Dispatches

Animal Behaviour: Emotion in Invertebrates?

Bees exposed to vigorous shaking designed to simulate a dangerous event judge ambiguous stimuli as predicting a negative outcome — a 'pessimistic' cognitive bias that is characteristic of anxious or depressed humans and other vertebrates in putative negative emotional states.

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Do non-human animals have emotions? If so, how can we measure them? And why should we be interested? Society's concerns about animal welfare are rooted in the assumption that animals can experience negative sensations and emotions, and hence suffer. Furthermore, the development of therapeutic psychoactive drugs continues to rely on animal models of emotion. Clearly, there are pragmatic and societally important reasons for studying emotional states in animals.

But how can such states be measured? At present, we cannot measure the conscious experience of emotion — the *feeling* of anxiety or happiness — in other species. However, contemporary research conceptualizes emotions as comprising not just a conscious component, but also behavioural, neurophysiological and cognitive components [1,2]. Changes in these latter three components can be measured objectively, may correlate with verbal report of conscious emotions in people and, therefore, may be useful proxy indicators of such states.

Recently there has been considerable interest in the possibility that the decisions animals make in ambiguous situations reliably reflect the valence (positivity or negativity) of their emotional (affective) state [3,4]. As in depressed or anxious humans, animals in putative negative affective states are more likely to make negative ('pessimistic') judgments about ambiguous stimuli than those in positive states [4]. These 'cognitive biases' may thus be useful measures of animal affect. So far, published studies have been of mammals and birds only (for example, [5–9]). In this issue of *Current Biology*, however, Bateson and colleagues [10] provide the first evidence that a similar relationship between putative affective state and cognitive bias exists in an invertebrate species, the honeybee, raising interesting questions about the interpretation of such studies, and their implications for invertebrate 'emotion' (Figure 1).

To measure cognitive biases, Bateson *et al.* [10] adapted a paradigm (Figure 2) initially developed for studying rats [5]. Honeybees were trained on a discrimination task in which one combination of two odours in a 1:9 ratio was presented with a rewarding sucrose solution, while another combination of the odours in a 9:1 ratio was presented with a less rewarding (more dilute) sucrose solution or, in separate experiments, an aversive (punishing) quinine solution [11]. In just 12 training trials, many of the bees learnt to extend their probosces to the 1:9 odour combination in order to drink the associated reward, and to withhold their probosces when the 9:1 odour combination was presented to avoid the punishing or less rewarding outcome.

The next stage of the experiment allowed investigation of cognitive biases by presenting ambiguous odour cues which were intermediate between the two trained odour combinations (odour ratios of 3:7, 1:1, 7:3). Bees responding to these ambiguous stimuli by extending their probosces (in anticipation of a sucrose reward) could be categorized as showing a more 'optimistic' response than those that did not. Before bees were tested, half were subjected to one minute of vigorous shaking to simulate a dangerous event such as a predatory attack on the hive, and to induce something akin to a negative affective state through exposure to this naturally aversive stimulus. The shaking caused decreases in dopamine, octopamine and serotonin, all of which function as hormones as well as neurotransmitters involved in learning and memory in insects, thus suggesting a potential mechanism to link state-related changes and decision-making [10].

The hypothesis that bees in this shaken state would show a more 'pessimistic' response to ambiguous odour cues than non-shaken bees was supported. They were less likely to extend their probosces to the trained 9:1 odour combination predicting punishment, and to the most similar ambiguous 7:3 odour combination. A lack of differences between the treatment groups in their responses to the 1:9 odour combination predicting reward indicates that the findings were not simply the result of shaking inhibiting subsequent proboscis extension, or decreasing general activity. The predicted cognitive bias was observed in three separate experiments using different combinations of sucrose rewards

Figure 1. High anxiety for a bee?

Insects are often viewed as simple reflex machines, but new research on the honeybee shows that their decisions in ambiguous situations appear to be state-dependent — they interpret ambiguous stimuli more negatively following experience of an aversive event. Depressed or anxious people, and other vertebrates in putatively negative affective states, also interpret ambiguous stimuli pessimistically, raising the possibility that bees, like these other species, possess affective states that help guide decision-making. However, whether these states are consciously experienced remains an open question.

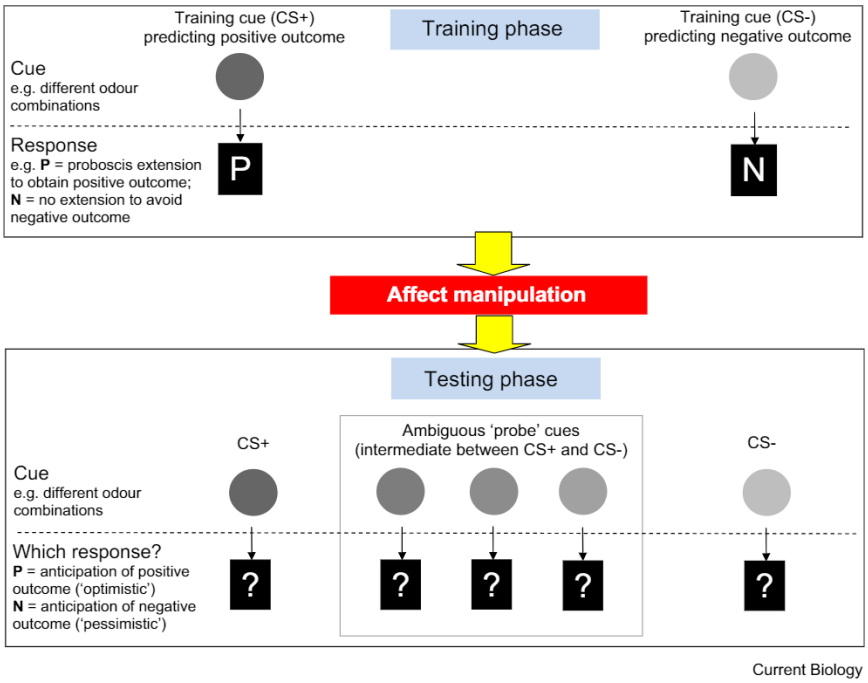

Figure 2. A generic method for testing affect-induced cognitive bias in decision-making under ambiguity.

Training phase: the subject is trained to make one response (P) to a CS+ cue predicting a positive outcome (e.g. palatable food) in order to obtain that outcome, and a different response (N) to a CS- cue (in the same sensory dimension as CS+) to avoid a negative outcome. Affect manipulation: once trained, the subject is exposed to a short- or long-term treatment designed to induce a relatively positive or negative affective state (e.g. exposure to an aversive event or environment). Testing phase: the subject is presented with CS+, CS-, and ambiguous cues with sensory properties that are intermediate between CS+ and CS-, to test the hypothesis that individuals in a putatively negative affective state are more likely to make response N to these cues — indicating a 'pessimistic' cognitive bias — than individuals in a relatively positive state. (See [5].)

and unrewarded or punishing stimuli, confirming a robust effect of the shaking treatment.

The effects are as clear as those from comparable vertebrate studies (for example, [5–9]), and remind us to be cautious about assuming that the cognitive processes of bigger-brained animals are necessarily richer or more sophisticated than those of smaller-brained ones [12]. Modulation of decision-making by affective state may occur in bees, as in humans and other vertebrates. The neurophysiological mechanisms are likely to be different, but the resultant decision-making behaviour looks strikingly similar.

Does this mean, then, that bees have emotional states, in the sense of conscious experiences of feelings? This question is as hard to answer as it is for vertebrate animals [13]. Indeed, the inference of conscious experience of emotions from observed behaviour is problematic even in humans [14]. Pain, unlike basic nociception, is a sensory and emotional experience that is consciously perceived. Yet symptoms typically associated with pain (withdrawal reflexes, grimacing, surges in stress hormones levels) occur even in anaesthetized humans under surgery, brain-dead patients, and anencephalic babies [15]. In animals, we must be even more cautious about deducing conscious emotional states from overt behaviour [16]. For example, in the 18th century, the honeybee dance was interpreted as an expression of 'joy', exhibited by bees that had discovered a particularly rich foraging bonanza [17]. If one defines happiness by behavioural criteria such as exuberant movement patterns following a rewarding experience, then one might indeed classify the waggle dance in this fashion. However, such an interpretation clearly misses the actual function of the honeybee dance, to communicate the precise coordinates of a food source.

It is, of course, legitimate to label behaviour patterns as equivalent to those associated with certain emotions in humans, and to consider their adaptive benefits. Consider aggression — the question of whether bees can be aggressive is quite apart from whether they consciously 'feel angry'. We can analyse the behavioural features, adaptive significance, physiology and genetics of aggression without implicating experiential components. In this vein, it is interesting to contemplate the adaptive function of the cognitive bias that Bateson *et al.* [10] have discovered in bees. Their findings raise the possibility that, perhaps like other species, bees possess a system that tracks their experiences of rewarding and punishing events in the environment, influencing the decisions that they make in an adaptive way [18]. A positively valenced state, resulting from experience of a generally rewarding environment, may act to bias judgement of an ambiguous stimulus as being more likely to herald a rewarding rather than a punishing event, whilst a negatively valenced state, resulting from experience of a punishing or dangerous environment, should favour the opposite judgement to avoid the higher probability of an unfavourable outcome. This (affective) state may thus act as a cumulative measure of experience in the environment — a proxy Bayesian prior — to aid decision-making, and be manifest by various neural mechanisms, perhaps as a result of convergent evolution, across diverse taxa. Bateson *et al.* [10] provide a first indication that the interface between such states and decision-making has similarities across vertebrate and invertebrate taxa, and raise fascinating questions about the processes that underlie ambiguous judgements.

Biased decision-making under ambiguity may thus reliably reflect the valence of an animal's affective state, but the question of whether (and which) animals have an actual awareness of such states (conscious emotions) remains open. It might be possible to address this question by borrowing conceptual tools from, for example, the study of metacognition — research into the question of whether animals know what they know [19]. Useful information might also emerge from a comparative exploration of what the adaptive benefits of such an awareness might be for a variety of animal species in their natural environment.

References

1. Lang, P.J. (1993). The three system approach to emotion. In The Organization of Emotion, N. Birbaumer and A. Öhman, eds. (Toronto: Hogrefe-Huber), pp. 18–30.
2. Clore, G.L., and Ortony, A. (2000). Cognition in emotion: always, sometimes or never? In Cognitive Neuroscience of Emotion, R.D. Lane and L. Nadel, eds. (Oxford: Oxford University Press), pp. 24–61.
3. 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.
4. 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.
5. Harding, E.J., Paul, E.S., and Mendl, M. (2004). Cognitive bias and affective state. Nature *427*, 312.
6. Bateson, M., and Matheson, S.M. (2007). Performance on a categorisation task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). Anim. Welfare *16*, 33–36.
7. Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E., Blackwell, E., and Casey, R. (2010). Dogs showing separation-related behaviour exhibit a 'pessimistic' cognitive bias. Curr. Biol. *20*, R839–R840.
8. Enkel, T., Gholizadeh, D., von Bohlen und Halbach, O., Sanchis-Segura, C., Hurlemann, R., Spanagel, R., Gass, P., and Vollmayr, B. (2010). Ambiguous-cue interpretation is biased under stress and depression-like states in rats. Neuropsychopharmacology *35*, 1008–1015.
9. Doyle, R.E., Hinch, G.N., Fisher, A.D., Boissy, A., Henshall, J.M., and Lee, C. (2011). Administration of serotonin inhibitor p-Chlorophenylalanine induces pessimistic-like judgement bias in sheep. Psychoneuroendocrinology *36*, 279–288.
10. Bateson, M., Desire, S., Gartside, S.E., and Wright, G.A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. Curr. Biol. *21*, 1070–1073.
11. Chittka, L., Dyer, A.G., Bock, F., and Dornhaus, A. (2003). Bees trade off foraging speed for accuracy. Nature *424*, 388.
12. Chittka, L., and Niven, J. (2009). Are bigger brains better? Curr. Biol. *19*, R995–R1008.
13. Dawkins, M.S. (1998). Through Our Eyes Only? The Search for Animal Consciousness (Oxford: Oxford University Press).
14. Berridge, K.C., and Winkielman, P. (2003). What is an unconscious emotion? (the case for unconscious 'liking'). Cogn. Emotion *17*, 181–211.
15. Lee, S.J., Ralston, H.J.P., Drey, E.A., Partridge, J.C., and Rosen, M.A. (2005). Fetal pain – a systematic multidisciplinary review of the evidence. JAMA *294*, 947–954.
16. Doering, T.F., and Chittka, L. (2011). How human are insects, and does it matter? Formosan Entomologist *31*, 85–99.
17. Leadbeater, E.A., and Chittka, L. (2009). Social information use in foraging insects. In Food Exploitation by Social Insects: Ecological, Behavioral, and Theoretical Approaches, S. Jarau and M. Hrncir, eds. (Boca Raton: CRC Press), pp. 135–146.
18. 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. Roy. Soc. B *277*, 2895–2904.
19. Smith, J.D., Shields, W.E., and Washburn, D.A. (2003). The comparative psychology of uncertainty monitoring and metacognition. Behav. Brain Sci. *26*, 317–339.

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DOI: 10.1016/j.cub.2011.05.028

Thermal Robustness: Lessons from Bacterial Chemotaxis

Temperature changes affect reaction kinetics. How do signaling pathways cope with such global perturbation? A recent study dissects the solution found by bacterial chemotaxis.

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Biological systems living in complex environments must perform well across a wide range of environmental conditions. Therefore, signaling pathways and regulatory networks controlling cellular functions are expected to operate in the presence of environmental perturbations [1]. Temperature fluctuations in the cellular environment affect the rate of all chemical reactions in a cell. How cells remain functional in the face of such global perturbations remains relatively unexplored. A recent study published in *Cell* by Oleksiuk *et al.* [2] takes us a step closer to understanding the molecular mechanisms used by signaling pathways to cope with temperature change. Using the *Escherichia coli* chemotaxis pathway as model system [3], the authors present a comprehensive picture of the various strategies evolved by this system to compensate for the effect of temperature change on two key functional parameters: the steady state of the system output and the rate of adaptation to a constant stimulus.

Like many flagellated bacterial species, *E. coli* biases its random walk (runs and tumbles) toward favorable conditions by making temporal comparisons of environmental signals and by suppressing changes in swimming direction accordingly [3]. The basic functional ingredients necessary to swim up a signal gradient are high sensitivity to stimuli and the capability to adapt to constant stimulus. In both regards, bacterial populations of *E. coli* perform extremely well, operating close to the theoretical limit of sensitivity [4] while exhibiting nearly perfect adaption over five orders of magnitude in signal intensity [5,6]. High sensitivity is achieved through the cooperative activity of receptors that form clusters in the cell membrane [7,8], and adaptation results from the slow methylation or demethylation of these receptors by the antagonistic enzymes CheR and CheB, respectively (Figure 1A). The activity of the receptors is transmitted to the flagellar motors through a phosphorylation cascade initiated by the receptor-bound histidine kinase CheA and relayed by the cytoplasmic response-regulator CheY. The switching rate of the flagellar motors, which determines the mean run length, is ultra-sensitive to the level of the phosphorylated form of CheY (CheY-P) [9]. CheY-P steady-state levels are maintained in the sensitive range of the motor by the basal level of CheA kinase activity and constitutive dephosphorylation by the phosphatase CheZ. Despite its simplicity, the bacterial chemotaxis pathway exhibits rich functions and continues to reveal remarkable properties of biological pathways to researchers.

Probing how *E. coli* chemotaxis might be affected by temperature variation represents a significant technical challenge. Thanks to current theoretical understanding of the behavior of the pathway and a clever use of genetic backgrounds, Oleksiuk *et al.* [2] were able to monitor the activities of different components of the system *in vivo.* Taking advantage of the rapid kinetics of the phosphorylation cascade, they monitored the Förster resonance energy transfer (FRET) between CheY-P and CheZ, both labeled with fluorescent proteins, as a readout of the kinase activity [8]. Using ordinary