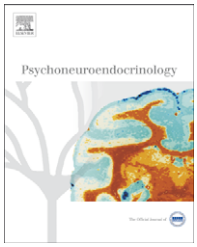
Psychoneuroendocrinology (2008) 33, 810–820

Available at www.sciencedirect.com

ScienceDirect

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



Examining cortisol rhythmicity and responsivity to stress in children with Tourette syndrome

B.A. Corbett[a,b,g,*], S.P. Mendoza[c], C.L. Baym[d], S.A. Bunge[e,f,g], S. Levine[a]

[a]*Department of Psychiatry and Behavioral Sciences, University of California at Davis, 2825 50th Street, Sacramento, CA 95817, USA*
[b]*M.I.N.D. Institute, University of California at Davis, 2825 50th Street, Sacramento, CA 95817, USA*
[c]*Department of Psychology, University of California at Davis, USA*
[d]*Department of Psychology, University of Illinois at Urbana-Champaign, USA*
[e]*Helen Wills Neuroscience Institute, University of California at Berkeley, USA*
[f]*Department of Psychology, University of California at Berkeley, USA*
[g]*Center for Mind and Brain, University of California at Davis, USA*

Received 4 January 2008; received in revised form 10 March 2008; accepted 20 March 2008

KEYWORDS
Tourette syndrome;
Cortisol;
HPA axis;
Stress;
Anxiety;
Diurnal rhythm

Summary
Background: Tourette syndrome (TS) is characterized by motor and vocal tics, which are often exacerbated by stress. The hypothalamic–pituitary–adrenocortical (HPA) axis, a major stress response system is thus of interest for understanding TS.
Methods: Diurnal cortisol rhythms were estimated in medication-free children 7–13 years with TS ($N = 20$) and healthy age-matched controls ($N = 16$). Salivary samples were collected on 3 consecutive days from the home. HPA responsivity was assessed by examining cortisol in response to a mock and real MRI scan.
Results: The results of diurnal rhythmicity revealed a trend showing marginally lower evening cortisol for the TS group. By contrast, the TS group had higher cortisol levels in response to the stressor. There were strong, negative correlations between evening cortisol and tic severity as well as diurnal cortisol and anxiety.
Conclusions: The children with TS showed increased cortisol in response to the MRI environment, supporting a model of enhanced HPA responsivity. The lower evening cortisol may be the result of chronic daily stress. Alternatively, the negative associations between cortisol and reported anxiety and tics may reflect biologically based anxiolytic properties of tic expression. Taken together, the results clearly implicate involvement of the HPA axis in the neuropathology of TS.

*Corresponding author at: Department of Psychiatry and Behavioral Sciences, University of California at Davis, 2825 50th Street, Sacramento, CA 95817, USA. Tel.: +1 916 703 0232; fax: +1 916 703 0244.
E-mail address: blythe.corbett@ucdmc.ucdavis.edu (B.A. Corbett).

0306-4530/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.psyneuen.2008.03.014

1. Introduction

Gilles de la Tourette syndrome (TS) is a complex, highly heritable neurodevelopmental disorder characterized by the expression of motor and phonic tics. The diagnostic criteria require the expression of tics defined as sudden, rapid, recurrent, nonrhythmic, stereotyped motor movements and vocalizations (APA, 1994). TS usually has a childhood onset with waxing and waning symptoms and response to medication over the course of development (Surwillo et al., 1978). Many individuals present with a mild-to-moderate symptom profile that is treated with education, behavioral techniques, and follow-up (Coffey et al., 1994). Patients with more severe symptoms often require treatment that may involve pharmacotherapeutic intervention and cognitive–behavioral therapy. TS is often accompanied by a range of co-occurring symptoms, including inattention, impulsivity, and impaired executive function (Bruun and Budman, 1992). There is also a high preponderance of symptoms of obsessive–compulsive disorder (OCD) in TS (McElroy et al., 1994; Steingard and Dillon-Stout, 1992; Swedo and Leonard, 1994). Further, anxiety disorders are often comorbid with TS and may be related to tic severity (Coffey et al., 1992).

It is commonly reported that stress-related fluctuations in symptom severity occur in all phases of TS illness (Peterson, 1996), often in response to fatigue, emotional trauma, anxiety, or stress (Shapiro et al., 1988). Premonitory urges are a sensory phenomenon that often precede motor tics (Banaschewski et al., 2003; Cohen and Leckman, 1992; Jankovic, 1997; Kwak et al., 2003; Leckman and Peterson, 1993; Woods et al., 2005), such that patients frequently describe their tics as voluntary and intentionally produced, as a way of relieving unpleasant involuntary premonitory sensations. Voluntary tic suppression can result in a buildup of tension that some claim leads to a paradoxical rebound or outburst of tics (Bliss, 1980; Jankovic, 1997). However, some studies involving behavioral observation methods (Himle and Woods, 2005; Meidinger et al., 2005; Woods et al., 2007) and intervention techniques (Verdellen et al., 2004) do not support the presence of a rebound effect.

A diathesis stress model proposed by Leckman et al. (1984) suggested that TS is the product of a genetic predisposition coupled with unknown environmental factors occurring at a vulnerable stage of development. An example of an environmental trigger is pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS) that arise after exposure to streptococcal (group A beta-hemolytic streptococcal [GABHS]) infections (Mell et al., 2005; Swedo et al., 1998; Trifiletti and Packard, 1999). However, other research does not support the notion that PANDAS and TS are the result of autoantibody exposure (Harris and Singer, 2006; Kurlan, 1998; Loiselle et al., 2004; Singer et al., 2005).

Aside from these specific etiological factors, anxiety and stress-related symptoms are consistently and intimately involved in both the maintenance and exacerbation of tics (Lombroso et al., 1991; Peterson, 1996). Given this strong relationship between symptoms and stress-related reactivity, the hypothalamic–pituitary–adrenocortical (HPA) axis is of interest in the study of TS.

Regulation of the HPA axis involves three interrelated processes: the maintenance of a diurnal rhythm, activation in response to challenge or threat (stress response), and the restoration of basal activity via negative feedback mechanisms. One or more of these processes could be affected in TS. Additionally, the consistency of the diurnal rhythm has been considered an important factor in some neuropsychiatric disorders (Corbett et al., 2008; Yehuda et al., 1996).

Cortisol, the primary glucocorticoid in humans, is a principal homeostatic regulator. Its secretion follows a circadian rhythm, with high concentrations in the morning and a decline throughout the day, with the lowest levels in the evening and at night. This pattern is already well-developed by the third month of infancy (Price et al., 1983; Vermes et al., 1980). It has been suggested that individual differences in cortisol secretion, especially in the morning, may be an important variable for typically developing children (Bartels et al., 2003) as well as children with neuropathology, such as autism (Corbett et al., 2006; Corbett et al., 2008).

Arguably, the best-studied aspect of the HPA axis pertains to the response to stress, and one of the most widely used biological markers of stress is an increased concentration of circulating cortisol secreted by the adrenal gland. The stress response is initiated by the release of corticotrophin releasing hormone (CRH) from the hypothalamus, which in turn stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which ultimately stimulates release of glucocorticoids, including cortisol, from the adrenal gland. Elevation in cortisol secretion occurs in response to novel, threatening, and unpredictable situations (Gunnar and Donzella, 2002; Hennessey and Levine, 1979).

Despite the observation that tics associated with TS may be exacerbated by stress (Lombroso et al., 1991; Peterson, 1996), there has been a dearth of research exploring the HPA axis in TS (Chappell et al., 1994; Sandyk, 1988; Sandyk and Bamford, 1988; Young et al., 1981), and no such work in children, who are typically most strongly affected by TS.

An early case study of a patient with TS with midbrain involvement showed a normal endocrine evaluation, which included plasma cortisol (Sandyk, 1988). A report of six TS patients evidenced a significant rise in plasma cortisol secretion in response to naloxone challenge (Sandyk and Bamford, 1988). The investigators hypothesized that noradrenergic locus coeruleus receptors involved in the release of corticotropic releasing factor are hypersensitive in response to chronic and excessive opiod-noradrenergic activity in TS (Sandyk and Bamford, 1988). However, given the lack of a direct comparison to control participants, this hypothesis awaits further investigation.

Another study (Chappell et al., 1994) examined the stressful effects of lumbar puncture on plasma ACTH and cortisol, urinary catecholamines, and self- and clinician ratings of anxiety in 13 medication-free TS patients and 10 normal controls, ages 17–41 years. The TS patients secreted significantly more ACTH than the control subjects following lumbar puncture, as manifested by greater mean and peak ACTH levels. Using a similar protocol, adult patients with TS showed elevated corticotrophin-releasing factor (CRF) in response to lumbar puncture when compared to individuals with OCD and normal control subjects (Chappell et al.,

1996). Taken together, these findings suggest that TS patients show heightened reactivity of the HPA axis.

In the current investigation, we sought to characterize the diurnal rhythmicity and reactivity of the HPA axis in children with TS. We examined: (1) the diurnal pattern of cortisol secretion in children with and without TS using in-home sampling of salivary cortisol, (2) between-subject variability in diurnal rhythms, (3) the response to stress following initial exposure to a mock MRI, and following response to a real functional MRI (fMRI) scan, and (4) the association between cortisol and clinical symptoms of tics and anxiety. We predicted that children with TS would exhibit elevated stress-related cortisol levels relative to age-matched control children.

2. Methods

2.1. Subjects

The subjects consisted of 36 unmedicated, English-speaking, predominantly male children between 7 and 13 years of age. Twenty of these children were diagnosed with TS (17 males, 3 females) and 16 were neurotypical (NT) (11 males, 5 females) children. For demographic information, see Table 1. Informed written consent was obtained from parents, and verbal assent was obtained from all research subjects prior to inclusion in the study. This study was approved by the Institutional Review Board of the University of California, Davis.

All subjects underwent a rigorous clinical assessment. Participants in the experimental group were recruited via clinical referrals, the Tourette Syndrome Association, local advertisements, physician referrals, and through the University of California at Davis M.I.N.D. Institute. The diagnosis of TS was based on DSM-IV criteria (APA, 1994) and established by: (1) a previous diagnosis of TS by a psychologist, psychiatrist or behavioral pediatrician, (2) clinical judgment by the first author (B.A.C.), and (3) confirmation of the presence of motor and phonic tics based on the Yale Global Tic Severity Scale (YGTSS). The parents of all participants also completed a semi-structured interview (see DISC below) to determine the extent of symptomology across other related disorders (i.e., OCD, ADHD, and anxiety).

Age-matched typically developing children were recruited through area schools, flyers, and recreational centers. Following initial contact, potential subjects were screened for neurodevelopmental disorders via parent interviews. Subjects with a history of serious physical illness (e.g., endocrine, cardiovascular, or neurological disorders) were not enrolled in the study.

2.2. Assessment

Testing was completed following informed consent procedures on the first of two visits.

2.2.1. Diagnostic and inclusion measures

Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) was used as an estimate of IQ. This measure was administered to all subjects unless an IQ score from a more comprehensive measure had been completed less than 2 years previously. Participants with a score of less than 75 on the WASI were excluded from the study.

Diagnostic Interview Schedule for Children (DISC; Shaffer et al., 2000) is a semi-structured, computer-assisted interview. For this study, 15 subsections of the DISC were administered and included: Tic Disorders [Tourette syndrome, Chronic Tic Disorder, Transient Tic Disorder], Obsessive–Compulsive Disorder, ADHD, Social Phobia, Separation Anxiety Disorder, Specific Phobia, Panic Disorder, Agoraphobia, Generalized Anxiety Disorder, Post-traumatic

Table 1 Demographic means, standard deviations, and differences across groups.

Clinical variable	NT mean (S.D.)	TS mean (S.D.)	*t*-Score	*p*-Value
Estimated IQ	115 (14.97)	107 (15.90)	1.66	0.11
Age	10.46 (1.96)	10.38 (1.80)	0.13	0.89
No. of motor tics	0.10 (0.31)	2.95 (1.18)	−11.02	0.001
No. of phonic tics	0.00 (0.00)	1.67 (1.02)	−7.33	0.001
Frequency of motor tics	0.15 (0.54)	3.69 (1.11)	−14.78	0.001
Frequency of phonic tics	0.00 (0.00)	2.72 (1.39)	−10.01	0.001
Intensity of motor tics	0.19 (0.69)	3.00 (0.89)	−12.97	0.001
Intensity of phonic tics	0.00 (0.00)	2.14 (1.09)	−9.96	0.001
Complexity of motor tics	0.00 (0.00)	2.17 (1.23)	−9.02	0.001
Complexity of phonic tics	0.00 (0.00)	1.38 (1.15)	−6.13	0.001
Interference of motor tics	0.00 (0.00)	2.00 (1.34)	−7.62	0.001
Interference of phonic tics	0.00 (0.00)	1.55 (1.40)	−5.63	0.001
Impairment	0.00 (0.00)	18.28 (9.67)	−9.64	0.001
YGTSS total score	0.42 (1.50)	23.76 (7.83)	−14.94	0.001
CY-BOCS	0.45 (1.28)	10.90 (8.35)	−5.53	0.001
SCQ	3.27 (2.43)	8.72 (5.86)	−3.36	0.001
Conners ADHD Index	46.13 (4.09)	62.83 (11.73)	−5.24	0.001

Note: CY-BOCS: Children's Yale-Brown Obsessive Compulsive Scale; SCQ: Social Communication Questionnaire; NT: neurotypical; TS: Tourette syndrome.

Stress Disorder, Trichotillomania, Major Depressive Episode, Dysthymic Disorder, Oppositional Defiant Disorder, and Conduct Disorder. The DISC was used to better characterize the diagnostic profile of the participants.

The *Yale Global Tic Severity Scale* (YGTSS; Leckman et al., 1989) is a clinical rating instrument that provides an assessment of the number, frequency, intensity, complexity, and interference of motor and phonic symptoms in individuals with TS and related tic disorders.

Child Yale-Brown Obsession and Compulsion Scale (CY-BOCS; Scahill et al., 1997) is a semi-structured interview administered jointly to the child and his/her parent. The scale is divided into separate subscales for obsessions and compulsions, each with a maximum score of 20. Considered the gold standard for assessment of OCD symptoms, the CY-BOCS was used to document the presence, rate and severity of these symptoms.

2.2.2. Questionnaires

The parents of the participants completed the following questionnaires.

Conners' Parent Rating Scale-Revised (Short) (Conners, 2001). The CPRS-R:S is a parental rating scale that provides a narrow range of information about behaviors associated with attention deficits and/or hyperactivity, as well as oppositional behavior. The Conners' is considered a standard and valid measure frequently used in the assessment of ADHD. The *t*-scores are generated with a mean of 50 and a standard deviation of 10. Scores ranging from 60 to 65 fall in the at-risk range and scores >70 are considered clinically significant.

The *Social Communication Questionnaire* (SCQ; Rutter et al., 2003) is used as a screening tool for autism spectrum disorders (ASD). Scores of 15 or higher are highly suggestive of ASD and scores of 22 and higher are suggestive of autism. Participants scoring $\geqslant 15$ were excluded from the study.

Multidimensional Anxiety Scale for Children (MASC; March, 1999) was used as a parental and self-report measure of anxiety. The results are presented as *t*-scores with a mean of 50 and a standard deviation of 10.

Parenting Stress Index (PSI; Abidin, 1995) is a 101-item questionnaire used to identify caregiver stress experienced in the child–parent relationship and family.

2.3. Cortisol sampling methods

We used the current preferred procedure in human stress research, collecting salivary samples for subsequent assay of cortisol concentrations. A total of 13 salivary samples were collected from each research participant: 9 home samples collected to measure the cortisol diurnal rhythm, and 4 laboratory samples collected to evalute the participant's response to stress.

Parents were trained on the collection procedures, and were provided with sample tubes and preprinted labels. Tubes were stored in a container fitted with a Trackcap™ (distributed by Aprex®, Union City, CA), described below. The participant was given Trident® Original Sugarless chewing gum, which acts as a saliva stimulant. After 30 s, the child was asked to spit the gum out into a tissue. The test tube was then removed from the Trackcap™ container. The child was given a large straw and asked to put it in his/her mouth while the lower end of the straw was placed into the collection tube. The participant was then asked to deposit saliva into the tube by spitting. This method of direct sampling into a collection container does not have any measurable effects on the results of the assays (Schwartz et al., 1998). Once completed, a pre-typed, peel-off label (coded with the date and time of collection) was placed on the tube. For home collection, the samples were placed in the home refrigerator for temporary storage.

A critical concern with respect to the home samples is the danger of parental noncompliance with collection of multiple salivary samples. We attempted to address this concern by monitoring compliance via careful instruction and parental diaries. As an additional means of measuring parental compliance to the outlined experimental procedures, the empty collection tubes were contained within a bottle fitted with a TrackCap™ (distributed by Aprex®, Union City, CA). A TrackCap is a bottle cap containing microelectronics that records the date and time for each occasion that the bottle is opened. This record was compared with the parent record of each sample collection time.

To evaluate the accuracy of recording and adherence to the sampling protocol, we computed Pearson correlations between the parent report (PR) sampling times and the Trackcap report (TR) times. For any disagreements greater than 30 min, the log and cortisol values were scrutinized for accuracy. A discrepancy was observed in fewer than 1% of the samples. Exclusion of these data points did not alter the results; thus, the samples remained in the analysis.

2.3.1. Diurnal rhythmicity

Basal levels of salivary cortisol were collected for three diurnal cycles. Research assistants instructed parents on the basic saliva sampling procedure described above for in-home collection. Parents were provided with a supply packet containing a TrackCap™-fitted container with collection tubes, Trident® Original Sugarless gum, large straws, storage bag, diary forms and a calendar with pre-numbered, peel-off labels that corresponded to the collection schedule. Research staff regularly called parents to ensure adherence and to answer questions regarding the sampling procedures. Within 48 h of the final saliva sample, the test tube kits were collected by the research assistant, placed in a cooler and brought to the Endocrine Laboratory.

Home samples were collected by parents three times per day for 3 consecutive days beginning with the morning sample on the first day, followed by mid-afternoon and evening samples. For each participant, the samples were collected at approximately the same time each day. Specifically, the morning sample was collected within a half-hour of waking prior to the participant eating, drinking or brushing his teeth. The afternoon sample was collected between 13:00 and 15:00 h, and children were to avoid eating for a minimum of one hour prior to sampling. The evening sample was collected within 30 min of bedtime, prior to brushing teeth and again avoiding eating for an hour before sample collection.

2.3.2. Environmental stress

This portion of the study was conducted at the UC Davis Imaging Research Center (IRC). To evaluate response to environmental challenges to an environmental stressor, we obtained salivary samples from the participants before and after exposure to the mock scanner (MRI simulator), and before and after a real MRI scan session. The exposure is conceptualized as a model of environmental stress insofar as it is a novel event that involves mild restraint and exposure to previously recorded unpleasant noises generated by the MRI scanner (Corbett et al., 2006; Corbett et al., 2008). It is important to note that the children exposed to this procedure were not provided with additional strategies (e.g., watching a videotape of an MRI) to help them habituate to the mock scanning procedures. All mock scanning sessions occured between 13:00 and 15:00 h. A total of four saliva samples were collected which included: Arrival (Arr): immediate collection upon arrival to the IRC, PostMock: 20 min post-exposure to the stressor, PreMRI: 40 min post-exposure and before MRI, and PostMRI: approximately 1 h after beginning the real MRI scans. The neuroimaging data have been published elsewhere (Baym et al., 2008). Saliva samples from three typically developing children were dropped from the analysis, because scanning took place too early in the day to allow for comparison of cortisol levels to the TS group.

2.3.3. Mock scanning procedure

During the mock scan sessions, participants were asked to lie on the scanning bed and, once situated, the research assistant (RA) moved them into the bore of the mock scanner. They were instructed to remain still and to not move their head or extremities during the simulator session. Throughout the simulation, participants were asked whether they were comfortable. A 2-min mock scan session was initiated to familiarize the children with the sights and sounds of the procedure including three separate previously recorded scanner noises that they would later experience in the real scanner. The RA then sat behind the scanner and monitored head and extremity movement. Approximately 15–20 min after initial exposure to the mock scanner environment, participants were removed from the scanner and provided a salivary sample. A Postmock/PreMRI sample was taken approximately 40-min after exposure. A final sample was collected after the functional MRI scanning (Baym et al., 2008).

2.3.4. Cortisol storage and assays

Cortisol assays were conducted in the Endocrine Laboratory of the University of California, Davis California National Primate Research Center under the direction of Dr. Sally Mendoza. Once all of the samples were collected, they were logged and stored in a −20 °C freezer until assayed. Immediately prior to assay, samples were centrifuged at 3000 rpm for 20 min to separate the aqueous component from mucins and other suspended particles. Salivary concentrations of cortisol were estimated in duplicate using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA).

Assay procedures were modified to accommodate overall lower levels of cortisol in human saliva relative to plasma as follows: (1) standards were diluted to concentrations ranging from 2.76 to 345 nmol/L; (2) sample volume was increased to 200 μL, and (3) incubation times were extended to 3 h. Serial dilution of samples indicates that the modified assay displays a linearity of 0.98 and a least detectable dose of 1.3854 nmol/L. Intra- and inter-assay coefficients of variation are 2.53 and 4.05, respectively.

2.4. Statistical analysis

Statistical analysis was performed with the following objectives: to characterize diurnal patterns of cortisol and response to stress, to compare these patterns between diagnostic groups (TS/NT), and to examine the association between cortisol and diagnostic variables. To assess diurnal cortisol and pre- and post-scan cortisol concentrations, two-way repeated measures analysis of variance ANOVA were conducted. Within group paired *t*-tests were conducted between afternoon values and arrival to the imaging center. Finally, relationships between cortisol and clinical variables were examined with Pearson product moment correlations.

3. Results

Independent samples *t*-tests revealed no statistically significant group differences for age ($t = -0.04$, $p > 0.05$) or estimated IQ ($t = 1.79$, $p > 0.05$). As expected, there were significant differences for many clinical variables including tic severity, obsessive–compulsive symptoms, and ADHD symptoms (all $p < 0.05$; see Table 1). In addition, there were significant group differences on the MASC anxiety scale (all $p < 0.05$; see Table 2).

Cortisol measurements are displayed by diagnostic group and time point in Figure 1 (home samples) and Figure 2 (imaging center samples) and Table 3. The results of the repeated measures ANOVAs and subsequent correlations are presented below.

There were no observable differences in the natural diurnal pattern, as both groups showed expected diurnal rhythms with higher cortisol concentration in morning followed by a sharp decline and lower evening cortisol (see Figure 1). Further, based on repeated measures ANOVA, there was no effect of Group for the morning or afternoon (all $F < 1$). However, there was a trend towards an effect of Group for the evening ($F(1,32) = 3.18$, $p = 0.08$), showing that the TS group tended to have lower cortisol values in the evening than the NT group.

In regards to the response to stress, a multivariate repeated measures ANOVA revealed a main effect for Stress (Arrival, PostMock, PreMRI, PostMRI) $F(1,27) = 3.50$, $p = 0.033$, and a main effect for Group $F(1,27), = 6.83$, $p = 0.014$, but no Stress × Group interaction ($F < 1$). The between-group ANOVAs were significant for Arrival $F(1,28) = 4.26$, $p = 0.048$, PostMock $F(1,28) = 6.42$, $p = 0.02$, and PreMRI $(1,28) = 5.22$, $p = 0.030$, and there was a trend for PostMRI $F(1,27) = 3.54$, $p = 0.07$ (see Table 4). Thus, the children with TS exhibited significantly higher cortisol levels in response to the MRI environment compared to the NT children.

In order to more thoroughly evaluate differences in the TS group between regulation vs. reactivity, analyses were

Table 2 MASC scales means, standard deviations, and differences across groups.

MASC	NT mean (S.D.)	TS mean (S.D.)	*t*-Score	*p*-Value
Tension/restlessness	41.33 (4.69)	51.00 (8.25)	−5.353	0.0001
Somatic/autonomic	39.47 (6.29)	47.00 (9.41)	−3.371	0.002
Physical symptoms	39.20 (5.36)	49.00 (8.62)	−4.965	0.0001
Perfectionism	46.60 (10.89)	51.59 (7.62)	−2.080	0.044
Anxiety coping	46.47 (13.51)	54.47 (6.58)	−2.471	0.018
Harm/avoidance	45.33 (13.14)	53.65 (7.05)	−2.673	0.011
Humility/rejection	46.67 (9.50)	55.12 (11.19)	−2.071	0.045
Perfection/fear	44.80 (8.34)	54.41 (10.47)	−2.161	0.037
Social anxiety	45.07 (9.02)	55.35 (10.78)	−2.418	0.020
Separation/panic	47.07 (9.17)	61.82 (12.41)	−4.005	0.0001
Total	41.33 (8.01)	55.88 (9.31)	−5.044	0.0001
Anxiety Disorder Index	46.93 (10.22)	56.94 (10.81)	−2.823	0.007

Note: Multidimensional Anxiety Scale for Children (MASC). NT: Neurotypical; TS: Tourette syndrome; S.D.: standard deviation.

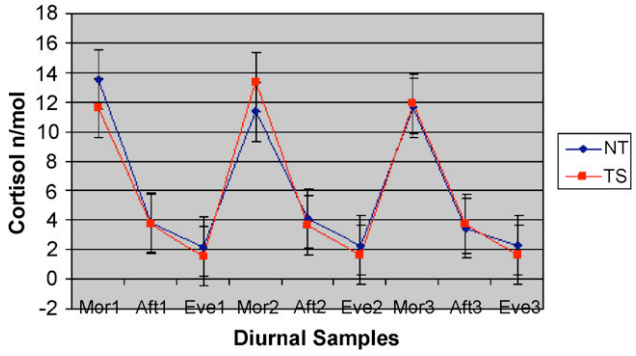


Figure 1 Cortisol diurnal values across the groups. Log-transformed salivary cortisol presented in n/mol per liter for each group sampled three times per day for 3 consecutive days over 2 consecutive weeks. NT: neurotypical; TS: Tourette syndrome; Mor: morning; Aft: afternoon; Eve: evening; 1: 1st day; 2: 2nd day; 3: 3rd day.

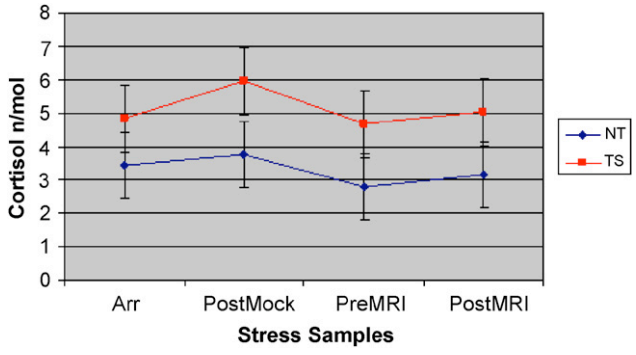


Figure 2 Cortisol stress paradigm samples. Log-transformed salivary cortisol samples obtained upon arrival to Imaging Research Center (Arr), 20 min post-exposure to mock MRI (PostMock), before the real MRI (PreMRI) and following a real MRI (PostMRI).

conducted between average afternoon cortisol compared to the stress protocol and arrival to the imaging center using within group paired sample *t*-tests. The results showed a

Table 3 Diurnal cortisol values by group and time.

Day sample time	Time	NT mean	NT S.D.	TS mean	TS S.D.
Mor1	Morning	13.97	6.36	12.75	4.71
Aft1	Afternoon	4.08	1.76	3.54	1.09
Eve1	Evening	2.18	1.75	1.56	0.46
Mor2	Morning	11.64	5.51	13.37	5.72
Aft2	Afternoon	4.08	1.51	3.98	2.90
Eve2	Evening	1.77	0.87	1.58	0.25
Mor3	Morning	11.68	5.26	11.78	6.74
Aft3	Afternoon	3.31	1.20	3.50	1.26
Eve3	Evening	1.64	0.38	1.60	0.27

Note: NT: neurotypical; TS: Tourette syndrome; Mor: morning; Aft: afternoon; Eve: evening; 1: 1st day; 2: 2nd day; 3: 3rd day; S.D.: standard deviation.

Table 4 Cortisol values before and after scan across the groups.

Sample	NT mean	NT S.D.	TS mean	TS S.D.
Arrival	3.18	1.35	5.29	4.70
PostMock	2.97	1.04	6.11	6.37
PreMRI	2.54	0.94	4.70	4.39
PostMRI	2.69	1.66	4.68	4.97

Note: NT: neurotypical; TS: Tourette syndrome. Cortisol salivary samples obtained upon arrival to Imaging Research Center, 20 min post-exposure to mock MRI (PostMock), before real MRI (PreMRI) and following a real MRI (PostMRI). S.D.: standard deviation.

significant difference between average afternoon cortisol and the stress protocol $t(20) = 5.59$, $p < 0.001$. The afternoon average compared to the arrival approached significance $t(20) = -1.36$, $p = 0.09$ suggesting that the anticipatory stress may have been a contributory factor, but not

sufficient to explain the pronounced reactivity in the TS group to the stress protocol. Further, cortisol slopes across the groups parallel one another, including a drop following the mock MRI exposure, indicative of good HPA regulation. Thus, the elevated cortisol response to the stressor is suggestive of enhanced reactivity rather than reduced regulation.

To evaluate potential relationships between cortisol levels and clinical variables for the TS children, within-group Pearson Product correlations were conducted. The first morning cortisol sample was highly correlated with the number of motor tics $r = 0.643$, $p = 0.005$. Evening cortisol variables were negatively correlated with the number, intensity or interference of motor tics, global impairment, and overall tic severity (see Table 5).

The Arrival cortisol value was significantly negatively correlated with the number and intensity of motor tics, number of phonic tics and overall tic severity (see Table 5). The PostMRI cortisol values were not correlated with tic severity. In other words, greater tic severity within the TS group was related to lower cortisol levels upon arrival to the MRI session but not to cortisol values after the mock or real MRI. Further, the diurnal and stress cortisol values were not correlated with the OCD or ADHD symptom variables ($p > 0.05$). Thus, the observed correlations appear specific to level of TS symptoms and anxiety.

Since children with TS reported greater levels of anxiety across all MASC subscales (all $p < 0.05$; see Table 2), correlations were conducted in an exploratory fashion to examine the relationship between anxiety and cortisol in the TS group. Several of the MASC anxiety domains were significantly negatively correlated with Morning, Afternoon and Evening cortisol (see Table 6), but Pre- and PostMRI cortisol were not correlated with general levels of anxiety as measured by the MASC (all $p > 0.05$). Thus, contrary to expectations in the TS group, lower diurnal cortisol levels were associated with greater symptoms of anxiety.

There was a significant difference between the groups regarding overall reported parenting stress $F(1,36) = 8.40$, $p < 0.008$, with the parents of children with TS reporting higher levels of stress. Even so, parental stress was not associated with their childrens' cortisol levels except immediately following exposure to the mock scanner $r = 0.38$, $p = 0.023$.

4. Discussion

The primary aims of the current investigation were to examine the diurnal pattern and responsivity of cortisol

Table 6 MASC scales correlated with cortisol variables in TS group.

MASC	Cortisol sample	*r*-Score	*p*-Value
Perfectionism	Mor2	−0.48	0.042
Anxiety coping	Eve1	−0.60	0.008
	Aft3	−0.48	0.046
Harm/avoidance	Eve1	−0.46	0.053*
	Aft3	−0.49	0.038
Humility/rejection	Aft1	−0.53	0.034
	Mor2	−0.48	0.042
Perfection/fear	Aft2	−0.62	0.006
Social anxiety	Aft1	−0.54	0.029
	Aft3	−0.55	0.017
Separation/panic	Eve2	−0.47	0.055*
Total	Eve1	−0.45	0.060*
Anxiety Disorder Index	Mor3	−0.50	0.030
	Aft3	−0.53	0.023

Note: NT: neurotypical; TS: Tourette syndrome; Mor: morning; Aft: afternoon; Eve: evening; 1: 1st day; 2: 2nd day; 3: 3rd day; S.D.: standard deviation; MASC: Multidimensional Anxiety Scale for Children; ADI: Anxiety Disorder Index. Data uncorrected for multiple comparisons. Only the Perfection/fear and Anxiety Coping survive correction. Neurotypical children showed a positive correlation with the Aft3 sample for Humility/rejection $r = 0.68$, $p < 0.016$ and a negative correlation with the Aft2 sample and tension and restlessness $r = -0.61$, $p < 0.026$.

*Trend.

Table 5 Cortisol correlations with tic severity for TS group.

YBTSS scales	Mor1		Eve1		Eve2		Arr	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
No. of motor tics	0.643	0.005	−0.553	0.014	−0.559	0.016		
No. of phonic tics							−0.493	0.032
Intensity of motor tics			−0.649	0.003	−0.569	0.014	−0.530	0.020
Intensity of phonic tics					−0.529	0.024		
Complexity of motor tics					−0.513	0.029		
Interference motor tics					−0.601	0.008		
Global impairment			−0.483	0.036				
YGTSS total			−0.564	0.012	−0.690	0.004	−0.496	0.031

Note: Mor: morning; Eve: evening; 1: 1st day; 2: 2nd day; 3: 3rd day; Arr: arrival; S.D.: standard deviation; YGTSS: Yale Global Tic Severity Scale.

secretion in children with TS compared to neurotypical (NT) peers, and to explore the association between cortisol levels and clinical symptoms of tics and anxiety in TS.

Regarding the clinical characterization of the sample, the children with TS showed increased levels of anxiety across all domains on the MASC compared to the typically developing children. In addition, they showed higher rates of OCD and ADHD symptoms. Although there was a statistical difference between the groups on the SCQ, a screener for autism, none of the children came close to the clincal range for an autism spectrum disorder. There were no statistically significant differences between groups in regards to age, IQ, or gender.

Initially, we simply plotted the patterns of cortisol indicating that both groups showed expected diurnal rhythm with higher cortisol concentration in the morning, a sharp decline throughout the day, and lowest cortisol levels in the evening. Statistically, there were no significant differences in the diurnal rhythm for morning or afternoon between or within the TS and NT groups. The evening values for the groups revealed a trend level for group in that individuals with TS tended to have lower evening cortisol. A lower evening cortisol profile has been reported in conditions of chronic stress (Nickel et al., 2007; Nicolson and van Diest, 2000; Ockenfels et al., 1995), including chronic fatigue syndrome (Strickland et al., 1998), and some cases of severe depression (Secunda et al., 1986). The reduced evening cortisol levels in the children with TS may thus be the result of chronic daily stress. Lower cortisol, which includes evening values, has been observed in posttraumatic stress disorder (PTSD) (Yehuda et al., 1993a, b, 1994, 1995a, b).

Previously, using a comparable sample collection methodology, we reported that children with autism exhibited higher evening cortisol and greater variability compared to age-matched typically developing peers (Corbett et al., 2006). These observed differences across the clinical populations speak to the importance of exploring diurnal rhythms in neurodevelopmental disorders.

Regarding the response to our stress manipulation, children with TS showed significantly higher cortisol levels than typically developing peers. These findings seem to reflect heightened responsivity of the HPA axis in response to stress in the TS group a finding consistent with previous research and clinical observation. For example, in response to lumbar puncture, adult patients with TS showed elevated ACTH compared to control subjects (Chappell et al., 1994) and higher levels of CRF than individuals with OCD and control participants (Chappell et al., 1996). The enhanced cortisol level in children with TS was observed especially in response to the stressor, but also in anticipation of the MRI environment. The pattern remained when comparing the cortisol stress values to diurnal afternoon cortisol values taken from home. While these findings could reflect heightened reactivity of the HPA axis in children with TS, alternatively TS may lead to disturbances in the HPA axis. Although these results do not specify a causal relationship between HPA axis reactivity and the symptomatology of TS, they do indicate that stress-related neurobiological mechanisms are involved in the neuropathology of TS. Furthermore, the data support a model of enhanced HPA reactivity rather than reduced regulation.

To explore the relationships between cortisol levels and clinical variables, comparisons were made between cortisol, anxiety, and tics. There was a single positive correlation between the first morning cortisol and number of motor tics, which may reflect a direct stress response to the sampling regimen. Significant negative correlations were found between cortisol and several MASC anxiety scales. In addition, evening cortisol and arrival to the MRI environment were highly negatively associated with greater tic severity. These inverse relationships may initially seem counterintuitive since higher cortisol levels might be expected to correlate with greater tic severity and anxiety. However, this association may be the result of the persistent, chronic nature of the disorder such as in PTSD. In other words, the chronicity of stress, concomitant anxiety, and persistent tendency to suppress tics on a daily basis may take a biological toll, resulting in reduced levels of cortisol by the end of the day. As noted, lower evening cortisol has been reported in conditions of chronic stress (Nickel et al., 2007; Nicolson and van Diest, 2000; Ockenfels et al., 1995; Secunda et al., 1986), and reduced circadian rhythms have been rather consistently reported in PTSD (Yehuda et al., 1993a, b, 1994, 1995a, b). It may be the case that an enhanced negative feedback mechanism is part of the pathophysiology in some individuals with TS, as has been proposed for PTSD (Yehuda et al., 1995a). To test this hypothesis, similar investigations in large samples utilizing dexamethasone or more thoroughly examining cortisol rhythms will be necessary to see if this is a robust, reproducible finding.

Alternatively, it is plausible that an increase in the expression of tics results in a decrease in cortisol secretion and experienced anxiety. In other words, the performance of tics may have anxiolytic properties leading to stress reduction and subsequently lower cortisol levels. There is some evidence in the TS literature to support this notion. There are numerous reports documenting sensory phenomena referred to as premonitory urges that precipitate the performance of a tic, which are alleviated to some degree following the behavior (Banaschewski et al., 2003; Cohen and Leckman, 1992; Jankovic, 1997; Kwak et al., 2003; Leckman and Peterson, 1993; Woods et al., 2005). Individuals with TS are often driven to decrease the discomfort of these urges through the expression of a specific tic or compulsion. Evers and van de Wetering (Evers and van de Wetering, 1994) proposed a model in which tics are characterized as tension-reducing responses to a specific sensory stimulus. In this intervention paradigm, tics are not suppressed, but instead are redirected to more socially acceptable alternative responses (Evers and van de Wetering, 1994).

Additionally, in the TS group there was a negative correlation between Arrival (higher cortisol levels) and the number and intensity of motor tics (fewer tics). Again, this finding is similar to the notion above that tic expression can result in stress reduction subsequently leading to less cortisol secretion. In other words, individuals with lower levels of tic severity (number and intensity) may show increased cortisol responsiveness under certain conditions of acute stress. Despite these provocative findings, the aforementioned associations are based on correlational analysis, and as such, no causal relationship can be made.

There are additional limitations to highlight regarding the current investigation. Although the patient population was well-characterized, medication-free, endorsed distress from symptoms, and included a fairly restricted age range, the fact that the children were unmedicated meant that their tic symptoms tended to be mild-to-moderate compared to some individuals with the disorder. As a result, the findings may not generalize to more severe cases of TS, to those who do not experience distress from symptoms, or to individuals with TS who are prescribed medication. We also acknowledge that our sample size was relatively small and large samples are clearly warranted. Further, the focus of the study and subsequent interpretation of the data arises from a Tourette syndrome lens which may not account for the influence of comorbid conditions on the HPA axis. It is apparent that other contributory factors, such as underlying symptoms of OCD, anxiety or ADHD may be influential. Thus, an enhanced study would include well-defined comparison groups of these disorders without tics to better characterize the independent influence of TS.

In spite of the aforementioned limitations, the findings may have important therapeutic implications. First, the enhanced reactivity of the HPA axis provides clues for neuropsychopharmacotherapy. For example, it has been shown that mecamylamine blocks the neuroendocrine effects of acetylcholine, thereby stimulating the HPA axis and ostensibly targeting the underlying stress and anxiety associated with TS (Newman et al., 2001; Sanberg et al., 1998; Silver et al., 2000). In addition, the observed relationship between tic performance and reduced cortisol in patients suggests that behavioral therapy which aims to redirect rather than suppress motor and vocal tics may be preferred (Evers and van de Wetering, 1994). Finally, it will be important to evaluate the diurnal rhythm and stress responsivity of cortisol in a cohort of children with more debilitating symptoms to see if our findings generalize to more severe cases of TS. Furthermore, the examination of the HPA axis in patients with TS prior to and following medication trials may be helpful in order to ascertain biological efficacy.

Role of the funding sources

Funding was provided by a NIH Career Development Award to Blythe Corbett (5K08NMHO72958) and a Merck Scholarship in the Biology of Developmental Disorders to Silvia Bunge. The NIH and the Merck Foundation had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the manuscript, or in the decision to submit the paper for publication.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgments

The authors wish to thank the children and families for participating in this study, the Tourette Syndrome Association for assistance with patient recruitment, the staff of the Endocrine Core of the California National Primate Research Center, Rashel Decant, and Lisa Laughlin, for running the cortisol assays, and Debra Galik, Vanessa Carmean and Samantha Wright for assisting with participant enrollment, parent training, and data collection and entry, respectively. We are grateful to Frank Sharp for his ongoing collaboration on a larger project of Tourette syndrome. Most of all we thank Seymour ''Gig'' Levine, Ph.D. for his extraordinary mentorship and years of dedication to the field of neuroendocrinology.

References

Abidin, R.R., 1995. The Parenting Stress Index. Psychological Assessment Resources, Inc., Lutz, FL.

APA, 1994. Diagnostic and Statistical Manual of Mental Disorders, fourth ed. American Psychiatric Association, Washington, DC.

Banaschewski, T., Woerner, W., Rothenberger, A., 2003. Premonitory sensory phenomena and suppressibility of tics in Tourette syndrome: developmental aspects in children and adolescents. Dev. Med. Child. Neurol. 45 (10), 700–703.

Bartels, M., de Geus, E.J., Kirschbaum, C., Sluyter, F., Boomsma, D.I., 2003. Heritability of daytime cortisol levels in children. Behav. Genet. 33 (4), 421–433.

Baym, C.L., Corbett, B.A., Wright, S.B., Bunge, S.A., 2008. Neural correlates of tic severity and cognitive control in children with Tourette syndrome. Brain 131 (Pt 1), 165–179.

Bliss, J., 1980. Sensory experiences of Gilles de la Tourette syndrome. Arch. Gen. Psychiatry 37 (12), 1343–1347.

Bruun, R.D., Budman, C.L., 1992. The natural history of Tourette syndrome. Adv. Neurol. 58, 1–6.

Chappell, P., Riddle, M., Anderson, G., Scahill, L., Hardin, M., Walker, D., et al., 1994. Enhanced stress responsivity of Tourette syndrome patients undergoing lumbar puncture. Biol. Psychiatry 36 (1), 35–43.

Chappell, P., Leckman, J., Goodman, W., Bissette, G., Pauls, D., Anderson, G., et al., 1996. Elevated cerebrospinal fluid corticotropin-releasing factor in Tourette's syndrome: comparison to obsessive compulsive disorder and normal controls. Biol. Psychiatry 39 (9), 776–783.

Coffey, B., Frazier, J., Chen, S., 1992. Comorbidity, Tourette syndrome, and anxiety disorders. Adv. Neurol. 58, 95–104.

Coffey, B.J., Miguel, E.C., Savage, C.R., Rauch, S.L., 1994. Tourette's disorder and related problems: a review and update. Harv. Rev. Psychiatry 2 (3), 121–132.

Cohen, A.J., Leckman, J.F., 1992. Sensory phenomena associated with Gilles de la Tourette's syndrome. J. Clin. Psychiatry 53 (9), 319–323.

Conners, K.C., 2001. Conners' Rating Scales Revised Manual. MHS, Tonawanda, New York.

Corbett, B.A., Mendoza, S., Abdullah, M., Wegelin, J.A., Levine, S., 2006. Cortisol circadian rhythms and response to stress in children with autism. Psychoneuroendocrinology 31 (1), 59–68.

Corbett, B.A., Mendoza, S., Wegelin, J.A., Carmean, V., Levine, S., 2008. Variable cortisol circadian rhythms in children with autism and anticipatory stress. J. Psychiatr. Neurosci. 33 (3), 227–234.

Evers, R.A., van de Wetering, B.J., 1994. A treatment model for motor tics based on a specific tension-reduction technique. J. Behav. Ther. Exp. Psychiatry 25 (3), 255–260.

Gunnar, M.R., Donzella, B., 2002. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology 27 (1/2), 199–220.

Harris, K., Singer, H.S., 2006. Tic disorders: neural circuits, neurochemistry, and neuroimmunology. J. Child. Neurol. 21 (8), 678–689.

Hennessey, J.W., Levine, S., 1979. Stress, Arousal, and the Pituitary–Adrenal System: A Psychoendocrine Hypothesis, vol. 8.
Himle, M.B., Woods, D.W., 2005. An experimental evaluation of tic suppression and the tic rebound effect. Behav. Res. Ther. 43 (11), 1443–1451.
Jankovic, J., 1997. Tourette syndrome. Phenomenology and classification of tics. Neurol. Clin. 15 (2), 267–275.
Kurlan, R., 1998. Tourette's syndrome and 'PANDAS': will the relation bear out? Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. Neurology 50 (6), 1530–1534.
Kwak, C., Dat Vuong, K., Jankovic, J., 2003. Premonitory sensory phenomenon in Tourette's syndrome. Mov. Disord. 18 (12), 1530–1533.
Leckman, J.F., Peterson, B.S., 1993. The pathogenesis of Tourette's syndrome: epigenetic factors active in early CNS development. Biol. Psychiatry 34 (7), 425–427.
Leckman, J.F., Cohen, D.J., Price, R.A., 1984. The pathogenesis of Gilles de la Tourette's syndrome: a review of data and hypothesis. In: Shah, A.B., Shah, N.S., Donald, A.G. (Eds.), Movement Disorders. Plenum Press, New York, pp. 257–272.
Leckman, J.F., Riddle, M.A., Hardin, M.T., Ort, S.I., Swartz, K.L., Stevenson, J., et al., 1989. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. J. Am. Acad. Child Adolesc. Psychiatr. 28 (4), 566–573.
Loiselle, C.R., Lee, O., Moran, T.H., Singer, H.S., 2004. Striatal microinfusion of Tourette syndrome and PANDAS sera: failure to induce behavioral changes. Mov. Disord. 19 (4), 390–396.
Lombroso, P.J., Mack, G., Scahill, L., King, R.A., Leckman, J.F., 1991. Exacerbation of Gilles de la Tourette's syndrome associated with thermal stress: a family study. Neurology 41 (12), 1984–1987.
March, J., 1999. Multidimensional Anxiety Scale for Children. Multi-health Systems, Inc., North Tonawanda, NY.
McElroy, S.L., Phillips, K.A., Keck Jr., P.E., 1994. Obsessive compulsive spectrum disorder. J. Clin. Psychiatry 55 (Suppl.), 33–51 (discussion 52–33).
Meidinger, A.L., Miltenberger, R.G., Himle, M., Omvig, M., Trainor, C., Crosby, R., 2005. An investigation of tic suppression and the rebound effect in Tourette's disorder. Behav. Modif. 29 (5), 716–745.
Mell, L.K., Davis, R.L., Owens, D., 2005. Association between streptococcal infection and obsessive–compulsive disorder, Tourette's syndrome, and tic disorder. Pediatrics 116 (1), 56–60.
Newman, M.B., Nazian, S.J., Sanberg, P.R., Diamond, D.M., Shytle, R.D., 2001. Corticosterone-attenuating and anxiolytic properties of mecamylamine in the rat. Prog. Neuropsychopharmacol. Biol. Psychiatry 25 (3), 609–620.
Nickel, C., Tanca, S., Kolowos, S., Pedrosa-Gil, F., Bachler, E., Loew, T.H., et al., 2007. Men with chronic occupational stress benefit from behavioural/psycho-educational group training: a randomized, prospective, controlled trial. Psychol. Med. 37 (8), 1141–1149.
Nicolson, N.A., van Diest, R., 2000. Salivary cortisol patterns in vital exhaustion. J. Psychosom. Res. 49 (5), 335–342.
Ockenfels, M.C., Porter, L., Smyth, J., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1995. Effect of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. Psychosom. Med. 57 (5), 460–467.
Peterson, B.S., 1996. Considerations of natural history and pathophysiology in the psychopharmacology of Tourette's syndrome. J. Clin. Psychiatry 57 (Suppl. 9), 24–34.
Price, D.A., Close, G.C., Fielding, B.A., 1983. Age of appearance of circadian rhythm in salivary cortisol values in infancy. Arch. Dis. Child 58 (6), 454–456.
Rutter, M., Bailey, A., Lord, C., 2003. The Social Communication Questionnaire. Western Psychological Services, Los Angeles, CA.
Sanberg, P.R., Shytle, R.D., Silver, A.A., 1998. Treatment of Tourette's syndrome with mecamylamine. Lancet 352 (9129), 705–706.
Sandyk, R., 1988. A case of Tourette's syndrome with midbrain involvement. Int. J. Neurosci. 43 (3/4), 171–175.
Sandyk, R., Bamford, C.R., 1988. Heightened cortisol response to administration of naloxone in Tourette's syndrome. Int. J. Neurosci. 39 (3/4), 225–227.
Scahill, L., Riddle, M.A., McSwiggin-Hardin, M., Ort, S.I., King, R.A., Goodman, W.K., et al., 1997. Children's Yale–Brown Obsessive Compulsive Scale: reliability and validity. J. Am. Acad. Child Adolesc. Psychiatr. 36, 844–852.
Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R., Laird, B., 1998. Assessing salivary cortisol in studies of child development. Child Dev. 69 (6), 1503–1513.
Secunda, S.K., Cross, C.K., Koslow, S., Katz, M.M., Kocsis, J., Maas, J.W., et al., 1986. Biochemistry and suicidal behavior in depressed patients. Biol. Psychiatry. 21 (8/9), 756–767.
Shaffer, D., Fisher, P., Lucas, C.P., Dulcan, M.K., Schwab-Stone, M.E., 2000. NIMH Diagnostic IntervieSchedule for Children Version IV (NIMH DISC-IV): description, differences from previous versions, and reliability of some common diagnoses. J. Am. Child Adolesc. Psychiatr. 39, 28–38.
Shapiro, A.K., Shapiro, E.S., Young, J.G., Feinberg, T.E., 1988. Gilles de la Tourett Syndrome. Raven Press, New York, pp. 127–193.
Silver, A.A., Shytle, R.D., Sanberg, P.R., 2000. Mecamylamine in Tourette's syndrome: a two-year retrospective case study. J. Child Adolesc. Psychopharmacol. 10 (2), 59–68.
Singer, H.S., Hong, J.J., Yoon, D.Y., Williams, P.N., 2005. Serum autoantibodies do not differentiate PANDAS and Tourette syndrome from controls. Neurology 65 (11), 1701–1707.
Steingard, R., Dillon-Stout, D., 1992. Tourette's syndrome and obsessive compulsive disorder. Clinical aspects. Psychiatr. Clin. North Am. 15 (4), 849–860.
Strickland, P., Morriss, R., Wearden, A., Deakin, B., 1998. A comparison of salivary cortisol in chronic fatigue syndrome, community depression and healthy controls. J. Affect. Disord. 47 (1–3), 191–194.
Surwillo, W.W., Shafii, M., Barrett, C.L., 1978. Gilles de la Tourette syndrome: a 20-month study of the effects of stressful life events and haloperidol on symptom frequency. J. Nerv. Ment. Dis. 166 (11), 812–816.
Swedo, S.E., Leonard, H.L., 1994. Childhood movement disorders and obsessive compulsive disorder. J. Clin. Psychiatry 55 (Suppl.), 32–37.
Swedo, S.E., Leonard, H.L., Garvey, M., Mittleman, B., Allen, A.J., Perlmutter, S., et al., 1998. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. Am. J. Psychiatry 155 (2), 264–271.
Trifiletti, R.R., Packard, A.M., 1999. Immune mechanisms in pediatric neuropsychiatric disorders. Tourette's syndrome, OCD, and PANDAS. Child Adolesc. Psychiatr. Clin. N Am. 8 (4), 767–775.
Verdellen, C.W., Keijsers, G.P., Cath, D.C., Hoogduin, C.A., 2004. Exposure with response prevention versus habit reversal in Tourettes's syndrome: a controlled study. Behav. Res. Ther. 42 (5), 501–511.
Vermes, I., Dohanics, J., Toth, G., Pongracz, J., 1980. Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. Horm. Res. 12 (5), 237–244.
Wechsler, D., 1999. Wechsler Abbreviated Scale of Intelligence. Psychological Corporation, San Antonio, TX.
Woods, D.W., Piacentini, J., Himle, M.B., Chang, S., 2005. Premonitory Urge for Tics Scale (PUTS): initial psychometric results and examination of the premonitory urge phenomenon in youths with Tic disorders. J. Dev. Behav. Pediatr. 26 (6), 397–403.

Woods, D.W., Himle, M.B., Miltenberger, R.G., Carr, J.E., Osmon, D.C., Karsten, A.M., et al., 2007. Durability, negative impact, and neuropsychological predictors of tic suppression in children with chronic tic disorder. J. Abnorm. Child. Psychol.

Yehuda, R., Resnick, H., Kahana, B., Giller, E.L., 1993a. Long-lasting hormonal alterations to extreme stress in humans: normative or maladaptive? Psychosom Med 55 (3), 287–297.

Yehuda, R., Southwick, S.M., Krystal, J.H., Bremner, D., Charney, D.S., Mason, J.W., 1993b. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am. J. Psychiatry 150 (1), 83–86.

Yehuda, R., Teicher, M.H., Levengood, R.A., Trestman, R.L., Siever, L.J., 1994. Circadian regulation of basal cortisol levels in posttraumatic stress disorder. Ann. NY Acad. Sci. 746, 378–380.

Yehuda, R., Boisoneau, D., Lowy, M.T., Giller Jr., E.L., 1995a. Dose–response changes in plasma cortisol and lymphocyte glucocorticoid receptors following dexamethasone administration in combat veterans with and without posttraumatic stress disorder. Arch. Gen. Psychiatry 52 (7), 583–593.

Yehuda, R., Kahana, B., Binder-Brynes, K., Southwick, S.M., Mason, J.W., Giller, E.L., 1995b. Low urinary cortisol excretion in Holocaust survivors with posttraumatic stress disorder. Am. J. Psychiatry 152 (7), 982–986.

Yehuda, R., Teicher, M.H., Trestman, R.L., Levengood, R.A., Siever, L.J., 1996. Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. Biol. Psychiatry 40 (2), 79–88.

Young, J.G., Cohen, D.J., Hattox, S.E., Kavanagh, M.E., Anderson, G.M., Shaywitz, B.A., et al., 1981. Plasma free MHPG and neuroendocrine responses to challenge doses of clonidine in Tourette's syndrome: preliminary report. Life Sci 29 (14), 1467–1475.