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Effects of early life adversity on cortisol/salivary alpha-amylase symmetry in free-ranging juvenile rhesus macaques

Lauren A. Petrullo [a], Tara M. Mandalaywala [b,c], Karen J. Parker [d], Dario Maestripieri [c,e], James P. Higham [a,*]

[a] Department of Anthropology, New York University, New York, NY, USA
[b] Department of Psychology, New York University, New York, NY, USA
[c] Institute for Mind and Biology, The University of Chicago, Chicago, IL, USA
[d] Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA
[e] Department of Comparative Human Development, The University of Chicago, Chicago, IL, USA

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ABSTRACT

Early life adversity (ELA) affects physiological and behavioral development. One key component is the relationship between the developing Hypothalamic-Pituitary-Adrenal (HPA) axis and the Sympathetic Nervous System (SNS). Recent studies suggest a relationship between early life adversity and asymmetry in cortisol (a measure of HPA activation) and salivary alpha-amylase (sAA: a correlate of SNS activation) responses to stress among human children, but to our knowledge there have been no comparable studies in nonhumans. Here, we investigate the responses of these two analytes in "low stress" and "high stress" situations in free-ranging juvenile rhesus macaques (*Macaca mulatta*) on Cayo Santiago, Puerto Rico. Behavioral data on maternal maltreatment were collected during the first 3 months of life to determine individual rates of ELA, and saliva samples were collected from subjects noninvasively during juvenility. Irrespective of ELA, salivary alpha-amylase levels were lower in low stress situations and higher in high stress situations. For cortisol however, high ELA subjects exhibited higher low stress concentrations and blunted acute responses during high stress situations compared to moderate and low ELA subjects. Cortisol and sAA values were positively correlated among low ELA subjects, suggesting symmetry, but were uncorrelated or negatively correlated among moderate and high ELA subjects, suggesting asymmetry in these individuals. These findings indicate dysregulation of the stress response among juveniles maltreated during infancy: specifically, attenuated cortisol reactivity coupled with typical sAA reactivity characterize the stress response profiles of juveniles exposed to higher rates of ELA during the first 3 months of life.

Introduction

Maternal care is the most fundamental of all environmental influences during mammalian development. Decades of developmental research have shown that early life adversity (ELA) related to the quality of maternal care can have a programming effect on neuroendocrine development. The role of maternal care in modulating phenotypic differences in nonhumans has its foundation in rodent studies (e.g. Francis et al., 1999; Liu et al., 1997). These provided some of the earliest evidence of maternal programming of offspring neuroendocrine function and specifically, a link between early life stressors and Hypothalamic-Pituitary-Adrenal (HPA) axis function (see Meaney and Szyf, 2005; Plotsky and Meaney, 1993). Development of the HPA axis has generated enduring interest in part for its relevance to human child development (Cicchetti, 1989; Heim et al., 1997; Liu et al., 1997; Sapolsky, 1994; Shea et al., 2005), and there is evidence for attenuation of the HPA response in relation to early life stress in both nonhuman primates (Dettling et al., 2002; Johnson et al., 1996; Levine and Mody, 2003; McCormack et al., 2003; see Sanchez, 2006, for review) and humans (Carpenter et al., 2007, 2011; de Bellis et al., 1994; Elzinga et al., 2008; Ouellet-Morin et al., 2011).

Interest in the coordination of the HPA and a second major component of stress physiology, the Sympathetic Nervous System (SNS), has grown as a result of advances in salivary bioscience and increased appreciation of multi-systems approaches to stress studies (Bauer et al., 2002). Bauer et al. (2002) proposed a complementary model in which a symmetrical relationship between the HPA and SNS (i.e. synchronized reactivity in the same direction in both systems) would be linked to optimal behavioral outcomes, while asymmetry would be associated with behavioral adjustment problems (e.g., anxious and depressed symptoms: Allwood et al., 2011; antisocial behaviors: Shenk et al., 2010). Although the mechanisms underlying the relationship between these two systems remains unclear, emerging research has since supported the idea that early life experience plays a role in mediating how the HPA and SNS interact.

* Corresponding author at: 25 Waverly Place, New York University, New York, NY, 10013, USA.
E-mail addresses: lauren.petrullo@nyu.edu (L.A. Petrullo), jhigham@nyu.edu (J.P. Higham).

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There is evidence that asymmetry in these systems, as measured by the relationship between salivary cortisol and salivary alpha-amylase (sAA), a correlate of SNS function, may have deleterious consequences for physical and emotional health (Ali and Pruessner, 2012; Allwood et al., 2011; Fortunato et al., 2008; Gordis et al., 2006; Keller et al., 2012; Koss et al., 2014; Vigil et al., 2010).

While previous studies have explored the HPA-SNS relationship among humans, to our knowledge no studies have applied this to nonhuman models. Animal models are valuable for understanding the relationships between maternal care and developing stress physiology because one can control and account for a number of confounding relationships present in humans (e.g., socioeconomic status, diet, access to health care, and so on). Further, studies of ELA in humans often rely on self-reporting of past experiences, which are prone to error and can be influenced by factors such as age and current psychological state.

Nonhuman primate models in particular can offer insight into the evolution of the stress response due to several key similarities between nonhuman primates and humans. These include that primate childhoods are long and complex, with infant and adolescent periods providing extended periods of sensitivity to environmental stimuli. Nonhuman primates have been historically used as models in studies emphasizing the nature, causes, and consequences of mother-infant interactions and variation in maternal parenting style (Altmann, 1980; Fairbanks, 1996).

One species that has been particularly well-studied with respect to ELA and maternal care is the rhesus macaque (*Macaca mulatta*). Rhesus infants spend the first month of life in almost constant ventro-ventral contact with their mothers and continue to remain in physical contact for the next year. As in humans, rhesus mothers engage in a gradient of parenting styles, with variations in protectiveness and sensitivity that mimic the scope of differences found in human parenting (Maestripieri and Carroll, 1998a; Mandalaywala et al., 2014). This variation extends to abusive behaviors as well; approximately 2–10% of mothers are abusive, exhibiting violent behaviors (e.g. dragging, crushing, inappropriate carrying, throwing) that are rarely exhibited by non-abusive mothers (Maestripieri and Carroll, 1998b). Infant abuse typically occurs in the first 3 months of life, and is transmitted intergenerationally (Maestripieri, 2005b; Maestripieri and Carroll, 1998a, 1998b), similar to patterns seen in humans (Berlin et al., 2011). Variation in maternal care patterns during this critical time has been shown to affect development of cognitive bias to threat (Mandalaywala et al., 2014) and HPA function, such that abused rhesus infants show higher basal and stress response cortisol levels than nonabused infants at 12 months of age but not later in life, suggesting an effect of both ELA and age on HPA axis function (Koch et al., 2014). However to our knowledge, no study has investigated SNS functioning in rhesus macaques in the context of ELA, or has examined the relationship between the HPA and the SNS in the same study.

This study examines the effects of maternal maltreatment on developing stress physiology in juvenile free-ranging rhesus macaques on Cayo Santiago, Puerto Rico. We used salivary cortisol as an index of HPA activity and salivary alpha-amylase (sAA) as an index of SNS activity, as these two markers, and in particular their relative symmetry versus asymmetry in response to stress, have been a particular focus of human work (e.g. Gordis et al., 2008). We collected saliva samples that represented "high stress" and "low stress" scenarios from each individual. We predicted that when compared to juveniles exposed to low rates of ELA in the form of maternal neglect and abuse during the first three months of life, juveniles that were exposed to higher rates of ELA would exhibit:

1) Attenuated cortisol patterns, consisting of higher basal concentrations, and reduced responsiveness to stressors;
2) sAA patterns consistent with other individuals, consisting of low basal concentrations and strong responsiveness to stressors;
3) Asymmetry in HPA-SNS reactivity to stress, as a consequence of 1 and 2.

Methods

Research described in this manuscript was approved by the Institutional Animal Care and Use Committees of New York University, the University of Chicago, and the University of Puerto Rico, and complied with the legal requirements of both Puerto Rico and the United States.

Study site and subjects

Data collection took place on Cayo Santiago, a small 15-hectare island located 1 km east of mainland Puerto Rico. A population of rhesus macaques was formed in 1938 on this island from free-ranging macaques captured in and transported from India (Rawlins and Kessler, 1986). The population is provisioned daily with commercial monkey chow and rainwater and trapped annually for identification purposes. Beyond this, the macaques are not regularly handled or manipulated in any way. The subjects of this study were 13 male and 7 female juvenile rhesus macaques (age mean ± SEM: 2.89 ± 0.01 years, range: 2.74–2.98 years) belonging to two naturally formed social groups (Groups R and S) living on Cayo Santiago. These juveniles were part of a larger cohort that had been previously studied during their first 3 months of life.

Behavioral data collection

Infants were followed in a random order on Cayo Santiago from 7:00 to 14:30, five days a week, from August to December 2011 using continuous focal animal sampling methods and a handheld event recorder. Individuals were observed for two 30-minute periods each week for the first 12 weeks of life, and were part of a larger dataset containing 542.5 h of behavioral observation (behavioral observation mean ± SEM across all infants: 11.8 ± 0.05 h, range: 10.5–12.0 h). Observational behaviors of mother-infant interactions were recorded, including rates of maternal abuse and rejection (see Table 1). Data were converted to mean hourly rates and abuse and neglect behaviors were averaged together to provide a single overall frequency of maternal maltreatment for each subject. Subjects were subsequently assigned to one of three categories characterizing their relative rates of ELA—high, moderate, or low—based on a three-way split in maternal maltreatment frequencies, following Mandalaywala et al., 2014. The number of individuals falling into each of these categories differs slightly for separate analyses due to variation in the quantity of useable samples and availability of paired values for comparison (see Assay protocols and Tables 2–3).

Saliva collection protocol

Saliva collection followed Higham et al., 2010, in which the authors found rhesus macaques to be most responsive to orange, citrus-flavored drink crystals. Following Higham et al., 2010, collection devices consisted of approximately eight feet of nylon rope hand-sewn with cotton thread to a Salimetrics® Oral Swab (www.salimetrics.com). The rope was wrapped approximately five times around the center of the swab, and then coated in a viscous solution of orange Tang® drink crystals and water (Kraft Foods Inc.). The sugared solution was never

Table 1
Ethogram of maternal maltreatment.

ELA variable	Description
Maternal rejection	Mother prevents contact by holding, pushing infant away, or biting, or in other ways
Maternal abuse	Mother slaps, hits, shoves, bites, drags, throws, steps on, sits on, or inappropriately carries the infant

Table 2
Breakdown of maternal maltreatment frequencies across ELA categories for cortisol analyses.

Maternal maltreatment frequencies (cortisol)				
ELA category	N	Mean ± SEM	Min	Max
High	7 (4 F, 3 M)	4.55 ± .40	3.26	6.42
Moderate	4 (1 F, 3 M)	1.58 ± .08	1.42	1.74
Low	9 (2 F, 7 M)	0.62 ± .12	0.16	1.26
Total	20	2.19 ± .43	0.16	6.42

applied directly to the swab itself, and collection devices were left to completely dry before use.

Saliva samples were collected between 7:00 and 14:00, Monday through Friday, from June 1, 2014 to July 23, 2014. Individuals that had been observed in conflict with conspecifics within 15 min prior where the individual was not the aggressor were sought out and samples collected were classified as "high stress". Samples from individuals that had been observed resting and not engaging with conspecifics for at least 30 min prior were classified as "low stress". Individuals that had eaten within 10 min prior or were currently eating were avoided, as mastication and some foods have been shown to affect the flow and secretion rates of sAA in humans (Lo Piparo et al., 2008; Mackie and Pangborn, 1990). The swab was presented to subjects by placing it on a rock, hanging it from a tree, or dangling it through nearby caging. The experimenter stepped back while holding on to the other end of the rope. The subject approached and placed the swab into his/her mouth and began to chew, while the experimenter recorded the amount of time in seconds the swab was in the individual's mouth. The device was collected once the animal abandoned the swab or once 2 total minutes of chewing had passed. The swab was separated from the nylon rope using scissors, placed into a centrifuge tube with retainer, and immediately placed on ice into a cooler filled with ice packs. Samples were then centrifuged for 15 min upon returning to the mainland. Saliva was transferred using 1 mL disposable Pasteur pipettes into 2 mL centrifuge tubes. Sample volumes (volume mean ± SEM: 0.298 ± 0.01 mL, range: 0.05 mL to 0.65 mL) were recorded using centrifuge tube gradations. Samples were then frozen at −80 **°C** until shipped on dry ice to the Department of Anthropology, New York University in September 2014. All samples arrived frozen. On day of testing, all sample tubes were centrifuged at 3000 rpm for 15 min to remove mucins.

Assay protocols

Saliva samples were assayed for cortisol (N = 141) using a commercial enzyme-linked immunosorbent assay from Salimetrics, Inc. (State College, PA), previously analytically validated for use in rhesus macaques (Higham et al., 2010). This assay utilizes 50 μL of saliva to be run in duplicate, with a lower limit of sensitivity of 0.007 μg/dL, and inter-assay coefficients of variation (CV) computed from high and low controls of 2.4% and 3.9%, respectively. Intra-assay CVs determined from the mean of 20 replicates each across five replicate tests for high and low controls was 7% and 4%, respectively. Samples ($N = 144$) were diluted 1:4 to offset viscosity of saliva and enable more accurate pipetting. Only data falling within the range of the linear curve of the assay were used; samples falling outside this range were re-run at a different dilution. Samples used in later analyses reflect subjects with at least two paired samples.

Table 3
Breakdown of maternal maltreatment frequencies across ELA categories for sAA and Spearman's rank correlation coefficient analyses.

Maternal maltreatment frequencies (sAA)				
ELA category	N	Mean ± SEM	Min	Max
High	ϕ 6 (3 F, 3 M)	4.73 ± .43	3.26	6.42
Moderate	ϕ 2 (2 M)	3.1 ± .13	1.42	1.68
Low	6 (1 F, 5 M)	0.49 ± .12	0.16	0.83
Total	14	2.46 ± .25	0.16	6.42

ϕ Spearman's rank correlation analysis utilizes this same breakdown except for the removal of one sample for an individual in the moderate ELA category because there was no paired cortisol value, and the removal of an individual from the high ELA category because that individual was represented by only two samples and so an intra-individual correlation was not viable. Spearman's analysis had an adjusted high ELA category mean ± SEM of 4.70 ± 0.53. All other values remained the same.

We assayed for sAA ($N = 144$) using a commercial kinetic reaction assay (Salimetrics, State College PA.), previously analytically validated for use in rhesus macaques (Higham et al., 2010). This kit utilizes 10 μL of saliva, and has an inter-assay CV of 2.5%, calculated from 10 replicates of high controls, and 7.2% computed from the mean of 10 replicates of low controls. The intra-assay CV computed from the mean of 8 high and 8 low controls was 3.6% and 5.8%, respectively. Final sAA concentrations are calculated by the following formula: [absorbance difference per minute × total assay volume (0.300 mL) × dilution factor (200)] / [millimolar absorptivity of 2-chloro-*p*-nitrophenol (12.9) × sample volume (0.008 mL) × light path of the plate (0.97)]. Note that following discussion with Salimetrics® support staff total assay volume run was 0.300 mL rather than the suggested 0.328 mL stated in the kit protocol to account for fast and accurate delivery of the heated substrate to the wells before insertion into the plate reader. As there remain concerns that saliva flow rate may have an effect on interpretation and analysis of sAA levels (Beltzer et al., 2010; Bosch et al., 2011), sAA values were corrected for flow rate by multiplying final calculated concentrations by the volume (mL) of saliva deposited on swab over the time (min) spent chewing on the swab during collection. Final values were expressed in U/min.

Of the 144 samples run on this assay, 38 samples were not detectable by the assay despite re-running at multiple dilutions, suggesting these samples may have had sAA concentrations below the lower limit of assay sensitivity. Additionally, 26 samples had absolute value changes <0.1, and were excluded due to the possibility that such small changes may reflect chemical actions in the substrate rather than sAA values. Two additional samples were excluded, as they became the sole data point representing an individual.

Statistical analyses

Outliers ($n = 3$) ± 3 SD from the mean were excluded prior to cortisol analyses. Cortisol data (final sample size: $N = 138$, samples per juvenile mean ± SEM: 6.90 ± 0.82, range: 2–15) were log transformed and sAA data (final sample size: $N = 78$, samples per juvenile mean ± SEM: 10.4 ± 0.75, range: 2–12) were square-root transformed to achieve an approximately normal distribution. For Spearman's correlation analysis, three samples were excluded as two were the only points representing an individual, and the third did not have a paired cortisol value. The final sample size for Spearman's coefficient calculations was $N = 75$, representing 13 individuals.

We utilized Linear Mixed Models (LMM) to examine the relationship between the physiological data and a number of variables. Two sets of LMMs were run: the first included ELA data specified as a continuous variable (raw frequency data), while the second models included ELA data defined categorically (binned into "High", "Moderate", "Low"). We undertook this second set of analysis to enable comparison with previous studies in this population, which treated ELA as a categorical variable (Mandalaywala, 2014; Mandalaywala et al., 2014), and because treating ELA as a categorical variable allowed us to examine whether the effect of ELA on physiological outcomes were graded or qualitatively different between high, medium, and low ELA experiences. First, to determine the influence of juvenile sex, date of collection,

maternal rank, and parity on cortisol/sAA output as continuous variables, a preliminary set of LMMs was run with these variables as fixed effects. Juvenile ID, and time of collection were included as random effects. Date of collection, maternal rank, and parity were not found to affect cortisol/sAA output and were therefore excluded from subsequent analyses. The second set of LMMs then tested the relationship between ELA, stress condition, and the interaction of these two fixed effects on sAA and cortisol specified as categorical variables, also including sex as a fixed factor, and juvenile ID and collection time as random effects. Pairwise comparisons compared overall differences in the means of each ELA category for sAA and cortisol during both "high stress" and "low stress" states.

To examine the nature of the relationship between sAA and cortisol values by ELA category, Spearman's rank correlation coefficients for each individual were calculated, including all data for each individual. Individual coefficients were then averaged together to produce overall mean coefficients for each ELA category. These coefficients were used to characterize the symmetry/asymmetry of HPA-SNS reactivity, with positive coefficients indicating relative symmetry and coefficients closer to zero indicating relative asymmetry. A Kruskal-Wallis test determined if there were significant differences in coefficients among ELA categories, and Mann-Whitney U post-hoc tests determined which groups differed. All analyses were undertaken in SPSS 21.0 using Restricted Maximum Likelihood (REML), and were two-tailed with a $p < 0.05$ considered significant.

Results

Salivary cortisol

In the model treating ELA as a continuous variable (model $R^2 = 0.457$), stress was found to have a significant effect on cortisol, [$F_{(1,120.195)} = 46.451$, $p < 0.001$.], such that stress level regardless of ELA predicted cortisol output. The interaction between ELA and stress state was found to have a significant effect on cortisol output, [$F_{(1,118.858)} = 15.050$, $p < 0.001$]. The effect of ELA alone on cortisol output was nonsignificant.

In the categorical model (model $R^2 = 0.456$), stress was similarly found to have a significant effect on cortisol, [$F_{(1,118.595)} = 23.297$, $p < 0.001$], such that stress level regardless of ELA predicted cortisol output. Again, matching the continuous results, the interaction between ELA and stress state was also found to have a significant effect on cortisol output, [$F_{(2,118.710)} = 7.117$, $p = 0.001$]. The effect of ELA alone on cortisol output was nonsignificant. High ELA juveniles exhibited higher "low stress" and lower "high stress" cortisol levels than moderate and low ELA juveniles (Fig. 1). Low ELA juveniles showed the greatest increase in cortisol levels from "low stress" to "high stress" states (cortisol concentration mean ± SEM: 0.47 ± 0.18 ng/dL), while high ELA juveniles showed the smallest increase (0.03 ± 0.14 ng/dL), and moderate ELA juveniles showed an intermediate increase (0.28 ± 0.20 ng/dL). Pairwise comparisons show a significant difference between mean cortisol output in high and low ELA juveniles only in "high stress" data (df = 15.736, $p = 0.027$, Cohen's d = 0.968). Together, these results indicate that that stress level combined with ELA has a predictive effect on cortisol reactivity. Consistent with this, high ELA juveniles exhibit a marginal increase in cortisol from "low stress" to "high stress" states, while low ELA juveniles exhibit a relatively greater increase.

Salivary alpha-amylase

In the model treating ELA as a continuous variable (model $R^2 = 0.485$) stress level was found to have a significant effect on sAA [$F_{(1,67.203)} = 28.508$, $p < 0.001$,]. Neither ELA nor the interaction between ELA and stress level was significant. Similarly. in the categorical model, stress was found to have a significant effect on sAA [$F_{(1,72)} = 44.791$, $p < 0.001$, model $R^2 = 0.474$], and the interaction between ELA and stress state was nonsignificant. Low ELA juveniles had the highest "low stress" and "high stress" sAA levels, with moderate ELA juveniles exhibiting the smallest "high stress" sAA levels, and high ELA juveniles exhibiting the lowest "low stress" sAA levels (Fig. 2). Pairwise comparisons of mean sAA values across ELA category show no significant differences among the three categories. Taken together, these results indicate that, unlike cortisol, sAA output is not influenced by ELA. Specifically, sAA output is not influenced by the interaction between ELA and stress level, and despite minor differences in sAA concentrations between groups/stress states, differences between means of the three groups are not significant.

Relationship between analytes

Using the categorical data, Spearman's rank correlation coefficients were calculated across both "low stress" and "high stress" data for each individual who had at least three paired sAA and cortisol samples. Low ELA juveniles (mean ± SEM: 0.47 ± 0.08, range: 0.23–0.70) had an average positive coefficient ($r_s = 0.47$, $N = 6$), while moderate ELA (mean ± SEM: −0.35 ± 0.15, range: −0.49–0.20) and high ELA (mean ± SEM: −0.10 ± 0.23, range: −0.80–0.50) juveniles had an average negative coefficient (moderate ELA: $r_s = -0.35$, $N = 2$; high ELA:

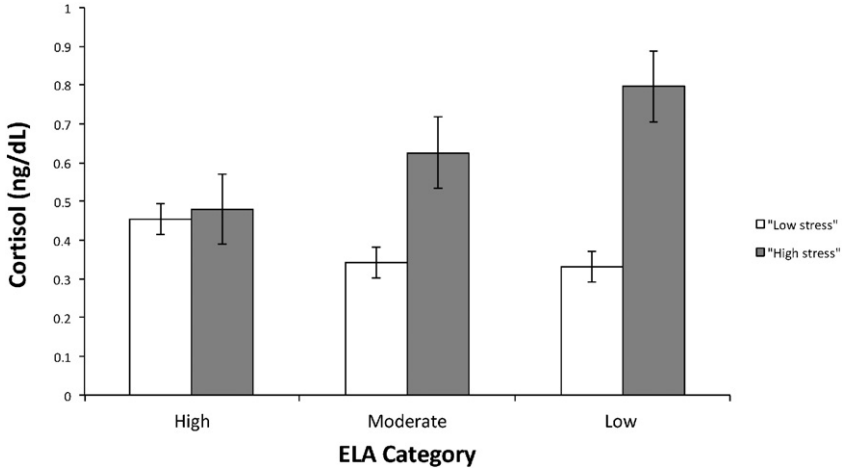

Fig. 1. Relationship between ELA category and cortisol concentrations. Graph depicts mean ± SEM cortisol concentrations in ng/dL for "low stress": high ELA (0.454 ± 0.09, range: 0.21–0.78, $N = 7$), moderate ELA (0.34 ± 0.05, range: 0.26–0.49, $N = 4$), and low ELA (0.33 ± 0.03, range: 0.21–0.46, $N = 9$), and for "high stress": high ELA (0.48 ± 0.05, range: 0.24–0.62, $N = 7$), moderate ELA (0.62 ± 0.15, range: 0.39–1.0, $N = 4$), and low ELA (0.80 ± 0.15, range: 0.32–1.72, $N = 9$). Graph represents raw unlogged cortisol concentrations, though values were logged for analyses to meet model assumptions.

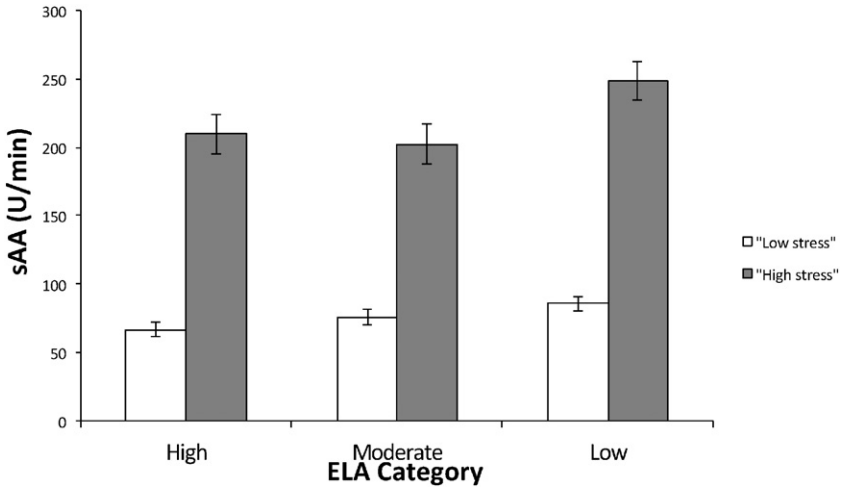

Fig. 2. Relationship between ELA category and sAA concentrations corrected for flow rate (U/min). Graph depicts mean ± SEM sAA concentrations in U/min for "low stress": high ELA (66.63 ± 15.18, range: 19.50–131.10, $N = 6$), moderate ELA (75.66 ± 7.06,range: 68.99–82.72, $N = 2$), and low ELA (85.67 ± 10.00, range: 53.41–120.98, $N = 6$),and for "high stress": high ELA (209.48 ± 13.10, range: 155.19–249.02, $N = 6$), moderate ELA (202.22 ± 53.59, range: 148.64–255.81, $N = 2$), and low ELA (248.54 ± 65.23, range: 170.90–328.06, $N = 6$). Graph represents raw unlogged sAA concentrations, though values were square root transformed for analyses.

$r_s = -0.10$, $N = 5$), with the moderate ELA juveniles having the most negative average coefficient of the three (Fig. 3). A Kruskal-Wallis H test found a significant difference in Spearman's rank correlation coefficients among the three ELA categories, $H(2) = 6.989$, $p = 0.030$, $\eta^2 = 0.582$. A series of Mann-Whitney U post-hoc tests revealed significant differences between high ELA and low ELA juveniles ($U = 3.00$, $N_1 = 5$, $N_2 = 6$, $p = 0.028$, $r = 0.661$), as well as low ELA and moderate ELA juveniles ($U = 0.00$, $N_1 = 6$, $N_2 = 2$, $p = 0.046$, $r = 0.707$). The difference between high ELA and moderate ELA juveniles was nonsignificant ($p = 0.439$).

Discussion

The present study sought to determine whether there was an association between ELA, assessed through frequency of maternal maltreatment in infancy, and asymmetry in stress response systems. To our knowledge, this study is the first to collect saliva non-invasively from a free-ranging population of juvenile primates and provides the first evidence of an association between ELA and HPA-SNS asymmetry in a nonhuman animal. Our data indicate that moderate to high rates of ELA have an attenuating effect on HPA activity, consisting of higher basal cortisol concentrations and lower cortisol rises in response to stressors. In contrast, SNS responses to stressors, as measured by sAA concentrations, were not affected by ELA. Our results indicate that maternal maltreatment during the first 3 months of life has a significant effect on the coordination of the HPA and SNS during juvenility, with the stress response becoming dysregulated in individuals exposed to greater rates of ELA. The lack of positive correlations between HPA and SNS concentrations among individuals experiencing moderate to high levels of ELA suggests that ELA has a disorganizing effect on the developing stress response.

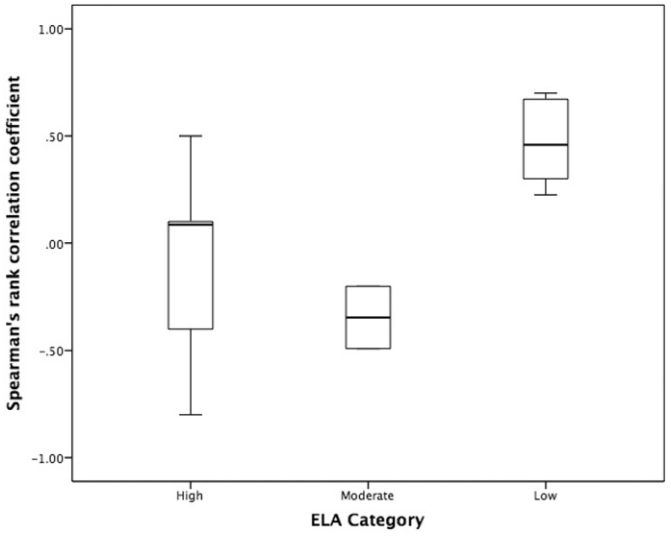

Fig. 3. Box-and-whisker plot showing relationship between ELA category and Spearman's correlation coefficients.

The marginal increase in cortisol concentrations in response to stressors in high ELA juveniles indicates a blunting effect of ELA on the HPA, consistent with previous research on rodents and rhesus macaques (rodents: Meaney and Szyf, 2005; rhesus macaques: Sanchez, 2006). This implies that the developing HPA is sensitive to early chronic stress and that maternal abuse and rejection specifically can result in the impairment of HPA function during juvenility. By down-regulating the HPA response, an individual may be better protected from energetic exhaustion due to chronic arousal (Susman, 2006). Considering the detrimental effects of chronically elevated cortisol levels, a blunted HPA response to stress among high ELA juveniles might best be considered a protective device by suppressing chronic activation of the stress response.

sAA concentrations did not significantly differ across the three groups, suggesting that early life stress may have differential effects on HPA and SNS functioning. Effects of chronic stress on HPA and SNS functioning may also be dependent on the nature of the stressor and psychological state of the individual, and so the relationship between ELA and the two systems may be subject to a number of factors not included here. Whether over-activation of the SNS has harmful effects similar to those seen with over-activation of the HPA axis is not well known—it is possible that the rapid response and recovery of the SNS to a stressor means that chronically elevated sAA, unlike chronically elevated cortisol, might not contribute significantly to overall allostatic load.

The finding that high and moderate ELA juveniles exhibited smaller increases in cortisol but more typical increases in sAA during a stress response indicates an asymmetry in stress system reactivity. Activity in the HPA and SNS is expected to be symmetrical based on the neural connectedness of these two systems, but the exact nature of their relationship remains unclear. One possible mechanism underlying this connection is the functioning of corticotropin-releasing factor (CRF), a

hormone messenger that is part of the HPA axis and is involved in regulating the release of cortisol. The locus coeruleus and the brain stem both receive CRF input from neurons of the hypothalamus, and are both involved in sympathetic regulation (Engert et al., 2011). Experimental studies show that central administration of exogenous CRF produces behavioral and physiological responses consistent with those seen in response to sympathetic activation, while administration of CRF antagonists suppresses these sympathetic-like responses (Valentino et al., 1993). Further experimental studies have demonstrated that norepinephrine stimulates production of CRF (Engert et al., 2011). Taken together, the neural and experimental data support the mediating role of CRF between the HPA and SNS. Beyond this, other work suggests that chronic stress early in life, which results in sustained elevation of CRF during development, influences HPA responses to future stress challenges (Heim and Nemeroff, 2001). CRF function may therefore contribute to the degree of symmetry between the HPA and SNS by differentially responding to environmental threat. As such, CRF-HPA pathways may be more vulnerable to psychosocial stress, resulting in HPA attenuation, and CRF-SNS pathways more resilient, resulting in typical SNS responses (Malarkey et al., 1995.)

The amygdala has also been suggested as another pathway through which symmetry in HPA-SNS reactivity is achieved. The locus coeruleus, in addition to receiving CRF input from the hypothalamus, can activate the SNS through direct input received from the amygdala. HPA activation by the amygdala occurs through communications from the central amygdala to the HPA axis through brain stem intermediary neurons (Herman et al., 2005). Further, circulating GCs act on the amygdala by increasing expression of CRF in the central amygdala and thus have been hypothesized to potentiate HPA response to stress in a feed-forward manner (Smith and Vale, 2006). Amygdala volume of juvenile rhesus macaques has been shown to be positively correlated with maternal abuse rates during the first three months of life, and a number of studies suggest that structural-developmental effects of ELA on amygdala volume may emerge during juvenility (Howell et al., 2014; McEwen and Gianaros, 2010; Nelson, 2013; Tottenham and Sheridan, 2010). Therefore the amygdala may represent another possible mediator of the HPA-SNS link—particularly in maltreated juveniles—although more work into the relationship between amygdala volume and acute stress response is needed to understand this association.

The HPA and SNS systems also differ in the timing of their respective responses to stress, which may influence the appearance of symmetry of their reactivity. Upon stimulation of the SNS, sAA enters into the saliva immediately and thus represents acute stress response with no time lag. The SNS has a faster recovery time than the HPA axis, with sAA returning more quickly to baseline levels after a stress response than cortisol (Allwood et al., 2011; Granger et al., 2007). In contrast, the HPA responds more slowly, with cortisol levels gradually rising to a peak before being suppressed by a negative feedback loop. With our results, it remains possible then that high and moderate ELA juveniles might have eventually reached post-stressor cortisol concentrations similar to low ELA juveniles but were unable to mount an HPA response as quickly. Our study was limited by using single-sample assessments of stress level rather than collections of multiple time points to account for baseline, stress, and recovery periods. As advances in salivary bioscience continue, the use of multiple time point assessment in future studies of free-ranging animals will allow us to better account for differences in reactivity and recovery. Nonetheless, our results indicate at minimum that ELA modulates the speed at which the HPA mounts a stress response.

Our findings support our hypothesis that exposure to high, and to a lesser extent, moderate, levels of maternal maltreatment are associated with long-term changes in neuroendocrine symmetry. Although our data do not explain precisely how the HPA-SNS connection becomes disrupted in juveniles with histories of maternal maltreatment, nor can we infer a causal relationship, we provide evidence that a relationship between ELA and HPA-SNS asymmetry well-documented in humans also occurs in a nonhuman primate, the rhesus macaque. These findings demonstrate the continued utility of rhesus macaques as a nonhuman primate model of human stress and disease, as asymmetry in HPA-SNS responsiveness to stress has been found to predict a variety of negative outcomes (e.g., depressive and antisocial behaviors: Shenk et al., 2010; aggressive behavior: Gordis et al., 2006; poor cognitive functioning: Berry et al., 2012). Our results highlight the importance of multi-systems approaches to stress studies and future developmental research in nonhuman primates under free-ranging conditions should continue to utilize these approaches to investigate the development of the stress response. The consideration of individual differences in psychology, health, and life history when assessing physiological responses to chronic stress will also aid in understanding differences in developmental outcomes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2016.05.004.

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