The development of the nociceptive brain

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Abstract

This review addresses the fundamental question of how we first experience pain, at the beginning of our lives. The brain is activated by peripheral tissue damaging stimulation from birth, but unlike other sensory systems, the pain system in healthy individuals cannot rely upon a prolonged activity dependent shaping through repeated noxious stimulation. Considering the importance of pain, remarkably little is known about when and how the nociceptive cortical network activity characteristic of the mature adult brain develops. We begin this review by considering the underlying framework of connections in the infant brain. Since this developing brain connectome is necessary, if not sufficient, for pain experience, we discuss the structural and functional development of cortical and subcortical networks that contribute to this network. We then review specific information on the development of nociceptive processing in the infant brain, considering evidence from neurophysiological and haemodynamic measures separately, as the two are not always consistent. Finally we highlight areas that require further research and discuss how information gained from laboratory animal models will greatly increase our understanding in this area.

Introduction

Pain has many dimensions and is processed at multiple different levels of the nervous system but how these processes develop in the newborn is incompletely understood (Fitzgerald, 2005, 2015). When tissue is injured, nociceptive pathways in the peripheral and central nervous system (CNS) trigger essential behaviours, mediated by reflex motor circuits in the spinal cord and brainstem, to ensure that the body is protected from further harm. In addition, brainstem and hypothalamic autonomic circuits are activated, altering the cardiovascular, respiratory, and endocrine systems to maintain homeostatic control of the body. However for the tissue injury to cause pain, the sensory discriminative, cognitive-evaluative, and motivational-affective components of the painful sensation must be encoded in the brain, creating the uniquely unpleasant and stressful qualities that define pain and suffering. While we have learned much about newborn behavioural and autonomic reactions to noxious stimulation, key questions in neuroscience remain as to when and how the brain develops the ability to encode noxious stimuli and create the experience of pain.

Noxious information is not processed in the brain in the same way as other sensory modalities. There is no dedicated **primary 'pain' cortex** analogous to the primary somatosensory or visual cortices; rather, noxious stimulation evokes a diffuse pattern of activity in many brain areas, including primary (SI) and secondary (SII) somatosensory cortices, anterior and mid cingulate cortex (ACC/MCC), insular cortex, amygdala, and regions of the prefrontal cortex (PFC). Traditionally, the somatosensory cortices (SI and SII) are proposed to play a role in the sensory-discriminative aspect of pain (Vierck et al., 2013), while the anterior cingulate cortex (ACC), insular cortex, and amygdala are associated with the affective-motivational components and the prefrontal cortex with the cognitive-evaluative components (Bushnell et al., 2013; Nakata et al., 2014; Veinante et al., 2013). **This network, previously referred to as the 'pain matrix', was thought to** provide a unique representation of the intensity and unpleasantness of the perception elicited by a nociceptive stimulus (Apkarian et al., 2005; Tracey and Mantyh, 2007), but this view has been challenged (Legrain et al., 2011). The current view of pain is that it arises from a distributed network of brain activity, none of which is unique to pain, but when coordinated or synchronised results in the sensory, emotional, motivational, and cognitive experience that is pain. Other brain regions may be recruited to exacerbate or reduce the dimensions of intensity and unpleasantness (Tracey and Johns, 2010). **Anatomical 'pain centres' or 'pain matrices' in the brain have been replaced by the concept of** the **'dynamic pain connectome'** and it is now recognized that the conscious experience of pain arises from a dynamic change in a distributed network of brain activity (Davis et al., 2015; Kucyi and Davis, 2015; Mano and Seymour, 2015; Woo et al., 2015).

Consistent with these findings, fMRI studies in adult rodents also display a distributed pattern of pain related activity across the brain (Borsook and Becerra, 2011; Thompson and Bushnell, 2012) and stimulation of the

hindpaw with noxious electrical or mechanical stimulation elicits BOLD changes in the primary sensory cortex, anterior cingulate cortex, septal nucleus, and retrosplenial cortex as well as subcortical regions (Amirmohseni et al., 2015). Furthermore, incisional and inflammatory induced hyperalgesia in adult rodents causes normally innocuous stimulation to engage pain related brain regions (such as anterior cingulate cortex and periaqueductal grey) beyond the sensory areas (Amirmohseni et al., 2015), and widespread changes within the somatosensory and cingulate cortices and subcortically within the thalamus and the periaqueductal grey are reported in neuropathic pain models (Hubbard et al., 2015).

How and when this complex brain network develops to encode noxious stimuli and create the experience of pain is an important area of current research. This information has clear clinical implications for devising analgesic strategies in hospitalised newborn infants, tailored to the developmental stage of the individual. It is also important for understanding how noxious input at an early stage of development might affect the development of the nociceptive system. For instance, sensory systems and their associated perceptive abilities are established during specific developmental time windows called "critical periods", during which deprivation of normal external inputs or disruption of physiological neuronal activity causes long lasting breakdown of sensory cortical maps and sensory impairment. While this phenomenon has been well characterised in animal models for the visual, auditory, and somatosensory systems (Hensch, 2004), it is difficult to define a critical period for nociception because of the absence of a primary nociceptive cortex and because such stimuli are already normally absent during development. Nevertheless, both animal models and clinical studies have shown that early exposure to noxious procedures causes long term alterations of pain perception and brain function and structures (Ranger and Grunau, 2014; Schwaller and Fitzgerald, 2014; Vinall and Grunau, 2014; Walker, 2013; Walker et al., 2016).

The pain system in healthy individuals cannot rely upon prolonged activity dependent shaping of connections through repeated patterns of noxious sensory stimulation; pain processing in the newborn infant brain must, to some extent, rely on an existing set of brain connections. We assume that the developing brain connectome is necessary, if not sufficient, for our first pain experience and so we discuss the structural and functional development of cortical and subcortical networks that participate in both intrinsic and somatosensory activity in the developing brain. We then review the evidence for specific, nociceptive evoked activity in the infant brain, separating evidence from neurophysiological and haemodynamic measures, as the two are not always consistent. Finally we discuss how information gained from laboratory animal models will increase our knowledge in this area.

Laying down a framework for nociception in the infant brain

The maturation of nociceptive brain function is likely to be underpinned by the development of the brain areas that will go on to form the adult nociceptive network. No study has addressed the development of this

network as an ensemble, so here we review evidence related to the emergence of function of the relevant brain areas over the last trimester of gestation. Figure 1 summarises the timeline of key developmental events in the human brain that lay down the frameworks for nociceptive processing.

Structural development

The somatosensory pathways signalling stimulation of the body, that is the sensory receptors, afferent connections to the spinal cord, and spinal sensory connections, emerge well before the third trimester of gestation, with connections to the thalamus in place by 20 weeks GA (Lowery et al., 2007). These will not be reviewed here as the focus is on the development of brain structures and connections that could underlie cortical processing of external inputs in the premature and term neonatal brain.

A key feature of the foetal brain is the subplate, a transient brain structure with a key role in cortical development (Kanold and Luhmann, 2010). In humans, this forms at 12-18 weeks and is at its maximal thickness (up to 4 times that of the cortical plate) between 22 and 34 weeks GA, after which it begins to disappear **(Kostovic and Rakic, 1990; Kostović and Judaš, 2002)**. In the primary somatosensory cortex, the subplate disappears by the first postnatal month, although traces of it can still be found at 6 months (Kostovic and Rakic, 1990); by contrast, its disappearance in the prefrontal cortex is more gradual over 6 postnatal months, probably related to protracted cortical development such as growth of short cortico-cortical pathways in this region **(Kostović and Jovanov-Milošević, 2006; Kostović and Judaš, 2010)**.

One of the roles of the subplate is to guide afferent input from the thalamus, basal forebrain, and cortex to their appropriate cortical targets. This is important as the presence of thalamocortical connections is considered a requirement for cortical processing of external inputs to occur. Thalamocortical afferents begin to reach the superficial subplate of sensory and associative cortices by 20-22 weeks GA and the cortical plate by 23-26 weeks, and develop at a similar time frame in all sensory cortices (Hevner, 2000; Kostovic and Goldman-Rakic, 1983; Kostovic and Rakic, **1984; Kostović and Judaš, 2002, 2010)**. The immaturity of thalamocortical connections prior to 25 weeks GA suggests that cortical processing of external input is unlikely in infants younger than 25 weeks; at this stage, infants may be considered capable of endogenous spontaneous activity only **(Kostović and Judaš, 2010)**. Migration of neurons from the subplate to the cortical plate begins at around 24 weeks and continues to occur throughout the third trimester, with neurons in place by term age (Burkhalter et al., **1993; Kostović and Judaš, 2010)**. The first synapses appear in the deep part of the cortical plate between 26 and 28 weeks GA, in parallel with the arrival to the cortical plate of the thalamic afferents **(Kostović and Jovanov-Milošević, 2006; Kostović and Judaš, 2010)**. At this age, evoked potentials can also be recorded from SI, visual & auditory cortices (Vanhatalo and Kaila, 2006; Vanhatalo and Lauronen, 2006), as described in greater detail below. Gyrification develops rapidly during the third trimester of gestation (Dubois et al., 2008) and the six-layered laminar structure of the cortex is fully

apparent from 32 weeks gestational age (GA) **(Burkhalter et al., 1993; Kostović and Judaš**, 2010). At this point, thalamocortical axons begin to synapse onto layer IV neurons **(Kostović and Judaš, 2010)**. Growth of interhemispheric callosal connections occurs from 35 weeks GA and both these and intra-hemispheric associative connections continue to mature at term **(Kostović and Judaš, 2010)**. The relocation of afferent fibres from the subplate to the cortical plate and the growth of callosal and long associative connections contribute to an increase in cortical grey matter of 50% between 31 weeks GA and term **(Kostović and Judaš,** 2010).

Much less is known about the parallel development of limbic structures that process "emotional" responses, such as the amygdala. The amygdala and other parts of the limbic system are already distinguishable at 6-7 weeks GA **(Müller and O'Rahilly, 2006)** which suggests a much earlier maturation than the cortex. An anatomical study using KCC2 expression to map the relative maturation of different brain areas (Sedmak et al., 2015) suggests that the amygdala, cerebellar cortex, and the mediodorsal nucleus of the thalamus mature first (10–13 weeks GA) followed by the hippocampus, striatum, and the motor cortex (16–19 weeks), the majority of neocortical areas (at 19–24 weeks) and finally the dorsolateral frontal cortex, and primary visual cortex (24 weeks). However caution must be taken in interpreting these expression studies as evidence of function. Despite its early formation, the punctate GAP-43 (growth associated protein) immunostaining that is characteristic of synaptogenesis does not appear in the amygdala until 20 weeks, peaking at 28 weeks and complete at 36 weeks (Ulfig et al., 2003), suggesting that the anatomical framework for limbic and cortical sensory areas may develop in parallel.

Therefore, by term age, much of the anatomical framework for cortical processing of external input, including thalamocortical and cortico-cortical connections, is present. Next we consider the evidence for these connections being functional.

The infant brain connectome

Over the last trimester of gestation the functional and anatomical macroscopic brain networks undergo substantial maturation leading to an almost adult-like arrangement at term, which further develops postnatally throughout childhood and adolescence (Hagmann et al., 2010; Hwang et al., 2012; Khundrakpam et al., 2013). These changes can be monitored in humans with non-invasive brain imaging techniques such as Diffusion Tensor Imaging (DTI, anatomical) and functional connectivity MRI (fcMRI, functional). Anatomically, the small-world modular architecture characteristic of the adult brain is already present at 30 weeks gestational age (GA), but strengthen until term age (Brown et al., 2014; van den Heuvel et al., 2014). Over the same period white matter microstructures become more refined and delineated (increase in fractional anisotropy (FA) and decrease in mean diffusivity (MD) and transverse diffusivity (TD), implying a

more efficient signal transmission (van den Heuvel et al., 2014; Hüppi et al., 1998). This is more evident for associative tracts just below the cortex than for commissural and deep projections from the thalamus and between hemispheres, which are mostly already formed (Neil et al., 1998; Partridge et al., 2004), but is weaker in premature born infants studied at term compared to term born infants (Anjari et al., 2007), resulting in impaired thalamocortical connectivity, particularly to the prefrontal cortex, but also, within other regions, to bilateral insula and middle cingulate (Ball et al., 2013a). Moreover, cortical gyration, decrease in FA, and increase in grey matter volume, possibly representing (i) the arborization of basal dendrites, (ii) the arrival to the cortical plate of thalamocortical and corticocortical connections, and (iii) synaptogenesis, occur earlier in the perirolandic than in the associative prefrontal cortex and parieto-temporal areas (Ball et al., 2013b; Delpolyi et al., 2005; Dubois et al., 2008; Mewes et al., 2006). Together these results indicate that while thalamic inputs can reach the somatosensory cortex as early as 28-30 weeks gestational age, and while this area is already quite mature at this stage, the capability of the brain to integrate information develops over the last trimester of gestation in parallel with the development of the prefrontal and temporal cortices. The premature brain also presents an anatomical hub structure and in particular a rich-club topology, where a few specific nodes are involved in the majority of the connections and are also strongly interlinked with each other. Interestingly many of the centres of the nociceptive brain network are part of this rich-club: the left superior frontal cortex and left lateral orbitofrontal gyrus (part of the prefrontal cortex), left postcentral gyrus (primary somatosensory cortex), bilateral cingulate gyrus and bilateral insula (Ball et al., 2014; van den Heuvel et al., 2014).

While DTI provides information about the anatomical aspects of the developing human connectome, fcMRI offers an insight into its functional features. Functional brain networks can be studied in the absence of a task, when the brain is 'at rest', and such studies find that multiple brain regions that are known to be functionally related in the performance of a task are also temporally synchronised at rest. Several such 'resting state networks' have been identified in adults. This approach has also proved useful in assessing functional connectivity in the newborn brain, where resting state networks are both present (Fransson et al., 2007, 2009) and adult-like by term age (Doria et al., 2010), indicating that long-range functional connectivity is already operational by the time of birth and parallel the anatomical development (van den Heuvel et al., 2014). Such networks include the somatosensory network and are not limited to primary areas, encompassing also the default mode and executive control networks (Doria et al., 2010). Furthermore, most of the resting state networks identified in adults can also be detected in preterm infants as young as 26 weeks GA (Doria et al., 2010; Smyser et al., 2010). Most of these are fragmented at 30 weeks GA, although the auditory and visual resting state networks already appear mature at that time (Doria et al., 2010). The thalamus contributes to somatosensory, auditory, motor, and salience networks at term (Alcauter et al.,

2014; Doria et al., 2010), again indicating functional thalamocortical connections by this age, although they continue to develop until at least 2 years of age (Alcauter et al., 2014).

Importantly for this review, the salience network identified in term infants includes the bilateral anterior insula, anterior cingulate cortex, and bilateral prefrontal cortex, while the somatosensory network includes the primary somatosensory cortex (Alcauter et al., 2014). This indicates that at least four of the structures that are implicated in nociceptive processing participate in functional brain networks by term age.

Functional development of the somatosensory cortex

The changes in anatomical and functional brain networks described above are accompanied by the development of specific sensory evoked activity in the somatosensory cortex, and these are illustrated in Figure 2. The somatosensory cortex has received particular attention in human and animal studies because it is relatively easy to engage in task-based experiments. It is relevant here because it is likely to play a key role in early life nociceptive brain activity. Analysis of the development of somatosensory cortical activity evoked by non-noxious tactile or low intensity electrical stimulation has been provided by electroencephalography (EEG) and magnetoencephalography (MEG) measurements in infants. Electrical or mechanical stimulation of newborn infant limbs suggests activation of the infant somatosensory cortex characterised by an early component (Somatosensory Evoked Potential, SEP) and a late component (Event Related Potential, ERP) (see Figure2). The SEPs, occurring less than 100 ms post stimulus, are the most studied as they seem to provide valuable prognostic information of long term neurological outcome (Klimach and Cooke, 1988; Pike and Marlow, 2000; Willis et al., 1989). Their topographical distribution and latency suggest that they are generated by the primary somatosensory cortex: the scalp appearance is consistent with a contralateral frontal-occipital positive-negative gradient when stimulating the hand (van den Heuvel et al., 2014; Lauronen et al., 2006; Nevalainen et al., 2015; Vanhatalo and Lauronen, 2006) and follows the expected somatotopic representation of the stimulated site, being more lateral or central when stimulating the upper or lower limbs respectively (Hrbek et al., 1968). The SEPs are present from early prematurity, decrease in latency with maturation and become sharper and larger in amplitude (Hrbek et al., 1973; Karniski et al., 1992; Taylor et al., 1996; Tombini et al., 2009). In adults, cortical SEP latencies are compatible with the conduction velocity of the peripheral fibers stimulated, which in turn depends on their diameter and myelination (Mauguiere, 2004). Selective electrical stimulation of large diameter myelinated Aβ (30-65 m/s (Vallbo et al., 1979) evokes a faster response (20 msec when stimulating the medial nerve (Mauguiere, 2004) compared to painful laser stimulation of small myelinated Aδ (4-30 m/s; response at 160 msec (Treede et al., 1988) or unmyelinated C fibers (0.4-1.8 m/s; response at 1000 msec (Bromm and Treede, 1987). The decrease in latency and sharpening (and consequently increase in amplitude) of the SEPs during development is therefore likely to mirror the increase in myelination and the decreased synaptic delay

throughout the somatosensory nervous system (Brody et al., 1987; Cracco et al., 1979; Gutrecht and Dyck, 1970). This is confirmed by the parallel decrease in the peripheral and central conduction time estimated by comparing the latency between the stimulus, the nuchal (or cervical) N13 (arising from the cervico-medullary junction) and the SEP (Bongers-Schokking et al., 1990; Trollmann et al., 2010). This decrease in latency is not affected by postnatal age (Tombini et al., 2009; Trollmann et al., 2010) but the amplitude of this component is smaller in ex-premature infants studied at term compared to full-term controls, suggesting an environmental influence (Tombini et al., 2009). At term the early SEP response is followed by a slower NP (negative-positive) ERP at 200 – 500 ms post stimulus, which is maximal at the vertex (Fabrizi et al., 2011; Hrbek et al., 1973; Nevalainen et al., 2015). The source of this activity is less clear, but bilateral secondary somatosensory cortex has been suggested (Nevalainen et al., 2015). This is much slower and wider in premature infants and is most prominent over the contralateral hemisphere (but also appears at the vertex and ipsilaterally) and could represent an immature delta brush following the initial SEP (Fabrizi et al., 2011; Milh et al., 2007), rather than an event generated by associative cortices (Hrbek et al., 1973). Evidence suggests that the initial SEP and the late ERP have distinct neuronal generators and therefore characterise different stages of the somatosensory processing: (i) different topographies (Karniski et al., 1992); (ii) SEPs are larger during active sleep, while ERPs during quiet sleep (Hrbek et al., 1968, 1973; Pihko et al., 2004); (iii) different rates of latency decrease with maturation (Karniski et al., 1992). This hypothesis is supported by the developmental trajectory of the hemodynamic responses to proprioception assessed with fMRI. A positive blood oxygen level–dependent functional response is consistently present from 30 weeks GA in the contralateral SI (Allievi et al., 2015; Arichi et al., 2012). This response becomes more localized and also extends to the ipsilateral SI and sensori-motor associative areas (including SII) in the following weeks (Allievi et al., 2015; Arichi et al., 2012; Erberich et al., 2006; Heep et al., 2009). Clear activation of the primary somatosensory cortex following vibration stimulation in term neonates has also been reported using multichannel NIRS (Shibata et al., 2012).

The development of nociceptive activity in the human infant brain

The evidence above suggests that the newborn brain contains the framework of connections required for somatosensory input from thalamic and other subcortical nuclei to be processed. As discussed earlier, we propose that brain processing of the first, postnatal externally applied noxious inputs requires a functional somatosensory framework. In this section we discuss the evidence for such early life noxious stimuli generating specific nociceptive activity in the human infant brain and the nature of this activity. The pattern of this activity is illustrated in Figure 2.

Neurophysiological evidence

Nociceptive related brain activity can be studied non-invasively in human neonates using scalp electroencephalography (EEG). This technique records ongoing and stimulus related electrical brain activity at different scalp locations providing an overall topographical representation of cortical neuronal activity. In adults, these recordings can be conducted in a controlled research environment and in response to experimental stimulation, such as cutaneous laser, mechanical and electrical stimuli, allowing for a tailored characterization of the effect of various peripheral stimulus parameters on the cortical responses and verbal report of the subjects (Baumgärtner et al., 2012). In neonates, EEG recordings have to be conducted in concurrence with clinically-required procedures due to regulatory and ethical considerations in the challenging neonatal care environment (Worley et al., 2012). Most of the studies to date have taken advantage of acute procedures such as heel lance and inoculation. These interventions provide an almost instantaneous event to which the EEG recording can be synchronised, enabling study of how the developing brain processes procedural pain.

Heel lance in term infants evokes a characteristic waveform made of two consecutive event related potentials (ERPs) maximal at the vertex (Slater et al., 2010a). The first ERP ('tactile') occurs between 100-400 ms post stimulation and can also be evoked just by tapping the heel and therefore is likely to represent a sensory response generated by the non-noxious aspects of the lance; the second ERP ('nociceptive') occurs between 300-750 ms and is only present following skin breaking stimulation. It is possible that these ERPs are preceded by an SEP similar to that evoked by somatosensory stimulation, although this has not yet been reported. These results suggest that the brain of a term infant is able to discriminate innocuous and noxious stimuli and processes separately the somatosensory and nociceptive information related to a skin breaking event. The long latencies indicate that these ERPs are not a direct readout of the incoming afferent volley, but because of the limited number of electrodes that can be placed on the scalp of a neonate it is not possible to estimate the sources of these signals. Nevertheless, nociceptive ERPs obtained using experimental non-tissue damaging graded pinpricks are correlated in amplitude with the pressure of the mechanical stimulus and the elicited withdrawal reflex, suggesting an encoding of the peripheral noxious stimulus intensity (Hartley et al., 2015). On the other hand, the nociceptive ERP does not seem to be related to activity in somatic motor and autonomic circuits underlying the behavioural and physiological response to procedural pain in neonates, as sucrose administration reduces this activity, but not the nociceptive ERP (Slater et al., 2010b).

Even if they share a similar topographical distribution, the tactile and nociceptive ERPs are likely to reflect different cortical generators and mechanisms as the tactile ERP is affected by the vigilance state of the subject while the nociceptive ERP is not (Slater et al., 2010a). Moreover, term infants born prematurely

have a larger nociceptive, but not tactile, ERP compared to control full-term infants, indicating that the experience in the neonatal intensive care unit has affected their nociceptive pathways, but not their tactile circuits (Slater et al., 2010c). It is important to note, however, that tactile sensitivity is high in the newborn infant and withdrawal reflexes can be easily evoked by non-noxious stimulation, particularly when repeated, causing increased reflex magnitude or 'wind-up'(Cornelissen et al., 2013). It is not yet known whether this windup is reflected in the tactile ERP, or whether this property is restricted to tactile activity at the spinal cord level (Koch and Fitzgerald, 2013). It would also be interesting to know whether the domination of A fibre versus C fibre nociceptive input at early ages, as described in the developing rodent (Fitzgerald, 2005), impacts upon the human preterm ERP profile.

These specific patterns and the associated ability to discriminate between different sensory modalities develop over the last trimester of gestation. Infants at 28 weeks gestational age (GA) respond to an innocuous and a noxious stimulus with similar non-specific neuronal activity bursts, or 'delta brushes', while modality specific ERPs become more prevalent only around 35-37 weeks GA, which is just before an infant would normally be born (Fabrizi et al., 2011). Nevertheless noxious stimulation is more likely to trigger these bursts than innocuous stimulation in premature infants, suggesting that even in the absence of any specific discrimination of stimulus modality the very immature brain is already capable of encoding stimulus intensity.

The tactile and nociceptive ERPs are still both present at one year of age following routine inoculation, indeed the nociceptive ERP is significantly larger in one year olds than in younger infants (Verriotis et al., 2015). As for the SEP, the decrease in latency of the early ERP is likely to be related to synaptic refinement and myelination processes occurring during development. Importantly, while the tactile response is still present in adulthood, with shorter latencies, the nociceptive component disappears (Fabrizi et al., 2013). This response is therefore characteristic of the early postnatal period and it is not known when, how, or why it disappears. The later response, instead, could be specific to developing networks and related to the experience dependent formation of the nociceptive pathways, alternatively it could represent a core nociceptive response that is increasingly masked by other complex patterns of brain activity, such as filtering by expectations of pain (Lobanov et al., 2014), as brain function matures.

Importantly the human infant brain encodes noxious information with different neuronal patterns compared to adults (Fabrizi et al., 2016). Comparing EEG responses to the same time-locked noxious skin lance in newborn infants and adults using time-frequency analysis reveals that while some features of adult nociceptive network activity are present in infants at longer latencies, including beta-gamma oscillations, infants display a distinct, long latency, noxious evoked 18-fold energy increase in the fast delta band (2-4 Hz) that is absent in adults (Fabrizi et al., 2016). The differences in activity between infants and adults have a

widespread topographic distribution across the brain indicating further important postnatal changes in the encoding of mechanical pain in the human brain that we still need to understand.

Haemodynamic evidence

The development of nociceptive cortical activity can also be studied non-invasively using techniques such as near-infrared spectroscopy (NIRS) and functional magnetic resonance imaging (fMRI). These techniques provide an indirect measure of neuronal activation by monitoring changes in blood flow within the brain in response to a stimulus. Such haemodynamic changes reflect the balance between the increased oxygen consumption of activated cells and the ensuing increase in cerebral blood flow (CBF) that brings more oxygenated blood to the activated region. The advantage of such techniques is their potential for greater spatial localisation of the cortical response than typically possible with EEG. Therefore, techniques based on haemodynamic measures can provide complementary information about brain function.

Although both techniques measure haemodynamic activity, they have important complementary differences and are therefore both useful in their own right. For instance, NIRS has greater tolerance for movement and can be applied at the cotside to awake, non-sedated babies, eliciting a clinically relevant response. Furthermore, NIRS enables measurement of changes in both oxygenated and deoxygenated haemoglobin, which is important in infant research, and has potentially higher temporal resolution than fMRI (Lloyd-Fox et al., 2010; Safaie et al., 2013). On the other hand, fMRI provides greater spatial localisation than NIRS, and can provide information about both cortical and subcortical regions, unlike NIRS which is limited to more superficial areas of cortex with a maximum penetration depth typically less than 2cm in infant studies.

It is important to note that maturational changes in both energy use and neurovascular coupling during development make interpretation of haemodynamic changes more challenging in the developing brain, particularly when comparisons are made across different ages. For instance, a greater increase of neural activity evoked energy use relative to blood flow may underlie the appearance of inverted haemodynamic responses in infants and young children, compared with neonates and adults; furthermore, it is plausible that there is some stage at which these might cancel each other out such that a haemodynamic response is not detectable but does not imply lack of neuronal activation (Harris et al., 2011). Here we focus on the neonatal brain where inverted haemodynamic responses are not typically observed; however other developmental changes could influence haemodynamic responses. Harris et al., advocate the construction of a database containing haemodynamic responses to standard stimuli at key developmental stages, particularly within reasonably well understood 'low level' neural circuits (such as visual and SI cortices), to improve our understanding of developmental changes in, and interpretation of, haemodynamic responses. We suggest here that simultaneous recordings of haemodynamic and electrophysiological responses will

further improve our understanding of haemodynamic responses in the developing brain (Verriotis et al., 2016).

NIRS studies

As in EEG studies, ethical considerations mean that most NIRS studies of infant cortical responses to noxious stimuli have taken advantage of acute, clinically required skin-breaking procedures. Specifically, studies have focussed on heel lance (Bembich et al., 2015; Slater et al., 2006; Verriotis et al., 2016), venepuncture (Bartocci et al., 2006), and chest drain removal (Ranger et al., 2013), and have reported changes in oxy- ([HbO_2]), deoxy- ([HHb]), and/or total ([HbT]) haemoglobin concentrations. While discrepancies between the results of these studies exist, all studies report clear haemodynamic responses in the infant brain, showing that processing of the noxious input occurs at a cortical level in both preterm and term neonates. Clear, localised increases in haemodynamic activity have been reported over the contralateral SI following heel lance (Slater et al., 2006), and bilaterally over SI following venepuncture (Bartocci et al., 2006). Importantly, significant increases in [HbO_2] and [HbT] following noxious heel lance can be observed in single trials (Slater et al., 2006; Verriotis et al., 2016) and are clear even in the youngest infants (Bartocci et al., 2006; Slater et al., 2006). Furthermore, the haemodynamic response to heel lance reflects cortical activation related to the stimulus rather than to movement, as innocuous von Frey hair stimulation eliciting clear withdrawal reflex does not elicit significant haemodynamic responses (Slater et al., 2006). Haemodynamic responses to noxious heel lance in term neonates have also been reported in channels anterior to the vertex (Cz) and therefore putatively in the motor region, rather than in SI (Bembich et al., 2013, 2015). Lack of activity in SI in these studies may have been due to grouping of infants regardless of stimulation side, potentially masking a response that is not bilateral, as previous work has shown thatsome infants exhibit decreased haemodynamic responses over the ipsilateral SI together with increased responses over the contralateral SI (Slater et al., 2006).

Consistent with the ability to record distinct neural responses to noxious and innocuous cutaneous stimulation using EEG, haemodynamic responses to noxious and innocuous stimuli recorded with NIRS are distinguishable, but in this case based on their magnitude. Noxious heel lance elicits haemodynamic activity nearly 10 fold larger than the response to tactile stimulation (Verriotis, Fabrizi, Lee, Cooper, Fitzgerald, Meek unpublished observation). This is also consistent with larger amplitude haemodynamic responses over the sensory-motor region to noxious vs. innocuous electrical stimulation in adults (Yücel et al., 2015), although this difference is considerably smaller in adults. A similar pattern is also observed when comparing longer lasting (up to 60s) noxious and innocuous stimuli in neonates (Bartocci et al., 2006).

Cortical responses to noxious heel lance recorded with NIRS correlate well with pain behaviour measured using the PIPP scale, which is based on facial and physiological responses (Slater et al., 2008). However, it is

possible on some occasions to detect clear cortical responses in the absence of behavioural changes (Slater et al., 2008). This dissociation between cortical and behavioural responses suggests, as with the ERP recorded in EEG studies (Slater et al., 2006), that cortical activity is not directly related to the motor circuits underlying behavioural changes. Subsequent studies have failed to identify an association between cortical and behavioural responses (Bembich et al., 2015).

Both near-infrared spectroscopy (NIRS) and electroencephalography (EEG) have both provided fundamental new information about how the newborn brain processes innocuous and noxious somatosensory information but results derived independently from these two techniques are not entirely consistent, raising questions about the relationship between hemodynamic and electrophysiological responses in the study of touch and pain processing in the newborn (Verriotis et al., 2016). A recent study has recorded NIRS and EEG responses simultaneously in the human infant following noxious (time-locked clinically required heel lances) and innocuous tactile cutaneous stimulation in newborn infants. This required a new quantitative approach to the analysis of NIRS responses, which should form the basis of such haemodynamic-based measures in the future. The study showed that noxious stimulation elicited a peak hemodynamic response that is 10-fold larger than that elicited by an innocuous stimulus and a simultaneous distinct nociceptive-specific N3P3 waveform in electrophysiological recordings. When these co-occur they are significantly correlated in magnitude. However, single-trial analysis revealed that hemodynamic and electrophysiological responses do not always co-occur at an individual level. Thus important individual differences remain between these two modes of recording and it is concluded that multimodal, haemodynamic and electrophysiological brain monitoring is required to fully understand cortical touch and pain processing in the newborn (Verriotis et al., 2016).

fMRI studies

Although many fMRI studies of cortical pain responses have been performed on adults, only two fMRI studies exploring cortical processing of noxious stimuli exist in neonates. As fMRI cannot be done at the cotside, it is not possible to take advantage of clinically required noxious procedures. Therefore, these studies have relied on experimental low intensity non tissue damaging punctate and pinprick stimuli. Both studies show that a number of brain regions are activated by cutaneous noxious stimulation of the foot, including the primary somatosensory cortex (Goksan et al., 2015; Williams et al., 2015), consistent with NIRS studies and also with adult fMRI studies (Apkarian et al., 2005). Specifically, graded low intensity punctate PinPrick stimulators which stimulate Aδ fibre nociceptors elicit widespread cortical activity in neonates that mostly overlaps with cortical regions activated in adults following similar PinPrick application to the heel (Goksan et al., 2015). This data shows that functional thalamocortical connections similar to those in the adult are already present at birth. There are some differences: (1) the activity is more widespread in the neonate, possibly reflecting immature cortico-cortical connections; and (2) the orbitofrontal cortex and

amygdala, two of the regions implicated in the processing of the affective components of noxious stimulation in adults, were not activated in the neonates, although there was clear activation of the anterior cingulate cortex, which is also implicated in affective processing (Goksan et al., 2015).

Application of graded von Frey hair filaments at intensities likely to stimulate Aβ mechanosensitive fibres to the foot elicits activity in similar cortical regions in the neonate, although the activity elicited by PinPrick is more widespread (Williams et al., 2015). Importantly, activity is somatotopically organised in the somatosensory representation area of the foot for both stimuli, and also following brushing of the foot (Williams et al., 2015). Clear intensity coding has been demonstrated in the neonate using von Frey hairs, particularly in the ipsilateral SI, bilateral SII, and contralateral insula (Williams et al., 2015). Although in adults multiple brain regions exhibit responses that are modulated according to PinPrick intensity (Goksan et al., 2015), intensity coding was not significant following PinPrick stimulation in the neonates. Sedation is often required for fMRI studies in infants. Importantly, Williams et al showed that it is possible to detect clear cortical responses in sedated infants, although these infants exhibited significantly reduced activation relative to unsedated infants (Williams et al., 2015).

The overlap between neonatal and adult fMRI activity in response to pinprick has been argued as evidence that the infant pain experience closely resembles that seen in adults (Goksan et al., 2015). Another interpretation is that this indicates that the brain regions receiving nociceptive thalamic input overlap in neonates and adults (Fitzgerald, 2015). Analysis of real time evoked EEG neural activity is required to recognise the important postnatal changes that take place in encoding mechanical pain in the human brain (Fabrizi et al., 2016).

Neurophysiological and imaging studies in infant animal models

The limited options for investigating pain processing in the human infant makes the need for animal research in this area particularly strong. The period between postnatal day (P)7 and P14 is particularly relevant to the developing human infant cortex. The most useful insights from animal models come from direct recordings of neuronal activity in infant rodent cortex following sensory stimulation or specific tasks, allowing analysis of the excitatory and inhibitory connections that determine cortical circuit activity over development. There are few such studies in the pain field, in marked contrast to, for instance, the whisker barrel field (Erzurumlu and Gaspar, 2012). In the adult rat, nociceptive evoked potentials have been recorded in S1 (Xia et al., 2016) and an increase in theta oscillations (4-8 Hz) has been observed following noxious stimulation (Leblanc et al., 2014), but such investigationa are only just beginning in infant rats (Chang et al., 2016). Below we highlight some of the developmental changes taking place in specific brain regions of the rodent related to pain, illustrating the potential for new discoveries in animal models.

Somatosensory and prefrontal cortex While most developmental research in rodent somatosensory cortex has focussed upon the whisker system there is increasing focus upon responses to cutaneous tactile and noxious stimulation of the body. Multiunit recordings from layer IV of the primary somatosensory cortex in anaesthetised rats in response to mechanical taps and brushes show initially nonspecific overlapping receptive fields that become more specific and topographically organized in the developmental period postnatal day 5-20 (P5-P20) (Seelke et al., 2012). A study comparing fMRI measurements with electrophysiological recordings following somatosensory stimulation from postnatal day 13 to adulthood showed that the regional BOLD response in rodents undergoes a systematic decline in latency and growth in amplitude over this period. Importantly, using these infant BOLD response characteristics, revealed that interhemispheric and higher-order cortical somatosensory stimulus responses are enhanced during the first weeks after birth (Colonnese et al., 2008). Electrocorticography (ECoG) at P5-7 shows no ongoing activity under anaesthesia and no response to a noxious tail clamp, while transdermal EEG at P12-14 and P21-22 shows developmental changes in the frequency content of the response to the same stimulus (Diesch et al., 2009). At P20 responses to innocuous and noxious (evoking a withdrawal reflex) thermal stimulation of the hindpaw can be recorded from the corresponding representation area in SI with ECoG. While the response to the innocuous stimulus did not change at P40, the response to noxious stimulation had a larger theta frequency component, suggesting a maturational change in nociceptive processing in SI (Devonshire et al., 2015). We have recently recorded nociceptive evoked field potentials (SEPs) and prolonged network oscillations using intracortical electrodes in SI in animals aged P7, P14, P21 and P30 (Chang et al., 2016). While this study focussed primarily upon the sensitivity of spontaneous and noxious evoked intracortical network activity to isoflurane in the neonatal brain, it is evident from this study and from our as yet unpublished data, that major changes can be recorded in noxious evoked network activity in SI between P7 and P21 (Chang, Fabrizi and Fitzgerald, unpublished observation).

Prefrontal Cortex The rodent prefrontal cortex (PFC) receives mediodorsal thalamocortical terminals early in development but the density of these projections continues to increase postnatally, peaking at P10, and then decreasing to a stable level by P16 (Rios and Villalobos, 2004). However the growth and reorganisation of the PFC continues for a protracted postnatal period until P24 to P30 making it especially susceptible to changing sensory inputs. During the postnatal period, there is a lot of cell loss in the mediodorsal thalamus and it has been suggested that disturbances in this process could result in PFC-dependent cognitive affective abnormalities (Ferguson and Gao, 2015).

Insula and anterior cingulate The adult insular cortex contributes to dynamic interactions between separate brain networks, and integration of sensory information, but intrinsic imaging experiments of the insula in postnatal mice show that overlapping multisensory (tactile and auditory) activation in the insular

cortex is absent before P16, whereupon it transiently involves a large widespread portion of the insula and gradually confines when the animals reach adulthood (Gogolla et al., 2014). Furthermore, the postnatal emergence of integrative properties in the insular cortex relies on the maturation and strengthening of inhibitory circuits as multisensory integration in the insula reflects an optimal excitatory/inhibitory circuit balance.

The anterior cingulate cortex (ACC) is critical in integration of neuronal circuitry for affect and motivational regulation, such as the affective component of pain. It has been demonstrated in rodents that ACC processing of pain affect is distinct from the processing of sensory information as noxious stimuli can still be perceived as unpleasant in ACC, in the absence of somatosensory activation (Fuchs et al., 2014). During postnatal development, the superficial and deep components of the anterior cingulate dopaminergic field develop at different rates. In the deep supragenual area, dopaminergic innervation is already present at birth, whereas in the superficial field, it emerges from postnatal day 3 onward and becomes adult-like at P21–P30. Moreover, negative socio-emotional experiences such as maternal deprivation and chronic isolation during the first three postnatal weeks strongly influence the morphology of the neurons in ACC and their synapses (Helmeke et al., 2001).

Amygdala Traditionally the amygdala represents the central component of a functional brain system regulating fear and emotional behaviours. Distinct behaviours related to amygdala activation emerge at different time points during postnatal development, and continue to mature during late postnatal development (Tallot et al., 2015). For example, infant rats do not show freezing behaviour until about P10, the age at which they begin to make brief excursions outside the nest, coincident with emergence of activity within the amygdala. The fear startle response only appears after weaning (Sullivan, 2001). Anatomical connections between the amygdala and the prefrontal cortex develop during the second postnatal week, whereas connections with subcortical structures, such as the thalamus are already established within the first week (Bouwmeester et al., 2002). Recordings from neurons in lateral amygdala brain slices from infant up to adult mice, show that spontaneous and evoked excitatory and inhibitory synaptic transmissions mature into adolescence. At adolescence, increased inhibitory activity and signalling has the ability to restrict the function of excitation by presynaptic modulation, and may thus enable precise stimulus associations to limit fear generalization from adolescence onward (Ehrlich et al., 2013).

Conclusions

How and when infants begin to experience pain is a fundamental question in sensory, emotional and cognitive neuroscience. It is also a matter of great clinical importance. Here we have taken the view that to understand these questions we need to take a wider view of the developing human brain connectome and study the functional development of brain areas that will go on to form the adult nociceptive network. No

study has specifically addressed the development of the pain network as an ensemble, so here we have explored how and when other brain connectivity may become ready to encode noxious stimuli at birth. Novel electrophysiological and haemodynamic recordings of nociceptive activity in the human infant brain reveal that the newborn brain is capable of processing noxious and skin breaking stimulation of the body surface and these techniques promise new insights into the postnatal development of pain processing and how this differs between individuals and in different contexts. In future, research should extend beyond brief noxious stimuli to the development of cortical processing of persistent pain states. There is a clear need for neurophysiological and imaging research in infant animal models to help us interpret cortical recordings collected in human infants.

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Figure legends

Figure 1. Developmental timeline of structural and connectivity brain features potentially involved in pain and somatosensory processing. Th-Cx, thalamocortical connections; CP, cortical plate; Cx-Cx, cortico-cortical connections;

Figure 2. Developmental timeline of electrophysiological and hemodynamic somatosensory and nociceptive evoked activity. The somatosensory evoked potential (SEP) in the diagram is that evoked by tactile stimulation of the hand. The SEP following foot stimulation has a more central distribution.

The development of the nociceptive brain

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Abstract

This review addresses the fundamental question of how we first experience pain, at the beginning of our lives. The brain is activated by peripheral tissue damaging stimulation from birth, but unlike other sensory systems, the pain system in healthy individuals cannot rely upon a prolonged activity dependent shaping through repeated noxious stimulation. Considering the importance of pain, remarkably little is known about when and how the nociceptive cortical network activity characteristic of the mature adult brain develops. We begin this review by considering the underlying framework of connections in the infant brain. Since this developing brain connectome is necessary, if not sufficient, for pain experience, we discuss the structural and functional development of cortical and subcortical networks that contribute to this network. We then review specific information on the development of nociceptive processing in the infant brain, considering evidence from neurophysiological and haemodynamic measures separately, as the two are not always consistent. Finally we highlight areas that require further research and discuss how information gained from laboratory animal models will greatly increase our understanding in this area.

Introduction

Pain has many dimensions and is processed at multiple different levels of the nervous system but how these processes develop in the newborn is incompletely understood (Fitzgerald, 2005, 2015). When tissue is injured, nociceptive pathways in the peripheral and central nervous system (CNS) trigger essential behaviours, mediated by reflex motor circuits in the spinal cord and brainstem, to ensure that the body is protected from further harm. In addition, brainstem and hypothalamic autonomic circuits are activated, altering the cardiovascular, respiratory, and endocrine systems to maintain homeostatic control of the body. However for the tissue injury to cause pain, the sensory discriminative, cognitive-evaluative, and motivational-affective components of the painful sensation must be encoded in the brain, creating the uniquely unpleasant and stressful qualities that define pain and suffering. While we have learned much about newborn behavioural and autonomic reactions to noxious stimulation, key questions in neuroscience remain as to when and how the brain develops the ability to encode noxious stimuli and create the experience of pain.

Noxious information is not processed in the brain in the same way as other sensory modalities. There is no dedicated **primary 'pain' cortex** analogous to the primary somatosensory or visual cortices; rather, noxious stimulation evokes a diffuse pattern of activity in many brain areas, including primary (SI) and secondary (SII) somatosensory cortices, anterior and mid cingulate cortex (ACC/MCC), insular cortex, amygdala, and regions of the prefrontal cortex (PFC). Traditionally, the somatosensory cortices (SI and SII) are proposed to play a role in the sensory-discriminative aspect of pain (Vierck et al., 2013), while the anterior cingulate cortex (ACC), insular cortex, and amygdala are associated with the affective-motivational components and the prefrontal cortex with the cognitive-evaluative components (Bushnell et al., 2013; Nakata et al., 2014; Veinante et al., 2013). **This network, previously referred to as the 'pain matrix', was thought to** provide a unique representation of the intensity and unpleasantness of the perception elicited by a nociceptive stimulus (Apkarian et al., 2005; Tracey and Mantyh, 2007), but this view has been challenged (Legrain et al., 2011). The current view of pain is that it arises from a distributed network of brain activity, none of which is unique to pain, but when coordinated or synchronised results in the sensory, emotional, motivational, and cognitive experience that is pain. Other brain regions may be recruited to exacerbate or reduce the dimensions of intensity and unpleasantness (Tracey and Johns, 2010). Anat**omical 'pain centres' or 'pain matrices' in the brain have been replaced by the concept of** the **'dynamic pain connectome'** and it is now recognized that the conscious experience of pain arises from a dynamic change in a distributed network of brain activity (Davis et al., 2015; Kucyi and Davis, 2015; Mano and Seymour, 2015; Woo et al., 2015).

Consistent with these findings, fMRI studies in adult rodents also display a distributed pattern of pain related activity across the brain (Borsook and Becerra, 2011; Thompson and Bushnell, 2012) and stimulation of the

hindpaw with noxious electrical or mechanical stimulation elicits BOLD changes in the primary sensory cortex, anterior cingulate cortex, septal nucleus, and retrosplenial cortex as well as subcortical regions (Amirmohseni et al., 2015). Furthermore, incisional and inflammatory induced hyperalgesia in adult rodents causes normally innocuous stimulation to engage pain related brain regions (such as anterior cingulate cortex and periaqueductal grey) beyond the sensory areas (Amirmohseni et al., 2015), and widespread changes within the somatosensory and cingulate cortices and subcortically within the thalamus and the periaqueductal grey are reported in neuropathic pain models (Hubbard et al., 2015).

How and when this complex brain network develops to encode noxious stimuli and create the experience of pain is an important area of current research. This information has clear clinical implications for devising analgesic strategies in hospitalised newborn infants, tailored to the developmental stage of the individual. It is also important for understanding how noxious input at an early stage of development might affect the development of the nociceptive system. For instance, sensory systems and their associated perceptive **abilities are established during specific developmental time windows called "critical periods"**, during which deprivation of normal external inputs or disruption of physiological neuronal activity causes long lasting breakdown of sensory cortical maps and sensory impairment. While this phenomenon has been well characterised in animal models for the visual, auditory, and somatosensory systems (Hensch, 2004), it is difficult to define a critical period for nociception because of the absence of a primary nociceptive cortex and because such stimuli are already normally absent during development. Nevertheless, both animal models and clinical studies have shown that early exposure to noxious procedures causes long term alterations of pain perception and brain function and structures (Ranger and Grunau, 2014; Schwaller and Fitzgerald, 2014; Vinall and Grunau, 2014; Walker, 2013; Walker et al., 2016).

The pain system in healthy individuals cannot rely upon prolonged activity dependent shaping of connections through repeated patterns of noxious sensory stimulation; pain processing in the newborn infant brain must, to some extent, rely on an existing set of brain connections. We assume that the developing brain connectome is necessary, if not sufficient, for our first pain experience and so we discuss the structural and functional development of cortical and subcortical networks that participate in both intrinsic and somatosensory activity in the developing brain. We then review the evidence for specific, nociceptive evoked activity in the infant brain, separating evidence from neurophysiological and haemodynamic measures, as the two are not always consistent. Finally we discuss how information gained from laboratory animal models will increase our knowledge in this area.

Laying down a framework for nociception in the infant brain

The maturation of nociceptive brain function is likely to be underpinned by the development of the brain areas that will go on to form the adult nociceptive network. No study has addressed the development of this

network as an ensemble, so here we review evidence related to the emergence of function of the relevant brain areas over the last trimester of gestation. Figure 1 summarises the timeline of key developmental events in the human brain that lay down the frameworks for nociceptive processing.

Structural development

The somatosensory pathways signalling stimulation of the body, that is the sensory receptors, afferent connections to the spinal cord, and spinal sensory connections, emerge well before the third trimester of gestation, with connections to the thalamus in place by 20 weeks GA (Lowery et al., 2007). These will not be reviewed here as the focus is on the development of brain structures and connections that could underlie cortical processing of external inputs in the premature and term neonatal brain.

A key feature of the foetal brain is the subplate, a transient brain structure with a key role in cortical development (Kanold and Luhmann, 2010). In humans, this forms at 12-18 weeks and is at its maximal thickness (up to 4 times that of the cortical plate) between 22 and 34 weeks GA, after which it begins to disappear **(Kostovic and Rakic, 1990; Kostović and Judaš, 2002)**. In the primary somatosensory cortex, the subplate disappears by the first postnatal month, although traces of it can still be found at 6 months (Kostovic and Rakic, 1990); by contrast, its disappearance in the prefrontal cortex is more gradual over 6 postnatal months, probably related to protracted cortical development such as growth of short cortico-cortical pathways in this region **(Kostović and Jovanov-Milošević, 2006; Kostović and Judaš, 2010)**.

One of the roles of the subplate is to guide afferent input from the thalamus, basal forebrain, and cortex to their appropriate cortical targets. This is important as the presence of thalamocortical connections is considered a requirement for cortical processing of external inputs to occur. Thalamocortical afferents begin to reach the superficial subplate of sensory and associative cortices by 20-22 weeks GA and the cortical plate by 23-26 weeks, and develop at a similar time frame in all sensory cortices (Hevner, 2000; Kostovic and Goldman-**Rakic, 1983; Kostovic and Rakic, 1984; Kostović and Judaš, 2002,** 2010). The immaturity of thalamocortical connections prior to 25 weeks GA suggests that cortical processing of external input is unlikely in infants younger than 25 weeks; at this stage, infants may be considered capable of endogenous spontaneous activity only **(Kostović and Judaš, 2010)**. Migration of neurons from the subplate to the cortical plate begins at around 24 weeks and continues to occur throughout the third trimester, with neurons in place by term age **(Burkhalter et al., 1993; Kostović and Judaš,** 2010). The first synapses appear in the deep part of the cortical plate between 26 and 28 weeks GA, in parallel with the arrival to the cortical plate of the thalamic afferents **(Kostović and Jovanov-Milošević, 2006; Kostović and Judaš, 2010)**. At this age, evoked potentials can also be recorded from SI, visual & auditory cortices (Vanhatalo and Kaila, 2006; Vanhatalo and Lauronen, 2006), as described in greater detail below. Gyrification develops rapidly during the third trimester of gestation (Dubois et al., 2008) and the six-layered laminar structure of the cortex is fully

apparent from 32 weeks gestational age (GA) **(Burkhalter et al., 1993; Kostović and Judaš, 2010)**. At this point, thalamocortical axons begin to synapse onto layer IV neurons **(Kostović and Judaš, 2010)**. Growth of interhemispheric callosal connections occurs from 35 weeks GA and both these and intra-hemispheric associative connections continue to mature at term **(Kostović and Judaš, 2010**). The relocation of afferent fibres from the subplate to the cortical plate and the growth of callosal and long associative connections contribute to an increase in cortical grey matter of 50% between 31 weeks GA and term **(Kostović and Judaš,** 2010).

Much less is known about the parallel development of limbic structures that process "emotional" responses, such as the amygdala. The amygdala and other parts of the limbic system are already distinguishable at 6-7 weeks GA (Müller **and O'Rahilly, 2006)** which suggests a much earlier maturation than the cortex. An anatomical study using KCC2 expression to map the relative maturation of different brain areas (Sedmak et al., 2015) suggests that the amygdala, cerebellar cortex, and the mediodorsal nucleus of the thalamus mature first (10–13 weeks GA) followed by the hippocampus, striatum, and the motor cortex (16–19 weeks), the majority of neocortical areas (at 19–24 weeks) and finally the dorsolateral frontal cortex, and primary visual cortex (24 weeks). However caution must be taken in interpreting these expression studies as evidence of function. Despite its early formation, the punctate GAP-43 (growth associated protein) immunostaining that is characteristic of synaptogenesis does not appear in the amygdala until 20 weeks, peaking at 28 weeks and complete at 36 weeks (Ulfig et al., 2003), suggesting that the anatomical framework for limbic and cortical sensory areas may develop in parallel.

Therefore, by term age, much of the anatomical framework for cortical processing of external input, including thalamocortical and cortico-cortical connections, is present. Next we consider the evidence for these connections being functional.

The infant brain connectome

Over the last trimester of gestation the functional and anatomical macroscopic brain networks undergo substantial maturation leading to an almost adult-like arrangement at term, which further develops postnatally throughout childhood and adolescence (Hagmann et al., 2010; Hwang et al., 2012; Khundrakpam et al., 2013). These changes can be monitored in humans with non-invasive brain imaging techniques such as Diffusion Tensor Imaging (DTI, anatomical) and functional connectivity MRI (fcMRI, functional). Anatomically, the small-world modular architecture characteristic of the adult brain is already present at 30 weeks gestational age (GA), but strengthen until term age (Brown et al., 2014; van den Heuvel et al., 2014). Over the same period white matter microstructures become more refined and delineated (increase in fractional anisotropy (FA) and decrease in mean diffusivity (MD) and transverse diffusivity (TD)), implying a

more efficient signal transmission (van den Heuvel et al., 2014; Hüppi et al., 1998). This is more evident for associative tracts just below the cortex than for commissural and deep projections from the thalamus and between hemispheres, which are mostly already formed (Neil et al., 1998; Partridge et al., 2004), but is weaker in premature born infants studied at term compared to term born infants (Anjari et al., 2007), resulting in impaired thalamocortical connectivity, particularly to the prefrontal cortex, but also, within other regions, to bilateral insula and middle cingulate (Ball et al., 2013a). Moreover, cortical gyration, decrease in FA, and increase in grey matter volume, possibly representing (i) the arborization of basal dendrites, (ii) the arrival to the cortical plate of thalamocortical and corticocortical connections, and (iii) synaptogenesis, occur earlier in the perirolandic than in the associative prefrontal cortex and parieto-temporal areas (Ball et al., 2013b; Delpolyi et al., 2005; Dubois et al., 2008; Mewes et al., 2006). Together these results indicate that while thalamic inputs can reach the somatosensory cortex as early as 28-30 weeks gestational age, and while this area is already quite mature at this stage, the capability of the brain to integrate information develops over the last trimester of gestation in parallel with the development of the prefrontal and temporal cortices. The premature brain also presents an anatomical hub structure and in particular a rich-club topology, where a few specific nodes are involved in the majority of the connections and are also strongly interlinked with each other. Interestingly many of the centres of the nociceptive brain network are part of this rich-club: the left superior frontal cortex and left lateral orbitofrontal gyrus (part of the prefrontal cortex), left postcentral gyrus (primary somatosensory cortex), bilateral cingulate gyrus and bilateral insula (Ball et al., 2014; van den Heuvel et al., 2014).

While DTI provides information about the anatomical aspects of the developing human connectome, fcMRI offers an insight into its functional features. Functional brain networks can be studied in the absence of a task, when the brain is 'at rest', and such studies find that multiple brain regions that are known to be functionally related in the performance of a task are also temporally synchronised at rest. Several such 'resting state networks' have been identified in adults. This approach has also proved useful in assessing functional connectivity in the newborn brain, where resting state networks are both present (Fransson et al., 2007, 2009) and adult-like by term age (Doria et al., 2010), indicating that long-range functional connectivity is already operational by the time of birth and parallel the anatomical development (van den Heuvel et al., 2014). Such networks include the somatosensory network and are not limited to primary areas, encompassing also the default mode and executive control networks (Doria et al., 2010). Furthermore, most of the resting state networks identified in adults can also be detected in preterm infants as young as 26 weeks GA (Doria et al., 2010; Smyser et al., 2010). Most of these are fragmented at 30 weeks GA, although the auditory and visual resting state networks already appear mature at that time (Doria et al., 2010). The thalamus contributes to somatosensory, auditory, motor, and salience networks at term (Alcauter et al.,

2014; Doria et al., 2010), again indicating functional thalamocortical connections by this age, although they continue to develop until at least 2 years of age (Alcauter et al., 2014).

Importantly for this review, the salience network identified in term infants includes the bilateral anterior insula, anterior cingulate cortex, and bilateral prefrontal cortex, while the somatosensory network includes the primary somatosensory cortex (Alcauter et al., 2014). This indicates that at least four of the structures that are implicated in nociceptive processing participate in functional brain networks by term age.

Functional development of the somatosensory cortex

The changes in anatomical and functional brain networks described above are accompanied by the development of specific sensory evoked activity in the somatosensory cortex, and these are illustrated in Figure 2. The somatosensory cortex has received particular attention in human and animal studies because it is relatively easy to engage in task-based experiments. It is relevant here because it is likely to play a key role in early life nociceptive brain activity. Analysis of the development of somatosensory cortical activity evoked by non-noxious tactile or low intensity electrical stimulation has been provided by electroencephalography (EEG) and magnetoencephalography (MEG) measurements in infants. Electrical or mechanical stimulation of newborn infant limbs suggests activation of the infant somatosensory cortex characterised by an early component (Somatosensory Evoked Potential, SEP) and a late component (Event Related Potential, ERP) (see Figure2). The SEPs, occurring less than 100 ms post stimulus, are the most studied as they seem to provide valuable prognostic information of long term neurological outcome (Klimach and Cooke, 1988; Pike and Marlow, 2000; Willis et al., 1989). Their topographical distribution and latency suggest that they are generated by the primary somatosensory cortex: the scalp appearance is consistent with a contralateral frontal-occipital positive-negative gradient when stimulating the hand (van den Heuvel et al., 2014; Lauronen et al., 2006; Nevalainen et al., 2015; Vanhatalo and Lauronen, 2006) and follows the expected somatotopic representation of the stimulated site, being more lateral or central when stimulating the upper or lower limbs respectively (Hrbek et al., 1968). The SEPs are present from early prematurity, decrease in latency with maturation and become sharper and larger in amplitude (Hrbek et al., 1973; Karniski et al., 1992; Taylor et al., 1996; Tombini et al., 2009). In adults, cortical SEP latencies are compatible with the conduction velocity of the peripheral fibers stimulated, which in turn depends on their diameter and myelination (Mauguiere, 2004). Selective electrical stimulation of large diameter myelinated Aβ (30-65 m/s (Vallbo et al., 1979)) evokes a faster response (20 msec when stimulating the medial nerve (Mauguiere, 2004)) compared to painful laser stimulation of small myelinated Aδ (4-30 m/s; response at 160 msec (Treede et al., 1988)) or unmyelinated C fibers (0.4-1.8 m/s; response at 1000 msec (Bromm and Treede, 1987). The decrease in latency and sharpening (and consequently increase in amplitude) of the SEPs during development is therefore likely to mirror the increase in myelination and the decreased synaptic delay

throughout the somatosensory nervous system (Brody et al., 1987; Cracco et al., 1979; Gutrecht and Dyck, 1970). This is confirmed by the parallel decrease in the peripheral and central conduction time estimated by comparing the latency between the stimulus, the nuchal (or cervical) N13 (arising from the cervico-medullary junction) and the SEP (Bongers-Schokking et al., 1990; Trollmann et al., 2010). This decrease in latency is not affected by postnatal age (Tombini et al., 2009; Trollmann et al., 2010) but the amplitude of this component is smaller in ex-premature infants studied at term compared to full-term controls, suggesting an environmental influence (Tombini et al., 2009). At term the early SEP response is followed by a slower NP (negative-positive) ERP at 200 – 500 ms post stimulus, which is maximal at the vertex (Fabrizi et al., 2011; Hrbek et al., 1973; Nevalainen et al., 2015). The source of this activity is less clear, but bilateral secondary somatosensory cortex has been suggested (Nevalainen et al., 2015). This is much slower and wider in premature infants and is most prominent over the contralateral hemisphere (but also appears at the vertex and ipsilaterally) and could represent an immature delta brush following the initial SEP (Fabrizi et al., 2011; Milh et al., 2007), rather than an event generated by associative cortices (Hrbek et al., 1973). Evidence suggests that the initial SEP and the late ERP have distinct neuronal generators and therefore characterise different stages of the somatosensory processing: (i) different topographies (Karniski et al., 1992); (ii) SEPs are larger during active sleep, while ERPs during quiet sleep (Hrbek et al., 1968, 1973; Pihko et al., 2004); (iii) different rates of latency decrease with maturation (Karniski et al., 1992). This hypothesis is supported by the developmental trajectory of the hemodynamic responses to proprioception assessed with fMRI. A positive blood oxygen level–dependent functional response is consistently present from 30 weeks GA in the contralateral SI (Allievi et al., 2015; Arichi et al., 2012). This response becomes more localized and also extends to the ipsilateral SI and sensori-motor associative areas (including SII) in the following weeks (Allievi et al., 2015; Arichi et al., 2012; Erberich et al., 2006; Heep et al., 2009). Clear activation of the primary somatosensory cortex following vibration stimulation in term neonates has also been reported using multichannel NIRS (Shibata et al., 2012).

The development of nociceptive activity in the human infant brain

The evidence above suggests that the newborn brain contains the framework of connections required for somatosensory input from thalamic and other subcortical nuclei to be processed. As discussed earlier, we propose that brain processing of the first, postnatal externally applied noxious inputs requires a functional somatosensory framework. In this section we discuss the evidence for such early life noxious stimuli generating specific nociceptive activity in the human infant brain and the nature of this activity. The pattern of this activity is illustrated in Figure 2.

Neurophysiological evidence

Nociceptive related brain activity can be studied non-invasively in human neonates using scalp electroencephalography (EEG). This technique records ongoing and stimulus related electrical brain activity at different scalp locations providing an overall topographical representation of cortical neuronal activity. In adults, these recordings can be conducted in a controlled research environment and in response to experimental stimulation, such as cutaneous laser, mechanical and electrical stimuli, allowing for a tailored characterization of the effect of various peripheral stimulus parameters on the cortical responses and verbal report of the subjects (Baumgärtner et al., 2012). In neonates, EEG recordings have to be conducted in concurrence with clinically-required procedures due to regulatory and ethical considerations in the challenging neonatal care environment (Worley et al., 2012). Most of the studies to date have taken advantage of acute procedures such as heel lance and inoculation. These interventions provide an almost instantaneous event to which the EEG recording can be synchronised, enabling study of how the developing brain processes procedural pain.

Heel lance in term infants evokes a characteristic waveform made of two consecutive event related potentials (ERPs) maximal at the vertex (Slater et al., 2010a). The first ERP ('tactile') occurs between 100-400 ms post stimulation and can also be evoked just by tapping the heel and therefore is likely to represent a sensory response generated by the non-noxious aspects of the lance; the second ERP ('nociceptive') occurs between 300-750 ms and is only present following skin breaking stimulation. It is possible that these ERPs are preceded by an SEP similar to that evoked by somatosensory stimulation, although this has not yet been reported. These results suggest that the brain of a term infant is able to discriminate innocuous and noxious stimuli and processes separately the somatosensory and nociceptive information related to a skin breaking event. The long latencies indicate that these ERPs are not a direct readout of the incoming afferent volley, but because of the limited number of electrodes that can be placed on the scalp of a neonate it is not possible to estimate the sources of these signals. Nevertheless, nociceptive ERPs obtained using experimental non-tissue damaging graded pinpricks are correlated in amplitude with the pressure of the mechanical stimulus and the elicited withdrawal reflex, suggesting an encoding of the peripheral noxious stimulus intensity (Hartley et al., 2015). On the other hand, the nociceptive ERP does not seem to be related to activity in somatic motor and autonomic circuits underlying the behavioural and physiological response to procedural pain in neonates, as sucrose administration reduces this activity, but not the nociceptive ERP (Slater et al., 2010b).

Even if they share a similar topographical distribution, the tactile and nociceptive ERPs are likely to reflect different cortical generators and mechanisms as the tactile ERP is affected by the vigilance state of the subject while the nociceptive ERP is not (Slater et al., 2010a). Moreover, term infants born prematurely

have a larger nociceptive, but not tactile, ERP compared to control full-term infants, indicating that the experience in the neonatal intensive care unit has affected their nociceptive pathways, but not their tactile circuits (Slater et al., 2010c). It is important to note, however, that tactile sensitivity is high in the newborn infant and withdrawal reflexes can be easily evoked by non-noxious stimulation, particularly when repeated, causing increased reflex magnitude or 'wind-up'(Cornelissen et al., 2013). It is not yet known whether this windup is reflected in the tactile ERP, or whether this property is restricted to tactile activity at the spinal cord level (Koch and Fitzgerald, 2013). It would also be interesting to know whether the domination of A fibre versus C fibre nociceptive input at early ages, as described in the developing rodent (Fitzgerald, 2005), impacts upon the human preterm ERP profile.

These specific patterns and the associated ability to discriminate between different sensory modalities develop over the last trimester of gestation. Infants at 28 weeks gestational age (GA) respond to an innocuous and a noxious stimulus with similar non-specific neuronal activity bursts, or 'delta brushes', while modality specific ERPs become more prevalent only around 35-37 weeks GA, which is just before an infant would normally be born (Fabrizi et al., 2011). Nevertheless noxious stimulation is more likely to trigger these bursts than innocuous stimulation in premature infants, suggesting that even in the absence of any specific discrimination of stimulus modality the very immature brain is already capable of encoding stimulus intensity.

The tactile and nociceptive ERPs are still both present at one year of age following routine inoculation, indeed the nociceptive ERP is significantly larger in one year olds than in younger infants (Verriotis et al., 2015). As for the SEP, the decrease in latency of the early ERP is likely to be related to synaptic refinement and myelination processes occurring during development. Importantly, while the tactile response is still present in adulthood, with shorter latencies, the nociceptive component disappears (Fabrizi et al., 2013). This response is therefore characteristic of the early postnatal period and it is not known when, how, or why it disappears. The later response, instead, could be specific to developing networks and related to the experience dependent formation of the nociceptive pathways, alternatively it could represent a core nociceptive response that is increasingly masked by other complex patterns of brain activity, such as filtering by expectations of pain (Lobanov et al., 2014), as brain function matures.

Importantly the human infant brain encodes noxious information with different neuronal patterns compared to adults (Fabrizi et al., 2016). Comparing EEG responses to the same time-locked noxious skin lance in newborn infants and adults using time-frequency analysis reveals that while some features of adult nociceptive network activity are present in infants at longer latencies, including beta-gamma oscillations, infants display a distinct, long latency, noxious evoked 18-fold energy increase in the fast delta band (2-4 Hz) that is absent in adults (Fabrizi et al., 2016). The differences in activity between infants and adults have a

widespread topographic distribution across the brain indicating further important postnatal changes in the encoding of mechanical pain in the human brain that we still need to understand.

Haemodynamic evidence

The development of nociceptive cortical activity can also be studied non-invasively using techniques such as near-infrared spectroscopy (NIRS) and functional magnetic resonance imaging (fMRI). These techniques provide an indirect measure of neuronal activation by monitoring changes in blood flow within the brain in response to a stimulus. Such haemodynamic changes reflect the balance between the increased oxygen consumption of activated cells and the ensuing increase in cerebral blood flow (CBF) that brings more oxygenated blood to the activated region. The advantage of such techniques is their potential for greater spatial localisation of the cortical response than typically possible with EEG. Therefore, techniques based on haemodynamic measures can provide complementary information about brain function.

Although both techniques measure haemodynamic activity, they have important complementary differences and are therefore both useful in their own right. For instance, NIRS has greater tolerance for movement and can be applied at the cotside to awake, non-sedated babies, eliciting a clinically relevant response. Furthermore, NIRS enables measurement of changes in both oxygenated and deoxygenated haemoglobin, which is important in infant research, and has potentially higher temporal resolution than fMRI (Lloyd-Fox et al., 2010; Safaie et al., 2013). On the other hand, fMRI provides greater spatial localisation than NIRS, and can provide information about both cortical and subcortical regions, unlike NIRS which is limited to more superficial areas of cortex with a maximum penetration depth typically less than 2cm in infant studies.

It is important to note that maturational changes in both energy use and neurovascular coupling during development make interpretation of haemodynamic changes more challenging in the developing brain, particularly when comparisons are made across different ages. For instance, a greater increase of neural activity evoked energy use relative to blood flow may underlie the appearance of inverted haemodynamic responses in infants and young children, compared with neonates and adults; furthermore, it is plausible that there is some stage at which these might cancel each other out such that a haemodynamic response is not detectable but does not imply lack of neuronal activation (Harris et al., 2011). Here we focus on the neonatal brain where inverted haemodynamic responses are not typically observed; however other developmental changes could influence haemodynamic responses. Harris et al., advocate the construction of a database containing haemodynamic responses to standard stimuli at key developmental stages, particularly within reasonably well understood 'low level' neural circuits (such as visual and SI cortices), to improve our understanding of developmental changes in, and interpretation of, haemodynamic responses. We suggest here that simultaneous recordings of haemodynamic and electrophysiological responses will

further improve our understanding of haemodynamic responses in the developing brain (Verriotis et al., 2016).

NIRS studies

As in EEG studies, ethical considerations mean that most NIRS studies of infant cortical responses to noxious stimuli have taken advantage of acute, clinically required skin-breaking procedures. Specifically, studies have focussed on heel lance (Bembich et al., 2015; Slater et al., 2006; Verriotis et al., 2016), venepuncture (Bartocci et al., 2006), and chest drain removal (Ranger et al., 2013), and have reported changes in oxy- ([HbO_2]), deoxy- ([HHb]), and/or total ([HbT]) haemoglobin concentrations. While discrepancies between the results of these studies exist, all studies report clear haemodynamic responses in the infant brain, showing that processing of the noxious input occurs at a cortical level in both preterm and term neonates. Clear, localised increases in haemodynamic activity have been reported over the contralateral SI following heel lance (Slater et al., 2006), and bilaterally over SI following venepuncture (Bartocci et al., 2006). Importantly, significant increases in [HbO_2] and [HbT] following noxious heel lance can be observed in single trials (Slater et al., 2006; Verriotis et al., 2016) and are clear even in the youngest infants (Bartocci et al., 2006; Slater et al., 2006). Furthermore, the haemodynamic response to heel lance reflects cortical activation related to the stimulus rather than to movement, as innocuous von Frey hair stimulation eliciting clear withdrawal reflex does not elicit significant haemodynamic responses (Slater et al., 2006). Haemodynamic responses to noxious heel lance in term neonates have also been reported in channels anterior to the vertex (Cz) and therefore putatively in the motor region, rather than in SI (Bembich et al., 2013, 2015). Lack of activity in SI in these studies may have been due to grouping of infants regardless of stimulation side, potentially masking a response that is not bilateral, as previous work has shown thatsome infants exhibit decreased haemodynamic responses over the ipsilateral SI together with increased responses over the contralateral SI (Slater et al., 2006).

Consistent with the ability to record distinct neural responses to noxious and innocuous cutaneous stimulation using EEG, haemodynamic responses to noxious and innocuous stimuli recorded with NIRS are distinguishable, but in this case based on their magnitude. Noxious heel lance elicits haemodynamic activity nearly 10 fold larger than the response to tactile stimulation (Verriotis, Fabrizi, Lee, Cooper, Fitzgerald, Meek unpublished observation). This is also consistent with larger amplitude haemodynamic responses over the sensory-motor region to noxious vs. innocuous electrical stimulation in adults (Yücel et al., 2015), although this difference is considerably smaller in adults. A similar pattern is also observed when comparing longer lasting (up to 60s) noxious and innocuous stimuli in neonates (Bartocci et al., 2006).

Cortical responses to noxious heel lance recorded with NIRS correlate well with pain behaviour measured using the PIPP scale, which is based on facial and physiological responses (Slater et al., 2008). However, it is

possible on some occasions to detect clear cortical responses in the absence of behavioural changes (Slater et al., 2008). This dissociation between cortical and behavioural responses suggests, as with the ERP recorded in EEG studies (Slater et al., 2006), that cortical activity is not directly related to the motor circuits underlying behavioural changes. Subsequent studies have failed to identify an association between cortical and behavioural responses (Bembich et al., 2015).

Both near-infrared spectroscopy (NIRS) and electroencephalography (EEG) have both provided fundamental new information about how the newborn brain processes innocuous and noxious somatosensory information but results derived independently from these two techniques are not entirely consistent, raising questions about the relationship between hemodynamic and electrophysiological responses in the study of touch and pain processing in the newborn (Verriotis et al., 2016). A recent study has recorded NIRS and EEG responses simultaneously in the human infant following noxious (time-locked clinically required heel lances) and innocuous tactile cutaneous stimulation in newborn infants. This required a new quantitative approach to the analysis of NIRS responses, which should form the basis of such haemodynamic-based measures in the future. The study showed that noxious stimulation elicited a peak hemodynamic response that is 10-fold larger than that elicited by an innocuous stimulus and a simultaneous distinct nociceptive-specific N3P3 waveform in electrophysiological recordings. When these co-occur they are significantly correlated in magnitude. However, single-trial analysis revealed that hemodynamic and electrophysiological responses do not always co-occur at an individual level. Thus important individual differences remain between these two modes of recording and it is concluded that multimodal, haemodynamic and electrophysiological brain monitoring is required to fully understand cortical touch and pain processing in the newborn (Verriotis et al., 2016).

fMRI studies

Although many fMRI studies of cortical pain responses have been performed on adults, only two fMRI studies exploring cortical processing of noxious stimuli exist in neonates. As fMRI cannot be done at the cotside, it is not possible to take advantage of clinically required noxious procedures. Therefore, these studies have relied on experimental low intensity non tissue damaging punctate and pinprick stimuli. Both studies show that a number of brain regions are activated by cutaneous noxious stimulation of the foot, including the primary somatosensory cortex (Goksan et al., 2015; Williams et al., 2015), consistent with NIRS studies and also with adult fMRI studies (Apkarian et al., 2005). Specifically, graded low intensity punctate PinPrick stimulators which stimulate Aδ fibre nociceptors elicit widespread cortical activity in neonates that mostly overlaps with cortical regions activated in adults following similar PinPrick application to the heel (Goksan et al., 2015). This data shows that functional thalamocortical connections similar to those in the adult are already present at birth. There are some differences: (1) the activity is more widespread in the neonate, possibly reflecting immature cortico-cortical connections; and (2) the orbitofrontal cortex and

amygdala, two of the regions implicated in the processing of the affective components of noxious stimulation in adults, were not activated in the neonates, although there was clear activation of the anterior cingulate cortex, which is also implicated in affective processing (Goksan et al., 2015).

Application of graded von Frey hair filaments at intensities likely to stimulate Aβ mechanosensitive fibres to the foot elicits activity in similar cortical regions in the neonate, although the activity elicited by PinPrick is more widespread (Williams et al., 2015). Importantly, activity is somatotopically organised in the somatosensory representation area of the foot for both stimuli, and also following brushing of the foot (Williams et al., 2015). Clear intensity coding has been demonstrated in the neonate using von Frey hairs, particularly in the ipsilateral SI, bilateral SII, and contralateral insula (Williams et al., 2015). Although in adults multiple brain regions exhibit responses that are modulated according to PinPrick intensity (Goksan et al., 2015), intensity coding was not significant following PinPrick stimulation in the neonates. Sedation is often required for fMRI studies in infants. Importantly, Williams et al showed that it is possible to detect clear cortical responses in sedated infants, although these infants exhibited significantly reduced activation relative to unsedated infants (Williams et al., 2015).

The overlap between neonatal and adult fMRI activity in response to pinprick has been argued as evidence that the infant pain experience closely resembles that seen in adults (Goksan et al., 2015). Another interpretation is that this indicates that the brain regions receiving nociceptive thalamic input overlap in neonates and adults (Fitzgerald, 2015). Analysis of real time evoked EEG neural activity is required to recognise the important postnatal changes that take place in encoding mechanical pain in the human brain (Fabrizi et al., 2016).

Neurophysiological and imaging studies in infant animal models

The limited options for investigating pain processing in the human infant makes the need for animal research in this area particularly strong. The period between postnatal day (P)7 and P14 is particularly relevant to the developing human infant cortex. The most useful insights from animal models come from direct recordings of neuronal activity in infant rodent cortex following sensory stimulation or specific tasks, allowing analysis of the excitatory and inhibitory connections that determine cortical circuit activity over development. There are few such studies in the pain field, in marked contrast to, for instance, the whisker barrel field (Erzurumlu and Gaspar, 2012). In the adult rat, nociceptive evoked potentials have been recorded in S1 (Xia et al., 2016) and an increase in theta oscillations (4-8 Hz) has been observed following noxious stimulation (Leblanc et al., 2014), but such investigationa are only just beginning in infant rats (Chang et al., 2016). Below we highlight some of the developmental changes taking place in specific brain regions of the rodent related to pain, illustrating the potential for new discoveries in animal models.

Somatosensory and prefrontal cortex While most developmental research in rodent somatosensory cortex has focussed upon the whisker system there is increasing focus upon responses to cutaneous tactile and noxious stimulation of the body. Multiunit recordings from layer IV of the primary somatosensory cortex in anaesthetised rats in response to mechanical taps and brushes show initially nonspecific overlapping receptive fields that become more specific and topographically organized in the developmental period postnatal day 5-20 (P5-P20) (Seelke et al., 2012). A study comparing fMRI measurements with electrophysiological recordings following somatosensory stimulation from postnatal day 13 to adulthood showed that the regional BOLD response in rodents undergoes a systematic decline in latency and growth in amplitude over this period. Importantly, using these infant BOLD response characteristics, revealed that interhemispheric and higher-order cortical somatosensory stimulus responses are enhanced during the first weeks after birth (Colonnese et al., 2008). Electrocorticography (ECoG) at P5-7 shows no ongoing activity under anaesthesia and no response to a noxious tail clamp, while transdermal EEG at P12-14 and P21-22 shows developmental changes in the frequency content of the response to the same stimulus (Diesch et al., 2009). At P20 responses to innocuous and noxious (evoking a withdrawal reflex) thermal stimulation of the hindpaw can be recorded from the corresponding representation area in SI with ECoG. While the response to the innocuous stimulus did not change at P40, the response to noxious stimulation had a larger theta frequency component, suggesting a maturational change in nociceptive processing in SI (Devonshire et al., 2015). We have recently recorded nociceptive evoked field potentials (SEPs) and prolonged network oscillations using intracortical electrodes in SI in animals aged P7, P14, P21 and P30 (Chang et al., 2016). While this study focussed primarily upon the sensitivity of spontaneous and noxious evoked intracortical network activity to isoflurane in the neonatal brain, it is evident from this study and from our as yet unpublished data, that major changes can be recorded in noxious evoked network activity in SI between P7 and P21 (Chang, Fabrizi and Fitzgerald, unpublished observation).

Prefrontal Cortex The rodent prefrontal cortex (PFC) receives mediodorsal thalamocortical terminals early in development but the density of these projections continues to increase postnatally, peaking at P10, and then decreasing to a stable level by P16 (Rios and Villalobos, 2004). However the growth and reorganisation of the PFC continues for a protracted postnatal period until P24 to P30 making it especially susceptible to changing sensory inputs. During the postnatal period, there is a lot of cell loss in the mediodorsal thalamus and it has been suggested that disturbances in this process could result in PFC-dependent cognitive affective abnormalities (Ferguson and Gao, 2015).

Insula and anterior cingulate The adult insular cortex contributes to dynamic interactions between separate brain networks, and integration of sensory information, but intrinsic imaging experiments of the insula in postnatal mice show that overlapping multisensory (tactile and auditory) activation in the insular

cortex is absent before P16, whereupon it transiently involves a large widespread portion of the insula and gradually confines when the animals reach adulthood (Gogolla et al., 2014). Furthermore, the postnatal emergence of integrative properties in the insular cortex relies on the maturation and strengthening of inhibitory circuits as multisensory integration in the insula reflects an optimal excitatory/inhibitory circuit balance.

The anterior cingulate cortex (ACC) is critical in integration of neuronal circuitry for affect and motivational regulation, such as the affective component of pain. It has been demonstrated in rodents that ACC processing of pain affect is distinct from the processing of sensory information as noxious stimuli can still be perceived as unpleasant in ACC, in the absence of somatosensory activation (Fuchs et al., 2014). During postnatal development, the superficial and deep components of the anterior cingulate dopaminergic field develop at different rates. In the deep supragenual area, dopaminergic innervation is already present at birth, whereas in the superficial field, it emerges from postnatal day 3 onward and becomes adult-like at P21–P30. Moreover, negative socio-emotional experiences such as maternal deprivation and chronic isolation during the first three postnatal weeks strongly influence the morphology of the neurons in ACC and their synapses (Helmeke et al., 2001).

Amygdala Traditionally the amygdala represents the central component of a functional brain system regulating fear and emotional behaviours. Distinct behaviours related to amygdala activation emerge at different time points during postnatal development, and continue to mature during late postnatal development (Tallot et al., 2015). For example, infant rats do not show freezing behaviour until about P10, the age at which they begin to make brief excursions outside the nest, coincident with emergence of activity within the amygdala. The fear startle response only appears after weaning (Sullivan, 2001). Anatomical connections between the amygdala and the prefrontal cortex develop during the second postnatal week, whereas connections with subcortical structures, such as the thalamus are already established within the first week (Bouwmeester et al., 2002). Recordings from neurons in lateral amygdala brain slices from infant up to adult mice, show that spontaneous and evoked excitatory and inhibitory synaptic transmissions mature into adolescence. At adolescence, increased inhibitory activity and signalling has the ability to restrict the function of excitation by presynaptic modulation, and may thus enable precise stimulus associations to limit fear generalization from adolescence onward (Ehrlich et al., 2013).

Conclusions

How and when infants begin to experience pain is a fundamental question in sensory, emotional and cognitive neuroscience. It is also a matter of great clinical importance. Here we have taken the view that to understand these questions we need to take a wider view of the developing human brain connectome and study the functional development of brain areas that will go on to form the adult nociceptive network. No

study has specifically addressed the development of the pain network as an ensemble, so here we have explored how and when other brain connectivity may become ready to encode noxious stimuli at birth. Novel electrophysiological and haemodynamic recordings of nociceptive activity in the human infant brain reveal that the newborn brain is capable of processing noxious and skin breaking stimulation of the body surface and these techniques promise new insights into the postnatal development of pain processing and how this differs between individuals and in different contexts. In future, research should extend beyond brief noxious stimuli to the development of cortical processing of persistent pain states. There is a clear need for neurophysiological and imaging research in infant animal models to help us interpret cortical recordings collected in human infants.

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Figure legends

Figure 1. Developmental timeline of structural and connectivity brain features potentially involved in pain and somatosensory processing. Th-Cx, thalamocortical connections; CP, cortical plate; Cx-Cx, cortico-cortical connections;

Figure 2. Developmental timeline of electrophysiological and hemodynamic somatosensory and nociceptive evoked activity. The somatosensory evoked potential (SEP) in the diagram is that evoked by tactile stimulation of the hand. The SEP following foot stimulation has a more central distribution.

Figure 1

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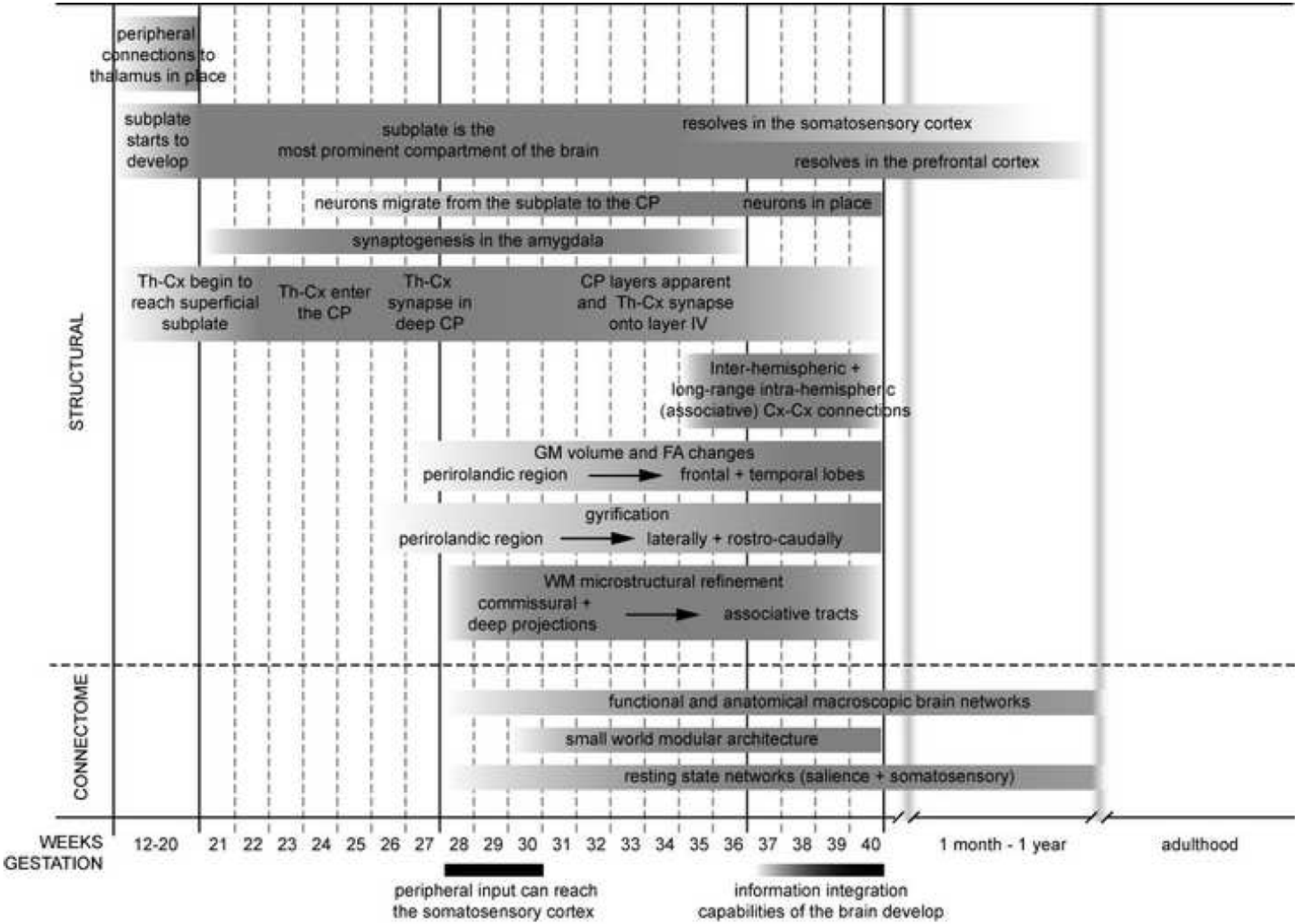

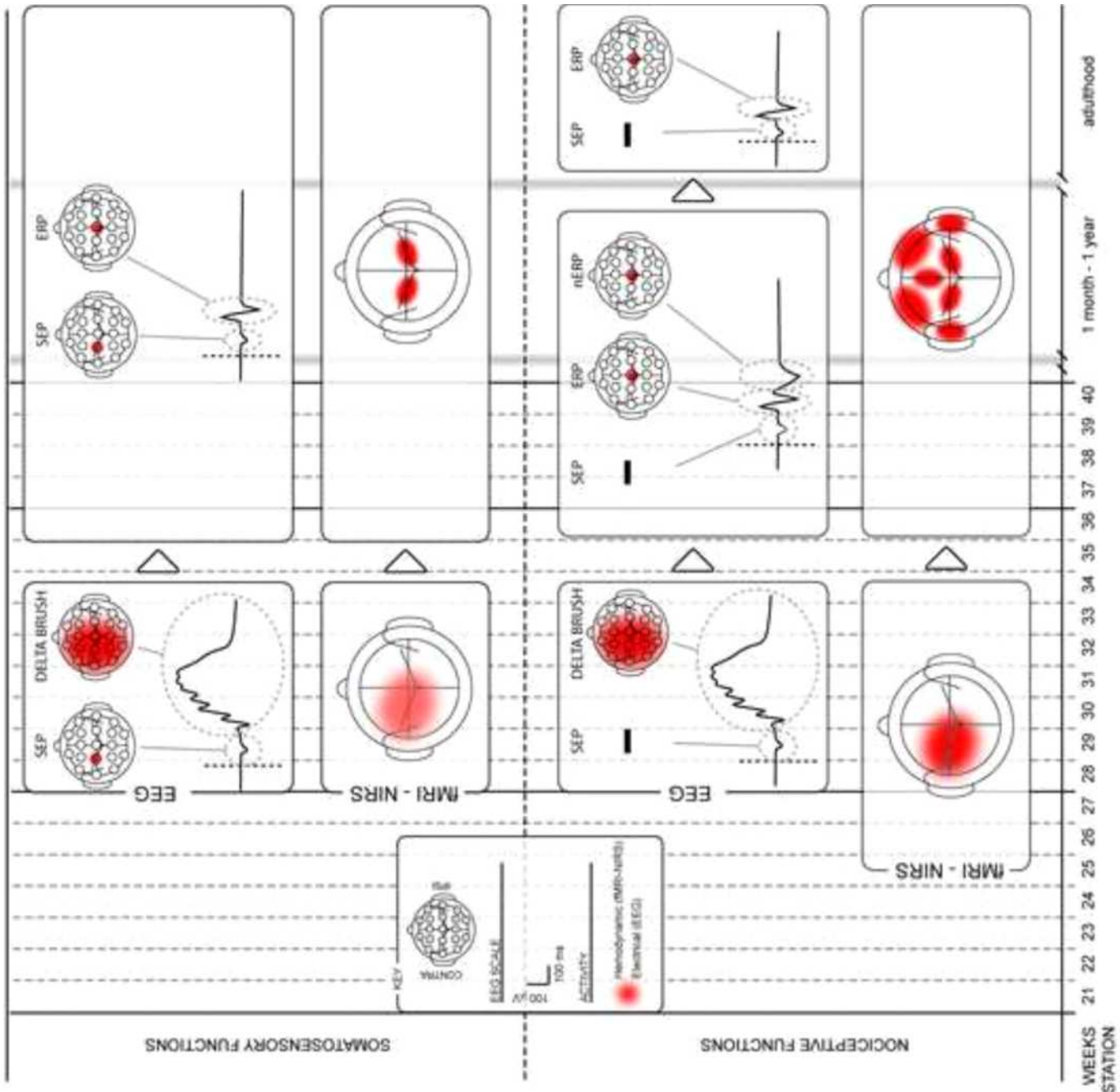

Figure 2
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