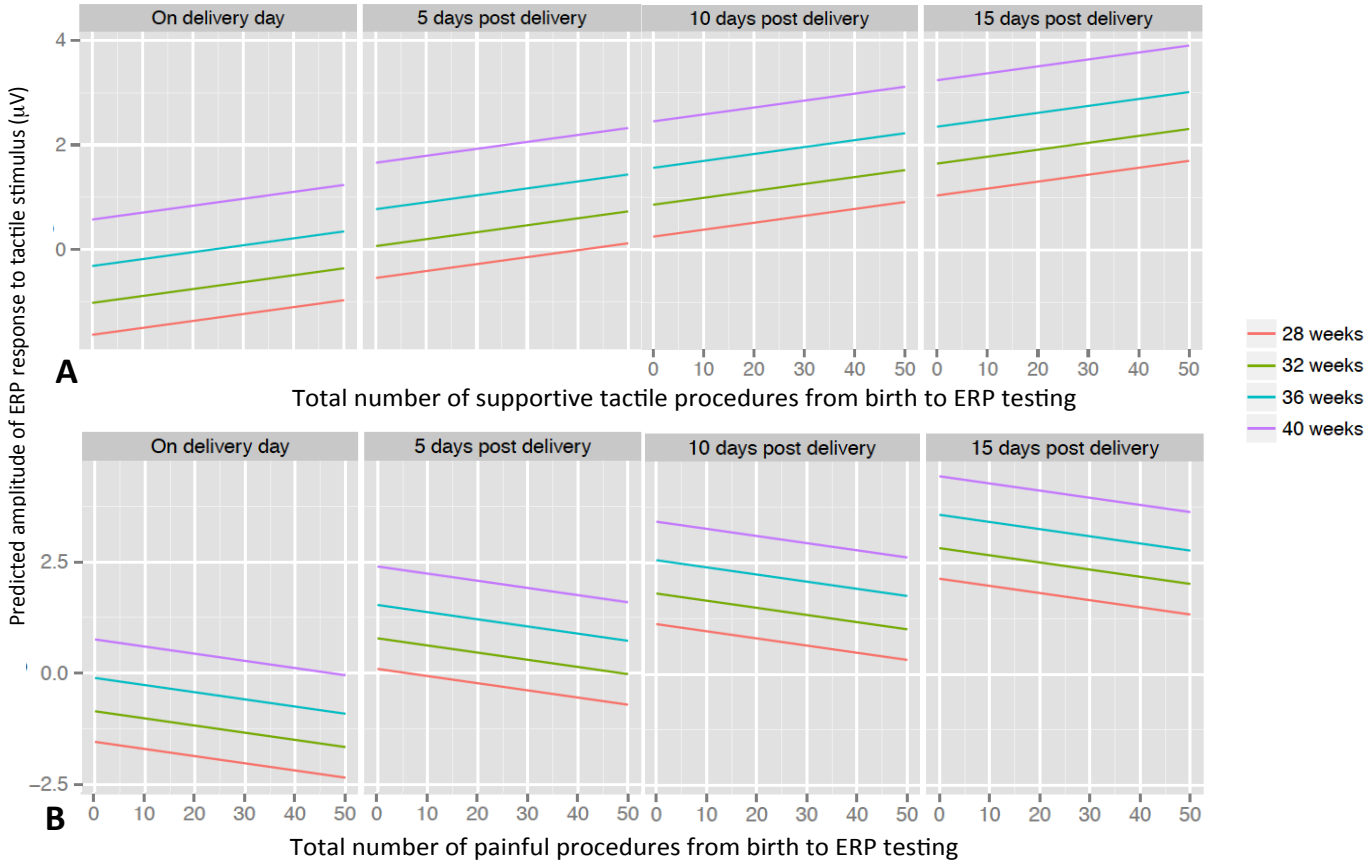

Figure S1: Associations between ERP responses to touch and gestation age, post-natal days, and supportive or painful procedures

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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