Published in final edited form as:

Psychoneuroendocrinology. 2018 July ; 93: 103–106. doi:10.1016/j.psyneuen.2018.04.021.

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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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.

Keywords

cortisol; hypothalamic pituitary adrenal axis; infant; stress

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)

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Contributions Disclosures

D.A.K. designed and oversaw the study and wrote the manuscript. H.S.K. collected data and participated in data analysis and manuscript writing. J.L. participated in data collection. S.S.B. assayed the salivary cortisol. M.K. participated in the study design and oversaw the clinical care of participants. All authors contributed to revising the final manuscript.

Conflicts of Interest

All authors verify that they have no conflicts of interest to declare.

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.

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 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 8:00am–4:00pm. 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.

Results

Upon inspection of the data, two cases were excluded due to infants consuming milk within 30 minutes 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 $> .05$).

Cortisol levels at the three assessments were: baseline $M = 0.19 \mu\text{g/dL}$, $SD = 0.15$; +20 min $M = 0.31 \mu\text{g/dL}$, $SD = 0.21$; and +40 min $M = 0.28 \mu\text{g/dL}$, $SD = 0.24$. 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, $F(1.49, 125.16) = 20.41$, $p < .001$. Within-subjects contrasts showed that both the cortisol rise from baseline to +20 min, $F(1, 82) = 31.82$, $p < .001$, and the cortisol drop from +20 to +40 min, $F(1, 82) = 6.85$, $p < .05$, were significant.

As is typically observed in cortisol studies, the magnitude of change showed individual variability. Using a liberal criteria of positive ($+0.0 \mu\text{g/dL}$) 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 $\times$ 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, $F(1, 68) = 7.57$, $p < .01$. 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, $F(1, 81) = 5.39$, $p < .05$, and a smaller rise from baseline to +20 min, $F(1, 81) = 3.58$, $p < .10$.

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, $F(1.60, 106.95) = 6.30$, $p < .01$. Within-subject contrasts showed that cortisol response from baseline to +20 min remained significant, $F(1, 67) = 9.71$, $p < .01$, with a large effect size (Cohen's $d = 0.80$). There was no significant change from +20 to +40 min post-stressor, $F(1, 67) = 2.58$, $p > .05$. Figure 1 displays cortisol levels at baseline, +20 min, and +40 min, after adjusting for covariates.

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 that older infants do experience a meaningful cortisol response to stress with developmentally appropriate stressors. Indeed, a large effect size was observed.

When considering infants as responders vs. non-responders by a criteria of a 10% rise above baseline, 68% showed a cortisol response. The magnitude of the cortisol rise reported here met was comparable to prior reports using either a heel lance or immunization stressor among infants aged 0–6 mos in which the mean cortisol response ranged from -0.12 to 0.95 $\mu\text{g/dL}$ (Cohen's $d = .17$ to 4.22 ; Jansen et al., 2010) and compared favorably to results with infants aged 7–24 mos which typically reported non-significant changes in cortisol to those individual stressors (Gunnar et al., 1996; Davis and Granger, 2009).

The success of the method reported here may reflect subtle but potentially important methodological modifications. First, samples were collected using a finger stick instead of a heel lance. Finger sticks involve holding the infant's arm straight, reminiscent of arm restraint paradigms, and thus may also evoke mild infant frustration; however, they are also clinically considered the ethically preferred blood draw method for infants once they begin standing or walking. Moreover, whereas stressors typically consist of either immunizations or a blood draw, this is the first study we know of with infants in which both procedures were administered in tandem, which is standard at the one year well-child checkup. The combination may have been more effective at evoking an acute cortisol response while retaining ecological validity. In addition, although there is a standard set of recommended immunizations by the Centers for Disease Control and Prevention for infants aged 12–15 mos (eight total as of 2018; Centers for Disease Control and Prevention (CDC), 2018), there is state-by-state variability in the specific timing at which those immunizations are administered, with the majority recommended at 12 mos (Centers for Disease Control and Prevention (CDC), 2017). Thus, it will be important for future studies to confirm that this stressor continues to be effective at other infant ages or under conditions in which a greater number of immunizations are administered. Although replication is warranted given the novelty of the modified stress paradigm, we are optimistic that future studies can usefully build upon the methodology described here to more sensitively examine underlying contributors to cortisol response at this formative age.

We also assessed potential confounds in research with infants using this protocol. Very few significant associations were observed. Time since morning awakening was associated with cortisol, in accordance with expectations based on the cortisol diurnal rhythm. Infants of older mothers had a smaller cortisol stress response than did infants of younger mothers. Although we did not assess maternal behavior in this study, caregiver sensitivity has previously been associated with infant cortisol responses (Gunnar et al., 1996; McLaughlin

et al., 2015). It is possible that older, presumably more experienced, mothers were familiar with the one year checkup which may have affected consoling behavior towards their infants. This possibility is worthy of investigation in future research.

In summary, this report demonstrates the efficacy of a naturalistic stressor – a finger stick blood draw and immunization conducted as part of children’s routine well-child checkup – in eliciting a cortisol response in one-year-old children. Although some modest associations were observed with covariates, they were few in number and in line with studies of cortisol reactivity in infants and children. The method presented here provides a new alternative for cortisol research at an age at which successfully eliciting a cortisol response has historically been difficult. This stress protocol moves the field forward by offering an effective methodology for assessing cortisol reactivity in older infants, which will in turn facilitate researchers’ ability to document factors influencing variation in infants’ HPA stress response.

Acknowledgments

The authors gratefully acknowledge the families who participated in this study and the staff at the University of Florida Pediatrics, especially Lindsay Thomson, MD and Christine Villamor, RN. This research was supported by the National Institutes of Health CTSA grant 1UL1RR029890 with funds awarded to Darlene A. Kertes (Principal Investigator).

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Highlights

- Finger stick blood draw and immunization successfully elicited a cortisol stress response among one-year-old children
- Demographic and clinical characteristics were largely unrelated to infant cortisol
- Time since awakening and maternal age were associated with infant cortisol
- Routine medical procedure at the well-child checkup is an effective naturalistic stressor for older infants

Figure 1. Cortisol response following finger stick blood draw and immunization stressor
Note: Levels are shown controlling for maternal age and time since morning awakening.
Error bars denote standard error. Baseline M = 0.19 µg/dL, SD = 0.15; +20 min M = 0.31 µg/dL, SD = 0.22; +40 min M=0.25 µg/dL, SD = 0.16.

Table 1

Tests of potential confounds on cortisol stress response to blood draw/immunization stressor at 12 months of age.

Variable	Mean or Percent	SD	F	p
<i>Infant characteristics</i>				
Sex (percent female)	46%		0.46	.58
Hours per week in child care	14.26	15.79	0.30	.94
Number of child care arrangements	1.27	0.99	1.13	.32
Current tobacco smoke exposure	18%		1.44	.22
Breastfed ever	90%		0.75	.44
<i>Family characteristics</i>				
Maternal age	32.14	4.80	5.06	.01
Mother education (college degree)	78%		0.23	.72
Father education (college degree)	60%		0.32	.65
Single parent household	11%		0.94	.37
Number of people living in household	3.98	1.16	0.93	.37
Household income (median)	25–50K		0.55	.53
Has private insurance (vs. Medicaid/CHIP)	60%		1.26	.28
<i>Pregnancy/birth data</i>				
Birth weight (lb)	7.40	1.22	0.59	.51
Birth length (in)	20.05	1.48	1.22	.29
Smoked during pregnancy	5%		0.84	.40
Medications taken during pregnancy	46%		0.91	.38
Primiparous	39%		2.04	.15
Pregnancy/birth complications	27%		0.49	.56
<i>Testing day variables</i>				
Infant current weight (lb)	21.06	2.24	0.85	.40
Infant current length (in)	30.04	2.10	0.58	.52
Infant currently taking medications	17%		0.21	.75
Currently teething	70%		0	.99
Fever in past 24 hours	4%		0.07	.89
Clinic site (site 1)	77%		0.53	.54
Stressor duration (min)	2.39	1.28	0.60	.50
Number of immunizations	3.14	0.61	0.94	.37
Time since morning wake (hr)	4.06	2.74	4.57	.02
Time since last feeding (hr)	2.18	1.35	2.54	.10

Note: F values shown indicate Covariate $\times$ Time Interaction in the RM ANOVA with Greenhouse-Geisser correction. Significant covariates are shown in bold.