Motivational trade-offs and potential pain experience in hermit crabs

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Motivational trade-offs and potential pain experience in hermit crabs

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ABSTRACT

One criterion of pain experience is that the emotional response to pain may be traded-off against other motivational requirements. This was tested in hermit crabs, housed in either preferred or unpreferred species of shells, by subjecting their abdomens to electric shocks of gradually increasing intensity. The first observable response was not affected by shell species but those in preferred shells evacuated at a higher shock level than those in poor quality shells. Thus, they seem to trade-off the requirement to retain a high quality shell with that of avoidance of the noxious stimulus. Some crabs returned to their shells and those that got back into the preferred species did so with less probing of the aperture before getting in and subsequently thrust their abdomen in and out less often in further investigation, thus confirming their shell species preference. Not all crabs returned to the vicinity of the shell and some attempted to climb the wall of the experimental chamber. Others engaged in shell rapping as if in a fight and grooming of the abdomen was noted. These findings are consistent with the idea of a pain experience rather than a nociceptive reflex.

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1. Introduction

Pain is defined as "an aversive sensation and feeling associated with actual or potential tissue damage" (Broom, 2001) and consists of two components. First, the noxious stimulus has to be perceived and a reflex can be elicited independent of awareness of any feeling. This is called nociception and can be found throughout all animal taxa (Sneddon, 2004). The second component is the feeling of pain involving awareness, interpretation and assessment of the perception and it may act as a reinforcer in avoidance learning (Broom, 1998). There has been debate as to the taxonomic groups that might experience pain, however, there is increasing acceptance that it occurs in all vertebrates, including birds (Gentle et al., 1991), amphibians (Machin, 1999; Stevens, 2004) and fish (Sneddon et al., 2003). However, this acceptance is not generally

extended to invertebrates despite behaviour that appears to be analogous to that of vertebrates being shown in response to noxious stimuli (Sherwin, 2001).

Experiments with prawns showed that they are aware of the location of a noxious stimulus and exhibit grooming and rubbing of an antenna treated with noxious chemicals and these responses are reduced by application of a local anaesthetic (Barr et al., 2008). Other protective motor reactions that might result from pain include reduced use of the affected area, limping, holding or autotomy. Autotomy responses, for example, were initiated by injecting bee venom, which induces pain in vertebrates, in legs of spiders (Eisner and Camazine, 1983). Further, avoidance learning has been observed in various invertebrates, including bees, molluscs and decapods (Denti et al., 1988; Sherwin, 2001), and there is a connection between nociceptors and learning centres in decapods (Sandeman et al., 1992). Further, defensive responses of the crab (Chasmagnathus granulatus) are elicited by electric shock but these are inhibited by morphine in a dose dependent manner (Lozada et al., 1988). Opioid binding sites have

been found in various taxa including decapod crustaceans (Sherwin, 2001), which also have nociceptors that respond to potentially painful stimuli (Broom, 2007). In vertebrate studies, these findings would be taken as evidence of a feeling of discomfort or pain by the animal (Sherwin, 2001).

Another approach that might distinguish nociceptive reflex from pain experience may be made by testing trade-offs between stimulus avoidance and other motivational requirements. This may be shown by examining responses to noxious stimuli when coupled with either a high or low motivation to perform another activity. For example, fish were seen to remain longer in an area at which they could feed but also receive an electric shock if they had been deprived of food (Millsopp and Laming, 2008) and accepted a higher shock level in an area if a conspecific was present (Dunlop et al., 2006). That is the requirement for food or social contact suppressed the escape response to the shock indicating that there was a trade-off between motivational systems. Thus some form of central processing is likely to be involved rather than the response being a reflex.

In the present study we used hermit crabs, Pagurus bernhardus, to examine if crustaceans show similar behavioural trade-offs when noxious stimuli are coupled with competing motivational demand. Hermit crabs live in empty gastropod shells, which are an important resource for them because they provide protection from predators, cannibalism, desiccation and extremes of salinity. Different qualities of shells lead to different degrees of motivation to keep the shell or to leave it (Elwood, 1995). Results of shell choice experiments show that P. bernhardus prefers Littorina obtusata over Gibbula cineraria shells and a higher proportion of uninhabited Gibbula shells occur on the shore compared to Littorina shells (Elwood et al., 1979). Further, crabs in Gibbula shells are more likely to escalate fights to obtain new shells compared to those in Littorina shells (Dowds and Elwood, 1983). We used hermit crabs in both shell species and delivered electrical shocks of increasing intensity, which may be noxious, potentially painful stimuli, through electrodes inside the shells. This might merely elicit a nociceptive reflex response at a particular threshold causing the crab to evacuate the shell. However, if pain mediates the response, there should be some central involvement, and the decision to evacuate is expected to be traded-off against the motivation to retain shells of high quality. That is we predict that crabs should accept higher levels of shock when they inhabit the preferred Littorina

shells if pain mediates the response. The subsequent behaviour might also be influenced by shell quality. For example, once the crab has evacuated the shell we might expect to see activities such as avoidance of less preferred shells but swift reentry, with little investigation, into preferred shells (Elwood and Stewart, 1985). In this way we may examine if conflicting motivational demands shape the responses to noxious stimuli in a way that is consistent with the idea of pain experience.

2. Methods

Hermit crabs (P. bernhardus) were collected from rock pools at the shore at Ballywalter, Co Down, Northern Ireland (54°32′0″N, 5°29′0″W) in February and March 2008 at low tide. They were transported to Queen's University Belfast and housed in aerated seawater tanks (changed every three days), maintained between 11 and 13 °C on 12:12 h light:dark cycle. They were cracked out of their shells using a bench vise before the day of testing and given an experimental shell, the species of which was randomly selected.

The experimental shells were modified so we could deliver shocks while the hermit crab was inside the shell. Two small holes (diameter 1 mm), approximately 2 mm apart perpendicular to the long axis of the shell whorl, were drilled into either L. obtusata or G. cineraria shells. We scraped off the resin at each end of two resin insulated copper wires (diameter 0.23 mm), put each of them through the holes in the shell, fixed the wires with resin and bent them flush with the interior wall of the shell (Fig. 1). The aim was to have these wires either touching or being very near to the dorsal surface of the abdomen when the crab was in normal active posture. The abdomen is uncalcified and sensitive to touch and heat (RWE personal observation). A range of shells was used to cover the size range of crabs used in the experiments (0.1–0.5 g) and each experimental shell was the optimum weight size for that shell species for the size of crab (Jackson and Elwood, 1989). They remained in these shells overnight prior to being used in the experiment between 09.00 and 14.00 the following day.

Hermit crabs were taken in random order and positioned alone in a 16 × 25 cm glass tank behind a one-way-mirror and the coiled wires were fixed at the side of the tank, leaving enough wire for them to move around the whole tank, and connected randomly to the positive and negative outputs of a GRASS stimulator. Crabs were
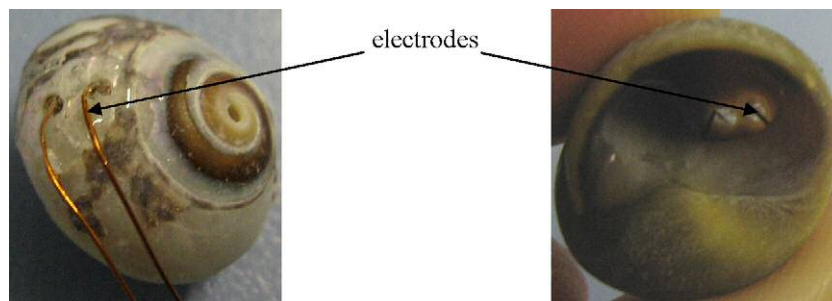

Fig. 1. On the left is the exterior of a Gibbula cineraria shell and on the right the interior of a Littorina obtusata shell showing electrode attachment.

allowed to become active, with limbs and eyes outside the shell (minimum: 8 min) before delivery of electrical shocks. The shock frequency was 200 Hz, shock duration 1 s and the inter shock interval 2 s. The shock intensity was increased with every shock by 0.2 V until 10 V when voltage was increased at 0.5 V intervals. If the hermit crab withdrew deep inside the shell, no shock was given until it assumed normal active posture. This was to ensure that the electrodes would always be near to the anterior part of the abdomen when shock was delivered. Seventy crabs were tested, 34 in *Littorina* and 36 in *Gibbula* shells.

Our continuous measures were the voltage at which the hermit crab reacted first by withdrawing or shrugging (crabs bend their body towards the ground with retracted appendages); the voltage at which the crab evacuated the shell (got completely out of the shell so the entire abdomen was visible); the time to reenter the shell (maximum 15 min) from the time it evacuated; the number of "chelae probes" (putting one or two chelipeds into the shell); and the number "abdomen thrusts" (putting abdomen in and out of the shell very quickly after reentering the shell). Our categorical measures were if the crab stayed within 1 cm of the shell after evacuation or if it moved further away; if the crab "climbed wall" (had four appendages simultaneously at the wall of the container); "rapped the abdomen" (repeatedly hit the abdomen onto the shell exterior in the manner used in shells fights (Dowds and Elwood, 1985)) and "head in shell" (the naked crab investigated the interior of the shell such that the head was placed within the shell, the eyes no longer being visible).

Differences between the two experimental groups for continuous data were determined by Mann–Whitney U tests and for categorical data Fisher exact probability tests (Statview). One tailed tests were used when the specific predictions outlined in the introduction were tested otherwise two tailed tests were used.

3. Ethical considerations

Crustaceans are not included in the Animal Laboratory Procedures Act (1986) of the United Kingdom and thus no licence is required for this work. Our method involved small increases in electric shocks starting at a level where there was no response. The shocks ceased when the hermit crab evacuated from its shell and thus the crab determined the maximum shock severity to which it was exposed. Crabs recovered from the experience and were returned to the shore in appropriate non-experimental shells.

4. Results

Seven crabs showed autotomy of a leg or cheliped during the procedure, four of which were in *Gibbula* shells and three in *Littorina* shells. Six of these did not evacuate from the shell at the time of autotomy and, as we did not apply further shock to elicit evacuation, cannot be analysed. One crab evacuated the shell at the same time as it autotomised, leaving 31 in *Littorina* and 33 in *Gibbula* shells that evacuated. The voltage of their first reaction to the electric shock was not significantly different between the two groups (means $\pm$ SE $L = 9.0 \pm 0.42$, $G = 9.1 \pm 0.34$)
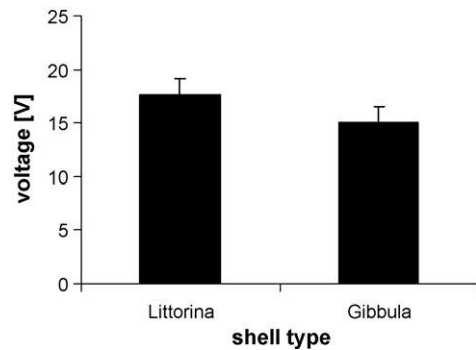

Fig. 2. Mean and SEM of 'voltage to evacuate' (*Littorina*: $n = 31$, $x = 17.7$, SEM = 1.52; *Gibbula*: $n = 33$, $x = 14.99$, SEM = 1.54). 'Voltage to evacuate' was significantly higher for crabs in *Littorina* shells (Mann–Whitney U test, (one tail), $z = 1.68$, $P = 0.0465$).

(Mann–Whitney U test (two tail), $z = 0.48$, $P = 0.6316$). However, as specifically predicted if pain is involved, hermit crabs in *Littorina* shells left the shells at significantly higher voltages than those in *Gibbula* shells (Mann–Whitney U test (one tail), $z = 1.68$, $P = 0.0465$, Fig. 2).

Crabs in *Littorina* shells did not reenter the shell significantly more quickly than those in *Gibbula* shells after evacuation (Mann–Whitney U test (one tail), $z = 0.25$, $P = 0.4022$). Neither did they differ in the probability that they reentered the shell (Fisher's exact test, reentering *Littorina*: 18/31, *Gibbula*: 23/33, $P = 0.4358$) nor in staying near to the shell after evacuation (Fisher's exact test, *Littorina*: 12/31, *Gibbula*: 15/33, $P = 0.621$) or in wall climbing (Mann–Whitney U test (one tail), $z = 1.165$, $P = 0.2439$). However, for those that reentered the shell, crabs in *Littorina* shells performed fewer 'cheliprobes' when investigating the shell prior to reentering (means $\pm$ SE $L = 3.7 \pm 1.14$, $G = 5.7 \pm 1.67$) (Mann–Whitney U test (one tail), $z = 1.77$, $P = 0.0383$). Further, crabs in *Littorina* shells made fewer abdominal thrusts than those in *Gibbula* shells (means $\pm$ SE $L = 0.13 \pm 0.06$, $G = 0.85 \pm 0.25$) (Mann–Whitney U test (one tail), $z = 2.15$, $P = 0.0164$).

Crabs that reentered the shell did not differ in the voltage to first reaction (Mann–Whitney U test (two tail), $z = 0.65$, $P = 0.10$) or in the voltage to evacuate compared to those that remained out of the shell (Mann–Whitney U test (two tail), $z = 1.64$, $P = 0.51$). Rapping the abdomen against the shell was performed by one crab in a *Gibbula* and three in *Littorina* shells after evacuation. Three crabs in *Littorina* and one in a *Gibbula* shell put their head into the shell while investigating it. Grooming of the abdomen was also observed in one individual. The numbers performing these activities are too small for meaningful analysis. Further, there were no effects of the size of the crab on any responses.

5. Discussion

Hermit crabs were not influenced by shell species with respect to the voltage at which they first reacted. This is important as it indicates that the shell species did not influence the nature of the electric current and that the electrodes were in similar positions relative to the crab's

abdomen in the two species of shell. This first reaction may be likened to a reflex rather than involving a decision on the part of the animal. It is thus similar to the tail flick reflex response in prawns when subject to a noxious chemical stimulus (Barr et al., 2008). It seems to depend on a physiological threshold and belong to the nociception part of responding to noxious stimuli rather than indicating a pain experience. It is possible that evacuating the shell might also be a reflex. If so, we would expect no effect of shell species. However, as specifically predicted if pain is involved, crabs evacuated the less preferred *Gibbula* shells at significantly lower voltages than did those in the preferred *Littorina* shells. This indicates that evacuating the shell is not simply a reflex response to a noxious stimulus because there is a trade-off between the quality of the shell species and the avoidance of shock. This finding is supported by a subsequent experiment using the same species and technique, except that a fixed voltage was used rather than the increasing voltage used here, which showed that significantly more crabs evacuated from the less preferred species than the preferred species (Elwood and Appel, 2009). There are several criteria that should be fulfilled to demonstrate pain experience and a trade-off of avoidance with other motivational requirements is one such criterion (Elwood et al., 2009). It demonstrates an interaction between different motivational systems with an evaluation of priority. Thus, we would expect some higher neural processing being involved in a similar manner to that suggested in trade-off experiments in vertebrates (Dunlop et al., 2006; Millsopp and Laming, 2008).

Unexpectedly, the crabs in the two species did not differ in the time to reenter the shell after evacuation. Previous work using housed crabs that had not been shocked and offered a preferred shell typically move to a new shell more quickly compared to when a lower gain is made (Elwood and Stewart, 1985). However, in the present case crabs were shocked and some seemed to remain naked for some time, apparently taking time to recover from the noxious stimulus. Indeed, many did not reenter the shell at all during the 15 min observation after shock but shell species did not influence that decision either. However, for those that returned to the shell there was less investigation of the preferred species prior to reentry, i.e. they were accepted more quickly as predicted by previous studies of information gathering, motivation and decision making (e.g. Jackson and Elwood, 1989; Elwood and Stewart, 1985; Elwood, 1995). Further, once the crabs moved back to their shells, those in the preferred species thrust their abdomen in and out of the shell less often than did those in the unpreferred species. This activity is frequently seen when crabs take a new shell and has been considered to be part of a continued evaluation of the shell (Elwood and Briffa, 2001). Thus, as predicted, crabs showed less investigation of the preferred species of shell prior to accepting them. However, we should note that the investigation of shells in which the crab had been shocked tended to be prolonged when compared to the very brief investigation of crabs that had been cracked out of shells and offered a shell (Elwood and Stewart, 1985) or one that had been evicted in a fight and then gained access

to a vacant shell (Dowds and Elwood, 1983). Some crabs in the present experiment reached deep inside the shell, often using both chelipeds at the same time such that the head was placed into the shell. It is possible that the shock produced a low motivation to reoccupy the shell and results in a prolonged investigation (Jackson and Elwood, 1989). Alternatively, it could be that the crab associates the shock with some adverse object in the shell that it attempts to remove. If sand is placed in a shell offered to a naked crab it often attempts to manipulate and scrape out the sand by use of chelipeds (Elwood and Briffa, 2001). Either interpretation suggests an awareness of the site of a problem.

The aim of this experiment was to shock on the abdomen and thus autotomy of thoracic appendages by seven crabs was not expected. Perhaps these crabs had not fully emerged to a normal posture when a shock was given, and thus it was delivered near to the chelipeds, or perhaps the shell had constrained and channeled the electric field and thus affected the chelipeds. Autotomy is commonly seen in crustaceans and has been elicited by injecting noxious, potentially painful substances into legs of crabs, *Carcinus maenas* (Barr et al., unpublished data).

Hermit crabs that lack shells are exposed to predation and physical damage and thus crabs do not normally leave shells (Elwood and Neil, 1992). They emerge briefly for mating, but even here they hold on to the shell with the tip of the abdomen (Elwood et al., 1995). They may evacuate a shell after being attacked in a shell fight, however, a loser of a fight stays near to the scene and waits for the victor to take its choice of shell. It then swiftly takes whichever shell remains (Dowds and Elwood, 1983). However, in the present study, some of the animals went away from the shell after being shocked. These crabs often climbed the wall of the container, which might be an attempt to escape from the area. This indicates the aversive nature of the experience and attempting to escape from the source of a noxious stimulus is another criterion for demonstrating pain (Elwood et al., 2009). This would have been demonstrated just by evacuation from the shell but moving away from the shell in a vulnerable, naked state is a much more extreme escape response.

A small number of crabs rapped their abdomen against the shell after evacuation, a behaviour not seen in naked crabs examining shells under normal conditions. Rapping against a shell, however, typically occurs when hermit crabs fight over shells (Briffa et al., 1998; Elwood and Glass, 1981). It is performed by one crab, the attacker, against the shell of the defender. It normally occurs when the attacker's abdomen is still in its original shell and when a real opponent exists, which the attacker is attempting to evict from the shell. It has been observed, however, with naked attackers, which had been experimentally cracked out of their shell, attempting to evict a housed defender (Dowds and Elwood, 1985). In the present case, the electric shock seems to have produced a response suitable if another crab was still inside the shell defending itself. In certain respects it is similar to the shock-induced aggression seen in rats where, if one of two rats is shocked, it may launch an attack on the other rat (Hutzell et al., 1972).

Rubbing or grooming by vertebrates at the site of a noxious stimulus has been suggested as a criterion for pain (Weary et al., 2006: Barr et al., 2008; Elwood et al., 2009). Grooming or rubbing at the specific point of stimulation indicates that the animal is aware of the location of the noxious stimulus (Barr et al., 2008). That a crab showed grooming/rubbing of the abdomen after shock is similar to the rubbing of lips of trout when injected in the lip with a noxious, pain-inducing substance (Sneddon, 2004) and is also consistent with the idea of the shock inducing pain in crabs. We have not seen this activity before in hermit crabs during three decades of study.

We conclude that hermit crabs are capable of nociception because a reflex response (shrugging) was observed in response to an electric shock. The voltage threshold did not differ between crabs in different shell species. However, evacuation of shells did differ in a manner consistent with a trade-off between motivational priorities. That indicates further neural processing of a sensation comparable to pain, which competes with the motivation to keep the shell as a shelter. Additionally we can say that hermit crabs are able to locate the source of the shock as they evacuate and often investigate the inside of the shell carefully. Some show extreme escape responses by attempting to climb the walls of the observation chamber, some show rubbing/ grooming of the site of the stimulus and others engage in shell rapping that is normally seen in a shell fight. All of these responses are consistent with criteria suggested to indicate pain (Sherwin, 2001; Elwood et al., 2009) and add further weight to the suggestion that pain is felt by crustaceans (Lozada et al., 1988; Broom, 1998; Sherwin, 2001; Barr et al., 2008).

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