CAN INVERTEBRATES SUFFER? OR, HOW ROBUST IS ARGUMENT-BY-ANALOGY?

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Abstract   *Animal Welfare 2001, 10:* S103-118

It is a popular notion that, compared to vertebrates, invertebrates have a reduced capacity to experience suffering. This is usually based on arguments that invertebrates show only simple forms of learning, have little memory capacity, do not show behavioural responses to stimuli that would cause 'higher' vertebrates to exhibit responses indicative of pain, and have differences in their physiology that would preclude the capacity for suffering. But, how convincing is this 'evidence' of a reduced capacity to suffer? Suffering is a negative mental state – a private experience – and, as such, it cannot be measured directly. When assessing the capacity of an animal to experience suffering, we often compare the similarity of its responses with those of 'higher' animals, conceptualized in the principle of argument-by-analogy. By closely examining the responses of invertebrates, it can be seen that they often behave in a strikingly analogous manner to vertebrates. In this paper, I discuss published studies that show that invertebrates such as cockroaches, flies and slugs have short- and long-term memory; have age effects on memory; have complex spatial, associative and social learning; perform appropriately in preference tests and consumer demand studies; exhibit behavioural and physiological responses indicative of pain; and, apparently, experience learned helplessness. The similarity of these responses to those of vertebrates may indicate a level of consciousness or suffering that is not normally attributed to invertebrates. This indicates that we should either be more cautious when using argument-by-analogy, or remain open-minded to the possibility that invertebrates are capable of suffering in a similar way to vertebrates.

Keywords: *animal welfare, argument-by-analogy, invertebrates, pain, suffering*

Introduction

Humans often treat invertebrates in a manner that indicates we believe they do not experience negative mental states (eg pain, distress and suffering), or that they have a reduced capacity for these compared with vertebrates. For example, scientists conduct invertebrate dissections without anaesthetic, veterinarians administer insecticides they believe cause pain when ingested by vertebrates, householders keep homes free of invertebrate pests by methods most of us would consider inhumane if used for vertebrates. This disparity in the way that we regard the capacity of invertebrates and vertebrates to experience negative mental states is considerably reinforced by legislation. In the United Kingdom, animal experiments are regulated by the Home Office by means of the Animals (Scientific Procedures) Act 1986 (GB Parliament 1986). This government department (and most comparable legislation departments of other countries throughout the world) decrees that any procedure which may

cause pain, distress or suffering must be licensed, but this is only applicable to vertebrates and the common octopus (*Octopus vulgaris*), implying that all other invertebrates are incapable of experiencing pain, distress or suffering. But, is this assumption correct?

Negative mental states are a private experience – we cannot measure them, even in other humans. In essence, then, we must rely largely on behavioural responses in assessing whether another animal is experiencing these, usually summarized in the concept of argument-by-analogy. This is the process in which we observe whether an animal responds to a putatively negative stimulus in a similar way to ourselves, and if it does, we assume the animal's experience must also be analogous. So, if a chimpanzee withdraws her hand after receiving an electric shock, we conclude the chimpanzee's experience was similar to ours, ie she felt pain. If we are to be consistent when using argument-by-analogy to assess suffering in non-human animals, we should apply the same reasoning when observing the responses of invertebrates, unless there is good evidence to the contrary. So, if we observe that a cockroach quickly withdraws her leg after receiving an electric shock, she has behaved in an analogous fashion to the chimpanzee and human, and therefore her subjective experience may be analogous. I stress here that the experience may be 'analogous' – not necessarily 'identical' – in the same way that vision, olfaction, respiration, etc may be analogous but not identical. Invertebrates have different sensory organs and nervous systems and so might perceive nociception or pain in an entirely different way to vertebrates, but still experience a negative mental state. Having to state such reasoning might seem patronizing to some readers, but it is remarkable how conveniently the rationale of argument-by-analogy can be overlooked or even dismissed when it results in our having to think that invertebrates might suffer in a like manner to vertebrates. For instance, it has been argued (Eisemann *et al* 1984 pp 165-166) that:

> Vigorous cleaning and violent struggling by insects which are restrained in spider's webs typify more complex stereotyped reactions. The occurrence of these reactions does not in itself indicate any pain (sic) experience ... The hyperexcitability, ataxia and convulsions characteristic of insects poisoned by DDT and pyrethroid insecticides, for example, have been attributed directly to abnormal repetitive discharges in a variety of neurones – no underlying motivation for this behavior, based on a perception of pain, seems called for.

Whilst this type of argument is strictly correct if the principle of Morgan's Canon is rigidly adhered to (ie 'in no case may we interpret an action as the outcome of the exercise of a higher psychical faculty if it can be interpreted as the outcome of one which stands lower on the psychological scale'), I doubt many people would agree with this line of reasoning if the same statements were made about the behaviour of a bear struggling to escape a hunter's net, or a dog convulsing after having eaten snail pellets.

So, why do we believe that invertebrates are less capable of experiencing negative mental states than vertebrates? 'Evidence' usually put forward is based on several generalizations, ie that invertebrates have largely pre-programmed behaviour patterns, have little memory capacity, show only simple forms of learning, lack behavioural responses to stimuli that would cause us pain, and have qualitative and quantitative differences in their physiology that would preclude, for example, pain (eg Wigglesworth [1980]; Eisemann *et al* [1984]; Fiorito [1986]). In this paper, I review published studies which contradict these generalizations and show that invertebrates can express highly plastic behaviour, have the capacity for advanced forms of learning and memory (perhaps including higher cognitive functions), often respond to noxious stimuli in the same manner as vertebrates, and have

relevant physiology strikingly more similar to vertebrates than we might expect. Based on these studies, it is my contention that we should either remain more open-minded to the possibility that invertebrates might have the capacity to experience suffering (or analogous experience), or we should question the rationale of argument-by-analogy and its sometimes ad hoc application across species. Whilst considering the studies below, it is worth remembering that absence of evidence is not evidence of absence. Animal welfare studies have been conducted almost exclusively on vertebrates – this does not mean that invertebrates would necessarily respond differently in similar studies if these studies were conducted. In addition, any apparent absence of evidence of negative mental states might be as much an indication of the limitations of our experimental techniques and knowledge – rather than an incapacity of the animals.

Memory and learning

It is sometimes stated that invertebrates have memories which are less complex or more short-term than vertebrates and this correlates with a reduced ability to experience suffering. This argument is disingenuous – few would argue that humans with memory impairments are less capable of experiencing pain, frustration and other negative mental states. This argument aside, however, there is evidence that invertebrate memory is considerably more complex and more long-term than is often assumed.

Memory

Like vertebrates, invertebrates have both short- and long-term memory. Dickel *et al* (1998) showed that when cuttlefish (*Sepia officinalis*) were presented with a shrimp in a glass tube, they would initially vigorously attack the shrimp but then quickly learn to inhibit their predatory response. The cuttlefish showed excellent retention between 2–8 min, recovery of the predatory response at around 20min, and good retention after 1h of the training phase. Dickel *et al* concluded that cuttlefish have a short-term memory of 5min which is fully operational at 8 days of age, whereas 60min retention increases progressively between 15 and 60 days of age. Memory has been widely investigated in foraging honeybees (*Apis mellifera*) which use both transient short-term working memory that is non-feeder specific and a feeder-specific long-term reference memory (Greggers & Menzel 1993; Menzel 1993; Wustenberg *et al* 1998). Hammer and Menzel (1995) stated that memory induced in a free-flying honeybee by a single learning trial lasts for days and, by three learning trials, for a lifetime. Yamada *et al* (1992) allowed slugs (*Limax flavus*) to learn the position of two different foods, one of which was laced with quinine. The slugs were then rapidly cooled which suppresses retention of the learned response if applied soon after the procedure, but has no effect if applied later. Thus it differentiates between short- and long-term memory. Using this cooling-induced retrograde amnesia, Yamada *et al* showed that slugs have a short-term memory of approximately 1min and long-term memory of 1 month. The authors suggested that although it is difficult to make inter-study comparisons because of animal and methodological differences, the short-term memory for slugs (and cited references for other invertebrates) was not unusually short, even compared to vertebrate species such as the rat and goldfish.

Declines in the effectiveness of memory as animals get older have been recorded in invertebrates, similar to those for vertebrates. Fresquet and Medioni (1993) trained flies to suppress the proboscis extension response to sucrose solution. Three groups were trained and tested at 7, 30 or 50 days of age. All groups learned, but acquisition of the suppression was slower in flies aged 30 and 50 days compared to flies aged 7 days.

In some invertebrates, memory can survive metamorphosis, paralleling a similar phenomenon observed in (vertebrate) amphibians (Miller & Berk 1977). Preimaginal induction is said to occur when emergent adults of larvae exposed to certain conditions show enhanced preferences or learning. In a review of the subject, Caubet *et al* (1992) described work in which adult fruit flies (*Drosophila* sp.) proceeding from larvae reared in a peppermint-scented medium displayed a preference for a perfumed over a non-perfumed environment. Conversely, if larvae were reared in a standard non-scented medium, the emergent adults showed a spontaneous avoidance of the peppermint-scented environment. Similarly, when grain beetle larvae (*Tenebrio molitor*) were trained in a T-maze using food rewards, there was a high degree of facilitation of learning in the adult beetles when re-tested on the same task; they learned to orient themselves in the maze more easily than adults from inexperienced, control larvae. Memory surviving metamorphosis has also been shown for host selection, species-specific recognition and colony recognition in parasitic wasps (Caubet *et al* 1992).

Regardless of the contention made that short or simple memory indicates an incapacity for suffering or negative mental states, many studies have clearly shown that invertebrate memory is complex and long-term (ie it can last the lifetime of the animal): it therefore has several fundamental characteristics similar to those of vertebrate memory.

Learning

Invertebrates exhibit several forms of complex learning. Although this does not prove the ability to experience negative mental states, it is sometimes argued that invertebrates show only simple forms of learning and that this indicates a reduced capacity for these states.

Conditioned suppression

If an animal can be taught to suppress a response, this indicates its behaviour is not rigidly fixed. Several studies have shown that the behaviour of invertebrates can be influenced in this way. Dethier (1964) cited experiments showing that honeybees can be trained to arrive at feeding places at specific times. The bees suppressed flying activity during the normal periods of flying until the appointed hour, indicating they have 'voluntary' control over what most people might consider to be a relatively fixed pattern of behaviour. Balaban (1993) showed that terrestrial snails (*Helix* spp.) could be trained to associate the acidity of water with receiving electric shocks and suppressed radular rasping on the substrate, and Lukowiak and Syed (1999) showed that aerial breathing attempts by water snails (*Lymnaea stagnalis*) in a hypoxic environment were subject to conditioned suppression by eliciting the pneumostome withdrawal response.

When the foreleg tarsi of a fruit fly come into contact with sucrose, the insect automatically extends its proboscis. It will continue to repeat this time after time, for as long as it is hungry. However, if presentation of the sugar is followed immediately by exposure to a solution of quinine, the fly begins to suppress this response over trials. Fresquet and Medioni (1993) trained flies in a differential conditioning procedure in which a white stimulus signalled a quinine reinforcer whereas a black stimulus was non-reinforced. Three groups were trained and then sequentially tested at 7, 30 or 50 days of age – all groups learned to suppress the proboscis extension response. Similarly, Smith *et al* (1991) showed that honeybees learnt to discriminate between two odours and suppress the proboscis extension response when one of the odours was paired with an electric shock.

All these examples show that responses we might think of as being automatic and rigidly fixed are under at least some voluntary control by the animals, in a manner that would usually be taken as indicative of a degree of consciousness if performed by vertebrates.

Social learning
Social learning is said to occur when social interaction facilitates the acquisition of a novel behaviour pattern. This is well described in vertebrates (eg Heyes & Galef [1996]). Fiorito and Scotto (1992) tested the ability of octopus to learn in this manner by allowing naive individuals (observers) to watch others (demonstrators) conditioned to attack one of two balls that differed only in colour. After being placed in isolation, the observers consistently attacked the same coloured ball as did the demonstrators. This learning occurred irrespective of the colour of the ball attacked by the demonstrators and was more rapid than the learning that occurred during the conditioning of the demonstrator octopus. Fiorito and Scotto concluded that observational learning had occurred (but see Biederman & Davey [1993]; Suboski *et al* [1993]). Among vertebrates, alarm calls or food signals by demonstrators result in facilitation of avoidance or approach behaviour in observers. Suboski *et al* (1993) suggested a similar form of learning occurred in freshwater snails in that feeding behaviour was regulated by food pheromones. Hungry snails, exposed overnight to effluent from non-observable conspecifics feeding on a novel food, approached or avoided that novel food depending on the density of the feeding snails that produced the effluent. Too few snails (0–4) produced no preference for the novel food, an intermediate number (8) produced attraction, and too many (16) produced aversion. It was claimed that demonstrators responded to the novel food by feeding and modulating their release of feeding pheromone.

Perhaps the best-known example of social learning in invertebrates is the 'waggle dance' of bees in which individuals communicate information about the quality, distance and direction of food sources to other members of the hive (see, for example, Hammer & Menzel [1995]; Rohrseitz & Tautz [1999]; Weidenmuller & Seeley [1999]). Furthermore, Seeley and Buhrman (1999) described how, when a honeybee swarm is about to move nest site, scouts locate potential sites in all directions up to several kilometres away, then return and initially advertise a dozen or more of the sites by dancing in the swarm. Some of the dancers progressively stop dancing and others switch allegiance from one site to another until, eventually, the scouts advertise only one site. Within an hour of unanimity amongst the dancers, the swarm lifts off to the chosen site – this is not necessarily the first one that is advertised to the swarm.

Studies on social learning in vertebrates are often taken to indicate a level of awareness. This can be either self-awareness, or awareness of the consequences of the actions of the other animal. Such awareness is not usually attributed to invertebrates, although these studies and others (see below) indicate this reticence might be unjustified.

Generalization of discrimination tasks
Giurfa *et al* (1996) described how honeybees could be trained to discriminate bilaterally symmetrical from non-symmetrical patterns, and that this could be applied to novel stimuli. This shows an ability to detect and generalize the concept of symmetry and asymmetry, but also intriguingly would indicate a high level of intelligence when intelligence is defined as the ability to utilize acquired information in a novel situation.

The learning and memory of invertebrates has been widely investigated. It has been shown that invertebrates are capable of learning in several ways very similar to vertebrates: for example, slugs are capable of first- and second-order conditioning, blocking, one-trial

associative learning and appetitive learning (Yamada *et al* 1992). In a comprehensive review of invertebrate learning and memory, Carew and Sahley (1986 p 473) were so impressed by the learning capabilities of invertebrates they were moved to write:

> In fact, the higher-order features of learning seen in some invertebrates (notably bees and *Limax*) rivals that commonly observed in such star performers in the vertebrate laboratory as pigeons, rats, and rabbits.

Spatial awareness and cognitive maps

Some invertebrates appear to have a great awareness of their environment and their spatio-temporal position within that environment, indicating plasticity in behaviour and the ability to monitor and memorize both time and motion. This is perhaps demonstrated at its simplest level in maze learning by cockroaches (*Periplaneta* sp.; cited by Geissler & Rollo [1987]) which can be explained by relatively simple rules-of-thumb, but other forms of spatial awareness, or how invertebrates find their way about the environment are not so easily explained.

Earwigs (*Labidura riparia*) taken from the wild and removed to the laboratory initially oriented themselves correctly toward their home shore by using the sun and moon as orienting cues (Ugolini & Chiussi 1996). However, after 1 week this direction had been forgotten, indicating landward direction was learnt and not genetically determined.

The possible ability of insects to form cognitive maps has received much attention (see, for example, Beugnon *et al* [1996]). Menzel *et al* (1998) displaced bees caught either at feeders or at the hive entrance. They found that the bees' return journey sometimes included novel shortcuts, indicating formation of a cognitive map, but this was in one direction only (to the hive) and only when the bees had been displaced from the hive, not the feeders. The authors therefore suggested it would be premature to conclude that bees can form a cognitive map.

Some spiders appear to be highly cognisant of their surroundings and their movements in time and space. Wandering spiders (*Cupiennius salei*) have been shown to use highly developed visual systems when locating and chasing prey. Seyfarth *et al* (1982) painted over the eyes of wandering spiders then placed them into an arena and allowed them to briefly encounter prey. The experimenters then removed the prey and chased the spider away in a straight line to a distance of up to 75cm from where the prey had been encountered. Despite the lack of visual cues, the spiders were able to move back accurately (ie within 5cm) to the area where the prey had been caught, at which point they would often commence searching behaviour. More surprisingly, if after encountering prey the spiders were chased through a semicircular corridor, they did not simply retrace this curvilinear route. Rather, they chose a straight, direct path to the site of the prey encounter although there was some bias in starting toward the corridor and the shape of some return paths reflected the curved shape. These experiments showed that spiders use idiothetic orientation, ie they memorized information about their previous movements. Seyfarth *et al* (1982) cite other studies showing that spiders use this ability for egg-sac retrieval and prey recapture in the wild. These indicate that some spiders have not only proprioreceptive capabilities, but they also appear to be aware of these in relation to space and time in the form of a simple cognitive map.

Some invertebrates show detour behaviour, in which an animal chooses to take an indirect route to a goal, rather than the most direct route. This is pertinent because it indicates flexibility in behaviour and route planning, and possibly insight learning. Jackson and

Wilcox (1993a) reported that jumping spiders (*Portia* sp.) in the wild scanned the environment surrounding the web of potential prey before moving to capture the prey, but sometimes chose an indirect route on up to four occasions during a single attack. The median time spent out of sight of the prey was 17min and the detouring distance 38cm, although in some attacks this was as great as 209cm. It is unlikely the spiders were simply wandering away and then inadvertently relocating the web because those spiders which did not scan the environment did not find the web, whereas all those spiders which scanned did find the web. Controlled studies on detour behaviour have been conducted under laboratory conditions. Spiders will successfully navigate an apparatus that requires them initially to move away from a prey item before reversing direction (Tarsitano & Jackson 1992, 1994; Carducci & Jakob 2000). Successful navigation was dependent on the presence of a prey item in the goal area, indicating the detour behaviour was not simply aimless wandering by the spiders. The spiders would stop and scan their environment prior to a detour being required, much as if they were planning which route was the next best – possibly indicating a capacity for insight learning. Certainly, such behaviour indicates these spiders have a great ability to comprehend the complex spatial relationship between themselves, their prey and possible routes to a goal.

Deception

Portia also uses an intriguing method of capturing prey which appears to involve deception (Jackson & Wilcox 1993b). A hungry *Portia* invades other spiders' webs and then makes a wide range of vibratory behaviours, including twitching its abdomen, and plucking, striking and fluttering movements using virtually any combination of legs and pedipalps at various phases, rates and amplitudes. When the prey spider moves or performs pull-ups (its normal response to web invasion by a conspecific), the *Portia* repeats the vibration given immediately prior to this until the prey moves close enough to be attacked. Jackson and Wilcox (1993b) cite categorization of deception into four levels: level 1 – mimicry in which deception is effected by appearance; level 2 – deception is effected by co-ordinated perception followed by action; level 3 – deception effected by learning; and level 4 – planned deception. The authors suggest this is an example of level 3 deception, apparently a rare ability in invertebrates.

Effect of environmental enrichment

There have been many studies on the effects of environmental enrichment on the behaviour and physiology of vertebrates. In general, these studies report that environmental enrichment leads to changes such as enhanced learning ability, advanced neuronal development, and reduced abnormal behaviours, eg stereotypies. Carducci and Jakob (2000) showed that the learning behaviour of jumping spiders (*Phidippus audax*) was significantly affected by rearing environment in ways similar to those recorded for vertebrates. Field-caught spiders were more likely than laboratory-reared spiders to react to videotaped prey, to progress further on a detour test, and to be less stereotactic in an open field. Larger cage size and the presence of dowel as an enrichment also improved performance in several tests. Increases in the volume of mushroom bodies (implicated in memory) in insect brains have been linked to more varied experience in bees (Durst *et al* 1994). Carducci and Jakob (2000) concluded that rearing conditions 'which are commonly employed by behavioural researchers, may profoundly influence the behaviour of adult spiders', a remarkably similar conclusion to that reached by many researchers working on the effects of environment on the behaviour and welfare of vertebrate species.

Preference tests and consumer demand studies

Preference tests and consumer demand studies are used to develop our understanding of what animals prefer and the strength of their motivation about aspects of their environment. Although it is possible for choices to be made without requiring consciousness, this is not usually considered to be the case for vertebrates and it has been suggested that these tests can tell us about animals' 'feelings'. Invertebrates also exhibit many preferences: woodlice quickly seek darkness when the rock they are sheltering under is removed; and entire industries have been built on the notion that insect repellents are effective. However, they also exhibit many more complex learned preferences. It could be argued that if invertebrates exhibit preferences or perform similarly in consumer demand studies, they must experience something analogous to vertebrate 'feelings' and 'motivations'.

Preimaginal learning of preferences has been reported in various species, for example, preimaginal feeding preferences by *Drosophila*, lepidopterans and dipterans, preimaginal habitat preferences by grain beetles, *Drosophila*, and formacine and ponerine ants, and preimaginal host selection preferences by hymenopterans (Caubet *et al* 1992).

Adult invertebrates also exhibit preferences. Slugs showed a preference for food items that contained nutrients missing in previous diets, indicating diet selection is based on their internal nutritional status (Cook *et al* 2000). Dethier (1964) described the several changes in preferences of flies for concentrated sucrose solution that occur throughout the lifecycle.

Learned aversions have been described for a variety of invertebrates under many circumstances (eg see Cook *et al* [2000]). The rapidity with which these aversions can be learnt is rather surprising: for instance, Yamada *et al* (1992), Sahley *et al* (1981) and Gelperin (1975) all reported that slugs were able to learn to avoid food after only one pairing with an aversive experience (quinine ingestion or CO_2 toxicosis). Carew and Sahley (1986) and Krasne and Glanzman (1995) comprehensively reviewed learning and memory in invertebrates. They cited a plethora of studies showing associative learning with subsequent preferences in species such as bees, slugs, marine molluscs, pond and land snails, leeches, locusts, cockroaches, fruit flies, blowflies, *Aplysia* and *Hermissenda*. It is poignant to note that when such studies, especially learned aversions, are conducted using 'higher' vertebrate species, we almost unquestioningly accept that this learning involved subjective states or 'feelings' such as 'sickness', 'hunger' or perhaps 'fear'.

Consumer demand studies measure the strength of motivation an animal has for a resource. The principle is that an animal will work harder for a resource that it considers more important, or when it is more highly motivated for the resource. For example, we might expect a hungry animal to work harder to get food than would a satiated animal. There is no doubt that invertebrates respond differently to varying levels of internal state. Papini and Bitterman (1991) trained octopuses (*Octopus cyanae*) to move down a runway to gain access to pieces of squid. A large reward produced faster acquisition when reinforcement was consistent, and better subsequent performance on a partial reinforcement schedule than did a small reward. Similar results have been gained with bees (eg Lee & Bitterman [1990]) and flies (Dethier 1964). Walker *et al* (1999) showed that wolf spiders changed locomotion rates with increasing food deprivation, but this was species dependent: *Pardosa milvana* increased its locomotion and was suggested to be an active forager, whereas *Hogna helluo* decreased locomotion and was deemed to have a sit-and-wait strategy. This dichotomy of strategy amongst comparatively similar animals suggests we must be cautious when interpreting non-activity as an indicator of the absence of a hunger state or lack of motivation in invertebrates. I am unaware of any studies using invertebrates to construct formal consumer demand

curves; this could prove a most fruitful area in understanding the perceptions and motivations of these creatures.

Operant responding

Operant studies using vertebrates have been conducted for many years. In such studies, an animal operates a manipulandum or changes the environment in some way to gain a positive reinforcement or avoid a negative one. Operant responses indicate a voluntary act; the animal exerts control over the frequency or intensity of its responses, making these distinct from reflexes and complex fixed motor patterns. A number of studies have revealed surprising similarities between vertebrates and invertebrates in their capacity to use operant responses to gain positive, or avoid negative, reinforcement.

Dethier (1964) stated that flies could not be trained to bar-press, but *Drosophila* can operantly learn specific flight or locomotor patterns under the control of an external reinforcer (see Kisch & Erber [1999]). More convincingly, cockroaches and locusts can be conditioned to hold one leg in a certain position to prevent punishment (Hoyle 1979 cited by Kisch & Erber [1999]) or receive a reward (Horridge 1962 cited by Kisch & Erber [1999]).

An operant learning protocol was developed by Kisch and Erber (1999) in which honeybees (with their eyes painted black) were fixed in small tubes such that they could touch a small silver plate with an antenna. The spontaneous frequency with which the bees touched the plate was recorded. When touching of the plate at a frequency above the spontaneous frequency was reinforced with sucrose solution being fed to the bee, there was a significant increase in touching of the plate during the subsequent test session. In a second test, the bees could touch either of two plates, one above and one below the antenna. Initially, the plate which was touched least frequently during determination of the spontaneous frequency was reinforced, which resulted in it being touched more frequently than the other, ie discrimination learning. Even more surprisingly, when the position of the plate being reinforced was swapped, this was soon learnt by the bees, ie reversal learning. In this series of experiments, control animals showed none of these forms of learning.

In another highly interesting experiment, it has been shown that snails will operate a manipulandum to electrically self-stimulate areas of their brain. Balaban and Maksimova (1993) surgically implanted fine wire electrodes in two regions of the brains of snails (*Helix* sp.). To receive electrical stimulation of the brain, the snail was required to displace the end of a rod. When stimulation was delivered to the parietal ganglion, the snails decreased the frequency of touching the rod compared to the baseline spontaneous frequency of operation. But, when self-stimulation was delivered to the mesocerebrum (which is involved in sexual activity) the snails increased the frequency of operating the manipulandum compared to the baseline spontaneous frequency. These are negative and positive reinforcement responses typical of those seen with vertebrates. It is interesting to reflect that if this experiment had been conducted with a vertebrate species, we would almost certainly ascribe these responses as being due to the animal experiencing sensations of pain or discomfort when self-stimulating the parietal ganglion, and pleasure when self-stimulating the mesocerebrum.

Nociception and pain

Pain presumably evolved to cause animals to behave to protect themselves from further harm after injury. The capacity to experience pain would therefore have a great selective advantage to invertebrates, which indicates that they might also have evolved a sensation of pain or a similar protective mechanism. Doubt about the ability of invertebrates to feel pain has been

expressed (Eisemann *et al* 1984; see also Wigglesworth [1980]), perhaps most recently by Varner (1999) who wrote:

> Although I agree with Rollin that it would be implausible to deny that any nonhuman animals can feel pain, when we look closely at the relevant behavioural and neurophysiological evidence, we see that a good case can be made for saying that invertebrates (with the possible exception of cephalopods) probably cannot feel pain.

Because pain is a negative mental state, it too is a private experience and we must again turn to argument-by-analogy in assessing its possible existence in other species. When we do this for invertebrates, it is apparent they often respond in a similar way to vertebrates, indicating a similar, or analogous, subjective experience.

Pain in invertebrates – physiological evidence

The physiological responses of some invertebrates to putative pain-inducing stimuli can be remarkably similar to those of vertebrates. At the cellular level, injury or wounding of invertebrates leads to the directed migration and accumulation of haematocytes (defence cells) and neuronal plasticity, much the same as the responses of human patients undergoing surgery or after injury (Clatworthy 1996; Stefano *et al* 1998). There are also similarities between vertebrates and invertebrates in the neurochemistry involved in vertebrate pain reception or mediation. It has been found that molluscs (Kream *et al* 1980 cited by Greenberg & Price [1983]) and insects (Stefano & Scharrer 1981 cited by Eisemann *et al* [1984]; Nunez *et al* 1983; Zabala *et al* 1984 cited by Fiorito [1986]) have opioid binding sites or opioid general sensitivity. Certainly there are many examples of neuropeptides involved in vertebrate pain responses being found in invertebrates (Clatworthy 1996; Stefano *et al* 1998), for example, enkephalin/endorphin has been found in platyhelminthes, molluscs, annelids, crustaceans and insects (Greenberg & Price 1983; Fiorito 1986). As pointed out by Greenberg and Price (1983), the occurrence of vertebrate pain-related neuropeptides in invertebrates does not necessarily mean that invertebrates experience pain; analogous physiological roles in different classes or phyla are rarely carried out by homologous peptides, but it does at least indicate that many invertebrates might have the physiological capacity to experience pain or an analogous sensation. Perhaps more convincing is that, in molluscs, naloxone injections (but not other neuroactive substances) into the sites of severed nerves counteract the migration of haematocytes in response to the injury, indicating the involvement of opioid peptides in this response (Clatworthy 1996). In support of this, injection of a synthetic analogue of Met-enkephalin induces the directed migration of haematocytes to the site of injection. Furthermore, Clatworthy, in discussing the responses of nociceptors to damaging or potentially damaging stimuli, wrote:

> The enhancement of responsiveness in these sensory neurones following injury or the induction of a foreign body response is therefore functionally similar to hyperalgesia, ie a heightened sensitivity to painful stimuli, in mammal(s).

More recently, Stefano *et al* (1998) reported that invertebrates contain an opioid precursor, proenkephalin. Enkelytin, an antibacterial peptide, is found in proenkephalin, exhibiting 98 per cent sequence identity with mammalian enkephalin. Stefano *et al* suggested that the function of enkelytin is to attack bacteria and allow time for the immunocyte-stimulating capabilities of the opioid peptides to emerge. Furthermore, based on the similarity of the

biochemical and physiological responses, they proposed that pain itself may be a component of this response.

Pain in invertebrates – behavioural evidence

Eisemann *et al* (1984) dismissed the possibility of pain experience in invertebrates (or at least insects) by stating:

> No example is known to us of an insect showing protective behaviour towards injured body parts, such as by limping after leg injury or declining to feed or mate because of general abdominal injuries.

But we must be careful in interpreting the absence of a response to injuries as indicating an animal is insensitive to pain. For vertebrate prey species, there is often a selective advantage in not showing pain or injury as this might cause the individual to be targeted by a predator. Similarly, many vertebrates have adopted tonic immobility as a 'last-ditch' strategy to avoid death after being captured by a predator so, despite having injuries we expect cause considerable pain, some vertebrates will show little behavioural disturbance – ie show no discernible evidence of experiencing pain. The same reasoning might also be adopted to explain a lack of response by invertebrates to noxious stimuli when this occurs.

Having said this, there is substantial evidence that invertebrates sometimes do respond overtly to putatively painful stimuli. Adult and larval *Drosophila* (Carew & Sahley 1986), snails (Balaban 1993), leeches (*Hirudo medicinalis*; Sahley 1995), locusts (Horridge 1962), bees (Smith *et al* 1991) and various marine molluscs (Carew & Sahley 1986) all learn to withdraw from, or alter their behaviour in response to, a conditioned stimulus when this has been previously paired with an electric shock; if a vertebrate species is used in such studies, it is usually taken for granted that the learning process is based on the animal experiencing a sensation of pain or discomfort from the electric shock.

Autotomy is a behavioural response in which a limb or part of the body is shed in response to capture (eg Punzo [1997]) or sometimes to stop the spread of potentially harmful stimuli to the rest of the body. Fiorito (1985) reported that crabs exposed to a hot-plate show leg autotomy. There is evidence that, in spiders, this response might be invoked by a sensation similar to human pain. Under natural conditions, orb-weaving spiders (*Argiope* spp.) undergo autotomy if they are stung in a leg by wasps or bees. Under experimental conditions, when spiders were injected in the leg with bee or wasp venom, they shed this appendage. But if they were injected with only saline, they rarely autotomized the leg, indicating it is not the physical insult or the ingress of fluid per se that causes autotomy. Even more interestingly, spiders injected with venom components which cause injected humans to report pain (serotonin, histamine, phospholipase A_2 and melittin) autotomized the leg, but if the injections contained venom components which don't cause pain to humans autotomy did not occur (Eisner & Camazine 1983).

Mental states – learned helplessness

Many vertebrates placed under conditions in which they cannot avoid a repeated negative stimulus (eg electric shock) sometimes show learned helplessness, ie they become lethargic and do not attempt to avoid the negative stimulus. In humans, this condition is associated with chronic frustration and depression. Eisenstein *et al* (1997) stated the major criteria of learned helplessness are: i) following inescapable shock training, the animals often become passive and still when confronted with an escapable shock; ii) failure to escape shock when it

is possible to do this following non-escapable shock; iii) reversion to non-escape behaviour even after successful escape. Balaban (1993) in a study on the learning of terrestrial snails wrote:

> Ten (or in several experiments up to 25) food presentations and electrical shocks presented in an unpaired manner evoked no apparent changes in feeding behaviour in 18 animals (one snail was inactive after unpaired presentation of stimuli).

The behaviour of this one individual has similarities to that of a vertebrate experiencing learned helplessness. Studies have been conducted using 'yoked control' animals in which animal A is able to escape the shock whereas animal B is subjected to the same stimulus contingency but is unable to avoid the shock. Horridge (1962) used this method on decapitated insects. Corresponding legs of two individuals were connected in a series electrical circuit which was completed whenever animal A lowered its leg into a saline bath. When this occurred, both animals received shocks until animal A lifted its leg. Animal B therefore received shocks regardless of its leg position and could do nothing to avoid these whereas animal A only received shocks when its leg was extended and could therefore learn to avoid the shocks. Following a 45min training period, both animals were reconnected for testing. Each animal now received a shock independently of the other when its leg was extended into its own saline bath. If any association between leg extension and shock had occurred during the initial training, then during the test period animal A would be expected to initiate fewer shocks than the now rewired animal B. Horridge (1962) presented data for cockroaches and locusts that showed that A type animals took significantly fewer shocks than B types during the test period. In addition, it was noted that a large proportion of the B type animals hung their legs limply, sometimes for several hours during re-testing and received almost the maximum number of shocks. Out of 20 B type cockroaches of one series, 11 failed to show any signs of leg raising for the duration of the test whereas among the A type animals there was a typical proportion of 5 in 20 that failed to improve – proportions stated as typical of those seen in vertebrate studies. This (lack of) response fulfils the three criteria of learned helplessness listed above, as has similar work on slugs cited by Eisenstein et al (1997). The fact that decapitated animals responded in this way indicates that any neural centres involved (eg pain centres) are not necessarily located in the head region. Even more intriguing is work showing similar non-responses by isolated thoracic ganglia of the cockroach (Eisenstein et al 1997), perhaps indicating that the response is mediated peripherally and might not require consciousness – unless the thoracic ganglia themselves have consciousness. These observations indicate either that slugs, cockroaches and locusts are capable of experiencing learned helplessness similar to that described in vertebrate studies or, alternatively, the criteria of learned helplessness should be re-defined. It is certainly worth considering the ethical nature of performing such experiments on invertebrates if they are able to experience a subjective state similar to learned helplessness in vertebrates.

A possible way forward

Vertebrates are often used as animal models in the development of analgesics and psychoactive drugs, presumably indicating these species experience similar pain or mental states to the humans for which the drugs are intended. I am unaware of any studies that have looked at the effects of analgesics or psychoactive drugs on the behaviour of invertebrates. These might prove profitable in assessing the capacity of invertebrates to also experience similar mental states. For example, recent studies (eg Pickup et al [1997]; Danbury et al

[2000]) have shown that animals in apparent discomfort will selectively choose diets containing analgesics, presumably indicating they experience pain in an analogous manner to humans. It would be a fascinating experiment to determine whether invertebrates will also self-select analgesics or psychoactive drugs in the same way it has been shown they self-select brain stimulation (Balaban & Maksimova 1993).

Final comments and animal welfare implications

Currently, there is much research aimed at differentiating between vertebrate species in their capacity for higher cognitive abilities, for example self-awareness amongst primates (Shillito *et al* 1999). It is sometimes suggested, implicitly or explicitly, that a demonstrated capacity for advanced cognitive abilities makes such species more similar to humans and therefore more likely to experience 'higher' forms of negative mental states such as frustration, depression or boredom. But, such advanced research begs the more fundamental question of whether consciousness and negative mental states are common to both vertebrate and invertebrate species. Precious little work has been directed to specifically investigating whether the widely perceived dichotomy between vertebrates and invertebrates with respect to their experiencing negative mental states has a justifiable basis. If it can be shown that invertebrates are capable of experiencing negative mental states, our treatment of them at all levels of society (eg domestic, agricultural and scientific) would have to change radically if we are to behave in an ethical and responsible manner, and treat them with the same regard we treat 'higher' vertebrates. In addition, if we accept that invertebrates experience negative mental states, then we would have to reconsider our attitudes towards other vertebrate species that we currently treat as if they have a reduced capacity to suffer (eg fish, reptiles and amphibians). Perhaps it is the thought of us having to radically and inconveniently change our attitudes and behaviour that compels us to so quickly accept arguments indicating that invertebrates do not experience pain, distress or suffering, and dismiss evidence to the contrary. After all, if we accept that chimpanzees are capable of frustration, depression and other negative mental states, this is unlikely to have much impact on most of our everyday lives. But, if we have to accept that insecticidal sprays cause immense pain to flies or that treading on an ant causes as much pain as treading on a mouse, few people would wish to acknowledge they were responsible for causing such suffering on a regular basis.

Because mental states are a private experience, we cannot be certain whether other species, vertebrate or invertebrate, have the same subjective experience as humans. For vertebrates, we almost unthinkingly employ argument-by-analogy and accept with little further evidence of consciousness or similarity of nervous function that if an animal responds similarly to the same stimuli, it is presumably having an analogous experience. But, with relatively little justification, we do not apply the same to invertebrates. This has led to the current widely held belief that, whereas vertebrates are capable of experiencing pain, distress and suffering, most invertebrates are not. But, if we employ argument-by-analogy using the evidence presented above, invertebrates often respond to potentially painful or distressing stimuli in a manner strikingly similar to vertebrates, seemingly indicating the capacity for analogous experiences. To dismiss this without irrefutable evidence is to arbitrarily move the goalposts of argument-by-analogy, which then raises the question of how robust is this concept? If it should not apply to invertebrates – why not? Should it apply to all vertebrates – if not, to which ones is it not applicable, and why? If we are to use argument-by-analogy in our interpretation of behavioural and physiological evidence relating to pain, distress and suffering, the evidence above indicates one of three conclusions: i) we should use argument-by-analogy consistently and accept that invertebrates might have experiences analogous to

those of vertebrates; ii) we should accept that argument-by-analogy is fundamentally flawed and cease to use it (raising the question of what is the evidence for negative mental states in other non-human vertebrates?); or iii) we should decide that argument-by-analogy is not robust across all species and that it can only be used for some species in some circumstances. This third option raises the obvious question of how do we decide for which species and under what circumstances argument-by-analogy can be used.

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