PNAS
www.pnas.org

Supplementary Information for

Pubertal Stress Recalibration Reverses the Effects of Early Life Stress in Post-Institutionalized Children

Megan R. Gunnar (gunnar@umn.edu), Carrie E. DePasquale (depas010@umn.edu), Brie M. Reid (reidx189@umn.edu), Bonny Donzella (donzella@umn.edu)

Corresponding Author: Megan R. Gunnar; Institute of Child Development, University of Minnesota - Twin Cities, 51 E. River Road, Minneapolis MN, 55455
Email: gunnar@umn.edu

This PDF file includes:

Supplementary text
Figs. S1 to S2
Tables S1 to S3
References for SI reference citations

Supplementary Information Text

Materials and Methods

Participants and Study Design.

Each annual session included two laboratory visits approximately one week apart: one assessed cortisol stress reactivity and another assessed pubertal stage. Of the 299 total participants, 280, 241, and 232 have pubertal stage and cortisol reactivity data for the first, second, and third sessions, respectively.

Post-institutionalized youth in this study were recruited through the International Adoption Project, a registry for families adopting children internationally. Families typically join this registry via an online survey sent to them after adopting internationally via a local agency. Non-adopted comparison youth were recruited through a registry of families who have previously demonstrated interest in participating in research. These families were typically mailed a letter describing the registry after giving birth at a hospital in the same state as the University, who then provided their contact information to be approached for future studies.

Measures.

Salivary Cortisol Reactivity. At every annual session, participants completed a modified version of the Trier Social Stress Test for Children (TSST-C) (1,2; see (3) for additional details on this procedure) while being filmed by an obvious camera in front of a two-way mirror. Novel experimenters were used each year in order to prevent familiarity. The participants were told that there were judges behind the mirror and that their film would be shown to other students to evaluate. The participants then judged their own performance and listed what they wanted to improve next time. These self-analyses were shown to the participants during the second and third session as an additional social evaluation factor to minimize habituation. After a 40-minute adaptation period, time 0 represents the beginning of the speech preparatory period, because the increase in cortisol production in response to stress is believed to start in anticipation of the speech rather than the speech itself (4) with peak cortisol levels approximately 20-40 minutes later (sample 4 or 5 in Figure S1) (5).

Seven whole, unstimulated saliva samples were collected throughout the two-hour laboratory visit, as shown in Figure S1 (3), to later be assayed for cortisol concentration. Saliva samples for each participant within a session were included on the same assay batch to control for inter-assay variability. Intra- and inter-assay coefficients of variation ranged from 4.0-7.9%. To focus on reactivity and recovery and not the anticipatory stress of arriving at the lab, the present analyses only included the six post-adaptation samples (samples 2-7 in Figure S1). Because the HPA axis has a strong diurnal rhythm, all TSST-C sessions began between 3:00 and 4:30 pm. Participants were asked to refrain from eating and drinking (including water and especially caffeine) during the visit. An index of daily medication usage (following guidelines in (6)) was created with regard to their potential impact on adrenal cortisol production and was examined as a potential covariate. Biologically implausible values (>2 μg/dL; n=11, 0.3%) were removed and values were $\log_{10}$-transformed to resolve positive skew. A sensitivity analysis was conducted excluding values greater than 4 standard deviations from the mean (n=5, 0.1%), which did not change the pattern of results (data available upon request).

To measure the subjective experience of stress during the TSST-C, upon completion of the TSST-C participants were asked to rate their feelings of stress at five points throughout the laboratory session (arrival, preparation, speech, math, after completion) on a scale from 1 (*Not at all*) to 5 (*A whole lot*). These ratings were averaged to create a single index of youth-reported subjective stress during the TSST-C.

Tanner Pubertal Staging. Each year, pubertal staging was conducted by a trained nurse and scored according to Marshall and Tanner criteria (7,8) on a different day from when they

completed the TSST-C. A subsample of participants underwent the physical exam with two of the three nurses to examine nurse agreement (κ=0.74-1.0 for all three annual sessions). If a participant reached stage 5 before their third annual session, they were presumed to remain at stage 5 and did not complete Tanner staging again. Missing Tanner stage values were imputed based on the participants' response to the Peterson Pubertal Development Scale (9,10) as an alternative assessment of pubertal stage. Items were assessed on a scale from 1=*not yet started* to 4=*seems complete* including growth in height, body hair and skin changes for both genders. Additionally, deepening of voice and facial hair were assessed for boys and breast development and onset of menarche were assessed for girls. Fifty-six (17.4%) participants refused Tanner staging and their values were imputed based on parent- and child-reported Peterson score. Specifically, 23, 21, and 34 refused Tanner for sessions 1-3 respectively – 15, 12, and 19 of whom were in the NA group. Because the two measures are on different scales, the Peterson scores were converted to Tanner scores following guidelines in (11). The previous year's score was used in cases when estimation was lower (n = 2) under the assumption that a child cannot regress in pubertal development. Tanner and Peterson scores were significantly highly correlated at all sessions (r=0.85-0.88, p's<.001).

Youth-Reported Life Stress. Participants completed a modified version of the Youth Life Stress Interview (12), a semi-structured interview assessing life challenges in eight domains (academic, behavioral, same-sex peer, opposite sex peer, romantic, sibling, parent-child, and marital). This interview takes approximately one hour and inquires about life events over the past 12 months, which were then rated on a scale from 1 (*Superior/No stress*) to 5 (*Extreme/Severe stress*), including half-points. Scores were given separately for each domain by two trained raters and conferenced to consensus. The conferenced scores were averaged to create one score of youth-reported chronic stress in the past year.

Data Analysis.

A hierarchical linear mixed-effects model (13) was used to examine change in cortisol reactivity over time. The model was fit using the 'nlme' package in R (14,15). Time since start of TSST-C prep and the quadratic effect of time were used as predictors to model cortisol reactivity and recovery, with random intercepts and random effects of time and quadratic time nested within session and within participant. Sex, as well as sex x group and sex x pubertal stage interactions were included as covariates. Age at adoption, medication usage, youth-reported past-year life stress, and youth-reported subjective stress were examined as potential covariates associated with cortisol reactivity, but these variables did not change the pattern of results, and so are not included in the final model.

Pubertal change (within-individual change in pubertal stage) was modeled using pubertal stage centered around each participant's mean across all sessions and between-individual change in puberty was modeled as each participant's mean pubertal stage. Each pubertal stage variable was then moderated by group (PI vs. NA) to examine whether changes in pubertal stage predicted changes in cortisol reactivity differently for the two groups.

Pubertal stage was used as the longitudinal "time" variable rather than session 1-3 to better represent the accelerated longitudinal design. Pubertal stage is ordinal; however, because model assumptions were still met it was analyzed continuously to simplify the model. Within- and between-individual effects were separated by including two pubertal stage scores in the same model: 1) all available time points of pubertal stage centered around each individual's mean (pubertal change; up to three data points per individual) and 2) each individual's mean pubertal stage (one data point per individual), respectively. The final model for sample k in session j for individual i is represented in Equation S1 below.

Level 1 (sample): (*Equation S1*)

$$\log_{10} \text{cortisol}_{ijk} = \beta_{0jk} + \beta_{1jk}\text{sample time}_{ijk} + \beta_{2jk}\text{sample time}^2_{ijk} + \varepsilon_{ijk}$$

Level 2 (session):

$$\beta_{0jk} = \gamma_{00k} + \gamma_{01k}\text{pubertal change}_{jk} + \upsilon_{0jk}$$
$$\beta_{1jk} = \gamma_{10k} + \gamma_{11k}\text{pubertal change}_{jk} + \upsilon_{1jk}$$
$$\beta_{2jk} = \gamma_{20k} + \gamma_{21k}\text{pubertal change}_{jk} + \upsilon_{2jk}$$

Level 3 (individual):

$$\gamma_{00k} = \delta_{000} + \delta_{001}\text{sex}_k + \delta_{002}\text{mean pubertal stage}_k + \delta_{003}\text{group}_k + \delta_{004}\text{sex*mean pubertal stage}_k + \delta_{005}\text{sex*group}_k + \delta_{006}\text{mean pubertal stage*group}_k + \nu_{00k}$$
$$\gamma_{10k} = \delta_{100} + \delta_{101}\text{mean pubertal stage}_k + \delta_{102}\text{group}_k + \delta_{103}\text{group*mean pubertal stage}_k + \nu_{10k}$$
$$\gamma_{20k} = \delta_{200} + \delta_{201}\text{mean pubertal stage}_k + \delta_{202}\text{group}_k + \delta_{203}\text{group*mean pubertal stage}_k + \nu_{20k}$$
$$\gamma_{01k} = \delta_{010} + \delta_{011}\text{group}_k + \nu_{01k}$$
$$\gamma_{11k} = \delta_{110} + \delta_{111}\text{group}_k + \nu_{11k}$$
$$\gamma_{21k} = \delta_{210} + \delta_{211}\text{group}_k + \nu_{21k}$$

Specifically, a significant effect of pubertal change would indicate that changes in pubertal stage within individuals, regardless of the actual stage, predict changes in cortisol reactivity over time. A significant effect of mean pubertal stage would suggest that between-individual differences in pubertal stage are associated with differences in cortisol reactivity. If both are significant, this would indicate that both within-individual change and the actual pubertal stage are significantly associated with cortisol reactivity. Group (PI vs. NA) was included as a moderator to examine whether the association between pubertal stage and cortisol reactivity differs as a function of exposure to early institutional care. The coefficients δ_{211} and δ_{203} in Equation S1 represent the within- and between-individual group x pubertal stage interactions on cortisol reactivity, respectively.

Pubertal Changes in Self-Reported Stress

In conjunction with the focal analyses described above, a supplemental analysis was conducted to examine the longitudinal changes in self-reported subjective stress during the TSST-C that may parallel the changes in cortisol reactivity. This was accomplished using a hierarchical mixed-effects model with sessions nested within participants, including both pubertal change (within-individual) and mean pubertal stage (between-individual) as predictors of self-reported stress over time. Group was examined as a possible moderator, given previous evidence in this sample (3) that self-reported subjective stress may differ across puberty between PI and NA youth. Sex was included as a covariate. As shown in Table S3, mean pubertal stage did not significantly predict self-reported stress over time but pubertal change did. Specifically, within-individual increases in pubertal stage were associated with within-individual *decreases* in self-reported subjective stress over time. There were no interactions with group, suggesting this relation does not differ for PI and NA children. Thus, even though PI children show increased cortisol reactivity across puberty (Figure S1), participants are reporting less subjective stress over time (perhaps due to decreased novelty following repeated exposure to the TSST-C).

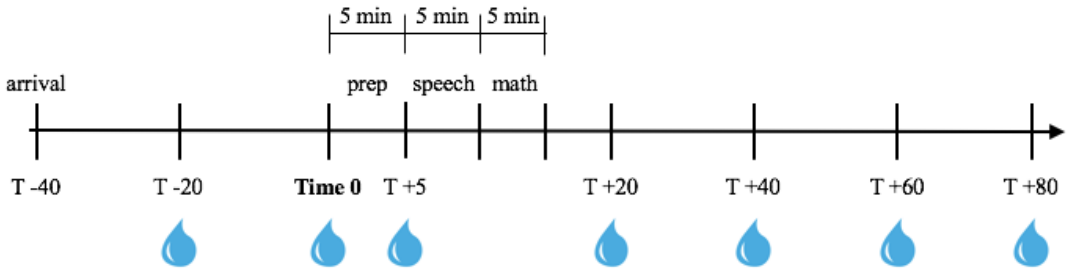

Fig. S1. TSST session timeline. Saliva samples are indicated by water droplets beneath the timeline. Adapted with permission from ref (3).

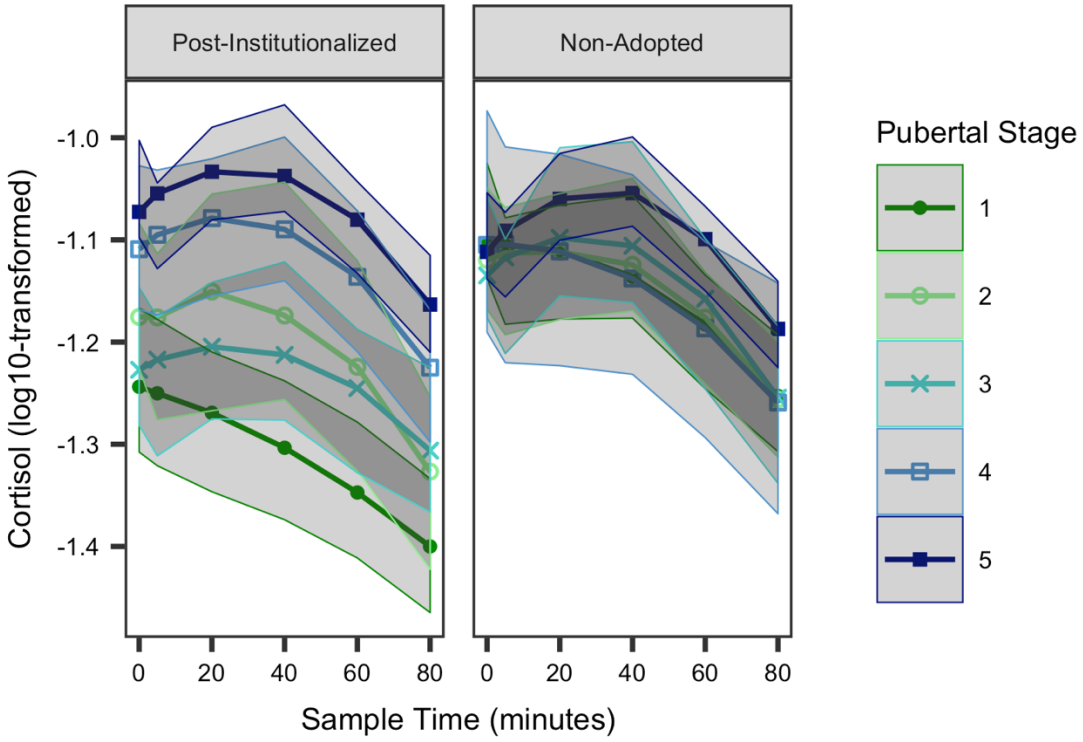

Fig. S2. The same data as is presented in Figure 1, with the addition of 95% confidence envelopes. For post-institutionalized youth, the confidence envelope for pubertal stage 1 never overlaps with that of stages 4 or 5. For non-adopted youth, all confidence envelopes overlap at all points.

Table S1. Descriptive statistics and intercorrelations between focal variables.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
1. S1 child age (years)	—												
2. S1 pubertal stage	**0.84*****	—											
3. S2 pubertal stage	**0.89*****	**0.89*****	—										
4. S3 pubertal stage	**0.83*****	**0.81*****	**0.90*****	—									
5. S1 T0 cortisol	0.11	0.03	-0.03	-0.03	—								
6. S1 T+40 cortisol	**0.22*****	**0.19****	**0.17****	**0.13***	**0.66*****	—							
7. S1 T+80 cortisol	**0.16****	**0.13***	0.11	0.09	**0.62*****	**0.78*****	—						
8. S2 T0 cortisol	**0.18****	**0.15***	0.12	0.12	**0.39*****	**0.31*****	**0.27*****	—					
9. S2 T+40 cortisol	**0.26*****	**0.21*****	**0.17****	**0.17****	**0.33*****	**0.50*****	**0.38*****	**0.68*****	—				
10. S2 T+80 cortisol	**0.25*****	**0.22*****	**0.18****	**0.15***	**0.31*****	**0.39*****	**0.35*****	**0.62*****	**0.79*****	—			
11. S3 T0 cortisol	**0.20****	**0.13***	**0.13***	0.10	**0.25*****	**0.28*****	**0.28*****	**0.34*****	**0.39*****	**0.30*****	—		
12. S3 T+40 cortisol	**0.28*****	**0.21*****	**0.23*****	**0.24*****	**0.24*****	**0.36*****	**0.29*****	**0.26*****	**0.48*****	**0.35*****	**0.56*****	—	
13. S3 T+80 cortisol	**0.30*****	**0.26*****	**0.26*****	**0.25*****	**0.24*****	**0.27*****	**0.27*****	**0.27*****	**0.41*****	**0.40*****	**0.53*****	**0.79*****	—

Mean (SD) or *Median*	11.28 (2.31)	2	3	4	-1.13 (0.31)	-1.12 (0.34)	-1.28 (0.33)	-1.12 (0.27)	-1.13 (0.29)	-1.25 (0.24)	-1.09 (0.23)	-1.09 (0.27)	-1.21 (0.26)

Note. * $p<.05$, * $p<.01$, *** $p<.001$. Significant values are indicated in bold. All correlations are Pearson correlations except those with pubertal stage, which are Spearman's rank correlations. Cortisol descriptive statistics and correlations are using $\log_{10}$-transformed values. Time 0, +40, and +80 cortisol values represent post-adaptation baseline, peak reactivity, and recovery, respectively. Medians are reported for pubertal stage rather than means and standard deviations. S1=Session 1, S2=Session 2, S3=Session 3.

Table S2. Final linear mixed-effects model examining the association between pubertal stage and cortisol stress reactivity, as moderated by group.

Fixed effects	Estimate (*SE*)	*t*-value
Intercept, δ_{000}	**-1.27 (0.04)**	**-31.30*****
Time, δ_{100}	-0.00 (0.00)	-1.89
Time^2, δ_{200}	-0.00 (0.00)	-0.52
Sex, δ_{001}	0.01 (0.05)	0.29
Group (PI vs. NA), δ_{003}	**0.14 (0.05)**	**2.91****
Mean pubertal stage, δ_{002}	**0.03 (0.01)**	**2.99****
Pubertal change, δ_{010}	**0.05 (0.02)**	**2.18***
Sex x Group, δ_{005}	0.05 (0.04)	1.30
Sex x Mean pubertal stage, δ_{004}	0.01 (0.01)	0.52
Group x Mean pubertal stage, δ_{006}	**-0.04 (0.01)**	**-2.96****
Group x Pubertal change, δ_{011}	**-0.08 (0.03)**	**-2.87****
Time x Group, δ_{102}	0.00 (0.00)	0.92
Time x Mean pubertal stage, δ_{101}	**0.00 (0.00)**	**4.31*****
Time x Pubertal change, δ_{110}	0.00 (0.00)	0.26
Time^2 x Group, δ_{202}	-0.00 (0.00)	-0.62
Time^2 x Mean pubertal stage, δ_{201}	**-0.00 (0.00)**	**-3.54*****
Time^2 x Pubertal change, δ_{210}	-0.00 (0.00)	-0.57
Time^2 x Group x Mean pubertal stage, δ_{203}	-0.00 (0.00)	-0.77
Time^2 x Group x Pubertal change, δ_{211}	**0.00 (0.00)**	**2.74****
Random effects - session	Variance	*SD*
Intercept	0.00	0.01

Time	0.00	0.00
Time^2	0.00	0.00
Random effects - participant	Variance	*SD*
Intercept	0.07	0.26
Time	0.00	0.00
Time^2	0.00	0.00
Residual	0.01	0.09

Note. *p<.05, ** p<.01, *** p<.001. Significant values are indicated in bold. PI is the reference group for "Group". Results and corresponding parameter labels are based on model described in Equation S1. "Mean pubertal stage" refers to the between-individual puberty variable (at the level of the individual, averaged across all sessions), while "Pubertal change" refers to the within-individual change in puberty variable (at the level of session, centered on each individual's mean pubertal stage).

Table S3. Linear mixed-effects models examining the association between pubertal stage and self-reported subjective stress.

Fixed effects	Model 1		Model 2	
	Estimate (*SE*)	*t*-value	Estimate (*SE*)	*t*-value
Intercept	**3.05 (0.16)**	**18.54*** **	**3.34 (0.32)**	**10.32*** **
Sex	-0.05 (0.09)	-0.55	-0.05 (0.09)	-0.64
Group (PI vs. NA)	0.05 (0.08)	0.55	-0.13 (0.19)	-0.70
Mean pubertal stage	-0.02 (0.03)	-0.53	-0.11 (0.09)	-1.15
Pubertal change	**-0.09 (0.04)**	**-2.34***	-0.01 (0.13)	-0.05
Group x Mean pubertal stage	—	—	-0.05 (0.08)	-0.68
Group x Pubertal change	—	—	0.06 (0.06)	1.04
Random effects	Variance	*SD*	Variance	*SD*
Intercept	0.71	0.84	0.71	0.84
Session	0.10	0.32	0.10	0.32
Residual	0.34	0.58	0.34	0.58

Note. *$p<.05$, ** $p<.01$, *** $p<.001$. Significant values are indicated in bold. PI is the reference group for "Group". "Mean pubertal stage" refers to the between-individual puberty variable (at the level of the individual, averaged across all sessions), while "Pubertal change" refers to the within-individual change in puberty variable (at the level of session, centered on each individual's mean pubertal stage).

References

1. C.E. Hostinar, A.E. Johnson, M.R. Gunnar, Early social deprivation and the social buffering of cortisol stress responses in late childhood: An experimental study. *Developmental Psychology*. **51**, 1597-1698 (2015).
2. I.S. Yim, J.A. Quas, L. Cahill, C.M. Hayakawa, Children's and adults' salivary cortisol responses to an identical psychosocial laboratory stressor. *Psychoneuroendocrinology*. **35**, 241-248 (2010).
3. C.E. DePasquale, B. Donzella, M.R. Gunnar, Pubertal recalibration of cortisol reactivity following early life stress: a cross-sectional analysis. *Journal of Child Psychology and Psychiatry*. **60**, 566-575 (2019).
4. S.R. Sumter, C.L. Bokhorst, A.C. Miers, J. Van Pelt, P.M. Westenberg, Age and puberty differences in stress responses during a public speaking task: Do adolescents grow more sensitive to social evaluation? *Psychoneuroendocrinology*. **35**, 1510-1516 (2010).
5. C. Kirschbaum, D.H. Hellhammer, Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*. **19**, 313-333 (1994).
6. D.A. Granger, L.C. Hibel, C.K. Fortunato, C.H. Kapelewski, Medication effects on salivary cortisol: Tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology*. **34**, 1437-1448 (2009).
7. W.A. Marshall, J.M Tanner, Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood.* **44**, 291-303 (1969).
8. W.A. Marshall, J.M. Tanner, Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood.* **45**, 13-23 (1970).
9. M.A. Carskadon, C. Acebo, A self-administered rating scale for pubertal development. *Journal of Adolescent Health.* **14**, 190-195 (1993).
10. A.C. Petersen, L. Crockett, M. Richards, A. Boxer, A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence.* **17**, 117-133 (1988).
11. E.A. Shirtcliff, R.E. Dahl, S.D. Pollak, Pubertal development: Correspondence between hormonal and physical development. *Child Development*. **80**, 327-337 (2009).
12. K.D. Rudolph, C. Hammen, Age and gender as determinants of stress exposure, generation, and reactions in youngsters: A transactional perspective. *Child Development*. **70**, 660-677 (1999).
13. J. Pinheiro, D. Bates, *Mixed-effects models in S and S-PLUS*. Springer Science & Business Media. (2006).
14. J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, R Core Team, nlme: linear and nonlinear mixed effects models. R package version 3.1-128. *Bioinformatics*. **26**, 1463-1464 (2016).
15. R Core Team, R: A language and environment for statistical computing [Computer software manual]. Vienna, Austria. (2016).