Psychoneuroendocrinology 93 (2018) 103–106

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Putting a finger on the problem: Finger stick blood draw and immunization at the well-child exam elicit a cortisol response to stress among one-year-old children

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ARTICLE INFO

Keywords:
Cortisol
Hypothalamic pituitary adrenal axis
Infant
Stress

ABSTRACT

Research examining stress reactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis in young children has historically been hampered by a lack of reliable methods to invoke a cortisol stress response. This report details an effective method of eliciting a cortisol rise in one-year-old children (N = 83) by modifying and combining two naturalistic stressors previously used with infants and children. Salivary cortisol levels were collected from children before and after a finger stick blood draw and immunizations performed during their one year well-child checkup at their pediatrician's office. Results indicated that the stressor was successful at eliciting a significant cortisol response. An extensive set of potential demographic and clinical confounds were also assessed in order to identify methodological considerations important in studies of infant cortisol. The stress paradigm presented here provides a promising alternative for studies of infant HPA activity to enable investigators to more effectively evaluate early functioning of the biological stress system during this developmentally important life stage.

1. Introduction

Blood draws and immunizations are naturalistic, mild pain stressors that have been used extensively to quantify stress reactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis via elevations in circulating cortisol. Up through six months of age, these stressors reliably provoke an HPA response. Their impact reduces after six months, however, such that by one year of age there is scant evidence that either stressor elicits an HPA response (Gunnar et al., 2009; Jansen et al., 2010). A dearth of paradigms that reliably evoke a cortisol response during the period of late infancy and early childhood poses a problem for psychoneuroendocrinology research because of evidence that the HPA axis is highly sensitive to early life stress with long-term effects on the system (Lupien et al., 2009).

In this report, we detail a method that successfully elicits a cortisol response among one-year-old children. We capitalize on a common and naturalistic stressor for infants, namely, a routine blood draw and immunizations occurring at the one year well-child checkup. Our method modifies existing blood draw and immunization stress protocols by converting the blood draw from a heel lance, which is typically used in cortisol research, to a finger stick. Finger sticks are commonly used with older infants, children, and adult patient groups. Even among typically developing children, finger sticks have ethical and empirical advantages. They are preferred over heel lances as a more ethical procedure once a child has reached an age at which they are standing or walking to minimize post-procedural discomfort (World Health Organization, 2010). However, finger stick requires brief arm, hand, and finger restraint, which often elicits mild frustration among infants and children (Calkins et al., 2002; Moscardino and Axia, 2006; Schuetze et al., 2008). We hypothesized that a naturally occurring event at the one year well-child checkup involving a finger stick blood draw and immunization stressor would significantly elevate cortisol levels. We further examined demographic, health, and testing day events for associations with cortisol levels to aid in the use of this procedure in research studies.

2. Methods

Ninety-five participants were recruited from two pediatric clinics from advertisements distributed via flyers, mailings, and patient visit packets. Interested families contacted the research laboratory and were screened for eligibility. Eligibility criteria included maternal age > 18

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https://doi.org/10.1016/j.psyneuen.2018.04.021
Received 29 January 2018; Received in revised form 19 April 2018; Accepted 20 April 2018

years and infants in generally good health with no major developmental disorder and not taking oral steroid medications. Parents identified their child's race as: Caucasian/non-Hispanic (54.2%), Asian (13.3%), Caucasian/Hispanic (12.0%), African American (12.0%), and more than one race (8.4%). These sample characteristics are comparable to data reported in the most recently available census for Gainesville, FL (US Census Bureau, 2016).

The study was approved by the human subjects review committee at the University of Florida. Parents were provided a general overview of the study procedures verbally over the phone prior to study participation. The mother and infant first met the study team at the research laboratory when infants were 9 months of age (M = 9.40 mos; SD = 0.65). Written informed consent was conducted by a research experimenter at this meeting. The consent process indicated that participation was voluntary, could be withdrawn at any time, and would not affect their clinical care or relationship with their pediatric practice. During the visit, mothers provided detailed information on demographics, family characteristics, infant health and medical history, and prenatal health history. After the visit, the mother and study team member maintained phone contact for purposes of scheduling the one year assessment.

A research team member accompanied families to their regularly scheduled well-child examination at the pediatric clinic when infants were 12 months of age (M = 12.21 mos, SD = 0.33). As appointments were constrained by physicians' availability, start times varied between 8:00 a.m.–4:00 p.m. While waiting for the medical exam to begin, mothers completed a daily diary to report on the child's sleep, feeding, and health to check for potential confounds for cortisol analyses. At this time, the experimenter collected the infant's baseline saliva sample.

Following the physician's physical exam, the clinic nurse administered a routine blood draw via finger stick immediately followed by immunizations. The finger stick was a brief procedure during which the nurse extended and restrained the infant's arm while the infant was held by the parent. The nurse applied a retractable lancet to the skin and massaged the finger to collect a small blood sample. Following the finger stick, infants received their scheduled immunizations in rapid succession via intramuscular injection to the thigh as per standard clinical care. The vast majority (90%) received 3–4 shots (range 1–5). The stressor was operationalized as the time from finger prick onset to withdrawal of the last immunization. The mean duration of the stressor was 2.35 min (SD = 1.21). Caregivers were present throughout the session and able to interact with their child as they saw fit.

The research team member remained in the exam room for collection of the two post-stressor saliva samples at +20 min and +40 min relative to the onset of the finger stick. No medical tests were performed during this time. Mothers completed the parent questionnaire while the infant had access to a box of eight age-appropriate toys.

To collect saliva samples, infants mouthed an absorbent eye spear (Roche Diagnostics, Indianapolis, IN) for 1 min. Mothers were requested, to the extent possible, to avoid feeding the child in the 30 min immediately preceding study onset to avoid contaminants. Samples were kept at −20 °C until assay. Samples were assayed at the University of Florida research lab using a commercially available enzyme-linked immunoassay kit (ELISA; Salimetrics, State College, PA). Intra- and inter-assay coefficients of variation were 4.0 and 6.4%, respectively. Statistical analyses were conducted using SPSS v22.0. The distribution of cortisol values at all three time points was positively skewed and thus log10 transformed prior to analyses.

3. Results

Upon inspection of the data, two cases were excluded due to infants consuming milk within 30 min prior to saliva collection and two cases because immunizations were not performed. Of the remaining 91 infants, 83 (87.4% of total) had cortisol data at all three time points and constituted the final analytic sample. Comparison using *t*-test and chi-square analyses showed that there were no significant differences on any of the covariates tested in this study between infants with and without missing data (all *p*'s > 0.05).

Cortisol levels at the three assessments were: baseline M = 0.19 μg/dL, SD = 0.15; +20 min M = 0.31 μg/dL, SD = 0.21; and +40 min M = 0.28 μg/dL, SD = 0.24. On average, infants' cortisol levels rose 63.2% above baseline 20 min after stressor onset. Cortisol dropped by an average of 9.7% from +20 to +40 min, consistent with a diurnal decline. To address the key question as to whether cortisol levels would show a significant stress response, a repeated-measures (RM) ANOVA was conducted with Greenhouse-Geisser correction. The within-subject main effect of time was significant, $F(1.49, 125.16) = 20.41$, $p < 0.001$. Within-subjects contrasts showed that both the cortisol rise from baseline to +20 min, $F(1, 82) = 31.82$, $p < 0.001$, and the cortisol drop from +20 to +40 min, $F(1, 82) = 6.85$, $p < 0.05$, were significant.

As is typically observed in cortisol studies, the magnitude of change showed individual variability. Using a liberal criteria of positive (+0.0 μg/dL) change score, 72.3% of infants showed a cortisol rise from baseline to +20 min. Based on a more conservative criteria of a 10% increase in post-stressor cortisol above baseline (Kertes et al., 2009), 67.5% of infants met criteria for a stress response.

RM ANOVAs were then conducted to identify potential covariates of infant cortisol using this paradigm. These included maternal characteristics (age, marital status, education), family demographics (insurance status, household income, number of people in household, paternal education), infant characteristics (sex, age, race/ethnicity, weight, length, breastfeeding history, hours per week in child care, medication use, current tobacco exposure), pregnancy/birth history (prenatal medication, prenatal tobacco, primiparity, delivery/birth complications, birth weight and length), and testing day (time since morning wake and last feeding, illness, medications, teething) and clinic visit (stressor duration, number of immunizations, clinic site) characteristics. Results are shown in Table 1.

The majority of the covariates tested were unrelated to infant cortisol. Maternal age was significantly associated with cortisol change (see Table 1 for time x covariate interactions). Within-subjects contrasts showed that infants of younger mothers had higher cortisol reactivity to the finger stick/immunization compared to infants of older mothers, $F(1, 68) = 7.57$, $p < 0.01$. As expected, time since morning awakening was also significantly associated with infant cortisol change. Within-subjects contrasts showed that more hours since morning awakening (i.e. testing later in the day) was associated with lower cortisol at baseline, $F(1, 81) = 5.39$, $p < 0.05$, and a smaller rise from baseline to +20 min, $F(1, 81) = 3.58$, $p < 0.10$.

A final RM ANOVA was run to verify that the finger stick/immunization stressor was successful at eliciting a cortisol response while accounting for significant covariates. Controlling for maternal age and time since waking, a significant within-subject main effect of time was observed, $F(1.60, 106.95) = 6.30$, $p < 0.01$. Within-subject contrasts showed that cortisol response from baseline to +20 min remained significant, $F(1, 67) = 9.71$, $p < 0.01$, with a large effect size (Cohen's d = 0.80). There was no significant change from +20 to +40 min post-stressor, $F(1, 67) = 2.58$, $p > 0.05$. Fig. 1 displays cortisol levels at baseline, +20 min, and +40 min, after adjusting for covariates.

4. Discussion

Given that acute stressors typically fail to evoke a significant cortisol response after six months of age (Gunnar et al., 2009; Jansen et al., 2010), results of this study are notable for demonstrating that a finger stick/immunization stressor is capable of triggering a significant cortisol rise at one year of age. It has long been debated whether the lack of cortisol rise to mild stressors is a result of protective mechanisms occurring at this age or methodological challenges (Egliston et al., 2007; Gunnar et al., 2009). The rise in cortisol seen in this study is evidence