Case Study of Salivary Cortisol as a Stress and Relaxation Marker

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Introduction

Based on recent development in molecular analysis techniques for tiny amounts of human secretory substances, several attempts have evaluated the physiological effects induced by mental stress and/or relaxation. Such psychophysiological studies have drastically advanced over the last decade, forming such inter-disciplinary scientific fields called “Psychoneuroendocrinology” and/or “Psychoneuroimmunology” (Ader, Felten & Cohen, 2001).

This new field of study is important for two aspects. One is as science; it is truly an interdisciplinary scientific field covering psychology, physiology, and especially cognitive science. Recent studies have revealed that such effects on human secretions are more than mere reflections of given psychological stimuli, because higher cognitive processes including individual personality can mediate those effects (Valdimarsdottir & Stone, 1997; Bosh, Ring, de Geus et al., 2002). The second reason is purely for newly developing methodology; human secretory substances can serve as new physiological indices to evaluate human mental states, applicable with conventional methods of electrophysiological indices, such as EEGs, ECGs, and EOGs.

However, even though previous studies successfully observed physiological changes in the human body caused by mental stress, such as an enhancement of hormonal and/or immune secretion against various stressful tasks and its reduction by relaxing factors; those reported results were still less consistent with each other, especially related to relaxing factors. Several reasons might explain the inconsistencies. For example, the modulation of secretory substances within the human body has turned out much more sensitive in terms of reaction speed and the variation of stressful or relaxing factors than previously thought. Therefore, for further investigation, designing systematic, well-controlled, and reproducible experiments is quite important.

In this study, we introduced human salivary cortisol, one major possible physiological index for the evaluation of mental stresses, and targeted the following two issues: Is salivary cortisol a useful physiological index for 1) a stressful workload and for relaxing with music?

Salivary Cortisol

Cortisol is the most potent glucocorticoid produced and secreted by the adrenal cortex. Activation of the hypothalamic-pituitary-adrenal axis (HPA axis) is considered a major biological pathway against acute mental stress, which increases glucocorticoid secretion from the adrenal cortex; therefore, cortisol is assumed to be one possible mental stress marker. Cortisol levels can be measured in serum, urine, and saliva. Past cortisol in saliva studies preferred investigating the physiological changes induced by psychological states because cortisol in saliva reflects free cortisol concentrations in serum. In addition, this approach has several methodological advantages, including the noninvasive collection of saliva, reduced stress and repeatable sampling unlike blood. Several review articles concluded that, for both scientists and clinicians, cortisol in saliva is a useful index for investigations of chronic and acute stress reflected in HPA axis activity (e.g., Kirschbaum & Hellhammer, 1994).

However, although most previous studies, which focused on physiological responses induced by mental stress, have reported increases of salivary cortisol levels, results are still inconsistent. Moreover, those reported responses induced by relaxing factor are rather controversial because of fewer studies, varieties of stressors and experimental conditions, uncontrolled possible mediators, the quantitative determination of salivary cortisol, and statistical analysis. Thus, to advance this field of study, we designed a well-controlled experiment that focused on the relaxing treatment of music assessed after acute stressful workloads.

Method

For this study ten healthy male students were recruited, ranging from 25 to 33 years old. Subjects were instructed to conduct 30-minute stressful workload of a simple calculation task; after that they were exposed to seven minutes of music, noise, or silence. Each subject daily experienced one of the three types of experimental procedures: calculation-music, calculation-noise, and calculation-silence. The order of types of experiment was counterbalanced. All experiments were individually conducted in a dark, soundproof room.
Saliva samples were taken by salivette cotton before/after calculation tasks, and after each music, noise, or silence experience. Subjects were instructed to place the cotton under their tongues without chewing, for three minutes. Cortisol level was determined by enzyme-linked immunosorbent assay (ELISA) (Cortisol Elisa Kit #EA 65, Oxford Biomedical Research, Inc., USA).

**Results**

The mean salivary cortisol of the pre- and post-stress levels significantly decreased (no figure shown). The mean (standard deviation) of pre- and post-stress is 0.78 (0.45) and 0.66 (0.39). The salivary cortisol level of post-stress was significantly smaller than pre-stress by paired samples t test (p = .044).

The change of mean salivary cortisol from post-stress to post-treatment of music, noise, and silence is shown in Figure 1., where the error bar represents standard error. A two-way analysis of variance (ANOVA) on 2 × 3 (time × treatment) indicated the significant main effects of treatment (p = .0061). As a further analysis, a post hoc Least Significant Difference (LSD) test was applied that indicated significant differences between music and silence conditions (p = .0021) and between noise and silence conditions (p = .0155). The salivary cortisol mean also showed a tendency to decrease after music treatment.

**Discussion**

The results of our decreasing salivary cortisol experiments after 30 minutes of stressful workload as an acute stressor are inconsistent with past studies that reported an increase of salivary cortisol levels after stressful interventions. However, several studies reported decrease or no-change of cortisol levels. In addition, some studies reported controversial results, e.g., serum cortisol increased equally in response to both negative and positive mood inductions (Brown, Sirota, Niaura, et al., 1993). Far more research on the effects of stress on human salivary and/or serum cortisol are necessary to conclude whether cortisol is a useful stress marker.

Another observed result of our experiments is the differences of salivary cortisol levels among treatments after stressful tasks. The reduction of cortisol levels by music is consistent with several past studies. In addition, it seems very consistent with purely psychological studies on the effects of music that mostly relied on subjective questionnaires, literally and empirically, suggesting cortisol’s usefulness as a relaxing marker.

Note that the significant differences of salivary cortisol levels might reflect the relatively short seven-minute treatment period. Physiological change induced by psychological induction thus might occur much faster than previously thought. Generally, studies targeting psychophysiological correlation, treatment (and/or intervention) last about 10 to 30 minutes, and pre- and post-physiological indices are taken for evaluation. Considering the drastic cortisol changes observed in our experiments, more frequently sampling and shorter treatment should be assessed.

![Figure 1: Change of mean salivary cortisol from post-stress to post-treatment.](image)

**Conclusion**

We admit the possibility of an uncountable potential mediator in our experiment, e.g., personality, chronic stresses, sex, age, or methodological varieties. In addition there are varieties of possible stress markers, such as amylase (Yamaguchi, Kanemori, Kanemaru, et al., 2004; note that α-amylase is suggested to responds only for distress, not for eustress.), free MHPG, and chromogranin.

Additional systematic and integrated research promises to create a reasonable criterion for human mental stress and relaxation, but it is not necessary to believe that changes of the human mental state are continuous in time series.

**References**


