SUPPLEMENTARY INFORMATION

The Association Between Maternal Cortisol and Infant Amygdala Volume Is Moderated by Socioeconomic Status

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Supplementary Methods

Participant Inclusion/Exclusion Criteria

Exclusion criteria for the current study included women with multiple gestations, diagnosed maternal infections known to cause congenital disease, premature birth (<37 weeks estimated gestational age), low infant birthweight (<2,000 grams), infant spending more than 7 days in the neonatal intensive care unit, MRI-identified brain injury in the infant, maternal alcohol use during pregnancy, maternal drug use during pregnancy (excluding marijuana and tobacco), and maternal steroid use in any form as steroids are known to impact cortisol production. Participant questionnaires and chart review were used to collect medical data by trained staff.

Maternal tobacco and/or maternal cannabis use during pregnancy was not an exclusion for study enrollment nor inclusion in the analyses presented here. The reasoning for including participants who reported tobacco and/or cannabis use during pregnancy and covarying for their use in the statistical models was twofold: First, the population included in this sample has lower socioeconomic status than the vast majority of other research and has a higher degree of substance use. Excluding participants who used tobacco and cannabis during pregnancy would limit the number of high disadvantage included in the analysis, which we believe perpetuates a lack of research in very low-income populations. Second, we believe that our results are more generalizable to other low-income populations as a result of the inclusion of dyads reporting maternal tobacco and/or marijuana use. As such, we believe that their inclusion (with appropriate covariate treatment) is an important aspect of our study.

Socioeconomic disadvantage score measures.

The socioeconomic disadvantage score was generated by reverse-coding the social advantage factor score generated by (1). This factor score was calculated using the following variables:

Income-to-needs ratio was calculated at each trimester and utilized self-reported family income and household size compared to federal poverty thresholds for families of the corresponding size. An income-to-needs ratio of 1.0 represents the poverty line.

Insurance status was collected at study enrollment via medical record review and was confirmed during the third trimester of pregnancy or at delivery. Possible insurance categories included Individual/Group, Medicaid, Medicare, VA/Military, or Uninsured.

Maternal education level was self-reported at study enrollment and included options for less than a high school degree, high school degree, college graduate, and post-graduate degree.

Area deprivation index was used to rank neighborhoods by socioeconomic disadvantage compared to the national average compared to census data and includes information about area income, education, employment, and housing quality (2,3).

Maternal nutrition was assessed during the third trimester or at delivery using the Healthy Eating Index. The Healthy Eating Index is a validated assessment available from the National Institutes of Health to measure diet quality based on U.S. Dietary Guidelines for Americans (4). The data used to calculate the Healthy Eating Index for each participant was collected using the Diet History Questionnaire (5,6).

Maternal psychosocial stress measures

Maternal perceived stress Maternal perceived stress was measured once per trimester using Cohen's Perceived Stress Scale (7). The scale asked participants to rate the frequency of stressful events in the past month on a scale ranging from 0 = "Never" to 4 "Fairly Often." Items included, "*In the last month, how often have you been upset because of something that happened unexpectedly?"* and *"In the last month, how often have you felt confident about your ability to handle your personal problems?"* among others. Sum scores were created for each trimester, reverse scoring items as needed. Thus, possible scores ranged from 0 – 40 in each trimester.

Maternal depression symptoms The Edinburgh Postnatal Depression Scale was used to collect self-reported depression symptoms once per trimester (8). Participants were asked to report the frequency of depressive symptoms on a scale of 0 = "Hardly" at all to 3 = "Yes, quite a lot". Items included, "*I have been able to laugh and see the funny side of things"* and *"I have blamed myself unnecessarily when*

things went wrong", among others. Sum scores were calculated for each trimester with items reverse scored as instructed. Possible scores ranged from 0 to 30.

Stressful and traumatic life events Self-reported stressful and traumatic life events during mothers' lifetimes were collected using the Stress and Adversity Inventory for Adults (STRAIN; Slavich & Shields, 2018) at the time of the neonatal brain imaging session or at follow-up.

Experiences of discrimination The Everyday Discrimination Scale (10) was completed by caregivers at the time of the neonatal brain imaging visit and used to measure experiences of discrimination based on race.

Factor score generation

The maternal socioeconomic disadvantage factor score included measures of income to needs ratio, insurance status, mother's highest level of education, area deprivation index, and maternal nutrition. For maternal psychosocial stress, measures of perceived stress, self-reported depression symptoms, self-reported traumatic life events, and experiences of discrimination were included in the factor score. The confirmatory factor analysis that generated the latent score was completed in MPlus (version 8.4; 11) and the full model can be seen in Figure S3. Fit indices indicated good fit to the data and the two-factor model outperformed one-factor and three-factor alternatives. More detailed methods of the confirmatory factor analysis can be found in (1).

Cortisol Sampling, Processing, and Data Preparation

Cortisol Sampling. Participants provided salivary samples (Salimetrics, United Kingdom) collected every four hours for 24 hours each trimester, starting at 18:00 hr. Participants received seven Salivette tubes (Sarstedt SAR-511534500) and the following instructions on how to collect saliva specimens at home. Participants were asked to collect clear saliva samples free of contamination with food, lipstick, blood, or other extraneous materials at least 30 – 60 minutes after a meal, oral intake of pharmaceutical drugs, or tooth cleaning. To collect saliva, participants were asked to remove the swab from the tube without touching it with their hands, insert it into their mouths, and gently chew until the

mouth salivated. Swabs were then returned to the suspended insert in the tube using their teeth to avoid touching it with their hands. Research staff called or texted participants once on the day of specimen collection to remind them of instructions for saliva collection and to bring completed saliva kits to subsequent visits. Participants labeled samples with the date and time upon sample completion, sealed them, and stored samples in a -20°C freezer at their home until delivered to study staff. Once received, the samples were timestamped, frozen, and processed for analysis of cortisol concentrations by ELISA (Salimetrics Melatonin EIA kit and Salimetrics Cortisol ELISA kits) at Washington University School of Medicine. Figure S1 shows the distribution of the difference between actual collection time and the instructed collection time. Participant compliance with collection time was high, as can be seen in the figure. Additionally, the data reduction technique used for the cortisol data (Area Under the Curve with Respect to Ground, see below) is agnostic to sample timing and uses the actual interval between samples when estimating total cortisol production.

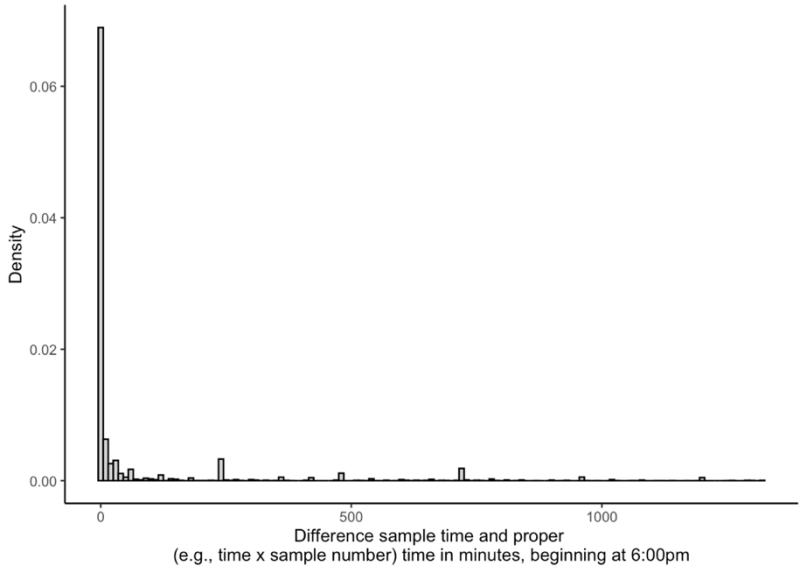

Figure S1. Density plot of the difference in minutes between instructed cortisol sample time and participant-reported sample time. Participants generally collected the samples within an hour of the appointed time.

Cortisol Processing. Upon receipt of the saliva, samples were thawed and centrifuged for two minutes at 1000x g. The one mL samples were aliquoted into cryovials (Corning 2.0 ml external thread #430659) and stored at -80°C until assayed. Prior to assay the aliquoted saliva samples were thawed, spun at 1500g for 15 minutes, and analyzed with a Salimetrics Saliva Cortisol ELISA kit (Salimetrics 1-3002-5) using manufacturer instructions. All samples were assayed in duplicate with measurements read on a BioTek Eon plate reader using Gen5 (version 2.07) software. Absorbance was measured at 450 nm and 620 nm. Sample concentrations were calculated according to a standard curve with cortisol concentrations between 0.12 ng/mL and 30 ng/mL. The lower limit sensitivity (i.e., the lowest cortisol concentration that can be distinguished from 0) was 0.07 ng/mL. Samples with concentrations above 30 ng/mL were diluted and re-assayed until values fit within the standard curve and then the concentration values were corrected based on dilution. The intra- and inter-assay CV in the duplicate assays were 4.6% and 6%, respectively. Individual sets of saliva samples with 5 or more samples were included in subsequent analyses, resulting in the exclusion of 17 sets of saliva samples (first trimester=7 exclusions, second trimester=6 exclusions, third trimester=4 exclusions). Participants provided the requested saliva samples at a very high rate, generally, resulting in very little missingness: 60 of 1,467 possible samples were missing in trimester 1; 92 of 2,518 were missing in trimester 2; and 89 of 2,384 in trimester 3.

Cortisol Data Preparation. Raw cortisol data were hand-cleaned to ensure accuracy and outliers were winsorized to the highest value less than 5 standard deviations from the mean (18.47ng/mL) if they fell outside this cut-off. This winsorization was completed due to some values falling outside the range of physiological plausibility and included 25 total data points, 2 in the first trimester, 8 in the second trimester, and 15 in the third trimester. This cut-off value was chosen to include as much original data as possible while allowing for variability that may be due to physiological changes during pregnancy (the normal range is approximately 11–12 ng/mL; Laudat et al., 1988). Area under the curve with respect to ground (AUCg) was then calculated as a measure of total cortisol production over the 24-hour saliva collection period (13). One AUCg value was calculated for each trimester in most cases. However, some participants completed two sets of saliva samples within the same trimester. In these cases, the average of

the two AUCg values within the same trimester was used for data analysis (Table S1 includes the number of averaged samples and the number of participants providing cortisol samples in each trimester). Descriptive statistics for cortisol AUCg by trimester can be found in Table 2. The resulting distribution of AUCg values was right-skewed and exhibited a high degree of positive kurtosis. To address these issues and meet the normality assumptions of the planned data analysis, AUCg values were log-10 transformed and one outlier AUCg observation, more than 5 standard deviations below the sample mean, was removed. The distribution met normality assumptions following transformation as skewness and kurtosis estimates fell within acceptable ranges (skewness=-0.02; kurtosis =1.41; Curran et al., 1996). Model results did not differ significantly with alternative outlier specifications (e.g. removal of AUCg observations +/- 3sd from the mean, no outliers removed) as can be seen in Tables S2 and S3.

Supplementary Results

Number of averaged AUCg values by trimester

Table S1. Number of participants providing saliva samples for each trimester or set of trimesters and the number of participants for whom two AUCg values were averaged in the same trimester. For example, 21 participants provided samples in trimester 1 and trimester 3 only, while 80 participants provided samples in trimester 2 and trimester 3 only. One hundred eight participants provided samples in all trimesters.

	T1	**T2**	**T3**	**T1, T2, & T3**	**Averaged AUCg Values**
T1	4				0
T2	5	15			29
T3	21	80	8		23
T1, T2, & T3				108	52

Differences between the full sample and the final model sample

Table S2. Demographics of participants excluded from the final model for providing only one set of saliva samples across pregnancy and those included in the model (e.g., provided two or more sets of saliva samples). P-values in the right-hand column were generated using t-tests for continuous variables and chi-square tests for categorical variables.

	Excluded (N=19)	Included (N=222)	P-value
Infant Sex			
Male	8 (42.1%)	120 (54.1%)	0.346
Female	11 (57.9%)	102 (45.9%)	
Infant Age at Scan			
Mean (SD)	41.6 (1.21)	41.3 (1.26)	0.282
Median [Min, Max]	42.0 [39.0, 43.0]	41.0 [38.0, 45.0]	
Infant Birthweight			
Mean (SD)	3180 (503)	3250 (487)	0.578
Median [Min, Max]	3160 [2370, 4130]	3190 [2270, 4610]	
Race/Ethnicity			
Black	12 (63.2%)	127 (57.2%)	0.448
White	6 (31.6%)	87 (39.2%)	
Asian	1 (5.3%)	3 (1.4%)	
Native Hawaiian/Pacific Islander	0 (0%)	0 (0%)	
American Indian/ Alaskan Native	0 (0%)	0 (0%)	
Other	0 (0%)	5 (2.3%)	
Unknown	0 (0%)	0 (0%)	
First Trimester INR			
Mean (SD)	2.46 (3.00)	3.13 (3.10)	0.364
Median [Min, Max]	1.24 [0.500, 11.8]	1.54 [0.430, 12.2]	
Missing	0 (0%)	2 (0.9%)	
Socioeconomic Disadvantage Factor Score			
Mean (SD)	0.109 (0.981)	-0.144 (0.983)	0.294
Median [Min, Max]	0.379 [-1.90, 1.37]	0.263 [-2.15, 1.47]	
Psychosocial Stress Factor Score			
Mean (SD)	0.245 (1.24)	-0.175 (0.848)	0.165
Median [Min, Max]	-0.109 [-1.21, 2.61]	-0.351 [-1.68, 3.66]	
Maternal Insurance Status			
Medicaid	8 (42.1%)	70 (31.5%)	0.62
Medicare	0 (0%)	6 (2.7%)	

	Excluded (N=19)	Included (N=222)	P-value
Individual or Group	10 (52.6%)	122 (55.0%)	
Uninsured	1 (5.3%)	24 (10.8%)	
Maternal Age at Delivery			
Mean (SD)	27.7 (6.32)	29.3 (5.23)	0.297
Median [Min, Max]	26.9 [19.0, 40.5]	29.0 [18.7, 41.8]	
Amygdala Volume			
Mean (SD)	926 (70.2)	911 (99.8)	0.382
Median [Min, Max]	936 [819, 1030]	905 [627, 1340]	
Total Gray Matter Volume			
Mean (SD)	123000 (13200)	121000 (15200)	0.497
Median [Min, Max]	124000 [95700, 142000]	120000 [79600, 168000]	

Robustness to outlier specification

To confirm that the significant interaction between socioeconomic disadvantage and cortisol slope across pregnancy predicting neonatal amygdala volumes was not due to the treatment of the outlier observation, the model was re-run using two alternative outlier treatments: 1) removing all observations ±3 standard deviations from the mean and 2) no outlier removal. The results of these models did not differ from the model presented in the main manuscript, in which one observation five standard deviations from the mean was removed. Full model results for these alternative model specifications are presented in Tables S3 and S4.

Table S3. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume. Only outliers ±3sd from the mean of cortisol slope were removed in this model specification and results are consistent with those presented in the main manuscript.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	-0.01	[-0.08, 0.07]	3.31	0.001
Cortisol Slope	0.04	[-0.03, 0.12]	0.65	0.517
Disadvantage Factor	-0.13	[-0.22, -0.04]	2.36	0.019
Infant Age at Scan	-0.03	[-0.12, 0.06]	-0.64	0.525
Infant Sex	-0.09	[-0.17, -0.01]	-2.15	0.033
Infant Birthweight	-0.06	[-0.15, 0.02]	-1.44	0.150
Total Gray Matter Volume	0.78	[0.67, 0.89]	14.19	<0.001
Maternal Tobacco Use	-0.08	[-0.16, -0.01]	-2.12	0.035
Maternal Cannabis Use	0.07	[-0.01, 0.16]	1.70	0.090
Cortisol Slope x Disadvantage Factor	-0.13	[-0.21, -0.04]	-3.03	0.003

Table S4. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume. No outliers were removed in this model specification and results are consistent with those presented in the main manuscript.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.06, 0.08]	3.58	<0.001
Cortisol Slope	0.04	[-0.03, 0.12]	0.60	0.550
Disadvantage Factor	-0.15	[-0.24, -0.06]	1.53	0.128
Infant Age at Scan	-0.03	[-0.12, 0.06]	-0.72	0.474
Infant Sex	-0.10	[-0.18, -0.02]	-2.54	0.012
Infant Birthweight	-0.07	[-0.15, 0.02]	-1.53	0.127
Total Gray Matter Volume	0.77	[0.66, 0.88]	14.27	<0.001
Maternal Tobacco Use	-0.08	[-0.16, 0]	-2.06	0.040
Maternal Cannabis Use	0.08	[0, 0.16]	1.88	0.062
Cortisol Slope x Disadvantage Factor	-0.16	[-0.24, -0.08]	-3.90	<0.001

Multi-level Model Output

Table S5. Results of multi-level model assessing cortisol production by trimester with random effects for intercept and slope

Effect Type	Grouping Variable	Predictor	B	SE	t	CI Lower Bound	CI Upper Bound
Fixed		Intercept	8.52	0.07	129.78	8.39	8.65
Fixed		Trimester	0.02	0.00	8.55	0.02	0.03
Random	subID	Intercept SD	0.49				
Random	subID	Cor: Intercept x Trimester	-0.66				
Random	subID	Trimester SD	0.02				
Random	Residual	Residual	0.63				

Left- and right-amygdala volume model

The pattern of results is consistent when models are fit to models with left- and right-amygdala volumes separately. Model results are in Tables S6 and S7 below.

Table S6. Coefficient-level estimates for a model fitted to estimate variation in left amygdala volume. One outlier -5 sd from the mean of cortisol AUCg was removed.

Outcome = Left Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.07, 0.08]	2.98	0.003
Cortisol Slope	0.05	[-0.03, 0.13]	0.85	0.398
Disadvantage Factor	-0.14	[-0.24, -0.04]	1.81	0.072
Infant Age at Scan	-0.01	[-0.11, 0.08]	-0.31	0.760
Infant Sex	-0.10	[-0.18, -0.02]	-2.36	0.019
Infant Birthweight	-0.09	[-0.18, 0]	-1.98	0.049
Total Gray Matter Volume	0.74	[0.63, 0.86]	12.74	<0.001
Maternal Tobacco Use	-0.09	[-0.17, -0.01]	-2.10	0.037
Maternal Cannabis Use	0.07	[-0.02, 0.16]	1.47	0.144
Cortisol Slope x Disadvantage Factor	-0.14	[-0.23, -0.06]	-3.35	<0.001

Table S7. Coefficient-level estimates for a model fitted to estimate variation in right amygdala volume. One outlier -5 sd from the mean of cortisol AUCg was removed.

Outcome = Right Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.07, 0.08]	3.61	<0.001
Cortisol Slope	0.03	[-0.05, 0.1]	0.19	0.851
Disadvantage Factor	-0.14	[-0.23, -0.04]	1.79	0.075
Infant Age at Scan	-0.05	[-0.14, 0.04]	-1.03	0.303
Infant Sex	-0.09	[-0.17, -0.01]	-2.18	0.031
Infant Birthweight	-0.04	[-0.12, 0.05]	-0.80	0.425
Total Gray Matter Volume	0.77	[0.66, 0.88]	13.82	<0.001
Maternal Tobacco Use	-0.07	[-0.15, 0.01]	-1.77	0.079
Maternal Cannabis Use	0.08	[0, 0.17]	1.93	0.055
Cortisol Slope x Disadvantage Factor	-0.14	[-0.22, -0.06]	-3.35	<0.001

Table S8. Coefficient-level estimates for a model fitted to estimate variation in bilateral amygdala volume with gestational age as a covariate. One outlier -5 sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.06, 0.08]	2.74	0.007
Cortisol Slope	0.04	[-0.04, 0.11]	0.48	0.632
Disadvantage Factor	-0.14	[-0.23, -0.04]	2.08	0.038
Infant Age at Scan	-0.02	[-0.11, 0.08]	-0.38	0.702
Infant Sex	-0.10	[-0.18, -0.02]	-2.45	0.015
Infant Gestational Age	-0.02	[-0.1, 0.06]	-0.45	0.652
Total Gray Matter Volume	0.74	[0.64, 0.84]	14.57	<0.001
Maternal Tobacco Use	-0.08	[-0.15, 0.00]	-1.99	0.048
Maternal Cannabis Use	0.08	[0, 0.17]	1.99	0.048
Cortisol Slope x Disadvantage Factor	-0.15	[-0.23, -0.07]	-3.68	<0.001

Table S9. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume. Outliers 5 sd from the mean (N = 1) and participants with winsorized cortisol samples (N = 10) removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.07, 0.08]	3.33	0.001
Cortisol Slope	0.06	[-0.02, 0.13]	0.99	0.324
Disadvantage Factor	-0.14	[-0.24, -0.05]	1.79	0.075
Infant Age at Scan	-0.02	[-0.11, 0.07]	-0.40	0.692
Infant Sex	-0.11	[-0.19, -0.03]	-2.68	0.008
Infant Birthweight	-0.08	[-0.16, 0.01]	-1.79	0.076
Total Gray Matter Volume	0.76	[0.65, 0.87]	13.77	<0.001
Tobacco Use	-0.08	[-0.16, 0]	-2.04	0.043
Cannabis Use	0.08	[0, 0.17]	1.95	0.052
Cortisol Slope x Disadvantage Factor	-0.14	[-0.22, -0.06]	-3.43	<0.001

Hippocampus, caudate, and medial prefrontal cortex models

There were no significant interactions of cortisol slope across pregnancy and socioeconomic disadvantage in the hippocampus, caudate, or medial prefrontal cortex. Full model results are below in Tables S10 – S12.

Table S10. Coefficient-level estimates for a model fitted to estimate variation in average hippocampus volume. One outlier -5 sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Hippocampus Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.1, 0.1]	1.92	0.057
Cortisol Slope	0.11	[0.01, 0.22]	1.93	0.055
Disadvantage Factor	-0.10	[-0.23, 0.03]	0.82	0.413
Infant Age at Scan	0.00	[-0.13, 0.13]	0.01	0.994
Infant Sex	0.01	[-0.1, 0.12]	0.13	0.896
Infant Birthweight	-0.06	[-0.18, 0.06]	-1.03	0.304
Total Gray Matter Volume	0.63	[0.48, 0.78]	8.18	<0.001
Maternal Tobacco Use	-0.06	[-0.16, 0.05]	-1.02	0.307
Maternal Cannabis Use	-0.00	[-0.12, 0.11]	-0.08	0.936
Cortisol Slope x Disadvantage Factor	-0.09	[-0.2, 0.02]	-1.63	0.106

Table S11. Coefficient-level estimates for a model fitted to estimate variation in average caudate volume. One outlier -5 sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Caudate Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.1, 0.1]	0.27	0.784
Cortisol Slope	-0.05	[-0.15, 0.06]	-0.95	0.345
Disadvantage Factor	-0.15	[-0.28, -0.02]	-0.78	0.437
Infant Age at Scan	0.07	[-0.06, 0.2]	1.09	0.278
Infant Sex	0.01	[-0.1, 0.12]	0.13	0.899
Infant Birthweight	0.01	[-0.11, 0.13]	0.20	0.845
Total Gray Matter Volume	0.53	[0.38, 0.68]	6.94	<0.001
Maternal Tobacco Use	-0.10	[-0.21, 0.01]	-1.87	0.062
Maternal Cannabis Use	0.07	[-0.05, 0.19]	1.16	0.249
Cortisol Slope x Disadvantage Factor	-0.02	[-0.13, 0.09]	-0.30	0.762

Table S12. Coefficient-level estimates for a model fitted to estimate variation in average medial prefrontal cortex volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral mPFC Volume				
Predictor	β	95% CI	t	p
Intercept	-0.00	[-0.07, 0.06]	3.40	<0.001
Cortisol Slope	0.04	[-0.03, 0.11]	1.36	0.174
Disadvantage Factor	0.12	[0.03, 0.2]	0.16	0.877
Infant Age at Scan	-0.16	[-0.24, -0.08]	-3.83	<0.001
Infant Sex	0.04	[-0.04, 0.11]	0.96	0.338
Infant Birthweight	0.02	[-0.06, 0.1]	0.47	0.639
Total Gray Matter Volume	1.00	[0.9, 1.09]	19.91	<0.001
Maternal Tobacco Use	-0.00	[-0.07, 0.07]	-0.03	0.977
Maternal Cannabis Use	-0.05	[-0.12, 0.03]	-1.18	0.239
Cortisol Slope x Disadvantage Factor	0.04	[-0.03, 0.12]	1.20	0.232

Trimester-specific cortisol x amygdala volume models

Uncorrected trimester-specific models for the relationship between prenatal maternal cortisol and neonatal amygdala volume are presented below (Tables S13 – S15). Significant interactions were present in trimester 2 and 3 only.

Table S13. Coefficient-level estimates for a model fitted to estimate variation in neonatal amygdala volume using first trimester AUCg. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	-0.01	[-0.1, 0.08]	2.76	0.007
AUCg T1	-0.04	[-0.13, 0.06]	-0.18	0.855
Disadvantage Factor	-0.10	[-0.22, 0.02]	-2.47	0.015
Infant Age at Scan	-0.04	[-0.16, 0.08]	-0.66	0.511
Infant Sex	-0.07	[-0.17, 0.02]	-1.50	0.136
Infant Birthweight	-0.06	[-0.17, 0.06]	-0.99	0.326
Total Gray Matter Volume	0.80	[0.66, 0.94]	11.47	<0.001
Maternal Tobacco Use	-0.12	[-0.22, -0.02]	-2.42	0.017
Maternal Cannabis Use	0.03	[-0.08, 0.14]	0.55	0.582
AUCg T1 x Disadvantage Factor	0.09	[-0.01, 0.19]	1.79	0.076

Table S14. Coefficient-level estimates for a model fitted to estimate variation in neonatal amygdala volume using second trimester AUCg. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.02	[-0.06, 0.1]	3.87	<0.001
AUCg T2	0.06	[-0.03, 0.15]	0.88	0.381
Disadvantage Factor	-0.17	[-0.27, -0.07]	-0.04	0.968
Infant Age at Scan	-0.07	[-0.16, 0.03]	-1.39	0.167
Infant Sex	-0.08	[-0.17, 0]	-1.97	0.050
Infant Birthweight	-0.07	[-0.16, 0.02]	-1.43	0.155
Total Gray Matter Volume	0.79	[0.68, 0.9]	13.93	<0.001
Maternal Tobacco Use	-0.07	[-0.16, 0.01]	-1.71	0.089
Maternal Cannabis Use	0.09	[0, 0.18]	1.97	0.050
AUCg T2 x Disadvantage Factor	-0.10	[-0.19, -0.01]	-2.16	0.032

Table S15. Coefficient-level estimates for a model fitted to estimate variation in neonatal amygdala volume using third trimester AUCg. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.06, 0.09]	3.81	<0.001
AUCg T3	0.01	[-0.07, 0.09]	-0.30	0.762
Disadvantage Factor	-0.16	[-0.25, -0.06]	1.07	0.286
Infant Age at Scan	-0.05	[-0.14, 0.05]	-0.98	0.330
Infant Sex	-0.08	[-0.16, 0]	-1.91	0.057
Infant Birthweight	-0.07	[-0.16, 0.02]	-1.49	0.139
Total Gray Matter Volume	0.78	[0.67, 0.89]	13.84	<0.001
Maternal Tobacco Use	-0.09	[-0.17, -0.01]	-2.24	0.026
Maternal Cannabis Use	0.08	[-0.01, 0.16]	1.77	0.078
AUCg T3 x Disadvantage Factor	-0.16	[-0.25, -0.07]	-3.47	<0.001

Cortisol intercept models

There were no significant effects when cortisol intercepts were used in place of cortisol slopes. Cortisol intercept represents first trimester cortisol AUCg for each participant as the multilevel models used centered trimester as the time variable such that the first trimester was equal to zero. Full model results for each brain region of interest using the outlier specifications presented in the main manuscript (e.g., removing one observation ± 5 standard deviations from the mean) are below in Tables S16 – S19.

Table S16. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.07, 0.08]	2.33	0.021
Cortisol Intercept	0.01	[-0.07, 0.08]	0.13	0.896
Disadvantage Factor	-0.13	[-0.23, -0.04]	0.49	0.628
Infant Age at Scan	-0.04	[-0.13, 0.05]	-0.82	0.416
Infant Sex	-0.10	[-0.18, -0.02]	-2.34	0.020
Infant Birthweight	-0.08	[-0.16, 0.01]	-1.72	0.086
Total Gray Matter Volume	0.79	[0.68, 0.9]	14.15	<0.001
Maternal Tobacco Use	-0.09	[-0.17, -0.01]	-2.25	0.026
Maternal Cannabis Use	0.06	[-0.02, 0.15]	1.43	0.156
Cortisol Intercept x Disadvantage Factor	-0.02	[-0.1, 0.06]	-0.57	0.572

Table S17. Coefficient-level estimates for a model fitted to estimate variation in average hippocampus volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Hippocampus Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.1, 0.11]	1.72	0.086
Cortisol Intercept	-0.03	[-0.14, 0.07]	-0.67	0.506
Disadvantage Factor	-0.08	[-0.22, 0.05]	0.56	0.574
Infant Age at Scan	0.01	[-0.11, 0.14]	0.21	0.834
Infant Sex	0.01	[-0.11, 0.12]	0.13	0.898
Infant Birthweight	-0.07	[-0.19, 0.06]	-1.06	0.290
Total Gray Matter Volume	0.63	[0.48, 0.78]	8.14	<0.001
Maternal Tobacco Use	-0.06	[-0.17, 0.05]	-1.03	0.305
Maternal Cannabis Use	-0.01	[-0.13, 0.11]	-0.16	0.875
Cortisol Intercept x Disadvantage Factor	-0.03	[-0.15, 0.08]	-0.60	0.550

Table S18. Coefficient-level estimates for a model fitted to estimate variation in average caudate volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Caudate Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.1, 0.11]	-0.17	0.868
Cortisol Intercept	0.04	[-0.07, 0.14]	0.63	0.532
Disadvantage Factor	-0.16	[-0.29, -0.03]	0.59	0.557
Infant Age at Scan	0.06	[-0.07, 0.18]	0.90	0.370
Infant Sex	0.00	[-0.11, 0.11]	0.02	0.982
Infant Birthweight	0.00	[-0.11, 0.12]	0.08	0.939
Total Gray Matter Volume	0.54	[0.39, 0.69]	7.04	<0.001
Maternal Tobacco Use	-0.11	[-0.21, 0]	-1.96	0.052
Maternal Cannabis Use	0.07	[-0.05, 0.18]	1.10	0.272
Cortisol Intercept x Disadvantage Factor	-0.04	[-0.15, 0.07]	-0.66	0.511

Table S19. Coefficient-level estimates for a model fitted to estimate variation in average medial prefrontal cortex volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral mPFC Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.05, 0.08]	2.68	0.008
Cortisol Intercept	-0.01	[-0.08, 0.06]	-0.55	0.585
Disadvantage Factor	0.11	[0.03, 0.2]	1.96	0.051
Infant Age at Scan	-0.15	[-0.23, -0.07]	-3.68	<0.001
Infant Sex	0.03	[-0.04, 0.1]	0.75	0.451
Infant Birthweight	0.02	[-0.06, 0.1]	0.51	0.610
Total Gray Matter Volume	0.99	[0.89, 1.08]	19.83	<0.001
Maternal Tobacco Use	0.00	[-0.07, 0.07]	0.13	0.899
Maternal Cannabis Use	-0.04	[-0.11, 0.04]	-0.95	0.345
Cortisol Intercept x Disadvantage Factor	-0.07	[-0.14, 0]	-1.88	0.061

Specificity analyses

The maternal psychosocial stress factor score was also used as a predictor of neonatal amygdala volume to assess the specificity of maternal cortisol production as a predictor of neonatal brain volumes. Maternal psychosocial stress was not a significant predictor of the relationship between maternal cortisol AUCg and neonatal amygdala volume. Table S20 contains the results for this model.

Table S20. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Predictor	β	95% CI	t	p
Intercept	-0.01	[-0.09, 0.07]	3.27	0.001
Psychosocial Stress Factor	0.04	[-0.05, 0.13]	1.01	0.315
Disadvantage Factor	-0.13	[-0.22, -0.03]	-2.48	0.014
Infant Age at Scan	-0.02	[-0.11, 0.07]	-0.33	0.738
Infant Sex	-0.12	[-0.2, -0.05]	-3.12	0.002
Infant Birthweight	-0.06	[-0.15, 0.03]	-1.37	0.172
Total Gray Matter Volume	0.77	[0.66, 0.88]	14.27	<0.001
Psychosocial Stress Factor x Disadvantage Factor	0.02	[-0.07, 0.11]	0.49	0.622

Assessment of Sex Differences. Given previous research reporting female-specific effects of prenatal maternal cortisol on offspring amygdala volumes (34), we also investigated an outcome model with a cortisol by infant sex interaction term. There was no evidence of an infant sex difference in the relationship between prenatal maternal cortisol and neonatal amygdala volumes (interaction p=0.89). Further, when both the cortisol by sex interaction term and the interaction of cortisol with the socioeconomic disadvantage factor were included in the same model, the focal interaction term remained significant with no evidence in support of a sex differentiated effect (see Table S21). Finally, neither the interaction between sex and socioeconomic disadvantage nor a three-way interaction between sex, socioeconomic disadvantage, and cortisol slope were associated with amygdala volume (see Tables S22 and S23).

Table S21. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume including interaction terms for both infant sex and socioeconomic disadvantage factor. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.06, 0.08]	3.72	<0.001
Cortisol Slope	0.04	[-0.04, 0.11]	-1.00	0.317
Disadvantage Factor	-0.15	[-0.24, -0.05]	2.20	0.029
Infant Age at Scan	-0.03	[-0.12, 0.06]	-0.75	0.456
Infant Sex	-0.10	[-0.18, -0.02]	-2.11	0.036
Infant Birthweight	-0.07	[-0.15, 0.02]	-1.57	0.117
Maternal Tobacco Use	0.77	[0.67, 0.88]	14.25	<0.001
Maternal Cannabis Use	-0.08	[-0.16, 0]	-2.05	0.041
Total Gray Matter Volume	0.08	[-0.01, 0.16]	1.84	0.068
Cortisol Slope x Infant Sex	0.05	[-0.03, 0.12]	1.23	0.220
Cortisol Slope x Disadvantage Factor	-0.16	[-0.24, -0.08]	-3.81	<0.001

Table S22. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume including and interaction term for infant sex and socioeconomic disadvantage factor. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.07, 0.07]	3.54	<0.001
Cortisol Slope	0.01	[-0.07, 0.08]	0.25	0.802
Disadvantage Factor	-0.13	[-0.22, -0.04]	-0.88	0.377
Infant Age at Scan	-0.04	[-0.13, 0.05]	-0.80	0.422
Infant Sex	-0.09	[-0.17, -0.01]	-2.29	0.023
Infant Birthweight	-0.07	[-0.16, 0.01]	-1.68	0.094
Maternal Tobacco Use	0.79	[0.68, 0.9]	14.09	<0.001
Maternal Cannabis Use	-0.09	[-0.17, -0.01]	-2.23	0.027
Total Gray Matter Volume	0.06	[-0.02, 0.15]	1.41	0.161
Disadvantage Factor x Child Sex	-0.01	[-0.08, 0.07]	-0.24	0.811

Table S23. Coefficient-level estimates for a model fitted to estimate variation in average amygdala volume including and a three-way interaction term with infant sex, socioeconomic disadvantage factor, and cortisol slope included. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Bilateral Amygdala Volume				
Predictor	β	95% CI	t	p
Intercept	0.01	[-0.06, 0.08]	3.74	<0.001
Cortisol Slope	0.05	[-0.03, 0.13]	-1.06	0.289
Disadvantage Factor	-0.15	[-0.24, -0.05]	0.26	0.794
Infant Age at Scan	-0.04	[-0.13, 0.05]	-0.80	0.427
Infant Sex	-0.09	[-0.17, -0.02]	-2.18	0.030
Infant Birthweight	-0.07	[-0.15, 0.02]	-1.53	0.127
Total Gray Matter Volume	0.78	[0.67, 0.89]	14.10	<0.001
Maternal Tobacco Use	-0.08	[-0.15, 0]	-2.01	0.046
Maternal Cannabis Use	0.08	[-0.01, 0.16]	1.85	0.066
Cortisol Slope x Disadvantage Factor	-0.17	[-0.26, -0.08]	-0.67	0.502
Cortisol Slope x Child Sex	0.06	[-0.03, 0.14]	1.34	0.181
Disadvantage Factor x Child Sex	-0.01	[-0.08, 0.06]	0.51	0.614
Cortisol Slope x Disadvantage Factor x Child Sex	-0.03	[-0.12, 0.06]	-0.61	0.543

Hemisphere-specific Models in Additional Regions of Interest. To ensure that the decision to use bilateral volumes of the regions of interest did not affect our pattern of results, we also tested the primary outcome model in the left and right hemispheres separately for the hippocampus, caudate, and medial prefrontal cortex (mPFC). The results from these models can be found in Tables S24 – S29, below.

Table S24. Coefficient-level estimates for a model fitted to estimate variation in left hippocampus volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Left Hippocampus Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.1, 0.11]	0.85	0.396
Cortisol Slope	0.09	[-0.02, 0.2]	1.41	0.161
Disadvantage Factor	-0.08	[-0.21, 0.06]	0.73	0.468
Infant Age at Scan	0.05	[-0.08, 0.19]	0.78	0.434
Infant Sex	-0.00	[-0.12, 0.11]	-0.06	0.953
Infant Birthweight	-0.04	[-0.17, 0.08]	-0.69	0.491
Total Gray Matter Volume	0.54	[0.38, 0.71]	6.64	<0.001
Maternal Tobacco Use	-0.03	[-0.15, 0.08]	-0.58	0.564
Maternal Cannabis Use	-0.01	[-0.13, 0.12]	-0.15	0.884
Cortisol Slope x Disadvantage Factor	-0.08	[-0.2, 0.04]	-1.32	0.187

Table S25. Coefficient-level estimates for a model fitted to estimate variation in right hippocampus volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Right Hippocampus Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.1, 0.11]	2.62	0.010
Cortisol Slope	0.12	[0.01, 0.23]	2.03	0.044
Disadvantage Factor	-0.11	[-0.24, 0.03]	0.72	0.475
Infant Age at Scan	-0.06	[-0.19, 0.07]	-0.86	0.391
Infant Sex	0.02	[-0.1, 0.13]	0.31	0.757
Infant Birthweight	-0.07	[-0.2, 0.05]	-1.15	0.252
Total Gray Matter Volume	0.63	[0.47, 0.78]	7.82	<0.001
Maternal Tobacco Use	-0.07	[-0.18, 0.04]	-1.26	0.208
Maternal Cannabis Use	0.00	[-0.12, 0.12]	0.01	0.990
Cortisol Slope x Disadvantage Factor	-0.09	[-0.21, 0.02]	-1.55	0.123

Table S26. Coefficient-level estimates for a model fitted to estimate variation in left caudate volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Left Caudate Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.1, 0.11]	0.98	0.326
Cortisol Slope	-0.01	[-0.12, 0.1]	-0.31	0.755
Disadvantage Factor	-0.18	[-0.31, -0.04]	-0.74	0.462
Infant Age at Scan	0.03	[-0.1, 0.17]	0.48	0.630
Infant Sex	0.02	[-0.1, 0.14]	0.36	0.721
Infant Birthweight	-0.03	[-0.16, 0.1]	-0.46	0.644
Total Gray Matter Volume	0.52	[0.36, 0.68]	6.40	<0.001
Maternal Tobacco Use	-0.09	[-0.2, 0.02]	-1.54	0.125
Maternal Cannabis Use	0.07	[-0.05, 0.19]	1.11	0.269
Cortisol Slope x Disadvantage Factor	-0.03	[-0.15, 0.09]	-0.46	0.646

Table S27. Coefficient-level estimates for a model fitted to estimate variation in right caudate volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Right Caudate Volume				
Predictor	β	95% CI	t	p
Intercept	0.00	[-0.1, 0.1]	-0.47	0.639
Cortisol Slope	-0.08	[-0.18, 0.03]	-1.52	0.129
Disadvantage Factor	-0.12	[-0.25, 0]	-0.77	0.445
Infant Age at Scan	0.10	[-0.02, 0.22]	1.62	0.106
Infant Sex	-0.01	[-0.11, 0.1]	-0.12	0.907
Infant Birthweight	0.05	[-0.07, 0.17]	0.85	0.394
Total Gray Matter Volume	0.52	[0.37, 0.67]	6.97	<0.001
Maternal Tobacco Use	-0.11	[-0.22, -0.01]	-2.08	0.039
Maternal Cannabis Use	0.06	[-0.05, 0.18]	1.12	0.265
Cortisol Slope x Disadvantage Factor	-0.01	[-0.11, 0.1]	-0.12	0.904

Table S28. Coefficient-level estimates for a model fitted to estimate variation in left mPFC volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Left mPFC Volume				
Predictor	β	95% CI	t	p
Intercept	-0.00	[-0.08, 0.07]	2.61	0.010
Cortisol Slope	0.02	[-0.06, 0.1]	0.82	0.414
Disadvantage Factor	0.16	[0.06, 0.25]	-0.27	0.788
Infant Age at Scan	-0.16	[-0.26, -0.07]	-3.34	<0.001
Infant Sex	0.07	[-0.01, 0.15]	1.63	0.106
Infant Birthweight	-0.01	[-0.1, 0.08]	-0.16	0.876
Total Gray Matter Volume	0.98	[0.87, 1.1]	17.05	<0.001
Maternal Tobacco Use	0.03	[-0.05, 0.11]	0.64	0.525
Maternal Cannabis Use	-0.05	[-0.13, 0.04]	-1.04	0.299
Cortisol Slope x Disadvantage Factor	0.08	[0, 0.16]	1.86	0.064

Table S29. Coefficient-level estimates for a model fitted to estimate variation in right mPFC volume. One outlier -5sd from the mean of cortisol AUCg was removed.

Outcome = Right mPFC Volume				
Predictor	β	95% CI	t	p
Intercept	-0.00	[-0.08, 0.08]	2.93	0.004
Cortisol Slope	0.05	[-0.02, 0.13]	1.41	0.161
Disadvantage Factor	0.06	[-0.03, 0.16]	0.53	0.598
Infant Age at Scan	-0.14	[-0.24, -0.05]	-2.90	0.004
Infant Sex	-0.00	[-0.09, 0.08]	-0.07	0.941
Infant Birthweight	0.04	[-0.05, 0.13]	0.93	0.354
Total Gray Matter Volume	0.89	[0.78, 1.01]	15.35	<0.001
Maternal Tobacco Use	-0.03	[-0.11, 0.05]	-0.69	0.489
Maternal Cannabis Use	-0.04	[-0.13, 0.05]	-0.88	0.380
Cortisol Slope x Disadvantage Factor	0.00	[-0.08, 0.09]	0.08	0.939

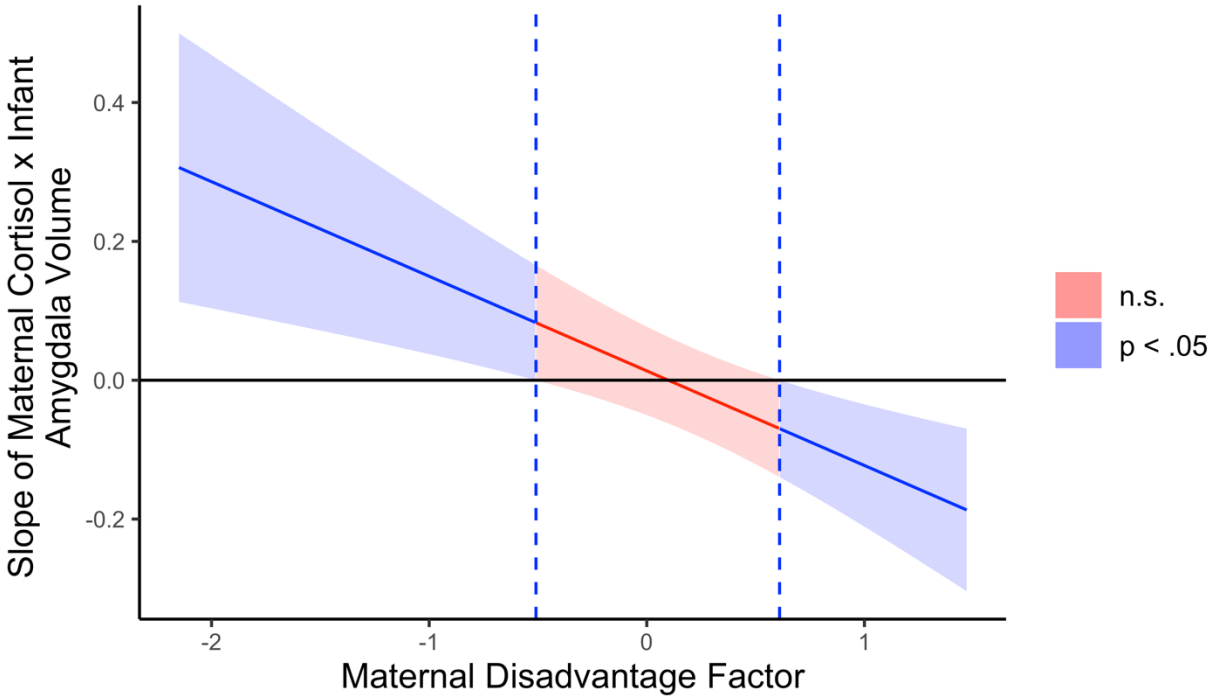

Figure S2. Johnson-Neyman plot of the interaction between socioeconomic disadvantage factor and cortisol slope across pregnancy.

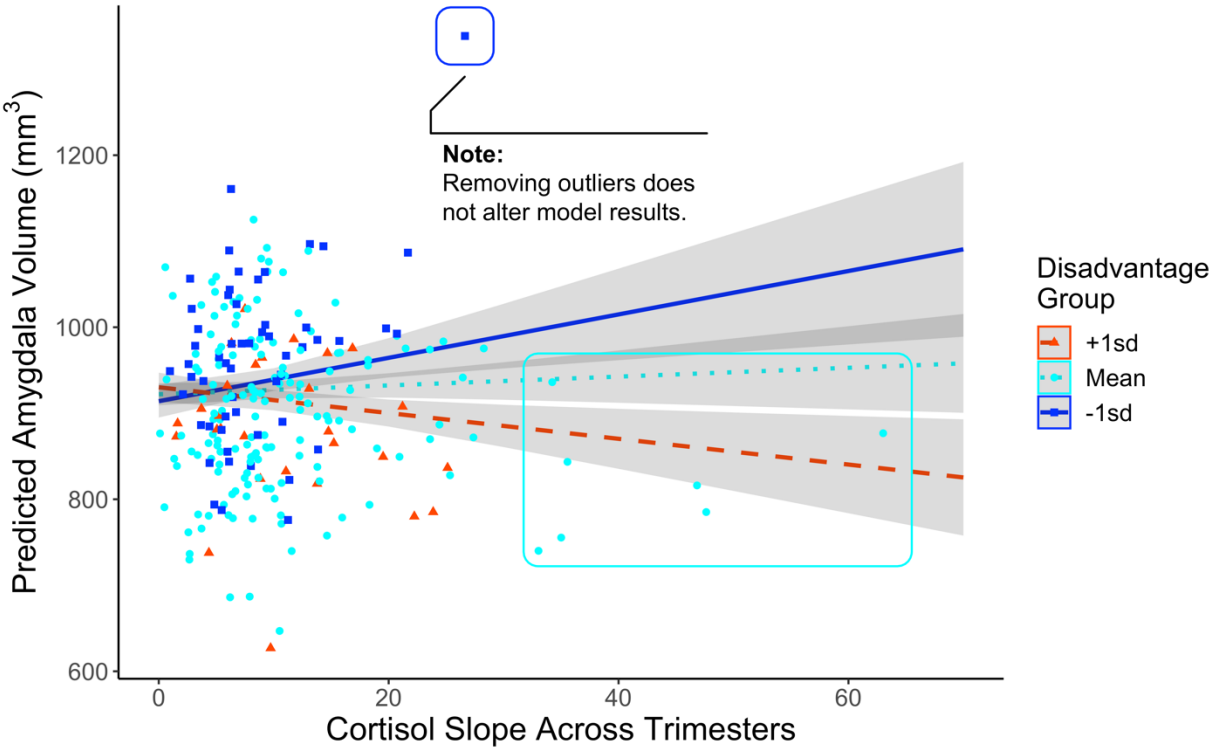

Figure S3. Neonatal amygdala volumes as a function of total maternal cortisol output slope across trimesters and socioeconomic disadvantage group with all data included. Lines represent model predicted results and points are the raw data included in the regression model. Gray shading indicates a 95% confidence interval. Categorical treatment of disadvantage group is for data visualization only. A continuous predictor was used to assess socioeconomic disadvantage in the regression model.

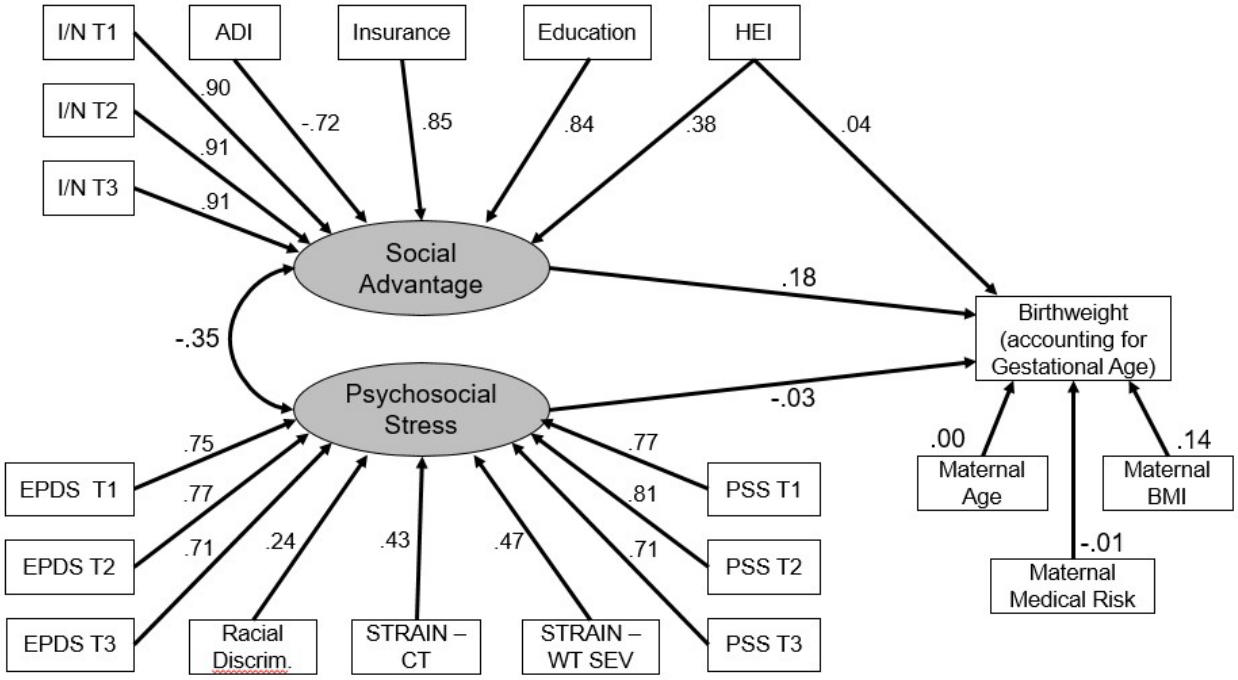

Figure S4. The structural equation model used to generate scores for social advantage and psychosocial stress. The social advantage score was reverse coded and used as the socioeconomic disadvantage score in these analyses. Some covariances are not included for clarity. (ADI = Area Deprivation Index; BMI = Body Mass Index; EPDS = Edinburgh Postpartum Depression Scale; HEI = Healthy Eating Index; I/N = Income/Needs; STRAIN-CT = Stressful Life Events Count; STRAIN_WT SEV = Stressful Life Events Weighted Severity; PSS = Perceived Stress Scale; T1 – T3 = Trimester 1 – 3). Reprinted with permission from (1).

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