Chapter 7
Assessing the Potential for Pain in Crustaceans and Other 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 behaviour modification based on memory of the unpleasant nature of pain. In this review, I consider various criteria that might help to distinguish nociception from pain. Because nociceptors are so taxonomically widespread, simply demonstrating their presence is not sufficient. Further, 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. Behaviour of the organism provides the greatest insights. Rapid avoidance learning and prolonged memory indicate central processing rather than mere reflexes and are consistent with the experience of pain. Complex, prolonged grooming or rubbing may demonstrate an awareness of the specific site of stimulus application. Trade-offs 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. Recent evidence of fitness enhancing, anxiety-like states is also consistent with the idea of pain. Thus, available data go beyond the idea of just nociception, but the impossibility of total proof of pain means they are not definitive. Nevertheless, more humane care for invertebrates is suggested.

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7.1 Pain

7.1.1 Introduction to Nociception and Reflexes

The soft tissues of animals are fragile and easily damaged, which could result in disease or death. If not so extreme, the tissue will usually be replaced, and resources are diverted from maintenance, growth and reproduction. Thus, minimising damage is important for fitness (Bateson 1991; Elwood 2011). This can be achieved by several means. One common method is by production of hard coverings that protect soft tissues, but the animal still needs to interact with the environment to obtain food and reproduce and exposes soft tissue to achieve those aims. Further, the hard tissues are costly to develop and may impede movement or at least increase the cost of movement. Another, more common, method is to develop sensory systems that detect tissue damage so that the animal can escape from whatever is causing the damage. This can be the whole animal moving away or just the part that is being damaged can be moved.

The specific receptors that are sensitive to injury are called nociceptors, and they mediate protective reflexes (Sherrington 1906). These receptors are simple in that there are no specialised or complex sensory organs. Rather the nociceptors have bare endings that respond to chemical, mechanical or thermal stimuli or sometimes a combination of these. They are found in virtually all phyla of multicellular animals, including nematodes, annelids, molluscs, chordates and arthropods. This wide occurrence of nociceptors indicates that they evolved very early and certainly before the Cambrian explosion that gave rise to major existing taxonomic groups. Conserved cellular processes thus control the responses of a wide range of animals to noxious stimuli (Burrell 2017).

Nociception is the process of encoding and processing noxious stimuli (Loeser and Treede 2008) or the detection and reaction to stimuli that may compromise their integrity (Besson and Chaouch 1987). Thus, nociception is the perceptual mechanism coupled with the organisation of reflex responses that typically take the animal away from the stimulus or, at least, are effective in terminating the perception. Nociception, however, does not eliminate tissue damage. Nociceptors only respond to the onset of damage and do not enable detection of distant stimuli that have the potential for tissue damage should contact occur. Further, there is no suggestion that nociception necessarily involves higher-level neural processing and the animal may be unaware of the nociceptive responses. Nevertheless, nociception confers major benefits in that tissue damage is clearly reduced. Indeed, even those animals with hard, protective coverings still possess nociceptors in those body regions that are exposed when gathering resources, moving or mating.

7.1.2 Concept and Function of Pain

In humans, and possibly other taxa, there is a second system that we call pain. This is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP 1979, p. 250). Pain typically follows nociception, but with pain, there are inputs to the central nervous system, resulting in awareness of the tissue damage and the stimuli that caused it. In humans, for example, unexpected damage to the hand may result in a nociceptive reflex that moves the hand away from the cause of the damage and a negative sensation follows shortly after. Because the experience is unpleasant, there is a high motivation to terminate the stimuli that caused the pain by escape (Bateson 1991). These attempts to escape might go beyond the reflexive response seen with nociception and might be more successful than is possible with a mere reflex. Further, because the event has a high salience, it is likely to be remembered, and the situation that gave rise to it is avoided in the future. Stimuli associated with pain may be perceived from a distance by various sensory systems so that there is no requirement for close contact to trigger a further nociceptive response. Thus, there is no repeat of tissue damage, which might occur should only nociception be available. Pain might also result in anxiety so that the animal becomes wary of various stimuli, possibly even stimuli that have not been encountered previously, and avoids further damage. Thus, pain offers protection from future tissue damage beyond that available with just nociception (Bateson 1991). Pain might also inhibit specific activities or, indeed, inhibit all activities that might otherwise delay recovering, and might result in guarding behaviour to protect the wound (Wall 1979; Bateson 1991).

7.1.3 Pain in Animals

The generally accepted definition of human pain noted above is not applicable to animals because animals cannot tell us what they experience. Instead, Broom (2001) defines pain as "an aversive sensation and feeling associated with actual or potential tissue damage". It uses the term "aversive" rather than "unpleasant" (IASP 1979, p. 250) because the former is more easily identified by the actions of the animal (Broom 2001). There is no doubt that this definition grasps the essence of the subject and provides a focus on what the implications are for the welfare of the animal (Broom 2014). However, it does not enable easy identification of pain as opposed to a non-pain response in animals for two key reasons. First, we cannot access animal feelings, and thus focusing on feelings might inhibit the identification of pain (Stamp Dawkins 2012). Second, if an animal moves away from a stimulus, we may describe that stimulus as being aversive, yet the movement might be due to a nociceptive reflex rather than pain. An alternative definition that provides some guidance on how to identify 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 behaviours, including social behaviour" (Zimmerman 1986). Sneddon (2009) adds to this definition in suggesting that an animal in pain should "quickly learn to avoid the noxious stimulus and demonstrate sustained changes in behaviour that have a protective function to reduce further injury and pain, prevent the injury from reoccurring, and promote healing and recovery". However, as the definitions include more potential indicators of pain, they assume a rather unwieldly, list-like quality. Nevertheless, the list approach turns the focus on how to detect when pain is likely to be occurring in an individual and to detect which species are likely to experience pain (Sneddon et al. 2014).

The list approach provides a set of criteria that would be expected to be fulfilled should an animal experience an aversive sensation and unpleasant feeling. But we need to be clear that although fulfilling criteria of pain is consistent with the idea of pain, it is not the same as proof of pain (Elwood and Adams 2015; Magee and Elwood 2016a). There may be alternative explanations for the behaviour following noxious stimulation (Rose et al. 2014; Key 2016), and alternative explanations are often more firmly promoted for invertebrates than for vertebrates (Sherwin 2001). Nevertheless, if many of the expected criteria are fulfilled for a species, we can say that it is possible or even highly probable that pain occurs in that species. Further, because of the close similarity between related species, if pain is considered highly probable in one species, we may view it as highly probable in closely related taxa. Alternatively, if the criteria are not fulfilled, or very few are fulfilled, then the possibility of a pain experience must be considered low. This is a somewhat unsatisfactory outcome of scientific investigations, which normally aim at more definite conclusions, but it is in keeping with other investigations of private mental attributes such as consciousness (Stamp Dawkins 2012).

Finally, attempts to define animal pain face a major hurdle because we relate to those definitions via our own individual experience of pain. We are so familiar with our own experience that despite attempts to avoid introspection, we naturally think of pain as something like our own feelings. To avoid thinking of animal pain in that way, phrases are used about the subjective experience as not necessarily being the same as human experiences (Molony 1992) or that an animal's pain might be totally different from a human's, reflecting its different way of life and differences in body function (Bateson 1991). In many ways, this is unsatisfactory because if the pain felt by an animal species is not necessarily the same, or indeed very different to humans, then what is it? Further, if pain in one species is very different from that in humans, it is unlikely that it would be similar to species from different broad taxa. Thus, as noted above, we have no way of determining what a particular species "feels" when subject to noxious stimuli. We can, however, judge if the behavioural and physiological responses to injury serve the same protective function across phyla (Rutherford 2002). We refer to those responses in humans as those indicating pain, and it seems reasonable to use the same term for animals, be they vertebrates or invertebrates (Sherwin 2001).

7.1.4 Lists of Criteria for Pain

Bateson (1991) proposed eight criteria for animals to be considered as having a subjective experience of pain. The rationale for these eight derived to some extent from the difficulty of judging pain in humans, and the criteria tend to be oriented primarily, although not exclusively, towards vertebrates. The first three refer to morphology and propose that for pain, an animal should possess nociceptors, brain structures analogous to the human cerebral cortex and nervous pathways that connect the two. Additionally, there should be receptors for opioid substances, and analgesics should modify the response to noxious stimuli. Also included is a suggestion that animals should select analgesics to self-administer when they are exposed to noxious stimuli. The last three criteria are entirely behavioural and suggest that the animal should avoid noxious stimuli and minimise damage to the body, that the avoidance should be relatively inelastic, that the responses should be persistent and that the animal should learn to associate neutral events with noxious stimuli (Bateson 1991). These criteria have been modified, particularly when attempting to consider if invertebrates might experience pain (Sherwin 2001; Broom 2007; Elwood 2011). The review by Elwood (2011) did not consider connections between nociceptors and the brain. It added responsiveness to anaesthetics and physiological changes in response to noxious stimulation. It also suggested that trade-offs should occur between stimulus avoidance and other motivational requirements. This was rather different to Bateson's (1991) view of a relatively inelastic response, with Elwood taking the view that pain could be thought of as a motivation and that all motivational requirements tend to trade-off. There was the inclusion of rubbing and attending to the site of a wound. Finally, high cognitive ability and sentience were considered. The most recent and extensive list that might be applied to all taxa is that of Sneddon et al. (2014), which suggests 15 criteria (Table 7.1).

Broom (2001) notes that identification of pain by fulfilling criteria might face problems with some species. First, overt responses to noxious stimuli might serve a function of warning close kin of a danger. If that occurs, then it makes it easy for us to assess that the noxious stimulus has at least been perceived by that animal. If the species is not social, then the selection promoting overt responses may be reduced. Indeed, there are situations where showing overt behavioural responses to tissue damage might be disadvantageous. It might pay the animal to avoid providing information about injury because doing so might make the animal more vulnerable to predation (Broom 2001) or possible defeat in aggressivee interactions. Hiding pain might thus be important in some situations for some animals, and that makes it more difficult for us to assess pain in those species, as animals hiding pain would be judged incorrectly as not feeling pain.

Another problem arises from difference between individuals in personality types. For example, in horses, certain personality types, e.g. extroverted, show increased overt responses to injury, and thus pain is relatively easy to identify (Ijichi et al. 2014). However, pain may be missed in less extroverted individuals. While this

Table 7.1 The 15 key criteria for pain in animals as suggested by Sneddon et al. (2014)

1. Evidence of central processing of nociception involving brain areas that regulate motivated behaviour (including learning and fear)
2. Nociceptive processing sensitive to endogenous modulators (e.g. opioids in vertebrates)
3. Nociception activates physiological responses (one or a combination of the following: change in respiration, heart rate or hormonal levels (e.g. cortisol in some vertebrates)
4. Evidence that responses are not just a nociceptive reflex (i.e. not simply moving away)
5. Alterations in behaviour over longer term that reduce encounters with the stimulus
6. Protective behaviour such as wound guarding, limping, rubbing or licking
7. All of the above reduced by analgesia or local anaesthetics
8. Self-administration of analgesia
9. Pay a cost to access analgesia
10. Selective attention whereby the response to the noxious stimulus has high priority over other stimuli; the animal does not respond appropriately to concurrent events (e.g. presentation of predator; reduced performance in learning and memory tasks)
11. Altered behaviour after noxious stimulation where changes can be observed in conditioned place avoidance and avoidance learning paradigms
12. Relief learning
13. Long-lasting change in a suite of responses especially those relating to avoidance of repeat noxious stimulation
14. Avoidance of the noxious stimulus modified by other motivational requirements as in trade-offs
15. Evidence of paying a cost to avoid the noxious stimulus

phenomenon has not been noted in invertebrates, given that they too show personality types (Briffa et al. 2015), there is the scope for identifiers of pain to be missed in some individuals. This variation in responses makes identification in some species more difficult.

It is clear from the above examples that testing criteria is not without problems. Nevertheless, that approach offers the best chance of identifying taxonomic groups that might experience pain. The aim of the following sections is to give an overview of investigations that test criteria in invertebrates. To improve clarity, some of the following sections comprise more than one criterion.

7.2 Experimental Evidence

7.2.1 Avoidance Learning

Invertebrates have received considerable attention with respect to their learning abilities, and there are many studies on avoidance learning (Sherwin 2001 for a review). Recent examples have specifically tested the criteria of pain experience that avoidance learning should be rapid. Speed of learning is important as swift learning brings about a marked reduction in the number of direct encounters with the noxious

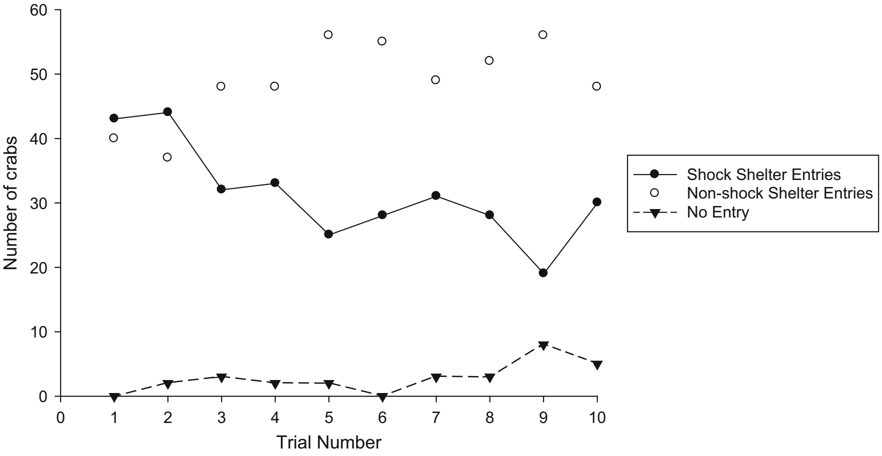


Fig. 7.1 The number of crabs that entered the shock and non-shock shelters in each of the ten trials, as well as the number of crabs that did not enter either shelter (from Magee and Elwood 2013)

stimulus and hence reduction in tissue damage. Magee and Elwood (2013), for example, repeatedly placed shore crabs, *Carcinus maenas*, in a brightly lit rectangular enclosure, at each end of which was a dark shelter, and the crabs typically used one to escape from the light (Barr and Elwood 2011). On the first such choice, all crabs swiftly entered one of the shelters, indicating a high motivation of shore crabs to avoid bright light. Some crabs had previously been selected to receive small electric shocks at 5 s intervals within their first chosen shelter, whereas the remaining crabs only received shocks in subsequent trials if they went to the alternative shelter. After each 2 min trial, the crabs were briefly removed from the enclosure and then returned to the centre so that they could again make a choice of the two shelters. Ten such trials were completed. On the first trial, the crabs had no information about potential shock. The first opportunity for the crabs to use information in their choice was in trial 2, but being shocked had no effect on the choice in trial 2, and most crabs simply went to the same shelter they used in trial 1. However, on trial 3, significantly more crabs that were shocked in the previous trial switched their choice of shelter compared to those not shocked. That is just two trials in which information might be gained resulted in significant avoidance of the shock shelter, and over the ten trials, there was a decrease in selecting the shock shelter (Fig. 7.1). The crabs were free to exit their chosen shelter, and about 55% of those being shocked did so in the first of the ten trials ($n = 41$). By contrast, no crab moved out of a safe shelter in any of the ten trials. In later trials, crabs entering the shock shelter became increasingly likely to move out of that shelter during the trial. Thus, crabs appeared to use a second method of avoiding shock.

Magee and Elwood (2013) placed striped cards at either end of the rectangular enclosure; one card had vertical stripes, while the other had horizontal, and the same stripe positions were used for the ten training trials. Further, the crab was always placed at the start of a trial facing in the same direction, perpendicular to the axis between the two shelters. Thus, throughout the ten training trials, they would always have to walk in one direction and to one stripe orientation (either left or right and either horizontal or vertical) to get to the safe shelter. The 11th trial was designed to test what the crab had learned. In that test, 50% of the crabs were tested with the same stripes used in training, but the others had the cards switched. Further, 50% the crabs started in the same position, but the other half had the start position turned 180°. There was no effect of visual stimuli on the choice made in the 11th trial indicating that visual information was not associated with the safety (or shock). By contrast, crabs turned 180° were more likely to go to the shock shelter than were those not turned. Thus, crabs used response learning and not place learning.

Although the crabs did not use the visual cues that might have been due to the cards being insufficiently different, both had the same number of stripes of identical width. Other studies, however, have shown associations between visual cues and avoidance. For example, spiders discriminate between black and white cues to avoid shock in a place learning paradigm (Peckmezian and Taylor 2015), and honey bees associate colours with shock (Agarwal et al. 2011). Further, non-visual cues can be used as with crayfish that learn to avoid shock associated with either a hard or soft substrate in an otherwise similar paradigm (Bhimani and Huber 2016). Thus, allocentric cues can be utilised by invertebrates as well as the egocentric cues noted by Magee and Elwood (2013). Nevertheless, the speed of learning in that experiment is impressive and consistent with the idea of pain. Further, spiders showed avoidance of the shock side of a petri dish within the first 5 min trial (Peckmezian and Taylor 2015), and crayfish avoided the shock areas within the first 10 min of training, during which time they received about nine shocks (Bhimani and Huber 2016).

In these place avoidance experiments, the choice was simultaneously presented on each trial. An alternative approach is to train animals with just one place at each trial, but that is a more cognitively challenging task (Dyer and Neumeyer 2005). This was attempted with shore crabs (Magee and Elwood 2016b) using the same enclosure as in Magee and Elwood (2013) but with an opaque partition separating the two dark shelters. For each 2 min trial, the crab was placed on one side of the partition and allowed to sample one shelter. As before, the crab was in the same orientation for each of ten training trials, and the horizontal and vertical stripes were placed above each dark shelter. All crabs were shocked on the first trial and in the second trial were placed on the other side of the partition, and the shelter on that side was always safe. The sides in which the crabs were placed alternated between tests. On an 11th trial, each crab was tested without the partition, thus having the first simultaneous choice of shelters but without shock in either shelter. On a 12th trial, the crabs were again tested without the partition, but half the crabs had their orientation changed by 180°, and half had the striped cards switched. With this paradigm, however, the crabs showed no preference for the safe shelter on the 11th trial. In the 12th trial, most crabs went to the same shelter chosen on the 11th trial. However, they were more likely to go in the same direction, i.e. to the crab's left or right, irrespective of their orientation but did not show a preference for stripe orientation. Nevertheless, the crabs showed other methods of reducing the number of shocks. Over the five training trials involving shock, there was an increase in the number of crabs exiting that shelter after receiving shock (as happened in Magee and Elwood 2013). Further, they exited the shelter after fewer shocks in later trials. This is consistent with the idea of avoidance learning and pain, but increased sensitivity might play a role (sensu Crook et al. 2011, 2013; Alupay et al. 2014). However, the main conclusion from Magee and Elwood (2016b) is that no associations were formed between the location of shock and either egocentric or allocentric cues. In this respect, there is no support for the idea of pain. This, however, seems to be because the paradigm requires a cognitive ability that is beyond the ability of these animals rather than their ability to experience pain. With more simple paradigms, discrimination avoidance learning is evident (Magee and Elwood 2013; Peckmezian and Taylor 2015; Bhimani and Huber 2016).

A different paradigm to investigate avoidance learning involves crabs raising one leg out of shallow water to avoid an electric shock to the leg when in the water (Hoyle 1976; Dunn and Barnes 1981; Punzo 1983). This results in a decrease in lowering of the leg compared with non-shocked controls or yolked animals that receive shock whenever a "master" crab is shocked. The technique has been employed in investigations of brain regions involved in this avoidance leaning (Punzo 1983). Remarkably, however, crabs that have the brain destroyed also show the capacity for learning, thus casting doubt on the idea that the learning is driven by a pain-like state (Dunn and Barnes 1981). However, there is reason to believe that the type of learning in these preparations is very different from the place avoidance learning noted above. Intact mud crabs, *Eurypanopeus depressus*, received a mean of 680 shocks during the first minute of training, which reduced to 140 per minute after 10 min of training (Punzo 1983). Decerebrate shore crabs, *C. maenas*, received over 1500 shocks in the first minute, which declined to 400 after 10 min of training (Dunn and Barnes 1981). That is the number of shocks required to train both intact and decerebrate crabs vastly exceed the numbers required for avoidance of locations (Magee and Elwood 2013). Even the intact crabs do not fulfil the criterion of swift avoidance learning, and decerebrate crabs presumably could not experience pain even if intact crab might. This appears to be a very different type of association, possibly involving ventral ganglia and less so with the brain. It tells us little about the possibility of pain in decapods.

There have been many studies on avoidance learning and the CNS mechanisms involved in short and long-term memory in molluscs, particularly *Aplysia* and *Octopus*. In one study, *Aplysia* were exposed to an odour (shrimp extract) either just before electric shocks to the head (paired) or 90 min after the shock (unpaired) (Walters et al. 1981). During training, the shock caused several reactions, including withdrawal of the head and ink release. They were then tested 1 or 2 days later with just the odour. The paired animals showed more head withdrawal than did the unpaired animals, but the two groups did not differ for the other measures when tested with just the odour. However, when the odour was paired with a small shock to the tail, other responses were much greater in paired animals. The siphon was withdrawn for longer in the paired than unpaired groups. The paired group showed inking at a lower intensity of shock than did the unpaired subjects, and there were more escape responses in the paired group and less feeding. That is, a range of responses was now associated with a combination of the odour and small shock, and defensive responses were enhanced and appetitive responses depressed. The authors concluded that the training stimuli had conditioned a fear response that included the anticipation of danger, similar to the fear responses noted in mammals.

One example for *Octopus vulgaris* is a study in which subjects were trained to attack a white ball and received food if they did so (Shomrat et al. 2008). Once trained, the subjects were shown a red ball, which was also attacked. However, some animals received a small electric shock when they attacked, and on subsequent trials, the number of animals attacking declined. Those not shocked continued to attack. By the fourth trial, after just three experiences of shock being associated with the red ball, about 50% stopped attacking.

As noted above, the key to understanding these experiments, with respect to the possibility of pain, is to see if learning is swift. For some experiments, the data comprise the time spent in areas in which shock and no shock occurs, but this does not give information on the number of choices. The data required are those showing the number of entries to the shock area (or area that will produce another noxious stimulus) prior to avoidance. That is, we can judge how quickly the animal changes its choice of action in a way that might protect it from tissue damage. An alternative is to determine if the animal is slower to move to an area in which a required resource is located if that is also associated with a noxious stimulus. Unfortunately, in many studies on avoidance conditioning, these data are not available.

However, experiments designed to investigate avoidance learning have used differing paradigms, and these may be a major source of variation in conclusions. For example, in Magee and Elwood (2013), the animal had to choose between two shelters that were identical except one was associated with shock. The safe shelter was available on each trial, and thus, not taking the shock shelter should cost little to the animal. However, the data show that animals typically return in trial 2 to their originally selected shelter so any change of use must overcome this preference. In an experiment with cuttlefish preying on their preferred food item (prawn or crab) tainted with quinine, there was a take it or leave it choice (Darmaillacq et al. 2004). Leaving it involved not feeding on the preferred prey type so any learning must involve an unlearning of food preference and giving up a valuable food resource. Thus, one might predict slower avoidance in the latter situation because there is a substantial cost in avoiding the noxious stimulus. Nevertheless, the squid showed markedly longer attack latencies in the second trial and reached learning criterion of not attacking in successive trials in eight trials. This avoidance was retained after 3 days, and the normal preference of individual cuttlefish was switched to the originally less preferred prey. In comparing experiments, we need to be aware of such cost incurred when avoiding the noxious stimulus.

Another factor that appears to determine speed of learning is the nature of the response that will enable the animal to avoid the noxious stimulus. This was shown with crayfish placed in a shuttle box when a light onset signalled that a shock would be delivered (Kawai et al. 2004). The animals could avoid the shock if they moved to the other side of the shuttle box within 10 s. Some animals were facing the direction that would lead to safety whereas others faced away. Two reactions were noted. At first, all animals showed a tail flick escape response to the shock. This resulted in those facing towards the safe area to dart backwards and further into the shock compartment. Those facing away from the safe compartment rapidly darted backwards into the safe area. Learning to avoid the shock by responding to the light was very different between the two groups. Those that faced towards the safe area slowly learned to use the signal and walk to safety. By contrast, those that were facing away when the light signal occurred and could have tail flicked to move to safety failed to do so. They only tail flicked when shocked and appeared to show no learning. However, when this group had their position reversed, they showed rapid learning and now walked to safety on the light signal (Kawai et al. 2004). The study shows that invertebrates, as well as mammals (Bolles 1970), are markedly influenced by the nature of the response.

The salience of cues might also influence the speed of avoidance learning. If the animals are being trained to avoid one half of an area and the cues are visual, then the cues for each half might be present all the time. Thus, when in the safe half the cues for the noxious half may be present so there is little salience for the cue, and this might result in slow learning. By contrast, if the onset of a light or odour signals the onset of the noxious stimulus, then the cue will likely have greater salience, and the association should be quicker. Further, if the arena is small, a shock might induce rapid walking, and the animal might move rapidly between the shock and no shock area and back to the shock area. Again, this might result in apparent slow learning. We need to consider what cues are easy for the animal to discriminate because what is obvious to us could be indistinct to the animal.

Various invertebrates can learn to predict shock if that is reliably preceded by a cue. For example, *Drosophila melanogaster* that are trained with a novel odour just prior to a shock will avoid that odour when given the opportunity (Yarali et al. 2008). That is, the odour predicts something that is “bad”, but when paired with the arrival of something “good” such as food, the odour will come to be preferred (Tempel et al. 1983). More surprisingly is the finding that an odour that arrives at the end of an electric shock comes to be preferred when tested against an alternative odour. That is, the odour signals safety or relief, and this learning has been termed “pain relief learning” (Gerber et al. 2014). However, it has different properties than when the odour precedes the shock. Relief learning typically takes more trials for the effect to be shown with 1, 2 or 4 trials not being effective but 6 and 8 trials resulting in odour preference (Yarali et al. 2008). With punishment learning, it can be as low as one trial for avoidance (Tully and Quinn 1985). Further, when the odour precedes shock, a strong avoidance is noted, but when it follows the shock, the preference is weak, with the size of the effect being about a fifth of that shown for avoidance (Yarali et al. 2008). The intensity of the shock used in training also has an effect with increasing learning seen with increasing intensity up to 100v but a marked decline thereafter. This latter finding was ascribed to the very high shocks inducing amnesia and/or damage to the fly. The relief learning in flies has many similarities to that found in rats and humans, but it remains relatively little understood, and studies are required in other species to aid in understanding how it might add to our understanding of pain (Gerber et al. 2014).

7.2.2 Giving Up a Valuable Resource and Motivational Trade-Offs

A nociceptive reflex might briefly interrupt an ongoing behaviour, but it unlikely to cause an animal to give up a valuable resource, e.g. food or shelter, for a prolonged period. The rationale for this is that once the reflex is completed, there is no need to presume an awareness and no need to presume a marked shift in motivational state. Thus, the animal should return swiftly to its original activity. However, there are cases when an animal responds to a noxious stimulus by giving up a valuable resource to remove itself from the scene of stimulation. The example noted above of shore crabs moving out of a dark shelter into a brightly lit area is one such situation (Magee and Elwood 2013). The dark shelter is important to shore crabs, and moving from a shelter to be exposed to light in natural situations will normally increase the risk of predation (Fathala and Maldonado 2011). Another example occurs with hermit crabs that are shocked within their shell, which causes crabs to evacuate from the shell and thus abandon the important protection the shell provides (Appel and Elwood 2009a, b). Often, a hermit crab will remain near the shell, and some investigate within the shell by probing it with their chelipeds (claws). Some crabs then move back into the shell, but a large number remain out of the shell for a prolonged period. Indeed, some move away from the shell and may scramble against the wall of the test arena, apparently attempting to escape from the location. This is not predicted by a nociceptive reflex; rather, it indicates a marked change in the motivational state of the animal. Hermit crabs are dependent upon shells, and abandoning the shell is an extreme reaction and clearly demonstrates the aversive nature of the electric shock.

A compelling argument for non-reflex responses can be made when motivational trade-offs occur. The rationale for this is that during normal decision-making processes, various motivational requirements affect the decision and patterning of behaviour. Thus, if we see the response to a noxious stimulus being affected by other motivational requirements, it must be due to a decision-making process, as opposed to a reflex (Elwood and Appel 2009). One example is seen when hermit crabs are induced to occupy either *Gibbula cineraria* or *Littorina obtusata* shells, the latter being considerably preferred to the former (Elwood 1995). Crabs receiving a small electric shock (10v) within their shell were more likely to get out of the less preferred species of shell, showing that the quality of the shell was traded-off against shock avoidance. A second example involves hermit crabs in *L. obtusata* shells that were subject to shocks of increasing intensity (up to 25v) and exposed to different odours (Magee and Elwood 2016a). When no odour was present, 95% of the crabs emerged from their shells, which was not significantly different to 80% emerging when the odour of a non-predator (mussels) was present, but these were both different from the group exposed to the odour of a predator in which only 41% emerged. That is, crabs were trading-off risk of predation with shock avoidance. Two other experimental groups received predator or non-predator odours that were 100× more concentrated. For the concentrated predator odour group, 47% got out of the shell, but this was not significantly different from 57% for the concentrated non-predator odour group. The response to extreme concentration of mussel odours was not statistically different from those to the predator odours, indicating the importance of using concentrations that might realistically be found in natural conditions. The conclusions are that crabs tend to remain in their shells when odours of either unnaturally high concentration or of potential predators are present. Thus, although evacuating from a shell might seem a relatively simple behaviour that might be reflexive, it is clearly influenced by other motivational requirements and thus a product of central decision-making. The data are consistent with the idea of pain.

7.2.3 Protective Behaviour: Prolonged Rubbing and Grooming/Wound Guarding

Wounded mammals may show activities that indicate some awareness of the site of the wound and some attempt to reduce further damage. Typical examples are rubbing, guarding of wounds and limping, and these activities are interpreted as being consistent with pain (Weary et al. 2006). Various examples of similar activities have been reported for invertebrates. For example, application of either 10% sodium hydroxide or 10% acetic acid to a single antenna of glass prawns, *Palaemon elegans*, resulted in prolonged grooming and rubbing of that specific antenna (Barr et al. 2008). The grooming involved repeatedly pulling that specific antenna through the small chelipeds (claws) or through the mouth parts, whereas rubbing was pressing and moving that antenna against the side of the tank. Pinching one antenna with forceps did not affect grooming rate but did increase rubbing. The responses were directed at the treated antenna significantly more than the untreated antenna, indicating an awareness of the specific location of the noxious stimulus. Further, application of sodium hydroxide to one eye of a glass prawn caused high levels of grooming of that specific eye with either one or both first walking legs. This behaviour was not seen if just sea water was applied (Barr 2009). Also, shore crabs scratch at their mouth parts if the latter is treated with acetic acid (Elwood et al. 2017), and hermit crabs will groom their abdomen if they had received abdominal shock, an activity not seen without the noxious stimulus (Appel and Elwood 2009a, b).

In some crab fisheries, the claws are twisted and pulled off, and the live animal then returned to the sea. McCambridge et al. (2016) compared the competitive ability of male crabs that had the claw forcibly removed with those induced to autotomise a claw, which does not cause a large wound. Apart from demonstrating a lower ability of the former to gain access to females, several observations suggested an awareness of the wound. These included holding the existing claw over the wound during the competition in a manner akin to guarding. Manually declawed crabs also touched their wound and picked at the broken exoskeleton with their remaining claw and sometimes then showed a "shuddering response". These manually declawed crabs showed a lower motivation to compete for the female and seemed to be more engaged in self-defence than were those induced to autotomise (McCambridge et al. 2016).

Similar behaviour directed at wounds is seen in the octopus *Abdopus aculeatus* that have had an arm crushed by forceps (Alupay et al. 2014). The wounded area was held in the beak for at least 20 min in some individuals. Some 6 h later, this behaviour was not observed after experimentally touching the wound area, but the animals held the wound close to the body and adjacent arms curled around. However, no such behaviour was noted when part of an arm was removed in the squid *Loligo pealeii* (Crook et al. 2011).

7.2.4 Autotomy

Autotomy is another protective motor response in arthropods, such as brown crabs *C. pagurus* (Patterson et al. 2007), and cephalopods, such as the octopus *A. aculeatus* (Alupay et al. 2014). It enables the animal to cast off an appendage that is damaged. For example, cutting a leg-joint membrane at a joint distal to the main body, causes immediate haemolymph loss and autotomy occurs within a few seconds, preventing further loss of fluid (Patterson et al. 2007). This autotomy leaves a clean break at the joint with the main body, which immediately seals to prevent loss of haemolymph. Crabs also autotomise limbs in situations that do not involve haemolymph loss, for example, if the whole animal is placed on a hot plate (Fiorito 1986), injected with formalin (Dyuizen et al. 2012) or injected with acetic acid (Barr 2009) or if the leg is subject to electric shock (Magee and Elwood 2013). The acetic acid treatment rapidly induces autotomy in a dose-dependent manner, and the results are consistent with the idea that pain mediates the autotomy response. In the octopus, *A. aculeatus*, autotomy is induced by crushing an arm with forceps.

Legs may also be autotomised in spiders, e.g. *Argiope aurantia* (Eisner and Camazine 1983). This occurred when these spiders attempted to capture ambush bugs (*Phymata fasciata*), usually when the bug grasped a spider leg and probed a joint with its proboscis (the venomous saliva is painful to humans). Eisner and Camazine (1983) examined the role of chemicals that induce pain in humans. Injected bee and wasp venom both induced autotomy, whereas penetration of the joint with a sterile pin did not. They found that when individual components of the bee venom were injected, some, but not all, produced autotomy. Effective components were histamine, serotonin, phospholipase and melittin, all of which induce pain in humans, whereas ineffective components were acetylcholine, bradykinin, hyaluronidase, adrenaline and dopamine. Acetylcholine and bradykinin induce pain in humans but not autotomy in spiders, and hyaluronidase, adrenaline and dopamine do not induce pain in humans. Thus, there is a concordance between pain effects in humans and autotomy in the spider.

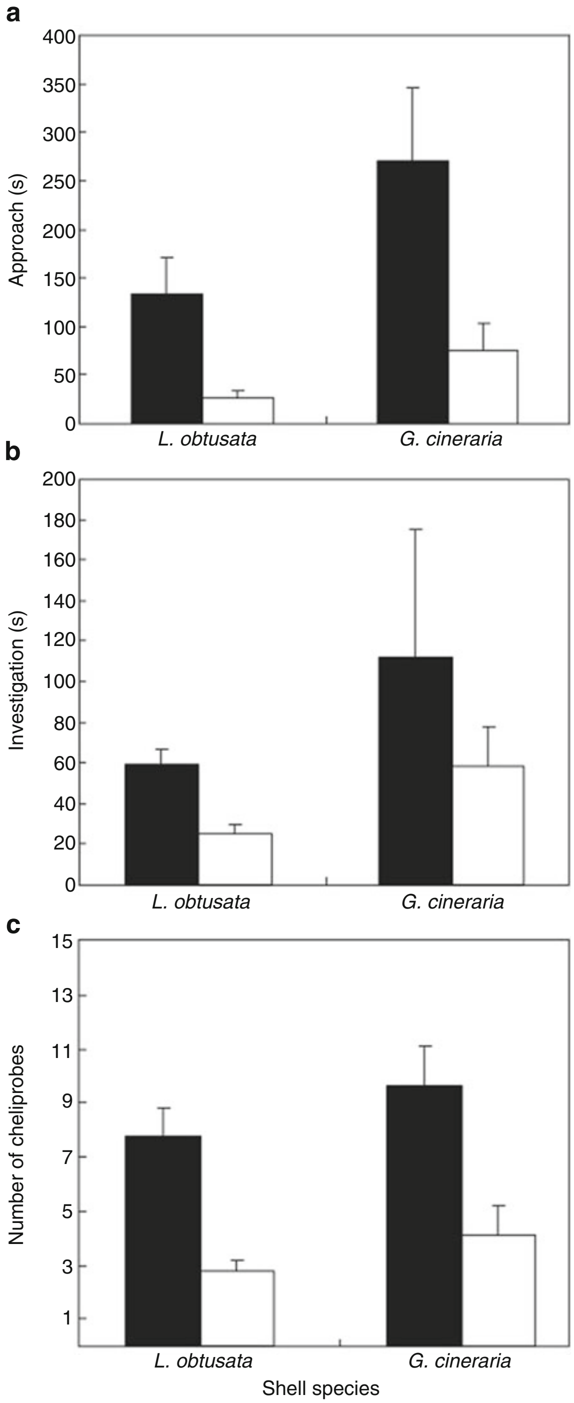
It is possible that at least some incidences of autotomy are mediated by a pain-like experience, but we need experiments to test the effects of anaesthetics and analgesics to determine if they reduce autotomy. Until those experiments are conducted, it is difficult to assess what autotomy tells us about possible pain.

7.2.5 Long-Term Motivational Change

As stated above, the advantage of pain over nociceptive reflex is that it may more easily result in a long-term change in behaviour, including avoidance learning discussed previously. There are, however, instances of long-term change in motivation that may not easily be shown as due to learning. One such example comes from studies in which hermit crabs were given small electric shocks within their shells. Those that did not evacuate from their shells were subsequently offered an empty shell 20 s after the last shock and the responses compared to crabs that were not shocked prior to offering a shell (Elwood and Appel 2009). Many crabs, in both treatment groups, moved toward the new shell, investigated and most then moved into the shell. However, crabs that were shocked were significantly more likely to approach and take the offered shell than were those that were not shocked. Of those that moved into the new shell, most did so after a single approach and investigation; those in the shock group approached more quickly (Fig. 7.2a), spent less time investigating the new shell prior to moving in Fig. 7.2b and used fewer insertions of their chelipeds during the investigation (“cheliprobes”) (Fig. 7.2c) compared to those not shocked. Shocked crabs thus showed a higher motivation to obtain a new shell and acted as if the shell they occupied was of very poor quality. They acted similarly to crabs housed in shells that are far too small and hence had a high motivation to change shells (Elwood and Stewart 1985; Elwood 1995).

To determine how long the motivation to obtain a new shell lasted, a subsequent experiment varied the time from the last shock (or control treatment) to the offering of a new shell (5 min, 30 min, 2 h and 24 h) (Appel and Elwood 2009b). Shocked crabs in the 30 min and 1 day groups were more likely to approach the new shell than were the non-shocked ones. Further, significantly more shocked than non-shocked crabs of the 30 min group moved into the new shell. Of those that contacted the new shell, shocked crabs from the 2 h group approached significantly quicker than the non-shocked group. Of the crabs that entered the new shell after first approach, shocked crabs used fewer cheliped probes in the 5 min, 2 h and 24 h groups. Further, after moving into shells, hermit crabs may thrust their abdomen in and out of the new shell, presumably in a continuation of the shell evaluation. Shocked crabs in the in the 2 and 24 h groups were less likely to show this behaviour than were the non-shocked crabs. That is, 24 h after the shock treatment, crabs still showed a higher motivation to obtain a new shell as evidenced by various activities. Clearly,

Fig. 7.2 Mean ± SE of (**a**) time taken to approach (s), (**b**) duration of investigation (s) and (**c**) number of cheliprobes by crabs in either *L. obtusata* or *G. cineraria* shells that had been shocked (open bar) or not shocked (black bar) (from Elwood and Appel 2009)



behavioural differences shown 24 h after the noxious treatment cannot be described as a reflex. Further, if a key function of pain is to change behaviour in the long term to avoid repeated tissue damage, the ability to remember is a prerequisite. The studies noted above on hermit crabs and shore crabs show such a memory. There are many other studies that demonstrate short- and long-term memory in a wide range of invertebrates (Sherwin 2001).

A key function of pain is to increase subsequent survival, and we expect animals subject to noxious, potentially painful, stimuli to be wary of stimuli that are associated with elevated predation risk. An example of this increased wariness comes from work on crayfish (Fossat et al. 2014). Crayfish were tested in cross mazes in which two arms of the maze were brightly lit and the other two were dark. Crayfish wandered throughout the maze but spent more time in the dark than the light. In natural conditions, crayfish stay in hiding during the day and are mostly active at night, presumably when predation risk is lower. In the main experiment, some crayfish were exposed to repeated short-duration electric fields that induced tail-flicking escape responses, indicating that this treatment provided a noxious stimulus. Animals thus treated spent far less time in the light arms of the maze than did those placed in the treatment area but without the shocks (Fig. 7.3). That is, the normal preference for the dark was considerably enhanced, and the shocked animals were described as showing “anxiety”.

A further example of increased wariness involves squid that had a small section of one arm removed with surgical scissors showing marked changes in response to approaching visual stimuli (Crook et al. 2011). The squid were touched at intervals with a bending filament attached to a long, thin handle to assess changes in sensitivity to tactile stimuli. However, the approaching filament caused the squid to respond by movement or colour change prior to contact. For control squid, there were no temporal changes in the distance between the filament and the animal before the first response. For injured squid, however, temporal changes in responsiveness were noted. Ten minutes after wounding, the squid appeared to be less responsive to the approaching stimulus, but thereafter, it was considerably more responsive than were controls. This increased wariness was apparent for up to 48 h after injury. Further, there was considerably more anticipatory jetting and protective ink plumes by injured squid, and these responses typically increased from the first trials 10 min after injury to the last trials 24 or 48 h later. Comparison of blindfolded squid and those that could see confirmed that the reactions were to visual stimuli rather than vibration, because the blindfolded squid did not respond during the approach of the filament (Crook et al. 2011).

While the increased wariness towards approaching visual stimuli by injured squid is consistent with a mechanism to reduce predation, it does not specifically demonstrate fitness enhancement. Another study on squid, however, clearly demonstrates fitness enhancement. Further, the enhanced fitness is dependent upon the nociceptive input from injury rather than just the injury itself (Crook et al. 2014). The approach was to give some squid a small wound whereas others were unharmed. Half of each of these treatments were also given a local anaesthetic that would block the nociceptive input of the wound. Giving the local anaesthetic to squid that were not

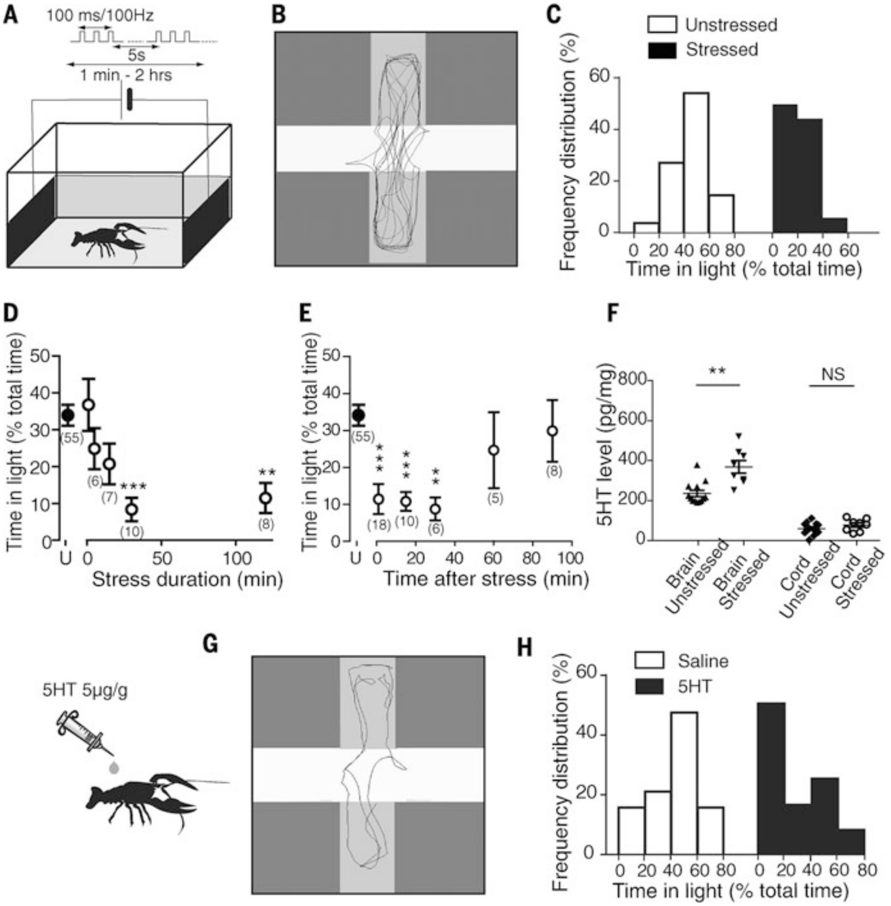


Fig. 7.3 Crayfish develop 5HT-dependent light avoidance after exposure to stress. (**a**) Experimental procedure for stress induction in crayfish (supplementary materials). (**b**) An example crayfish route after a 30 min exposure to an electric field. Walking occurred nearly exclusively in the dark arms. (**c**) Frequency distribution histograms of the percent time spent in light arms by stressed and unstressed crayfish. (**d**) Effect of stress duration on time spent in light arms (*U* unstressed; *P* vs. unstressed <0.001 after 30 min and *P* vs. unstressed <0.01, after 2 h of stress, Dunn's test). (**e**) Time course of behavioural changes (as measured by time spent in light arms) after exposure to a 30-min stressful experience. Crayfish recovered "normal" behaviour after 90 min (U = unstressed; *P* vs. unstressed >0.05, Dunn's test). The number of animals (*n*) is in parentheses in (**d**) and (**e**). (**f**) Serotonin concentrations (in picograms per milligrams of fresh weight) measured by means of HPLC in the brain and ventral cord of unstressed and stressed crayfish. Brain concentrations of 5HT were significantly higher in stressed than in unstressed animals. (**g**) After injection of 5 μg/g 5HT into the haemolymph, the crayfish route was similar to that of stressed crayfish. (**h**) The frequency distribution histograms of percent time in light arms for saline- and 5HT-injected crayfish were similar to those for (**c**) unstressed and stressed crayfish, respectively (from Fossat et al. 2014)

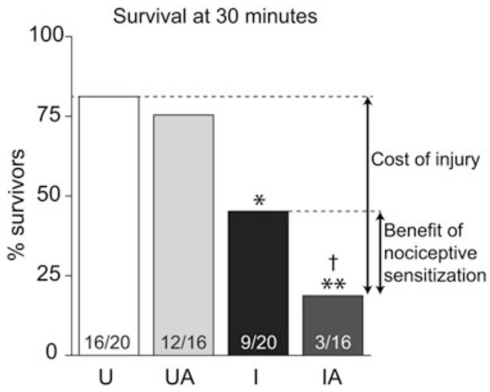


Fig. 7.4 Injured squid lacking nociceptive sensitisation had the lowest odds of survival. At the conclusion of a 30 min trial with free interaction of squid and fish, squid in the I and IA groups had lower overall survival than in the U group, and IA group squid were most likely to be killed. The difference in survival between the U and the IA group can be considered the cost of being injured, while the difference in survival percentage between the IA and I groups ($p = 0.05$) reveals the benefit that nociceptive sensitisation provides to injured animals. Odds ratios, $^{*}p \leq 0.05$, $^{**}p < 0.01$. *U* uninjured, *UA* uninjured with anaesthetic treatment, *I* injured, *IA* injured with anaesthetic treatment (from Crook et al. 2014)

wounded enabled the effect of the anaesthetic per se to be evaluated. The squid were then exposed in groups of four to predatory fish for 30 min and interactions between the squid and fish recorded. Fish could discriminate between injured and uninjured squid and oriented to the injured squid and started to pursue the injured squid at a greater distance than they did to the uninjured squid, irrespective of the application of local anaesthetic on the squid. The local anaesthetic had no effect on the behaviour of uninjured squid but did on the injured squid. Injured squid without local anaesthetic became alert and began to flee at a greater distance from the fish compared to all other groups. The squid that had the greatest chance of being captured by the fish were those with the injury and the local anaesthetic, and these were more likely to be predated than those that were injured but had no local anaesthetic (Fig. 7.4). The nociceptive input from the injury gives some protection, presumably because they were more reactive to the fish. This is consistent with the idea of pain resulting from the nociceptive input inducing a heightened awareness in the squid (Crook et al. 2014). The resulting fitness gain is clearly demonstrated in this study, and it is consistent with a key expectation of pain (Bateson 1991; Sneddon et al. 2014).

7.2.6 *Sensitisation of Touch Nociceptors*

The increased wariness after injury in squid is not restricted to visual stimuli. Similar changes in general sensitivity have been shown for tactile stimuli applied to areas distant from the site of experimental wounding as well as near to the wound (Crook et al. 2011, 2013). Squid that had a small portion of an arm cut off showed stronger responses to touch, in terms of increased latency to return to crypsis or settled behaviour, compared to unharmed controls (Crook et al. 2011). These responses and changes were similar for touching at different body locations, and responsiveness seemed to peak at 24 h after wounding. By contrast, unharmed squid showed only minor shifts over time, generally becoming slightly less responsive. In keeping with this increased responsiveness after injury was a decreased threshold required to elicit defensive behaviour, which again was not site-specific. Indeed, a wound on one fin increased sensitivity to touch not only on that fin but also on the contralateral fin (Crook et al. 2013). This is different from the site-specific increase in sensitivity following wounding in mammals (Treede et al. 1992) and other molluscs (Walters 1987). In the squid, the changes following wounding appear to be a generalised shift in sensitivity and increased wariness to a range of stimuli (Crook et al. 2011).

In the study of sensitisation of fins noted above, the sensitisation occurred even in fins that had been excised from the main body before crushing of the fin occurred (Crook et al. 2013). Further, if fin crushing was done to intact animals, the sensitisation was seen if those fins were excised 30 min and 24 h later. This increased sensitisation was also observed in the uncrushed contralateral fin after that was excised. Apart from the sensitisation, spontaneous firing of neurons in the fins was noted that did not depend on further tactile stimulation (Crook et al. 2013). When intact animals had a fin crushed, spontaneous firing was noted after the fins were excised not just in the crushed fin but also in the unharmed contralateral fin. However, this spontaneous firing in the contralateral fin did not occur when the other fin was crushed after being excised from the main body. This shows that the spontaneous firing is dependent upon intact neural or humoral connections at the time of crushing and thus suggests a role of the CNS in enabling the spontaneous firing.

The findings noted above are important as they cast severe doubt on the idea that nociceptors simply feed information about tissue damage to the CNS; rather, there is feedback to the nociceptor that is likely to influence their continued functioning (Crook et al. 2013). Such feedback occurs in mammals and occurs at numerous levels from the nociceptor to the brain and involves multiple excitatory and inhibitory process (Burrell 2017). It is thus beyond that expected of a simple reflex mechanism. Remarkably, this overall complexity and the specific modulatory mechanisms are found in at least four invertebrate phyla, e.g. arthropods, nematodes, molluscs and annelids (Burrell 2017). For example, in vertebrates, substance P and bradykinin sensitise nociceptive inputs, whereas opioid reduces the nociceptive signalling. Invertebrates have similar neuropeptides with tachykinins and opioid-like substances. Both vertebrates and invertebrates have glial cells that contribute to nociceptive function (Walters 2014) and have other mechanisms that relate to both nociception and learning/memory. Endocannabinoids are found widely in different phyla and are associated with nociception modulation (Elphik 2012). GABA and glycine inhibit nociceptive signals to the brain in vertebrates, and these substances are also found in invertebrates and inhibit nociceptive signalling (Burrell 2017). The complexity of these systems goes beyond the scope of the present chapter but is reviewed by Burrell (2017). Importantly, these studies demonstrate that the nociceptive mechanisms appear to be bound up with adaptive behavioural changes beyond a reflex. For example, both localised and widespread sensitisation appear to be important in reducing further tissue damage. Other systems regulate nociceptive input to the CNS and thus regulate pain experience, at least in vertebrates, and hence influence behaviour for a prolonged period.

7.2.7 Further Physiological Changes

The increased "anxiety" noted in crayfish exposed to repeated electric shocks has a physiological basis (Fossat et al. 2014, 2015). Stressed animals had higher levels of serotonin (5HT) in the brain, and unstressed animals injected with 5HT showed similar levels of anxiety to those that were stressed (Fig. 7.3). Crayfish, pretreated with a 5HT agonist, did not show the anxiogenic effect of 5HT (Fossat et al. 2015). There were also close correlations between 5HT levels and behavioural indicators of anxiety thus providing further evidence for a role of 5HT in anxiety. Dopamine also increased in stressed animals, but there was no correlation between this biogenic amine and behavioural indicators of anxiety (Fossat et al. 2015).

Fossat et al. (2015) also investigated the effects of chlordiazepoxide (CDZ), which is a drug used to reduce anxiety in humans. There was a marked effect of this anxiolytic treatment. Previously stressed animals, treated with CDZ, spent far more time in the light arms of the cross-maze than did the saline-treated controls. Indeed, they spent about as much time in the light arms of the maze as did animals that had had not been stressed. However, CDZ did not alter 5HT levels, suggesting that the effect of the anxiolytic is independent of the biogenic amine.

In crayfish and other decapods, serotonin also functions to release the crustacean hyperglycaemic hormone (CHH), which elevates haemolymph glucose concentrations (Webster 1996; Bergmann et al. 2001; Toullec et al. 2002). This occurs by mobilisation of intracellular glycogen, with liberated glucose either moving to extracellular fractions or being converted intracellularly to lactate via glycolysis (Stentiford et al. 2001; Verri et al. 2001), which is analogous to the stress responses of vertebrates. Removing one claw of edible crabs by twisting the claw had rapid physiological effects (Patterson et al. 2007). When compared to control animals that were handled, there was a significant increase in lactate and glucose but no decline in glycogen. However, the shift in the glucose to glycogen ratio indicated a marked mobilisation of glycogen to glucose. These physiological changes were not evident in crabs induced to autotomise (Patterson et al. 2007), suggesting that the effects were predominantly due to the tissue damage caused by manual declawing. Physiological changes were even more marked if the crabs were housed with an intact crab immediately after treatment, suggesting that being with a potential competitor was stressful for those animals without a claw. When male crabs were observed competing for a female, those with a claw removed by twisting showed marked deficits in their competitive ability compared to intact crabs and to crabs induced to autotomise a claw (McCambridge et al. 2016). Those with the tissue damage did not compete effectively and seemed to act in a defensive and submissive manner. Thus, it is not the lack of the claw that seems to have wide-ranging effects but those that had tissue damage showed marked physiological stress responses coupled with behavioural changes.

One problem that arises in interpreting studies on physiological change after noxious stimulation is that animals subject to the stimulation often engage in more vigorous behaviour than controls. Thus, there is a possibility that the effect is due to the activity rather than the stimulation (Elwood and Adams 2015). For example, in the studies of Fossat et al. (2014, 2015), crayfish were subject to electric charges repeated at 5 s intervals over a period of 30 min, which caused repeated vigorous tail-flipping escape responses. When the power of the electric charges was reduced so that flipping was not observed, there was no physiological change. At higher shock intensities, the tail flipping decreased over time, and this was suggested to be due to habituation (Fossat et al. 2015). It could, however, have declined due to exhaustion. This problem was examined in shore crabs by shocking at 10 s intervals for 2 min, i.e. less frequent, and for a much shorter time than for the crayfish (Elwood and Adams 2015). Shore crabs do not engage in the vigorous tail-flip response, but some show escape responses by attempting to climb the walls of the tank or showing a threat response. However, this was not seen in all shocked crabs, and some did not engage in behaviour more vigorous than walking. Many control animals also walked, but some remained still during the equivalent 2 min in the test tank. The key comparison in this experiment involved those shocked crabs and those control animals that walked. Lactate was significantly higher in shocked than non-shocked crabs, thus showing that the physiological stress response was caused by the noxious stimulus rather than the behaviour that it elicited (Fig. 7.5).

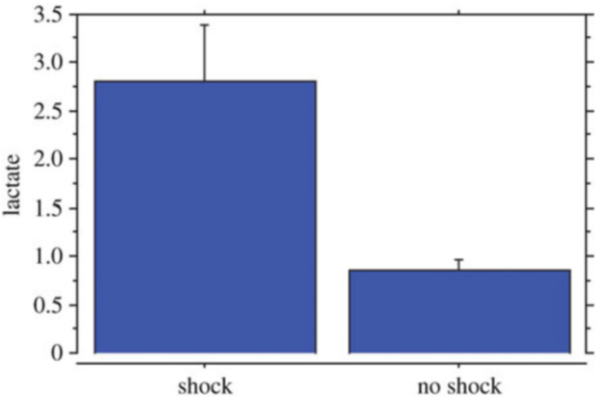


Fig. 7.5 Means and standard errors of lactate (mmol l^{-1}) for shock and control crabs that showed walking as most active response (from Elwood and Adams 2015)

7.2.8 Opioids and Local Anaesthetics

Opioids moderate responses to noxious stimuli among vertebrates such as fish (Sneddon 2003), amphibians (Machin 1999), birds (Gentle and Corr 1995) and mammals (Brownstein 1993). Opioid peptides and receptors also occur in various invertebrates and seem to be involved with pain or stress-induced analgesia (Harrison et al. 1994) but also affect feeding, aggression and protective behaviour (Dyakonova 2001). In mantis shrimps, *Squilla mantis*, and crabs, *Chasmagnathus granulatus*, morphine reduces the response to electric shock in a dose-related manner (Maldonado and Miralto 1982; Lozada et al. 1988). In the crab, this was reversed by the opioid antagonist, naloxone. In general, these effects required high doses of morphine, and they declined much more rapidly than in vertebrates. However, morphine also has other non-analgesic effects. For example, the escape response to a moving shadow in the crab, *C. granulatus*, is reduced by morphine (Tomsic and Maldonado 1990). This opens the possibility that the apparent analgesic effects of morphine simply reflect a reduction of responsiveness to all stimuli (Tomsic and Maldonado 1990). This possibility was tested by Barr and Elwood (2011) using the shore crab *Carcinus maenas*. Crabs were either given morphine or water injections and placed into a light area that had a single dark shelter into which the crabs moved. Some crabs received an electric shock within the shelter, but others did not. Each crab was tested for 20 trials; whether they entered the shelters and the latency to enter was recorded. The rationale was that if morphine had an analgesic effect then more crabs should move into the shelter when paired with a shock compared to those without morphine. However, this was not found. Irrespective of shock or not, crabs given morphine showed low numbers of shelter entries during the first ten trials and appeared to be unresponsive and limp. They soon recovered, and in the second ten trials, there was no difference between those given morphine and those given water injections. This supports the idea that the suggested analgesic effects noted in other studies were simply due to a general lack of response rather than analgesia (Barr and Elwood 2011). Local anaesthetics such as benzocaine, however, appear to be effective at eliminating nervous transmission and, hence, nociception. For example, the effects of noxious chemicals on grooming and rubbing of antennae in prawns were significantly reduced when the animal was pre-treated with benzocaine (Barr et al. 2008).

The use of cephalopods in science is now regulated by the EU Directive 86/609/EEC, and the use of analgesics and anaesthetics is demanded for procedures that have the potential to cause pain. However, there is little systematic study on the effectiveness and manner by which different chemicals act on the nervous system of these animals (Andrews et al. 2013). For example, magnesium chloride solution appears to block nociceptive transmission (Crook et al. 2014), but there is a suspicion that it acts as a muscle relaxant (Graindorge et al. 2008) and its use has been queried on welfare grounds (Andrews et al. 2013).

7.2.9 Self-Administration of Analgesics

In this approach, an animal is offered two types of food or fluid that are clearly distinguishable by colour of container, location or taste. With one, an analgesic is included, but the other lacks the drug. The aim is to determine if animals facing long-term pain learn to shift the intake to favour the container with the analgesic (Colpaert et al. 1980). This was achieved in a highly influential study on chickens in which lame chickens consumed more of the analgesic than did control chickens (Colpaert et al. 1980). However, not all such studies on vertebrates have shown an ability to associate the distinguishing features of the container and the effects of the analgesic. Indeed, this is a difficult association to achieve, especially if both containers are sampled within a short time and the analgesic is slow to work. In such a situation, there would be few clues as to which container produced a beneficial effect. To date, there appears to be one such study on invertebrates. Honey bees, *Apis mellifera*, had a single leg amputated compared to unharmed controls (Groening et al. 2017). They were then allowed access to two differently coloured feeders, one of which had sucrose solution and the other sucrose plus morphine. Amputated bees consumed more sucrose overall and thus more morphine than did control bees; however, there was no significant change in the ratio of sucrose/morphine to pure sucrose consumed. Thus, there was no evidence of an association between the colour of the container and effects of morphine, and, therefore, the data do not support the idea of pain in these animals. Nevertheless, this is an interesting approach to the study of potential pain, and further work is warranted. Paradigms should be used that enable temporal separation of the sampling of the containers, coupled with a swift-acting analgesic. Further work might also use different flavoured fluids or food as the vehicle for the drug and the control.

7.3 Specific Brain Structures

7.3.1 A Suitable Central Nervous System Analogous to the Human Cerebral Cortex?

We know much about human pain, and the complex pathways involved in human pain are well established (Key 2016). These pathways and neural structures differ in many other vertebrates and are absent from invertebrates. For this reason, it has been argued that fish and invertebrates are unable to experience pain (Rose et al. 2014; Key 2016). It is suggested that only animals having a system that closely matches that found in humans may reasonably be expected to have the potential for pain, e.g. primates. The argument rests on the idea of functional homologies being mapped onto structural homologies. Key (2016), for example, uses the example of the vertebrate visual system comprising a laminated optic tectum, and it is this structure that specifically enables vision.

However, it has been noted repeatedly that animals may show similar function with completely different neuronal structures (Elwood et al. 2009; Elwood 2012). The complex brains and eyes of humans, octopus and honey bees have separate evolutionary histories, each developing independently for many hundreds of millions of years. The result is that the eyes are very different, with the honey bee having a convex arrangement of numerous ommatidia and humans and the octopus having concave arrangements of light-sensitive pigments with a lens to focus the incoming light. These latter two have evolved independently, and the superficial similarities between the octopus and human eye are examples of convergent evolution. The structures are not homologous. Further, the brain structures of the honey bee and octopus are very different from each other, and both lack the optic tectum of vertebrates. Nevertheless, both have an ability to use light from distant sources to gather information about the world. That is, they have the same function as that in humans despite having different evolutionary histories and different structures.

Key (2016) notes that for pain to have survival value it must be mapped to specific body locations. Specific structures in the human cortex are presumed to enable such mapping. However, we have seen above that crabs, prawns and octopuses will attend to specific locations on the body that have been subject to noxious stimuli but they do this without the human cortex. Further, damage to the cortex in humans can lead to a loss of pain sensitivity (Key 2016), and thus there is no motivation to escape from the stimulus. We have demonstrated above that many invertebrates show high motivation to escape from or avoid noxious stimuli yet they have no human cortex. This demonstrates that at least some of the components of pain identified as requiring the cortex in humans nevertheless are found in animals that lack it. The conclusion is clear; those invertebrates are showing the same functions with different anatomy. Thus, we may conclude they must have brains that are in part analogous to those of mammals.

Of course, noting that different taxa have similar sensory abilities with different structures does not mean that they must experience pain. The point of the argument is to indicate that the possibility cannot be dismissed because of morphological differences. If pain confers such evolutionary advantages that it has developed in at least some vertebrates, then other taxa might well have developed a similar solution to long-term avoidance and protection from noxious stimuli.

Another argument that invertebrates do not experience pain is that their brains are too small. Indeed, the brain of a honey bee only has approximately 1 million neurons compared to about 68 million for a mouse (Klein and Barron 2016). Size and weight are particularly important to a flying animal, and there has clearly been considerable selection pressure to reduce unnecessary weight whenever possible. However, Klein and Barron (2016) query if neuron number is the key to understanding the capabilities of a brain. They maintain that functional organisation is the key and argue that bees and mammals are similar in that respect. Bees have surprising cognitive abilities, and the processing capacities of vertebrates and insects are not as different as the neuron numbers might suggest (Chittka and Niven 2009). It should be noted, however, that the brain of the octopus is large, complex and distinctly divided into specialised lobes (Young 1963; Crook and Walters 2011). The CNS comprises some

500 million cells and enables the highly complex behaviour of this animal, but many of these are in ganglia in the arms (Mather 2011). However, there seems to be no compelling argument to link brain size and or neuron number to the ability to experience pain (Broom 2007).

A key aspect of mobile animals is that they monitor internal states and external environment and prioritise actions in what has been termed the final common path (sensu McFarland and Sibly 1975). Most can distinguish between changes to their perceptual input that is caused by their own movement and those not affected by such movement. That is, they must have some basic awareness and sentience. In this respect, Klein and Barron (2016) argue that some fundamental aspects of brain structure of vertebrates, arthropods and molluscs, but not nematodes, are conserved. That is, the structures and abilities that enable decision-making likely predate the divergence of these major taxonomic groups. It is suggested that these groups share the ability of subjective experience, which is presumably a prerequisite for pain experience (Klein and Barron 2016).

7.4 Conclusions and Thoughts on Humane Treatment

Invertebrates have traditionally been considered to respond to noxious stimuli purely by nociceptive reflex, and some still argue that that remains true (Rose et al. 2014). It is clear from the evidence, however, that in many cases, the responses go far beyond reflex. They show rapid avoidance learning involving both egocentric and allocentric cues (Magee and Elwood 2013). They make behavioural decisions about responses to noxious stimuli, and the requirement to avoid the noxious stimuli is traded off against other requirements (Magee and Elwood 2016a). They show long-term motivational changes that reflect memory of their prior experiences (Elwood and Appel 2009; Appel and Elwood 2009b) and show similarities to the anxiety seen in vertebrates (Crook et al. 2011; Fossat et al. 2014). These changes are mediated by complex physiological processes that are analogous and, in many cases, homologous to those of vertebrates (Fossat et al. 2015; Elwood and Adams 2015). They have complex, compartmentalised brains that in some cases have more neurons than some vertebrates. They have complex cognitive abilities and show an awareness of the noxious stimulus and about the part of the body that was affected, and this improves survival (Crook et al. 2014).

This mass of evidence, in terms of criteria that are fulfilled, is consistent with the idea of pain and shows similarities to the available evidence for many classes of vertebrates. As pointed out by Sherwin (2001), however, the acceptance of experimental evidence is often guided more by our views of specific taxa than by the data. There is a public dislike of invertebrates because they are strange and alien and thus a marked lack of empathy (Kellert 1993). As Sherwin (2001) argues, however, the degree of empathy should not affect our acceptance of data. If data are accepted for vertebrates, they should also suffice for invertebrates.

This is not a trivial point because little thought or protection is provided to invertebrates, particularly with respect to their treatment in the human food chain (Elwood 2012). With respect to crustaceans captured or farmed for human consumption, the numbers are vast. The number of tiger prawns (*Penaeus monodon*) used in 2008 is estimated at 214 billion (i.e. 214 million, million). Further, this species comprises only about 12% of the number of crustaceans used per year, making the total number over 1600 billion animals. This greatly exceeds the combined numbers of chickens, pigs, sheep and cattle killed (Elwood 2012). Even if we conclude that the probability of these species being able to suffer is low, or that the degree of suffering is likely to be low, we should weigh that potential suffering by the vast numbers involved.

It is important to reiterate that there is no absolute certainty that any animal experiences pain. Nevertheless, our treatment of vertebrates is frequently influenced by the possibility that they feel pain and hence might be able to suffer. However, the current lack of control measures enables food processors to treat lobsters in the most extreme ways. In processing factories, live lobsters may have the appendages bearing the claws twisted off. The still living animal may be impaled on a spike to remove the abdominal (tail) muscle and the living head and thorax is discarded (PETA 2013). Further, in several crab fisheries, the claws are twisted off and retained, but the animal is returned alive to the sea. Here, it may be unable to feed (Patterson et al. 2009) and may show marked stress response and low survival (Patterson et al. 2007). In the light of evidence being consistent with the idea of pain, more humane methods to kill the animal before dismembering should be encouraged.

Other species in the food industry also need attention. Billions of live individuals are boiled, and we need to consider for which species death may be swift and suffering minimised and which species may die slowly. Some processes currently employed for crustaceans and cephalopods might result in slow death with a high possibility of pain, and these should be modified. Arguments that because we cannot prove pain in animals then those animals should not be protected must be rejected. We now have established criteria that we expect to be fulfilled should pain exist in these animals, and we now have numerous tests of those criteria. In many cases, the criteria have been fulfilled. It is possible that not all criteria are equal in indicating pain and we need to establish which of those are the more persuasive and how many of those should be fulfilled. It has been suggested that protection should be given if just one criterion is fulfilled (Birch 2017), but it is likely that the food industry will demand more. It is important to accept that closely related groups of species are likely to have the same capabilities and thus avoid delays in giving protection that could arise from demands that each species be tested (Birch 2017). The evidence is now extensive, and we should be in a position to use it to enhance the welfare of a considerable number of animals.

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