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Use of a resting control day in measuring the cortisol response to mental stress: Diurnal patterns, time of day, and gender effects

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Summary In laboratory studies of individual differences in stress reactivity, cortisol responses are typically measured by comparing a prestress baseline with values obtained at the end of the stressor. In the present study, we measured cortisol in this manner on a stress day, but we also took samples on a second day when the volunteers rested in the lab at the same time of day and for the same duration. We compared stress responses as the difference from pre- to poststress and also poststress vs. rest day control. The latter method allowed a greater appreciation of how stress perturbed the underlying diurnal baseline. Although the effect of stress was statistically significant when measured as the change from pre- to poststress, the magnitude of the effect was 54% larger when measured against the time-of-day control from the rest day. In particular the diurnal control method provided a wider range of stress values that potentially provide a greater range of response values in carrying out analyses of individual differences.
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Cortisol is highly reactive to events that may threaten the system's homeostasis, and as such the response of the hypothalamic-pituitary-adrenocortical axis is considered the core of the stress response (Selye, 1936). In keeping with this, a healthy, or normative, stress response is considered essential to homeostasis (de Kloet, 2003), and the integrity of the cortisol response to stress is considered an indicator of a normal state of health (McEwen, 2000). The assessment of cortisol stress responses in the laboratory is a common method for comparing stress reactivity in groups of individuals differing in significant characteristics such as sex, age, and health status (al'Absi et al., 1994; Kirschbaum et al., 1999; Lupien et al., 1999; Cacioppo et al., 2000; Burleson et al., 2003).

Studies examining cortisol stress reactivity usually obtain data on a single occasion in which levels of cortisol prior to a stressor are used as a baseline for levels obtained during or

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after stress exposure (Kirschbaum et al., 1999; Dickerson and Kemeny, 2004). However, cortisol's well characterized diurnal cycle shows that cortisol levels in blood or saliva are not stable over time, but are high just after awakening and decline over the waking hours (Czeisler and Klerman, 1999; Kunz-Ebrecht et al., 2004). This lack of a stable underlying baseline poses interpretive problems and also presents research opportunities if its effects are taken into account when evaluating stress reactivity effects.

In this paper we present cortisol responses to psychological stress derived from a large data set collected on healthy young adults, and we use these to illustrate the value of comparing data collected on a day of stress compared to a resting control day. The analyses illustrate how the measurement of stress reactivity may benefit from using a diurnal control as the baseline instead of the prestress value. We illustrate this by examining the presence of an anticipatory response on a single stress day and by comparing men and women and the effects of time of day of testing.

1. Methods

1.1. Overview

The present data are derived from the Oklahoma Family Health Patterns Project, an ongoing study of the psychological and physiological characteristics of young adults with and without a family history of alcoholism. The volunteers are screened for present substance use disorders and psychiatric conditions and are in current good health. As part of this study every volunteer who met inclusion criteria, visited the lab on two days, first on a stress day and then on a resting control day. Because of the number of volunteers (N = 324) and due to the use of a consistent protocol, the data set provides a useful resource for assessing the characteristics of the stress response in healthy young adults.

1.2. Subjects

The present sample includes 187 females and 137 males, 18–30 years of age and in good health, who were recruited through community advertisement from the general population of Oklahoma City, OK, USA. They were 78% European American, 12% African American, 4% Native American, 3% Hispanic, and 3% other race and ethnicity. The participants were (mean ± SEM) 23.7 ± .24 years of age, with males being 24.1 ± .43 years and females being 23.4 ± .22 years (p = .2, by Student's t test), and as a group had 15 ± .3 years of education. All participants signed a consent form approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center and the Veterans Affairs Medical Center in Oklahoma City, OK, USA, and were paid for participating.

1.3. Inclusion and exclusion criteria

Prospective volunteers were excluded if they had any of the following: a history of alcohol or drug dependence; met diagnostic criteria for substance abuse within the past 2 months; had current use of any abused drug assessed by a urine drug screen and alcohol breath test on each day of testing; had any current Axis I disorder as defined by the Diagnostic and Statistical Manual of Mental disorders, 4th ed. (American Psychiatric Association, 1994) and assessed by the Diagnostic Interview Schedule. All participants were in good health as determined by self-report, had a body mass index < 30, had normal hearing assessed by audiologic exam, were taking no prescription medications at the time of testing, and to had no reported history of serious medical disorder, including neurological disorders, cardiovascular diseases, or diabetes

Because cortisol secretion is dependent on the sleep-wake cycle (Czeisler and Klerman, 1999), all volunteers were required to have a normal work or school schedule and to have a nighttime sleep pattern. Also, because acute cortisol secretion is affected by prevailing blood sugar levels (Dallman, 2003), all volunteers ate a standard meal prior to beginning the protocol. This consisted of a small breakfast for morning volunteers and a small lunch for afternoon volunteers prepared by the campus General Clinical Research Center bionutrition core. Women were excluded if they were pregnant based on self-report and were required to have a negative urine pregnancy test on each day of testing. Smoking and smokeless tobacco use were not exclusionary.

1.4. Study design and procedure

After screening, subjects visited the lab two more times for behavioral and psychophysiological testing, and each subject was tested at the same time on both days, either in the morning at 0900 h or in the afternoon at 1300 h. In all cases, the first day of the study involved a stress procedure and the second day was designated a resting control day. Subjects were clearly briefed on this order of testing on their screening day in order to maximize the effect of anticipation of the stress procedure on day 1 and to ensure that day 2 was a rest day free of the effects of novelty or anticipation. The description of the stress procedure given on the screening day was that the subject should expect to deliver three short speeches to a member of the lab staff and that they would also undergo a mental arithmetic task. No further detail was provided until the stress day proper.

1.5. Stress protocol

The stress protocol consisted of a 30-min prestress baseline, during which time the subject sat quietly and read general interest magazines, followed by 45 min of behavioral stress. Stress included simulated public speaking (Saab et al., 1989) followed by mental arithmetic (al'Absi et al., 1997). The speech task (30 min) included three successive speeches prepared and delivered with no breaks. At the start of each speech period, the subject was given a card with a topic written on it and told that they had 4 min to prepare a speech without making notes and 4-min to deliver it from memory. To increase the sense of realism, the speech was observed by a white-coated experimenter holding a clipboard and with a nearby video camera set to the record mode. The subject was told that his or her speech would be shown to the laboratory staff and that they would judge the subject's fluency of delivery and how convincing their speech was. The speech topics included recounting an article on why hair turns gray,