Animal behaviour

Energetic cost of learning and memory can cause cognitive impairment in honeybees

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The energetic cost of cognitive functions can lead to either impairments in learning and memory, or to trade-offs with other functions, when the amount of available energy is limited. However, it has been suggested that, under such conditions, social groups such as honeybees might be able to ward off cognitive impairments in individual bees by adjusting resource allocation at the colony level. Using two complementary experiments, one that tests the effect of learning on subsequent energetic state and survival, and another that tests the effect of energetic state on learning and retention, we show that individual bees pay a significant energetic cost for learning and therefore suffer from significant cognitive deficits under energetic stress. We discuss the implications of such cognitive impairments for the recent observations of bees disappearing from their colonies as well as for social life in general.

1. Introduction
All biological processes require energy, and even cognitive functions such as learning and memory, which are generally discussed only with respect to their benefits, have energetic costs [1–3], which can either lead to their trade-offs with other functions [4,5] or to impaired cognition, when energy is limited [6,7]. In social animals, however, the negative effects of energetic stress on the cognitive ability of an individual can potentially be mitigated by changes in resource allocation patterns at the group level. In one such experiment, implementing a nutritional stress at the colony level in honeybees resulted in no impairments in learning and memory in individual bees, and the colony adjusted to the stress by reducing its brood production [8]. However, the assay used in that study, which involved the associative learning of an appetitive task, can confound the effect of energetic stress on learning with an increased motivation to learn such a task under those circumstances [9]. Moreover, energetic stress can apply at the level of the individual rather than the entire group when only specific individuals are targets of stressors such as parasitic infections [10], which are incidentally also known to cause cognitive deficits quite commonly [11–14]. The goal of this study was therefore to test if energetically stressed individuals in a social group show any trade-offs or deficits with respect to cognition.

We first determined if there is an energetic cost of learning in honeybees by testing the hypothesis that appetitve associative learning [15] leads to lower post-learning energy level and shorter survival. However, by not applying an energetic stress to the bees prior to the conditioning procedure, we avoided the confounding effects of any increase in motivation. Then, we tested if bees show any impairment in cognitive functions when they are at a lower energetic state, by assessing learning and retention in bees made to face a significant energetic stress through a combination of starvation and an energetically expensive immune challenge. In this case, by using an aversive learning assay [16], we...
could directly test the effect of energetic stress on learning without the confounding effects of increased motivation to learn appetitive tasks under such stress.

2. Material and methods

(a) Experiment 1
We randomly assigned a bee to one of three different groups: paired, unpaired and untreated. Paired bees were subjected to the conditioning assay such that they received an odour (a conditioned stimulus, CS) and a sucrose reward (an unconditioned stimulus, US) overlapping in time, allowing the bees an opportunity to associate the two. Unpaired bees received the same CS and US, but separately in time, not giving the bees an opportunity to associate the two (see the electronic supplementary material for details). A bee that showed a proboscis extension response (PER) to the CS before the US was delivered was given a score of one for exhibiting the conditioned response (CR), whereas a bee showing a PER later or not showing it at all was given a score of zero, and the resulting proportion of CR in each trial was used as a measure of learning. Untreated bees were not exposed to any conditioning but were fed with the same amount of sucrose received by bees in the other two groups during each trial in order to equalize the energetic states of all the three groups.

Just before beginning the conditioning trials, and again immediately after their completion, a subset of bees in each group was euthanized to assay their haemolymph trehalose (see the electronic supplementary material for details), which is a robust indicator of their energetic levels [8]. The other half of bees in each group was placed in an incubator maintained at 25°C and 70 per cent relative humidity, and their survival was monitored every 1 h. The experiment was terminated after 36 h and any bees still alive were recorded as surviving for 36 h. Twelve replicates of the experiment were conducted.

(b) Experiment 2
We challenged the immune system of bees by injecting into their thorax 5 μl of Ringer’s solution containing 4 per cent lipopolysaccharide, which is a non-pathogenic elicitor of the immune response. The bees in the control group were each injected with 5 μl of Ringer’s solution at the same time. Each group was then further divided into two subgroups, half the bees in each subgroup being fed with 50 per cent sucrose solution until satiation and the other half being fed with nothing. This resulted in four groups, satiated control, starved control, satiated immune-challenged and starved immune-challenged. Each bee was then subjected to an aversive conditioning assay using a sting extension reflex, which provides it with an opportunity to associate an odour with an electric shock. A retention test consisting of presenting only the CS was performed 1 h after the conditioning trials (see the electronic supplementary material for details). Learning and retention were measured as the proportion of CR displayed by the bees.

3. Results

(a) Experiment 1
Paired bees learned to associate the CS and the US (repeated-measures logistic regression, Wald’s $\chi^2 = 50.62, p < 0.0001$; electronic supplementary material, figure S1), whereas the unpaired bees did not (Wald’s $\chi^2 = 3.84, p = 0.42$). The survival of the different groups subsequent to the conditioning trials was significantly different (figure 1a; see the electronic supplementary material, table S1 for complete details). Dividing the bees in the paired group into learners (bees with number of CR $\geq 1$) and non-learners (bees with number of CR $= 0$), there was a significant difference in survival among the four groups, with the learners having a significantly shorter survival compared with the non-learners (Kaplan–Meier analysis followed by log rank test for paired comparisons, $\chi^2 = 22.43, p < 0.001$) and the unpaired bees ($\chi^2 = 41.15, p < 0.001$), which in turn did not differ significantly in survival from the untreated bees ($\chi^2 = 0.02, p = 0.88$). Moreover, for the paired bees, survival had a significant negative correlation with their learning score as given by the number of CRs they showed ($R = -0.42, n = 48, p = 0.003$, figure 1b). The difference in survival among the groups paralleled the difference in energetic levels among them following
conditioning, with the paired bees having significantly lower trehalose in their haemolymph compared with the unpaired bees (Mann–Whitney $U = 282, p = 0.04$, figure 1c) and the untreated bees ($U = 225, p = 0.01$), the latter two of which did not differ significantly from each other ($U = 467, p = 0.56$). The paired bees also showed a significant decrease in their trehalose levels compared with the bees before the conditioning trials began ($U = 653, p = 0.04$), a change not seen in the unpaired bees ($U = 1261, p = 0.92$).

(b) Experiment 2
Bees from all the four groups learned to associate the CS with the US (repeated-measures logistic regression, Wald’s $\chi^2 = 412.25, p < 0.001$, figure 2a). Each treatment, energetic state ($\chi^2 = 20.13, p < 0.001$) and immune challenge ($\chi^2 = 24.73, p < 0.001$) had a significant independent effect on learning and there was a significant additive interaction between the two treatments ($\chi^2 = 48.58, p < 0.001$). Similarly, both energetic state (logistic regression, Wald’s $\chi^2 = 5.10, p = 0.02$) and immune challenge ($\chi^2 = 5.05, p = 0.02$, figure 2b) had significant independent effects on a bee’s ability to retain the aversive association. In terms of both learning and retention, starved and immune-challenged bees had the poorest performance, whereas satiated bees that were not immune-challenged showed the highest performance.

4. Discussion
The results of this study show that individual honeybees pay a significant energetic cost for learning, and suffer learning and retention deficits when they are energetically stressed. This shows that while sociality can potentially mitigate the effect of nutritional stress on cognition by changing resource allocation within the colony [8], we see the same kind of costs and trade-offs related to cognitive functions in individual bees as is seen in solitary animals [4–7]. Our result showing that the learning performance of satiated immune-challenged bees is substantially higher than starved immune-challenged bees but is similar to starved control bees suggests that nutritional compromise might be a primary underlying mechanism for the cognitive impairment seen in diseased animals [17]. The energetic cost of learning observed here most probably arises from the cost of neural processing rather than from any locomotor activity that would normally accompany learning (since the bees were harnessed), although such costs are going to add up in the natural setting.

In honeybees, foragers both perform the most energetically expensive task and carry the highest infection loads in the colony. Given that they also must learn a variety of tasks such as navigating the resource landscape and choosing and handling different flowers, they are the ones most likely to show trade-offs between cognitive functioning and survival under conditions of nutritional stress. A cognitive impairment in foragers might have some implications for the recently observed phenomenon of colony collapse characterized by bees disappearing from their colonies. Colonies suffering from an energetic stress due to a range of factors such as disease [10], pesticide exposure [18] or poor habitats [19] could experience a twofold cost, a reduction not only in the total number of foragers, but also a disproportionate loss of the best among them, which could hasten the demise of a colony. This is the inherent risk in a system in which a select few, the high learners, disproportionately bear the cost of learning, while the entire group reaps its benefits. Our results also reveal the interesting paradox that, while cognitive processes such as learning and memory have particularly high ecological benefits during times of energetic stress, these are also the times when cognitive impairments are most likely.

We thank Morgan Petersen for her help. This work was supported by a NSF CAREER award to D.N. who also thanks Chris Mayack and H. S. Arathi for helpful discussions.

References


