Published in final edited form as:

Psychoneuroendocrinology. 2008 November ; 33(10): 1344–1356. doi:10.1016/j.psyneuen.2008.07.011.

Neuroticism and Introversion are Associated with Salivary Cortisol Patterns in Adolescents

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Abstract

Previous studies have yielded equivocal findings on the relationship between personality and cortisol activity. The present study examined associations between personality and cortisol activity in a large, diverse adolescent sample, while partialling the effects of relevant demographic and health-related covariates. A subsample of 230 participants (57% of whom reported elevated neuroticism) was selected from a larger sample of 16- to 18-year-olds involved in a study on risk factors for emotional disorders. Subsample participants completed a battery of personality questionnaires, and saliva collection was requested several months later on three consecutive days at six time points per day, from wakeup to bedtime. Associations between personality and cortisol rhythms were examined using multilevel growth curve modeling. Neuroticism (N) and introversion (I) were significantly and differentially associated with features of diurnal cortisol patterns. Specifically, a significant N by gender interaction was observed, demonstrating flatter cortisol rhythms across the waking day among male participants with higher N. Elevated I, however, was associated with lower cortisol awakening responses for both male and female participants, and higher cortisol at the time of waking for male participants only. The present study supports personality as a significant predictor of diurnal cortisol patterns in late adolescence, after accounting for the effects of demographic and health covariates, and suggests that gender plays a role in moderating associations between personality and cortisol.

Keywords

cortisol diurnal rhythms; HPA activity; personality; neuroticism; introversion; gender; adolescence

In the search for risk factors of mood and anxiety disorders, there has been a burgeoning interest in the role of neuroticism (N), a relatively enduring personality trait associated with a proneness to experience negative emotions and thoughts (e.g., Costa & McCrae, 1980; H. J. Eysenck, 1967)—including anxiety, hostility, depression, self-consciousness, impulsiveness, and

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vulnerability (Costa & McCrae, 1995). N has been shown prospectively to predict the development of emotional disorders, including major depression, posttraumatic stress disorder (PTSD), phobias, and panic attacks (e.g., Breslau, Davis, & Andreski, 1995; Clark, Watson, & Mineka, 1994; Hayward, Killen, Kraemer, & Taylor, 2000; Krueger, Caspi, Moffitt, Silva, & McGee, 1996). The personality trait of introversion (I) has also been of interest as a risk factor for social phobia and major depression chronicity (e.g., Clark et al., 1994; Trull & Sher, 1994). Introversion (i.e., low extraversion) is represented by low levels of warmth, gregariousness, assertiveness, activity, excitement-seeking, and positive emotions (Costa & McCrae, 1995).

Personality and HPA-Axis Function

Investigations of the biological correlates of N and I may contribute to our understanding of potential pathways by which N and I relate to emotional disorders. One important biological system that has been examined in relation to N and I is the hypothalamic-pituitary-adrenal (HPA) axis—one of the major neurobiological systems mediating the stress response. There are significant theoretical reasons why N and I may be related to variation in HPA-axis regulation. An essential aspect of personality involves individual differences in cognitive appraisal, which have known influences on HPA-axis activity (e.g., Gaab, Sonderegger, Scherrer, & Ehlert, 2006). For instance, a central element of N is the tendency to interpret events as harmful, which is associated with increased negative affect. Increases in negative affect in response to stress have also been associated with increases in cortisol, a primary product of the HPA (Adam, 2006; Schlotz, Schulz, Hellhammer, Stone, & Hellhammer, 2006). Furthermore, a central element of I is the tendency to withdraw socially, often due to the overestimation of social evaluation and threat. Such fear of negative social evaluation has been shown to elicit acute cortisol elevations (Dickerson & Kemeny, 2004).

Although associations between HPA-axis function and personality may seem intuitive, previous evidence for the presence of such associations has been mixed. Several researchers using adult samples have reported no significant associations between personality (including neuroticism and introversion) and various measures of cortisol, including: (a) average basal cortisol concentrations (Schommer, Kudielka, Hellhammer, & Kirschbaum, 1999); (b) responses to the Dexamethasone Suppression Task (DST), which measures HPA negative feedback sensitivity to a synthetic glucocorticoid (Roy, 1996); and (c) cortisol responses to standard laboratory stress tasks (Kirschbaum, Bartussek, & Strasburger, 1992; Schommer et al., 1999). Among studies that *have* found significant associations, the directions of these associations have been mixed—for example, high N has been associated with both increased and decreased responses to the DST (McCleery & Goodwin, 2001; Zobel et al., 2004).

Personality and diurnal cortisol patterns

Important indicators of cortisol functioning can be found both when gathering levels of cortisol across the whole day and when examining the pattern of changes across the day. Some of the most informative studies on personality and cortisol have obtained multiple cortisol samples across the waking day in order to examine diurnal cortisol patterns (e.g., Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005). Cortisol levels are typically high upon awakening, increase in the 30 to 40 minutes post-awakening (known as the *cortisol awakening response*, or *CAR*), and are followed by a steady decline to near zero values at bedtime (Kirschbaum & Hellhammer, 1989; Pruessner et al., 1997). The CAR is increasingly considered to be an important indicator of individual differences in HPA-axis activity (Clow, Thorn, Evans & Hucklebridge, 2004). Individual differences in the size of the CAR are thought to have a strong genetic component (Clow, Thorn, Evans, & Hucklebridge, 2004) but are also responsive to psychosocial experience. Specifically, increases in chronic psychosocial stress (Clow et al., 2004; Schmidt-Reinwald et al., 1999) and psychosocial experiences on the days of cortisol

testing (Adam, Hawkley, Kudielka & Cacioppo, 2006), have predicted a larger CAR. It has also been suggested, however, that the *absence* of a CAR under situations of stress may reflect HPA-axis dysregulation (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Gunnar & Vazquez, 2001).

The slope of cortisol rhythm across the waking day is also an important indicator of HPA axis function. It is thought to be highly subject to psychosocial influences, with flatter diurnal cortisol slopes having been associated with increased experience of negative affect on the days of cortisol testing (Adam et al., 2006) as well as with the impact of accumulated chronic stress—in particular, interpersonal stress and/or trauma (Adam & Gunnar, 2001; Gunnar & Vazquez, 2001). Evening cortisol levels, which are an important contributor to cortisol slopes, appear to have less of a genetic component than wake-up cortisol levels or the CAR (Clow et al., 2004).

Associations between cortisol diurnal patterns and personality have been observed as early as childhood. Dettling et al. (1999) found that, among preschool boys, flatter diurnal slopes were associated with general negative affect, sadness, and shyness. Another study reported similar findings, with increased social fear predicting flatter diurnal cortisol slopes among preschool boys and girls (Wataamura, Donzella, Alwin, & Gunnar, 2003). When interpreted together, these two empirical studies support the association between flat diurnal slopes and the childhood temperamental traits of negative affect, sadness, shyness, and social fear. Importantly, these childhood temperamental traits have been shown to predict adult personality characteristics, with the childhood traits of negative affect and sadness significantly associated with neuroticism (N) in adulthood (Rothbart, 2007), and the childhood traits of shyness and social fear significantly associated with introversion (I) in adulthood (Rothbart, 2007). However, it remains to be seen whether these associations between flatter diurnal slopes and childhood precursors to N/I develop into associations between flatter slopes and increased N/I in adulthood.

There are very few adult studies examining associations between personality and diurnal cortisol. To our knowledge, the only study that has considered diurnal cortisol in the examination of both N and I in adults has yielded complex results. Polk et al. (2005) found that high trait negative affect (NA) was associated with higher total salivary cortisol and greater morning rise in men but not in women. In addition, cortisol levels for men who were low in positive affect (PA) followed a relatively high, flat rhythm, whereas women high in PA tended to follow a low, flat rhythm. Although these results are informative, participants provided samples while in a hotel; additional research is needed to examine how personality is associated with diurnal cortisol rhythms in naturalistic settings. Such research would be particularly informative if the effect of daily psychosocial experiences (including appraisal of stressful situations) were assessed, providing a glimpse into possible pathways for the associations between personality and diurnal cortisol.

A critical observation regarding both the adult and childhood literature on personality and cortisol concerns the presence of gender differences. In addition to the studies cited above examining diurnal cortisol, gender differences have also been reported in studies measuring stress reactivity among adults in laboratory settings (Kudielka & Kirschbaum, 2005; Traustadottir, Bosch, & Matt, 2003), with males showing greater cortisol reactivity to laboratory-based stressors. In addition, recent theories have supported differing endocrinological and behavioral responses to stress among males and females (Taylor et al., 2000). The presence of these gender differences in studies of personality and diurnal cortisol rhythms, as well as adult responses to stress tasks, underscore the importance of examining gender as a potential moderating variable for the associations between these variables.

Most research on personality and cortisol activity has relied on samples of adults or very young children. To our knowledge, no study has examined these relationships in adolescents or young adults. Investigations focusing on this age group could provide an important bridge between child and adult studies. Late adolescence represents the period when many individual personality characteristics are first stabilized, including styles of environmental appraisal, emotional and behavioral responses to stress, coping methods, and preferred social environments (Arnett, 2000; Hoffman, Levy-Shiff, & Malinski, 1996). Late adolescence also represents the period before personality and cortisol patterns have been further affected by a long history of major life events or psychopathology. Thus, examining associations between personality and cortisol patterns during late adolescence may render a relatively “clean” observation of their relationship.

The Present Study

The present study examined associations between personality and the individual differences in various elements of diurnal cortisol patterns in a large late-adolescent sample. The role of gender in moderating these associations was also assessed. The original sample, while community-based, was oversampled for individuals with high levels of N. In the current analyses, all participants with currently diagnosed emotional (i.e., anxiety and depressive) disorders were excluded, in order to rule out the possibility that effects found for neuroticism were confounded with the associations between emotional disorder and cortisol. Other potentially confounding influences were statistically partialled, including demographic and health-related covariates. These covariates, which have been associated with individual differences in cortisol levels in prior studies (e.g., S. Cohen et al., 2006; e.g., DeSantis et al., 2007; Kudielka & Kirschbaum, 2005; Meulenberg, Ross, Swinkels, & Benraad, 1987), included: waking time, bed time, use of caffeine, use of nicotine, use of oral contraceptives, participant gender, and participant ethnicity.

The specific objectives of the present study were threefold: (1) To determine if the personality traits of N or I were associated with individual differences in diurnal cortisol patterns (i.e., cortisol level at each participant's wakeup time, cortisol awakening response, and slope across the waking day). (2) To test the potential influences of gender as a moderator of the association between personality and diurnal cortisol rhythms. (3) To determine if associations between personality, gender, and diurnal cortisol patterns were mediated by recent psychosocial experiences—in particular, by differences in participants' self-reported emotions and experiences during the days of cortisol testing.

In accordance with prior findings associating flatter cortisol rhythm with negative affect and shyness in children, we hypothesized that high N and high I would be associated with flatter diurnal cortisol slopes. Given previous evidence that males with lower positive affect or sociability showed significantly flatter diurnal cortisol slopes than did females (Dettling, Gunnar, & Donzella, 1999; Polk et al., 2005), it was further hypothesized that gender would moderate these effects, with high N or high I males showing even flatter diurnal cortisol slopes than females with increased N or I. Due to a lack of research on associations between personality and the remaining two measures of diurnal cortisol in the present study (i.e., cortisol level at wakeup time and the CAR), we did not have specific directional hypotheses regarding these variables. Given prior evidence that both the CAR and diurnal cortisol slope may be modified by psychosocial experiences on the days of testing (Adam et al., 2006), we further predicted that associations between N and cortisol could be mediated by negative emotion on the days of testing, and associations between I and cortisol could be mediated by differing amounts or perceptions of social interactions on the days of testing.

Method

Participants

Participants for the present study were high school juniors who were recruited through their local schools for an ongoing longitudinal study of personality, cognitive, biological, and life stress risk factors for emotional problems in late adolescence. All students in their junior year at two local high schools (in suburban Chicago and Los Angeles) were initially invited to participate. A total of 1,977 students completed the 22-item Neuroticism scale of the Eysenck Personality Questionnaire–Revised (EPQ-R; S. B. Eysenck, Eysenck, & Barrett, 1985), which was used to screen potential participants for level of neuroticism. Students scoring in the upper tertile of the EPQ-R N scale were over-selected as potential participants, in order to increase the number of participants at risk for future psychopathology (Alloy et al., 2006; Costello et al., 1996). Using this method, 923 individuals were invited to participate. Of the 923 invited students, 520 initially agreed to participate, of whom 491 completed baseline assessments, including assessment of Axis I psychopathology using the Structured Clinical Interview for DSM-IV-TR, non-patient edition (SCID-I/NP; First, Spitzer, Gibbon, & Williams, 2002). Of those participants completing baseline assessments, 375 were randomly selected to participate in a cortisol sampling assessment, 278 of whom eventually completed that portion of the study.

Additional exclusionary criteria were applied in selecting the final sample for analysis, and included the following: pregnancy in the third trimester; a past or present endocrine disorder (e.g. Cushing's syndrome, adrenal tumors, Addison's disease); presence of psychotic symptoms during the diagnostic clinical interview; use of corticosteroid-based medications; diagnosis of a current clinical mood disorder, PTSD, or generalized anxiety disorder; or extensive missing data. None of the participants in the current study reported being pregnant. Three individuals were removed due to psychotic symptoms and 13 due to the use of corticosteroid-based medications. Two participants were excluded due to poor reporting of cortisol sampling times, and five participants did not complete the personality questionnaires. Finally, 25 participants who met criteria for current major depression ($n = 12$), generalized anxiety disorder ($n = 13$), dysthymia ($n = 3$), or post-traumatic stress disorder ($n = 1$)¹ were excluded, to ensure that potential effects of personality could not be accounted for by the current psychopathology that is itself typically associated with HPA-axis dysregulation (e.g., Hirschfeld et al., 1989).^{2,3}

Despite the removal of participants with current emotional disorders, the final sample ($n = 230$) did not significantly differ from the larger study's original sample ($n = 491$) on neuroticism ($t[484] = 1.520, p = .129$) or on introversion ($t[467] = 0.880, p = .379$). There were also no significant differences between the current sample and the original sample on age ($t[482] = -0.335, p = .737$). Regarding race/ethnicity, the current sample of participants was more likely to be Hispanic than participants in the original sample ($t[484] = -1.959, p = .051$), and was less likely to be African American ($t[484] = 2.72, p = .007$). The current and original samples did not differ in terms of their representation of any other races/ethnicities (i.e., Caucasian, Asian, Native American, Pacific Islander). The current sample ($n = 230$) scored an average of 1.82 points higher ($t(1974) = -5.702, p < .000$) on the study's initial N measure (the EPQ-R; range = 0 – 22), as compared to the screening sample ($n = 1977$). This difference was likely due to the effect of oversampling for high N. Group differences between the current sample and the screening sample were not significant for age ($t[1966] = -0.444, p = .657$) or for any racial/ethnic group.

¹Four of these twenty-five participants were comorbid for two of the aforementioned diagnoses.

²All analyses were also completed including participants who met criteria for these disorders (but partialling the effect of present/absent diagnoses). The results obtained from these analyses do not differ significantly from the present results.

³It should be noted that cortisol was collected several months following the diagnostic interviews ($M = 58$ days, $SD = 43$ days). Thus, these diagnoses may not accurately reflect the participants' mental health during the exact time of testing.

The results reported herein were based on data from the remaining 230 participants (170 females and 60 males). The unequal gender representation in the present sample is attributable to several causes. First, females were slightly more likely than their male counterparts to complete the initial screening questionnaire (i.e., 56% female, 44% male). Second, females, on average, tend to score higher on measures of neuroticism than do males (Goodwin & Gotlib, 2004), and because the study overselected for high N individuals, more females were inherently invited to participate than males (i.e., 63% of those who were invited were female). Third, of those invited, females were more likely than males to agree to participate (i.e., 65% of the invited females agreed to participate, vs. 52% of the invited males).

At the time that cortisol sampling was completed, participants were 16–18 years old ($M = 17.1$, $SD = 0.4$). The sample was diverse, consisting of Caucasian Americans (47%), Hispanic or Latino Americans (23%), African Americans (15%), Asian Americans / Pacific Islanders (8%), and other ethnicities (7%). Fifty-eight percent scored in the upper tertile on the EPQ-R N scale, 23% were in the middle tertile, and 19% were in the bottom tertile on N.

Procedure

All participants in the present study were administered the Structured Clinical Interview for DSM-IV-TR, non-patient edition (First et al., 2002) following a semi-structured life stress interview (Hammen et al., 1987). Following the two interviews, participants completed a battery of questionnaires either following the interview session or during a meeting scheduled shortly thereafter. Participants received \$40 for their participation in these tasks.

Approximately 2 months ($M = 57.8$ days, $SD = 42.8$ days) following questionnaire completion, participants completed the three-day period of salivary cortisol collection. Participants were mailed cortisol collection supplies, written instructions, and programmable wristwatches. Students were instructed to provide a saliva sample immediately upon waking, 40 minutes after waking, immediately before bedtime, and in response to three wristwatch beeps that were programmed to sound approximately 3, 8, and 12 hours after waking. This schedule (totaling 18 collections) was devised to provide the most informative description of participants' cortisol rhythm, while avoiding the increase in cortisol levels following meals (Follenius, Brandenberger, & Hietter, 1982).

During the three days of cortisol assessment, students also completed Experience Sampling Method diary reports (ESM; Csikszentmihalyi & Larson, 1987). Students were instructed to report on the situations and emotions that they experienced immediately prior to the cortisol sampling. For the present analyses, particular variables from the ESM data were theoretically chosen as potential mediators of associations between personality and diurnal cortisol patterns. These ESM variables included: social environment at the time of the cortisol sample (alone, not alone, want to be alone, with family member, with peer, with friends, with significant other, with someone else); mood at the time of the cortisol sample (happy, friendly, cheerful, cooperative, alert, caring, relaxed, active, productive, tired, nervous, lonely, frustrated, worried, irritable, stressed, sad, determined, feeling good about self); and the presence, severity, and topic (self, family, peer, friend, significant other) of any current stressor at the time of sampling. Individual mood state items were averaged to form composite measures of momentary positive moods (i.e., happy, friendly, cheerful, cooperative, alert, caring, relaxed; $\alpha = .922$) and momentary negative moods (i.e., nervous, lonely, frustrated, worried, irritable, stressed, sad; $\alpha = .903$). Additional data and analyses based on the full range of ESM data are discussed elsewhere (Doane et al., 2008; Mor et al., 2007).

Personality Measures

Eysenck Personality Questionnaire-Revised (EPQ-R), Neuroticism scale—

Potential participants for the present study completed a 22-item⁴ version of the EPQ-R Neuroticism scale (S. B. Eysenck et al., 1985). Items included yes/no questions concerning mood, anxiety, and energy levels. In a 24-item version of the EPQ-R N scale, Eysenck et al. reported internal consistencies of .85 for females and .88 for males. Using Cronbach's alpha to assess reliability, the 22-item EPQ-R N scale in the present study yielded appropriate internal consistency for a trait measure ($\alpha = .78$). Coefficient omega_{hierarchical} (ω_h) was estimated at 0.66 for the present sample (Uliaszek et al., 2007).

International Personality Item Pool NEO-PI N scale (IPIP)—Participants also completed the 60-item IPIP NEO-PI N scale as a second measure of N (International Personality Item Pool, 2001). Individuals were asked to rate all items on a 5-point scale (1 = “Very Inaccurate”, 5 = “Very Accurate”), according to the items' accuracy in describing their own behaviors. Items include both positively and negatively coded statements such as “Get irritated easily”. All six IPIP NEO-PI subscales have been found to yield adequate internal consistency ($\alpha > .77$), and correlate substantially with their corresponding NEO-PI-R subscale ($r > .7$). Internal consistency was again estimated using Cronbach's alpha, indicating high reliability for the IPIP NEO-PI N scale ($\alpha = .94$), and coefficient omega_{hierarchical} was estimated at .67 (Mor et al., 2007).

Behavioral Inhibition System (BIS)—Students were also administered the Behavioral Inhibition System scale (Carver & White, 1994). The 7-item BIS scale is measured on a 4-point Likert scale and is a part of a larger 20-item BIS/BAS questionnaire, assessing both behavioral inhibition and behavioral activation characteristics. The BIS is generally considered to reflect the construct of trait anxiety, one of the known facets of neuroticism (Costa & McCrae, 1995). Anxiety and neuroticism in general have been positively associated with the BIS (Gray, 1994). Specifically, the BIS is associated with the tendency to fear and avoid potentially aversive situations, and to respond to threat with increased arousal and sensitivity (for a review, see Revelle, 1995). BIS scale items include statements such as “If I think something unpleasant is going to happen, I usually get pretty ‘worked up.’” Carver and White (1994) reported an 8-week test-retest reliability of .66 and demonstrated significant convergent/divergent validity of the BIS as a marker of neuroticism (also see Zinbarg & Mohlman, 1998). Internal consistency for the BIS scale was estimated with Cronbach's alpha ($\alpha = .75$).

Big Five Mini-Markers—To provide a fourth measure of neuroticism, as well as a single measure of introversion, participants in the current study completed the Big 5 Mini-Markers (referred to hereafter as the “Big 5”; Saucier, 1994). In the 40-item, homogenously keyed Big 5 questionnaire, each of the five personality factors is represented with 8 questionnaire items. Individuals rated all items on a 9-point Likert scale (1 = “Extremely Inaccurate”, 9 = “Extremely Accurate”), also according to the items' accuracy in describing the rater's behaviors. Items corresponding to N included such characteristics as “Moody” or “Relaxed”, and items representing I included traits such as “Energetic”, “Talkative”, “Bashful”, or “Withdrawn”. All of the five factors on this scale have been reported to exhibit strong reliability ($\alpha > .77$). For the Big 5 N scale in the present study, internal consistency was strong ($\alpha = .81$).

Although the present analyses assessed I using only the Big 5 I scale, there is evidence to support the reliability of this measure. Saucier reported alpha estimates of .80 for the Big 5 I

⁴The original EPQ-R Neuroticism Scale consists of 24 items. The item referring to suicidality was omitted based on recommendations from the Institutional Review Board. The item, “Do you worry about your health” was also omitted, because preliminary analyses indicated its failure to load on any factor.

scale (Saucier, 1994), and correlations between .91 and .96 with Goldberg's more extensive I scale have been reported (Goldberg, 1992). It is important to note, however, that the Big 5 Mini-Markers assessment of I includes only items that reflect characteristics of sociability and withdrawal. Items reflecting other putative facets of I, such as low positive emotionality (Costa & McCrae, 1995), are not examined with this measure. Using the present data, internal consistency for the Big 5 I scale was similarly strong ($\alpha = .83$).

Computations of N and I—To make use of the multiple measures of neuroticism (N) that were available for the present dataset, a composite measure of N was calculated. This was computed by averaging *z* scores from the EPQ-R N scale, IPIP, BIS, and Big 5 Mini-Markers N subscale⁵. To confirm that these four measures of N (EPQ-R N, IPIP, BIS, Big 5 N) could be regarded as reflecting a unitary construct, intercorrelations were computed. Correlation coefficients between pairs of measures ranged from: $r = 0.49$ (EPQ-R N and BIS; $p < .0001$) to $r = 0.76$ (IPIP and Big 5; $p < .0001$). To confirm that the BIS (a measure of trait anxiety—one of the facets of N) was appropriately included in the present measure of N rather than I, intercorrelations were computed between the BIS and the N and I scales of the Big 5 Mini-Markers. The correlation between the N scale on the Big 5 Mini-Markers and the BIS was significant, $r = .559$, $p < .0001$. The correlation between the I scale on the Big 5 Mini-Markers and the BIS was also significant ($r = -.288$, $p < .0001$) but was more comparable to the correlation between the N and I scales on the Big 5 Mini-Markers ($r = -.286$, $p < .0001$). A recent report (Griffith et al., 2007) determined that a unifactorial model provided a good fit to the observed covariances among the four scales of N (CFI = 1.0, RMSEA = .04, SRMR = .01). Introversion (I) was calculated by obtaining reverse scores of the raw values on the Big 5 E subscale, and then standardizing this measure.

Health and Demographic Measures

Participants in the present study completed a health questionnaire reporting on their personal medical history and health behaviors, including self-reported medical conditions, chronic medical conditions (particularly those that could affect cortisol levels), intake of caffeine and nicotine, waking and bed time, and use of prescribed medications (e.g., corticosteroids, oral contraceptives). Participants also provided information on health-related variables that are putatively associated with cortisol (e.g., S. Cohen et al., 2006; Kudielka & Kirschbaum, 2003; Meulenberg et al., 1987). These health-related variables (i.e., waking time and bed time; use of caffeine, nicotine, and oral contraceptives) were not considered exclusionary criteria but were included as covariates in statistical analyses, along with additional demographic variables (i.e., gender, ethnicity). Ethnicity was reported using dummy (0, 1) variables, for participants who identified as African-American, Asian-Pacific Islander, or Hispanic-Latino (Caucasian was the excluded group). Due to prior findings that diurnal cortisol slopes were flatter for African-American males (DeSantis et al., 2007), ethnicity by gender interactions were also included.

Cortisol Sampling and Assay Procedures

To obtain samples, participants expelled saliva through a straw into a sterile 2 mL cryogenic vial (Schwartz, Granger, Susman, Gunnar, & Laird, 1998). No salivary stimulants were used. Participants were asked to cap the vials of their saliva samples tightly, label them with date and time of day, and refrigerate them as soon as possible. Once returned via postal mail or by way of a frequently-checked drop box at participants' schools, saliva samples were stored at -20 degrees Celsius until assayed. Investigations have confirmed that cortisol concentrations remain stable when mailed in an unfrozen state (e.g., Clements & Parker, 1998).

⁵For 4 participants, the Neuroticism composite was comprised of only the EPQ-R N and the IPIP, due to missing data for those participants. One participant's Neuroticism composite was equal to the EPQ-R N score, due to missing data on the remaining N measures.

Samples were later shipped to the Biochemisches Labor at the University of Trier, Germany. Samples were assayed in duplicate, using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric endpoint detection (DELFI^A). Fluorescence was detected using a DELFI^A-Fluorometer (Wallac, Turku, Finland). Inter- and intra-assay coefficients of variation are 7.1% – 9.0% and 4.0% – 6.7%, respectively.

Data Analysis

Description of key variables—Key predictor variables were the composite N and I scores described previously. Because scores on these measures approximated normal distributions, no transformations were applied. Gender was also a predictor variable, but was considered a primary variable of interest only in the context of its interaction with personality. All values of personality variables (that were multiplied by gender to create interaction terms) were converted to *z* scores for subsequent analyses, in order to reduce multicollinearity due to non-essential ill-conditioning among interaction variables (J. Cohen, Cohen, West, & Aiken, 2003). As described earlier, demographic and health-related variables were regarded as covariates. In the final analyses, N and I were entered in models simultaneously, such that associations could be attributable to the unique effects of N and I, rather than the effects of their shared variance.

The criterion variables in the present analyses were 3 parameters characterizing the diurnal cortisol rhythm: *level of cortisol at wakeup time*, *size of cortisol awakening response (CAR)* and *slope of the diurnal curve*. Although the precise meanings of each cortisol parameter (wakeup level, CAR, and diurnal slope) are not yet fully understood, we consider these three parameters to be relatively distinct in their origins and interpretations. Cortisol level during wakeup is typically more genetically determined than cortisol measured in the afternoon or evening (Clow et al., 2004). The size of the CAR is thought to be heavily genetically influenced as well, but is also related to current chronic stress, in addition to perceived or expected stress on the specific days that the CAR is measured (Adam et al., 2006; Clow et al., 2004; Gunnar & Vazquez, 2001). Finally, a flattening of the diurnal cortisol slope can result, in part, from the experience of acute stress or negative affect on the specific days of cortisol testing (Adam et al., 2006). Flatter slopes also appear to be partially entrained over time by a history of chronic psychosocial strain (Adam et al., 2006; Gunnar & Vazquez, 2001).

Estimates of the three cortisol parameters of interest were obtained using multilevel growth curve modeling, as described below. In order to correct for positive skew, a base *e* logarithmic transformation was applied to the cortisol values prior to use in our models. No significant cortisol outliers were identified in the data.

Multilevel Modeling of Diurnal Cortisol Rhythms

A 3-level multilevel analysis (Raudenbush & Bryk, 2002; Singer & Willett, 2003) was performed to model the shape of the diurnal cortisol rhythm, and to examine the associations between the independent variables and the key parameters defining individual differences in diurnal cortisol rhythms (wakeup values, size of the CAR, and slope from wakeup to bedtime). The three levels represented data at the moment level (cortisol levels across the day), nested in the day level (waking times and bedtimes on the days of testing), nested in the person level (individual differences in personality, health, and demographic variables). Multilevel modeling was selected because it allowed us to simultaneously model the shape of the diurnal cortisol rhythm for each individual, and to examine predictors of those rhythms (Adam & Gunnar, 2001; Adam et al., 2006). This method also adjusts for correlated error within each level of data, whereas OLS models do not (Adam & Gunnar, 2001; Hruschka, Kohrt, & Worthman, 2005).

To provide estimates of diurnal cortisol rhythm from the moment-level cortisol data, a Level 1 model was fitted with the following parameters:

$$(1) \text{ Level-1 Model: Cortisol} = \pi_0 + \pi_1 * (\text{Time Since Waking}) + \pi_2 * (\text{Time Since Waking})^2 + \pi_3 * (\text{CAR}) + e$$

In the model above, the intercept term (π_0) represents the latent estimate of each participant's cortisol level at wakeup, estimated across all 3 days of cortisol collection. The π_1 coefficient (associated with the linear term of “Time Since Waking”) represents the latent estimate of the slope of each participant's diurnal cortisol curve at wakeup. The π_2 coefficient (associated with the quadratic term), was included to test for curvilinear relationships. The “Time Since Waking” variable represents the time of day that the cortisol sample was taken, modeled as the number of hours since the participant had woken on that particular day. Finally, the π_3 coefficient is a latent estimate of the size of the cortisol awakening response. By including a dummy variable (0, 1) indicating which samples were part of the CAR (taken 40 minutes post-awakening), π_3 reflects how much the 40 minute post-awakening samples deviated above the expected cortisol value at that time of day (based on the typical diurnal decline for that individual).

Demographic and health-related covariates were included at either the person level (Level 3) or the day level (Level 2). Covariates that varied with each *day* (including wakeup time and bedtime) were included at Level 2 of the hierarchical analysis. The general model for these day-level covariates was as follows:

$$(2) \text{ Level-2 Model: } \pi_0 \text{ to } \pi_3 = \beta_{i0} + \beta_{ij} * (\text{Day Level Covariates}) + r_{ij}$$

Covariates that varied with each *participant* (but remained consistent from day to day) were included at Level 3. These person-level covariates included participants' ethnicity, as well as use of caffeine, nicotine, and oral contraceptives. In addition, Level 3 included the primary predictor variables of N and I, as well as the N * gender and I * gender interaction terms (with male = 1). The general model for these person-level variables was as follows:

$$(3) \text{ Level-3 Model: } \beta_{i0} + \beta_{ij} = \gamma_{ij0} + \gamma_{ijk} * (\text{Person Level Covariates}) + \gamma_{ijk} * (\text{Personality Variables}) + \gamma_{ijk} * (\text{Gender by Personality Interaction Variables}) + u_{ijk}$$

To test for potential mediators of any significant associations between personality and diurnal cortisol patterns, ESM diary variables were added to the model (describing participants' average mood states and social experiences across the days of cortisol testing, as described above). ESM data were reported at the moment level but aggregated to the person level, in order to test their potential mediation of the person-level personality effects. All momentary ESM variables of interest were therefore averaged for each participant across all moments of cortisol sampling. These variables were added to the Level 3 model. The general model for these analyses is listed below.

$$(4) \text{ Level-3 Model: } \beta_{i0} \text{ to } \beta_{ij} = \gamma_{ij0} + \gamma_{ijk} * (\text{Person Level Covariates}) + \gamma_{ijk} * (\text{Personality Variables}) + \gamma_{ijk} * (\text{Gender by Personality Interaction Variables}) + \gamma_{ijk} * (\text{Averaged Daily Experiences}) + u_{ijk}$$

Results

Descriptive Analyses

Table 1 shows means, standard deviations, and ranges of the raw scores for all personality variables as well as for cortisol levels at each sampling time point. Note that the mean cortisol

values follow the expected diurnal rhythm: high upon waking, increasing 40 minutes after waking, and then declining steadily thereafter.

Simple associations—Simple associations were examined among the personality (independent) variables as well as among the various cortisol (dependent) variables. As would be expected, neuroticism and introversion were positively correlated ($r = .36, p < .0001$). A higher cortisol awakening response (CAR) was significantly associated with a lower cortisol level at wakeup ($r = -0.30, p < .0001$). This is conceptually consistent with the significant association between higher CARs and flatter cortisol slopes ($r = 0.62, p < .0001$) in the present sample, because the higher CARs were associated with lower wakeup levels, and lower wakeup levels were significantly associated with flatter slopes ($r = -0.86, p < .0001$). Due to significant correlations among variables in the present study, multicollinearity diagnostic tests were completed. Tolerance and variance inflation factor (VIF) statistics did not present concerns regarding multicollinearity.

Multilevel Analyses Predicting Diurnal Cortisol Measures

Participants in the present analyses generally reflected typical diurnal patterns of cortisol levels, as illustrated in Table 2. Cortisol levels, on average, were appropriately high at wakeup (π_0 intercept, $\gamma_{000} = -1.142 = 0.32 \mu\text{g/dl}$), and were followed by a 56% increase during the cortisol awakening response (π_3 intercept, $\gamma_{300} = 0.45$).⁶ As would be expected, cortisol levels decreased throughout the day at a rate of 12% per hour at wakeup time (π_1 intercept, $\gamma_{100} = -0.13$), with a declining rate of change thereafter due to the significant positive quadratic effect (π_2 intercept, $\gamma_{200} = .001$).

Neuroticism—Table 2 provides a summary of the multilevel model. All personality and gender coefficients are shown; the health and demographic variables that were included in levels 2 and 3 are not shown, in order to focus on the variables of interest and conserve space. There were no main effects of N on the diurnal slope ($\gamma_{101} = 0.000, p = .979$), on wakeup values ($\gamma_{001} = -0.021, p = .571$), or on the size of the CAR ($\gamma_{301} = 0.025, p = .690$).

Although N was not significant as a main effect, the interaction of N with gender was significant in predicting diurnal cortisol slope. As indicated in Table 2, increased N among males was significantly associated with a flatter diurnal cortisol slope ($\gamma_{103} = 0.017, p = .046$). Diurnal cortisol slopes were 1.7% flatter among men for every SD higher on N⁷. The interaction of N with gender was not significant in predicting wakeup values or the size of the CAR.

Due to the inclusion of the quadratic term, the linear slope reported herein represents the slope of the diurnal cortisol curve when the quadratic term equals zero (i.e., at wakeup). Follow-up analyses were conducted to determine whether the association between increased N in males and flattened slopes (centered at wakeup) would retain significance when time since waking was centered at midday or bedtime, thus providing slopes of the diurnal cortisol curve at those specific time points. Increased N among males was significantly associated with a flatter diurnal cortisol slope at both midday ($\gamma_{103} = 0.019, p = .040$) and bedtime ($\gamma_{103} = 0.019, p = .037$). For both midday and bedtime centering, slopes were 1.9% flatter for every SD higher on this factor. Thus, the effect of flattened slopes among high N males was consistent regardless of whether the intercept was centered at wakeup, midday, or bedtime.

⁶For interpretation of effect sizes, Level 1 coefficients can be viewed as the percent change in the outcome per unit change in the criterion variable (due to a unique property of a logarithmic outcome), after applying the following transformation: $B\% \text{ change} = [\exp(B_{\text{raw}})] - 1$.

⁷While these analyses indicate that N was a significant predictor of cortisol patterns, the causal direction of effect is unclear. Indeed, for regression analyses in which the cortisol indicators were used to predict the personality variables, flatter diurnal slopes significantly predicted increased N among males (the interaction of gender with N), even with the remaining two cortisol variables (i.e., CAR and wakeup level) entered simultaneously in the model.

Introversion—As indicated in Table 2, introversion (I) as a main effect was a significant predictor of the cortisol awakening response (CAR), when all demographic and health-related covariates were partialled. Higher I was significantly associated with a *lower* CAR ($\gamma_{302} = -0.118, p = .030$), with the CAR approximately 12% lower for every SD higher on this factor⁸. Introversion (I) was not significantly associated with wakeup cortisol levels or with the diurnal cortisol slope.

Increased levels of introversion among males were also significantly associated with increased cortisol levels at wakeup ($\gamma_{004} = 0.201, p = .007$), with wakeup cortisol measuring 22% higher for every SD higher on this factor⁹. However, higher introversion in general (among both males and females, γ_{002}) was not associated with differences in cortisol level at wakeup. The I by Gender interaction term predicted neither diurnal slope (γ_{104}) nor CAR (γ_{304}). Note that the main effect of I on CAR was significant even with the interaction term in the model, suggesting that both males and females with high I had significantly lower CARs.

Mediator effects—To consider one potential explanation for the associations between personality and diurnal cortisol patterns (i.e., mediation by individual differences in social and emotional experiences on the days of testing), variables from participants' ESM diary reports were examined. As noted above, variables tested included the proportion of time the participant was alone at the time of diary reporting, the proportion of time with family members or peers, the participant's average positive affect, average negative affect, and the presence, severity and content (related to self, family, peer, etc.) of stressors encountered at the time of each diary report. When each of these ESM variables was individually included in the model, none of the coefficients for the previously observed personality or personality by gender effects were reduced substantially in size. Although several of the ESM diary variables were significantly related to diurnal cortisol slopes (see Doane et al., 2008), the addition of these variables did not meaningfully alter the size of the coefficients for the personality and personality * gender associations, and none of the diary variables appeared to be significant mediators of the associations between personality and diurnal cortisol patterns (Krull & MacKinnon, 2001).¹⁰

Area Under the Curve (AUC)—One additional aspect of cortisol activity could not be assessed using the multilevel models described above—the area under the diurnal cortisol curve (AUC), an estimate of the overall cortisol secretion throughout the waking day. This variable was therefore computed separately by applying the trapezoid method, with the base of the trapezoid at zero (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). To ensure that this variable was independent of the measure of CAR, the cortisol concentration at 40 minutes after awakening was removed from the calculation of AUC. This variable was examined via multiple linear regression analyses, regressing AUC on neuroticism and introversion (as main effects and as interactions with gender). These analyses, which included the health-related and demographic covariates described previously, did not yield significant associations between AUC and personality or personality by gender.

⁸Again, it should be noted that the causal direction of effect is unclear in these analyses. Decreased CAR was also a significant predictor of increased introversion in OLS regression analyses, even after covarying the effects of the other two cortisol variables (i.e., wakeup level and slope).

⁹This association was not significant for OLS regression analyses in which the cortisol measures were used as the predictor variables. That is, increased wakeup cortisol levels did not significantly predict increased introversion among males (or females).

¹⁰A 2-2-1 Krull and MacKinnon multilevel mediation model was used to test mediators with a $\beta_a\beta_b$ MacKinnon method. The β_a was calculated through OLS, while the β_b was deduced through the HLM γ_b coefficients. As an example, the mediation test for the momentary emotion of sadness ("How sad were you feeling in the last hour?") was not significant, $\beta_a\gamma_b = .00056$.

Discussion

The present study examined associations between personality traits and aspects of the diurnal cortisol rhythm. To our knowledge, it is the first study to report significant relationships among these variables in an adolescent sample. Several significant associations were observed between personality variables (i.e., neuroticism, introversion) and diurnal cortisol patterns (i.e., wakeup level, cortisol awakening response, diurnal cortisol slope). Gender was an important moderator of some of these effects.

Neuroticism

Higher N was associated with a flatter diurnal cortisol slope among male adolescents. This pattern has some similarity to previous findings, if one considers that the trend for flatter cortisol slopes has been associated not only with reports of shyness in young boys but also with *additional* negative emotions, such as aggression and sadness in boys (Dettling et al., 1999). However, associations between neuroticism and flatter slopes have not been found among adult males (Polk et al., 2005). This discrepancy may be due to differences in cortisol measurement rather than to real differences between populations. In the present study, slope was defined without including the CAR period, which is thought to be regulated by biological processes that are distinct from the wakeup to bedtime diurnal rhythm (Clow et al., 2004). Polk and colleagues, however, defined slope as the period from 8:00 h to 14:00 h, thereby possibly including the CAR response in their computation of slope for some individuals.

It is unclear why the association of increased N and flattened slope is present only for males. One possible explanation is that men with high N encountered or perceived their experiences on the days of testing to be more stressful or negative than did women with high N, resulting in flatter slopes. Our mediational analyses, however, indicated that the perceived level of negative emotion and stress occurring on the days of testing did not account for the association between N and cortisol for men, suggesting that this effect is not solely due to differences in perceived concurrent negative experiences. An alternative explanation is that high N males reported similar perceived experiences but greater physiologic responding to those events. Prior findings have shown that males react to standardized laboratory stressors with higher cortisol responses than their female counterparts (Kudielka & Kirschbaum, 2005; Traustadottir et al., 2003); perhaps this is also the case for naturalistic stressors. Nonetheless, the fact that experiences on the days of testing showed no signs of mediating the N effect suggests that associations between N and cortisol slopes for males may not reflect effects of immediate experience, but rather, may be more enduring—either reflecting trait-like patterns of genetic origin, or an entrainment over time by a longer history of negative experiences. The latter hypothesis is supported by prior theory and data suggesting that flat slopes may emerge over time from a history of exposure to negative social experiences (Gunnar & Vazquez, 2001).

Introversion

Regression analyses indicated that higher I significantly predicted a lower CAR. Because the measure of I in the present study (i.e., Big Five Mini-Markers) was solely comprised of items reflecting withdrawal and low sociability, it is perhaps not surprising that the results do not replicate findings of flatter slopes which were based on only partially overlapping constructs, such as low positive affect (e.g., Polk et al., 2005). Furthermore, most of the literature on social fear in childhood has been limited by cortisol samples obtained during the times of day when the child is attending preschool, thereby omitting cortisol levels during the important periods of awakening, CAR, and bedtime (e.g., Kagan, Reznick, & Snidman, 1988; Watamura et al., 2003). The present study also reported a positive association between introversion in males and cortisol levels at wakeup, with high I males showing increased cortisol at waking, as compared to males with average I. The higher wakeup cortisol levels among high I males may

help to explain why CAR responses are lower for this group, given that cortisol levels at wakeup and the size of the CAR are negatively correlated. This explanation does not, however, extend to the finding of decreased CAR among females in the study, as wakeup levels were not significantly increased among high I females.

Associations between I and cortisol awakening response were not mediated by participants' anticipated or actual social experiences on the days of testing, as indicated by diary reports reporting social behavior (for example, the time spent alone or the time spent socializing during the three days of cortisol sampling). Although the CAR is thought to respond to social experience (Clow et al., 2004), it has also been found to have among the strongest genetic components of the diurnal cortisol pattern. Perhaps the causal pathways for the association between introversion and decreased CAR do not lie in differing immediate experiences, but are rather due to more long term environmental or genetic differences. The association between CAR and Introversion was significant among both males and females—lending further weight to the idea that there may be an organic origin for this relationship. Alternatively, if the size of the CAR does indeed respond to expectations of daily stress (Adam et al., 2006; Clow et al., 2004), examining individual differences in expected (rather than actual) experiences on the days of testing may be important to examine. In addition, experiences prior to the days of testing should be examined, to test the possibility that differing psychosocial experiences may have contributed to alterations over time in the size of the cortisol awakening response. Additional longitudinal evidence is required to test the latter hypothesis for both N and I.

Advantages, Limitations, and Future Directions

The present study benefited from a large, diverse sample; multiple days of cortisol sampling; meticulous measurement of N and I, using well-validated personality questionnaires; the use of sophisticated statistical strategies (i.e., multilevel modeling); and the incorporation of covariates for potential health and demographic confounds. It also benefited from the examination of associations between personality and cortisol in naturalistic settings, allowing us to observe these associations as they operated in the course of participants' everyday lives.

Interpretations of the present findings are limited by their cross-sectional nature. It cannot be established whether N and I personality characteristics influence cortisol patterns, whether individual differences in cortisol patterns contribute to the development and/or maintenance of N and I, or whether a third variable contributes to both N and I as well as to related cortisol patterns. Gathering longitudinal data on the stability of N, I, diurnal cortisol, and their interrelations, is a critical next step. In conducting prospective analyses of the personality variables that predict cortisol patterns, it is hoped that causal relationships may be better understood. A better understanding of the current associations would be gained by observing whether individual differences in diurnal cortisol patterns are stable attributes associated with enduring personality differences, or whether diurnal cortisol patterns change over time in relation to participants' changes in N and I (or to participants' differing histories of experience). An additional limitation concerns the lack of multiple measures for introversion. Although an aggregate measure was calculated to assess N, additional measures of I were unavailable in the present study. Furthermore, due to the diminished representation of males in the present analyses ($n = 172$ females, $n = 62$ males), the gender interactions reported herein would benefit from replication in an additional, larger sample. Finally, it is possible that the exclusion of participants who met criteria for a current mood or anxiety disorder resulted in reduced generalizability. On the other hand, including these participants and using a variable corresponding to diagnostic status as a covariate would have likely underadjusted for the potential confounding effects of psychopathology on cortisol (Zinbarg & Suzuki, 2007).

To develop effective preventive treatments or early interventions in mood and anxiety disorders, it may be valuable to understand the biological role of vulnerabilities for these

disorders. If personality risk factors for psychopathology do predict certain disruptions in the HPA axis, and if, in turn, these HPA-axis differences partially mediate the impact of personality traits on psychopathology, preventive attempts to target personality and HPA-axis function directly could be clinically relevant (Tafet & Bernardini, 2003). Longitudinal investigations on the relationships between personality risk factors, HPA-axis activity, gender, and the development of mood and anxiety disorders would therefore be valuable. Future research in this vein could potentially help illuminate both the origins and consequences of associations between personality and HPA-axis function.

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Table 1
Descriptive Statistics of Raw Data for Personality Predictor Variables and Diurnal Levels of Cortisol

	Minimum	Maximum	Mean	SD
EPQ-R N	0.00	22.00	11.27	4.46
IPIP	1.43	4.22	2.70	0.55
BIS	8.00	28.00	20.14	4.12
Big 5 - N	1.71	8.38	4.67	1.43
Big 5 - E	2.25	8.75	5.79	1.37
Age in years	15.00	17.00	16.11	0.43
Cortisol Level at Time 1	0.04	2.00	0.44	0.24
Cortisol Level at Time 2	0.03	1.38	0.61	0.29
Cortisol Level at Time 3	0.02	1.11	0.24	0.15
Cortisol Level at Time 4	0.02	1.54	0.17	0.15
Cortisol Level at Time 5	0.01	1.14	0.12	0.11
Cortisol Level at Time 6	0.01	1.10	0.09	0.12

Notes. Cortisol levels at times 1 – 6 are presented in µg/dl and represent average salivary cortisol levels at the following times: immediately upon waking (Time 1); 40 minutes after waking (Time 2); at approximately 3 (Time 3), 8 (Time 4), and 12 (Time 5) hours following waking; and immediately before bedtime (Time 6).

Table 2
Multilevel Model of Associations between Personality (Neuroticism, Introversion) and Diurnal Cortisol Parameters

Fixed Effect	β	SE	t	p
Model for Wakeup Cortisol Level, π_0				
Average Wakeup Cortisol Level, β_{00}				
Intercept, γ_{000}	-1.142	0.028	-40.279	.000
Neuroticism (N), γ_{001}	-0.021	0.037	-0.566	.571
Introversion (I), γ_{002}	-0.018	0.029	-0.618	.537
N x gender, γ_{003}	-0.102	0.079	-1.283	.201
I x gender, γ_{004}	0.201	0.073	2.748	.007
Gender, γ_{005}	-0.254	0.073	-3.461	.001
Model for Time Since Waking, π_1				
Average Effect of Time Since Waking, β_{10}				
Intercept, γ_{100}	-0.125	0.008	-15.493	.000
Neuroticism (N), γ_{101}	0.000	0.005	0.026	.979
Introversion (I), γ_{102}	-0.004	0.003	-1.404	.162
N x gender, γ_{103}	0.017	0.009	2.004	.046
I x gender, γ_{104}	-0.006	0.008	-0.761	.447
Gender, γ_{105}	0.004	0.009	0.470	.638
Model for Time Since Waking Squared, π_2				
For Intercept, β_{20}				
Intercept, γ_{200}	0.001	0.000	1.463	.143
Model for Cortisol Awakening Response, π_3				
Average Size of Awakening Response, β_{30}				
Intercept, γ_{300}	0.446	0.041	10.967	.000
Neuroticism (N), γ_{301}	0.025	0.063	0.398	.690
Introversion (I), γ_{302}	-0.118	0.054	-2.179	.030
N x gender, γ_{303}	0.096	0.090	1.065	.288
I x gender, γ_{304}	0.003	0.103	0.026	.980
Gender, γ_{305}	-0.042	0.105	-0.404	.687
Wakeup Time, β_{31}				
Intercept, γ_{310}	-0.107	0.047	-2.306	.021
Random Effect	Variance Component	df	χ^2	p
Level 1 Intercept, u00	0.044	213	320.619	.000
Time Since Waking, u10	0.001	213	368.862	.000
Awakening Response, u30	0.074	213	287.013	.001

Notes: All fixed effects are with robust standard errors. All Level 1 predictors are uncentered; Level 2 and Level 3 variables are grand mean centered. Covariates in Level 2 of the model, which are not presented here in order to conserve space, included wakeup time and bedtime. Additional variables in Level 3 which were not presented here included ethnicity (African American, Asian/Pacific Islander, Hispanic/Latino), African American x gender, and the use of caffeine, nicotine, or oral contraceptives. All coefficients were set as fixed, except the quadratic (time since waking squared) effect at levels 2 and 3, and the coefficients for the day-level covariates at Level 3, as we did not have the degrees of freedom to model day-to-day variation in the quadratic effect or between-person variation in effects of day-level covariates.