quevedo, 2007; Tarullo and Gunnar, 2006).

Intriguing observations are that disturbed anxiety and/or aggression regulation are frequently seen as components of depression. Nearly half of the patients meeting life time criteria for major depression also have met criteria for co-morbid anxiety disorders (e.g., Murphy et al., 2004; Regier et al., 1998). Aggression can be turned inward, manifesting itself as self-denigration or suicidality, or can be directed outward with symptoms such as irritability, short-temperedness, impatience, outburst

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of anger after only slight provocations (Van Praag et al., 2004). Sudden spells of anger have been observed in approximately 30–40% of both patients with major depression and patients with dysthymia. Depressed persons with anger attacks have higher anxiety scores in comparison to both, depressed patients without anger attacks and normal control subjects (Painuly et al., 2007). It has been suggested that anxiety and/or aggression co-morbidity may explain some of the differences in HPA-axis functioning among depressed patients (Van Praag et al., 2004). For instance, high cortisol levels are only seen in the subgroup of depressive patients who were also anxious (Kara et al., 2000; Korte, 2001; Young et al., 2004).

The aim of our study is twofold. First, we examine the influences of self-reported symptoms of depressed mood, anxiety and aggression on diurnal cortisol rhythm with univariate (longitudinal) repeated measurements regression in post-pubertal adolescents. Second, we question whether depressed mood, anxiety and aggression each make independent contributions to diurnal cortisol profiles above their shared variance. By doing so we also try to examine whether anxiety disorder co-morbidity might explain some of the differences in HPA-axis among post-pubertal adolescents with depressed mood.

Method

Participants

The current study involved a sample of 68 14- and 15-year olds, participating in the third wave of a prospective follow-up study, that included 86 pregnant mothers and their newborns in the first wave and 72 mother–infant pairs in the second wave, when the child was 8/9 years old (see Van den Bergh et al., 2006). The 58 participants who had complete data for all cortisol measures are included (29 boys and 29 girls, $M_{\text{age}}=15.06$ years, $SD=26$ years). Most of them had reached Tanner stage four of pubertal development ($M=4.13$; $SD=.526$). The local ethical committee approved the study and all adolescents and their parents gave their written informed consent.

Measures

Cortisol: short cortisol day-time profile

Saliva sampling, in the morning as well as during the day, is best done with strict reference to the timing of awakening rather than on exact, predefined hours; the latter underlies large inter- and intra-individual variation (Edwards et al., 2001; Pruessner et al., 1997, 2003). We used a short version of the day-time cortisol profile (cf. Van den Bergh et al., 2008; Wüst et al., 2000) and samples were collected at awakening, at approximately 4 h (noon) and 12 h (evening) after awakening, at home, on a typical week-end day. For reasons of feasibility, we planned the saliva collection on a week-end day (e.g., for an adolescent on a normal school day it is difficult to collect the noon sample 4 h after the awakening sample). Samples were collected by spitting in a small plastic tube (Sarstedt, Germany), without using swabs or aids to salivation. The tubes were kept refrigerated and brought along to the laboratory visit together with the questionnaires they had completed at home. All samples were stored at -60°C upon arrival. For assessment of dynamic HPA-axis activity, salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol (Gozansky et al., 2005). Two hundred microliters of saliva and diluted standards was used, with incubation for 3 h at room temperature, modified by an additional period overnight (15–18 h) at $+4^{\circ}\text{C}$ (cf. O'Connor et al., 2005). The method was sensitive to as low as .3 nmol/L; all cases below this threshold were set at zero.

Self-reported symptoms of mood and anxiety disorders and aggressive behavior

We used standardized Dutch versions of the scales. In Dutch reference populations, Cronbach's alphas were between .78 and .95 for Trait anxiety and CDI. For the aggressive behavior scale, Cronbach's alpha was .67 in the English-speaking reference population.

Depressed mood. The Child Depression Inventory (CDI, Kovacs, 1992; Timbremont and Braet, 2002) measures the severity of cognitive, affective and behavioral symptoms of depression over the last two weeks and contains 27 items, scored from 0 to 2. According to the authors of the CDI, a score of 19 best distinguishes between non-depressed and clinically depressed persons rated with DSM-IV criteria, whereas levels between 13 and 18 are regarded as subclinical or minor depressive episode.

Trait anxiety. The State Trait Anxiety Inventory for Children (STAIC; Bakker et al., 1989) comprises two subscales of 20 items rated on a 3 point scale. Although originally designed for use with children aged between 9 and 12 by Spielberger, the STAIC has been used to measure anxiety in children and adolescents falling outside of this age range (Lau et al., 2006). State anxiety refers to a transient emotional state, characterized by subjectively experienced tension and increased activity state of the autonomous nervous system. Trait anxiety refers to a disposition or proneness to react with anxiety. In this study we used only trait anxiety; it was used as a measure of predisposing anxiety trait. Measures of trait anxiety and neuroticism often

correlate between .60 (e.g., Gomez and Francis, 2003) and .80, or even more (Twenge, 2000).

Aggressive behavior. The Aggression subscale of the Early Adolescent Temperament Questionnaire-Revised (EATQ-R for ages 9–16, short form; Ellis and Rothbart, 2001; Rothbart and Capaldi, 1992) measures hostile and aggressive actions, including persons- and object-directed physical violence, direct and indirect verbal aggression and hostile reactivity. It contains 6 items rated on a 7 point scale. We used this scale as a measure of aggression regulation problems.

Statistical analyses

The associations among the cortisol measurements at different times of the day – i.e. at awakening (value 0), approximately 4 h (value 4) and approximately 12 h after awakening (value 12) – were best modeled using a heterogeneous first-order autoregressive covariance structure. First, repeated measurements (RM) regression analyses were done separately for the three variables (univariate analyses). Each of these analyses started with a model containing the main effect of the variable (to investigate whether the cortisol level depends on the severity of self-report symptoms), the linear and quadratic time effects of cortisol (to investigate whether the cortisol level evolves during the day and, if so, in what way it evolves), and the interaction effect of the symptom variable with the linear time effect (to investigate whether the linear diurnal cortisol evolution depends on the severity of the symptoms). Backward variable selection was performed by sequentially dropping non-relevant predictors, if any. We kept the model hierarchical in the sense that the inclusion of an interaction effect implied the inclusion of all its higher order effects. The multivariate RM analysis included the main effects of all three variables, the linear and quadratic time effects on cortisol, and the interaction of all three variables with the linear time effect. Again, backward variable selection was performed and the model was kept hierarchical. Thus, together with the intercept we started the backward selection with a model containing 9 predictors.

Some of the variables were positively skewed and were transformed. Cortisol levels were \log_e -transformed (after adding 1 to avoid log of zero) because mainly the noon and evening levels were skewed. The CDI benefited more from a square root-transformation to reduce skewness. Note that the results of the analyses for the untransformed variables (not reported) were nearly identical to those for the transformed ones.

In case ordinary least squares regression was performed (i.e. regression without repeated measurements), the ω^2 (omega squared) index was computed as a measure of effect size for each predictor. This index tries to give a population-based estimate of the proportion of variance in the outcome that is accounted for by each predictor (Olejnik and Algina, 2000). In case two unpaired medians are compared, the 95% CI for the difference of the medians is computed using the method by Bonett and Price (2002).

Results

Descriptive statistics, correlational and incomplete case analysis

Descriptives for all relevant variables are presented in Table 1. Using the cut-off scores of the CDI, we can tentatively conclude that 14 % ($N=8$; 4 females, 4 males) may suffer from a minor depressive episode (score range 13–18), while 9 % ($N=5$; 4 females, 1 male) may suffer from a major depressive period (score >18). Taking into account the range of scores obtained and the fact that the mean and median CDI scores (Timbremont and Braet, 2002) for girls (9.3 and 7 respectively) and boys (8.6 and 8 respectively) are situated at deciles 5–7 and the mean and median Trait anxiety scores (Bakker et al., 1989) for girls (31.7 and 31 respectively) and boys (31 and 31 respectively) are situated at deciles 6–8 of reference samples we can tentatively conclude that our sample covered the range of depressive and anxiety symptoms seen in non-clinical populations and that a substantial proportion scored in the higher range. Parents of the latter adolescents were contacted in an

Table 1
Descriptive statistics for key variables

Variable	Mean (SD)	Median	Q1–Q3	Min–Max
<i>Self-reported symptoms</i>				
Depressed mood (CDI)	8.9 (5.44)	7.5	5–12	1–25
Trait anxiety (STAIC)	33.3 (6.68)	32.0	29–38	22–49
Aggressive behavior (EATQ-R)	2.43 (.73)	2.42	1.83–2.83	1.17–4.33
<i>Cortisol (nmol/l)</i>				
Awakening cortisol level	12.2 (4.99)	11.8	9–15.4	1.5–25.4
Noon cortisol level	6.1 (4.24)	5.1	3.3–7.3	.4–22.2
Evening cortisol level	2.3 (3.26)	1.3	0–3.3	0.0

appropriate way. One of the adolescents had antidepressive medication; this case was included in the sample since removing it did not change the results.

No norms are available for the aggressive behavior measure that we used.

Spearman correlations, which were used since some of the variables are skewed, indicated that depressed mood was highly correlated with anxiety ($r = .58; p < .0001$) and aggressive behavior ($r = .43, p < .001$) and that anxiety was correlated with aggressive behavior ($r = .25, p < .05$).

No big differences are present between the 58 cases with complete cortisol data and the remaining 10 with incomplete cortisol data. For the CDI the median of the 58 cases was 7.5, the remaining 10 had a median of 5.10. The difference in medians is 2.40 (95% CI = -5.44 to 10.25; Mann-Whitney test (M-W): $p = .51$). For trait anxiety: medians are 32 vs. 32.7, difference in medians is -.69 (95% CI = -4.90 to 3.53; M-W: $p = .73$). For aggressive behavior: medians are 2.42 vs. 2.33, difference is .08 (95% CI = -.63 to .80; M-W: $p = .78$).

Uni- and multivariate associations of depressed mood, trait anxiety and aggressive behavior with diurnal cortisol profiles

In the univariate RM models of depressed mood, trait anxiety and aggression, the strong linear time effect indicates that cortisol levels decreased during the day; the quadratic time effect indicates that the decrease slowed down as the day progressed. More importantly, for the univariate RM models involving depressed mood and trait anxiety, a significant interaction effect between these variables and the linear cortisol evolution indicated that these variables affected the diurnal cortisol evolution ($p = .0459$ for depression, $p = .0038$ for trait anxiety). Higher scores for depressed mood and for trait anxiety resulted in a flattened cortisol profile. A similar, yet weaker effect was found for trait aggression in the univariate RM model ($p = .1381$).

In the multivariate analysis, the effects involving depressed mood and aggressive behavior were weak and could be dropped from the model. The final model from the multivariate analysis was thus similar to the univariate model for trait anxiety (Table 2). Fig. 1 illustrates the relationship between trait anxiety and diurnal cortisol profiles.

The RM analysis did not reveal whether trait anxiety is related to awakening or evening cortisol as such (e.g., Vedhara et al., 2003, 2006; Feder et al., 2004; Forbes et al., 2006). Post-hoc ordinary least squares regression analyses suggested that trait anxiety was mainly related to elevated evening cortisol ($\omega^2 = .07; p = .0281$) rather than to decreased awakening cortisol ($\omega^2 = .01; p = .2124$).

In the final model, two cases were identified as outliers as they had studentized residuals whose absolute value exceeded 2.5. Repeating the multivariate analysis without these cases yielded very similar

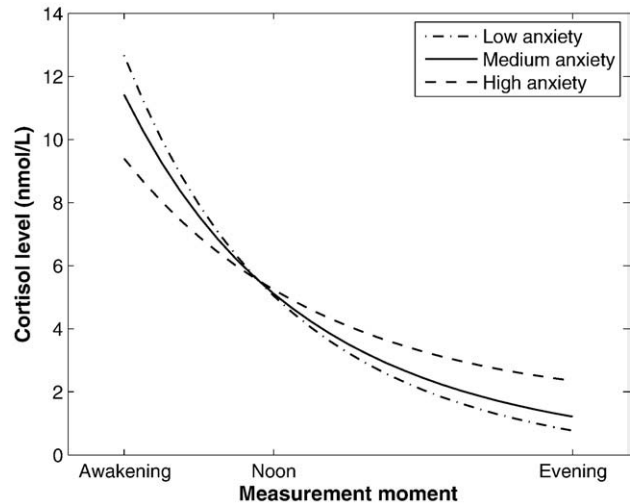


Fig. 1. Trait anxiety score and cortisol secretion across the day (the cortisol profile for adolescents with low (first decile; score 25), medium (median; score 32) and high anxiety (ninth decile; score 45) anxiety).

results. Four cases had a low flat cortisol profile in the sense that their cortisol levels were low at awakening (<5 nmol/L) and remained low throughout the day. Repeating the multivariate analysis without these four cases yielded very similar results as well.

In an extra analysis, we examined whether there is a trait anxiety threshold level above which cortisol secretion is different as compared to cortisol secretion for lower anxiety levels. We did not find any anxiety level above which awakening or noon cortisol was markedly different. However, adolescents with a trait anxiety score of at least 38 showed clearly higher evening cortisol. The low anxious (score <38, $n = 43$) adolescents' median cortisol levels were 12.2 nmol/L at awakening, 5.2 at noon, and .7 in the evening, whereas the high anxious ($n = 15$) adolescents' median levels were 11.8, 4.6, and 3.3 nmol/L, respectively. The difference in median evening cortisol was -2.6 nmol/L (95% CI = [-4.5, -.8]). The median CDI scores were 7 for low and 13 for high anxious adolescents.

Discussion

We studied the influence of self-reported symptoms of depressed mood, anxiety, and aggressive behavior on diurnal cortisol profiles in 58 post-pubertal adolescents. Univariate RM regressions revealed that, in our sample, depressed mood and trait anxiety were strongly associated with a flattened diurnal cortisol profile. For aggressive behavior, a similar yet much weaker trend was found. Emotional distress is associated with a flattened diurnal cortisol profile. Multivariate RM regressions, however, revealed that only trait anxiety made an independent contribution. Further analyses suggested that trait anxiety was mainly related to elevated evening cortisol rather than to decreased awakening cortisol and that from a trait anxiety score of 38 onwards, high anxious adolescents show clearly higher evening cortisol than low anxious adolescents.

Our data reveal several interesting findings. However, they clearly need further confirmation in larger samples before firm conclusions can be drawn.

First, it has been suggested that anxiety disorder co-morbidity might explain some of the differences in HPA-axis function among depressed patients (Kara et al., 2000; Korte, 2001; Young et al., 2004; Van Praag et al., 2004: 228). The results of our multiple regression analysis, showing that only trait anxiety independently influenced the diurnal cortisol profile, are in accordance with this suggestion. These results may indicate that it is 'the anxiety part of depressed mood' that is related to the flattened cortisol profile. Anxiety is a state of apprehension, tension, and uneasiness in response to a perceived threat. It is considered normal when it is temporary, e.g., during a stressful or

Table 2
Results of the multivariate regression of cortisol day-time profile on self-reported symptoms of depressed mood, trait anxiety and aggressive behavior

Effect	Estimate (SE)	df	t-value	p-value
<i>Full model</i>				
Intercept	2.50 (.061)	54	40.71	<.0001
Trait anxiety	-.081 (.076)	54	-1.07	.2873
Sqrt(CDI)	-.025 (.081)	54	-.31	.7607
Aggression	.025 (.066)	54	.039	.7017
Linear time effect	-.19 (.025)	111	-7.49	<.0001
Quadratic time effect	.0042 (.0019)	111	2.14	.0346
Trait anxiety*Linear time	.023 (.011)	111	2.17	.0319
Sqrt(CDI)*Linear time	.00025 (.012)	111	.02	.9831
Aggression*Linear time	.0081 (.0094)	111	.86	.3929
<i>Final model</i>				
Intercept	2.50 (.061)	56	41.34	<.0001
Trait anxiety	-.091 (.060)	56	-1.54	.1294
Linear time effect	-.19 (.025)	113	-7.54	<.0001
Quadratic time effect	.0042 (.0019)	113	2.15	.0338
Trait anxiety*Linear time	.025 (.0085)	113	2.96	.0038

uncertain situation. Our data seem to indicate that prolonged, intense, or inappropriate periods of anxiety possibly imposed by intense inner conflict, counterproductive defences and disorganized attempts at coping (Gold et al., 1988) are related to HPA-axis alterations.

Second, although we cannot really examine the supposed pathophysiological pathways of the hitherto hypothetical construct anxiety/aggression-driven depression (Van Praag et al., 2004), some of our data reveal tentative evidence that is in line with this construct.

The association between symptoms of anxiety and a flattened diurnal cortisol profile may indicate that they are primary symptoms and that disturbed anxiety regulation is a precursor dysfunction and a key component (i.e. a pacemaker) of a certain type of depression (Van Praag, 2001). Other evidence can be seen in the fact that symptoms of depressed mood, trait anxiety and aggressive behavior were correlated. Moreover, these symptoms were also correlated with a measure of emotional reactivity (additional analyses: depressed mood: $r=.58$, $p<.0001$; anxiety: $r=.61$, $p<.0001$; aggressive behavior: $r=.41$, $p<.001$). This subscale, taken from a questionnaire of Strelau and Zawadzki (1993), measures the tendency to react intensively to emotion-generating stimuli and can in fact be seen as a measure of neuroticism. This is in accordance with the observation of Van Praag et al. (2004) that individuals with anxiety/aggression-driven depression show a high prevalence of character-neurotic traits. It is supposed that this debilitating personality trait may thwart successful coping and enhance anxiety (and aggression) proneness.

The rather weak associations between aggressive behavior and a flattened cortisol profile may be related to the fact that our aggressive behavior measure covered only outward regression, while aggression regulation problems in depression can also be directed inward.

Third, post hoc univariate regressions revealed that trait anxiety was related to elevated evening cortisol level, but not to awakening and noon levels. This finding is in line with findings of elevated evening salivary cortisol in depression in 8- to 16-year olds (Goodyer et al., 1996), in 13- to 17-year olds (Goodyer et al., 2001), in adolescents having reached Tanner stage III (Dahl et al., 1991; Rao et al., 1996; Forbes et al., 2006) and in patients with severe depression or psychotic depression (Keller et al., 2006). However, these results do not corroborate the findings of Vedhara and colleagues (2003), who did not find associations between indices of emotional distress and absolute levels of cortisol on any time of the day, and the findings of Adam (2006) who, in a study of normal adolescents, did not found associations between trait anxiety and any of the diurnal cortisol parameters. Moreover, Goodyer et al. (2003) found raised morning cortisol in depressive adolescents. The inconsistency in these results may be related to the use of different measures to obtain and analyse cortisol and to the fact that the puberty stage is not always taken into account in studies with adolescents (e.g., Angold et al., 1998).

Our study shows limitations that warrant some caution in interpreting the results and their implications.

First, the sample was rather small which hampers a very reliable assessment of the effects.

Second, our cortisol measures showed some shortcomings. For reasons of feasibility, we planned the saliva collection on a week-end day in which schedules are typically more erratic and wake times later than adolescents' typical weekday schedules. Adolescents may sleep in on week-end days. Adolescents with depressed mood and/or high anxiety may sleep in more than those without depressed mood and/or anxiety. However, we do not think that this is a plausible explanation for our results. Sleeping in should mainly result in a lower cortisol level at awakening, whereas the observed flattening of the day-time profile was mainly a consequence of an elevated evening cortisol. Also, the type of procedure we used relies very heavily upon participant's adherence to the protocol (Clow et al., 2004). Our control for non-compliance was a rather crude one: we excluded cases in which no three saliva samples taken on the same day were available. We could have employed electronic monitoring devices which are now beco-

ming standard in ambulatory cortisol research (e.g., Jacobs et al., 2005; Schlotz et al., 2006). As we didn't use these devices we don't know whether adolescents reported their sampling times correctly.

Third, adjusting the analyses with regard to cortisol for exact time of sampling may be important (Hruschka et al., 2005). Even though only about 44 subjects had given exact hours for at least one of the three samples, in additional analyses we tried RM mixed-effects regression models (fitting random intercepts and random slopes for the linear time effect) using the exact hours. The results of these models were very similar to the results we presented: the aggressive behavior was less strongly related to cortisol than depressed mood or trait anxiety, and multivariate analysis retained only effects related to trait anxiety.

A *fourth* limitation is the lack of attention to potential confounding factors. Only time of awakening was measured and controlled for, but other factors such as nicotine and caffeine use, use of steroid based medications, presence/absence of a menstrual cycle and menstrual timing (for the girls), were not statistically controlled for in the analyses. However, the incidence of smoking was rather low in our sample of 14–15 year olds. Concerning menstrual cycle differences in girls, some authors have reported no cycle-related differences in 24 h cortisol secretion in either depressed or control women (Young et al., 2001).

Fifth, the HPA-axis is connected with other biological systems (e.g., monoamine neurotransmitters, immune system) which we did not measure. The simultaneous study of several systems may have given a more complete picture of pathophysiological pathways in subclinical and clinical mood and anxiety disorders. Moreover, as the role of serotonin (Van Praag et al., 2004) and of the corticotrophin releasing hormone (CRH) in mood and anxiety disorders (e.g., Korte, 2001; Risbrough and Stein, 2006; Schulkin et al., 1998; Steckler and Holsboer, 1999) has been understudied in children and adolescents, data on these measures could have been useful.

Sixth, our study had no genetic sensitive design. Therefore, we could not examine effects of genes or of gene–environment interaction (de Kloet et al., 2005).

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