

## REVIEW

# Studying emotion in invertebrates: what has been done, what can be measured and what they can provide

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## ABSTRACT

Until recently, whether invertebrates might exhibit emotions was unknown. This possibility has traditionally been dismissed by many as emotions are frequently defined with reference to human subjective experience, and invertebrates are often not considered to have the neural requirements for such sophisticated abilities. However, emotions are understood in humans and other vertebrates to be multifaceted brain states, comprising dissociable subjective, cognitive, behavioural and physiological components. In addition, accumulating literature is providing evidence of the impressive cognitive capacities and behavioural flexibility of invertebrates. Alongside these, within the past few years, a number of studies have adapted methods for assessing emotions in humans and other animals, to invertebrates, with intriguing results. Sea slugs, bees, crayfish, snails, crabs, flies and ants have all been shown to display various cognitive, behavioural and/or physiological phenomena that indicate internal states reminiscent of what we consider to be emotions. Given the limited neural architecture of many invertebrates, and the powerful tools available within invertebrate research, these results provide new opportunities for unveiling the neural mechanisms behind emotions and open new avenues towards the pharmacological manipulation of emotion and its genetic dissection, with advantages for disease research and therapeutic drug discovery. Here, we review the increasing evidence that invertebrates display some form of emotion, discuss the various methods used for assessing emotions in invertebrates and consider what can be garnered from further emotion research on invertebrates in terms of the evolution and underlying neural basis of emotion in a comparative context.

**KEY WORDS:** Affective neuroscience, Biogenic amines, Cognitive bias, Computational modelling, Emotional states, Insects

## Introduction

Invertebrate research has contributed immensely to our understanding of the brain. In terms of basic neuroscience, pioneering studies with invertebrates helped reveal the existence and structure of neurons (Ehrenberg, 1836; Nansen, 1886; Ramón y Cajal, 1909), and how information is transferred between them (Hodgkin and Huxley, 1939). Invertebrate research continued progress in the neurosciences with Kandel and colleagues' work showing the biochemical and neuroanatomical bases of learning and memory (Kandel, 2006).

Invertebrates are still actively used in many areas of neuroscience, particularly when it comes to the dissection of neural circuitry. This is because it has been possible to link the activity of single identified

neurons to behaviour. Hammer (1993) demonstrated in the honeybee that the activation of a single identified neuron could replace sucrose during reward learning. Subsequently, a range of invertebrate studies have pinpointed neural substrates responsible for both reward and punishment, indicating a network of interacting neuromodulators that is organized and functions similarly to the reward system of vertebrates (Perry and Barron, 2013). Using sophisticated genetic tools in *Drosophila*, researchers have analysed distinct elements in food valuation and mapped out neurons involved in certain features of food reward (e.g. sweetness versus caloric content) at a level of detail that is, as of yet, impossible in vertebrate animals (Das et al., 2016). This type of mapping has been applied to many other aspects of behaviour (Iliadi, 2009), including sleep (Donlea et al., 2014), courtship (Dickson, 2008) and social foraging (de Bono et al., 2002).

Invertebrates are often not considered to have the neural requirements for such sophisticated abilities as emotion (see Glossary) and are assumed to accomplish all they do through sensorimotor responses alone (Allen-Hermanson, 2008). However, accumulating evidence suggests a re-evaluation of these views (Perry et al., 2013, 2017). Invertebrate research has now shown a variety of cognitive phenomena that were previously thought to be restricted to vertebrates and, at one point of course, to the domain of humans uniquely, such as concept learning (Giurfa et al., 2001), numerical cognition (Pahl et al., 2013), categorization of stimuli (Benard et al., 2006), cognitive/behavioural flexibility (Loukola et al., 2017; Mather, 2007) and cultural transmission (Alem et al., 2016).

Darwin (1872) may have first suggested that invertebrates express emotions, but only recently has anyone empirically explored this. Before discussing these works, we will first define what we mean by emotions and consider the importance of emotion research in non-human animals, including invertebrates.

## Defining emotion

Emotions are transient central states comprising subjective, cognitive, behavioural and physiological phenomena that are triggered by appraisal of certain types of environmental stimuli (Anderson and Adolphs, 2014; LeDoux, 2012; Mendl et al., 2010; Nettle and Bateson, 2012; Scherer, 2001). For example, fear is an internal state that includes increased attentional bias towards potential sources of danger and physiological preparation for fight or flight responses, which is triggered by the appraisal that there is something dangerous in the environment (Nettle and Bateson, 2012). Our conceptual understanding of an emotion is heavily based on its subjective component: i.e. the experience or 'feeling' (see Glossary) of the emotion usually in terms of pleasure (valence, see Glossary) and intensity (arousal, see Glossary). The subjective part of emotion has been the main focus of emotion theories for quite some time (Nettle and Bateson, 2012; Box 1). Internal states that humans associate with a 'feeling' (e.g. joy, anger, surprise) are what we most strongly consider to be emotions. But these verbally reported subjective

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## Glossary

### Affective neuroscience

The study of the neural mechanisms of emotion.

### Arousal

A specific dimension of the emotional experience within the dimensional theories of emotions (Box 1). It describes the level of intensity (on a scale from low to high) of the reaction towards the stimuli.

### Cognitive bias

Information processing changes caused by a specific emotional state or mood. Three main categories of cognitive biases are: attentional bias (changes in perceptual vigilance), memory bias (changes in storage, consolidation and retrieval of memories) and judgement bias (changes in interpretations of ambiguous stimuli, expectations and risk-taking).

### Conspecific

An animal of the same species.

### Emotions

Transient brain states, triggered by appraisal of the environment and comprising cognitive, behavioural, physiological and subjective components.

### Feeling

The conscious awareness or subjective experience of emotions.

### Haemolymph

Fluid equivalent to blood in invertebrates, but with direct contact with all tissue.

### Proboscis

An elongated tubular mouthpart of many insects for consuming liquid.

### Stridulation

Production of sound by rubbing certain body parts together.

### Valence

A specific dimension of the emotional experience within the dimensional theories (Box 1). It describes the level of pleasantness (on a scale from pleasant to unpleasant) of the event experienced.

feelings are accompanied by cognitive (e.g. perceptual biases), behavioural (e.g. escape behaviour) and physiological (e.g. heart rate) changes (Box 1). By adapting non-verbal techniques used in human psychology, researchers have been able to examine the cognitive, behavioural and physiological components of emotions in a variety of animal species (Boissy et al., 2007; Désiré et al., 2002). We know of no direct evidence of subjective feelings in any animals, but we do not exclude the possibility. We, like others, assume that many animals other than humans, including invertebrates, experience some basic form of subjective experience (Barron and Klein, 2016; Mendl et al., 2010; Nettle and Bateson, 2012; Panksepp, 2005, 2011). What this entails and the ways in which it is like our own subjective experience are currently beyond our scientific capability to investigate. Experimental directions towards answering these questions will include and benefit from, in our opinion, the study of emotion in invertebrates. There is ample evidence now that invertebrates have some form of emotion and therefore future efforts studying all facets of emotion in invertebrates will help provide a more complete picture of how emotion differs across phyla, how emotion has evolved and the neurobiological underpinnings of emotion.

## The importance of animal emotion research

Whether and how invertebrates, or any animal other than humans, experience emotions is of immense societal and scientific concern. From a societal perspective, understanding whether animals have a subjective component of emotion at any level, will be crucial to and will undoubtedly guide how we interact with them (Mendl and Paul, 2004; Dawkins, 2008, 2015). Again, until we develop methods to directly assess the subjective component, we can learn a great deal about emotions from studying the cognitive, behavioural and physiological components (Dawkins, 2015; Mendl et al., 2010;

## Box 1. Theories of emotions

Here, we give a very brief overview of the contemporary cognitive theories of emotion for non-specialists. The philosophical analysis of emotion began with Plato and Aristotle. Both recognized that emotions (pathe) were responses within the body of animals to the outside world. Aristotle recognized 11 (Nicomachean Ethics) to 14 (Rhetoric) distinct emotions (e.g. fear, confidence, envy, joy). Most philosophers agreed that animals possessed emotions but disagreed on which ones they shared with humans and to what level cognitive capacities interacted with emotions. Since then, there have been many proposed theories of emotions that consider how emotions work and to what extent different animals have emotions. The contemporary theories of emotion can be separated into three main categories: basic emotions, dimensional and appraisal theories. A common feature is the idea that emotional processing is a multicomponent phenomenon that comprises subjective experience (the unique direct perception or feeling of the emotional state), cognitive evaluation, behaviour, neurophysiology and motivation (the desire to act or action tendency). The main differences between these theories are whether they emphasize the classification of emotions, the underlying mechanisms or the components of emotion (Désiré et al., 2002).

Basic emotion theories (e.g. Ekman, 1992; Oatley and Johnson-laird, 1987; Panksepp, 1982) are based on neural circuits or facial expressions (and body postures) that underlie/indicate each of the basic emotions (e.g. fear, anger, desire, distress). They postulate a limited set of evolutionarily selected, pre-programmed, universal, basic emotions. More-complex emotions supposedly arise from interactions of these fundamental emotions. Although it has been historically difficult for these theories to actually incorporate and integrate the wide range of experienced emotional states, they have made a substantial contribution to the study of emotions in non-human animals by bypassing the need for verbal reports.

Dimensional theories (Schlosberg, 1954; Russell, 1980) typically represent emotions depicted along two independent axes: the dimension of valence and arousal. These theories and their method of classification are entirely dependent on subjective experience and verbal reports, and they fail to address the causes of emotion. Therefore, they are of little to no utility for assessing non-human animal emotion. However, Mendl et al. (2010) attempted to integrate dimensional and basic theories, proposing methods for assessing a wide range of emotions in non-human animals using non-verbal measurements.

Appraisal theories (Frijda, 1987; Ellsworth, 1991; Lazarus, 1991; Scherer, 2001) focus on the components of emotion, and are based on the idea that emotions are generated by the cognitive evaluation of a situation (Scherer, 2001). Emotions are considered more a process, rather than a state, by which all components (i.e. neurophysiological responses, motor expressions, subjective feelings, motivation, appraisal itself, etc.) constantly influence each other over time and induce a subjective experience over time (i.e. emotional states, moods, psychological states, personality traits). Appraisal theories have been applied successfully to the study of emotions in animals given their reliance on multiple components and independence of verbal reports (Désiré et al., 2002, 2004; Greiveldinger et al., 2011).

A fruitful direction for emotion research will be to combine methods to assess multiple components simultaneously (Reefmann et al., 2009; Briefer et al., 2015; Perry et al., 2016). Anderson and Adolphs (2014) recently proposed a new framework for studying emotions across all animal species. Their approach assumes that emotions are central states of the brain that have properties that are expressed through cognitive, behavioural, physiological and subjective components. They attempt to distil the common features of emotion agreed upon across the different theories of emotion, rather than making comparisons with specific human emotions. These common features include scalability, persistence, valence and generalization to multiple contexts. Hence, despite early concerns about its apparent intractability and lack of suitability for such a human-centric subject, invertebrate research (compare with Gibson et al., 2015; Perry et al., 2016) provides an opportunity for such evaluation alongside powerful methods for unveiling the neural mechanisms responsible for all aspects of emotion.

Nettle and Bateson, 2012; Panksepp, 2011). Concurrently, studying elements of animal welfare, health and survival, which are important independently of whether animals are conscious or not, will garner vital information for how we interact with them and will ultimately affect animals' well-being (Dawkins, 2015). From a purely scientific perspective, it will be necessary to determine whether certain animals have emotions in order to understand how our own emotions have evolved and, even more importantly, this will allow neurobiological approaches to elucidate how the brain produces emotions (Panksepp, 1998, 2005, 2011). In addition, the information obtained from a neurobiological study of emotions in animals will be able to help develop potential treatments for emotion-related cognitive dysfunctions that affect all brains. The subcortical regions of the human brain that have been strongly implicated in emotion are, at present and for the foreseeable near future, almost impossible, ethically, to study in any real detail. What non-human animals offer, especially those of the miniature invertebrate kind, are much more limited and accessible neural architectures – not to replace but to complement human and other vertebrates – to study in much greater detail and with more powerful techniques, largely unrestricted by ethical constraints and allowing for a simultaneous whole-brain system approach.

Various methods have been used to successfully study emotional expression in a variety of mammalian species, and only very recently have some of these methods been adapted to a small number of invertebrate species. Here, we attempt to comprehensively review the studies performed so far that explicitly assess emotions in invertebrates from a multi-componential perspective (Table 1). We have not covered any works examining pain (the unpleasant feeling of tissue damage) in invertebrates because others have already discussed these at great length (e.g. Cooper, 2011; Eisemann et al., 1984;

Sherwin, 2001), but we do briefly describe the current debate within this field and its relation to animal welfare (Box 2).

We begin by considering cognitive approaches, before analysing behavioural approaches and finish by examining physiological methods, discussing the strength and weaknesses of each in turn. Finally, we consider how current and future research on invertebrate emotion might impact our understanding of the neural basis and evolution of emotions, in both invertebrates and vertebrates, and potentially provide opportunities for new avenues for emotion dysfunction research.

### Cognitive approach

Emotions are triggered by appraisals of stimuli within our environment and help us evaluate the importance of immediate situations (Scherer, 2001; Paul et al., 2005; Anderson and Adolphs, 2014). When we experience a change in emotion, there are concomitant changes in how we view the world, i.e. our cognitive processes and our perception of our environment. These links between cognitive processes and emotional states in humans have been demonstrated in numerous tasks involving attention, perception, memory, expectations and risk assessment (Christianson, 1992; Mathews and MacLeod, 1994; Mathews, 1995; Nygren et al., 1996; Cahill and McGaugh, 1998; Mogg and Bradley, 1998; Loewenstein et al., 2001). However, exploration of cognitive components of emotion in non-human animals is only just beginning. Most of the methods used with humans rely on language-based tasks; however, others do not, meaning that some of these non-verbal tasks lend themselves to being adapted to studies in non-human animals. The first measurement of cognitive components of emotion in a non-human animal came as recently as 2004 (Fig. 1). Harding and colleagues (2004) used a paradigm known as the 'judgement bias test'

**Table 1. Summary of studies assessing invertebrate emotions\***

Study	Species	Approach <sup>‡</sup>	Paradigm	Manipulation	Emotion	Amines <sup>§</sup>
Walters et al., 1981	<i>Aplysia californica</i>	B	Aversive conditioning	Shrimp extract + electric shock	Fear	
Kita et al., 2011	<i>Lymnaea stagnalis</i>	P	Aversive conditioning	Sucrose + KCl	Fear	
Bateson et al., 2011	<i>Apis mellifera</i>	C, P	Judgement bias	Shaking	Pessimism	DA** OA** 5HT** TH**
Yang et al., 2013	<i>Drosophila melanogaster</i>	B	Escape response	Heat shock	Learned helplessness	
Fossat et al., 2014	<i>Procambarus clarkii</i>	B, P	Dark/light test	Electric shock	Anxiety	5HT** <sup>††</sup>
Gibson et al., 2015	<i>Drosophila melanogaster</i>	B	Innate aversive reaction	Looming effect	Fear	
Hamilton et al., 2016	<i>Pachygrapsus crassipes</i>	B, P	Dark/light test	None	Anxiety	5HT**
Mohammad et al., 2016	<i>Drosophila melanogaster</i>	B, P	Exposure avoidance	Heat shock	Anxiety	5HT <sup>††</sup>
Cassill et al., 2016	<i>Solenopsis invicta</i>	B, P	Nest behaviour	None	Pleasure	
Hughes et al., 2016	<i>Lymnaea stagnalis</i>	B, P	Aversive conditioning	Various stressors	Anxiety	NE
Perry et al., 2016	<i>Bombus terrestris</i>	C, B, P	Judgement bias, predator attack	Sucrose	Optimism	OA <sup>†</sup> DA <sup>††</sup> 5HT <sup>†</sup>
Batsching et al., 2016	<i>Drosophila melanogaster</i>	B	Escape response	Electric shock	Learned helplessness	
Bacqué-Cazenave et al., 2017	<i>Procambarus clarkii</i>	B, P	Dark/light test	Social harassment	Anxiety	5HT <sup>††</sup>
Schlüns et al., 2017	<i>Apis mellifera</i>	C	Judgement bias	Shaking, formic acid	Pessimism	

\*Studies arranged in chronological order.

<sup>‡</sup>Approach: B, behaviour; C, cognitive; P, physiological.

<sup>§</sup>Biogenic amines: measured (\*\*), tested (<sup>†</sup>), and found to be involved in the emotional components measured (<sup>††</sup>); DA, dopamine; OA, octopamine; 5-HT, serotonin; TH, tyramine; NE, potentially norepinephrine (noradrenaline) via  $\beta$ -adrenergic receptor in *Lymnaea stagnalis*.



## Box 2. The study of pain in invertebrates

Others have reviewed the many works exploring pain in invertebrates (e.g. Cooper, 2011; Eisemann et al., 1984; Sherwin, 2001), so we briefly describe here the current state of and overall debate within the field.

Pain is defined as the unpleasant emotional subjective experience that is associated with actual or potential tissue damage (Sneddon et al., 2003). Although there is no direct evidence of subjective experience in any animal, many argue that comparative research suggests that a variety of animal species may experience pain to some degree (Dawkins, 2008, 2015; Sneddon et al., 2003). Whether invertebrates can experience pain is currently debated (for excellent commentaries on this debate, please see e.g. Adamo, 2016; Elwood, 2001), but many of the criteria used to assess pain in vertebrates have also been shown in invertebrates.

Nociceptors, the receptors that respond to noxious stimuli and initiate avoidance behaviour to protect from further damage, have been identified in several invertebrate species (Smith and Lewin, 2009). But nociception alone does not imply the experience of pain, as there must be a nervous system that is able to process this experience as painful. The nervous systems of many invertebrate species possess intricate neural architecture (Breibach and Kutsch, 1995) that allows them to perform seemingly complex cognitive behaviours (Perry et al., 2017). However, invertebrate brains are not enough like ours to make solid arguments by analogy, and we do not yet know the minimum neural criteria for subjective experience and so have no neurobiological methods of determining whether or not invertebrates can experience pain.

The behavioural responses to analgesics, anaesthetics and opioids, and physiological responses to noxious stimuli themselves, may indicate modulation of nociception via higher brain centres (Elwood, 2011) but many of these responses may be the result of simpler peripheral effects (Adamo, 2016). Rapid avoidance learning and protective motor reactions are also suggested to be evidence of pain in invertebrates, as these may indicate awareness of the location of pain, both the potential source and the actual location on the body (Elwood, 2011). However, it is possible that invertebrates solve seemingly complex problems using much simpler mechanisms (Adamo, 2016). The fact that robots can be programmed to mimic human-like responses to noxious stimuli, and thus show ‘evidence’ of pain without the actual experience of pain, is used to argue against pain in invertebrates (Adamo, 2016). The same argument comes from the finding that physiological preparations of animals detached from their brain will still withdraw from electric shocks (National Academies, ILAR., 2009) and similarly from the evidence that humans can sometimes be unconscious of their emotions (Berridge and Winkielman, 2003).

Understanding the function of subjective experience may very well aid us in determining which animals possess the cognitive capacity for pain or the subjective component of any emotion. Some argue that the conscious awareness of emotions helps mediate flexible behaviour, such as motivational effects (modifying the strengths of competing behaviours in relation to their costs and benefits) and approach/avoidance behaviour involving innovation and/or planning (Dolan, 2002; Mason, 2011; Rolls, 1999), and indeed there is strong evidence for such behavioural flexibility in a number of invertebrate species (Balaban and Maksimova, 1993; Tarsitano and Jackson, 1997). Although these are still not concrete evidences of pain or subjective experience in invertebrates, we should consider an animal’s behavioural flexibility, as well as their ecological heterogeneity and neuroanatomical structures, as mutually reinforcing sources of evidence that suggest the presence of more-complex cognitive mechanisms (Powell et al., 2016).

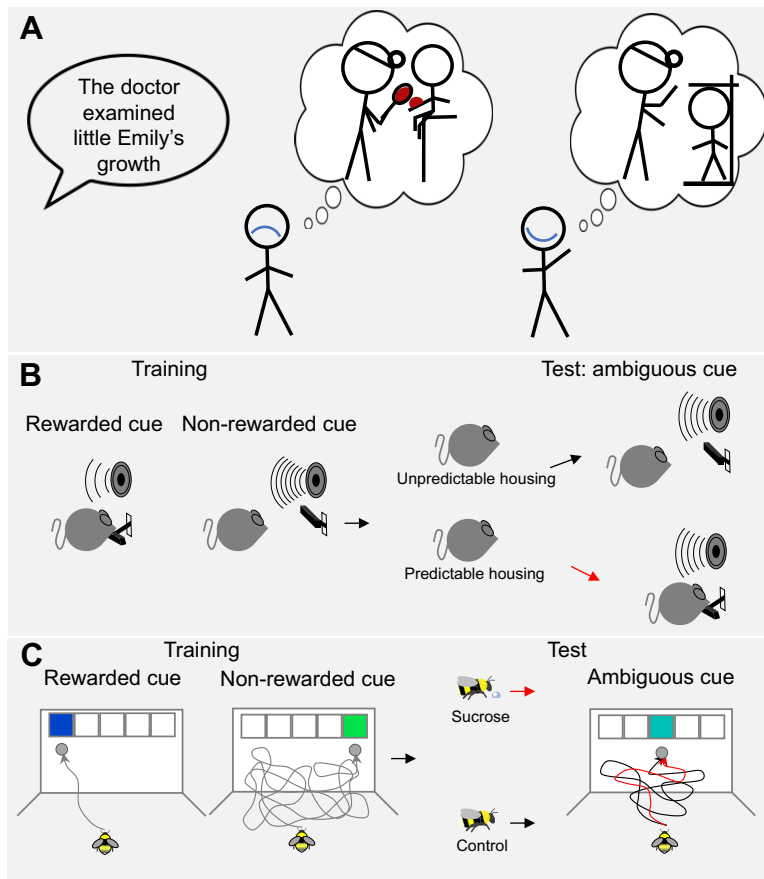
The debate within the field of animal pain research, which centres around the idea of subjective experience, illustrates the need to determine the neural architecture required to support subjective experience in order to ultimately determine which animals have the capacity for pain, or any type of emotion. Combined efforts in neuroscience, computer modelling and clever comparative cognition work will eventually help to determine the neural architecture required for subjective experience. Until then, these issues, resolved or not, have considerable social, political, economic and moral consequences, and those who have taken the time to consider pain in invertebrates advocate that, while balancing these considerations, we should take care of invertebrates, with at least the nervous systems to potentially support subjective experience, in a way that respects life (Adamo, 2016; Mason, 2011).

to assess negative emotions in rats. The judgment bias test is based on the fact that humans who report negative emotions tend to make negative judgements about ambiguous stimuli, whereas people reporting positive emotions make positive judgements about the same ambiguous stimuli (Fig. 1A) (Eysenck et al., 1991; Wright and Bower, 1992; MacLeod and Byrne, 1996; Gotlib and Joernmann, 2010). Rats in the Harding study (Harding et al., 2004) were trained to press a lever in response to a tone predicting a rewarding food pellet and to avoid pressing the same lever in response to a different tone predicting an aversive white noise burst (Fig. 1B). Once rats learned this discrimination, for several days they experienced either an unpredictable housing treatment designed to induce a negative/unpleasant state [e.g. at random times on any one day the cage might be tilted, the bedding wet or contain a strange conspecific (see Glossary)] or a predictably good housing treatment (control). Subsequently, the rats were exposed to ambiguous tones. As hypothesized, rats in unpredictable housing took longer to respond, and were less likely to respond to ambiguous tones, than rats in control conditions, showing behaviour indicating reduced anticipation of an appetitive/desirable outcome, similar to findings in depressed or anxious humans. The technique has since been applied to a number of different species, predominantly mammals, using different types of tasks (Go/NoGo, Active Choice, Spontaneous Behaviour), stimuli (visual, olfactory, auditory and position), and a variety of positive and negative manipulations (Mendl et al., 2009; Baciadonna and McElligott, 2015; Roelofs et al., 2016). To date, judgement bias paradigms have been employed in three studies in invertebrates. Bateson and colleagues (2011) and Schlüns and colleagues (2017)

assessed negative bias in honeybees, whereas Perry and colleagues (2016) assessed positive bias in bumblebees.

Bateson et al. (2011) and Schlüns et al. (2017) used an olfactory learning protocol to investigate the presence of negative judgement bias (Mendl et al., 2011). Bees will naturally extend their proboscis (see Glossary) when sugar solution is touched to their antennae. Honeybees were conditioned to extend their proboscis to a mixture of two odours (1:9 1-hexanol and 2-octanone) by pairing one odour mixture with sugar water (‘reward’) and to withhold their proboscis to another odour mixture (9:1 1-hexanol and 2-octanone) paired with a bitter quinine solution (‘punishment’). After training, half of the bees were subjected to vigorous shaking on a lab table-top vortex machine for 60 s to simulate a predator (e.g. honey badger) attack on the colony. After the shaking manipulation, bees were tested with ambiguous odour mixtures intermediate to the two mixtures used for training (3:7, 1:1 and 7:3 1-hexanol and 2-octanone). In both studies, honeybees subjected to shaking were less likely to respond to the ambiguous odour mixture closest in ratio to the odour mixture associated with quinine during training, suggesting that shaking induces a negative cognitive bias (see Glossary) to ambiguous odour cues. However, it has been argued that shaking may cause bees to become better discriminators – shaking increased haemolymph (see Glossary) concentrations of octopamine, which can modulate sensory function (Adamo, 2016; Giurfa, 2013).

Perry and colleagues (2016) broadened the scope of invertebrate emotion research to consider positive/pleasant states (Plowright, 2017). Bumblebees were first trained on a free-flying task, where they found a cylinder positioned under either a green card at the left on the



**Fig. 1. Cognitive approaches for assessing emotion: the judgement bias test.** (A) Measuring the effect of emotions on judgment in humans. When humans are in a negative emotional state, they tend to view ambiguous statements negatively; when in a more-positive state, they view the same ambiguous statements more positively. The illustrated example comes from Eysenck et al. (1991), where participants were given the ambiguous statement 'The doctor examined little Emily's growth'. Subjects with primary diagnosis of generalized anxiety disorder were more likely to interpret the statement as threatening, i.e. thinking that Emily had some type of disease. Both control and recovered anxious patients tended to view the same statement as non-threatening: i.e. that Emily was growing normally in height and weight. (B,C) Measuring emotions in non-human animals. (B) Harding and colleagues (2004) were the first to adapt the judgment bias test to non-humans. Rats were first trained to press a lever for reward upon hearing a tone at a certain frequency and avoiding the lever at a higher frequency. Rats that were housed in an unpredictable 'stressful' environment tended to react to ambiguous tones as though they were the unrewarded cue and rats kept in predictable 'calm' housing tended to react to the same ambiguous tone as though it was the rewarded cue. (C) Perry and colleagues (2016) tested free-flying bumblebees on the judgment bias test, first training them to approach and find a reward under a blue placard and to avoid an unrewarding green placard. Bees that received a small droplet of high-concentration sugar water just prior to a test tended to react to an ambiguous placard as though it was rewarding.

back wall or a blue card at the right on the back wall of the arena (Fig. 1C). Bees found a 30% sucrose solution reward under one of the colour-location combinations and water (unrewarding) under the other. Bees learned to approach the rewarding configuration faster and avoid entering the cylinder of the unrewarding configuration. After training, bees were tested with ambiguous configurations: intermediate colour (blue-green) and location. Prior to each test, to induce a positive emotion state, half of the trained bees received a small droplet of high-concentration sucrose solution. This unexpected reward amounted to less than 5% of the total crop load of a bee, similar perhaps to a small piece of chocolate for a human. Compared with controls, bees given the unexpected pre-test reward flew faster to intermediate cues, suggesting a positive judgement bias. Control experiments showed that after consumption of the small unexpected reward, bees did not increase their flight speed and were not more likely to explore novel stimuli, suggesting that the small reward did not simply increase the bees' general activity or exploration, but was indeed due to changes in their decision-making processes under ambiguity, thus resembling optimism in humans. Further work supporting this conclusion, in which the optimism response was manipulated pharmacologically through the dopamine pathway, is discussed later in this Review. Additional experiments, described below, showed that the positive effect of the pre-test reward generalized to an entirely different context.

### Behavioural approach

The study of emotional reactions in human psychology and the field of human affective neuroscience (see Glossary) has relied predominantly on verbal reports and subjective rating scales (Russell, 2003; Paul et al., 2005; Oatley and Johnson-Laird,

2014). However, many emotional states are recognized in human infants, and some emotions are accepted in several primate species, and even rats, through facial expressions in response to stimuli (Berridge et al., 2009; Sotocina et al., 2011; Waller and Micheletta, 2013; Steiner et al., 2001; Berridge and Kringelbach, 2008). Invertebrates lack the facial musculature for any real type of comparisons to be made in this regard; however, a substantial amount of work in mammals has utilized other bodily expressions and motor behaviour in response to stimuli to assess both valence (pleasantness) and intensity (arousal) of emotions (Désiré et al., 2004; van der Harst and Spruijt, 2007; Reefmann et al., 2009; Boissy et al., 2011; Briefer et al., 2015).

Humans and other animals can learn to predict aversive events if a neutral conditioned stimulus is paired with an emotion-producing unconditioned stimulus, such as a loud noise or electric shock. The first study showing any form of emotion in an invertebrate came from the Kandel lab, where sea slugs (*Aplysia californica*) were trained in an aversive classical conditioning paradigm (Walters et al., 1981). *Aplysia* received shrimp extract for 90 s occurring 1 min before the onset of an electric shock to the head for 30 s (unpaired controls received the extract after the electric shock). Two days after training, animals were tested by delivering shrimp extract to their head, and their behaviour was recorded. The sea slugs that received paired training expressed significant facilitation of four different defensive responses: head withdrawal, siphon withdrawal, inking and escape locomotion. In addition, the feeding behaviour of the sea slugs was markedly decreased in the presence of the shrimp extract. The observed behavioural responses to conditioned stimuli resemble the actions of conditioned fear in mammals (LeDoux, 2012).

Fear conditioning relies on innate fear-inducing stimuli. Fear can result in the expression of a variety of adaptive and defensive innate behaviours, which are aimed at escaping or avoiding a threatening or potentially dangerous stimulus. Reflex reactions serve to protect vulnerable parts of the body (e.g. the eyelid blink to protect the eye) or to facilitate escape from a sudden oncoming stimulus. The startle response, a generally unconscious defensive reaction to threatening stimuli, can even be expressed as entire body movements, such as jumping or perhaps aggression or freezing in position, when the threat is inescapable.

Gibson and colleagues (2015) attempted to examine fear in fruit flies using an innately aversive stimulus: an overhead shadow (Fig. 2A). *Drosophila* were confined to an enclosed arena and repeatedly exposed to a rotating opaque paddle. Repetitive stimulus exposure resulted in increases in locomotor velocity, hopping and freezing, and dispersed the flies from a food source. To distinguish emotional behaviour from other forms of environmentally induced states, perhaps driven by learning, it is necessary to quantify a range of responses similar to those studied in humans. The behavioural responses of flies to stimuli were both graded (i.e. the more passes of the stimulus, the greater the behavioural responses) and persistent (i.e. the behaviour lasted longer than the presence of the stimulus). The repeated stimulus also dispersed flies from a food source, suggesting negative valence and context generalization. These behavioural results are consistent with the idea of an internal emotional state similar to what we consider to be fear in humans and other vertebrates.

Clear and present sources of threat/danger induce fear and result in escape behaviour. Anxiety, a related negative emotion, is experienced in response to imagined or potential, but unclear, threats. There have been a variety of model paradigms developed to examine anxiety – both normal and pathological – in humans and other vertebrates, mostly rats and mice, and even in zebrafish (Graeff et al., 1998; Liebsch et al., 1998; Belzung and Griebel, 2001; Egan et al., 2009; Stewart et al., 2012). Most of these tests measure how the innate behaviour of an animal changes when exposed to unfamiliar aversive places or threats, such as predators. One such classical and widely used method is the elevated plus maze, a simple paradigm where an animal, usually a rat or mouse, is placed in a four-armed apparatus in the form of a ‘plus sign’. Two of the arms have walls, whereas the other two arms are open. The ratio of the time spent on the open arms to the time spent on the closed arms is taken to indicate the level of anxiety expressed by the animal (i.e. the more time spent in the open arms, compared with the walled arms, the less anxious the animal is). The plus maze relies on the proclivity of the animal towards dark, enclosed spaces and an unconditioned fear of heights/open spaces. This method has recently been adapted and used with crayfish, where the design relied on the preference of crayfish for dark places (Fossat et al., 2014). Crayfish were placed in a dark/light plus maze, submerged in water, where two arms were shaded and two arms were exposed to light. Crayfish that were exposed to a series of electric shocks before testing in the maze, compared with control crayfish, hardly explored and rapidly abandoned the light arms. In addition, the number of entries, mean duration per visit, latency to first visit into a light arm and ratio of retreats from light arms compared with dark arms were significantly different in stressed crayfish. These behavioural results fulfil criteria normally designated for anxiety in mammals, including being innate, being unconditioned, occurring in the absence of a stressor, and expressed in a novel context. A follow-up study from the same lab also showed that, after a fight, the aggressive acts that a winning conspecific displayed towards the

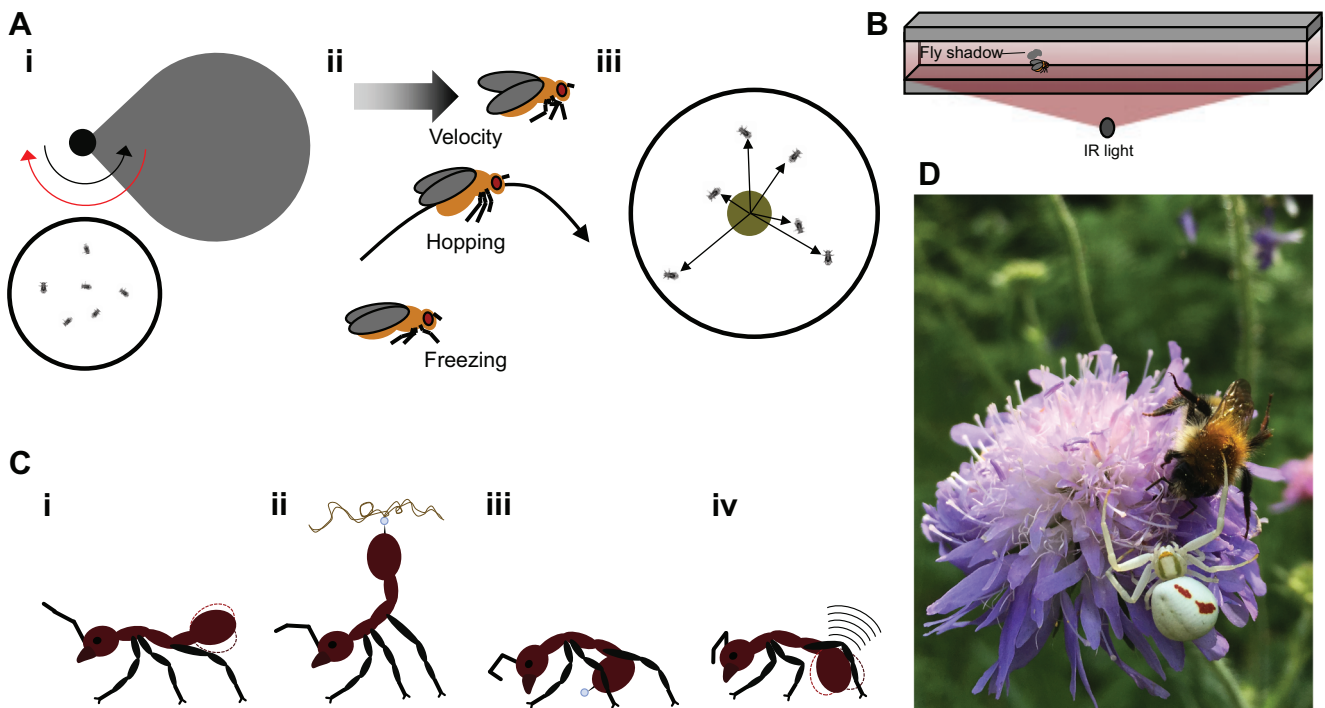
losing crayfish while both were in an aquarium (interpreted as harassment behaviour) induced anxious behaviour in the losing crayfish later in the dark/light plus maze (Bacqué-Cazenave et al., 2017), resembling the anxiety effects of psychological harassment in humans.

Repeating, uncontrollable stressful events can induce learned helplessness, where an animal exposed to an uncontrollable stressful situation becomes unable or unwilling to avoid subsequent encounters with stressful stimuli, considered an animal model of depression in humans (Eisenstein and Carlson, 1997; Willner, 1986). Yang et al. (2013) examined this phenomenon in *Drosophila*. Two flies were placed in separate dark walking chambers and exposed to the same sequence of heat pulses (Fig. 2B). Heat was applied to both when one, the ‘in-control’ fly, stopped moving. When the in-control fly started moving again, the heat ceased. Flies with no control over the heat pulses quit trying to escape as normal, began to walk slower and took more frequent rests, compared with ‘in-control’ flies, behaviours said to indicate a depressed state in flies. These results have also been replicated with electric shocks (Batsching et al., 2016). To support these interpretations, however, it would be valuable to know whether the flies without control were attempting other behaviours in the chamber, such as pushing or jumping, albeit unsuccessfully. Moreover, does the low activity of flies and their reluctance to escape transfer to new similar situations? In addition, how this state affects the flies physiologically (health and longevity), socially and neurobiologically [e.g. serotonin is known to play a crucial role in learned helplessness (Willner, 1986)] would be helpful in interpreting these findings.

Most studies on emotion involving vertebrates, including humans, have traditionally focused on negative emotions. It is argued that the reasons that positive emotions have been neglected in research are because they are few in number – reflected even in the imbalance of English-language words for negative over positive emotions – and are harder to differentiate (Fredrickson, 1998; Fredrickson and Branigan, 2011). The asymmetry might also stem from our understanding that natural selection has shaped emotions more for survival than for prosperity: there are many more ‘threats’ than ‘treats’ in our environment (Fredrickson, 1998). Furthermore, most of the psychological and clinical work with emotions in humans has been focused on solving problems, and therefore dealing with those emotions that pose more problems within our daily lives.

Although only a very limited number of studies have examined emotions in invertebrates, of those that have used a behavioural approach, two have addressed the possibility of positive emotions. Cassill and colleagues (2016) report a behaviour in fire ants (*Solenopsis invicta*) that, they argue, is similar to bodily expressions indicating pleasure in humans and other animals (Fig. 2C). Experimenters watched many hours of video-recordings from inside a fire ant nest observing ants’ behaviours during various situations. They found that ants performed what has been called ‘wagging’, where they position and move their abdomen up and down at around 45°, when they interacted with brood and consume sugar water. This behaviour was not a defensive posture as it was within the hive, their stinger was never extruded and no venom was ever observed on the tip of their abdomen, nor found to be dispersed during wagging. As no sounds were produced during wagging, natural nest conditions are completely dark, and nestmates did not react negatively or positively to wagging, it does not seem to be a form of communication. Interestingly, wagging occurred significantly more during two specific behaviours: tending to brood and consuming sugar water. Cassill and colleagues (2016) suggest that this in-nest behaviour might be analogous to facial





**Fig. 2. Behaviour approaches of assessing emotions in invertebrates.** (A) Gibson et al. (2015) assessed in fruit flies the innate fear behavioural responses to a looming shadow, produced by an opaque paddle passing overhead of the flies contained within a petri dish (i). (ii) Repetitive passing of the paddle induced graded and persistent increases in locomotor velocity, hopping and freezing behaviours, indicating scalability and persistence of response. (iii) The passing shadow also dispersed flies from a food resource, indicating negative valence and generalization. (B) Yang et al. (2013) examined learned helplessness in flies using a heat box apparatus. An infrared (IR) diode emitted IR light through the transparent front wall of the box, which cast a shadow of the fly onto an optical sensor on the back wall of the box. When the sensor detected no movement, i.e. the fly stopped walking, the bottom of the box would heat up immediately to an uncomfortable 37°C. Once the fly tried to escape the heat, i.e. began walking again, the heat would drop quickly to a comfortable 24°C. Flies that received the same sequence and amount of heat but had no control over the changes soon became less active and stopped trying to escape the heat, as if they were 'depressed'. (C) Common postures displayed by fire ant workers. Cassill and colleagues (2016) suggest that abdominal wagging at 45° (i) is behaviourally similar to an expression of pleasure. Unlike flagging (ii) or defence displays (iii), wagging did not occur outside the nest, nor was venom present during the display. Unlike stridulation when trapped and trying to communicate for help (iv), wagging did not produce any sounds. In addition, wagging occurred much more often when ants were in contact with brood or food, situations that are usually considered to be pleasure inducing in other animals. (D) Crab spider attack on a foraging bee. Crab spiders are sit-and-wait predators of foraging bees. Perry and colleagues (2016) simulated this, presumably stressful, event by immobilizing bees under a micro-controlled sponge, and asked whether an unexpected droplet of high-concentration sugar water would attenuate the bees' reaction. Bees consuming a 5 µl 60% sucrose solution droplet prior to the attack took much less time to reinstate foraging after the attack than control bees.

expressions and bodily postures of 'hedonic pleasure' in humans and other mammals during pleasurable events (Briefer et al., 2015; Proctor and Carder, 2014; Quaranta et al., 2007). However, to substantiate these claims, more experiments would be needed. For example, is this a simple mechanical response to manipulating any item with their mouthparts? Whether interactions with brood or consumption of sucrose cause cognitive biases or changes in neurotransmitter levels indicative of a positive state would also help support a claim for pleasure. At the moment, this work exemplifies how observations of natural behaviour can potentially provide valuable information about invertebrate emotions.

Having already shown that pre-test sucrose resulted in a positive cognitive bias (described above), Perry and colleagues (2016) tested the idea that emotions generalize across contexts (Anderson and Adolphs, 2014). They adapted a protocol used with infants to bumblebees. Infants receiving sugar water before a heel lance (a procedure to collect capillary blood for laboratory tests) tend to cry for a shorter duration and less loudly (Fernandez et al., 2003). In nature, bees are sometimes ambushed at flowers by 'sit-and-wait' predators, such as crab spiders, but often escape (Fig. 2D). This predator attack was mimicked using a trapping mechanism in which a constant pressure was applied for 3 s before the bee was released. Half the bees received a small droplet of high-concentration sucrose

prior to the attack. Bees that received the droplet of sucrose before the test took much less time to commence foraging, indicating the small pre-test reward was causing a positive emotional state change in the bee across behavioural contexts.

Voices are an important modality for emotional expression (Schirmer and Adolphs, 2017). The voice is produced when air flow from the lung passes the larynx, where air is converted into sound by the vibration of the vocal fold. Before being expelled into the environment, the voice is filtered by the pharynx, oral and nasal cavities (Fant, 1960; Titze, 1994). The structure of vocalisation in mammals depends on the physiology and anatomy of the respiratory, phonatory and filter systems (Fant, 1960; Titze, 1994; Juslin and Scherer, 2005), each of which can be altered by emotional states (Scherer, 2003). Emotions can affect the tension and action of the muscles responsible for sound production, via the somatic and autonomic nervous systems (Scherer, 1986). Vocalisations therefore can, and have been, used as non-invasive markers of animal emotions (Scherer, 2003; Briefer, 2012; Panksepp and Burgdorf, 2000). In many of these efforts, sound features have been quantified using a variety of acoustic parameters clearly linked with the intensity/arousal and valence of emotional experience (Briefer, 2012). Many invertebrates, especially insects, have developed specific features that allow acoustic communication

(e.g. for attracting mates or luring prey, defence against predators and territory defence) (Leonhardt et al., 2016). Darwin (1872) was the first to suggest that insects might potentially communicate emotions such as ‘anger’, ‘terror’, ‘jealousy’ and ‘love’ through their stridulation (see Glossary). However, again, this possibility has yet to be tested in invertebrates. Similar to vocalization in mammals, the acoustic signals used for communication in invertebrates could provide useful avenues for exploring the adaptive functions of emotions: e.g. does stress (or enrichment) induce changes in acoustic signalling in ways that might affect social dynamics, health or survival?

### Physiological approach

Physiological measures of emotion in humans have mostly relied on indices of activation within the sympathetic and parasympathetic nervous systems. Along these lines, studies adapted from what is known in humans to non-human animals have relied on similar methods (Appelhans and Luecken, 2006; von Borell et al., 2007; Kreibitz, 2010; Lench et al., 2011). Some of the most common measures include skin conductance levels, skin temperature, heart rate, heart rate variability, blood pressure, neuroendocrine activity, EEG and neuroimaging (Paul et al., 2005). Studies have not been able to reliably relate distinct physiological measures to discrete emotions in humans. Situations that give rise to different emotions can result in similar physiological responses: e.g. increased heart rate due to anxiety and joy (Paul et al., 2005). Instead, most of these measures have pointed to relationships between changes in physiological responses and the dimensional properties of emotion, i.e. valence (positive/negative) and arousal (intensity). However, combinations of physiological measures in humans have provided some support for differentiating basic emotional states (Cacioppo et al., 2000; Rainville et al., 2006). Rainville and colleagues (2006) proposed a heuristic tree to differentiate basic emotions based on the cardiac and respiratory pattern. For example, ‘anger’ is characterized by an increase in heart rate and no visible change in high frequencies of heart-rate variability, whereas ‘fear’ is characterized by an increase of the heart rate and a noticeable decrease of high frequencies of heart-rate variability concomitant with a change in the respiratory activity. Most of these types of measurements are quite difficult to apply to invertebrates, given their often-miniature size and hard carapace and, in the case of insects, an open circulatory system, where heart rate is not increased. It has been shown in some crustaceans and molluscs, however, that sudden changes in the surrounding environment can induce modifications of some physiological variables: e.g. heart and ventilator rate (Schapker et al., 2002; Belzung and Philippot, 2007). Similar to the experiments by Walters et al. (1981), described above, Kita and colleagues (Kita et al., 2011) explored fear conditioning in pond snails. Snails learned to associate the presence of sucrose, which normally elicits feeding behaviour, with an aversive stimulus, KCl, that caused a full body withdrawal. Subsequent exposure to sucrose failed to induce feeding behaviour, showing learning, and caused the hearts of the snails to skip a beat, suggesting physiological responses similar to fear in mammals (Randall and Hasson, 1981).

The similarity of neurochemicals within invertebrate and vertebrate brains has provided another valuable physiological element with which to assess emotions across distant species (Perry and Barron, 2013). In humans, the role of biogenic amines (neurotransmitters) in the regulation of emotion is illustrated in the fact that many of the drugs that affect emotional states target and change the biogenic amine systems (Handley and McBlane, 1993;

Dailly et al., 2004; López-Muñoz et al., 2011). A plethora of studies suggest that the three major biogenic amines – serotonin, dopamine and noradrenaline – are essential in the control of emotions (for excellent reviews/syntheses of much of this work, please see Fellous, 1999; Lövhheim, 2012). Although cells that produce these neurotransmitters are located within the midbrain, basal ganglia and brainstem regions of vertebrates (raphe nuclei for serotonin, ventral tegmental area and substantia nigra for dopamine; locus ceruleus for noradrenaline), their connections branch to practically all regions of the brain (Lövhheim, 2012). Growing evidence suggests that the biogenic amine systems might work concurrently as a final pathway for the simultaneous delivery of emotion-eliciting information to dispersed areas of the brain. The relationship between each amine and any specific emotion appears complex, but a recent model has suggested that the systemic levels of these three amines could potentially predict all of the basic emotions (Lövhheim, 2012). Studies of drugs of abuse in humans and animals show that animals respond in similar ways to these drugs and that these observed behaviours rely on similar brain systems, many within the biogenic amine pathways (Panksepp, 2005).

Invertebrate nervous systems contain corresponding biogenic amines that function similarly to neurotransmitters, neuromodulators and hormones. Of the studies assessing emotion in invertebrates, four have applied physiological approaches, and all of these have relied on measuring or manipulating systemic biogenic amine levels. Bateson and colleagues (2011) assessed how systemic biogenic amine levels changed in response to a presumed negative-emotional event. Haemolymph was collected from honeybees after simulating a predator attack (shaking bees on a vortex for 60 s). Analysis of the haemolymph using high-performance liquid chromatography (HPLC) showed that systemic levels of the biogenic amines dopamine, octopamine (chemically similar to noradrenaline) and serotonin all decreased in response to bees being shaken vigorously. In humans, it seems that a depletion of biogenic amines (serotonin, noradrenaline, and dopamine) is responsible for features of depression (monoamine hypothesis of depression) (Anderson and McAllister-Williams, 2015). Increasing the levels of these biogenic amines, either through serotonin or noradrenaline reuptake inhibitors, or noradrenaline- or dopamine-enhancing drugs, has anti-depressant effects (Anderson and McAllister-Williams, 2015). Furthermore, and perhaps similar to the observed cognitive bias observed in honeybees, deficient serotonin activity in humans leads to a bias towards negative over positive stimuli, so that negative stimuli have a greater impact on behaviour and cognition (Murphy et al., 2002). The results of Bateson et al. (2011) suggest that the general effects of biogenic amine levels influence emotional states in invertebrates in a manner similar to that found within humans.

Fossat and colleagues (2014) manipulated the systemic levels of serotonin to determine how changes in serotonin would affect anxiety behaviour in crayfish (Fig. 3A). Injection of serotonin into the haemolymph of unstressed crayfish caused avoidance behaviour similar to that exhibited by crayfish stressed with an electric shock. Injection of the anxiety-reducing (anxiolytic) drug chlordiazepoxide prevented anxious behaviours (avoidance of light areas) induced by electric shock or serotonin injection. Similar methods were used in a follow-up study by Bacqué-Cazenave and colleagues (2017), who showed that anxious behaviour induced by social harassment by crayfish conspecifics could be abolished with injection of the anxiolytic drug chlordiazepoxide or the serotonin-receptor antagonist methysergide (Fig. 3A). A separate group also showed that acute exposure to the serotonin reuptake inhibitor fluoxetine (crabs were



immersed in a container of seawater containing the drug) reduces anxious behaviour of crabs in a simple light/dark paradigm (Hamilton et al., 2016). Mohammad and colleagues (2016) used interventions of heat-shock stress and a benzodiazepine, diazepam, to verify that the behaviour of flies of staying close to a wall during spontaneous locomotion both relates to anxiety and is dependent on serotonin signalling, comparable to what has been found in a rodent model of anxiety (Fig. 3B). In humans, serotonin has long been implicated in a variety of emotional processes. The specific relationship between serotonin levels and anxiety, as well as with depression, is somewhat complex; however, in general, it seems that fluctuations in serotonin affect diverse neural systems in the control of negative emotions and aversive processing, likely through different receptors, and potentially in combination with the dopamine system (Cools et al., 2008). The results of the above-mentioned invertebrate studies again suggest that the serotonin system might have been conserved, or at least co-opted, through evolution to help regulate emotions such as anxiety in a variety of animal species.

Perry and colleagues (2016) examined the potential role of serotonin, octopamine and dopamine in the optimistic behaviours observed in bumblebees, using topical applications of receptor antagonists for each of these amines (Fig. 3C,D). They showed that dopamine played a role in the positive emotional state of bumblebees. The optimistic behaviour seen in the judgment bias test in response to a pre-test sugar reward was abolished when bees were topically treated with the dopamine-receptor antagonist fluphenazine. Similarly, topical treatment with fluphenazine eliminated the effect that pre-test sugar had on the response of bees to a simulated predator attack. In humans, it seems that the expression and regulation of positive emotions is dependent, at least partially, on the dopamine system. Similar cognitive effects have also been seen in humans, e.g. increasing dopamine levels in humans reduces negative expectations regarding future events in response to negative information (Sharot et al., 2012). Indeed, psychostimulants that increase dopamine levels cause euphoria and have a positive effect in humans, and are rewarding in other mammals (Burgdorf and Panksepp, 2006).

Hughes and colleagues (2016) tested whether propranolol ( $\beta$ -adrenergic receptor blocker) could block stress-related memories in the pond snail. Snails were exposed to various types and combinations of stressors. They found that memories formed during more stressful combinations, e.g. a mimicked encounter with a predator (crayfish effluent+potassium chloride, which induces defensive full-body withdrawal) could be abolished using propranolol after reactivation, but memories formed during less stressful events, e.g. presence of a distant predator (crayfish effluent alone), were not affected. These results suggest phenotypically similar memories were molecularly different, and may help explain the mixed results in human research on disrupting reconsolidation with propranolol in post-traumatic stress disorder. Future work would benefit from addressing these molecular differences. Consistent with similar results in rats, this work also suggests that modulation of memory by emotional events are highly conserved. All of these works indicate that, in invertebrates, similar to what is known in vertebrates, the biogenic amines play a central role in emotions.

The link between certain biogenic amines and emotions in humans and other animals, and recently including invertebrates, is strong. Why would we find biogenic amine involvement in such similar biological phenomena? We feel that it is unlikely that this indicates a true homology of emotion systems across phyla (Barron et al., 2010). We speculate that it is more likely that ancient roles for biogenic amines as neuromodulators of more basic functions, such as in motor response circuits, were co-opted (perhaps even

combining some of these more basic functions) for emotions through evolutionary pressure.

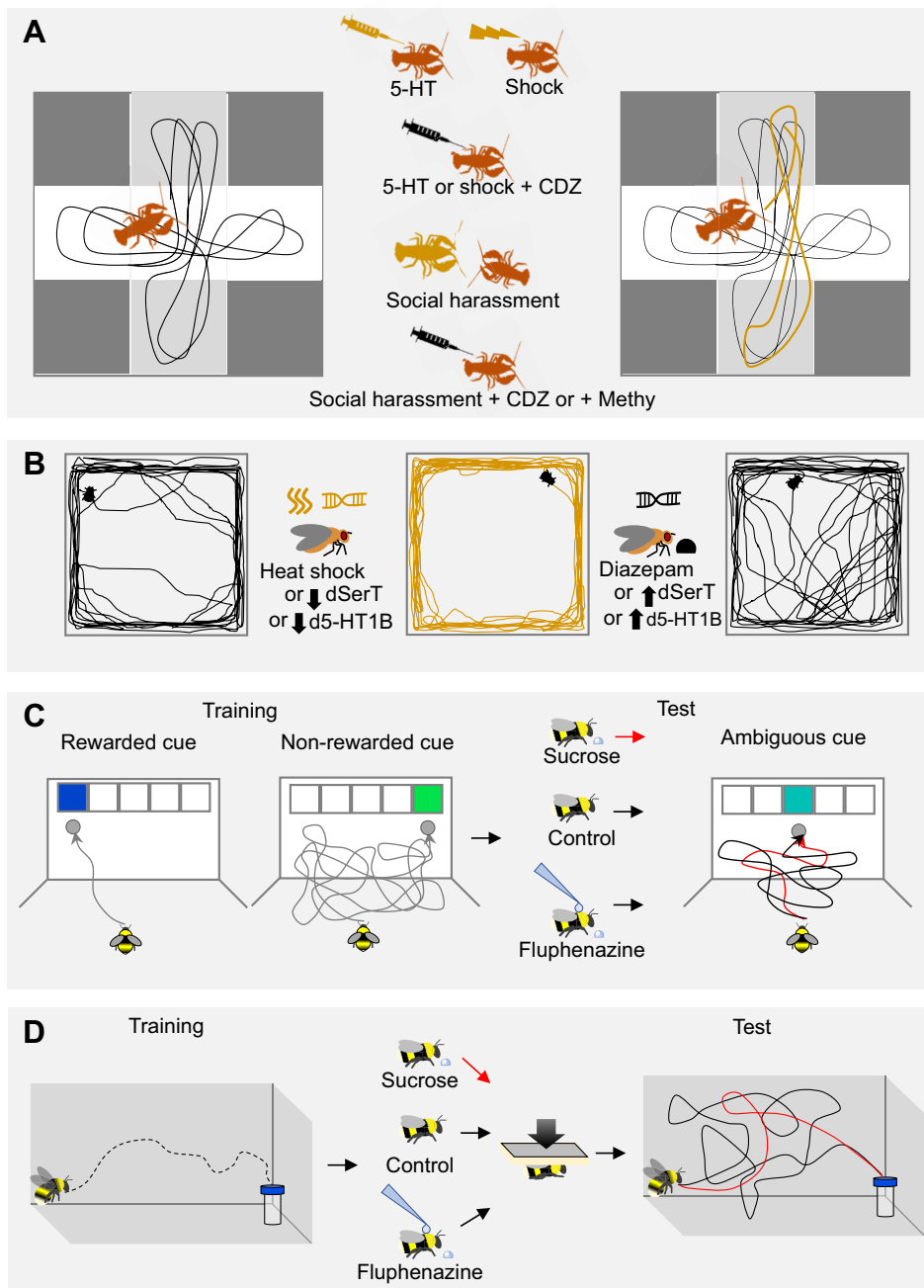
Many regions of the mammalian brain have been implicated in the regulation of emotion. However, it is important to note that emotional states have typically been much easier to illicit in humans through stimulation of subcortical circuits, especially those within the midbrain area and basal ganglia, which have also been found to mediate emotional behaviours in other vertebrates (Burgdorf and Panksepp, 2006; Panksepp, 2011). Strausfeld and Hirth (2013) have highlighted developmental, anatomical and genetic evidence arguing that the central complex of the insect brain is analogous to the vertebrate basal ganglia. In addition, Barron and Klein (2016) have made a strong argument that the insect brain is functionally analogous to that of the vertebrate midbrain and potentially capable of subjective experience, defined by the authors as a very basic level of conscious awareness. It does seem evident that the insect brain contains the neural structures able to support emotional states. Given these neurochemical and functional similarities, combined with what has already been shown in crayfish (Fossat et al., 2014), fruit flies (Mohammad et al., 2016) and pond snails (Hughes et al., 2016), it seems promising that pharmacological and genetic manipulations of some invertebrates may be able to provide models of human diseases and/or disorders linked to emotions.

It is also important to realize that emotions are brain states with a wide and vast neural architecture involved in their expression and regulation, and that analogous neuroanatomical features with vertebrates may not be necessary to support similar functions, as there are multiple ways of producing the same output. In studying emotions in invertebrates, as in all animals, we must consider the evolution and ecology of each species, and realize that any of the components of emotion may be expressed differently. Furthermore, given the limited neural architecture available in many invertebrates, it may be that the invertebrate brain evolved a simpler way of producing and regulating emotions. Efforts to understand how exactly this occurs are necessary and will no doubt inform us on the evolution of emotions and shed light on the nature of our own emotions.

### Concluding remarks

Traditionally, emotion research has been limited to large-brained animals, owing to an undeserved bias against small-brained invertebrates, with an assumption that possession of such a limited number of neurons might not lend itself to supporting the complex cognitive functions known to operate in vertebrates. This bias has likely hindered substantial progress in the field of cognitive science. However, a plethora of studies in the past few decades has unveiled a variety of impressive cognitive abilities in these miniature-brained organisms, and various types of neural network models have highlighted how seemingly complex cognitive tasks can sometimes be resolved by relatively limited circuits (e.g. Ardin et al., 2016; Peng et al., 2017). The handful of recent studies reviewed here have ignored this 'size bias' and have together provided corroborative evidence that several invertebrate species display emotions on cognitive, behavioural and physiological levels similar to those of vertebrates.

One of the biggest divisions of opinion in the field of emotion research is whether to call what is observed in invertebrates, or other non-human animals, 'emotions', as we cannot yet directly measure its subjective component. Because we are ignorant of its neurological basis, we cannot exclude the possibility that invertebrates have some basic form of subjective experience. Furthermore, it is not necessary to fully understand subjective experience in order to study emotions. Therefore, with the help of invertebrate research, studying all facets of



**Fig. 3. Assessing emotions in invertebrates through manipulation of biogenic amines: physiological approaches.** (A) Fossat et al. (2014) and Bacqué-Cavenave et al. (2017) manipulated the systemic levels of serotonin in the crayfish after electric shock or social harassment. Behaviour indicative of anxiety, avoidance of light areas in a plus maze increased after electric shock or social harassment by a conspecific. Injection of serotonin (5-HT) produced the same anxious behaviour and combining any of these manipulations with the benzodiazepine anxiolytic drug chlordiazepoxide (CDZ) or the serotonin-receptor antagonist methysergide (Methy) abolished the anxious behaviour. (B) Mohammad et al. (2016) showed that the behaviour of flies to stay close to the walls during spontaneous locomotion is related to anxiety. They used RNAi to reduce and ectopic expression to increase the expression of a serotonin receptor gene (d5-HT1B) or mRNA of a serotonin transporter (dSerT). Heat shock or reducing dSerT mRNA or d5-HT1B levels increased wall-following behaviour. Feeding flies a benzodiazepine (diazepam) or elevating levels of d5-HT1B gene expression or dSerT mRNA reduced the anxious behaviour. (C) Perry and colleagues (2016) topologically applied antagonists for dopamine receptors, serotonin receptors and adrenergic receptors (which respond to octopamine) prior to a judgment bias test. Bees were trained to approach and find reward under a blue placard and to avoid an unrewarding green placard. Bees that received a small droplet of high-concentration sugar water just prior to a test took less time and were more likely to approach an ambiguous placard, than control bees. This optimistic behaviour was abolished if the bees were topically treated with the dopamine-receptor antagonist fluphenazine. (D) Perry and authors (2016) simulated a stressful crab spider attack, by immobilizing bees under a micro-controlled sponge. Bees consuming a 5  $\mu$ l 60% sucrose solution droplet prior to the attack took much less time to reinitiate foraging after the attack than control bees. This attenuation of a negative reaction was abolished if bees were topically treated with the dopamine-receptor antagonist fluphenazine.

emotion will progress us towards answering the most-intriguing and evasive questions about emotion. How did emotions evolve? What are the adaptive functions of negative and positive emotions? In what ways do cognition and emotion interact? How is emotion produced within the brain?

Demonstrating specific emotions in a particular species of animal alone will not provide much useful information at this point. Future research directions should emphasize the mechanisms of emotions, because understanding the mechanisms that produce emotions will ultimately help to answer the questions above and will help expand our efforts to tasks such as building artificial intelligence with emotions and developing therapeutics for emotion-related disorders. A powerful and efficient tool that will support these endeavours will be the use of computational modelling. Computational models can use the data from separate neurobiological and behavioural

experiments to simulate and provide a functional account of specific cognitive phenomena (Abbott, 2008; Brodland, 2015). Of course, the smaller the number of neurons in a system, the easier it will be to model. Therefore, many invertebrates, with their limited neural architecture and impressive cognitive abilities, lend themselves well to computational modelling. Three areas of technological advancement will help tremendously in these collaborative efforts. Improved computational behavioural analysis allowing for automatic tracking and discovery of detailed behaviour (Egnor and Branson, 2016) will enable neural computational models to take advantage of massive behavioural data sets with extraordinary detail and help to determine the neural mechanisms of emotion-linked behaviour. Advances in the fabrication and application of electrophysiological recording of many neurons simultaneously in freely moving small animals will provide the temporal and spatial resolution necessary to

determine the contributions of specific regions and circuits to emotional processing. Ultimately, to be able to verify models we must be able to establish causal relationships. Establishing causal techniques such as optogenetics in more invertebrate species with rich behavioural repertoires will be vital in determining the causal neural links responsible for emotion and propel the field forward. Miniaturizing and adapting psychological methods for the study of emotions in invertebrates, combining cognitive, behavioural and physiological approaches, and applying computational behavioural analyses and neural network modelling techniques primed for small brains will be necessary to fully understand the evolutionary origins of and the neural mechanisms behind emotions.

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#### Competing interests

The authors declare no competing or financial interests.

#### References

- Abbott, L. F. (2008). Theoretical neuroscience rising. *Neuron* **60**, 489–495.
- Adamo, S. A. (2016). Do insects feel pain? A question at the intersection of animal behaviour, philosophy and robotics. *Anim. Behav.* **118**, 75–79.
- Alem, S., Perry, C. J., Zhu, X., Loukola, O. J., Ingraham, T., Søvik, E. and Chittka, L. (2016). Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. *PLoS Biol.* **14**, e1002564.
- Allen-Hermanson, S. (2008). Insects and the problem of simple minds. *J. Philos.* **105**, 389–415.
- Anderson, D. J. and Adolphs, R. (2014). A framework for studying emotions across species. *Cell* **157**, 187–200.
- Anderson, I. M. and McAllister-Williams, R. H. ed. (2015). *Fundamentals of Clinical Psychopharmacology*. Boca Raton, FL: CRC Press.
- Appelhans, B. M. and Lueken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Rev. Gen. Psychol.* **10**, 229–240.
- Ardin, P., Peng, F., Mangan, M., Lagogiannis, K., Webb, B. and Wolf, R. (2016). Using an insect mushroom body circuit to encode route memory in complex natural environments. *PLoS Comput. Biol.* **12**, e1004683.
- Baciadonna, L. and McElligott, A. G. (2015). The use of judgement bias to assess welfare in farm livestock. *Anim. Welf.* **24**, 81–91.
- Bacqué-Cazenave, J., Cattaert, D., Delbecq, J.-P. and Fossat, P. (2017). Social harassment induces anxiety-like behaviour in crayfish. *Sci. Rep.* **7**, 39935.
- Balaban, P. M. and Maksimova, O. A. (1993). Positive and negative brain zones in the snail. *Eur. J. Neurosci.* **5**, 768–774.
- Barron, A. B. and Klein, C. (2016). What insects can tell us about the origins of consciousness. *Proc. Natl. Acad. Sci. USA* **113**, 4900–4908.
- Barron, A. B., Søvik, E. and Cornish, J. L. (2010). The roles of dopamine and related compounds in reward-seeking behavior across animal phyla. *Front. Behav. Neurosci.* **4**, 163.
- Bateson, M., Desire, S., Gartside, S. E. and Wright, G. A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Curr. Biol.* **21**, 1070–1073.
- Batsching, S., Wolf, R. and Heisenberg, M. (2016). Inescapable stress changes walking behavior in flies - learned helplessness revisited. *PLoS ONE* **11**, e0167066.
- Belzung, C. and Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: A review. *Behav. Brain Res.* **125**, 141–149.
- Belzung, C. and Philippot, P. (2007). Anxiety from a phylogenetic perspective: Is there a qualitative difference between human and animal anxiety? *Neural Plast.* **2007**, 59676.
- Benard, J., Stach, S. and Giurfa, M. (2006). Categorization of visual stimuli in the honeybee *Apis mellifera*. *Anim. Cogn.* **9**, 257–270.
- Berridge, K. and Winkelman, P. (2003). What is an unconscious emotion? (The case for unconscious "liking"). *Cogn. Emot.* **17**, 181–211.
- Berridge, K. C. and Kringelbach, M. L. (2008). Affective neuroscience of pleasure: Reward in humans and animals. *Psychopharmacology* **199**, 457–480.
- Berridge, K. C., Robinson, T. E. and Aldridge, J. W. (2009). Dissecting components of reward: "liking", "wanting", and learning. *Curr. Opin. Pharmacol.* **9**, 65–73.
- Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J. et al. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiol. Behav.* **92**, 375–397.
- Boissy, A., Aubert, A., Désiré, L., Greiveldinger, L., Delval, E. and Veissier, I. (2011). Cognitive sciences to relate ear postures to emotions in sheep. *Anim. Welf.* **20**, 47–56.
- Breidbach, O. and Kutsch, W. ed. (1995). *The Nervous Systems of Invertebrates: An Evolutionary and Comparative Approach*. Basel: Birkhäuser Basel.
- Briefer, E. F. (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* **288**, 1–20.
- Briefer, E. F., Tettamanti, F. and McElligott, A. G. (2015). Emotions in goats: mapping physiological, behavioural and vocal profiles. *Anim. Behav.* **99**, 131–143.
- Brodland, G. W. (2015). How computational models can help unlock biological systems. *Semin. Cell Dev. Biol.* **47–48**, 62–73.
- Burgdorf, J. and Panksepp, J. (2006). The neurobiology of positive emotions. *Neurosci. Biobehav. Rev.* **30**, 173–187.
- Cacioppo, J., Berntson, G., Larsen, J., Poehlmann, K. and Ito, T. (2000). The psychophysiology of emotion. In *The Handbook of Emotion* (ed. M. Lewis and J. Haviland-Jones), pp. 173–191. New York: Guilford Press.
- Cahill, L. and McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci.* **21**, 294–299.
- Cassill, D., Ford, K., Huynh, L., Shiffman, D. and Vinson, S. B. (2016). A study on abdominal wagging in the fire ant, *Solenopsis invicta*, with speculation on its meaning. *J. Bioeconomics* **18**, 159–167.
- Christianson, S. A. (1992). Emotional stress and eyewitness memory: a critical review. *Psychol. Bull.* **112**, 284–309.
- Cools, R., Roberts, A. C. and Robbins, T. W. (2008). Serotonergic regulation of emotional and behavioural control processes. *Trends Cogn. Sci.* **12**, 31–40.
- Cooper, J. E. (2011). Anesthesia, analgesia, and euthanasia of invertebrates. *ILAR J.* **52**, 196–204.
- Dailly, E., Chenu, F., Renard, C. E. and Bourin, M. (2004). Dopamine, depression and antidepressants. *Fundam. Clin. Pharmacol.* **18**, 601–607.
- Darwin, C. (1872). *The Expression of the Emotions in Man and Animals*. London: John Murray.
- Das, G., Lin, S. and Waddell, S. (2016). Remembering components of food in *Drosophila*. *Front. Integr. Neurosci.* **10**, 4.
- Dawkins, M. S. (2008). The science of animal suffering. *Ethology* **114**, 937–945.
- Dawkins, M. (2015). Animal welfare and the paradox of animal consciousness. *Adv. Study Behav.* **47**, 5–38.
- de Bono, M., Tobin, D. M., Davis, M. W., Avery, L. and Bargmann, C. I. (2002). Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. *Nature* **419**, 899–903.
- Désiré, L., Boissy, A. and Veissier, I. (2002). Emotions in farm animals: a new approach to animal welfare in applied ethology. *Behav. Proc.* **60**, 165–180.
- Désiré, L., Veissier, I., Després, G. and Boissy, A. (2004). On the way to assess emotions in animals: Do lambs (*Ovis aries*) evaluate an event through its suddenness, novelty, or unpredictability? *J. Comp. Psychol.* **118**, 363–374.
- Dickson, B. J. (2008). Wired for sex: the neurobiology of *Drosophila* mating decisions. *Science* **322**, 904–909.
- Dolan, R. J. (2002). Emotion, cognition, and behavior. *Science* **298**, 1191–1194.
- Donlea, J. M., Pimentel, D. and Miesenböck, G. (2014). Neuronal machinery of sleep homeostasis in *Drosophila*. *Neuron* **81**, 860–872.
- Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavalle, P. R., Elegante, M. F., Elkhayat, S. I., Bartels, B. K., Tien, A. K., Tien, D. H. et al. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behav. Brain Res.* **205**, 38–44.
- Egnor, S. E. R. and Branson, K. (2016). Computational analysis of behavior. *Annu. Rev. Neurosci.* **39**, 217–236.
- Ehrenberg, C. G. (1836). *Betrachtung einer auffallenden bisher unerkannten struktur des seelenorgans* Berlin: Königliche Akademie der Wissenschaften.
- Eisemann, C. H., Jorgensen, W. K., Merritt, D. J., Rice, M. J., Cribb, B. W., Webb, P. D. and Zalucki, M. P. (1984). Do insects feel pain?—A biological view. *Experientia* **40**, 164–167.
- Eisenstein, E. M. and Carlson, A. D. (1997). A comparative approach to the behavior called "learned helplessness." *Behav. Brain Res.* **86**, 149–160.
- Ekman, P. (1992). Are there basic emotions? *Psychol. Rev.* **99**, 550–553.
- Ellsworth, P. C. (1991). Some implications of cognitive appraisal theories of emotion. In *International Review of Studies on Emotion*, vol. 1, pp. 143–161. New York: John Wiley and sons.
- Elwood, R. W. (2011). Pain and suffering in invertebrates. *ILAR J.* **2**, 175–184.
- Eysenck, M. W., Mogg, K., May, J., Richards, A. and Mathews, A. (1991). Bias in interpretation of ambiguous sentences related to threat in anxiety. *J. Abnorm. Psychol.* **100**, 144–150.
- Fant, G. (1960). *Acoustic Theory of Speech Production*. Mouton: The Hague.
- Fellous, J.-M. (1999). Neuromodulatory basis of emotion. *Neuroscientist* **5**, 283–294.
- Fernandez, M., Blass, E. M., Hernandez-Reif, M., Field, T., Diego, M. and Sanders, C. (2003). Sucrose attenuates a negative electroencephalographic response to an aversive stimulus for newborns. *J. Dev. Behav. Pediatr.* **24**, 261–266.



- Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecq, J.-P. and Cattaert, D. (2014). Anxiety-like behavior in crayfish is controlled by serotonin. *Science* **344**, 1293–1297.
- Fredrickson, B. L. (1998). What good are positive emotions. *Rev. Gen. Psychol.* **2**, 300–319.
- Fredrickson, B. L. and Branigan, C. (2011). Positive emotions broaden the scope of attention and thought-action repertoires. *Cogn. Emot.* **19**, 1–19.
- Frijda, N. H. (1987). Emotion, cognitive structure, and action tendency. *Cogn. Emot.* **1**, 115–143.
- Gibson, W. T., Gonzalez, C. R., Fernandez, C., Ramasamy, L., Tabachnik, T., Du, R. R., Felsen, P. D., Maire, M. R., Perona, P. and Anderson, D. J. (2015). Behavioral responses to a repetitive visual threat stimulus express a persistent state of defensive arousal in drosophila. *Curr. Biol.* **25**, 1401–1415.
- Giurfa, M. (2013). Cognition with few neurons: Higher-order learning in insects. *Trends Neurosci.* **36**, 285–294.
- Giurfa, M., Zhang, S., Jenett, A., Menzel, R. and Srinivasan, M. V. (2001). The concepts of 'sameness' and 'difference' in an insect. *Nature* **410**, 930–933.
- Gottlib, I. H. and Joormann, J. (2010). Cognition and depression: current status and future directions. *Annu. Rev. Clin. Psychol.* **6**, 285–312.
- Graeff, F. G., Ferreira Netto, C. and Zangrossi, H., Jr (1998). The elevated T-maze as an experimental model of anxiety. *Neurosci. Biobehav. Rev.* **23**, 237–246.
- Greiveldinger, L., Veissier, I. and Boissy, A. (2011). The ability of lambs to form expectations and the emotional consequences of a discrepancy from their expectations. *Psychoneuroendocrinology* **36**, 806–815.
- Hamilton, T. J., Kwan, G. T., Gallup, J. and Tresguerres, M. (2015). Acute fluoxetine exposure alters crab anxiety-like behaviour, but not aggressiveness. *Sci. Rep.* **6**, 19850.
- Hammer, M. (1993). An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* **366**, 59–63.
- Handley, S. L. and McBlane, J. W. (1993). 5HT drugs in animal models of anxiety. *Psychopharmacology* **112**, 13–20.
- Harding, E. J., Paul, E. S. and Mendl, M. (2004). Animal behaviour: cognitive bias and affective state. *Nature* **427**, 312.
- Hodgkin, A. L. and Huxley, A. F. (1939). Action potentials recorded from inside a nerve fibre. *Nature* **144**, 710–711.
- Hughes, E., Shymansky, T., Sunada, H. and Lukowiak, K. (2016). Qualitatively different memory states in *Lymnaea* as shown by differential responses to propranolol. *Neurobiol. Learn. Mem.* **136**, 63–73.
- Iliadi, K. G. (2009). The genetic basis of emotional behavior: Has the time come for a *Drosophila* model? *J. Neurogenet.* **23**, 136–146.
- Juslin, P. and Scherer, K. (2005). Vocal expression of affect. In *The New Handbook of Methods in Nonverbal Behavior Research* (ed. J. Harrigan, R. Rosenthal and K. Scherer), pp. 65–135. Oxford: Oxford University Press.
- Kandel, E. R. (2006). *In Search of Memory: the Emergence of a New Science of Mind*. New York, NY: W.W. Norton & Co.
- Kita, S., Hashiba, R., Ueki, S., Kimoto, Y., Abe, Y., Gotoda, Y., Suzuki, R., Uraki, E., Nara, N., Kanazawa, A. et al. (2011). Does conditioned taste aversion learning in the pond snail *Lymnaea stagnalis* produce conditioned fear? *Biol. Bull.* **220**, 71–81.
- Kreibitz, S. D. (2010). Autonomic nervous system activity in emotion: a review. *Biol. Psychol.* **84**, 14–41.
- Lazarus, R. S. (1991). Progress on a cognitive-motivational-relational theory of emotion. *Am. Psychol.* **46**, 819–834.
- LeDoux, J. (2012). Rethinking the emotional brain. *Neuron* **73**, 653–676.
- Lench, H. C., Flores, S. A. and Bench, S. W. (2011). Discrete emotions predict changes in cognition, judgment, experience, behavior, and physiology: a meta-analysis of experimental emotion elicitation. *Psychol. Bull.* **137**, 834–855.
- Leonhardt, S. D., Menzel, F., Nehring, V. and Schmitt, T. (2016). Ecology and evolution of communication in social insects. *Cell* **164**, 1277–1287.
- Liebsch, G., Montkowski, A., Holsboer, F. and Landgraf, R. (1998). Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behav. Brain Res.* **94**, 301–310.
- Loewenstein, G. F., Weber, E. U., Hsee, C. K. and Welch, N. (2001). Risk as feelings. *Psychol. Bull.* **127**, 267–286.
- López-Muñoz, F., Álamo, C. and García-García, P. (2011). The discovery of chlordiazepoxide and the clinical introduction of benzodiazepines: Half a century of anxiolytic drugs. *J. Anxiety Disord.* **25**, 554–562.
- Loukola, O. J., Perry, C. J., Coscos, L. and Chittka, L. (2017). Bumblebees show cognitive flexibility by improving on an observed complex behavior. *Science* **355**, 833–836.
- Lövheim, H. (2012). A new three-dimensional model for emotions and monoamine neurotransmitters. *Med. Hypotheses* **78**, 341–348.
- MacLeod, A. K. and Byrne, A. (1996). Anxiety, depression, and the anticipation of future positive and negative experiences. *J. Abnorm. Psychol.* **105**, 286–289.
- Mason, G. J. (2011). Invertebrate welfare: where is the real evidence for conscious affective states? *Trends Ecol. Evol.* **26**, 212–213.
- Mather, J. A. (2007). Cephalopod consciousness: behavioural evidence. *Conscious. Cogn.* **17**, 37–48.
- Mathews, A. (1995). Effect of psychological treatment on cognitive bias in generalized anxiety disorder. *Behav. Res. Ther.* **33**, 293–303.
- Mathews, A. and MacLeod, C. (1994). Cognitive approaches to emotion and emotional disorders. *Annu. Rev. Psychol.* **45**, 25–50.
- Mendl, M. and Paul, E. (2004). Consciousness, emotion and animal welfare: Insights from cognitive science. *Anim. Welf.* **13**, 17–25.
- Mendl, M., Burman, O. H. P., Parker, R. M. A. and Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Appl. Anim. Behav. Sci.* **118**, 161–181.
- Mendl, M., Burman, O. H. P. and Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. *Proc. R. Soc. B* **277**, 2895–2904.
- Mendl, M., Paul, E. S. and Chittka, L. (2011). Emotion in invertebrates? *Curr. Biol.* **21**, R463–R465.
- Mogg, K. and Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behav. Res. Ther.* **36**, 809–848.
- Mohammad, F., Aryal, S., Ho, J., Stewart, J. C., Norman, N. A., Tan, T. L. and Eisaka, A. and Claridge-Chang Correspondence, A. (2016). Ancient anxiety pathways influence drosophila defense behaviors. *Curr. Biol.* 981–986.
- Murphy, F., Smith, K., Cowen, P., Robbins, T. and Sahakian, B. (2002). The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology* **163**, 42–53.
- Nansen, F. (1886). Preliminary communication on some investigations upon the histological structure of the central nervous system in the Ascidia and in Myxine glutinosa. *J. Nat. Hist. Ser.* **5** **18**, 209–226.
- National Academies, ILAR., P.W.G. (2009). *Recognition and Alleviation of Pain in Laboratory Animals*. Washington, D.C: National Academies Press.
- Nettle, D. and Bateson, M. (2012). The evolutionary origins of mood and its disorders. *Curr. Biol.* **22**, R712–R721.
- Nygren, T. E., Isen, A. M., Taylor, P. J. and Dulin, J. (1996). The influence of positive affect on the decision rule in risk situations: Focus on outcome (and especially avoidance of loss) rather than probability. *Organ. Behav. Hum. Decis. Process.* **66**, 59–72.
- Oatley, K. and Johnson-Laird, P. N. (1987). Towards a cognitive theory of emotions. *Cogn. Emot.* **1**, 29–50.
- Oatley, K. and Johnson-Laird, P. N. (2014). Cognitive approaches to emotions. *Trends Cogn. Sci.* **18**, 134–140.
- Pahl, M., Si, A. and Zhang, S. (2013). Numerical cognition in bees and other insects. *Front. Psychol.* **4**, 162.
- Panksepp, J. (1982). Toward a general psychobiological theory of emotions. *Behav. Brain Sci.* **5**, 407–467.
- Panksepp, J. (1998). *Affective neuroscience: the Foundations of Human and Animal Emotions*. New York: Oxford University Press.
- Panksepp, J. (2005). Affective consciousness: core emotional feelings in animals and humans. *Conscious. Cogn.* **14**, 30–80.
- Panksepp, J. (2011). The basic emotional circuits of mammalian brains: do animals have affective lives? *Neurosci. Biobehav. Rev.* **35**, 1791–1804.
- Panksepp, J. and Burgdorf, J. (2000). 50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: Effects of social housing and genetic variables. *Behav. Brain Res.* **115**, 25–38.
- Paul, E. S., Harding, E. J. and Mendl, M. (2005). Measuring emotional processes in animals: the utility of a cognitive approach. *Neurosci. Biobehav. Rev.* **29**, 469–491.
- Peng, F., Chittka, L., Sandoz, J. C., Rössler, W., Devaud, J. M., Rössler, W., Waddell, S., Robie, A. A., Yamagata, N., Schnaitmann, C. et al. (2017). A Simple computational model of the bee mushroom body can explain seemingly complex forms of olfactory learning and memory. *Curr. Biol.* **27**, 224–230.
- Perry, C. J. and Barron, A. B. (2013). Neural mechanisms of reward in insects. *Annu. Rev. Entomol.* **58**, 543–562.
- Perry, C. J., Barron, A. B. and Cheng, K. (2013). Invertebrate learning and cognition: Relating phenomena to neural substrate. *Wiley Interdiscip. Rev. Cogn. Sci.* **4**, 561–582.
- Perry, C. J., Baciadonna, L. and Chittka, L. (2016). Unexpected rewards induce dopamine-dependent positive emotion-like state changes in bumblebees. *Science* **353**, 1529–1537.
- Perry, C. J., Barron, A. B. and Chittka, L. (2017). The frontiers of insect cognition. *Curr. Opin. Behav. Sci.* **16**, 111–118.
- Plowright, C. M. S. (2017). Bumblebees at work in an emotion-like state. *Learn. Behav.* **45**, 207–208.
- Powell, R., Mikhalevich, I. and Logan, C. (2016). Is behavioural flexibility evidence of cognitive complexity? How evolution can inform comparative cognition. *Interface Focus* **7**, 20160121.
- Proctor, H. S. and Carder, G. (2014). Can ear postures reliably measure the positive emotional state of cows? *Appl. Anim. Behav. Sci.* **161**, 20–27.
- Quaranta, A., Siniscalchi, M. and Vallortigara, G. (2007). Asymmetric tail-wagging responses by dogs to different emotive stimuli. *Curr. Biol.* **17**, R199–R201.
- Rainville, P., Bechara, A., Naqvi, N. and Damasio, A. R. (2006). Basic emotions are associated with distinct patterns of cardiorespiratory activity. *Int. J. Psychophysiol.* **61**, 5–18.
- Ramón y Cajal, S. (1909). *Histologie du système nerveux de l'homme & des vertébrés*. Paris: Maloine.

- Randall, D. C. and Hasson, D. M. (1981). Cardiac arrhythmias in the monkey during classically conditioned fear and excitement. *Pavlov. J. Biol. Sci.* **16**, 97–107.
- Reefmann, N., Wechsler, B. and Gygas, L. (2009). Behavioural and physiological assessment of positive and negative emotion in sheep. *Anim. Behav.* **78**, 651–659.
- Roelofs, S., Boleij, H., Nordquist, R. E. and Van Der Staay, F. J. (2016). Making decisions under ambiguity: Judgment bias tasks for assessing emotional state in animals. *Front. Behav. Neurosci.* **10**, 1–16.
- Rolls, E. T. (1999). *The Brain and Emotion*. Oxford, UK: Oxford University Press.
- Russell, J. A. (1980). A circumplex model of affect. *J. Pers. Soc. Psychol.* **6**, 1161–1178.
- Russell, J. A. (2003). Core affect and the psychological construction of emotion. *Psychol. Rev.* **110**, 145–172.
- Schapker, H., Breithaupt, T., Shuranova, Z., Burmistrov, Y. and Cooper, R. L. (2002). Heart and ventilatory measures in crayfish during environmental disturbances and social interactions. *Comp. Biochem. Physiol. Part A* **131**, 397–407.
- Scherer, K. R. (1986). Vocal affect expression: a review and a model for future research. *Psychol. Bull.* **99**, 143–165.
- Scherer, K. R. (2001). Appraisal considered as a process of multilevel sequential checking. In *Appraisal Processes in Emotion: Theory, Methods, Research* (ed. K. Scherer, A. Schorr and T. Johnstone), pp. 92–120. New York and Oxford: Oxford University Press.
- Scherer, K. (2003). Vocal communication of emotion: a review of research paradigms. *Speech Commun.* **40**, 227–256.
- Schirmer, A. and Adolphs, R. (2017). Emotion perception from face, voice, and touch: comparisons and convergence. *Trends Cogn. Sci.* **21**, 216–228.
- Schlosberg, H. (1954). Three dimensions of emotion. *Psychol. Rev.* **61**, 81–88.
- Schlüns, H., Welling, H., Federici, J. R. and Lewejohann, L. (2017). The glass is not yet half empty: agitation but not Varroa treatment causes cognitive bias in honey bees. *Anim. Cogn.* **20**, 233–241.
- Sharot, T., Guitart-Masip, M., Korn, C. W., Chowdhury, R. and Dolan, R. J. (2012). How dopamine enhances an optimism bias in humans. *Curr. Biol.* **22**, 1477–1481.
- Sherwin, C. M. (2001). Can invertebrates suffer? Or, how robust is argument-by-analogy? *Anim. Welf.* **10**, 103–118.
- Smith, E. S. J. and Lewin, G. R. (2009). Nociceptors: a phylogenetic view. *J. Comp. Physiol. A* **195**, 1089–1106.
- Sneddon, L. U., Braithwaite, V. A. and Gentle, M. J. (2003). Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proc. R. Soc. B* **270**, 1115–1121.
- Sotocina, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S., Mapplebeck, J. C., Wei, P., Zhan, S., Zhang, S. et al. (2011). The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol. Pain* **7**, 55.
- Steiner, J. E., Glaser, D., Hawilo, M. E. and Berridge, K. C. (2001). Comparative expression of hedonic impact: Affective reactions to taste by human infants and other primates. *Neurosci. Biobehav. Rev.* **25**, 53–74.
- Stewart, A., Gaikwad, S., Kyzar, E., Green, J., Roth, A. and Kalueff, A. V. (2012). Modeling anxiety using adult zebrafish: A conceptual review. *Neuropharmacology* **62**, 135–143.
- Strausfeld, N. J. and Hirth, F. (2013). Deep homology of arthropod central complex and vertebrate basal ganglia. *Science* **340**, 157–161.
- Tarsitano, M. S. and Jackson, R. R. (1997). Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. *Anim. Behav.* **53**, 257–266.
- Titze, I. R. (1994). *Principles of Voice Production*. Upper Saddle River, NJ: Prentice Hall.
- van der Harst, J. E. and Spruijt, B. M. (2007). Tools to measure and improve animal welfare: Reward-related behaviour. *Anim. Welf.* **16**, 67–73.
- von Borell, E., Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A. et al. (2007). Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals - a review. *Physiol. Behav.* **92**, 293–316.
- Waller, B. M. and Micheletta, J. (2013). Facial expression in nonhuman animals. *Emot. Rev.* **5**, 54–59.
- Walters, E. T., Carew, T. J. and Kandel, E. R. (1981). Associative learning in Aplysia: evidence for conditioned fear in an invertebrate. *Science* **211**, 504–506.
- Willner, P. (1986). Validation criteria for animal models of human mental disorders: Learned helplessness as a paradigm case. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **10**, 677–690.
- Wright, W. F. and Bower, G. H. (1992). Mood effects on subjective probability assessment. *Organ. Behav. Hum. Decis. Process.* **52**, 276–291.
- Yang, Z., Bertolucci, F., Wolf, R. and Heisenberg, M. (2013). Flies cope with uncontrollable stress by learned helplessness. *Curr. Biol.* **23**, 799–803.