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RESEARCH ARTICLE

Effect of stimulus height on cockroach optomotor response

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ABSTRACT

Using tethered American cockroaches walking on a trackball in a spherical virtual reality environment, we tested optomotor responses to horizontally moving black-and-white gratings of different vertical extent under six different light intensities. We found that shortening the vertical extent of the wide-field stimulus grating within a light level weakened response strength, reduced average velocity and decreased angular walking distance. Optomotor responses with the vertically shortened stimuli persisted down to light intensity levels of 0.05 lx. Response latency seems to be independent of both the height of the stimulus and light intensity. The optomotor response started saturating at a light intensity of 5 lx, where the shortest behaviourally significant stimulus was 1 deg. This indicates that the number of vertical ommatidial rows needed to elicit an optomotor response at 5 lx and above is in the single digits, maybe even just one. Our behavioural results encourage further inquiry into the interplay of light intensity and stimulus size in insect dim-light vision.

KEY WORDS: Visual behaviour, Dim-light vision, Virtual reality, Optomotor reaction, Periplaneta

INTRODUCTION

When it comes to seeing, night-active insects do not have it easy. Compared with their day-active counterparts, they have a fraction of the number of photons at their disposal. Thanks to the stochastic nature of photon incidence and absorption into the photoreceptor membranes, visual signals produced in dim light are fundamentally noisy with a low signal-to-noise ratio (SNR) (Rose, 1942; de Vries, 1943; Honkanen et al., 2017). Anatomical adaptations to the compound eye optics can only take a species part of the way into acquiring a visual system able to perform in low-light conditions. Neural adaptations, such as temporal and spatial summation, are often necessary in addition to optical modifications for enhancing the sensitivity of the visual system (Warrant, 1999).

Temporal summation allows the visual system more time to collect photons, and it is detectable in physiology as slow response dynamics in photoreceptors and motion-sensitive neurons (Heimonen et al., 2012; Stöckl et al., 2016a), slow dynamics and lower contrast efficiency in lamina monopolar cells (LMCs) (van Hateren, 1992), and in behaviour as deterioration of the responses to fast movement (Baird et al., 2011; Honkanen et al., 2014).

Spatial summation is a strategy for enhancing the SNR of a visual system by pooling together responses from several visual channels

(here, visual channels mean photoreceptors and motion-sensitive neurons of the optic lobe). The existence of spatial summation has long been predicted theoretically on the basis of anatomy (Strausfeld and Blest, 1970; Ohly, 1975; Ribi, 1977; van Hateren, 1992; Warrant, 1999; Greiner et al., 2004, 2005), and it is thought to take place in LMCs of the first visual neuropil of the compound eye (Dubs et al., 1981; van Hateren, 1992; Stöckl et al., 2016a,b), where it probably arises through increasing the relative weight of synaptic inputs from the most distal parts of the LMC dendrites (Stöckl et al., 2020).

The American cockroach (*Periplaneta americana*, Dictyoptera: Blattodea) has been a workhorse of vision research for decades. The structure of its apposition-type compound eyes is known (Butler, 1973a,b), its visually guided behaviour has been studied (Autrum and Stöcker, 1952; Roberts, 1965; Okada and Toh, 1998; Ye et al., 2003; Lent and Kwon, 2004), and special interest has been paid to its ability to see in low light (Heimonen et al., 2006, 2012; Salmela et al., 2012; Honkanen et al., 2014, 2018; Immonen et al., 2014; Zhukovskaya et al., 2017).

In recent studies, we have used a spherical panoramic virtual reality (VR) setup (Takalo et al., 2012) to assess the visual capabilities of the American cockroach in dim light (Honkanen et al., 2014, 2018). We have previously shown that their optomotor response persists down to light levels where each compound eye photoreceptor captures photons at a rate of 0.1 s^{-1} (Honkanen et al., 2014), and that ocelli improve the sensitivity of the optomotor response at low light intensities (Honkanen et al., 2018). Estimating the extent of spatial summation at different light intensities based on visual performance is possible when the average number of absorbed photons at each intensity is known. We made tentative calculations of the extent of spatial pooling in Honkanen et al. (2014) using wide-field stimuli presented on a spherical surface around the cockroach. The behavioural effect of gratings that are restricted in size either vertically (Duistermars et al., 2007) or horizontally (Tammero et al., 2004) has been reported, but the combined effect of the two on behaviour and visual system physiology has not been studied. We planned the behavioural experiments presented here to be the first in a series aimed at defining the smallest receptive field sizes required to evoke optomotor responses under different illuminations and identifying the correlated physiological changes in the retina and the optic lobes. Unfortunately, we were not able to perform the follow-up experiments; therefore, we present these behavioural results as a stand-alone publication.

The objective of this study was to examine the vertical receptive fields for wide-field stimuli by varying the vertical extent (height) of the horizontally moving grating while keeping the other stimulus properties constant. This changes the area of the stimulus without shifting its temporal properties, thus allowing an estimation of the number of ommatidial rows recruited at each light intensity. Specific questions we set out to answer are: what is the vertically shortest stimulus that can elicit an optomotor turning response?; and how does the optomotor turning in response to each stimulus height change as a function of light intensity?

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MATERIALS AND METHODS

Equipment

The spherical panoramic VR system used in these experiments was originally presented in Takalo et al. (2012). In brief, the system consists of two computers, one for controlling the stimuli with Ogre3D graphics engine (http://www.ogre3d.org/) and the other for recording the trackball movements; a modified DepthQX2 projector (Lightspeed Design Inc., Bellevue, WA, USA) with a 360 Hz frame rate after the removal of the colour filter from the image engine; a fisheye lens able to project the stimuli on the inside of a hollow spherical anti-reflection-painted screen covering 270 deg horizontally; and an air-suspended trackball (radius 4.7 mm, mass 4.8 g), the movements of which are detected with infrared sensors and logged by the recording computer. The system, excluding the computers, is located inside an anti-vibration Faraday cage with a blackout curtain. Light intensity inside the projection screen is about 500 lx and it can be dimmed with -1, -2, -3, -4 and -5 decade neutral density filters (NE Series, Thorlabs, Newton, NJ, USA) (see Table 1).

Animals and preparation

Adult male wild-type American cockroaches, *Periplaneta americana* (Linnaeus 1758) (Blades Biological Ltd, Cowden, Kent, UK) were used in this study. All applicable Finnish laws and guidelines for the care and use of animals were followed. Only cockroaches with fully intact bodies were accepted for the experiments; special care was given to the condition of antennae and legs. Experimental cockroaches were housed individually in small plastic containers with wood chips as a substrate, a hiding place, and food and water *ad libitum*, at 25°C in an inverse 12 h:12 h light:dark rhythm. Experiments were done during the subjective night, when cockroaches were most active.

Preparation of the cockroaches for experiments started with CO_2 anaesthesia followed by cleaning of the animal's pronotum with 70% ethanol and attachment of a metal wire holder onto it with a 1:1:1 mixture of beeswax, resin and paraffin. The animal was then fixed onto the trackball in a natural walking position and allowed 20 min to recover and dark adapt. If after this time the cockroach was not moving normally or looked damaged in any way, it was discarded from the experiments. After the experiments, the cockroach was anaesthetised for removal of the wire holder and either returned to its individual container or eliminated.

Experiments were completed on 16 cockroaches. Data from 11 individuals were included in the analyses (rejection rate 31.25%). Four of the discarded cockroaches were frequently too inactive during the experiments (see 'Data analysis and statistical testing', below) and one individual damaged its legs during experimentation and was therefore excluded.

Stimulus parameters and protocol

The American cockroach has eight photoreceptors in each ommatidium; five green and three UV sensitive (Butler, 1971).

Table 1. Illuminance levels used in the experiment

Illuminance (lx)	Relative illuminance	ND filter (decade)	Corresponding to
500	10^0	No filter	Average office lighting
50	10^{-1}	-1	Dark overcast day
5	10^{-2}	-2	Civil dusk
0.5	10^{-3}	-3	Full moon
0.05	10^{-4}	-4	Half moon
0.005	10^{-5}	-5	Moonless clear night sky with airglow

The VR projector does not emit UV wavelengths (Takalo et al., 2012), so the stimuli excite the green receptors exclusively. The angular period of the stimulus grating, i.e. the horizontal width of one black and white repeat in the stimulus pattern, was held constant at 60 deg throughout the experiments. Three temporal frequencies for the optomotor stimulus movement were chosen based on our earlier study (Honkanen et al., 2014), where cockroach optomotor response was found to be strongest at 0.4, 2.4 and 4 Hz. The vertical extent of the stimulus grating varied from 90 to 1 deg (90, 60, 30, 20, 10, 4, 2, 1 deg) and was centred on the equator of the spherical screen so that an equal share of it was visible on the upper and lower hemisphere. Vertical pixel size at the equator of the projection sphere is about 1.3 deg (Takalo et al., 2012), so the shortest stimulus was only one pixel tall. The rest of the screen outside the stimulus grating was as dark as the dark stripes of the grating.

The eight different vertical lengths of stimulus were shown at the three temporal frequencies for each of the six light intensities shown in Table 1, yielding a total of 144 unique stimulus combinations per cockroach. The stimulus protocol was as follows: 30 s control (stimulus is visible but does not move); 30 s rotation to the left; 15 s control; 30 s rotation to the right; 15 s dark screen for each stimulus combination. Rotation direction was always first left, then right. Stimulus order (the combination of vertical length and temporal frequency) was randomized for each individual cockroach and light intensity. Experiments always began with the dimmest light intensity (0.005 lx) and proceeded gradually by changing the ND filters until finally reaching 500 lx.

The projection surface of the VR system has an anti-reflection coating, which lowers the contrast of the projected image when full-screen images are being used (Takalo et al., 2012). The Michelson contrast attainable with an optomotor grating extending vertically across the entire projection surface is 0.33 at all ambient intensities (Honkanen et al., 2014); however, the contrast intensifies when using smaller stimuli on a dark background. Therefore, because of the lower contrast, movement of large stimulus patterns could in theory produce weaker optomotor responses than smaller patterns would. In bright light, optomotor responses to rotating gratings should not be very sensitive to changes in these contrast ranges (Duistermars et al., 2007). Contrast efficiency (response amplitude per unit contrast) of LMCs is higher with higher SNR in brighter background light (van Hateren, 1992); therefore, higher image contrast of the smaller patterns might counteract the lower contrast efficiency at dim intensities.

Data analysis and statistical testing

Data were analysed in Matlab (MathWorks Inc., Natick, MA, USA). Total angular distance travelled by the cockroach on the trackball, ϕ_{tot} (deg), was calculated as the sum of all recorded forward and turning movements during stimulus rotation. Average velocity of each individual cockroach was calculated from the total angular distance by dividing by 30 (the duration of stimulus rotation in seconds).

Turning tendency of the cockroaches was quantified as relative strength of the turning response, R, for each individual cockroach, and scaled between -1 and 1 as in Honkanen et al. (2014):

$$R = 2(\phi_{rot}/\phi_{tot}) - 1, \qquad (1)$$

where ϕ_{rot} is the angular distance the cockroach moved in the same direction as the stimulus. $R=0$ when the cockroach has no turning preference, $R=1$ denotes perfect positive turning response, and $R=-1$ marks perfect negative turning response. For the control

sequences, R was calculated as if the stimulus were moving in the same direction as during the rotation sequence following that control. R was calculated separately for rightward rotation, leftward rotation and the two control sequences. These parameters could not be calculated for cockroaches that did not move during the stimulus presentation. Therefore, cockroaches that had repeated bouts of immobility during the experiments were excluded from the data, but random immobility readings in the data were tolerated and replaced with a '0' denoting no turning preference.

Latency from the beginning of the stimulus movement to the beginning of the turning response was calculated as the first time that the insect's turning velocity in the direction of the rotating stimulus exceeded a threshold. The threshold velocity was defined as the 80th percentile of the velocity distribution of a trial. Trials in which the cockroaches were not already moving at the onset of the rotating stimulus and trials in which the cockroaches did not exceed the threshold velocity in the correct direction were excluded from statistical analyses of response latency.

Data from these experiments were not normally distributed (see also Honkanen et al., 2014, 2018), nor did they follow other assumptions of parametric tests. Therefore, the non-parametric Friedman test was used to inspect the statistical significance of stimulus effects. Wilcoxon signed-rank test was used for comparing rotation and control values in optomotor response strengths, pairwise comparisons during preliminary testing of data, and *post hoc* multiple comparisons between total angular distances travelled. Holm's multiple test procedure (Holm, 1979) was used for redefining the significance values of multiple comparisons.

Friedman test could not be performed on the latency data because of unequal sample sizes (see the exclusion criteria). Instead, a generalized linear mixed-effects regression model was implemented as a substitute. The regression model was fitted to a gamma distribution using the maximum pseudolikelihood (MPL) method. Identity link function was selected for the model as Lo and Andrews (2015) have recommended for the analysis of reaction time data. Based on the Akaike information criterion (AIC), the fixed effects selected for the model were the vertical extent of the stimulus, temporal frequency of stimulus rotation, $\log_{10}$ of light intensity and the interactions between these three. Individual identity of the insect was added as a random covariate in the model. As the computation of the regression did not accept any zero values in data, the occasional zero values were replaced with the smallest non-zero value found in the data in order to execute the regression analysis.

RESULTS

Turning responses of the cockroaches were not statistically different between the three temporal frequencies of the optomotor stimulus (P=0.14, Friedman test) and between leftward and rightward stimulus rotation (P=0.54, Wilcoxon signed-rank test). Also, in the case of total travelled angular distance, there was no statistically significant difference between the temporal frequencies (P=0.18, Friedman test) or the directions of rotation (P=0.12, Wilcoxon signed-rank test). Therefore, data from different temporal frequencies and directions of stimulus rotation were pooled, which enabled a greater sample size for the analyses.

Response strength

The strength of optomotor turning responses as a function of light intensity and vertical extent of the stimulus is presented in Fig. 1A. During the control sequences, the average response strengths were close to zero, suggesting that, on average, there were no turning preferences. All the moving gratings were able to elicit optomotor responses at light intensities of 5–500 lx, and the turning responses were strongest at 500 lx. Response strength became weaker as the vertical length of the stimulus shortened. For instance, at 500 lx, the response strength for a 90 deg stimulus was 0.81, whereas for 1 deg stimulus, it was 0.43. Under 0.5 lx illumination, cockroaches could no longer distinguish the movement of the 1 deg stimulus from the control. A tenfold decrease in light intensity (0.05 lx) elicited statistically significant optomotor responses only for the three tallest stimuli (30, 60 and 90 deg). At a light intensity of 0.005 lx, the dimmest condition, all optomotor responses were absent, except for one statistically significant rotation–control pair when the vertical extent of the stimulus was 10 deg (P<0.05, Wilcoxon signed-rank test).

Fig. 1B shows the effect of light intensity (left panel) and vertical extent of the stimulus pattern (right panel) on the response strength. Optomotor response strength during the rotation sequence increased with increasing light level, and with increasing vertical extent of the stimulus. At 0.005 lx, there was no statistically significant difference between rotation and control sequences (P=0.23, Wilcoxon signed-rank test).

Angular distance and average velocity

Fig. 2A presents the total angular distance walked by the cockroaches at different light intensities and vertical lengths of stimuli as box plots. Cockroaches may be active even when the response strength is low if they turn to the left and right equally or run straight; therefore, a separate analysis of activity parameters such as distance is needed. However, the results resemble the response strengths of the optomotor turnings. Generally, travelled distance and its variance decreased with decreasing light intensity and vertical length of the stimulus (Fig. 2A). The travelled distance was highest (median ca. 1900 deg, or 1.6 m) under the brightest light intensity and with 60 deg tall stimuli. At 0.005 lx, the total distance did not change significantly as a function of the vertical length of the stimulus (P=0.56, Friedman test). The effect of the vertical length on the total distance travelled by the cockroaches was statistically significant from 500 lx down to 0.05 lx (each light intensity level: P<0.001, Friedman test). Multiple comparisons of the medians are presented in Table S1. Similar observations can be made from Fig. 2B, which shows pooled angular distance within light intensity level (left panel; P<0.001, Friedman test) and vertical extent of the stimulus (right panel; P<0.001, Friedman test). Multiple comparisons of the medians in Fig. 2B are shown in Tables S2 and S3.

Average velocity of the trials was calculated from the angular distance (Fig. 2C) and shared the same statistical test results (Table S1). Velocity increased with increasing brightness and vertical length of the stimulus. At its highest, the average velocity was 64.31 deg s^{-1} at 500 lx and 90 deg. At 0.005 lx, the average velocity no longer changed significantly as a function of the vertical length of the stimulus. Variation in average velocity seemed to decrease with dimming light levels.

Response latency

The latency from the beginning of the stimulus movement to the beginning of the turning response as a function of light intensity and vertical extent of the stimulus is illustrated in Fig. 3A. All median latencies were under 5 s, and the median latency seemed to decrease slightly with increasing illuminance and vertical extent of the stimulus pattern. Although scattering of the data points in Fig. 3A grew towards the dimmer light intensities, the median latencies and variation of data seemed to be relatively independent of light
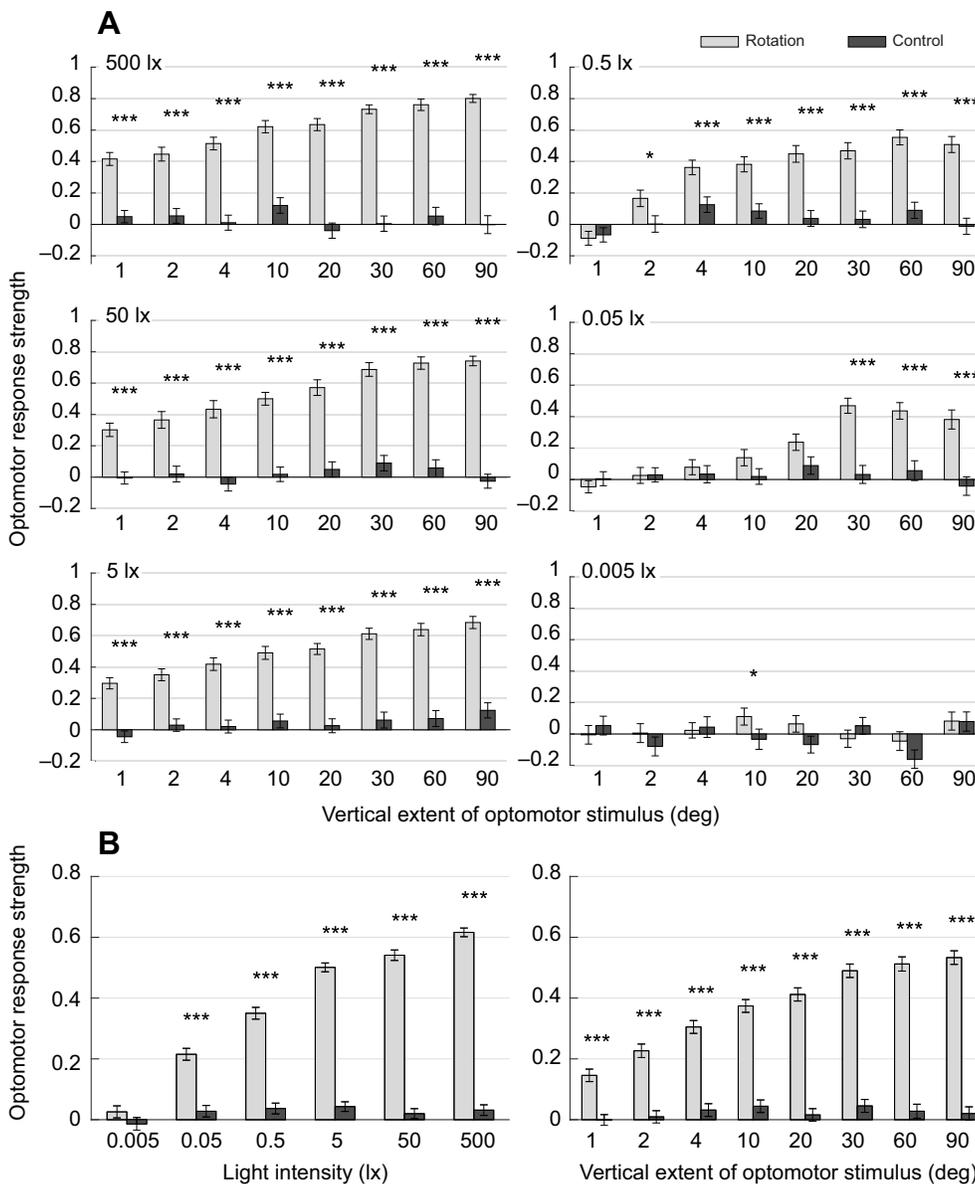
Fig. 1. Optomotor stimulus strength (*n*=66). (A) Strength of the optomotor response (mean±s.e.m.) as a function of the vertical extent of the stimulus at different light intensities (upper left corner of each panel). (B) Left: effect on response strength of light intensity alone. Right: effect of vertical extent of the stimulus alone. During control sequences (dark grey), the turning response was always close to zero, i.e. cockroaches walked equally far to the right and left when the stationary stimulus was presented. When the stimulus rotated (light grey), response strength became weaker in response to both shorter stimuli and dimmer light intensities. Statistical significance between each rotation and its control value is denoted with asterisks (**P*<0.05, ****P*<0.001; Wilcoxon signed-rank test).

intensity and vertical extent of the stimulus patterns when the data were pooled within light intensity and vertical extent of the stimulus patterns (Fig. 3B). In Fig. 3B, all median latencies were under 2 s and most cockroaches turned within 13 s.

Latency did not seem to be dependent on the vertical extent of the stimulus, temporal frequency of rotation, tenfold change in light intensity or the interactions between these three, as the estimated generalized linear mixed-effects regression coefficients were small, and statistically not different from zero (Table S4). Estimated intercept of the regression equation was 4.09±0.77 s (95% confidence interval). Standard deviation between the intercepts of individual insects was estimated to be ~0.99 s.

Variation in response latency decreased with increasing light intensity (Fig. 3A). When data from all stimulus vertical extents were combined within light level, the highest median latency, ~1.9 s, was found at 0.05 lx (Fig. 3B, left). This indicates that although cockroaches could not follow the stimulus motion very well at this intensity, they were not moving directly forward all the time. Presumably, they were making random fast turns left and right, which resulted in a weak response strength of optomotor turning.

DISCUSSION

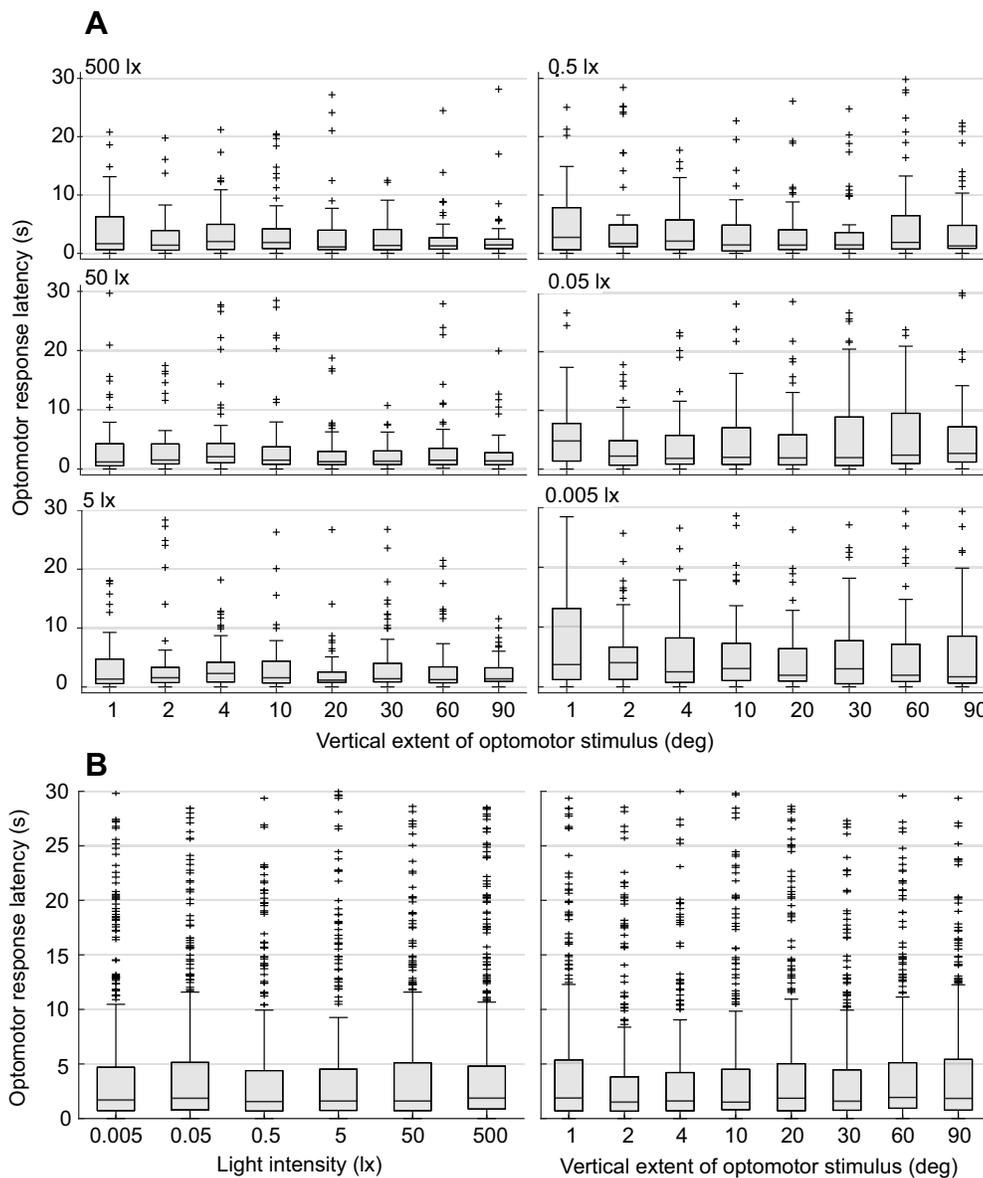### Effect of stimulus vertical length on response strength

All vertically shortened stimuli at 5 lx and brighter were adequate for producing an optomotor response (Fig. 1A). At intensities below 5 lx, gradually taller stimuli were needed to elicit a response. At 0.5 lx illumination, cockroaches failed to respond to 1 deg tall stimuli. At 0.05 lx, the ability to respond to 10 deg tall stimuli decreased dramatically, and statistically significant differences to controls were found only in response to stimuli of 30 deg and taller. Under the dimmest condition of 0.005 lx, the statistically significant difference (*P*<0.05) between the response to rotation and control 10 deg tall stimuli shown in Fig. 1A was probably caused by the control being slightly negative. The left panel of Fig. 1B shows responses to stimuli of all vertical extents combined within each light intensity level, in which rotation and control values at 0.005 lx do not differ statistically from each other. Also, comparison with the statistically non-significant difference with 10 deg stimuli at 0.05 lx shows that at 0.005 lx, turning response strength is weaker. Therefore, we can confidently say that cockroaches did not exhibit any optomotor turning responses at 0.005 lx with the stimuli used here.
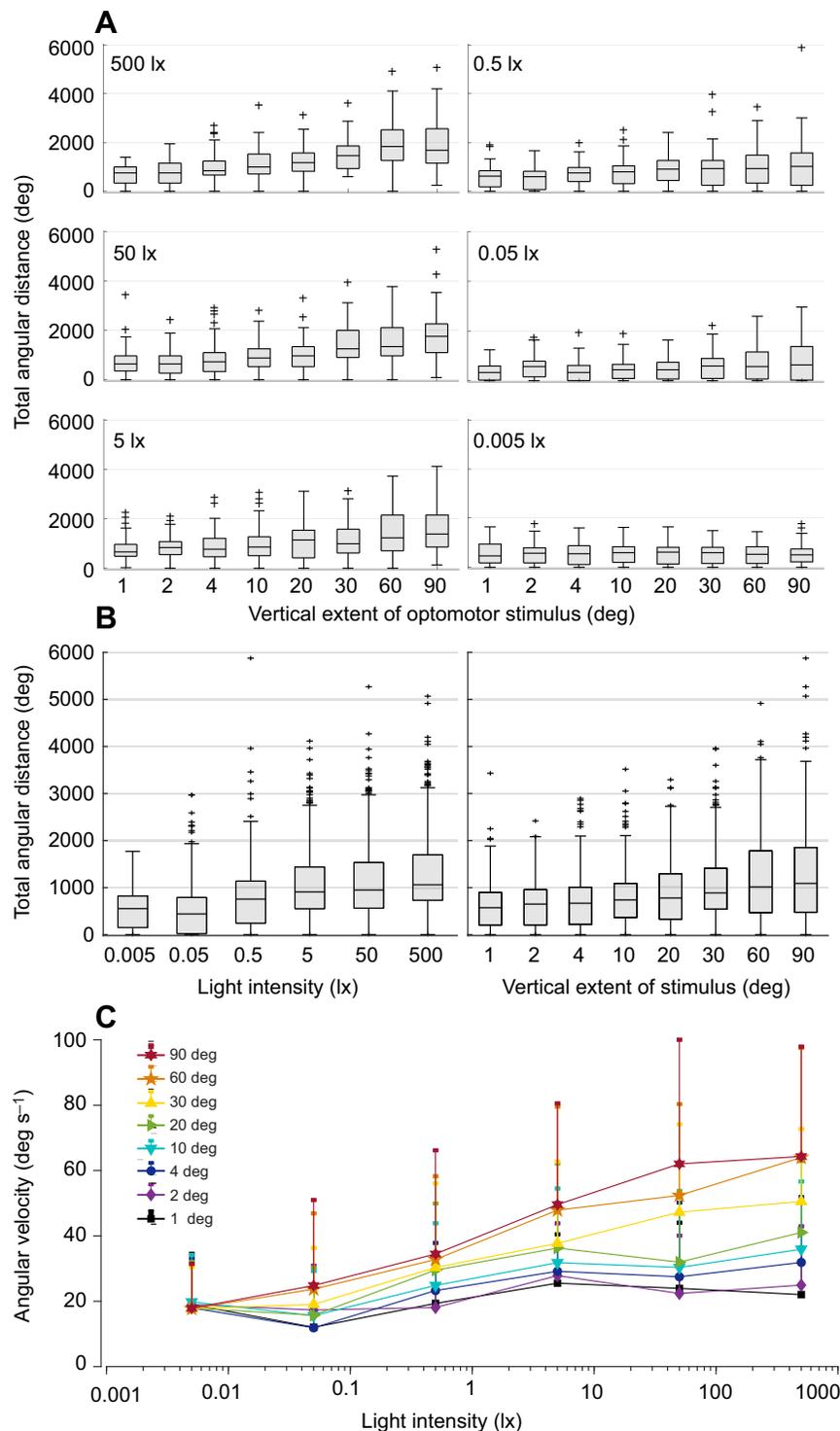
Fig. 2. Total angular distance and average angular velocity walked by the cockroaches during a trial as a function of light intensity and vertical length of the stimulus (*n*=66). (A) 2000 deg of trackball rotation corresponds to 1.64 m walking distance. In the box plots, the central mark is the median, the lower edge is the 25th percentile (q1) and the upper edge is the 75th percentile (q3) of the data. Boxes also represent the interquartile range (IQR=q3−q1). The whiskers cover data in range of 1.5×IQR below the 25th and above the 75th percentiles. Outliers are denoted by a plus sign (+). The vertical extent of the stimulus had a statistically significant effect within light intensity levels from 500 to 0.05 lx (*P*<0.001, Friedman test), but not within 0.005 lx illuminance (*P*=0.56, Friedman test). Multiple comparisons are presented in Table S1. (B) Left: effect on distance of light intensity alone. Right: effect of vertical extent of the stimulus alone. Both light intensity and vertical extent of the stimulus had a statistically significant effect on the total distance travelled (*P*<0.001, Friedman test). Multiple comparisons are presented in Tables S2 and S3. (C) Mean±s.d. angular walking velocity of cockroaches with different stimulus vertical lengths (see key) across different light intensities. To avoid clutter, s.d. is only shown in one direction.

The optomotor turning responses at each vertical extent were very similar at light intensities between 5 and 500 lx (Fig. 1A), indicating that the responses of cockroach photoreceptors and nervous system start to saturate at these intensities. The visual system of the American cockroach seems to be adapted to dim light environments, as previously suggested (Heimonen et al., 2006, 2012; Honkanen et al., 2014). The greatest changes in optomotor response strength occurred between 5 and 0.005 lx, which agrees with the notion that visual systems of insects are able to adapt over 4 decades of light intensity (Land and Chittka, 2012).

Comparison of the response strengths in Fig. 1A with results reported in Honkanen et al. (2014) reveals that the mean strengths with 90 deg stimuli at 500 lx are similar to the responses obtained with full-size (121 deg tall) stimuli, about 0.8 in both cases. At 5 lx, the 90 deg stimulus gives a slightly better average (ca. 0.7 versus 0.6), which may be caused by the better contrast with the smaller stimulus (Takalo et al., 2012), assuming, on the basis of saturated responses in Fig. 1A and Honkanen et al. (2014), that the contrast efficiency at this light level is still relatively high (van Hateren, 1992). Because of the better contrast with shorter stimuli, it is

Fig. 3. Latency from the beginning of stimulus movement to the beginning of the turning reaction as a function of light intensity and vertical length of the stimulus (n=66). (A) All latencies. (B) Left: effect on latency of light intensity alone. Right: effect of vertical extent of the stimulus alone. Box plots constructed as in Fig. 2A. Trials where cockroaches remained stationary at the time of stimulus onset or did not exceed the turning velocity threshold were excluded from the analysis. No statistically significant predictors of response latency were found with a generalized linear mixed-effect regression (Table S4).

important to note that the eight stimulus vertical extents (Fig. 1A) within a given light intensity level are not strictly comparable. Therefore also, to standardise the contrast, future studies with less than full-field stimuli in the VR sphere should use a grey background with the same irradiance as the full-size stimulus, instead of the dark background used here. At a light intensity of 0.05 lx, however, the full-size stimulus produces a stronger response: ca. 0.4 versus 0.6. At the dimmest intensity of 0.005 lx, only the full-size stimulus used by Honkanen et al. (2014) elicited an optomotor response.

Distance, velocity and latency

Insects are known to move slower in dim light than in bright light (Baird et al., 2011; Reber et al., 2015; Honkanen et al., 2018), and our results support this observation. The more modest distance travelled in dim light (Fig. 2A,B) could be connected to the increase in the visual integration time, i.e. temporal summation, because slower motion gives more time for image formation and motion detection in the dark (Baird et al., 2011), or it could be motivational, caused by a reduction of stress in dimmer light (Zhukovskaya et al., 2017). When

comparing Fig. 2C with the average velocities of unmanipulated cockroaches stimulated with full-screen grating (Honkanen et al., 2018), we can see that reducing the stimulus vertically to 90 deg reduced the average velocity at 500 lx by 30%, from 109 to 64 deg s^{-1}. Velocities obtained with the vertically shortest stimuli, 1 and 2 deg, are comparable to velocities of cockroaches whose compound eyes or compound eyes together with ocelli were covered, and which were most likely reacting to light reaching their photoreceptors through the cuticle (Honkanen et al., 2018).

In general, response latency seems to behave contrarily to the turning response strength, total distance and average velocity, i.e. it increases slightly with decreasing light intensity (Fig. 3A). However, neither the vertical extent of the stimulus nor light intensity had a statistically significant effect on response latency (Fig. 3B; Table S4). In relation to the optomotor response strength, a generally low median response latency might suggest that turning responses in the direction of moving stimuli could be initiated relatively quickly regardless of the intensity of light and size of the stimuli used, while maintaining the turning response would be a harder task in dim light (0.005–0.5 lx) and with vertically shorter

stimuli. However, this possible relationship between initiation and continuation of optomotor turning responses in dim light should be tested with a more specific set of experiments. A less speculative explanation is that the cockroaches were making fast random turns in the dark.

Slightly shorter latencies in bright light and with vertically larger stimulus patterns (Fig. 3A) could be explained by negative phototaxis or shelter seeking (Guthrie and Tindall, 1968; Zhukovskaya et al., 2017). Positioning of the cockroaches on the trackball prevents antennal contact to solid surfaces, so the effect of thigmotaxis on response latency can be ruled out (Laurent Salazar et al., 2018). At 5 lx intensity, the latency and its variation were smaller than at 50 and 500 lx (Fig. 3A), which may partly be explained by weariness of the animals, as experiments lasted for several hours and the brightest conditions were presented last. Alternatively, as 5 lx seems to be the threshold for saturation of the response (Honkanen et al., 2014), cockroaches might be most motivated to explore their surroundings at this light level.

In an ideal scenario, cockroaches would move constantly during the experiments. However, cockroaches were not forced to move during the trials, i.e. their walking behaviour was spontaneous, which may affect the measured response latencies and reduce the number of comparable trials for statistical analyses. While the visual stimuli and lack of antennal contact provide the cockroaches with a strong incentive to move, there are always occasional cases where cockroaches stop moving for example to groom their antennae in order to maintain their olfactory acuity (Böröczky et al., 2013). During these stops, the animals do not react to the moving stimuli, which may explain some of the latency distribution seen in Fig. 3. Considering that variation in responses is to be expected in behavioural experiments, the analysed response latencies seem reasonable.

The smallest functional size of a 'wide-field' stimulus
At the brightest light intensities used here (5–500 lx) cockroaches could discern the shortest wide-field stimuli, which were ~1 deg tall. These patterns stimulate only a few ommatidial rows in the compound eyes of the cockroach (Fig. 4A, top). As the cockroach experiences dimmer environments (less than 5 lx), the smaller and more random photoreceptor signals (mostly separate quantum bumps; see Honkanen et al., 2014) originating from these ommatidial rows are drowned by photon shot noise (Fig. 4B, top).

With taller patterns, more rows of ommatidia are stimulated and SNR remains adequate for producing an optomotor response in dimmer light intensities (Fig. 4A,B, bottom).

According to Honkanen (2014), in this VR system the American cockroach cannot discern the movement of optomotor grating with an angular period less than 15 deg (i.e. each black or white bar has to be at least 7.5 deg wide). Because the cockroach can, under adequate illumination, respond to just 1.3 deg tall stimuli (resolution limit of the VR setup; Takalo et al., 2012), the minimum area with which the cockroach could perceive wide-field motion in the VR should theoretically be 7.5 deg×1.3 deg. In our experiments, stimuli were centred on the equator of the projection sphere across the entire 270 deg of the horizontal extent of the projection surface (Takalo et al., 2012), so we cannot as yet say whether this would be enough to cause an optomotor turning response or elicit some other reaction (Tammero et al., 2004).

Where to go from here?
In this experiment the stimuli horizontally covered the entire panorama screen (270 deg). To assess the extent of spatial summation of photoreceptor responses in one compound eye, the optomotor stimulus must be restricted not only vertically but also horizontally by, for example, unilateral stimulation or covering one of the compound eyes. As horizontal interommatidial angles are smallest in the forward-facing parts of the eye (Butler, 1973a), covering only the anterior or posterior half of each eye could also be revealing. Electrophysiological recordings from the optic lobe could detect changes in the action potential coding as the light intensity decreases and stimulus size changes. Ideal recording sites would be the lamina monopolar cells (Stöckl et al., 2020) and the motion-sensitive neurons of the lobula, such as the descending contralateral movement detectors (Edwards, 1982a,b).

Conclusions
The optomotor response of the American cockroach with vertically restricted stimuli started saturating at a light intensity of 5 lx. Vertically extensive stimulus patterns elicited stronger optomotor turning responses than more restricted patterns, although results obtained in this VR setup with different vertical lengths of the stimulus within a light level are not directly comparable because of contrast differences. Under the brightest intensities (5–500 lx), only
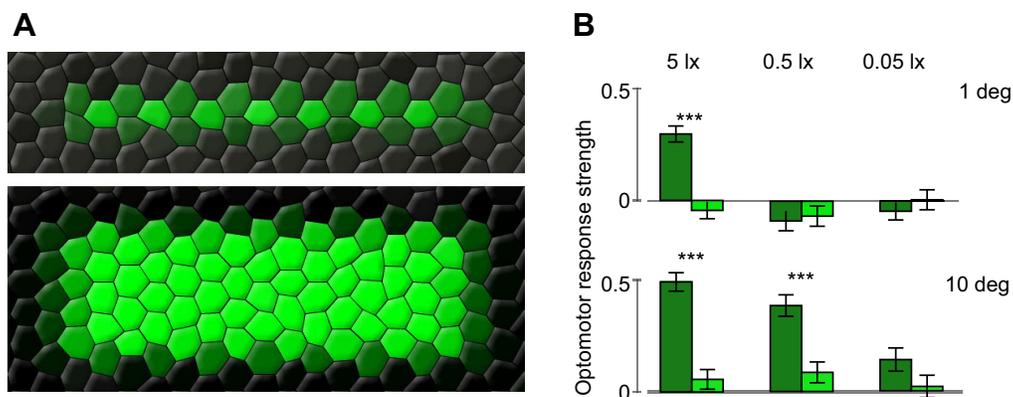
Fig. 4. Effect of stimulus height on the optomotor turning response at different light intensities. (A,B) To distinguish the direction of a moving fixed-width pattern in dim light, the cockroach must recruit more horizontal rows of ommatidia (A) to produce a brighter image (greater optomotor response strength; B). Short stripes (such as the 1 deg stimulus), stimulating only a few rows of ommatidia (A and B, top), disappear into the visual noise. To produce a comparable response in dim light, a taller pattern (e.g. 10 deg) is needed, stimulating more rows of ommatidia (A and B, bottom). Illustrations in A are not to scale. In B, light green represents control sequence; dark green represents stimulus rotation. Statistical significance between control and rotation is denoted with asterisks (***P<0.001; Wilcoxon signed-rank test).

a few ommatidial rows of the compound eye need to be stimulated to elicit a following response. At an intensity of 0.5 lx, cockroaches no longer responded to 1 deg tall patterns, and at 0.05 lx, responses could only be elicited by stimuli taller than 10 deg. Under the dimmest light intensity of 0.005 lx, even the tallest pattern, 90 deg, did not evoke a response (in contrast to a full-size pattern; see Honkanen et al., 2014). The average velocity and total distance moved during a trial decreased with declining vertical extent of stimuli, whereas response latency was independent of light intensity and vertical extent of the stimulus.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.204768.supplemental

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