Pain and Suffering in Invertebrates?

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Abstract

All animals face hazards that cause tissue damage and most have nociceptive reflex responses that protect them from such damage. However, some taxa have also evolved the capacity for pain experience, presumably to enhance long-term protection through behavior modification based on memory of the unpleasant nature of pain. In this article I review various criteria that might distinguish nociception from pain. Because nociceptors are so taxonomically widespread, simply demonstrating their presence is not sufficient. Furthermore, investigation of the central nervous system provides limited clues about the potential to experience pain. Opioids and other analgesics might indicate a central modulation of responses but often peripheral effects could explain the analgesia; thus reduction of responses by analgesics and opioids does not allow clear discrimination between nociception and pain. Physiological changes in response to noxious stimuli or the threat of a noxious stimulus might prove useful but, to date, application to invertebrates is limited. Behavior of the organism provides the greatest insights. Rapid avoidance learning and prolonged memory indicate central processing rather than simple reflex and are consistent with the experience of pain. Complex, prolonged grooming or rubbing may demonstrate an awareness of the specific site of stimulus application. Tradeoffs with other motivational systems indicate central processing, and an ability to use complex information suggests sufficient cognitive ability for the animal to have a fitness benefit from a pain experience. Available data are consistent with the idea of pain in some invertebrates and go beyond the idea of just nociception but are not definitive. In the absence of conclusive data, more humane care for invertebrates is suggested.

Key Words: behavior; discrimination learning; invertebrate; morphology; nociception; pain; physiology; stimulus avoidance

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Defining Pain versus Nociception

All species of animal are susceptible to a variety of naturally occurring hazards that can cause tissue damage. Sharp objects, such as teeth or mandibles of predators, or defensive thorns or spines in plants or animals, are common. Chemicals, blunt objects, and thermal extremes may also cause damage. Some plants (e.g., nettles) and animals (e.g., hymenoptera and coelenterates) have specialized structures that are sharp, penetrate the tissues, and transfer noxious, potentially damaging chemicals. However, animals have mechanisms that enhance their ability to maintain the integrity of their tissues through the detection of noxious stimuli and action to get away from them and/or minimize their deleterious effects.

The sensory systems that respond to noxious stimuli and mediate protective reflexes are termed *nociceptors* (Sherrington 1906). *Nociception* is defined as "the neural processes of encoding and processing noxious stimuli" (Loeser and Treede 2008, 475) or the detection and reaction "to stimuli that may compromise their integrity" (Besson and Chaouch 1987, 67). Thus nociception is the perceptual mechanism coupled with the organization of responses that typically take the animal away from the stimulus or at least are effective in terminating the perception. For example, *Drosophila* larvae attacked by a parasitoid wasp respond by rolling toward the stimulus, which causes the wasp's ovipositor to pull out and the wasp to leave (Hwang et al. 2007).

By contrast, the definition of pain in humans is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP 1979, 250). Various definitions have been used with respect to animals—for example, "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 behaviors, including social behavior" (Zimmerman 1986, 1). A shorter definition that excludes pain assessment criteria is "an aversive sensation and feeling associated with actual or potential tissue damage" (Broom 2001, 17).

It is clear that nociception is central to the concept of pain, as without it the experience of pain is unlikely. However, simply observing a nociceptive ability does not demonstrate pain. Nociception per se is an involuntary rapid reflex response and lacks the negative emotional response or feeling associated with pain (Bateson 1991; Broom 2001). Indeed, in humans the reflex response to touching something hot precedes the experience of pain.

Although the distinction between nociception and pain is widely accepted there are semantic issues that may cloud the issue. The term "pain perception" is frequently used in pain studies (e.g., Braithwaite 2010; Sneddon 2009) and nerve fibers are said to "transmit pain" (Weary et al. 2006). Even in studies that are overtly about nociception rather than pain, nociception is described as "pain sensing" and ascending tracts in the vertebrate spinal cord are said to carry "painful sensory information" (Hwang et al. 2007) or "pain information" (Sneddon 2009). Larval *Drosophila* that lack a particular functional gene fail to roll away from thermal or mechanical stimuli and the mutation has been called "painless" even though the gene function is in the activation of transducer channels by which a neural signal results from physical stimuli (i.e., a key part of nociception) (Tracey et al. 2003). The use of these terms blurs the critical distinction between nociception and pain.

I prefer to use terms such as "pain experience" to denote an internal awareness, coupled with a negative emotional state or feeling, that results from perception of actual or potential tissue damage. It is the damage that is perceived. There is no "pain" to be perceived and the information carried to the brain in vertebrates is not in itself "painful." The pain results from a powerful, unpleasant emotion that is part of, or coupled with, a strong motivation to terminate the experience that results from neural signals about tissue damage.

Clarity about the definitions of and distinctions between nociception and pain is essential for determining whether pain occurs in particular groups of animals.

Function and Evolution of Pain

As noted above, nociception provides a means of detecting and escaping from a stimulus that might continue to cause damage in the absence of action. Thus there are clear benefits in nociception and, presumably, they outweigh the costs of developing, maintaining, and using the system. What further advantage is gained by having an additional system that enables the experience of nociceptive inputs as an unpleasant emotion? It may be that the "emotional" component provides a long-lasting motivation that enables the animal to better maintain its tissue integrity (Bateson 1991).

A nociceptive response may be organized as a reflex (Sneddon et al. 2003) but may not be associated with a lasting memory and motivational change. Pain, on the other hand, might induce a long-term memory and be coupled with learning to avoid situations that gave rise to the original pain experience (Bateson 1991). The greater the tissue damage in the original experience the greater may be the unpleasant emotional response and the greater the motivation to avoid it in the future. Thus pain experience has a longer-lasting effect and protects the animal from future damage in a more effective manner than does nociception alone.

Nociceptive abilities are found in most of the major animal phyla and thus are presumably a product of very early evolution. They clearly predate the Cambrian "explosion," a period approximately 530–550 million years ago during which the major modern phyla evolved (Budd and Telford 2009). The next step in the development of pain was probably a link between nociception (with the associated reflex response) and a longer-term motivational change (with central processing and memory). But in which groups did this development take place and when? Some researchers are confident that it occurred only in vertebrates and that it is evident in fish and also perhaps the more primitive lamprey (Braithwaite 2010; Sneddon 2009). If correct then pain has been around since the Cambrian explosion because that is when many of the major invertebrate phyla evolved. But if it is accepted that pain occurs in fish and lamprey then the question is whether it is a novel development in the vertebrates or if the ability to experience pain predated the split of the vertebrates from the ancestors of some other phyla and could thus be present in some extant invertebrate groups? Alternatively, if there is an advantage in experiencing pain, it could be that it has evolved on more than one occasion (Elwood et al. 2009).

How Can Pain Be Identified?

The interest in the potential of invertebrates to experience pain lies in the quest to understand and improve welfare, as humans generally seek to avoid causing pain or suffering to animals (Broom 2007). If an animal responds to a noxious stimulus in an adaptive fashion via a nociceptive reflex and without any unpleasant experience, then welfare concerns are diminished. The key objective of this article is to examine the evidence that some invertebrates may or may not experience pain.

Inferring feelings or mental states in animals is fraught with difficulty (Dawkins 2006). A common approach is to use argument by analogy (Dawkins 1980; Sherwin 2001): if an animal responds to a potentially noxious stimulus in a manner similar to that observed to the same stimulus in humans then it is reasonable to argue that the animal has had an analogous experience (Sherwin 2001). However, Sherwin (2001) notes differences in the acceptance of this argument depending on the species rather than the behavior: observers of a dog or primate writhing in response to an electric shock accept that the animal is experiencing pain, whereas much the same response in an invertebrate is often dismissed as irrelevant to the question of pain. He suggests a more symmetrical approach when comparing vertebrates with invertebrates, with consistent acceptance or rejection of the argument by analogy (Sherwin 2001). However, empathy for invertebrates is typically low and some researchers believe that it would be "inconvenient" if these animals were believed to feel pain (Kellert 1993).

Coupled with analogy, various criteria have been proposed as collectively having the potential to demonstrate pain in mammals (Bateson 1991) and have been applied to pain in amphibians (Machin 1999; Stevens 2004), fish (Sneddon et al. 2003), and various invertebrates (Broom 2007; Elwood et al. 2009;

Fiorito 1986; Sherwin 2001). In the following sections I consider criteria that are similar (but not identical) to those used in previous work (Bateson 1991; Broom 2007; Sherwin 2001), assessing the presence of the following features:

- suitable receptors;
- a suitable central nervous system;
- responsiveness to opioids, analgesics, and anesthetics;
- physiological changes;
- avoidance learning;
- protective motor reactions;
- tradeoffs between stimulus avoidance and other activities; and
- cognitive ability and sentience.

Suitable Receptors

Sea anemones respond to mechanical stimuli and to the stings of other anemones but not to thermal stimuli (Mather 2011, in this issue). Annelids have nociceptors that respond to acid, capsaicin, and heat (although the sensitivity to acid is lower than that seen in vertebrates; Smith and Lewin 2009) and cells that respond to touch and pressure (Nicholls and Baylor 1968). Nociceptors and nociceptive behavior have been described in molluscs; for example, the snail (*Cepaea nemoralis*) responds to a hot plate at >40°C by lifting the anterior portion of its foot (Kavaliers et al. 1993). In *Aplysia californica* (Castellucci et al. 1970; Smith and Lewin 2009), once the stimulus threshold is reached, nociceptors increase firing in line with subsequent increase in stimulus strength and show maximal activity with the crushing or tearing of tissues.

Nociceptive systems have been described in particular detail in the nematode (*Caenorhabditis elegans*) and fruit fly (*D. melanogaster*) (reviewed by Smith and Lewin 2009) and molecular tools have been applied to elucidate the detailed development and functioning of their nociceptors (Goodman 2003; Tobin and Bargmann 2004). Nociceptive neurons of *Drosophila* larvae have multiple dendritic branches with naked endings attached to epidermal cells (Hwang et al. 2007); several classes of such multidendritic (md) neurons have been described but not all are involved in nociception. If all these md neurons are rendered inactive in particular genetic mutants, the larvae fail to respond to noxious stimuli, showing that at least one is nociceptive (Tracey et al. 2003). Selective silencing of particular classes of md neurons, however, showed that one class serves as the primary nociceptive system and that the silencing of this class of neurons eliminates the rolling response of larvae to thermal stimuli (Hwang et al. 2007). The nociceptive neurons are also responsive to attacks by parasitic wasps attempting to insert their ovipositor (as described above; Hwang et al. 2007), indicating that they are polymodal, as are those of vertebrates (Goodman 2003).

The common theme of these studies is the ability of a wide range of taxa to detect noxious stimuli and to translate them into neuronal signaling (Tobin and Bargmann 2004). The systems involved are complex but conserved across markedly different taxa, as is evident from the use of *Drosophila* in drug discovery for application in vertebrates, especially humans (Manev and Dimitrijevic 2005). In addition, these systems show adaptive, temporary, heightened, and reduced sensitivity and these features are also conserved across phyla (Babcock et al. 2009). One recent study, however, failed to detect nociceptors in decapod crustaceans and also noted little ability to respond to noxious stimuli (Puri and Faulkes 2010) despite organized responses to noxious chemical and electrical stimuli noted in other studies (Barr et al. 2007; Elwood et al. 2009; Elwood and Appel 2009).

Vertebrates have a variety of nociceptive fibers, some myelinated and others not. By contrast, those in invertebrate groups are only unmyelinated (Smith and Lewin 2009). However, this distinction reveals little about any taxonomic difference in pain experience because it is the unmyelinated C fibers in mammals that are most prevalent and are important in the perception of stimuli that give rise to pain (Smith and Lewin 2009). Because pain experience associated with tissue damage typically depends on nociception, a lack of nociceptors would suggest that the animal was insensitive to noxious stimuli and could not experience pain. This argument was central in recent work in fish that demonstrated nociceptors similar to those of mammals, allowing the conclusion that fish had apparatus that should be sufficient for them to experience pain (Sneddon 2003). However, that study was also right to state that the presence of nociceptors per se does not demonstrate that pain is experienced.

A Suitable Central Nervous System

Because it is clear that the human brain is suitable for pain experience there has been an assumption by some (Rose 2002) that only animals with structures very similar to those of humans have the capacity to experience pain. For example, the possibility of fish experiencing pain has been dismissed because human pain is experienced in parts of the cerebral cortex whereas fish lack this structure (Rose 2002). If one accepts this argument then the possibility of pain being experienced by any invertebrate must be dismissed because none has a central nervous system (CNS) built on the vertebrate plan. However, according to the same logic it could be suggested that because crustaceans or cephalopods lack any of the visual system found in humans they must be blind. This is not the case as both have a well-developed visual ability, each based on an entirely different CNS and receptors. Thus clearly the same function can arise in different animal taxa using different morphology, and it appears to be illogical to accept this reasoning for some experiences but to dismiss it for pain (Elwood et al. 2009).

In his review of pain criteria Bateson (1991, 834) avoided the idea that structures homologous to those of humans must be present and instead suggested that there should be "structures analogous to the human cerebral cortex." Because

many invertebrates have a remarkably complex brain structure, albeit rather different from that of humans, some might have structures analogous to the cortex (Smith 1991). For example, it has been suggested that specific brain areas in the octopus are specialized for sensory analysis, memory, learning, and decision making and thus may be considered analogous to the human cerebral cortex (Wells 1978).

A second argument for rejecting a pain experience in invertebrates is that their brains might be too small. However, the octopus brain is larger than that of most fish and reptiles when regarded as a ratio of body weight (Smith 1991) and even the brains of many decapod crustaceans (e.g., crabs, lobsters, shrimp) are likely to be considerably larger than those of many vertebrates when regarded in an absolute comparison (Elwood et al. 2009). Broom (2007) notes that brain size does not necessarily equate to complexity of function (Broom and Zanella 2004); indeed, the brains of some invertebrates have a surprising complexity (Sandeman et al. 1992; Wells 1978), with clear functional separation of distinct areas, and thus might be sufficiently complex in function to enable pain experience (Broom 2007).

Responsiveness to Opioids, Analgesics, and Local Anesthetics

Mammals have a system for regulating pain such that the same tissue damage may result in very different responses depending on the situation. For example, humans engaged in sports often report little pain in response to tissue damage. The physiological basis of this regulation is complex but in part is dependent on endogenous opioids, release of which reduces the pain experience. Injection of the opiate morphine also reduces the pain experience and the opiate antagonist naloxone reverses this effect. For these reasons the presence of opioid receptors and responses to analgesics has been regarded as an indicator that animals experience pain (Bateson 1991; Roughan and Flecknall 2001; Sneddon et al. 2003).

A particularly persuasive approach is to offer the animal a choice between water (or food) that does or does not contain an analgesic and to observe whether a preference develops for the analgesic when noxious stimuli are applied (Colpaert et al. 1980). Danbury and colleagues (2000) found that lame chickens consumed more feed containing an analgesic than did those that were not lame. As far as I am aware this approach has not been tried with invertebrates, but various studies have applied analgesics and local anesthetics and examined their effects on responses to noxious stimuli. For example, in the crab (*Chasmagnathus granulatus*) electric shock elicited a defensive threat display and the percentage of animals that showed this response rose with the voltage applied. Injection of morphine hydrochloride reduced the crabs' sensitivity to the shock in a dose-dependent manner and naloxone injection inhibited the effects of morphine (Lozada et al. 1988). Morphine also had inhibitory effects on the escape tail-flick response to electric shock in mantis shrimps (*Squilla mantis*) that was reversed by naloxone (Maldonado and Miralto 1982), and researchers observed a similar effect of opioids and naloxone in nematodes (Pryor et al. 2007) and snails (Kavaliers et al. 1983).

This approach, however, is problematic because analgesics might produce a general reduction in responsiveness to all stimuli. One way around this is to create a situation in which analgesia might increase particular responses. In one such study, fruit flies placed in a tube at the darker side of a light gradient moved toward the light. If the center of the tube was heated, however, the flies were inhibited from passing this section. The application of specific analgesics (agonists for $GABA_B$ that are effective analgesics in hot plate tests in rats; Thomas et al. 1996) reduced this inhibition and the flies passed through the heat to the lighter area (Manev and Dimitrijevic 2005).

A recent study on the glass prawn (*Palaemon elegans*) noted that the animal engaged in prolonged grooming of the antennae and rubbed them against the side of the tank when the antennae were treated with acetic acid or sodium hydroxide, but prior treatment with a local anesthetic (benzocaine) reduced the rubbing and grooming (Barr et al. 2008). There was no effect of benzocaine on the general locomotion of the prawn so the reduction in the two behaviors was not simply due to inactivity. However, the result with acid was not replicated in other decapod species (Puri and Faulkes 2010).

Both opioid analgesics and local anesthetics have effects that appear similar to those observed in vertebrates. But local anesthetics block sodium channels (Machin 2005) and, in crayfish (*Procambarus clarkia*; Leech and Rechnitz 1993), prevent the conduction of impulses from nociceptors, so it is the nociception that is reduced or eliminated. Furthermore, opioids may produce analgesia by acting on a modulatory system in the CNS (Tomsic and Maldonado 1990), but they might also have a peripheral effect (Del Seppia et al. 2007; Pryor et al. 2007). In all of these cases the nociception is or may be disrupted, so conclusions about the potential for the animal to experience pain are limited.

Physiological Changes

Noxious stimuli applied to vertebrates typically result in tachycardia, pupil dilation, and defecation. Changes in blood flow, respiratory patterns, arteriole blood gases, electrolyte imbalance, and endocrine changes are also common (Short 1998; Sneddon et al. 2003). The latter often involve corticosteroid release, which is used as a measure of stress (Stafford and Mellor 2005).

There has been limited examination of similar responses in invertebrates. Cephalopods are said to have an adrenal system that releases adrenal hormones when the animal is exposed to noxious, potentially painful stimuli, and noradrenaline and dopamine are released when the animal is disturbed (Stefano et al. 2002). Crustaceans have a stress hormone, the crustacean hypoglycemic hormone (CHH) (Chang 2005; Lorenzon et al. 2004), that functions to convert glycogen to glucose in a manner analogous to that of

cortisol in vertebrates. Glucose rose substantially in edible crabs when a claw was removed in a manner that caused a wound but not when the crab was induced to autotomize the claw (Patterson et al. 2007). However, this might be due to the tissue damage per se rather than any negative emotional state.[1]

There is a clear need for more studies on physiological aspects of invertebrate responses to noxious stimuli. Of particular use would be an examination of physiological changes during avoidance learning and during presentation of just the conditioned stimuli (without the noxious event) to determine whether features akin to anxiety are present.

Avoidance Learning

I have noted that nociception allows for an immediate escape by use of a reflex response whereas pain enables motivational change and avoidance learning. The key function of pain is thus to reduce damage over a relatively long term. One would therefore expect to see evidence of rapid avoidance learning coupled with a long memory in an animal that experiences pain.

Such evidence has been reported in *Drosophila* that learned to associate an odor that preceded or overlapped with an electric shock: after eight (Yarali et al. 2008) or twelve trials (Tully and Quinn 1985) they avoided the odor for up to 24 hours. Researchers have used this paradigm to examine genetic influence on learning and memory and the morphology involved in terms of brain region (de Belle and Heisenberg 1994) and to create mutants for dissecting biochemical pathways involved in learning and memory (Sokolowski 2001). Curiously, *Drosophila* also learn to associate the odor if it occurs after the shock has ended and in this case they show a mild preference for the odor, a feature termed pain relief learning (Yarali et al. 2008).

Similarly, the crab *C. granulatus* associated a shock with a particular location (Denti et al. 1988) after just a single trial and retained the association for 3 (but not 24) hours. Subsequent experiments involving multitrial training, however, showed retention after a 24-hour rest interval in a different environment from that used in training (Fernandez-Duque et al. 1992).

Experiments with crayfish (*P. clarkia*) demonstrated an association between a light and a shock given 10 seconds later: the animals learned to respond by walking to a safe area in which the shock was not delivered (Kawai et al. 2004). But the animal did this only if it was facing the area to which it could walk to avoid the shock; if it was facing away from the safe area it exhibited a tail-flick escape response, by which it moved away tail first. Despite repeated pairings of light and shock, the animal did not learn to avoid the shock by tail flicking in response to light. However, when the animals that had experienced shocks while facing away from the safe area were subsequently tested facing toward the safe area they showed a very rapid avoidance of the shock at the onset of the light. Thus they seemed to have learned the association although they had not previously used it to avoid the shock. This finding was explained by the specific associations between cues and particular responses that are also common in vertebrates.

Hermit crabs (*Pagurus bernhardus*) in their shell that were shocked on the abdomen demonstrated a long-term behavioral change compared with crabs that were not shocked. The shocked crabs were more likely to approach and enter a newly offered empty shell (Elwood and Appel 2009) and, compared to those not shocked, they moved into the new shell more quickly, spent less time investigating it, and inserted their chelipeds into its aperture less often before moving in.[2] This is consistent with the idea that shocked crabs assessed their original shells as being of very poor quality. Shocked crabs altered their behavior for up to a day after the initial shock (the maximum time tested), indicating a long-term shift in motivation about obtaining a new shell after the aversive experience (Appel and Elwood 2009a).

Taken together, these studies on learning and motivational change show abilities in arthropods that seem to fit this key criterion for pain experience.

Protective Motor Reactions

Protective motor reactions include reflex withdrawal from a noxious stimulus, but this is a basic feature of nociception and gives little indication of emotional state. Weary and colleagues (2006) argued that prolonged rubbing denoted an awareness of the site of the noxious stimulus and Sneddon and colleagues (2003) noted that rainbow trout (*Oncorhynchus mykiss*) that had noxious chemicals injected into the lip showed rubbing of the lip on the substrate, consistent with the idea of pain.

Hermit crabs induced to evacuate their shells by electric shock to the abdomen demonstrated sustained grooming by use of claws on the abdomen (Appel and Elwood 2009a,b; Elwood and Appel 2009), a response not seen when the crabs are cracked out of their shell or evicted in a shell fight. Further, when either sodium hydroxide or acetic acid solution was applied to one antenna of a glass prawn there was a significant increase in grooming of that antenna during which it was pulled repeatedly through the animal's small pincers and mouth parts (Barr et al. 2008), and there was an increase in rubbing of that antenna against the side of the tank. The animal seemed to be aware of the specific location of the noxious stimulus and directed its attention to the treated antenna. (However, work on three species of prawn found no significant increase in directed grooming of treated antennae; Puri and Faulkes 2010.) In addition, when acetic acid was applied to an eye of a glass prawn there was a marked increase in grooming that involved both pincers moving

[1]Crabs lacking a claw showed a higher level of glucose when an intact crab was housed in the same tank, an effect that might be due to fear or increased alertness (Patterson et al. 2007).

[2]Such a minimum of investigation of the new shell is otherwise characteristic of crabs in shells of inadequate size (Elwood and Stewart 1985).

simultaneously and in very different and complex ways. The grooming was directed specifically to that eye, again demonstrating that the animal was aware of the location of the noxious event (Barr et al., unpublished data). The complexity of these prolonged responses is beyond that expected from a nociceptive reflex response and consistent with the idea of pain.

Another protective motor response in arthropods is autotomy, in which an appendage is cast off from the body. In the spider *Argiope aurantia*, legs may be autotomized when damaged (Eisner and Camazine 1983). This was seen during attempts by these spiders to capture ambush bugs (*Phymata fasciata*), typically when the bug grasped a spider leg and probed a joint with its proboscis (the venomous saliva is painful to humans)—autotomy occurred within 5 seconds (Eisner and Camazine 1983). Simple experimental penetration of the joint with a sterile pin did not cause autotomy, indicating that the saliva had an effect. Eisner and Camazine (1983) also injected bee and wasp venom, both of which induced autotomy. They found that when individual components of the venom were injected, some, but not all, produced autotomy; effective components were histamine, serotonin, phospholipase, and melittin, all of which induce pain in humans; ineffective components were acetylcholine, bradykinin, hyaluridase, adrenaline, and dopamine. Acetylcholine and bradykinin induce pain in humans but not autotomy in spiders, and hyaluridase, adrenaline, and dopamine do not induce pain in humans, suggesting a concordance between pain effects in humans and autotomy in the spider.

Autotomy in crustaceans typically leaves a clean break at a specific joint close to the main body, which immediately seals to prevent loss of hemolymph. Cutting a membrane at a joint distal to the autotomy plane, causing hemolymph loss, elicits rapid autotomy (within a few seconds) of that appendage, preventing further loss of fluid (Patterson et al. 2007). Crabs also autotomize limbs in situations that do not involve hemolymph loss, for example if the whole animal is placed on a hot plate (Fiorito 1986) or if the leg is subject to electric shock or injected with acetic acid (Barr and Elwood, unpublished observations). The acetic acid treatment rapidly induces autotomy in a dose-dependent manner and the results are consistent with pain mediation of the autotomy response.

Tradeoffs between Stimulus Avoidance and Other Activities

Bateson (1991) suggests that one criterion for pain should be a relatively inelastic response (*sensu* Dawkins 1990), but a response that is purely mediated by nociception is an inelastic reflex—it should be the same regardless of other motivational priorities. Thus an animal that is hungry or satiated would likely exhibit the same reflex avoidance to a noxious stimulus, even if food is present. By contrast, pain is a negative emotional state, typically coupled with a very high motivation to escape that state, and thus should be given a high priority and might appear to be inelastic. Thus it seems difficult to discriminate pain from nociception by this criterion.

However, if variation in the response to noxious stimuli is dependent on other motivational requirements then there must be some higher-level interaction between competing motivational systems (McFarland and Sibly 1975). In fish, for example, those deprived of food are less likely to respond to an electric shock in a feeding area than those that are not food deprived (Millsopp and Laming 2008). And lame hens stop limping in the period leading up to egg laying but limp again after laying (Gentle 2001). Competition between different activities for expression or requirements is the essence of motivational tradeoffs. Such competition is important for pain research as it is a strong indicator that the response to the noxious stimulus is not purely reflexive: tradeoffs clearly involve some form of processing in which different needs are weighed.

Tradeoffs were the subject of two experiments in which hermit crabs were given shells with two small holes drilled and electrodes inserted so the crab could be shocked on the abdomen. When shocks of a single intensity were applied, at a level that was hoped would not cause evacuation, some crabs evacuated and were more likely to do so from a less preferred shell species (Elwood and Appel 2009). Similarly, when the shocks increased in intensity, crabs evacuated the shell at a lower shock intensity if they were in a less preferred shell species (Appel and Elwood 2009b). Thus, the animals' response to the shock was determined in part by their normal preference for particular species of shell. Further, they were much less likely to evacuate after being shocked when the odor of a predator was present, suggesting a tradeoff between shock avoidance and predator avoidance (Wilson and Elwood, unpublished observations). These responses cannot be a reflex response as they required information from sources other than the noxious stimulus to have an effect on the response.

This approach of determining what is "traded off" against avoidance of the noxious stimulus may give insights into an animal's priorities (Dawkins 1990)—that is, what it might "pay" to avoid the noxious stimulus in terms of lost opportunities to satisfy other motivational demands. For example, among the shocked hermit crabs that evacuated their shell some stayed near the shell and many got back into it; others, however, walked away and even attempted to climb the walls of the observation chamber (Appel and Elwood 2009a,b). This is remarkable because the shell is a vital resource and abandoning it indicates the aversive nature of the shock.

Octopuses provide another example of tradeoff in their avoidance of stinging sea anemones. Octopuses readily prey on hermit crabs but experiments have shown that when the crabs placed an anemone on their shell as protection the octopuses dramatically changed their tactics. They tried a variety of approaches such as moving below the anemone, blowing jets of water at it, and using a single outstretched arm. Thus they seemed to try to avoid the stings while attempting to maintain food intake, albeit with tactics that are less efficient for food capture (MacLean 1983; Mather 2008).

High Cognitive Ability, Consciousness, and Sentience

Several authors have considered a high cognitive ability coupled with consciousness or sentience a prerequisite for a pain experience or at least have suggested that such abilities make pain experience more likely. Bateson (1991, 832), for example, suggests that "if the animal can be shown to be conscious of what it is doing, then most people would conclude that it could experience pain." Particular cognitive abilities are also considered important in assessing the welfare status of animals (e.g., Braithwaite 2010; Chandroo et al. 2004; Duncan 1996; Duncan and Petherick 1991). At the very least sentience probably involves awareness of internal and external stimuli (Chandroo et al. 2004; Duncan 1996), and "primary consciousness" involves the ability to generate a mental scene in which diverse information is integrated for the purpose of integrating behavior (Chandroo et al. 2004; Edelman and Tononi 2000). I consider here several examples of invertebrate abilities in integrating information from different sources to make "informed" decisions.

Spiders exhibit a variety of complex behaviors that appear to illustrate a capacity for information integration. Jumping spiders, for example, are known to adjust hunting methods depending on the type of prey and its ability to escape (Bartos 2008). The hunting spider (*Portia labiata*), when it hunts spitting spiders (*Scytodes pallidus*), which are themselves predators of spiders and thus dangerous, gathers information as to whether the spitting spider is carrying eggs in the mouth and thus less dangerous; if so, the hunting spider modifies its attack (Jackson et al. 2002). Furthermore, when hunting prey in complex environments, *Portia* appears to plan routes with detours that initially take it away from the prey item to avoid obstructions (Tarsitano 2006). Such behavior suggests an ability to comprehend the complex spatial relationships between itself and the prey and possible routes to a goal (Sherwin 2001).

Male giant cuttlefish (*Sepia apama*) exhibit a remarkable ability to change shape and color to switch between the appearance of a female and that of a male in order to foil the mate-guarding attempts of larger males. Norman and colleagues (1999) showed that small males that assumed the body shape and patterns of a female were not attacked by the larger mate-guarding male. When the larger male was distracted by another large male intruder, the small males changed body pattern and behavior to those of a male in mating display and successfully mated.

Squid use surprisingly complex color patterns for courtship and protection (Hanlon et al. 1994). A male can display a courtship coloration on one side of the body toward a female while at the same time displaying a completely different pattern on the other side to ward off an intruding male (Mather 2004, 2008). And he can switch the sides of the body showing the two displays as soon as the relative positions of the other two animals change.

Octopuses also show complex learning abilities (Edelman et al. 2005). When confronted with a maze in which the experimenter frequently changed the nature of the obstacles octopuses readily solved the maze, apparently considering the maze before proceeding (Moriyama and Gunji 1997), suggesting a level of ability that might signal consciousness.

A final example concerns hermit crabs, which when deciding to change shells systematically evaluate various components of potential new shells to determine whether they offer a gain in terms of size, shape, and weight before moving in (Elwood and Stewart 1985). The evaluation continues even after moving in and a crab may switch between the two before making a final decision (Elwood 1995). Hermit crabs that fight to take the shell from another hermit crab not only evaluate the opponent's shell (Dowds and Elwood 1983) but also take in information about the opponent (e.g., its size and power; Briffa and Elwood 2002; Dowds and Elwood 1985). In addition, they monitor their own physiological state during the encounter in order to make effective fight decisions (Briffa and Elwood 2000, 2002, 2005)—for example, when lactate increases the attack rate slows and the attacker then stops fighting (Briffa and Elwood 2002, 2005). Hermit crabs can remember particular opponents for up to 4 days after an encounter (Gherardi and Artema 2005).

It is clear from the above examples and others (Broom 2007; Mather 2008) that some invertebrates are capable of integrating information from various sources, both internal and external, to enable complex decisions. Also apparent is a fine discrimination learning ability (Mather 2008), indicating a high cognitive ability. For example, honeybees can learn a complex learning task in which they have to select from previously unseen shapes on the basis of whether they are symmetrical or not (Benard et al. 2006; Giurfa et al. 1996) and cephalopods appear particularly adept at a range of learning tasks (Mather 2008).

Good discrimination learning may not necessarily indicate an ability to experience pain, but one might expect to see such discrimination when pain is experienced—simple nociceptive reflex avoidance results in an immediate withdrawal but does not imply any long-term motivational change. To benefit from pain experience the animal needs to be able to discriminate between the specific situation that led to the pain and other situations that did not. Animals that cannot make fine discriminations may avoid potentially harmless or even useful situations or objects. Thus it seems reasonable to speculate that the evolution of pain experience developed hand in hand with enhanced discrimination learning.

Conclusion

It is clear that the various criteria I have described differ in their usefulness in discriminating pain from nociception. Because of the wide taxonomic occurrence of functional nociceptors, the demonstration of their presence does not indicate the capacity to experience pain, and investigation of the central nervous system provides limited clues of what is or is not suitable for pain experience. The use of opioids and other

analgesics might indicate a central modulation of responses, but potential peripheral effects may explain the analgesia. Physiological changes might prove useful but, to date, the study of their appearance in invertebrates is limited and reveals little about their pain experience.

It is thus behavior that provides the greatest insights into the likely experience of pain. Rapid avoidance learning, coupled with a prolonged memory, indicates central processing and is consistent with pain, but it is more convincing after one stimulus than after numerous repetitions. Complex, prolonged grooming or rubbing might indicate an awareness of the specific site of stimulus application and seems to be more than a reflex reaction. Tradeoffs with other motivational systems indicate central processing and may be useful to determine what an animal will "pay" to avoid the noxious stimulus. An ability to use information from various sources might indicate sufficient cognitive ability for the animal to have a fitness benefit from a pain experience.

Evidence from behavioral studies is entirely consistent with the idea that some invertebrates, particularly crustaceans and molluscs, experience pain. However, more studies must use a variety of imaginative techniques to confirm that invertebrates do indeed experience pain. Substantial research on various taxa is necessary to assess which, if any, show (1) rapid avoidance learning of noxious stimuli, (2) prolonged responses directed to the specific site on their body where the noxious stimulus was applied, and/or (3) tradeoffs between avoidance and other activities that would indicate central decision making rather than reflex reaction. Studies that demonstrate marked physiological stress responses to conditioned stimuli that herald the imminent application of a noxious stimulus would also be helpful.

Clearly, a start has been made on some of these approaches but much more is needed. Recently, Braithwaite (2010) was confident enough to state that fish feel pain but invertebrates do not. I do not share the confidence to make that discrimination. Neither do I feel confident in stating unequivocally that some of them do feel pain, although it is clear that the responses described above cannot be explained just by nociceptive reflexes. While awaiting the results of further relevant studies, perhaps all who use invertebrates should consider the possibility that at least some might suffer pain and, as a precaution, ensure humane care for these animals.

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