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Repeated measurement of salivary cortisol within and across days among shy young adults

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ABSTRACT

Temperamental shyness emerges early in childhood and remains relatively stable throughout development and has been associated with high and low levels of the stress hormone cortisol. Studies examining the relation between shyness and cortisol have been limited because they have traditionally collected only one measure of cortisol on a single day in the laboratory, restricting the reliability and diurnal profile of the measure in the participant's everyday environment. We collected 15 saliva samples across three separate days (i.e., upon waking, +60 min post-waking, +8 h post-waking, +10 h post-waking, and bedtime) in a sample of healthy young adults selected for high and low shyness in order to characterize a portion of the diurnal cortisol rhythm. Overall, shy individuals demonstrated relatively lower cortisol across the day and across multiple mornings than non-shy adults. Higher self-reported social anxiety across multiple measures was also related to lower total cortisol levels across all participants. The present study replicates and extends our previous findings of low salivary cortisol measured in the laboratory in shy adults to repeated measurement in their everyday environments.

1. Introduction

Shyness is characterized by behavioral inhibition, anxiety, and preoccupation with the self in real or imagined social situations. While a common and transient experience in the general population of reports of over 90% experiencing the phenomenon at some point in their lives (Zimbardo, 1977), 10–15% of people are characterized by temperamental shyness (Kagan, 1994). Temperamental shyness is a stable feature of personality that has its origins in early infancy and is associated with a number of stable baseline and stress-reactive behavioral and physiological patterns across development, including right frontal EEG asymmetry, a high and stable heart, and high morning and day time cortisol levels (e.g., Beaton et al., 2006; Beaton et al., 2008a; Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Kagan, Reznick, & Snidman, 1987; Kagan, Reznick, & Snidman, 1988; Schmidt, 1999; Schmidt & Fox, 1994; Schmidt, Fox, Schulkin, & Gold, 1999; Schmidt, Santesso, Schulkin, & Segalowitz, 2007; Schmidt et al., 1997; Theall-Honey & Schmidt, 2006; Tyrka et al., 2008). Shyness in childhood is also associated with greater risk of anxiety disorders such as social phobia in adolescence and adulthood (Beidel & Turner, 1998; Hirshfeld et al., 1992), but this is not a forgone developmental trajectory (e.g., Stein, Chavira, & Jang, 2001).

We previously found that shy and socially anxious adults demonstrated relatively lower, rather than an expected higher, salivary cortisol response than their non-shy counterparts when preparing for a videotaped speech, even though the shy participants reported a higher degree of anticipatory anxiety (Beaton et al., 2006). These findings of low salivary cortisol levels in shyness were evidenced across the two studies: one study involved adults who were selected for extreme shyness; and the second study involved adults who were not selected for shyness (Beaton et al., 2006). We argued that relatively lower cortisol levels observed in shy adults may have reflected experience-driven HPA axis dysregulation as a result of coping with a life-long history of shyness and social anxiety (Beaton et al., 2006). Interestingly, the adults in these two studies were healthy and functioning in a highly social environment (i.e., attending classes and presumably interacting with peers, faculty, and staff) even though those in the selected study met diagnostic criteria for social phobia. However, these findings and much of the previous work in the area have been limited by exclusively

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using laboratory measurements of cortisol collected on a single laboratory visit and on a single day, restricting the reliability of the findings, given the known diurnal rhythms in cortisol and differences in measures collected in the laboratory versus everyday environments.

The purpose of the present study was to extend the ecological validity of our previous findings (e.g., Beaton et al., 2006). We repeatedly measured daily salivary cortisol within and across days in a nonclinical sample of young adults who were selected for high and low shyness. Saliva was collected in their everyday environments of home, school, and work. Participants were asked to collect their own saliva samples at five separate time points: upon waking but before arising from bed, +60 min post-waking, +8 h post-waking, corresponding to late afternoon, +10 h post-waking, corresponding to early evening, and bedtime over three separate days for a total of 15 samples to allow for the characterization of cortisol output over time.

These daily time points were selected for several reasons. The morning time points were selected because cortisol peaks in the morning within 60 min of wakening in most people. We were also particularly interested in examining the cortisol awakening response (CAR). The CAR refers to the consistent daily rapid elevation in cortisol production that occurs in a 20–60 min period upon awakening that appears to be responsive to the expected demands of the day, although the purpose of this increase is not entirely clear even though the literature investigating this phenomenon is extensive. Some researchers have demonstrated that populations with high self-perceived stress such as low socioeconomic status, chronic worry, and burn-out exhibit a high CAR (see Fries, Dettenborn, & Kirschbaum, 2009, for a review). However, atypical diurnal cortisol output with a reduction in morning CAR have been reported in a variety of child and adult population samples exposed to prolonged psychological and/or physical stressors (Badanes, Watamura, & Hankin, 2011; Carlson & Earls, 1997; Fries et al., 2009) and the late afternoon time points were selected because they coincided with the end of the work/school day and before and after dinner in most people. Participants were told to keep their normal sleep-wake schedule and to make saliva collections at times relative to their normal waking times.

Given that shyness is a complex phenomenon maintained by a blend of high negative affectivity (e.g., fear and anxiety) and low positive affectivity, we predicted that, compared to non-shy adults, shy adults would have an atypical diurnal salivary cortisol rhythm, but did not make specific predictions for the direction of the cortisol patterns between shy and non-shy adults, given the inconsistencies in the literature and our prior findings (Beaton et al., 2006; Fries et al., 2009). A recent meta-analysis of 147 studies of HPA-axis activation and psychosocial stress that included the cortisol awakening response suggests that general life and job stress is associated with elevated awakening cortisol (Chida & Steptoe, 2009). In contrast post-traumatic stress, fatigue, burnout, and exhaustion predict lower awakening cortisol. Thus, one possibility was that awakening and morning cortisol levels in shy versus non-shy adults would be relatively higher in anticipation of coping with the social demands of school and work. Alternatively, and equally plausibly, shy versus non-shy participants could demonstrate a relatively lower awakening and morning cortisol levels that might be evidence of a life history of coping with social anxiety.

2. Methods

2.1. Participants

Twenty-four (12 shy: 7 males/5 females; and 12 non-shy: 8 males/4 females) participants were drawn from a larger sample of 152 undergraduate university students (61 males, *M*age = 19.74 years; and 91 females, *M*age = 20.41 years) as part of a larger study examining the psychophysiological and neural correlates of social anxiety (Beaton, Schmidt, Schulkin, & Hall, 2010; Beaton et al., 2008b; Beaton et al., 2009). Participants received partial credit for their voluntary participation in the initial screening procedure and $100 remuneration for taking part in the larger study. All procedures were approved by the McMaster University Health Sciences Research Ethics Board.

Participants were screened for high and low shyness using the Cheek Shyness Scale (Cheek, 1983; Cheek & Buss, 1981). Shy and non-shy groups did not differ in age, $t(22) = 0.67$ ns, or sex composition, $X^2(1) = 1.50$ ns. These selected participants also completed self-report measures of social phobia (Social Phobia Inventory [SPIN]; Connor, Davidson, Churchill, Sherwood, & Weisler, 2000; and Social Phobia Scale [SPS]; Mattick & Clarke, 1998) in order to examine whether they approached clinical levels of social anxiety and to provide converging evidence of group classification.

2.2. Salivary cortisol collection

Participants were given saliva collection kits that consisted of 15 sterile 1.5 ml color-coded Nalgene cryotubes, a form for recording perceived stressful life events during the collection period, and a Palm III personal data assistant (PDA; Palm Inc., Sunnyvale, CA). Each tube was color coded according to requested saliva sampling time. To increase participant compliance, participants were asked to estimate their typical wake/sleep schedule to input reminder alarms into the PDA to prompt saliva collection. Participants were prompted both for time and color-code. Participants were asked to expectorate at least 0.75 ml of saliva on five occasions: (1) upon waking, but before arising from bed, (2) +60 min post-waking, (3) +8 h post-waking, corresponding to late afternoon, (4) +10 h post-waking, corresponding to early evening, and (5) bedtime – on each of 3 days that they were attending school. Samples were collected over multiple days to better characterize trait versus state factors of cortisol production and the CAR (Hellhammer et al., 2007). Participants were told not to modify their typical sleep schedules as an attempt to maintain ecological validity.

These procedures resulted in a high degree of variability both in terms of the start-of-day and length-of-day measurement periods, which is likely typical of young adults attending college. Twenty participants collected samples on Tuesday, Wednesday, and Thursday, and two participants collected samples on Tuesday, Wednesday, and Friday.

All participants were medication free and were asked to refrain from consuming excessive amounts of alcohol over the three days of participation and to refrain from smoking or consuming any caffeine, dairy, or citrus juices 2 h before collecting any of the saliva samples. Participants recorded the time of each sample and daily events that they perceived as stressful. While under participants' control, samples were stored in their home freezer. Following the last day of collection, saliva kits were returned to the laboratory, and samples were stored at −80 °C until assayed.

2.3. Enzyme-linked immunoassay (EIA)

Hormone assays from saliva were conducted at the Neuroendocrinology Laboratory at St. Joseph's Healthcare in Hamilton, Ontario. Samples were thawed, mixed, and centrifuged for 15 min at 1500*g*. Salivary cortisol concentrations were determined with a commercial competitive enzyme immunoassay kit that was optimized for saliva (HS-Cortisol High Sensitivity, Salimetrics®, LLC, State College, PA). Standards, controls, and samples were assayed in triplicate at a volume of 25 µl. All samples with a coefficient of variability that exceeded 15 percent were repeated ($n = 9$) as a