Trade-offs between predator avoidance and electric shock avoidance in hermit crabs demonstrate a non-reflexive response to noxious stimuli consistent with prediction of pain

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ABSTRACT

Arthropods have long been thought to respond to noxious stimuli by reflex reaction. One way of testing if this is true is to provide the animal with a way to avoid the stimulus but to vary the potential cost of avoidance. If avoidance varies with potential cost then a decision making process is evident and the behaviour is not a mere reflex. Here we examine the responses of hermit crabs to electric shock within their shell when also exposed to predator or non-predator odours or to no odour. The electric shocks start with low voltage but increase in voltage with each repetition to determine how odour affects the voltage at which the shell is abandoned. There was no treatment effect on the voltage at which hermit crabs left their shells, however, those exposed to predator odours were less likely to evacuate their shells compared with no odour or low concentrations of non-predator odour. However, highly concentrated non-predator also inhibited evacuation. The data show that these crabs trade-off avoidance of electric shock with predator avoidance. They are thus not responding purely by reflex and the data are thus consistent with predictions of pain but do not prove pain.

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1. Introduction

Pain in animals has been 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). The initial perception involves nociceptors and it is possible for these to trigger a reflex response to move part or the whole of the organism away from the stimulus without the emotional experience (Elwood et al., 2009). Thus, mere withdrawal from the stimulus is not evidence of pain because it is easily explained as a nociceptive reflex. Therefore other approaches are required by which various behavioural and physiological criteria (Bateson 1991; Elwood 2012; Sneddon et al., 2014) may be tested and only if fulfilled can pain be deemed a possibility. In particular, responses that cannot be just a reflex are required before the idea of pain can be entertained.

For example, octopus show prolonged, apparently non-reflexive activities directed at the site of a wound (Alupay et al., 2014). Squid show greater responsiveness to approaching stimuli after an experimentally induced localised injury (Crook et al., 2011). Further, squid that have the sensory input of a localised injury blocked by local anaesthetic do not survive an interaction with a predator as well as those not blocked. However, just the local anaesthetic without the wound had no detrimental effect (Crook et al., 2014). That is, perception of the input provides long-term protection and increases survival.

With respect to decapod crustaceans, long term rubbing in prawns (Barr et al., 2008) and hermit crabs (Appel and Elwood, 2009a,b) indicates an awareness of the location of the wound site (Weary et al., 2006) and the prolonged, complex rubbing appears to be beyond a reflex (Elwood, 2011). Further, avoidance and discrimination learning has been demonstrated in shore crabs where they avoided a shelter in which they received a noxious stimulus and developed a preference for a similar shelter, in which no shock was given, offered during the same trial (Magee and Elwood, 2013). This long-term reduction of tissue damage by avoiding the noxious stimuli in the future is a key criterion for pain (Bateson, 1991). Further, hermit crabs that have been shocked within their shell show an increased likelihood of changing shells that lasts at least 24 h (Appel and Elwood, 2009a; Elwood and Appel, 2009) indicating a long-term shift in motivation to avoid the shell in which shock was experienced. Further evidence consistent with pain is a general anxiety after noxious stimuli, as evidenced by crayfish avoiding brightly lit areas of a maze after being repeatedly subject

to an aversive electric field in another arena (Fossat et al., 2014, 2015). Physiological change consistent with stress after stimulation has also been noted (Dyuizen et al., 2012; Fossat et al., 2014, 2015; Elwood and Adams, 2015).

Because a reflex reaction should be fairly consistent in magnitude regardless of other behavioural priorities a demonstration of trade-offs between avoidance of the noxious stimulus and access to another resource indicates that some central processing has occurred and that the response is not reflexive. Thus fish will continue to feed for longer in an area in which they receive shocks if they have been deprived of food (Millsopp and Laming, 2008). Similar trade-offs have been demonstrated in hermit crabs (Elwood and Appel, 2009), which may evacuate their shells if shocked within the shell. However, they are more likely to evacuate from *Gibbula* spp. than *Littorina* spp. shells. Female hermit crabs in *Gibbula* shells have a lower production of eggs than those in *Littorina* spp. shells (Elwood et al., 1995) and *Gibbula* shells are perceived by crabs of both sexes as being of lower quality (Elwood, 1995). Thus hermit crabs appear to trade-off shell quality with shock avoidance. The preference in shells is not due to their weight to volume ratio but, rather, to the internal shape (Elwood et al., 1979). However, the difference in shape might provide an alternative explanation for the apparent trade-off. It is possible that the wires fixed to the inner whorls of the shells to deliver the shock made closer contact with the crab's abdomen when in the *Gibbula* shells than in the *Littorina* shells and thus the effect of the shock might have been physically greater in the *Gibbula* shells. Even if the wires and the abdomen had similar contact it is possible that the different shape of the shell might have modified the electric field and altered shock intensity. If the shock was of greater intensity in the *Gibbula* shells then crabs would be more likely to evacuate from the shells (Appel and Elwood, 2009a). Thus the previous claims of motivational trade-offs between avoidance of shock and retention of high quality shells (Appel and Elwood, 2009a; Elwood and Appel, 2009) might be due to an artefact of the experimental approach. Here we present an alternative method to test if hermit crabs trade-off shock avoidance with another motivational requirement.

Specifically, we test for a trade-off between shock avoidance and predator avoidance. There are numerous studies on a wide range of taxa that have demonstrated the detection and avoidance of predator odours (Kats and Dill, 1998). However, the response to predator stimuli depends on other factors such as the requirement to feed (Lima, 1998). Here, we shock hermit crabs that are all housed in one species of shell in the presence of odours of a potential predator and compare their responses with hermit crabs shocked when no odour or the odour of non-predators are present. If predator odours are normally avoided we anticipate that hermit crabs exposed to such odours should be less likely to leave their shells as the odour should indicate high predation risk (Elwood and Neil, 1992). We shock the hermit crabs, initially with low intensity shock, and increase the shock level until a previously determined maximum is reached. We examine if there is a difference in any initial response to electric shock to determine if the different odours affect minimum perception of electric shock. We examine if the intensity of the shock required to cause the hermit crab to exit the shell differs between the groups in a manner consistent with the idea of trade-off. Because we set a maximum intensity of shock to be applied we also examine if the proportion of hermit crabs exiting the shell differs between the odour treatments. We further examine if crabs that exit the shells are more likely to remain in contact with the shell in the predator odour groups. Because it is difficult to judge what would be a 'natural' concentration of odours in the field we vary the concentration of the odour of both predator and non-predators. Finally, because crustaceans show sex differences in response to noxious stimuli (Appel and Elwood, 2009a) we examine if sex affects responses in the present experiment.

2. Methods

Hermit crabs, *Pagurus bernhardus*, were collected at low tide from rock pools on the shore at Ballywalter, Co. Down, Northern Ireland (54°32′0″N, 5°29′0″W). European shore crabs, *Carcinus maenas*, were collected using baited crab pots from Bar Hall Bay, Strangford Lough, Co. Down (54°23′0″N, 5°33′0″W). Edible mussels, *Mytilus edulis*, were collected from the shore in Belfast Lough at Holywood, Co. Down (54°38′44″N, 5°49′51″W). They were all collected late February 2011 and then transported to Queen's University Belfast and housed in aerated seawater tanks, maintained at 11–13 °C on a 12:12 light:dark cycle, where they were allowed to acclimatise for 5 days prior to testing. All seawater used in the experiment was collected from Queen's University Belfast Marine Station in Portaferry, Co Down via a pump from the sea.

Shore crabs (N = 5) with a wet weight of 634.51 g were kept with 754.1 g of *Ascophyllum nodosum* (also collected from Bar Hall Bay) for shelter. Mussels (N = 41) with a wet weight of 635.6 g were also kept with *A. nodosum*, 754.15 g, for shelter and substrate. The seawater, 6 l, in the holding tanks of the shore crabs and mussels was changed 24 h before use in experiments. Goldfish food flakes, 0.250 g, were added for both mussels and crabs after the water was changed, for feeding the mussels the flakes were ground to a powder using a mortar and pestle. If there was no testing scheduled, either pre-experiment, over the weekend or post-experiment, the water was changed every 3 days and the crabs and mussels were fed.

The hermit crabs were also maintained in a tank with *A. nodosum* for shelter. The water was changed every 3 days and they too were fed fish flakes. Hermit crabs were cracked out of their shells using a bench vice the day before testing, weighed and offered an experimental *Littorina obtusata* shell. The experimental shells were previously prepared as per Elwood and Appel (2009), in that two small holes were drilled and electrodes inserted and cemented in place to allow the electric shock to be applied to the abdomen. After a shell had been used the electrode wires were replaced to reduce the effects of oxidation reducing conductivity on the exposed copper over time. The experimental shells were offered to the crab in a round observation dish (100 mm diameter × 50 mm height) filled with 100 ml of aerated seawater, with a layer of gravel on the base. The copper wires were coiled and fixed to the side of the observation dish to allow the crab movement around the dish. The crabs were allowed to habituate to the observation dish for 24–36 h. Testing occurred in early March 2011 between 0800 and 1700 h and following this crabs were sexed and then offered ordinary shells before transportation back to the shore.

On the day of testing hermit crabs and their observation dishes were placed in an observation chamber behind a one-way mirror, the wires were connected to a Grass S9 electric stimulator. Hermit crabs were randomly assigned to one of five treatment groups, shore crab odour, diluted shore crab odour, mussel odour, diluted mussel odour and a control of just seawater. The shore crab odour was presented by adding 1 ml of seawater taken via a syringe from the *C. maenas* holding tank. Diluted shore crab odour was created by mixing 1 ml of the shore crab odour with 99 ml of aerated seawater. Mussel and diluted mussel odours were created using the same method. The control treatment was 1 ml of aerated seawater added to the observation dish.

After a treatment had been added to the dish it was allowed to mix for 1 min before electric shock delivery began. Single shocks were delivered for 200 ms at a frequency of 180 Hz commencing at 1 V. The voltage increased by 1 V every 10 s until either the crab