

ORIGINAL RESEARCH ARTICLE



Cold anaesthesia decreases foraging recruitment in the New World bumblebee, *Bombus occidentalis*

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Summary

Cold narcosis is commonly used for immobilizing bees and has been found to have no effect on mortality and fecundity in honeybees. We present the first study examining the effect of cold narcosis on recruitment and foraging in bumblebees. In a controlled laboratory setting, we observed that the number of foraging *Bombus occidentalis*, a New World bumblebee, increased after the focal forager returned to the nest with rich 2.5 M sucrose solution. However, cold narcosis (~4° C, ~5 min) significantly reduced the ability of *B. occidentalis* foragers to incite nestmates to forage in a flight arena: 22% fewer total foragers and 56% fewer new recruits were present in the flight arena 10 min after the return of a cold-treated bee compared to the numbers present after the return of an unmanipulated bee. These results demonstrate unanticipated effects of cold anaesthesia on bumblebee foraging behaviour, suggesting that the use of cold anaesthesia should be treated with caution in bumblebee foraging studies.

Keywords: Cold anaesthesia, foraging, alerting, communication

Introduction

Cold-induced narcosis is frequently used to temporarily immobilize bees for marking or experimental treatment (Robinson and Visscher, 1984). In adult honeybees, cold anaesthesia significantly affects certain behaviours (Free and Williams, 1972; Mardan and Rinderer, 1980; Pankiw and Page, 2003) and short-term memory (Erber *et al.*, 1980) but causes no change in the number of trips per forager (Ebadi *et al.*, 1980), time per foraging flight (Robinson and Visscher, 1984) or mortality rate (Ebadi *et al.*, 1980). In fruit flies, cold anaesthesia disrupts some forms of memory (Tully *et al.*, 1994), increases copulation latencies (Barron, 2000) and has detrimental effects on some behaviours for 24 hours after treatment (Greenspan, 1997).

Many studies of foraging behaviour and communication in bees utilize cold anaesthesia for marking individuals, although there has been no explicit investigation of how this treatment may affect communication or recruitment within the colony. To respond to fluctuations in resources and colony requirements, social bees have developed complex communication systems to activate foraging (Seeley and Tovey, 1994). Honeybees (Anderson and Ratnieks, 1999) and bumblebees (Dornhaus and Chittka,

1999) both use forms of recruitment communication. Because recruitment plays a key role in the foraging strategies of social bees, we examine how cold anaesthesia affects the recruitment behaviour of the New World bumblebee, *Bombus occidentalis* Greene. We tested the hypothesis that short-term cold anaesthesia would have no effect on recruitment. Although recruitment and foraging studies often use cold-induced narcosis for the temporary immobilization of bees, the long-term effects of such cold temperatures on recruiting behaviours have largely been unexplored.

Materials and Methods

Four colonies of *B. occidentalis* with 81, 98, 113 and 206 foragers respectively were obtained from Biobest Biological Systems Ltd and studied in a temperature-controlled (21° C) laboratory at the University of California, San Diego. Each nest, built on a plastic substrate, was placed in a separate wooden nest box (24 × 36 × 15 cm) with a glass top. Wire mesh comprised the nest floor, which allowed for ventilation and for disposal of waste and

debris. A 2.5-cm diameter clear vinyl tube connected the nest to the foraging arena that consisted of a large plastic tub (40.6 × 40.6 × 81.3 cm) with mesh ventilation in the side panels and a glass top. We prepared 2.5 M unscented sucrose solution (Ultra Pure, ICN Biomedicals) with double distilled water and provided it daily *ad libitum* in a 10-ml plastic dish in the foraging arena. Bees were allowed to feed freely on the 2.5 M sucrose solution for one hour each morning and at the end of the day's trials. Fresh water and pollen were provided inside the nest as needed.

All bees (controls and cold-treated) were labelled individually with a unique plastic identifying tag glued to their thorax (Queen Marking Kit, www.beeworks.com). To mark an individual, we captured the bee with a plastic vial (10 ml volume) and placed her underneath a wire mesh screen (6 × 7 mm mesh) so as to expose her thorax. We then glued a numbered tag on her back with cyanoacrylate adhesive. To test the effects of cold anaesthesia on foraging behaviour, we randomly chose and cooled 22 foraging workers at ~4° C until they were sluggish (~5 min). Bees varied slightly in the amount of time that they took to become cold anaesthetized, perhaps because of forager size variation. Anaesthetized bees were subsequently placed in individual plastic vials and allowed to recover for 5 min before being returned to the nest. Foragers were tested within five days of their cold anaesthesia treatment. Control foragers ($n = 44$) experienced no chilling. All bees included in this study were tested within three weeks of their first foraging trip.

Food was withheld from the study colonies 1 h before each trial to control for immediate colony need. The focal forager was isolated in the foraging arena, and access into the arena was blocked with a plastic gate. After 5 min of isolation in the arena, 2.5 M sucrose solution was provided at the feeder, and the focal forager was permitted to feed. If she did not discover the food within 15 min, the trial was aborted. A total of two control trials were aborted. We tested each focal bee only once.

We measured the time and duration of feeding for each focal forager. The food source was removed after the focal bee ceased feeding and access to the nest was re-established. We used audio (Sony microcassette-recorder M-470) and video (Panasonic PV-DV4020) recording equipment to record the identity and time of any recruit entering or subsequently exiting the arena. We excluded from analysis two trials (out of 68) in which the focal forager did not regurgitate into a honey pot.

Data analysis

We analyzed foraging activity for 10 min following the focal forager's return. This time interval was chosen based on preliminary data that showed that the rate of foragers entering the arena did not change significantly after 10 min in 30 min trials ($F_{1,6} = 0.0952, P = 0.7681$). Because only a single forager was fed in any one trial, peaks in foraging would be expected immediately following the focal forager's successful return.

A bee visit was included in the analysis only if the foraging bee remained in the foraging arena for at least 10 sec. A recruit was defined as a bee that was foraging for the first time that day because she departed the nest in response to external information, such as the return of a nectar-laden focal bee (Biesmeijer and de Vries, 2001).

To assess whether *B. occidentalis* foragers alert nestmates to food availability outside the nest, we used repeated-measures

MANOVA to analyze the change in number of bees foraging in the arena over time for an effect of cold temperatures. To quantify foraging response, we calculated the cumulative number of recruits over time and tested for an effect of cold using repeated-measures MANOVA. The effect of the focal bee's treatment on the mean number of new recruits was analyzed using ANOVA. We used Wilcoxon paired-sample tests to assess the effect of cold anaesthesia on the duration of time focal bees spent searching for sucrose, feeding, visiting the nest and subsequently foraging in the arena. A Bonferroni adjustment was used to control for possible Type I error and the level was set to 0.01 for these tests. In addition, we used repeated-measures MANOVA to assess whether the location of the focal forager (ie, whether she was in the arena or in the nest) influences the number of new recruits. All analyses were performed using JMP v. 5.1 (SAS Institute).

Results

Alerting nest mates to food

If foragers do not alert nest mates to the presence of food, we would expect that the number of bees in the arena is unaffected by treatment and depends rather on colony size. Contrary to this null expectation, there was a statistical relationship between the increase in the number of foragers over time and treatment (repeated-measures MANOVA: $\chi^2_{32,179} = 0.3831, P = 0.0212$). We observed significantly more bees foraging in the arena during control trials compared to the number present during cold-treatment trials ($F_{1,55} = 5.9588, P = 0.0179$). Cold treatment of the focal bee significantly decreased the proportion of foragers: 20.9% of bees in the nest foraged when the focal bee was a control bee, while 17.8% of bees foraged when the focal bee was cold treated ($F_{1,60} = 5.784, P = 0.0193$).

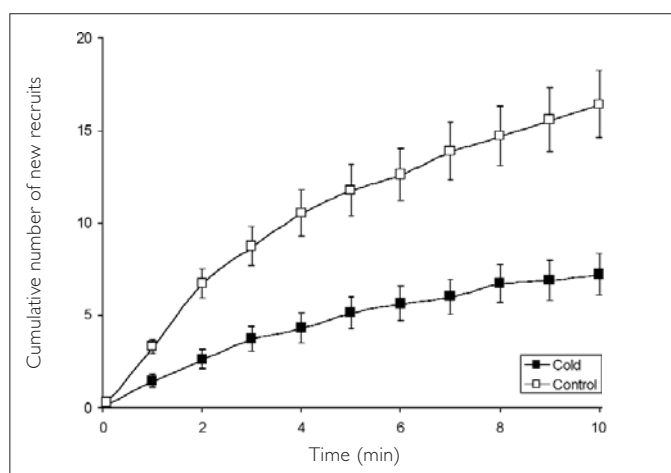


Fig. 1. Cumulative number of recruits over time by treatment (mean ± SE). The cumulative number of recruits was calculated each minute for 10 minutes following the focal bee's return to the nest and regurgitation of sucrose. Control bees recruit more nest mates to forage and do so at a higher rate than focal bees in the cold treatment group. Repeated-measures MANOVA: $F_{3,61} = 21.6799, P < 0.0001$. Effect of treatment: $F_{9,549} = 3.8549, P < 0.0001$.

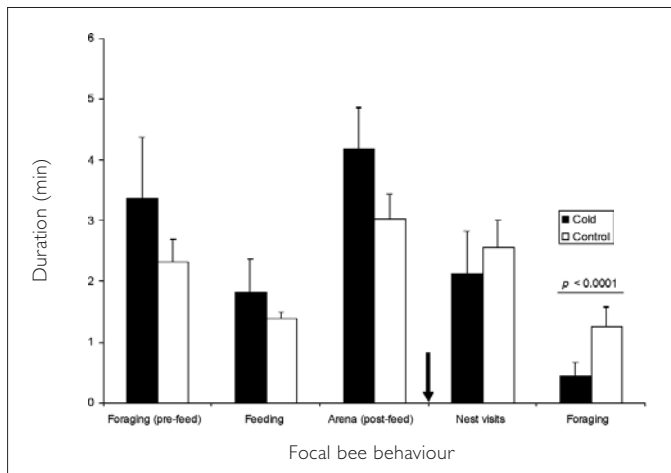


Fig. 2. Duration of time focal bees spent foraging, feeding and visiting the nest by treatment (mean \pm SE). The black arrow indicates the return of focal bees to the nest to deposit sucrose loads into honey pots. Compared to cold-treated bees ($n = 22$), control foragers ($n = 42$) spent more time in the arena foraging after regurgitating sucrose at the nest. Two control bees were excluded from analysis because feeding durations were not measured.

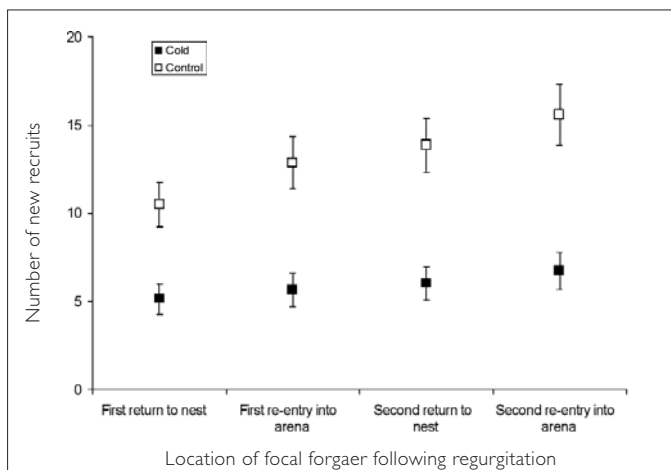


Fig. 3. Cumulative number of new recruits following the focal bee's return to the nest with sucrose (mean \pm SE). After sucrose is deposited at the nest, the number of new recruits is unaffected by location of focal bee: in the nest or in the foraging arena. Control bees activated significantly more recruits than did cold-treated bees. Repeated-measures MANOVA: $F_{2,62} = 5.9045$, $P = 0.0045$.

Effect of cold treatment on recruitment

We observed a significant decrease in the cumulative number of foragers per trial when bees were cold treated (Fig. 1). Repeated-measures MANOVA revealed a statistically significant effect of treatment on cumulative numbers of bees foraging over time ($\chi^2_{27,155} = 0.3337$, $P < 0.0001$). Chilled focal foragers had a significantly lower rate of recruitment over the entire trial period (7.23 ± 1.95 recruits) than did control foragers (16.42 ± 1.4 recruits) (ANOVA: $F_{3,61} = 19.9560$, $P < 0.0001$; effect of treatment: $F_{1,61} = 5.9874$, $P = 0.0173$).

Effect of focal forager behaviour

There was no effect of cold treatment on the time focal bees allocated to find, collect or return with the food resource. However, cold-treated foragers spent significantly less time foraging after having fed than did control bees (Fig. 2: $Z_{41} = 5.3729$, $P < 0.0001$). For both cold anaesthesia and control treatments, we observed a peak in the number of new recruits during or immediately after the focal forager returned to the nest. Repeated-measures MANOVA revealed that the mean numbers of recruits foraging was significantly correlated with the focal bee location (in nest or in arena) ($\chi^2_{3,186} = 0.7629$, $P = 0.0113$). Focal bees that experienced cold anaesthesia recruited fewer nest mates than did control focal bees (Fig. 3: $F_{1,62} = 11.0696$, $P = 0.0015$), although there was no effect of focal bee location on the number of recruits within treatments (Fig. 3: $F_{1,256} = 0.8486$, $P = 0.3578$).

Discussion

Cold anaesthesia significantly reduced recruitment in treated *B. occidentalis* foragers. Focal bees returned to the nest and alerted nest mates to food, as was shown by the significantly greater number of bees foraging during control trials as compared to cold-treatment trials. If these high foraging rates resulted from bees establishing an equilibrium between the arena and nest after being forced to remain in the nest prior to the trial, we would expect equal foraging rates for both control and cold treatments. Instead, significantly more bees foraged in the arena when the focal forager was a control bee than when the focal forager was cold treated. These results indicate that bees were responding to the focal forager and that focal bees treated with cold anaesthesia recruited fewer bees than did control foragers.

It is unlikely that the additional 10 min confinement in plastic vials (5 min chilling and 5 min recovery) that bees experienced during the cold exposure caused the observed difference in recruitment between treatments. Control bees were not subjected to confinement because previous work by Pankiw and Page (2003) demonstrated that stress from handling decreased sucrose sensitivity in non-anaesthetized bees and suggested that handling effects on cold-treated bees may have been blocked by anaesthesia. To allow for recovery from any stress from this short-term confinement, we waited several days after treatment before testing the bees.

A single episode of cold anaesthesia was sufficient to reduce subsequent recruitment several days after treatment. This long-term effect differs from published reports demonstrating lasting effects that result from extensive exposure to cold. For example, parasitic wasps exposed to 4°C for multiple weeks exhibited modified foraging strategies, reduced oviposition rates, increased rates of superparasitism, and difficulty learning (van Baaren *et al.*, 2005). In the present study, it is unlikely that low temperatures caused a disruption of normal development because only adult foragers were exposed to cold.

The impaired ability of chilled *B. occidentalis* foragers to recruit may be the result of behavioural changes induced by cold, such that foragers are unable to produce the appropriate behavioural or olfactory stimuli to elicit a recruitment response.

Cold treatment reduced the time focal bees spent foraging in the arena after regurgitating in the nest. It is unclear whether this change in time allocation may influence the recruitment of nest mates. Further investigations of the mechanisms of recruitment and the physiological effects of cold narcosis are needed.

Our results are consistent with the effect of cold on foraging behaviour in a re-analysis of data reported by Ebadi *et al* (1980) who investigated effects on memory, survival, and number of foraging trips per forager in *Apis mellifera*. Using Wilcoxon paired-sample tests on data presented in Ebadi *et al* (1980), we determined that their cold-treated colony had significantly fewer bees foraging ($Z_7 = 2.36, P < 0.01$) and fewer foragers returning with pollen loads ($Z_7 = 2.36, P < 0.01$) than did their control colony. In addition, we calculated the proportion of foraging bees returning with pollen loads in Ebadi *et al* (1980) and found that a significantly higher proportion of foraging bees carried pollen loads in the control colony than in the cold-treated colony ($\chi^2_1 = 9.04, P = 0.0026$). Although the low-temperature exposure used by Ebadi *et al* (1980) lasted only 3 min and did not affect mortality, the cold treatment significantly lowered colony foraging success in *A. mellifera*.

The negative effect on recruitment due to cold treatment may be irreversible. Nevěd *et al* (1998) found that chill injury was reversible in springtails if exposure to low temperatures was limited to two days. In addition, periodic interruptions of long-term cold exposure with warm temperatures allows for physiological recovery from chill injury in parasitoid wasps (Colinet *et al*, 2006). However, all bees in this study were tested within days of cold exposure. If the cold-induced reduction in recruitment was reversible, we would expect that treated foragers could recover from a 5-min cold treatment within 5 days, particularly if smaller organisms recover from extended cold exposure within this time period (Nevěd *et al*, 1998). Additional testing of cold-treated individuals after a longer recovery period (eg 14 days) is needed to assess whether foragers later regain the ability to recruit at high rates.

Cold-induced narcosis is often considered a non-invasive anaesthetic technique with no lasting neural or behavioural effects. However, this study demonstrates that a single treatment of cold anaesthesia affected the subsequent behaviour of treated *B. occidentalis* foragers by decreasing their ability to recruit nest mates to a food resource. We therefore suggest that cold anaesthesia should be treated with caution as a method of marking in bumblebee foraging studies, particularly if little to no foraging activation is observed after the bees have been cold treated.

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