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Early life pain experience changes adult functional pain connectivity in the rat somatosensory and the medial prefrontal cortex

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Abstract

Early life pain experience (ELP) alters adult pain behaviour and increases injury induced pain hypersensitivity, but the effect of ELP upon adult functional brain connectivity is not known. We have performed continuous local field potential (LFP) recording in the awake adult male rats to test the effect of ELP upon functional cortical connectivity related to pain behaviour. Somatosensory cortex (S1) and medial prefrontal cortex (mPFC) LFPs evoked by mechanical hindpaw stimulation were recorded simultaneously with pain reflex behaviour for 10 days after adult incision injury. We show that, post adult injury, sensory evoked S1 LFP delta and gamma energy and S1 LFP delta/gamma frequency coupling are significantly increased in ELP rats compared to controls. Adult injury also induces increases in S1-mPFC functional connectivity but this is significantly prolonged in ELP rats, lasting 4 days compared to 1 day in controls. Importantly, the increases in LFP energy and connectivity in ELP rats were directly correlated with increased behavioural pain hypersensitivity. Thus, early life pain (ELP) alters adult brain functional connectivity, both within and between cortical areas involved in sensory and affective dimensions of pain. The results reveal altered brain connectivity as a mechanism underlying the effects of early life pain upon adult pain perception.

Keywords: cortical pain networks, infant pain, experience dependence, brain development, pain development, early life adversity, nociception, nociceptive development.

Significance Statement

Pain and stress in early life has a lasting impact upon pain behaviour and may increase vulnerability to chronic pain in adults. Here we record pain-related cortical activity and simultaneous pain behaviour in awake adult male rats previously exposed to pain in early life. We show that functional connectivity within and between the somatosensory cortex and the medial prefrontal cortex is increased in these rats and that these increases are correlated with their behavioural pain hypersensitivity. The results reveal that early life pain alters adult brain connectivity, which may explain the impact of childhood pain upon adult chronic pain vulnerability.

Introduction

Exposure to pain and injury in early life pain (ELP) is associated with altered pain behaviour in adults. Evidence from both human and animal studies shows that repeated painful procedures or surgical incision during a critical period of early postnatal development has significant long-term effects on pain processing (Walker et al., 2009b, 2009a; Beggs et al., 2012b; Schwaller and Fitzgerald, 2014; van den Hoogen et al., 2018). The mechanisms underlying the effects of ELP involve changes in peripheral cutaneous innervation (Reynolds and Fitzgerald, 1995; De Lima et al., 1999; Beggs et al., 2012a; Boada et al., 2012), peripheral afferent sensitization (Walker et al., 2016; Liu et al., 2017; Dourson et al., 2021), spinal cord nociceptive circuitry (Torsney and Fitzgerald, 2003; Li and Baccei, 2019), early life spinal microglial activation (Moriarty et al., 2019) and altered descending brain stem pain control (Walker et al., 2015). There is also evidence from human studies of structural changes in the thalamus and cortex (Duerden et al., 2018) and functional changes in descending pain control from supraspinal sites (Walker et al., 2018). The importance of this extends into a wider area of the long-term consequences of early life stress and pain which, by inducing long-term alterations in brain function and behaviour may lead to higher susceptibility to chronic pain (Jones et al., 2009; Denk et al., 2014; Ririe et al., 2021; Melchior et al., 2022). However, as yet, there is no evidence that ELP has any effect upon adult cortical pain networks or upon functional connectivity between the key cortical regions involved in the sensory and emotional dimensions of pain.

A wide network of brain areas is involved in acute pain processing, including primary (S1) and secondary (S2) somatosensory cortices, the medial prefrontal cortex (mPFC), insula, thalamus, and prefrontal areas (Apkarian et al., 2005; Duerden and Albanese, 2013; Tan and Kuner, 2021). To address whether ELP impacts

upon cortical function from the sensory-discriminative and emotional/cognitive perspectives, the S1 and mPFC are attractive targets (Tan and Kuner, 2021). S1 is a functionally defined part of the somatosensory and nociceptive system and processes sensory nociceptive information about pain from an early age in both rodents and humans (Chang et al., 2016, 2020b; Jones et al., 2022). S1 encodes nociceptive intensity and perceived pain intensity (Mancini et al., 2012) and gamma-band oscillations in this area correlate with subjective pain perception (Ong et al., 2019). While mPFC is critically involved in numerous cognitive functions (Euston et al., 2012; Chang et al., 2020a) and emotion behaviour (Cao et al., 2018; Huang et al., 2020), this area also plays an important role in the emotional and affective aspects of pain, and could modulate pain sensation by controlling the flow of afferent sensory stimuli into the dorsal horn through descending control pathways (Zhang et al., 2015; Huang et al., 2020). Here we hypothesise that ELP alters pain related connectivity in the adult S1 and mPFC and that this is associated with increases in adult pain related behaviour.

In this study, we used a well-established model of injury and postoperative pain: hind-paw plantar incision of skin and underlying muscle (Brennan et al., 1996; Beggs et al., 2012b) to examine the impact of ELP upon pain behaviour and associated neural activity in S1 and mPFC. We recorded local field potentials (LFPs) in S1 and mPFC in awake, freely moving adult rats and analysed the oscillatory energy within those sensory evoked LFPs and the functional connectivity within and between these areas. Acute pain is associated with defined changes in cortical oscillations (Tan et al., 2021). In humans, gamma-band oscillations in S1 correlate with subjective pain perception (Heid et al., 2020; Yue et al., 2020a) and are strengthened in rodent S1 cortex during nociception and inflammatory pain in association with behavioural nociceptive hypersensitivity (Tan et al., 2019). We also analyse phase-amplitude coupling and coherence of neuronal oscillations as putative mechanisms of regional and inter-areal communication (Buzsaki, 2004; Peng and Tang, 2016). Together, our results provide new insights into how early life pain (ELP) alters adult cortical function underlying sensory and emotional dimensions of pain behaviour.

Materials and Methods

Experimental animals: All experiments were performed in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986. Reporting is based on the ARRIVE Guidelines for Reporting Animal Research developed by the National Centre for Replacement, Refinement and Reduction of Animals in Research, London, United Kingdom. Male Sprague-Dawley rat pups were obtained from the Biological Services Unit, University College London. Rats were housed in cages of five age-matched animals (>P21) or with the dam and littermates (P) 3 to 21 under controlled environmental conditions (24–25°C; 50–60% humidity; 12 h light/dark cycle) with free access to food and water. In the case of rat pups, handling and maternal separation were kept to a minimum. All animals were exposed to the same standard caging, handling and diet throughout development. The different experimental groups are represented in Figure 1A and protocol for probing the impact of nociceptive inputs in the early life on central pain processing and adult pain sensitivity is summarized in Figure 1B

Plantar hind-paw incision: Male rat pups on postnatal Day 3 were anaesthetized and plantar hindpaw incision performed. Under general anesthesia with 2% isoflurane in 100% oxygen (flow rate, 1–1.5 L/min), a midline longitudinal incision was made through the skin and fascia extending from the midpoint of the heel to the proximal border of the first footpad and the underlying plantar muscle elevated and incised. The same relative length of incision was performed in adult animals as previously described (Brennan et al., 1996; Walker et al., 2009b). Skin edges were closed with 5–0 nylon suture (Ethicon). The whole procedure took 3–5 min. After plantar hindpaw incision, rats were placed in a recovery chamber and allowed to recover from the general anesthesia before returning to their home cage.

Four experimental groups were used: **II:** neonatal incision on postnatal day 3 and repeat incision 2 months later in adulthood. **NI:** littermate control with equivalent anaesthesia, handling and maternal separation on post-natal day 3 and having incision in adulthood. Animals having neonatal incision and follow-up in adulthood **(IN)** and age-matched non-incised litter mates from the same colony **(NN)** were pooled data and used as control group **(Con)**, due to that there is no significant different between the two groups (Figure 1A)

Pain hypersensitivity testing: To test behavioural pain hypersensitivity following hind-paw incision, an electronic von Frey unit (EVF4, Bioseb) was used to measure hindpaw mechanical flexion withdrawal thresholds (Ferrier et al., 2015, 2016). Following habituation for 30 min on an elevated mesh platform, a mechanical stimulus was applied to the plantar surface of the hindpaw adjacent to the distal half of the incision. The von Frey (eVF) apparatus, whichhas a measurement range of 0-500 g with 0.1 g resolution, consists of a plastic tip fitted in a hand-held force transducer, which was applied to the rat hindpaw from below with force (g) gradually increased until paw withdrawal. The force that induced paw withdrawal was digitized and recorded automatically by the unit and used as the threshold for mechanical nociception. For each recording session, the eVF was applied 3-5 times at ~50 sec intervals. Simultaneous recording from both S1 and mPFC accompanied testing of eVF withdrawal thresholds (Figure 1 C, D).

Surgical Preparation and Transmitter Implantation for Long-term Recording: Rats were anaesthetised with 2.5-3 % isoflurane (Abbot, AbbVie Ltd., Maidenhead, UK) in 100% oxygen (flow rate of 1-1.5 litre/min) via gas anaesthesia mask (Model 906, David Kopf Instruments) from a recently calibrated vaporizer (Harvard Apparatus, Cambridge, MA). Body temperature was maintained with a heat blanket during surgery. A transmitter (A3028D-DDA, Open Source Instruments, Brandeis, Boston, USA)(Chang et al., 2011) was implanted subcutaneously with the depth recording electrodes (J-electrode (wire 125-µm dia 316SS 10kOhm impedance), a Teflon-insulated stainless steel electrode, Open Source Instruments, Brandeis, Boston, USA) positioned in mPFC (3.2 mm anterior, 0.5 mm lateral, 4 mm ventral) and primary somatosensory hindpaw cortex (1 mm posterior, 2.5 mm lateral, 2 mm ventral) (Paxinos et al., 2013; Chang et al., 2016). The reference electrode was implanted over the cerebellum posterior to lambda. The whole assembly was held in place with dental cement (Simplex Rapid, Acrylic Denture Polymer, UK). A subcutaneous injection of bupivacaine and metacam was provided for post-surgical pain management. At the end of surgery, enrofloxacin (5mg/kg, Baytril, Bayer health care) and pre-warm saline (0.5-1 ml) were administered subcutaneously. The animals were placed in a temperature controlled (25°C) recovery chamber until ambulatory and closely monitored at least 1-2 hours before returning to their home cage to allow recovery for at least 14 days after surgery.

The transmitter, which has no adverse effects (Chang et al., 2016), was implanted for data recordings. During all recording sessions, continuous LFP recordings were recorded (bandpass filter: 0.2 Hz to 160 Hz, 512Hz sampling rate with 16 bit resolution) using LWDAQ Software (Open Source Instruments, Brandeis, Boston, USA). Animals were carefully monitored daily and were euthanized at the end of experiment with carbon dioxide (CO_2). The brain was removed and immediately immersed in 4% paraformaldehyde for >24 hours before being transferred to 30% sucrose post-fixation solution. Brain sections (40-µm thick thickness) were cut using a microtome (Leica SM2000R, Leica Microsystems (UK) Ltd., United Kingdom) and stained with Cresyl violet to allow histological location of the electrode track. This procedure allowed us to verify recording electrode locations, and LFP data were only included in the study if electrode tips were located in mPFC and S1 (Figure 1D).

Analysis of electrophysiology data: Data analysis was performed with Brainstorm (Tadel et al., 2011), which is free and open source for electrophysiology data visualization and processing through a simple and intuitive graphical user interface (GUI) (http://neuroimage.usc.edu/brainstorm) and custom Matlab scripts (The Mathworks Inc.MA, USA).

Evoked LFP data processing: LFP Pre-processing: For our initial analyses, continuous LFP recordings from each region were segmented into 10s epochs that lasted from 5s before to 5s after the peak of evoked LFP. Each epoch was visually inspected for artefacts prior to further analysis. Any epochs that, upon visual inspection, exhibited electrode artifacts (ie, abrupt vertical transients that do not modify the background activity) were excluded from subsequent analysis. **Time-frequency Analysis:** Activity changes in LFP in different frequency bands were calculated using the Hilbert transform (Le Van Quyen et al., 2001; Bruns, 2004; Tadel et al., 2011). Each epoch was filtered in various frequency bands with bandpass filters for delta (2–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–90 Hz) band. The magnitude (μV/sqrt(Hz)) of the Hilbert transform of a narrow-band signal is a measure of the envelope of this signal, and therefore gives an indication of the activity in this frequency band. The energy magnitude data were then averaged across repetitions within each animal. Stimulus-induced changes in energy magnitude for each animal were then calculated by normalized to mean of baseline (-4 to -1s).

Time-resolved Phase-amplitude Coupling Analysis: This approach measures cross-frequency coupling between bursts of high-frequency oscillations and the phase of lower frequency rhythms, over a time-window, which slides along the electrophysiological data (Samiee and Baillet, 2017). mPFC and S1 time-courses were examined for changes in phase of slow oscillation at delta band (2-4 HZ) coupled to the amplitude of a faster rhythm at gamma (30-90 Hz) band. Phase and amplitude information were obtained via the Hilbert transform. The coupling between phase and amplitude was then quantified and Modulation Index values were calculated. To avoid edge artefacts, which can result in spurious phase-amplitude coupling (PAC) (Kramer et al., 2008), the first 2 s and last 2 s of each trial was used as buffer. These were then averaged across repetitions within each animal. Stimulus-induced Phase-Amplitude Coupling for each animal were then calculated by normalized to mean of baseline (-2.5 to -1 s). **Time-resolved Phase locking Analysis:** To evaluate the functional connectivity between mPFC and S1, we estimated phase-locking value between the LFPs simultaneously recorded at the two areas in different frequency bands (Lachaux et al., 1999). To do this we (i) band-pass filtered the LFPs at S1 and mPFC in the delta (2–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–90 Hz) frequency bands; (ii) applied Hilbert transform to the band-passed signals; (iii) calculate the instantaneous phase-locking value between mPFC and S1. PLVs were then averaged across repetitions within each animal. Stimulus-induced magnitude changes in LFP energy for each animal were then calculated by normalized to mean of baseline (-4 to -1s)

Quantification and Statistical Analysis: Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software), SPSS (Statistical Product and Service Solutions, IBM). All data are presented as mean ± SEM. Comparisons of means were performed using one way ANOVA with Tukey post hoc test if the data were normally distributed; Kruskal-Wallis test with post hoc Dunn's multiple comparisons test if the data were not normally distributed (with the Shapiro-Wilk test used to assess normality of the data distributions). Generalized linear model (GLM) Type III tests followed by Bonferroni post hoc tests were used for analysis of repeated-measures behaviour data. Differences were considered statistically significant at $p < 0.05$. Estimation statistics (open source estimation program available on https://www.estimationstats.com)(Ho et al., 2019) were used to compute the change of electrophysiological data in mPFC/S1 in response to eVF stimulation with days following injury (D0, D4, and D10), compared to pre-injury activity. Mean differences are shown using Cumming estimation plots, with each graphed as a bootstrap sampling distribution (5000 bootstrap samples). The p value(s) reported are the likelihood(s) of observing the effect size(s), if the null hypothesis of zero difference is true. For each permutation p value, 5000 reshuffles of the control and test labels were performed; $p < 0.05$ is considered a significant difference. Pearson correlation was applied to calculate the correlation between pain sensitivity and electrophysiological data. The significance threshold for all correlation tests was set at $p < 0.05$.

Results

Early life pain (ELP) increases injury induced hyperalgesia and pain in adult life

Behavioural pain threshold testing confirmed the impact of early life pain upon adult pain behaviour, as described previously (Beggs et al., 2012; Moriarty et al., 2019). We measured the amplitude and duration of hindlimb withdrawal reflexes in response to electronic von Frey hair stimulation following incision injury in adult male rats. Figure 1 shows von Frey hair pain thresholds in adult ELP male rats before and 10 days after an adult hindpaw incision **(II)**. This is compared to age- matched animals with no ELP, experiencing their first hindpaw incision in adulthood **(NI)** and control rats that have ELP only or no incisions at all **(Con)** (Figure 1).

Hindpaw incision injury caused von Frey hair thresholds to fall in both groups of adult rats (NI and II) compared to the control (Con) group, indicating a significant post injury pain and hyperalgesia. Consistent with previous reports (Beggs et al., 2012b; Moriarty et al., 2019), animals that experienced early life pain (ELP) (II, n=9) developed significantly lower paw withdraw thresholds (PWT), compared to injured animals with no ELP (NI, n=10). In addition, as in earlier studies (Walker et al., 2009b), ELP resulted in more prolonged as well as enhanced hyperalgesia, lasting up to 10 days after hindpaw incision, compared to 3-4 days in non ELP rats.

Early life pain (ELP) increases post injury evoked delta and gamma activity in adult S1

To test the impact of early life pain experience upon pain related neural activity in S1 and mPFC, we next investigated evoked potentials (EP) and oscillatory neural activity in S1 and mPFC evoked by mechanical stimulation (von Frey hair, eVF) following incision injury. Evoked potential (EP) amplitudes in S1 and mPFC did not differ between groups and so to gain further insight into the pattern and time-course of evoked cortical activity following incision injury, the EP energy was analysed in the delta (2–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–90 Hz) frequency bands. Fig 3 & Fig 4 show a significant increase in the eVF evoked delta energy (Fig 3C,D) and gamma energy (Fig 4C,D) in S1 following incision in ELP rats, which is not observed in the other adult rat groups, NI and Con. The sensory evoked data in Figs 3 and 4 has been normalized to baseline (a period before stimulation), removing any effect of increased gamma power in the S1 and PFC caused by the surgical pain alone, and revealing only eVF stimulus evoked energy changes. These stimulus evoked increases in delta and gamma energy in ELP rats were recorded in II groups only, in the 2-3 hours post injury (D0) and had recovered by 4 days post injury. They were not observed in mPFC

Importantly, the magnitude of S1 evoked delta and gamma activity was significantly correlated to pain sensitivity, or fall in behavioural von Frey hair paw withdrawal threshold (PWT), as indicated by the inverse correlation of S1 delta power (Fig 3E) and S1 gamma power (Fig 4E) with PWT in II male adult rats.

Early life pain (ELP) increases post injury evoked delta-gamma coupling in adult S1

Since delta and gamma energy evoked by mechanical stimulation (eVF) post injury is increased in S1 in ELP rats, we next asked whether ELP altered cross-frequency coupling (delta (2-4Hz) vs. gamma (30-90 Hz)) associated with the observed differences in pain sensitivity following hindpaw incision. Cross-frequency interaction (Florin and Baillet, 2015). Here, in order to evaluate event related changes in phase-amplitude coupling, we used time-resolved phase-amplitude coupling (tPAC). Fig 5 shows a significant enhancement of evoked delta-gamma coupling in S1 immediately post-incision (D0) in II rats (Figure 5 A-C). This increase in delta-gamma coupling was not seen in mPFC (Figure 5D). The enhanced evoked delta-gamma coupling in S1 coupling potentially provides a mechanism for investigating local-to-wide networks synchronization and was observed on the day of injury and return to pre-injury levels by 4 days (D4) post-incision in II rats. There was no significant alteration in S1 evoked delta-gamma coupling in NI and Con rats (Figure 5E). To determine whether this increase in evoked S1 delta-gamma coupling is associated with the enhanced pain sensitivity, we subsequently examined the correlation between the two measures. A significant inverse correlation was

found between delta-gamma coupling and paw withdrawal threshold in II rats, but not in NI and Con rats (Figure 5F). Thus, pain related stimulus evoked delta-gamma coupling in the somatosensory cortex and its association with pain behaviours is selectively increased in adult ELP rats.

Early life pain (ELP) increases post injury evoked S1-mPFC connectivity in adult rats

The increased pain related signal processing in ELP found in adult S1, was not observed in mPFC. Since alterations in pain processing in mPFC may depend upon connections with other areas of the cerebral cortex, we next examined the functional connectivity between the S1 and mPFC in ELP rats. To explore this, we used phase locking value (PLV), a statistical method used to investigate task-induced changes in long range synchronization of neural activity (Lachaux et al., 1999) which provides an index of phase synchrony between two signals over a specific time period.

On the day of injury (D0), 2-3 hours after the incision, a significant increase in S1-mPFC PLV in response to eVF stimulation occurred in both ELP and non ELP rats following hindpaw incision (NI and II). There was no significant difference between the two injured groups (Figure6 A-C). This increase in phase locking was restricted to the theta band and was not observed in other frequency bands (Delta: $F_{(2, 28)} = 0.16$, P=0.85 ; Alpha: $F_{(2, 28)} = 1.75$, P=0.19; Beta: $F_{(2, 28)} = 0.96$, P=0.39; Gamma: $F_{(2, 28)} = 2.41$, P=0.10). Importantly, a clear difference emerges upon inspection of the time course of this effect post injury, which reveals that the increased theta phase locking is maintained until 4 days post-injury in ELP (II) rats, compared to non-ELP (NI) groups (Figure 6D and 6E). We further examined correlation coefficients with pain behaviour to determine whether the increased S1-mPFC PLV in the theta band is associated with pain hypersensitivity. A significant inverse correlation between S1-mPFC PLV and PWT is seen in both NI and II rats, but not in uninjured Con rats (Figure 6F).

Discussion

The results presented here provide novel insights into the effects of ELP upon adult cortical pain networks. Using telemetric recording of local field potentials in the S1 and mPFC in awake adult mice we show that ELP results in significant changes in neural connectivity in the adult S1 and mPFC related to post-injury pain hypersensitivity.

We used a well-established model of ELP, incision on the plantar hindpaw, which when applied at a critical stage of development, is known to cause lasting changes in pain behaviour and increased post-injury pain hypersensitivity in adult life (Walker et al., 2009b; Beggs et al., 2012b; Schwaller and Fitzgerald, 2014). The effect is likely to be driven by altered peripheral nociceptor sensitization (Jankowski et al., 2014; Walker et al., 2016; Dourson et al., 2021) and microglial activation in the dorsal horn of the spinal cord (Beggs et al., 2012b; Moriarty et al., 2019) resulting in altered synaptic connectivity and reduced dynorphin inhibition in the dorsal horn of the spinal cord (Li and Baccei, 2016, 2019; Brewer et al., 2020). Brainstem descending pain control is also altered in adults following early life incision (Walker et al., 2015) but the current data is the first to show changes in functional cortical pain networks following ELP. By recording simultaneous behavioural and cortical LFP responses to the same mechanical stimulus, we show that following ELP delta and gamma energy and delta/gamma modulation are increased in S1, together with increased phase-locking connectivity with mPFC, all directly correlated with behavioural pain hypersensitivity.

The data provide new insight into the central mechanisms whereby exposure to painful sensory experience in early life alters adult pain experience. The mPFC and S1 have key roles in cortical pain processing (Tan and Kuner, 2021); mPFC receives ascending nociceptive input, but also exerts important top-down regulation of sensory and affective processes of pain (Kummer et al., 2020), whereas S1 is the first level of pain perception and encodes nociceptive intensity and perceived pain intensity (Fields, 2012; Mancini et al., 2012). Pain is a complex phenomenon that depends on communication between different brain areas, which is served by

neural oscillations and connectivity involving short-range and long-range communication processes (Baliki et al., 2011; Baliki and Apkarian, 2015; Kucyi and Davis, 2015; Ploner et al., 2017; Tan et al., 2021) and it is these oscillations that we have focussed on here.

The results reveal a significantly greater noxious-evoked gamma in S1 in injured rats with ELP compared to controls. In humans, gamma-band oscillations in the primary somatosensory cortex correlate with subjective pain perception (Zhang et al., 2012; Heid et al., 2020) and in mice they are specifically strengthened, independently of any motor component, in the S1 cortex during nociception and are elevated during pain hypersensitivity (Tan et al., 2019). Nociceptive C fibre stimulation drives gamma activity in adult rat S1 (Chang et al., 2020b) and gamma oscillations generated by optogenetic activation of parvalbumin-expressing inhibitory interneurons in the S1 cortex enhance nociceptive sensitivity and induce aversive avoidance behaviour, while activating a network of prefrontal cortical and subcortical centres including descending serotonergic facilitatory pathways (Tan et al., 2021). Recent evidence suggests that gamma oscillations reflect strongly coupling of neural activity with fast spiking interneurons in the superficial layers of the S1 contralateral to the stimulated side (Yue et al., 2020a). The increased energy of gamma oscillations, considered one of the most promising biomarkers of pain in the brain, is important evident for increased post injury pain perception in ELP animals.

Evoked activity in the delta frequency was also observed in the S1 of injured ELP rats. Event-related delta oscillations serve active sensory and cognitive functional roles across different sensory domains (Arnal and Giraud, 2012; Knyazev, 2012; Fardo et al., 2017) and play an crucial role in S1 sensory perception (Schroeder and Lakatos, 2009). Delta oscillations association with pain has been demonstrated elsewhere and may reflect coupling in thalamocortical loops (Sarnthein et al., 2006; Walton et al., 2010; Peng and Tang, 2016). The lack of delta frequency changes in mPFC supports the proposal that thalamo-S1 pathways are altered in ELP rats. Indeed, in human infants, ELP is associated with volume loss in the somatosensory thalamus accompanied by disruptions in thalamic metabolic growth and thalamocortical pathway maturation (Brummelte et al., 2012; Duerden et al., 2018).

Neural oscillations play an important role in the integration and segregation of brain regions that are important for pain processing. Low-frequency oscillations (e.g., delta, theta) mediate long-range communication at slow timescales across distant brain regions and are crucial for functional integration in large-scale brain networks. In contrast, high-frequency brain oscillations (e.g., gamma) are more transient and focal and thus important for local neuronal synchrony in cortical areas (Canolty and Knight, 2010). Understanding these spatiotemporal and oscillatory aspects in the context of pain-related neural responses will therefore inform the neural mechanisms underlying pain-sensation. Studies of neural oscillations related to pain have identified several functional bands, especially theta, delta, and gamma bands, implicated in nociceptive processing (Kim and Davis, 2021; Luo et al., 2021). Delta oscillations are changes in the thalamus and S1, as well as the coupling between the thalamus and S1, in laser-induced pain (Li et al., 2017) and in neuropathic disease (Walton et al., 2010). Furthermore, a recent study suggested that the delta combined with other oscillations is responsible for the coding of pain perception, indicating that perception as an overall reflection of the pain state may contain complex information and involve additional brain areas (Luo, 2021). On the other hand, gamma oscillations in S1 predict the pain intensity induced by laser stimulation in both humans and rodents (Hu and Iannetti, 2019; Yue et al., 2020b) and the pain level in chronic pain patients (Parker et al., 2020), indicating gamma oscillations may contain more specific information about pain. Therefore, the combination of neural oscillations is essential for encoding perceptive and sensory measures of pain. Our findings highlight that pain related sensory evoked neuronal activity in S1 which is associated with both low- and high-frequency oscillatory rhythms, mediating functional integration at both local and large-scale brain networks are altered by early life pain experiences.

Overall, these results indicate that the changes in delta and gamma activity in S1 are functionally linked to the behavioural hypersensitivity in injured rats with ELP. However, given the distinct intrinsic spatiotemporal properties of low- and high-frequency oscillations, we further examined the transient modulation of high-frequency amplitude (gamma) by low-frequency phase (delta) in relation to pain sensitivity and found enhanced evoked S1 delta-gamma modulation in injured rats with ELP. Because the high-frequency activity reflects local cortical processing, while low-frequency brain rhythms are dynamically entrained across distributed brain regions by both external sensory input and internal cognitive events, cross frequency modulation between low and high frequency is thought to contribute to information flow from large-scale brain networks to the fast, local cortical processing (Cardin et al., 2009; Canolty and Knight, 2010). Phase-amplitude cross-frequency coupling strength changes quickly in response to sensory, motor, and cognitive events (Schroeder and Lakatos, 2009) and abnormalities of cross frequency modulation may contribute to abnormal routing of information flow in chronic pain (Ploner et al., 2017). Our results suggest that such abnormal routing of information may occur in adults following ELP.

While the S1 reflects sensory discriminative aspects of pain, the prefrontal cortex is associated with the affective aspect of pain, providing top-down modulation of sensory and affective processes, including inhibition of both sensory and affective pain signals by descending projections to the various brain and spinal cord regions (Ji and Neugebauer, 2014; Bräscher et al., 2016; Kummer et al., 2020). Enhanced functional connectivity under procedural pain has been observed in several areas involved in pain perception: somatosensory cortices, anterior insula, anterior cingulate cortex and thalamus and mPFC (Bräscher et al., 2016; Galambos et al., 2019). Here we tested whether communication between S1 and mPFC was affected by ELP using synchronisation in the theta range as a measure of connectivity. Theta synchronization is proposed to be involved in large scale integration between long range multiple brain regions (von Stein and Sarnthein, 2000), especially in mPFC (Colgin, 2011; O'Neill et al., 2013; Esmaeili and Diamond, 2019), consistent with human data showing that prefrontal-sensorimotor connectivity is increased in tonic pain (Nickel et al., 2020). Our results show that adult incision injury does indeed produce a marked increase in evoked theta S1–mPFC connectivity, highly correlated to behavioural pain sensitivity in both ELP and control groups, but this increase is prolonged in ELP, lasting for 4 days compared to only one day in controls. Our data suggests that the connection between sensory and affective pain processing is enhanced in ELP rats which may underpin the wider social, emotional and cognitive life-long impact of ELP beyond increased pain perception (Ranger et al., 2018; de Kort et al., 2021; Ririe et al., 2021) .

Our demonstration that ELP affects the cortical dynamics and connectivity underlying adult pain perception has important translational implications. Hospitalised infants exposed to ELP as a result of necessary clinical care, despite efforts to control that exposure (Laudiano-Dray et al., 2020; Eccleston et al., 2021), display long term structural and functional brain changes (Ranger and Grunau, 2014; Walker, 2019). Early life adversity, including stress and pain, has been reported to increase the risk of persistent pain in adults (Victoria and Murphy, 2016, 2016; Nelson et al., 2017) and it is possible that the changes reported here underlie an increased vulnerability to chronic pain in adults exposed to ELP. Pain is the perceptual consequence of the complex interactions of many cortical areas, including the somatosensory, prefrontal cortices, and limbic areas (e.g., thalamus) and both animal (Eto et al., 2011) and human (Geha et al., 2008; Ichesco et al., 2012) studies reveal functional and structural changes in these specific areas of the cerebral cortex in chronic pain conditions. Furthermore, S1 and mPFC closely interact in chronic pain (Kong et al., 2013; Jones and Sheets, 2020; Kummer et al., 2020). This reorganization of local cortical circuits provides a mechanism for abnormal activity underlying chronic pain and early life adversity, including stress and pain, may not only have long-term effects on nociceptive processing, but also increase the risk of persistent pain in the adult by altering normal brain development and function (Brummelte et al., 2012; Schneider et al., 2018; Chau et al., 2019).

In conclusion, we have demonstrated that painful sensory experiences in early life have a significant effect on the function of adult pain-related cortical circuits. This change is likely driven by altered peripheral nociceptor and spinal cord circuit function following early life injury. Changes in regional and interregional neural oscillations in S1 and mPFC caused by painful experience in early life play a key role in altered nociceptive processing and may predispose an adaptive mechanism during chronification of pain. Understanding the effects of ELP upon developing cortical pain networks will increase our understanding of individual susceptibility to pain in adult life (Denk et al., 2014).

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Author contributions

MF and PC conceived the study, PC collected the behavioural and electrophysiology data; PC and LF analysed the data; all authors interpreted the data and contributed to the manuscript.

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Figure Legends

Figure 1. Experiment design (A) Schematic of experimental groups. II: neonatal incision on postnatal day 3 and repeat incision 2 months later in adulthood (ELP model). NI: littermate control with equivalent anaesthesia, handling and maternal separation on post-natal day 3 and first incision in adulthood. Con: pooled data from animals having neonatal incision only and from age-matched non-incised litter mates from the same colony. (B) Experimental protocol for probing the impact of early life pain (ELP) on adult cortical pain processing and pain behaviour. Upper scale: timeline for recording cortical local field potentials (LFP) and pain behaviour, where * marks days of simultaneous electronic von Frey hair (eVF) stimulation and LFP recording. Lower box: Detail of testing protocol for recording resting LFPs and eVH evoked LFP recording. (C) Schematic of the experimental set-up for simultaneous recording of neural LFP activity in medial prefrontal cortex (mPFC) and primary somatosensory cortex (S1) in awake adult rats using wireless telemetry while applying electronic von Frey hairs (eVF) to the plantar hindpaw. (D) Sample traces of simultaneous S1 and mPFC evoked potentials evoked by mechanical eVF stimulation of the plantar hindpaw.

Figure 2: Early life pain (ELP) increases hyperalgesia following incision injury in adult rats. (A) Electronic von Frey hair (eVF) testing of the plantar hindpaw adjacent to the wound (B) Plot of contralateral mechanical paw withdrawal thresholds (PWT), before (Pre) and up to 10 days (D) after hindpaw incision in adult rats. Mean ± SEM with individual data superimposed. (B) Statistical differences between groups using generalized linear models (C) Summary the post-hoc pairwise comparisons with Bonferroni correction. *P<0.05, **P < 0.01 ***p < 0.001. Non-incised controls (Con, n=18), incision in adults without neonatal incision (NI, n=10) and incision in adults with neonatal incision (II, n=9).

Figure 3: Stimulus-evoked delta energy in SI increases after adult incision injury only in animals who experienced early life pain. Electrophysiological responses in the (A) somatosensory cortex (S1) and (F) medial prefrontal cortex (mPFC) to mechanical (eVF) stimulation of the hindpaw following adult injury in ELP rats (II, red) and non-ELP rats (NI, blue) and controls (Con, black). Peristimulus normalised delta frequency (2-4 Hz) oscillations (mean ± SEM) in S1 (B) and mPFC (G) on the day of adult incision injury (D0). Comparison of the injury-induced changes in stimulus-evoked delta energy in S1 (C) and mPFC (H), expressed as a ratio of normalized magnitude (D0/Pre), between groups. (D) The enhancement of injury-induced changes in sensory

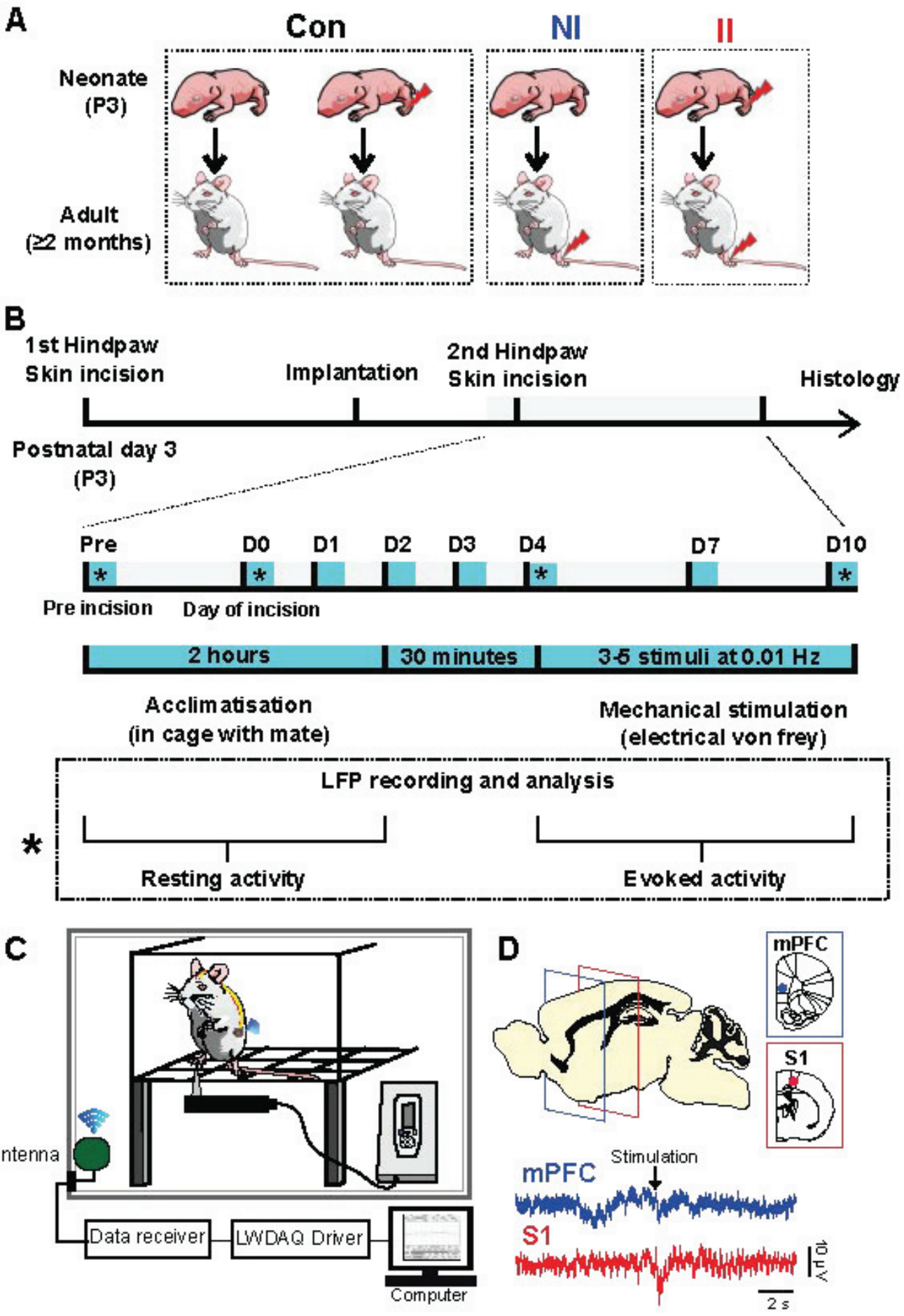
evoked S1 delta energy returned to pre-injury level by 10 days (D10) following injury. The paired mean difference for comparisons is shown as Cumming estimation. Each paired mean difference is plotted as a bootstrap sampling distribution; 95% confidence intervals are indicated by the ends of the vertical error bars. Statistical analysis was performed using a permutation t-test (Randomization: 5000). (E) Correlations between paw withdrawal threshold (PWT) and stimulus-evoked S1 delta activity (normalized magnitude). The scatter plots represent the correlations between PWT and normalized energy (Pre to D10) with continuous lines showing the linear regression. Pearson correlation coefficient (R) with significance (P value) is presented in the figures. Non-incised adult controls (Con, n=15), incision in adults without neonatal incision (NI, n=8), and incision in adults with neonatal incision (II, n=8).

Figure 4: Stimulus-evoked gamma energy in SI increases after adult incision injury only in animals who experienced early life pain. Electrophysiological responses in the (A) somatosensory cortex (S1) and (F) medial prefrontal cortex (mPFC) to mechanical (eVF) stimulation of the hindpaw following injury in ELP adult rats (II, red), non-ELP rats (NI, blue) and controls (Con, black). Peristimulus normalised gamma frequency (30-90 Hz) oscillations (mean ± SEM) in S1 (B) and mPFC (G). Comparison of changes in stimulus-evoked gamma energy in S1 (C) and mPFC (H), expressed as a ratio of normalized magnitude (D0/Pre), between groups. (D) The enhancement of injury-induced changes in sensory evoked S1 gamma energy returned to pre-injury level by 4 days (D4) following injury. The paired mean difference for comparison is shown as Cumming estimation. Each paired mean difference is plotted as a bootstrap sampling distribution; 95% confidence intervals are indicated by the ends of the vertical error bars. Statistical analysis was performed using a permutation t-test (Randomization: 5000). (E) Correlations between paw withdrawal threshold (PWT) and stimulus evoked S1 gamma activity (normalized magnitude). The scatter plots represent the correlations between PWT and normalized energy (Pre to D10) with continuous lines showing the linear regression. Pearson correlation coefficient (R) with significance (P value) is presented in the figures. Non-incised adult controls (Con, n=15), incision in adults without neonatal incision (NI, n=8), and incision in adults with neonatal incision (II, n=8).

Figure.5. Stimulus-evoked delta-gamma cross-frequency coupling in SI increases after adult injury only in animals who experienced early life pain. (A) Sample trace of LFP recorded in S1 during hindpaw mechanical stimulation (eVF) and a diagram illustrating the principle of cross-frequency coupling. Peristimulus normalised time-resolved delta-gamma coupling in S1 (B) and mPFC (D) on the day of adult injury (D0), data are presented as mean ± SEM. (C) Comparison of the injury-induced changes in stimulus-evoked delta-gamma coupling in S1, expressed as a ratio of normalized magnitude (D0/Pre), between groups. (E) The enhancement of pain-induced changes in stimulus-evoked delta-gamma coupling in S1 returned to pre-injury level by 4 days (D4) following injury. The paired mean difference for comparisons is shown as Cumming estimation. Each paired mean is plotted as a bootstrap sampling distribution; 95% confidence intervals are indicated by the ends of the vertical error bars. Statistical analysis was performed using permutation t-test (Randomization: 5000). (F) Correlations between paw-withdraw threshold (PWT) and delta-gamma modulation in S1 expressed as normalized modulation index. The scatter plots represent correlations between PWT and normalized delta-gamma coupling with continuous line as linear regression. Pearson correlation coefficient (R) with significance (P value) *$p < 0.05$, **$P<0.01$. Non-incised adult controls (Con, n=15), incision in adults without neonatal incision (NI, n=8) and incision in adults with neonatal incision (II, n=8).

Figure 6: Stimulus-evoked S1-mPFC beta phase coupling is enhanced after adult injury and is prolonged in animals who experienced early life pain (A) An example of simultaneous recording of stimulus evoked LFPs in S1 and mPFC, before (left) and after (right) filtering for phase coupling measurement at theta frequency (B) Peristimulus normalised S1-mPFC phase-locking value (PLV) at theta frequency following injury, presented as mean ± SEM. (C) Comparison of changes in S1-mPFC PLV at theta on the day of injury (D0) and

(D) 4 days following injury (D, D4), expressed as a ratio of normalized PLV (D0/Pre), between groups. (E) The enhancement of injury induced changes in sensory evoked S1-mPFC PLV at theta returned to pre-injury level by 4 days (D4) in the NI group, whereas a longer lasting increase in S1-mPFC PLV at theta was found in II. as a bootstrap sampling distribution, 95% confidence intervals are indicated by the ends of the vertical error bars. Statistical analysis was performed using a permutation t-test (Randomization: 5000). (F) Correlations between paw-withdraw threshold (PWT) and stimulus evoked S1-mPFC phase lock theta oscillations. The scatter plots represent correlations between PWT and normalized delta-gamma coupling with continuous line as linear regression. Pearson correlation coefficient (R) with significance (P value) *$p < 0.05$, **$P<0.01$. Non-incised adult controls (Con, n=15), incision in adults without neonatal incision (NI, n=8) and incision in adults with neonatal incision (II, n=8).



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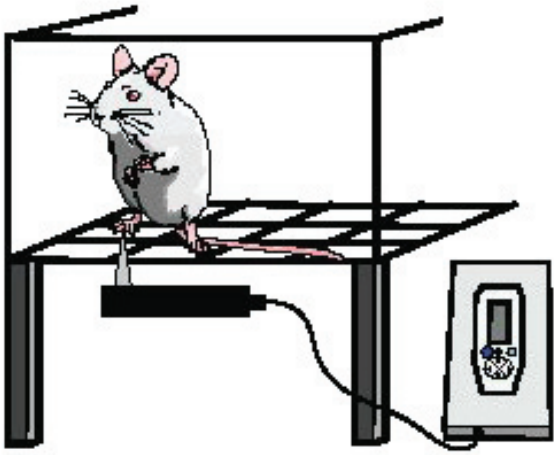

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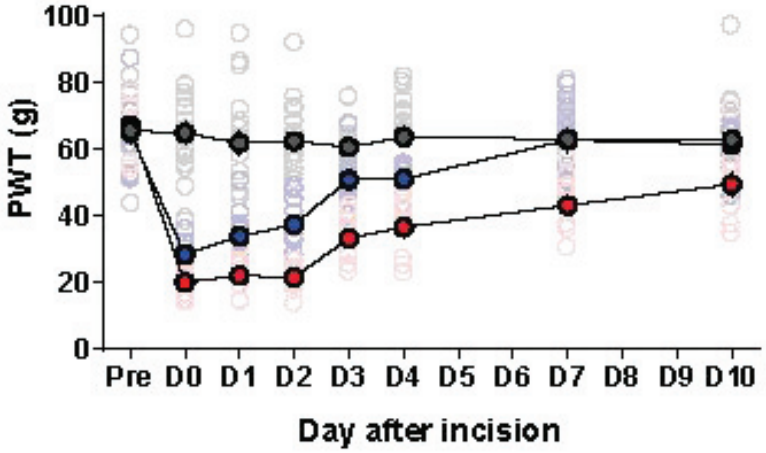

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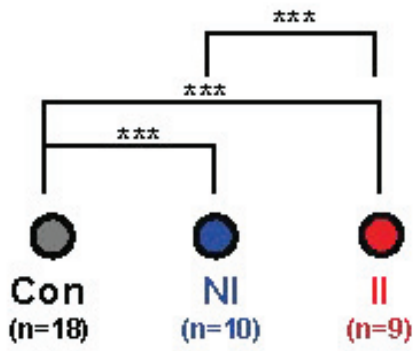

D

	Pre	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
CON vs NI	ns	***	***	***	***	ns			ns			ns
CON vs II	ns	***	***	***	***	***			***			ns
NI vs II	ns	ns	ns	ns	*	ns			**			ns