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Conditional Perception Under Stimulus Ambiguity: Polarization- and Azimuth-Sensitive Neurons in the Locust Brain Are Inhibited by Low Degrees of Polarization

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Pfeiffer K, Negrello M, Homberg U. Conditional perception under stimulus ambiguity: Polarization- and azimuth-sensitive neurons in the locust brain are inhibited by low degrees of polarization. *J Neurophysiol* 105: 28–35, 2011. First published October 20, 2010; doi:10.1152/jn.00480.2010. Sensory perception often relies on the integration and matching of multisensory inputs. In the brain of desert locusts, identified neurons that signal the sun's direction relative to the animal's head integrate information about the polarization pattern of the sky with information on the color and intensity contrast of the sky. The cloudless blue sky exhibits a gradient from unpolarized sunlight to strongly polarized light at 90° from the sun. Therefore the percentage of polarized light in the sky is highest at dusk and dawn and lowest when the sun is in the zenith. We investigated the effect of different degrees of polarization on neurons of the anterior optic tubercle of the desert locust through intracellular recordings. Whereas dorsal presentation of strongly polarized light largely excited the neurons, weakly polarized light, i.e., a blend of polarized light of many orientations, led to inhibition. The data suggest that the polarization input to these neurons is inhibited within a radius of 50° around the sun, thereby avoiding conflicting input from the polarization and direct sunlight channels. These properties can be regarded as sensory filters to avoid ambiguous signaling during sky compass orientation.

INTRODUCTION

For many animal species, the horizontal position of the sun (solar azimuth) is the most prominent reference cue in spatial orientation and navigation. It can either be assessed directly or through indirect cues arising from scattering of sunlight in the atmosphere. An intensity gradient, a gradient of UV/green color contrast, and a pattern of polarization along the sky (Fig. 1*A*) are all directly linked to the position of the sun and can be used to infer the solar azimuth (Coemans et al. 1994; Coulson 1988).

Orientation and navigation require neuronal representation of an animal's position relative to its environment. Grid cells, place cells, and head direction cells serve this task in the rat brain (Hafting et al. 2005; O'Keefe and Dostrovsky 1971; Taube and Bassett 2003). The relatively small brains of insects offer the possibility to scrutinize the neuronal basis of sky-compass orientation at the level of single identified neurons. In the brain of desert locusts (*Schistocerca gregaria*), several identified types of visual interneuron, branching in the anterior optic tubercle (AOTu) have been proposed to signal the solar azimuth relative to the animal's head (Pfeiffer and Homberg 2007). In most sensory systems, different aspects of the same stimulus are processed in spatially segregated pathways to avoid ambiguous signaling. In the insect visual system, color and polarization information are perceived by different regions of the compound eye and are usually processed in different brain regions (Homberg and Paech 2002; Labhart and Meyer 1999; Wehner and Bernard 1993). In contrast, identified neurons of the locust AOTu are sensitive to dorsal polarized light and to the color and azimuth of unpolarized light (Pfeiffer and Homberg 2007; Kinoshita et al. 2007). The integration of these cues is likely to happen in line tangential neurons in the medulla. These neurons have branches in the dorsal rim area of the medulla, which is the projection site of polarization-sensitive photoreceptors, and in the serpentine layer of the medulla (Homberg et al. 2003).

At dawn and dusk, when the sun is near the horizon, the angle between the orientation of polarized light (*E*-vector) and the solar azimuth is 90° throughout sky (Fig. 1*B*). Therefore the polarization pattern of the sky, the color of scattered light from the sky, and the direction of direct sunlight provide the animal with redundant information leading to an unambiguous, robust signal of solar azimuth. This way of multisensory processing requires less neurons and is therefore more energy efficient than parallel processing. As the sun rises in the course of the day, the angle between *E*-vector and solar azimuth increasingly depends on the direction of view (Fig. 1*B*). This could lead to conflicting information provided by the polarization and unpolarized light channels. Neurons in the AOTu partly resolve this problem by a daytime-dependent change in their *E*-vector tuning that matches the change in the sky polarization pattern associated with changing solar elevations. However, this mechanism is only accurate for a single direction of view. For the rest of the visual field, there is a mismatch of information conveyed by the dorsal polarization vision channels and the lateral color/intensity channels that is correlated with solar elevation (Pfeiffer and Homberg 2007).

The degree of polarization (d), i.e., the percentage of light that is polarized, depends on the angular distance from the sun. In the natural sky, d ranges from unpolarized direct sunlight ($d = 0$) to strongly polarized light ($d \leq 0.75$) at 90° from the sun (Fig. 1*A*) (Brines and Gould 1982). Consequently, the scattered light of the sky in general and specifically the light in the zenith, i.e., directly above the animal, become less polarized the higher the elevation of the sun. To learn how AOTu neurons cope with high solar elevations, we tested their re-

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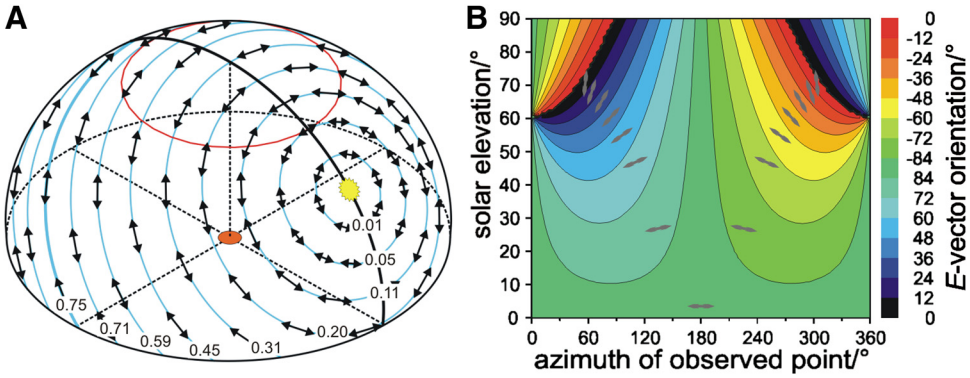

FIG. 1. The sky polarization pattern. *A*: pattern of polarization according to single scattering Rayleigh model (Strutt 1871) as seen from the center of the sphere (red spot) at a solar elevation of 40°. *E* vectors are arranged tangentially to concentric circles around the sun. Blue iso lines indicate degree of polarization. Values are adjusted to a maximum of 0.75 according to the natural sky polarization pattern (Brines and Gould 1982). Light in the vicinity of the sun is weakly polarized. The highest degree of polarization occurs along the solar equator at 90° from the sun. Red line indicates elevation of 60°, corresponding to the elevation of the viewing direction of LoTu1 and TuTu1 neurons. *B*: *E*-vector orientation with respect to solar azimuth as a function of azimuth of the observed point and solar elevation, calculated for 60° elevation of viewing direction according to the single scattering Rayleigh model. At low solar elevations, when the sun is near the horizon and along the solar meridian, *E*-vector orientation is invariant of the azimuth of the point under observation (dark green). At higher solar elevations, especially when the sun is higher than the direction of view, *E*-vector orientation is strongly dependent on viewing direction. Double arrows indicate mean *E*-vector orientation in respective area.

sponses to polarized light at different degrees of polarization. We found that totally polarized light ($d = 1$) had excitatory effects for at least half of all *E*-vector orientations. Unpolarized light, which is a blend of polarized light rays of all possible *E*-vector orientations, in contrast, inhibited the neurons. The data show that AOTu neurons code polarized and unpolarized light as two different entities that are likely combined for sky compass orientation. Based on the observations we discuss a possible mechanism that might explain the seemingly paradoxical results through the dynamics of transmitter release.

METHODS

Animals

Experiments were performed on adult gregarious desert locusts (*Schistocerca gregaria*) of both sexes. Animals were obtained from crowded laboratory colonies at the University of Marburg and were kept at 28°C with a photoperiod of 12 h light and 12 h dark.

Preparation and electrophysiology

Animals were cooled at 4°C for ≥20 min. Wings, legs, abdomen, and the entire gut were removed to minimize movements. Locusts were waxed to a metal holder using beeswax/rosin. The head capsule was opened frontally, and air sacs and fat tissue were removed to expose the brain. The neural sheath above the target area was removed with forceps. During the preparation and later during the experiment, the brain was submerged in locust saline (Clements and May 1974).

Sharp microelectrodes with resistances between 80 and 150 MΩ in the tissue were drawn from omega-shaped borosilicate glass (Hilgenberg, Malsfeld, Germany) using a Sutter P-97 puller (Sutter Instrument, Novato, CA). The tips of the electrodes were filled with 10 mM Alexa488 (Invitrogen, Darmstadt, Germany) in 200 mM KCl, the shanks were filled with 1 M KCl. Signals were amplified and filtered (low-pass 2 kHz) with a custom built filter/amplifier. Spike trains were observed visually with a digital storage oscilloscope (HM 205-2, Hameg, Frankfurt/Main, Germany) and acoustically with a custom built audiomonitor. The amplified and filtered signal was digitized at 25 kHz with a CED 1401 plus (Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer using an automated script written in the scripting language of the data acquisition and evaluation software *spike2 v6* (Cambridge Electronic Design). Some of the neurons were iontophoretically injected by applying constant hyperpolarizing current of ≤5 nA for ~3 min. Dye-injected brains were dissected out of the head capsule and examined with a Zeiss Axioskop fluorescence microscope (Zeiss, Oberkochen, Germany). Owing to the superficial location of the recorded neurons in the anterior optic tubercle, Alexa488-injected neurons could be identified without further histochemical processing of the brains.

Stimulation

Unpolarized light from a blue LED (Luxeon LED emitter, LXHL-BB01, 1 W, peak wavelength 470 nm, spectral half-width 25 nm, Philips Lumileds Lighting, San Jose, CA) was linearly polarized by a high quality dichroic polarizer sheet (HNP'B, Polaroid, Cambridge, MA). It was then elliptically polarized by a transparency (Copier/Laser Transparencies, P/N 003R96019, Xerox, Norwalk, CT) that served as an optical retarder and was rotated with the polarizer during stimulation (Fig. 2). By changing the angle of the principal axis of the transparency relative to the *E*-vector orientation of the linear polarizer, the ellipticity of the light could be varied between 0.06 (0 would be circularly polarized light) and 0.991 (1 would be linearly polarized light). Changes in the ellipticity of the light, however, also changed the orientation of the polarized light emerging from the stimulus device. Thus the *E*-vector orientation of each stimulus differed with the degree of polarization, and the angles in Figs. 3 and 4 refer to the orientation of the polarizer rather than to the *E*-vector orientation that was seen by the animal. At the highest degree of polarization, the initial *E*-vector orientation was parallel to the longitudinal axis of the animal, and this orientation was defined as 0° (Fig. 2). To monitor the state of polarization, we used a custom built polarimeter that was positioned close to the animal's head. It consisted of a fixed HNP'B polarization filter as analyzer and an integrated photodiode/transimpedance amplifier (OPT101, Texas Instruments, Dallas, TX) with linear output voltage/light intensity correlation. Whereas light that oscillated parallel to the polarization axis of the analyzer was transmitted and led to high light intensity at the photodiode, light oscillating in the perpendicular plane was blocked by the analyzer, leading to low light intensities at the photodiode (Fig. 2). This resulted in sinusoidal output of the polarimeter as the stimulus device was rotated in the course of the experiment. Ellipticity of the polarized light was calculated from the output voltage of the polarimeter as $d = (V_{max} - V_{min})/(V_{max} + V_{min})$. To a photoreceptor (and to our polarimeter), elliptically polarized light and partially linearly polarized light as

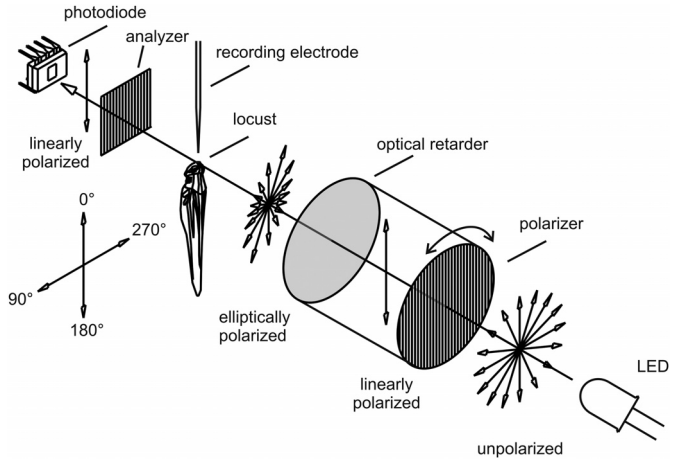

FIG. 2. Experimental setup. Unpolarized light emitted by a light-emitting diode (LED) was linearly polarized by a polarizer and then elliptically polarized by a transparency that served as an optical retarder. The ellipticity, i.e., the degree of polarization, could be varied between 0.06 and 0.991 by changing the orientation between the polarizer and the transparency. The orientation of the retarder also affected the angle of polarization. During stimulation, polarizer and retarder were rotated through 360°. In the starting position (0° = 360°), the polarization axis of the polarizer was parallel to the longitudinal axis of the animal. To assess the degree of polarization, a fixed polarization filter (analyzer) was mounted in front of a photodiode. During rotation of the stimulus device, the light intensity detected by the photodiode varied in a sinusoidal way.

occurring in the blue sky appear identical if they have the same d value (Labhart 1996). At each degree of polarization, we stimulated with a clockwise and a counterclockwise 360° revolution of the polarizer at 30°/s. The angular extent of the stimulus at the locust's eye was 20°. The stimulus intensity was 52.6 μW/cm^2, measured with a radiometer (Photodyne 18 XTA, silicium detector). This is at the lower end of light intensities occurring in the blue sky during the day (65–190 μW/cm^2, Marburg, Germany, September, personal measurements).

Data analysis

Spike trains were analyzed using a semi-automatic *spike2* script. Action potentials were identified using the *spike2* threshold function. The response strength to polarized light was calculated as described by Labhart (1996). In brief, action potentials during rotation of the polarizer were counted in 18 consecutive bins (each 20° wide). The absolute response value A was calculated as

$$A = \sum_{i=1}^{i=18} \left| n_i - \bar{n} \right| \quad (1)$$

where n_i is the number of spikes in bin i and $\bar{n}$ is the number of spikes during the 360° rotation divided by the number of bins. Thus A is the sum of absolute differences between E-vector specific spike counts and mean spike counts and hence expresses the absolute amplitude of spike FM during stimulation.

To calculate the preferred E-vector orientation of a neuron (Φ_{max}), we calculated the angle between the E-vector orientation and the longitudinal axis of the animal at the moment of each action potential that occurred during stimulation. From these angles, the mean was calculated using *Oriana 2.02c* (Kovach Computing Services, Anglesey, UK). To calculate the mean of a set of angles, Oriana treats each angle as a vector of unit length and calculates the mean vector r. The angle of this mean vector is the mean angle, while its length describes the directedness of the response, i.e., how concentrated the observed angles are around the mean angle (Batschelet 1981; p. 31, chapt. 2.1).

Statistics

Linear regressions were calculated using *Origin 6.0* (Microcal, Northhampton , CA). For all other statistics, we used the open source software R (www.r-project.org). Specifically, to test whether responses (mean activity, A, and r) of LoTu1 and TuTu1 neurons differed in their dependency on the degree of polarization, we pooled the respective data and employed a linear model with multiple predictors (cell type and interaction between cell type and degree of polarization). The residuals between data and model were tested for normality using the Shapiro-Wilk test and were plotted against the degree of polarization to examine if they exhibited a trend. If the residuals did not follow a normal distribution or showed a trend along the x axis, the data were logarithmically transformed and the model was applied to the transformed dataset. After transformation the residuals of all datasets were distributed normally and showed no trend.

RESULTS

This study is based on 20 intracellular recordings from neurons with ramifications in the AOTu, a neuropil in the central brain of the locust, receiving input from the second and third optic ganglion (medulla and lobula). Most of the data were obtained from two identified cell types termed LoTu1 ($n = 9$) and TuTu1 ($n = 8$). These neurons have large neurites of $\leq$6 μm in diameter. Neurons of the AOTu-LAL tract that connects the AOTu with the lateral accessory lobe (LAL) have much smaller fiber diameters and were only encountered three times (TuLAL1a: $n = 1$, TuLAL1b, $n = 2$). These neurons showed similar responses to varying d values as the other two types but were not analyzed further due to the small number of observations. All cell types have been characterized before and could be distinguished on the basis of their physiology (Pfeiffer and Homberg 2007; Pfeiffer et al. 2005). The neurons are part of the anterior polarization vision pathway that connects the polarized-light sensitive photoreceptors of the dorsal rim area of the compound eye with the central complex in the central brain of the locust (Homberg et al. 2003). LoTu1 and TuTu1 neurons cross the midline of the brain and interconnect both AOTus. LoTu1 has additional ramifications in the anterior lobe of both lobulae. The degree of polarization (d) used for stimulation ranged from 0.06 to 0.991. In half of the preparations, we were able to stimulate the same neurons with at least four different degrees of polarization.

E-vector dependent modulation of spiking and overall activity are smaller at low degrees of polarization

In both LoTu1 and TuTu1 neurons, we observed two effects of the degree of polarization on spiking behavior. First, at high d values, the amplitude of spiking activity modulation was higher than at low d values (Figs. 3 and 4) and second, the mean spiking activity during stimulation was higher at high d values (Fig. 5). To test if the changes in spiking activity changed significantly at different degrees of polarization, we calculated a linear model on the pooled data of LoTu1 and TuTu1 neurons, using the neuron type and the interaction between the neuron type and the degree of polarization as predictors. Because the residuals of this model were not distributed normally (Shapiro-Wilk test, $W = 0.908$, $P = 0.0003$), we recalculated the model using the logarithmically transformed dataset. The residuals were then normally distributed

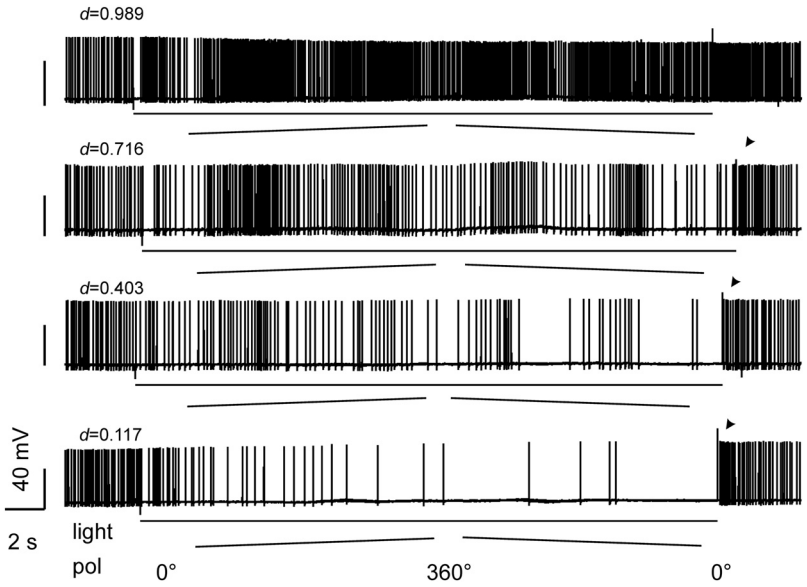

FIG. 3. Responses of a LoTu1 neuron to four different degrees of polarization. The duration of the polarized light stimulus (light) is indicated by a bar. The rotation of the polarizer (pol) is indicated by ramps. Angles (0°, 360°) indicate the orientation of the polarizer rather than *E*-vector orientation, which is shifted due to the optical retarder (see METHODS). The neuron's activity strongly depended on the degree of polarization. While at the highest degree of polarization ($d = 0.989$) spiking activity was above background, the neuron was inhibited irrespective of *E*-vector orientation at the lowest d value. Rebound excitation was observed on release from inhibition (arrowheads). Negative and positive artifacts at lights on and off result from voltage peaks that occurred during switching of the LED.

and showed no trend. The model revealed that the mean activity of the two types of neuron differed significantly ($P = 0.0076$). We therefore calculated simple regressions separately for each type of neuron (Fig. 5). As indicated by the slopes of the fits, LoTu1 neurons showed a stronger increase in activity with increasing d values than TuTu1 neurons.

E-vector dependent modulation of spiking activity and directedness of response are correlated with the degree of polarization

To quantify the response strength of the neurons, we used the absolute response value A, which is a measure of the amplitude of spike frequency modulation (for calculation of A, see METHODS) (see also Labhart 1996). In all recordings, A depended on the degree of polarization. With increasing degree of polarization, the response amplitude of the neurons and, thus A became larger (Fig. 6). To test for linearity of the relationship between the degree of polarization and the response amplitude, we fitted straight lines through the d/A plots if at least 4 degrees of polarization had been tested (not shown). This was the case in 10 of 17 recordings from LoTu1 and TuTu1 neurons. In all cases, we found a high degree of linearity. The mean of the coefficients of determination (R^2) from the linear fits was 0.891 ± 0.114 (mean $\pm$ SD; $n = 10$). Although all recordings exhibited a linear relation between the degree of polarization (d) and the absolute response value A, the slope of the fitting lines differed considerably between individuals, which partly explains the scatter in Fig. 6. Another factor that contributed to scatter was inter-individual differences in overall response strength (i.e., the y intercept of the fitting lines). To investigate possible differences in the responsiveness of LoTu1 and TuTu1 neurons, we pooled the datasets and calculated a linear model using the neuron type and the interaction between the degree of polarization and the neuron type as predictors. Because the residuals did not follow a normal distribution (Shapiro-Wilk test, $W = 0.8735$, $P = 1.6 \times 10^{-8}$) and showed a clear trend along the x axis, the dataset was logarithmically transformed. The linear model of the transformed data revealed that the

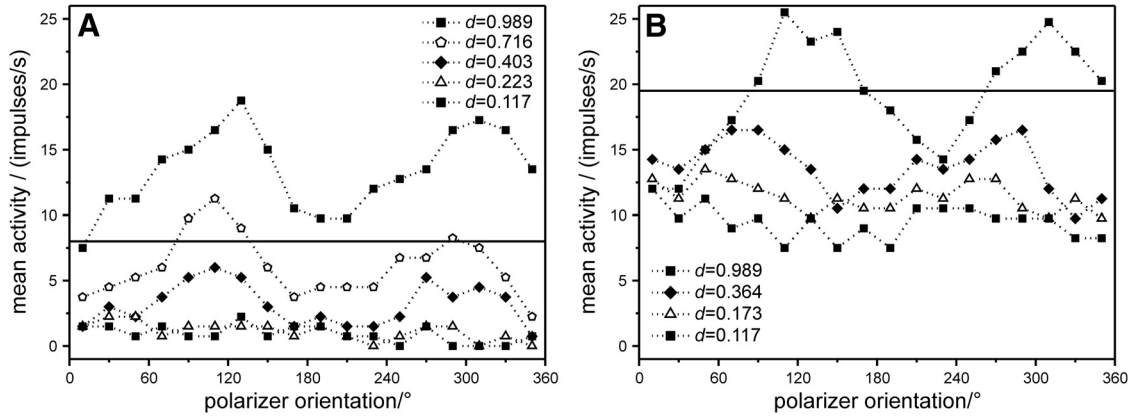

FIG. 4. *E*-vector response curves from the recordings of the LoTu1 neuron shown in Fig. 3 and from a TuTu1 neuron. Data points show mean activity within 20° bins. Different symbols denote different degrees of polarization (d). Solid lines indicate background activity in darkness. Both the response amplitude and the overall activity depended on the degree of polarization. *A*: LoTu1 neuron. At $d = 0.989$, the neuron was excited above background level at all *E*-vector orientations. With decreasing d values, the neuron was increasingly inhibited even at the preferred *E*-vector orientation. *B*: TuTu1 neuron. At $d = 0.989$, the activity of the neuron was modulated around the background level. Similar to LoTu1, the overall activity of TuTu1 decreased at low degrees of polarization, falling below background level but remained higher than that of LoTu1 at all levels of d.

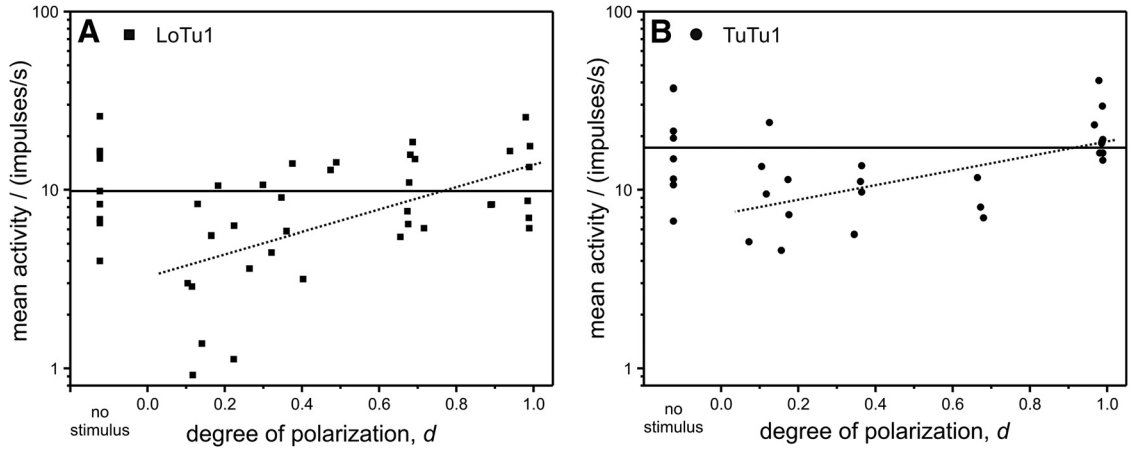

FIG. 5. Mean spiking activity of LoTu1 and TuTu1 neurons at different degrees of polarization. Spikes during stimulation were counted and divided by the stimulus duration. Mean spiking activity without stimulation (no stimulus) is based on activity during 12 s of darkness. Solid lines show median of no-stimulus data. Dashed lines show linear regressions fitted to the logarithmically transformed datasets (see METHODS). *A*: LoTu1 neurons. Mean activity $= 10^{0.51 + 0.63d}$, $R^2 = 0.342$. *B*: TuTu1 neurons. Mean activity $= 10^{0.87 + 0.41d}$, $R^2 = 0.385$.

neuron type had no influence on the response ($P = 0.482$) and that there was no significant interaction between the type of neuron and the degree of polarization ($P = 0.250$). We therefore excluded these parameters from the model and fitted a simple linear regression to the dataset (Fig. 6). The regression shows that the response of the neurons, as measured by the absolute response amplitude A, becomes stronger with increasing degrees of polarization ($A = 10^{0.55d + 1.13}$, $R^2 = 0.468$).

The absolute response value A is a measure of spike FM. It does not provide information about the directedness of the response, i.e., whether the frequency modulation is correlated with the stimulus orientation. This information is provided by the length of the mean vector r (see METHODS for details on r). It measures the concentration of action potentials around the mean E-vector tuning angle Φ_{max}. If spiking frequency fluctuates randomly, r is small. If spiking modulation is regular, with a periodicity of 180° (or multiples thereof) and thus depends on E-vector orientation, r is large. Figure 7 shows the length of the mean vector r, which was calculated from consecutive clockwise and counterclockwise 360° rotations as a function of d.

To test whether the degree of polarization, the neuron type, or the interaction between the two affected r, we calculated a linear model. The residuals between the model and data were normally distributed (Shapiro-Wilk test, $W = 0.965$, $P = 0.089$) and showed no trend along the x axis. Like the absolute response value A, the length of the mean vector r only depended on the degree of polarization ($P = 0.013$) and was not

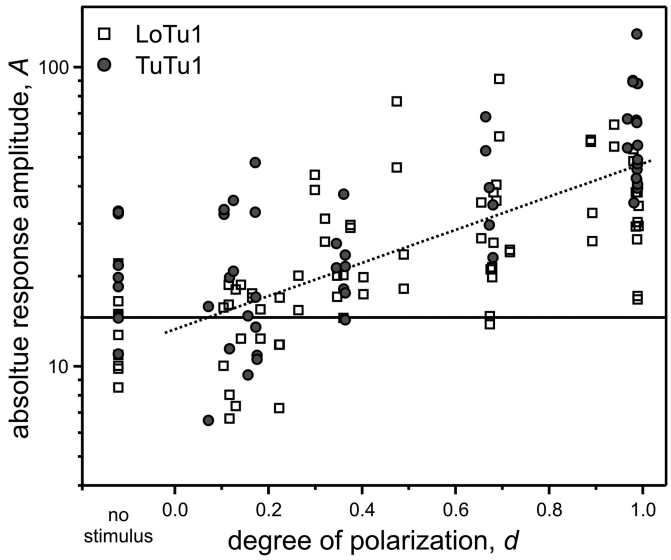

FIG. 6. Absolute response amplitude A (see text for definition) as a function of the degree of polarization. Data points are from single 360° rotations of the polarizer. Activity without stimulation (no stimulus) was determined during 12 s of darkness. Solid line, median background activity at darkness (no stimulus). Dotted line, linear regression through the logarithmically transformed stimulus data. The absolute response amplitude A was dependent on the degree of polarization ($A = 10^{0.55d + 1.13}$, $R^2 = 0.468$).

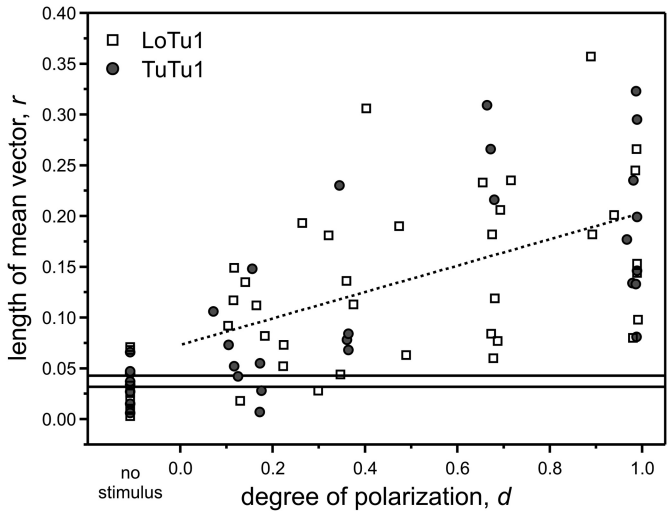

FIG. 7. Directedness of the neuronal responses measured by the length of the mean vector r (see text for details). Directedness increased in both types of neuron with increasing degree of polarization. Data points were obtained by calculating the mean vector of the angles of the polarizer at which spikes occurred during a clockwise and a counterclockwise rotation. Data at no stimulus were calculated identically for 12 s of darkness. Dotted line shows the linear regression through the data obtained during stimulation ($r = 0.13d + 0.07$, $R^2 = 0.264$). Solid lines indicate the mean and the upper 95% confidence limit of the no stimulus data. All datapoints with d values >0.3 lie above the 95% confidence interval, indicating the threshold for E-vector coding for these neurons around 30% polarization.

influenced by the neuron type ($P = 0.446$) or the interaction between neuron type and degree of polarization ($P = 0.377$). We therefore omitted these two parameters and fitted a simple linear regression to the dataset. The fitline indicates that *E*-vector responses contain stronger directional information with increasing degree of polarization ($r = 0.13d + 0.07$, $R^2 = 0.264$).

Threshold for reliable E-vector coding

To determine the threshold for reliable coding of directions we compared the mean vector length r obtained during stimulation with the upper 95% confidence limit (i.e., 1.96 × SE) of r obtained without stimulation (Fig. 6). For d values >0.3, all r values exceeded the upper 95% confidence limit of the dark control. This indicates the ability of the neurons to discriminate *E*-vector orientations down to d values of 0.3.

Theoretical considerations

Given the threshold degree of polarization, it is possible to estimate the minimum angular distance of a patch of sky from the sun that is required to provide detectable polarization information for each individual neuron studied here. According to the single scattering Rayleigh model (Strutt 1871; Coulson 1988), the dependency between the angular distance θ from a light source and the degree of polarization (d) is

$$d = \frac{1 - \cos^2\theta}{1 + \cos^2\theta} \quad (2)$$

The d values calculated with this term describe a theoretical ideal and lie between 0 and 1. While the polarization pattern of the blue sky is well described by the single scattering Rayleigh model in terms of *E*-vector orientation (Suhai and Horváth 2004), the actual degree of polarization is always significantly lower than the theoretical maximum of 1. The highest degree of sky polarization measured in a polarimetric study did not exceed d values of 0.75 (Brines and Gould 1982). To account for this, we multiplied the term with 0.75. The resulting d values are shown in Fig. 1*A*. At a threshold of $d = 0.3$, the angular extent of the "*E*-vector blind" area around the sun can be calculated by resolving the equation

$$0.3 = 0.75 \cdot \frac{1 - \cos^2\theta}{1 + \cos^2\theta} \quad (3)$$

The angle θ then becomes 49.1. This means that a circular area around the sun with a radius of ~50° does not provide useful *E*-vector information and is able to inhibit the neurons.

DISCUSSION

Our results show that potentially unreliable information (low degree of polarization) acts to shut down neurons of the AOTu in the locust, thus preventing ambiguous signaling. We suggest that this inhibition is mediated through the same set of polarization-sensitive photoreceptors that signal relevant stimuli, i.e., the plane of polarization, by causing excitation.

Integration of sky compass signals by neurons of the AOTu

The neurons reported here signal solar azimuth relative to the animal's head and exploit all relevant sources of visual information to do so: orientation of polarized light from dorsal direction and the position and color of lateral, unpolarized light (Pfeiffer and Homberg 2007). The most prominent of these natural stimuli during daytime is direct sunlight. With its high object-to-background contrast, the retinal image of the sun disc is conceivably the most important information for neurons of the AOTu. This view is supported by the finding that maximum spiking rates in LoTu1 neurons always occurred during stimulation with lateral unpolarized light rather than with dorsal polarized light (Pfeiffer et al. 2005).

A neuronal system that integrates multiple sources of information has to be laid out in a way that ensures that all input channels convey congruent information. As judged from the optical axes of dorsal-rim photoreceptors, LoTu1 and TuTu1 neurons have visual fields that are centered at an elevation of ~60° (Pfeiffer and Homberg 2007). Therefore a fixed geometrical relationship between *E*-vector orientation of polarized light and azimuth of lateral unpolarized light only exists at low solar elevations (Fig. 1*B*). At higher solar elevations, *E*-vector orientation is increasingly dependent on viewing direction and is therefore no longer a reliable cue for orientation. Low degrees of polarization that accompany high elevations of the sun inhibit the neurons reported here. We suggest that this inhibition selectively shuts down the polarization channel, while direct assessment of the solar azimuth through lateral nonpolarization sensitive photoreceptors would still be available. Lindauer (1957) showed that honeybees are able to detect the azimuth of the sun even if it is as close as 2.5° to the zenith. At such extreme solar elevations, the first-order interneurons (lamina neurons) in the locust polarization vision pathway might be totally inhibited. However, other neurons in the medulla called line tangential neurons (Homberg et al. 2003) might still receive and transmit excitatory input from lateral nonpolarization sensitive photoreceptors or first order interneurons signaling solar azimuth. These neurons are the most likely site of integration of dorsal polarized and lateral unpolarized light information. At higher solar elevations, it is less likely that direct view of the sun is obstructed by mountains or vegetation, and therefore locusts might be able to afford suppressing the polarization channel in favor of an unambiguous direct azimuth signal from the sun.

Threshold for signaling E-vector orientation

We determined the threshold for reliable signaling of polarized light information to be at $d = 0.3$. However, both the responses to polarized and to unpolarized light are sensitive to light intensity (Kinoshita et al. 2007). Therefore the threshold might be different at different light levels. In nature, the degree of polarization of scattered skylight roughly follows the single scattering Rayleigh model under optimal conditions but is strongly reduced by clouds or haze (Pomozi et al. 2001; Suhai and Horváth 2004). Crickets have a similar array of polarization-sensitive photoreceptors in the dorsal rim of their eye but detect polarized light at d values as low as 0.05 (Labhart 1996). Behavioral experiments determined the threshold at $d = 0.07$ (Henze and Labhart 2007). The physical properties of the locust dorsal rim should also allow for detection of polarized light orientations at low levels of polarization. Therefore it seems reasonable to assume that locusts are able to dynami-

cally adjust the threshold sensitivity. This could be achieved by altering the efficacies of the photoreceptor/lamina synapses.

Hypothetical model underlying the responses to polarized and unpolarized light

A particularly puzzling aspect of our data is the contrast between the strong decrease in spiking activity of LoTu1 when stimulated with unpolarized light versus an increase in spiking activity when stimulated with strongly polarized light (Figs. 3 and 4*A*). Because both stimuli were identical in photon flux, wavelength and site of stimulation, we assume that the same sets of polarization-sensitive dorsal rim photoreceptors mediated these responses. Two types of interneuron, unidentified lamina monopolar neurons and medulla line tangential neurons identified in mass stainings (Homberg et al. 2003) are likely to be intercalated between photoreceptors and LoTu1 and TuTu1 neurons. Insect photoreceptors release histamine, which opens ligand-gated chloride channels (Nässel 1999) and, therefore causes hyperpolarization of lamina cells that is followed by rebound excitation when transmitter action is over. In darkness, thermal noise in the photoreceptors leads to transmitter release. For our model to work single vesicle fusions would have to be temporally and spatially spaced widely enough, perhaps in the order of 10–20 events per second, to allow for rebound excitation to occur. This would lead to background activity in darkness. Unpolarized light causes all polarization-sensitive photoreceptors to be equally excited and to constantly release histamine. The continuity of histamine release under these conditions causes a constant hyperpolarization of lamina neurons. Thus the LoTu1 neuron is silent. Rebound excitation would only occur on termination of the stimulus (see Fig. 3). Polarized light, in contrast, activates only a fraction of all polarization-sensitive photoreceptors. The temporal and spatial pattern of histamine release under these conditions would allow for rebound excitations between single vesicle releases to occur, exciting the LoTu1 neuron. Rebound excitations have, indeed, been described in the lamina of flies on termination of a light stimulus or a hyperpolarizing current injection (Laughlin and Osorio 1989; Uusitalo et al. 1995; Weckström et al. 1992). With decreasing degree of polarization, more and more transmitter is released at any given time increasingly impeding rebound excitation and promoting inhibition of the lamina neurons and thus LoTu1 (Fig. 4).

The sinusoidal *E*-vector dependency of the polarization response may be owed to two asymmetries at the receptor level. First, the cross-sectional areas of the rhabdomeres of the two orthogonal microvillar orientations found in each ommatidium are strongly asymmetric (Homberg and Paech 2002). Second the distribution of preference directions of the 360 ommatidia of the dorsal rim area is nonuniform but dominates around a mean angle of 35° (calculated for microvilli of photoreceptor R7 in the left eye) (unpublished data). In summary, we propose that the stimulus-dependent temporal patterns of histamine release by polarization-sensitive photoreceptors, together with the dynamic membrane properties of lamina neurons might explain both the excitation by polarized light and the inhibition by unpolarized light.

Excitation of the neuron only occurs if the temporal pattern of transmitter release from the photoreceptors contains enough gaps to allow for rebound spikes. Therefore one would expect that at sufficiently high levels of irradiance excitation by polarized light would turn into inhibition. Preliminary results from intracellular recordings in fact revealed complete inhibition of LoTu1 neurons by polarized light under high light intensity (B. el Jundi and U. Homberg, unpublished results). While our hypothesis was outlined here specifically to fit the properties of LoTu1, we believe that it is of general significance for explaining the characteristics of AOTu neurons and with slight changes in parameters might underlie the properties of TuTu1 as well.

Comparison with the cricket POL1 neuron

Previous models of polarization-sensitive neurons in crickets (POL1 neurons) were built using a completely different approach. Inputs to POL1 neurons were assumed to be provided by inhibitory and excitatory synapses from orthogonally oriented polarization-sensitive photoreceptors and emphasize the network connectivity, rather then its dynamics (Labhart 1988; Lambrinos et al. 1997; Sakura et al. 2008). This layout is based on the observation that POL1 neurons show polarization opponency, i.e., they are maximally excited by a certain *E*-vector orientation and maximally inhibited by the orthogonal *E*-vector orientation (Labhart 1988). In addition, POL1 neurons are insensitive to unpolarized light and to the intensity of polarized light. Such models are well suited to explain the observation made in cricket neurons but cannot explain our findings. Both LoTu1 and TuTu1 neurons show polarization opponency at certain degrees of polarization but are sensitive to the intensity of polarized and unpolarized light (Kinoshita et al. 2007). Our proposed mechanism, therefore not only explains the seemingly paradoxical responses to polarized and unpolarized light but also offers an alternative view on how polarization opponency can be produced by the nervous system, using solely inhibitory receptor outputs.

Our study shows how ambiguity arising from multisensory integration can be avoided through the dynamics of transmitter release, effectively acting as a sensory filter. This allows the nervous system to separate accurate, task-specific information from conflicting cues to obtain an unambiguous signal.

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