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Neonatal Stress, Health, and Development in Preterms: A Systematic Review

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abstract

CONTEXT: An overview of the full range of neonatal stressors and the associated clinical, laboratory, and imaging outcomes regarding infants' health and development may contribute to the improvement of neonatal care.

OBJECTIVE: To systematically review existing literature on the associations between all kinds of neonatal stressors and the health and development of preterm infants.

DATA SOURCES: Data sources included Embase, Medline, PsycINFO, the Cumulative Index to Nursing and Allied Health Literature, and reference lists.

STUDY SELECTION: Studies were eligible if they included a measure of neonatal stress during the NICU stay, reported clinical, laboratory, and/or imaging outcomes regarding health and/or development on discharge from the NICU or thereafter, included preterm infants, and were written in English or Dutch.

DATA EXTRACTION: Two reviewers independently screened the sources and extracted data on health and development. Study quality was assessed by using the Newcastle-Ottawa Quality Assessment Scale.

RESULTS: We identified 20 articles that reported on neonatal stress associated negatively with clinical outcomes, including cognitive, motor, and emotional development, and laboratory and imaging outcomes, including epigenetic alterations, hypothalamic-pituitary-adrenal axis functioning, and structural brain development. We found no evidence regarding associations with growth, cardiovascular health, parent-infant interaction, the neonatal immune system, and the neonatal microbiome.

LIMITATIONS: The studies were all observational and used different definitions of neonatal stress.

CONCLUSIONS: Neonatal stress has a profound impact on the health and development of preterm infants, and physicians involved in their treatment and follow-up should be aware of this fact.

REVIEW ARTICLE

During their stay in a NICU, preterm infants (gestational age of <37 weeks) are exposed to a variety of potential stressors. These stressors include noise and bright lights, medical interventions, and skin-breaking procedures, but routine handling by physicians and nursing staff and maternal separation also constitute stress.[1] Regarding painful skin-breaking procedures, for example, averages of 7.5 to 17.1 per day have been reported.[2] Because these procedures are known to elicit obvious pain responses, they have been studied most often. Other, perhaps less obvious stressors, such as handling by medical staff, maternal separation, and environmental stress, evoke similar responses[3] (a phenomenon known as sensitization[4]), adding to the extensive range of neonatal stress. All procedures experienced by preterm infants during their stay in a NICU should therefore be taken into consideration. For the purpose of this study, we defined stress as "an adverse circumstance that disturbs, or is likely to disturb, the normal physiological or psychological functioning of an individual," thereby basing ourselves on the definition given by the Oxford English Dictionary.[5] Although a few overviews of literature on the topic of neonatal stress exist,[4,6] to date, a comprehensive systematic literature review on the broad range of neonatal stressors, which extends beyond skin-breaking procedures, is lacking.

The environment of the NICU, and the stress associated with it, is thought to have major long-lasting effects on neonatal health and development.[7] These effects may include poorer clinical outcomes on cognitive, psychomotor, and emotional and behavioral development, all representing domains in which preterm children run a higher risk of

impairment.[8–12] Effects may also include laboratory and imaging findings because these may reflect pathways between stressors and concurrent or later clinical outcomes. First, for example, the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the production of glucocorticoids, including cortisol (dubbed the "stress hormone"), is likely to be involved in shaping reactions to neonatal stress.[4] Second, evidence emerging rapidly from the field of behavioral epigenetics is that epigenetic alterations (changes to the DNA profile without changing the nucleotide sequence) may play a role in development.[13] Third, the role of the neuroendocrine system and microbiome is receiving increasing attention, especially in the field of psychiatry. This discipline associates early life experiences with proinflammatory immune profiles, changes to the gut-brain axis, and psychiatric disorders, such as anxiety, depression, or dementia.[14,15] Finally, imaging studies show that the preterm brain develops differently from that of term infants[16,17] because synaptic connections are being formed and cortical networks are being established during this period.[18]

An overview of literature on the associations between neonatal stress in the broad sense, as outlined here, and various health and developmental outcomes is important because it may well contribute to improvement of neonatal care.[4,7] We therefore aimed to systematically review the literature dealing with the assessment of associations between all kinds of neonatal stress and clinical, laboratory, and imaging outcomes in the domains of health and development of preterm infants.

METHODS

Search Strategy

We performed our search strategy according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for systematic reviews.[19] We used Endnote X8 as a reference manager. Two researchers (N.H.v.D and M.L.A.d.K.) conducted the independent systematic searches in the Embase, Medline, PsycINFO, and the Cumulative Index to Nursing and Allied Health Literature databases for all literature up to and including April 2021. In addition, the same 2 authors independently screened the reference lists of all the retrieved articles for potential inclusion of relevant publications.

Our search comprised 3 main elements: (1) stress, (2) preterm birth, and (3) clinical, laboratory, and imaging outcomes of health and development. Regarding the element stress, we included all kinds of stress neonates might experience during their NICU stay, such as pain-related (skin-breaking) procedures, nursing and handling, noise and light, and maternal separation. We did not include studies that measured an immediate reaction to neonatal stress because our interest lay in collective circumstances during the NICU stay. Regarding the element preterm birth, we included studies with infants born at a gestational age of <37 weeks with or without a term control group. Regarding the element outcomes of health and development, we first searched for clinical outcomes: growth, cardiovascular health, motor development, cognitive development, emotional and behavioral development, language development, and parent-infant interaction. Second, we searched for laboratory and imaging outcomes, including epigenetic

alterations (such as DNA methylation and telomere length), alterations of the microbiome, immunologic changes, alterations in the functioning of the HPA axis as expressed in cortisol levels, and changes in structural brain development. These outcomes had to be reported on discharge from the NICU or thereafter to encompass the effects of the entire NICU stay. We excluded studies that regarded animal research and studies written in languages other than English and Dutch. The full search strategy is presented in Table 1.

Quality Assessment

To assess the quality of the studies, we used the Newcastle-Ottawa Quality Assessment Scale for case-control and cohort studies.[20] This scale covers 3 domains: selection, comparability, and exposure in case of case-control studies or outcome in cohort studies. Selection focused on representativeness of the cases and the selection of controls in case-control studies or representativeness of the cohort and the selection of a nonexposed group in cohort studies. Comparability focused on the factors controlled by the study in the statistical analyses. Exposure, in the case of case-control studies, focused on ascertaining exposure and nonresponse rates in follow-up assessments, whereas outcome, in cohort studies, focused on the length and adequacy of follow-up. The total quality assessment score consists of the sum of these 3 domains and ranges from 0 to 9, in which 9 indicates the highest quality.[20]

Analysis and Reporting

As far as analysis and reporting is concerned, we first present the flow of studies and the characteristics of the included studies and their quality. Second, we provide a detailed summary of the studies we included regarding associations between neonatal stress and clinical, laboratory, and imaging outcome measures of health and development.

RESULTS

Flow of Studies and Study Characteristics

Our search resulted in 20 485 articles. Figure 1 is a flowchart of the study selection procedures. After removing duplicates, we screened 13 670 articles on the basis of their titles and abstracts and found 66 relevant studies. Of these, we excluded 45 because they were conference abstracts, doctoral theses, comments, or books (n = 22); because they did not meet our inclusion criteria regarding the moment at which neonatal stress occurred (n = 1); because they did not meet our inclusion criteria regarding the moment at which the outcome had been measured (n = 12); or because they did not involve original research or fell outside the scope of our search (n = 10). The latter, for example, dealt with an immediate response to a painful stimulus, with parenting or parental psychosocial stress, or with the predictive value of epigenetic alterations for outcomes later in life, without presenting a measure of neonatal stress. We selected an additional 5 articles from the reference lists, 2 of which fulfilled the inclusion criteria.

Finally, our review consisted of 20 studies. Most studies involved very preterm infants with gestational ages of <32 weeks, as well as control groups of term infants, and examined pain-related stress. In general, the measure used to assess neonatal stress during the NICU stay was skin-breaking procedures. Eleven studies were part of a large cohort study by Grunau and colleagues.[21–31] A mere 7 studies extended the scope of neonatal stress beyond pain-related stressors by including exposure to all stressors during the NICU stay: the quality of developmental care, medical stressors (such as all potentially stressful events during the NICU stay associated with medical care based on the Neonatal Infant Stressor Scale[3]), and maternal separation.[32–38] We did not identify studies fulfilling our inclusion criteria that investigated noise or light exposure as stressors. Two studies addressed both clinical and laboratory or imaging outcomes.

TABLE 1 Search Strategy

| Search Strategy |
| --- |
| ("Stress, Psychological"[Mesh] OR stress[tiab] OR noise OR light OR skin-breaking procedure* OR medical intervention* OR handling OR maternal separation*) AND ("Premature Birth"[Mesh] OR premature[tiab] OR prematurity[tiab] OR preterm[tiab] OR gestational age[tiab] OR "Gestational Age"[Mesh]) AND (cardiovascular dis*[tiab] OR "Cardiovascular Diseases"[Mesh] OR cerebral palsy[tiab] OR telomere*[tiab] OR methylation[tiab] OR epigenetic*[tiab] OR language disorder*[tiab] OR language impairment[tiab] OR developmental[tiab] OR brain[tiab] OR pain[tiab] OR microbio*[tiab] OR education*[tiab] OR executive function*[tiab] OR neurobehavior[tiab] OR neurobehavior[tiab] OR behavior[tiab] OR behavior[tiab] OR cogniti*[tiab] OR intelligence[tiab] OR growth[tiab] OR weight[tiab] OR height[tiab] OR bmi[tiab] OR BMI[tiab] OR head circumference[tiab] OR "Growth"[Mesh] OR neurodevelopment*[tiab] OR cortisol[tiab] OR "Parent-Child Relations"[Mesh] OR parent infant interaction[tiab] OR mother-child dyad[tiab] OR "Intensive Care Units, Neonatal"[Mesh] OR NICU[tiab] OR NICU*[tiab] OR "Neurodevelopmental Disorders"[Mesh] OR "Pain"[Mesh] OR "Language Disorders"[Mesh] OR "Cerebral Palsy"[Mesh] OR "Epigenomics"[Mesh] OR "Child Behavior"[Mesh] OR attention deficit disorder*[tiab] OR developmental coordination disorder*[tiab] OR dcd[tiab] OR adhd[tiab] OR motor[tiab]) |

Mesh, Medical Subject Headings.
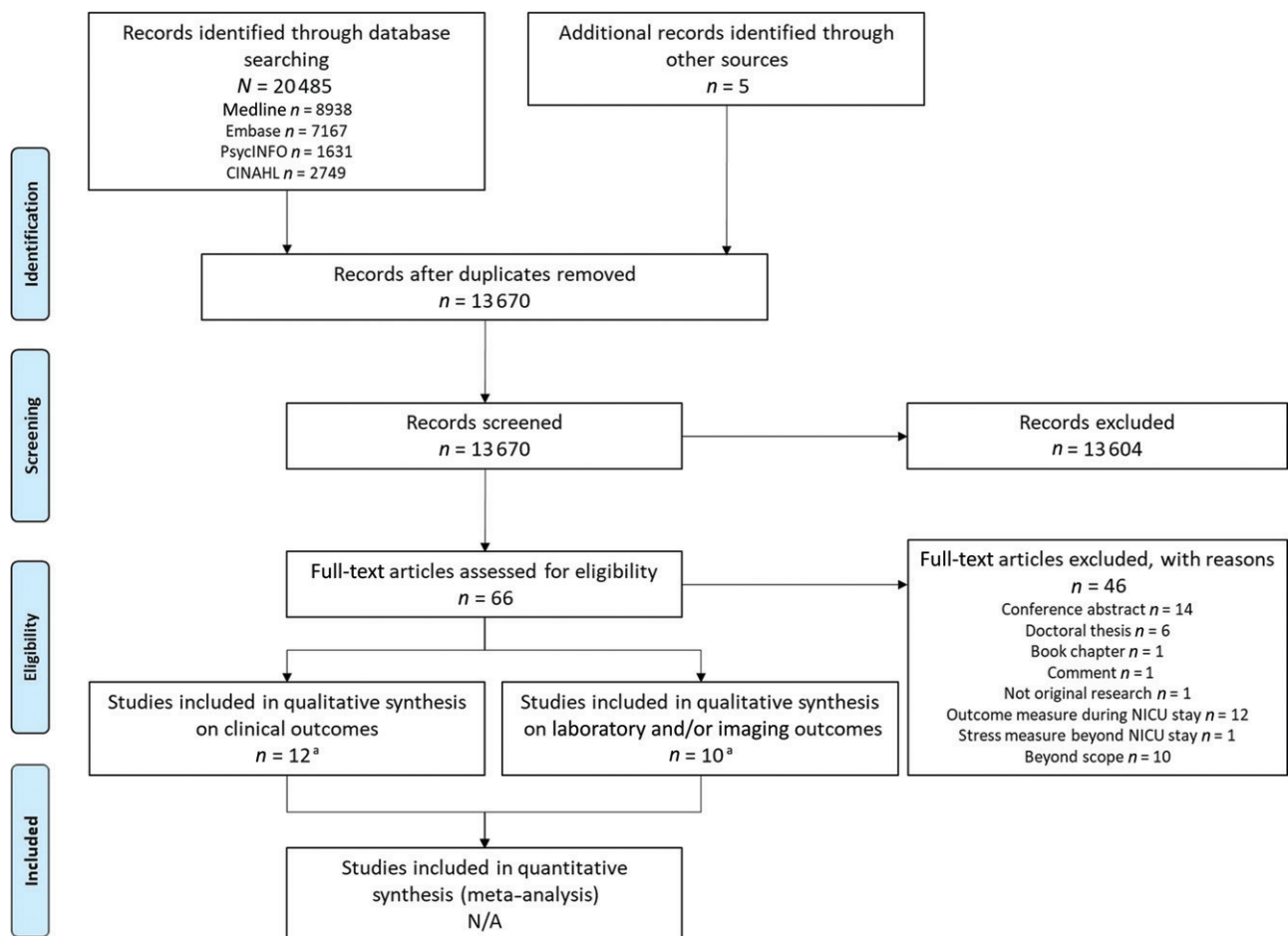
FIGURE 1
Flow of studies. [a] Two studies included an association with a clinical outcome and a laboratory or imaging outcome, bringing the total number of studies included in qualitative analysis to $n = 20$. CINAHL, Cumulative Index to Nursing and Allied Health Literature; N/A, not applicable.

One study assessed cortisol levels and cognitive and motor development,[35] and the other addressed epigenetic alterations and emotional and behavioral development.[22]

Quality of Studies

By and large, the quality of the studies was moderate. In most studies, a preterm cohort was combined with a nonexposed group, mainly term children, who had been selected from either the same or a different hospital.[21–29,33–35,37,39,40] A few studies did not include a nonexposed group.[30–32,36,38] In 5 studies, the statistical analyses had not been adjusted for confounding factors, and only unadjusted

associations between the stressor and outcome were reported.[24,27,33,34,36] Most studies failed to report nonresponse and the characteristics of the participants lost to follow-up.[21–29,31,35,40] The authors of 3 studies, in particular, reported on blind assessment of the outcome measures.[25,30,38] In Supplemental Table 4, we present detailed quality scores of all the studies, and the total scores are summarized in Tables 2 and 3.

Associations Between Neonatal Stress and Clinical Outcomes

Our search yielded studies on cognitive, motor, behavioral, and emotional development, which we summarize in Table 2. We found no

studies on language development, parent-infant bonding, or interaction. Nor did we find studies on the associations between neonatal stress and growth indicators, such as weight, height, BMI, or energy expenditure. Cardiovascular outcomes, such as blood pressure, were not reported either.

Cognitive and Motor Development

In 2 studies, cognitive and motor development were assessed as outcome measures in early infancy and toddlerhood by using the Bayley Scales of Infant and Toddler Development, Second Edition (BSID-II).[24,35] One study assessed maternal separation as a neonatal stressor

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TABLE 2 Findings on Associations Between Neonatal Stress and Clinical Outcomes in Preterm Infants

Study	Design	Participants	Stress Measure	Outcome Measure	Findings	Quality Score (Maximum of 9)
Cognitive and motor development						
Grunau et al[24]	Cohort study	$N = 211$: $n = 137$ preterm infants (48% boys), $n = 74$ terms infants (48% boys)	No. skin-breaking procedures	BSID-II at 8 mo, 18 mo	Higher stress → ↓ mental and psychomotor subscales at 8 mo and 18 mo	7
Mello et al[35]	Case-control study	$N = 42$ <37 wk and >1000 g; incubator group: $n = 15$ (27% boys); experimental group: $n = 27$ (44% boys)	Full-time incubator care versus continuous kangaroo care	BSID-II at 6–7 mo	No differences in mental and psychomotor subscales	7
Emotional and behavioral development						
Chau et al[22]	Cohort study	$N = 111$: $n = 69$ <32 wk (59% boys), $n = 50$ term infants (percentage of boys unknown)	No. skin-breaking procedures	CBCL at 7 y	Higher stress → ↑ total problems on CBCL, no association with subscales	6
Dimitrova et al[37]	Cohort study	$N = 58$: $n = 36$ <34 wk (44% boys), $n = 22$ term infants (36% boys)	Perinatal risk inventory: 18-item scale to measure severity of neonatal illness	Symptom Checklist at 18 mo, CBCL at 11 y	Higher stress → ↑ emotional scores at 18 mo, higher stress → ↑ internalizing problems at 11 y, interaction effect between higher stress and emotional scores at 18 mo	6
Gaspardo et al[38]	Cohort study	$N = 62$ <37 wk and/or <1500 g and admitted to the NICU, 49% boys	Neonatal Infant Stressor Scale, only the pain-related stress items	CBCL and ECBQ at 18–36 mo	No association between Neonatal Infant Stressor Scale and CBCL, higher stress → ↓ effortful control subscale	6
Montirosso et al[34]	Cohort study	$N = 257$: $n = 134$ <30 wk and/or <1500 g (54% boys), $n = 123$ term infants (51% boys, matched by sex, maternal age, and SES)	Quality of care: 29-item questionnaire operationalized in ICC (scale 0–8) and IPM (scale 0–10) indices	CBCL at 18 mo, internalizing and externalizing subscales only	No association between ICC and internalizing or externalizing subscales; lower IPM → ↑ internalizing subscale, no association with externalizing subscale	5
Ranger et al[29]	Cohort study	$N = 101$ <32 wk: $n = 44$ nonventilated (39% boys), $n = 57$ ventilated (58% boys)	No. skin-breaking procedures	CBCL at 7 y, internalizing subscale only	High stress → ↑ internalizing subscale in nonventilated infants; no independent effect established in ventilated infants because of high correlation between the 2	7

ECBQ, Early Childhood Behavior Questionnaire; ICC, infant-centered care; IPM, infant pain management; SES, socioeconomic status; → associated with; ↑ increased; ↓ decreased.

TABLE 3 Findings on Associations Between Neonatal Stress and Laboratory and Imaging Outcomes in Preterm Infants

Study	Design	Participants	Stress Measure	Outcome Measure	Findings	Quality Score (Maximum of 9)
HPA axis functioning: cortisol levels						
Brummelte et al[21]	Cohort study	$N = 129$: $n = 27$ <28 wk (63% boys), $n = 57$ <32 wk (39% boys), $n = 45$ term infants (40% boys)	No. skin-breaking procedures	Salivary cortisol levels at 7 y, before, during, and after test as well as diurnal patterns from 2 non–school days	Higher stress → ↓ cortisol levels during cognitive assessment, higher stress → ↓ diurnal cortisol, especially early morning	6
Grunau et al[25]	Cohort study	$N = 133$: $n = 91$ <32 wk (46% boys), $n = 42$ term infants (36% boys)	No. skin-breaking procedures	Hair cortisol, psychometric assessment, and cytokine levels at 7 y	Higher stress → ↓ cortisol levels for boys only	5
Grunau et al[26]	Cohort study	$N = 225$: $n = 64$ <28 wk (59% boys), $n = 81$ <32 wk (47% boys), $n = 80$ term infants (50% boys)	No. skin-breaking procedures	Salivary cortisol levels at 18 mo	Higher stress → ↑ cortisol levels, especially in the group with extremely low gestational ages	7
Mello et al[35]	Case-control study	$N = 42$ <37 wk and >1000 g; incubator group: $n = 15$ (27% boys); experimental group: $n = 27$ (44% boys)	Full-time incubator care versus continuous kangaroo care	Salivary cortisol levels on waking up, 30 min after awakening, and at 4:00 PM on the day before cognitive testing	No differences in cortisol levels; no differences in wt; incubator → ↓ height; kangaroo → ↑ BMI	7
McLean et al[31]	Cohort study	$N = 124$ <32 wk, 60% boys	No. skin-breaking procedures	Salivary cortisol levels 15 min before test, during test, and after test and sensory processing questionnaire at 4 y	No association with cortisol levels; higher stress → ↑ sensory processing problems in girls only; sensory processing problems and cortisol levels correlated	6
Epigenetic alterations Chau[22]	Cohort study	$N = 111$: $n = 69$ <32 wk (59% boys), $n = 50$ term infants (percentage of boys unknown)	No. skin-breaking procedures	Salivary DNA methylation of *SLC6A4* at 20 CpG sites at 7 y	↑ methylation of CpG sites 1–5 in preterm infants compared with term infants ↓ methylation of CpG sites 1–5 in preterm infants with *Met/Met COMT158* genotype	6
Giarraputo et al[36]	Cohort study	$N = 63$, all <1500 g: $n = 38$ high medical risk[a] (45% boys), $n = 25$ low medical risk[a] (40% boys)	High versus low medical risk; medical risk assessed by using a therapeutic intensity or neonatal illness severity score	Salivary DNA methylation of *NR3C1* at 5 CpG sites on discharge from NICU	High medical risk → ↓ methylation at CpG site 1, no effects on CpG sites 2–5	5
Montirosso et al[33]	Cohort study	$N = 78$: $n = 48$ <32 wk and/or <1500 g (52% boys), $n = 30$ term infants (50% boys)	Length of NICU stay	Blood sample DNA methylation of *SLC6A4* at 20 CpG sites at birth and on discharge from NICU	No differences at birth; NICU stay versus term infants → ↑ methylation at CpG site 5; NICU stay versus birth in preterm → ↑ methylation at CpG sites 5 and 7	6.5

TABLE 3 Continued

Study	Design	Participants	Stress Measure	Outcome Measure	Findings	Quality Score (Maximum of 9)
Provenzi et al[39]	Cohort study	N = 77: n = 46 <32 wk (48% boys), n = 31 term infants (52% boys)	No. skin-breaking procedures	Blood sample telomere lengths	Longer telomeres for preterm infants versus term infants at birth; no differences NICU discharge versus term infants; high stress → ↓ telomere length on discharge from NICU	7
Provenzi et al[40]	Cohort study	N = 88: n = 56 <32 wk and/or 1500 g (52% boys), n = 32 term infants (53% boys)	No. skin-breaking procedures	Blood sample DNA methylation of *SLC6A4* at 20 CpG sites at birth and on discharge from NICU	No differences at birth; high stress → ↑ methylation at CpG sites 5 and 6 on discharge from NICU	7
Structural brain development Chau et al[30]	Cohort study	N = 57 <32 wk, 45% boys	No. skin-breaking procedures	MRI at 8 y	Higher stress → ↓ brain volumes → ↓ cognitive, visual-motor, and behavioral outcomes; No. skin-breaking procedures: amygdala and thalamus; mechanical ventilation: several subcortical regions; surgeries: several subcortical regions	5
Doesburg et al[23]	Cohort study	N = 54: n = 22 <28 wk (55% boys), n = 32 <32 wk (34% boys), n = 25 term infants (32% boys)	No. skin-breaking procedures	Magnetoencephalography, WISC, and Beery-Buktenica developmental test for visual-perceptual scores at 7 y	Higher stress → ↓ visual-perceptual abilities; atypical magnetoencephalography for children <28 wk → ↓ visual-perceptual abilities	6
Kozhemiako et al[27]	Cohort study	N = 100: n = 24 <28 wk (58% boys), n = 37 <32 wk (35% boys), n = 39 term infants (38% boys)	No. skin-breaking procedures	Magnetoencephalography, MRI, WISC, CBCL, BRIEF, and Beery-Buktenica developmental test for visual-perceptual scores and Flanker task at 7 y	Higher stress → ↑ connectivity, especially in θ band (frontal), especially in the group of infants of extremely low gestational age	4.5
Ranger et al[28]	Cohort study	N = 41 preterm infants, 38% boys	No. skin-breaking procedures	MRI at 7 y	Higher stress → ↓ cortical thickness in several brain regions; No. skin-breaking procedures: frontal and parietal; mechanical ventilation: frontal and parietal; infections: frontal and parietal; surgeries: parietal, temporal, and occipital; SNAP-II: frontal and parietal; after adjusting for all others, No. skin-breaking procedures → ↓ cortical thickness in 21 of 66 brain regions	6
Smith et al[32]	Cohort study	N = 44 < 30 wk, percentage of boys is unknown	Neonatal Infant Stressor Scale	MRI at term equivalent age	Higher stress → ↑ cerebellar hemorrhage and total brain injury, no association with white and gray matter injury scores	6.5

BRIEF, Behavioral Inventory of Executive Functioning; CpG, 5'—C—phosphate—G—3' site; SNAP-II, Score for Acute Neonatal Physiology version II; WISC, Weschler Scale of Intelligence; → associated with; ↑ increased; ↓ decreased.
[a] Medical risk assessed by using a therapeutic intensity or neonatal illness severity score.

and found no differences between the separated and nonseparated groups on the BSID-II mental and motor subscales.[35] The other study found an association between higher numbers of skin-breaking procedures and lower scores on the mental and motor subscales of the BSID-II at 8 and 18 months.[24]

Emotional and Behavioral Development

Five studies assessed emotional and behavioral outcomes, and all 5 used the Child Behavior Checklist (CBCL) as an outcome measure.[22,29,34,37,38] In addition, one study used the Symptom Checklist,[37] and another used the Early Childhood Behavior Questionnaire, which is focused on attention problems in toddlers.[38] In 3 studies, emotional and behavioral problems were assessed during early childhood, 18 to 36 months,[34,37,38] and at school age, 7 to 11 years.[22,29,37] Studies consistently reported more emotional and behavioral problems with neonatal stress assessed as either skin-breaking procedures,[22,29,38] the quality of infant-centered care regarding pain management (assessed by using a 29-item quality of care questionnaire operationalized in these indices),[34] or medical stress (assessed by using the perinatal risk inventory, an 18-item scale on severity of perinatal complications).[37]

Associations Between Neonatal Stress and Laboratory and Imaging Outcomes

Table 3 provides a summary of the laboratory outcomes we found in studies on the functioning of the HPA axis, as expressed in cortisol levels, and epigenetic alterations, both in DNA methylation and telomere length. Our search also yielded studies on structural brain development, which are included in Table 3. None of the studies we found reported on associations between neonatal stress and changes to the neonatal microbiome or immune system.

HPA Axis Functioning: Cortisol Levels

We identified 5 studies that examined the association between neonatal stress and cortisol levels as an indicator of the functioning of the HPA axis. Measurements were performed in early childhood, 6 to 18 months,[26,35] and at school age, 4 to 7 years.[21,25,31] Cortisol levels were measured in saliva in 4 studies[21,26,31,35] and in hair in another.[25] Findings were contradictory as far as the association between neonatal stress and cortisol levels was concerned. Studies on skin-breaking procedures[21,25,26,31] either found lower levels in diurnal cortisol patterns and during cognitive assessments,[21,25] higher levels (particularly in the case of children who were born at extremely low gestational ages),[26] or no relation at all.[31] One study on maternal separation reported no differences in cortisol levels.[35]

Epigenetic Alterations

We found 5 studies that assessed associations between neonatal stress and epigenetic alterations, such as changes in DNA methylation[22,33,36,40] and in telomere length.[39] Four of these studies assessed epigenetic changes between birth and discharge, thus incorporating the full extent of neonatal stress during the NICU stay,[33,36,39,40] whereas one study assessed DNA methylation at the age of 7 years.[22] Three studies assessed DNA methylation of the serotonin transporter gene, SLC6A4, with consistent results of hypermethylation across different sites.[22,33,40] In one study, DNA methylation of the glucocorticoid receptor gene, NR3C1, was assessed, and the authors reported that infants with more neonatal stress showed hypomethylation at one specific site.[36] For NR3C1, the reported hypomethylation is most likely associated with a decrease in gene transcription of the glucocorticoid receptor, whereas for SLC6A4, hypermethylation is most likely associated with an increased transcription of the serotonin transporter gene. Strikingly, none of the studies reported differences at birth between preterm and term infants in DNA methylation of these 2 genes. Finally, one study reported that in very preterm infants, more neonatal stress was associated with shorter telomere lengths across the epigenome on discharge from the NICU.[39]

Structural Brain Development

Five studies assessed structural brain development as an imaging outcome. Two of these studies used magnetoencephalography,[23,27] whereas the other 3 used MRI.[28,30,32] In the studies using magnetoencephalography, the authors reported that more neonatal stress, assessed as skin-breaking procedures, was associated with increased connectivity, especially in the frontal lobe of the brain.[27] This atypical magnetoencephalography pattern of increased connectivity was in turn associated with decreased visual-perceptual abilities, especially in children born before 28 weeks' gestation.[23] The studies using MRI reported that more neonatal stress, assessed as the total number of medical stressors during the NICU stay and measured by using either the Neonatal Infant Stressor Scale[32] or skin-breaking procedures,[28,30] was associated with increased cortical thickness across the entire brain, more cerebellar hemorrhages, reduced brain volumes in several areas of the brain (including the amygdala and thalamus), or diffuse injury throughout the brain.[28,30,32]

DISCUSSION

Our systematic review demonstrates that neonatal stress is associated with a variety of clinical, laboratory, and imaging outcomes directly after an infant's stay in a NICU and beyond. A higher level of neonatal stress is associated with poorer cognitive, motor, emotional, and behavioral development. In addition, a higher level of neonatal stress is associated with changes in structural brain development in several areas of the brain. We also identified associations between neonatal stress and the functioning of the HPA axis and epigenetic alterations. Our review provides no evidence for other health and developmental outcomes, such as growth, cardiovascular health, language development, parent-infant interaction, and alterations in the neonatal immune system and neonatal microbiome.

We found that neonatal stress is associated with clinical outcomes, that is, cognitive, motor, emotional, and behavioral development. For cognitive and motor development, we identified only 2 studies in early life,[24,35] whereas for emotional and behavioral development, several studies over the course of childhood were identified.[22,29,34,37,38] Internalizing behavioral and attentional problems in particular seem to increase after higher stress exposure during the NICU stay.[29,34,37] For preterm infants, neonatal stress during the NICU stay thus seems to be an additional factor that increases the risk of poorer outcomes in these domains. We think that laboratory and imaging outcome measures provide clues regarding the lines along which associations between neonatal stress and these clinical outcomes may be explained.

One intermediate outcome supporting the association between neonatal stress and clinical presentations of neurodevelopmental issues regards structural brain development. In our review, we found stress to be associated with a variety of changes in the structure of the brain.[23,27,28,30,32] For example, neonatal stress was associated with alterations in the structure of the frontal lobe, a part of the brain that is essential for social, attentional, and executive development and processing.[27] Moreover, stress was associated with more cerebellar hemorrhages,[32] which may result in coordination disorders, cognitive dysfunction, and behavioral problems because the cerebellum is involved in those functions.[41] Furthermore, the authors of several studies reported that cortical thickness across diffuse brain regions was affected.[27,28,32] An increase in cortical thickness may lead to a variety of problems involving intellect and other high-order cognitive functions.[42] Previously, many of the regions affected were reported to be involved in spatial orientation and memory.[30] The findings underline the hypothesis that structural brain development may be affected by stress occurring in the neonatal period, during which the brain is extremely sensitive to environmental stimuli. The mediating role of structural changes to the brain is also confirmed by the fact that, particularly, internalizing and attention problems were found to be associated with neonatal stress. Functions relating to emotion and attention indeed originate in the frontal lobe, cerebellum, and cortical areas throughout the brain, which are reported to be affected by neonatal stress.

In our review, laboratory findings, particularly those related to the functioning of the HPA axis, were other outcomes we identified that supported the associations between neonatal stress and clinical presentations of neurodevelopmental problems. These alterations in function of the HPA axis (ie, cortisol dysregulation) had conflicting directions (ie, either increased[26] or decreased levels[21,25]). Both directions may contribute to a deterioration of the infants' health.[43] In preterm-born children, altered cortisol levels are associated with attention problems.[21] In studies on other areas of stress during early life, including maltreatment or traumatic events, alterations in HPA axis settings were also reported.[43] This phenomenon is labeled stress sensitization and is hypothesized to be a mechanism responsible for the observed association between exposure to stressors in early life and emotional and behavioral development, specifically anxiety and depression.[43,44] Additionally, altered cortisol levels are associated with growth and cardiovascular health.[45] Alterations in the settings of the HPA axis may therefore also be a mechanism in the association between neonatal stress and clinical outcomes.

Moreover, we identified several studies reporting that epigenetic alterations of the two most studied genes, NR3C1 and SLC6A4, were associated with exposure to neonatal stressors.[22,33,36,40] These alterations are also associated with social-emotional development,[46] poorer neurodevelopmental outcomes,[13] and brain growth.[47] Several studies on other circumstances in which stress occurs during early life also reported associations between epigenetic alterations and problems later on, including stress hypersensitivity,[48] neuroendocrine dysregulation,[39] anxiety, and depression.[49] In addition, cross-links exist with the HPA axis because epigenetic alterations have been

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associated with altered cortisol reactivity in preterm-born infants.[50] Thus, epigenetic alterations may be the molecular mechanism underlying the functioning of the HPA axis and associated clinical findings. Moreover, these alterations may also be the basis for structural changes in brain development because these receptors are widely expressed in the human brain and are involved in the early processes of neuronal formation.[47]

By using the Newcastle-Ottawa Scale for Quality Assessment[20] the quality of the included studies appeared to be moderate. All included studies were observational, that is, either cohort or case-control studies. This cannot be considered a real limitation nor can it be avoided because, for ethical reasons, observational studies are the only type of study on stress exposure possible in human neonates. Studies further differed regarding their definition of neonatal stress, which hampered comparisons between them. Many studies only investigated skin-breaking procedures as neonatal stress, thus possibly underestimating the accumulation of stressors infants are exposed to during the NICU stay. The kind of neonatal stress may also have influenced the results. In the cognitive and motor outcomes, for example, maternal separation was not associated with lower BSID-II scores,[35] whereas this was the case for skin-breaking procedures.[24] Finally, we were surprised that we did not find any studies investigating sound and light as stressors during the NICU stay in relation to long-term outcomes. A definition of neonatal stress incorporating the full extent of exposure to neonatal stressors therefore warrants urgent attention.

Strengths and Limitations

This is the first systematic review of the literature on a wide range of health and developmental outcomes, including clinical, laboratory, and imaging outcome measures associated with all kinds of neonatal stress. The strengths of the review lie in the extensive search algorithm deployed and in the full assessment of the studies by two reviewers. A limitation is that given the limited number of eligible studies, we were unable to provide a meta-analysis. Second, we only included studies in English and Dutch, which may have led to missing some studies.

Implications

Our review implies that a wide range of neonatal stressors should be taken into consideration during neonatal care and follow-up of preterm children. Follow-up programs for preterm NICU graduates should address the impact of neonatal stress and should highlight the need for structural recording of neonatal stress during the NICU stay. Physicians and NICU nursing staff alike should watch for any early signs of developmental and health-related effects of neonatal stress that may warrant early intervention aimed at improving day-to-day functioning.[51–53] Minimizing the exposure of neonates to stress during the NICU stay is therefore of the utmost importance and is achieved either through improving individualized developmental care or through initiating nonpharmacologic interventions. In animal studies, for example, the effect of the exposure to neonatal pain-related stress is buffered by maternal licking and grooming,[54,55] behavior that may also hold true for human neonates.[56]

For the purpose of future research, the definition of neonatal stress should be expanded to include stressors beyond skin-breaking procedures to determine if a composite of stressors represent the total burden of neonatal stress in preterm infants more accurately. Unraveling these various types of neonatal stress and their associations with clinical outcomes is key for improvement of neonatal care. With this review, we show that only few long-term studies exist beyond skin-breaking procedures. Studies are needed on the full extent of exposures during the NICU stay, including noise, light, handling, maternal separation, nursing procedures, and medical procedures. A broader definition of neonatal stress is also key to expanding our knowledge of the effects of neonatal stress on health and development in preterm-born infants and to further elucidating the pathways between neonatal stress and laboratory, imaging, and clinical outcomes, as was conceptualized in a recent study.[57] In such research, several outcome domains and potential mechanisms may be of interest because of their likely associations with neonatal stress. Outcome domains should also include growth and cardiovascular health,[58,59] whereas a potential mechanism may be the role of the neonatal immune system.[14]

CONCLUSIONS

Our review underscores that neonatal stress is associated with a wide range of outcomes, including clinical (neurologic development as well as emotional and behavioral development), laboratory (altered HPA axis settings and epigenetic alterations), and imaging (structural brain development), after preterm birth. Nevertheless, to date evidence is lacking regarding several other clinical outcomes, such as growth and cardiovascular health, language development, and parent-infant interaction, as well as outcomes related to potential mechanisms,

such as functioning of the neuroendocrine and immune systems and the neonatal microbiome. Pediatricians and neonatologists should try to minimize exposure to stressors during the NICU stay as much as possible. Physicians and other caregivers involved in neonatal care and follow-up should be consciously aware of the impact of neonatal stress on preterm children.

ABBREVIATIONS

BSID-II: Bayley Scales of Infant and Toddler Development, Second Edition
CBCL: Child Behavior Checklist
HPA: hypothalamic-pituitary-adrenal

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