Immune response impairs learning in free-flying bumble-bees

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Parasites can influence different host behaviours including foraging, mate choice and predator avoidance. Several recent papers have shown reduced learning abilities in infected insects. However, it is difficult to separate the effects of the immune response from the direct effects of the parasite. Using a free-flying learning paradigm, this paper shows that learning performance is impaired in bumble-bees (Bombus terrestris) that are not infected but whose immune system is stimulated non-pathogenically. This demonstrates that before it is assumed that a parasite has a direct effect on a host’s behaviour, the effect of the immune response stimulated by the parasite must first be quantified.

Keywords: psychoneuroimmunology; cross-talk; social insects

1. INTRODUCTION
Parasites can influence different host behaviours including foraging, mate choice and predator avoidance (Moore 2002). Several recent papers have shown reduced cognitive abilities in infected insects (Gegear et al. 2006; Iqbal & Mueller 2007). In nature, this would have severe fitness costs (Raine & Chittaka 2008). However, although parasites act directly on hosts, a growing number of general pathologies have been shown to be the result of the immune response elicited by the parasite (Moret & Schmid-Hempel 2000). Bumble-bees (Bombus impatiens) infected by a protozoan parasite (Crithidia bombi) have an impaired ability to learn the colour of rewarding flowers (Gegear et al. 2006). Although this infection-induced change to cognitive function in bumble-bees could be the direct effect of the parasite, this seems unlikely as Crithidia is restricted to the gut of the bumble-bee (Gegear et al. 2006). It seems more likely that this behaviour is caused by the actions of the immune response.

There is extensive communication between the central nervous system and the immune system (Dantzer 2004). Many behavioural responses to infectious agents, such as fever, increased slow-wave sleep, reduced activity, exploration and sexual behaviour in mammals, are orchestrated by immune products called proinflammatory cytokines that are released in response to the detection of antigens (Maier & Watkins 1998). Links between the nervous and immune systems are not unique to vertebrates.
at least five consecutive foraging bouts were chosen for training. During the training phase, each rewarding flower was filled with 15 μl sugar water (50% w/v) and the unrewarding flower was filled with 15 μl water to ensure that the bees could not discriminate by sight alone. Once a bee began foraging only it was allowed in and out of the flight arena. We recorded the colour of the flowers visited for a total of 90 successive visits for each bee. Once a bee temporarily left the flight arena, the flowers were refilled manually with a pipette. After each individual bee’s visits, the flight arena was cleaned using 70% industrial methylated spirit. The colour of the rewarding flower was alternated between the bees. It was noted that the bees often walked between the flowers in the same Petri dish.

(ii) Training method B
Training method B, which was used for colony 2, is similar to the method outlined in Raine et al. (2006). We found this to be a simpler way of recording free-flying learning ability, specifically the bees were required to land on each flower and could not walk rapidly between the flowers. During the pretraining, the bees foraged from 20 randomly placed blue or yellow flowers, both containing 15 μl of sugar water. The flowers consisted of an inverted Eppendorf tube on a small platform (20 mm in diameter), 50 mm from the floor of the arena. The bees completing at least five consecutive foraging bouts were selected for training. Ten blue flowers and 10 yellow flowers were placed at random locations within the arena. The yellow flowers were always rewarding, as the bees show an innate preference for the blue flowers (Raine et al. 2006). For each bee, we recorded 89 flower choices after the bee first chose a rewarding flower. That is, each bee visited a minimum of 90 flowers.

(c) Statistical analysis
The method of generalized estimating equations was used to account for correlations among observations from the same subject in a repeated measures logistic regression (Hardin & Hilbe 2003). The dependent variable was ‘visit to rewarding flower’ (yes/no). The independent variables were visit (1–90) and treatment (LPS or Ringer).

3. RESULTS
We tested 31 bees injected with LPS (colony 1: 16 and colony 2: 15) and 27 injected with Ringer’s solution (colony 1: 12 and colony 2: 15). As each colony was trained in a different way, each colony was analysed separately. The probability of both LPS and Ringer bees choosing the rewarding flowers increases over subsequent visits, i.e. the bees learn (visit: colony 1 $z = 14.59, n = 2520, p < 0.0001$ and colony 2 $z = 7.84, n = 2690, p < 0.001$; figure 1). However, we found that LPS bees take a longer time to learn the colour of the rewarding flowers (treatment: colony 1 $z = -3.37, n = 2520, p = 0.001$ and colony 2 $z = 9.3, n = 2690, p < 0.0001$; figure 1). This is especially obvious in the results of colony 2 where LPS bees have a much lower probability of choosing a rewarding flower in earlier visits when compared with the Ringer bees. In both colonies, figure 1 shows that by the eighth visit block, the proportion of the rewarding flowers is the same for both LPS and Ringer bees.

For colony 2, treatment (LPS versus Ringer) had no effect on how quickly the bees first found a rewarding flower (Mann–Whitney: $U = -1.378, n = 30, p = 0.1681$, median value = 7 visits). This suggests that treatment does not affect the naive colour choice of bees. Owing to the design of training method A, this information is not available for colony 1.

4. DISCUSSION
We found that bees whose immune systems were stimulated non-pathogenically by LPS had an impaired ability to learn the colour of the rewarding flowers. This result mirrored the effects found in both PER assays using LPS (Riddell & Mallon 2006) and the performance of bumble-bees in a flower choice assay when infected by Crithidia (Gegear et al. 2006).

Previous work has shown a correlation between the performance of honeybees in the PER assays and in the free-flying experiments (Laloi et al. 2000). One difference that we found between our PER work and our current study involves protein consumption. In both honeybees and bumble-bees, immune-induced PER impairment was only recorded when protein consumption was restricted (Riddell & Mallon 2006). From this, we suggested that increased protein consumption ameliorated the effects of the immune response on memory. However, in this current free-flying experiment, protein was not controlled, yet a clear effect was present. A recent study has shown that an immune response increases the food intake of bumble-bees (Tyler et al. 2006). It is also known that if a bumble-bee is allowed to forage, its immune response is decreased (Konig & Schmid-Hempel 1995). We hypothesize that during the PER assays, the harnessed bees have reduced protein requirements, therefore to expose the immune-induced reduction in memory we must control protein consumption. In the free-flying experiment, foraging as a physical act has a protein cost, therefore the protein requirements of the bee are increased. The bee then exhibits the immune-induced cognitive impairment without artificial external protein reduction.

Although both the experimental assays show a reduction in the ability to choose the correct flower type, the degree of immune-induced impairment in colony 2 (figure 1b) seems to be much stronger than in colony 1. It has been shown that bumble-bee colonies differ in both the level of their immune response (Mallon et al. 2003b) and their performance.

in associative learning tasks (Raine et al. 2006). It is possible that the colonies vary in how much their cognitive abilities are affected by their immune response. However, we feel this is a question for future work. Our two colonies’ results cannot be directly compared as the assays used to test them were different. The larger impairment seen in colony 2 is still within the range of abilities found naturally in colonies (Raine & Chittka 2008).

This paper shows that the previously found decreased PER ability in immune-stimulated bees can be generalized to a more natural learning paradigm and a more realistic nutritional status. Recently, it has been shown that learning ability has a direct effect on bumble-bee colony fitness (Raine & Chittka 2008). This opens up the possibility that this cross-talk between the immune and the nervous systems could have vital fitness costs. Our results clearly demonstrate that before it is assumed that a parasite has a direct effect on a host’s behaviour, the effect of the immune response stimulated by the parasite must first be quantified.

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