Developmental alterations in noxious-evoked EEG activity recorded from rat primary somatosensory cortex

Ian M. Devonshire, Charles M. Greenspon, and Gareth J. Hathway*

School of Life Sciences, Nottingham University Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

*Corresponding author: +44 (0) 115 82 30152,

gareth.hathway@nottingham.ac.uk

Running title: Noxious-evoked EEG changes over development

Pages = 17; figures = 3

Total words = 5444

Key words: EEG, nociception, somatosensory cortex, pain, development.

Abstract

Primary somatosensory cortex (S1) contains a nociceptive map that localizes potential tissue damage on the body and encodes stimulus intensity. An objective and specific biomarker of pain however is currently lacking and is urgently required for use in non-verbal clinical populations as well as in the validation of pre-clinical pain models. Here we describe studies to see if the responses of the primary somatosensory cortex (S1) in juvenile rats are different to those in the adult. We recorded EEG responses from S1 of lightly-anaesthetised Sprague-Dawley rats at either postnatal day 21 or postnatal day 40 during the presentation of noxious (55^0C) or innocuous (30^0C) thermal stimuli applied to the plantar surface of the left hindpaw. The total EEG power across the recording period was the same in both ages after stimulation but the frequency distribution was significantly effected by age: noxious heat evoked a significant increase in theta band (4-8 Hz) activity in adults only ($P<0.0001$ compared to baseline; $P<0.0001$ compared to juveniles). There were no significant differences in EEG responses to innocuous thermal stimuli. These data show that there are significant alterations in the processing of nociceptive inputs within the maturing cortex and that cortical Theta activity is involved only in the adult cortical response to noxious stimulation.

Introduction

Pain is a subjective experience arising from both sensory and affective centres in the brain and spinal cord. Forebrain structures consciously determine the location and magnitude of the noxious stimulus as well as assessing the valence of pain and assigning affective responses to it (ref). More caudal midbrain and brainstem structures are involved in autonomic and subconscious responses to pain, for example controlling nociceptive inputs to the CNS by increasing or decreasing spinal excitability which, in turn, influence reflex sensitivity and nociceptive thresholds (ref). The appreciation of where on the body potential damage has occurred is primarily processed within the somatosensory cortex, particularly the primary somatosensory cortex (S1). This is demonstrated by lesions of S1 selectively impairing sensory/discriminative aspects of pain whilst sparing the affective component [ML Uhelski et al., 2012 Pain]. S1 neurons have discrete cutaneous receptive fields that produce a somatotopic map of pain [U Bingel et al. 2004 NI; S Omori et al., 2013 Clin Neurophys; Y Ogino et al., 2005 Anesth] (Tracey and Mantyh, 2007, Garcia-Larrea and Peyron, 2013) and can also rate stimulus intensity (Mancini et al., 2012).

The development of S1 is complex and functional consequences of a range of structural modifications that take place during maturation are poorly understood. Cortical sensory areas (including S1) are "developmentally privileged" (Finlay and Uchiyama, 2015): amongst thalamic nuclei, sensory nuclei appear first, thalamic innervation of the cortex occurs before birth and, once innervation begins, a basic topographic map is immediately created (Erzurumlu and Jhaveri, 1992). This map undergoes many changes, however; cortical neurogenesis continues postnatally with the ultimate functional and topographic development of S1 determined by the interaction extrinsic inputs to the cortex and intrinsic firing patterns of cortical neurons (O'Leary and Nakagawa, 2002). Furthermore, it has recently been shown early in development that ascending inputs have transient access to superficial cortical layers (I-III) – layers that receive only from neighboring cortical areas when mature – which shapes the balance of afferents between different interneurons (De Marco Garcia et al., 2015). Rapid increases in myelination occur over the first few years of life,

an increase in synaptic density, and a protracted stage of synaptic pruning until mid-adolescence (T Paus et al., 2001 BRB; Huttenlocher & Dabholkar, 1997 JCompN). The ultimate age at which maximal cortical thickness occurs is both age- and region-specific (JN Giedd & JL Rapoport, 2010 Neuron). Thus, cortical areas have an unusually-protracted maturation period and remain sensitive to environmental changes for a long period of time. (L Krubitzer & JC Dooley, 2013 Front Hum Neurosci)

Whilst at least some of these stages of structural cortical maturation can be mapped onto changes in brain activity [PJ Uhlhaas et al., 2009 PNAS; T Gasser et al., 1988 EEG-CN], a more thorough characterization of nociceptive-specific S1 activity during development and adulthood is required. For example, although a recent fMRI study has shown that heel-prick evoked pain activates comparable brain regions in infants and adults (Goksan et al., 2015), the use of more mobile techniques with greater temporal resolution, such as electrophysiology, may be able to detect differences in the processing of noxious sensory information in different age groups. Such knowledge has a range of potential applications, including as a bio-marker of pain, especially in non-verbal populations such as neonates and young children (C Hartley et al., 2014 Pain), a means of ensuring adequate anaesthesia in surgical procedures (C Hartley et al., 2014 Pain) and as a measure of spontaneous pain in pre-clinical models (BW Leblanc et al., 2014 Pain), an essential measurement in order to validate such models and improve their translatability.

Increased activity within S1 can be electrophysiologically assessed either invasively via microelectrodes or non-invasively using a variety of techniques including EEG, MEG, or fMRI. EEG represents the most convenient method for the assessment of electrical activity in cortical areas and changes in S1 power of EEG frequency bands have been linked with subjective rating of intensity of pain sensation [Gross et al., 2007; Zhang et al., 2012]. The aim of the current study was to examine whether EEG recordings from S1 in lightly-anesthetized rats could discriminate age-dependent differences in processing of noxious stimuli. Here we show that S1 responses to noxious thermal stimulation of the foot are

significantly different in adults compared to juvenile rats. This is not the result of differences in the overall level of cortical activity but reflects a lack of refinement in the frequencies employed in cortical activity in early life.

Experimental Procedures

Juvenile (post-natal day (P)21 ± 2 days; n=23) and adult (P38-P47 days; n=27) male Sprague Dawley (Charles River, Margate, UK) rats were used weighing between 52-92 g (P21; juvenile) and 159-256 g (P40; adult) and kept in a 12-hr dark/light cycle in closed, ventilated cages in a holding room kept at a temperature of 22°C and 55% humidity; food and water were available *ad libitum*. All experiments were approved by the local University ethical committee and all procedures were performed and specifically licensed following approval by the UK Home Office and in accordance with the Animals (Scientific Procedures) Act 1986.

Rats were anesthetized with urethane (0.4 g/kg, i.p.) and isoflurane. A tracheostomy was performed followed by endotracheal intubation to enable artificial ventilation. Urethane was included in the anaesthetic mix as it allows isoflurane concentration to be decreased which has the advantage of minimally influencing cortical EEG whilst maintaining spinal reflexes [25]. Isoflurane was vaporized in oxygen at a concentration of 1.5-2.5% during surgical preparation and 0.65-1.2% during data acquisition (juvenile: 0.79 ± 0.05%; adult: 0.84 ± 0.17%). Animals were artificially ventilated using a volume-controlled ventilator (60-80 breaths per minute, 1-1.8 ml/kg; Model 683 Small Animal Ventilator, Harvard Apparatus, Edenbridge, UK). Body temperature was measured with a rectal probe and maintained at 37°C using a thermostatic heating pad (Harvard Apparatus, Edenbridge, UK). Animals were transferred to a stereotaxic frame (Stoelting, Dublin, Ireland) and small trepanations were drilled over the hind-limb area of the primary somatosensory cortex (S1HL; AP = -1.5 mm, ML = 2.0 mm) and the left frontal cortex to allow the sub-cranial insertion of loop-tipped silver wire EEG recording and differential electrodes respectively (diameter=0.2 mm; Intracel, Royston, UK). EEG was monitored continually throughout the experiment. A craniotomy was also performed overlying the rostroventral medulla (RVM; AP = 8.0 to 12.5 mm, ML = -2.0 to 2.0mm) and the dura resected using a 31-gauge hypodermic needle (Becton-Dickinson, Oxford, UK).

Sensory Stimulation

Noxious (55°C) and innocuous (30°C) thermal stimuli were delivered to the plantar surface of the hindpaw contralateral to the EEG recording site using a custom-built device: briefly, a small, convex-shaped aluminium block was heated using an etched foil resistance heater encapsulated in polyamide housing running from a stabilized 24V DC supply. Temperature was controlled via a three wire platinum resistance sensory device giving control accuracy of ± 0.5°C. The left hindpaw of the rat was secured with the plantar surface facing upwards and the heater block gently applied to the entire foot-pad upon release of a pressurized pneumatic valve. The stimulus block was retracted manually upon commencement of the withdrawal reflex (in response to noxious stimuli typically < 4 s) or after 5 s (following innocuous stimuli). Stimuli were presented with an interval of at least 180 seconds. The heater control device communicated with a microCED1401 data acquisition unit (Cambridge Electronic Design, Cambridge, UK) so that the presentation and removal of stimuli were time-stamped on acquired data.

Electrophysiology

The silver wire EEG electrodes were connected to a NeuroLog head-stage (NL100AK; Digitimer, Welwyn Garden City, UK), signals amplified x2000 (NL104A), band-pass filtered between 0.5-1000 Hz (NL125) before being recorded at 2kHz using Spike2 software via a microCED1401 data acquisition unit (Cambridge Electronic Design). Single-unit recordings from the RVM were made with either glass or glass-coated tungsten microelectrodes. Glass capillaries (GC120F-10; Harvard Apparatus, Edenbridge, UK) were heated and pulled to with a taper between 8.5-9.0 mm using a Sutter P-97 puller (Novato, CA, USA), broken back with a glass rod to reach a final tip diameter of 1.5-2.5 μm and filled with 0.5M NaCl and 20mM Chicago blue 6B (Sigma Aldrich, Gillingham, UK). Glass-coated tungsten microelectrodes were used and inserted into the RVM to reach the final co-ordinates: AP = 9.0 to 11.5mm, DV = 10.2 to 11.0, ML = -1.0 to 1.0mm; in vivo impedance of the glass microelectrodes was typically 8-14 MΩ. Microelectrode signals were amplified x100 with a NeuroLog AC-DC amplifier (NL106), filtered between 300-10kHz (NL125) and the data sampled at 20kHz. Signals from glass microelectrodes were initially passed through a

NeuroData IR183 headstage and amplifier (x10; Cygnus Technology Inc., Delaware, USA). Electrodes were advanced using a motorized in vivo manipulator (IVM-1000; Scientifica, Uckfield, UK) linked to LinLab software until a responsive cell was found (i.e. in which a noxious stimulus elicited either an increase or decrease in basal firing). The total number of spikes in each 500 ms time period was used as our measure of single-unit activity (SUA) and converted into an instantaneous firing rate (Hz). Electrode recording sites were marked by iontophoretically injecting Chicago Blue 6B from the glass capillary or by passing current through the glass-coated tungsten electrode (<100 μA) and sites marked on a para-sagittal atlas image [Devonshire 2015].

In addition to supraspinal responses, recordings were also made of electromyographic (EMG) activity from the left ipsilateral flexor muscle (biceps femoris) as an assay of reflex sensitivity. The fur overlying the biceps femoris muscle was trimmed and a custom-built bipolar concentric needle EMG recording electrode (comprising a modified 27 gauge hypodermic needle) was inserted into the belly of the muscle. The EMG signal was amplified in the same way as EEG signals, band-pass filtered between 10-1000 Hz and sampled at 2kHz. Note that only a summary of SUA and EMG data are presented here; full analysis and interpretation of these data has already been provided in [Devonshire et al., 2015].

EEG Data Analysis

Data analysis was performed using Matlab 2014b using fast Fourier transform (FFT) functions of Matlab to analyse the frequency composition of the EEG. Multiple stimuli were presented to each animal, analysed separately and averaged for each animal. EMG onset time, rather than stimulus onset time, was used to align all data; this is because EMG onset is a reliable indicator of when the neural circuit registers a stimulus as noxious. EMG onset time was calculated as the time at which activity was 10% of the maximum. In some EEG recordings, movement artefacts were detected produced by the contact of the thermal stimulator with the rat's paw. These artefacts were removed and data interpolated between the start and end point of the artefact.

EEG data was divided into 5 s bins and for each animal 23 normalised FFTs generated from 115 s of data which included 30 s pre-EMG response data; data was normalised between 0 and 80 Hz. For some analyses, data were converted into fractional change of the baseline. In these instances, a 5 s period immediately before EMG onset was avoided because transient changes in frequency components were observed at this time. For additional analyses, data were divided into traditional frequency bands: delta (0.1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-32 Hz) and gamma (32-80 Hz) and power in each range averaged. In order to compare the total power at different time points before and after stimulation, as opposed to the distribution between frequency bands, non-normalised FFTs were generated from three periods of data: baseline (-30 to -10 s), peri-stimulus (-10 to 0 s) and response (0 to 20 s). Is this correct?

Statistical analysis

Similarity of resting state (baseline) EEGs between juvenile and adult rats was assessed with a two-way ANOVA (age x frequency band) and Sidak's post-hoc test (???). To examine changes in EEG frequency bands over time following thermal stimulation, a two-way ANOVA was performed (frequency band x time) for each age group using BASELINE PERIOD?; this was followed by Dunnett's post-hoc tests. A similar analysis was performed to examine differences between age groups using a two-way ANOVA (age x time) for each frequency band followed by Sidak's post-hoc tests. To compare total power during baseline, pre-response and post-response periods, a two-way ANOVA was performed (age x response period) followed by POST-HOC TEST? – is this bit correct?. Data are shown as mean ± standard error of the mean.

Results

Before thermal stimuli were presented, the distribution of power in the five frequency bands was not different between juvenile and adult rats (no interaction between age and frequency band, $F_{X,X}$ = X, P = X; Figure 1). This indicates that both age groups were in similar anaesthetic states. Noxious thermal stimulation of the plantar hindpaw skin triggered hindlimb withdrawal reflexes in both age groups and concurrent changes in firing rates of ON and OFF cells in the RVM. A comprehensive analysis of ON and OFF cell and EMG responses has already been published (Devonshire et al., 2015) and is shown here for comparison purposes alongside previously unreported changes in EEG parameters (Figure 2). All data have been aligned to the onset of EMG responses. With respect to EMG onset time, the average onset time of adult ON and OFF cell responses was -1.42 ± 0.21 s and -1.24 ± 0.25 s, respectively. In juveniles, the average onset time of ON and OFF cell responses was -0.46 ± 0.09 s and 0.05 ± 0.24 s.

Noxious thermal stimulation of the skin evokes changes in EEG frequency bands in both juvenile and adult rats

Noxious stimulation of the hindpaw plantar skin caused significant changes in different EEG frequencies (Figure 2; upper panels). The *total power* during baseline, peri-stimulus, and response is the same between the age-groups (no interaction between age and response period; $KW_{6,147}$ = 1.996, P = 0.849) but the frequencies over which this cortical activity is distributed is significantly different. In juvenile animals no interaction was found between band and time ($F_{88,2310}$ = 1.043, P = 0.372), but in the adult animals a significant interaction was found ($F_{88,2860}$ = 4.494, P < 0.0001). Analysis of individual bands revealed that immediately before onset of the EMG response there was an increase in Gamma activity relative to baseline in juvenile rats only (STATS). After the EMG response there was a transient decrease in Delta activity in both juveniles and adults relative to baseline (Figure 3). The magnitude of decrease is similar in both groups: 16.6% decrease in the juveniles (q_{2310} = 3.064, P = 0.034) and 19.7% decrease in the adults (q_{2860} = 3.58, P = 0.006). In adult rats only noxious thermal

stimulation evoked a large increase in Theta power (Figure 3) peaking at 143.8% of baseline 15 seconds after the EMG response. This increase in power is highly significant from both baseline (q_{2860} = 7.965, P < 0.0001) and juvenile Theta responses at corresponding time points (is this an interaction statistic? The implication is that it's post-hoc stats $F_{22,1034}$ = 8.416, P < 0.0001; see Figure 3 for timings and significance levels). This increase in adult Theta power was accompanied by decreases in the power of other bands that reached significance in Beta only (stats).

Non-noxious stimulation does not evoke changes in EEG frequency bands

To ensure that the recorded responses are due to thermal stimulation and not the mechanical stimulation associated with the block touching the rat's paw, non-noxious thermal stimulation of the plantar hindpaw was applied via the same heating block in the exactly the same manner to noxious stimuli. Analysis of FFTSs after non-noxious stimulation (stimulation that does not meet the threshold to produce a reflex withdrawal of the hindlimb and is therefore non-noxious) showed no interaction between band and time in either juvenile or adult animals ($F_{88,770}$ = 0.625, P = 0.996; $F_{88,330}$ = 0.797, P = 0.898 respectively).

Discussion

Here we have shown that noxious stimulation of the footpad produces changes in S1 EEG frequency bands that are significantly different between juvenile and adult rats. We show that although the overall level (power) of EEG activity within S1 is the same in both ages, post-stimulus cortical activity is restricted to theta frequencies in older animals, whereas more distributed whole spectrum activity is observed in juveniles.

Considerably less is known about the maturation of the ascending component of the "pain pathway" than the maturation of either the spinal cord (Fitzgerald, 2005) or the descending component of the pain pathway (Hathway et al., 2009, Hathway et al., 2012, Kwok et al., 2014). Dorsal horn projection neurons are found in the rodent spinal cord by E16 (Nissen et al., 2005) and grow up the spinal cord before birth (Bice and Beal, 1997). In the rat, spinothalamic fibres connections are present by E19 (Higashi et al., 2002). What is most striking is that there has not been a thorough physiological assessment of the integration of noxious and innocuous inputs into the ascending aspect of the pain pathway over post-natal development, especially at sensory cortical levels, that would allow a mechanistic explanation of clinical observations to be made.

Sensory cortex remains highly plastic throughout life, being able to modify cortical territories after major loss of input such as in sensory denervation (V Schubert et al., 2013 J Neurosci; M Kossut, 1998 Exp Brain Res) as well refining receptive fields and enhancing response amplitudes after receiving enriched input in naturalistic, or enriched, environments (Devonshire et al., 2010 Neuroscience; DB Polley et al. 2004 Nature). And, whilst the changing pattern of cortical somatotopy in response to non-noxious stimuli have been mapped over post-natal development (Seelke et al., 2012), a nociceptive map has not. Seelke and colleagues (Seelke et al., 2012) included in their stimulus repertoire a stimulus identified as a "hard-tap", but it is unclear how this was presented or whether it was of noxious intensity. Furthermore, this study mapped cortical neuronal responses only in as much determining somatotopic cortical representation, rather than how the intensity of that stimulus is encoded within

S1 and whether this changes with age; this leaves important unanswered questions regarding the development of functional responses to nociceptive stimuli in the cortex.

We have shown that whilst total EEG activity in juvenile and adult S1 following a thermal stimulus is quantitatively similar, the distribution of EEG activity in the is significantly different. Whereas in adults significant increases in Theta band frequencies occur, in juveniles this post-stimulus activity is distributed across the entire frequency spectrum. Further work will be required to determine if the lack of theta activity in juvenile animals is associated with larger and faster reflex responses that are observed in this age group (Figure 2; Devonshire et al., 2015). Further work is also required to determine the precise origin of the Theta band activity and how such oscillatory activity continues in the cortex for up to 70 s following the hindlimb withdrawal response (Figure 3). Acute injection of capsaicin into the hindpaw has also been shown to induce Theta oscillations in S1 (BW LeBlanc et al., 2014). However, in this study no Theta oscillations were detected in the ventroposterior lateral (VPL) thalamic nucleus from which S1 receives its input, suggesting a non-thalamic origin of Theta. The oscillatory correlations between sensory cortex and the hippocampus are well known (A Sirota et al., 2003 PNAS) and, therefore, Theta oscillations may originate in the hippocampus. Indeed, Raghavachari and colleagues have pointed to hippocampal and cingulate origins of theta oscillations (Raghavachari et al., 2006). This is supported by the activation of the hippocampus in the acute response to pain (S Khanna et al., 1992 Exp Neurol; S Khanna et al., 1997 Neurosci), stimulating the hippocampus eliciting pain in humans (E Halgren et al., 1978 Brain) as well as morphological alterations to hippocampal circuitry in both adult and neonatal rodents in chronic pain (AA Mutso et al., 2012 J Neurosci; AT Leslie et al., 2011 Neurosci Lett; JM Malheiros et al., 2014 Hippocampus). It is noteworthy here that patients with chronic pain also exhibit an enhancement in cortical theta oscillations [J Sarnthein et al., 2006 Brain; KD Walton et al., 2010 Pain].

We have demonstrated a significant increase in Theta band power following noxious stimulation but only in adults. In juvenile rats there were no significant

increases in Theta. The significance of this age-related difference is unclear, as are the mechanisms that underpin it. In humans the presence of Theta band activity is associated with the perception of pain (Walton et al., 2010, Schulman et al., 2011, Wang et al., 2015) and Theta activity has recently been linked to the synchronization of neuronal activity between the anterior cingulate cortex and thalamic structures in an experimental model of visceral pain (Wang et al., 2015). Furthermore, the phase of Theta oscillations have been shown to modulate Gamma oscillations, something that has been proposed to be important in cognitive processing (Canolty et al., 2006, Jensen et al., 2007) as well as in the subjective pain perception (Zhang et al., 2012). The consequences of an immature cortical response to noxious stimulation in juveniles requires further investigation, especially whether it may play a role in, for example, phenomena that are associated specifically with injury at young ages such as long-term abnormal pain sensitivity (REF).

In conclusion we have shown that post-stimulus responses of S1 to noxious, but not innocuous, stimuli are significantly different between adult and juvenile rats and that this most-likely reflects immaturity in the integration of noxious inputs into supraspinal sites.

Acknowledgements

This work was supported by a grant from BBSRC to GJH (BB/I001565/1) and a BBSRC Doctoral Training Programme studentship to CMG.

References

Berry M, Rogers AW (1965) The migration of neuroblasts in the developing cerebral cortex. J Anat 99:691-709.

Bice TN, Beal JA (1997) Quantitative and neurogenic analysis of the total population and subpopulations of neurons defined by axon projection in the superficial dorsal horn of the rat lumbar spinal cord. JComp Neurol 388:550-564.

Canolty RT, Edwards E, Dalal SS, Soltani M, Nagarajan SS, Kirsch HE, Berger MS, Barbaro NM, Knight RT (2006) High gamma power is phase-locked to theta oscillations in human neocortex. Science 313:1626-1628.

Catania KC, Henry EC (2006) Touching on somatosensory specializations in mammals. Curr Opin Neurobiol 16:467-473.

De Marco Garcia NV, Priya R, Tuncdemir SN, Fishell G, Karayannis T (2015) Sensory inputs control the integration of neurogliaform interneurons into cortical circuits. Nat Neurosci 18:393-401.

Devonshire IM, Kwok CH, Suvik A, Haywood AR, Cooper AH, Hathway GJ (2015) A quantification of the relationship between neuronal responses in the rat rostral ventromedial medulla and noxious stimulation-evoked withdrawal reflexes. Eur J Neurosci.

Erzurumlu RS, Jhaveri S (1992) Emergence of connectivity in the embryonic rat parietal cortex. Cereb Cortex 2:336-352.

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

Finlay BL, Uchiyama R (2015) Developmental mechanisms channeling cortical evolution. Trends Neurosci 38:69-76.

Fitzgerald M (2005) The development of nociceptive circuits. Nat Rev Neurosci 6:507-520.

Garcia-Larrea L, Peyron R (2013) Pain matrices and neuropathic pain matrices: a review. Pain 154 Suppl 1:S29-43.

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

Gross J, Schnitzler A, Timmermann L, Ploner M (2007) Gamma oscillations in human primary somatosensory cortex reflect pain perception. PLoS biology 5:e133.

Hathway GJ, Koch S, Low L, Fitzgerald M (2009) The changing balance of brainstem-spinal cord modulation of pain processing over the first weeks of rat postnatal life. J Physiol 587:2927-2935.

Hathway GJ, Vega-Avelaira D, Fitzgerald M (2012) A critical period in the supraspinal control of pain: opioid-dependent changes in brainstem rostroventral medulla function in preadolescence. Pain 153:775-783.

Hicks SP, D'Amato CJ (1968) Cell migrations to the isocortex in the rat. Anat Rec 160:619-634.

Higashi S, Molnar Z, Kurotani T, Toyama K (2002) Prenatal development of neural excitation in rat thalamocortical projections studied by optical recording. Neuroscience 115:1231-1246.

Hlinka J, Alexakis C, Diukova A, Liddle PF, Auer DP (2010) Slow EEG pattern predicts reduced intrinsic functional connectivity in the default mode network: an inter-subject analysis. Neuroimage 53:239-246.

Jensen O, Kaiser J, Lachaux JP (2007) Human gamma-frequency oscillations associated with attention and memory. Trends Neurosci 30:317-324.

Kwok CH, Devonshire IM, Bennett AJ, Hathway GJ (2014) Postnatal maturation of endogenous opioid systems within the periaqueductal grey and spinal dorsal horn of the rat. Pain 155:168-178.

Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: General considerations. Experientia 42:109-114.

Mancini F, Haggard P, Iannetti GD, Longo MR, Sereno MI (2012) Fine-grained nociceptive maps in primary somatosensory cortex. J Neurosci 32:17155-17162.

Meerwijk EL, Ford JM, Weiss SJ (2015) Resting-state EEG delta power is associated with psychological pain in adults with a history of depression. Biological psychology 105:106-114.

Nissen UV, Mochida H, Glover JC (2005) Development of projection-specific interneurons and projection neurons in the embryonic mouse and rat spinal cord. J Comp Neurol 483:30-47.

O'Leary DD, Nakagawa Y (2002) Patterning centers, regulatory genes and extrinsic mechanisms controlling arealization of the neocortex. Curr Opin Neurobiol 12:14-25.

Parnavelas JG, Uylings HB (1980) The growth of non-pyramidal neurons in the visual cortex of the rat: a morphometric study. Brain Res 193:373-382.

Raghavachari S, Lisman JE, Tully M, Madsen JR, Bromfield EB, Kahana MJ (2006) Theta oscillations in human cortex during a working-memory task: evidence for local generators. J Neurophysiol 95:1630-1638.

Schulman JJ, Cancro R, Lowe S, Lu F, Walton KD, Llinas RR (2011) Imaging of thalamocortical dysrhythmia in neuropsychiatry. Front Hum Neurosci 5:69.

Seelke AM, Dooley JC, Krubitzer LA (2012) The emergence of somatotopic maps of the body in S1 in rats: the correspondence between functional and anatomical organization. PLoS One 7:e32322.

Slater R, Worley A, Fabrizi L, Roberts S, Meek J, Boyd S, Fitzgerald M Evoked potentials generated by noxious stimulation in the human infant brain. Eur J Pain 14:321-326.

Sur M, Leamey CA (2001) Development and plasticity of cortical areas and networks. Nat Rev Neurosci 2:251-262.

Tracey I, Mantyh PW (2007) The cerebral signature for pain perception and its modulation. Neuron 55:377-391.

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

Walker SM, Franck LS, Fitzgerald M, Myles J, Stocks J, Marlow N (2009) Long-term impact of neonatal intensive care and surgery on somatosensory perception in children born extremely preterm. Pain 141:79-87.

Walton KD, Dubois M, Llinas RR (2010) Abnormal thalamocortical activity in patients with Complex Regional Pain Syndrome (CRPS) type I. Pain 150:41-51.

Wang J, Cao B, Yu TR, Jelfs B, Yan J, Chan RH, Li Y (2015) Theta-frequency phase-locking of single anterior cingulate cortex neurons and synchronization with the medial thalamus are modulated by visceral noxious stimulation in rats. Neuroscience 298:200-210.

Welker E, Van der Loos H (1986) Quantitative correlation between barrel-field size and the sensory innervation of the whiskerpad: a comparative study in six strains of mice bred for different patterns of mystacial vibrissae. J Neurosci 6:3355-3373.

Zhang ZG, Hu L, Hung YS, Mouraux A, Iannetti GD (2012) Gamma-Band Oscillations in the Primary Somatosensory Cortex—A Direct and Obligatory Correlate of Subjective Pain Intensity. The Journal of Neuroscience 32:7429-7438.

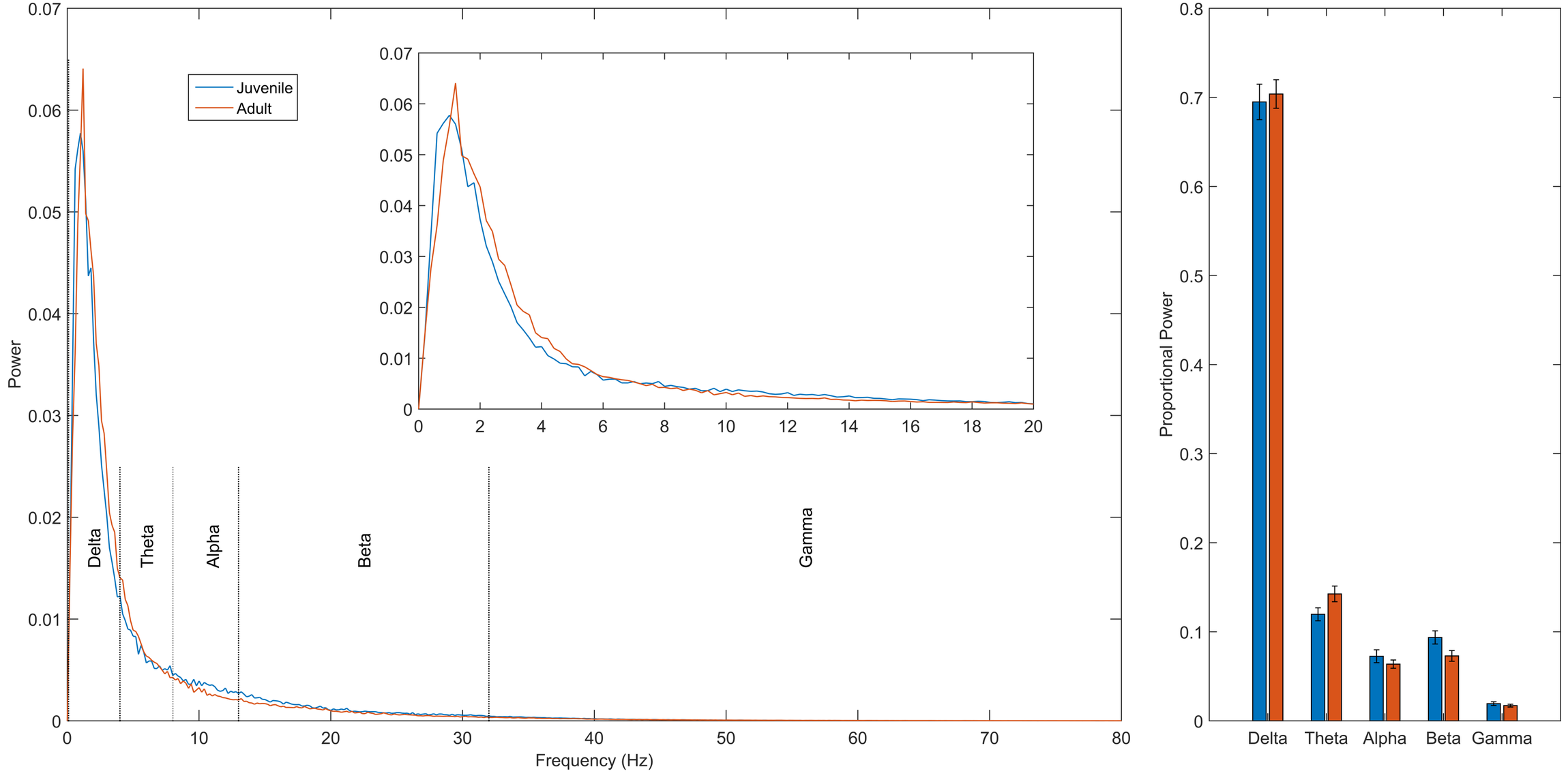
Figure Legends

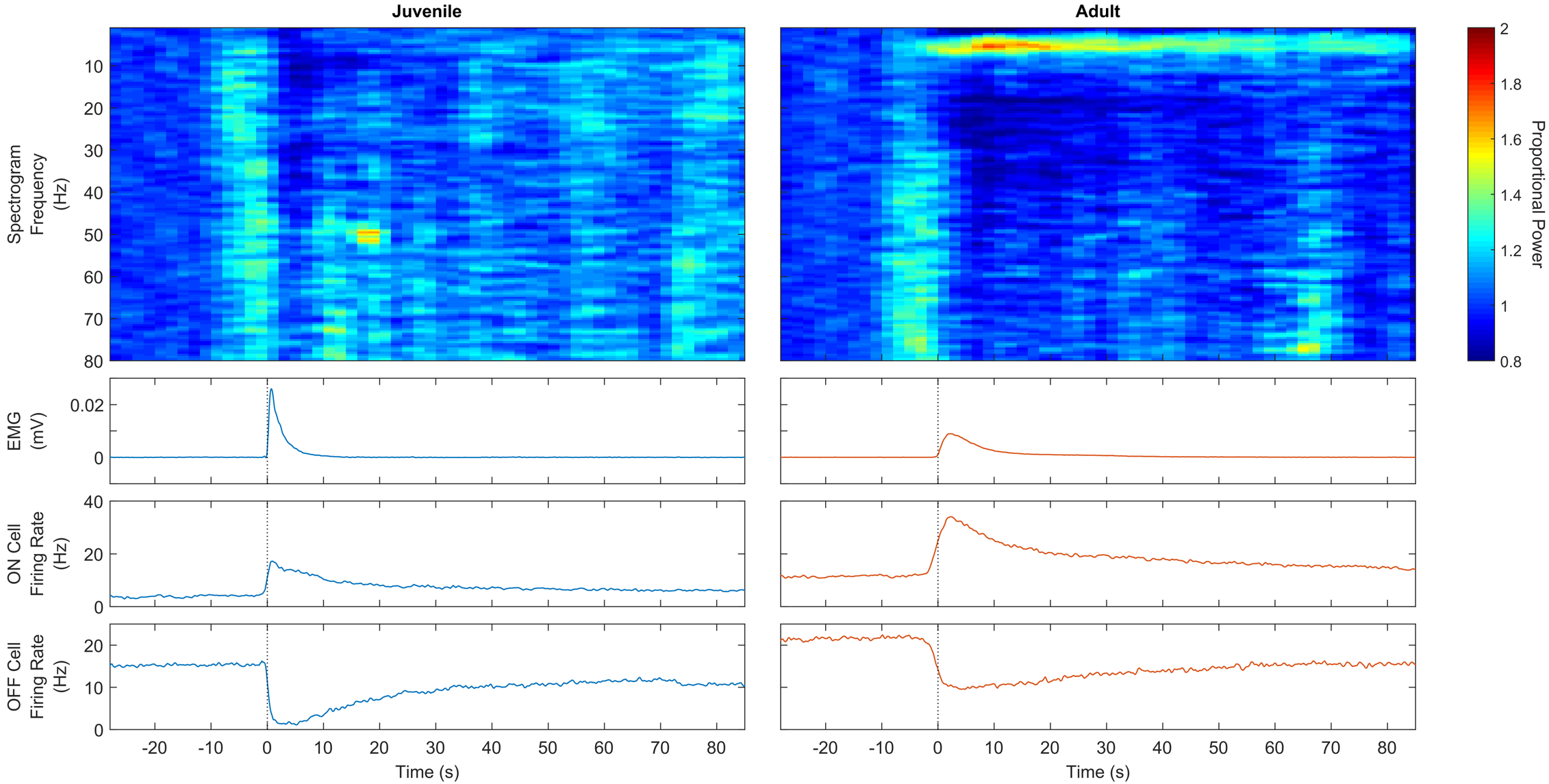
Figure 1.

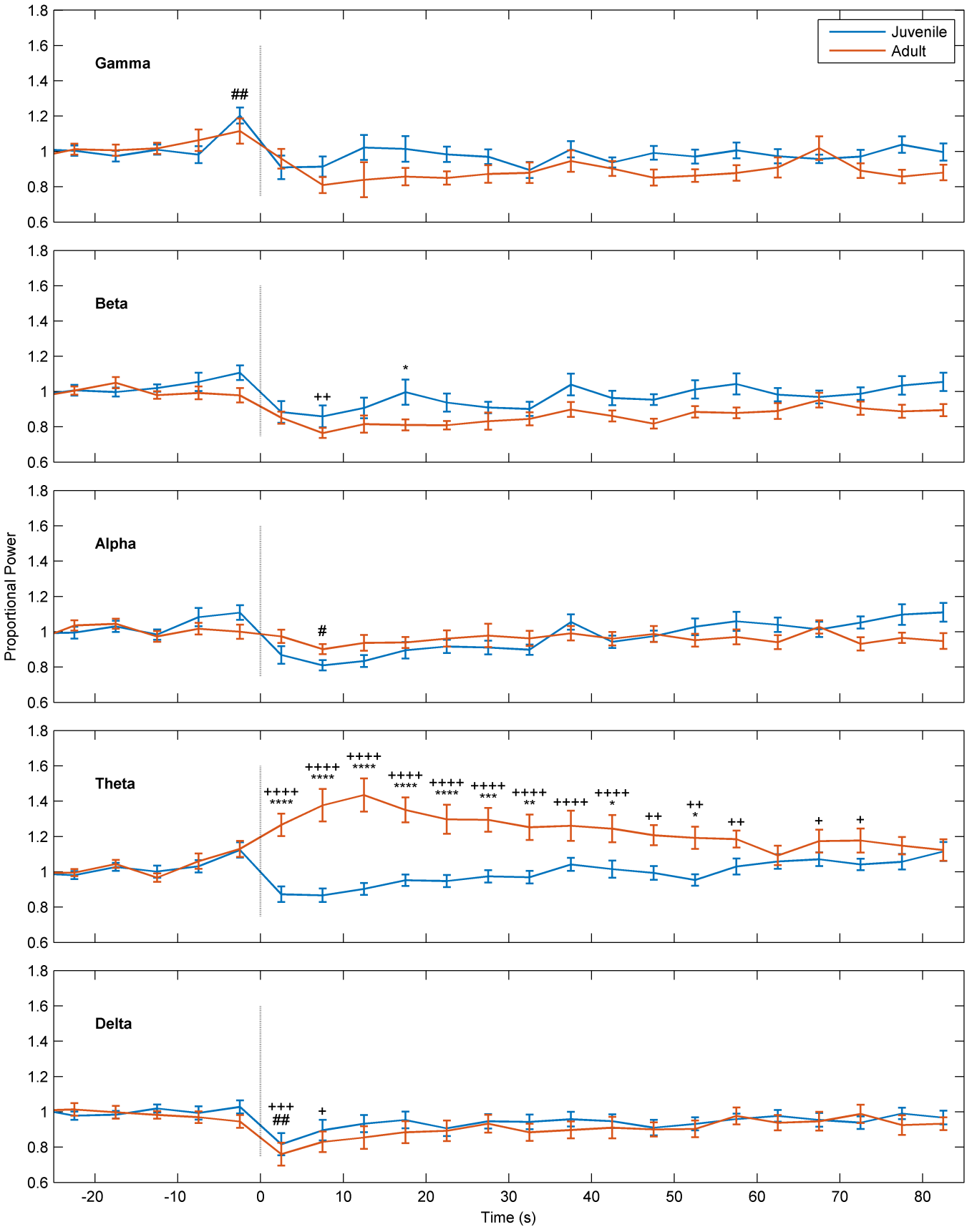
Left: Resting normalized distribution of S1 EEG frequencies of juvenile and adult animals is shown between 0 and 80 Hz. Inlay: Same data shown for frequencies between 0 and 20 Hz. Right: Resting power divided into bands as a proportion of total power.

Figure 2. Age significantly influences cortical EEG responses to noxious heating of the hind paw. Frequency specific EEG response normalised to baseline activity (top) recorded from all animals in each age-group. Data is aligned to the onset of the EMG response (middle). EMG (Juvenile n = 21; Adult n = 27) onset is concurrent with RVM ON (Juvenile n = 13; Adult n = 15) and OFF (Juvenile n = 9; Adult n = 12) cell activity (bottom).

Figure 3. Significant changes in specific EEG bands following noxious heating of the paw. There are significant post-stimulus changes in Theta power in the post-stimulus period. Blue lines denote responses from juvenile rats whereas orange lines are responses from adults. Asterisks denote differences between age-groups (*P<0.05, ** P<0.01, ***P<0.001, ****P<0.0001), hash denotes juvenile difference from baseline (#P<0.05, ## P<0.01, ###P<0.001, ####P<0.0001) whilst plus denotes adult difference from baseline ((+P<0.05, ++ P<0.01, +++P<0.001, ++++P<0.0001).


Juvenile
Adult
Power
Frequency (Hz)
Delta
Theta
Alpha
Beta
Gamma
Proportional Power
Delta
Theta
Alpha
Beta
Gamma


Juvenile
Adult
Spectrogram Frequency (Hz)
10
20
30
40
50
60
70
80
Proportional Power
2
1.8
1.6
1.4
1.2
1
0.8
EMG (mV)
0.02
0
ON Cell Firing Rate (Hz)
40
20
0
OFF Cell Firing Rate (Hz)
20
10
0
-20
-10
0
10
20
30
40
50
60
70
80
Time (s)


Juvenile
Adult
Gamma
Beta
Alpha
Theta
Delta
Proportional Power
Time (s)
1.8
1.6
1.4
1.2
1
0.8
0.6
-20
-10
0
10
20
30
40
50
60
70
80