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Tactics for modeling multiple salivary analyte data in relation to behavior problems: Additive, ratio, and interaction effects

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Summary Individual differences in the psychobiology of the stress response have been linked to behavior problems in youth yet most research has focused on single signaling molecules released by either the hypothalamic—pituitary—adrenal axis or the autonomic nervous system. As our understanding about biobehavioral relationships develops it is clear that multiple signals from the biological stress systems work in coordination to affect behavior problems. Questions are raised as to whether coordinated effects should be statistically represented as ratio or interactive terms. We address this knowledge gap by providing a theoretical overview of the concepts and rationales, and illustrating the analytical tactics. Salivary samples collected from 446 youth aged 11—12 were assayed for salivary alpha-amylase (sAA), dehydroepiandrosterone-sulfate (DHEA-s) and cortisol. Coordinated effect of DHEA-s and cortisol, and coordinated effect of sAA and cortisol on externalizing and internalizing problems (Child Behavior Checklist) were tested with the ratio and the interaction approaches using multi-group path analysis. Findings consistent with previous studies include a positive association between cortisol/DHEA-s ratio and internalizing problems; and a negative association between cortisol and externalizing problems conditional on low levels of sAA. This study highlights the importance of matching

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analytical strategy with research hypothesis when integrating salivary bioscience into research in behavior problems. Recommendations are made for investigating multiple salivary analytes in relation to behavior problems.

1. Introduction

Numerous studies link individual differences in the psychobiology of the stress response to variation in the degree of behavior problems in youth (Granger et al., 1996; McBurnett et al., 2000). These advances are due, at least in part, to improved measurement methodologies that allow biomarkers and analytes to be assessed from children noninvasively through collection of oral fluid specimens (see review Granger et al., 2012). As our understanding about biobehavioral relationships has developed it is clear that multiple signals from the physiological systems involved in the stress response have the potential to be associated with behavior problems in children (Goodyer et al., 1998; Chen et al., in press). However, in practice, most research in child behavior problems that includes salivary measures has focused on single signaling molecules released by either the hypothalamic–pituitary–adrenal axis (e.g., cortisol, dehydroepiandrosterone) or the sympathetic branch of the autonomic nervous system (alpha-amylase). It has been argued that the focus on single salivary analytes limits conclusions because it is likely that multiple components of stress responsive systems work in coordination to relate to behavior problems (Granger et al., 2012).

Largely for this reason, a ''multiple system measurement approach'' now reflects the current state of the art for salivary bioscience in the study of child behavior problems. When multiple salivary analytes are assessed, however, investigators are faced with a new set of challenges. The capacity to assess multiple measurements of biological systems raises questions as to whether the combined effects of the measured parameters should be represented in statistical models as simple additive effects, or as coordinated effect as captured by ratios or interactive terms. Surprisingly, the literature provides little guidance on this set of issues. In this report, we address this specific knowledge gap and provide recommendations for best practices related to analytical strategy and tactics.

1.1. Stress system specialization and connections

The two main components of the physiological stress response are the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic branch of the autonomic nervous system (ANS) (Chrousos and Gold, 1992). The ANS is designed to produce a rapid ''fight or flight'' response, preparing the body to actively cope with a stressor through effects on the cardiovascular and respiratory systems and the release of stored catecholamines. The HPA system activates a slower cascade of secretory signals that culminate in the release of secretory products from the adrenal gland, adapting the body to stress conditions by inhibiting non-emergency vegetative processes such as sleep, sexual activity, and growth (Weiner, 1992).

Salivary cortisol and dehydroepiandrosterone (DHEA) or dehydroepiandrosterone-sulfate (DHEA-s) offer noninvasive measures of HPA activity (Granger et al., 1999; Hellhammer et al., 2009), and sAA, an enzyme produced in response to activation of ANS innervation of the salivary glands, is used as a surrogate marker of autonomic activity (Granger et al., 2007; Nater and Rohleder, 2009). Our review of the literature reveals that most researchers who study the combined effects of DHEA(-s) and cortisol use ratios (Goodyer et al., 2003; Markopoulou et al., 2009), and most who study the combined effects of sAA and cortisol use interactive terms (Gordis et al., 2006; El-Sheikh et al., 2008; Chen et al., in press). Generally, there is operational consensus in these methods but scientific justification (with few exceptions) at the conceptual level is largely absent.

1.2. Additive, interaction, and ratio approach: theoretical overview

The simplest approach to examine the combined effects of multiple salivary analytes is to assume that the effects of analytes on the outcome are additive in a linear way. Their effects can either be independent or overlap to a certain degree. The additive effect can be represented as a linear combination of the two analytes. It can be easily tested by examining the main effects of the two analytes on behavioral outcome within one model.

In comparison to a linear additive effect, coordinated effect assumes that the two analytes work in coordination rather than in a linear sum fashion. That is, the strength and/or the direction of the relation between one analyte (X) and the behavioral outcome (Y) is qualified or conditional on the strength of the other analyte (Z) as shown in Eqs. (1) and (2). Coordinated effect can be captured by ratio or interactive term. The two approaches differ in terms of their statistical formation and their applicability to test different research hypotheses.

$$Y = a_0 + d_0 \times \frac{X}{Z} + \varepsilon_0 = a_0 + \left(d_0 \times \frac{1}{Z}\right) \times X + \varepsilon_0 \tag{1}$$

$$\begin{aligned} Y &= a_1 + b \times X + c \times Z + d_1 \times X \times Z + \varepsilon_1 \\ &= a_1 + (b + d_1 \times Z) \times X + c \times Z + \varepsilon_1 \end{aligned} \tag{2}$$

For a ratio approach (see Eq. (1)), one unit change in X corresponds to $d_0 \times 1/Z$ unit change in behavioral outcome, and such change (i.e., $d_0 \times 1/Z$) is a non-linear function of Z, as seen in Fig. 1a. In contrast, for an interaction approach (see Eq. (2)), a one unit change in X corresponds to $b + d_1 \times Z$ unit change in behavioral outcome, and such change (i.e., $b + d_1 \times Z$) is a linear function of Z. In other words, although the relation between X and Y is not linear, the change of the slope is a linear relation, as shown in Fig. 1b. Moreover,