The Representation of Nociception and Pain in the Developing Brain

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ABSTRACT

Pain is a fundamental human experience, but how does it begin? Noxious stimuli elicit strong behavioural and physiological responses, even in the youngest newborns, reflecting early subcortical engagement, but the experience of pain requires higher brain processing. This review summarizes current knowledge on how pain is represented in the newborn brain after tissue injury. It explores the nature of nociceptive information reaching the infant brain, how immature networks process it, and how biological and external factors influence this process. We outline current methods for recording infant brain activity during clinical tissue-damaging procedures, review collected data and address common misconceptions in the field. We also discuss how the sensory, emotional, and cognitive brain systems involved in pain mature at different rates and propose a model of pain representation that evolves as neural networks develop and the infant learns from their environment.

Keywords

pain connectome, neonatal pain, nERP, microstates, cortical pain activity

INTRODUCTION

Pain is a fundamental part of human experience. Although the evolutionary importance of pain in preserving life explains its unique salience, in many situations repeated or persistent pain causes suffering that demands relief. But how do we learn what pain feels like and what it means? And how does the newborn infant nervous system process pain for the first time?

Throughout history, the pains of infancy and childhood have been acknowledged, if not understood. 'As soon as man is born into this world, then he is made subject to endure pains' wrote the physician Felix Wurtz in 1656 (1), but the question we address here is how the experience of pain is established in newborn infants. The current drive to measure infant pain and pursue a rational approach to infant pain relief emerged in the 1980's, triggered by advances in neonatal intensive care and associated increases in medical interventions. An earlier, adult centric approach of defining newborn infant pain in terms of whether infants feel more, less or the same pain as adults has been superseded by a developmental neuroscientific approach which recognises the functional importance of each stage of development. Now, the focus of infant pain research is upon measuring immature sensory physiology and behaviour, mapping developing connections within the brain and understanding the complex interaction between the immature nervous system and the environment (2–5). We now know that the relationship between injury and pain is not fixed from birth, but depends on the state of maturity of the central nervous system (4). The first tissue damaging events in an infant's life are arguably triggers or 'single-shot learning' events prompting synaptic reconfiguration in central circuits subserving pain experience (6). This is followed by a lifetime of biological, psychological, and social factors and it is through these broader life experiences that individuals learn the concept of pain and its applications (7).

The subjectivity of pain makes it difficult to measure, and, when possible, is typically evaluated through self-report both in adults and children (8). However, this is not an option in preverbal infants, meaning pain cannot be directly measured or quantified – only inferred through indirect means. The concept of analgesia is similarly limited to the reduction of these proxy signs. As a result, most pain management procedures focus on minimizing what can be observed and measured. This creates a circular challenge, where the validity of a pain measure is judged by its responsiveness to an intervention presumed to be analgesic, and the effectiveness of the intervention is confirmed by a change in that same measure. Given these limitations, one of the few viable approaches to studying pain in non-verbal populations is to examine responses to tissue injury – a defined and clinically necessary noxious stimulus. In neonates, this involves assessing physiological and neural responses to tissue-breaking procedures. While this inherently does not capture the full subjective experience of pain, it provides a standardized and ethically justifiable model for probing pain-related processes in the absence of self-report.

This review aims to summarise our current knowledge of the representation of nociception and pain in the newborn infant brain following tissue injury. The emphasis is on pain in preterm and full-term human infants, but data is drawn from laboratory animals for mechanistic insight and interpretation. We ask what tissue injury related nociceptive information reaches the infant brain, how this nociceptive information is accessed and processed by immature brain networks, and how it is affected by biological and external factors. We explain the methods that are currently used to record human infant brain activity during clinically required tissue breaking procedures

and review the data collected with these methods while addressing some misconceptions in the field. Finally, we propose a model of pain representation in the developing human brain that changes rapidly as neural networks mature and as the infant adapts and learns from the adult world.

THE MATURATION OF NOCICEPTIVE INFORMATION TRANSMITTED TO THE INFANT CORTEX

The brain is essential for the experience of pain because it is where sensory signals are interpreted and given meaning. When the body encounters a harmful stimulus, the afferent signal travels through the spinal cord and the brainstem to the brain. However, the actual feeling of pain does not occur until this signal is processed in the cortex. Without the brain's involvement, these signals would remain just data – only the brain integrates them with other factors, such as expectation, mood and attention to transforms them into the sensation we recognize as pain (9). Noxious, tissue damaging stimulation evokes strong behavioural and physiological reactions in the youngest infant (10–12). These are commonly used as composite measures of infant pain in clinical settings, but they are mediated subcortically and do not necessarily reflect representation of pain in the brain. Nevertheless this 'pain behaviour' provides important information about the functional development of peripheral nociceptor connections to the spinal cord and brainstem and their ability to recruit motor, autonomic, and endocrine systems in the newborn (3). These signals, if they are transmitted centrally to the thalamus and cortex, will be critical factor in shaping the early representation of pain in infants.

Reflex behaviours evoked by tissue damage in preterm and term infants can be measured using electromyography and video analysis. These behaviours include not only defined limb reflexes but also body movements (e.g., startling), facial expressions, vocalizations (e.g., screaming, crying), and disruptions in ongoing behaviours (e.g., irritability, restlessness, sleep disturbances) (10–16). Together with laboratory-based electrophysiological analysis of developing nociceptive pathways in infant rodent models, they indicate that the pattern of centrally transmitted nociceptive information is very different in neonates and adults. This difference is evident in terms of selectivity, magnitude, spatial coding and patterns of sensitization (3, 17, 18) (Figure 1).

Selective Information

Tissue damage is signalled by unmyelinated C fibre and thinly myelinated Aδ fibre nociceptors. The activity of these two nociceptor groups in adult human subjects can be distinguished by verbal descriptors (19) which are correlated with distinct patterns of cortical activity (20). Importantly, the nociceptive information they provide is distinct and different from innocuous tactile information. A sense of touch, mediated largely by Aβ myelinated mechanoreceptors, does not normally overlap with a sense of pain. Nociceptors develop as a distinct physiological group, separate from mechanoreceptive tactile afferents, before birth. However, the central tactile and nociceptive sensory circuits in the spinal cord dorsal horn and brainstem are much slower to develop. Indeed, detailed behavioural and electrophysiological studies in newborn laboratory rodents and preterm human infants reveal a clear overlap between tactile and nociceptive spinal processing (3, 21, 22). Newborn rat pups are exceptionally sensitive to touching the skin and neurons in the newborn dorsal horn

are dominated by widespread termination of A-fibre tactile afferents, underpinned by immature glycinergic inhibition, such that neurons 'wind-up' to repeated tactile stimulation (3). In the very youngest preterm infants, tactile stimulation evokes limb withdrawal electromyographic (EMG) activity that is indistinguishable from that evoked from noxious stimulation (4, 15). Even at term age and into the first postnatal year, tactile stimulation causes reflex withdrawal of the limb, albeit significantly smaller than that elicited by noxious stimulation (10, 23). Facial reactions to identical noxious stimuli vary between individuals and, as a result, do not show consistent average changes following venipuncture (2, 4, 24). Tissue damage also changes blood pressure, heart rate and heart rate variability, oxygen saturation, and breathing rate in infants (25, 26) which are largely mediated by the sympathetic adrenal medullary system (27). However, similar responses occur in response to other stimuli which are not noxious such as background noises (28, 29), handling (30), bathing (31, 32) and unswaddled weighing (33). Another critical part of the physiological reaction to a tissue-breaking procedures is the hormonal response, such as cortisol changes which represent the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol levels, measured through salivary samples, have been reported to increase after a tissue-break (34), but the data are extremely variable. In fact, changes in cortisol levels might not be associated with tissue-damaging events themselves especially in very preterm infants (35), but rather reflect the level of background stress due to the environment (36).

Thus, in newborn infants, local and centrally transmitted information is not always tightly coupled to the intensity or modality of the stimulus.

Proportionate Information

The lack of selectivity does not detract from the fact that both preterm and term infants can mount a robust and long-duration flexion reflex (>4 seconds) to a single noxious skin lance (10). The younger the infant, the more exaggerated and chaotic the response, which is not restricted to the affected limb and involves also the contralateral limbs (10, 23). Injury-induced behaviour in humans is paralleled in newborn laboratory rodent pups, where noxious and injury-evoked reflexes are also exaggerated and poorly directed (37, 38).This apparently disproportionate response arises from immature inhibitory control systems both within the spinal cord (39) and descending from the brainstem (40, 41). In adults, the responses of dorsal horn neurons to peripheral noxious stimuli are dependent not only upon nociceptive input to the spinal cord and local circuit modulation but also upon powerful descending control from supraspinal centres (42, 43). The importance of this control for spinal nociceptive function cannot be overemphasized, being the route by which all aspects of brain function, from attention to anticipation, emotion to expectation, can influence pain perception. Studies in rodents have demonstrated that these descending controls, from brain to spinal cord, mature much later than pathways ascending from spinal cord to brain (3, 40, 41). Thus, centrally transmitted noxious information in the newborn is exaggerated compared to adults, and this is linked to lack of descending modulatory control from the brain.

Spatial Information

Important too is the relatively slow maturation of spatial discrimination of a stimulus on the body surface, as a result of immature dorsal horn inhibitory circuits. Infant rodent dorsal horn cells, have large, overlapping receptive fields and lack of noxious evoked

contralateral inhibition, coinciding with poor spatial discrimination (21, 44, 45). This is reflected in the widespread effect of tactile and noxious stimulation on newborn infant, such that stimulation of the heel will frequently evoke whole body movements (10, 15). In preterm human infants at 28 weeks, a withdrawal reflex from the whole limb can be evoked by stimulation as far up as the top of the thigh and buttock, with the same stimulus intensity. After 30 weeks of age, a sensitivity gradient emerges, decreasing progressively from the sole of the foot towards the knee (23). Thus, centrally transmitted noxious information in the newborn provides poor spatial discrimination with respect to the location of the stimulus on the body surface.

Sensitization

Human infants display behavioural signs of sensitization for variable amounts of time following repeated heel lancing or referred visceral conditions (46–48). Furthermore, healthy newborns, when exposed to repeated heel lances in the first 24 to 36 hours of life display greater behavioural reactions to venipuncture in another body area (49). Importantly, the degree of central sensitization is related to the number of preceding heel lances and is not prevented by commonly used soothers. Sensitization is also observed in rodent dorsal horn cells which display clear changes following tissue injury, depending upon the type of injury and age. In rodent pups, local tissue inflammation rapidly increases spontaneous activity and the response to noxious stimuli, consistent with behavioural hypersensitivity (45). Local skin incision, known to cause behavioural nociceptive hypersensitivity in young rats, is accompanied by increases in dorsal horn cell receptive field area, spontaneous firing, evoked spike activity (50, 51) and alterations in synaptic connections (52). Thus, centrally transmitted noxious information in the newborn can be sensitized, that is increased in amplitude and duration and reduced in activation threshold, following repeated tissue injury.

Together these data provide a picture of the information transmitted from the newborn spinal cord to higher centres following tissue damage: stimulus specificity, localization and intensity are not tightly coded, but repeated injury causes clear sensitization.

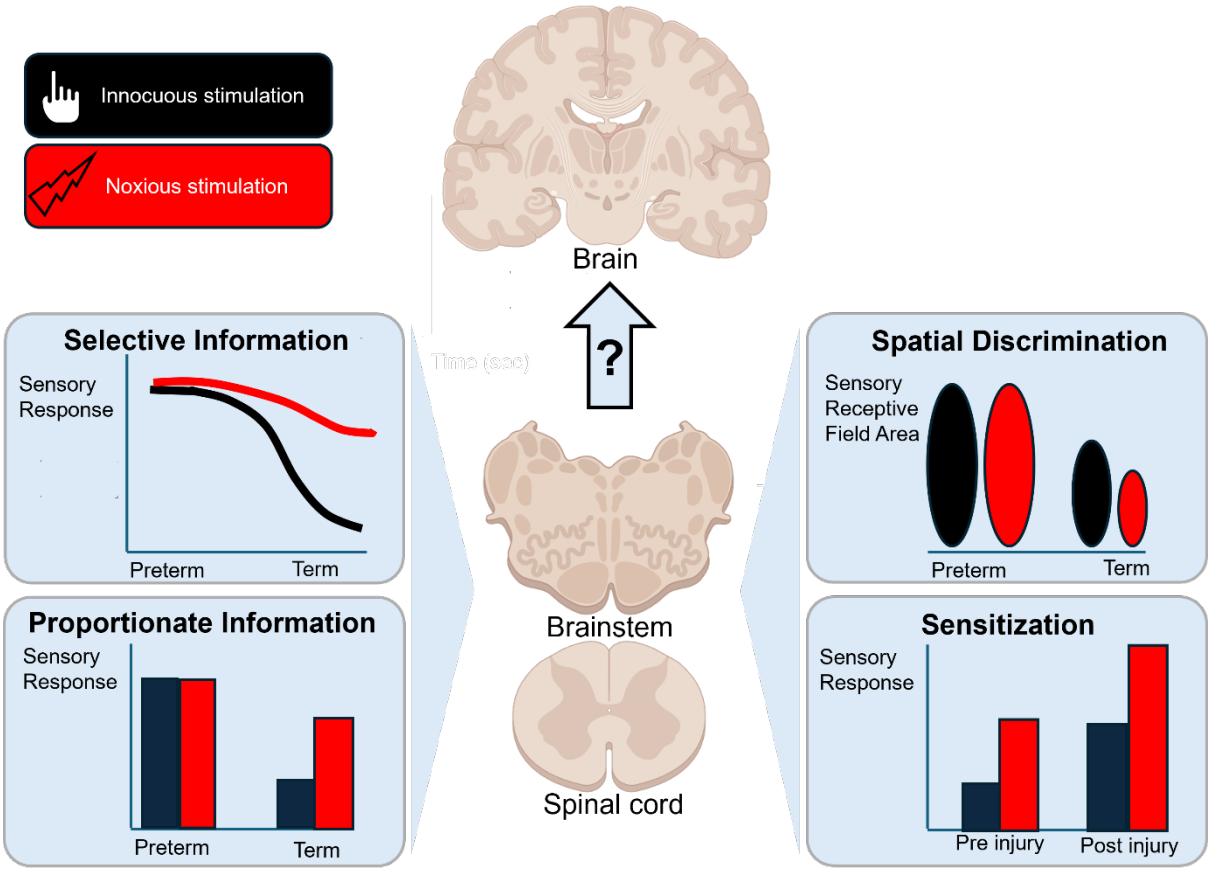

Figure 1: Summary diagram showing that innocuous touch (black) or noxious stimulation (red) stimulation of the skin evoke strong reactions in newborn rodent and human infants which change both qualitatively and quantitatively with maturity, from preterm to term equivalent age. These reactions, measured using observational and neurophysiological recordings of reflex limb movements, facial expressions and other motor outputs, are generated subcortically by circuits in the spinal cord and brainstem. Together they provide a picture of the changing nature of the information transmitted from subcortical centres to the brain following tissue damage. Top left: Ability to display selective responses to innocuous and noxious stimulation increases with age. Data from References (10, 15, 37, 53). Bottom left: Ability of response amplitudes to reflect stimulus intensity emerges with age. Data from References (15, 23, 54). Top right: Ability to localize stimuli on the body surface increases with age, as sensory neuron receptive field size declines. Data from References (21, 23, 44, 45). Bottom right: In contrast to the slow maturation of stimulus coding shown in the first three plots, robust injury-induced sensitization to both innocuous and noxious stimulation is present from early in development. Data from References (46, 47, 50, 51).

THE MATURATION OF BRAIN INFRASTRUCTURES

While pain experience requires the brain, there is no single identifiable brain area that is responsible for it. Imaging studies in healthy adult participants suggest that the experience of pain arises from highly distributed activity patterns, including both serial and parallel processes (55). Neural activity and areas of the brain associated with painfulness and intensity/saliency are spatially distributed across the brain (56, 57) and multiple cortical and subcortical systems are needed to decode pain intensity and representation of pain experience (58).

Thalamocortical Projections

The thalamus acts as a central hub for sensory input from the spinal cord, relaying information to the cortex. In humans, histological mapping and diffusion tensor imaging (DTI)-based tractography reveals that the early projections outgrowth from the thalamus towards the cortex is followed by a "waiting" phase in the cortical subplate (14–22 weeks), two weeks earlier in the somatosensory cortex than in the visual and other cortices, and then by final ingrowth into the cortical plate (23–24 weeks) (59). In mice, synaptic connections between thalamic afferents and early-generated, largely transient, subplate neurons are formed during this waiting period (60). While these synapses are unlikely to provide a basis for sensory experience as they are not yet part of the cortex, they may play an instructional role in forming a functional template for developing thalamocortical networks and cortical architecture (61, 62).

The Developing Cerebral Cortex

Benchmarking developing human brain morphology derived from a metanalysis of over 100000 MR images, reveals a striking increase in grey matter and white matter volume from mid-gestation through to early childhood (63). The formation of the cortex is a relatively protracted process across gestation. The migration of neural progenitor cells from the ventricular and outer- subventricular zones along a scaffolding of radial glial cells is largely complete by 30 weeks gestation, although continues up to 2 years of age in some areas (64, 65). It is maximal at around 23 weeks in the parietal lobes where the primary somatosensory cortex is located (66). Lamination, a marker of cortical organisation is present in the primary sensory and motor cortices at 25 weeks of gestation, with the full adult complement of distinct lamina by 32 weeks (67). High-resolution in utero diffuse tensor imaging (DTI) has mapped the increasing innervation of the cortical plate by thalamocortical axons, increasing soma volume and dendritic branching of neurons and synaptogenesis over 26–31.5 weeks (68). These studies suggest that preterm infants have formed early thalamic connections and a simple cortical structure by 26 weeks gestation, ready to receive sensory information transmitted from the spinal cord and brainstem.

The Developing Connectome and Resting State Networks

The Developing Human Connectome Project has provided a large sample of neonatal functional MRI data with high temporal and spatial resolution. This enabled the mapping of intrinsic functional connectivity between spatially distributed brain regions in the developing human brain. At rest, the brain can be organized into patterns of temporally coordinated activity, known as resting-state networks (RSNs). These networks represent distributed patterns of synchronized hemodynamic activity that occur when the brain is not engaged in a specific task, reflecting its intrinsic functional organization. These networks are thought to support fundamental cognitive and sensory functions and include well-characterized systems such as the default mode network, sensorimotor network, and visual network (69). RSNs begin as fragmented patterns in the early equivalent of the third trimester of gestation, typically confined to regions within a single hemisphere (70, 71). Over time, long-range interhemispheric and anterior–posterior connectivity patterns emerge. By term age, RSNs associated with primary networks—such as medial and lateral motor, somatosensory, auditory, and visual systems—exhibit an adult-like, bilateral spatial distribution, the connectivity of which is particularly strong between homologous regions. In contrast, RSNs related to association networks remain underdeveloped (72), reflecting the brain's progression from primary to higher-order functional

organization. More recently, it has become clear that RSNs are not static; rather, they exhibit dynamic temporal properties that can be described in terms of six recurring "brain states". Three of these reflect global whole-brain synchronization, while the remaining three are more regionally localized: one in the occipital cortex, one in the sensorimotor cortex, and one in the frontoparietal cortex (73). The fundamental properties of the functional connectome are already present even in the foetus at 21-40 weeks gestation, suggesting the presence of both primary motor and sensory networks as well as the first signs of higher order networks that are pruned in later life (74, 75).

In rodents the first activity in the somatosensory cortex evoked by tactile stimulation appears in the first postnatal week (76) but in human infants the exact timing is less clear. From the time of the first arrival of tactile information to the somatosensory cortex, there is a rapid maturation until birth and beyond. Hemodynamic and electrophysiological responses to a somatosensory stimulus can be recorded from the beginning of the third gestational trimester. Cortical hemodynamic responses are initially confined to the contralateral primary somatomotor cortex, which already exhibits its characteristic somatotopic organization (77). Over the course of the third gestational trimester, these responses show decreased latency and increased amplitude, accompanied by progressive integration of the ipsilateral hemisphere and sensorimotor associative areas (78). These changes are paralleled by changes in the electrophysiological responses to mechanical stimuli from a single high amplitude wave (delta brush), to more mature event-related potentials which increase in complexity over the late preterm and term period, reflecting the progressive involvement of first primary and then associative cortical areas (53, 79).

MEASURING THE CORTICAL REPRESENTATION OF NOCICEPTION IN THE INFANT BRAIN

In the last section, we discussed the development of the brain and its readiness to receive sensory information from the body. To understand the development of cortical pain representation, we need direct, real-time measures of cortical activity evoked by tissue damaging stimulation in preterm and full-term infants. These measures must be available at the cot side during clinically required procedures, be acceptable to caregivers and parents, and cause minimal discomfort to the infants themselves (80). Below, we describe the most used methods for this purpose. Each method captures different aspects of brain activity and relies on distinct analytical techniques, which we briefly explain.

EEG: Event-Related Potentials

Event-Related Potentials (ERPs) are measured using electroencephalography (EEG) to assess cortical activity in response to stimuli. They are small voltage deflections in the brain's electrical activity that are time- and phase-locked to an event, such as a sensory, cognitive, or motor stimulus. Normally ERPs are obtained by averaging multiple EEG epochs over repeated trials to filter out background noise (which can be environmental or physiological) and highlight stimulus-specific responses. Different ERP deflections are meant to provide insights into the timing and nature of neural processing in response to the stimulus (81). Traditionally, these deflections are recorded at a single channel and named according to their direction (N for negative and P for positive deflections), their order of occurrence (e.g. P1 is the first positive

deflection) or their latency from stimulus onset (e.g. P100 is a positive deflection 100 ms after the stimulus). The fundamental measures of an ERP include: (i) *peak amplitude* – the maximum voltage change (in microvolts, μV), which supposedly reflects the strength of neural activation, and (ii) *peak latency* – the time delay (in milliseconds, ms) between stimulus onset and the peak of an ERP component. These measures are generally robust and reliable when multiple trials per subject are available under the same stimulus conditions. However, in studies of pain representation in the infant brain, data collection is often limited to a single event (e.g. a clinically required heel lance) per subject.

To address this limitation, most research in this field has relied on *Principal Component Analysis (PCA)* to characterize the nociceptive response. Rather than focusing on a single peak, PCA considers the entire waveform shape, offering an analysis more resilient to noise at a single time point. PCA decomposes the ERP into fundamental waveforms, known as *principal components (PCs)*, which capture systematic variations in signal amplitude across time points within a cluster of electrodes or trials. Each individual EEG trace is then assigned a *weight* for each PC, representing the degree to which the trace resembles that component. The larger the weight, the more similar the individual ERP is to the given PC. Event-related Potentials have been used to investigate the ability of the neonatal brain to discriminate noxious from innocuous stimulation, the dependency of nociceptive cortical representation on brain maturation and the influence of biological and environmental factors on infant pain processing as described in the next section.

EEG: Time-Frequency Analysis

While ERPs provide information about neural activity that is time- and phase-locked to a stimulus, they miss non-phase-locked activity that may also be relevant to sensory and cognitive processing (82). Averaging the signal in the time-frequency domain addresses this limitation by decomposing it into different frequency bands, capturing both types of activity over time. Techniques such as wavelet transforms and short-time Fourier transforms break down the signal into power across frequencies at each time point, revealing transient neural oscillations that might be missed in traditional ERP analysis. This is important because different frequency bands are thought to encode distinct types of neural processes – for example, higher frequencies (gamma) are often associated with feedforward information routing, while lower frequencies (alpha/beta) may reflect top-down modulation, cognitive control, or predictive coding (83). By capturing these dynamics, time-frequency analysis provides a more complete picture of neural responses beyond that which traditional ERP analysis can offer. Time-frequency analysis has been used to study the dependency of nociceptive cortical representation on brain maturation and frequency-specific differences from adult nociceptive processing as described in the next section.

EEG: Global Topographic and Microstate Analysis

More recently, the recognition of the complex relationships between the nociceptive ERP, other responses to the same noxious stimulus, and various biological factors – together with an appreciation that pain processing is not merely a brief sensory event, but a multidimensional experience shaped by contextual, intrinsic, and sensory factors over time – has prompted a shift in ERP analysis approaches. Traditional single-channel vertex ERP analysis can be overly reductive, failing to capture the

temporospatial dynamics of cortical activity. Single-channel analysis is also limited by its reliance on a single spatial sample, making it noisier, reference-dependent, and susceptible to erroneous magnitude differences due to latency shifts or changes in voltage field distribution (topography). In contrast global topographic and EEG microstate analysis provide a more comprehensive and physiologically meaningful description of brain activity by examining the spatial and temporal dynamics of neural responses (84). Global topographic analysis assesses the distribution of EEG activity across the scalp, identifying consistent topographic patterns associated with sensory or cognitive processes rather than relying on isolated electrode signals. EEG microstate analysis further extends this approach by segmenting brain activity into short, metastable topographic states (microstates), which are thought to represent fundamental building blocks of neural processing (85). One key advantage of this approach is the use of global field power (GFP), a reference-independent measure that quantifies the overall strength of EEG activity across all electrodes. This allows for the distinction between changes in topography (i.e., shifts in the configuration of active brain sources) and changes in signal magnitude, which single-channel ERP analysis cannot resolve. Global topographic and microstate analysis offers a more comprehensive view of the multifaceted relationship between nociceptive cortical representation and behavioural and physiological responses to a noxious stimulus and of the effect of contextual factors (such as parental contact) as described in the next section.

Functional Near-Infrared Spectroscopy

Functional Near-Infrared Spectroscopy (fNIRS) measures changes in cerebral oxygenation and hemodynamics by detecting variations in oxyhemoglobin and deoxyhemoglobin concentrations. It takes advantage of the relative transparency of biological tissue to near-infrared light and the distinct absorption properties of these hemoglobin states. NIRS systems can be either single-channel or multichannel, with single-channel NIRS providing measurements from a single location and multichannel systems enabling broader mapping of hemodynamic responses across cortical regions. Unlike EEG, which captures electrical activity with high temporal resolution but limited spatial localization, NIRS provides better spatial localization of brain hemodynamic activity by measuring changes within a restricted volume directly beneath the probes. While hemodynamic changes are not a direct measure of neural activity, they are tightly linked through neurovascular coupling, which is present in neonates but continues to mature with age (86). Functional near-infrared spectroscopy allows the mapping of nociceptive cortical activation in the neonatal brain as described in the next section.

UNDERSTANDING PAIN REPRESENTATION IN THE DEVELOPING BRAIN

The Neonatal Brain Can Discriminate Noxious and Innocuous Information at Term Age

Noxious tissue damaging stimulation (heel lance or venipuncture) elicits significant changes in the hemodynamic and electrophysiological activity of the neonatal brain in

term aged infants (14, 53, 87–89). A brief tissue breaking stimulus elicits an increase in total hemoglobin concentration in the primary somatosensory cortex contralateral to the stimulated site, with a response significantly larger than that evoked by tactile stimulation (88, 89). The same tissue damage elicits a nociceptive-specific ERP, originally identified by the research group at University College London as a PC whose weight was, on average, significantly larger following a heel lance than after a control, innocuous stimulus (90). The weight of this component was maximal at the vertex electrodes (Cz and CPz). This was the first evidence that the neonatal brain can discriminate noxious from tactile information. This finding was later replicated in independent samples at other two UK centres (91, 92). Over the years, this component/ERP has been called the N420–P560 (according to the approximate latency from stimulus onset (90)); the N3-P3 (‘3’ because it follows other somatosensory-related deflections (36, 79)), the nociceptive or nociceptive-specific ERP (nERP) (36, 53), template of nociceptive brain activity (92) and, more recently, noxious neurodynamic response function (n-NRF) (91). Principal component analysis (PCA) is a data-driven approach; thus, initial studies redefined the nociceptive specificity of a component for each new dataset by comparing traces from heel lances to control traces or other non-noxious stimuli (53, 90, 92–94). The consistency of this response across different studies and samples (always in response to a heel lance) has led to the adoption of the nociceptive PC as a template – or 'signature' – of neonatal pain in term infants (91, 92), in line with similar efforts in adult fMRI research (95). The idea is that responses with larger template weights indicate more pain, while smaller weights signify less pain, although this is likely to be overly reductive.

Indeed, topographic and microstate analysis, has identified a sequence of 5–6 distinct microstates following a single heel lance (2, 96, 97), each reflecting a distinct configuration of active cerebral sources. While the previously described P3 – characterized by a symmetrical central topography maximal at the vertex – was confirmed, microstate analysis revealed additional spatiotemporal patterns of activity that would have been missed using traditional ERP analysis, as they do not correspond to distinct peaks at Cz.

Overall, these results suggest that noxious information can reach the term neonatal brain, can engage the primary somatosensory cortex, can be distinguished from innocuous stimulation, and activates a multi-step sequence of cortical processes. The ability of the brain to discriminate between noxious and innocuous stimuli is a necessary, though not sufficient, condition for generating the experience of pain.

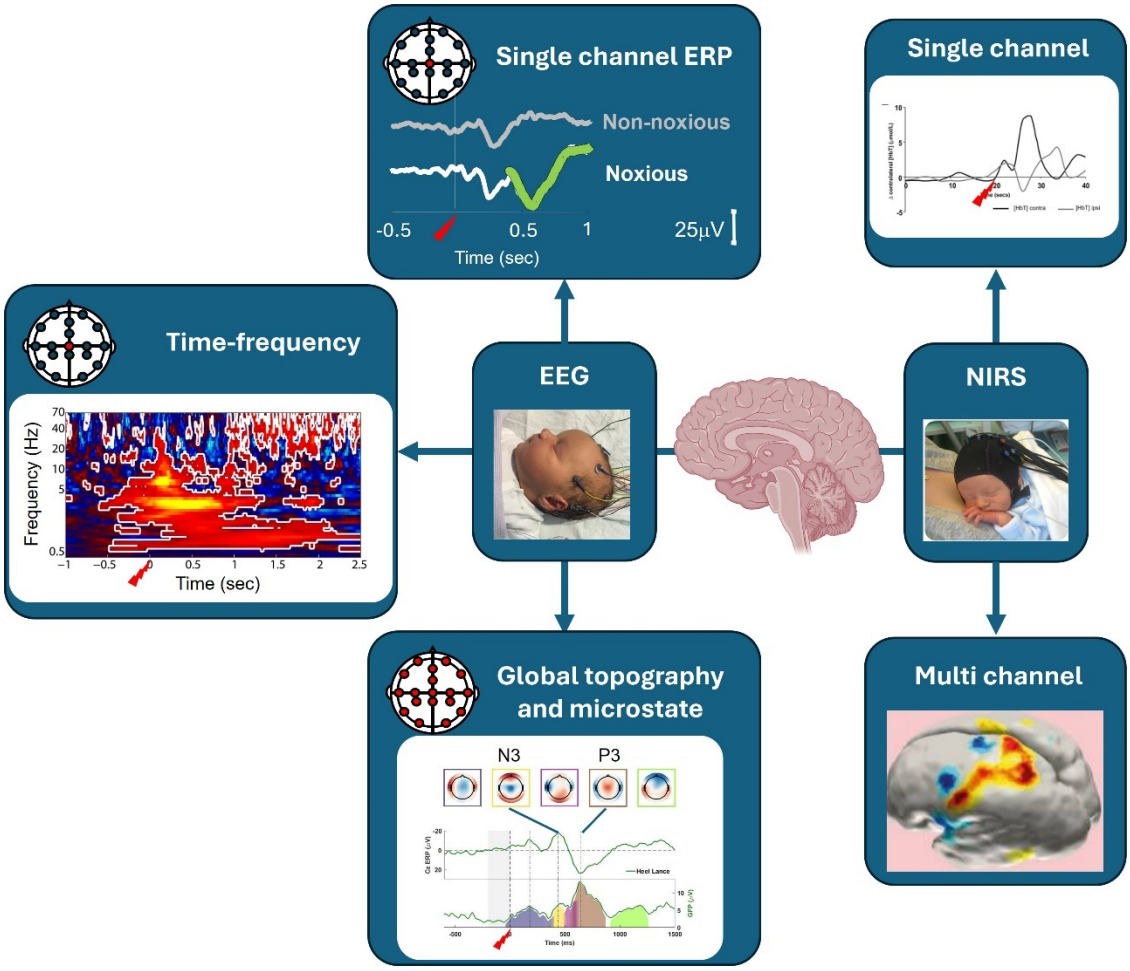

Figure 2. Overview of methods used to assess cortical hemodynamic and neurophysiological responses to acute tissue-damaging stimuli in term infants. Hemodynamic responses were measured using near-infrared spectroscopy (NIRS), while neurophysiological responses were assessed via electroencephalography (EEG). Single-channel event-related potentials (ERPs) recorded from Cz (marked in red) demonstrated a nociceptive component – a principal component (green trace) corresponding to an N3–P3 deflection – elicited more strongly by noxious than non-noxious stimuli, indicating early cortical discrimination of noxious input. Panel adapted with permission from Reference (90); copyright 2012 John Wiley and Sons. Time-frequency analysis revealed post-stimulus increases in beta-gamma (20–70 Hz) activity and a pronounced, long-latency 18-fold energy increase in the fast delta band (2–4 Hz), with significant changes marked in white (warm colors: energy increase; cool colors: decrease). Panel adapted from Reference (98) (CC BY 4.0). Global topographic and microstate analysis showed that a single noxious event elicits a sequence of discrete topographic brain states. While some correspond to the nociceptive ERP (N3-P3), others are not evident in single-channel recordings, highlighting the complexity of neonatal nociceptive processing. Microstate occurrences are represented by colour blocks below the global field power trace. Panel adapted from Reference (96) (CC BY 4.0). Hemodynamic responses were measured using single-channel recordings placed over the primary somatosensory cortex, both contralateral and ipsilateral to the lanced leg. A clear increase in total hemoglobin concentration was observed contralateral to the stimulated leg, but not ipsilaterally. Panel adapted from Reference (88); copyright 2006 Society for Neuroscience. Using multichannel NIRS, a broader mapping of hemodynamic responses across the contralateral somatosensory cortex revealed that the response to a heel lance is not limited to the topographic regions associated with foot input. Instead, it extends to areas typically involved in hand representation. Panel adapted from Reference (99) (CC BY 4.0).

Cortical Pain Representation Is Highly Dependent on Brain Maturation

Cortical activity – including hemodynamic activity, evoked potentials, and oscillatory patterns – undergoes dramatic changes over the equivalent of the third gestational trimester. Over this period, the hemodynamic response to a noxious stimulus increases in magnitude and decreases in latency, suggesting ongoing maturation of nociceptive cortical processing (88, 89). In preterm infants, EEG time-frequency analysis has shown that a heel lance is more likely to elicit widespread delta brush activity, a hallmark preterm EEG pattern, rather than a distinct nociceptive ERP (14, 53). Similar delta brushes are evoked by tactile stimulation (53, 100), as well as by other sensory modalities, with visual stimulation eliciting occipital delta brushes (101) and auditory stimulation eliciting temporal delta brushes (102). The fact that both noxious and tactile stimuli elicit similar delta brush responses in preterm infants suggests that, at this developmental stage, the brain has limited ability to differentiate between innocuous and noxious somatosensory input. Instead, sensory stimulation appears to trigger a general cortical response indicating an immature sensory processing system. However, the incidence of delta brush responses declines over the equivalent of the third trimester of gestation as cortical processing matures. These responses are progressively replaced by distinct nociceptive and tactile ERPs, reflecting the brain's developing ability to differentiate between nociception and touch (53, 54). Although this differentiation in EEG activity is poor in preterm infants, including limb withdrawal, heart rate, and facial expressions in a classifier can distinguish between responses to noxious and tactile somatosensory stimulation, suggesting that the input from subcortical regions is a greater contributor than cortical circuit activity at this stage (87).

Even at term age, neonatal nociceptive processing remains distinct from that of adults (98, 99). EEG time-frequency analysis has shown that some features of adult nociceptive activity, such as beta-gamma oscillations, are already present in infants, but at longer latencies (98). However, the neonatal nERP is not present in adults and neonates also exhibit a distinct, long-latency 18-fold energy increase in the fast delta band (2–4 Hz) which is absent in adults (98). Notably, these EEG differences are broadly distributed across the scalp, suggesting more widespread cortical involvement than in adults. Similarly, multichannel near-infrared spectroscopy (NIRS) has demonstrated that the hemodynamic response to tissue-damaging stimulation is more extensive than expected, spreading into areas of the somatosensory cortex associated with hand representation. This widespread activity indicates immature and diffuse localization of noxious input, in contrast to the highly refined and overlapping nociceptive and tactile maps in adults (99, 103).

Overall, these results suggest that the way the brain processes noxious information evolves significantly throughout the equivalent of the third trimester of gestation. Even at term age, the response remains distinct from that of adults. This indicates that pain representation in the developing brain is not just an immature version of the adult system, but fundamentally different. While the neonatal brain begins to show signs of more refined nociceptive processing, it still lacks the spatial specificity of the mature brain. These findings highlight the importance of further postnatal changes in brain circuits that encode and represent pain

Pain Representation in the Developing Brain Cannot Be Inferred from Behaviour and Physiology Alone

Reflexive motor, behavioural, physiological and hormonal responses to tissue damaging stimulation are related to cortical responses, which is not surprising, considering that they are all elicited by the same afferent input. However, the recent developmental, brain-led approaches reviewed here have highlighted that these indicators, although informative, are unlikely to fully capture the representation of pain in the immature brain. The weight/amplitude of the nERP and change in oxygenated haemoglobin concentration are overall significantly associated with facial behavioral or composite reactivity scores (54, 92, 104). However, high background stress conditions, as measured by salivary cortisol and heart rate variability, disrupt this relationship (36) and some infants who do not exhibit a facial response to tissue damage may still display a clear nociceptive ERP (36, 92, 93, 104) suggesting that even in subjects that cannot mount a behavioural reaction, because of underlying high stress or other reasons, the brain still receives nociceptive input. Moreover, global topographic analysis revealed that the relationship between noxious behavioural and cortical responses is more complex than a simple one-to-one correspondence, where greater behavioural responses directly reflect greater cortical activity. Rather than simply reflecting the degree of brain activation, facial activity is linked to differences in the nociceptive microstate sequence (2). The noxious response appears to involve two distinct and interleaved sub-sequences of microstates, one which is independent from behavioural scores and another one which is entirely different in those infants with high facial reactivity from those with subclinical facial responses (2). This implies that the relationship between behavioural reactivity and brain processes are far from straightforward and that there is parallel engagement of different processing cortical pathways in representing pain, only one of which is directly linked to behaviour.

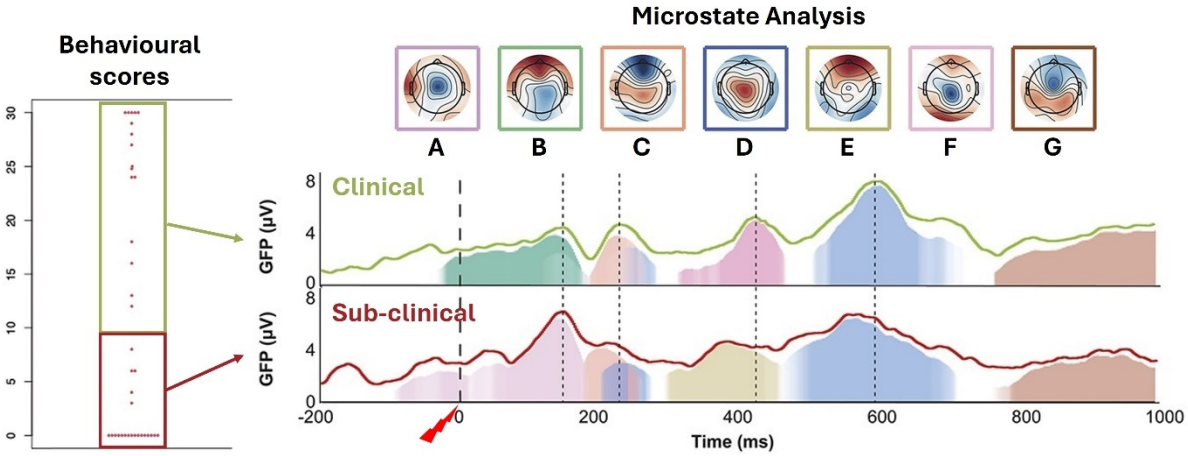

Figure 3. Differences in microstate engagement patterns in full-term neonates with sub-clinical vs. clinically significant behavioural responses (NFCS-P-3, Neonatal Facial Coding System) during the first second following a heel lance. Left panel: Distribution of post-stimulus NFCS-P-3 total scores (range: 0–30). Each dot represents an individual neonate, highlighting the high variability in behavioural responses to the same noxious stimulus. Top right panel: Microstates derived from the grand average across all participants (n = 37). Bottom right panel: Temporal sequence of microstate engagement following a heel lance, shown separately for the NFCS-subclinical and NFCS-clinical groups. Microstates C, D, and G are present in both groups, while microstates A, B, E, and F appear uniquely in either the sub-clinical or clinical group—indicating distinct neural processing of the noxious input. Solid lines represent the global field power (GFP), a measure of overall cortical response strength across the scalp.

Red lightning indicates the moment of heel lance. Figure adapted from Reference (2) (CC BY 4.0).

Biological and Environmental Factors Modulate Infant Pain Processing

Despite its immaturity, the representation of pain in the infant cortex is related to biological factors and sensitive to interventions and environment. The nERP is more widespread over the scalp in term female than male infants, suggesting a prepubertal sex difference in nociceptive processing linked to broader brain connectivity in females which could be a precursor of the higher sensitivity to noxious stimulation in adult female (105). The nERP is also larger in the presence of underlying background stress (36) or suspected inflammation (106), suggesting early interactions between the stress, immune, and nociceptive systems that may lead to sensitization, even at this early stage of life (107). Stress can influence nociceptive processing through activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of cortisol and other stress mediators that modulate neuronal excitability (108). Simultaneously, immune activation – such as from inflammation – can lead to the release of pro-inflammatory cytokines, which sensitize peripheral and central nociceptive pathways (109). These processes can converge at subcortical and cortical pathways, amplifying cortical responses to noxious stimuli and contributing to the increase in nERP amplitude.

The weight/amplitude of the nociceptive ERP is reassuringly reduced by topical anaesthetic (4% tetracaine gel) (92); it has been shown to both decrease and remain unchanged following gentle leg stroking at 3 cm/s before a heel lance, a stimulus thought to activate tactile C fibers involved in pleasant touch and pain modulation in adults (110, 111); however, it is unaffected by sucrose or morphine administration (93, 112). Other external factors such as parental contact and stimulus repetition also affect the nociceptive microstate sequence (96, 97). Parental skin-to-skin contact reduces the energy of the microstate related to the N3 (97), but does not alter the initial sequence of microstates engaged, suggesting that the initial arrival of the signal to the somatosensory cortex and the basic processing of stimulus features occur consistently, regardless of parental contact. In contrast, later microstates, which are thought to reflect higher-order processes within the hierarchy of stimulus processing, do change depending on the level of parental contact, suggesting a modulatory effect on cortical responses beyond the initial sensory encoding of the stimulus.

Noxious stimulus repetition also alters the nociceptive microstate sequence in an age-dependent manner (96). Habituation to recurrent, non-threatening, or unavoidable noxious stimuli is a key adaptive mechanism in pain processing. In term infants, stimulus repetition dampens the engagement of early microstates, along with associated behavioral and autonomic responses, suggesting an ability to regulate initial reactivity to a noxious stimulus. In contrast, late preterm infants do not show signs of habituation, indicating that these regulatory mechanisms are not yet fully functional. However, in both late preterm and term infants, longer-latency cortical microstates are different following each lance, likely reflecting changes in higher-level stimulus processing with repeated stimulation. These findings suggest that while both age groups can encode contextual differences in pain, the preterm brain lacks the ability to regulate its initial cortical, behavioral, and autonomic responses to repeated noxious stimuli. This might explain why the nERP is larger in preterm born infant at term equivalent age compared to full-term control, indicating sensitizing effect of the

neonatal intensive care experience (94), although this finding was not replicated in another sample (113).

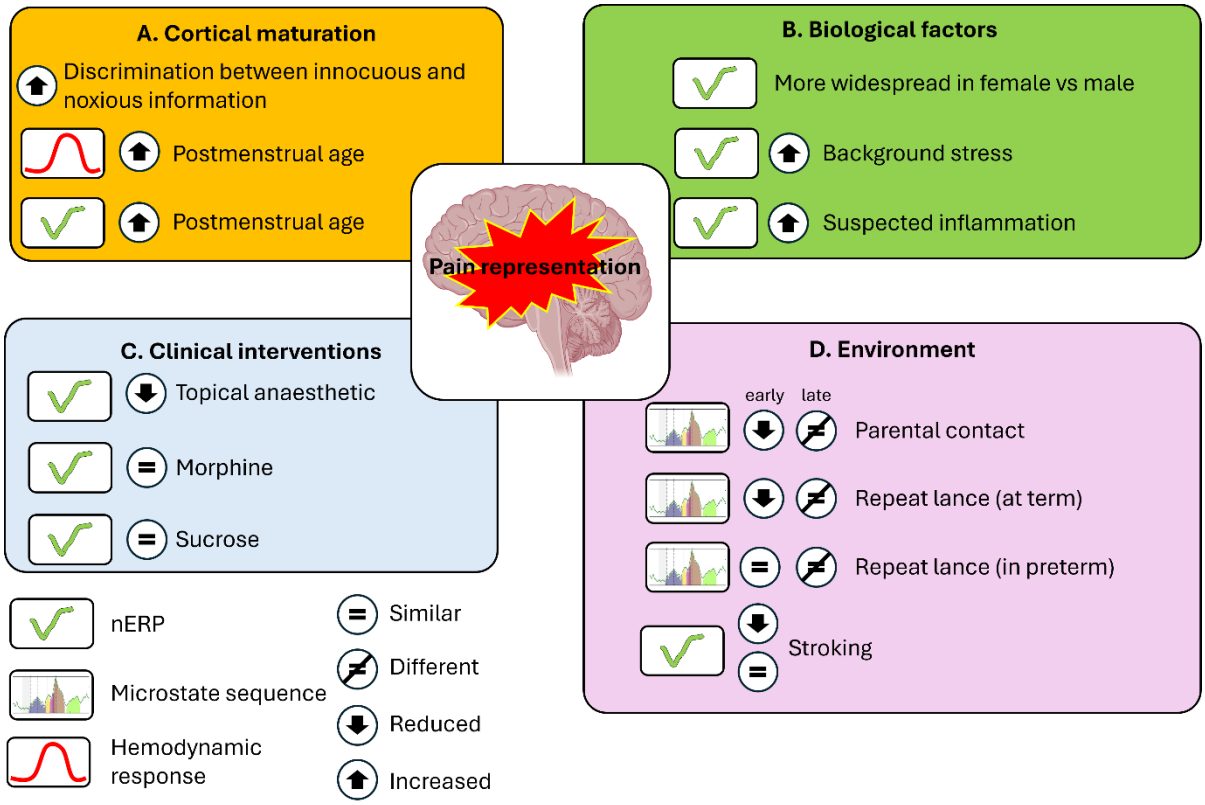

Figure 4. Summary diagram of the influence of cortical maturation, biological factors, environment and clinical interventions on cortical pain representation in the neonatal brain. A. Cortical responses to noxious and tactile stimulation become increasingly distinct and hemodynamic and nociceptive event-relation potentials (nERPs) become larger with postmenstrual age. Data from References (14, 53, 54, 87, 88). B. nERPs are more widespread in female than in male infants, are larger in the presence of background stress - as measured by salivary cortisol - or suspected systemic inflammation. Data from References (36, 105, 106). C. Application of topical anaesthetic decrease the amplitude of the nERP, which instead is not altered by morphine or sucrose. Data from References (92, 93, 112) D. Microstate analysis reveals that the early part of the response to a noxious stimulus is dampened by parental contact while the later engages different microstates. The early part of the microstate sequence is also dampened with repetition in term, but not preterm infants, while the later part engages different microstates at both ages. Data from References (96, 97, 110, 111).

Sensory Pain Networks Develop Early, While Affective and Evaluative Circuits Remain Immature at Birth

Although recent advances have improved our understanding of pain in the developing brain, research has largely focused on brief, phasic responses to acute noxious stimuli. However, pain is not simply a brief sensory response, but it is a state resulting from a complex interplay between contextual, intrinsic and sensory factors over a period of time. Pain perception in adults is underpinned by the activation of a widespread network of brain regions, which together are responsible for the encoding of the sensory-discriminative and cognitive-affective qualities of pain (95, 114, 115).

These include the insula, thalamus, primary and secondary somatosensory cortices, anterior and posterior cingulate cortex, dorsolateral and ventrolateral prefrontal cortex, amygdala, orbitofrontal cortex and periaqueductal grey. To function in a coordinated manner, these brain regions establish preferential connections, forming a network known as the pain connectome (116). This framework must be intact for healthy pain processing. However, we have recently shown that at the start of the equivalent of the third gestational trimester, the pain connectome is significantly weaker than in adults, follows an uneven developmental trajectory, and does not reach adult-like configuration even by term age (5). The sensory subnetwork develops more rapidly than the others and is hyperconnected compared to adults at term age, offering an explanation for the widespread responses to noxious stimuli described above (98, 99). The affective subnetwork develops more slowly but still becomes hyperconnected at term, whereas the cognitive subnetwork – including the prefrontal cortex (PFC) – lags behind and does not reach adult levels within the equivalent of the third gestational trimester. The PFC plays a crucial role in modulating sensations and emotions, assigning meaning to experiences (117), and is considered essential for conscious perception and self-report (118). However, even in the absence of conscious awareness, unconscious sensory registration can still trigger autonomic and behavioural survival responses and may have long-term consequences through implicit memories of aversive stimuli (119). Because the PFC remains largely unconnected throughout the third gestational trimester, neonates may lack conscious awareness or cognitive control of pain but could still form implicit memories through sensory and limbic activation.

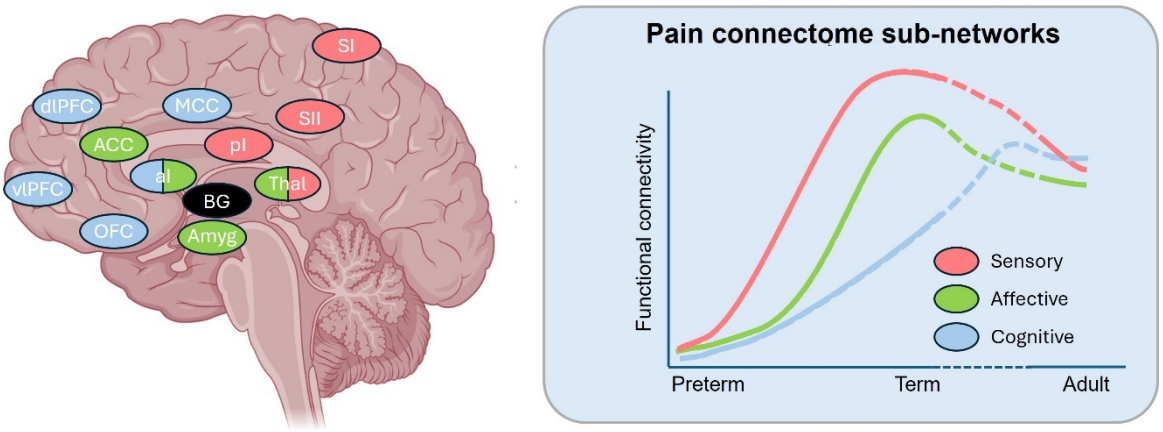

Figure 5. Developmental trajectories of pain connectome sub-networks (sensory, red; affective, green; cognitive, blue). The sensory subnetwork involves the Thalamus (Thal), Primary Somatosensory Cortex (SI), Secondary Somatosensory Cortex (SII), posterior Insula (pI); the affective subnetwork involves the Thalamus (Thal), anterior Insula (aI), Amygdala (Amyg) and Anterior Cingulate Cortex (ACC); the cognitive subnetwork involves the anterior Insula (aI), Medial Cingulate Cortex (MCC), dorso-lateral and ventro-lateral Prefrontal Cortex (dlPFC and vlPFC), Orbito-Frontal Cortex (OFC). The Basal Ganglia (BG) contribute to all three sub-networks. Compared to adults, functional connectivity within the neonatal pain connectome is initially weak, develops unevenly, and remains immature at term age. Sensory connections mature earliest, followed by affective, both showing signs of hyperconnectivity at term, while cognitive connectivity lags behind and remains underconnected even at term. Data from Reference (5).

In Summary

Recordings of brain activity in infants undergoing clinically required, tissue-damaging procedures have informed us that nociceptive information reaches the brain and is processed at the cortical level from at least the beginning of the last trimester of gestation. However, both the nature of the transmitted information and the way it is represented in the brain change significantly during this period due to structural and functional maturation of the peripheral input, the spinal cord, the brainstem, the thalamus and the cortex. The ability to distinguish between touch and noxious-evoked cortical activity, a necessary but not sufficient condition for experiencing pain as distinct from innocuous stimulation, does not become clear until 34 weeks gestation, and even at term age tissue damage evoked cortical activation and its spatial specificity is clearly different than in adults. Although behavioural and physiological signs like facial expressions and heart rate often correlate with cortical pain activity, they don't fully capture the brain's pain representation, particularly under stress or in cases of subclinical responses. Global topographic analyses have revealed that this relationship is more complex than a simple one-to-one correlation; rather, differences in behavioural reactivity correspond to distinct pathways within the brain's hierarchy of nociceptive processing. Pain processing in neonates is also shaped by biological factors (e.g., sex, inflammation, stress levels) and modifiable by environmental influences such as parental contact, analgesic interventions, and stimulus repetition. All the maturational changes and differential responses to tissue damaging stimuli in young infants are likely underpinned by changes in the pain connectome. Since the subcomponents of the pain connectome mature at different rates, their relative connectivity shifts from week to week, creating a constantly changing neural landscape. Consequently, both the processing and subjective representation of pain in early life are dynamic and developmentally unique. At no point during this period does the neonatal pain connectome mirror that of an adult, strongly suggesting that pain is a qualitatively different experience for neonates, even at term age.

References

1. McGrath PJ, Unruh AM. 1987. *Pain in Children and Adolescents*, Vol. 1. Amsterdam: Elsevier
2. Bucsea O, Rupawala M, Shiff I, Wang X, Meek J, et al. 2023. Clinical thresholds in pain-related facial activity linked to differences in cortical network activation in neonates. *Pain*. 164(5):1039–50
3. Fitzgerald M. 2024. The Bayliss-Starling Prize Lecture: The developmental physiology of spinal cord and cortical nociceptive circuits. *J Physiol*. 602(6):1003–16
4. Fitzgerald M. 2024. On the relation of injury to pain-an infant perspective. *Pain*. 165(11S):S33–38

5. Jones L, Batalle D, Meek, J, Edwards AD, Fitzgerald M, et al. 2025. Differential maturation of the brain networks required for the sensory, emotional and cognitive aspects of pain in human newborns. *Pain*. in press:
6. Barabási DL, Ferreira Castro A, Engert F. 2025. Three systems of circuit formation: assembly, updating and tuning. *Nat. Rev. Neurosci.* 26(4):232–43
7. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, et al. 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *PAIN*. 161(9):1976–82
8. Tsze DS, von Baeyer CL, Bulloch B, Dayan PS. 2013. Validation of Self-Report Pain Scales in Children. *Pediatrics*. 132(4):e971–79
9. Tracey I, Mantyh PW. 2007. The Cerebral Signature for Pain Perception and Its Modulation. *Neuron*. 55(3):377–91
10. Cornelissen L, Fabrizi L, Patten D, Worley A, Meek J, et al. 2013. Postnatal Temporal, Spatial and Modality Tuning of Nociceptive Cutaneous Flexion Reflexes in Human Infants. *PLoS One*. 8(10):
11. Grunau RVE, Craig KD. 1987. Pain expression in neonates: facial action and cry. *PAIN*. 28(3):395
12. Owens ME, Todt EH. 1984. Pain in infancy: neonatal reaction to a heel lance. *Pain*. 20(1):77–86
13. Andrews K, Fitzgerald M. 2000. Flexion reflex responses in biceps femoris and tibialis anterior in human neonates. *Early Human Development*. 57(2):105–10
14. Hartley C, Moultrie F, Gursul D, Hoskin A, Adams E, et al. 2016. Changing Balance of Spinal Cord Excitability and Nociceptive Brain Activity in Early Human Development. *Current Biology*. 26(15):1998–2002

15. Cornelissen L, Underwood E, Gabard-Durnam LJ, Soto M, Tao A, et al. 2022. Tactile sensitivity and motor coordination in infancy: Effect of age, prior surgery, anaesthesia & critical illness. *PLoS One*. 17(12):e0279705

16. Stevens BJ, Pillai Riddell, RR, Oberlander TE, Gibbins S. 2007. Assessment of pain in neonates and infant. In *Pain in neonates and infants*, pp. 67–90

17. Fitzgerald M. 2015. What do we really know about newborn infant pain? *Experimental Physiology*. 100(12):1451–57

18. Fitzgerald M. 2005. The development of nociceptive circuits. *Nature Reviews Neuroscience*. 6(7):507–20

19. Beissner F, Brandau A, Henke C, Felden L, Baumgärtner U, et al. 2010. Quick Discrimination of Adelta and C Fiber Mediated Pain Based on Three Verbal Descriptors. *PLOS ONE*. 5(9):e12944

20. Fabrizi L, Williams G, Lee A, Meek J, Slater R, et al. 2013. Cortical activity evoked by an acute painful tissue-damaging stimulus in healthy adult volunteers. *Journal of Neurophysiology*. 109(9):2393–2403

21. Fitzgerald M, Jennings E. 1999. The postnatal development of spinal sensory processing. *Proc. Natl. Acad. Sci. U.S.A.* 96(14):7719–22

22. Koch SC, Fitzgerald M. 2013. Activity-dependent development of tactile and nociceptive spinal cord circuits. *Ann. N.Y. Acad. Sci.* 1279(1):97–102

23. Andrews K, Fitzgerald M. 1994. The cutaneous withdrawal reflex in human neonates: sensitization, receptive fields, and the effects of contralateral stimulation. *Pain*. 56(1):95–101

24. Ahola Kohut S, Pillai Riddell R. 2009. Does the Neonatal Facial Coding System Differentiate Between Infants Experiencing Pain-Related and Non-Pain-Related Distress? *The Journal of Pain*. 10(2):214–20

25. Maxwell LG, Fraga MV, Malavolta CP. 2019. Assessment of Pain in the Newborn: An Update. *Clinics in Perinatology*. 46(4):693–707

26. Sakthivel M, Su V, Nataraja RM, Pacilli M. 2024. Newborn and Infant Parasympathetic Evaluation (NIPE™) Monitor for Assessing Pain During Surgery and Interventional Procedures: A Systematic Review. *J Pediatr Surg*. 59(4):672–77

27. Goldstein DS, Kopin IJ. 2008. ADRENOMEDULLARY, ADRENOCORTICAL, AND SYMPATHONEURAL RESPONSES TO STRESSORS: A META-ANALYSIS. *Endocr Regul*. 42(4):111–19

28. Bremmer P, Byers JF, Kiehl E. 2003. Noise and the Premature Infant: Physiological Effects and Practice Implications. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*. 32(4):447–54

29. Zahr LK, Balian S. 1995. Responses of Premature Infants to Routine Nursing Interventions And Noise in the NICU. *Nursing Research*. 44(3):179

30. Sweeney JK, Blackburn S. 2013. Neonatal Physiological and Behavioral Stress During Neurological Assessment. *The Journal of Perinatal & Neonatal Nursing*. 27(3):242

31. Lee H-K. 2002. Effects of sponge bathing on vagal tone and behavioural responses in premature infants. *Journal of Clinical Nursing*. 11(4):510–19

32. Peters KL. 1998. Bathing premature infants: Physiological and behavioral consequences. *American Journal of Critical Care*. 7(2):90–100

33. Neu M, Browne JV. 1997. Infant physiologic and behavioral organization during swaddled versus unswaddled weighing. *J Perinatol*. 17(3):193–98

34. Jansen J, Beijers R, Riksen-Walraven M, de Weerth C. 2010. Cortisol reactivity in young infants. *Psychoneuroendocrinology*. 35(3):329–38

35. Olszewska M, Pointinger-Tomasik S, Kwinta P. 2023. Assessment of salivary cortisol concentrations for procedural pain monitoring in newborns. *Journal of Perinatal Medicine*. 51(4):564–72

36. Jones L, Fabrizi L, Laudiano-Dray M, Whitehead K, Meek J, et al. 2017. Nociceptive Cortical Activity Is Dissociated from Nociceptive Behavior in Newborn Human Infants under Stress. *Current Biology*. 27(24):3846-3851.e3

37. Fitzgerald M, Shaw A, MacIntosh N. 1988. Postnatal development of the cutaneous flexor reflex: comparative study of preterm infants and newborn rat pups. *Dev Med Child Neurol*. 30(4):520–26

38. Waldenström A, Thelin J, Thimansson E, Levinsson A, Schouenborg J. 2003. Developmental learning in a pain-related system: evidence for a cross-modality mechanism. *J. Neurosci.* 23(20):7719–25

39. Baccei ML. 2007. Development of Pain: Maturation of Spinal Inhibitory Networks. *International Anesthesiology Clinics*. 45(2):1

40. Hathway GJ, Koch S, Low L, Fitzgerald M. 2009. The changing balance of brainstem-spinal cord modulation of pain processing over the first weeks of rat postnatal life. *J. Physiol. (Lond.)*. 587(Pt 12):2927–35

41. Schwaller F, Kwok C, Fitzgerald M. 2015. Postnatal maturation of the spinal-bulbo-spinal loop: brainstem control of spinal nociception is independent of sensory input in neonatal rats. *Pain*

42. Bannister K, Dickenson AH. 2016. What the brain tells the spinal cord. *PAIN*. 157(10):2148

43. Gebhart GF. 2004. Descending modulation of pain. *Neurosci Biobehav Rev*. 27(8):729–37

44. Bremner LR, Fitzgerald M. 2008. Postnatal tuning of cutaneous inhibitory receptive fields in the rat. *J. Physiol. (Lond.)*. 586(6):1529–37

45. Torsney C, Fitzgerald M. 2002. Age-dependent effects of peripheral inflammation on the electrophysiological properties of neonatal rat dorsal horn neurons. *J. Neurophysiol.* 87(3):1311–17

46. Andrews KA, Desai D, Dhillon HK, Wilcox DT, Fitzgerald M. 2002. Abdominal sensitivity in the first year of life: comparison of infants with and without prenatally diagnosed unilateral hydronephrosis. *Pain*. 100(1–2):35–46

47. Fitzgerald M, Millard C, McIntosh N. 1989. Cutaneous hypersensitivity following peripheral tissue damage in newborn infants and its reversal with topical anaesthesia. *Pain*. 39(1):31–36

48. Fitzgerald M, Millard C, MacIntosh N. 1988. Hyperalgesia in premature infants. *Lancet*. 1(8580):292

49. Taddio A, Shah V, Gilbert-MacLeod C, Katz J. 2002. Conditioning and hyperalgesia in newborns exposed to repeated heel lances. *JAMA*. 288(7):857–61

50. Ririe DG, Bremner LR, Fitzgerald M. 2008. Comparison of the immediate effects of surgical incision on dorsal horn neuronal receptive field size and responses during postnatal development. *Anesthesiology*. 109(4):698–706

51. Walker SM, Tochiki KK, Fitzgerald M. 2009. Hindpaw incision in early life increases the hyperalgesic response to repeat surgical injury: critical period and dependence on initial afferent activity. *Pain*. 147(1–3):99–106

52. Brewer CL, Baccei ML. 2020. The development of pain circuits and unique effects of neonatal injury. *J Neural Transm (Vienna)*. 127(4):467–79

53. Fabrizi L, Slater R, Worley A, Meek J, Boyd S, et al. 2011. A Shift in Sensory Processing that Enables the Developing Human Brain to Discriminate Touch from Pain. *Current Biology*. 21(18):1552–58

54. Green G, Hartley C, Hoskin A, Duff E, Shriver A, et al. 2019. Behavioural discrimination of noxious stimuli in infants is dependent on brain maturation. *Pain*. 160(2):493–500

55. Coghill RC. 2020. The Distributed Nociceptive System: A Framework for Understanding Pain. *Trends in Neurosciences*. 43(10):780–94

56. Liang M, Su Q, Mouraux A, Iannetti GD. 2019. Spatial Patterns of Brain Activity Preferentially Reflecting Transient Pain and Stimulus Intensity. *Cereb Cortex*. 29(5):2211–27

57. Tracey I, Woolf CJ, Andrews NA. 2019. Composite Pain Biomarker Signatures for Objective Assessment and Effective Treatment. *Neuron*. 101(5):783–800

58. Petre B, Kragel P, Atlas LY, Geuter S, Jepma M, et al. 2022. A multistudy analysis reveals that evoked pain intensity representation is distributed across brain systems. *PLoS Biol*. 20(5):e3001620

59. Krsnik Ž, Majić V, Vasung L, Huang H, Kostović I. 2017. Growth of Thalamocortical Fibers to the Somatosensory Cortex in the Human Fetal Brain. *Frontiers in Neuroscience*. 11:

60. Wess JM, Isaiah A, Watkins PV, Kanold PO. 2017. Subplate neurons are the first cortical neurons to respond to sensory stimuli. *Proceedings of the National Academy of Sciences*. 114(47):12602–7

61. Molnár Z, Luhmann HJ, Kanold PO. 2020. Transient cortical circuits match spontaneous and sensory-driven activity during development. *Science*. 370(6514):eabb2153

62. Ohtaka-Maruyama C, Okamoto M, Endo K, Oshima M, Kaneko N, et al. 2018. Synaptic transmission from subplate neurons controls radial migration of neocortical neurons. *Science*. 360(6386):313–17

63. Bethlehem R a. I, Seidlitz J, White SR, Vogel JW, Anderson KM, et al. 2022. Brain charts for the human lifespan. *Nature*. 604(7906):525–33

64. Cadwell CR, Bhaduri A, Mostajo-Radji MA, Keefe MG, Nowakowski TJ. 2019. Development and Arealization of the Cerebral Cortex. *Neuron*. 103(6):980–1004

65. Paredes MF, James D, Gil-Perotin S, Kim H, Cotter JA, et al. 2016. Extensive migration of young neurons into the infant human frontal lobe. *Science*. 354(6308):aaf7073

66. Trivedi R, Gupta RK, Husain N, Rathore RKS, Saksena S, et al. 2009. Region-specific maturation of cerebral cortex in human fetal brain: diffusion tensor imaging and histology. *Neuroradiology*. 51(9):567–76

67. Kostović I, Sedmak G, Judaš M. 2019. Neural histology and neurogenesis of the human fetal and infant brain. *NeuroImage*. 188:743–73

68. Wilson S, Pietsch M, Cordero-Grande L, Christiaens D, Uus A, et al. 2023. Spatiotemporal tissue maturation of thalamocortical pathways in the human fetal brain. *eLife*. 12:e83727

69. van den Heuvel MP, Sporns O. 2013. Network hubs in the human brain. *Trends Cogn Sci*. 17(12):683–96

70. Doria V, Beckmann CF, Arichi T, Merchant N, Groppo M, et al. 2010. Emergence of resting state networks in the preterm human brain. *Proc. Natl. Acad. Sci. U.S.A.* 107(46):20015–20

71. Smyser CD, Snyder AZ, Neil JJ. 2011. Functional connectivity MRI in infants: exploration of the functional organization of the developing brain. *Neuroimage*. 56(3):1437–52

72. Eyre M, Fitzgibbon SP, Ciarrusta J, Cordero-Grande L, Price AN, et al. 2021. The Developing Human Connectome Project: typical and disrupted perinatal functional connectivity. *Brain*. 144(7):2199–2213

73. França LGS, Ciarrusta J, Gale-Grant O, Fenn-Moltu S, Fitzgibbon S, et al. 2024. Neonatal brain dynamic functional connectivity in term and preterm infants and its association with early childhood neurodevelopment. *Nat Commun*. 15(1):16

74. Turk E, van den Heuvel MI, Benders MJ, de Heus R, Franx A, et al. 2019. Functional Connectome of the Fetal Brain. *J Neurosci*. 39(49):9716–24

75. van den Heuvel MI, Turk E, Manning JH, Hect J, Hernandez-Andrade E, et al. 2018. Hubs in the human fetal brain network. *Dev Cogn Neurosci*. 30:108–15

76. Khazipov R, Milh M. 2018. Early patterns of activity in the developing cortex: Focus on the sensorimotor system. *Semin. Cell Dev. Biol.* 76:120–29

77. Dall'Orso S, Steinweg J, Allievi AG, Edwards AD, Burdet E, Arichi T. 2018. Somatotopic Mapping of the Developing Sensorimotor Cortex in the Preterm Human Brain. *Cereb Cortex*. 28(7):2507–15

78. Allievi AG, Arichi T, Tusor N, Kimpton J, Arulkumaran S, et al. 2016. Maturation of Sensori-Motor Functional Responses in the Preterm Brain. *Cereb Cortex*. 26(1):402–13

79. Whitehead K, Papadelis C, Laudiano-Dray MP, Meek J, Fabrizi L. 2019. The Emergence of Hierarchical Somatosensory Processing in Late Prematurity. *Cerebral Cortex*. 29(5):2245–60

80. Whitehead K, Jones L, Laudiano Dray P, Meek J, Fabrizi L. 2018. Full 10-20 EEG application in hospitalised neonates is not associated with an increase in stress hormone levels. *Clinical Neurophysiology Practice*. 3:20–21

81. Woodman GF. 2010. A brief introduction to the use of event-related potentials in studies of perception and attention. *Attention, Perception, & Psychophysics*. 72(8):2031–46

82. Mouraux A, Iannetti GD. 2008. Across-trial averaging of event-related EEG responses and beyond. *Magnetic Resonance Imaging*. 26(7):1041–54

83. Ploner M, Sorg C, Gross J. 2017. Brain Rhythms of Pain. *Trends in Cognitive Sciences*. 21(2):100–110

84. Murray MM, Brunet D, Michel CM. 2008. Topographic ERP Analyses: A Step-by-Step Tutorial Review. *Brain Topogr*. 20(4):249–64

85. Michel CM, Koenig T. 2018. EEG microstates as a tool for studying the temporal dynamics of whole-brain neuronal networks: A review. *NeuroImage*. 180:577–93

86. Arichi T, Fagiolo G, Varela M, Melendez-Calderon A, Allievi A, et al. 2012. Development of BOLD signal hemodynamic responses in the human brain. *NeuroImage*. 63(2):663–73

87. van der Vaart M, Hartley C, Baxter L, Mellado GS, Andritsou F, et al. 2022. Premature infants display discriminable behavioral, physiological, and brain responses to noxious and nonnoxious stimuli. *Cereb Cortex*. 32(17):3799–3815

88. Slater R, Cantarella A, Gallella S, Worley A, Boyd S, et al. 2006. Cortical pain responses in human infants. *Journal of Neuroscience*. 26(14):3662–66

89. Bartocci M, Bergqvist LL, Lagercrantz H, Anand KJS. 2006. Pain activates cortical areas in the preterm newborn brain. *PAIN®*. 122(1):109–17

90. Slater R, Worley A, Fabrizi L, Roberts S, Meek J, et al. 2010. Evoked potentials generated by noxious stimulation in the human infant brain. *European Journal of Pain*. 14(3):321–26

91. Aspbury M, Mansfield RC, Baxter L, Bhatt A, Cobo MM, et al. 2024. Establishing a standardised approach for the measurement of neonatal noxious-evoked brain activity in response to an acute somatic nociceptive heel lance stimulus. *Cortex*. 179:215–34

92. Hartley C, Duff EP, Green G, Mellado GS, Worley A, et al. 2017. Nociceptive brain activity as a measure of analgesic efficacy in infants. *Science Translational Medicine*. 9(388):eaah6122

93. Slater R, Cornelissen L, Fabrizi L, Patten D, Yoxen J, et al. 2010. Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. *The Lancet*. 376(9748):1225–32

94. Slater R, Fabrizi L, Worley A, Meek J, Boyd S, Fitzgerald M. 2010. Premature infants display increased noxious-evoked neuronal activity in the brain compared to healthy age-matched term-born infants. *NeuroImage*. 52(2):583–89

95. Wager TD, Atlas LY, Lindquist MA, Roy M, Woo C-W, Kross E. 2013. An fMRI-Based Neurologic Signature of Physical Pain. *New England Journal of Medicine*. 368(15):1388–97

96. Rupawala M, Bucsea O, Laudiano-Dray MP, Whitehead K, Meek J, et al. 2023. A developmental shift in habituation to pain in human neonates. *Current Biology*. 33(8):1397-1406.e5

97. Jones L, Laudiano-Dray MP, Whitehead K, Meek J, Fitzgerald M, et al. 2021. The impact of parental contact upon cortical noxious-related activity in human neonates. *European Journal of Pain*. 25(1):149–59

98. Fabrizi L, Verriotis M, Williams G, Lee A, Meek J, et al. 2016. Encoding of mechanical nociception differs in the adult and infant brain. *Sci Rep*. 6(1):28642

99. Jones L, Verriotis M, Cooper RJ, Laudiano-Dray MP, Rupawala M, et al. 2022. Widespread nociceptive maps in the human neonatal somatosensory cortex. *eLife*. 11:e71655

100. Milh M, Kaminska A, Huon C, Lapillonne A, Ben-Ari Y, Khazipov R. 2007. Rapid Cortical Oscillations and Early Motor Activity in Premature Human Neonate. *Cereb. Cortex*. 17(7):1582–94

101. Colonnese MT, Kaminska A, Minlebaev M, Milh M, Bloem B, et al. 2010. A Conserved Switch in Sensory Processing Prepares Developing Neocortex for Vision. *Neuron*. 67(3):480–98

102. Kaminska A, Delattre V, Laschet J, Dubois J, Labidurie M, et al. 2018. Cortical Auditory-Evoked Responses in Preterm Neonates: Revisited by Spectral and Temporal Analyses. *Cerebral Cortex*. 28(10):3429–44

103. Mancini F, Haggard P, Iannetti GD, Longo MR, Sereno MI. 2012. Fine-Grained Nociceptive Maps in Primary Somatosensory Cortex. *J. Neurosci.* 32(48):17155–62

104. Slater R, Cantarella A, Franck L, Meek J, Fitzgerald M. 2008. How Well Do Clinical Pain Assessment Tools Reflect Pain in Infants? *PLOS Medicine*. 5(6):e129

105. Verriotis M, Jones L, Whitehead K, Laudiano-Dray M, Panayotidis I, et al. 2018. The distribution of pain activity across the human neonatal brain is sex dependent. *NeuroImage*. 178:69–77

106. Cobo MM, Green G, Andritsou F, Baxter L, Evans Fry R, et al. 2022. Early life inflammation is associated with spinal cord excitability and nociceptive sensitivity in human infants. *Nat Commun*. 13(1):3943

107. Ren K, Dubner R. 2010. Interactions between the immune and nervous systems in pain. *Nat Med*. 16(11):1267–76

108. Jennings EM, Okine BN, Roche M, Finn DP. 2014. Stress-induced hyperalgesia. *Progress in Neurobiology*. 121:1–18

109. Maier SF. 2003. Bi-directional immune–brain communication: Implications for understanding stress, pain, and cognition. *Brain, Behavior, and Immunity*. 17(2):69–85

110. Gursul D, Goksan S, Hartley C, Mellado GS, Moultrie F, et al. 2018. Stroking modulates noxious-evoked brain activity in human infants. *Current Biology*. 28(24):R1380–81

111. Hauck AGV, Vaart M van der, Adams E, Baxter L, Bhatt A, et al. 2024. Effect of parental touch on relieving acute procedural pain in neonates and parental anxiety (Petal): a multicentre, randomised controlled trial in the UK. *The Lancet Child & Adolescent Health*. 8(4):259–69

112. Hartley C, Moultrie F, Hoskin A, Green G, Monk V, et al. 2018. Analgesic efficacy and safety of morphine in the Procedural Pain in Premature Infants (Poppi) study: randomised placebo-controlled trial. *The Lancet*. 392(10164):2595–2605

113. Schmidt Mellado G, Pillay K, Adams E, Alarcon A, Andritsou F, et al. 2022. The impact of premature extrauterine exposure on infants' stimulus-evoked brain activity across multiple sensory systems. *NeuroImage: Clinical*. 33:102914

114. Duerden EG, Albanese M-C. 2013. Localization of pain-related brain activation: a meta-analysis of neuroimaging data. *Hum Brain Mapp*. 34(1):109–49

115. Wiech K. 2016. Deconstructing the sensation of pain: The influence of cognitive processes on pain perception. *Science*. 354(6312):584–87

116. Kucyi A, Davis KD. 2015. The dynamic pain connectome. *Trends Neurosci*. 38(2):86–95

117. Bingel U, Tracey I. 2008. Imaging CNS Modulation of Pain in Humans. *Physiology*. 23(6):371–80

118. Bastuji H, Mazza S, Perchet C, Frot M, Mauguière F, et al. 2012. Filtering the reality: Functional dissociation of lateral and medial pain systems during sleep in humans. *Human Brain Mapping*. 33(11):2638–49

119. Garcia-Larrea L, Bastuji H. 2018. Pain and consciousness. *Prog Neuropsychopharmacol Biol Psychiatry*. 87(Pt B):193–99