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Short Communication

Validation of autonomic and endocrine reactivity to a laboratory stressor in young children

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ABSTRACT

The validation of laboratory paradigms that reliably induce a stress response [including hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) activation], is critical for understanding how children's stress-response systems support emotional and cognitive function. Early childhood research to date is markedly limited, given the difficulty in establishing paradigms that reliably induce a cortisol response. Furthermore, research to date has not included a control condition or examined concurrent ANS reactivity. We addressed these limitations by characterizing the extent to which a modified matching task stressor paradigm induces HPA and ANS activation, beyond a closely matched control condition. Modifications include an unfamiliar and unfriendly assessor to increase the stressful nature of the task.

Results validate the matching task as a laboratory stressor, with significant differences in HPA and ANS responsivity between conditions. The Stressor group exhibited a cortisol increase post-stressor, while the Control group was stable over time. Children in both conditions exhibited reduced parasympathetic activity to the first-half of the task, but in the second-half, only children in the Stressor condition, who were experiencing exaggerated signals of failure, exhibited further parasympathetic decline. The Stressor condition induced higher sympathetic activity (versus Control) throughout the task, with exaggerated second-half differences. Within the Stressor condition, responsivity was convergent across systems, with greater cortisol reactivity correlated with the magnitude of parasympathetic withdrawal and sympathetic engagement. Future research employing the matching task will facilitate understanding the role of HPA and ANS function in development.

Research on children's acute stress-response has grown markedly, with function of both the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) linked to numerous health and well-being domains. Such work has been facilitated by standardized laboratory paradigms, which are key to establishing how flexible biological engagement supports emotional and cognitive function (Gunnar et al., 2009). A paradigm's effectiveness in eliciting temporal cortisol increases, consistent with HPA activation, has been considered a benchmark for stressor validation. Utilizing such benchmark measures is important to ensure engagement across systems when examining the interplay between biological markers of stress-reactivity.

Though numerous paradigms induce frustration or anxiety, few reliably elicit mean elevations in cortisol. There is a dearth of well-validated laboratory paradigms for early childhood (~3–6 years), during which children undergo rapid development and important transitions such as school-entry (Blair, 2002). Following a comprehensive review, Gunnar et al. (2009) identified only two promising paradigms in this age range.[1] One has limited practical utility given an 80-min implementation time and complex task battery (van Goozen et al., 1998). The second, which employed both success and failure trials during a cognitively-demanding 'matching task,' only induced a cortisol response in children exhibiting embarrassment or shame (Lewis and Ramsay, 2002). After modifying this

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[1] Since this review, one paradigm was developed for 5–6 year old children that induced a cortisol response, but research did not include ANS measures or a control condition (De Weerth et al., 2013).

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paradigm with blocked reward and repeated failure, Kryski et al. (2011) demonstrated a mean cortisol response, when assessed in home. Tolep and Dougherty (2014) modified this in the laboratory using an assessor familiar to the child, but did not elicit a mean cortisol response (though small increases were noted, adjusting for individual differences in highest cortisol measurement 20–50 min post-stressor).

Although this matching task paradigm is promising, research to date has not included a control condition to demonstrate that the matching task engages the HPA-axis significantly more than other non-stressful tasks, which is critical given that cognitively-demanding tasks engage the HPA-axis for some children (e.g. Piccolo et al., 2016). Research in adults emphasizes the need for control conditions matched for cognitive and motor demands to understand the effects of psychosocial stress, as opposed to cognitive load or fatigue, on subsequent behavior (Het et al., 2009).

Furthermore, prior matching task research has not characterized ANS responsivity, perhaps due to the relative focus on cortisol within the stressor literature (Kryski et al., 2011; Tolep and Dougherty, 2014; Gunnar et al., 2009). Given that the ANS is critical for the short-term mobilization of the 'fight or flight' response as well as arousal regulation, we argue that ANS activation should be on par with HPA activation as a benchmark for laboratory stressors. Here, we used a modified matching task to assess children's stress-reactivity, as measured by both HPA-axis and ANS (parasympathetic and sympathetic) function, versus a closely controlled non-stressful condition. Prior matching task research conducted in-home has utilized some of the key cortisol-inducing task features (i.e. unpredictability, uncontrollability, social-evaluative threat; Dickerson and Kemeny, 2004) through techniques such as blocking the child's ability to succeed and providing feedback about the child's failure to earn a preferred toy. However, when this task was employed in-lab, it failed to produce a robust cortisol response (Tolep and Dougherty, 2014). Consistent with social-evaluative threat paradigms in older samples (i.e. the Trier Social Stress Task; Kudielka et al., 2007), we hypothesized that employing an unfamiliar, unfriendly assessor who used non-reinforcing language and stern, flat affect would be essential to inducing a cortisol response signal over and above the HPA-activating demands of entering a novel lab environment. We also modified the task by providing repeated feedback, throughout the task, about the child's failure to meet task demands to constrain individual differences in children's perceptions about their own performance. Results lend insight into both HPA-axis and ANS responsivity to a laboratory stressor in early childhood and will facilitate future examinations of acute stress effects on behavior.

1. Methods

1.1. Participants and procedure

Eighty-four children and their mothers volunteered to participate through community recruitment. Participants were randomly assigned to a Control ($N = 26$, 14 female) or Stressor condition ($N = 58$, 33 female), with more children in the Stressor condition to permit examination of within-condition individual differences. Participants (age 4.20–6.71 years, $M = 5.38$, $SD = 0.65$) represented a wide range of household incomes (median = $25,000–$29,999; range <$4999–$100,000+) and maternal education (median = some college or associate's degree; range < high school – graduate or professional degree), with no significant sociodemographic (age, sex, household income, maternal education) differences between conditions (all $ps > 0.05$).

Participants completed one 2-h laboratory visit (start times 9a.m.–3p.m.; with children awakening a least 1 h prior and not eating for 1 h prior). Visits included mother and child watching a 5-min peaceful ocean video to assess baseline ANS (45 min post lab-entry; Piferi et al., 2000). The mother then left the room, and children completed one cognitive task (a Go/NoGo Task, not reported here) followed by the matching task (60 min post-lab-entry) and additional assessments (Go/NoGo Task, ocean video, mother-child interaction task; not reported here). Saliva samples were collected following consent and immediately, 20, 40, and 50 min post-matching task. Notably, with the exception of the matching task, all laboratory procedures were identical regarding assessments and children's interactions with their mothers and assessors.

1.2. Matching task

In both Stressor and Control conditions, children played a game in which they were instructed to match colored stickers to transportation types on a worksheet with 30 squares using a key. Children performed this task on three consecutive two-minute trials. (Two minuates is insufficient for children of this age to complete the worksheet). Thus, cognitive and motor demands were equated between conditions.

1.2.1. Control

This condition was facilitated by a friendly, familiar assessor. After two minutes, the assessor told the children it was time to work on the next worksheet. There was no mention of prizes, winning, or losing.

1.2.2. Stressor

The stressor was based on a previous study (Kryski et al., 2011). Children picked a desired prize to win for successfully completing a worksheet. The assessor operated a stoplight that was set to green (90 s), to yellow (30 s), and finally to red, accompanied with a loud beep, signaling the end of a trial. Modifications included use of an unfamiliar assessor who used stern, flat affect (non-smiling, non-encouraging) and negative feedback twice each trial (e.g. *you're not going fast enough*). After three failures, children were told they failed to earn their prize. Next, the assessor left the room and a friendly, familiar assessor returned. Children received the desired prize at the end of the visit (60 min post-stressor).

1.3. Cortisol

Salivettes were used to collect saliva (Sarstedt, Inc., Newton, NC), with samples frozen (−20 °C) and sent to University of Trier to be assayed in duplicate [coefficients of variance for inter-assay (7.1–9.0%) and intra-assay (4.0–6.7%)]. Time-from-awakening was calculated as the difference between children's first cortisol sample and the time they awoke ($M = 5.28$ h; $SD = 2.91$; *Range* 1.17–10.00 h). Ten participants (4 Control, 6 Stressor) were excluded due to refusal ($N = 5$), eating/drinking <60 min prior to lab visit ($N = 4$), and oral infection ($N = 1$). Extreme values (>3SDs condition mean; $N = 10$ (of 370 total) were winsorized.

1.4. Autonomic physiology

An 11-electrode configuration was used for assessment of respiratory sinus arrhythmia [RSA; parasympathetic activity (PNS)] and pre-ejection period [PEP; sympathetic activity (SNS)]. Three electrodes in a lead II arrangement assessed electrocardiogram (ECG). An 8-electrode tetrapolar montage recorded cardiovascular impedance. Data were acquired via Biopac wireless transmitters (Biopac Systems Inc, Goleta, CA). RSA was derived from natural log-transformed values of high frequency (0.24–1.04 Hz) ECG spec-