The spectral input to honeybee visual odometry

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Summary

Bees returning from a feeder placed in a narrow tunnel that is lined with a chequered pattern will strongly overestimate travel distance. This finding supports the view that their distance estimation is based on integrating optic flow experienced during flight. Here, we use chequered tunnels with various colour combinations as a tool to identify the spectral channel used by bees to gauge travel distance. The probability of bees performing waggle dances after a short travel distance correlates only with the low range of the green contrast of the pattern in the tunnel. But it does not correlate with the pattern's chromatic contrast or brightness contrast. Distance estimation is therefore colour blind. We also evaluated the waggle runs as a function of colour pattern. Their duration is the code for the food source distance. Waggle run duration is entirely independent of the colour pattern used, implying that once green contrast is above detection threshold, distance estimation depends solely on the angular motion of the landscape passed in flight.

Key words: colour vision, dance language, motion vision, optical flow, waggle dance, odometry, honeybee, *Apis mellifera*.

Introduction

Contrary to most other animals, honeybees can inform us directly about some of their perceptual experience, by means of their dance language (von Frisch, 1967; Waddington, 2001). The dance notifies nestmates about the location and quality of food. Round dances are performed when food is discovered in the immediate vicinity of the hive; no specific location information is transmitted in these dances. Above a certain distance threshold, bees perform figure-eight-shaped waggle dances with highly specific location information. By analysing the dances of successful foragers, it has been shown that honeybees estimate the distance between their hive and a food source by integrating retinal image flow along the flight path (Esch and Burns, 1995; Esch et al., 2001; Si et al., 2003; Srinivasan et al., 1997, 2000). When bees are trained to forage at the end of a chequered tunnel, they experience a much greater image flow than during flight in open territory. As a result, they overestimate actual travel distance by more than an order of magnitude. Under these conditions, they also display waggle dances after short-distance flights where they would usually perform only round dances (Esch et al., 2001; Srinivasan et al., 2000). Here, we ask which spectral channel is used by bees to analyse image flow.

Do bees use their trichromatic colour vision to measure the optic flow? In fact, in several types of motion-related behaviours, such as the optomotor response, edge detection and motion parallax, bees behave as if they were colour blind (Srinivasan, 1989). These behaviours are driven entirely by a single class of receptor: the long-wave or green receptor (Giurfa and Lehrer, 2001; Horridge, 2000; Spaethe et al., 2001). Here, we investigate the spectral input channel that drives the bee odometer. We evaluate not only the probability of waggle dances as a function of the visual contrast experienced during flight but also waggle run duration, which in the honeybee codes for the distance to a food source.

Materials and methods

Bees (*Apis mellifera ligustica* Spin) were kept in observation hives with transparent side walls, so that all dances could be monitored. Individually marked bees were trained to collect sucrose solution from a feeder placed at the far end of a tunnel 6 m long, 8 cm wide and 18 cm high (Fig. 1). The only entrance to the tunnel was at the near end. Between the hive and the feeder, bees flew through open space for 2.75 m, so that the total distance to the feeder was slightly less than 9 m. The top of the tunnel was covered with black fly-screen mesh; the walls and floor were lined with a Julesz pattern, i.e. a random arrangement of squares of two colours (Srinivasan et al., 2000). Each square measured 1.1 cm². Eleven combinations of colours were used: these were (to humans) blue–yellow; turquoise–blue; green–white; light yellow–dark yellow; black–white; two different red–grey combinations; two different green–grey combinations; two different blue–grey combinations. Colour patterns were generated using a laser printer (Hewlett Packard Design Jet 5000). Spectral reflectance functions of the colours employed were measured using a

Fig. 1. Experimental set-up. Bees were trained to forage from a feeder placed at the end of a tunnel 6 m long, 8 cm wide and 18 cm high. The only entrance to the tunnel was at the near end. Between the hive and the feeder, bees flew through open space for 2.75 m, so that the total distance to the feeder was slightly less than 9 m. The top of the tunnel was covered with black fly-screen mesh; the walls and floor were lined with a random arrangement of squares of two colours. Each square measured 1.1 cm^2.
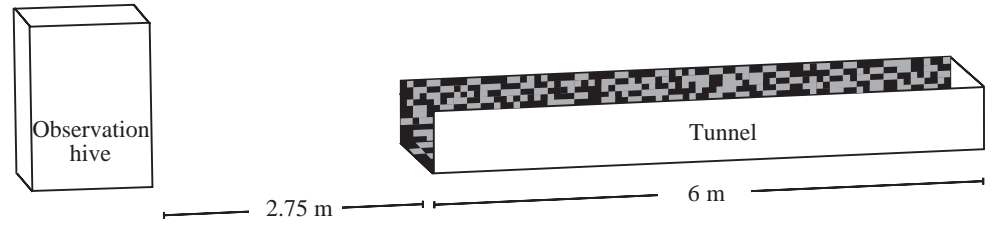
spectrometer (Ocean Optics S2000 with a deuterium/halogen light source). The colour parameters [relative excitation values in the bees' UV, blue and green receptors (Table 1), colour contrast and brightness contrast] were calculated as follows. The relative quantum catch Q for each photoreceptor colour type is:

$$Q = k \int_{300}^{700} I_S(\lambda)S(\lambda)D(\lambda)d\lambda . \qquad (1)$$

$I_S(\lambda)$ is the spectral reflectance function of the stimulus; $S(\lambda)$ is the spectral sensitivity function of the receptor (we used the functions of Peitsch et al., 1992 for the *Apis mellifera* UV, blue and green receptors); $D(\lambda)$ is the illuminant (in our case, standard daylight function D65; Wyszecki and Stiles, 1982). No experiments were performed near dusk or dawn to avoid purplish light conditions, which might not fully be compensated for by colour constancy (Endler, 1993; Kevan et al., 2001).

The scaling factor k in equation 1 is given by:

$$k = 1/ \int_{300}^{700} I_B(\lambda)S(\lambda)D(\lambda)d\lambda . \qquad (2)$$

$I_B(\lambda)$ is the spectral reflection function of the background to which the receptors are adapted. We assumed the receptors to be adapted to a mixture of the two colours presented in the tunnel. However, assuming that the receptors were adapted to

an equireflectant grey background (reflectance 20%) or to green foliage did not alter the main results. By definition, our model assumes that the quantum catch is 1 for the background. When the maximum excitation (E_{max}) of the photoreceptors is normalized to unity, the photoreceptor excitation can be described by:

$$E = Q / (Q + 1) , \qquad (3)$$

so that for $Q=1$, $E=0.5$ (i.e. half the maximum potential; for details, see Chittka et al., 1994). Relative receptor excitation ranges from 0 to 1; therefore, the contrast in each receptor type, such as green contrast, can have values up to 1. Stimulus brightness is defined as the sum of all three photoreceptor excitations, so it can have any value from 0 to 3. Colour contrast is assessed here by evaluating distance between colour loci in the colour hexagon. It can have values of up to 2, since the distance from the centre to each of the corners of the colour hexagon is unity.

When *Apis mellifera ligustica* workers fly through open territory, they typically perform round dances (which are unspecific with respect to direction) when the food sources are within a range of 50 m. Above this threshold, bees switch to the figure-eight-shaped waggle dance, where the duration of the central 'waggle run' indicates flight distance as perceived by the bees. When bees fly through a textured tunnel, they massively overestimate flight distance. This is because flying through the narrow tunnel induces translatory optic flow of a

Table 1. *Receptor excitation values in the honeybee's UV, blue and green receptors calculated for the two colours in each pattern*

Pattern	Colour 1	UV	Blue	Green	Colour 2	UV	Blue	Green
1	Black	0.369	0.344	0.343	White	0.828	0.931	0.912
2	Red	0.426	0.619	0.428	Gray1	0.424	0.389	0.346
3	Green	0.372	0.442	0.642	Gray2	0.439	0.439	0.379
4	Blue1	0.479	0.657	0.457	Gray3	0.506	0.461	0.409
5	Blue2	0.421	0.614	0.366	Yellow	0.459	0.414	0.851
6	Blue3	0.587	0.847	0.782	Turquoise	0.423	0.632	0.699
7	Green	0.348	0.404	0.656	White	0.828	0.931	0.912
8	Light yellow	0.442	0.379	0.809	Dark yellow	0.471	0.449	0.859
9	Red2	0.453	0.410	0.505	Gray4	0.478	0.480	0.412
10	Green2	0.470	0.502	0.605	Gray5	0.526	0.535	0.477
11	Blue4	0.403	0.619	0.428	Gray6	0.431	0.456	0.412

λ_{max}=344 nm, 436 nm and 544 nm for the UV, blue and green receptors, respectively (Peitsch et al., 1992).

Table 2. *Descriptive statistics of behavioural analysis*

Pattern	Colour	*N* bees	*N* dances	*N* dance circuits	% waggle dance
1	Black–white	27	168	1541	56.2±17.5
2	Red1–gray1	12	42	770	48.3±14.8
3	Green1–gray2	8	22	944	66.0±14.1
4	Blue1–gray3	7	66	604	45.7±27.0
5	Blue2–yellow	5	40	811	51.6±21.5
6	Blue3–turquoise	6	43	982	53.8±20.0
7	Green–white	3	50	573	50.7±7.9
8	Light yellow–dark yellow	8	53	804	25.9±15.0
9	Red2–gray4	5	39	690	56.2±14.3
10	Green2–gray5	4	29	257	63.6±22.8
11	Blue4–gray6	7	74	866	37.5±22.5

Numbers of bees, dances and dance circuits analysed per colour pattern; mean ± S.D. of the percentage of waggle dances.

magnitude that is much higher compared with a situation when the bee flies at the same speed but in the open field (Srinivasan et al., 2000). Thus, the tunnel can be used as a tool to investigate the colour parameters used as input to the bee odometer. We determined the probability of waggle dances performed for each colour pair displayed in the tunnel, as well as waggle durations depending on the colours used. We tested whether any single receptor (UV, blue or green) was solely responsible for driving the bee odometer or whether bees use colour contrast for this purpose. We also explored the possibility that bees might use the sum of the three photoreceptors (brightness) as the input to the mechanisms that evaluate optic flow.

A new group of 3–27 workers was trained for each new Julesz pattern (Table 2). The linings in the tunnel were replaced every 3 h to prevent bees from using scent marks or bee excrement as an additional visual cue. Dances of trained bees were videotaped with a digital Camcorder (Panasonic NV-DS35EG) at 25 frames s^{-1}. Dancers typically switched between waggle dance circuits and round dance circuits during their dances; for each individual bee, we counted the complete waggle dance and round dance circuits. We determined the mean percentage of waggle runs performed for each Julesz pattern. This means that we first calculated the percentage of waggle runs for each individual, then calculated the mean of individual percentages. This procedure ensured that each individual bee entered the analysis with equal weight, regardless of how long it was observed. We also analysed the waggle duration of bees as a function of colour pattern in the tunnel using frame-by-frame video analysis.

Results

For all patterns presented in the tunnels, bees overestimated travel distance, so that waggle dances were performed in addition to round dances, even though bees had flown less than 10 m. But the probability of performing waggle dances depended strongly on the colour patterns used to line the flight tunnel (Table 2); it ranged from 26% in the light yellow–dark

yellow pattern to 66% in the green–grey pattern. The waggle dance probability correlated strongly with green contrast (Spearman's rank correlation coefficient r_s=0.724, P=0.011, N=11) but not with predicted signal differences in any of the other receptors (blue receptor: r_s=0.239, P=0.477, N=11; UV receptor: r_s=0.401, P=0.225, N=11) nor with colour contrast (r_s=0.205, P=0.545, N=11) or brightness contrast (r_s=–0.009, P=0.545, N=11) (Fig. 2). However, the correlation between green contrast and waggle dance probability falls slightly short of being significant when the significance level of α=0.05 is adjusted by the Bonferroni method (in which case α=0.01).

Correlation with green contrast is particularly strong in the range up to 0.15 and flattens off at higher values (Fig. 2C). The correlation coefficients above are determined for the entire range of contrast values; if we calculate r_s for the range of green contrast from 0 to 0.15, the correlation becomes even more pronounced (r_s=0.893, P=0.005, N=7). Note that this remains highly significant even after Bonferroni correction. For the other colour parameters, there is no such correlation even when only the lower range of contrast values (up to 0.15) is considered (UV contrast: r_s=0.45, P=0.26, N=8; blue contrast: r_s=–0.8, P=0.2, N=4; colour contrast: r_s=–0.4, P=0.6, N=4). Since brightness contrast can vary over three times the range of the colour parameters, we recalculated the correlation between brightness contrast and waggle dance probability for contrast values up to 0.45; again, the correlation is not significant (r_s=–0.196, P=0.61, N=9).

We therefore conclude that the decision to perform waggle dances rather than round dances is based on visual odometry driven by the green receptor. Because the effect of green contrast on dance probability is especially pronounced over low contrast values, we conjecture that bees have difficulties perceiving the contrast between adjacent squares in the tunnel when green contrast is low. We assume that the signal-to-noise ratio increases continuously over the range of contrast values from 0 to 0.15 and that at all values above 0.15, contrast is reliably perceived, so that waggle dance probability increases no further.

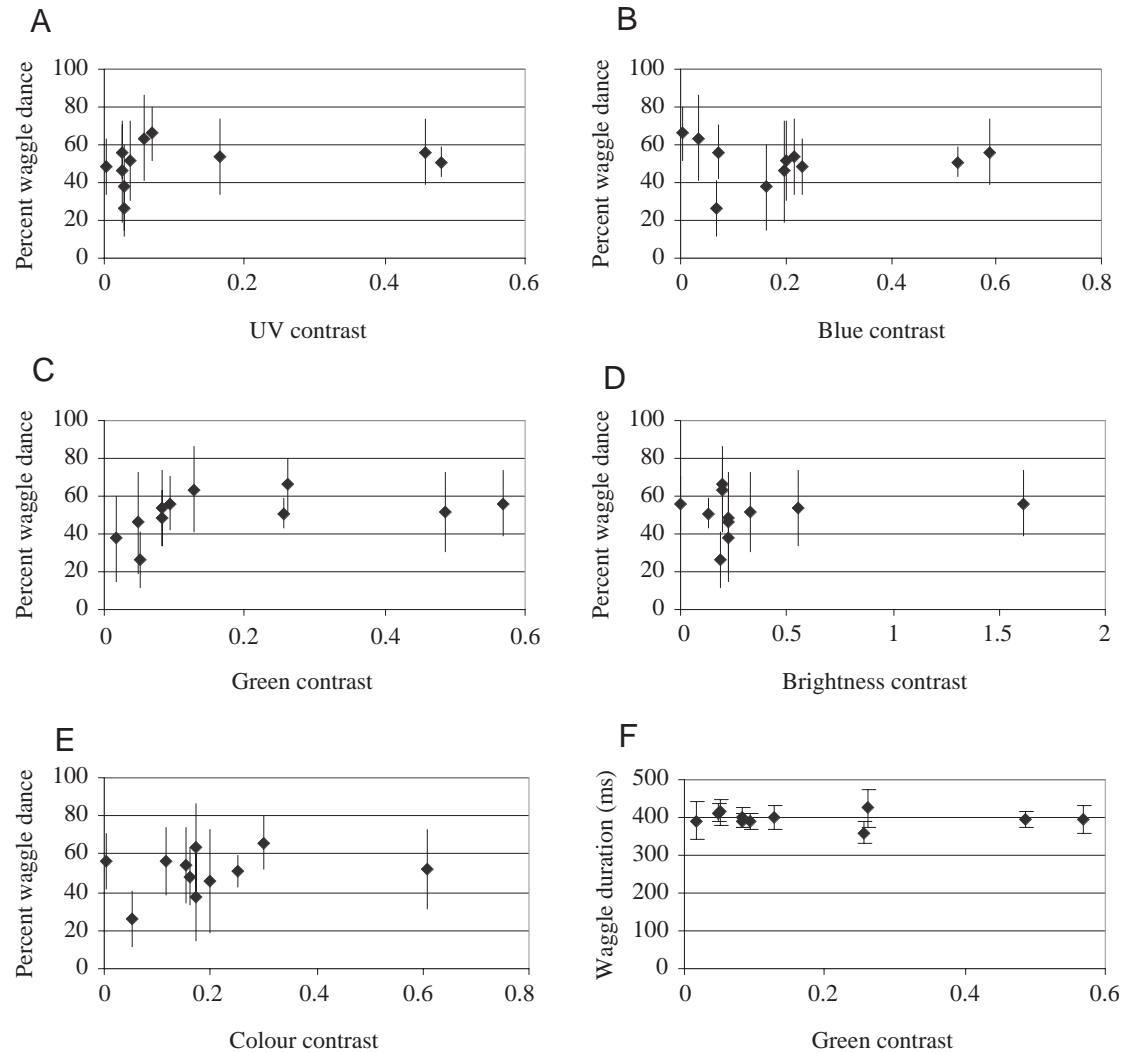The evaluation of waggle run duration is consistent with this

Fig. 2. Percentage ± S.D. of waggle dances as a function of various types of visual contrast (A–E), and waggle run duration ± S.E.M. (F) as a function of green contrast in the 11 colour patterns presented in the tunnel.

interpretation. Whenever bees experienced green contrast sufficiently strong for the waggle dance to be triggered, waggle duration was entirely independent of the particular colour pattern presented in the tunnel (Fig. 2F). There was no significant difference in waggle duration between the different patterns (Kruskal–Wallis test; K=10.35, P=0.41, N=92). Waggle runs invariably lasted about 400 ms. For bees foraging in natural landscapes, this waggle run duration codes for food sources 100–200 m from the hive, depending on the particular territory passed in flight (Esch et al., 2001). We interpret this to mean that the bee odometer is sensitive only to the integration of image motion over time, not to the *amount* of contrast present in the image. This applies, of course, only if contrast is sufficiently high to be detected.

Discussion

Earlier studies on bee visual odometry (Esch et al., 2001; Srinivasan et al., 2000) demonstrated that waggle dance probability depends on angular motion of the scene passed by the flying bee. We identify the bees' long-wavelength receptor

as the spectral input to visual odometry. Colour contrast and brightness contrast (quantified by the sum of the UV, blue and green receptor signals), as well as contrast present in any other spectral receptor type, were disregarded by bees in this context. An interesting additional experiment might be to use a pattern presenting 0% green contrast but a strong contrast in one or two of the other spectral channels. Our prediction would be that waggle dance probability would decrease drastically when faced with such a pattern. It would probably not drop to zero, since bees flying through a tunnel even perform 40% waggle dances when presented with horizontal stripes in the tunnel (Si et al., 2003). Nevertheless, our approach of using suprathreshold stimuli with a wide range of visual contrast clearly identifies the spectral channel underlying distance estimation. There is a strong correlation between green contrast and waggle dance probability over the low contrast range, and there is no correlation between any other spectral parameters and waggle dance probability. We conclude that the green receptor signal is used to estimate travel distance in honeybees.

While the probability of waggle dances can clearly be influenced by the visual scene passed *en route*, the duration of

the waggle phase (a component of the distance code) remains unaffected. This might be because waggle phase duration (as opposed to waggle dance probability) is independent of optic flow above a certain distance. However, in a new study, Si et al. (2003) showed that waggle dance duration *can* be influenced by the visual pattern passed in flight and is influenced by distance beyond 6 m. We therefore conjecture that the visual odometer relies only on the magnitude of optic flow (Srinivasan et al., 2000) not the amount of contrast presented within the scene passed *en route*.

Whether or not bees overestimate flight distance as a response to experimentally increased optic flow depends, of course, on whether contrast in the visual scene is detected in the first place. We assume that over the range of low green contrast values from 0 to 0.15, several sources of noise determine whether or not contrast between adjacent squares in the tunnel is detected. It is noteworthy that in all patterns, even those with very low green contrast, waggle dances are elicited at least occasionally. In the blue4–grey6 pattern, green contrast is only 3.7%, which corresponds to a predicted difference in green receptor voltage signal of V/V_{max}=0.016. Vorobyev et al. (2001) report a noise standard deviation of 0.2–0.4 mV for the honeybee green receptor at daylight intensity, and a V_{max} of 38 mV for that receptor type. The predicted signal difference for the blue4–grey6 pattern is therefore 0.016×38 mV=0.61 mV, which is outside the noise standard deviation. We would therefore predict that if receptor noise were the only limiting factor, even the patterns with the lowest green contrast would be above detection threshold. But noise of subsequent neuronal processing units, such as lamina monopolar cells, can be substantially stronger than in photoreceptors (de Souza et al., 1992), so limiting contrast detection more strongly than the peripheral (receptor) level. In addition, the bees' flight speed and proximity to the tunnel walls will determine whether or not low contrast between adjacent squares will be detected. Small fluctuations in illumination might introduce additional variation, although we expect them to be mostly compensated for by photoreceptor adaptation (Laughlin, 1989) and colour constancy (Kevan et al., 2001). We assume that at a green contrast of 0.15, the signal reliably exceeds all forms of noise, and contrast is reliably detected.

Our results are in line with those on other behaviours in bees controlled by motion vision, all of which appear to be colour blind and entirely driven by the long-wavelength or green receptor (Giurfa and Lehrer, 2001; Horridge, 2000; Kaiser, 1974; Spaethe et al., 2001; Srinivasan, 1989). Even though bees have excellent trichromatic colour vision, which they use, for example, in flower identification (von Frisch, 1967; Chittka et al., 2001), colour is not used for motion vision. Apparently, in bees, colour and motion are processed strictly in parallel (Lehrer, 1993; Zhang and Srinivasan, 1993).

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