Learning Performance of Foraging Bumblebees under Manipulation of Inter-visit Time Intervals (Hymenoptera: Apidae: Bombus terrestris)

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Short-term information affects feeding choices in bees. When making rapid successive choices, bees tend to forage in a flower-constant manner, and perform well in delayed matching-to-sample learning tests. Flower constancy and learning performance decline when consecutive choices are more than five seconds apart. It was suggested that visual stimuli associated with the last-encountered food source are retained in the bees’ working memory, and prime their next choice. The contents of the working memory may either fade, or be replaced by other visual input, within a few seconds. To distinguish between these possibilities, the visual input available to bees between consecutive feeding choices was controlled. Bombus terrestris (Linnaeus 1758) bumblebees, foraging on differentially-rewarding artificial feeders of different colors, were exposed to either 1.5 seconds or 15 seconds of darkness following each feeder visit. The bees did not fly in the dark, therefore these treatments differed in the time elapsed between successive feeding choices, but not in the visual input available to the foragers during this time. The bees’ feeder choices were compared to the choices of untreated controls. Control bees chose rewarding feeders significantly more often than bees from both darkness treatments. Shifts between feeders of different colors, and choices of non-rewarding feeders, were associated with long time-intervals between visits in the control treatment, but not in the darkness treatments. The results support previous evidence that short-term information, when available, improves the learning performance of bees. The similarity in the bees’ foraging performance in the two darkness treatments suggests that the decay of working memory may involve acquisition of new visual data. Alternative interpretations for the similar results in the two darkness treatments are discussed.

Key words: Bombus terrestris (Linnaeus 1758) – color – conditioning – darkness – flight duration – working memory


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Introduction

Bees integrate long-term information with recent information to forage efficiently [CHITTKA & THOMSON 2001]. For example, bees learn and retain odor-reward and color-reward associations for several days [reviewed by MENZEL et al 1993, MENZEL 1999], but also modify their floral choices according to the last-encountered rewards [CRESSWELL 1990, DEMAS & BROWN 1995, KADMON & SHMIDA 1992, KEASAR et al 1996a, OHYAMA et al 1995, FÜLÖP & MENZEL 2000]. Similarly, feeder handling durations are affected by bees’ foraging experience on previous days [KEASAR et al 1996b], but also by the reward levels in the last-encountered feeders [GREGGERS & MENZEL 1993].

Moves between flowers of the same species or type are correlated with shorter flight durations than transitions between flower types [CHITTKA et al 1997, GREGGERS & MENZEL 1993]. It has been suggested that this correlation reflects a short-term memory effect [MEYER & SCHVANEVELDT 1971] of the last-visited flower, which gradually fades as flight durations increase. In other words, bees may use mainly recent data in their flower-choice decisions when flight durations are short, and primarily long-term data when flight durations are long. It was further proposed that recently acquired information is first stored in a short-term working memory, where it is rapidly accessible for retrieval. The information is later integrated into a longer-term memory, which is retained for a longer time, but allows slower retrieval [MENZEL 1999].

The properties of visual working memory in honeybees were recently investigated using a delayed matching-to-sample paradigm. Bees flying in a tunnel were first trained to match a sample visual stimulus to an identical comparison stimulus that they encountered further along the tunnel. The time delay between encounters with the two stimuli was then experimentally manipulated. The bees’ accuracy in matching the sample stimulus with the comparison stimulus decreased exponentially, as the delay between the stimuli was increased. When the delays exceeded 5 seconds, the bees chose the comparison stimulus and a distractor stimulus with equal frequency. These findings suggest that a visual cue can be retained in the working memory for a period as long as 5 sec [COLLETT 2005, ZHANG et al 2005]. This estimate agrees well with field observations on flight constancy (frequency of flights between flowers of the same species) in bumblebees.
Constancy is high at inter-visit intervals of up to 2 s, drops to half-maximum accuracy at 3–4 sec, and disappears when inter-visit intervals exceed 9 sec. As pointed out above, a possible interpretation is that a ‘search image’ of the last-visited flower is transiently stored in working memory following each visit, priming the bees to approach a similar flower on their next visit. This effect lasts as long as the working memory is active (<5 sec), and does not affect the bees’ choices at longer inter-visit intervals [CHITTKA et al 1997, 1999].

Why is visual information lost from working memory within a few seconds? One possible answer is that working memory is frequently and regularly cleared of data, regardless of the bee’s activity. A second possibility is that new incoming visual stimuli gradually overwrite the old ones in working memory, eventually erasing them. It is difficult to distinguish between these possibilities in natural foraging situations, since increased delays between flower visits usually involve flight and increased exposure to novel visual stimuli. In a laboratory setting, on the other hand, it is possible to manipulate inter-visit intervals while controlling the visual input available to the bees.

In the present study, the inter-visit intervals of bumblebees that foraged on sucrose-solution feeders were extended by darkening the flight room, for 1.5 sec or 15 sec, between successive visits. Bees were not able to fly and obtain new visual information during the darkness periods. The two darkness treatments therefore differed in the durations of inter-visit intervals, but not in the visual information available to the bees during these intervals. The bees’ performance in a simple task of discrimination learning was measured and compared to the performance of a control group that did not experience darkness. The short-darkness group is expected to outperform the long-darkness group on the learning task, if working memory increasingly fades with time. Both darkness-treatment groups are expected to perform equally on the task, if working memory mainly fades as a result of new incoming visual data.

2 Material and methods

2.1 Experimental conditions

Experiments were carried out in a 3X4 m flight room. Temperature ranged 26–30 °C and relative humidity was 40–70%. The room was illuminated by six pairs of D-65 fluorescent lights of 100-Hz frequency between 0630–1830 hours. No external light source illuminated the room. Observations were conducted between 0800–1700 hours.

Colonies of naive Bombus terrestris (Linnaeus 1758) bumblebees were obtained from kibbutz Yad Mordechai, Israel. All adults in the colony were marked within three days of emergence. Pollen was supplied ad libitum, directly to the colony. 32 workers from two colonies were used for experiments.

2.2 Feeders

Forty morphologically identical computer-controlled feeders were used for experiments. Each feeder included a photo detector, which allowed automatic recording of feeder number, the time of first head insertion by a foraging bee, and the time of head withdrawal. A 30% w/v sugar solution was used as nectar imitation in the feeders. Rewarding feeders dispensed ca 1 μl of sucrose solution each time they were probed by a bee, while non-rewarding feeders provided no sucrose solution at all. Measurement error for the 1 μl volume was ±20%. Feeders were refilled ca 2 s after the forager’s departure, and did not interfere with the bees’ activity. See KEASAR [2000] for further details on feeder operation and data recording.
A round removable plastic landing surface of 3.7 cm diameter was placed on the top of each feeder. Twenty of the landing surfaces were human-blue, and the other twenty were human-yellow. A physiological chromaticity diagram for these colors is provided in Keasar et al [1997]. The blue and yellow feeders were placed on a 6x20 position grid on a 1.40x2.40 m hardwood table. A semi-random protocol was used to assign locations to the feeders to prevent feeder clustering. The mean distances between feeders were 89.95±42.53 (SD) cm and 86.89±44.15 cm for feeders of the same color and feeders of different colors, respectively. For 15 bees, the rewarding feeders were blue and the non-rewarding feeders were yellow; 17 other bees were rewarded when visiting yellow feeders and were not rewarded when visiting blue.

2.3 Experimental design

A naive worker, foraging singly, was allowed to forage in the array of feeders. Preliminary observations indicated that darkness induced inexperienced foragers to stop feeding and return to the colony. All bees were therefore allowed to complete their first ten visits to feeders without interruption. The bees were then arbitrarily assigned to one of three experimental treatments:

(a) Short darkness (12 bees): bees in this treatment experienced dark period of 1.5 sec immediately after they departed from each feeder. The bees could not fly at dark, and typically stayed on the experimental table until illumination was resumed. Bees that were in flight dropped to the floor when the lights were turned off.

(b) Long darkness (10 bees): this treatment differed from the short-darkness treatment only in the length of the dark period, which was 15 sec.

(c) Control (10 bees): bees in this treatment were allowed to forage without interference.

Foragers were caught and removed from the experiment after they completed at least 150 feeder visits, in addition to the first ten training visits. Each bee was only used once. Data from the first 150 feeder visits were used for analysis. Two of the bees disappeared from the floral array after they completed only 111 and 134 visits. The available data from these bees were included in the analysis as well.

2.4 Data analysis

Changes in the bees’ inter-visit intervals and feeder-choice performance over time were calculated. Inter-visit intervals were defined as the time between head withdrawal from one feeder and head insertion into the next feeder. Consecutive inter-visit intervals for a given bee are not independent variables, since the duration of a bee’s early flights may affect its later flight durations. The calculation of standard errors was therefore based on cluster sampling methods [Steel & Torrie 1981]. Two inter-visit intervals that were longer than 1000 s were treated as outliers, and were excluded from the analysis. Seventy seven inter-visit intervals that involved return into the bee colony were excluded as well.

Changes in the bees’ choice performance were analyzed by calculating, separately for each bee, the proportion of choices of rewarding feeders for blocks of consecutive 25 visits and for all visits together. Repeated-measures ANOVA, followed by planned comparison tests, was used to test for the effects of treatment on the bees’ proportions of correct choices.
3 Results

3.1 Choice performance

Choice frequencies of rewarding feeders over all 150 foraging visits were 90.57±3.01% in the control treatment, 82.39±2.62% in the short-darkness treatment, and 85.13±2.11% in the long-darkness treatment. 60–70% of the bees’ first 25 visits were directed to rewarding feeders, regardless of treatment. The frequency of choice of rewarding feeders increased over time, and stabilized after ca 100 feeder visits (Fig 1). The variance in choice frequencies was significantly affected by treatment (repeated-measures ANOVA, \( F_{2,27} = 6.68, p = 0.004 \)). Bees in the control treatment chose rewarding feeders significantly more frequently than bees that experienced either short or long darkness (planned comparison test, \( F_{1,27} = 11.49, p = 0.002 \)). No statistically significant difference in choice accuracy was found between the long-darkness and the short-darkness treatments (planned comparison test, \( F_{1,27}=1.20, p=0.28 \)) along the whole experiment. However, the long-darkness group chose rewarding feeders significantly more frequently than the short-darkness group during the last 25 recorded visits (Fig 1, \( F_{1,20}=13.37, p=0.002 \)).

\[
\begin{align*}
\text{Visit no.} & \quad 1-25 & \quad 26-50 & \quad 51-75 & \quad 76-100 & \quad 101-125 & \quad 126-150 \\
\% \text{choices of rewarding feeders} & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \%
\end{align*}
\]

Fig 1: Mean (+SE) choice frequencies of rewarding feeders by *Bombus terrestris* (Linnaeus 1758) [Hymenoptera: Apidae] in a color-discrimination learning task. Choices were grouped into blocks of 25 visits. ◆ – Control, ■ – short darkness, ▲ – long darkness.

3.2 Inter-visit intervals and color-shift frequencies

The mean durations of inter-visit intervals decreased over the first 40 feeder visits, and then stabilized, in a similar manner to earlier studies [KEASAR et al 1996b, LAVERTY & PLOWRIGHT 1988] (Fig 2). Flights between feeders of different colors were associated with longer inter-visit intervals than flights between feeders of the same color in the control treatment (Fig 3). However, this trend marginally misses statistical significance, while the variability in flight durations among bees is highly significant (logit regression, Inter-visit interval: df = 1, Wald statistic = 3.26, \( p = 0.07 \); Bees: df = 9, Wald statistic = 30.86, \( p = 0.0003 \)).
This point was further examined by calculating the mean frequency of color transitions in the shortest inter-visit intervals (lower quartile) and longest intervals (upper quartile) of each treatment (Fig 4). Color shifts were significantly more frequent during long inter-visit intervals than during short intervals in the control treatment (paired t-test $t_9 = -2.686$, $p = 0.012$). Flight durations did not affect the bees’ decision whether to shift color in the two darkness treatments (Fig 3). Individuals within each treatment, on the other hand, varied significantly in their frequencies of feeder-color switches (logit regressions; Short-darkness – Bees: $df = 11$, Wald statistic = 68.26, $p<0.0001$; Inter-visit interval: $df = 1$, Wald statistic
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= 0.09, p = 0.78; Long-darkness – Bees: df = 9, Wald statistic = 57.77, p<0.0001; Inter-visit interval: df = 1, Wald statistic = 0.01, p = 0.93). Color-shift frequency was similar following the flights of shortest (lowest quartile) and longest (upper quartile) durations in these treatments as well (Fig 4, t11 = -0.709, p = 0.246 for short darkness, t9 = -1.545, p = 0.078 for long darkness).

Mean (±SD) color-shift frequencies were 0.132±0.338, 0.230±0.421 and 0.202±0.402 for the control, short-darkness and long-darkness treatments, respectively. Bees from the darkness treatments may have shifted colors more frequently than control bees because of their longer inter-visit intervals, or because they experienced different visual inputs during these intervals as compared to the controls.

Fig 4: Top – Mean (+SE) inter-visit intervals (in seconds) of short (dotted bars) and long (hatched bars) flights by Bombus terrestris (Linnaeus 1758) [Hymenoptera: Apidae] in all experimental treatments. Bottom – Mean (+SE) frequency of feeder color shifts in short and long flights. Inter-visits intervals were sorted in ascending order. Short flights comprise the lower quartile of the list, and long flights comprise the upper quartile.
To evaluate these possibilities, color-shift frequencies in the control vs. short-darkness treatment were compared, following the same inter-visit intervals. Inter-visit intervals were sorted in ascending order for each treatment, and the frequencies of color shift were calculated for 1-s bins of inter-visit intervals (Fig 5). Inter-visit intervals in the long-darkness treatments could not be matched with inter-visit intervals in the control treatment, and were therefore not included in this analysis. If color shifts result directly from long inter-visit intervals, then their frequency is expected to be similar across treatments for flights of identical durations. No such similarity is expected if color-shift frequencies are affected by the bees’ visual experience during flight. In support of this possibility, Fig 5 indicates that color-shift frequencies are generally higher in the short-darkness treatment than in the control, even when identical for inter-visit durations are compared.

3.3 Inter-visit intervals and choice of rewarding feeders

Visits to rewarding feeders were preceded by shorter inter-visit intervals than visits to non-rewarding feeders in the control treatment (logit regression; Bees: df = 9, Wald statistic = 18.876, p = 0.026; Inter-visit interval: df = 1, Wald statistic = 5.448, p = 0.020). Choices of rewarding feeders were not significantly associated with short inter-visit intervals in the two darkness treatments (Fig 5, Short-darkness – Bees: df = 11, Wald statistic = 195.10, p = 0.001; Inter-visit interval: df = 1, Wald statistic = 0.042, p = 0.84; Long-darkness – Bees: df = 9, Wald statistic = 57.08, p<0.0001; Inter-visit interval: df = 1, Wald statistic = 0.527, p = 0.468).

**Fig 5:** Color-shift frequencies by *Bombus terrestris* (Linnaeus 1758) [Hymenoptera: Apidae] for inter-visit intervals of equal duration in the control (■) and short-darkness (▲) treatments. The numbers of flights used for calculations for each data point are indicated.
4 Discussion

4.1 Choice performance on the learning task

Exposure of bumblebees to darkness periods between foraging visits increased inter-visit intervals, and decreased the bees’ choice performance in a learning task by 5–10%. These results are consistent with previous experiments, which indicate an association between increased delays, reduced short-term information, and poorer decision-making by foraging bees [ZHANG et al 2005]. ZHANG et al’s [2005] results were obtained in a delayed matching-to-sample learning task. The present results extend these findings to a color-conditioning task that involved no time delay.

Individuals in both darkness treatments experienced different inter-visit intervals, but were exposed to similar visual input during these intervals. Both groups of bees performed similarly on the learning task, suggesting that the duration of inter-visit intervals did not, in itself, affect their choice performance. Several interpretations for the similarity between the darkness treatments come to mind. First, following the working hypotheses, the results may indicate that working memory is modified as bees encounter new visual stimuli. According to this interpretation, bees in both darkness treatments experienced similar fading of their working memories, because they were exposed to similar visual cues. Alternatively, the decreased choice performance of the darkness-treated bees may reflect the general stress effects of repeated light-off periods (regardless of the length of these periods), rather than specific effects of reduced short-term information. Several other non-specific stress agents (e.g. narcosis, cooling and weak electrical brain stimulation) have been shown to interfere with short-term memory in bees [ERBER 1975]. It may be that the short- and long-darkness treatments had a similar stress effect, i.e. they not only reduced the amount of available short-term information, but also interfered with the bees’ ability to recall this information. This interpretation is not ruled out in the present experiment, and requires further study. Finally, the short-darkness delay of the present study (1.5 sec) may have been long enough to allow cause complete fading of working memory. This interpretation is less likely, because the span of working memory in bees has been estimated at several seconds [CHITTKA et al 1999, MENZEL 1999, ZHANG et al 2005] or even minutes [BROWN et al 1998].

Fig 6: Mean (+SE) inter-visit intervals (in seconds) preceding visits to rewarding feeders (black bars) and non-rewarding feeders (white bars) by Bombus terrestris (Linnaeus 1758) [Hymenoptera: Apidae].
Interestingly, bees in the long-darkness treatment (inter-visit intervals of ca 28 s) performed slightly better in the learning task than bees that were exposed to short darkness, and had inter-visit intervals of 4–5 s (Fig 1). Although this difference was not statistically significant (except during the last 25 visits), it may hint at a time-lag which is associated with the retrieval of long-term experience from memory [MENZEL et al 1993]. That is, bees in the long-darkness treatments may have had more time to retrieve long-term information between visits than bees that experienced short darkness.

Choice frequencies of rewarding feeders increased during the course of the experiment in all treatment to over 85%, following a typical learning curve [MENZEL 1968, BITTERMAN 1996]. Some learning of the rewarding feeders occurred already within the first 25 visits, placing the first point of the learning curve above 50%. This suggests that the learning task used for the present experiment was fairly easy for the bees. A more difficult learning task may have resulted in larger behavioral differences between controls and darkness-treated bees.

### 4.2 Color shifts and inter-visit intervals

Bees in the control treatment switched between feeders of different colors less frequently than bees in the darkness treatments. Color shifts were more common during long flights than during brief flights in the control treatment, but not in the darkness treatment (Fig 3, 4). These results are compatible with the hypothesis that the last-visited feeder has a short-lasting effect on the bees’ decision-making: when short-term memory is active, the forager is likely to choose a feeder of the same type as it had just visited. After the effect of short-term memory fades (as in the long flights of the control treatments and all flights of the darkness treatments) color-shift frequencies are expected to increase, and to be determined by the bee’s long-term experience.

Choices of non-rewarding feeders were also more frequent following long inter-visit intervals than following short intervals in the control treatment (Fig 5). This trend probably arises from the higher frequency of visits, and longer runs of consecutive visits, in rewarding flowers as compared to non-rewarding ones. Color-shift flights, associated with long inter-visit intervals, constituted a smaller proportion of flights directed to rewarding feeders in the control treatment than in the darkness treatments. The mean inter-visit intervals preceding rewarded visits were therefore shorter than inter-visit intervals preceding non-rewarded flights for the control group. In the darkness treatments, on the other hand, color-shifts were not correlated with long inter-visit intervals. Following the same reasoning, choice of non-rewarding feeders is not predicted to correlate with long intervals in these treatments. The findings reported here agree with this prediction.

In summary, the current study provides circumstantial evidence regarding the role of visual working memories in bee foraging, and the factors involved in the fading of these memories. Additional experimental manipulations are needed to rule out possible additional sources of interference with decision-making during the time intervals between visits. A further limitation of the present experimental design is that the bees were rewarded by one feeder type only throughout the experiment, so that they had identical short-term and long-term information on the association between feeder color and reward. A more complex learning task could create different color-reward associations in the short-term vs the long-term memories, helping to elucidate their respective roles in choice behavior.
5 References

BITTERMAN M E [1996]: Comparative analysis of learning in honeybees. – Anim Learn Behav 24: 123–141; London/UK.
DEMAS G E & BROWN M F [1995]: Honeybees are disposed to win-shift but can learn to win-stay. – Anim Behav 50: 1041–1045; London/UK.
KEASAR T [2000]: The spatial distribution of non-rewarding artificial flowers affects pollinator attraction. – Anim Behav 60: 639–646; London/UK.
KEASAR T, SHMIDA A & MOTRO U [1996a]: Innate movement rules in foraging bees: flight distances are affected by recent rewards and are correlated with choice of flower type. – Behav Ecol Sociobiol 39: 381–388; Heidelberg/Deutschland.
KEASAR T, MOTRO U, SHURY Y & SHMIDA A [1996b]: Overnight memory retention of foraging bumblebees in imperfect. – Anim Behav 52: 95–104; London/UK.
LAVERY T M & PLOWRIGHT R C [1988]: Flower handling by bumblebees: a comparison of specialists and generalists. – Anim Behav 36: 733–740; London/UK.
MENZEL R [1999]: Memory dynamics in the honeybee. – J Comp Physiol A 185: 323–340; Berlin/Deutschland.
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