RESEARCH ARTICLE

Shock avoidance by discrimination learning in the shore crab (*Carcinus maenas*) is consistent with a key criterion for pain

Barry Magee and Robert W. Elwood*

School of Biological Sciences, Queen's University, Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK

*Author for correspondence (r.elwood@qub.ac.uk)

SUMMARY

Nociception allows for immediate reflex withdrawal whereas pain allows for longer-term protection *via* rapid learning. We examine here whether shore crabs placed within a brightly lit chamber learn to avoid one of two dark shelters when that shelter consistently results in shock. Crabs were randomly selected to receive shock or not prior to making their first choice and were tested again over 10 trials. Those that received shock in trial 2, irrespective of shock in trial 1, were more likely to switch shelter choice in the next trial and thus showed rapid discrimination. During trial 1, many crabs emerged from the shock shelter and an increasing proportion emerged in later trials, thus avoiding shock by entering a normally avoided light area. In a final test we switched distinctive visual stimuli positioned above each shelter and/or changed the orientation of the crab when placed in the chamber for the test. The visual stimuli had no effect on choice, but crabs with altered orientation now selected the shock shelter, indicating that they had discriminated between the two shelters on the basis of movement direction. These data, and those of other recent experiments, are consistent with key criteria for pain experience and are broadly similar to those from vertebrate studies.

INTRODUCTION

Recent studies, such as those investigating pain in fish (Sneddon et al., 2003; Braithwaite, 2010) and birds (Gentle, 2011) and depression-like states in mammals (Mendl et al., 2010), appear to support ideas about negative affective states and suffering in animals (Dawkins, 1980). The conclusions are often based on argument by analogy, which suggests that if behavioural responses to particular situations are similar in animals and humans then they are probably mediated by similar affective states. However, the argument by analogy is not applied equally to different taxa. For example, when vertebrates and invertebrates provide similar evidence suggestive of negative affective states, the argument is often accepted for the former but rejected for the latter (Sherwin, 2001). Recent work, however, indicates that invertebrates show pessimistic cognitive biases similar to those shown by vertebrates (Bateson et al., 2011), and results from studies on decapod crustaceans (Barr et al., 2008; Elwood et al., 2009; Elwood and Appel, 2009) and other invertebrates (Elwood, 2011; Crook et al., 2011) are consistent with predictions about pain.

Pain in humans is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (IASP, 1979). However, because animals cannot describe their emotional experiences, pain in animals is defined as 'an aversive sensory experience caused by actual or potential injury that elicits protective and vegetative reactions, results in learned behaviour, and may modify species specific behaviour' (Zimmerman, 1986). Sneddon (Sneddon, 2009) refines this definition, suggesting that animals 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 recurring, and promote healing and recovery.'

There are two distinct components of pain: the sensory component called 'nociception' and the aversive negative affective state. Nociception allows detection of noxious stimuli and results in a reflex response to move an appendage or whole body away from the source (Sherrington, 1906), and it is found across all major animal taxa (Sneddon, 2004). There is no inference of an aversive experience, long-term behavioural change or awareness of this nociceptive response (Sneddon, 2009). What we typically refer to as 'pain' is the unpleasant experience or 'feeling' (Broom, 2001) that involves awareness, interpretation and long-term behavioural change (Sneddon, 2009; Broom, 1998). If indicators of strong long-term motivational change after noxious stimulation are observed, then it may be assumed that they are mediated by an aversive experience or 'feeling' rather than just nociception (Braithwaite, 2010; Gentle, 2011; Sherwin, 2001).

Nociception is clearly adaptive in that it allows the animal to escape from the noxious stimulus and thus reduce further tissue damage (Bateson, 1991). If nociception gives that protection then we may enquire what further advantage is provided by the pain experience. Pain is a powerful motivating factor and it enables the organism to learn to avoid the situation that led to tissue damage (Zimmerman, 1986; Sneddon, 2009). That is, nociception offers immediate protection, but pain facilitates long-term protection because of the ease with which animals learn to avoid that situation and avoid future damage (Sneddon, 2009; Broom, 2001; Bateson, 1991). Pain thus increases fitness by changing behaviour in a way that enables the animal to live for long enough to produce young (Bateson, 1991; Broom, 2001) and may be widespread in the animal kingdom (Elwood, 2012).

Assessing pain in animals is difficult because of their lack of verbal communication. Simply noting a response to noxious stimuli is not sufficient, as that might be indicative of nociception rather than pain. However, various criteria have been proposed (Bateson, 1991) and reviewed with respect to invertebrates (Elwood, 2011). Some authors have suggested the inclusion of criteria relating to brain size, complexity or similarity to the human brain (e.g. Bateson, 1991). However, knowing that particular neurons are active when a noxious stimulus is applied tells us nothing about a possible unpleasant experience (Stamp Dawkins, 2012). For this reason, others have argued for criteria based on behaviour (e.g. Elwood 2012). Because pain, as opposed to nociception, is presumed to facilitate swift avoidance learning of noxious, potentially tissue-damaging stimuli (Bateson, 1991; Sneddon, 2009), we test that key criterion in shore crabs.

Avoidance learning in crustaceans has been investigated in the crab *Chasmagnathus granulatus* (Denti et al., 1988). Each crab was placed in a dark compartment of a double chamber and, despite a natural preference to be in the dark (Fathala and Maldonado, 2011), many moved to a light compartment, where some received an electric shock. They were then allowed to return to the dark chamber. When retested up to 3 h later, those that received a shock showed a greater latency to enter the light chamber. Crabs were also slow to enter the light chamber after a 24 h rest period if three training sessions had been given (Fernandez-Duque et al., 1992). Thus shock increased a natural reluctance to enter the light area but the results could be explained by an inhibition of walking rather than learning. In another study, crayfish *Procambarus clarkii* were subject to one noxious event per trial and showed evidence of avoidance learning, but this was slow because numerous trials were required (Kawai et al., 2004). To benefit from avoidance learning, not only should it be rapid (Sneddon, 2009), but it is also important that animals discriminate between specific situations that give rise to the unpleasant experience and those that do not. Failure to discriminate might result in avoidance of benign or even favourable conditions and hence loss of feeding, shelter or mating opportunities.

In the present study we offered shore crabs, *Carcinus maenas*, a choice of two dark shelters in an otherwise brightly lit tank and determined whether they learned to avoid one shelter in which shock was delivered and preferentially use the alternative shelter in which no shock was given. We used repeated trials and analysed specifically whether and how quickly the learning was evident by noting whether crabs were more likely to switch shelter choice in the following trial if they had received shock. Another useful technique to assess the aversive nature of a stimulus is to test whether the animal will give up a valuable resource to avoid that stimulus (Dunlop et al., 2006). Shore crabs show a strong preference for a dark shelter rather than being in the light (Barr and Elwood, 2011), so emergence from the dark shelter into the light requires them to give up a valuable resource. Thus we noted whether crabs emerged from the shock shelter more often than the non-shock shelter and whether the probability and speed of emergence changed over trials. If our data indicate no learning or only slow learning and crabs failed to emerge from shelters when shocked, it would substantially reduce the case for pain in these animals. However, if swift avoidance and discrimination are coupled with emergence from the shelter when shocked, this would be consistent with the case for pain but would not prove that case. The results are discussed in the light of other studies that have attempted to disprove the notion of pain in crustaceans (Elwood, 2011).

Finally, we investigated the cues that might be used to discriminate between the shock and non-shock shelter (should such discrimination be found). We placed distinctive visual cues over each shelter and these were switched around in some cases in a final test. Further, we always had crabs facing in one particular direction during the trials so the crabs could preferentially use the non-shock shelter by learning to walk to their left or right. This possibility was tested by changing the orientation of some crabs by 180 deg in a final test. In this way we could discriminate whether visual cues or left or right movement was used. If either one of these proves positive, it would eliminate the possibility of magnetic or odour cues being used. For example, if the orientation of the crab was changed and the crab then selected the shock shelter, then it must have previously learned to walk to the left or right to get to the non-shock shelter. That the crab now goes to the other shelter would show that it had not used odour cues or cues related to the compass bearing because those hypotheses would predict that the crab would continue to go to the non-shock shelter. The same argument applies if they switch shelter use after the visual stimuli are switched.

MATERIALS AND METHODS
Collection and design
European shore crabs, *Carcinus maenas* (Linnaeus 1758), were collected from Barr Hall Bay, Strangford Lough, Co Down, UK, using baited pots. Fully intact crabs with carapace width of 5–8 cm were transported to Queen's University, Belfast, and housed in plastic tanks (76×38×17 cm) filled with aerated seawater to a depth of 5 cm and maintained at 11–13°C with a 12 h:12 h light:dark regime. Seaweed (*Ascophyllum nodosum*) from the collection site was provided as shelter within the holding tanks and crabs were fed with Tetra Pond Floating Food Pellets (Melle, Germany). The water was changed every 3 days.

Crabs (N=90) were tested individually in a glass tank (62×25×25 cm) with dark shelters, made from dark sheet plastic positioned at opposite ends of the tank (each 11×25 cm), leaving an open area (40×25 cm) between the shelters. We randomly positioned two distinctive patterned cards to cover the end walls of the tank above the shelters. One pattern consisted of vertical and the other horizontal black-and-white stripes of equal width (1.8 cm) and total area. Gravel was placed on the floor of the tank, seawater was added to a depth of 1 cm above the bottom of the tank and the tank was placed into an observation chamber (71×36×39 cm) behind a one-way mirror. Two energy-saving bulbs, each equivalent to 100 W (3430 lx, measured by a Precision Gold N76CC Light Meter, Yorba Linda, CA, USA), were suspended over the tank.

Each crab had a loop of insulated copper wire (0.2 mm diameter) placed around each of the fifth walking legs, with the other end of each wire attached to a Grass S9 electric stimulator (West Warwick, RI, USA); the insulation was removed at both ends of the wire. The left and right legs had wires that were randomly attached to the positive and negative terminals of the stimulator, which was set to deliver a shock of 10 V at 180 Hz for 200 ms. Each subject was placed into the centre of the tank between the two shelters so that it faced towards the observer behind the one-way mirror.

The crab could choose which shelter to enter, but whether electric shock was delivered was randomly decided before the first trial commenced. If the crab was shocked, the shelter it selected served as the shock shelter throughout the experiment. If the crab was not shocked on first entry, the shelter it selected acted as a non-shock shelter throughout the experiment. In either case, the alternative shelter resulted in the alternative treatment for that crab. If the shelter entered was the non-shock shelter, then the crab was allowed to stay inside the shelter for 2 min before being removed from the tank.

For shock shelter entries, the crab received a shock 5 s after its entire carapace was under the shelter and received another shock every 5 s for 2 min or until it exited the shelter. If the crab did not exit within the 2 min, it was removed from the tank. If the crab exited the shelter, it remained inside the tank for 2 min before being removed. If during this time it re-entered the shock shelter, it was shocked again; however, if it entered the non-shock shelter, it was removed after residing for 2 min in that shelter. If the crab did not enter either shelter within 10 min it was removed from the tank.

We monitored the crabs for autotomy, a defensive reaction by which an appendage is cast off at a specific breakage plane, leaving a sealed limited wound (Patterson et al., 2007). If autotomy occurred, the wire was attached to the adjacent walking leg in preparation for the next trial. Seven of these crabs subsequently autotomised a second appendage and were excluded from the analysis. However, the 10 that autotomised just one appendage during the course of the experiment did not differ in their subsequent behaviour and these were retained in the analysis.

Between trials, each crab was placed for 2 min in an adjacent seawater-filled container with a loosely fitted lid. The room was dark for these 2 min apart from the final 10 s, when the lid was removed and the energy-saving bulbs were turned on, to allow for the bulbs to attain full brightness. This treatment was repeated nine times, allowing the crab a total of 10 choices between shelters. Note, however, that in trial 1 the experimenters selected whether the crab would receive a shock and thus only in trial 2 could the crabs make a choice based on experience.

Following these 10 trials, a test then determined what aspects of the situation might have been learned. To test whether the visual stimulus (horizontal or vertical lines) was involved, some crabs received the stimuli in the same location whereas for others they were switched. To test whether the direction of movement was involved, some crabs were placed again facing the observer whereas others were placed facing away from the observer. Now, if previously they had walked to the left to the non-shock shelter, they would have to walk to the right to reach that same shelter. The shelter that the crab entered was recorded with reference to previous shock or non-shock status, although no shocks were delivered in this test. If the crab did not enter a shelter within 5 min, it was removed from the tank. Upon removal, each crab was sexed and carapace width was measured.

Ethical note

No licence was required for this work as crustaceans are not included in UK scientific legislation. We used electric shock as the stimulus because this has been shown to be aversive in a wide variety of taxa and it can be delivered to freely moving well-protected animals such as the shore crab in a reliable manner. Crabs are prone to autotomise walking legs, and crabs with missing appendages are common in the field; those with one or two legs missing do not appear to be impeded in movement. We sought to avoid autotomy during the experiment by using only intact crabs and setting the voltage below that which a single shock caused autotomy. However, some crabs autotomised during shock trials, causing the circuit to break. Wires were subsequently attached to the fourth walking leg and trials continued. Crabs could terminate the shock by exiting the shock shelter and could avoid shock either by not entering either shelter or by selecting the non-shock shelter. The original sample size (N=90) was determined by the number of groups (four) required in the final test and the fact that some crabs failed to make a choice between shelters or autotomised two legs and thus were dropped from the analysis. All crabs were returned to a suitable shore near to the collecting site after the experiments were conducted.
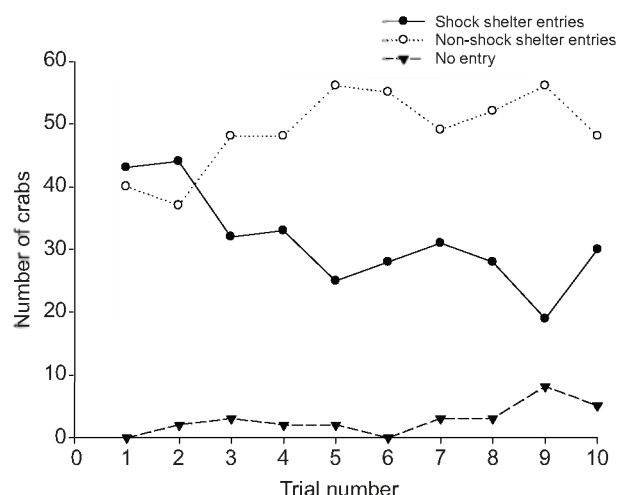

Fig. 1. Number of crabs that entered the shock and non-shock shelters in each trial, as well as the number of crabs that did not enter either shelter.

Statistical analyses

All analyses used the Statview package (Version 5, SAS Institute, Cary, NC, USA). For contingency analyses we used χ^2 for large samples with d.f. >1 but used G-tests for 2×2 analyses or Fisher's exact tests. We used independent t-tests or paired t-tests to compare means as appropriate.

Males for which data were used (N=64) were larger than females (N=19; t-test, male: 64.7±1.1 mm, female: 58.5±1.5 mm, t_{82}=2.93, P=0.0044). However, sex and body size had no effect on the dependent variables and results of these analyses are not reported.

RESULTS
Shelter entry

All crabs entered a shelter in the first trial but not on subsequent trials (χ^2=18.33, d.f.=9, P=0.032; Fig. 1). More crabs failed to enter a shelter following a shock trial than following a non-shock trial in trial 9 (4/28 vs 1/52, Fisher's exact test, P=0.048). Furthermore, those that were shocked in trial 10 were less likely to enter a shelter in the following test for cues (Fisher's exact test, shocked no-entries: 12/18, non-shocked no-entries: 4/44, P=0.0012).
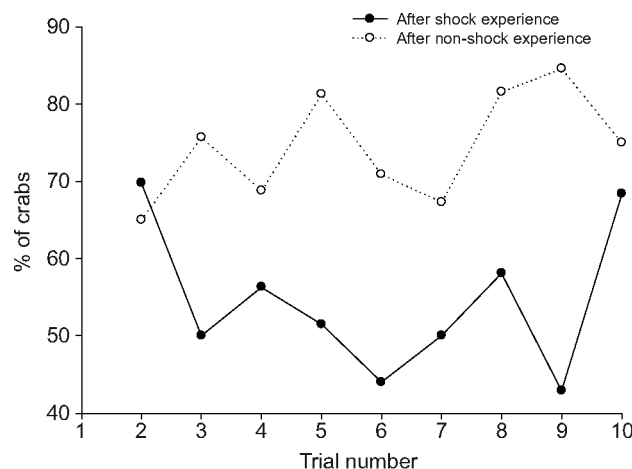

Fig. 2. Percentage of crabs entering the same shelter in the subsequent trial following a shock or non-shock shelter experience.

Table 1. The number of crabs that leave the shock shelter and do not subsequently enter a shelter, the number that enter another shelter during the same trial and, of those, whether that shelter is the original shock shelter or the non-shock shelter

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
No entry	15	16	14	11	13	13	16	14	10	14
Total that enter	6	8	3	6	4	9	8	9	7	10
Shock shelter	1	1	0	1	0	1	2	0	0	0
Non-shock shelter	5	7	3	5	4	8	6	9	7	10

In trial 1, whether a crab was to receive a shock was decided randomly, irrespective of which shelter was selected, and thus ~50% of the crabs were shocked (shock=43, non-shock=40). Subsequently, of those crabs that entered a shelter, the proportion that selected the shock shelter declined over the next 9 trials (χ^2=23.50, d.f.=9, P=0.0052; Fig. 1).

On the first trial, 54 crabs moved to their left whereas 29 moved to their right (binomial test: P=0.008). In trial 2 more crabs returned to the shelter that they entered in trial 1 (N=56) than the alternative shelter (N=25; binomial test: P=0.0008). We asked whether receiving a shock was more likely to induce the crabs to switch shelter choice in the following trial, but there was no effect of receiving a shock in trial 1 on shelter choice in trial 2 (G^2=0.63, P=0.43; Fig. 2). However, crabs were more likely to change shelter choice in trial 3 if they had been shocked in trial 2 (G^2=5.57, P=0.0182). This effect of shock on change in shelter choice in the following trial also occurred in trial 4 (G^2=9.14, P=0.0025), trial 5 (G^2=4.74, P=0.0294), trial 7 (G^2=4.37, P=0.0347) and trial 8 (G^2=10.83, P=0.001). Some crabs shocked in trial 2 had also received a shock in trial 1, but others had experienced the non-shock shelter in trial 1. We asked whether receiving two shock experiences caused more crabs to switch shelters, but there was no significant effect, with 12 of 29 crabs changing shelter after two shock shelter choices and 8 of 13 crabs changing shelter after one non-shock and one shock shelter choice (G^2=1.47, P=0.23).

Shelter exit

No crab emerged from the non-shock shelter, but some emerged from the shock shelter (Fisher's exact test, P<0.0001 for each of the 10 trials). Further, the proportion of crabs exiting the shock shelter increased over the 10 trials (G^2_9=24.21, d.f.=9, P=0.004; Fig. 3). We asked whether crabs became quicker at exiting the shock shelter by comparing the number of shocks received prior to their first exit with the number of shocks on the last trial during which they exited the shock shelter, but these did not differ (paired t-test, first exit: 3.69±0.40, last exit: 3.64±0.54, t_{44}=0.005, P=0.94).

When crabs exited the shock shelter, they often went into a shelter again during that trial. The probability of this occurring did not differ across the 10 trials (G^2=5.16, d.f.=9, P=0.82; Table 1). Also, if a crab chose to enter a shelter after exiting there was no effect of trial number on the choice made between the shelters (χ^2=7.08, d.f.=9, P=0.63; Table 2). However, after leaving the shock shelter, significantly more crabs chose to enter the non-shock shelter than the shock shelter in trial 6 (binomial test P=0.0391), trial 8 (P=0.0039), trial 9 (P=0.0156) and trial 10 (P=0.0020). In the other trials, although most crabs chose the non-shock shelter after emerging from the shock shelter, there was no significant preference (binomial tests, trials 1–5 and 7, P>0.07).

Test for cues

In the test for cues used in discrimination, 63 of 83 crabs entered a shelter. There was no significant effect of test conditions (cue configuration) on whether crabs entered a shelter (G^2=2.57, d.f.=3, P=0.46). There was no overall preference for the previous non-shock shelter (N=29) compared with the previous shock shelter (N=34; binomial test: P=0.6). However, the treatment conditions markedly affected the choice of shelter (χ^2=25.07, d.f.=3, P<0.0001; Table 2). Those tested with just a change in the patterned cards typically went to the previous non-shock shelter whereas those that just had their body orientation changed typically went to the previous shock shelter (χ^2=19.71, P<0.0001).

DISCUSSION

Crabs were randomly allocated to either receive a shock or no shock on their first trial, independent of the shelter selected, and that specific shelter either resulted in shock or no shock on each subsequent entry. Although receiving a shock was randomly decided by the experimenter, the first shelter was not a random choice by the crabs. More crabs moved to their left than to their right, suggesting a possible lateralisation of movement in this species, but conclusive evidence for this would require controls for magnetic or some other cue. During the second trial there was a significant bias for moving in the same direction as before, and thus crabs tended to use the same shelter as in the first trial. This shelter fidelity was not affected by shock status in the first trial. Those crabs not receiving shock in the second trial tended to persist with their choice of shelter; however, those receiving shock in the second trial were significantly more likely to switch choice of shelter. Interestingly, this switching did not depend on whether they had been shocked in the first trial. Having one non-shock trial and then one shock trial seemed to be as effective as having two shock trials. In further trials, those not receiving a shock again tended to select that shelter, whereas crabs that received shock were more likely to switch their choice in the next trial. In this way the majority of crabs came to use the non-shock shelter (Fig. 1). These data show swift avoidance learning and discrimination that is consistent with expectations
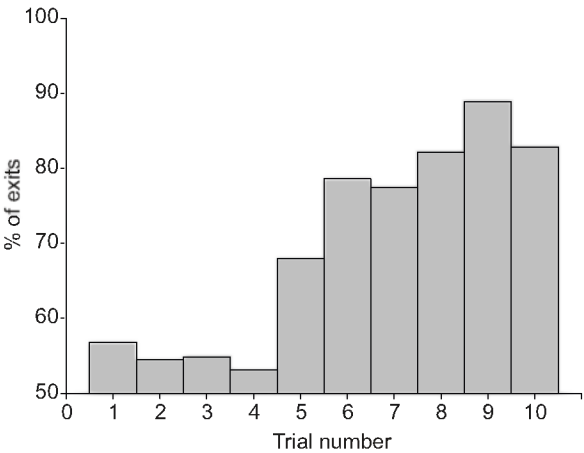

Fig. 3. Percentage of crabs that exited from the shock shelter for each trial.

Table 2. Non-shock and shock shelter entries by crabs, arranged by treatment conditions during the test for cues used in learning

	Non-shock	Shock
Same	7	7
Background changed	17	2
Orientation changed	2	13
Background and orientation changed	3	12

should these animals experience pain (Elwood, 2011; Bateson, 1991; Sneddon, 2009). The small proportion of subjects that failed to discriminate between the shock and non-shock shelters may not have discovered that an alternative, safe shelter was available.

The present study contrasts with one on crayfish *P. clarkii*, which 'gradually developed avoidance behaviour' following 20 trials per day for 32 days (Kawai et al., 2004). Those crayfish were only shocked once per trial whereas in the present study shocks were delivered every 5 s whilst the crab was in the shock shelter. However, crabs (*Eurypanopeus depressus*) have been shown to learn to avoid shock by moving a single leg, and this is associated with changes in RNA synthesis in the protocerebrum (Punzo, 1983). Single trial association with shock was claimed using the crab *C. granulatus* (Denti et al., 1988), but the dependent variable was the speed with which a subject moved from one location to another and thus there might have been a general reduction of movement induced by the shock rather than a specific learning. By contrast, in the present study, shock caused crabs to move out of a preferred dark location and in later trials alter the preferred direction of movement so that the alternative shelter was used. Thus we demonstrate discrimination learning whereby one shelter of the two is avoided and the other is preferred. We argue that our approach reflects likely field conditions when a crab attempts to use a shelter that has a noxious stimulus such as a predator and is thus subsequently avoided.

Other responses to shock indicate the aversive nature of the stimulus. Some crabs showed autotomy, and this only occurred during shock trials and always involved a leg with wire attached. Autotomy is a common response of arthropods where the limb is severed at a specific point and the wound is immediately sealed to avoid loss of haemolymph (Patterson et al., 2007); in the present study, a single instance of autotomy did not alter the crabs' subsequent behaviour.

Crabs commonly emerged from a shelter during a trial and thus entered the brightly lit central area. No crab did this without being shocked and because shock caused crabs to give up an otherwise desired dark shelter, this further indicates the aversive nature of the shock. However, exiting the shelter terminated the shock and it was evident that an increasing proportion of crabs exited the shock shelter with subsequent trials. This might be viewed as another aspect of avoidance learning, but the number of shocks within a trial required to cause the crab to exit did not decline with experience. Leaving a shelter in the field, even when being severely disturbed by a potential predator, is likely to be a tactic of last resort. Hermit crabs abandon their shell if the abdomen is shocked and some even walk away from that essential resource (Elwood and Appel, 2009; Appel and Elwood, 2009a; Appel and Elwood, 2009b). When vertebrates are induced to give up a valuable resource by an aversive experience, the idea of pain is typically invoked (Dunlop et al., 2006; Millsopp and Laming, 2008).

Crabs that exited the shock shelter frequently re-entered a shelter during that trial, and in the vast majority of cases (64/70) the shelter that was entered was the alternative shelter. Thus crabs receiving a shock could gain experience of both shelters during a single trial,

and this may have facilitated the discrimination learning noted above. On exiting the shock shelter the shock ceased, but, at that moment, the crab was closer to the shock shelter than to the non-shock shelter. Nevertheless, such crabs were more likely to cross the brightly lit arena to access the alternative shelter. Thus there must be at least a short-term memory of which shelter produced the shock.

All crabs entered a shelter in the first trial and this indicates the strong motivation to avoid bright light and seek a dark shelter (Barr and Elwood, 2011). Subsequently, some crabs failed to enter either shelter in some trials and tests. Non-entries increased over the course of the experiment, and more crabs failed to enter a shelter if they had received a shock in the previous trial. This latter effect was significant in trial 9. Furthermore, fewer crabs entered a shelter during the final test to determine cue use if they had received a shock in trial 10 compared with those that had not been shocked. In these later trials, the small proportion of crabs receiving shock had clearly not learned to discriminate between shelters and might be wary about entering either shelter. The crabs might thus learn to avoid the shock by staying in the light area, but this requires the crab to overcome the previous strong motivation to avoid the light. This further indicates the aversive sensory experience induced by the shock.

Our final test was to alter the cues available to the crabs. Some had the visual cues in the form of vertical or horizontal stripes switched around and some other crabs were oriented the other way around. If the crab had associated a particular pattern of stripes with the shock, then changing them around should have caused the crab to move to the previous shock location, but this did not occur. If the crabs simply learned a direction of movement, e.g. they associated moving to their right with reaching the non-shock shelter, then turning the crab around should cause the crab to move to the shock shelter. The data show that crabs learned to walk either to their left or to their right to get to the non-shock shelter and avoid the shock. There is no evidence that crabs use cognitive maps (Vannini and Cannicci, 1995) or magnetic sense (Boles and Lohmann, 2003) to find the non-shock shelter, because if either was used then changing the crabs' orientation should not have altered their choice of shelter. Further, there is no evidence for olfactory cues (Vickers, 2000). Had olfactory alarm cues been used to avoid the shock shelter, then changing the orientation should have had no effect on choice. Also, had the crabs initially used olfactory cues, but if those cues became dispersed then selection of shelter in the final test should have been random. Neither outcome occurred in these tests. Instead, the results show that the crabs simply used left/right directional movement or the specific leg movements that generate left or right movement as their method of selecting the non-shock shelter. Foraging fiddler crabs have been shown to maintain a specific lateralised orientation to their burrows and thus use direction of movement to enable them to quickly run to a position close to the burrow and only then are visual cues used (Zeil, 1998). However, we are not aware of any studies that show discrimination learning by the direction of walking in crustaceans.

In conclusion, the current work suggests that crabs discriminated between two shelters by walking either to their left or to their right and thus avoided the specific shelter in which they were shocked. Thus avoidance required altering a previously preferred direction of locomotion, and this became apparent after the second trial. These findings indicate swift avoidance learning, which is a key criterion/expectation for pain experience, but this alone does not prove that crabs can experience pain. Other studies have shown prolonged rubbing after noxious stimuli (Barr et al., 2008; Elwood and Appel, 2009; Dyuizen et al., 2012), motivational trade-offs

between shock avoidance and holding resources of different values (Elwood and Appel, 2009; Appel and Elwood, 2009a), prolonged memory of shock and giving up vital resources to avoid shock (Appel and Elwood, 2009b), which are all consistent with the concept of pain. Furthermore, injury results in marked physiological changes that mirror those related to corticosteroid release in vertebrates (Patterson et al., 2007). Thus, multiple studies have had the potential to disprove the notion that invertebrates experience pain, and their results have been consistent with the notion of pain. Our data are consistent with the definition for animal pain proposed by Zimmerman (Zimmerman, 1986) and the expectation of Sneddon (Sneddon, 2009). In particular, various studies on crustaceans have shown sustained changes in behaviour that have a protective function. In this sense, these studies are similar to various studies on fish that had the potential to counter the idea of fish pain but instead were consistent with pain (e.g. Braithwaite, 2010).

In conclusion, the data from this and other studies (e.g. Elwood, 2012) go beyond the idea of crustaceans responding to noxious stimuli simply by nociceptive reflex. Instead, long-term motivational change that enables discrimination learning has been demonstrated. Perhaps such motivational changes and learning can arise without any an unpleasant experience, although that is doubted by Gentle (Gentle, 2011) for birds. However, if we accept that possibility for invertebrates, we should also accept the same possibility for at least some vertebrates (Sherwin, 2001).

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