Inferring the infant pain experience: a translational fMRI-based signature approach

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Abstract

An infant's lack of verbal communication makes it challenging to infer their sensory and emotional experiences. Improving our interpretation of their perceptions will enhance our understanding of human brain development and improve the clinical care of infants. Multivariate neuroimaging signatures have been validated and developed in adults, which capture aspects of the human pain experience. Here we translate these fMRI signatures to infants in order to draw inferences about their experience of nociceptive events. We find that the Neurologic Pain Signature (NPS), which reflects the nociceptive and intensity encoding aspects of pain experience, is consistently activated in a cohort of adults (n=10) and in two cohorts of infants (n=15 & n=22). However, evidence of higher-level cognitive modulation of the nociceptive input, characterised by the Stimulus Intensity Independent Pain Signature (SIIPS1), is

observed in adults but not observed in infants. This study expands the use of brain signatures to infer and deconstruct the experience of pain in non-verbal populations and provides a framework to better understand the early development of human sensory and emotional experience.

Introduction

In the absence of verbal communication, the perceptions of infants are enigmatic. Behavioural cues such as grimacing and vocalisation are relied upon by caregivers and clinicians to make inferences regarding an infant's experience of pain [1–5], which are broadly based on experiences of older children and adults capable of verbal report. Nevertheless, behaviours have limited sensitivity and specificity and their interpretation is subjective. Behavioural responses attributed to pain can be elicited by non-noxious stimuli in premature infants[6] and infants can have high behavioural pain scores in response to innocuous procedures such as the changing of a nappy[7,8]. While pain scoring systems such as the Premature Infant Pain Profile-Revised (PIPP-R) have some construct validity [9], the extent to which these scores track pain perception is unknown. Given that pain perception manifests in the brain, neuroimaging provides a proxy measure of neural activity that can be compared between infants and adults in order to make valuable inferences regarding the experience of pain in this non-verbal population.

Pain has no specific nexus in the human brain. Instead, a disperse mode of neural activity across sensory, limbic and other areas, with complex temporal and stochastic characteristics is thought to underlie an individual's subjective perception [10]. The adult experience of pain involves complex cognitive and emotional processes, which are influenced by anticipation, attention, memory, salience, attribution of causality, fear and anxiety [11,12]. However, many of these modulatory factors may not influence pain perception in the youngest infants. As the complexity of brain circuitry develops in terms of both structural and functional connectivity, a richer experience of pain will likely evolve [1,4]. Functional magnetic resonance imaging (fMRI) has been extensively used to characterise the nociceptive system in adults, and in infants noxious stimulus-evoked neural activity has been reliably detected [13,14]. Regional analysis of adult and infant responses has previously revealed broadly similar patterns of noxious-evoked activity [13], with infants displaying more bilateral activation, which may be due to exuberant cortico-cortical and interhemispheric

connectivity of the immature brain. Additional brain regions are also activated in infants, such as the auditory cortices, hippocampus and caudate, and more limited activity is reported in some brain regions such as the amygdala and orbitofrontal cortex. However, inferring the perceptual consequences of functional activity or inactivity within individual anatomical regions remains challenging.

Regionalised assessments of activity can be combined to make more robust inferences from data. Multivariate spatial patterns of fMRI activity have been identified that can predict specific aspects of a reported experience [15–17]. Recently, a series of studies have characterised fMRI response signatures associated with pain and negative affect [18–21]. The primary pain template was the Neurologic Pain signature (NPS), which predicts reported pain intensity [19]. The NPS was trained on responses to varying levels of heat pain, and has subsequently been shown to predict variations in pain in over 40 independent published cohorts [15,18,19,22] including noxious thermal [19,23] electrical [24] and mechanical [24] stimuli. This signature is sensitive to nociceptive pain and does not correspond to activity evoked by non-noxious control stimuli[19], vicarious pain[24], threat[24], negative affect[25] or social pain[19]. A related signature, the Stimulus Intensity Independent Pain Signature (SIIPS1), was subsequently developed to explain additional variation in reported pain beyond the NPS and stimulus intensity, relating to modulatory factors such as expectation, motivation, context and perceived control [26,27].

Verbal report is the gold standard measure of pain in adults, but as infants cannot use language to describe their pain, there is great value in identifying alternative objective surrogate measures that can be used to quantify pain experience in infants. Here, following the application of graded acute nociceptive stimuli to infants and adults, we project functional neuroimaging signatures of pain onto infant noxious-evoked brain activity to detect and quantify neural patterns of activity underpinning pain experience. We determine whether infant fMRI responses express several adult pain-related signatures and how their expression across stimulus intensities compares to signature expression in adults. This work provides the proof of concept for an approach to building more specific infant brain-derived biomarkers of pain.

Results

fMRI brain-derived signatures predict reported pain in adults

We studied a single cohort of adults (Prisma scanner: n=10; age range 23-36 years) and two cohorts of healthy newborn infants within 10 days of birth (Verio scanner: n=15; Prisma scanner: n=22). Acute noxious experimental punctate stimuli of various intensities were applied to the foot of each participant (applied force = 64 and 128 mN (infants); 64, 128, 256 and 512 mN (adults); 10 trials per force, Fig 1A) and the evoked brain activity was recorded using fMRI. Both adult and infant response parameter maps were registered to the Montreal Neurological Institute (MNI) template space for the application of signatures (Fig 1B). The punctate stimuli were reported in adults to evoke a low to moderate pain sensation that adults most frequently described as pricking (n = 8 of 10) and sharp (n = 6 of 10), and verbal pain scores increased with stimulus intensity (Spearman r=0.89; $p < 0.001$; Fig 1C)[13].

For each adult participant, 6 brain signatures were applied to the fMRI response data and cosine similarity was used to measure the presence of the signature activity in response to the nociceptive input. The signatures included the Neurologic Pain Signature (NPS) and the Stimulus Intensity Independent Pain Signature (SIIPS1), and 4 control signatures, of which 3 correspond to aspects of negative affect (the vicarious pain (VPS) [24]; picture induced negative affect (PINES) [25]; and social rejection (SR) [21]) and one which measures global signal (GS). The NPS and SIIPS1 showed robust expression in the adults, whereas the 4 negative control signatures were not significantly expressed, confirming that the nociceptive responses specifically expressed pain-predictive signatures (Fig 1D). The NPS signature tracked the reported pain intensity scores ($r = 0.61$, $p<0.001$), while the SIIPS1 did not show a significant association with the reports ($r=0.14$, $p=0.37$).

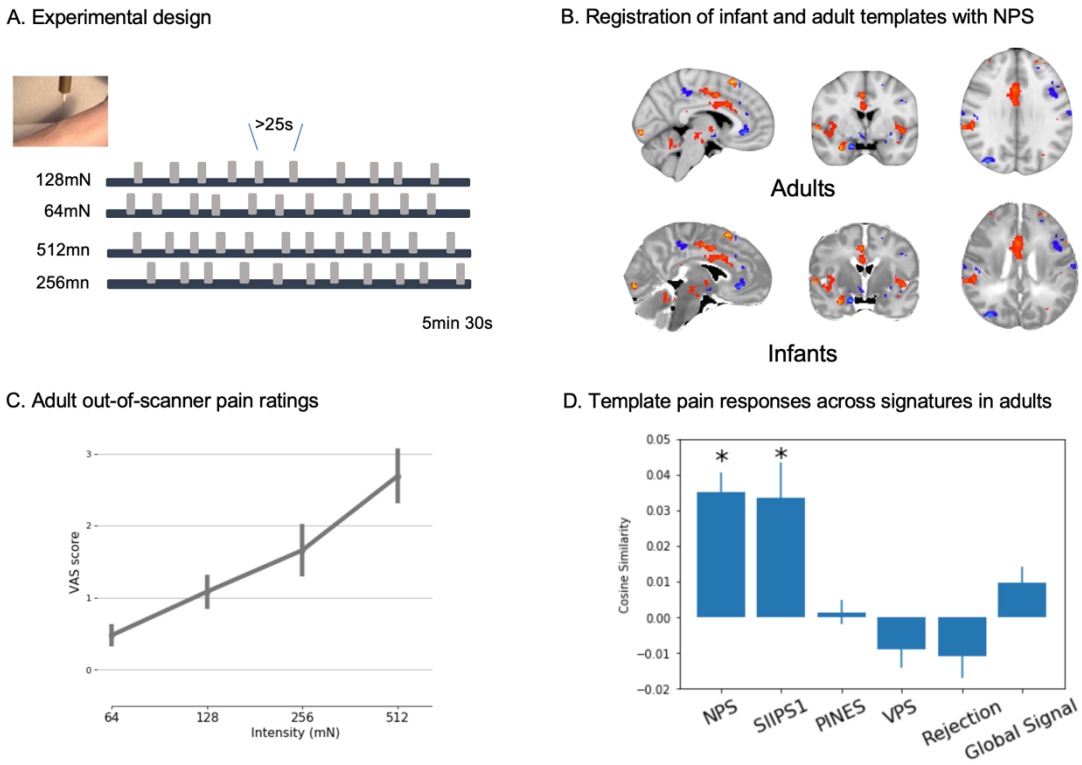

Fig 1. A. Experimental design: stimuli were applied with a minimum inter-stimulus interval of 25s across a range of intensities in a randomised order. 4 stimulus intensities were used in adults and the lowest 2 stimulus intensities were used in infants. B. NPS signature overlaid on registered adult and infant templates, with both templates transformed into MNI template space[28]. C. Adult Cohort: the intensity of the verbal pain report (Visual Analogue Score (VAS)) significantly increased with increasing stimulus intensity ($r=0.89$; $p < 0.001$) D. Adult cohort: quantification of the presence of six signatures in response to the nociceptive input. Only the NPS and SIIPS1 show significant expression. * indicates that cosine similarity is significantly greater than zero.

Correspondence between adults and infant expression of the Neurologic Pain Signature

We considered the comparative expression of the NPS in the adult and infant participants. The NPS was consistently expressed in the adults (mean cosine similarity 0.01-0.063), and showed significant intensity encoding between 64 - 512 mN ($p <.001$, Fig. 2A), consistent with its correlation with verbal pain report. Similarly, in both infant data sets (Prisma and Verio scanner) there was strong evidence of the presence of the NPS at 128 mN ($p=0.001$ Prisma; $p<0.05$ Verio, Fig 2A). Mean cosine similarity levels in the infants (at both 64 mN and 128 mN) were

148 similar to adult values. With the reduced number of force levels, intensity coding (i.e.
149 a difference between 64 mN and 128 mN responses) was not significant. Overall,
150 these results suggest that the neural activity patterns that are indicative of intensity-
151 modulated pain in adults (evidenced by verbal report) are also present in infants.

152

153 Subregion analysis of NPS responses in adults and infants

154 The NPS signature extends across numerous brain regions and the strength of the
155 expression of the pain signature may vary across different spatial areas, particularly
156 in the immature brain[26,29]. At a gross anatomical level the NPS can be divided into a
157 "pro-pain" component, where the signature has positive weights (indicating a
158 positive relationship with pain report) in regions such as the insular cortex,
159 secondary somatosensory cortex, and anterior cingulate cortex (ACC), and an "anti-
160 pain" component, where the signature has negative weights (indicating a negative
161 relationship with pain report) in regions such as the inferior parietal lobule and
162 lateral occipital cortex[29]. These components can be further divided into spatially
163 contiguous subregions.
164
165 In the "pro-pain" NPS brain component both adults and infants showed strong
166 intensity-dependent activation (Fig 2B, red lines), with infants having greater average
167 expression compared to adults in response to the same intensity of stimulation.
168 "Pro-pain" subregions identified in adults also showed positive responses in infants
169 (Fig 2C, red bars), and activity generally increased with stimulus intensity (Fig 2D). In
170 adults the "anti-pain" NPS brain component responses showed weak cosine
171 similarity with the signature (Fig 2B adults, blue line). Weaker responses in these
172 areas may be a result of the brief transient nature of the punctate stimulus
173 compared to other experimental noxious stimuli. Infants showed similarly negligible
174 cosine similarity at 64mN intensity, and negative cosine similarity at 128mN.
175
176 When considering the "anti-pain" NPS on a regional basis, a divergence between
177 infant and adult responses becomes clear. In adults, many constituent NPS "anti-
178 pain" brain regions, including the posterior cingulate cortex, the inferior parietal
179 lobule and the lateral occipital cortex, showed significant expression of the NPS in
180 response to the stimuli (Fig 2C, 2D adults – blue bars). However, these brain regions
181 did not show positive expression in the infant cohort (Fig 2C, 2D infants – blue bars).

182 Instead an inverted response relative to the NPS template was observed,
183 corresponding to increased activation in response to the stimulus.

184 Overall, these results indicate that "pro-pain" NPS brain regions, primarily targeted
185 by ascending nociceptive afferents[19,30,31], show similar activation patterns in adults
186 and infants, whereas, "anti-pain" regions, which are not directly targeted by
187 nociceptive afferents, show divergent responses between the adult and infant.

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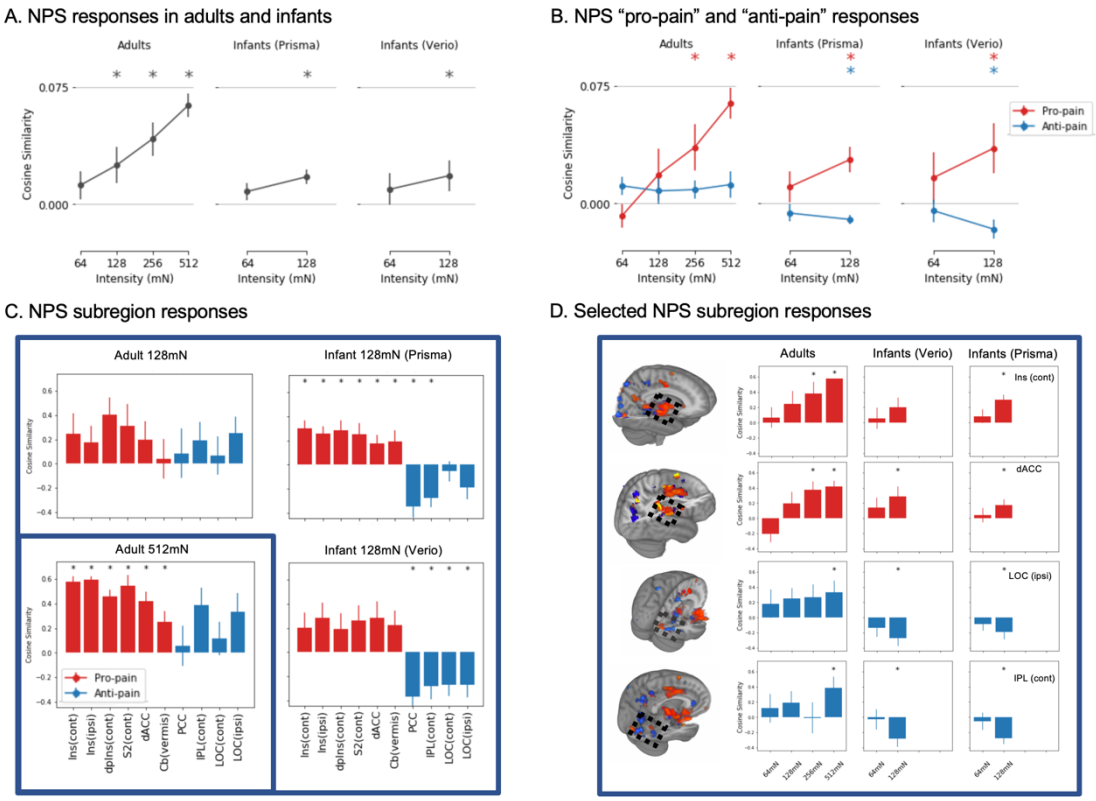

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190 **Fig 2** A. Neurologic Pain Signature (NPS) responses to the brief experimental noxious
191 stimuli for adults and the two infant cohorts, measured by cosine similarity. All three
192 cohorts showed positive cosine similarities with evidence of intensity encoding. B.
193 Plots of the NPS separated into "pro-pain" and "anti-pain" components. C. NPS
194 subregion analysis showing mean subregion responses following the application of
195 128 mN force (infants and adults) and 512 mN force (adults only). Adults and infants
196 show concordance in "pro-pain" NPS subregions (red); only the adults show
197 concordance in "anti-pain" NPS subregions (blue) D. Subregion analysis across
198 intensities for selected subregions. Abbreviations: ipsi – ipsilateral; cont –
199 contralateral; Ins – insula; dpIns – dorsal posterior insula; S2 –secondary
200 somatosensory cortex; dACC – dorsal Anterior Cingulate Cortex; cerebellum (vermis);

PCC – posterior cingulate cortex; IPL – inferior parietal lobule; LOC – lateral occipital cortex. * indicates cosine similarity is significantly greater than zero.

The Stimulus Intensity Independent Pain Signature (SIIPS1) is not present in infants

We next assessed the expression of the SIIPS1 in infants. The SIIPS1 is designed to predict variability in subjective pain report that is not predicted by stimulus intensity or the NPS, and is known to be driven by factors such as expectation, motivation, context and perceived control. [26] The SIIPS1 was reliably expressed in adults (cosine similarity: 0.023-0.062). As expected, it did not show significant intensity encoding (Fig. 3A, adults). In contrast, noxious-evoked brain activity in infants did not positively express the SIIPS1 signature (Fig. 3A, infants). The infant cohort imaged with the Verio scanner showed a negative concordance at 128 mN.

SIIPS1 can also be divided into “pro-pain” and “anti-pain” components. [26] “Pro-pain” components active in the adults include SIIPS1 specific patterns within the insula and central operculum, and have been reported to exhibit intensity encoding[26]. “Anti-pain” subregions include areas of the somatomotor cortex, hippocampus and cuneus and have been reported to not show intensity encoding.

In the “pro-pain” brain component both adults and infants showed a similar pattern of SIIPS1 related activation (Fig 3B, red lines). Adults (p=0.006) and infants from the Prisma cohort (p=0.04) showed significant intensity encoding (Verio cohort p=0.20). A subregion decomposition of the SIIPS1 “pro-pain” component found those subregions reflecting the signature in adults showed similar responses in infants (Fig 3C, red bars).

While the “anti-pain” SIIPS1 component was consistently present in adult responses a positive expression was not seen in infants (Fig. 3B). A significant negative contribution of SIIPS1 was observed when a force of 128 mN was applied in both cohorts of infants. This divergence in the adult and infant responses to the nociceptive input was observed across all subregions that responded in adults, and follows a similar pattern to that observed with the NPS (Fig. 3C and Fig 3D).

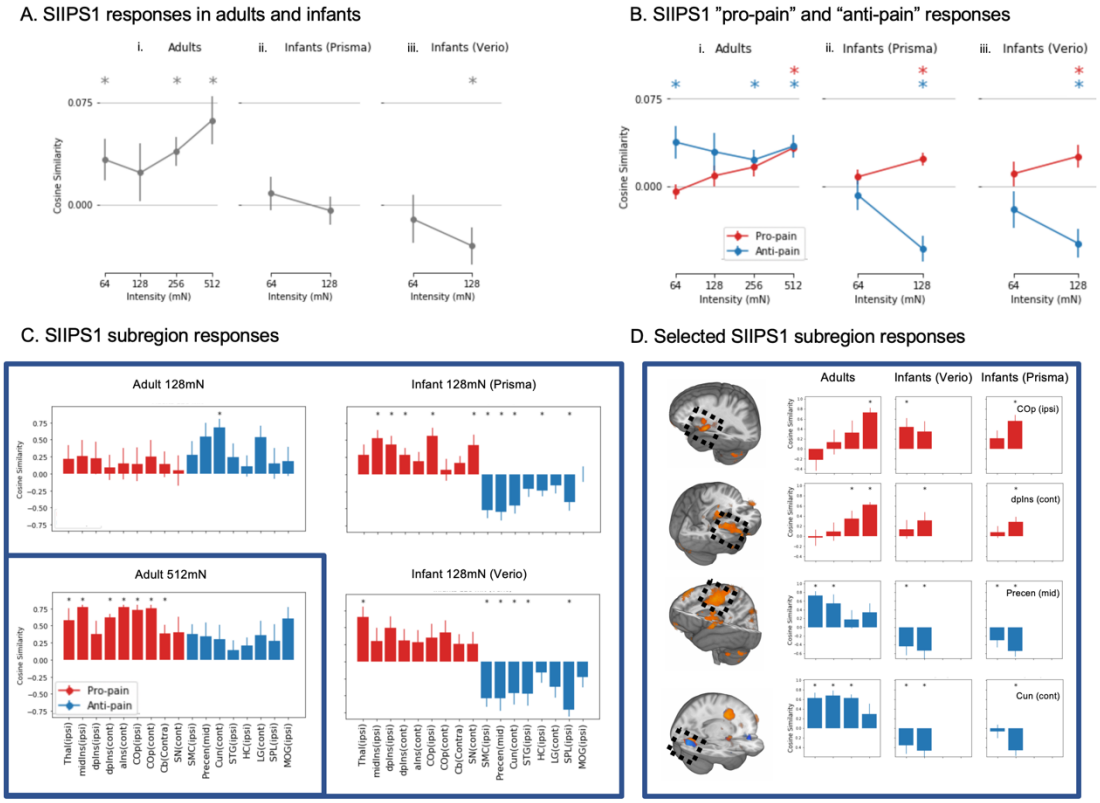

Fig 3. A. SIIPS1 signatures responses across stimulus intensity levels for the adult cohort and two infant cohorts. Adults show consistent positive concordance with the SIIPS1, while infants do not. B. Plots of SIIPS1 separated into reliable "pro-pain" and "anti-pain" subcomponents. C. SIIPS1 subregion analysis showing mean subregion responses following the application of 128 mN force (infants and adults) and 512 mN force (adults only). Consistent with the NPS, both adults and infants show concordance in "pro-pain" NPS subregions (red), but only adults show concordance in "anti-pain" SIIPS subregions (blue) D. Subregion analysis across intensities for selected subregions. Adults show some evidence of intensity encoding in "pro-pain" subregions. ipsi – ipsilateral; cont – contralateral; Thal – thalamus; midIns – middle insula; dpIns - dorsal posterior insula; aINS – anterior insula; ; COp – central operculum; Cb – cerebellum; SN - substantia nigra; SMC – somatomotor cortex; Precen – precentral gyrus; Cun – Cuneus; STG – superior temporal gyrus ; HC – hippocampus; LG – lingual gyrus; SPL - superior parietal lobule; MOG - middle occipital gyrus. * indicates cosine similarity is significantly greater than zero.

Discussion

Human language provides an exquisite means by which subtle sensory and affective states can be communicated. However, in non-verbal populations, such as newborn infants, inference about sensory and emotional experiences are guided by behavioural and physiological observations. For the quantification and treatment of infant pain, this approach is relied upon in clinical practice but these measures lack specificity and the inferences that can be drawn are subjective. How behaviours relate to a conscious experience of pain is ultimately unknown. Neuroimaging provides more direct insight into the neural processes underlying infant experience[32,33] and has the potential to improve the inferences we make about pain in nonverbal populations. Multivariate fMRI signatures aim to robustly link features of brain activity to specific experiences [15,19], and a key potential application of these signatures is to make valuable inferences in challenging patient populations with limited communication.

In infants, noxious-evoked behaviour and brain activity have been studied and correlated[34–36]. However, determining a quantitative association between behaviour, or brain activity, and perception has remained elusive. Here we translate whole-brain fMRI signatures that predict aspects of pain perception in adults to the newborn infant brain, in order to advance our understanding of how infants experience noxious events in early life. Two pain-related brain signatures (NPS and SIIPS1) that represent different aspects of the adult pain experience were applied to two independent cohorts of infants. The NPS is a multivariate template that tracks reported pain intensity associated with a variety of stimuli[19,23,24] and is robust to potential confounds such as non-physical pain and other salient aversive and sensory events [19,24,25]. The SIIPS1 signature aims to quantify further cerebral contributions to reported pain experience, beyond features of brain activity that directly relate to nociceptive input and intensity encoding [26]. Unlike the NPS, it is found to explain the effects of psychological factors on reported pain, such as expectation and control. These signatures identify combinations of regional activity rather than activity in single brain subregions, which allows for more accurate and specific prediction of pain reports [17,26].

Although caution is advised when translating and interpreting adult signatures in the immature brain[37], there is considerable evidence to support the validity of this approach. Core structural features of the mature adult brain are developed in term-aged infants[38,39]. We see functional responses[40–43], connectivity patterns, and resting

285 state activity [44,45] exhibit similar features to adult brain activity. Furthermore, strong
286 neural activity overlap between adult and infant sensory-evoked and noxious-evoked
287 brain activity patterns have been reported across a variety of brain imaging
288 modalities [13,36,46].

289 Our results provide quantitative evidence that noxious stimulation in infants evokes
290 changes in brain activity that would be attributed to the sensory discriminative
291 aspects of pain experience in an adult. We found the NPS to be expressed similarly
292 across infants and adults. This result aligns with a recent EEG study comparing pain
293 responses of adults and infants[36]. Here, infants displayed noxious evoked potentials
294 and gamma oscillations, understood in adults to be markers of primary nociceptive
295 processing and subjective pain[47]. In contrast to the NPS results, we also show that
296 endogenous cerebral contributions to pain reports that are observed in adults but
297 indirectly relate to the nociceptive input, are not significantly represented in infants.
298 In adults this activity, which is characterised by the SIIPS1, is reported to reflect
299 variation in expectation, motivation, context and perceived control [26]. The
300 observation that nociceptive input in newborn infants does not evoke patterns of
301 cerebral activity that relate to these complex psychological constructs may reflect a
302 lack of contextual awareness. As infants mature they become more engaged with
303 their external environment but it is not known at what developmental stage they are
304 capable of interpretation, judgement and modulation of their subjective experience
305 of pain.

306 A lack of concordance of infant brain activity with the SIIPS1 was primarily driven by
307 divergent responses in “anti-pain” brain regions, which occurs when a brain region
308 that is normally de-activated with increasing pain intensity is instead activated, for
309 example in fibromyalgia patients [29]. This divergence of responses in “anti-pain”
310 regions, including the cuneus, hippocampus, and areas of the precentral gyrus, was
311 also seen in the NPS signature, and was the most striking axis of differentiation
312 between infants and adults. A number of sources could potentially contribute to
313 this observation. It is possible that immaturities in the hemodynamic response in
314 infants produce altered BOLD responses in these areas. However, while differences
315 in BOLD mechanisms have been observed in infant rats[48], we used a hemodynamic
316 response function derived from somatosensory responses in newborn human
317 infants, where differences compared to adults appear more subtle[49]. Alternatively,
318 the differences in “anti-pain” responses may be underpinned by differences in the
319 noxious-evoked neural activity. Slower maturation of association and attentional

networks is well established in infants[50,51], and has been observed in resting state fMRI[52]. Some differences between infant and adult EEG responses to nociceptive stimuli have been reported, including delays in event-related potentials (ERPs), and a late ERP component in infants corresponding to an increase in delta band energy[36]. The positive BOLD response association with the nociceptive stimuli in the "anti-pain" regions of infants could be due to exuberant connections in the infant brain[50], coupled with a potentially underdeveloped GABAergic inhibitory system[53]. However, it is speculative to consider the exact mechanisms that underpin differences in the evoked haemodynamic activity in the infant and adult brain[54].

The translational application of validated adult brain signatures to the immature brain can be further expanded and refined. Applying these methods to a cohort of young children who can reliably report their experiences would reveal the extent to which these signatures are maintained once they appear during development. Developmentally varying neural signatures could be characterised, with alternate trajectories for infants born prematurely. Potential confounding factors could also be further addressed. These signatures can be optimised for infants by including testing and validation in contexts designed to replicate the reduced knowledge of the experimental context of infants [55]. Despite its advantages, it is important to appreciate that a single modality, such as fMRI, cannot capture the full complexity of the processes that underlie a conscious experience. Therefore, we strongly advocate a multimodal measurement approach to provide the best proxy of infant pain[56,57]. Infant behavioural pain scores have been extensively studied, and brain-derived EEG signatures have been linked to infant nociception [34,58–63]. Linking these approaches to validated fMRI signatures will enhance our confidence in these methods.

Given that infants are non-verbal, finding the best proxy to quantify their pain experience is critical if we are to minimise pain exposure in clinical care and mitigate the potential long-term and short-term impact of pain in early life[1,4,63]. Improving the validity of inferences we make about infant perceptions will also advance our understanding of human cognitive development. This study expands the use of brain signatures to infer and deconstruct the experience of pain in non-verbal populations, and provides a framework to better understand the early development of human sensory and emotional experience.

Methods

This work extends previous work that used a subset of the data analysed here, which identified qualitative correspondences between adult and infant fMRI pain responses [64].

Experimental Data

All data were acquired at the FMRIB Centre, University of Oxford. Data were acquired in three studies. Informed written consent for the studies was provided by adult participants or by the infant's parent before the study commenced. The infants were studied under ethical approval given by the National Research Ethics Service. The adult study was approved by the University of Oxford Central University Research Ethics Committee. Both studies were carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

The adult study used a 3T Siemens Verio scanner with a 32-channel adult head coil to measure pain responses in 10 healthy adults (mean age = 28.3 years; range: 23–36; number of females: 5). BOLD images were acquired using a T2*-weighted EPI acquisition (sequence parameters: TR/TE = 3280/40ms; flip angle = 90°; FOV = 192×192 mm; imaging matrix 64×64; resolution 3×3×3 mm; slices = 50, collected in descending order; average total volumes = 142).

The second study, *Infants-Verio,* used the same protocol to study 15 healthy infants scanned within 7 days of birth (GA 38 - 43 weeks; number of females: 6) [13]. The infants were wrapped, fed and fitted with appropriate ear protection before being placed in the scanner. These infant data were acquired with the same sequence as the adults except using 33 slices, and an infant optimised TR/TE of 2500/40ms [65].

The final experiment studied 22 healthy term infants within 10 days of birth (GA 35-42 weeks; number of females: 12) using a Siemens Prisma 3T scanner with an adult 32 channel receive coil (Infants-Prisma). Infants were fed, wrapped and fitted with appropriate ear protection before being placed in the scanner. BOLD images were acquired using a T2* BOLD-weighted, GRE acquisition with EPI readout, 70° flip angle, TE= 50 ms[65], TR= 1,300 ms, mean TA= 6 mins (approx.), multiband 4 [66,67], 90 x 90 in-plane matrix size, 56 slices, 2 mm isotropic voxels.

Participants were scanned while receiving trains of 10 very brief non-tissue damaging punctate stimuli of a specific intensity, applied to the left foot by a trained

experimenter with a minimum inter-stimulus interval of 25s (Fig 1A). Participants had scans with 64mN and 128mN force, and adults additionally had scans with 256mN and 512mN stimuli. Some subjects also had 32mN scans, which were not studied here. The order of scans was randomised across subjects. The noxious stimuli were time-locked to the fMRI recording using Neurobehavioural Systems (Presentation, www.neurobs.com) software.

After the scanning session, application of the stimuli was repeated in the adults and each participant was asked to lie down on a patient bed and verbally state a pain score following application of each stimulus. Once all stimuli had been presented, the participants were asked to describe the type of pain they experienced by completing the McGill Pain Questionnaire[68].

Data Preprocessing

All infant FMRI data were processed using the DHCP functional and structural pipelines[45,69,70]. Boundary-based-registration (BBR) to register functional data to high-resolution structural data. Dynamic motion-by-susceptibility distortions were corrected using FSL's EDDY[71,72], and ICA-based denoising was used to remove additional artefactual signals [73,74]. 3mm spatial smoothing and 100s high-pass temporal filtering was applied.

Time series modelling

The pain responses were modelled using a general linear model (GLM) in FEAT with a regressor defined for responses at each stimulus intensity. The experimental design (Fig 1A) was convolved with term-infant-specific optimal basis functions generated by Arichi and colleagues [49]. Additional regressors modelling periods of excessive motion-related signal as identified by DVARS [75] were also included. Parameter maps were generated for each subject at each stimulus intensity and transformed into MNI space.

The adult data were processed using FSL 6.0 [76]. BOLD data were high pass filtered with a cut-off of 100s, and 5mm spatial smoothing was applied. FIX clean-up was employed and motion parameters were regressed from the data [74].

Registration

Parameter estimate maps for individual subject and intensity levels were transformed into adult MNI space via a series of transformations generated using

418 ANTs's SyN (Advanced Normalization Tools Symmetric image Normalization method)
419 [77]. Multi-modal non-linear registration was first used to register structural images T2
420 to the age-matched T2 template [78]. This was combined with the appropriate atlas
421 week-to-week nonlinear transforms to transform to 44 week template, and then to
422 MNI standard space with the NIHPD2 lifespan atlases (ages birth to 21 years) used as
423 intermediate transform targets [79,80]. For adults, FNIRT nonlinear registration was
424 used to transform data to MNI space [81].

425 Signature Analysis

426 Parameter map data derived from responses to specific intensities were analysed for
427 their correspondences to nociceptive signatures using a modified pipeline from the
428 CANlab toolbox [19,27,82](canlab.github.io), using additional MATLAB and Python code.
429 The CANLAB toolbox provides a pipeline for processing, QC and visualization of
430 parameter maps for the signature-based analysis of group fMRI data. This includes
431 the extraction, assessment, regression and of white matter and CSF components, the
432 calculation of signature similarity measures, estimation of contrasts and SVM
433 classifier maps, and NPS subregion analysis. Concordances between brain responses
434 and signatures are based on cosine-similarity scores – equivalent to correlation
435 without centring – between the response parameter maps and the predefined
436 spatial signatures. A cosine similarity of 1 represents perfect alignment of the data
437 with the signature and a cosine similarity of 0 indicates that the signature and the
438 data are orthogonal. To assess whether the signature responses tracked stimulus
439 intensity, we used a linear modelling across stimulus levels. We used the adult study
440 to validate the signature responses for our specific experimental protocol, to identify
441 responsive subregions for the infant analysis, and for direct comparison of adult and
442 infant responses.

443 The primary signature employed was the Neurologic Pain Signature (NPS) [19]. This
444 signature was developed using a regularised regression procedure that identified a
445 pattern of brain regions whose activity tracks pain ratings across trials of painful
446 thermal stimuli. The pattern has been validated across a variety of datasets, and has
447 been shown to exhibit specificity to pain relative to non-painful warm stimuli,
448 romantic rejection, and other emotions of negative affect[15,20]. Presence of the NPS in
449 infant responses to nociceptive stimuli, and any evidence of intensity encoding,
450 constitutes evidence for the experience of pain in infants. We also performed
451 analyses of subregions of the NPS and SIIPS1. The subregions were obtained using
452 the Canlab toolbox, and previously used in assessments of NPS [19] and SIIPS1 [26]. We

excluded regions that showed negligible or negative signature responses to our nociceptive stimulus in the adult dataset (average cosine similarity <0.01).

We also assess the Stimulus Intensity Independent Pain signature (SIIPS1) [26], a signature associated with factors affecting reported pain in adults independent of stimulus intensity and the NPS. The SIIPS1 signature was trained using data from which the NPS had been removed via regression. We performed the same procedure here. If pain is processed in a similar fashion in adults and infants, we would expect infant brain responses to painful stimuli to be concordant with the NPS. On the other hand, correlation with the SIIPS1 signature might be expected to vary depending on the level of development of the neurobiological systems captured (e.g., ventromedial prefrontal cortex and nucleus accumbens figure prominently) and individual infants' capacity to engage these systems, which are associated with both descending modulation[83,84] and self-regulation[27]. Therefore, in addition to the mean response across subjects, we assessed the variability of responses across individuals, measuring responses of individual trials to identify confidence intervals. We adapted CANLAB code to include regression of the NPS prior to SIIPS1, and analysis of SIIPS1 subregions and single trial variability.

In addition, to determine the specificity of signature responses, we looked for the presence of a number of other validated negative affect signatures, namely, picture induced negative affect (PINES) [25]; signature of vicarious pain (VPS) [24], and social rejection (SR) [21], and a marker of global signal (GS).

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