Low salivary cortisol levels among socially anxious young adults: Preliminary evidence from a selected and a non-selected sample

Elliott A. Beaton a,b, Louis A. Schmidt a,*, Andrea R. Ashbaugh a, Diane L. Santesso a, Martin M. Antony b,c,d, Randi E. McCabe b,c, Sidney J. Segalowitz e, Jay Schulkin f,g

a Department of Psychology, Neuroscience and Behaviour, McMaster University, 1280 Main Street West, Hamilton, Ont., Canada L8S 4K1
b Department of Psychiatry and Behavioural Neuroscience, McMaster University, Hamilton, Ont., Canada
c Anxiety Treatment and Research Centre, St. Joseph’s Healthcare, Hamilton, Ont., Canada
d Department of Psychology, Ryerson University, Toronto, Ont., Canada
e Department of Psychology, Brock University, St. Catharines, Ont., Canada
f Clinical Neuroendocrinology Branch, NIMH, NIH, Bethesda, Maryland, USA
g Department of Physiology and Biophysics, Georgetown University, School of Medicine, Washington, DC, USA

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Abstract

We investigated salivary cortisol levels among socially anxious adults in two separate non-clinical samples using different participant designs. Study 1 examined salivary cortisol, heart rate, and subjective measures of anxiety in response to a self-presentation task in undergraduate students meeting DSM-IV criteria for social phobia. Adults with social phobia displayed significantly lower salivary cortisol compared to their non-socially phobic counterparts, despite being more anxious. Study 2 examined the relation between trait shyness and salivary cortisol in a different sample of undergraduate students who were not selected for individual differences in personality. High trait shyness was related to low salivary cortisol. We speculate that
relatively low levels of salivary cortisol in socially anxious people may reflect how the adrenocortical system responds to social stress, allowing socially anxious individuals to cope and adapt to their environment.

Keywords: Salivary cortisol; Heart rate; Social stress; Shyness; Social anxiety; Social phobia

1. Introduction

Social phobia (also referred to as social anxiety disorder) is characterized by a marked and persistent fear of one or more social and performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others (American Psychiatric Association, 2000). It is a relatively common affliction with a lifetime prevalence of about 7% (Stein, Walker, & Forde, 1994), with fear of public speaking being a common feature of the disorder (Pollard & Henderson, 1988). Social phobia has been also linked to a number of distinct patterns of psychophysiological responses during baseline conditions and in response to social stress, including raised right frontal brain electrical activity (EEG) and increases in heart rate (see Davidson, Marshall, Tomarken, & Henriches, 2000; Schmidt & Schulkin, 1999).

Basal and reactivity measures of the stress hormone cortisol have been used to examine individual differences in clinical and non-clinical socially anxious profiles of children, adolescents, and adults. Much of the work in this area has found that high basal and reactive cortisol characterizes socially anxious profiles. For example, temperamentally shy and inhibited preschoolers (Kagan, Reznick, & Snidman, 1988; Schmidt et al., 1997), seven-year-olds (Schmidt et al., 1999b), and young (Windel, 1994) and older adults (Bell et al., 1993) are known to have high basal and/or reactive cortisol responses. Socially phobic adolescent females have been shown to exhibit increases in cortisol in anticipation of a socially stressful task (Martel et al., 1999). Still others have found increased cortisol reactivity in response to social and/or anticipatory stress (e.g., Antoni et al., 1990; Cameron & Nesse, 1988; Kirschbaum, Wüst, & Hellhammer, 1992). However, the relation between socially anxious profiles and high cortisol is not a foregone conclusion (Condren, O’Neill, Ryan, Barrett, & Thakore, 2002; Dabbs & Hopper, 1990; Hubert & deJong-Meyer, 1992; Kirschbaum et al., 1992; Schmidt, Fox, Schulkin, & Gold, 1999a; van Goozen et al., 1998). For example, some researchers have reported no differences in baseline cortisol between socially phobic adults and their same age- and sex-matched controls (Potts, Davidson, Krishnan, & Doraiswamy, 1991; Uhde, Tancer, Gelenter, & Vittone, 1994), but rather a hyper-reactive cortisol release in response to a social stressor (Condren et al., 2002). And still others have found lower cortisol reactivity in people with social phobia than in controls during a psychological stress test, despite the fact that the test produced subjective reports of anxiety (Furlan, DeMartinis, Schweizer, Rickels, & Lucki, 2001).

We attempted to clarify and extend previous findings examining the relation between social anxiety and salivary cortisol in young adults by using two separate convenience samples and participant designs. In Study 1, undergraduate students selected for high social anxiety and who met DSM-IV criteria for social phobia were compared with non-socially phobic adults on salivary cortisol, heart rate, and subjective anxiety measures during a baseline condition and in response to a social stress task. In Study 2, the relations between a measure of trait shyness and baseline salivary
cortisol were examined in a sample of undergraduate students who were not selected for individual differences in social anxiety and who had no history of psychiatric problems. We predicted high baseline and reactive salivary cortisol levels among socially anxious adults compared to non-socially anxious adults.

2. Study 1

2.1. Method

2.1.1. Participants
Participants were 330 (84 males, 246 females; \( M \) age = 20.3 yrs, SD = 4.2 yrs) undergraduate students enrolled in psychology courses at McMaster University. Participants completed a series of pre-screening questionnaires as part of a larger study examining the psychological and physiological correlates of social anxiety.

2.1.1.1. Participant selection. Of the 330 participants, 20 were selected based on their responses to the Social Phobia Inventory (SPIN; Connor et al., 2000): 10 most extreme and 10 least extreme, as determined by \( \pm 2 \) SD above and below the mean, respectively. Of these 20 students, 19 agreed to participate in the study. These 19 students were interviewed over the phone using the Social Phobia section of the Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon, & Williams, 1996). The SCID is a commonly used, clinician-administered, semi-structured interview that has been successfully administered over the telephone. This measure was administered by a trained clinician.

A chi-square analysis with SPIN (high, low) × SCID (clinical range, non-clinical range) confirmed that all 10 students originally selected for social phobia reported symptoms consistent with a DSM-IV diagnosis of social phobia using the SCID. None (0/9) of the students selected for non-social phobia met DSM-IV criteria for social phobia \([\chi^2(1) = 19.00, p < .00001]\). The socially phobic and non-socially phobic groups did not differ in sex composition \([\chi^2(1) = 1.31, \text{n.s.}]\), with two males and eight females in the socially phobic group and three males and six females in the non-socially phobic group.

2.1.2. Self-report measures

2.1.2.1. Social Phobia Inventory. The Social Phobia Inventory measure is a 17-item self-report measure of fear, avoidance, and physiological symptoms associated with social phobia and social anxiety (Connor et al., 2000). SPIN items focus on fears of talking and socializing with others, and fears of experiencing and exhibiting physiological signs of anxiety such as blushing or sweating. Good test–retest reliability and validity data for this measure have been established with clinical samples of individuals with social phobia (Connor et al., 2000).

2.1.2.2. Cheek and Buss Shyness Scale. Trait shyness was assessed using the five highest-loaded (Bruch, Gorsky, Collins, & Berger, 1989) items from the original Cheek and Buss Shyness Scale (Cheek, 1983; Cheek & Buss, 1981) to ensure that the groups also differed on a trait measure of social anxiety. An example item includes: “I find it hard to talk to strangers”. Responses to items
were scored on a five-point scale ranging from 0 ("not at all characteristic") to 4 ("extremely characteristic"). Reliability and validity data are presented elsewhere (Bruch et al., 1989; Cheek & Buss, 1981).

2.1.2.3. Speech Preparation Anxiety Scale. Subjective experience of anxiety during the self-presentation task was measured using the Speech Preparation Questionnaire (PREP; Ashbaugh, McCabe, Antony, Schmidt, & Swinson, 2005), a five-item self-report questionnaire. The PREP assesses nervousness, confidence, calmness, and preparedness of the individual before he or she gives a presentation, using a five-point Likert scale from 1 (not at all) to 5 (extremely). The questionnaire also assesses how well the respondent anticipates he or she will do (i.e., job quality) on a five-point Likert scale from 1 (poor job) to 5 (excellent job).

2.1.3. Procedures

All participants were tested at the Child Emotion Laboratory at McMaster University, and all procedures were approved by the McMaster University Research Ethics Board. All participants were asked to refrain from consuming excessive amounts of alcohol 24 h prior to the study and to refrain from smoking or consuming any caffeine 2 h prior to the study. Upon the participants’ arrival to the laboratory, informed consent was obtained and they were asked if they had any nicotine or caffeine within the previous 2 h. No participant reported having either nicotine or caffeine 2 h prior to arrival or alcohol in the previous 24 h. All participants were medication free and reported that they were not experiencing any extraordinarily stressful life events (e.g., recent death in family or prolonged sickness). An initial saliva sample was collected from participants approximately 10 min after arrival and after the participants had a chance to acclimate to the laboratory (i.e., Time 1: Baseline). Approximately 15 min after the speech instructions and prior to giving the speech, a second saliva sample was collected (i.e., Time 2: Speech Preparation). Three minutes of heart rate were then recorded, using two disposable electrodes attached to the participant’s forearms, immediately preceding the self-presentation speech. The participants then gave the 3 min speech.

After debriefing, a third saliva sample was collected from participants approximately 15 min after the speech (i.e., Time 3: Post-Speech). Saliva samples were collected approximately 15 min after the stressors because it takes this amount of time to observe changes in salivary cortisol levels following stress (Stansbury & Gunnar, 1994). All measures were taken in the afternoon or early evening between 1600 and 2000 h as salivary cortisol measures are relatively stable at this time. Time of day did not systematically vary between or within group. Upon completion of the study, participants were given an honorarium of $10.

2.1.3.1. Self-presentation task. All participants were asked to prepare for 10 min and then give a self-presentation task in which they would talk for 3 min about their opinions about classroom presentations (e.g., Do you think that classroom presentations reflect the true ability of students? Are they useful learning experiences for students?). The PREP scale was administered immediately after the 10 min preparation period. During the self-presentation task, if the participant stopped talking for more than 5 s, the researcher asked them one of 10 prompting questions (e.g., "What characteristics do you think make a good presenter?"). This speech was video recorded, and the
participant was told that his or her speech would be shown to a number of other participants as part of another study.

2.1.4. Heart rate collection and reduction

We examined heart rate just prior to the anticipated speech because it is a well-established and reliable indicator of autonomic arousal due to stress (see Berntson & Cacioppo, 2004, for review). A SA Instrument Bioamplifier was used to amplify the heart rate signal, with the bandpass filters set at 1 Hz (high pass) and 100 Hz (low pass). The heart rate data were digitized on-line at a sampling rate of 512 Hz. The heart rate data were scanned visually for artifacts (e.g., missing beats) and reduced using software developed by the James Long Company (ECG Analysis Program, Caroga Lake, NY). This program calculates the mean inter-beat interval (i.e., heart period) in milliseconds (ms). Heart period was computed separately for baseline and during the speech preparation task. Mean heart period for six participants (three socially phobic, three non-socially phobic) was missing due to excessive artifacts, electrodes detaching, or equipment problems.

2.1.5. Salivary cortisol procedures and assaying

2.1.5.1. Saliva collection. Salivary cortisol was used because it is non-invasive to collect and highly correlated with serum cortisol (Vining, McGinley, Maksvytis, & Ho, 1983). Each participant was given a piece of Trident regular sugarless gum and instructed to chew it for approximately 1 min to induce salivation. Next, after removing the gum, the participant was asked to expectorate at least .75 ml of saliva into a sterile 1.5 ml Nalgene cryotube. The saliva samples were stored at −80 °C until assayed.

2.1.5.2. Enzyme-Linked Immunoassay (EIA). Hormone assays from saliva were conducted at the Behavioural Endocrinology Laboratory in the Department of Biology at Queen’s University (Kingston, Ontario). Samples were thawed, mixed, and centrifuged for 15 min at 1500g. 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% were repeated (n = 9) as a singleton on another plate. The average of the triplicates was then used in subsequent analyses. On each plate, controls included saliva pools from four to seven-year-old children and from manufacturer-supplied controls. The coated plate was incubated at room temperature for 1 h in the presence of 200 μl of enzyme conjugate. Plates were then washed four times using a plate washer (Skanwasher™ 400, Molecular Devices, Sunnyvale, CA). Colour was developed in the presence of 200 μl of TMB (tetramethylbenzidine), with 25 min incubation in darkness at room temperature. Within 10 min of adding 50 μl of ‘stop’ solution, the plate was read at 492 nm and 450 nm with the optical density as the difference (VERSAmax™, Molecular Devices, Sunnyvale, CA). No individuals were ever split across a plate, so only intra-assay variance applies to within individual calculations across repeated samples. Intra-assay variability was calculated across each plate—after the standard curve, in the middle, and at the end. This calculation is more rigorous and accurate than the more common intra-assay variability calculation based on placement immediately after the standard curve. Intra-assay variability was calculated at both .46 μg/dl and 1.03 μl/dl, yielding intra-assay
coefficients of variability of 5.4% and 13.8%, respectively. Comparable inter-assay coefficients of variability were 14.6% and 16.4%.

2.2. Results and discussion

2.2.1. Manipulation check

To ensure that the self-presentation task elicited stress, a series of between-groups independent t-tests were performed separately on the five self-rating items of the PREP. As predicted, adults in the socially phobic group reported significantly more self-perceived nervousness \( t(17) = 5.60, p < .0001 \), less confidence \( t(17) = -2.75, p < .01 \), less calmness \( t(17) = -2.51, p < .01 \), and more concern with the quality of their preparations \( t(17) = 3.33, p < .01 \) compared with the non-socially phobic group. However, the groups did not differ in their perceived feelings of preparedness \( t(17) < 1 \).

Next, we examined autonomic arousal to the anticipated speech. An analysis of variance (ANOVA) with Group (Socially Phobic, Non-Socially Phobic) as a between-subjects factor and Condition (Baseline, Speech Preparation) as a within-subjects factor was performed. The dependent measure was mean heart period (in ms). The analysis revealed a highly significant main effect for Condition \( F(1,11) = 26.79, p < .0003 \). Both groups displayed a significantly shorter heart period from baseline \( (M = 762.49, SD = 106.51) \) to speech preparation \( (M = 669.02, SD = 98.76) \), indicating that the speech preparation task was stressful for both groups (i.e., a shorter mean heart period reflects a higher heart rate). The main effect for Group and the Group \( \times \) Condition interaction were not significant.

2.2.2. Self-reported shyness differences

As expected, a significant between-subjects t-test indicated that adults in the socially phobic group \( (M = 11.40, SD = 2.07) \) reported significantly higher trait shyness than adults in the non-socially phobic group \( [(M = .78, SD = 1.09); t(17) = 13.77, p < .0005] \).

2.2.3. Salivary cortisol differences

An ANOVA with Group (Socially Phobic, Non-Socially Phobic) as a between-subjects factor and Condition (Baseline, Speech Preparation, Post-Speech) as a within-subjects factor was performed on salivary cortisol level (in \( \mu g/dl \)). The analysis revealed a statistically significant main effect for Group \( [(1,17) = 5.59, p = .03] \). Adults in the socially phobic group exhibited significantly lower salivary cortisol over all three conditions compared with adults in the non-socially phobic group (see Fig. 1). The Group \( \times \) Condition interaction, however, was not significant \( (F < 1) \), suggesting that the groups did not differ on salivary cortisol reactivity. Nor was the main effect for Condition significant \( (F < 1) \).

We found that, although the anticipated speech presentation produced increases in subjective experience of anxiety and heart rate in undergraduate students selected for high social anxiety and who met DSM-IV criteria for social phobia, the stressor did not elicit increases in their salivary cortisol. Rather, the socially phobic group exhibited relatively lower salivary cortisol levels overall across all conditions compared with their non-socially phobic counterparts. These results are similar to other recent findings in which adults with social phobia exhibited no increase in cortisol during a psychological stress test, despite reporting increases in anxiety (Furlan et al., 2001).
3. Study 2

The purpose of Study 2 was to examine the relations between trait shyness and baseline salivary cortisol in a non-clinical convenience sample of undergraduate students not selected for individual differences in social anxiety. Participants were part of a larger study on the psychophysiology of cognition and emotion in which regional EEG, salivary cortisol, and subjective measures of personality were collected. The present analyses focus on the salivary cortisol and subjective measures of personality collected.

3.1. Method

3.1.1. Participants

Participants were 35 (9 males, 26 females; M age = 23 yrs, SD = 3.6 yrs) undergraduate students enrolled in introductory psychology courses at McMaster University. Participation was limited to students between 18 and 30 years of age. Participants received experimental course credit for their participation.

3.1.2. Procedures

All participants were tested at the Child Emotion Laboratory at McMaster University, and all procedures were approved by the McMaster University Research Ethics Board. Upon the participants’ arrival to the laboratory, the procedures were explained and informed consent obtained. After the participants had a chance to acclimate to the laboratory (10 min), two baseline saliva samples were collected: an initial saliva sample was collected (i.e., Time 1), and a second saliva...
sample was collected 15 min following the initial sample (i.e., Time 2) while the participants were waiting for the study to commence. A third saliva sample was collected after the baseline measures, following a visual discrimination task (i.e., Time 3, 15 min post-task), at which time they completed the same trait shyness self-report measure used in Study 1. The saliva collection procedures, sampling times, and participant restrictions were identical to those used in Study 1 as well as the cortisol assaying procedures. Time of day was not related to shyness. As well, there were no differences between males and females on the salivary cortisol or shyness measures ($p$’s < .05).

3.2. Results and discussion

Because the two initial baseline salivary cortisol samples were highly related [$r = .71, p < .0005$], they were averaged to form a composite measure of baseline average cortisol level. Pearson correlations were then computed between the Cheek and Buss trait shyness measure and baseline and

![Figure 2](image_url)

Fig. 2. Scatterplot of the relation between trait shyness and (A) baseline and (B) 15 min post-task salivary cortisol level (in µg/dl) in a sample of undergraduate students unselected for individual differences in personality.
15 min post-task salivary cortisol measures. Significant relations between trait shyness and salivary cortisol collected at baseline \( r(35) = -0.47, p = 0.002 \) and 15 min post-task \( r(35) = -0.35, p = 0.045 \) were found. Adults who self-reported high trait shyness had low salivary cortisol levels at baseline (see Fig. 2A), and this relation remained at 15 min post-task (see Fig. 2B).

Given the findings from Study 1 involving young adults selected for extreme social anxiety, we next examined the relation between individual differences in shyness and salivary cortisol in Study 2. We computed high versus low shy groups and high versus low cortisol groups using a median split on the trait shyness and baseline salivary cortisol measures, respectively. A chi-square analysis with Trait Shyness (high, low) \( \times \) Baseline Salivary Cortisol (high, low) revealed that, of the 17 adults classified as high shy, 71% (12/17) exhibited low salivary cortisol levels, while of the 18 adults classified as low shy, only 33% (6/18) exhibited low salivary cortisol levels \( \chi^2(1) = 4.86, p < 0.03 \). These preliminary results extend, in principle, the findings of Study 1 to a sample of young adults not selected for individual differences.

4. General discussion

What do relatively low levels of salivary cortisol in social anxiety reflect? There is a growing literature suggesting that low cortisol may reflect coping and adaptation following repeated stress (see Fries, Hesse, Hellhammer, & Hellhammer, 2005, for a recent review). A lack of cortisol reactivity is seen in individuals with post-traumatic stress disorder (PTSD; e.g., Heim, Ehlert, & Hellhammer, 2000; Yehuda et al., 1991). Gunnar and Vazquez (2001) have argued that traumatic experiences during early development could result in decreased cortisol responsiveness in adulthood, termed hypo-active cortisol response. We and others speculate that the pattern of relatively low salivary cortisol levels in non-clinical adults with social phobia or trait shyness may reflect a change in how their adrenocortical system responds to stress as a result of the individual coping with a life-long history of shyness and social anxiety, resulting in a high allostatic load (Schulkin, Gold, & McEwen, 1998). One coping strategy of a dysregulated adrenocortical system may be reflected in a hyporesponsivity of the system, allowing these individuals to effectively mount successful immunological defences in the face of prolonged glucocorticoid-driven immunosuppression (see Fries et al., 2005, for a review). The socially phobic and shy adults in Studies 1 and 2 were non-clinical and functioning at a high level (i.e., attending classes and presumably interacting with peers, faculty, and staff). Suppression of so-called ‘sickness behaviours’ (Hart, 1988; Maier & Watkins, 1998) such as fatigue, pain susceptibility, anorexia, and decreased activity may allow these individuals to successfully cope with the complex social demands and responsibilities of a university setting. We further suggest that the socially phobic and shy individuals in the present study could represent a hypothetical adult cross-section of temperamentally shy children, such as those observed in studies by Schmidt and his colleagues (for a review, see Schmidt & Schulkin, 1999). Although we do not know if the adults tested in the present study were temperamentally shy children, it is well-documented that many adults with social phobia and high trait shyness were temperamentally and extremely shy as children (Beidel & Turner, 1998).

Given that these preliminary findings were based on only one time point (early afternoon saliva collection) as well as a relatively small number of participants and failure to control oral contraceptives in females which are known to influence cortisol levels (Kirschbaum, Pirke,
Hellhammer, 1995), future work needs to incorporate a longitudinal design with repeated measurements, a larger number of participants, and control for oral contraceptives than used in the present study to ensure the reliability and generalizability of these preliminary results. Because cortisol is highest upon waking in temperamentally shy children (e.g., Kagan et al., 1988; Schmidt et al., 1997), future research should do repeated cortisol measurements throughout the day in shy adults to see if levels vary throughout the day. Also because these findings are from non-clinical undergraduate samples, they may differ from a clinical treatment-seeking sample, so extending these findings to a clinical sample of social phobics is necessary.

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