Contextual modulation of visuomotor associations in bumble-bees (*Bombus terrestris*)

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We performed the following experiment to investigate whether contextual cues can prevent interference during the acquisition of potentially competing visuomotor associations in the bumble-bee (*Bombus terrestris*). Bees discriminated between horizontally and vertically orientated gratings of black and white stripes in order to reach a feeder and between different diagonally orientated gratings to gain access to their nest. Once bees were well trained on these two tasks, the discrimination task at the nest was changed so that bees had to distinguish between horizontal and vertical gratings at both sites. Whereas they still approached the horizontal grating to reach food, they now had to approach a vertical grating in order to return to their nest. The new task was learnt rapidly. Errors at the feeder did not increase during or after the acquisition of this potentially competing behaviour at the nest entrance. For a brief period during acquisition, bees showed some hesitation at the feeder and hovered for slightly longer before choosing between horizontals and verticals. After acquisition was complete, bees showed a slight increase in their preference for verticals over a more ambiguous stimulus of an array of dots. These findings are consistent with the hypothesis that different contextual signals are associated with approaching the nest or approaching the feeder, and that these contextual signals facilitate learnt associations between orientation detectors and motor commands.

Keywords: bumble-bees; contextual cues; visual pattern learning; orientation detectors

1. INTRODUCTION

Observations on freely foraging honeybees have long suggested that spatial and temporal contexts play a significant role in helping a bee select and return to a good foraging site (Ribbands 1953). One way in which contextual cues can act is by focusing an insect's 'attention' onto relevant local visual cues. Gould (1987), for instance, showed that honeybees can be trained to land on one petal of an artificial flower at one time of day and on another petal of the same flower at another time of day. The insect treats the same global pattern in different ways at different times, approaching the appropriate local region as required. Similarly, Schatz *et al.* (1994) trained ants to forage in an arena with three distinct visual shapes fixed to the wall. Each shape signalled access to a feeding area that was only baited at a particular time of day. Individual ants learned to approach the appropriate shape at the correct time. Kelber (1989) trained honeybees to discriminate between simultaneously presented vertical and horizontal gratings affixed to a vertical surface. Approaches to the horizontal grating were rewarded when the patterns faced in one compass direction, and approaches to the vertical grating were rewarded when the pattern faced in the opposite direction. Cues signalling whether bees should approach the horizontal or vertical stripes could have come either from the sky compass or from the different panoramic

views seen when bees faced in one or other direction (Collett *et al.* 1997). Fourth, Srinivasan *et al.* (1998) trained honeybees to discriminate between differently orientated gratings (or between different colours) to reach a feeder and between the same gratings (or colours) to reach their hive. Access to the feeder was through a hole in one grating (or colour) and access to the hive through a hole in the other one. Various contextual cues were available to distinguish between the two situations: the panorama surrounding the patterns, the direction in which the bees faced, and whether the bees were motivated to collect sucrose or to fly home. In all four of these studies, bees or ants responded differently to the same visual stimuli in different contexts suggesting that associations between particular visual stimuli and motor commands are modulated in a context-dependent manner. It should be clear from this survey that defining what constitutes a contextual cue is a somewhat 'knotty' problem and one that we do not attempt to unravel here.

In this paper we ask what effect acquiring a new response to a pair of distinguishable visual stimuli in one context has on a well-learned visuomotor association involving the same pair of stimuli in another context. We have seen that contextual signals allow insects to respond differently to the same stimuli in different situations. What happens while these associations are being learnt? Can contextual signals prevent interference during the acquisition of potentially competing visuomotor associations in different situations?

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To explore this question, we trained bumble-bees to approach horizontal and not vertical stripes to reach a sucrose reward and to approach 45° rather than 135° diagonal stripes to enter a passage leading to their nest. Once bees performed stably on both discriminations, we changed the task on the homeward route, but left conditions unaltered at the feeder. Bees now had to approach vertical but not horizontal stripes to gain access to their nest. Any reconfiguring of old associations that is required to accommodate this new response at the nest entrance might be detectable in the transient disruption or permanent degradation of discrimination at the feeder.

To reduce the need for bees to take note of contextual signals in the first phase of training, we performed a variant of this experiment. Bees were trained initially to approach horizontal rather than vertical stripes in order to reach both the feeder and the nest. In the second phase of the experiment, training conditions remained the same at the feeder, but were reversed at the nest so that bees had to approach vertical rather than horizontal stripes there. If context were ignored during the first phase of training, we would expect more disruption of the bee's behaviour at the feeder on reversing the discrimination at the nest than occurred at the changeover in the first experiment.

2. MATERIALS AND METHODS

(a) *Animals and apparatus*

Queenright bumble-bee (*Bombus terrestris*) colonies were obtained commercially and housed in a darkened nest-box. Freeze-dried pollen was placed daily directly into the nest-box. Workers had access from the nest-box to a second transparent box. A sucrose feeder was placed in this feeding area whenever experiments were not being performed. The nest-box was also connected via a tunnel with sliding doors to the experimental area, which consisted of a third solid box with a transparent roof ($80\text{ cm} \times 50\text{ cm}$ floor area and 30 cm high). The tunnel ended in a compartment placed outside and halfway up one wall of the experimental box. Bees could move between the compartment and the experimental box through one of a pair of horizontally placed holes 2.5 cm in diameter and 15 cm above the floor. The holes were positioned towards the end of one of the long walls. A reward box could be entered through a similar pair of holes placed diametrically opposite on the other long wall (figure 1a). Bees that had been individually marked with number plates on their backs were trained to enter the experimental box, to collect sucrose from the reward box, and then return to their nest through one of the pair of holes. Bees varied greatly in their diligence. As far as possible, we worked with foragers that made frequent trips (approximately every 5 min).

(b) *Visual patterns*

One of the holes to the reward box was surrounded by a positive pattern (F^+). Entry through this hole allowed the bees to feed at a bottle cap filled with sucrose. The other hole was also open and surrounded by a negative pattern (F^-). It gave access to a similar bottle cap filled with sucrose and covered with wire mesh so that bees could not feed from it. At the nest side, one hole in the centre of the positive pattern (N^+) was open; the other hole was closed and surrounded by the negative pattern (N^-). All the training patterns (figure 1b) were square gratings

Figure 1. Apparatus and visual patterns. (a) Plan view of apparatus. The entrance to the feeding area and the tunnel to the experimental area could be closed by sliding doors. Time that bees spent in solid or dotted rectangles was used to compute relative and absolute hovering times, respectively. (b) Visual patterns used in (i) experiment I and (ii) experiment II. F^+ and N^+ are rewarded patterns at feeder and nest, respectively. F^- and N^- are unrewarded patterns. Patterns at the nest differed in the two phases of each experiment, whereas those at the feeder were the same throughout the experiments. (c) Feeder side; patterns during probe tests. Dots were paired with F^- or with F^+ .

($10.5\text{ cm} \times 10.5\text{ cm}$) of high-contrast black and white stripes (each 1.5 cm wide). The patterns were prepared on computer, photocopied, and stuck onto card.

Both experiments consisted of two phases. Throughout both experiments, F^+ was always a horizontal (90°) grating and F^- was a vertical (0°) grating. The patterns at the nest side differed

between the first and second phases. In the first phase of experiment I, the gratings were diagonal ($N^+ = 45^\circ$, $N^- = 135^\circ$), and in the first phase of experiment II they were the same as at the feeder ($N^+ = 90^\circ$, $N^- = 0^\circ$). In the second phases of both experiments, the patterns at the nest side were the reverse of those at the feeder ($N^+ = 0^\circ$, $N^- = 90^\circ$). The sequence of patterns used in the two experiments is shown in figure 1*b*.

Bees trained with these patterns were given occasional tests in which one feeder hole was marked by F^+ or F^- and the other hole by an array of dots (figure 1*c*), which was ambiguous in that it contained elements of both F^+ and F^- .

(c) Training

It took up to three days for bees to learn to approach the correct patterns at both the food and the nest holes. Bees were trained individually to approach a feeder. The sides of F^+ and F^- were swapped after every second trial and the sides of N^+ and N^- were swapped on intervening trials. To avoid bees learning odour cues rather than visual cues, the patterns were rotated through 90° , rather than switched, when sides were changed.

(d) Testing

We were interested both in the bee's initial choice of hole on each trial and in the relative and absolute hovering times in front of the positive and negative stimuli at the feeder and in relative hovering times at the nest holes. The bee's behaviour while it flew singly in the experimental box was recorded on videotape so that these behavioural parameters could be measured. Once bees performed well on phase 1 of the experiment (15 or more consecutive trials in which the initial choice was at least 80% correct), we introduced probe tests. We recorded the bee's behaviour when it was presented with the array of dots versus F^+ or F^- at the feeder holes. Food was then absent. The bee was given 30–45 s to make a choice. At the end of this time, the room lights were extinguished. The bee stopped flying and the probe stimuli were replaced by the normal training arrangement. Once bees performed well in phase 2 they were tested in the same way with dots versus F^- .

(e) Data analysis and statistics

Bees were deemed to have chosen the pattern on which they first landed. Relative hovering time in front of each pattern was scored as the time bees spent flying within a $10\text{ cm} \times 13\text{ cm}$ rectangular area centred on and in front of the pattern (solid lines in figure 1*a*). It was calculated as the hovering time in front of the positive stimulus divided by the total hovering time in front of both stimuli. Total hovering time at the feeder was the total time spent close to both patterns within the area bounded by the dotted line in figure 1*a*. We used this larger area so that we could include flights between F^+ and F^- . Times were recorded with a stopwatch to a resolution of 1 s.

Hovering times in different situations were compared using the Wilcoxon test for paired comparisons (training stimuli) and the Mann–Whitney test for unpaired comparisons (probe tests). The significance of choice behaviour was assessed with the χ^2 -test.

3. RESULTS

(a) Responses to training stimuli before and after the switch

In phase 1 of both experiments, trained bees chose reliably the correct patterns at both the feeder and the nest

Figure 2. Initial choice of pattern. (*a*, *c*) At the nest; (*b*, *d*) at the feeder in experiments I (*a*, *b*) and II (*c*, *d*), respectively. Graphs plot the number out of a total of eight bees in each experiment that chose the correct pattern on each trial. Data are given as percentages. The numbering of trials is reinitialized immediately after the switch of patterns at the nest.

(figure 2). Bees also hovered significantly more in front of F^+ and N^+ than they did in front of F^- and N^- , respectively (figures 3 and 4). After the switch of patterns at the nest, the bee's preference for horizontal over vertical stripes at the feeder was unchanged. There was no significant change either in choice frequency or in relative hovering times (figures 2–4).

In experiment I, total hovering time at the feeder showed a transient and significant increase after the switch (figure 3). Total times were averaged over blocks of three trials, and the duration of the block immediately after the switch was significantly longer than the durations of the surrounding blocks (comparison of block before the switch with block after: $Z = -2.57$, $p = 0.01$; comparison of adjacent blocks after the switch: $Z = -2.449$, $p = 0.014$; Wilcoxon test with Bonferroni correction). In experiment II, total hovering time at the feeder increased after the switch ($Z = -5.622$, $p = 0.001$), but showed no decline with continued training. Similar tests on measurements of relative hovering times revealed no statistical differences between phase 1 and phase 2. Nonetheless, there was a transient small and non-significant reduction in relative hovering times in experiment I that occurred just after the switch.

After the switch of patterns at the nest, bees initially made frequent mistakes there. In experiment I, choice frequency for N^+ rose from *ca.* 50% on trials immediately after the switch to an asymptote of about 90% correct after ten trials. The score in the block of three trials before the switch was significantly greater than the block

Figure 3. Hovering times in front of patterns at feeder and nest during experiment I plotted over successive trials. (a) Relative hovering time in front of nest patterns (time in front of N^+ /time in front of N^+ and N^- combined). (b) Relative hovering time in front of feeder patterns (time in front of F^+ /time in front of F^+ and F^-). (c) Total hovering time at feeder. Data are means of the scores of eight bees. Error bars are ± 1 s.d. Other conventions as in figure 2.

after the switch (χ^2 (1 d.f.) = 8.71, $p = 0.0032$). In experiment II, the initial frequency of the correct choice was lower (ca. 40%) and oscillated around 80% correct after ten trials. Again, the score in the block of three trials before the switch was significantly greater than the block after the switch (χ^2 (1 d.f.) = 24.168, $p = 0.0001$). Relative hovering times followed a parallel course in experiment I. In experiment II, relative hovering times in front of N^+ dropped but did not regain their initial level of discrimination. These results show that the acquisition of a new visuomotor association at the nest had little influence on preference scores at the feeder, either in experiment I or in experiment II.

(b) Responses to dot patterns

The bees' choice between dot patterns and vertical or horizontal gratings was tested at the feeder in order to discover whether leaning to approach a vertical grating at the nest spilled over into a greater attraction for verticals at the feeder. In phase 1, we examined the bees' choices when they were presented either with an array of dots and a horizontal grating or with an array of dots and a vertical grating. Each bee was given between 3 and 11 tests on the two comparisons. After bees had acquired the new visuomotor association at the nest, we retested the bees' choices between dots and verticals at the feeder.

Bees in phase 1 showed a strong preference for horizontal over dots, but they preferred dots over verticals. These preferences are seen best in the relative hovering

Figure 4. Hovering time in front of patterns at feeder and nest during experiment II plotted over successive trials. Conventions as in figure 3.

times (hovering time in front of grating/total hovering time in front of both grating and dots). Because of the small amount of data from each bee, we pooled the data from all the tested bees. Mean relative hovering time in front of horizontal was 85.26% (s.d. $\pm 25.53\%$, 28 tests from seven bees) and in front of verticals it was 25.17% (s.d. $\pm 27.41\%$, 52 tests from 12 bees). On retest in phase 2, there was a small increase in the relative amount of time spent hovering in front of the vertical grating (mean relative hovering time in front of verticals is: 35.09%, s.d. $\pm 28.51\%$, 59 tests from 12 bees). The increase in preference for verticals from phase 1 to 2 is barely significant (Z score = 2.02, $p = 0.043$, Mann-Whitney U -test). The change suggests that training to verticals at the nest slightly enhances the attractiveness of verticals at the feeder.

4. DISCUSSION

The major conclusion from these experiments is that bees can acquire a new response to a pair of stimuli in one context without disturbing an established and potentially conflicting response to the same stimuli in another context. Bees that were accustomed to approach horizontal but not vertical bars to reach sucrose in context F (food) were trained to approach vertical but not horizontal bars to gain access to their nest (context N). Learning this new task in context N had no measurable influence on the bees' preference for horizontal over vertical bars in context F, or on their relative hovering times in front of the two patterns. The only significant change seen in context F, when tasks were switched in context N, was an increase in the total amount of time

Figure 5. Scheme showing how contextual signals might modulate learnt connections between three orientation detectors and the bee's approach to orientated gratings.

spent hovering in front of the vertical and horizontal patterns. It seems as though the change in context N induces some hesitation in context F, but it does not degrade the bee's ability to choose the correct pattern. The lack of interference when bees are trained in different contexts should be contrasted with the marked interference that is found when bumble-bees are trained on two visuomotor tasks in the same context (Chittka & Thomson 1997; Chittka 1998).

We interpret our findings in terms of the simple scheme shown in figure 5, in which a bee's behaviour is governed by a mixture of contextual and visual signals. The differential approach to horizontal bars in context F and to diagonal or vertical bars in context N is achieved by having contextual signals facilitate the appropriate association between orientation detectors and motor commands. Following Srinivasan *et al.* (1994), we assume that orientation is encoded in the response of three broadly tuned interneurons that are optimally sensitive to orientations at 60° intervals (with arbitrarily chosen peak sensitivities at 0°, 60° and 120°). The 60° and 120° detectors are active for 90° gratings and 0° and 60° for 45° gratings. Bees might thus be expected to have difficulties in learning to discriminate simultaneously 90° from 0° gratings and 45° from 135° gratings, if the task is presented in a single context. In this case, all three detectors would be linked both to approach and to avoidance, making the positive and negative patterns hard to distinguish. Indeed, Horridge (1999) used such pairs of stimuli and attempted to train honeybees to make both discriminations at the same time and in the same place. He found that, without contextual signals to label the two

pairs of stimuli, bees performed very badly. According to this particular scheme, the task of learning two discriminations should be less severe if the positive stimuli were 0° and 45°. The 120° detector would then contribute to the negative but not to the positive patterns. Further experimental analysis along these lines may reveal the preferred orientations of the bee's orientation detectors.

We illustrate, in figure 5, that contextual signals can be used to facilitate the connections between approach and the 60° and 120° detectors in context F, and between approach and the 0° and 60° detectors in context N. This contextual modulation will limit confusion when bees must approach differently orientated gratings in contexts F and N. Unless the contextual signals act as a perfect switch that turns on or off the connection between orientation detectors and approach, this simple scheme makes a testable prediction. The bees' tendency to approach verticals in context F will be enhanced when vertical gratings become the positive pattern in context N. We detected such a change in the bees' slight increase in preference for vertical bars over an array of dots. Before bees had been trained to approach vertical bars in context N, they preferred horizontals to dots and dots over verticals. Once the bees had learnt to approach vertical bars and avoid horizontal bars in context N, there was a slight and just significant increase in the bees' preference for vertical stripes in context F. Taken together, these findings suggest that contextual cues act to modulate the pathway connecting specific visual patterns to a specific response. These experiments do not allow us to say where along the pathway modulation occurs. It could either be on the input connections to specific orientation detectors, or on the connections between orientation detectors and specific motor commands.

Does such contextual modulation always occur, or does it develop only when required by a particular situation? In the first experiment, the initial pairs of patterns to be distinguished in contexts F and N were different, but both pairs consisted of orientated stripes. The tasks in the two contexts are likely to have interfered with each other (Horridge 1999), and this interference could be lessened by contextual modulation. In the second experiment, bees initially made exactly the same discrimination in both contexts, so that no *prima facie* advantage was gained by attending to contextual cues. Contextual modulation only became necessary when the task in context N was switched, and the vertical grating signalled access to the nest. As with the first experiment, the bees' preference for horizontals in context F was unaffected by the switch. The lack of even a transient change in preference suggests that contextual modulation is always applied, even when to us it seems to be unnecessary.

Taking the argument one step further, it seems that when the same discrimination is made at both the feeder and the nest, the approach to horizontals is enhanced by signals from both context N and context F. Correspondingly, when bees switch tasks, and approach verticals in context N, facilitation of the approach to horizontals in context N should be reduced. Contextual signals, it seems, can both facilitate and suppress visuomotor connections. The slower recovery of relative hovering times at the nest in experiment II compared with

experiment I (figures 3 and 4) suggests that suppression may be less easy to accomplish than enhancement. In conclusion, we have found that contextual signals can isolate potentially competing associations and so prevent them from interfering with each other both during learning and after learning is complete. Our data suggest that interference is avoided by the local action of contextual signals within visuomotor pathways.

If we suppose that all the elements of a pattern within one context are primed by the same contextual cues, then those pattern elements should be bound together by this common context. Support for the view that context is a powerful means of grouping together pattern elements comes from studies on insect place learning. A particular site seems to be specified in part by the pattern of nearby visual landmarks viewed from there. When insects arrive at a place that has become familiar because of the distant panorama, they will expect to encounter there a particular array of local landmarks or a particular visual pattern (Collett & Kelber 1988; Collett *et al.* 1997).

M.C. is in receipt of a UK Medical Research Council studentship, K.F. is supported by the Fyssen Foundation, Paris, France. Additional financial support comes from the UK Biotechnology and Biological Sciences Research Council and Human Frontier Science Program, Strasbourg, France. We thank Lars Chittka and Charlie Ellington for advice on maintaining and experimenting on bumble-bees in the laboratory. We are grateful to Paul Graham for a critical reading of the typescript.

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