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Common.SubmissionDetails.Abstract:	Summary Every year 15 million preterm infants are born, and most spend their first weeks in neonatal intensive care units (NICUs)[1]. Although essential for the support and survival of these infants, NICU sensory environments are dramatically different from those in which full-term infants mature, and, thus, likely impact the development of functional brain organization[2]. Yet, the integrity of sensory systems determines effective perception and behaviour[3,4]. In neonates, touch is a cornerstone of interpersonal interactions and sensory-cognitive development[5-7]. NICU treatments used to improve neurodevelopmental outcomes rely heavily on touch[8]. Yet, we understand little of how brain maturation at birth (i.e. prematurity) and quality of early-life experiences (e.g. supportive vs. painful touch) interact to shape the development of the somatosensory system[9]. Here, we identified the spatial, temporal and amplitude characteristics of cortical responses to light touch differentiating them from sham stimuli in full-term infants. We then utilized this data-driven analytical framework to show that the degree of prematurity at birth determines the extent to which brain responses to light touch (but not sham) are attenuated at the time of discharge from the hospital. Building on these results, we showed that when controlling for prematurity and analgesics, supportive experiences (e.g. breastfeeding, skin-to-skin care) are associated with stronger brain responses, whereas painful experiences (e.g. skin punctures, tube insertions) are associated with reduced brain responses to the same touch stimuli. Our results shed crucial insights into the mechanisms through which common early perinatal experiences may shape the somatosensory scaffolding of later perceptual, cognitive and social development.

The dual nature of early-life experience on somatosensory processing in the human infant brain

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Summary

Every year 15 million preterm infants are born, and most spend their first weeks in neonatal intensive care units (NICUs)[1]. Although essential for the support and survival of these infants, NICU sensory environments are dramatically different from those in which full-term infants mature, and, thus, likely impact the development of functional brain organization[2]. Yet, the integrity of sensory systems determines effective perception and behaviour[3,4]. In neonates, touch is a cornerstone of interpersonal interactions and sensory-cognitive development[5-7]. NICU treatments used to improve neurodevelopmental outcomes rely heavily on touch[8]. Yet, we understand little of how brain maturation at birth (i.e. prematurity) and quality of early-life experiences (e.g. supportive vs. painful touch) interact to shape the development of the somatosensory system[9]. Here, we identified the spatial, temporal and amplitude characteristics of cortical responses to light touch differentiating them from sham stimuli in full-term infants. We then utilized this data-driven analytical framework to show that the degree of prematurity at birth determines the extent to which brain responses to light touch (but not sham) are attenuated at the time of discharge from the hospital. Building on these results, we showed that when controlling for prematurity and analgesics, supportive experiences (e.g. breastfeeding, skin-to-skin care) are associated with stronger brain responses, whereas painful experiences (e.g. skin punctures, tube insertions) are associated with reduced brain responses to the same touch stimuli. Our results shed crucial insights into the mechanisms through which common early perinatal

experiences may shape the somatosensory scaffolding of later perceptual, cognitive and social development.

Keywords: tactile, pain, infant, preterm, sensory, development

Results and Discussion

In a large cohort of 125 preterm (24-36 weeks' gestational age, GA) and full-term infants (38-42 weeks GA) before discharge from the hospital, we recorded high-density 128-channel EEG and event-related potentials (ERPs) to calibrated light touch (see Supplemental Experimental Procedures for full exclusion criteria and Supplemental Table S1 for subject characteristics). Parents consented prior to testing using Vanderbilt IRB-approved protocols. Participant data on hospitalizations and experiences were extracted from medical and nursing records. Cumulative nociceptive exposure was measured as in seminal work in this field "in the absence of an empirical basis for assigning weights to every procedure" [10,11]. All surgical procedures involved the use of opioids, whereas non-surgical procedures involved oral sucrose administration, per unit protocols. The cumulative number of positive tactile experiences was measured in the absence of empirical evidence that one type of stimulus is more positive than another or that one duration is more optimal than another [10,11]. Only touch with the purpose of providing supportive and positive tactile experience for the infant beyond usual nursing care was recorded.

Characterization of full-term responses to light touch

We first objectively characterized full-term infants' brain responses to light touch (Figure 1a) compared to the sham stimulus (see Supplemental Experimental Procedures). Fifty-five full-term infants (median GA: 39.5 weeks; 49% female) were tested at a median of 2 post-natal days (range 1-3 days). To characterize temporal and topographic responses, we applied a normative approach wherein brain activity of full-term infants was presumed to reflect typical tactile processing. These analyses were implemented with Cartool freeware[12]. We first compared ERPs as a function of time across the entire electrode montage. We only considered as reliably "tactile" those time intervals when >10% of the electrode montage exhibited a significantly different response from that to the sham stimulus for a minimal duration of 40ms (non-parametric randomization test at each time sample of the ERP; $p<0.05$; temporal extent >40ms)[13]. This analysis identified the 184-500ms post-stimulus interval as showing differential responses to touch vs. sham in full-term infants (Figure 1b-c).

Because it cannot be assumed that this entire period reflects a singular brain process that is stable over time, we next submitted the group-averaged ERP data from full-term infants in response to touch and sham stimuli to a hierarchical topographic cluster analysis[14]. This process identified time intervals of stable ERP topography, a data-driven manner to identify the series of ERP components. This data-driven approach identified four time windows comprising distinct ERP topographies in response to touch: (1) 171-240 ms, (2) 241-340 ms, (3) 341-400 ms, and (4) 401-500 ms (Figure 1d). Using the time intervals of these components and the loci of topographic maxima (fronto-

central scalp sites F3/F4, C3/C4, an F7/F8; Figure 1e), we derived mean amplitudes at a subset of electrodes used in all subsequent analyses. This provided an objective validation of the choice of electrodes used in our (and others') prior studies of tactile processing[4,15-17], allowing the convergence of our data-driven approach with existing methodological knowledge.

Characterization of preterm responses to light touch

The above results obtained from full-term infants served as a normative framework within which to compare the brain responses to touch by preterm infants (N= 61; median GA = 31 weeks, range 24-36; 51% female; median age at time of EEG recording = 28 post-natal days, range 2-103, or 36 weeks' post-menstrual age, range 35-43). The mean amplitude values for each time interval of stable ERP topography described above were compared between full-term and preterm infants using Wilcoxon rank sum tests. Two time-windows demonstrated differences (Table 1; Figure 2), as measured using the selected subset of electrodes identified objectively above. Full-term infants' cortical responses to light touch were of higher amplitude in the 171-240ms window than those of preterm infants (0.67 μV *difference;* CI 0.45-1.09 μV; $p < 0.001$). Next, we compared the topographic distribution of the ERPs from full-term and preterm infants, to determine whether differences were due to changes in the active configuration of brain circuitry[14]. This was achieved by quantifying and analyzing global dissimilarity between the topography of ERPs, independent of their strength. Global dissimilarity equals the square root of the mean of the squared differences

between the potentials measured at each electrode (versus the average reference), each of which is first scaled to unitary strength by dividing by the instantaneous global field power (i.e. the standard deviation across the electrode montage). This measure can range from 0 to 2, with 0 meaning topographies are identical and 2 meaning that topographies are inverted. It is therefore directly converted to correlation; i.e. spatial correlation equals 1 minus the squared value of global dissimilarity divided by 2). Global dissimilarity was statistically tested here using non-parametric randomization. Effects were considered reliable for $p \leq 0.05$ and if also temporally sustained for ≥40ms.

Combining these topographical and temporal amplitude analyses, we confirmed the presence of differential responses to touch (but not sham) between the two groups as early as the P2 component (171-240 and 241-340ms) (Table 1 and Figure 2). Infants' responses to median nerve stimulation over this period have been previously linked with their neuro-developmental outcomes at 18 months [18]. Our analytical framework revealed that brain responses to touch in our cohort of preterm infants prior to discharge from the NICU not only were of significantly lower amplitude, but also differed in their topographic distribution relative to full-term infants (Figure 2a-c). No attenuation of the sham response was seen between preterm infants and no correlation was present between amplitude/response to sham and degree of prematurity, underlining the possible specificity of our results to touch.

Prematurity can contribute to these differences in somatosensory functional activity via altered post-natal experiences, interruptions in the normal sequence of brain maturation by preterm birth itself, or interactions between the two [9,12]. In animal

models, somatosensory map formation via thalamo-cortical afferent axon organization[19] is initiated at both full-term or preterm birth and appears experience independent. Studies of human infants' auditory system however show that postnatal experience does not appear to compensate for immaturity at birth during the first months of life[20]. In our current cohort of preterm infants, the amplitude of responses to touch in the 171-240 ms time window increased by 0.08 μV ($p<0.001$) for each week of GA (sham responses did not). GA and post-natal days were strongly correlated ($r^2_{(59)}= -0.757$, $p<0.001$). Therefore, we used the Akaike Information Criterion (AIC) and likelihood ratio tests to summarize overall model fits and identify independent effects[21]. A model with both GA and post-natal days explained significantly more variance in the amplitude of light touch responses than post-natal days alone (AIC 323 vs. 329; $p =0.004$). Conversely, including the number of post-natal days did not enhance the fit of the model with GA alone (AIC 323 vs. 321, $p = 0.58$). Thus, preterm infants cared for in NICUs exhibit decreased touch responses when they are discharged home compared to full-term infants, and these decreases are proportional to their degree of immaturity at birth. This finding may superficially contrast observations [22] that somatosensory responses to a non-noxious stimulus appeared equivalent between term and term-equivalent preterm infants. However, Slater et al. used stimuli ~29-145 times stronger than ours, which in all likelihood activated deep pressure receptors (Pacinian corpuscles). While processing of deep pressure as measured in central locations may be typically developed at term equivalent in preterm infants, more complex processing of light touch in frontal and central locations appears to still be attenuated.

Associations between painful and supportive experiences in the NICU and touch response in preterm infants

While the number of post-natal days was not strongly associated with amplitude of touch response, length of stay is an imprecise surrogate for multiple components of intensive care, which could potentially impact somatosensory development. In particular, painful experiences are associated with childhood problems in somatosensory function and socio-emotional development[9]. Conversely, developmental care approaches (e.g. Newborn Individualized Developmental Care and Assessment Program) often include varied tactile components purported to improve neuro-developmental outcomes[8], but rarely studied in association with quantitative changes in somatosensory processing.

Therefore, we next determined dose-response associations between both positive tactile experiences (beyond usual nursing care) as well as painful procedures and the tactile ERP amplitudes in preterm infants at the time of discharge from the NICU (related to Supplemental Experimental Procedures and Figure S1). All analyses were performed with and without the inclusion of minor surgical procedures without a change in conclusions. Supportive tactile experiences were associated with increased amplitude of cortical responses to light touch, even when controlling for GA and postnatal days ($r^2_{(110)} = 0.177$; $p < 0.001$). Additionally, we provide the first demonstration that nociceptive exposures are associated with decreased amplitude of cortical responses to light touch after controlling for variations in GA and PND ($r^2_{(110)} =$

0.153; p=0.002). In preterm infants, painful exposures may have a negative impact on typical processing of non-painful tactile stimuli. Importantly, the preterm cohort had no known conditions associated with severe illness (necrotizing enterocolitis, sepsis, severe bronchopulmonary dysplasia, abnormalities on cranial imaging). Between birth and time of EEG recording, the preterm infants had a median 32 (range 10-103) painful procedures and a median 4 (range 0-46) supportive tactile experiences, with no discernible associations between these two types of experiences. This cross-modal association between exposure to pain and attenuated light touch response supports previous findings showing that repeated exposure to painful procedures is associated with decreased responses across multiple other modalities (e.g. temperature[23]) and systems (autonomic nervous system[24]).

Sensory processing throughout infancy and early childhood enables learning from experiences, and constitutes a foundation for the construction of higher-level perceptual and cognitive representations. Among the sensory systems, the somatosensory system is the earliest to develop, with physiological responses first observable at 14 weeks of gestational age (GA) and detectable cortical responses at 24 weeks[3,4]. The somatosensory system mediates biological and social interactions with the mother[7] during early life and, thus, scaffolds the development of other sensory systems (e.g., vision, hearing). Yet, objective and quantitative metrics of the consequences of an early-life NICU experience on light touch - including those associated with medical procedures and even potentially therapeutic interventions - were previously missing.

Our collective results now extend current understanding of the development of the somatosensory system and its susceptibility to the quality of early-life experiences. The emergence of evoked brain activity alongside the disappearance of unstructured and spontaneous “neural bursts” is considered an index of the maturation of neural circuits across sensory systems[25,26]. In the case of the human somatosensory system, this shift occurs at 35-37 weeks GA[11] with discrimination of different types of somatosensory inputs (e.g. nociceptive vs. touch) perhaps maturing along a similar trajectory[11]. The case could have been made that greater amount of any somatosensory experience would have resulted in an enhanced response to somatosensory stimuli, thus compensating for immaturity at birth through amount of experience. This appeared to be the case for deep pressure processing[22]. However, in the case of complex processing of light touch, our results suggest that repeated painful experiences in early life attenuate the formation of later typical responses perhaps through cross-modal inhibition established from non-specific neuronal bursts to both light touch and to nociception. Previous work has also demonstrated that nociceptive experiences can alter the perception of multiple somatosensory modalities (pain, touch, temperature) at the site of a procedure or in other parts of the body[23]. These findings, along with observed long-term dysesthesias of preterm infants undergoing surgical procedures[23] also argue in favor of cross-modal interactions at both peripheral and central levels. Individual modalities within the somatosensory system may be differentially affected by maturation, experience, and the complexity of connections between modalities.

One limitation of this study was that controlling for opiate use was infeasible, as all infants undergoing painful procedures received some form of analgesia. Recent evidence supports conventional anesthetics and sucrose as both contributing to altered brain maturation[27-29]. Additional analyses were conducted in the preterm infants that considered cumulative total opiate exposure. In particular, we examined the robustness of the significant associations between GA and ERP amplitude of touch response (Table 2) and associations of pain and touch with ERP amplitudes, controlling for postnatal days and GA (Figure S1). Results of these analyses are consistent with pain being significantly associated with ERP response to light touch after accounting for opiate exposure. The reported association with total supportive touch exposure was unchanged when controlling for cumulative opiate exposure (see Supplemental Experimental Procedures). Together, our results support the hypothesis that exposure to painful procedures in preterm infants, even when analgesics are used to mitigate pain and when sucrose is used as a sedative to mask the behavioral expression of pain, may contribute to attenuated responses to non-noxious tactile stimuli at discharge to home.

With respect to painful procedures themselves, our study faced the same challenges as groundbreaking work in the field: we could not quantify the intensity of each painful stimulus: EEG data on each experience throughout the entire hospital stay for each infant would have been infeasible due to methodological (EEG nets are not designed for infants <30 weeks PMA) and time limitations (interrupting care to measure intensity of stimulus). Behavioral manifestations of pain would have been difficult to prospectively collect on an hourly basis, and are not always reliable in preterm infants.

Furthermore, our study was limited in its focus on the processing of pain in the brain, and did not address the subjective and emotional experience of pain. Similarly, our analysis of supportive tactile experiences was limited by feasibility considerations, such as controlling for individual variations in nursing handling and parent education; we relied instead on the consistent and extensive training - disseminating knowledge-base and following protocols -implemented by nursing leaders and the intent to provide support, rather than the behaviors elicited in response to support.

The results of our study have important clinical implications for infants cared for in NICUs and for those aiming to improve their neurodevelopmental outcomes. Current efforts aim to minimize the number and intensity of painful procedures, especially through non-pharmacological pain management[30]. Concurrently, family-centered initiatives and therapeutic interventions may remedy the relative paucity of supportive tactile experiences; a common problem in referral center NICUs, where geography, socioeconomic conditions and support systems impact parents' direct involvement with their infants[31,32]. Simultaneously, our study raises concerns with regards to subjectively inferring positive or negative experiences for infants hospitalized in the neonatal period without first examining their impact on brain processing. However, regardless of these concerns, a greater one remains: infants currently discharged to their homes from NICUs have decreased cortical processing of touch compared to their full-term counterparts. This creates an altered learning scaffold for motor, tactile and multisensory exploration of the environment and self, as well as for social-emotional interactions. Abnormal tactile processing and neurological thresholds are associated

with worse cognitive, motor and language outcomes in preterm infants[5,6]. Promoting optimal development and function in newborns hospitalized in NICUs may help establish the sensory building blocks of cognition, behavior and communication[33].

Table1: Differences in ERP amplitudes between full-term and preterm infants: Comparison of responses to touch between term and preterm infants in four time windows of significance. *Wilcoxon Rank Sum test. All results expressed as median amplitude (interquartile range).
**Increase in microvolts ERP response per 1week unit in the predictor

Table2: Associations between ERP amplitudes to touch, gestational age at birth (GA) and postnatal days (PND) *Wilcoxon Rank Sum test. All results expressed as median amplitude (interquartile range).
**Increase in microvolts ERP response per 1week unit in the predictor
Table 2 is related to Figure S1

Figure 1. Normative analysis of ERPs from full-term infants. **A**. Photos of a full-term infant undergoing EEG recording (left) and the tubing and nozzle for delivering calibrated light touch to the hand (right). **B**. Superimposed ERPs to touch and sham stimuli (black and red traces, respectively). **C**. Significant ERP differences began at 184ms post-stimulus onset (percentage of significant electrodes across time shown). **D**. Hierarchical topographic clustering identified a series of touch-related ERP components (shaded boxes); the earliest, 171-240ms, was the focus of the present analyses. **e**. 24 bilateral electrodes were at the maxima/minima of the blue ERP topography, and measures from these were used in subsequent analyses. For term patient characteristics see Table S1.

Figure 2. Impaired ERP responses to light touch in preterm infants. **A**. Group-averaged ERPs from full-term and preterm infants (black and red traces, respectively; s.e.m. shown) at a left frontal scalp site. **B**. Overlay of ERPs from the entire electrode montage. Insets show mean ERP topographies over the 171-240ms period (top view) when significant differences were observed (Table 1). **C**. The orange curve displays the spatial correlation between ERPs from full-term vs. preterm infants. The blue area displays statistically significant differences in ERP topography, indicative of differences in the active brain circuits in responses from full-term vs. preterm infants. Corresponding data and analyses in response to sham stimuli are shown in **d-f**. No statistically reliable differences were observed. For preterm patient characteristics see Table S1.

Table 1. Immaturity at birth alters amplitudes of ERP touch responses

Tactile processing mean amplitude in full-term and preterm infants in identified time intervals post-stimulus (μV; 95% confidence interval indicated)				
Stimulus	**Time (ms)**	**Full-term**	**Preterm**	**p**
Touch	171-240	0.33 (-0.09,1.16)	-0.24 (-0.60,0.26)	**<0.001***
Touch	241-340	0.75 (0.19,1.37)	-0.10 (-0.53,0.56)	**<0.001***
Touch	341-400	0.27 (-0.40,1.14)	-0.17 (-0.73,0.70)	**0.051***
Touch	401-500	0.07 (-0.71,0.92)	-0.18 (-0.93,0.62)	0.212
Sham	171-240	0.32 (-0.35,0.73)	0.12 (-0.24,0.26)	0.291
Sham	241-340	0.14 (-0.31,0.81)	0.05 (-.37,0.41)	0.319
Sham	341-400	0.09 (-0.46,0.66)	-0.11 (-0.56,0.51)	0.603
Sham	401-500	0.24 (-0.61,1.05)	-0.07 (-0.72,0.52)	0.212

Table 2. Improved tactile processing amplitude is associated with increasing gestational age at birth in preterm infants

Univariable models				
Predictor	**Stimulus**	**Slope****	**Confidence Interval**	**p**
GA	Touch	0.08	(0.05, 0.12)	**<0.001***
PND	Touch	-0.02	(0.07, 0.04)	0.524
GA	Sham	0.01	(-0.02, 0.05)	0.530 [-0.684]
PND	Sham	0.01	(0.04, 0.06)	0.684
Multivariable model for tactile stimulus				
	Value	**Standard Error**	**t-value**	**p**
Intercept	-3.19	1.31	-2.43	0.02
GA	0.10	0.03	2.85	0.01
PND	0.02	0.04	0.54	0.59

Author Contributions: NLM identified the link between sensory dysfunction and prematurity, directed intensive care research methods, performed preliminary analyses and proposed connections between tactile processing and nociceptive and positive experiences, was the primary author of the text and created all tables. AK directed ERP acquisition and pre-processing and ensured paradigm integrity. OC directed all intensive care protocols, data acquisition and tested all subjects. JCS conceived and performed all biostatistics data analyses. MMM directed all ERP post-processing and analyses, interpreted the results, generated all figures, and was involved in all stages of the writing and editing of the text. PJM interpreted the results, and was involved in all stages of the writing and editing of the text, as well as in editing the figures MTW was involved in the early conceptualization of the study and provided input during the writing and editing of the manuscript.

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Figure1

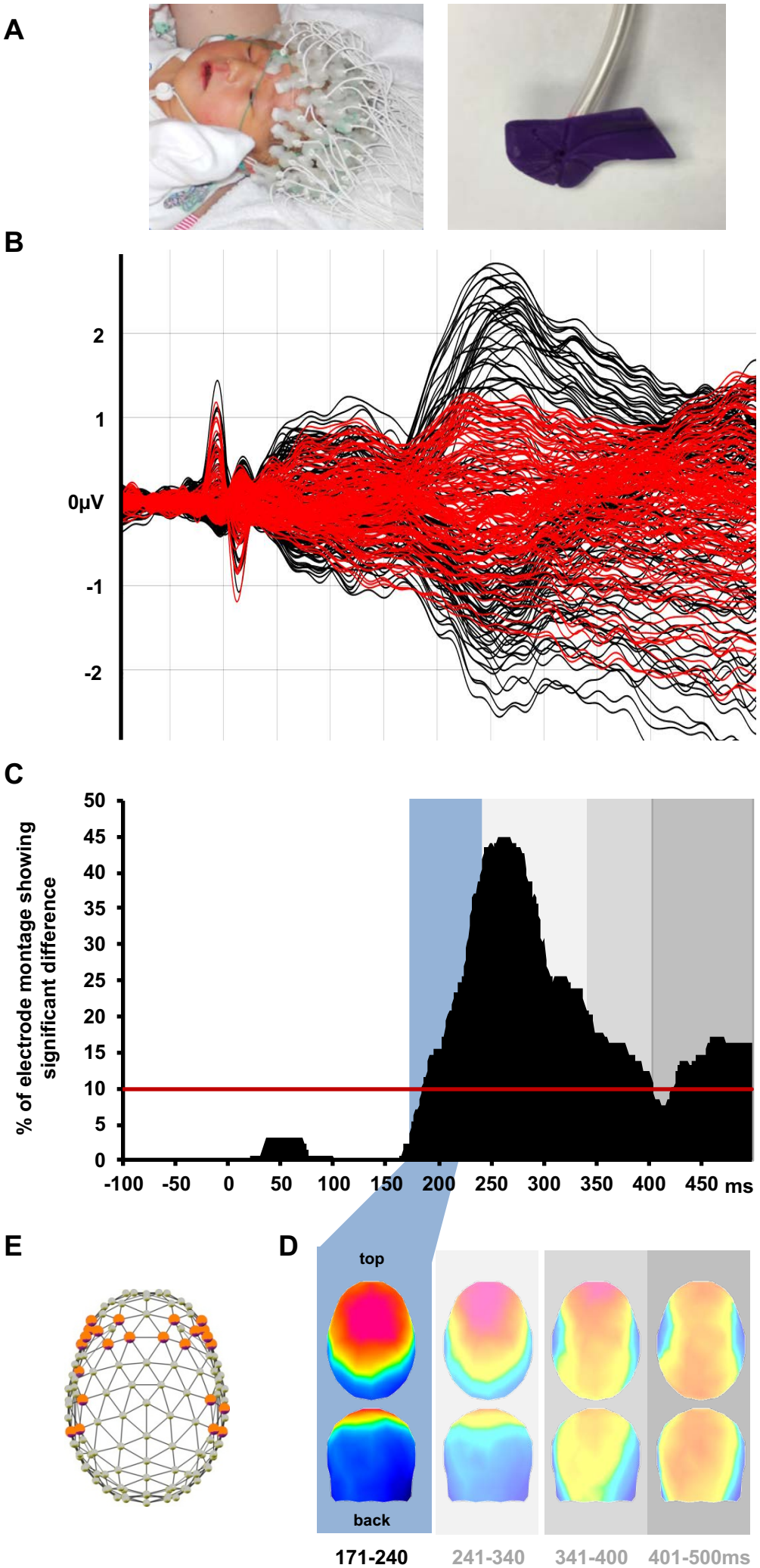
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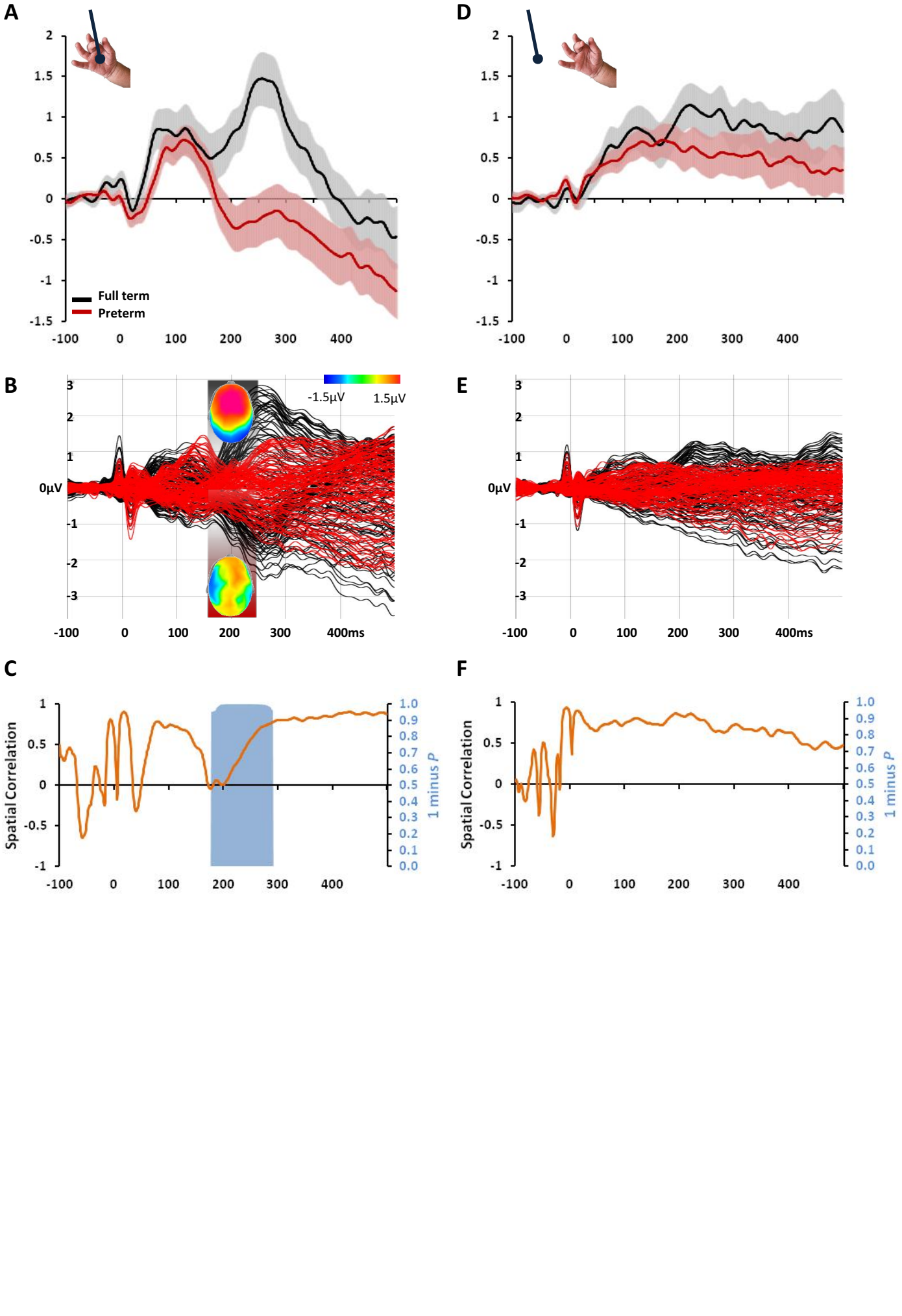
Figure2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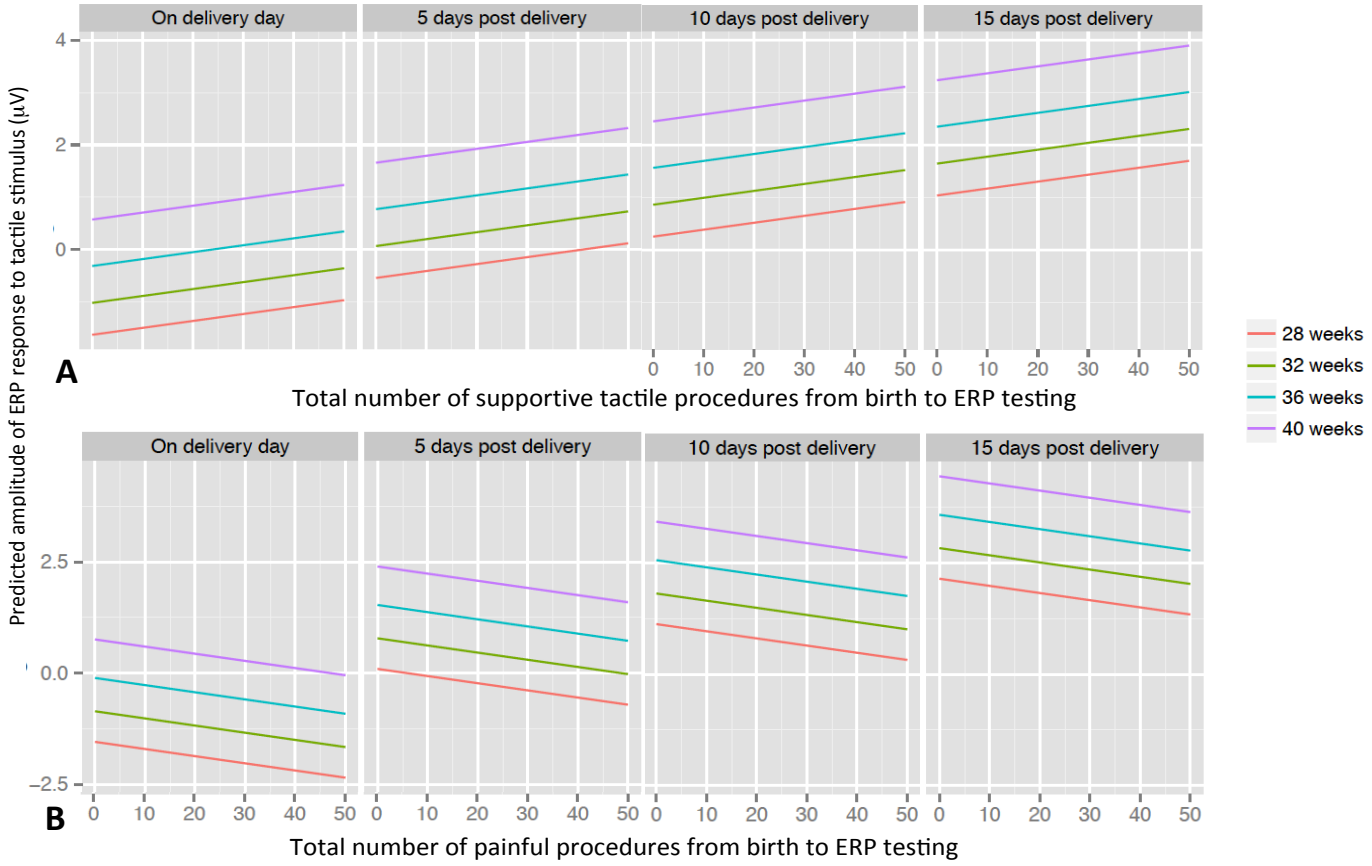

Figure S1: Associations between ERP responses to touch and gestation age, post-natal days, and supportive or painful procedures

Figure S1 is related to Table 2 and section Associations between painful and supportive experiences in the NICU and touch response in preterm infants, of the main document.
Modelling associations between GA, post-natal days and supportive (**A**) or painful (**B**) exposures in preterm infants on the amplitude of light touch responses over the 171-240ms window. Mathematical models based on the cohort's data illustrate that as GA and post-natal days increase, so does amplitude of the tactile response. At comparable GA and post-natal days, increased exposure to supportive tactile experiences (X-axis) results in increased mean amplitudes. For painful procedures, modelling shows an inverse relationship, with decreased mean amplitudes in the light touch response at comparable GA and post-natal days.

Table S1: Subject Characteristics. Related to Figure 1.

	Term (n = 59)	Pre-term (n = 57)
GA at birth (median, IQR)	39 (39; 40)	31 (30; 33)
Sex, N (%) female	28 (24.1)	27 (23.3)
Race:		
Black, N	8 (0)*	17 (4)*
White, N	43 (5)*	38 (7)*
Other, N	3	0
Race/Ethnicity unknown	2	5
Supportive procedures:		
Skin-to-Skin care (median, IQR)	N/A	1 (0; 5)
PT/OT massage	N/A	0 (0; 2)
Painful procedures:		
Surgical procedures(median, IQR)	0	1 (0;1)
Skin breaks(median, IQR)	0	27 (19; 37)
Tube insertions (median, IQR)	0	3 (1; 4)
Total painful procedures (median, IQR)	0	32 (21; 41)

GA: gestation age
*Hispanic/Latino ethnicity
IQR: Interquartile range, 25thand 75th percentile
PT: Physical Therapy
OT: Occupational Therapy

Supplemental Experimental Procedures

Touch Experience Data Collection

Painful procedures: Each attempt at a procedure was included. Thus, the total sum reflected all procedures, regardless of pain intensity[S1]. Types of nociceptive occurrences were defined as in published work by neonatal pain specialists. Numbers of heel lances, intravenous or central line insertions, intramuscular injections, endotracheal or chest tube insertions, gastrostomy tube insertions, tape removals, as well as nasogastric tube insertions and surgical procedures were quantified.

Positive tactile experiences: Included were breastfeeding, skin-to-skin care, massage, physical or occupational therapy sessions or parental holding. In the NICU, parent touch started at birth with expert education from nurses on providing supportive touch (containment, pressure, skin-to-skin) while hands-on therapist involvement started at 32-33 weeks PMA.

Exclusion Criteria for study population

All pre-term infants were cared for at the Vanderbilt University Medical Center between 05/01/2013 and 05/30/2014. We excluded any full-term infants with documented circumcision, maternal opiate use within 48 hours of testing time or concerns for intrauterine drug exposures from the medical team, any preterm infants receiving opiates or sedatives within 48 hours of testing time or those with any antiepileptic use since birth. We also excluded any infants with lethal congenital abnormalities or severe abnormalities on any cranial imaging (cerebellar hemorrhage, intraventricular hemorrhage grade III or IV, periventricular leukomalacia, ischemia or stroke) or infants who had failed their auditory brainstem response testing performed at 34 weeks' postmenstrual age (PMA). No infant required treatment with either dexamethasone or nitric oxide in this cohort. Therefore, these were not further considered.

Light Touch and Sham Stimuli

Published studies of somatosensory function in preterm infants and neonates have often used direct electrical stimulation of the median nerve, providing invaluable data on large nerve conduction and maturation[S2-4]. Additionally, these studies focused on the latency of the N1 component; a cortical somatosensory response whose latency decreases with PMA. In contrast, the goal of the current study was to objectively measure a clinically relevant somatosensory stimulus, the light touch experienced by infants during routine NICU care. This meant calibrating the stimulus for activation of Meissner's corpuscles at the lowest possible threshold (estimated at 0.13 gm/mm2 or 4.5 psi[S5]), having a rigorously consistent stimulus throughout 60 trials, using a relevant sham control stimulus and a protocol that review boards for the protection of human subjects would consider minimal to no risk, for generalizability purposes.

Therefore, tactile stimulation approximating light touch was delivered by means of air puffs emanating from a nozzle positioned 5 mm above the skin of the palmar surface of the right hand using a mold holder (Figure 1). During each trial, a touch or a sham was randomly generated. The puff delivered a consistent calibrated pressure of 4.5 psi over a 3 mm^2 area as measured in our previous studies[S6]. This is equivalent to the force exerted by the smallest monofilament used to evaluate loss of light touch sensation in patients with neuropathies. The sham stimulus was the identical puff delivered with a nozzle pointed away from the infant hand, to account for any concurrent auditory stimulation from the sound of the puff. Over a 5-minute trial, 60 sham and 60 touch stimuli were delivered at random inter-trial intervals, with a minimum of 2500 ms between puffs and no greater than 2 consecutive touches, to prevent habituation.

EEG Acquisition and Pre-processing

Continuous EEG data were acquired at 1000Hz using published protocols as near to discharge as possible in preterm infants and 1-3 days after birth for full-term infants. Briefly, a high-density array net of 128 electrodes embedded in soft sponges (Geodesic Sensor Net, EGI, Inc., Eugene, OR) recorded the EEG using NetStation software (v. 4.3; EGI, Inc., Eugene, OR). All infants were tested in his/her patient room while lying on their backs in the bassinet/crib or being held in the supine position by a caregiver. No restraint was used, and infants were tested in quiet alert to drowsy states. ERP data were pre-processed according to published protocols using NetStation algorithm. An infant was deemed to have analyzable ERP data whenever there were more than 10 usable trials per condition, with every usable trial also having more than 108/128 electrodes with artifact-free signals. In the present study ERP, data from 4 full-term and 5 preterm infants were excluded.

Analysis of associations between ERP amplitude and GA, post-natal days, and total pain or touch

Generalized least squares methodology[S7] was used to estimate the association between ERP mean amplitude to touch with GA, post-natal days and total pain or touch events. GA and post-natal days were modeled using restricted cubic splines (3 d.f.) to allow for a non-linear association with ERPs. In models where multiple observations were taken on the same subject, we used an unstructured covariance to account for any within-subject correlation. Sensitivity analyses confirmed the unstructured model provided a much better penalized fit compared to other covariance models. Likelihood ratio tests were used to calculate p-values.

Controlling for opiate usage:

Infants in the preterm cohort were occasionally exposed to fentanyl as the analgesic of choice, but never within 48 hrs preceding EEG testing. Because this was a cohort of predominantly healthy infants (see exclusion criteria) 32 of the 61 infants had no exposure to analgesics, and the median cumulative analgesic exposure for the preterm group was 0 mcg/kg (IQR 0-1.2 and range 0-15.5). Additional analyses were conducted in the preterm infants that considered cumulative total analgesic exposure. In particular, we examined the robustness of the significant results presented in Table 1b (associations between GA and ERP amplitude of touch response) and Figure 3 (associations of pain and touch with ERP amplitudes, controlling for postnatal days and GA).

For the univariable model we reported a significant association between EGA and amplitude of ERP touch response (slope=0.08, $p<0.001$) unadjusted for other covariates. When we adjusted for total analgesic exposure in this model, the slope was still 0.08 with $p=0.003$, which is consistent with a negligible confounding effect of total analgesic exposure on the association. Therefore, exposure to analgesics did not change the finding that immaturity at birth results in attenuated response to touch at discharge to home.

Not surprisingly, there was a significant correlation between cumulative analgesic exposure and total number of painful procedures ($r=0.37$; $p=0.003$) but no evidence of a correlation between cumulative analgesic exposure and total supportive touch exposure ($r=0.02$, $p=.87$). We were thus primarily concerned with the robustness of the results presented for the association of pain with ERP when controlling for postnatal days and GA. We first created a similar set of models with cumulative analgesic exposure on the x-axis; when controlling for GA and postnatal days, there was a negative association (slope=-0.016, $p=0.01$) with ERP amplitude of touch response.

This association was slightly smaller but in the same direction as the one between total painful procedures and ERP amplitude of touch response (slope=-0.018, p=0.02) between total painful procedures. Next, we fit a multivariable model that included both cumulative analgesic exposure total painful procedures, controlling for EGA and postnatal days as before. In this model, the analgesic slope was attenuated and no longer significant (new slope=-0.010, p=0.11) while the painful procedure slope, while attenuated, remained significant (new slope=-0.014, p=0.05). These results are consistent with pain being significantly associated with ERP response to light touch after accounting for analgesic exposure. The reported association with total supportive touch exposure was unchanged when controlling for cumulative analgesic exposure.

Together, our results support the hypothesis that exposure to painful procedures in preterm infants, even when analgesics are used to mitigate pain and when sucrose is used as a sedative to mask the behavioral expression of pain, may contribute to attenuated responses to non-noxious tactile stimuli at discharge to home.

Supplemental References

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