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Persistent changes in peripheral and spinal nociceptive processing after early tissue injury

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Abstract

It has become clear that tissue damage during a critical period of early life can result in long-term changes in pain sensitivity, but the underlying mechanisms remain to be fully elucidated. Here we review the clinical and preclinical evidence for persistent alterations in nociceptive processing following neonatal tissue injury, which collectively point to the existence of both a widespread hypoalgesia at baseline as well as an exacerbated degree of hyperalgesia following a subsequent insult to the same somatotopic region. We also highlight recent work investigating the effects of early trauma on the organization and function of ascending pain pathways at a cellular and molecular level. These effects of neonatal injury include altered ion channel expression in both primary afferent and spinal cord neurons, shifts in the balance between synaptic excitation and inhibition within the superficial dorsal horn (SDH) network, and a 'priming' of microglial responses in the adult SDH. A better understanding of how early tissue damage influences the maturation of nociceptive circuits could yield new insight into strategies to minimize the long-term consequences of essential, but invasive, medical procedures on the developing somatosensory system.

Keywords

neonatal; surgical incision; inflammation; microglia; pain; spinal cord; dorsal horn; synapse; patch clamp; DRG; primary afferent; glutamate; GABA; glycine; membrane excitability

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Impact of early life injury: Clinical studies

Neonates and infants who require major surgery or management in a neonatal intensive care unit (NICU) are exposed to significant painful stimuli at a time when the developing nervous system is sensitive to changes in sensory experience (Fitzgerald and Walker, 2009). Evidence for associations between early life pain and adverse neurodevelopmental outcomes, persistent changes in sensory processing, and altered responses to future pain is increasing. However, differences in study methodology, included populations, and outcome measures mean that reported effects of early life pain vary in degree, direction (increase or decrease in pain sensitivity), and functional impact. Sources of variability (Walker, 2013) can include:

- **i.** the type and intensity of initial pain and injury. This may encompass variable numbers and types of procedures or surgery in otherwise healthy neonates or infants, through to repeated interventions in preterm-born neonates with multiple co-morbidities.
- **ii.** developmental stage at the time of the initial insult can range from neonates born extremely preterm (i.e. before 26 weeks gestation) through to infancy.
- **iii.** age at subsequent assessment. At older ages more detailed outcomes can be assessed, but the increased time interval may also increase the potential for other family, social, environmental or health-related factors to influence reported associations between early life experience and current pain.
- **iv.** the outcome measured, and particularly the intensity of subsequent test stimuli. Examples include: observer ratings and evaluation of behavioural responses to future clinical procedures (e.g. observer pain score and duration of cry following immunization); psychophysical measures of sensory detection/pain thresholds at baseline or in response to noxious experimental stimuli (e.g. prolonged heat stimulus, cold pressor test, pressure algometry); or cortical EEG or fMRI responses to noxious stimuli.

These factors and related studies are discussed in recent reviews (Walker, 2013; Ranger and Grunau, 2014; Vinall and Grunau, 2014). Here, the focus is on clinical studies evaluating changes in sensory processing and spinal reflex thresholds, and their parallels with laboratory studies investigating the pattern, mechanisms and age-related susceptibility to long-term effects following neonatal injury.

Acute injury-related changes in sensory threshold

Noxious stimuli produce acute physiological and behavioral responses even in preterm neonates, and increases activity in spinal and cortical nociceptive circuits (Fitzgerald and Walker, 2009; Slater et al., 2010; Walker, 2013). Although less well-tuned than at older ages, spinal reflex thresholds encode stimulus intensity in neonates and infants (Andrews and Fitzgerald, 1994; Slater et al., 2010) and intense or repeated stimuli lead to sensitization. Following repeated heel lance blood sampling, the hindlimb mechanical withdrawal threshold is reduced, but sensitivity is reversed by topical local anesthesia (Fitzgerald et al., 1988). By contrast, sucrose does not alter acute hindlimb reflex responses to heel lance

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(Slater et al., 2010) or prevent enhanced behavioral responses following repeated blood sampling (Taddio et al., 2009). Reductions in abdominal skin reflex thresholds quantify sensitization around surgical wounds in infants, with partial reversal by bolus doses of opioids (Andrews et al., 2002). In infants with unilateral hydronephrosis, referred visceral hyperalgesia was quantified by reductions in the threshold of the ipsilateral abdominal skin reflex (Andrews et al., 2002).

Persistent changes in sensory threshold following neonatal injury

Although peripheral tissue injury acutely reduces sensory thresholds, a more complex pattern of sensory changes is seen at older ages, with effects dependent on the intensity of the stimulus. Quantitative Sensory Testing (QST) has identified persistent changes in sensory processing following neonatal intensive care and/or surgery. Mechanical light touch detection thresholds around neonatal thoracotomy wounds were reduced 10–12 years following surgery, (Schmelzle-Lubiecki et al., 2007) with a trend to greater change than around smaller scars related to chest drain insertions (Walker et al., 2009a). Baseline sensitivity to thermal stimuli was also reduced at different body sites (thenar eminence, face, or chest) in 9–12 year old children following NICU (Hermann et al., 2006; Walker et al., 2009a). The degree of change compared to healthy term-born controls was more marked in those born preterm (Hermann et al., 2006) and those who also required surgery (Walker et al., 2009a) suggesting that both the degree of injury and age at the time of initial injury may be contributing factors.

Although baseline thermal thresholds were higher (i.e. reduced sensitivity), a prolonged heat stimulus at pain threshold (Hermann et al., 2006) or repeated trials (Hohmeister et al., 2010) unmasked enhanced perceptual sensitization in preterm born children who had required NICU management. This dual pattern of generalized baseline *hypo*algesia, but *hyper*algesia in response to a more intense noxious stimulus, has also been identified in laboratory studies (see subsequent section). Sensitivity to a noxious mechanical stimulus (reduced pressure pain threshold) and an increased number of tender points was reported in adolescents 12–18 years following NICU (Buskila et al., 2003). The number of studies utilizing QST and experimental pain tasks in control and clinical populations of children is increasing, although there is some variation in methodology and in reported effects of age and sex (Myers et al., 2006; Blankenburg et al., 2011; Birnie et al., 2012; de Graaf et al., 2012; Birnie et al., 2014). Ongoing follow-up and sensory testing of NICU cohorts at older ages, when more complex psychophysical tests can be performed, will further clarify the pattern or persistence of sensory change.

Impact of early life injury: Laboratory studies

Despite increasing clinical evidence for associations between early life experience and altered sensory processing or nociceptive sensitivity, attributing causation is more problematic, and there have been limited studies evaluating the effect of the same insult at different ages. Preclinical studies can control for potential confounding factors and provide essential information that cannot be achieved with clinical studies alone, by:

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i. comparing the impact of the same injury across a range of postnatal ages to establish a critical period for altered response;

ii. identifying underlying age- and injury-dependent mechanisms; and

iii. evaluating prevention or modulation by analgesia to inform subsequent clinical research and practice.

A range of injury models have been used to investigate long-term effects of early life pain and injury (Walker, 2013; Schwaller and Fitzgerald, 2014). Hindpaw inflammation is a well-established model, but the degree and duration of inflammation varies with the type and volume of hindpaw injectate; for example, relatively large volumes of complete Freund's adjuvant (CFA) on the first postnatal day (P1) (Ruda et al., 2000) produce chronic inflammation rather than a specific neonatal insult (Walker et al., 2003; Lim et al., 2009). Similarly, persistent effects following formalin are influenced by the concentration and frequency of the initial injections (Anand et al., 2007; Rovnaghi et al., 2008; Walker et al., 2008; Negrigo et al., 2011). Neonatal full thickness wounds with skin removal produce persistent hyperalgesia (Reynolds and Fitzgerald, 1995; Alvares et al., 2000; Torsney and Fitzgerald, 2003; Young et al., 2008), with hyperinnervation within the wounded area mediated by local release of neurotrophic factors including nerve growth factor (NGF) and neurotrophin-3 (NT-3) (Constantinou et al., 1994; Reynolds et al., 1997; Beggs et al., 2012a) and reduced expression of the short-range neural inhibitory cue ephrin-A4 (Moss et al., 2005). Other forms of injury aim to reproduce clinical stimuli; such as repeated needle injection (Anand et al., 1999; Miranda et al., 2006) to model procedural pain in the NICU; or laparotomy (Sternberg et al., 2005) and hindpaw incision (Ririe et al., 2003; Walker et al., 2009b) to investigate surgical injury. Here we will focus on inflammation and surgical injury, as the pattern of change in baseline sensory thresholds and reflex responses to subsequent noxious stimuli parallels clinical reports.

Hindpaw inflammation

Hindpaw injection of CFA in neonatal rodents (25 μl in P1 rat or 20 μl in P1 mouse) has resulted in persistent hyperalgesia in some (Blom et al., 2006; Roizenblatt et al., 2010) but not all studies (Ruda et al., 2000; Walker et al., 2003; Lim et al., 2009). However, as these large volumes produce histological signs of chronic inflammation (Walker et al., 2003; Lim et al., 2009), effects may not relate to a specific neonatal insult. Hindpaw carrageenan (0.5–2 μl/g of 0.25–2% solution) produces a robust but short duration acute inflammatory hyperalgesia (Torsney and Fitzgerald, 2002; Walker et al., 2003; Ren et al., 2004), and has consistently been associated with higher reflex thresholds for mechanical and thermal stimuli that emerge after the fourth postnatal week and persists until adulthood (Lidow et al., 2001; Ren et al., 2004; Wang et al., 2004; Anseloni et al., 2005). Importantly, this hypoalgesia is generalized and not restricted to the previously injured paw, suggesting that centrally mediated changes in baseline reflex sensitivity are involved (Wang et al., 2004). Enhanced inhibition from the rostroventral medulla (RVM) (Zhang et al., 2010) and alterations in opioid-mediated response in the periaqueductal gray (PAG) (Laprairie and Murphy, 2009) have been noted in adults following neonatal hindpaw carrageenan.

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The first postnatal week in the rodent represents a critical period for inducing persistent effects following hindpaw inflammation that are not seen following injury at older ages. Changes in the structure and function of nociceptive pathways follow hindpaw CFA at P1, but not at P14, in rats (Ruda et al., 2000; Hohmann et al., 2005) and mice (Blom et al., 2006), although this comparison is based on the assumption that the same degree of injury is achieved by varying the volume of injectate. Body weight-adjusted volumes of carrageenan (1 μl/g) at ages between P0 and P5, but not at P8 and beyond, altered adult mechanical and thermal withdrawal thresholds (Ren et al., 2004).

Neonatal hindpaw inflammation increases the response to noxious stimuli in adulthood. Prior CFA (25 μl) at P1 increased behavioral hyperalgesia following repeat CFA (Ruda et al., 2000; Lim et al., 2009) or capsaicin (Hohmann et al., 2005), the response to formalin was more rapid, and dorsal horn neuronal responses to peripheral brush or pinch were increased (Ruda et al., 2000). Neonatal carrageenan inflammation also increases the degree of behavioral hyperalgesia to subsequent inflammation (Ren et al., 2004) or incision (Chu et al., 2007) of the previously injured paw. The enhanced responses to noxious stimuli following early life injury differ in both time course and distribution from the baseline hypoalgesia reported above. Enhanced re-injury responses are apparent soon after the initial injury, and are segmentally restricted to the previously inflamed paw (Ren et al., 2004; Chu et al., 2007). Systemic morphine at the time of the initial insult reduces the long-term impact of neonatal carrageenan inflammation on sensory processing (Rahman et al., 1997; Laprairie et al., 2008).

Surgical Incision

Surgical injury produces inflammation, skin wounding and peripheral nerve injury (Kehlet et al., 2006). A midline abdominal incision in mice on the day of birth has been used to model neonatal laparotomy (Sternberg et al., 2005), and plantar hindpaw incision (Brennan et al., 1996) produces acute hyperalgesia and increased hindlimb reflex sensitivity at all postnatal ages in the rat (Ririe et al., 2003; Walker et al., 2009b).

Neonatal laparotomy in mice was associated with a generalized decrease in baseline sensitivity to thermal and visceral stimuli in adulthood (Sternberg et al., 2005). Similarly, and as seen following carrageenan inflammation, neonatal hindpaw incision produces a generalized hypoalgesia to mechanical and thermal stimuli that emerges after the 4th postnatal week (Walker et al., 2015). Descending modulation from the rostroventral medulla is altered, with a predominance of descending inhibition rather than the usual bimodal pattern of facilitation and inhibition control. Effects are generalized, as the same pattern of RVM stimulation-evoked inhibition of hindlimb reflex sensitivity is seen if the neonatal incision had been performed in the same paw, contralateral paw or forepaw. Blocking afferent activity from the injured area with peri-operative sciatic nerve local anesthetic prevents long-term changes in RVM signaling (Walker et al., 2015).

Hindpaw incision in the first postnatal week (P3 or P6) but not at older (P10, P21, P40) ages enhanced the response to future incision (Walker et al., 2009b). Both the degree and duration of hyperalgesia are increased when the same paw is re-injured, either 2 weeks later or in adulthood (P60) (Walker et al., 2009b; Beggs et al., 2012b). Repeat hindpaw incision

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at P3, P10 and P17 also increased the degree of incision-related hyperalgesia at P90 (Low and Fitzgerald, 2012).

Administration of systemic morphine at the time of neonatal laparatomy prevented the long-term changes in sensory response (Sternberg et al., 2005). The impact of plantar hindpaw incision can be modulated by either preventive strategies at the time of neonatal injury, or more selectively targeted by mechanism-based interventions at the time of subsequent re-injury. A single pre-operative sciatic nerve block with local anesthetic reduced acute hyperalgesia, but more prolonged perioperative blockade throughout the first 6–8 hours also prevented the enhanced response to subsequent incision (Walker et al., 2009b). In adults with prior neonatal incision, the enhanced hyperalgesia was effectively targeted by inhibiting spinal microglial reactivity with intrathecal minocycline (Beggs et al., 2012b) or an inhibitor of p38 mitogen-activated kinase in adult male rats (Schwaller et al., 2015). Underlying neuroglial mechanisms will be discussed later in the review.

Nociceptive signaling in the DRG and superficial dorsal horn after early tissue injury

Using preclinical models which allow for precise control over the timing, location and nature of the tissue injury, the above behavioral studies have clearly demonstrated that tissue damage during a critical period of early life evokes a prolonged ‘priming’ of developing pain pathways, as evidenced by an exacerbated degree of hypersensitivity following a second injury later in life (Ren et al., 2004; Chu et al., 2007; Walker et al., 2009b). The observation that the priming effect is site-specific, namely that it requires that the subsequent injury occur within the same area as the initial insult (Ren et al., 2004), suggests that neonatal tissue damage evokes localized changes within the periphery and/or the spinal superficial dorsal horn (SDH) which increases the gain of ascending nociceptive transmission to the brain. It should be noted that these localized changes in spinal sensory processing occur in combination with long-term alterations in descending modulatory pathways from the brain, as neonatal injury is known to increase the strength of descending inhibition from the RVM (Zhang et al., 2010) and modulate the endogenous opioidergic tone in the adult CNS (Laprairie and Murphy, 2009). However, this review will focus on plasticity at the level of the sensory neuron and SDH network following tissue damage during early postnatal development.

Altered primary afferent function after neonatal tissue damage

Early tissue injuries could prime developing pathways by persistently enhancing the response of sensory neurons to subsequent noxious stimulation. This could be achieved via multiple mechanisms, including changes in the transduction of noxious stimuli into receptor potentials and/or long-term shifts in the intrinsic membrane excitability of those primary afferent neurons innervating the injury site.

Sensory neurons in the dorsal root ganglion (DRG) can be sensitized to inflammatory stimuli from the embryonic period onwards (Koltzenburg and Lewin, 1997), although the precise subtypes of DRG neurons which are affected depends on the age at which the injury

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occurs (Jankowski et al., 2014). The sensitization of immature DRG neurons following peripheral inflammation likely involves changes in the function of various transient receptor potential (TRP) cation channels which are expressed in these cells throughout development (Hjerling-Leffler et al., 2007). Interestingly, recent evidence suggests that neonatal injury can lead to long-term changes in TRP channel expression and function within sensory neurons. Transient inflammation of the colon in neonatal mice causes an increase in the functional expression of TRPA1, a key transducer of mechanical stimuli in viscera (Brierley et al., 2009), within adult colonic afferents (Christianson et al., 2010). This may relate to inflammation-induced changes in growth factor expression, as NGF, NT-3, and GDNF were upregulated in the colon for at least one week following the injury (Christianson et al., 2010), and growth factors are known to profoundly regulate TRP channel function in primary afferent neurons (Ernsberger, 2009). Importantly, earlier studies have shown that manipulating growth factor signaling in the periphery during the neonatal period can permanently alter the phenotype of developing sensory neurons (Ritter et al., 1991; Lewin et al., 1992).

Evidence also suggests that early tissue damage can cause a persistent enhancement in the intrinsic membrane excitability of primary afferent neurons. For example, neonatal colonic inflammation led to a > 2-fold increase in the density of voltage-gated Na^+ currents in adult sensory neurons projecting to the colon, which was accompanied by a significant up-regulation of $Na_V1.7$ and $Na_V1.8$ protein in the adult DRG (Qu et al., 2013). In addition, noxious stimulation of the colon (via acetic acid infusion) between postnatal days (P) 8 and 21 led to a significant reduction in the expression of A-type voltage-gated K^+ currents, and decreased levels of the $K_V4.3$ subunit, in adult DRG neurons (Qian et al., 2009). The colonic insult also evoked a negative shift in the voltage-dependence of steady-state inactivation for both A-type and delayed-rectifying K^+ channels (Qian et al., 2009), which further reduces the availability of these voltage-gated K^+ channels near the resting potential of the afferent neuron. Therefore, downstream of any neonatal injury-induced changes in sensory transduction, these alterations in the expression of voltage-gated channels within DRG neurons would be predicted to elevate the firing of mature DRG neurons in response to noxious stimulation. Nonetheless, much work is still needed to fully understand how early injury persistently influences the intrinsic membrane properties of developing sensory neurons. For example, while it is clear that voltage-independent (i.e. “leak”) ion channels are essential for controlling the excitability of DRG neurons (Cooper et al., 2004; Rau et al., 2006; Liu et al., 2013), nothing is known about whether neonatal tissue damage modulates the expression of these channels during adulthood.

In considering how these animal studies might relate to the clinical treatment of infants in the NICU, it is important to keep in mind that these infants experience a very high number of stressors during their hospital stay in addition to the tissue injuries sustained as a consequence of essential medical interventions. Interestingly, recent work has shown that neonatal stress alone can evoke marked changes in the excitability of mature primary afferent neurons innervating muscle, as evidenced by a reduced mechanical threshold in these afferents following the administration of a limited bedding stressor during early life (Green et al., 2011). The complex interactions between stress and painful experiences during

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early life, and how they shape nociceptive processing throughout development, remain an important topic for future investigation.

Reorganization of primary afferent input to the dorsal horn following neonatal nerve or tissue damage

Peripheral nerve damage during the neonatal period leads to the collateral sprouting of nearby intact primary afferents into the denervated regions of the spinal cord (Fitzgerald et al., 1990), where they establish functional synaptic contacts onto dorsal horn neurons (Shortland and Fitzgerald, 1991). Further studies demonstrated a role for both A-fibers and C-fibers in this collateral sprouting within the dorsal horn (Shortland and Fitzgerald, 1994). Damage to primary afferent fibers occurring at later ages fails to elicit the same robust anatomical plasticity within the spinal cord (Schwegler et al., 1995). While the reasons for this difference remain to be fully elucidated, it should be noted that the myelination of the rodent spinal cord occurs during early postnatal life (Kapfhammer and Schwab, 1994) and myelin in the CNS is known to include numerous inhibitors of axonal growth (Hu and Strittmatter, 2004). Interestingly, a significantly increased degree of collateral sprouting has been observed in the myelin-deficient spinal cord (Schwegler et al., 1995).

The degree to which early inflammation persistently alters the pattern of primary afferent projections to the spinal dorsal horn has proven less straightforward. Hindpaw inflammation during the neonatal period was first reported to evoke an expansion in the central terminal fields of sensory afferents in the adult rat spinal cord (Ruda et al., 2000). However, subsequent studies found that this expansion was transient and did not outlast the time course of the injury itself (Walker et al., 2003). Nonetheless, it is becoming clear that distinct classes of sensory neurons can make highly specific connections with subpopulations of spinal dorsal horn neurons (Lu and Perl, 2003; Seal et al., 2009; Zheng et al., 2010; Li et al., 2011). Given that the earlier investigations primarily used bulk-labeling techniques to visualize afferent projections to the spinal cord, one cannot completely exclude the possibility that early inflammation persistently influences the precise pattern of synaptic connections formed by individual subtypes of sensory afferents onto second-order nociceptive neurons in the dorsal horn.

Intrinsic membrane excitability of mature dorsal horn neurons following early tissue injury

In vivo extracellular recordings have demonstrated that transient peripheral inflammation in the neonate evokes hyperexcitability in spinal dorsal horn neurons that persists into adulthood, as manifested by enhanced spontaneous firing, larger receptive fields and prolonged spike after-discharge in response to sensory input (Peng et al., 2003; Torsney and Fitzgerald, 2003; Ness and Randich, 2010). These firing properties could be explained by an increase in the intrinsic membrane excitability of mature SDH neurons, and/or persistent changes in synaptic transmission within the SDH network, as a result of early inflammatory injury. However, recent evidence using in vitro patch clamp recordings in spinal cord slices suggests that neonatal tissue damage actually reduces the intrinsic excitability of dorsal horn neurons during adulthood. Surgical incision of the hindpaw (Brennan et al., 1996) in P3 mice expressing GFP selectively within GABAergic neurons (Oliva, Jr. et al., 2000) evoked a hyperpolarizing shift in the resting potential (V_{rest}), reduced membrane resistance,

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elevated rheobase levels and dampened repetitive firing frequency in both GFP and adjacent non-GFP (i.e. presumably glutamatergic) neurons within lamina II of the adult SDH (Li and Baccei, 2014). Nonetheless, the persistent reduction in intrinsic firing in these populations may arise from distinct underlying ionic mechanisms, as a long-term increase in the level of conductance through classic inward-rectifying potassium ($K_{ir}2$) channels was observed only in the GABAergic population after early surgical injury (Li and Baccei, 2014). The potential long-term effects of neonatal trauma on the intrinsic membrane properties of ascending projection neurons, which serve as the output of the spinal nociceptive circuit and are essential for the generation of inflammatory and neuropathic pain in adults, has yet to be carefully studied although a recent report failed to find any differences in V_{rest} in lamina I projection neurons from naïve vs. neonatally-incised mice (Li et al., 2015). Collectively, the above studies suggest that the most likely explanation for the enhanced activity seen in adult dorsal horn neurons in vivo following neonatal injury is a persistent shift in the balance between synaptic excitation and inhibition within the spinal nociceptive network.

Neonatal injury alters synaptic function within adult spinal nociceptive circuits

An increase in the overall excitability of pain networks in the CNS (i.e. central sensitization) after nerve or tissue damage can result from a strengthening of excitatory synaptic transmission (Wang et al., 2005; Torsney, 2011; Ruscheweyh et al., 2011), deficits in inhibitory signaling (Moore et al., 2002; Coull et al., 2003; Muller et al., 2003; Harvey et al., 2004; Coull et al., 2005), or a combination of both. In the days immediately following neonatal tissue injury, we have previously documented an activity-dependent enhancement in glutamatergic signaling within lamina II of the ipsilateral SDH which occurs in the absence of changes in synaptic inhibition (Li and Baccei, 2009; Li et al., 2009). However, a very different complement of synaptic changes emerges at later time points after the injury. Interestingly, neonatal surgical injury reduces both phasic and tonic glycinergic signaling within lamina II during adulthood without modulating spontaneous glutamatergic transmission to the same population (Li et al., 2013), suggesting that early tissue damage can compromise synaptic inhibition in the SDH long after the initial trauma has resolved. Although the somatotopy of these synaptic modifications has not yet been fully elucidated, the available evidence points to some degree of site specificity, as the lack of a similar effect in the contralateral SDH (Li et al., 2013) argues against a global decrease in inhibitory tone within spinal nociceptive circuits after neonatal injury.

While lamina II is thought to consist almost entirely of local circuit interneurons and propriospinal neurons (Bice and Beal, 1997a; Bice and Beal, 1997b), cells in this region receive direct input from nociceptive primary afferents and can strongly influence the output of the SDH network via their excitatory and inhibitory synapses onto ascending projection neurons located in lamina I and deeper laminae (i.e. ‘feed-forward’ excitation and inhibition). Importantly, the level of $GABA_A$R-mediated and GlyR-mediated feed-forward inhibition of adult lamina I projection neurons is significantly lower when preceded by neonatal tissue damage (Li et al., 2015). The reduced inhibition cannot be explained a structural loss of inhibitory synaptic terminals onto the mature projection neurons (Li et al., 2015), and may instead reflect the lower membrane excitability of GABAergic neurons in

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the mature SDH after early injury (Li and Baccei, 2014), which is expected to result in the activation of fewer inhibitory interneurons following sensory input to the adult spinal cord.

In addition, the strength of direct primary afferent input to adult lamina I projection neurons is potentiated by surgical injury during the neonatal period (Li et al., 2015), further disrupting the normal balance between synaptic excitation and inhibition onto these key output neurons of the spinal nociceptive circuit. Mature projection neurons were also more likely to receive glutamatergic input from low-threshold A-fiber afferents when preceded by early tissue damage (Li et al., 2015), thus providing further support for a persistent reorganization in the pattern of sensory projections to the dorsal horn following neonatal injury. As predicted from an enhanced efficacy of primary afferent synapses coupled with a reduction in feed-forward inhibition, adult projection neurons from neonatally-incised mice exhibited significantly higher levels of action potential discharge in response to dorsal root stimulation compared to naïve littermate controls (Li et al., 2015). These findings indicate that early tissue damage leads to a prolonged increase in the ‘gain’ of the SDH circuit, which represents a novel potential mechanism by which the developing spinal nociceptive network can be primed by neonatal injury.

The influence of spinal neuroimmune interactions on the long-term consequences of neonatal injury

The spinal cord dorsal horn is the site of the first level of nociceptive processing within the spinal cord and transmission of the nociceptive information from the cord to the brain is a neuronally mediated process. However, the processing of the nociceptive signal is more complex. Neuronal function in the CNS is not simply restricted to neuron-neuron signaling but rather a complex interaction influenced by other cell types that make up the CNS parenchyma, including elements of the immune system, specifically microglia (Beggs et al., 2012c). In the adult, microglia are reactive cells, surveilling the CNS and ready to mount a response to injury or disease with either pro- or anti-inflammatory consequences (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011). Damage to peripheral nerves in the adult elicits a specific response in spinal dorsal horn microglia that surround the central terminals of peripherally-axotomised sensory axons that is a key mediator leading to the onset and maintenance of neuropathic pain (Coull et al., 2005; Beggs et al., 2012c).

In the immediate postnatal period the situation is more complex. It is becoming increasingly evident that the immune system, and specifically microglia, is intrinsically active in the normal development of the central nervous system (Pont-Lezica et al., 2011; Kettenmann et al., 2013). Importantly, rather than being solely reactive to injury or disease, microglia have a normal developmental instructive role in synaptic maturation and the refinement of neuronal circuitry (Kettenmann et al., 2013; Salter and Beggs, 2014). It is likely that this function of microglia is important in the development of spinal nociceptive circuitry where considerable postnatal refinement of connectivity and removal of inappropriate structures occurs, as well as the strengthening and maturation of developmentally appropriate synaptic connectivity (Fitzgerald, 2005). During this time microglia may be driving these developmental processes in preference to the defined immune role in the adult, and it has been suggested that this prevents the risk of auto-inflammatory response to the clearance of

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normal developmental axonal debris (Costigan et al., 2009). Certainly the spinal proliferative microgliosis associated with nerve injury is very much muted in younger animals as compared to the adult (Moss et al., 2007), and a consequent absence of typical associated pain behaviors is observed. However, rather than failing to develop, pain hypersensitivity has now been shown to be delayed, with the normal adult level of sensitivity developing as the animals reached an adolescent equivalent age (Vega-Avelaira et al., 2012). This phenomenon is a direct consequence of altered postnatal neuroimmune responsivity to peripheral nerve damage. Recent work (reviewed by Fitzgerald and McKelvey in this special issue) showed that following spared nerve injury (SNI) at P10, the initial lack of pain behavior is due to the adoption of an anti-inflammatory response by the immune system, characterized by increases in the anti-inflammatory cytokines IL-4 and IL-10 (McKelvey et al., 2015). As the animals mature into adolescence, this anti-inflammatory profile shifts to the more typically adult pro-inflammatory profile, now characterized by the presence of pro-inflammatory mediators TNF-alpha and BDNF, and the consequent emergence of pain behaviors more typical of the injury in the adult animal (McKelvey et al., 2015). It is important to note that the delayed onset of behavioral sensitivity to nerve injury in juvenile animals is not due to a lack of pro-inflammatory immune factors per se, but is instead driven by an actively anti-inflammatory immune response to the injury, as pain behaviors can be elicited earlier by spinal pharmacological blockade of IL-10 (McKelvey et al., 2015).

There is a further level of complexity in the neuroimmune response to surgical injury if the initial injury occurs within the described critical period of plasticity that spans the first postnatal week in the rodent. While the immune response may appear muted in terms of immediate spinal microglial reactivity (i.e. proliferation and microgliosis in the dorsal horn), there is a priming of the microglial capacity to respond to re-injury in later life (Walker et al., 2009b; Beggs et al., 2012b). This increased reactivity, manifested as a more rapid and widespread proliferation, results in an increased behavioral pain response, both in terms of the degree and duration of hypersensitivity (Beggs et al., 2012b). A role of microglia was first shown by the attenuation of the primed behavioral response by the administration of the non-specific glial inhibitor minocycline at the time of adult re-incision and has now been shown functionally by the pharmacological block of the microglial-specific p38 MAP kinase (Schwaller et al., 2015).

The molecular control of this apparent ‘pain memory’ (Ransohoff and Perry, 2009; Kettenmann et al., 2011) has yet to be fully elucidated. As a result, the degree to which microglia play a causative role in the retention of the ‘pain memory’ throughout life, or contribute solely to the expression of the priming effect during adulthood, remains unknown. Since the molecular signature of both pro-and anti-inflammatory states has been revealed in the juvenile animal (McKelvey et al., 2015) but not in the immediate neonatal period, a comprehensive characterization of the microglial phenotype throughout postnatal development may yield insight into this important issue. Nonetheless, it is clear that the development and subsequent maintenance of chronic pain is dependent, at least in part, on the spinal dorsal horn neuroimmune profile, as subsequent injury in later life following neonatal incision unmasks an enhanced immune component that amplifies pain sensitivity. Collectively, the two phenomena of critical period priming and the late onset pain responses

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to peripheral nerve injury highlight the potential for a latent sensitization of nociceptive circuits subsequent to pain exposure in early life, and that these effects can persist into adulthood.

Future Directions

Although convincing evidence now points to persistent modifications in nociceptive processing within primary sensory neurons, as well as the SDH network, after early life injuries, many key questions have yet to be addressed. For example, does neonatal tissue damage exert a long-term impact on activity-dependent synaptic plasticity within the mature spinal cord? What is the functional relationship, if any, between the observed changes in spinal neuroimmune signaling and the documented alterations in synaptic transmission within the SDH? Answers to questions such as these will not only significantly improve our understanding of how early tissue injury can shape nociceptive processing throughout life, but may yield valuable insight into the potential link between pediatric and adult chronic pain conditions.

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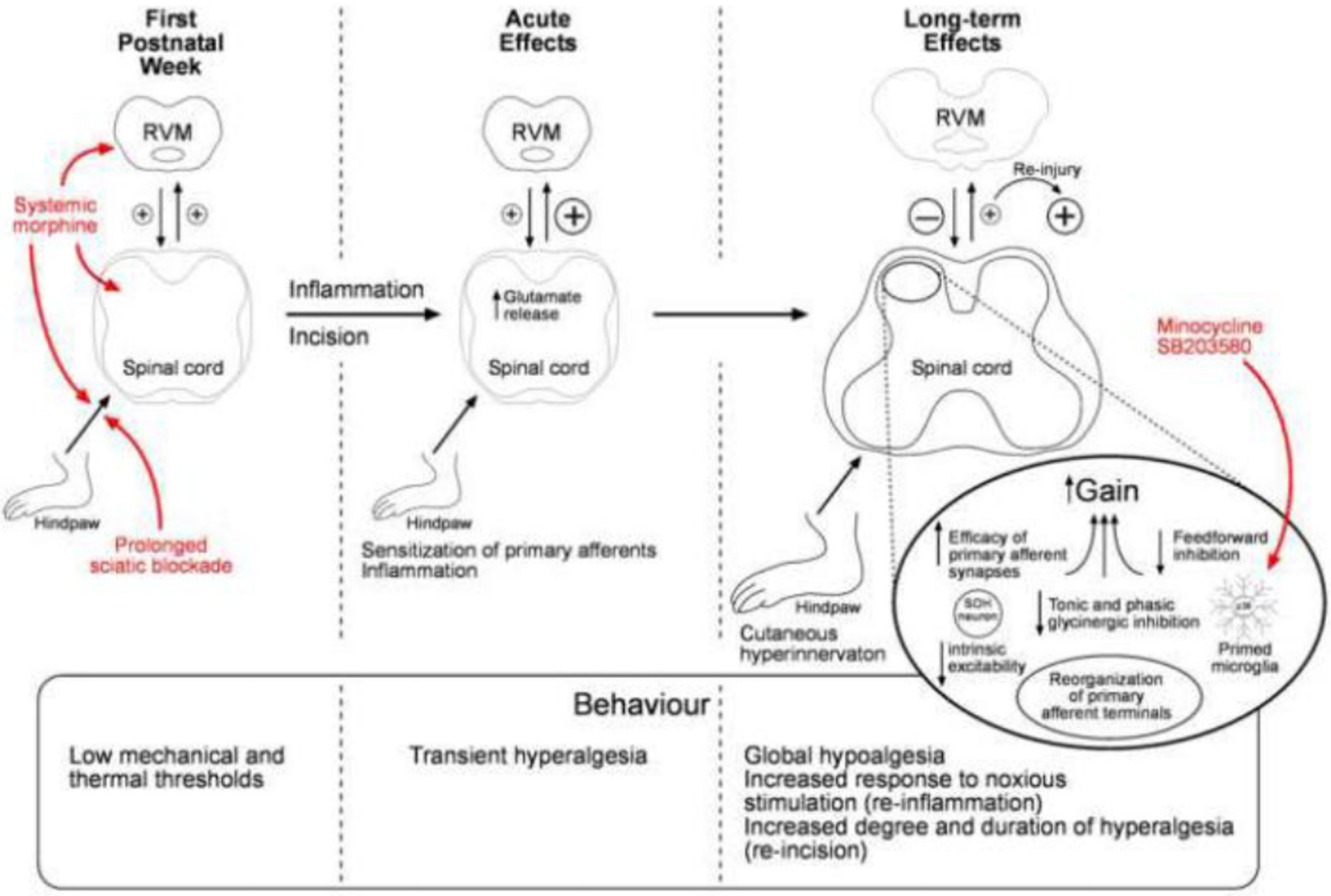

Figure 1.

Summary of the acute (*middle panel*) and long-term (*right*) effects of early tissue damage on nociceptive signaling, including alterations in descending modulatory pathways originating from the rostroventral medulla (RVM), in comparison to the naïve state in the neonate (*left*). The corresponding consequences for pain behavior are indicated in the box at the bottom. Red text indicates the interventions administered either at the time of neonatal injury (*left*) or during adulthood (*right*) that have been shown to ameliorate the persistent changes in pain sensitivity following early tissue damage. See text for further details.