Biological Psychiatry

Archival Report

Neonatal Hippocampal Volume and Parent Structuring at 18 Months in Relation to Cortisol Stress Regulation Across Early Childhood and Executive Functions at Age 4 Years in Children Born Very Preterm

Mia A. McLean, Joanne Weinberg, Jillian Vinall Miller, Ting Guo, Anne R. Synnes, Steven P. Miller, and Ruth E. Grunau

ABSTRACT

BACKGROUND: Children born very preterm (≤32 weeks gestational age) display altered neonatal brain maturation and physiological stress regulation that may be related to executive function difficulties. Supportive parent behaviors are related to better neurodevelopment, but children may vary in their susceptibility to such behaviors. We investigated whether supportive parenting during toddlerhood (1.5, 3 years) moderated the relationship between neonatal hippocampal volume, stress dysregulation across childhood, and executive functions.

METHODS: In a prospective longitudinal cohort study (N = 188, 100 male), infants born very preterm underwent magnetic resonance imaging at term-equivalent age. At 1.5, 3, and 4.5 years, saliva samples were collected during challenging cognitive tasks and assayed for cortisol; area under the curve with respect to ground (AUCg) measured total cortisol output at each age. Parent interaction was rated from films at 1.5 and 3 years, and parents reported on child executive functions at age 4.5 years.

RESULTS: Longitudinal modeling revealed no direct relationship between hippocampal volume and cortisol trajectories. Children with smaller neonatal hippocampal volume showed greater susceptibility to poorer parent structuring at 1.5 years, associated with altered AUCg trajectories across age, and displayed fewer executive functions in the face of optimal structuring. Lower AUCg across age was related to more positive parenting (sensitivity, nonhostility, nonintrusiveness) at 3 years and better child executive function at 4.5 years.

CONCLUSIONS: In children born very preterm, smaller neonatal hippocampal volume may represent a neurophenotype of susceptibility to parent provisions of structure related to stress regulation across early childhood. Conversely, supportive parenting may benefit the development of stress regulation across early childhood and executive functions at preschool age.

https://doi.org/10.1016/j.biopsych.2025.06.005

As early as the neonatal period, infants born very preterm display altered stress regulation as indexed by salivary cortisol levels (1), widespread brain dysmaturation (2–4), and elevated executive function difficulties (5), in part related to early adverse exposures during neonatal intensive care unit (NICU) stay at a time of rapid neuroendocrine and brain development (3,6,7). The hippocampus is a brain region that forms part of a highly interconnected corticolimbic system. Importantly, the hippocampus is a region rich in glucocorticoid receptors, and it plays a major role in feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis (8,9). In addition to playing a key role in the regulation of stress response by exerting an inhibitory effect on HPA axis activity (8), the hippocampus is also particularly vulnerable to the effects of early-life stress. Animal studies have demonstrated coupling of the hippocampus and HPA axis (10). Research in children exposed to adversity early in life has demonstrated associations between HPA axis functioning (diurnal cortisol, cortisol reactivity) and concurrent hippocampal volume (11,12) and later function (13). Using an accelerated longitudinal cross-lag design, Van Tieghem *et al.* (14) found that previously institutionalized youths (n = 93) displayed reduced childhood hippocampal volumes compared with a comparison group (n = 161) across 4 to 20 years. Lower hippocampal and amygdala volumes (~11 years; n = 115) were associated with greater morning diurnal cortisol and steeper slopes 2 to 4 years later (~13 years; n = 72). The small sample with repeat cortisol and imaging data likely limited detection of group differences, highlighting the need for further research with early adversity cohorts. Moreover, executive functions are related to hippocampal structure (15) and appear

SEE COMMENTARY ON PAGE 353

© 2025 Society of Biological Psychiatry. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ISSN: 0006-3223

to be sensitive to both short- and longer-term cortisol elevations that result from acute (16) and chronic (17,18) stress, respectively. However, it is not known how these systems influence each other across early development and, in turn, executive functions in children born very preterm.

Early childhood is a period of heightened neurobiological plasticity. HPA activity is partially regulated by parental care, and supportive parenting appears to buffer physiological reactivity to stressors (19), particularly for those exposed to early adversity (20) including those born very preterm (21). Variability in neurobiology is said to underlie developmental susceptibility frameworks (22); however, research in this population is sparse (23). Examination of neural markers in early life may provide an understanding of the neural basis of susceptibility to the postnatal parenting environment with respect to HPA axis functioning and executive functions across childhood (24,25).

Hippocampal volume is of particular interest as a possible neurophenotype of environmental sensitivity (22) given that hippocampal structure and function are related to aspects of temperament (26,27) and physiological stress regulation (14). The hippocampus is involved in the consolidation and storage of distress- or emotion-related memories (28), may shape infant threat perception and distress regulation (29), as well as being involved in executive functioning (15). Across studies in normative samples (28–30), parent sensitivity and environmental enrichment at 6 months were related to better concurrent cognition (28), less disorganized attachment at 1.5 years (30), and greater working memory and cognition in early childhood (30), but only for those with larger neonatal hippocampal volumes. Findings have been consistent across studies, although with small effect sizes [10% variance accounted for in (30)], demonstrating increased susceptibility to different aspects of parenting for those with larger neonatal hippocampi. It has been shown that children exposed to maltreatment with smaller hippocampal volumes have lower diurnal cortisol levels, but this is not true for unexposed counterparts (11). Therefore, in the context of preterm birth—an adverse exposure that disrupts the development of the hippocampus—smaller hippocampi may moderate the association between negative parent environments and physiological stress regulation.

To our knowledge, the interrelationship of early hippocampal maturation, trajectories of cortisol levels, and subsequent executive functions across early childhood has not yet been studied in children born very preterm. We recently found that cortisol levels in response to age-appropriate cognitive challenge decreased across ages 1.5, 3, and 4.5 years among children born extremely preterm (24–28 weeks gestation) and very preterm (29–32 weeks gestation) in the present cohort (30). However, neonatal hippocampal volume and parent behaviors in early childhood have not yet been investigated in relation to total cortisol output at these ages. In the current prospective longitudinal cohort study, we investigated whether, in children born very preterm, 1) hippocampal volume at term equivalent age is related to cortisol output in response to a cognitive challenge across ages 1.5, 3, and 4.5 years; 2) those who display lower neonatal hippocampal volume are at heighted susceptibility to parent behaviors at 1.5 and/or 3 years in relation to longitudinal cortisol output; 3) trajectories of cortisol output are related to executive functions at age 4.5 years; and 4) changes in cortisol output a) mediate between or b) interact with neonatal hippocampal volume, executive functions, and parent behaviors. See Figure 1.

METHODS AND MATERIALS

Participants

Families in the current study were part of a larger prospective longitudinal cohort study of children born very preterm ($N = 234$; 24–32 weeks gestational age [GA]) recruited from the Level III NICU at BC Women's Hospital in Vancouver, Canada, between 2006 and 2013. A total of 188 children were included in the current study: 167 at 1.5 years corrected age, 165 at 3 years, and 152 at 4.5 years. See the Supplement for exclusions. Informed written parent consent was obtained at every age. The study was approved by the University of British Columbia Clinical Research Ethics Board and the BC Children's and Women's Hospitals Research Ethics Board (H05-70579) and was conducted in accordance with the Declaration of Helsinki (1964).

Measures

Full details on measures are provided in the Supplement.

Neonatal Clinical Factors. Neonatal research nurses conducted detailed prospective day-by-day medical and nursing chart reviews from birth to NICU discharge or term-equivalent age (TEA), whichever came first.

Demographics. Demographic information was reported by parents at 18 months.

Neonatal Hippocampal Volume. Methods for neonatal magnetic resonance imaging (MRI) acquisition in this cohort have been reported in detail previously (31,32) and are detailed in the Supplement. In the current study, we examined bilateral hippocampal volumes at TEA. We created a residual score for hippocampal volume (hippocampal volume adjusted for total cranial volume [TCV]) and used this in subsequent analyses.

Child Cortisol

Saliva samples were collected at 3 time points (pretest, during assessment, end) on the study day using salivettes (Salimetrics LLC). Pretest samples were collected after the child was settled following arrival at the center. The second sample (during) was collected after children completed the cognitive assessment/challenge, which was the Cognitive and Language sections of the Bayley Scales of Infant Development, 3rd Edition (33) and the Wechsler Preschool and Primary Scale of Intelligence-Fourth Edition (34) at 4.5 years. The final saliva sample (end) was collected on completion of all assessments. Saliva was stored at −20 °C and assayed in duplicate using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit for quantitative determination of salivary cortisol (Salimetrics LLC). The intra- and interassay coefficients of variation were 5.04 and 6.58, respectively. Total cortisol production was computed as the area under the curve with respect to ground (AUCg), accounting for the duration of time across assessments (35).

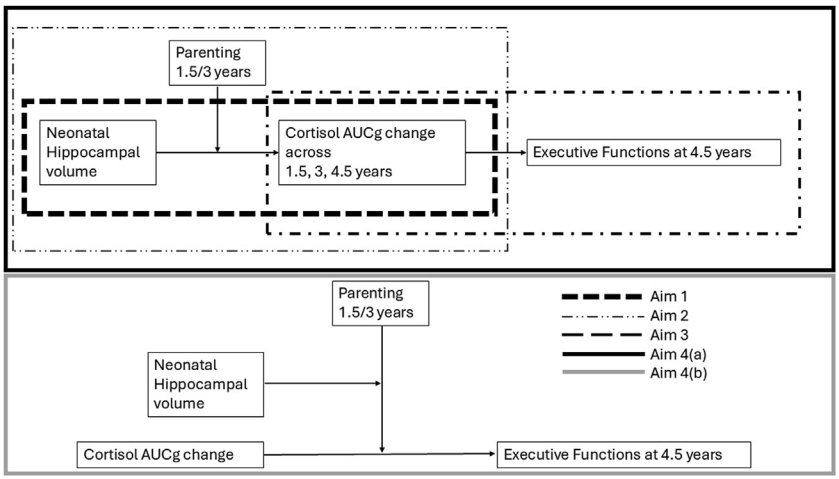

Figure 1. Study aims. AUCg, area under the curve with respect to ground.

Parenting Behaviors

At ages 1.5 and 3 years, the primary caregiver (~85% mothers at each assessment) engaged in a 5-minute semistructured teaching interaction with their child [described in (36) and the Supplement].

The sessions were videotaped and later scored using the Emotional Availability (EA) Scale 4th edition (37), which captures 4 dimensions of parent behavior: sensitivity (appropriateness/authenticity of affect), structuring (guidance), nonintrusiveness (lack of overprotection, allows autonomy), and nonhostility (nonthreatening, akin to warmth). Scores on each dimension range from 7 to 29, with higher scores denoting greater EA. Scores of 7 to 17 are indicative of nonoptimal, 18 to 26 are termed inconsistent, and 27 to 29 are termed optimal on each parenting dimension. We used continuous scores in all analyses. The interrater reliability of blinded coders on 20% of data was high (intraclass correlation coefficient [ICC] = 0.84–0.93 at 1.5 years, ICC = 0.83–0.90 at 3 years).

Child Executive Functions

At 4.5 years, parents reported on their child's executive function on the Behavior Rating Inventory of Executive Function–Preschool Version (BRIEF-P) (38). The BRIEF-P comprises 63 items that measure aspects of executive function in day-to-day life: inhibit, shift, emotional control, working memory, and plan/organize. We used the overall composite global executive index T score (mean = 50, SD = 10) in the current study. Higher scores indicate greater difficulties in executive functions, with T scores $>$ 64 indicating elevated difficulties.

Statistical Analysis

Analyses were conducted in R version 4.1.6 (39). Due to the non-normal distribution, physiological data were log transformed, and outliers for cortisol values were winsorized (40). Outliers on the BRIEF-P Global Executive Index and residual hippocampal volume were winsorized. We ran multilevel models using the *lme4* (41) and *lmerTest* (42) packages in R to examine effects of predictors on cortisol AUCg across ages 1.5, 3, and 4.5 years (aims 1, 2, and 3). Model estimates were tested using the restricted maximum likelihood estimation and Satterthwaite method of degrees of freedom (43). To address aim 4, we examined the relationship between change in cortisol, factors that related to trajectories of cortisol longitudinally (aims 1 and 2), and executive functions, first using moderated mediation models and then using moderation models via PROCESS (44). An alpha of 0.05 was used; analyses involving parent behaviors were adjusted for multiple tests using false discovery rate (FDR) at each age. We followed up interactions with contrasts in *emmeans* (45) and simple slopes in *reghelper* (46).

For sensitivity analyses, we subsequently ran all multilevel models with bilateral thalamic volumes and cerebellar volumes (after regressing out TCV); see the Supplement.

RESULTS

Descriptive Statistics

Neonatal characteristics, demographics, and measures are shown in Table 1. Of the 188 children included, 100 (53%) were assigned male sex at birth, and 64% were extremely low GA ($<$29 weeks GA).

As reported previously (30), at each age, pretest cortisol levels were the highest, followed by a decrease through during and end of assessment (Figure S1). AUCg decreased across age as shown in Figure S2. Parent domains of behavior within each age were highly correlated; however, parent behaviors demonstrated small to moderate correlations across time (Table S2). Moreover, this low level of stability was not associated with changes in child cortisol across time (Table S3), but that consistency within a particular parent behavioral domain (e.g., sensitivity across 1.5–3 years) was highly correlated with consistency across another behavioral domain (e.g., structuring).

Aim 1

Neonatal hippocampal volume (residual) did not predict AUCg on average (B = 0.00, SE = 0.00, $t_{108.2}$ = 0.42, p = .675), and the relationship between hippocampal volume and AUCg did not differ by age at assessment ($F_{178.46}$ = 0.18, p = .839).

Table 1. Descriptive Statistics

	Mean (SD) or *n* (%)	Range
Neonatal Clinical Variables		
Gestational Age at Birth, Weeks	28.0 (2.3)	24.0–32.3
Number of Invasive Procedures Across NICU Stay	119.4 (78.3)	14.0–385.0
SNAP-II Score	12.8 (12.5)	0.0–57.0
Morphine, Cumulative Dose [mg] Adjusted for Daily Weight	4.2 (10.2)	0.0–58.3
Days on Respiratory Support	48.4 (36.5)	0.0–111.0
Postnatal Infection (Positive Culture Confirmed)	92 (49%)	–
Demographics		
Maternal Age at Birth, Years	32.4 (5.3)	21.8–45.9
Maternal Marital Status		
Married/common law	168 (92%)	–
Single/divorced/separated	14 (8%)	–
Maternal Ethnicity		
Black	0	–
East Indian	10 (6%)	–
Filipino	13 (7%)	–
First nations	6 (3%)	–
Oriental	21 (12%)	–
Other	21 (11%)	–
White Caucasian	110 (61%)	–
Maternal Education, Years	16.1 (2.7)	10.0–27.0
Maternal Level of Education		
Partial or complete undergraduate degree	124 (69%)	–
Postgraduate university degree	32 (18%)	–
Primary or secondary school graduation	25 (14%)	–
Study Variables		
Hippocampal Volume at TEA, mm^3	941 (148)	366–1298
Total Cranial Volume at TEA, mm^3	347,694 (63,538)	197,420–502,403
Days of Life at TEA Scan	87.2 (20.5)	45.0–150.0
Parent Interaction at 1.5 Years		
Parent Sensitivity	20.2 (4.0)	10.5–29.0
Parent Structuring	21.0 (3.4)	13.0–28.5
Parent Nonintrusiveness	19.2 (4.9)	8.0–29.0
Parent Nonhostility	21.7 (3.5)	12.0–29.0
Parent Interaction at 3 Years		
Parent Sensitivity	21.4 (3.6)	12.5–29.0
Parent Structuring	22.5 (3.6)	13.0–29.0
Parent Nonintrusiveness	20.7 (4.4)	10.0–29.0
Parent Nonhostility	22.3 (3.1)	15.0–29.0
4.5-Year Global Executive Index[a]	48.8 (10.9)	34.0–78.0

NICU, neonatal intensive care unit; SNAP-II, Score for Neonatal Acute Physiology-II; TEA, term equivalent age.

[a]Behavior Rating Inventory of Executive Function–Preschool Version.

Aim 2

Parenting at 1.5 Years. Interactions across child age for hippocampal volume and parent sensitivity (p_{FDR} = .122), parent nonhostility (p_{FDR} = .122), and parent nonintrusiveness (p_{FDR} = .264) in relation to AUCg were not significant. A significant 3-way interaction showed that 1.5-year parent structuring interacted with neonatal hippocampal volume to predict AUCg across cognitive assessments ($F_{2,186.67}$ = 5.32, p_{FDR} = .023), such that changes in AUCg across 1.5, 3, and 4.5 years differed depending on neonatal hippocampal volume and parent structuring. Simple slope analyses identified shifts of significance at the 50th percentile for neonatal hippocampal volume and a parent structuring score of 18. In defining simple slopes of EA parent structuring, we used established cutoffs and terminology. Therefore, we report and graph simple slopes and contrasts of 1.5-year parent structuring at scores of 17 and 24, indicative of nonoptimal parent structuring and inconsistent and optimal parent structuring, respectively, and for individuals with neonatal hippocampal volume at the sample 16th percentile (−1 SD from the mean) ("smaller neonatal hippocampus," residual of −68) and the 50th percentile ("average neonatal hippocampus," residual of 5). Simple slopes are described with reference to Figures 2 and 3 below with complete reporting in the Supplement.

Children with average neonatal hippocampus showed the expected decrease in AUCg from 1.5 to 3 years, remaining stable through 4.5 years, regardless of parent structuring level (Figure 2). In contrast, children with smaller neonatal hippocampus displayed susceptibility to parent structuring. In the face of inconsistent and optimal parent structuring at 1.5 years, children with smaller neonatal hippocampus displayed the same pattern: a decrease from 1.5 to 3 years, stable through 4.5 years, and an overall decrease in AUCg across ages. However, in the face of nonoptimal parent structuring, children with smaller neonatal hippocampus showed no change in AUCg from 1.5 to 3 years, decreasing through 4.5 years, with no overall change in AUCg from 1.5 to 4.5 years.

Figure 3 illustrates the interaction, with alternate simple slopes of parenting and hippocampus across ages. As shown in Figure 3, with inconsistent and optimal parent structuring, regardless of neonatal hippocampal volume, cortisol demonstrated the expected pattern from 1.5 to 4.5 years. By contrast, cortisol pattern differed by hippocampal volume with nonoptimal parent structuring behaviors. Children with smaller neonatal hippocampus showed no decrease in cortisol from 1.5 to 3 years but an overall decrease from 1.5 to 4.5 years, driven by a decrease from 3 to 4.5 years. In contrast, children with average neonatal hippocampus showed a significant decrease from 1.5 to 3 years but no change from 3 to 4.5 years.

Pattern differences between smaller neonatal hippocampus and average neonatal hippocampus were driven by a significant difference in AUCg for nonoptimal parent structuring at age 3 years, which was not evident for inconsistent and optimal parent structuring. Taken together, these findings support a diathesis-stress model of susceptibility to parent structuring. That is, in the context of poorer parenting, infants born very preterm with smaller residual hippocampal volumes display different cortisol profiles, but in the context of more optimal parenting behaviors, these children display cortisol profiles like those of their less-susceptible counterparts (i.e., those with larger hippocampal volumes).

Parenting at Age 3 Years. Parenting measures at age 3 years, hippocampal volume, and child age did not interact to

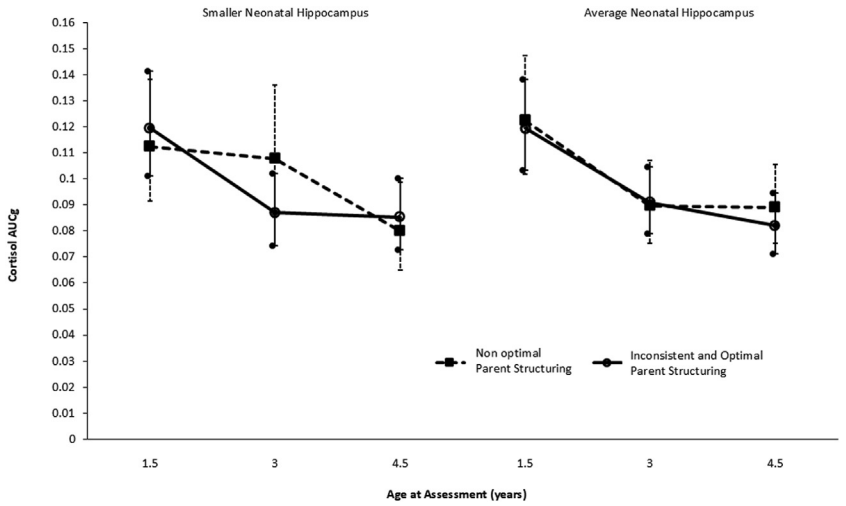

Figure 2. Cortisol area under the curve with respect to ground (AUCg) across 1.5, 3, and 4.5 years differed by neonatal hippocampal volume and parent structuring at 1.5 years ($F_{2,186.67}$ = 5.32, p_{FDR} = .023). For children with smaller neonatal hippocampal volume, observed nonoptimal parent structuring at age 1.5 years was associated with a pattern of cortisol ouput of no change in AUCg from 1.5 to 3 years, and a decrease through 4.5 years, with no overall decrease in AUCg from 1.5 to 4.5 years. All other children displayed a decrease in AUCg from 1.5 to 3 years, stability through 4.5 years, and an overall decrease in AUCg across ages. FDR, false discovery rate.

predict AUCg. Greater parent nonintrusiveness, sensitivity, and nonhostility, but not structuring, at age 3 years were related to lower AUCg across ages (2%–3% variance) (Table 2).

Aim 3

Higher scores (greater difficulties) on the Brief Global Executive Composite (GEC) at 4.5 years were related to higher AUCg across ages 1.5, 3, and 4.5 years (Table 3). The model accounted for 23% of the variance in the outcome; executive functions accounted for 4% of 10% of the fixed effect variance in the outcome.

Aim 4

Moderated mediation analysis was not significant. A 3-way interaction demonstrated that parent structuring at age 1.5 years interacted with neonatal hippocampal volume and change in cortisol (1.5–3 years) in relation to child executive functions at 4.5 years (B = −0.01, SE = 0.005, p = .021). Only for children with smaller neonatal hippocampus volume exposed to optimal parent structuring (EA score of 25), change in AUCg from age 1.5 to 3 years was significantly related to the Brief GEC, such that a greater decrease from ages 1.5 to 3 years was associated with fewer executive function difficulties at age 4.5 years.

DISCUSSION

To our knowledge, this is the first study to examine the interrelationship of early hippocampal maturation, trajectories of cortisol levels, and subsequent executive functions across early childhood in children born very preterm.

On average, lower total cortisol output across ages 1.5, 3, and 4.5 years was associated with more supportive (nonintrusiveness, sensitivity, nonhostility) parenting at age 3 years and fewer difficulties in executive functions at age 4.5 years. These findings are novel, extending a larger body of normative

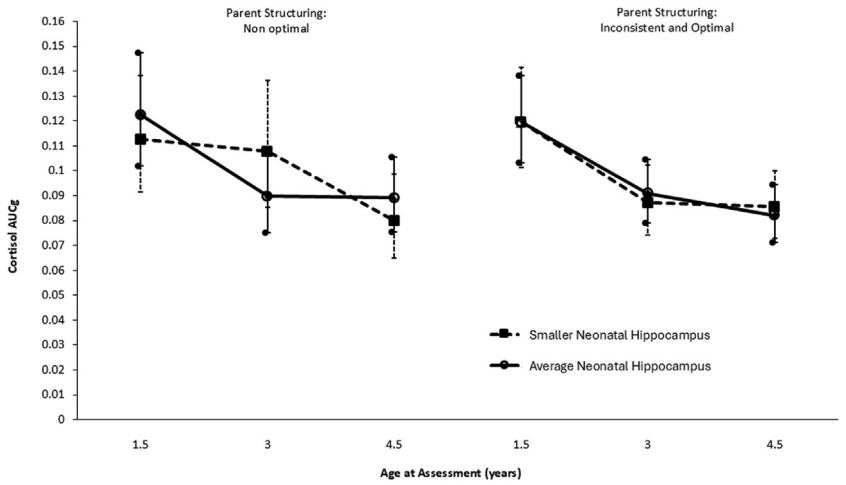

Figure 3. Neonatal hippocampus and parent structuring at 1.5 years interacted with child age to predict pattern of cortisol area under the curve with respect to ground (AUCg) at 1.5, 3, and 4.5 years. Children with smaller neonatal hippocampus showed no decrease in cortisol AUCg from 1.5 to 3 years but an overall decrease from 1.5 to 4.5 years.

Table 2. Relationships Between 3-Year Parent Behaviors and Average Total Cortisol Output (AUCg) Across Ages 1.5, 3, and 4.5 Years

	Cortisol AUCg	Cortisol AUCg	Cortisol AUCg	Cortisol AUCg
Parent Sensitivity 3 Years	−0.03 (−0.04 to −0.01)	–	–	–
Parent Structure 3 Years	–	0.001 (−0.02 to 0.02)	–	–
Parent Nonintrusiveness 3 Years	–	–	−0.02 (−0.03 to −0.01)	–
Parent Nonhostility 3 Years	–	–	–	−0.03 (−0.05 to −0.01)
Child Age: 3 Years[a]	−0.26 (−0.39 to −0.12)	−0.26 (−0.40 to −0.13)	−0.26 (−0.40 to −0.12)	−0.26 (−0.39 to −0.12)
Child Age: 4 Years[a]	−0.32 (−0.45 to −0.18)	−0.32 (−0.46 to −0.19)	−0.32 (−0.46 to −0.19)	−0.32 (−0.45 to −0.18)
GA Group[b]: VLGA	−0.10 (−0.23 to 0.03)	−0.13 (−0.26 to 0.003)	−0.11 (−0.24 to 0.02)	−0.10 (−0.23 to 0.03)
Time of Cortisol Collection	−0.001 (−0.003 to −0.0001)	−0.001 (−0.003 to −0.0000)	−0.001 (−0.003 to −0.0001)	−0.001 (−0.003 to −0.0001)
Constant	−0.77 (−1.61 to 0.07)	−1.37 (−2.24 to −0.50)	−0.89 (−1.70 to −0.07)	−0.68 (−1.56 to 0.20)
No. of Observations	333	333	333	333
Log Likelihood	−263.30	−267.43	−264.04	−263.51
Akaike Information Criterion	542.59	550.86	544.09	543.03
Bayesian Information Criterion	573.06	581.33	574.55	573.49

Values are presented as unstandardized B (95% CI) except where otherwise indicated.

AUCg, area under the curve with respect to ground; VLGA, very low gestational age.

[a]Reference group = age 1.5 years.

[b]Reference group = extremely low gestational age (<29 weeks gestation).

research, which has established that early maternal care is critical for physiological stress regulation and behavior (19) and previous research in preterm dyads demonstrating better neurodevelopment and behavior in the context of observed parent behavior (36,47). Executive functions suffer disproportionately under stress (48,49). At each age, children displayed elevated cortisol levels prior to testing, followed by a decrease and then stable levels through the end of assessment. Therefore, higher cortisol levels during cognitive assessment may reflect decreased task-based attention and regulatory control of the stress response via other- and/or self-regulation. Supportive parenting may lower child cortisol levels in response to challenging situations and therefore may be related to improved ability to shift attention across tasks, inhibit behaviors, and hold and process information. However, it is important to note that, different from cognitive testing, parent ratings of executive functions primarily assess behavioral disruption; this is a potential limitation of the current study. Future research should examine cognitive tests of executive functions in relation to cortisol levels.

Table 3. Relationship Between Average Total Cortisol Output (AUCg) Across Ages 1.5, 3, and 4.5 Years and Child Executive Functions at 4.5 Years

	Cortisol AUCg
Executive Functions 4.5 Years	0.01 (0.0003 to 0.01)
Child Age[a]: 3 Years	−0.25 (−0.39 to −0.11)
Child Age[a]: 4.5 Years	−0.32 (−0.46 to −0.18)
GA Group[b]: VLGA	−0.21 (−0.33 to −0.09)
Time of Cortisol Collection	−0.001 (−0.002 to 0.0001)
Constant	−1.69 (−2.49 to −0.89)
No. of Observations	323
Log Likelihood	−249.57
Akaike Information Criterion	515.14
Bayesian Information Criterion	545.36

Values are presented as unstandardized B (95% CI) except where otherwise indicated.

AUCg, area under the curve with respect to ground; VLGA, very low gestational age.

[a]Reference group = age 1.5 years.

[b]Reference group = extremely low gestational age (<29 weeks gestation).

Our most novel findings suggest that parent provision of structuring during early toddlerhood may be particularly important for stress regulation and executive functioning through early childhood for children born very preterm who had smaller neonatal hippocampal volumes. Children with smaller neonatal hippocampal volumes displayed greater vulnerability to poorer parent structuring at 1.5 years corrected age, as evidenced by poorer physiological stress regulation with nonoptimal parent structuring. Moreover, for children with smaller neonatal hippocampal volumes who were exposed to more optimal parent structuring at 1.5 years, the greater their decrease in cortisol from ages 1.5 to 3 years, the fewer executive function difficulties reported by the parent at age 4.5 years. Together, the findings suggest that smaller hippocampal volumes may render children both more susceptible to poorer parent structuring and ensure that they benefit more from optimal parent structuring, potentially in a for-better-or-worse fashion. While research with normative populations has shown that individuals with larger infant hippocampal volumes are susceptible in a for-better-or-worse fashion to parent behaviors (50–52), studies that have investigated effects of early-life stress on brain development have commonly identified smaller hippocampal volume as reflecting a vulnerability factor for poorer functioning (14,53,54). Broadly similar to the current findings, we recently found potential evidence that neonatal cortical fractional anisotropy renders children more susceptible in a for-better-or-worse fashion to parent sensitivity at age 3 years in relation to concurrent cognition and language in the

current cohort (24,55). In contrast, Vanes *et al.* (25) found no evidence of differential susceptibility to cognitively stimulating parenting in relation to cognition or behavioral outcomes when considering neonatal structural connectivity networks. Disparate findings from those of previous studies are likely due to the variation in the specific neural markers examined, the measurement tools and observational settings used to measure parent behaviors and child outcomes, and varying age of assessment across studies. The current study is also longitudinal in nature.

Parent structuring has mostly been studied in relation to, and shown to relate positively to, child cognitive abilities and executive functions (56,57). Parent scaffolding and promotion of self-driven problem solving may also benefit child emotion regulation behaviors (58), potentially due to contingent responding and rich language stimulation via mutually regulatory exchanges, thereby aiding in child-focused attention and engagement (59). Furthermore, those born preterm appear to benefit more than their full-term counterparts (58). The hippocampus contributes to emotional processes, such as the processing and consolidation of contextual sensory input of emotional information and emotion learning and regulation (60). Children with smaller hippocampal volumes may have greater fear generalization that results in higher cortisol in relation to the foreign testing environment. Therefore, they may require more structure for self-regulation during challenging tasks than their counterparts with larger hippocampal volumes (58,59).

The hippocampus continues to mature across the first few years of life (61) and is highly susceptible to early stress (62). In this cohort, we previously found that neonatal exposure to the sedative midazolam and surgery were associated with slower growth of the hippocampus across the NICU stay (31). For those with smaller hippocampal volumes in the neonatal period, poorer parenting (less structure) during early childhood may maintain reduced growth of the hippocampus related to the degree of prematurity and clinical exposures (31,63), resulting in stress dysregulation across early childhood and ultimately downregulation of the HPA axis (64,65) as well as poorer brain development in stress-sensitive regions long term (23). Improved physiological stress regulation and executive functioning in the face of more parent structuring may be a result of, or occur in tandem with, accelerated growth/greater maturation in the hippocampus (23,66–68). Provision of parent structure during infancy at a time of significant growth in this brain region (61) may help these children catch up, leading to lowering of cortisol profiles across 3 to 4.5 years and/or a decrease in executive functions, in a similar fashion to those with larger neonatal hippocampal volumes. Importantly, the parent-child relationship is bidirectional. Parent behaviors were not highly correlated across ages, and change in parent behavior across age 1.5 to 3 years was not associated with change in cortisol across these ages. Repeated concurrent MRI and cortisol measures across childhood would enable examination of how early supportive parenting behaviors may shape brain development and physiological stress responses in tandem. Nevertheless, MRI data are difficult to obtain in young children (69). An additional limitation is that we did not assess parent behaviors at 4.5 years.

Assessment of different parent behaviors at multiple ages afforded us the opportunity to examine and demonstrate specificity of domain and timing of parent behaviors. The fact that structured parenting at 1.5 and not 3 years may be associated with long-term trajectories of stress regulation is consistent with work that suggests that early infancy is a sensitive period for HPA axis calibration (70) and hippocampal maturation (62,67), both following early-life stress and in normative populations. At age 3 years, greater parent sensitivity, nonintrusiveness, and nonhostility, but not structuring, were associated with lower cortisol AUCg across ages. This finding is consistent with our previous work in which we demonstrated that a more positive parent environment, indexed by low parent stress and higher levels of all 4 parent behaviors assessed here was associated at age 3 but not at age 1.5 years with lower child internalizing behaviors from ages 1.5 through 8 years (71). We postulate that supportive, reciprocative parenting that promotes independence and autonomy may be most beneficial for long-term stress regulation during late toddlerhood, when children shift from emotional coregulation strategies dependent on their primary caregiver toward activities of exploration and independent learning. In addition, despite differences at age 3 years, by age 4.5 years, children displayed similar cortisol levels to their younger counterparts, suggesting a temporal and age-dependent effect of parenting and perhaps also reflecting other unmeasured environmental influences (e.g., day care) at age 3 years—a limitation of the current study.

Contrary to our hypothesis and research that has examined early caregiving adversity (14), neonatal hippocampal volume was not directly related to trajectories of cortisol output. The hippocampus is part of a large and complex network of limbic and frontal brain regions that together mediate cortisol output during early childhood and beyond, including the prefrontal cortex, amygdala, and hypothalamus (9). Extending our current findings, cortisol may be related to executive functioning because stress hormones modulate synaptic activity in the site for executive functions—the prefrontal cortex.

Limitations of the current study include the absence of a full-term control group and limited ethnic and socioeconomic diversity among participating families. Frequency and duration of parental visitation prior to infant discharge was not routinely tracked in our NICU at the time of data collection and is difficult to collect; however, this could be considered as a positive confounder in future studies. Work by members of our team has previously demonstrated that parental engagement in NICU care promotes improved child behavior via decreased parental cumulative physiological stress at 1.5 years of age (72). Finally, we did not control for antenatal corticosteroid exposure in this study. Steroids are very commonly administered in this population, with mixed findings of associations with child cortisol levels beyond early infancy (71). Future research should investigate brain-cortisol-executive function relationships through school age in children born very preterm, given that executive functioning is a core adaptive process that impacts child social and academic success as well as mental well-being (5).

Conclusions

Our work is the first to examine interrelationships among early brain dysmaturation, physiological stress, and parenting to advance understanding of divergent neurodevelopmental

pathways in populations of preterm children. Mapping of developmental pathways is central to our identification of risk and mitigation factors in this population, and yet there is a scarcity of published work. The current work goes a substantive way toward advancing this important scientific endeavor.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the Canadian Institutes of Health Research (CIHR) Grant Nos. MOP86489 [to REG], MOP68898 [to SPM], and MOP79262 [to SPM and REG]). REG is supported by a salary award from the BC Children's Hospital Research Institute, and MAM was supported by a CIHR Postdoctoral Fellowshipand a Michael Smith Foundation for Health Research Trainee Award.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada (MAM, ARS, SPM, REG); BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada (MAM, ARS, SPM, REG); Department of Psychology and Neuroscience, Auckland University of Technology, Auckland, New Zealand (MAM); Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada (JW); Department of Anesthesiology, Perioperative & Pain Medicine, University of Calgary, Calgary, Alberta, Canada (JVM); Child Brain and Mental Health, Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada (JVM); and SickKids Research Institute, Toronto, Ontario, Canada (TG).

SPM and REG are co-senior authors.

Address correspondence to Ruth E. Grunau, Ph.D., at rgrunau@bcchr.ca.

Received May 2, 2024; revised Jun 3, 2025; accepted Jun 6, 2025.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2025.06.005.

REFERENCES

1. Grunau RE, Holsti L, Haley DW, Oberlander T, Weinberg J, Solimano A, *et al.* (2005): Neonatal procedural pain exposure predicts lower cortisol and behavioral reactivity in preterm infants in the NICU. Pain 113:293–300.
2. Brummelte S, Grunau RE, Chau V, Poskitt KJ, Brant R, Vinall J, *et al.* (2012): Procedural pain and brain development in premature newborns. Ann Neurol 71:385–396.
3. Lammertink F, Benders MJNL, Hermans EJ, Tataranno ML, Dudink J, Vinkers CH, van den Heuvel MP (2022): Vulnerability of the neonatal connectome following postnatal Stress. J Neurosci 42:8948–8959.
4. Duerden EG, Grunau RE, Guo T, Foong J, Pearson A, Au-Young S, *et al.* (2018): Early procedural pain is associated with regionally-specific alterations in thalamic development in preterm neonates. J Neurosci 38:878–886.
5. Taylor HG, Clark CAC (2016): Executive function in children born preterm: Risk factors and implications for outcome. Semin Perinatol 40:520–529.
6. Boggini T, Pozzoli S, Schiavolin P, Erario R, Mosca F, Brambilla P, Fumagalli M (2021): Cumulative procedural pain and brain development in very preterm infants: A systematic review of clinical and preclinical studies. Neurosci Biobehav Rev 123:320–336.
7. Grunau RE, Vinall Miller J, Chau CMY (2021): Long-term effects of pain in infants. In: Stevens BJ, Hathway G, Zempsky WT, editors. Oxford Textbook of Pediatric Pain. New York: Oxford University Press, 38–46.
8. Herman JP (2016): Limbic pathways to stress control: Regulation of the HPA axis. In: Liberzon I, Ressler KJ, editors. Neurobiology of PTSD: From Brain to Mind. New York, NY: Oxford University Press, 148–168.
9. McEwen BS, Nasca C, Gray JD (2016): Stress effects on neuronal structure: Hippocampus, amygdala, and prefrontal cortex. Neuropsychopharmacology 41:3–23.
10. Myers B, Mark Dolgas CM, Kasckow J, Cullinan WE, Herman JP (2014): Central stress-integrative circuits: Forebrain glutamatergic and GABAergic projections to the dorsomedial hypothalamus, medial preoptic area, and bed nucleus of the stria terminalis. Brain Struct Funct 219:1287–1303.
11. Dahmen B, Puetz VB, Scharke W, von Polier GG, Herpertz-Dahlmann B, Konrad K (2018): Effects of early-life adversity on hippocampal structures and associated HPA axis functions. Dev Neurosci 40:13–22.
12. Wiedenmayer CP, Bansal R, Anderson GM, Zhu H, Amat J, Whiteman R, Peterson BS (2006): Cortisol levels and hippocampus volumes in healthy preadolescent children. Biol Psychiatry 60:856–861.
13. Pagliaccio D, Luby JL, Bogdan R, Agrawal A, Gaffrey MS, Belden AC, *et al.* (2014): Stress-system genes and life stress predict cortisol levels and amygdala and hippocampal volumes in children. Neuropsychopharmacology 39:1245–1253.
14. VanTieghem M, Korom M, Flannery J, Choy T, Caldera C, Humphreys KL, *et al.* (2021): Longitudinal changes in amygdala, hippocampus and cortisol development following early caregiving adversity. Dev Cogn Neurosci 48:100916.
15. Rubin RD, Watson PD, Duff MC, Cohen NJ (2014): The role of the hippocampus in flexible cognition and social behavior. Front Hum Neurosci 8:742.
16. Shields GS, Sazma MA, Yonelinas AP (2016): The effects of acute stress on core executive functions: A meta-analysis and comparison with cortisol. Neurosci Biobehav Rev 68:651–668.
17. Reul JMHM, de Kloet ERD (1985): Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology 117:2505–2511.
18. Hakamata Y, Komi S, Sato E, Izawa S, Mizukami S, Moriguchi Y, *et al.* (2019): Cortisol-related hippocampal-extrastriate functional connectivity explains the adverse effect of cortisol on visuospatial retrieval. Psychoneuroendocrinology 109:104310.
19. Gunnar MR, Hostinar CE (2015): The social buffering of the hypothalamic-pituitary-adrenocortical axis in humans: Developmental and experiential determinants. Soc Neurosci 10:479–488.
20. Koss KJ, Gunnar MR (2018): Annual Research Review: Early adversity, the hypothalamic-pituitary-adrenocortical axis, and child psychopathology. J Child Psychol Psychiatry 59:327–346.
21. Brummelte S, Grunau RE, Zaidman-Zait A, Weinberg J, Nordstokke D, Cepeda IL (2011): Cortisol levels in relation to maternal interaction and child internalizing behavior in preterm and full-term children at 18 months corrected age. Dev Psychobiol 53:184–195.
22. Belsky J, Pluess M (2016): Differential susceptibility to environmental influences. Developmental Psychopathology. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119125556.devpsy202. Accessed June 6, 2018.
23. Treyvaud K, Thompson DK, Kelly CE, Loh WY, Inder TE, Cheong JLY, *et al.* (2021): Early parenting is associated with the developing brains of children born very preterm. Clin Neuropsychol 35:885–903.
24. Miller JV, Chau V, Synnes A, Miller SP, Grunau RE (2022): Brain development and maternal behavior in relation to cognitive and language outcomes in preterm-born children. Biol Psychiatry 92:663–673.
25. Vanes LD, Hadaya L, Kanel D, Falconer S, Ball G, Batalle D, *et al.* (2021): Associations between neonatal brain structure, the home environment, and childhood outcomes following very preterm birth. Biol Psychiatry Glob Open Sci 1:146–155.
26. Karim HT, Perlman SB (2017): Neurodevelopmental maturation as a function of irritable temperament: Insights From a Naturalistic Emotional Video Viewing Paradigm. Hum Brain Mapp 38:5307–5321.
27. Oler JA, Fox AS, Shelton SE, Rogers J, Dyer TD, Davidson RJ, *et al.* (2010): Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. Nature 466:864–868.
28. Yassa MA, Stark CEL (2011): Pattern separation in the hippocampus. Trends Neurosci 34:515–525.
29. Lecei A, van Winkel R (2020): Hippocampal pattern separation of emotional information determining risk or resilience in individuals exposed to childhood trauma: Linking exposure to neurodevelopmental alterations and threat anticipation. Neurosci Biobehav Rev 108:160–170.
30. McLean MA, Weinberg J, Synnes AR, Miller SP, Grunau RE (2024): Relationships between cortisol levels across early childhood and

processing speed at age 4.5 years in children born very preterm. Child Neuropsychol 30:1215–1233.
31. Duerden EG, Guo T, Dodbiba L, Chakravarty MM, Chau V, Poskitt KJ, *et al.* (2016): Midazolam dose correlates with abnormal hippocampal growth and neurodevelopmental outcome in preterm infants. Ann Neurol 79:548–559.
32. Guo T, Winterburn JL, Pipitone J, Duerden EG, Park MTM, Chau V, *et al.* (2015): Automatic segmentation of the hippocampus for preterm neonates from early-in-life to term-equivalent age. Neuroimage Clin 9:176–193.
33. Bayley N (2005): Bayley Scales of Infant and Toddler Development, 3rd ed. San Antonio, TX: Psychological Corporation.
34. Wechsler D (2002): Wechsler Preschool and Primary Scale of Intelligence, 3rd ed. Toronto, ON, Canada: Pearson Clinical Assessment.
35. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003): Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28:916–931.
36. Vinall J, Miller SP, Synnes AR, Grunau RE (2013): Parent behaviors moderate the relationship between neonatal pain and internalizing behaviors at 18 months corrected age in children born very prematurely. Pain 154:1831–1839.
37. Biringen Z (2000): Emotional availability: Conceptualization and research findings. Am J Orthopsychiatry 70:104–114.
38. Gioia G, Isquith P, Guy S, Kenworthy L (2000): Behavior Rating Inventory of Executive Function. Child Neuropsychology 6:235–238.
39. R Core Team (2024): R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
40. Miller R, Plessow F (2013): Transformation techniques for cross-sectional and longitudinal endocrine data: Application to salivary cortisol concentrations. Psychoneuroendocrinology 38:941–946.
41. Bates D, Mächler M, Bolker B, Walker S (2015): Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48.
42. Kuznetsova A, Brockhoff PB, Christensen RHB (2017): lmerTest package: Tests in linear mixed effects models. Available at: http://www.jstatsoft.org/v82/i13/. Accessed June 11, 2021.
43. Satterthwaite FE (1946): An approximate distribution of estimates of variance components. Biometrics 2:110–114.
44. Hayes AF (2018): Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach, 2nd ed. New York, NY: The Guilford Press.
45. Lenth RV, Bolker B, Buerkner P, Giné-Vázquez I, Herve M, Jung M, *et al.* (2024): emmeans: Estimated Marginal Means, aka Least-Squares. Available at: https://cran.r-project.org/web/packages/emmeans/index.html. Accessed April 28, 2024.
46. Hughes J, Beiner D (2023): reghelper: Helper Functions for regression analysis. Available at: https://cran.r-project.org/web/packages/reghelper/index.html. Accessed April 28, 2024.
47. Treyvaud K, Doyle LW, Lee KJ, Ure A, Inder TE, Hunt RW, Anderson PJ (2016): Parenting behavior at 2 years predicts school-age performance at 7 years in very preterm children. J Child Psychol Psychiatry 57:814–821.
48. Arnsten AFT (2009): Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci 10:410–422.
49. Diamond A, Ling DS (2016): Conclusions about interventions, programs, and approaches for improving executive functions that appear justified and those that, despite much hype, do not. Dev Cogn Neurosci 18:34–48.
50. Nolvi S, Rasmussen JM, Graham AM, Gilmore JH, Styner M, Fair DA, *et al.* (2020): Neonatal brain volume as a marker of differential susceptibility to parenting quality and its association with neurodevelopment across early childhood. Dev Cogn Neurosci 45:100826.
51. Overfeld J, Entringer S, Rasmussen JM, Heim CM, Styner MA, Gilmore JH, *et al.* (2020): Neonatal hippocampal volume moderates the effects of early postnatal enrichment on cognitive development. Dev Cogn Neurosci 45:100820.
52. Rifkin-Graboi A, Tan HM, Shaun GKY, Sim LW, Sanmugam S, Chong YS, *et al.* (2019): An initial investigation of neonatal neuroanatomy, caregiving, and levels of disorganized behavior. Proc Natl Acad Sci U S A 116:16787–16792.
53. Frodl T, O'Keane V (2013): How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol Dis 52:24–37.
54. Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, *et al.* (2002): Childhood trauma associated with smaller hippocampal volume in women with major depression. Am J Psychiatry 159:2072–2080.
55. Lean RE (2022): Sensitive parenting: A key moderator of neonatal cortical dysmaturation and neurodevelopmental outcomes in children born very preterm. Biol Psychiatry 92:609–611.
56. Bernier A, Carlson SM, Whipple N (2010): From external regulation to self-regulation: Early parenting precursors of young children's executive functioning. Child Dev 81:326–339.
57. Koşkulu-Sancar S, van de Weijer-Bergsma E, Mulder H, Blom E (2023): Examining the role of parents and teachers in executive function development in early and middle childhood: A systematic review. Dev Rev 67:101063.
58. Erickson SJ, Duvall SW, Fuller J, Schrader R, MacLean P, Lowe JR (2013): Differential associations between maternal scaffolding and toddler emotion regulation in toddlers born preterm and full term. Early Hum Dev 89:699–704.
59. Landry SH, Taylor HB, Guttentag C, Smith KE (2008): Chapter 2 Responsive parenting: Closing the learning gap for children with early developmental problems. International Review of Research in Mental Retardation 36:27–60.
60. Gongora M, Teixeira S, Martins L, Marinho V, Velasques B, Moraes L, *et al.* (2019): Neurobiological evidences, functional and emotional aspects associated with the amygdala: From "What Is It?" to "What's to be done?". Neuropsychiatry 9:749–751.
61. Gilmore JH, Shi F, Woolson SL, Knickmeyer RC, Short SJ, Lin W, *et al.* (2012): Longitudinal development of cortical and subcortical gray matter from birth to 2 years. Cereb Cortex 22:2478–2485.
62. Humphreys KL, King LS, Sacchet MD, Camacho MC, Colich NL, Ordaz SJ, *et al.* (2019): Evidence for a sensitive period in the effects of early life stress on hippocampal volume. Dev Sci 22:e12775.
63. Chau CMY, Ranger M, Bichin M, Park MTM, Amaral RSC, Chakravarty M, *et al.* (2019): Hippocampus, amygdala, and thalamus volumes in very preterm children at 8 years: Neonatal pain and genetic variation. Front Behav Neurosci 13:51.
64. Brummelte S, Chau CMY, Cepeda IL, Degenhardt A, Weinberg J, Synnes AR, Grunau RE (2015): Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. Psychoneuroendocrinology 51:151–163.
65. Grunau RE, Cepeda IL, Chau CM, Brummelte S, Weinberg J, Lavoie PM, *et al.* (2013): Neonatal pain-related stress and NFKBIA genotype are associated with altered cortisol levels in preterm boys at school age. PLoS One 8(9):e73926.
66. Luby JL, Barch DM, Belden A, Gaffrey MS, Tillman R, Babb C, *et al.* (2012): Maternal support in early childhood predicts larger hippocampal volumes at school age. Proc Natl Acad Sci U S A 109:2854–2859.
67. Luby JL, Belden A, Harms MP, Tillman R, Barch DM (2016): Preschool is a sensitive period for the influence of maternal support on the trajectory of hippocampal development. Proc Natl Acad Sci U S A 113:5742–5747.
68. Whittle S, Yap MBH, Sheeber L, Dudgeon P, Yücel M, Pantelis C, *et al.* (2011): Hippocampal volume and sensitivity to maternal aggressive behavior: A prospective study of adolescent depressive symptoms. Dev Psychopathol 23:115–129.
69. Tamnes CK, Bos MGN, van de Kamp FC, Peters S, Crone EA (2018): Longitudinal development of hippocampal subregions from childhood to adulthood. Dev Cogn Neurosci 30:212–222.
70. Gunnar MR, Chapter MA (2022): Calibration and recalibration of stress response systems across development: Implications for mental and physical health. Adv Child Dev Behav 63:35–69.
71. McLean MA, Scoten OC, Chau CMY, Synnes A, Miller SP, Grunau RE (2022): Association of neonatal pain-related stress and parent interaction with internalizing behaviors across 1.5, 3.0, 4.5, and 8.0 years in children born very preterm. JAMA Netw Open 5:e2238088.
72. Mclean MA, Scoten OC, Yu W, Ye XY, Petrie J, Church PT, *et al.* (2022): Lower maternal chronic physiological stress and better child behavior at 18 months: Follow-up of a cluster randomized trial of Neonatal Intensive Care Unit family integrated care. J Pediatr 243:107–115.e4.