What do we really know about newborn infant pain?

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Abstract

Increased awareness of pain in the newborn has led to the development of numerous assessment tools for use in neonatal intensive care units. Here I argue that we still know too little about the neurophysiological basis for infant pain to interpret data from clinical observational measures. With increased understanding of how the neural activity and CNS connections which underlie pain behaviour and perception develop in the newborn will come better measurement and treatment of their pain. This review focusses upon two interconnected nociceptive circuits, the spinal cord dorsal horn and the somatosensory cortex in the brain, to highlight what we know and what we do not know about infant pain. The effectiveness of oral sucrose, widely used in clinical practice to relieve infant pain, is discussed as a specific example of what we do not know. This hot topic highlights the importance of new laboratory based neurophysiological research for the treatment of newborn infant pain.

Introduction

Pain is the most urgent and demanding of all our senses. It is essential for survival but when it persists it can become a significant clinical problem. Individual variability in the nature of pain makes it difficult to measure and treat and this is an particular problem for newborn infants, who not only lack the ability to verbalise their experiences, but also have an immature central nervous system, including pathways and networks involved in somatosensory and emotional processing.

The study of postnatal central pain processing is of both biological and clinical interest, but these interests do not always entirely overlap. The physiologist or neuroscientist wants to know how thoughts, feelings and behaviour arise from activity in individual neurons and neural networks and so understand how pain perception emerges in the developing brain. This information will, they hope, provide a biological basis for treatment of pain in infants in the future. The clinician, on the other hand, faces infants in pain every day in the clinic and, quite reasonably, seeks a reliable measure of infant pain as an output measure in order to trial

effective methods of pain relief and improve clinical practice as soon as possible. All agree that the underlying science is the key.

Studying the spinal cord and brain activity of infants in the Neonatal Intensive Care Unit (NICU) provides a unique opportunity to study the development of human nociceptive circuits. Epidemiological studies report that infants in the NICU are subjected to an average of 11 painful procedures a day, not including the high numbers of failed attempts (up to 15)(Carbajal *et al.*, 2008; Roofthooft *et al.*, 2014). These clinically required procedures can be incorporated into ethically approved investigative studies of CNS pain activity. Pioneering studies of the distinct pain behavioural and physiological responses to such stimuli (Craig *et al.*, 1993) formed the basis of clinical assessment measures and indicators in this field, resulting in greater clinical awareness and improved clinical practice (Maxwell *et al.*, 2013; Lee & Stevens, 2014). Thus the Neonatal Facial Coding System (NFCS), which analyses detailed facial expression in infants following tissue damaging stimuli, provided important and objective evidence of pain in infants at a time when many doubted the ability of infants to perceive pain (Grunau *et al.*, 1998; Ahola Kohut & Pillai Riddell, 2009). An unfortunate side-effect of these studies was a perception among some clinical practitioners of 'job done' and a resistance to healthy scientific questioning which was seen as a form of infant 'pain denial' (Rodkey & Pillai Riddell, 2013).

Recently, the field has turned to exciting new evidence of pain induced activity in the brain of human infants which offer real insight into how pain is processed at higher levels of the newborn CNS (Slater *et al.*, 2006, 2010*c*; Fabrizi *et al.*, 2011; Williams *et al.*, 2015; Goksan *et al.*, 2015). This has provided previously unknown information about how much noxious information reaches the newborn brain and has opened the door to new research into the functional development of early nociceptive circuits in the somatosensory cortex and other brain areas in man. This new field will benefit enormously from the fast moving adult research in this area, but should also learn from the mistakes of the past, avoiding simplistic notions of specific 'pain centres' or 'pain matrices' in the brain (Davis *et al.*, 2015). Before we assume that these novel cortical measurements will produce a new 'read-out' of clinical pain in infants, we need to make sure that we understand what we are doing.

In this review, I discuss the results of recent 'brain' led studies of infant pain, focussing upon the developing connectivity and neural activity which underlie them and which determine pain behaviour and perception in infants. First, spinal and brainstem nociceptive reflexes in the newborn are discussed, and how research on developing nociceptive circuits in laboratory animals has helped us to interpret these behaviours in the newborn. Next, we review the evidence for newborn cortical pain processing, emphasising how increased knowledge of the developmental neurophysiology of cortical nociceptive circuits in laboratory animals is needed to better interpret this data. This is a 'hot topic' of great importance, not only for a fundamental

understanding of how pain is first processed in the human brain but also for developing more sophisticated methods of assessing and treating infant pain in the future.

Infant pain and reflex behaviour

Both preterm (<37 weeks gestational age, GA) and term (≥ 37 weeks GA) newborn infants display clear nociceptive reflexes, measurable as lower limb withdrawal and flexor muscle activity, in response to clinically required tissue damaging stimuli, such as heel lances. In adults, such heel lances are considered painful and excite cutaneous A delta and C fibre nociceptors (Fabrizi *et al.*, 2013*a*). These basic protective reflexes remove an affected region from the source of potential injury and can be quantified in terms of thresholds, amplitude and duration using surface electromyographic (EMG) recordings from flexor muscles. All infants display a robust and long duration flexion reflex (>4 seconds) to a single noxious skin lance, the magnitude of which decreases significantly with gestational age (Cornelissen *et al.*, 2013). In adults, these nociceptive reflexes are evoked by stimulation of small diameter afferent nociceptive fibres and are a reliable and objective reflection of an individual's pain experience (Skljarevski & Ramadan, 2002). We might reasonably expect the same to be true of infants, but only if we assume that the infant nervous system functions in the same way as that of adults. Studying spinal cord pain neurophysiology in infant humans and laboratory rat pups casts important light on this question.

(i) Important differences between adult and infant spinal nociceptive reflexes

The properties of infant nociceptive reflexes differ from those in adults in several important ways. Firstly, the temporal pattern is very different. In healthy adults, the duration of this reflex is relatively short: high intensity cutaneous nerve A delta and C fibre stimulation produces a distinct burst in biceps femoris, which is linearly correlated with pain sensation and returns to baseline 100–120 ms poststimulus (Chan & Dallaire, 1989). Thermal laser stimulation of the foot also elicits a very brief (~100 ms), long latency nociceptive reflex that is well correlated with pricking pain sensation (Willer *et al.*, 1979). In contrast, the infant flexion reflex is remarkably long duration. A single heel lance evokes biceps femoris activity for at least 2–4 seconds, decreasing significantly between the preterm and term period, but remaining longer in duration than adult reflexes (Cornelissen *et al.*, 2013).

Secondly, while this is a purely nociceptive reflex in adults, this is not so in newborn infants, where it can also be evoked by tactile and punctate innocuous skin stimulation. Within individual infant comparisons show that in 29% of preterm infants, tactile stimulation evokes flexor reflex EMG activity that is indistinguishable from noxious stimulation, while in 40% of term infants, tactile responses are also present but significantly smaller than nociceptive reflexes (Cornelissen *et al.*, 2013). Thirdly the spread of the response is much greater in infants such that the contralateral flexion response to noxious stimulation is as large as the ipsilateral response, while in adults it is entirely ipsilateral (Sandrini *et al.*, 2005). Finally, repeated innocuous stimulation

in infants causes a significant increase in reflex magnitude and a drop in threshold, which are the hallmarks of sensitization (Andrews & Fitzgerald, 1994; Cornelissen *et al.*, 2013). In contrast, flexion reflex temporal summation or sensitization occurs in adults only after repeated C fibre or noxious stimulation and is associated with increased pain ratings (Price, 1972).

(ii) Interpreting spinal cord processing of tactile and nociceptive information.

Since newborn laboratory rodents display the same spinal reflex properties as human infants, with lower thresholds, in the innocuous range, greater amplitudes and durations than adult rats, they make an excellent model for investigating the dorsal horn sensory circuits that underlie these behaviours. Numerous anatomical and electrophysiological studies in rat pups have demonstrated that dorsal horn nociceptive circuits differ in early postnatal life from those in adults (Fitzgerald, 2005; Fitzgerald & Walker, 2009). A key feature is the predominant excitation in immature dorsal horn circuits: larger cutaneous excitatory receptive fields (Torsney & Fitzgerald, 2002), mismatched inhibitory receptive fields (Bremner & Fitzgerald, 2008), excitatory tone from the brainstem (Hathway *et al.*, 2009; Schwaller *et al.*, 2015) and pacemaker activity in lamina I cells (Li & Baccei, 2011). Also important is the relatively stronger synaptic input from low and high threshold mechanoreceptor A fibre afferents relative to C fibre inputs and sensitization of dorsal horn cells by repeated tactile or A fibre stimulation in neonates (Koch & Fitzgerald, 2013). The developmental reversal of dorsal horn GABA currents is complete by birth and GABA signalling in newborn spinal cord circuits is inhibitory (Baccei & Fitzgerald, 2004) but notably less targeted to specific cell groups than in adults (Koch *et al.*, 2012). Importantly there is very weak glycinergic inhibitory signalling in the newborn dorsal horn (Baccei & Fitzgerald, 2004; Koch *et al.*, 2012). These findings not only explain why human infant flexion reflexes differ in temporal, modality and spatial characteristics from those in adults, but also reveal the nature of the first stage processing of sensory information that will be transmitted to higher centres in the newborn brain. The newborn dorsal horn facilitates sensory inputs and thereby strengthens activity-dependent synaptic connections, but this is at the cost of reduced sensory discrimination and poor spatial localization (Cornelissen *et al.*, 2013). Increased excitability is traded for selectivity and focus. Reflex magnitude and tactile sensitivity decreases and nociceptive specificity and spatial organisation increases with gestational age.

Does the lack of inhibitory processing at the dorsal horn level mean that infants feel more pain than adults to a defined noxious stimulus? Not necessarily, but it may mean that reflex behaviour in newborn infants is not tightly linked to the input modality: low intensity mechanical stimuli, such as light or firm touch can trigger the same behaviour newborn infants. Reflexes provide an enormously useful gateway into studying the development and plasticity of sensory and motor circuits but cannot be assumed to be a read-out of infant 'pain' behaviour.

Facial expression of pain – a brain stem reflex

Facial expression of pain is a more complex behaviour than lower limb withdrawal, but it is still a spontaneous reflexive reaction to noxious stimuli (Chambers & Mogil, 2015) and, as described above, there is a danger of misinterpreting the effectiveness (or lack of) pain relieving procedures that are based upon infant behaviour without an appreciation of the neural basis of that behaviour and its underlying development. Facial 'pain' reflexes develop early in fetal life in the absence of any noxious stimulation (Reissland *et al.*, 2013) and can continue postnatally in the presence of extensive cerebral white matter damage (Oberlander *et al.*, 2002). While in later life these reflexes become under the control of brain areas responsible for emotions, this is not necessarily so in the newborn.

The neural mechanisms underlying facial expression is especially important when considering the effects of a commonly used analgesic in the newborn, sucrose. Small amounts of oral sucrose (24%) administered to newborn infants before a clinically required tissue breaking procedure, has shown to be a safe and effective way of transiently reducing the behavioural and physiological response to pain (Stevens *et al.*, 2013). The anti-nociceptive effect of sucrose is mediated by the brainstem and persists following midcollicular transection in rats (Anseloni *et al.*, 2005). Indeed physiologists recognise sucrose is an example of ingestion analgesia that functions to defend eating from ending and stops when eating is over. This type of analgesia requires ingestion of hedonic foods: NaCl is an effective analgesic if an animal is sodium depleted (Foo & Mason, 2011).

Does this make sucrose an effective analgesic? In a double-blind, randomised controlled trial of 59 newborn infants undergoing a clinically required heel lance, infants were assigned to receive sucrose or sterilised water(Slater *et al.*, 2010*a*). While observational pain (PIPP) scores were significantly lower in infants given sucrose than in those given sterile water and significantly more infants had no change in facial expression after sucrose administration, as reported previously (Stevens *et al.*, 2013), there was no difference in spinal nociceptive reflex withdrawal activity in the two groups. Furthermore, there was no significant difference in the brain activity evoked by the heel lance, recorded with EEG electrodes (see below), between the sucrose and sterile water groups. Thus oral sucrose acts at the level of the brainstem and does not significantly affect activity in neonatal brain or spinal cord nociceptive circuits, and as such might not be an effective analgesic drug. The ability of sucrose to reduce clinical observational scores after noxious events in newborn infants should not be interpreted as pain relief. While noxious inputs activates both brainstem reflexes and higher cortical areas, such that pain reflexes and pain perception are frequently correlated in adults (Mancini *et al.*, 2015), the two can function independently in infants (Slater *et al.*, 2008, 2010*a*) and need not be causally linked. Understanding the underlying physiology of these different pain responses is therefore essential to assess the efficacy of analgesic management.

Infant pain and the cerebral cortex

While reflex measurements provide interesting insights into the maturation of human nociceptive behaviours, to understand newborn pain we need to know whether the information processed in the spinal cord and brainstem is transmitted centrally to the cerebral cortex. This is a necessary, if not sufficient, requirement for true pain experience. The discovery that noxious sensory information is indeed transmitted to the newborn infant cerebral cortex was demonstrated only a decade ago, using real-time near-infrared spectroscopy, (Slater *et al.*, 2006) and has proved a robust finding.

Haemodynamic measures

Noxious stimulation, in the form of a clinically required heel lance produces a clear cortical response, measured as an increase in total hemoglobin concentration [HbT] in the contralateral somatosensory cortex. This is observed in even the youngest premature infant of 25 weeks gestational age and increases in amplitude with gestational age at study (Slater *et al.*, 2006). Time-locking a heel lance stimulus reveals an predominant response in the contralateral somatosensory cortex (Slater *et al.*, 2006) but longer duration noxious events (venepuncture) are linked also to the ipsilateral cortex and the motor cortex (Bartocci *et al.*, 2006; Bembich *et al.*, 2015), and no doubt many other areas in time, most likely at a longer latency, as would be expected from intracortical connections.

Clinically required, tissue breaking stimuli are hard to adapt to magnetic imaging so fMRI studies have relied on experimental brushing, von Frey hair (vFh) punctate and pinprick stimulation of the skin (Williams *et al.*, 2015; Goksan *et al.*, 2015). In a study of 19 term infants with a mean age of 13 days, distinct patterns of functional brain activation were evoked by brush and vFh punctate stimulation, which were reduced, but still present, under chloral hydrate sedation. Brain activation increased with increasing von Frey hair stimulus intensity (Williams *et al.*, 2015) revealing the ability of the newborn brain to code stimulus intensity. A feasibility study in a single full-term infant (Williams *et al.*, 2015) laid the foundation for a fMRI study of ten newborn infants, which identified the network of brain regions that are active following pinprick stimulation (Goksan *et al.*, 2015). The blood-oxygen-level dependent (BOLD) response in newborn infants was compared to that observed following the same pinprick stimulation in adults and significant infant brain activity was observed in 18 of the 20 active adult brain regions, including primary somatosensory cortices, anterior cingulate cortex (ACC), bilateral thalamus, and all divisions of the insular cortices, but not in the infant amygdala or orbitofrontal cortex. It should be noted that most of these areas are also activated by any salient stimulus (Iannetti & Mouraux, 2010). Several areas were only active in infants: bilateral parietal lobe, pallidum, and precuneus cortex (only contralateral in adults), bilateral auditory cortices, hippocampus, and caudate (Goksan *et al.*, 2015).

What do these BOLD studies from tell us about newborn infant pain? They tell us that information about cutaneous noxious stimulation activates a wide range of brain regions, both cortical and subcortical in newborn infants. Some of these are brain regions that encode sensory and affective components of pain in adults, but others are not. While this data has been interpreted as evidence that the infant pain experience closely resembles that seen in adults (Goksan *et al.*, 2015), it more likely reflects the existence of thalamocortical connections at both ages. The increased bilateral activity and greater number of active regions in infants suggest that these connections are more widespread in the newborn. The known immaturity of cortico-cortical and interhemispheric pathways at this time (Kostović & Jovanov-Milošević, 2006) will have fundamental consequences upon cortical function in the newborn. FMRI studies have been interpreted as measures of conscious sensory perception of pain. While some minimal consciousness and sensory awareness has been convincingly argued in the newborn (Lagercrantz, 2014), brain activity cannot simply be equated with human perception in infants or adults (Ranger & Grunau, 2015). Furthermore, comparative haemodynamic studies need to be interpreted in the context of developing brain (Colonnese *et al.*, 2008). Particularly important is the high energy use in infant brains, correlated to new synaptic connections and cortical thickening, which decreases as the cortex thins, connections are pruned and the brain reaches its mature state (Harris *et al.*, 2011). This is due to energy-consuming synapses, ATP consumption in synthesizing cellular components, and changes in ligand gated receptor function (Harris *et al.*, 2012) all of which will be reflected in the infant BOLD response.

(ii) Electroencephalography

BOLD responses are a surrogate measure of the neuronal activity within the brain. Electroencephalography, on the other hand is a non-invasive neurophysiological method that records the electrical activity of the brain at the scalp, largely from superficial regions, such as the somatosensory cortex, as voltage changes dissipate from deeper subcortical structures over a certain distance. It is possible to observe event-related changes in the EEG traces following certain stimuli including noxious laser, pinprick and heel lance (Zhang *et al.*, 2012; Fabrizi *et al.*, 2013*b*; Iannetti *et al.*, 2013). Recently, studies have identified an event-related potential (ERP) in newborn infants and infants up to one year old, that is specific to noxious stimulation (Slater *et al.*, 2010*c*, 2010*b*; Fabrizi *et al.*, 2011; Verriotis *et al.*, 2015). This consists of an early negative-positive (NP) waveform that is also present following tactile stimulation, which is followed by a second NP waveform (N420-P560) that is only present following noxious stimulation. This ERP is found at central (Cz) and central parietal (CPz) electrodes suggesting that it might be related to the sensory-discriminative aspect of pain that arises in the somatosensory cortex. It should be emphasised that most sensory evoked EEG recordings involve multiple repetitions of the same stimulus to increase the signal to noise and pick out a significant potential. This would be unethical in newborn studies, but in any case are not necessary as a tissue breaking lance can cause a 50µV evoked potential that is easily detectable in a single trial.

Furthermore, EEG recording of noxious-evoked activity in infants of different gestational ages has revealed how the brain begins to distinguish noxious information from innocuous tactile stimulation in early life (Fabrizi *et al.*, 2011). Recording the brain neuronal activity in response to time-locked touches and clinically essential noxious lances of the heel in infants aged 28 to 45 weeks' gestation reveals a transition in brain response following tactile and noxious stimulation from non-specific, evenly dispersed neuronal bursts to modality-specific, localized, evoked potentials. These recordings suggest that the haemodynamic recordings in premature infants could reflect an increase in spontaneous burst responses, which is greater with noxious than tactile stimulation, but that the specific neural circuits necessary to distinguish the qualitative difference between touch and nociception emerge from 35 to 37 weeks' gestation in the human brain (Fabrizi et al., 2011).

What do these EEG studies from tell us about newborn infant pain? They tell us there is a temporal and spatial pattern of brain activity that follows a noxious stimulus in the newborn that can be characterised and which changes with age. They show that infant cortical neurons and networks are directly activated by noxious information and promise to tell us much more in the future about the distributed networks, frequency patterns and coherence of brain activity that underlie pain experience.

How can we discover more about newborn infant pain?

Too much of our current knowledge of infant pain is based upon observations of pain behaviour. While acknowledging the pioneering work on infant pain behaviour, this review has emphasised the importance of understanding developing spinal and brainstem sensory and nociceptive circuits to interpret this behaviour. Failure to appreciate the changing properties of nociceptive spinal and brainstem circuits may lead to behaviour being misinterpreted. The correlation between spinal reflexes and cortical responses evoked by experimental pinprick in individual infants (Hartley *et al.*, 2015) confirm that the spinal cord and cortex are sharing a nociceptive input but failure to appreciate the excitability of neonatal somatosensory circuits, whereby nociceptive behaviours can be triggered by light handling (Cornelissen *et al.*, 2013), could lead to reflex behaviour being misinterpreted as pain in a clinical setting. Much has been learned from comparing infant behaviour with the NIRS or EEG activity recorded from the brain following a clinically required lance. Importantly, some infants show no behavioural reaction to heel lance, but their cortex is strongly activated (Slater *et al.*, 2008), a fact that would be lost in an observational study. Observational behavioural scoring of needle prick pain associated with inoculation proves to be a blunt tool compared to EEG recording which reflects age related and individual differences in cortical pain activity (Verriotis *et al.*, 2015).

The future of this field therefore lies in a better understanding of developing cortical pain circuitry which supports the perception of pain. Currently we have only a rudimentary understanding of infant brain activity

following a noxious stimulus. We are at the beginning of big discoveries in this field and it is privilege to be part of the exploration of the very first processing of pain in the newborn human brain. We are entering the world of distributed networks and dynamic pain connectomes (Kucyi & Davis, 2015) but too much theoretical modelling can be avoided if we turn to animal research to help us to interpret the how, when and why of nociceptive cortical activity in the newborn. To this end, our laboratory is beginning to record nociceptive activity in the rat pup cortex with the aim of contributing to a brain based understanding and treatment of pain in infants in the future (Chang *et al.*, 2015) – this is indeed a hot topic.

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References

Ahola Kohut S & Pillai Riddell R (2009). Does the Neonatal Facial Coding System differentiate between infants experiencing pain-related and non-pain-related distress? *J Pain Off J Am Pain Soc* **10,** 214–220.

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

Anseloni VCZ, Ren K, Dubner R & Ennis M (2005). A brainstem substrate for analgesia elicited by intraoral sucrose. *Neuroscience* **133,** 231–243.

Baccei ML & Fitzgerald M (2004). Development of GABAergic and glycinergic transmission in the neonatal rat dorsal horn. *J Neurosci Off J Soc Neurosci* **24,** 4749–4757.

Bartocci M, Bergqvist LL, Lagercrantz H & Anand KJS (2006). Pain activates cortical areas in the preterm newborn brain. *Pain* **122,** 109–117.

Bembich S, Brovedani P, Cont G, Travan L, Grassi V & Demarini S (2015). Pain activates a defined area of the somatosensory and motor cortex in newborn infants. *Acta Paediatr*n/a – n/a.

Bremner LR & Fitzgerald M (2008). Postnatal tuning of cutaneous inhibitory receptive fields in the rat. *J Physiol* **586,** 1529–1537.

Carbajal R, Rousset A, Danan C, Coquery S, Nolent P, Ducrocq S, Saizou C, Lapillonne A, Granier M, Durand P, Lenclen R, Coursol A, Hubert P, de Saint Blanquat L, Boëlle P-Y, Annequin D, Cimerman P, Anand KJS & Bréart G (2008). Epidemiology and treatment of painful procedures in neonates in intensive care units. *JAMA J Am Med Assoc* **300,** 60–70.

Chambers CT & Mogil JS (2015). Ontogeny and phylogeny of facial expression of pain: *PAIN* **156,** 798–799.

Chan C & Dallaire M (1989). Subjective pain sensation is linearly correlated with the flexion reflex in man. *Brain Res* **479,** 145–150.

Chang P, Walker SM & Fitzgerald M (2015). Neonatal rat primary somatosensory cortical pain activity is resistant to isoflurane anesthesia. *Anesthesiology*.

Colonnese MT, Phillips MA, Constantine-Paton M, Kaila K & Jasanoff A (2008). Development of hemodynamic responses and functional connectivity in rat somatosensory cortex. *Nat Neurosci* **11,** 72–79.

Cornelissen L, Fabrizi L, Patten D, Worley A, Meek J, Boyd S, Slater R & Fitzgerald M (2013). Postnatal Temporal, Spatial and Modality Tuning of Nociceptive Cutaneous Flexion Reflexes in Human Infants. *PloS One*.

Craig KD, Whitfield MF, Grunau RV, Linton J & Hadjistavropoulos HD (1993). Pain in the preterm neonate: behavioural and physiological indices. *Pain* **52,** 287–299.

Davis KD, Bushnell MC, Iannetti G, St. Lawrence K & Coghill R (2015). Evidence against pain specificity in the dorsal posterior insula. *F1000Research*; DOI: 10.12688/f1000research.6833.1.

Fabrizi L, Slater R, Worley A, Meek J, Boyd S, Olhede S & Fitzgerald M (2011). A shift in sensory processing that enables the developing human brain to discriminate touch from pain. *Curr Biol CB* **21,** 1552–1558.

Fabrizi L, Williams G, Lee A, Meek J, Slater R, Olhede S & Fitzgerald M (2013*a*). Cortical activity evoked by an acute painful tissue-damaging stimulus in healthy adult volunteers. *J Neurophysiol*; DOI: 10.1152/jn.00990.2012.

Fabrizi L, Williams G, Lee A, Meek J, Slater R, Olhede S & Fitzgerald M (2013*b*). Cortical activity evoked by an acute painful tissue-damaging stimulus in healthy adult volunteers. *J Neurophysiol* **109,** 2393–2403.

Fitzgerald M (2005). The development of nociceptive circuits. *Nat Rev Neurosci* **6,** 507–520.

Fitzgerald M & Walker SM (2009). Infant pain management: a developmental neurobiological approach. *Nat Clin Pract Neurol* **5,** 35–50.

Foo H & Mason P (2011). Ingestion analgesia occurs when a bad taste turns good. *Behav Neurosci* **125,** 956–961.

Goksan S, Hartley C, Emery F, Cockrill N, Poorun R, Moultrie F, Rogers R, Campbell J, Sanders M, Adams E, Clare S, Jenkinson M, Tracey I & Slater R (2015). fMRI reveals neural activity overlap between adult and infant pain. *eLife* **4,** e06356.

Grunau RE, Oberlander T, Holsti L & Whitfield MF (1998). Bedside application of the Neonatal Facial Coding System in pain assessment of premature neonates. *Pain* **76,** 277–286.

Harris JJ, Jolivet R & Attwell D (2012). Synaptic Energy Use and Supply. *Neuron* **75,** 762–777.

Harris JJ, Reynell C & Attwell D (2011). The physiology of developmental changes in BOLD functional imaging signals. *Dev Cogn Neurosci* **1,** 199–216.

Hartley C, Goksan S, Poorun R, Brotherhood K, Mellado GS, Moultrie F, Rogers R, Adams E & Slater R (2015). The relationship between nociceptive brain activity, spinal reflex withdrawal and behaviour in newborn infants. *Sci Rep* **5,** 12519.

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* **587,** 2927–2935.

Iannetti GD, Baumgärtner U, Tracey I, Treede R-D & Magerl W (2013). Pinprick-evoked brain potentials (PEPs): a novel tool to assess central sensitisation of nociceptive pathways in humans. *J Neurophysiol*; DOI: 10.1152/jn.00774.2012.

Iannetti GD & Mouraux A (2010). From the neuromatrix to the pain matrix (and back). *Exp Brain Res* **205,** 1–12.

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

Koch SC, Tochiki KK, Hirschberg S & Fitzgerald M (2012). C-fiber activity-dependent maturation of glycinergic inhibition in the spinal dorsal horn of the postnatal rat. *Proc Natl Acad Sci U S A* **109,** 12201–12206.

Kostović I & Jovanov-Milošević N (2006). The development of cerebral connections during the first 20–45 weeks' gestation. *Semin Fetal Neonatal Med* **11,** 415–422.

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

Lagercrantz H (2014). The emergence of consciousness: Science and ethics. *Semin Fetal Neonatal Med* **19,** 300–305.

Lee G & Stevens B (2014). Neonatal and infant pain assessment. In *Oxford Textbook of Paediatric Pain*, Eds PJ McGrath, BJ Stevens,SM Walker, WM Zempsky., pp. 353–369. OUP.

Li J & Baccei ML (2011). Pacemaker neurons within newborn spinal pain circuits. *J Neurosci Off J Soc Neurosci* **31,** 9010–9022.

Mancini F, Beaumont A-L, Hu L, Haggard P & Iannetti GD (2015). Touch inhibits subcortical and cortical nociceptive responses. *Pain*; DOI: 10.1097/j.pain.0000000000000253.

Maxwell LG, Malavolta CP & Fraga MV (2013). Assessment of pain in the neonate. *Clin Perinatol* **40,** 457–469.

Oberlander TF, Grunau RE, Fitzgerald C & Whitfield MF (2002). Does parenchymal brain injury affect biobehavioral pain responses in very low birth weight infants at 32 weeks' postconceptional age? *Pediatrics* **110,** 570–576.

Price DD (1972). Characteristics of second pain and flexion reflexes indicative of prolonged central summation. *Exp Neurol* **37,** 371–387.

Ranger M & Grunau RE (2015). How do babies feel pain? *eLife* **4,** e07552.

Reissland N, Francis B & Mason J (2013). Can healthy fetuses show facial expressions of "pain" or "distress"? *PloS One* **8,** e65530.

Rodkey EN & Pillai Riddell R (2013). The infancy of infant pain research: the experimental origins of infant pain denial. *J Pain Off J Am Pain Soc* **14,** 338–350.

Roofthooft DWE, Simons SHP, Anand KJS, Tibboel D & van Dijk M (2014). Eight years later, are we still hurting newborn infants? *Neonatology* **105,** 218–226.

Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G & Willer JC (2005). The lower limb flexion reflex in humans. *Prog Neurobiol* **77,** 353–395.

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

Skljarevski V & Ramadan NM (2002). The nociceptive flexion reflex in humans -- review article. *Pain* **96,** 3–8.

Slater R, Cantarella A, Franck L, Meek J & Fitzgerald M (2008). How well do clinical pain assessment tools reflect pain in infants? *PLoS Med* **5,** e129.

Slater R, Cantarella A, Gallella S, Worley A, Boyd S, Meek J & Fitzgerald M (2006). Cortical pain responses in human infants. *J Neurosci Off J Soc Neurosci* **26,** 3662–3666.

Slater R, Cornelissen L, Fabrizi L, Patten D, Yoxen J, Worley A, Boyd S, Meek J & Fitzgerald M (2010*a*). Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. *Lancet* **376,** 1225–1232.

Slater R, Fabrizi L, Worley A, Meek J, Boyd S & Fitzgerald M (2010*b*). Premature infants display increased noxious-evoked neuronal activity in the brain compared to healthy age-matched term-born infants. *NeuroImage* **52,** 583–589.

Slater R, Worley A, Fabrizi L, Roberts S, Meek J, Boyd S & Fitzgerald M (2010*c*). Evoked potentials generated by noxious stimulation in the human infant brain. *Eur J Pain Lond Engl* **14,** 321–326.

Stevens B, Yamada J, Lee GY & Ohlsson A (2013). Sucrose for analgesia in newborn infants undergoing painful procedures. *Cochrane Database Syst Rev* **1,** CD001069.

Torsney C & Fitzgerald M (2002). Age-dependent effects of peripheral inflammation on the electrophysiological properties of neonatal rat dorsal horn neurons. *J Neurophysiol* **87,** 1311–1317.

Verriotis M, Fabrizi L, Lee A, Ledwidge S, Meek J & Fitzgerald M (2015). Cortical activity evoked by inoculation needle prick in infants up to one-year old. *Pain* **156,** 222–230.

Willer JC, Boureau F & Berny J (1979). Nociceptive flexion reflexes elicited by noxious laser radiant heat in man. *Pain* **7,** 15–20.

Williams G, Fabrizi L, Meek J, Jackson D, Tracey I, Robertson N, Slater R & Fitzgerald M (2015). Functional magnetic resonance imaging can be used to explore tactile and nociceptive processing in the infant brain. *Acta Paediatr Oslo Nor 1992* **104,** 158–166.

Zhang ZG, Hu L, Hung YS, Mouraux A & Iannetti GD (2012). Gamma-band oscillations in the primary somatosensory cortex--a direct and obligatory correlate of subjective pain intensity. *J Neurosci Off J Soc Neurosci* **32,** 7429–7438.