Psychoneuroendocrinology (2009) **34**, 633—643

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Salivary alpha-amylase as a longitudinal predictor of children's externalizing symptoms: Respiratory sinus arrhythmia as a moderator of effects

Peggy S. Keller [a,*], **Mona El-Sheikh** [b]

[a] *Department of Psychology, University of Kentucky, KY 40506, United States*
[b] *Department of Human Development and Family Studies, Auburn University, AL 36849, United States*

Received 29 July 2008; received in revised form 9 December 2008; accepted 29 December 2008

KEYWORDS
Alpha-amylase;
Sympathetic nervous system;
Parasympathetic nervous system;
Respiratory sinus arrhythmia;
Externalizing;
Vagal tone

Summary Salivary alpha-amylase (sAA) was examined as a predictor of children's externalizing symptoms cross-sectionally when children were in the 3rd grade (T1; *N* = 64) and again in the 5th grade (T2; *N* = 54) and longitudinally over two years. Parasympathetic nervous system (PNS) activity, indexed by respiratory sinus arrhythmia (RSA), was examined as a moderator of the sAA and child externalizing link. Participants were healthy, typically developing children, 34% of whom were African American and the rest European American. At each time point, saliva samples were collected during afternoon laboratory visits and assayed for sAA. Children's RSA was measured during baseline conditions and in response to an inter-adult argument and a star-tracing task. Cross-sectional associations between sAA and externalizing symptoms at T1 and T2 were moderated by PNS functioning. Longitudinally, sAA was directly associated with changes in externalizing symptoms in a non-linear fashion. Specifically, lower externalizing symptoms were predicted for children with moderate levels of sAA, but higher externalizing was predicted for children with higher or lower levels of sAA. Findings highlight the importance of the contemporaneous assessment of SNS and PNS functioning in the prediction of child psychopathology, and the need to examine curvilinear relations between ANS functioning and behavior.

Individual differences in sympathetic (SNS) and parasympathetic (PNS) nervous system activity are important for elucidating children's adjustment and developmental psychopathology. Externalizing symptoms represent a particularly important form of maladjustment for investigation. Externalizing behaviors include aggression, delinquency, and hyperactivity (Liu, 2004). Low levels of externalizing behaviors are often common in children; however, high levels of externalizing symptoms may meet criteria for clinical diagnoses such as a conduct disorder or antisocial personality. Externalizing problems in childhood predict violence and criminal behavior in adulthood (Farrington, 1989), and thus the elucidation of factors contributing to their development is critical.

Certain patterns of arousal and regulation that may be either inherited and/or acquired through experience may contribute to or protect against externalizing problems. In this study, we focus on the two main branches of the autonomic nervous system (ANS): the sympathetic (SNS) and

* Corresponding author. Tel.: +1 334 844 3294; fax: +1 334 844 4515.
E-mail address: elshemm@auburn.edu (P.S. Keller).

0306-4530/$ — see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.psyneuen.2008.12.016

parasympathetic (PNS) nervous systems. Individual differences in SNS activity can now be measured non-invasively in children's saliva, and were examined in this study via salivary alpha-amylase (sAA; Granger et al., 2006, 2007). Salivary AA is an enzyme produced by the salivary gland, and is a marker of the adrenergic component of the stress response. Salivary AA levels increase under stressful conditions known to increase plasma catecholamines, heart rate, systolic blood pressure, cardiac output, and PEP (Chatterton et al., 1997; Skosnik et al., 2000; West et al., 2006), and thus are linked to SNS activation.

SNS activity has been linked empirically to externalizing behavior problems, but most of these studies have relied on electrodermal activity or pre-ejection period (PEP) to index SNS activity. According to the results of a recent meta-analysis (Lorber, 2004), individuals with conduct problems exhibit lower resting electrodermal activity or underarousal within the same time point; longitudinal research is also supportive of this association (Raine et al., 1990). Children with lower baseline SCL than controls in middle childhood have greater externalizing symptoms in adolescence (van Bokhoven et al., 2005). Cross-sectional research shows that preschoolers, elementary-age children, and adolescents with clinical levels of conduct problems exhibit attenuated baseline levels of SNS-linked cardiac activity (i.e., lengthened cardiac pre-ejection period, PEP) at baseline and during reward conditions (Beauchaine et al., 2007; Crowell et al., 2006).

Very few studies have utilized sAA as the SNS marker, and no study has so far reported longitudinal relations between sAA and child externalizing behavior. The main objective of this study is the examination of relations between sAA and externalizing behavior in children cross-sectionally and longitudinally over two years. Given the individual specificity phenomenon in physiological responses (Stern and Sison, 1990), in which some individuals tend to exhibit arousal in some particular physiological domains, ascertaining biobehavioral connections with different SNS indices (cardiac, electrodermal, and salivary) is of importance. Emerging cross-sectional evidence linking resting sAA with functioning in infants, preschoolers, children, and adolescents (El-Sheikh et al., 2008; Granger et al., 2006, 2007) is supportive of further investigation of sAA as a correlate and predictor of child functioning. Notably, resting sAA has been more frequently examined in prior research than sAA reactivity, and the focus of the current study is on measures of sAA obtained during a visit to the laboratory that is not in response to challenge protocol.

The current study examines linear and curvilinear associations. Examination of curvilinear associations between physiological and behavioral outcomes is very important yet understudied in the literature. Non-linear and U-shaped associations have been found linking physiological responses such as cortisol (e.g., Abercrombie et al., 2003; McBurnett et al., 2000), and physiological arousal including heart rate and skin conductance (e.g., Gilbert, 1998), with behavioral and cognitive outcomes, at least cross-sectionally. Examination of curvilinear associations among variables is therefore likely to shed light on the nature of relations between sAA and children's adjustment.

Although examining the activity of individual physiological systems is important for clarification of developmental psychopathology processes, physiological systems do not operate in isolation. A better understanding of links between children's adjustment and physiological processes is likely attained by considering the joint influence or interaction between SNS and PNS activity (El-Sheikh et al., in press). Thus, another goal of the present study is to examine PNS activity, indexed by both RSA and RSA reactivity to challenge (RSA-R), as a moderator of the relation between sAA and child externalizing behavior cross-sectionally and longitudinally.

RSA is the rhythmic fluctuation of heart rate during spontaneous breathing and is considered an index of the impact of the myelinated vagus nerve on the heart (Porges, 1995). RSA is an appropriate index of vagal activity for research with normally developing children (Grossman and Taylor, 2007). RSA reflects the status of the PNS at rest, and perhaps the ability to focus attention, engage in social communication, and maintain homeostasis under normal circumstances (Porges, 1991, 2007). Lower RSA has been associated with externalizing behaviors in clinical samples of children (see Beauchaine et al., 2001) but such associations are not often found in community samples (Calkins et al., 2007; El-Sheikh, 2001, 2005a; Graziano et al., 2007). These inconsistent findings highlight the need for additional research. RSA withdrawal (lower RSA) in response to environmental challenges represents parasympathetic inhibition and reflects awareness of environmental demands and the mobilization of physiological and attentional resources to mount an active stress response (Bornstein and Suess, 2000; Porges, 2007). Conversely, RSA augmentation (higher RSA) in response to challenge reflects PNS activation, and may index a failure to generate physiological resources that promotes engagement with stressors. Less pronounced RSA withdrawal (or increased augmentation) has been associated with externalizing behavior problems in children (El-Sheikh et al., 2001; Calkins and Dedmon, 2000). Both RSA and RSA-R have been examined extensively in prior research, and both appear to have important links with child behavior. However, these associations are not always similar in nature, and thus the current study includes measures of both RSA and RSA-R.

Researchers have developed frameworks for understanding the joint operation of PNS and SNS activity. Beauchaine (2001) posited that aggression may be characterized by low SNS activity accompanied by either low vagal tone or abnormally high vagal withdrawal, which both reflect PNS inhibition. Berntson and colleagues (Berntson et al., 1991; Berntson and Cacioppo, 2004) proposed another model concerning the joint action of the SNS and PNS in which reactivity across these systems can be characterized as reciprocal or nonreciprocal. Reciprocal activation refers to conditions under which the two ANS branches facilitate the same directional response in a target organ or system (e.g., cardiovascular system). *Reciprocal sympathetic activation* involves SNS activation and PNS inhibition, both of which upregulate physiological processes such as cardiovascular arousal. Conversely, *reciprocal parasympathetic activation* is characterized by SNS inhibition in conjunction with PNS activation, both of which down regulate physiological arousal and have calming functions. Nonreciprocal activation refers to conditions under which ANS branches promote opposing responses. Specifically, *coactivation* refers to increased SNS and PNS activity, and *coinhibition* refers to decreased activity in both ANS branches. Because the SNS and PNS serve opposing