Cite this article: Walters ET, Williams ACdC. 2019 Evolution of mechanisms and behaviour important for pain. Phil. Trans. R. Soc. B 20190275. http://dx.doi.org/10.1098/rstb.2019.0275

Evolution of mechanisms and behaviour important for pain

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Summary

Our understanding of the biology of pain is limited by our ignorance about its evolution. We know little about how states in other species showing various degrees of apparent similarity to human pain states are related to human pain, or how the mechanisms essential for pain-related states evolved. Nevertheless, insights into the evolution of mechanisms and behaviour important for pain are beginning to emerge from wide-ranging investigations of cellular mechanisms and behavioural responses linked to nociceptor activation, tissue injury, inflammation, and the environmental context of these responses in diverse species. In May 2019 an unprecedented meeting on the evolution of pain hosted by the Royal Society brought together scientists from disparate fields who investigate nociception and pain-related behaviour in crustaceans, insects, leeches, gastropod and cephalopod molluscs, fish, and mammals (primarily rodents and humans). Here we identify evolutionary themes that connect these research efforts, including adaptive and maladaptive features of pain-related behavioural and neuronal alterations - some of which are quite general, and some that may apply primarily to humans. We also highlight major questions, including how pain should be defined, that need to be answered as we seek to understand the evolution of pain.

This article is part of the Theme Issue 'Evolution of mechanisms and behaviour important for pain'.

1. Introduction

No human experience is more compelling than that of intense pain. It seems safe to assume that pain did not appear de novo in humans; that the functions and mechanisms of human pain are products of prior evolution. While the control of human pain has long been a medical priority, and experimental animals have played a large role in preclinical efforts to develop new analgesics, remarkably little is known about the evolution of pain. This reflects, in part, relatively little interest in evolution among most clinically oriented pain researchers, and little attention paid to pain by most evolutionary biologists. It also reflects the complexity of human pain and problems in applying the most widely accepted definition of human pain to other species. Nevertheless, a small but growing number of scientists is seeking a better understanding of the biology of human pain by considering the mechanisms and functions of pain from an evolutionary perspective.

This Theme Issue resulted from the first international meeting on the evolution of pain, which brought together experts in disparate areas of neuroscience, psychology, medicine, and evolutionary biology. The focus was on the evolution of pain behaviour and associated mechanisms as revealed by comparisons of pain-related phenomena across diverse invertebrate and vertebrate species, including humans. The single meeting and resulting Theme Issue could not accommodate all the relevant topics. For example, we have not included articles focused on the evolution of neuroanatomical structures important for pain or the evolution of endogenous opioids and their receptors. However, we are confident the following articles will stimulate much-needed discussion and research on the topics explicitly covered as well as related topics on the evolution of pain that could not be addressed directly in this pioneering Theme Issue.

2. How should pain be defined by biologists?

In trying to understand the broader biology of pain, a major problem immediately arises. The most influential definition of pain neglects all species but our own. The vast majority of pain researchers around the globe have accepted the following definition of pain from the International Association for the Study of Pain (IASP): "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (https://www.iasp-pain.org/terminology?navItemNumber=576#Pain). This definition was developed by clinicians and clinically oriented pain researchers. It has proven useful for defining human pain, albeit with some limitations, such as the lack of reference to cognitive and social components of pain that are important clinically (Williams and Craig, 2016). Currently, the IASP is seeking public comment on a revised definition of pain as "An aversive sensory and emotional experience typically caused by, or resembling that caused by, actual or potential tissue injury" (https://www.iasp-pain.org/PublicationsNews/NewsDetail.aspx?ItemNumber=9218&navItemNumber=643). A critical part of both IASP definitions is "emotional experience," a phrase that in everyday English and for many researchers (e.g., Izard, 2009) is closely linked to conscious, subjective feeling. This intent is confirmed by the first note appended to the new IASP definition stating that "pain is always a subjective experience." For a biologist, a major limitation of these definitions is that defining pain in terms of subjective experience limits conclusive assessment of pain to the one species that can provide verbal reports of subjective experience: humans (and not even all humans). Further, the focus on subjective experience eclipses the motivational functions of pain that are key to an evolutionary understanding (Elwood REF; Finlay REF; Sneddon REF; Walters, 2018; Williams REF).

Two definitional approaches have been used to address pain in non-verbal animals. One is to accept definitions that emphasize conscious experience, such as the IASP definition, and to argue on the basis of sufficient neuroanatomical, physiological/pharmacological, and behavioral analogies that certain species are likely to experience pain similarly to humans (while admitting that compelling proof of the existence of conscious pain in other species may be impossible). This approach has profound ethical and legal implications, as persuasive evidence for conscious pain (and thus for potential suffering) is the basis for including selected species for protection under animal welfare laws. Argument by lists of apparent analogies has been used to conclude that mammals and other large-brained animals are likely to experience conscious pain (e.g., Bateson, 1991). As explained in review articles in this issue by Elwood (REF) and by Sneddon (REF), this approach has provided systematic arguments for the likelihood of pain and the possibility of suffering in crustaceans and fish (see also Sneddon et al., 2014). Given the enormous scale and economic impact of the human use of fish and crustaceans, it is not surprising that such arguments have met with strong resistance from some quarters.

A second approach for defining animal pain has been favoured (often implicitly) by many investigators of pain-like phenomena in invertebrates, for which argument by analogy is necessarily much weaker than for mammals. This is to define pain on the basis of functional rather than subjective properties, as advocated by Elwood (REF). For example, researchers studying injury-induced sensitization of defensive behaviour in *Drosophila* assume the functions of such sensitization are the same as for mammalian allodynia and hyperalgesia (types of conscious evoked pain as defined by the IASP) and use these terms and sometimes the word "pain" to refer to injury-related states in this insect --without explicitly arguing that flies might have conscious pain experience. This approach is illustrated in the articles by Lopez-Bellido *et al*. (REF) and by Khuong et al. (REF). In contrast, investigators of molluscs such as Howard *et al.* (REF) and annelids such as Paulsen & Burrell (REF) have generally preferred to use terms such as nociception and nociceptive sensitization that do not imply conscious pain, rather than stating that these invertebrates exhibit pain, allodynia, or hyperalgesia (Walters, 2018). Nonetheless, virtually all investigators of invertebrates, including all of those represented in this Theme Issue (Adamo & McMillen; Elwood; Himmel *et al*.; Howard *et al*.; Khuong *et al.*; Lopez-Bellido *et al*.; Mihail *et al*.; Paulsen and Burrell) assume that some of the mechanisms and functions under investigation in invertebrates are also likely to be involved in mammalian pathways that promote or suppress conscious pain.

Central states linked to nociceptive plasticity in many species meet many of the criteria of an early functional definition in which pain is "an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species-specific behaviour, including social behaviour" (Zimmermann, 1986). This definition is consistent with behavioural observations described in this issue for crustacea (Elwood [REF]) and fish (Sneddon [REF]), as well as insects and molluscs (Walters 2018). It ties pain to a complex aversive state that 1) is induced by noxious stimulation during injury or imminent injury, 2) has a presumed protective function involving overt defensive behavior, internal physiological alterations, and an aversive motivational state that can promote avoidance learning, and 3) can be revealed by operant tests such as avoidance learning that demonstrate the aversive state. Assuming that the word "experience" encompasses unconscious as well as conscious experience, all of these features are amenable to experimental investigation in non-human animals. In a few non-mammalian species, operant tests have provided some evidence for the existence of pain-like aversive states, as reviewed here by Elwood (REF) and Sneddon (REF) as well as by Walters (2018), but this evidence would be strengthened by the addition of conventional conditioned place avoidance and conditioned place preference tests that increasingly are used in rodent studies to reveal the affective-motivational component of pain. One part of Zimmermann's pain definition that will not apply to many animals (vertebrate and invertebrate) is "including social behavior" because functionally defined pain also is likely to occur in asocial animals. While functional definitions of pain encourage comparative studies of pain-related processes across diverse taxa, providing valuable evolutionary insights, purely functional definitions of pain do not include what for many is the core feature of human pain -- its distressing subjective component.

In sum, at least two types of pain definition can serve as foundations for enquiries into the evolution of pain. One type emphasizes the distressing subjective experience that defines human pain but is not directly accessible for study in other species. The other emphasizes the protective and motivational functions of aversive pain states induced by bodily injury without reference to subjective experience. These views of pain should be complementary, but they have been difficult to integrate (Bateson, 1991, Sneddon et al., 2014, Walters, 2018). This

discordance has contributed to the contentious debate about which species feel pain (e.g., see the target article by Key (2016) and accompanying commentary on whether fish feel pain).

3. Evolution of mechanisms important for nociception and pain

Whether their emphasis is on the subjective experience of pain or pain-related functions, clinicians and researchers agree that pain is closely (although not always) linked to nociceptive activity in the nervous system that normally is produced by bodily injury and inflammation. Thus, while nociception is distinct from the experience of pain (Sherrington, 1906), it is clear that activity in nociceptive pathways usually drives pain, and that enhanced nociceptive activity increases pain (Basbaum et al., 2009, Gold and Gebhart, 2010). This makes comparisons of the mechanisms of nociception and of nociceptive alterations across diverse species useful for enquiries into the evolution of pain.

Nociception begins when energy that produces or threatens to produce imminent injury (a noxious stimulus) is transduced into neural activity, leading to organized responses that defend the tissue under threat and aid in its repair. In addition to strong mechanical and thermal stimuli, caustic chemical stimuli such as acids can threaten tissue integrity and homeostasis. In their review article, Pattison and St. John Smith (REF) show that acid nociception is a feature of many primary nociceptors, and it has been documented in several phyla. Prominent proton-sensing molecules in diverse mammals include acid-sensing ion channels (ASICs), transient receptor potential vanilloid 1 (TRPV1), and specific 2-pore K^+ channels and G protein-coupled receptors, which are often expressed in polymodal nociceptors that also are sensitive to other noxious stimuli and/or inflammatory signals. As reviewed by Sneddon (REF), ASICs occur in fish, and vigorous defensive behavior is evoked in teleost fish by either injection or bath application of weak acids. Interestingly, while the nociceptor molecules that detect protons in phyla other than Chordata have yet to be identified, Lopez-Bedillo *et al.* in this issue (REF) demonstrate in *Drosophila* that multiple classes of primary sensory neurons, including nociceptors, are specifically required for defensive responses evoked by cutaneous application of strong acid. The availability of powerful genetic tools in *Drosophila* coupled with access to known proton-sensing sensory neurons in this fly should begin to answer the question of whether arthropods share ancient acid-nociception mechanisms with vertebrates, or they have independently evolved different mechanisms. As described by Himmel et al. (REF), these genetic tools combined with neurophysiological and behavioral tests in *Drosophila* revealed that another chemical stimulus, menthol, activates nociceptors to evoke defensive responses. The receptors involved are channels in the TRPM and TRPA families, and their phylogenetic analysis suggests the TRPM family was present in ancestral Precambrian blilaterians.

A notable feature of nociception and pain compared to other senses is the complex modulation that can occur at all stages of sensory transmission. That this feature is not unique to mammalian pain pathways is emphasized by two articles in this Theme Issue. In a broad review of cannabinoid signaling related to nociception in mammals and invertebrates, Paulsen and Burrell (REF) provide evidence of molecular conservation across chordates and several other phyla at the level of cannabinoid receptors (both GPCRs and TRP channels) and the enzymes that synthesize and degrade endocannabinoids. Unexpected functional parallels have been found between rodents and a leech species in the opposing effects of endocannabinoids at different neural loci; these can depress transmission at nociceptor synapses while potentiating (by disinhibition) transmission at synapses of other primary sensory neurons. This suggests that a complex pattern of endocannabinoid modulation of nociceptive responses has been conserved for over half a billion years, and/or that this pattern is a product of convergent evolution. Among the types of mammalian pain that respond to endocannabinoids (and many

other intracellular signals) is inflammatory pain. While chemicals released by peripheral and central inflammatory and immune cells are known to be important for many types of pain in mammals (Cook et al., 2018, Grace et al., 2014, Ji et al., 2016), little is known about inflammatory and immune modulation of nociception and pain-like behavior in invertebrates. For example, it was not known whether any invertebrate exhibits generalized allodynia-like hypersensitivity to tactile stimulation during a systemic immune challenge. In this issue, Adamo and McMillan (REF) report that larvae of the hawkmoth, *Manduca sexta*, display a reduction in threshold for defensive responses to punctate mechanical stimuli 2 hours after ingestion or injection of heat-killed bacteria. This effect resembles the allodynia that often accompanies sickness behaviour in mammals, and it may represent a widespread adaptation to maintain anti-predator vigilance when an animal is weakened by infection. The large larvae of *Manduca* offer many advantages for neurophysiological investigations into mechanisms of pain-like behavioural plasticity (Tabuena et al., 2017), which could be especially useful when combined with molecular predictions obtained from genetic manipulations of *Drosophila* larvae (e.g., see Himmel et al. [REF], Khuong et al. [REF] and Lopez-Bedillo et al. [REF]).

Equally notable is the remarkable capacity of nociception and pain to become chronically enhanced after injury, inflammation, toxin exposure, or other bodily stresses. Long-term sensitization (lasting a week or longer) of defensive responses in an invertebrate was first demonstrated in the large marine snail, *Aplysia,* after noxious shock (Pinsker et al., 1973) or peripheral injury (Walters, 1987). Transection or strong artificial depolarization (mimicking a major consequence of cellular injury) of peripheral axons of primary nociceptors caused persistent hyperexcitability of nociceptor somata (Walters et al., 1991) and axonal segments that had been transiently depolarized (Weragoda et al., 2004). Mihail et al. (REF) have now demonstrated that the hyperexcitability of an axonal segment persisting after transient depolarization depends upon a core signaling pathway that has also been shown to drive hyperexcitability in mouse nociceptors. Previous evidence indicated that local protein synthesis dependent on mechanistic target of rapamycin (mTOR) signaling was required for long-lasting hyperexcitability of axons in *Aplysia* nociceptors (Weragoda et al., 2004) and for various injury-related responses of primary afferent neurons in mammals (Price and Géranton, 2009). Here, Mihail et al. (REF) describe a further requirement in *Aplysia* axons for signaling by mitogen-activated protein kinase interacting kinase (MNK) to eukaryotic translation initiation factor (eIF) 4E, which is known to regulate mTOR. Their findings indicate that eIF4E phosphorylation by MNK is an ancient and highly conserved mechanism for maintaining sensory hyperexcitability.

Chronic pain in humans is often caused by peripheral nerve injury, which also is used experimentally in rodents to investigate chronic pain mechanisms. Khuong and colleagues (Khuong et al., 2019) recently reported that amputation of a leg in adult *Drosophila* produces allodynia-like mechanical hypersensitivity that lasts at least 3 weeks, far longer than the allodynia-like alterations previously described in larval *Drosophila*. In this Theme Issue, Khuong et al. (REF) now show that this hypersensitivity requires the expression of an alpha2 delta3 Ca^{2+} channel auxiliary subunit in primary nociceptors. In mammals, closely related subunits are targeted by gabapentinoid analgesics, which are among the most effective treatments available for neuropathic pain. Remarkably, both the amputation-induced hypersensitivity and an associated apparent delayed loss of central inhibitory neurons (which should produce permanent hypersensitivity) were attenuated by early treatment with gabapentin or pregabalin. These findings encourage the use of *Drosophila* to help discover drugs that target fundamental mechanisms important for persistent pain.

Unlike other sensory systems, nociceptive systems can sometimes undergo very long-lasting, even permanent, enhancement of function following sufficiently intense activation. This feature

has long been assumed to contribute to chronic pain conditions and to involve transcriptionally dependent neuronal alterations (Ji and Woolf, 2001, Walters, 1994). Until now, the longest lasting sensitizing effects of noxious stimulation in any invertebrate had been reported for *Aplysia,* where nociceptor sensitization after nerve injury persisted for over a month (Gasull et al., 2005), a duration similar to that of behavioral alterations recently reported after leg amputation in *Drosophila* (Khuong et al., 2019) (Khuong et al. REF). For the first time in any invertebrate, Howard et al. (REF) describe lifelong (13 weeks) sensitization of probable primary afferent neurons. This was produced by mechanical trauma to the fins of newly hatched squid (using the small, relatively short-lived Hawaiian bobtail squid). Interestingly, the primary afferent sensitization did not correlate directly with complex alterations in defensive behavior or aversive learning, suggesting that potent effects of injury also occurred within the central nervous system that could override the permanent peripheral sensitization. Some of the effects of early-life injury in this squid resemble lifelong effects of early-life stress in mammals. Work reviewed by Geranton in this issue (REF) has shown that early-life stress in mammals produces a lifelong predisposition to chronic pain that involves the increased expression of a regulatory protein within the stress axis, FKBP51. Importantly, this molecular response depends upon a persistent epigenetic change, reduced methylation of the *FKBP5* gene that encodes FKB51. While epigenetic regulation has been implicated in allodynia-like alterations in *Aplysia* (Bédécarrats et al., 2018), different roles of methylation in this example compared to known examples in mammalian chronic pain models (Geranton REF), as well as differences in DNA methylation in *Drosophila* compared to mammals (Deobagkar, 2018), raise questions about how conserved the epigenetic mechanisms important for persistent pain-like alterations are across different phyla.

As reviewed by Mogil in this issue (REF), there has been substantial evolutionary divergence in some traits related to pain -- documented within closely related mammals, such as rats and mice, and even between different strains of the same species of mouse. These differences may have contributed to the limited translational success in human clinical trials of candidate analgesics developed largely on the basis of efficacy tests in rodents. On the other hand, many of the papers in this Theme Issue add to accumulating evidence of strong conservation of fundamental molecular mechanisms of neuronal plasticity that induce and maintain pain-like alterations across the Animal Kingdom (Walters, 2018). To optimize the chances of discovering drug targets important for pain that have been conserved both in humans and rodents, Mogil (REF) provides strong arguments for coordinated, essentially identical studies on humans and rodents, with the results from each species being used to adjust the design of the experiments for both species. He provides three successful examples of this combined experimental strategy, involving a stress-induced analgesia gene, emotional contagion of pain (see below), and context-dependent pain hypersensitivity.

4. Adaptive and maladaptive features of pain behaviour and its mechanisms

Understanding how evolution shaped the mechanisms and behaviours important for pain requires that the evolutionarily adaptive, neutral, and maladaptive aspects of pain be identified. This is not straightforward. For example, the protective functions of acute nociception and pain have always seemed obvious, an assumption supported by the high morbidity of humans with congenital insensitivity to pain (Nagasako et al. 2003) and by direct experimental support in flies (Robertson et al., 2013). However, the general assumption of pain researchers and evolutionary psychologists that chronic pain is necessarily maladaptive is being questioned. Several papers in this Theme Issue show that combining an evolutionary perspective with sophisticated pain studies can modify entrenched assumptions about adaptive and maladaptive pain behaviour.

While pain is often associated with pathology in the body, this certainly does not mean that pain itself is pathological. As Nesse and Schulkin write in this issue (REF), “Pain always seems like a problem, but usually it is part of the solution”, and they emphasise that it is not pain but the capacity for pain that is subject to natural selection. They discuss Tinbergen’s four questions that need to be answered to explain any behaviour, including pain: What is its adaptive significance, its phylogeny, its mechanisms, and its ontogeny? Each question demands different methods to answer, and the answers are largely independent. For example, despite the enormous progress made in identifying mechanisms related to pain, this knowledge provides limited insight into adaptive functions of pain-related behaviour, Moreover, mechanistic investigators tend to assume dysfunction rather than adaptation when investigating pain-related phenomena in which protective benefits are not immediately obvious.

Nesse and Schulkin (REF) are concerned with explanations based in evolutionary medicine for why pain is often expressed inappropriately. One explanation, common to many defensive traits, is the "smoke detector principle" in which evolution has traded off the relatively small cost of numerous false alarms against the very high cost of failing to respond to a threat of serious injury or infection. Another is that a system that sensitizes readily has a cost in the inherent vulnerability of such systems to runaway positive feedback that may lead to chronic pain. A third explanation is the mismatch between the modern human lifestyle and the environment to which the pain system was adapted (e.g., lower back and joint pain resulting from sedentary habits, chronic pelvic pain from more numerous menstrual cycles in the modern world). In her review, Williams (REF) finds unexpected evidence that chronic pain results from a mismatch of the pain system with the modern environment. She notes that virtually all documented observations of chronic pain have come either from reports of humans or observations of their dependent farm and companion animals, or from dependent laboratory animals. She suggests that chronic pain expressed in the wild is probably maladaptive for most species because it interferes with necessary physical activity (whereas a timely return to normal activity appears to suppress persistent pain), and that it may occur only under conditions where inactive individuals experiencing chronic pain can be ministered to by human caretakers.

Finlay (REF) also seeks evolutionary explanations for pain, especially for apparently inappropriate degrees of pain experienced by humans. By analogy to other perceptual systems (especially vision), she suggests that pain evolved to guide adaptive behavior, and that this involves complex processing in the brain to assess contingent relationships between noxious stimuli and behavioral actions. This predictive processing enables pain to be minimized during voluntary activities such as extreme exercise, cosmetic procedures, and self-harm in humans. Conversely, Finlay suggests that evolution has amplified the experience of pain in women beginning labour (beyond the degree of pain expected from the amount of tissue damage early in labour) because the resulting pain behaviour provides an "honest signal" that effectively solicits help and protection from partners and relatives during childbirth, thereby enhancing the survival of mother and child.

Whether chronic pain and its mechanisms can be adaptive is addressed by several papers in the Theme Issue. As mentioned, Williams (REF) argues that chronic pain, or at least the behaviour that is taken to indicate ongoing pain, can be maladaptive and may be much less common in the wild than has been suggested by human clinical, preclinical, and veterinary experience. Enhanced survival during predatory encounters resulting from the induction of potentially long-lasting sensitization and hypervigilance induced by injury or electric shock has been shown in squid (Crook et al. 2014) and amphipod crustaceans (Perrot-Minnot et al., 2017) (see also Howard et al., REF; Elwood REF). Long-lasting, pain-related hypervigilance is also likely to influence estimate of risk and thus behavioural decisions in mammals (Williams REF;

Walters REF). The opposite effect, discounting of potentially painful experience in humans pursuing a valued goal, is discussed by Finlay (REF) in human behaviours rarely subject to scientific research, including self-harm and cosmetic procedures. She recommends further exploration of habituation to pain (De Paepe et al., 2019), and of the variance in human pain attributable to expectation and to agency.

Nesse (REF) and Walters (REF) also emphasise the importance of mechanisms that turn off pain when not needed so that chronic pain does not occur. On the other hand, several articles consider evidence that chronic pain-like alterations can be adaptive under appropriate conditions. Howard et al. (REF) suggest that permanent sensitization of primary afferent neurons in bobtail squid after traumatic early-life injury represents an adaptation to what is likely to be a highly dangerous environment, extending previous evidence and suggestions for the adaptiveness of long-lasting nociceptive sensitization induced in adults (Crook et al., 2013, Crook et al., 2014, Walters, 1994). A similar suggestion for the adaptiveness of amputation-induced allodynia mediated by loss of inhibitory interneurons in flies has been made by Khuong et al. (Khuong et al., 2019), although it will be important to show that large-scale, permanent neuronal loss (Khuong et al., [REF]) does not also have major maladaptive consequences in this species. For extremely long-lasting alterations, Geranton (REF) even considers the possibility that trans-generational effects of stress mediated by changes in DNA methylation can increase the resilience of offspring to stress and pain.

The adaptiveness of persistent pain is also consistent with evidence from fossils, behavioural observations in the field, and distinctive specializations of nociceptors. Dangers from predators and aggressive conspecifics have probably been major selection pressures for persistent alterations in nociceptive systems since the Precambrian era. Presenting a systematic meta-analysis of fossil evidence from the Mesozoic era, Hearn and Williams (REF) conclude that dinosaurs could survive long after severe injuries, during which time guarding behaviour appears to have been present, possibly accompanied by persistent pain. Walters (REF) argues on the basis of such evidence and from field and laboratory observations of living species that some forms of chronic pain and persistent nociceptor hyperactivity are adaptations that promote survival after injuries severe enough to cause permanent disfigurement and impairment of motor function. Such injuries greatly increase the risk of, and vulnerability to, subsequent attack. Walters also shows that the "ectopic activity" of primary sensory neurons caused by neural injury in mammals, which always was assumed to be a purely pathological effect, instead exhibits properties expected of an evolutionary adaptation to promote ongoing pain and hypervigilance (anxiety) under the conditions of heightened vulnerability that follow severe injury. In particular, the complex, functionally coherent set of mechanisms that enable persistent ongoing activity in nociceptors after severe injury shows non-random organization and coordination that satisfy the "design criterion" for an evolutionary adaptation (Andrews et al., 2002, Stearns and Medzhitov, 2016).

5. Evolutionary aspects of pain-related social behaviour

While true social behaviour is found in a minority of all animal species (Wilson, 2012), it appears important for nociceptive function and pain in humans and in the rodents that are employed for most preclinical studies of pain (Williams and Craig, 2016). Further, the environmental context of any animal contains challenges (Mobbs and Kim, 2015): predators seen or unseen, behaving in a threatening or non-threatening way; a potential mate or competitor for a mate, to be impressed or deterred by appearing healthy and strong. Several papers in this issue address questions about pain-related social behaviour that involve evolutionary considerations. Hearn and Williams (REF) discuss evidence from fossils (including fossilized track patterns) and from the behaviour of contemporary archosaurs (birds and crocodiles) that is consistent with parental

and family care by dinosaurs. This may have extended to help with injured family members expressing pain. Mogil (REF) describes a rudimentary form of empathy in mice, emotional contagion, in which a mouse receiving painful stimulation displays higher levels of pain behaviour in the presence of familiar mice than with strangers. He describes the results of coordinated experiments on mice and humans that have revealed for the first time in either species that the lack of emotional contagion of pain between strangers is caused by stress, as shown by analogous procedures that reduce stress in each species and enable pain contagion between strangers. Whether emotional contagion has adaptive functions, and whether these functions could be similar between mice and humans is an interesting question. As mentioned, Finlay (REF) (Finlay and Syal, 2014), building upon the capability of expressed pain to elicit helping behaviour in humans (Williams, 2002), has proposed that evolution has enhanced the pain experienced by women during the initial stages of labour in order to more effectively obtain help and protection from others during childbirth.

Social context is likely to determine whether pain is expressed overtly and by which behaviours. For example, some behaviours are detectable at long range, including by predators and competitors, while others require proximity, which is more likely for kin and allies. This prediction has important implications for questions about the adaptive significance of pain expression (Williams, 2002), and it has been supported by observations in rodents (e.g., Mogil, this issue), in companion and farm animals (Williams, this issue), and in humans (Kappesser, this issue, REF). However, conclusive experimental evidence for pain expression altering the behaviour of conspecifics is surprisingly sparse for any species, including humans (Mogil, this issue). This is illustrated by Kappesser's review (REF) of findings on social threat and facial expression of pain in humans, which reveals inconsistent findings that may be attributable to differing theories and models (most of which do not include evolutionary premises), different methodologies, limits on the degree of pain and social threat permissible in ethically acceptable experimental paradigms, and experimentally uncontrolled complexities in social relationships that may influence the expression of pain and related anxiety. How the social context influences pain behaviour raises questions about what is socially threatening in humans, which have potentially important implications; for example, for more effectively treating patients for whom nonverbal behaviour is the only route of communication. Most of the studies reviewed by Kappesser are experimental studies using healthy subjects and brief evoked pain. Manipulation of threat value, and of social relationship between participants, as is possible in some common clinical procedures that are painful or that exacerbate significant pain, may well be more informative for revealing the nature and functions of differences in the facial expression of pain in different social contexts.

5. Conclusions

A biological understanding of pain in humans and other species requires knowledge of pain's evolutionary context. This requires extensive comparisons of the behavioural functions, cellular mechanisms, and sequences of involved genes and/or gene products important for pain across diverse living species representing the major phyla. Two major obstacles stand in the way of such enquiries into the evolution of pain. One is the difficulty in defining pain in a way that allows pain and its possible evolutionary antecedents to be recognized and compared across species, a task that is especially challenging for attempted comparisons of the conscious component of pain. Because of our personal experience, human pain is most familiar and most important, so the most relevant comparisons are between other species and our own. Such comparisons bring up a second obstacle, which is the special status of the human species. This reflects the enormous complexity of pain-linked behaviour (including uniquely complex social behaviour in humans) and of pain's intricate substrates involving large parts of the human nervous system, plus the ethical impermissibility of controlled studies on severe and/or chronic pain induced

experimentally in healthy human volunteers. Despite impressive recent progress with human imaging and neural stimulation methods, identifying the neuronal populations critical for even transient pain experience in the human nervous system is incomplete at best. Compounding these obstacles is the fact that pain is an inferred internal state, rather than an obvious external behaviour, and thus is extremely difficult in any species to assess accurately using behavioural or neural activity measures. Consequently, there is considerable uncertainty about which behavioural features, neural circuits, cell types, and molecules to compare across taxa when defining evolutionary relationships (homologous and analogous traits). In addition, behavioural states leave very little fossil evidence (although some has been found) for making inferences about when, and in which types of animal, pain states appeared during evolution.

In spite of these obstacles, substantive insights into the evolution of pain are emerging, as illustrated by the following articles. Regarding the evolution of mechanisms important for pain, there is no doubt that human pain is usually initiated and often maintained by electrical activity in primary nociceptors. In contrast to other cell types involved in nociceptive and pain-related processing, the basic functions and anatomical locations of nociceptive primary afferents are known in humans, other mammals, and in some representatives from other major taxa, including the fish, annelids, arthropods and molluscs discussed in this issue, as well as nematodes. This has enabled direct comparisons of cellular and molecular traits important for nociception and nociceptive plasticity in nociceptors across invertebrate and mammalian species. These comparisons have revealed similar roles of conserved proteins, including TRPA, TRPV, TRPM, and ASIC channels, as well as alpha2 delta auxiliary subunits of Ca^{2+} channels; many protein kinases, including MNK and mTOR for regulating local protein synthesis; and transcription factor families such as PRDM and CREB (see also Walters, 2018). Divergent mechanisms are certain to be involved as well, including possible differences across phyla in the roles of epigenetic mechanisms, and various differences even within closely related mammalian taxa in pain-related effects (e.g., on gene expression). At the functional level, some primary afferent neurons in different phyla have been found to exhibit similar hyperactivity long after strong noxious stimulation, contributing to hypersensitive states resembling allodynia and hyperalgesia in mammals. The degree to which conserved and convergent mechanisms in nociceptors contribute to these functional similarities in pain-like states across diverse taxa is a fascinating question.

Given our limited knowledge about the evolution of pain, particularly important questions concern the evolutionary adaptiveness, or lack thereof, of various forms of pain. Whereas acute pain is universally agreed to be adaptive, only recently have possible benefits been recognized for some forms of chronic pain or very severe pains that seem out of proportion to the existing state of tissue damage. Some of these pains are likely to represent mismatches between evolved pain systems and the modern environment, or trade-offs with other adaptations, such as effective immune function. However, plausible arguments also suggest that evolution selected mechanisms in diverse species that can persistently maintain pain-related hypervigilance as adaptations to especially dangerous environments and to enhanced vulnerability persisting long after disfiguring injury. Identification of evolutionarily adaptive and maladaptive features of pain behaviour, in relation to environmental (including social) variables and the state of the organism, may require investigatory frameworks and experimental paradigms that are novel for the pain research field. For some species, evolution has selected processes that allow the expression of pain to be strongly modulated by social context. In humans, evolution may have amplified the experience of pain under conditions, such as labour, in which an "honest signal" can solicit aid that enhances reproductive success. While adaptive arguments are speculative, they and their alternatives have scientific and clinical implications

that can be tested rigorously by behavioural and mechanistic pain research that is informed by evolutionary principles.

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