The Lizard Celestial Compass Detects Linearly Polarized Light in the Blue

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Abstract

The present study first examined whether ruin lizards *Podarcis sicula* are able to orientate using plane polarized light produced by a LCD screen. Ruin lizards were trained and tested indoors, inside an hexagonal Morris water maze, positioned under the LCD screen producing white polarized light with a single E-vector, which provided an axial cue. White polarized light did not include wavelengths in the UV. Lizards orientated correctly either when tested with E-vector parallel to the training axis or after $90^\circ$ rotation of the E-vector direction, and thus validating the apparatus. Further experiments examined whether in ruin lizards there is a preferential region of the light spectrum to perceive the E-vector direction of polarized light. For this purpose, lizards reaching learning criteria under white polarized light were subdivided into 4 experimental groups. Each group was respectively tested for orientation under a different spectrum of plane polarized light (named red, green, cyan and blue) with equalized photon flux density. Lizards tested under blue polarized light orientated correctly, whereas lizards tested under red polarized light were completely disoriented. Green polarized light was barely discernible by lizards, and thus insufficient for a correct functioning of their compass. When exposed to cyan polarized light, lizard orientation performances were optimal, indistinguishable from lizards detecting blue polarized light. Overall, the present results demonstrate that perception of linear polarization in the blue is necessary - and sufficient - for a proper functioning of the sky polarization compass of ruin lizards. This may be adaptively important, since detection of polarized light in the blue improves functioning of the polarization compass under cloudy skies, i.e. when the alternative celestial compass based on detection of the sun disk is rendered useless because the sun is obscured by clouds.

Keywords:

Ruin lizard, orientation, Morris water maze, polarization compass, wavelength of light, *Podarcis sicula*
1. Introduction

The first investigation establishing a relationship between compass orientation and linearly polarized light was carried out by von Frisch (von Frisch, 1949) in honey bees (Apis mellifera). As von Frisch was capable to show, when the sun’s position is obscured by vegetation or clouds bees can use the E-vector direction of polarized light in the form of a sky polarization compass. Since then, the capability of using a sky polarization compass in orientation behaviour was demonstrated in a variety of insects, spiders, crabs and also in many taxa of vertebrates including fish, lizards and birds (Horvath and Varjú, 2004, for an exhaustive review). In lizards, the existence of a sky polarization compass was first demonstrated in the fringe-toed lizard Uma notata (Adler and Phillips, 1985), in the sleepy lizard Tiliqua rugosa (Freake, 1999), and most recently confirmed in the ruin lizard Podarcis sicula (Beltrami et al., 2010). In insects, the detection of polarized skylight is mediated by a group of anatomically and physiologically specialized ommatidia in an upward-pointing narrow dorsal rim area (DRA) of the compound eye (Labhart and Meyer, 1999). In the ruin lizard it was shown for the first time that the parietal eye, a component of the reptile pineal complex, plays a central role in the functioning of a sky polarization compass (Beltrami et al., 2010). Species in which orientation behaviour was systematically examined under selected wavelengths of polarized light, such as the honey bee A. mellifera, the desert ant Cataglyphis bicolor, and the scarab beetles Lethrus spp., were shown to use a sky polarization compass only in presence of light in the ultraviolet (UV) range (von Helversen and Edrich, 1974; Duelli and Wehner, 1973; Edrich and von Helversen, 1987; Frantsevich et al., 1977). In contrast, in ruin lizards a sky polarization compass was demonstrated to work in the absence of UV light (Beltrami et al., 2010).

The present experiments were aimed at testing whether there is a preferential region of the light spectrum to perceive the E-vector direction of polarized light used by ruin lizards for compass orientation. For this purpose, lizards were trained inside a Morris water maze positioned indoors while exposed to plane polarized light with a single E-vector, which provided an axial cue. Lizards meeting learning criteria under white polarized light were then tested for orientation under plane polarized light of different wavelengths. Plane polarized light was produced and regulated by an LCD screen connected to a computer and dedicated software (Glantz and Schroeter, 2006). For the first time here an LCD system was used to study compass orientation behaviour in a terrestrial vertebrate (Parretta et al., 2011).
2. Materials and methods

2.1 Animals.

Ruin lizards *Podarcis sicula* (Rafinesque-Schmaltz 1810; adults only) were collected from the area of Ferrara (Italy; longitude: 12°21'44''E, latitude: 45°03'72''N) under the authority of the Parco Delta del Po-Emilia Romagna (Department of Wildlife and Fisheries). After capture, lizards were transported to the lab where they were exposed to natural daylight (thus natural photoperiodic and light intensity conditions). Food (*Tenebrio molitor* larvae) and water were supplied *ad libitum*. The captive maintenance procedures and research protocols were approved by the University of Ferrara Institutional Animal Care and Use Committee and by the Italian Ministry of Health.

2.2 Experimental apparatus.

The Morris water maze was the same previously utilized (Foà et al., 2009; Beltrami et al., 2010). The vertical walls of the maze, 40 cm high, were made of mat, whitish gray PVC to reduce as much as possible selective reflection of linearly polarized light. The maze was filled with water to a depth of 15±0.5 cm and the water temperature was maintained at 29±1.0°C. Water was obscured by the addition of fossil flour (Clarcel, Ceca, Honfleur, France). The goals consisted of two identical plexiglas, transparent rectangular platforms (23.7 × 16 × 2.5 cm thick), each mounted upon a pedestal (11.5 cm high from the maze bottom). The goals were in direct contact with the centre of two opposite vertical walls of the maze along the axis 0°-180°. At a distance of 60 cm from its vertical walls, the maze was surrounded by a mat black, thick, fence cloth to a height of 190 cm to prevent the lizards from seeing laboratory features. The top of the fence was closed with a mat wood roof (diameter 266 cm). The maze was illuminated with plane polarized light (single E-vector) produced by a LCD display (42'' TFT-LCD-TV, Daewoo Electronics Corporation, Seoul, Korea) placed in a hole (diameter 48 cm) cut around the centre of the roof (Fig. 1A). Before use in the test apparatus, the LCD screen was characterized in a specialized optical laboratory. The luminance $L_v(\theta,\varphi)$ of the screen was derived, as function of the polar and azimuthal angles (see an example in Fig. 1B), by illuminance measurements performed by isolating a small central region of the screen (4-cm diameter) and moving the sensor head of a luxmeter (Konica Minolta T-10, Konica Minolta Sensing Inc., Tokyo, Japan) on the surface of a hemispherical plastic globe (30 cm radius), centred on the light zone, whose spectral transmittance was measured in advance. The knowledge of angle-resolved luminance $L_v(\theta,\varphi)$ allows to calculate, by integration over the 48-cm diameter full screen window, the illuminance produced over any surface element faced to the screen, in particular the surface elements of the water maze. An alternative, simpler way for obtaining the distribution of illuminance on the water maze at the different light spectra, was the
direct measure of it in laboratory by the luxmeter moved on a planar wall kept parallel to the screen. The distribution of simulated illuminance at the water level on the maze is shown in Fig. 1C. It is highly symmetric, as it is expected from the circular shape of light window opened on the LCD screen. The intensity, degree and direction of the polarization of light on the horizontal plane at the level of the goals were measured also in situ in some points of the maze by the Konica Minolta luxmeter, equipped with a linear polarizing filter (PL-C, Canon Inc., Tokyo, Japan). Rotation of the LCD display by 90° did not change the profile and intensity of the illuminance inside the Morris water maze at the level of the goals (Fig. 1D; mean±SEM: 4.1±0.1 and 3.9±0.1 lx, respectively; Student’s t-test: t(36)=1.4, P>0.1). The degree of polarization of light was always 100% at all tested points in the maze. The LCD display was connected to a computer and a dedicate software was used to regulate the intensity and colour, through RGB coordinates, of the emitted plane polarized light.

The spectrum of the plane polarized light was measured by a spectrometer operating in the visual–near infrared (Vis-NIR) region (Jeti 1211 UV, Photo Analytical, Milano, Italia). From the spectra and the illuminance data we have calculated the photon flux density integrated over the full Vis-NIR spectrum (Fig. S1). We generated 5 different plane polarized lights with equalized photon flux density (≈10^{17} photons/m^2·sec·nm), but different spectra: 1. light with similar percentages of the photon flux in the short (400-495 nm), medium (495-590 nm) and long (590-780 nm) wavelength range (RGB: 95,95,95; Fig. S1A); 2. light with 92% of photon flux in the long wavelength range (RGB: 221,0,0; Fig. S1B); 3. light with 77% of photon flux in the medium wavelength range (RGB: 0,190,0; Fig. S1C); 4. light with 58% of photon flux in the medium and 35% in the short wavelength range (RGB: 0,255,255; Fig. S1D); 5. light with 93% of photon flux in the short wavelength range (RGB: 0,0,221; Fig. S1E). For the sake of brevity, through the rest of the paper the reported lights from 1 to 5 were respectively named white, red, green, cyan, and blue, as their corresponding RGB colour names. The reflection of linearly polarized light on the vertical walls was measured to test for the existence of differences in illuminance. No differences in illuminance were found between vertical walls, and no changes in illuminance pattern were detected on each of these walls after 90° rotation of the E-vector. Some unevenness in illuminance (<0.3 lx) was measured between different points along the same wall, whose spatial distribution remained the same after 90° rotation of the E-vector. The experimental apparatus is covered by an Italian patent Number RM2011A000123 (Parretta et al., 2011).

2.3 Experimental protocol.

All lizards (n = 81) used in experiments were subjected to axial pre-training and training under white polarized light with a single E-vector (Beltrami et al., 2010). All lizards were trained along
of the axis 0° - 180°, which was parallel to the E-vector direction. In each trial, the compass direction of the first point of a side wall touched by each lizard was measured from the center of the maze by means of an azimuth compass (Wayfinder Outback ES, Sphere Innovative Technologies, Kingsford, NSW, Australia). This compass direction was recorded as the directional choice of the lizard in the current trial. Lizards reaching one of the two goals (±5° from platforms) were rewarded, and their trials were scored 1.5 (Fig. S2). The reward consisted in immediately lowering the water level in the maze, so that the goal and the lizard placed on it could emerge completely from water within 5-6 s. The lizard was kept there 30 s before recapture. Lizard reaching the correct side walls, but not the goal platforms, were not rewarded and their trials were scored 1 (Fig. S2). Lizards reaching one of the four side walls not containing a goal within 15° from the edge of a side wall containing the goal were scored 0.5, and beyond this angle were scored 0 (Fig. S2). To reach learning criteria each lizard had to award a score of 6 or higher within 6 consecutive trials, with a maximum of 1 trial scoring ≤0.5, and with the last trial scoring ≥0.5. Lizards failing to reach criteria were excluded from experiments.

To validate the apparatus, a group of lizards (N=10) was tested under white polarized light after 90° rotation of the E-vector. Other lizards (N=54) were subdivided into four experimental groups (red: N=16; green: N=15; cyan: N=10; blue: N=13). Each group was respectively tested for orientation under a different spectrum (red, green, cyan or blue) of plane polarized light. First, the orientation of all lizards of each group was tested under light with E-vector parallel to the training axis. Subsequently, the orientation of lizards of each group was tested after 90° rotation of the E-vector. Since at least 7 days passed between first and second test, a refreshing training was carried out, which consisted of two trials under white light with E-vector parallel to the training axis. Only lizards scoring ≥1.0 in both trials were admitted to the orientation test with 90° rotated E-vector.

2.4 Data analysis and statistics.

In most training and orientation tests lizards’ directional choices were distributed in an approximately bimodal fashion. In all those situations mean vector length would approach zero and no mean angle (mean direction) could be determined (Batschelet, 1981, p.17; Zar, 1999, p. 607). One can get meaningful results from such bimodal bearing distributions only by doubling the angles and then reducing to modulo 360°: in this way unimodal distributions are obtained on which statistical tests can be applied (Batschelet, 1981). In the present study we doubled the angles (directions) chosen by lizards during the last training trial and used the obtained data to calculate the training mean vector. We also doubled the angles chosen by the same lizards during the one trial orientation test and used the obtained data to calculate the test mean vector. The V test was used to
test whether the directions chosen by the lizards deviated from uniform: in other words, to test whether the distribution of these directions was statistically different from a random distribution (Batschelet, 1981). The \( V \) test takes into account the expected direction, which was 0° for training, and 90° for testing after rotation of the E-vector. When bearing distributions were not bimodal, angles chosen by lizards were not doubled. This happened when directions chosen by the lizards did not deviate from uniform. For each treatment, the Hotelling test for paired data was applied to test for differences between the directions chosen by lizards in the last training trail and the directions chosen by the same lizards in the respective one trial orientation test (Batschelet, 1981). The Watson \( U^2 \)-test was applied to test for differences between distributions of directional choices of different groups of lizards (Batschelet, 1981).

3. Results

3.1 White light validation test (Fig. 2A,B).

We tested whether lizards could orientate by using a single E-vector of white plane polarized light produced by a LCD display (Fig. S1A). Ten lizards were used, whose directional choices were symmetrically bimodally distributed along the training axis (0°-180°). After doubling the angles, the directional choices of the group in the last training trial was found to deviate from uniform (\( V \) test: \( u=4.32, P<0.0005 \)) (Fig. 2A). After 90° rotation of the E-vector direction with respect to the E-vector direction during training the directional choices were also found to deviate from uniform (\( N=10; V \) test: \( u=1.91, P<0.05 \)) (Fig. 2B). The directions chosen by lizards after 90° rotation of the E-vector were significantly different from those the same lizards chose in the last training trial before rotation (Hotelling test for paired data: \( F(2,8)=11.76, P<0.005 \)).

3.2 Red light test (Fig. 3A,B).

Sixteen lizards reaching criteria were tested under red plane polarized light (Fig. S2B). In the last test under white light their directional choices deviated from uniform (\( V \) test: \( u=4.96, P<0.0005 \)) (Fig. 3A), but under red light with E-vector parallel to the training axis the directional choices of these lizards did not deviate from uniform (\( V \) test: \( u=0.65, P>0.25 \)) (Fig. 3B). The directions chosen by lizards in the red light test with E-vector parallel to the training axis were significantly different from those the same lizards chose in the last training trial under white light (Watson \( U^2 \)-Test: \( U^2(16,16)=0.44, P<0.001 \). Since under red light with E-vector parallel to the training axis lizards were disoriented, no test with 90° rotated E-vector was performed.

3.3 Green light test (Fig. 4A,B).

A group of 15 lizards was tested under green plane polarized light (Fig. S2C). The directional choices of lizards tested with E-vector parallel to the training axis deviated from uniform (\( V \) test:
After refreshing training, seven lizards were admitted to the orientation test with 90° rotated E-vector. Their directional choices did not deviate from uniform (V test: \( u=0.51, \) \( P>0.25 \)).

**Cyan light test (Fig. 4C,D).**

We tested whether lizards could orientate under cyan plane polarized light (Fig. S2D). Ten lizards were tested for orientation under cyan light with E-vector parallel to the training axis. Their directional choices deviated from uniform (V test: \( u=1.87, \) \( P<0.05 \)). Nine lizards passed refreshing training and were then subjected to the orientation test with 90° rotated E-vector. In this new condition lizards’ directional choices deviated from uniform (V test: \( u=1.82, \) \( P<0.05 \)). The directions chosen by lizards in the cyan light test with E-vector parallel were significantly different from directions chosen after 90° rotation of the E-vector (Watson \( U^2 \)-test: \( U^2(9,10)=0.19, P<0.05 \)).

**3.4 Blue light test (Fig. 4E,F).**

Thirteen lizards were tested for orientation under blue plane polarized (Fig. S2D). Lizards’ directional choices with E-vector parallel to the training axis deviated from uniform (V test: \( u=2.00, \) \( P<0.02 \)). Ten lizards passed refreshing training and were then tested for orientation with 90° rotated the E-vector. In this new conditions lizards’ directional choices deviated from uniform (V test: \( u=2.60, \) \( P<0.005 \)). The directions chosen by lizards in the blue light test with E-vector parallel to the training axis were significantly different from the directions chosen after 90° rotation of the E-vector (Watson \( U^2 \)-test: \( U^2(13,10)=0.24, P<0.02 \)).

**3.5 Blue vs. Cyan.**

The directions chosen by lizards in blue light test with E-vector parallel to the training axis did not differ from those of cyan light test with E-vector parallel (Watson \( U^2 \)-test: \( U^2(10,13)=0.096, P>0.20 \)) (Fig. 4C,E). Similarly, the directions chosen by lizards in blue light test after 90° E-vector rotation did not differ from those of cyan light test after 90° E-vector rotation (Watson \( U^2 \)-test: \( U^2(9,10)=0.037, P>0.50 \)) (Fig. 4D,F).

**4. Discussion.**

The present results first showed that ruin lizards (*Podarcis sicula*) can learn a training direction when trained under white polarized light produced by an LCD and E-vector parallel to the training axis (Fig. 2A). Following 90° rotation of the E-vector direction, lizard orientation rotated correspondingly (Fig. 2B). This validates functioning of our LCD screen as an optimal source of plane polarized light, and further confirms the capability of ruin lizards to use polarized light for compass orientation (Beltrami et al., 2010; Parretta et al., 2011).
We further tested whether there are preferential regions of the light spectrum detected by ruin lizards for an optimal working of their sky polarization compass. When tested under blue and cyan polarized light lizards were actually capable of correct orientation both with E-vector parallel to the training axis and after 90° E-vector rotation (Fig. 4C-F). Conversely, lizards tested under red polarized light could not even be trained to orientate with E-vector parallel: they were completely disoriented (Fig. 3B). The results of the orientation tests carried out under green polarized light were not completely clear, since lizards orientated correctly with E-vector parallel, but were disoriented after 90° rotation of the E-vector. Although not statistically significant, the distribution of the directional choices shows that these lizards mainly behave as if the 90° rotation of the E-vector would have not occurred (Fig. 4B). In other words, lizards tested in the green light did not seem to perceive the 90° rotation of the E-vector. The fact that green polarized light was somehow sufficient to orientate along the training axis but inadequate in a new E-vector axis orientation suggests that the polarization in the green we have presented was not clearly discernible to lizards, and thus insufficient for a correct functioning of their sky polarization compass. To get an explanation of the results obtained with red, green, cyan and blue plane polarized lights, we need to look in the detail at the different spectra to which the different groups of lizards were exposed (Fig. S1). First of all, the red light has 92% of photon flux in the long wavelength range (590-780 nm). Lizards were completely disoriented already during training. It is clear that long wavelengths completely prevent functioning of the sky polarization compass of ruin lizards. Differently, the blue plane polarized light that we presented to ruin lizards has 93% of photon flux in the short wavelengths range (400-495 nm) (Fig. S1E). In this situation the sky polarization compass is working properly. The same is true for cyan plane polarized light having a 35% of photon flux in the short wavelength range (Fig. S1D). Green plane polarized light has only 18% of photon flux in the short wavelength range (Fig. S1C), and it is not sufficient for a correct functioning of their sky polarization compass (these lizards do not perceive 90° rotation of the E-vector). Thus, an increase of 17% of short wavelengths (cyan versus green) is sufficient to warrant orientation by means of sky polarization compass. Overall, the present results show that: i) long wavelengths (590-780 nm) are not involved in the lizard sky polarization compass; ii) short wavelengths (400-495 nm) are necessary and sufficient for a proper functioning of the sky polarization compass.

Previous investigations demonstrated that in ruin lizards an intact parietal eye plays a central role in mediating functioning of the sky polarization compass (Beltrami et al., 2010). The parietal eye exhibits a chromatic response to light mediated by different photopigments, such as short, medium and long wavelength-sensitive opsins, rod opsin, pinopsin and parietopsin (Kawamura and
Yokoyama, 1997, Frigato et al., 2006, Su et al., 2006). Electrophysiological studies carried out in the desert night lizard *Xantusia vigilis* and the common side-blotched lizard *Uta stansburiana* showed higher spectral sensitivity of their parietal eyes for both blue (short wavelengths) and green (medium wavelengths) lights (Solessio and Engbretson, 1993, 1999; Su et al., 2006). While sensitivity to the blue is compatible with the present results in ruin lizards, the sensitivity to the green is unforeseen, due to the marginal role of polarization in the green for compass orientation performances. Although interspecific differences in spectral sensitivity among lizards may be due to the different ecological niches in which they evolved, it is also possible that the spectral sensitivity to green light would be used in a behavioural or physiological context different from the detection of polarized skylight for compass orientation. For instance, a chromatic antagonism between green and blue sensitivity was discovered in parietal eye photoreceptors of *X.vigilis* and *U.stansburiana* that may provide lizards with a “photometric mechanism” that processes diurnal light intensity and spectral composition to detect the beginning and end of the daily photophase (Solessio and Engbretson, 1993).

Although perception of polarized light in the UV range (<400 nm) cannot be ruled out in ruin lizards, the present results confirm those of a previous investigation already showing that the sky polarization compass of these lizards doesn’t need UV light to work (Beltrami et al., 2010). A similar situation was found in field crickets (*Gryllus campestris*) and desert locusts (*Schistocerca gregaria*), in which sky polarization compass mainly uses linear polarization in the blue ($\lambda_{\text{max}}$=433 and 450 nm, respectively) and not in the UV (Herzmann and Labhart, 1989; Eggers and Gewecke, 1993). In several other species, however, such as the honey bee *Apis mellifera*, the desert ant *Cataglyphis bicolor*, and the scarab beetles *Lethrus* spp., the sky polarization compass does not work in absence of linear polarization in the UV (von Helversen and Edrich, 1974; Edrich and von Helversen, 1987; Duelli and Wehner, 1973; Frantsevich et al., 1977). In an attempt to explain that discrepancy, Zufall et al. (Zufall et al., 1989) proposed that highly polarization-sensitive blue receptors may be a common adaptation for insects active not only during the day, but also during crepuscular periods and at night, such as field crickets, as opposed to exclusively day-active insects - honeybees, desert ants and flies - which predominantly use UV receptors to detect skylight polarization. Importantly, however, ruin lizards do not support the hypothesis above, since they are day-active animals equipped with a sky polarization compass working in the absence of UV light. Apart from this unsolved question, it is important to point out here that blue and UV wavelengths are both well suited to detect polarized light under cloudy skies. Pomozi and collegues (Pomozi et al., 2001) measurements carried out by using a full-sky imaging polarimeter demonstrated that
under partly cloudy skies the shorter the wavelength, the greater the proportion of the celestial polarization pattern for use in animal orientation. In the detail, the extension of the E-vector pattern of clear sky into celestial areas covered by clouds is more useful for a polarization compass when skylight is perceived in the blue or in the UV rather than in the green or the red. The fact that detection of polarized light both in the UV and the blue substantially improves and stabilizes functioning of the polarization compass under partly cloudy skies is a crucial issue here: the polarization compass becomes the only celestial compass available if some clouds obscure the sun disk completely. In such a situation the sun azimuth compass is useless, and thus the adaptive value for an animal of being equipped with an alternative celestial compass mechanism - the sky polarization compass - becomes immediately clear. If our interpretation is correct, blue or UV photopigments should have been selected to serve the polarization compass mechanism simply because they enhance detection of polarized light under cloudy skies. On the other hand, if the sky polarization compass would be mainly used for orientation under clear skies, the importance of selecting some wavelengths with respect to others should be substantially reduced. In fact under clear skies there is no favoured wavelength for the perception of skylight polarization, because the proportion of celestial polarization pattern useful for orientation is large enough at all wavelengths including the UV (Brines and Gould, 1982, Pomozi et al., 2001; Barta and Horváth, 2004).

Future investigations in ruin lizards should include molecular studies aimed at identifying the different photopigments expressed in the parietal eye, and electrophysiological studies to characterize their functioning in response to administration of polarized light of different wavelengths.

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**Figure captions.**

**Figure 1.**

A. Schematic representation of the experimental apparatus (1). The mat black cloth (3) and the roof (7) surrounded the water maze (4), illuminated from the top window (5) by the polarized light (2) of the LCD monitor (9) placed face down on the roof. The change of E-vector direction was done acting on the lever (13) of the annulus (6,14) that supports the screen. The screen color was set at the computer (10) by operating outside the box. Inside the maze the two goals (16) mounted on their pedestals were placed just beneath the opaque water surface (11). B. Luminance curves obtained by fitting the $L_v(\theta,\varphi)$ data ($\Delta \theta \approx 10^\circ; \Delta \varphi = 10^\circ$) measured after setting the azure colour (RGB: 187,224,227) on the LCD screen. The black dots are the luminance values averaged over the azimuthal angle. C. Map of illuminance $E_v(x,y)$ produced by the 48 cm diameter screen window on the maze at water level, obtained for white light (RGB: 255,255,255) from the simulated laboratory measurements. The maze profile is also shown. D. Intensity and direction of the polarization of light on the horizontal plane at the level of the goal platforms, measured on some points along the three directions connecting opposite vertical walls of the hexagonal maze. Each arrow indicates the direction of the plane of polarization (E-vector).

**Figure 2.**

White light validation test. Orientation behaviour of lizards trained and tested under white polarized light with a single E-vector produced by a LCD screen. Each symbol indicates the directional choice of a single lizard identified by its number. In each hexagon the inner arrow represents the mean vector of the group calculated after doubling the angles. In each hexagon the mean vector length ($r$) and the mean direction ($\alpha$) of the group are reported. Solid line mean vector: the bearings distribution deviated from uniform. For the hexagon in A, the two outer solid arrows mark the expected axis of orientation of lizards during training trials (0-180°), while in B mark the expected axis of orientation after 90° rotation of the E-vector (90-270°). Lizards orientated correctly either when tested with E-vector parallel to the training axis or after 90°rotation of the E-vector direction.

**Figure 3.**

Red light test. Lizards which orientated correctly under white polarized light with E-vector parallel to the training axis (A) became completely disoriented when tested under red polarized light (B). The dotted mean vector in B indicates a bearings distribution which did not deviate from uniform. Further information in Fig. 2.
Figure 4.

Orientation behaviour of 3 groups of lizards, which were respectively tested under green (A, B), cyan (C, D), or blue (E, F) plane polarized light. Lizards tested under green polarized light orientated correctly with E-vector parallel to the training axis (A), but were disoriented after 90° E-vector rotation (B). Lizards tested under cyan polarized light orientated correctly both with E-vector parallel to the training axis (C) and after 90° E-vector rotation (D), and the same was true for the lizards tested under blue polarized light (E, F). Further information in Fig. 2 and 3.
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