Receptive field properties and intensity-response functions of
polarization-sensitive neurons of the optic tubercle in gregarious
and solitarious locusts

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POL-neurons of the AOTu in gregarious and solitarious locusts

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ABSTRACT

Many migrating insects rely on the plane of sky polarization as a cue to detect spatial directions. Desert locusts (*Schistocerca gregaria*), like other insects, perceive polarized light through specialized photoreceptors in a dorsal eye region. Desert locusts occur in two phases, a gregarious swarming phase that migrates during the day and a solitarious nocturnal phase. Neurons in a small brain area, the anterior optic tubercle (AOTu), are critically involved in processing polarized light in the locust brain. While polarization-sensitive intertubercle cells, LoTu1 and TuTu1, interconnect the AOTu of both hemispheres, TuLAL1 neurons transmit sky compass signals to a polarization compass in the central brain. To better understand the neural network underlying polarized-light processing in the AOTu and to investigate possible adaptations of the polarization vision system to a diurnal vs. nocturnal life style we analyzed receptive field properties, intensity/response relationships, and daytime dependence of responses of the AOTu neurons in gregarious and solitarious locusts. Surprisingly, no differences in the physiology of these neurons were found between the two locust phases. Instead, clear differences were observed between the different types of AOTu neurons. Whereas TuTu1 and TuLAL1 neurons encoded $E$-vector orientation independent of light intensity and would thus be operational in bright daylight, LoTu1 was inhibited by high light intensity and provided strong polarization signaling only at dim light conditions. The presence of a high and low intensity polarization channel might, therefore, allow solitarious and gregarious locusts to use the same polarization coding system despite their different activity cycles.

Keywords:

Insect brain; visual system; orientation; polarization vision; desert locust
INTRODUCTION

Many navigating animals rely on external visual signals for spatial orientation. Insects use mainly two mechanisms to calculate moving directions during flight or walking. In familiar areas they are able to use visual landmarks as directional cues while in unknown terrains and during long-distance migrations, compass signals from the sky are more relevant (Giurfa and Capaldi 1999; Collett and Collett 2000). Besides the direct position of the sun, the plane of sky polarization serves as a crucial reference for spatial directions during seasonal migration or homing (Wehner and Labhart 2006). Celestial polarized light signals are detected by photoreceptors in a specialized region of the compound eye, the dorsal rim area (DRA) (Labhart and Meyer 1999). While diurnal insects including ants, bees and monarch butterflies refer to polarized light generated by the sun (Frost and Mouritson 2006; Wehner 1984), nocturnal dung beetles rely on the dim polarization pattern produced around the moon (Dacke et al. 2003; 2004).

Desert locusts (*Schistocerca gregaria*) perform long-distance migrations in huge swarms throughout North Africa and the Middle East and have been used as model organisms to analyze neural networks underlying the processing of sky compass signals in the brain. Behavioral experiments on tethered flying locusts suggest that they are able to use polarized light signals from the blue sky to define their course during migration (Mappes and Homberg 2004). Like other locust species, desert locusts occur in two phases, a gregarious and a solitarious phase, that show substantial differences in appearance and behavior (Uvarov 1966, Simpson et al. 1999). While gregarious locusts migrate in swarms during the day, solitarious locusts are nocturnal and preferentially fly as individuals during the night (Waloff 1963; Roffey 1963). Both phases can fly long distances, and their movements can be oriented in consistent directions for periods of time. Movement directions are strongly influenced by wind direction, but there is some evidence for diurnal gregarious locusts that flight directions may be influenced by a sun-compass mechanism (Kennedy 1951; Baker 1978). Recent
experiments showed that solitarious locusts have significantly larger eyes compared to gregarious locusts (Rogers et al. 2010), a common way to increase the sensitivity of the visual system to the nocturnal lifestyle (Warrant 2004; Warrant and Dacke 2011). In the brain, considerable differences in the size and proportion of brain areas underlying the processing of visual signals were found between both phases (Ott and Rogers 2010), and, at the neural level, differences were demonstrated in the size of the receptive field of a looming-sensitive interneuron (Rogers et al. 2010). However, how the neural network in the brain allows a navigation of solitarious locusts at night and how this network is adapted to dramatically lower light conditions is completely unknown.

Polarized light information is processed in distinct areas in the locust brain (Homberg 2004; Homberg et al. 2011). The anterior optic tubercle (AOTu) is a major relay station for processing polarized light information and transfers polarized light signals from the optic lobe to the central complex (Homberg et al. 2003). The tubercle receives signals from the dorsal rim area of the medulla and layer 4 of the distal medulla via transmedulla neurons (el Jundi et al. 2011). Two classes of polarization-sensitive (POL)-neurons, intertubercle cells and neurons of the tubercle-lateral accessory lobe tract, were identified in the AOTu (Pfeiffer et al. 2005). The intertubercle neurons connect the AOTus of both hemispheres and consist of three neurons per brain hemisphere, a single LoTu1 cell and two TuTu1 cells (Pfeiffer et al. 2005). Neurons of the tubercle-lateral accessory lobe tract, termed TuLAL1, transfer polarization signals to input neurons of the central complex (Träger et al. 2008) and consist of about 40-50 neurons per brain hemisphere (Homberg et al. 2003). Spiking activity in most POL-neurons is modulated sinusoidally during zenithal stimulation with a rotating polarizer (Labhart 1988). Except for the LoTu1 cell, all POL-neurons of the AOTu show polarization opponency, i.e. they are maximally activated at a distinct $E$-vector orientation ($\Phi_{\text{max}}$) and are maximally inhibited at an orthogonal orientation ($\Phi_{\text{min}}$) (Pfeiffer et al. 2005). The LoTu1 neuron lacks an inhibitory part at $\Phi_{\text{min}}$, suggesting a particular role in the neural network of the AOTu.
Detailed characterization of POL-neurons of the AOTu has focused on the intertubercle neurons, LoTu1 and TuTu1. Pfeiffer et al. (2005) showed that both types of intertubercle neuron receive polarization information via the ipsilateral dorsal rim area of the compound eye. Corresponding to anatomical evidence, $E$-vector tuning in LoTu1 was clustered around a $\Phi_{\text{max}}$ of $134^\circ$ (soma in left hemisphere) whereas two tuning types around $\Phi_{\text{max}} = 135^\circ$ and $175^\circ$ were found for TuTu1 neurons (somata in left hemisphere). Neurons with somata in the right hemisphere showed mirror symmetric tuning (Pfeiffer et al. 2005). In addition to polarized light, both cell types are sensitive to unpolarized light stimulation. Whereas zenithal unpolarized light stimulation, especially at high light intensities, inhibits the neurons (Kinoshita et al. 2007; Pfeiffer et al. 2011), stimulation with chromatic light spots at an elevation of $45^\circ$ leads to azimuth-dependent excitations and inhibitions, suggesting that the neurons use chromatic cues of the sky to distinguish between the solar and antisolar hemispheres (Kinoshita et al. 2007; Pfeiffer and Homberg 2007). While receptive fields structures of neurons in several stages of the polarization vision pathway have been determined (Heinze et al. 2009; el Jundi and Homberg 2010; Träger et al. 2011; el Jundi et al. 2011), we still know little about the receptive field properties of AOTu neurons. In addition, the tuning and response characteristics of TuLAL1 neurons to polarized light are still poorly understood.

The present study had two aims, first to close these gaps in physiological data on AOTu POL-neurons and second, to reveal adaptations to different lifestyles by comparing data from gregarious and solitarious locusts. We show that receptive fields of the intertubercle neurons are large ($> 100^\circ$) and centered in the contralateral visual field, whereas those of TuLAL1 neurons are very heterogeneous. Intensity-response characteristics show that TuTu1 and TuLAL1 neurons are adapted to signal $E$-vector orientation during the day independent of light intensity, while LoTu1 showed an increased sensitivity and response strength during the night suggesting optimal signaling of $E$-vector contrast under twilight conditions.
Surprisingly, we found no differences between solitarious and gregarious locusts in the physiological parameters of these neurons. Therefore, gregarious and solitarious locusts might possess similar adaptations for high and low light intensity detection of the sky polarization pattern.

**METHODS**

*Locus rearing*

Gregarious desert locusts (*Schistocerca gregaria*) were raised under crowded conditions at a constant temperature of 28°C on a 12h:12h light/dark cycle. Rearing conditions for solitarious animals followed the procedures of Roessingh et al. (1993). Animals were kept individually in small boxes at 26.5°C, 60% humidity and 12 h light/dark photoperiod and had neither visual nor olfactory contact. In general, full transition to the solitarious phase required three generations of animals kept in isolated conditions. A number of morphological markers were used as indicators for successful generation of solitarious animals. Solitarious nymphs had a bright green coloration in contrast to a yellow-dark brown patterning of gregarious nymphs (Simpson et al. 1999). Freshly hatched adults were light green in the solitarious state but had a pinkish coloration when they were gregarious. Sexually mature males were of yellow color with black patches in the gregarious state and were more uniformly brown-grey colored as solitarious animals. Another marker for solitarious adults was a light midline stripe along the dorsal thorax, which was less prominent in gregarious animals.

*Preparation and electrophysiology*

Only sexually mature locusts (1-3 weeks after imaginal molt) were used for the experiments. Recordings were performed from AOTu neurons during the subjective night (zeitgeber time ZT 12-24) and subjective day (zeitgeber time ZT 0-12) of the animals. In both
cases preparation of the animals was performed under identical conditions using a cold light source (Leica, KL 1500, Leica Microsystems, Wetzlar, Germany) for illumination.

Animals were cold anesthetized for at least 30 min. Legs and wings were cropped and stumps were closed with glue or wax. Mouthparts were sealed with wax, and animals were mounted with tape to a metal holder held by a ball joint in a vertical orientation. The holder was carefully adjusted so that the light stimulus (see below) had an exact zenithal position relative to the locust head as shown by Pfeiffer et al. (2005). A ridge of wax was brought up frontally between the mouthparts and the anterior edge of the compound eyes. The head capsule was opened anteriorly, and fat and trachea surrounding the brain were removed. To obtain stable recordings, the esophagus was cut, the abdomen was opened posteriorly, and the gut was removed from the opened abdomen. The abdomen was sealed with a tightly knotted thread. A wire platform was inserted between the esophageal connectives and was fixed at the ridge of wax to increase stabilization. Electrode penetration was facilitated by removing the neural sheath at the right anterior optic tubercle. During the whole preparation procedure, lasting for about 45 minutes and during recording of neurons, the brain was immersed in locust saline (Clements and May 1974).

Neurons of the AOTu were recorded intracellularly using sharp microelectrodes (resistance: 60-190 MΩ). The electrodes were drawn from borosilicate capillaries (inner diameter: 0.75 mm; outer diameter: 1.5 mm; Hilgenberg, Malsfeld, Germany) using a Flaming/Brown horizontal puller (P-97, Sutter, Novata, CA). Tips of the glass micropipettes were filled with 4% Neurobiotin (Vector Laboratories, Burlingame, UK) in 1 M KCl and shanks, with 1 M KCl. A silver wire inserted into the hemolymph solution served as reference electrode. Neural activity of neurons of the AOTu was amplified (10×) with a custom-made amplifier and monitored with an oscilloscope (Hameg HM 205–3, Frankfurt/Main, Germany).

After digitizing at a sampling rate of 5 kHz (CED 1401 plus, Cambridge Electronic Design, UK), signals were stored on a personal computer using Spike2 software (version 6.02;
Cambridge Electronic Design, UK). After recording, a constant depolarizing current was used to inject Neurobiotin iontophoretically into the neurons (2-3 nA, 1-5 min).

Stimulation

Locusts of both phases were stimulated with polarized monochromatic blue light obtained from a xenon lamp (XBO 150W, LOT-Oriel Group; Darmstadt, Germany, photon flux 6.9 \times 10^{13} \text{ photons/cm}^2\text{s}), after passing a monochromatic filter (450 nm), a light guide (Schölly Fiberoptic, Denzingen, Germany) and a motor-driven linear polarizer (HNP’B, Polaroid, Cambridge, MA). The polarization filter was rotated through 360° in clockwise (0°-360°) and counter clockwise (360°-0°) directions with a constant speed of 30°/s. A set of neutral density filters between the light guide and the xenon lamp allowed changing the light intensity in logarithmic steps. The polarization filter and the end of the light guide were attached to a perimeter device that enabled to test the neuronal responses to stimulation from various points along the left-right meridian. In one experiment, ocular dominance was tested by shielding one eye from the light source with a handheld piece of cardboard during stimulation with zenithal polarized light. Recordings were performed under dim ambient light conditions.

During intensity/response measurements background light was reduced further by covering the front of the Faraday cage with a light-tight curtain.

Zenithal stimulation of the animal was defined as 90° elevation, lateral stimulations at an angular distance of 90° from the zenith were defined as 0° ipsilateral or contralateral stimulation. The terms ipsi- and contralateral refer to the position of the soma of the recorded neuron. The angular size of the stimulus at the locust eye was about 4.7°. For stimulation with high intensity polarized white light the 450 nm-monochromatic filter was moved out of the light beam. Maximum light intensity was 1.68 \times 10^{16} \text{ photons/cm}^2\text{s}, measured in the range of 350-880 nm, using a USB 2000+ fiber optic spectrometer (Ocean Optics, Dunedin, Florida).
Brains with Neurobiotin-injected neurons were dissected out of the head and were fixed overnight in 4% paraformaldehyde at 4°C. Then, brains were washed 4 × 15 min with 0.1 M phosphate buffered saline (PBS, pH 7.4) and were incubated with streptavidin conjugated to Cy3 (1:1000; Dianova, Hamburg, Germany) in 0.1 M PBS containing 0.3% Triton X-100 (PBT). After an incubation period of three days, brains were again rinsed two times in 0.1 M PBT and then in 0.1 M PBS and were dehydrated in an ascending ethanol series (25%-100%, 15 min each). After treatment with a solution of ethanol/methyl salicylate (1:1, 15 min), brains were cleared in methyl salicylate for at least 35 min. The wholemount preparations were finally embedded in Permount (Fisher Scientific, Pittsburgh, PA, USA) between two glass coverslips using ten reinforcement rings as spacers (Zweckform, Oberlaindern, Germany). Neurons were examined and identified using a Zeiss Axioskop epifluorescent light microscope.

For detailed 3D reconstructions of selected neurons, brains were rehydrated, sectioned and again treated with Cy3-streptavidin as described by el Jundi et al. (2010). Briefly, Permount around the brains was removed by incubation with xylene (2-4 h). Then, brains were rehydrated in a descending ethanol series, were embedded in albumin-gelatin (4.8% gelatin, 12% ovalbumin in demineralized water), and were fixed in 4% formaldehyde solution overnight at 4°C. Subsequently, brains were cut into 130 - 250 µm sections by using a vibrating-blade microtome (Leica VT1200 S, Leica Microsystems). The brain sections were preincubated with 5% normal goat serum (NGS; Jackson ImmunoResearch) in 0.1 M PBT overnight at 4°C. Then they were incubated for 6 days with a monoclonal mouse antibody against synapsin I (SYNORF1, dilution 1:50; Klagges et al., 1996; kindly provided by Dr. E. Buchner, Würzburg) and with Cy3-streptavidin (1:1000) in 0.1 M PBT containing 1% NGS. Finally, after a treatment of the brain sections with a secondary antibody, goat anti mouse conjugated to Cy5 (Cy5-GAM, 1:300; Jackson ImmunoResearch) and with Cy3-streptavidin
Confocal imaging and 3D reconstruction

Brain sections were scanned with a confocal laser scanning microscope (CLSM, Leica TCS SP5) using a 20× (HCX PL APO 20×/0.70 Imm UV, working distance: 260 µm; Leica) oil objective. The Cy3 signal was scanned by using a DPSS (561 nm) laser and Cy5-fluorescence was detected with a HeNe (633 nm) laser. All neurons were scanned in several image stacks with a resolution of 1024 × 1024 (voxel size: 0.75 × 0.75 × 1.5µm). The obtained image stacks were processed on a personal computer using the software Amira 5.3.3 (Visage Imaging). The procedure of merging of corresponding image stacks and the 3D reconstruction of brain areas based on anti-synapsin staining were described by el Jundi et al. (2009). 3D reconstructions of the neurons were performed by using the SkeletonTree tool (Schmitt et al. 2004).

Data analysis

The sampled spike trains were evaluated by using the Spike2-software with a custom designed script (kindly provided by Dr. K. Pfeiffer, Halifax, Canada). Action potentials were detected through threshold-based event detection. Events were visualized as mean spiking frequency using a gliding average algorithm (moving average of firing rate in window size: 1s). With few exceptions, background activities of the recorded cells were measured by counting of spikes divided by the respective time (12 seconds) in a part of the spike train without stimulation (at dim ambient light conditions). To determine the $E$-vector tuning of the neurons, events during clockwise and counter clockwise rotations of the polarizer were assigned to the corresponding $E$-vectors and lists of these angles were analyzed using Oriana 2.02 software (Kovach Computing Services, Anglesey, UK). The angle of the mean vector $\mathbf{r}$
averaged from equal numbers of clockwise and counter clockwise rotations of the polarizer was defined as the $E$-vector tuning ($\Phi_{\text{max}}$) of that neuron. The length of $r$ describes the concentration of action potentials around $\Phi_{\text{max}}$ and is, thus, a measure for the directedness of the response during rotation of the polarizer (Batschelet 1981; Pfeiffer et al. 2011).

To quantify the modulation strength of the neurons during polarized-light stimulation, we calculated the response strength $R$ (Labhart 1996). The stimulation period of the rotating polarizer was divided into 18 consecutive bins of 20°. In each bin we calculated the difference between the actual spike frequency and the mean spike frequency during the total stimulation period. The sum of the absolute values of all 18 bins was defined as the response strength $R$.

Background variabilities of the cells were calculated in the same way in a section of the spike train without stimulation. Relative $R$ values were obtained by normalizing the response strength at a given position of the visual field to the maximum value ($R_{\text{norm}}$). The widths of the receptive fields were determined by analyzing the elevations of half-maximal response strength in relation to the background variability. For visualization, data points of the receptive fields were connected by lines. $\Phi_{\text{max}}$-distributions within the receptive field were obtained by subtracting the absolute deviation of the $\Phi_{\text{max}}$ value at each elevation from the zenithal $\Phi_{\text{max}}$.

In intensity/response diagrams, the response strengths were normalized against the response strength $R$ at log I= 0 ($R_{\text{norm}}$). Intensity/response curves were fitted by applying a modified Naka-Rushton function to the data (Naka and Rushton 1966)

$$R_{\text{norm}} = R_{\text{norm(max)}} \cdot \frac{I^\nu}{(I^\nu + K^\nu)}$$

where $I$ is the intensity of the stimulus, $K$ is the intensity of the stimulus at 50% $R_{\text{norm(max)}}$, and $\nu$ is an exponent.

Box plots were created with the software Origin 6.0 (Microcal, Northhampton, CA, USA).

The median value was indicated through a horizontal line and boxes denoted the 25% and
75% quartiles of the data. The 5% and 95% range of the data were visualized through whiskers.

Statistics

Circular statistics were performed in Oriana 2.02. Responses of neurons to polarized light were analyzed statistically through the Rayleigh test for axial data (Batschelet 1981). Neurons were defined as polarization sensitive if the distribution of angles was significantly different from randomness (significance level, 0.05). The distribution of the preferred orientations of different recordings from the same neuron type was analyzed through Rao’s spacing test (significance level, 0.05). To test whether the $\Phi_{\text{max}}$-distribution of corresponding neurons differed between solitarious and gregarious animals, the Watson-Williams $F$ test (significance level, 0.05) was used.

Further quantitative comparisons of the data were made by using the SPSS software (Version 11.5). The Shapiro-Wilk test (significance level, 0.05) was used to test for normality of data and the Levene test (significance level, 0.05) to test for homogeneity of variance. For data that were not distributed normally or if the variance was inhomogeneous the Mann-Whitney $U$ test (significance level, 0.05) was applied. In the case of a normal distribution of the data and homogeneity of variance, the two samples were analyzed through a student’s $t$ test (significance level, 0.05). If data were compared from the same recorded neuron, quantitative analysis was performed through a paired student $t$ test (significance level, 0.05).

For statistical evaluation of multiple groups a one-way ANOVA combined with Tukey-honestly significant difference (HSD) post hoc test was applied (significance level, 0.05). If the Shapiro-Wilk test or the Levene test were significant, ANOVA with Games-Howell post hoc test was used (significance level, 0.05). Linear regressions were calculated using Origin 6.0. The correlation coefficient ($R_{\text{corr}}$) was measured and the significance of regression was tested through a $t$ test against a slope of 0 (significance level, 0.05).
RESULTS

This study presents electrophysiological data from 113 intracellular recordings from polarization-sensitive (POL) neurons of the anterior optic tubercle (AOTu) in the locust brain. Four types of neuron were analyzed. The tubercle-tubercle neuron 1 (TuTu1) innervates the lower units of the AOTus and transfers polarization information from the ipsilateral to the contralateral AOTu (Fig. 1A). The second type of intertubercle neuron, the lobula-tubercle neuron 1 (LoTu1) has additional ramifications in the ipsi- and contralateral anterior lobulae (Fig. 2A; Vitzthum et al. 2002). The other two types of neuron, termed tubercle-lateral accessory lobe neurons (TuLAL) connect the lower unit of the AOTu via the tubercle-accessory lobe tract to the lateral accessory lobe. TuLAL1a neurons connect the AOTu with a subunit of the lateral accessory lobe, the lateral triangle (Fig. 3A). TuLAL1b neurons ramify in the anterior lobula, the lower unit of the AOTu and in the median olive of the lateral accessory lobe (Fig. 4A; Pfeiffer et al. 2005).

Receptive field structure and general tuning of AOTu neurons in gregarious and solitarious locusts

TuTu1 intertubercle neurons were analyzed in 20 recordings from gregarious animals and 13 recordings from solitarious locusts (Fig. 1). TuTu1 neurons responded with polarization opponency to a dorsally rotating polarizer with excitation at $\Phi_{\text{max}}$ and inhibition at $\Phi_{\text{min}}$ relative to the background activity (Fig. 1, B and C). TuTu1 neurons from gregarious locusts had a background activity of 25.5 ± 11.4 (SD) imp/s and a background variability of 38.9 ± 15.0 (SD). They showed an average absolute response strength R of 176.2 ± 98.9 (SD) to polarized light stimulation. Although visual inspection suggested clustering of $\Phi_{\text{max}}$ values of the gregarious TuTu1 neurons between 10° and 60° and between 100° and 180° (Fig. 1E), the distribution of preferred orientations was statistically not significantly different from a
uniform distribution (Rao’s spacing \( U \) test, \( 0.9 > p > 0.5 \)). Receptive field properties of TuTu1 neurons of gregarious animals were analyzed in 19 recordings. In 16 recordings the bilateral expansion of the receptive fields was analyzed during the subjective day (ZT 0-12), while in three gregarious locusts receptive field properties were analyzed at ZT 12-24 (subjective night). No obvious differences between the receptive fields of TuTu1 cells recorded at night or during the day were noted. The averaged receptive field of all 19 TuTu1 neurons had a width of about 110° and was centered eccentrically at an elevation of 60° in the contralateral hemisphere (Fig. 1D).

TuTu1 neurons of solitarious animals had a mean background activity of 29.1 ± 10.9 (SD) imp/s and a mean background variability of 44.9 ± 23.5. Both values did not differ significantly between solitarious and gregarious animals (student’s \( t \) test, \( p = 0.38, p = 0.33 \), respectively). TuTu1 neurons from solitarious locusts had an averaged response strength \( R \) of 144.20 ± 61 (SD), which was not significantly different from that of TuTu1 cells from gregarious animals (Mann-Whitney \( U \) test, \( p = 0.55 \)). As in gregarious TuTu1 cells, \( \Phi_{\text{max}} \) orientations of TuTu1 neurons were distributed randomly in solitarious locusts (Fig. 1F).

Receptive field properties were analyzed in 11 neurons at night and 2 neurons during the day, but as in gregarious animals no obvious differences were observed between the two groups. The averaged receptive field of all TuTu1 cells of solitarious animals had a width of about 120°. Similar to the receptive field in gregarious locusts, it was centered eccentrically between 60° and 30° in the contralateral hemisphere (Fig. 1D). No significant differences were observed between solitarious and gregarious locusts at any tested position in the visual field.

The LoTu1 neuron was analyzed in 69 experiments (Fig. 2). In contrast to TuTu1 neurons, LoTu1 was activated at \( \Phi_{\text{max}} \) (Fig. 2,B and C) but lacked an inhibition at \( \Phi_{\text{min}} \) relative to background activity (Fig. 2.C). In 43 recordings, LoTu1 properties were tested in gregarious animals. The neurons had a background activity of 13.3 ± 10.1 (SD) imp/s, a mean background variability of 21.3 ± 7.3 (SD), and a response strength \( R \) of 72.4 ± 27.8 (SD) in
the center of the receptive field. The $\Phi_{\text{max}}$ orientations of the recorded neurons in gregarious animals showed a non-random distribution (Rao’s spacing $U$ test, $p < 0.01$) and ranged – with three exceptions – from about $76^\circ$ to $176^\circ$, with a mean $\Phi_{\text{max}}$ orientation at $128.4^\circ \pm 31.6$ (SD) (Fig. 2E). The receptive field structure of LoTu1 was analyzed in 33 gregarious animals. 26 recordings were obtained during the subjective day and in seven animals recordings were performed during the night. No significant differences were found between receptive fields of LoTu1 cells in gregarious animals recorded during the day and at night at any of the tested elevations (ANOVA analysis with Games-Howell post hoc test). Similar to TuTu1 cells, the gregarious LoTu1 neuron had an eccentric receptive field with the strongest response at an elevation of $60^\circ$ contralaterally (Fig. 2D). The width of the receptive field was about $130^\circ$ along the left-right meridian.

Physiological properties of the LoTu1 cell in solitarious locusts were analyzed in 26 animals. The averaged background activity of $14.9 \pm 8.1$ (SD) imp/s and the mean background variability of $19.4 \pm 6.1$ (SD) was not different from the corresponding firing properties in gregarious animals (Mann-Whitney $U$ test, $p = 0.34$, $p = 0.25$, respectively). The neurons showed an absolute response strength of $85.9 \pm 33.16$ (SD) in the center of the receptive field which did not differ significantly from the response strength in gregarious locusts (student $t$ test, $p = 0.1$). $\Phi_{\text{max}}$ orientations of LoTu1 neurons in solitarious animals were distributed randomly (Fig. 2F), but statistically the mean preferred directions between solitarious and gregarious locusts did not differ (Watson-Williams $F$ test, $p = 0.07$). The receptive field properties of LoTu1 neurons from solitarious animals were studied in 25 recordings (9 neurons during the subjective day and 16 neurons during the subjective night). Again, no significant differences were found in the receptive field properties between gregarious and solitarious locusts that were recorded during the subjective night or the subjective day (ANOVA analysis with Games-Howell post hoc test). In all groups, LoTu1 neurons had a receptive field of highly similar width (about $135^\circ$) and shape (Fig. 2D). As in gregarious
locusts, the strongest response of LoTu1 in solitarious locusts was centered at an elevation between the zenith and 60° contralaterally.

Owing to the small diameter of TuLAL1 neurites, recordings from these neurons were relatively difficult and, thus, in previous work these types of neuron were analyzed only rarely. We studied TuLAL1 neurons in eleven recordings (Figs. 3,4). The size of the receptive field along the left-right meridian of TuLAL1a cells was analyzed in seven recordings (two gregarious and five solitarious locusts). In all recordings, TuLAL1a neurons showed polarization opponency (Figs. 3, B and C). The background activity of the two gregarious TuLAL1a neurons ranged from 36.2 to 45.5 imp/s and the background variability ranged from 16.5 to 34. Both receptive fields were zenith-centered and quite narrow (about 60°) (Fig. 3E). The response strength R of both cells ranged from 105.49 to 115.74. The $\Phi_{\text{max}}$ orientation of both neurons was around 30° whereas the $\Phi_{\text{max}}$ orientations of the five TuLAL1a neurons from solitarious animals were distributed randomly (Fig. 3G). Without stimulation, neurons in solitarious animals had a mean background activity of 38.7 ± 19.7 (SD) imp/s and a mean background variability of 38.5 ± 9.2 (SD). Solitarious TuLAL1a neurons had an averaged response strength R of 101.9 ± 57.79 (SD). No obvious differences were observed in response strength, background activity, and background variability of TuLAL1a neurons between solitarious and gregarious locusts. The receptive fields of the solitarious TuLAL1a neurons varied considerably in bilateral size and position and had centers in the contralateral or ipsilateral hemisphere (Fig. 3F). In one TuLAL1a cell from a gregarious locust, ocular dominance was tested by monocular stimulation of the ipsi- and contralateral eye (Fig. 3D). In contrast to the intertubercle neurons (Pfeiffer et al. 2005), the neuron responded with similar response strength to ipsilateral, contralateral, and bilateral polarized-light stimulation (Fig. 3D).

Recordings from TuLAL1b neurons were obtained from four gregarious animals (Fig. 4). Three of the four neurons showed polarization opponency (Figs. 4, B and C), while one
TuLAL1b neuron showed $E$-vector dependent differences in activity with maximum activation at $\Phi_{\text{max}}$ but no inhibition at $\Phi_{\text{min}}$. The TuLAL1b neurons arborized exclusively in the median olive of the lateral accessory lobe (Fig. 4A) or showed additional ramifications in the lateral triangle of the lateral accessory lobe. The four neurons had a background activity of $18.5 \pm 5.6$ (SD) imp/s and a background variability of $34.24 \pm 16.9$ (SD) in darkness.

TuLAL1b neurons had a mean response strength of about $155 \pm 46.8$ (SD) in the center of the receptive field. As in TuLAL1a neurons, receptive field structures of individual TuLAL1b cells varied substantially in bilateral extension and position of the receptive field along the left-right meridian (Fig. 4D). The cells had receptive field centers in the zenith, the ipsilateral or the contralateral hemisphere. Preferred $E$-vector orientations in the receptive field center were between $130^\circ$ and $180^\circ$ in three neurons and about $5^\circ$ in one neuron (Fig. 4E).

Taken together, no differences in the general physiological properties and in receptive field structures of POL-neurons of the AOTu between gregarious and solitarious locusts were observed. Both intertubercle neurons had large receptive fields centered to the contralateral hemisphere. In contrast, the receptive fields of TuLAL1 neurons varied substantially in bilateral size, shape and position with strongest responses at elevations ranging from $30^\circ$ contralaterally to $30^\circ$ ipsilaterally.

**Intensity-response functions of AOTu neurons in solitarious and gregarious locusts**

While gregarious locusts migrate during the day, solitarious animals preferentially migrate during the night (Walloff 1963; Roffey 1963). We were therefore interested to see whether these different lifestyles are reflected in the polarization vision network in the locust AOTu. Intensity/response (I/R) functions were obtained by changing the intensity of the polarized blue light stimulus in the center of the receptive field over a range of 4 log units (Fig. 5). Neurons recorded during the subjective day (ZT 0-12) were treated separately from neurons recorded during the subjective night (ZT 12-24).
TuTu1 neurons were analyzed during the subjective day in 7 gregarious and 6 solitarious animals (Fig. 5A). The response strengths of the gregarious TuTu1 neurons were saturated between log I = 0 and log I = -2 and showed a sharp drop to background levels between log I = -3 and -4 (Fig. 5A). The I/R function of TuTu1 in solitarious animals was intensity-independent between log I = 0 and -3, but at a logarithmic step of -4 the response broke down to background levels (Fig. 5A). Statistically, no differences were observed at each intensity step of the I/R function of TuTu1 neurons between gregarious and solitarious locusts.

I/R functions of LoTu1 neurons are based on 24 recordings in gregarious locusts and 18 recordings from solitarious animals. 18 LoTu1 neurons of gregarious locusts and five LoTu1 neurons of solitarious animals that were recorded during the day (Fig. 5B, left) showed similar I/R curves that gradually decreased to background levels between log I= 0 and log I = -4. In addition, recordings from seven LoTu1 neurons in gregarious animals and 13 LoTu1 neurons of solitarious animals during the night revealed similar sensitivity curves (Fig. 5B; right). As in TuTu1 neurons, I/R curves from LoTu1 did not differ significantly between solitarious and gregarious locusts.

As mentioned earlier, the response value R is a measure for the modulation strength of firing activity during stimulation but does not give information about the directedness of the response. Therefore we tested whether the length of the mean vector r, which serves as a measure for the directedness of the response to polarized light (Pfeiffer et al. 2011), differed between both locust phases (Fig. 5, C and D). No significant differences in the directedness of TuTu1 (Fig. 5C) and LoTu1 neurons (Fig. 5D) between solitarious and gregarious locusts were found. Taken together the data suggest that there are no differences in the neural network of the AOTu underlying the processing of polarized light between both locust phases.

Differences in neural responses between AOTu neurons
Because no differences between solitarious and gregarious locusts were found in general tuning characteristics and light intensity dependence, we pooled data from both forms to compare the neural response properties of the different AOTu cell types in detail. Interestingly, the I/R curves between both types of intertubercle neurons differed substantially. Whereas the response strength to polarized light of the TuTu1 neurons remained relatively constant between log I = 0 and log I = -3 but declined to background levels within the final log unit (Fig. 6A), the response strength in the LoTu1 neuron decreased gradually from one logarithmic intensity step to the next (Fig. 6B). This is also reflected in the statistical analysis: In TuTu1 neurons the response at log I = 0 differed only from the response at the lowest light intensity step (log I = -4, Fig. 6A), whereas in the LoTu1 neuron the response to the highest analyzed light intensity differed significantly from all other light intensities (Fig. 6B). Furthermore, the responses at several light intensity steps in LoTu1 differed significantly among each other. The I/R curves of the TuLAL1a and TuLAL1b neurons were similar to the I/R function of TuTu1 neurons, but showed a slightly more shallow decline between log I = -2 and log I = -4 to baseline levels (Fig. 6, C and D). Thus, in contrast to TuTu1 and TuLAL1 neurons which may signal E-vector orientation above threshold levels independent of light intensity, the response in LoTu1 is strongly dependent on the intensity of the polarized light throughout all intensities tested.

We compared the response properties of the neurons in greater detail to further characterize the distinct roles of the different cell types in the processing of polarized light. LoTu1 neurons showed a significantly lower background firing rate than TuTu1 and TuLAL1a neurons (Fig. 7A). Furthermore the background spiking rate in darkness was significantly lower in TuLAL1b cells than in TuLAL1a neurons. While LoTu1 neurons showed low background variability and response strength to polarized light stimulation, TuTu1 neurons showed significantly higher background firing variability and a higher response strength R (Fig. 7, B and C). No statistical differences were observed between...
TuLAL1a and TuLAL1b neurons in background variability and response strength R and between the intertubercle cells and the TuLAL1 neurons. Finally, the directedness of the response showed no differences between all AOTu neuron types (Fig. 7D).

In the next step we analyzed possible correlations between the tuning characteristics. Not surprisingly, the response strength of all cell types correlated significantly with the length of the mean vector $r$ (data not shown). However, in all other tuning properties, no significant correlations were found except for a correlation between the background activity and the length of mean vector $r$ in LoTu1. While in TuTu1 neurons no correlation between the background spiking activity and the length of the mean vector $r$ was found (Fig. 7E), a linear correlation was present in LoTu1 (Fig. 7F; $t$ test for slope = 0, $R_{corr}$: -0.5, $p = 0.0001$). Owing to the small number of recorded neurons a conclusion for the TuLAL1 neurons was not possible but statistically no correlation was found in TuLAL1a cells ($t$ test for slope = 0, $R_{corr}$: -0.54, $p = 0.212$) nor in TuLAL1b neurons cells ($t$ test for slope = 0, $R_{corr}$: -0.55, $p = 0.46$).

In the next analysis, the distributions of $\Phi_{max}$ within the receptive field were investigated (Fig. 8). In contrast to TuTu1 cells which did not show systematic changes in the preferred $E$-vector orientation within the receptive field (Fig. 8A), the preferred $E$-vector angle of LoTu1 increased within the receptive field from ipsi- to contralateral positions (Fig. 8B, $t$ test for slope = 0, $R_{corr}$: 0.23, $p = 0.005$). Likewise, in TuLAL1a neurons deviations from zenithal $\Phi_{max}$-values depended on the hemispheric side of the stimulus (Fig. 8C, $t$ test for slope = 0, $R_{corr}$: 0.61, $p = 0.015$), while in TuLAL1b cells no changes of the $\Phi_{max}$ values within the field of view were found (Fig. 8D).

To elucidate further differences between the AOTu neurons, we next analyzed the correlation between tuning characteristics and the time of day of the recording (Fig. 9). TuTu1 neurons did not show any systematic changes in background activity ($t$ test for slope = 0, correlation coefficient $R_{corr}$: 0.16, $p = 0.39$) or variability ($t$ test for slope = 0, $R_{corr}$: 0.31, $p = 0.09$) depending on the time of day (data not shown). We also did not observe a correlation.
between the directedness of the response to polarized light in the center of the receptive field and the time of day (t test for slope = 0, $R_{corr}$: 0.15, $p = 0.39$, data not shown) nor between the response strength $R$ in the center of the receptive field and the zeitgeber time (Fig. 9A; t test against slope = 0, $R_{corr}$: 0.09, $p = 0.63$). The neurons responded to polarized blue light during the subjective day and subjective night with similar response strength (Fig. 9D, Mann-Whitney $U$ test, $p = 0.68$). Also, the response directedness did not differ between TuTu1 neurons recorded at night and during the day (Mann Whitney $U$ test, $p = 0.94$). Similar to the conditions observed in TuTu1 neurons, no correlation between the general tuning characteristics (background activity, background variability), directionality, or response strength (Fig. 9C) and the time of day was observed in TuLAL1 cells. LoTu1 cells showed no correlation between background activity or background variability and the time of day (t test against slope = 0, correlation coefficients $R_{corr}$: 0.05 and -0.03 respectively, $p = 0.64$, $p = 0.83$, data not shown). Although the length of the mean vector $r$ in the center of the receptive field did not change systematically with daytime (t test against slope = 0, $R_{corr}$: 0.24, $p = 0.07$, data not shown), LoTu1 neuron showed an increased directedness in neurons that were recorded at night compared to neurons that were recorded during the day (Fig. 9E; Mann-Whitney $U$ test, $p = 0.042$). Although no significant differences were found between the intensity/response functions of LoTu1 neurons recorded during the day and LoTu1 cells analyzed at night (Fig. 5B, ANOVA analysis combined with Games-Howell post hoc or with Tukey-HSD post hoc test, $p > 0.05$), we found a correlation between the response strength $R$ in the center of the receptive field and the time of day of the recordings (Fig. 9B; t test for slope = 0, $R_{corr}$: 0.41, $p = 0.002$). LoTu1 also showed a significantly higher response strength to polarized blue light at night than during the day (Fig. 9E, two tailed t test, $p = 0.0004$).

Responses to high intensities of polarized light
After analyzing responses under low-light conditions, we were interested in how the interneurons of the AOTu responded to stimulation with polarized light at higher light intensities as occur, e.g., at noon on a cloudless sky. Therefore, we stimulated LoTu1 and TuTu1 neurons with high intensity polarized white light \(1.68 \times 10^{16} \text{photons/cm}^2 \text{s}\) that ranged in a similar order of magnitude as illumination from the sun at noon (Elvegård and Sjöstedt 1940). As shown by Kinoshita et al. (2007) both intertubercle neurons have broad spectral sensitivities and are excited at \(\Phi_{\text{max}}\) during presentation with different wavelengths of polarized light. Whereas LoTu1 was activated during stimulation with polarized blue light, high-intensity polarized white light resulted in strong inhibition of spiking activity without any action potentials in three out of 19 recorded LoTu1 neurons. The other neurons responded with reduced modulation of spiking activity (Fig. 10, A-D). Opposite lights on-responses to the different light intensities were particularly typical for LoTu1 neurons: Whereas the cells showed an increase in firing rate at low light intensities, (Fig. 10A) they were inhibited when turning light on at high intensities of light (Fig. 10C). Accordingly, the response strength of the LoTu1 neuron was significantly higher during stimulation with polarized blue light compared to stimulation with polarized bright white light (Fig. 10E).

TuTu1 cells were analyzed in 7 experiments. In contrast to LoTu1, TuTu1 cells showed no significant difference in the responses between blue light stimulation and bright white light stimulation (Fig. 10G). To further exclude the possibility that the effect of the reduced response strength in LoTu1 neurons is the result of the different spectral composition of the stimuli, we determined I/R relationships for different intensities of white light stimulation (Fig. 10, F and H). Five LoTu1 neurons showed a bell-shaped sensitivity curve with a maximum response strength at \(\log I = -2\) and reduced response strength at lower and higher intensities (Fig. 10F). Statistically, the response strength at \(\log I = 0\) differed significantly form the maximum response strength at \(\log I = -2\) (ANOVA analysis combined with Games-Howell post hoc, \(p=0.04\)). In individual LoTu1 neurons the response strength at \(\log I = 0\) was
reduced by 29% - 70% compared to their maximum response at lower light levels. In addition, we also analyzed the difference between the response strengths at each logarithmic step and the background variability. Because the background variability was calculated analogous to the response strength, however, from a section of the spike train without stimulation, it is a measure for the modulation of a neuron at darkness. The response strengths between log I = -1 and -3 were significantly higher than the background variability, whereas the response strengths at log I = 0 and -4 were not different from background variability. In one investigated TuTu1 neuron the response strength at log I = 0 was similar to the response strength at intensities between log I = -1 to -3 (6% reduction of the response strength at log I = 0), again exhibiting an intensity-independent response (Fig. 10H). Taken together, these data confirm that the reduced response strength in LoTu1 at bright white light is an effect of light intensity.

DISCUSSION

We have analyzed general neural activities, E-vector-tuning, receptive field properties, and intensity/response functions in four classes of polarization-sensitive neurons of the AOTu in the locust brain. To our surprise, we found no difference in these physiological parameters between gregarious and solitarious locusts despite their different life styles and activity patterns. Consistent differences in physiological parameters were, however, found when comparing the different neuronal cell types. LoTu1 was exclusively activated by polarized light at moderate light intensities, while TuTu1 and TuLAL1 neurons showed polarization opponency. Above a certain threshold of light intensity, TuTu1 and TuLAL1 cells were largely invariant to changing light intensities and daytime, while the E-vector response of LoTu1 was clearly dependent on light levels as well as on time of day. In addition, stimulation with polarized light intensities mimicking illuminance levels of midday sunlight led to strong reduction of E-vector dependent responses in LoTu1 but not in TuTu1 neurons. Taken
together, these observations suggest that TuTu1 and TuLAL1 neurons provide a robust compass signal throughout the day, while LoTu1 is tuned to signal polarization information at low light conditions during sunset or sunrise.

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598 General tuning properties

While the response strength between TuTu1 and LoTu1 neurons differed significantly, no differences were found in the averaged directedness of the response between any of the AOTu cell types. This indicates that the tuning width is similar in all AOTu neurons. The higher background variability of the TuTu1 neurons compared to the LoTu1 cell could mean that TuTu1 neurons receive synaptic input from a larger number of neurons than the LoTu1 cell. Furthermore, in LoTu1 and TuLAL1 neurons, a correlation between background activity and directedness was found. This implies that in these cell types the background activity has an effect on the tuning width around $\Phi_{\text{max}}$. For LoTu1 this is not surprising because its activity at $\Phi_{\text{min}}$ is similar to background levels. Experiments in flies showed that neural activities of visual neurons in the brain are modified during flight or walking (Rosner et al. 2010; Maimon et al. 2010; Chiappe et al. 2010). In visual neurons of locusts a change of firing activity dependent on the behavioral state was also observed (Homberg 1994). If the spiking rate of LoTu1 and TuLAL1 neurons were, likewise, modified during walking or flight, the directedness of the response to polarized light should also be modified in a behavior dependent context.

The $E$-vector tuning of LoTu1 in gregarious animals was significantly different from a random distribution. It had a mean value of about 128.4°, which is similar to the mean $\Phi_{\text{max}}$ of 134° reported for LoTu1 by Pfeiffer et al. (2005). In contrast, the distribution of $\Phi_{\text{max}}$ orientations of TuTu1 neurons was not significantly different from randomness unlike the two peaks around 135° and 175° reported by Pfeiffer et al. (2005) corresponding to the presence of two TuTu1 neurons per brain hemisphere. Therefore, we have to assume considerably larger
interindividual differences in $E$-vector tuning of TuTu1 neurons than in the population of animals studied by Pfeiffer et al. (2005). Like in TuTu1 neurons, the preferred $E$-vector orientations of the TuLAL1 neurons varied considerably. This might be explained by the existence of 40-50 TuLAL1 neurons as suggested from fiber counts (Homberg et al. 2003).

Receptive fields

The receptive fields of the intertubercle neurons are directed to the contralateral visual hemisphere with maximum response strength at an elevation of 60° along the left-right meridian. This fits very well to data from Pfeiffer et al. (2005), who showed that LoTu1 and TuTu1 receive polarization input from the ipsilateral eye (Pfeiffer et al. 2005) and to anatomical data showing contralaterally pointing visual axes of DRA ommatidia (Homberg and Paech 2002). The extent of the receptive fields of TuTu1 (110°-120°) and LoTu1 (130°-135°) along the left-right meridian was considerably wider than the receptive fields of DRA photoreceptor neurons (about 30°; Eggers and Gewecke 1993). This suggests that photoreceptor neurons with different spatial tuning are recruited by the intertubercle cells. In contrast to the intertubercle neurons, TuLAL1 neurons differed widely in receptive field properties including receptive field orientation and bilateral expansion. However, this may not be surprising in view of the high number of TuLAL1 cells per brain hemisphere (Homberg et al. 2003).

One of the main functions of the intertubercle neurons is probably to provide contralateral visual input to postsynaptic TuLAL1 neurons. As shown here in one example, TuLAL1a neurons do receive binocular input, and their postsynaptic partners, type TL2 tangential neurons with projections to the lower division of the central body (Träger et al. 2008), are likewise binocular (Heinze et al. 2009). TuLAL1b neurons, in contrast, might receive ipsilateral visual input only, because their likely postsynaptic partners, TL3 tangential neurons
of the lower division of the central body (Träger et al. 2008) are also dominated by ipsilateral visual input (Heinze et al. 2009).

In contrast to TuTu1 and TuLAL1b neurons, $E$-vector tuning in LoTu1 and TuLAL1a neurons changed systematically along the left-right meridian. In both types of neuron an increase in $E$-vector tuning from the ipsilateral to the contralateral hemisphere was observed. As already illustrated for neurons of the protocerebral bridge, this shift of $\Phi_{\text{max}}$ values within the receptive field suggests that neurons of the right AOTu respond more strongly when the sun is behind the animal and neurons of the left AOTu, when the locust faces the sun (Heinze et al. 2009). Together with input from the sky chromatic contrast, proposed by Pfeiffer and Homberg (2007), the $E$-vector tuning shift may further aid in distinguishing between the solar and antisolar hemisphere of the sky.

Responses of intertubercle neurons to different light intensities

Intensity/response (I/R) functions of the intertubercle cells were determined in the center of their receptive fields. The response threshold at which the neurons showed no differences to firing activity at darkness was at a light intensity of about $6.9 \times 10^9$ photons/cm$^2$s. This is similar to the sensitivity of the intertubercle neurons analyzed during zenithal stimulation (Kinoshita et al. 2007). Clear differences were observed in I/R functions between LoTu1 and the remaining AOTu neurons. In TuTu1, TuLAL1a and TuLAL1b neurons the response strength remained relatively constant and showed a sharp drop within the final 2 log intensity steps. This is in accordance to data from cricket POL1 neurons, which were also intensity independent above a certain threshold level of polarized light intensity (Labhart and Petzold 1993; Labhart et al. 2001; Petzold 2001). These neurons should, therefore, not be susceptible to changes in sky clouding conditions and are thus, ideally suited to process polarized light signals for spatial orientation. In contrast, the response of LoTu1 was intensity dependent over at least four log units of light levels.
LoTu1 but not TuTu1 neurons showed higher response strengths and increased
directedness at night than during the day. No difference, however, was observed in the
absolute sensitivity measured by the I/R curves of LoTu1 during the day and at night. As
shown by Pfeiffer et al. (2005), Kinoshita et al. (2007) and in this study, LoTu1 receives at
least two visual inputs from dorsal direction: (1) a low threshold excitatory input that is
polarization sensitive, and (2) a higher threshold inhibitory input that is insensitive to $E$-vector
orientation. The lack of inhibition by unpolarized light at low light levels leads to stronger
responses to polarized light at night than during the day and to a steeper I/R function of
LoTu1 at night without affecting its absolute threshold for polarized light. When increasing
the intensity of polarized light, however, the increasing contribution of the higher threshold
inhibitory input eventually dominates the response of the LoTu1 cell. It leads to a reduction
and finally to complete elimination of the $E$-vector dependent response (Fig. 10F). Based on
these characteristics, LoTu1 is probably adapted to signal polarized light at low light
conditions during sunset or sunrise ($1 \times 10^{10}$ photons/cm$^2$s; Johnsen et al. 2006), while high
light intensities at noon should inhibit LoTu1 and strongly reduce its polarization sensitivity.
A model proposed by Pfeiffer et al. (2011) suggests that the temporal dynamics of neural
responses might largely account for the reversal of responses of LoTu1 with increasing light
intensities. According to that model, stimulus-dependent release of histamine by DRA
photoreceptors combined with dynamic membrane properties of lamina neurons could explain
the opposing effects of high and low intensity polarized light on LoTu1.

In contrast, the responses of TuTu1 to high and low intensity polarized light did not differ
significantly. As shown in Kinoshita et al. (2007), an inhibition of TuTu1 neurons by dorsal
unpolarized light was observed in only one of a total of four recordings, and TuTu1 neurons
did not show significant responses to UV or green unpolarized light presented from dorsal
direction (Kinoshita et al. 2007). This correlates well with our data indicating intensity
independent responses of TuTu1 to zenithal polarized light.
Comparison between gregarious and solitarious locusts

The transformation in locusts from the solitarious to the gregarious form strongly depends on population density and is mediated by the level of serotonin in the thoracic ganglia (Anstey et al. 2009). Field experiments suggest that solitarious locusts prefer to migrate as individuals during the night (Waloff 1963; Roffey 1963). While Roffey (1963) observed flight activity at night of locusts ranging from solitarious to transiens and gregarious, Waloff (1963) reported that flight of solitarious locusts occurred exclusively at night. He observed spontaneous flight activity of solitarious locusts from sunset to about 5 hours after disappearance of the sun. In both field studies, however, migrating solitarious locusts were also observed during the day, but their flight activity was interpreted as forced flights or as exceptions.

Substantial differences in the size and proportion of brain areas involved in visual processing were observed between gregarious and solitarious locusts (Ott and Rogers 2010). Thus, gregarious locusts have larger brains than solitarious locusts, a noticeable larger optic lobe and central complex and, in addition, a smaller optic lobe to midbrain ratio than solitarious locusts (Ott and Rogers 2010).

We did not find significant differences in the physiological responses of POL-neurons of the AOTu between both forms. Receptive field structures as well as absolute sensitivities were analyzed extensively in the intertubercle cells and in both, no correlation between physiological responses and locust phase was found. In addition, no difference in E-vector tunings or in I/R functions were found between the two phases. The data therefore suggest that polarized light signals may be of similar significance as navigational cues in both forms. The presence of a high (TuTu1) and low (LoTu1) intensity polarization channel in both phases might, therefore, allow solitarious and gregarious locusts to use the same polarization coding system despite their different lifestyles. Although solitarious animals have larger eyes than gregarious locusts (Rogers et al. 2010), this difference is not reflected in the width and
alignment of the receptive fields of AOTu intertubercle cells. The size and orientation of
intertubercle receptive fields suggest that the size of the DRA as well as the number of DRA
photoreceptors may not be different between both locust phases and thus, the increased eye
size of solitarious locusts might be restricted to the main retina. It is, however, conceivable
that specific adaptations of AOTu neurons in the two locust phases may be present in the
neurons’ responses to unpolarized chromatic stimuli, which were not tested here.

Possible functional role of AOTu neurons

LoTu1 and TuTu1 neurons receive polarized light signals mainly from the ipsilateral eye
(Pfeiffer et al. 2005), thus an interconnection between the intertubercle cells of both
hemispheres can be excluded. Both intertubercle neurons likely transmit signals to TuLAL1
eurons which provide input to the sky compass in the central complex. Physiologically
TuTu1 neurons are similar to the TuLAL1 cells and could implement an early comparison of
E-vector signals of both eyes in TuLAL1a neurons. Owing to the strong dependence of the
firing activity of LoTu1 on changes of the light intensity and thus, on the solar elevation
LoTu1 might act as a gain modulator that controls the response strength of TuLAL1 cells in a
daytime dependent manner. Due to the higher absolute sensitivity of the LoTu1 neuron to
polarized light than to unpolarized light (Kinoshita et al. 2007), light intensity conditions
might be crucial in LoTu1 for the impact of the detected sky compass cue. As already
discussed by Kinoshita et al. (2007), high light intensities at noon will probably reduce the
contribution of polarized light input and simultaneously increase the significance of
unpolarized light input for detection of the azimuthal direction of the sun. Thus, LoTu1 could
control the balance in TuLAL1 neurons between input from the sky polarization pattern and
direct azimuthal input from unpolarized sunlight.

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**AUTHOR CONTRIBUTIONS**

The experiments were conceived and designed by Basil el Jundi and Uwe Homberg, Basil el Jundi performed the experiments and analyzed the data, and both authors wrote the manuscript.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
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FIGURE CAPTIONS

FIG. 1. Anatomy and physiology of TuTu1 neurons. 

A: Anterior view of a three-dimensional reconstruction of a TuTu1 neuron. lAOTu: lower division of the anterior optic tubercle; uAOTu: upper division of the anterior optic tubercle. Scale bar: 100 µm. 

B. Spike train of a TuTu1 neuron from a gregarious animal during dorsal stimulation with polarized blue light. The polarizer was rotated in clockwise direction; lower trace: spike train; upper trace: mean spiking frequency (moving average of spike rate in 1 s time window). 

C: Circular plot of the mean spiking rate of the neuron shown in A plotted against the E-vector orientation of the polarizer (bin size: 10°; n = 4; error bars = standard deviation, Φmax = 174°, Rayleigh test, p < 10^(-12)). Grey circle indicates the background activity of the neuron in darkness. 

D: Mean response strengths of TuTu1 neurons from gregarious (n = 19, black) and solitarious (n = 13, grey) locusts along the left-right meridian. Normalized response strength (Rnorm) was measured at different elevations in the ipsilateral (i) and contralateral (c) field of view. For better visualization, data points are connected by solid lines. Response amplitudes and mean background variabilities (dotted lines) were normalized to the maximum R value in the visual field of each neuron. In gregarious animals, eight neurons showed the strongest response at an elevation of 30° contralaterally (30c), seven cells at an elevation of 60° contralaterally (60c), one neuron during zenithal stimulation (90), and three neurons in the ipsilateral visual field (60i). Of a total of 13 receptive fields analyzed in solitarious animals, five neurons showed the strongest modulation at an elevation of 30° contralaterally (30c), four neurons at 60° contralaterally (60c), and three neurons during zenithal stimulation (90). Data are means ± SE. 

E: Distribution of Φmax orientations of TuTu1 neurons of gregarious locusts obtained during zenithal stimulation (n = 16; bin width: 10°). 

F: Distribution of zenithal E-vector orientations of TuTu1 neurons from solitarious animals plotted against the number of recorded neurons (n = 13; bin size: 10°). All values are plotted for cells with perikarya in the left brain.
hemisphere. $\Phi_{\text{max}}$ values of neurons with cell bodies in the right hemisphere were mirrored against the longitudinal axis of the animal.

FIG. 2. Analysis of LoTu1 neurons in gregarious and solitarious locusts. A: Anatomy of the LoTu1 neuron shown from frontal direction. ALO: anterior lobe of the lobula complex; lAOTu: lower division of the anterior optic tubercle; uAOTu: upper division of the anterior optic tubercle. Scale bar: 200 µm. B: Unfiltered spike train (lower trace) and mean firing frequency (upper trace) of a LoTu1 neuron during stimulation with polarized blue light (clockwise rotation) obtained from a gregarious animal (moving average, bin size: 1s). C: Circular diagram of the mean spike frequency of the neuron shown in A plotted against the orientation of the polarizer (bin size: $10^\circ$; $n = 6$; error bars = SD, $\Phi_{\text{max}} = 94^\circ$, Rayleigh test, $p = 5.74 \times 10^{-6}$). Grey circle shows the background activity of the LoTu1 neuron. D: Averaged receptive field width along the left-right meridian of LoTu1 analyzed in gregarious ($n = 33$, black) and solitarious ($n = 25$, grey) animals. Relative response strength ($R_{\text{norm}}$) is plotted at different elevations of the polarizer along the left-right meridian of the visual field. In each neuron, $R$ and the mean background variability (dotted lines) were normalized to the highest $R$ value in the visual field. In gregarious locusts, one neuron responded maximally to polarized light from the contralateral horizon (0c), two neurons at an elevation of $30^\circ$ contralaterally (30c), and 16 neurons at an elevation of $60^\circ$ contralaterally (60c). Ten neurons showed the strongest sinusoidal modulation during presentation of polarized light from dorsal direction (90), two neurons at $60^\circ$ ipsilaterally (60i), and two further cells at an elevation of $30^\circ$ in the ipsilateral hemisphere (30i). In solitarious animals, eight neurons responded maximally to polarized light at an elevation of $30^\circ$ in the contralateral field of view (30c), six neurons at a position of $60^\circ$ in the contralateral hemisphere (60c), and eight cells during zenithal stimulation with polarized light (90). Three LoTu1 neurons from solitarious animals showed the strongest responses in the ipsilateral hemisphere (two neurons at 60i, one cell at
Data are means ± SE. $E$: $\Phi_{\text{max}}$ distribution of LoTu1 neurons ($n = 29$) from gregarious animals. Only neurons that showed significant responses during zenithal stimulation were considered. The distribution of the preferred orientations to polarized light differed significantly from a uniform distribution (mean $\Phi_{\text{max}}$ angle: $128.4^\circ ± 31.6^\circ$ (SD); Rao’s spacing test, $p < 0.01$, bin size: $10^\circ$). $F$: The distribution of $\Phi_{\text{max}}$ from solitarious animals ($n = 24$) did not differ from randomness (Rao’s spacing test, $p > 0.05$; bin width: $10^\circ$). All values were treated as if originating from neurons with somata in the left brain hemisphere. For neurons with cell bodies in the right hemisphere, values were mirrored against the longitudinal axis of the animal.

FIG. 3. Polarization-sensitive TuLAL1a neurons recorded in gregarious and solitarious locusts. $A$: Morphology of the TuLAL1a neuron, anterior view. lAOTu: lower division of the anterior optic tubercle; LT: lateral triangle; MO: median olive; uAOTu: upper division of the anterior optic tubercle. Scale bar: 100 µm. $B$: Neural activity and mean firing rate of a TuLAL1a neuron during zenithal stimulation with a rotating polarizer (clockwise rotation, blue light, 450 nm); lower trace shows the spike train, whereas the mean spiking activity is visualized in the upper trace with a moving average bin size of 1s. $C$: Circular diagram of mean frequencies of action potentials of the neuron in $A$ plotted against $E$-vector orientation of the polarizer ($n = 4$, error bars = SD, bin size: $10^\circ$; $\Phi_{\text{max}} = 158^\circ$; Rayleigh test, $p < 10^{-12}$). Grey circle indicates background firing activity without stimulation. $D$: Ocular dominance test of a TuLAL1a neuron from a gregarious locust. Grey bar shows the background variability of the neuron. The response strength $R$ for zenithal monocular stimulation of the ipsilateral eye (ipsi) and contralateral eye (contra) with a rotating polarizer was normalized to the response strength (dotted line) for binocular stimulation. Error bars = SD. $E, F$: The normalized response strength ($R_{\text{norm}}$) of two gregarious ($E$) and five solitarious ($F$) animals plotted against the elevation of the stimulus along the left-right meridian. In each neuron, the $R$ value was...
measured at different elevations and was normalized to the strongest response of the neuron in
the visual field. Normalized variabilities of firing activity in darkness are shown as dotted
lines. G: The distribution of $\Phi_{\text{max}}$ orientation analyzed in the center of the receptive fields of
seven TuLAL1a neurons recorded from gregarious (black bars, $n = 2$) and solitarious (grey
bars, $n = 5$) animals plotted against the number of recorded neurons (bin size: $10^\circ$). All values
are plotted as if originating from neurons with somata in the left brain hemisphere.

FIG. 4. Physiological and anatomical analysis of TuLAL1b neurons from gregarious animals.
A: Frontal view of a 3D- reconstructed TuLAL1b neuron. ALo: anterior lobula; lAOTu:
lower division of the anterior optic tubercle; LT: lateral triangle; MO: median olive; uAOTu:
upper division of the anterior optic tubercle. Scale bar: 200 µm. Spike train (upper trace) and
mean spiking frequency (lower trace) of a TuLAL1b neuron during zenithal stimulation with a
polarizer that rotated in counter clockwise direction (moving average, bin width: 1s). B:
Circular plot of the mean firing rate of the TuLAL1b neuron in A plotted against the
orientation of the polarized light-stimulus ($n = 4$, error bars = SD, bin size: $10^\circ$; $\Phi_{\text{max}} = 7^\circ$;
Rayleigh test, $p = 1.22 \times 10^{-5}$). Grey solid circle shows the background activity of the neuron.
C: Receptive field properties along the left-right meridian of four neurons from gregarious
locusts. For each neuron, the response strength $R$ was measured at different elevations of the
ipsilateral (i) and contralateral (c) field of view and was normalized to the maximum $R$ value
in the receptive field ($R_{\text{norm}}$). Mean background variability is denoted as dotted line. D: The
distribution of preferred $E$-vector orientations of the four TuLAL1b neurons in the center of
the receptive fields. Data are plotted as if originating from neurons with perikarya in the left
brain hemisphere of the animal.

FIG. 5. Normalized intensity/response (I/R) functions of intertubercle neurons of gregarious
and solitarious locusts to stimulation with polarized blue light in the center of their receptive
fields. Maximum light intensity (log I = 0) was $6.9 \times 10^{13}$ photons/cm$^2$s. The response
strength R ($A,B$) and the length of the mean vector $r$ ($C,D$) were calculated for each light
intensity. Solid curves in $A$ and $B$ are fitted through a modified Naka-Rushton function, and
dotted lines denote the background variability. In $C,D$ the data points are connected through
solid lines for better visibility; broken lines show the directedness of the cells without
stimulation. Data in all diagrams are means ± SE. $A$: No differences are observed in the I/R
function of TuTu1 neurons from gregarious locusts ($n = 7$, black fit, Naka-Rushton fitting
parameters, $R_{\text{norm(max)}} = 1.02$, $K = -3.5$ log units, $\nu = 0.74$) analyzed during the day (ZT 0-12)
and solitarious animals ($n = 6$, grey curve, $R_{\text{norm(max)}} = 1.01$, $K = -4.34$ log units, $\nu = 1.3$)
recorded during the night (ZT:12h-24h) (log I = -1, -3 and -4 are tested through a student $t$
test; log I = -2 tested through a Mann-Whitney $U$ test, $p > 0.05$). $B$: I/R functions of LoTu1.
Left figure shows the I/R functions from gregarious locusts ($n = 18$, black line, $R_{\text{norm(max)}} =
1.12$, $K = -2.6$ log units, $\nu = 0.29$) and solitarious animals ($n = 5$, grey line, $R_{\text{norm(max)}} = 1.27$, $K
= -0.19$ log units, $\nu = 0.26$) analyzed during the day. The right diagram shows I/R functions of
LoTu1 neurons from gregarious ($n = 7$, black plot, $R_{\text{norm(max)}} = 1.05$, $K = -1.95$ log units, $\nu =
0.29$) and solitarious animals ($n = 13$, grey fit, $R_{\text{norm(max)}} = 0.92$, $K = -3.99$ log units, $\nu = 0.69$)
measured at ZT 12h -24h. No differences were found between gregarious and solitarious
LoTu1 neurons and between the I/R curves at ZT 0-12 and ZT 12-24 (log I = -1 and log I = -4
tested through an ANOVA analysis with Games-Howell post hoc test, log I = -2/-3 analyzed
through an ANOVA combined with Tukey-HSD post hoc test, $p > 0.05$). $C,D$: Directedness of
the response at different intensities of polarized light; same set of neurons as in $A,B$. No
significant differences are present in TuTu1 neurons ($C$, log I = -1/-2 tested through a student
$t$ test; log I = -3/-4 tested by a Mann-Whitney $U$ test) and in LoTu1 neurons ($D$, ANOVA
analysis with Games-Howell post hoc test) between the two locust phases.
FIG. 6. Normalized intensity/response (I/R) functions of AOTu neuron types to stimulation in the center of the receptive fields. Maximum light intensity (log I = 0) was 6.9 × 10^{13} photons/cm² s. Solid curves are fitted through a modified Naka-Rushton function. Dotted lines indicate background variability. Data in A and B are means ± SE. A: I/R function from all analyzed TuTu1 cells (n = 13, data are taken from Fig. 5A, R_{norm(max)} = 1.01, K = -4 log units, υ = 0.9). B: I/R function from all measured LoTu1 neurons (n = 43, data are the same as in Fig. 5B, R_{norm(max)} = 1.01, K = -3.16 log units, υ = 0.36). Whereas in LoTu1 the response amplitude increases steadily through all intensity steps (B), the maximum response strength in TuTu1 (A) is already reached at log I = -2 (ANOVA analysis combined with Games-Howell post hoc test; significance levels, *p < 0.05, **p < 0.01, ***p < 0.001). C: I/R function from two TuLAL1a neurons of solitarious locusts (R_{norm(max)} = 1.02, K = -3.34 log units, υ = 0.67). D: I/R plot from three TuLAL1b neurons of gregarious locusts (R_{norm(max)} = 0.95, K = -2.9 log units, υ = 0.25).

FIG. 7. General tuning characteristics, response strengths, and directedness of the responses of the four AOTu neuron types. Box plots show the median (horizontal lines) and corresponding mean values (squares), and the 25% and 75% quartiles (boxes). The whiskers indicate the 5-95% range of the data. A: Background activities of TuTu1, LoTu1, TuLAL1a, and TuLAL1b neurons. B: Background variabilities of the neurons of the AOTu. C: Response strength of the AOTu neurons to polarized light presented in the center of the receptive fields. D: Directedness (length of mean vector r) of the polarization response in the AOTu neurons. In all cases (A-D), ANOVA analysis combined with Games-Howell post hoc test was applied for statistical analysis (significance levels, *p < 0.05, **p < 0.01, ***p < 0.001). E: Background spiking frequency of TuTu1 neurons plotted against the directedness of the response (length of mean vector r). Gregarious locusts are indicated as black circles, solitarious animals are shown as grey triangles. No correlation between background frequency and directedness of
the neurons was noted ($n = 31; R_{corr} = -0.17$, t test against slope of 0, $p = 0.32$). 

**F:** Correlation of background firing activity and directedness in LoTu1 neurons (black circles: gregarious locusts; grey triangles: solitary locusts; $n = 55; R_{corr} = -0.5$, t test against slope of 0, $p = 0.0001$; linear regression is shown as solid line, 95% confidence band is shown as dotted line).

**G:** Correlation between background activity and directedness in TuLAL1 neurons. TuLAL1a neurons are shown as circles, TuLAL1b cells are indicated as squares. Data from gregarious animals are shown in black and from solitary animals, in grey ($n = 11$).

**FIG. 8.** $\Phi_{\text{max}}$-distribution of AOTu neurons at different elevations along the left-right meridian. Data were obtained by subtracting the absolute deviation of the preferred orientation angle at each tested position from the $\Phi_{\text{max}}$ value in the zenith. Only neurons that showed a significant response to polarized light during zenithal stimulation (Rayleigh test, $p < 0.05$) were included. In these analyses we did not distinguish between solitary and gregarious locusts because we did not find any differences in the receptive field structures and the zenithal $\Phi_{\text{max}}$ values. 

**A:** The $\Phi_{\text{max}}$ distribution of TuTu1 cells ($n = 29$) does not change systematically along the left-right meridian ($R_{corr} = -0.03$, t test against slope of 0, $p = 0.76$).

**B:** LoTu1 neurons ($n = 44$) showed a significant correlation between $E$-vector tuning and corresponding position along the left-right meridian ($R_{corr} = 0.23$, $p = 0.005$). Linear regression is shown as solid line, confidence intervals (95%) are shown as dotted curves. For better visibility, only averaged preferred direction values are shown in A and B (error bars = SE).

**C:** Distribution of $\Phi_{\text{max}}$ values along the left-right meridian of TuLAL1a cells ($n = 5$). A correlation between the position of the polarized light-stimuli along the left-right meridian and $\Phi_{\text{max}}$ orientation of the neurons was observed ($R_{corr} = 0.61$, $p = 0.015$). Linear regression is shown as solid line, 95% confidence bands are shown as dotted lines. 

**D:** No correlation between observed $E$-vector tuning and elevation of the polarized light-stimuli was found in TuLAL1b cells ($R_{corr} = -0.03$, $p = 0.93$).
FIG. 9. Changes of the response strength $R$ and the directedness $r$ in the center of the receptive field of TuTu1 ($A, D$), LoTu1 ($B, E$) and TuLAL cells (C) during the time of day. In $D$ and $E$ the mean (squares) and median (horizontal lines) response strength $R$ and the 25% and 75% quartiles (boxes) are shown. The whiskers indicate the 5-95% range of the data.

Light intensity of the polarized blue light was $6.9 \times 10^{13}$ photons/cm$^2$ s. $A$: The response strength $R$ of TuTu1 neurons plotted against the time of the recording. Data from gregarious locusts are indicated as black circles, data from solitarious animals are shown as grey triangles. No correlation between response strength and recording time was observed ($n = 32$; $R_{corr} = 0.09$, $t$ test against slope of 0, $p = 0.63$).

$B$: Analysis of response strength $R$ of LoTu1 during the time of day. Data from gregarious animals are indicated as black circles, data from solitarious animals are shown as grey triangles. In LoTu1 the response strength $R$ correlated significantly with the time of day of the recording ($n = 58$; $R_{corr} = 0.41$, $t$ test against slope of 0, $p = 0.002$). The linear regression is shown as solid line, 95% confidence bands are denoted as dotted lines.

$C$: Response strength $R$ of TuLAL1 neurons plotted against the time of day of the recording ($n = 11$; $R_{corr} = -0.36$, $t$ test against slope of 0, $p = 0.27$). $D$: Comparison of the response strength (left panel) $R$ between TuTu1 neurons analyzed during the day (ZT 0-12) and TuTu1 neurons that were recorded at night (ZT 12-24). The data are the same as the data shown in $A$. TuTu1 cells recorded during the day ($n = 18$) do not differ significantly from TuTu1 neurons recorded during the night ($n = 14$; Mann-Whitney $U$ test, $p = 0.68$). Right plot: Analysis of the directedness of the same set of TuTu1 neurons as in $A$. The length of the mean vector $r$ does not differ statistically between TuTu1 cells recorded during the day and TuTu1 neurons recorded at night (Mann-Whitney $U$ test, $p = 0.94$). $E$: Left diagram: The response strength of LoTu1 is significantly higher at night ($n = 23$) than during the day ($n = 35$; student $t$ test, $p = 0.0004$). LoTu1 showed a higher directedness at night than during the day (right
plot; Mann-Whitney $U$ test, $p = 0.042$). The data are from the same set of LoTu1 neurons as in $B$.

FIG. 10. Response strength of intertubercle neurons to zenithal stimulation with polarized blue light ($6.9 \times 10^{13}$ photons/cm$^2$ s) and high intensity polarized white light ($1.68 \times 10^{16}$ photons/cm$^2$ s). $A$: Spike train (upper trace) and mean firing activity (lower trace) of a LoTu1 neuron during stimulation with polarized blue light (moving average, bin size: 1s). The duration of the polarized light stimulus is denoted by a solid line. The start and the direction of the rotation of the polarization filter are indicated by the ramps. $B$: Circular plot of the mean spiking rate of the neuron to polarized light shown in $A$ plotted against the $E$-vector orientation of the polarizer (bin size: $10^\circ$; $n = 2$; error bars = SD, $\Phi_{\text{max}} = 138^\circ$, Rayleigh test, $p = 1.08 \times 10^{-5}$). Grey circle indicates background activity of the neuron in darkness. $C$: The same neuron as in $A$ stimulated with high intensity polarized white light. $D$: Circular diagram from the recording in $C$ illustrates the mean firing rate as a function of $E$-vector orientation (bin size: $10^\circ$; $n = 2$; error bars = SD, $\Phi_{\text{max}} = 121^\circ$, Rayleigh test, $p = 0.008$). $E$, $G$: Comparison of the absolute response strength $R$ between LoTu1 ($E$) and TuTu1 neurons ($G$) to stimulation with zenithal polarized blue light and polarized bright white light. The median response strengths $R$ are shown as horizontal lines, the mean values as square, and the 25% and 75% quartiles boxes are indicated by boxes. The whiskers denote the 5-95% range of the data. $E$: LoTu1 neurons responded stronger to polarized blue light than to bright polarized white light ($n = 19$; paired student $t$ test, $p = 0.0007$). $F$: Normalized I/R function of the LoTu1 neuron ($n = 5$) to stimulation with zenithal white light. Maximum light intensity: $1.68 \times 10^{16}$ photons/cm$^2$ s. Data points are connected by solid lines for better visibility. Dotted line indicates background variability. Data are means ± SE. For statistical analysis ANOVA combined with Games-Howell post hoc test was used (significance levels, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). $G$: Response strength $R$ of TuTu1 neurons during zenithal stimulation
with polarized blue light and bright white light (n = 7). No differences are observed in the response strength (paired student t test, p = 0.42). H: Normalized I/R function of one TuTu1 neuron stimulated with white polarized light from dorsal direction (light intensity at log I = 1.68 × 10^{16} photons/cm^2 s). Data points are connected by solid lines for visualization. Dotted line indicates the background variability of the neuron.