mechanisms may vary between pathogenic proteins or even between cells (*13*). However, interfering with any stage has the potential to block the spreading and neurotoxicity of proteopathic lesions (*1*, *13*). It will be important to determine whether LAG3 is simply a cell-surface binding protein that mediates the uptake of pathogenic α-synuclein, or whether LAG3 might mediate further intracellular trafficking or signaling or propagation of aggregates. Another crucial step in transmission is the exit of pathogenic α-synuclein from cells. Strikingly, an unconventional deubiquitylase USP19-dependent secretion pathway for misfolded cytoplasmic proteins, including misfolded α-synuclein but not tau (*14*), suggests that the mechanisms for uptake and release may be very specific for given proteopathic aggregates.

In Parkinson's disease, α-synuclein aggregation in the brain begins many years before the onset of symptoms (*15*). Thus, interfering with the α-synuclein cascade is likely to be most effective when initiated at an early preclinical stage. LAG3-blocking antibodies were used by Mao *et al.*, and anticancer agents that block LAG3 have been developed to overcome immunosuppressive mechanisms within the tumor microenvironment (*9*). However, to minimize the risk of autoimmune or hyperimmune activation phenotypes, very specific suppression of LAG3 in the brain is needed if this protein is to be safely targeted in the treatment of brain diseases.

If LAG3 is confirmed to be a receptor for pathogenic α-synuclein, it will be important to define the structural requirements for binding and the nature of the pathogenic α-synuclein species that binds LAG3. Although many important issues remain to be resolved, the remarkable interaction of LAG3 and aggregated α-synuclein calls for additional research to determine the physiological function of LAG3 in the brain and to evaluate the potential of LAG3 as a therapeutic target for modifying the course of Lewy body dementia and Parkinson's disease. ■

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Decision-making behavior in bumblebees appears analogous to optimism in humans.

PHOTO: SMUDGE 9000/FLICKR

BEHAVIOR

Bee happy

Bumblebees show decision-making that reflects emotion-like states

By **Michael T. Mendl** *and* **Elizabeth S. Paul**

In his book, *The Expression of the Emotions in Man and Animals*, Charles Darwin noted that "Even insects express anger, terror, jealousy, and love by their stridulation." Almost 150 years later, spurred by an interest in the evolutionary roots of emotional (affective) processes and their underlying mechanisms, there has been a sudden upsurge of research into the question of whether insects and other invertebrates may indeed have emotion-like states (*1*–*4*). Recent work has focused on negative affect, but on page 1529 of this issue, Perry *et al.* (*5*) broaden the scope to consider positive emotions. The authors report decision-making behavior in bumblebees that is analogous to optimism in humans and may reflect positive affect in both humans and other species (*6*–*8*). Moreover, the behavior appears to depend on the activity of dopamine, a neurotransmitter involved in the processing of reward in humans.

Emotions are quintessentially subjective experiences—positive or negative feelings such as happiness or anger—so how can such private states be studied in nonhuman animals? One way is to provide an operational definition of emotion that allows researchers to identify what state an animal is in and hence to search for associated underlying mechanisms, and physiological, behavioral and cognitive markers that, unlike feelings, can be measured objectively. A recent suggestion is to use general properties of human emotion, including valence [positivity or negativity; a key defining characteristic of human emotion (*7, 9*)], arousal or scalability, persistence after a stimulus or event, and generalization across situations—so-called "emotion primitives" (*1*)–to identify affective states in other species.

Another operational definition is that emotions are states elicited by rewards and punishers, where a reward is something for which an animal will work and a punisher is something that it will work to avoid (*7, 9, 10*). On this basis, an animal exposed to a punisher is in a negative affective state. Even if researchers disagree, such definitions lay out assumptions that can be argued and improved, and make clear exactly why a particular method for inducing an emotion, or a particular measure of emotion, is being studied. Many scientists are agnostic about whether these operationally defined states are consciously experienced in animals, and it is possible that some species subjectively

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experience emotion whereas others exhibit behavioral and physiological indicators of emotion ("emotion-like") without any accompanying feeling (*11*).

Perry *et al.* used a rewarding sweet sucrose solution to induce a positive emotional state in bumblebees, in line with the rewards-punishers operational definition. They then confirmed this state with a cognitive bias test that is based on the finding that human emotions can bias decision-making under ambiguous conditions (*6*). Thus, happy people are more likely than unhappy people to make optimistic judgments about ambiguous situations (*7*). This cognitive bias test is now used widely in animal emotion research (*8*). Bumblebees were trained such that in any trial, a cylinder was placed either on one side of a foraging arena next to a green card or on the other side next to a blue card. One of the location-color combinations indicated that the cylinder contained a 30% sucrose solution reward, whereas the other location-color combination indicated that the cylinder contained just water. The bees learned to fly faster to the cylinder on trials with the sucrose reward configuration (see the figure). Occasional trials were then used to test their responses to ambiguity by presenting the cylinder between the two trained locations and next to an intermediately colored (blue-green) card. The prediction was that bees in a positive affective state would fly faster to intermediate cues, analogous with an optimistic judgment of ambiguity (*6*). Indeed, Perry *et al.* found that bees given an unexpected 60% sucrose reward to induce a positive affective state prior to an ambiguity trial, flew faster to the cylinder than nonrewarded bees.

Testing emotion-like states

A cognitive bias test examines affect-induced changes in decision-making under ambiguity. Bees are trained to associate one set of cues with a sucrose reward and another set of cues with no reward.

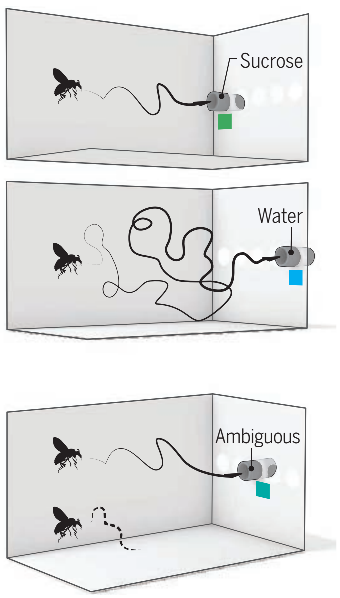
Training trials

With reward cue
Bee learns to fly quickly to one color/cylinder location to obtain sucrose.

With nonreward cue
Bee learns to fly slowly or not at all to a different color/cylinder location that contains only water.

Testing trial

With ambiguous cue
Bee in a positive affective state is more likely to fly to an ambiguous color/cylinder location.



Perry *et al.* then tested the idea that emotional states generalize across contexts (*1*), observing that the 60% sucrose solution also improved the speed of recovery (resumption of foraging) from a subsequent predator attack simulated by brief restraint. The effect of sucrose disappeared when a dopamine antagonist (fluphenazine) was topically applied before the predation test. Fluphenazine also prevented sucrose-induced optimistic-like responses to ambiguity in the cognitive bias test.

The findings of Perry *et al.* suggest that a sucrose reward induces a putatively positive state in an insect that persists for at least a short time, has effects across both positive (foraging) and negative (simulated predation) contexts, alters behavior as predicted in a cognitive bias test specifically designed to assess the valence of affective states, and is mediated by dopaminergic circuitry. Such a state satisfies a number of the criteria identified by the operational definitions of emotion (*1, 10*).

It is possible that the induced behavioral state of the bees, rather than having affective properties, was simply one of general increased activity resulting from the energizing effects of sucrose. However, Perry *et al.* showed that the sucrose-induced faster flight in the ambiguity test was not observed in other foraging contexts, and did not occur in response to novel stimuli, indicating that it was unlikely to be an effect of a general activity increase. In the absence of a specific control in the simulated predation context, it remains possible that sucrose may have exerted its effects via a general energizing effect on the speed of recovery after restraint.

The finding that a dopamine antagonist blocked the effects of sucrose in the foraging and predator tests indicates that the same underlying state was at work in both contexts. For example, increased activity of reward-sensitive dopaminergic neurons, which could be likened to a primitive positively valenced state, may bias action selection in favor of active or approach behavior in response to relevant stimuli (*12*). On its own, however, this mechanism would not explain the specificity of such a response to ambiguous as opposed to novel stimuli. Moreover, given that dopamine is also involved in punishment processing (*12, 13*), fluphenazine would have been expected to interfere with dopamine-mediated suppression of action in response to simulated predation in the absence of a sucrose reward, but this was not observed.

The study of Perry *et al.* extends recent work on invertebrate emotion, in particular by focusing on positive states and their impact across contexts. It also supports the hypothesis that one function of affective states is to act as a predictor of decision outcome probabilities that guides decision-making, particularly under ambiguity in which current information on outcomes is lacking (*9*). Given the likely adaptive value of such emotion-cognition interactions, it is not surprising that insects, like other taxa, possess emotion-like systems to implement them.

Many questions remain to be answered, including whether cross-context effects are indeed the result of an internal state that is mediated by the same underlying neural mechanisms. Likewise, do induced negative states also show cross-context effects and are these mediated by the same or different neural processes? The extent to which such states exhibit other operationally defined properties of emotion and hence warrant the label, is also unclear. Finally, whether "emotion-like" states in insects are accompanied by emotional feelings remains unanswered, but the possibility of insect consciousness is now the topic of exciting new theories and vigorous debate (*14, 15*). ■

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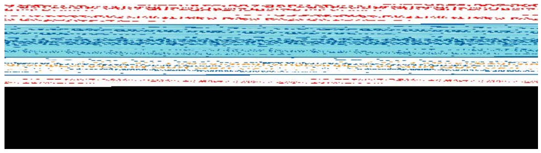

Bee happy
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Editor's Summary