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Behavioral responses to visual overstimulation in the cockroach *Periplaneta americana* L.

Marianna Zhukovskaya[1] · Ekaterina Novikova[1] · Paulus Saari[2] · Roman V. Frolov[2]

Abstract In the visual systems of insects, different types of photoreceptors contribute to specialized visual channels that mediate distinct functions and behaviors. Large compound eyes of *Periplaneta americana* contain photoreceptors of two spectral classes, broadband green-sensitive photoreceptors and narrow-band UV-sensitive photoreceptors. Here, we investigated how visual stimulation by UV and green light affects locomotor, resting, and grooming behaviors in *P. americana* under conditions when light avoidance is not possible. We show that green but not UV light stimulates locomotor activity, inducing paradoxical positive masking. Duration of resting and grooming decreased with increasing light intensity, consistent with development of behavioral stress in response to visual overstimulation. A reaction of full immobility is described under UV light and at higher intensities of green light, with relative periods of immobility and grooming strongly negatively correlated. Low-intensity UV was more effective than low-intensity green light in suppressing grooming and inducing immobility. Our results suggest that locomotor activity in *P. americana* is mainly regulated by green-sensitive photoreceptors, and that dim UV light can trigger behavioral immobility, whereas both wavelengths induce stress-like reactions at high intensities. Considering the intrinsic UV sensitivity of green-sensitive photoreceptors, the contrasting behavioral responses indicate antagonistic interactions between UV and green visual channels.

Keywords *Periplaneta americana* · Vision · Locomotor behavior · Grooming · Immobility

Introduction

The American cockroach, *Periplaneta americana*, is a nocturnal insect with large compound eyes. Under normal conditions, cockroaches are active during the night, engaging in exploration, feeding, and mating (Lipton and Sutherland 1970a, b; Seelinger 1984), while avoiding illuminated areas (Kelly and Mote 1990). One of the most salient behavioral features of *P. americana* is its escape reaction in response to sudden illumination: when a dark-adapted cockroach is exposed to light, it immediately bolts toward the nearest dark shelter. Such reactions to light depend on its intensity and wavelength (Kelly and Mote 1990) and are principally based on light input through the compound eyes (Okada and Toh 1998).

The visual system of *P. americana* consists of several sub-systems or channels morphologically based on the compound eyes and ocelli. The apposition-type compound eye contains: a powerful and highly sensitive broadband motion detection "scotopic" channel predominantly mediated by green-sensitive photoreceptors (Honkanen et al. 2014); a color vision channel as evidenced by the presence of ultraviolet/green color opponent neurons in the optic lobe (Kelly and Mote 1990); and a polarization-sensitive channel (Butler and Horridge 1973; Kelly and Mote 1990). The simple eyes, or ocelli, that appear to be involved in a myriad functions that vary from species to species, ranging from motion detection to circadian rhythm and flight

✉ Roman V. Frolov
rvfrolov@oulu.fi

[1] Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, 44 Thorez Pr., Saint Petersburg 194223, Russia

[2] Faculty of Science, Nano and Molecular Materials Research Unit, University of Oulu, P.O. Box 3000, 90014 Oulu, Finland

control regulation (Parry 1947), are also present in *P. americana*, where they modulate escape reactions in very dim light (Okada and Toh 1998). This role of the ocelli is corroborated by the morphological evidence of enormous spatial summation taking place in the ocellar plexus, where the axons of all ten thousand ocellar photoreceptors converge on just 4 second-order neurons (Mizunami 1995).

Spectroscopic and electrophysiological studies revealed that ommatidia in the compound eye of *P. americana* contain rhabdomeres of two spectral types, with maximal sensitivity to UV (365 nm) or green (ca. 510 nm) light (Mote and Goldsmith 1970). According to intracellular recording and patch-clamp studies, green-sensitive photoreceptors are at least ten times more numerous than UV photoreceptors (Heimonen et al. 2006). There is no apparent segregation of photoreceptor types into specialized regions or zones of the eye, as it is observed in other nocturnal insects (Frolov et al. 2014). Instead, UV- and green-sensitive photoreceptors appear to be interspersed in the ommatidia, although it is possible that densities of UV and green photoreceptors are dissimilar in different parts of the eye. Recent retinal transcriptome analysis with consecutive RNA interference experiments discovered the presence of three opsins in the compound eye retina, one UV and two green ones, with one green opsin more abundant than the other by several orders of magnitude (French et al. 2015).

The escape reaction in response to illumination that exceeds a certain intensity threshold can be a manifestation of stress in an animal, which is suddenly exposed to dramatic sensory overstimulation. However, despite extensive observations of behaviors associated with overstimulation of the visual system in *P. americana* and similar nocturnal insect species (Godden and Goldsmith 1972; Shimoda and Honda 2013; Shi et al. 2017), questions remain about relative contributions of different visual channels to visual overstimulation-induced reactions, and to specific behavioral reactions associated with stress. Grooming, a complex, often stereotypic process of cleaning body surfaces and sensory appendages, which has been extensively studied in both vertebrates and insects [for review see (Hlavac 1975; Smith and Valentine 1985)], is one such behavior that changes in stressed animals (Tinbergen 1951; Zhukovskaya et al. 2013; Kalueff et al. 2016).

In this work, we characterized behavioral responses in *P. americana* to UV and green light over a wide range of light intensities and describe a novel immobility reaction, which develops in progressively brighter light. As green-sensitive photoreceptors are characterized by high sensitivity to UV, our results strongly suggest the presence of antagonistic interactions between visual channels processing responses of UV- and green-sensitive photoreceptors in the higher visual centers of *P. americana* nervous system.

Materials and methods

Animals and the experimental setup

American cockroaches *P. americana* (L.) were reared locally (St. Petersburg, Russia) and maintained in reversed L/D 12:12 h illumination conditions at temperatures from 24 to 28 °C. A fluorescent lamp emitting a substantial amount of UV light was used as a light source during the day phase.

Behavioral experiments were performed in the following way. The setup consisted of three connected compartments (Fig. 1a), two of which, a constant dark "shelter" and a L/D 12:12 h illuminated "habitat" (Fig. 1b) supplied with food and water *ad lib*, were available to animals all the time, while the third one, a detachable testing chamber, was available only during testing. The testing chamber was equipped with a side panel for illumination with dark red light (Fig. 1b); two LEDs, green and UV, and a VSC-756 USB high-resolution monochrome video camera (EVS, Russia) 25 cm above the floor; and a moist air inlet. Narrowband 1W 019595 ARPL-1 W-EPL UV400 (UV, spectral peak at 400 nm) and 1W Green ARPL-Star (green, spectral peak at 530 nm) LEDs were used in experiments (Fig. 1c). LED emission spectra were measured using an Ocean Optics spectrometer (Ocean Optics, Dunedin, FL, USA). Use of forced wet air ventilation was essential to prevent development of dehydration-induced behavioral changes during the prolonged recordings, as well as odor accumulation. The flow of air was ca. 100 ml min^{-1}. Adult cockroaches (up to 2 weeks after the final molt) were placed in the living compartment 2 weeks prior to the experiments.

Experimental procedure

Experiments were performed in the beginning of the night, when cockroaches are most behaviorally active. During each experiment, a single cockroach was allowed to enter the testing chamber and explore it in red light for 10 min (Fig. 1d). After that, a 30-min video recording was performed. This recording session (henceforth "session 1" or S1) was followed by another 10-min period in red light without recording, after which a 30 min light exposure testing session was started (henceforth "session 2" or S2). Throughout the text (*n*) indicates sample size.

In control experiments (*n* = 11), during session 2, the animal was exposed to the same dark red light as during session 1. In light exposure experiments, the chamber was illuminated with one intensity of green or UV light. Three UV and three green light intensities were used in the experiments. When the light intensities were measured with a digital optical power meter Thorlabs PM100D equipped with a photodiode sensor S121C (Thorlabs Inc., Newton, NJ, USA) in the middle of the floor of the testing chamber, the photon fluxes
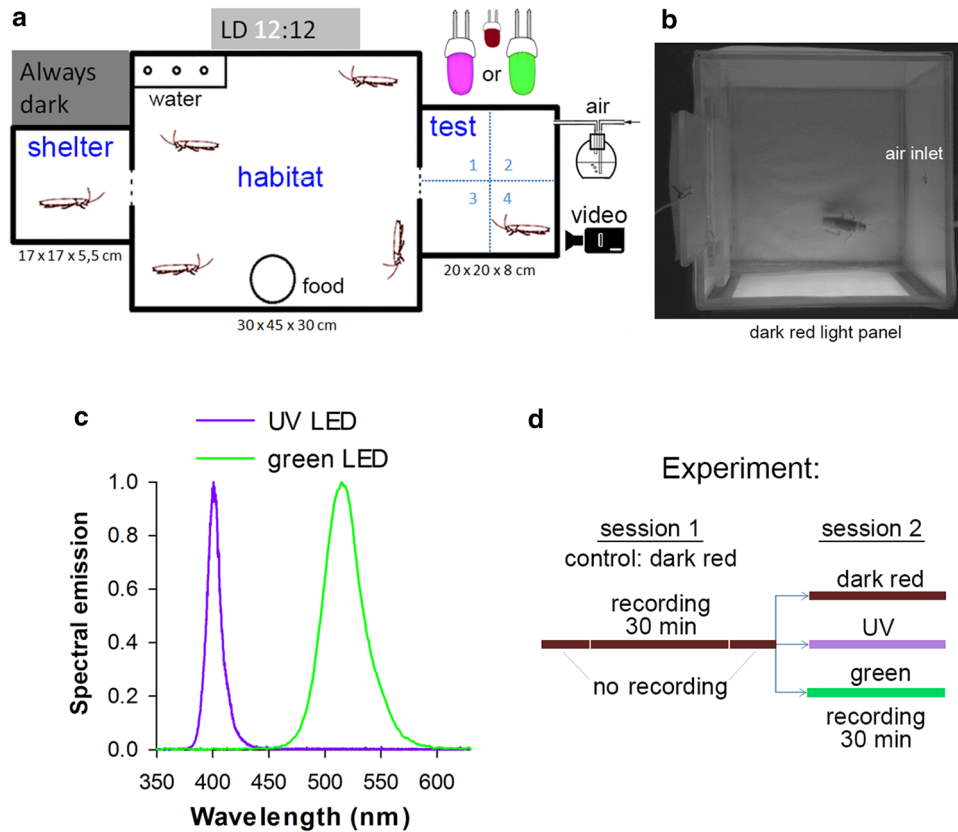
Fig. 1 The experimental setup and methodology. **a** The experimental setup consisted of three chambers: a small, constantly dark shelter, a larger living compartment containing water and food ad lib, and an adjacent testing chamber accessible only during the actual experiment. The test chamber was equipped with green and UV LEDs, and a dark red side panel (see photograph in panel **b**); it also had a moist air inlet and a camera in the middle of the ceiling. **b** A photograph of a cockroach in the testing chamber during an experiment in dark red light. **c** Spectra of UV and green LEDs used in experiments. **d** Schematic description of the experimental methodology. During S1, cockroach behavior was recorded for 30 min in dark red light, preceded and followed by 10-min periods without recording. During S2, in control experiments, only dark red light of the same intensity as during S1 was used; in light exposure experiments, cockroaches were presented with 30 min of steady green or UV light of three different intensities, so that each individual cockroach was exposed to one intensity only

were 2.8×10^{10}, 8.5×10^{12}, and 2.4×10^{14} photons s^{-1} cm^{-2} for the UV LED, and 4.1×10^{12}, 9.7×10^{13}, and 1.1×10^{15} photons s^{-1} cm^{-2} for the green LED. Each cockroach was used in experiments only once. Between the experiments, the test chamber was cleaned thoroughly. The numbers of experiments for the six different conditions were, respectively: 12, 10, 13, 12, 11, and 14.

Intracellular recordings

In vivo intracellular single-electrode recordings and light stimulation were performed as described previously (Saari et al. 2017). In brief, microelectrodes were manufactured from borosilicate glass (Harvard Apparatus, Holliston, MA, USA) with a laser puller (P-2000; Sutter Instrument Novato, CA, USA) and filled with 2 M KCl solution, pH 6.84, to the final resistance of 100–150 MΩ. The reference electrode was placed through an antenna. Signals were recorded with an intracellular amplifier (SEC-05L; npi electronic, Tamm, Germany). For light stimulation, a computer-controlled custom voltage-to-current LED driver was used to drive 14 LEDs (Roithner Laser Technik, Austria), with narrow-band emission peaks ranging from 355 to 625 nm (355, 385, 400, 435, 450, 462, 490, 505, 525, 545, 572, 594, 612, 625 nm), in combination with a series of neutral density filters (Kodak, New York, NY, USA).

Data analysis and statistics

Analysis of video files was performed manually frame by frame using VirtualDub (free software by Avery Lee). During analysis of grooming, a grooming sequence was defined as a sequential cleaning of several body parts without interruption by other behaviors. A grooming sequence included some of the following elements: cleaning of the antennal flagellum with the mouthparts; scraping of the antennal

base with a foreleg; cleaning of the forelegs, mid legs and hind legs with the mouthparts; and cleaning of the cerci and genitalia with the hind legs. Locomotion was measured by counting the number of quadrants the cockroach walked across during the recording sessions (Fig. 1a).

During statistical data analysis, the Shapiro–Wilk normality test was applied to data samples to determine if they could be analyzed using parametric statistical methods. If data in some samples describing a certain behavior type failed the normality test, or passed only marginally for a minority of experimental groups, such data were presented using medians and interquartile ranges (25% quartile:75% quartile), and compared using the Wilcoxon signed-rank test (WSRT) for paired results or the Mann–Whitney U test (MWUT) for independent samples. Such data are presented in the figures using box plots; the box plots show medians, interquartile ranges (error bars), and 5:95% outliers. The data that passed the normality test were presented as mean ± standard deviation, and compared using Student's t test as indicated. Pearson's correlation coefficient (r) was used in the analysis of correlations. In the figures, (*) stands for $0.05 < P < 0.001$, and (**) for $P < 0.001$.

Results

Behavioral experiments

Analysis of video recordings of cockroach behavior in the testing chamber during S1 and S2 allowed us to define and quantify four distinct behaviors: locomotion, grooming, resting behavior, and a novel immobility reaction. Resting was defined as a behavior without visible leg movement, which, however, was always accompanied by some movement of

the antennae, or the mouthparts. The immobility reaction is defined here as an inactive, immobile state resembling resting but without any movements. Immobility could occur after resting or grooming, and while its onset was straightforward to determine during resting, transition from grooming to immobility was more difficult to define. Grooming of the mouthparts was generally impossible to resolve in our video recordings and if immobility occurred after grooming, to account for this uncertainty, the duration of such an immobility episode was reduced by 1 min.

Figure 2 summarizes changes in locomotion. In control experiments, the median number of quadrants visited decreased from 92 (63:112) during S1 to 30 (14:51) during S2 ($P = 0.0036$, $n = 11$, WSRT), which obviously reflects a decrease in the exploratory activity due to adaptation of the cockroach to its new environment (Fig. 2a). Similar decreases in locomotor activity were observed in the animals exposed to UV light at all three intensities, dim ($P = 0.0024$, $n = 12$, WSRT), intermediate ($P = 0.0074$, $n = 10$, WSRT), and bright UV ($P = 0.004$, $n = 13$, WSRT, Fig. 2a). In contrast, cockroaches exposed to green light showed notably smaller, statistically insignificant decreases in the number of quadrants visited during S2 (Fig. 2a). The relative changes in locomotion are shown in Fig. 2b. Since no intensity-dependence of the locomotor activity decrease was observed, data within experimental green and UV groups were pooled together to facilitate statistical analysis. Figure 2c shows that under green light cockroaches moved significantly more than in control ($P = 0.004$, MWUT, $n = 37$ and 11, respectively) or in UV light ($P < 0.001$, MWUT, $n = 37$ and 35, respectively).

Figure 3 specifies how long the cockroaches were engaged in each particular behavior under different conditions. The pie charts were generated by summing up the durations of each behavior for the entire experimental
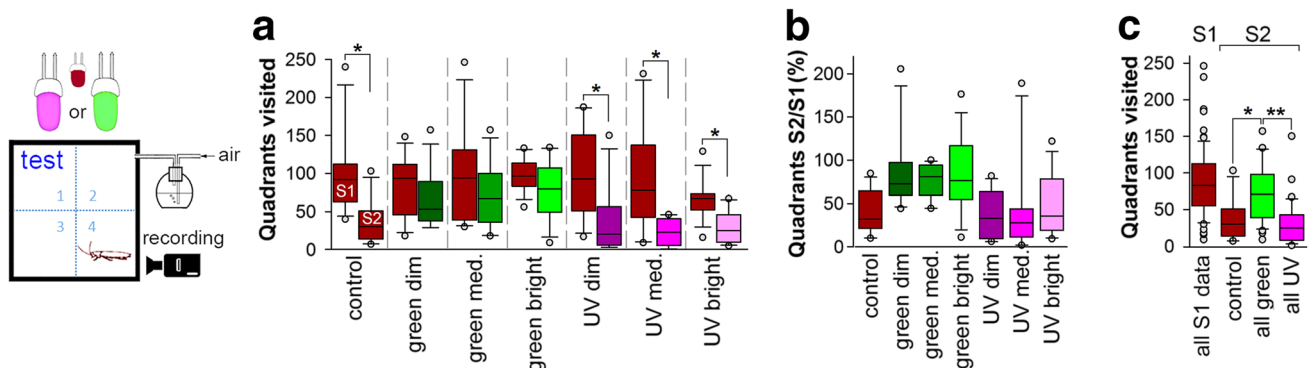

Fig. 2 Changes in locomotor activity under green and UV light. **a** Box plots describe the median numbers of quadrants of the testing chamber floor walked over by a cockroach during S1 (*left column in each pair*) or S2 (*right column*) experiments; light intensities are specified in "Methods", with the lowest intensity denoted here as "*dim*", intermediate one as "*med*", and the highest one as "*bright*"; the number of animals in each experimental group varied from 11 to

14 (see "Results"); *grey dashed lines* separate experimental groups. **b** The relative numbers of quadrants visited during S2; values were obtained by dividing the numbers of quadrants visited during S2 by those in S1 for each animal. **c** Since the differences in locomotor activity between three intensities for each LED were insignificant, all *green* and UV exposure data were pooled together and compared; locomotor activity increased significantly in *green* but not in UV light
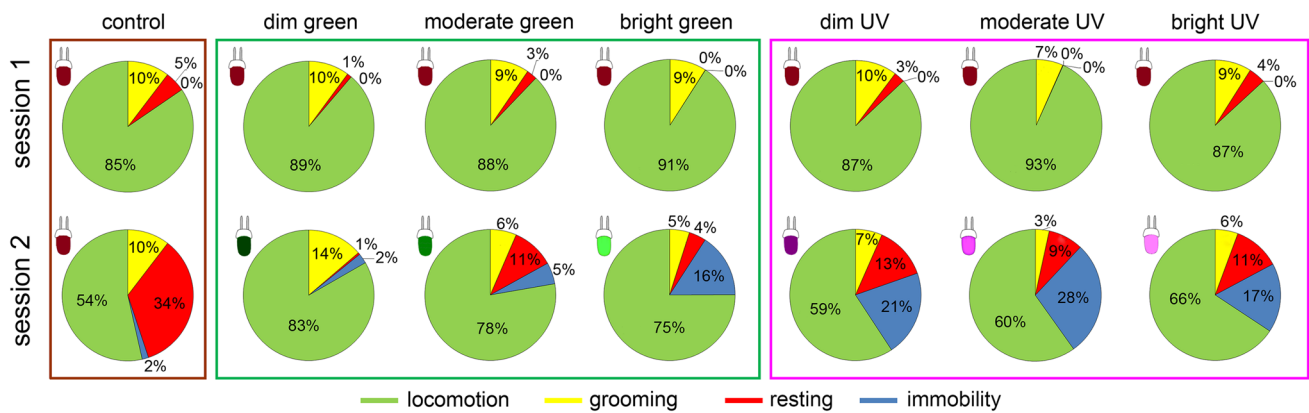
Fig. 3 Contributions of different behaviors change with light intensity. *Pie charts* show the relative periods cockroaches engage in each of four distinct behaviors (locomotion, resting, grooming, and immobility) during S1 and S2

group. Since locomotion was quantified in terms of quadrants visited (Fig. 2), while the periods of other behaviors were measured directly, the period of locomotion was estimated by subtracting the durations of three other behaviors from the total time of experimental observation. During S1 cockroaches were predominantly engaged in locomotion (85–93% of observation time). Remaining time was divided between grooming, and, to a much smaller extent, resting. No incidents of immobility were observed. During S2, locomotion was reduced notably in the control group, the time share of resting behavior increased, and cockroaches spent 2% of time in the completely immobilized state.

In the experiments involving illumination with green light, the behavior durations changed dramatically. In the dimmest green light, resting behavior was virtually abolished due to increased locomotion, while grooming duration increased slightly. At the next, intermediate green light level, 3 out of 11 animals exhibited episodes of immobility lasting from 16 to 882 s, which accounted for 5% of the total observation time (Fig. 3). In the brightest green light, 9 out of 14 cockroaches displayed episodes of immobility, altogether accounting for 16% of the recording period.

Under UV light, the time spent for locomotion was reduced to the same level as in control, but instead of resting and grooming, cockroaches were in the immobility state more than half of the locomotion-free time (Fig. 3). Specifically, 3 out of 11 cockroaches in dim UV light, 8 out of 9 at the intermediate UV intensity, and 9 out of 13 animals in bright UV light displayed immobility reactions of varying duration.

Figure 4 illustrates changes in the grooming behavior. Grooming sequences were characterized by variable durations, depending on the number of appendages cleaned on each occasion. We restricted our analysis to determining: the share of time spent for grooming for each experimental group (Fig. 3); the number and average duration of grooming

sequences (Fig. 4a, c); and the duration of two major and unambiguously definable grooming events, cleaning of the antennal flagella, and mid and hind legs combined (Fig. 4e).

In dim green light, the total grooming duration during S2 remained the same as in control. However, it decreased notably in brighter green and at all three intensities of UV light, due to a decrease both in the number of grooming sequences and in their average duration (Fig. 4a–c). A strong negative correlation was found between the amounts of time cockroaches spent grooming and in immobile state (Fig. 4d). The Pearson's r was -0.81 ($n = 7$, $P = 0.03$). To investigate how illumination altered the duration of specific grooming events, we compared the relative durations of antennal grooming and grooming of mid and hind legs during S2 (Fig. 4e). Whereas the antennal grooming was largely unaffected, exposure to progressively bright light led to reduction of leg cleaning.

Figure 5 illustrates dependencies of changes in the four behaviors on the actual photon fluxes during S2. Our data indicate that UV light is extremely effective at suppressing grooming and inducing immobility (Fig. 5a, b). Differential effects on locomotion and resting time shares were less prominent (Fig. 5c, d).

Intracellular recordings

Here we found contrasting behavioral responses to illumination by green and UV light. However, previous electrophysiological studies of visual pigments in insects established the presence of secondary higher-frequency absorbance bands, including the most physiologically relevant beta-band centering around 400 nm (Stavenga et al. 1993). Therefore, it was necessary to see if green-sensitive photoreceptors in the compound eyes of *P. americana* exhibit any substantial intrinsic sensitivity to UV light.

Figures 6a and b demonstrate typical action spectra of UV- and green-sensitive photoreceptors. As expected,

Fig. 4 Changes in grooming behavior with illumination. **a** Duration of grooming sequences decreased with increasing light intensity; *grey dashed lines* separate experimental groups; WSRT was used for data comparison. **b** The relative duration of average grooming sequences during S2; the values were obtained by dividing average durations of grooming sequences in S2 by those during S1 for each experimental group. **c** The number of grooming sequences; paired *t* test was used for data comparison. **d** Correlation between the total grooming and immobility behavior durations during S2; the numbers were obtained from Fig. 3 plots as indicated by the *inset*; *color coding* is as in panels **a–c**. **e** Relative durations of antennal grooming and grooming of mid and hind legs
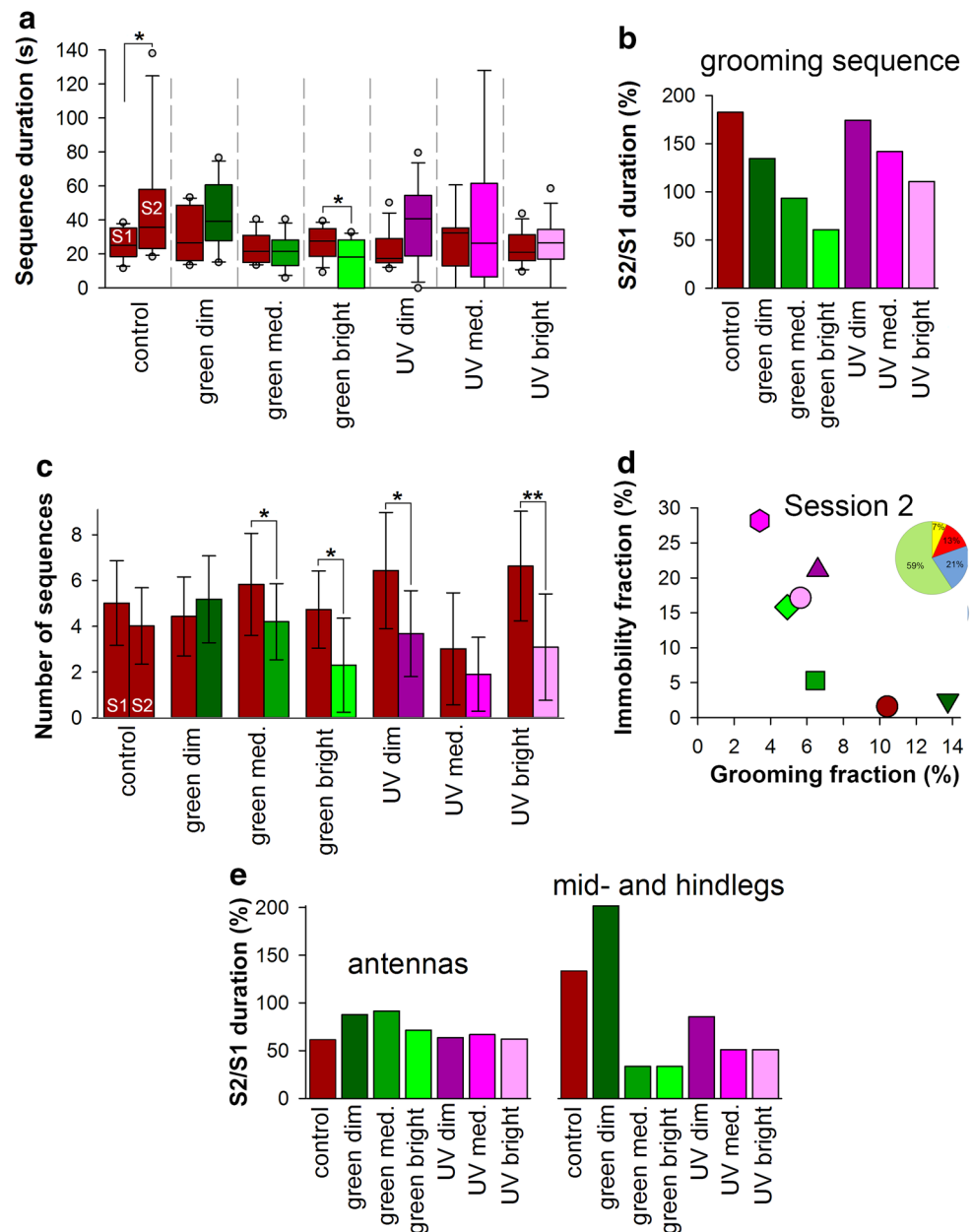
broadband photoreceptors had a secondary peak at 385 nm, indicating a substantial sensitivity to UV (Fig. 6b, c). When the actual spectral sensitivities of the two photoreceptors were calculated using a previously published data sample (Saari et al. 2017), for the green-sensitive photoreceptor the ratio of UV to green peaks was reduced to 0.35. The UV-sensitive photoreceptor was characterized by narrow sensitivity to UV light with a peak at 385 nm.

Discussion

In this work we characterized the effects of narrow-band UV and green light over a 100-fold intensity range on

several specific behaviors of *P. americana* and described a new type of behavior, a sleep-like immobility reaction. Although similar experiments have been performed previously (Kelly and Mote 1990), they were focused on the analysis of light avoidance behavior, whereas here we specifically investigated how illumination alters individual behavior under conditions when *avoidance is impossible*. Our research builds upon previous electrophysiological studies in *P. americana*, which found only two spectral types of photoreceptors in the compound eyes, UV- and green-sensitive ones (Heimonen et al. 2006; Kelly and Mote 1990) and determined that physiological avoidance reactions are principally mediated by the compound eyes (Okada and Toh 1998). It should be noted that we did
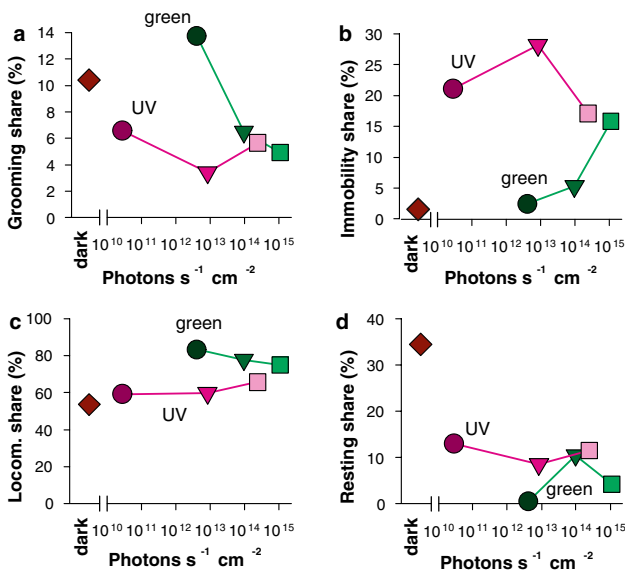
Fig. 5 Dependencies of changes in behavior on light intensity. Fractional durations of the four behaviors during session 2 are plotted against the photon fluxes during light stimulation; "*dark*" refers to dark red light illumination in S2 experiments in control; "locom." stands for "locomotion". The duration values are the same as in Fig. 3

not attempt to evaluate effects of illumination on other behaviors, such as feeding or mating, or any other social interactions among cockroaches.

The main individual behaviors of *P. americana* are locomotion, resting, and grooming. Locomotion is an explorative behavior, whereas resting and grooming are sedentary behaviors. Importantly, studies in both vertebrates and invertebrates showed that the duration of grooming decreases and its patterns change when animals are stressed (Tinbergen 1951; Zhukovskaya et al. 2013; Kalueff et al. 2016), which makes the duration of grooming a useful proxy for stress.

In natural environments small nocturnal animals rarely or never experience exposure to daylight levels of illumination, as they leave shelter in the dark and reenter it before sunrise. Here we described immobility as a behavioral reaction associated with an abnormal visual stimulation. Is there a sufficient experimental justification for distinguishing immobility from other behaviors, such as resting? Our findings suggest that immobility appears to be a distinct reaction. It was different from the normal resting since the cockroach became completely immobile. The relative duration of immobility was short under dim red or dim green light, and increased with green light intensity. However, immobility manifested prominently in dim UV light, when the photon flux was approximately two orders of magnitude lower than in dim green light.

Immobility observed in our experiments seems to be different from a "freezing" reaction, which could be triggered in insects with sudden illumination and could be seen as an

adaptive behavior during the initial overstimulation blindness, because an immobile target is less likely to be spotted by predators than a moving one. In our experiments immobility episodes were not associated with a sudden change in illumination but rather happened spontaneously and were also registered during S2 in control experiments under dark red illumination.

Although immobility appears to be a sleep-like condition, we did not attempt to characterize and compare the natural sleep with immobility in this work. However, the light-induced immobility is clearly different from thanatosis, a well-characterized tonic immobility behavior observed in a wide range of species across the animal kingdom, including crickets and stick insects (Godden and Goldsmith 1972; Nishino 2004): in contrast to thanatosis, the immobility observed here did not develop suddenly in response to the onset of light, but rather gradually; also, it ended and recurred spontaneously.

Immobility appears to be different from a behavior called "learned helplessness". Essentially, the behavior can be defined as tolerating a stressful situation without attempting to escape it, while the escape is possible. It develops in an animal, which was previously subjected to the same distressful factor but in a situation when avoidance was not possible (Eisenstein and Carlson 1997). The phenomenon is widespread and can be induced in vertebrates and invertebrates alike and does not require brain for establishing. However, as we report here, immobility reaction occurred without prior conditioning, its episodes ended and recurred spontaneously, and it occurred on few instances in control S2. In one study on learned helplessness in *P. americana*, no detailed description of cockroach behavior was provided (Brown and Stroup 1988), making it impossible to compare learned helplessness with the reaction of immobility.

Overall, although immobility seems to be different from resting, "freezing", and "learned helplessness" behaviors, more research is needed to properly establish its characteristics.

How do the overall patterns of the four behaviors change with illumination? We showed that locomotor activity of cockroaches decreased dramatically in control from S1 to S2, indicating that by the time of S2 cockroaches had more or less completed exploration of the testing compartment. In green light, however, cockroaches became agitated again and significantly increased their activity, possibly looking for escape from the test chamber. This response to light falls under the definition of the paradoxical positive masking as "an increase in activity after an increase in illumination in a nocturnal animal" by N. Mrosovsky (Mrosovsky 1999).

Whereas no evidence of stress was found under dim green light, the total grooming time, the number of grooming sequences and their average durations decreased in
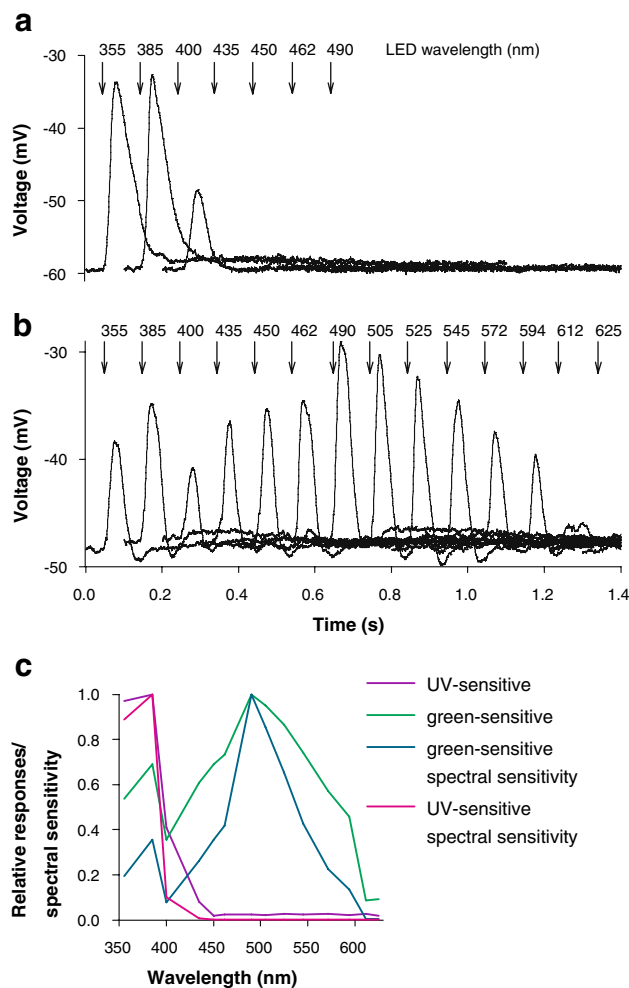
Fig. 6 Responses of UV- and green-sensitive photoreceptors. Action spectra were obtained by applying 20 ms isoquantal flashes of light (*arrows*) from 14 LEDs. **a** Responses of a typical UV-sensitive photoreceptor are shown for first seven LEDs; the superimposed 1 s voltage response traces are shifted relative each other in 100 ms steps for presentation purposes; the numbers stand for LED wavelengths. **b** Responses of a typical green-sensitive photoreceptor. **c** Dependencies of voltage transient amplitudes (relative responses) and spectral sensitivities of the photoreceptors from panels **a** and **b** on the peak LED wavelength; spectral sensitivities were calculated for the on the basis of the previously published control voltage response ranges (Saari et al. 2017)

green light of intermediate and bright intensities (Fig. 4), suggesting growing stress. Close examination of the grooming sequence revealed that grooming duration decreased largely by reduced grooming of mid- and hind legs, which usually takes place at the end of the grooming sequence. Increasing UV intensity caused similar changes in grooming, i.e., decrease in the number of grooming sequences and their durations, with shorter leg grooming, indicating increased stress level (Fig. 4). Still, the question remains why dim green light caused locomotor agitation. One possibility could be that selective stimulation of the green visual

channel represents an abnormal situation since UV is always present under natural conditions (see below).

At the same time, the total duration of immobility episodes increased in an intensity-dependent manner, from 2% in control and dim green to 16% in bright green light. In comparison, locomotor activity did not differ from control under UV light at all three intensities, which was a counterintuitive finding since UV illumination was previously shown to be more effective in evoking an escape reaction (Kelly and Mote 1990). It is of particular importance that exposure to the lowest UV intensity, roughly corresponding to UV fluxes at environmental irradiance approximately one order of magnitude higher than irradiance from the moon (Cronin et al. 2014), produced a decrease in grooming and an increase in immobility periods, which was similar to that induced by bright green light, at four orders of magnitude higher photon flux (Fig. 5a, b). This behavior might be classified as negative masking (Mrosovsky 1999). This disparity gets even more salient when one notices that UV photoreceptors are underrepresented in the compound eyes of *P. americana*. Because environmental levels of UV radiation depend less on weather conditions than visible light (Cronin et al. 2014), it is possible that the UV-sensitive visual channel has evolved as the main circadian switch controlling *P. americana* behavior.

The strikingly different behavioral reactions to UV and green illumination are particularly interesting in light of intrinsic sensitivity of green-sensitive photoreceptors to UV (Fig. 6b, c). It follows that when cockroaches are irradiated by green light, only green-sensitive photoreceptors respond. When, on the other hand, UV light is used, both UV- and green-sensitive photoreceptors activate. Therefore, the immobility behavior is triggered when both UV- and green-sensitive photoreceptor are active, whereas increased locomotion is observed when only green-sensitive photoreceptors are active. Such a situation implies antagonistic interactions between UV and green visual channels, with the physiological outcome depending on the strength of stimulation via each channel. Indeed, the presence of inhibitory interactions in the medulla between interneurons receiving innervations from specific receptor types was demonstrated in the previous study (Kelly and Mote 1990).

In conclusion, we have assessed the influences of the UV and green visual channels on individual behavior in *P. americana* and found substantially different patterns of responses. Our results suggest that excitation of the green but not UV channel stimulates locomotor behavior, while low-intensity UV stimulation triggers sleep-like immobility behavior. Thus, our results suggest that UV and green visual channels indeed mediate different visual functions, that bright illumination causes stress independent of wavelength, and that the two channels are mutually antagonistic.

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