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NO EVIDENCE FOR POLARIZATION SENSITIVITY IN THE PIGEON ELECTRORETINOGRAM

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Summary

The electroretinographical response to flashes of linearly polarized light directed at the pigeon's yellow field was compared with that to flashes of unpolarized light. This was carried out for white light and for monochromatic light of various wavelengths, including ultraviolet. In addition, responses to slow rotation of the *E*-vector of polarized light were measured. Neither the presence or absence of polarization, nor the orientation of the *E*-vector, influenced any of the electrophysiological variables that were monitored in these experiments.

Key words: polarization-sensitivity, electroretinography, pigeon, *Columba livia.*

Introduction

The homing pigeon is renowned, to the scientist as well as to the layman, for its exceptional orientational and navigational performance. Several sensory modalities, using a variety of environmental cues, such as the earth's magnetic field and the position of the sun, landmarks and odours, are generally believed to be involved in this process (Berthold, 1991).

It has been suggested that the celestial polarization pattern may also serve as a source of orientational information, because these patterns are geometrically coupled to the sun's position (Sekera, 1956; Brines and Gould, 1982; Wehner, 1989). Kreithen and Keeton (1974) and Delius *et al.* (1976) hypothesized that perception of these patterns could serve as an enhancement of, or as a supplement to, the pigeon's time-compensated sun compass mechanism (Schmidt-Koenig, 1958, 1979). For example, it has been argued that under partial cloud cover, when the sun is not visible, the polarization pattern of a patch of blue sky could be used to reconstruct the sun's position. Phillips and Waldvogel (1988) brought forward another model, in which information from polarized sky light near the horizon is involved in calibrating the absolute position of the azimuthal sun compass.

In the recent literature (for an overview, see Berthold, 1991), the ability of birds to orient relative to the celestial polarization pattern seems to be taken for granted. It is of interest, therefore, to examine whether one of the fundamentals on which this conviction rests, namely the positive outcome of laboratory experiments (see below) demonstrating the sensitivity to polarized light of the homing pigeon, is valid.

The first attempt to demonstrate polarization sensitivity in the pigeon had a negative outcome. Montgomery and Heinemann (1952) employed an apparatus in which polarized light was projected onto a pecking key. Their results, using a behavioural discrimination procedure, showed that pigeons could not indicate the difference between stimuli with orthogonal ***E***-vector orientations. Many years later, Kreithen and Keeton (1974) demonstrated, with a classical cardiac conditioning technique, that pigeons could be taught to discriminate between a stationary and a rotating linearly polarized light stimulus, thereby contradicting the conclusions of Montgomery and Heinemann. Delius *et al.* (1976; see also Delius and Emmerton, 1979) conducted a series of behavioural experiments to test whether pigeons are able to orient with respect to the orientation of the ***E***-vector. They used an octagonal box, equipped with four pecking keys, which were positioned in a perpendicular arrangement, and an overhead polarized light source. The pigeons could learn to peck a key at a specific azimuthal angle with respect to the ***E***-vector of the stimulus above them (but see Coemans *et al.* 1990, 1994). Delius *et al.* (1976) supplemented their findings with an electrophysiological examination. The stimulus consisted of flashes of white light that were polarized by means of a polaroid sheet. They reported a consistent relationship between the shape of the b-wave of the electroretinogram (ERG) and the orientation of the polaroid when the eye was stimulated axially. For a given angle of the polaroid with respect to a stereotactic baseline (the 'auditory meatus–palatine line'), the summit of the b-wave was reported to be sharp and single-peaked, whereas the wave became more flattened, or even double-peaked, when the polaroid was turned through 90°. This effect was described as being unmistakably recognizable

in four of the five pigeons tested (the other animal had an aberrant ERG, but this wave still showed a polarization-dependent shape change, though very small). Using control experiments, they excluded the possibility that this change in the shape of the ERG was brought about by small, unintentional intensity variations. This dependence of the shape of the ERG on the orientation of the ***E***-vector remained present within an intensity range of 2 log units. This type of response was even more pronounced when the retina was stimulated ventrally (40 ˚ below the optical axis of the eye). In addition, experiments using broadband colour filters showed that flashes of green or red light yielded ERGs whose shape depended on the orientation of the ***E***-vector, although in a less apparent, colour-specific manner. This was not found with broadband blue stimulation.

To incorporate sensitivity to polarized light into avian navigational models in a meaningful manner it is imperative to have detailed data concerning the sensitivity of the birds to this stimulus. Physiological variables, such as the just discernible angle between two linearly polarized light stimuli or the minimum perceptible degree of polarization, must be determined. Only on the basis of such knowledge is it possible to make quantitative predictions.

The objective of this electrophysiological study was to acquire this information and to gain insight into the mechanism underlying the pigeon's polarization sensitivity. Therefore, we set out to quantify the behaviour of certain variables of the ERG when pigeons were stimulated with light whose state of polarization, spectral composition and intensity could be varied. Because of the success of the electrophysiological experiments reported by Delius *et al.* (1976), we used basically the same techniques of stimulation and recording.

Exploratory pilot experiments were aimed at finding the appropriate experimental conditions to obtain the expected dependency of the ERG on the polarized light stimulus. As several different settings were tested, they are not detailed separately in the Materials and methods section.

Materials and methods

Animals

Nineteen male and female homing pigeons (*Columba livia* L.) were used. All had free-ranging experience and were proved successful homers. Their ages varied between 4 and 8 years.

Pigeons were anaesthetized by a subcutaneous injection of a mixture of Rompun (xylazine, 16 mg kg^{-1} body mass) and ketamine HCl (90 mg kg^{-1} body mass). The animal was placed in quiet surroundings for about 45 min before being mounted in a stereotactic holder. An infrared ceramic lamp kept the body temperature at a constant level. The eyes were treated with a local analgesic (0.4 % oxybuprocaine) and the pupil of the stimulated eye was dilated with a curare solution (Campell and Smith, 1962). Despite the presence of the corneal electrodes, the nictitating membrane could move freely, thereby lubricating the cornea to some extent. Nevertheless, normal saline was applied to the eyes at regular intervals.

Recording

Corneal electrodes consisted of a circular Perspex ring, with a small chlorided silver wire at one side. The electrode was placed between the eyelids, keeping the eye open, so that the silver wire was in electrical contact with the cornea. A stainless-steel needle electrode was used to ground the animal. The electroretinal potentials from the stimulated and the unstimulated (reference) eye were amplified differentially (Princeton Applied Research 113) and fed into an active filter (Krohn-Hite 3750; 0.3–150 Hz, 24 dB per octave). A microcomputer, equipped with an analogue-to-digital converter, was used to average the responses (throughout the text, the terms 'ERG' and 'response' refer to averaged ERGs or averaged responses).

Stimulus arrangement

Retinal illumination was achieved by using a single-channel Maxwellian view system (subtense 15 ˚) with all optical elements being transparent down to the deep ultraviolet. Using the data provided by Marshall *et al.* (1973), it can be calculated that, with this subtense, a retinal area of 3.5 mm^2 was illuminated. The light source was a 150 W xenon arc lamp, whose output was stabilized by means of an optical feedback amplifier (Stabilarc System, Ealing Corp). Spectral selection was obtained with the aid of interference filters (Balzers, 10 nm bandwidth). A heat-blocking filter (BG22; Schott) was inserted into the light path when white light flashes were given. This filter is a broad bandpass filter with the low and high 50 % cut-off points at 360 and 660 nm, respectively. The intensity of the stimulus was controlled by inserting neutral density filters (nickel alloy coating on a fused silica substratum). The light was polarized with a quartz, air-spaced, Glan-Thompson polarizer. Because the light leaving the monochromator was partially polarized, rotating the polarizer would give changes in intensity. To prevent this, a calcite depolarizer (Hanle-type; Ealing Corp), suitable for depolarizing monochromatic as well as broadband light, was placed between the monochromator and the polarizer. The polarizer and the depolarizer were placed in a collimated part of the light path. The polarizer could be rotated freely (the pivoting axis coinciding with the centre of the light path) and, in addition, could easily be moved into or out of the light path. The angle of the ***E***-vector of the stimulus was given with reference to the line defined by the earhole and the tip of the bill (see inset to Fig. 1). An electronically driven diaphragm shutter, placed at a focal point, was used to control the temporal characteristics of the stimulus. Flashes with a duration of 140 ms were administered to the test eye every 1030 ms. The pulse that triggered the shutter to open was also used to synchronize the averaging.

In experiments with rotating ***E***-vectors, a different stimulus apparatus was used. The stimulus entered the pigeon's eye in Maxwellian view with a subtense of 30 ˚, which means that the stimulated retinal area measured about 14 mm^2. The light

was projected onto the ventral part of the retina, 20 ° below the area centralis. A 450 W xenon light source (Zeiss) was used, whose out traversed on infrared-absorbing distilled water filter. A dichroic polarization sheet (HN38, Polaroid Corp.), located after the depolarizer, was rotated with a relatively low angular speed (0.5–4 Hz) by means of a servo-controlled d.c. motor. The signal to trigger the averaging was derived from the rotating polaroid holder. Care was taken to ensure that the light entered the polaroid at a normal angle of incidence so that the light spot, as projected onto the retina, would not follow a (tiny) circular trajectory. The light impinging on the polaroid also had to have a degree of polarization as low as possible, because any degree of polarization would modulate the intensity of the stimulus (the modulation depth thus obtained was 0.125 %). Both these artefacts could cause variations in the observed ERG, regardless of whether the pigeon is sensitive to polarized light. In control measurements, a second static polaroid was inserted into the light path, in front of the first one, yielding a stimulus whose intensity was modulated sinusoidally with a modulation depth of 100 %.

Radiation measurements

The radiant power of the light stimuli was measured with a photodiode (UV 444 BQ, EG&G; used in short-circuit mode), combined with a current-to-voltage amplifier (NF Circuit Design Block LI-76). The spectral sensitivity of the photodiode was obtained by comparison with a thermopile (Eppley, C-16), the output of which was fed into a sensitive voltmeter (Datron 1061A). We always captured the full light bundle, so that the magnitude of the stimulus was measured in radiant power units (μW). These measurements were converted to numbers of photons per flash (or per second when continuous stimulation was used).

Experimental regimens

In the experiments in which light flashes were presented (mostly 140 ms in duration), white or coloured (393, 477, 575 and 632 nm) stimuli, all with a radiant power of 6 μW, were used (with the numbers of photons per flash being 2.2×10^{12}, 1.7×10^{12}, 2.0×10^{12}, 2.5×10^{12} and 2.7×10^{12}, respectively). The spectral selection was obtained with interference filters (Balzers) with a bandpass of 10 nm. The 393 and 575 nm filters were selected because their transmission peaks are close to the two local maxima of the luminous sensitivity function of the ventral part of the pigeon's retina (Wortel *et al.* 1984). The 477 nm filter has a maximum transmission that coincides with a minimum of this sensitivity function. The polarized stimuli were given in the following order of angles: 0 °, 45 °, 90 °, 135 °, followed by the same set of stimuli given in reverse order. Each ERG response to a polarized stimulus was followed by a reference ERG measurement. After a complete set (comprising eight test ERGs interspersed with eight reference ERGs), the adaptation light (500 lx) was turned on for 5 min, and the interference filter was exchanged or, in the case of white light stimulation, omitted.

The ventral part of the retina, 20 ° below the horizontal axis, was stimulated (in some experiments, the area centralis was stimulated). To maintain a constant level of stimulation, the flashing was continued throughout the experiment.

Experiments with the rotating polaroid were all carried out with the fluorescent room illumination switched on (100 lx at pigeon eye level). Both white and monochromatic (494 or 575 nm) light were used. After each ERG response to a stimulus with a rotating ***E***-vector, a second one was recorded in response to an intensity-modulated stimulus.

Data processing

Several quantitative features of the flash ERG were analyzed: the amplitudes and the latencies of the a- and b-waves, the rise time of the b-wave, and the width and the centre of the b-wave (see Fig. 1). To check whether the differences between the shapes of ERG responses to stimuli with orthogonal ***E***-vectors were significant, we used the procedure described by Coemans *et al.* (1990).

In experiments in which higher light intensities were employed, so-called oscillatory potentials (OPs) were often superimposed on the ERGs. Fourier filtering (50–140 Hz) followed by (digital) amplification was used to enable us to assign sequence numbers to the individual OP wavelets. The latencies of the peak values of the OPs were also determined.

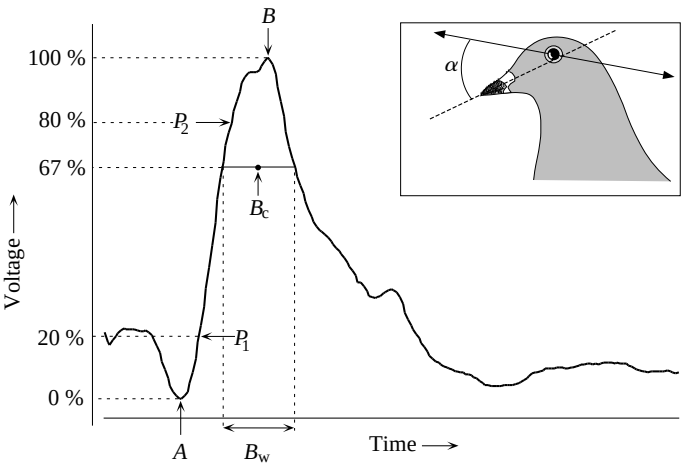

Fig. 1. The ERG variables examined. *A* indicates the a-wave minimum, *B* indicates the peak of the b-wave. Also determined were the response difference between the b-wave and the a-wave and their respective latencies. As a second indication of the b-wave latency, the centre point (B_c) was used; it was defined as the midpoint of the line that intersected the b-wave at the 67 % level (with *A* and *B* defining 0 % and 100 %, respectively). The length of this line was a measure of the b-wave width (B_w). The steepness of the rising flank of the b-wave was obtained by calculating the slope between P_1 and P_2; P_1 and P_2 are located at the 20 % and 80 % levels, respectively, of the height of the b-wave. The inset shows how the orientation of the polarized stimulus was defined: it was the angle α between the direction of vibration of the ***E***-vector (the solid line) and the line defined by the earhole and the tip of the bill (the dotted line).

To check whether the retinal responses to rotating stimuli contained components having the same periodicity as the stimulus, the ERGs were autocorrelated. We used this technique because it is an extremely sensitive tool for revealing periodic signals (Randall, 1977). The resulting autocorrelograms are graphs from which noise (uncorrelated signals) is virtually eliminated and in which the periodicities of the original signal are displayed.

Results

Pilot experiments

Because of the explorative nature of the pilot experiments, various conditions were used and different parts of the ventral part of the retina were stimulated. We measured responses to polarized and unpolarized flashes of light. The latter type of stimuli were, apart from the state of polarization, identical to the polarized stimuli. The ERG responses elicited by these flashes were evaluated by classifying the shape of the b-wave (see Fig. 1) in accordance with the method of Delius *et al.* (1976). In addition, we used quantitative ERG features to judge the effects of the state of polarization of the stimulus (see Materials and methods).

To determine how the orientation of the ***E***-vector affects the ERG, the polarizer was rotated in fixed steps of either 10° or 30° after each recording. In some experiments, the ***E***-vector orientations of the polarized light flashes were presented in random order. We also used an alternative scheme of stimulation, in which the orientations of the ***E***-vector for two subsequent polarized stimuli were at right angles. The latencies remained rather stable (Fig. 2A), whereas the difference in amplitude between the a- and the b-waves of the same ERGs varied strongly in this experiment (Fig. 2B, this was not always so). This also exemplifies what appeared to be a general property of the ERGs: the temporal characteristics of the a- and the b-waves (Fig. 1) are less influenced by physiological changes than are their amplitudes.

The ERG responses to more intense stimuli (approximately 4 μW or more) were usually superimposed with oscillatory potentials. The change in the shape of the ERG responses, as reported by Delius *et al.* (1976), is probably caused by the behaviour of oscillatory potentials, so we studied them carefully. An example of this kind of analysis is shown in Fig. 3. Fig. 3A,B shows that the use of control measurements is mandatory, because if, as in Fig. 3B, the responses to the test stimuli were plotted without control ERGs, some kind of periodicity could be inferred. This means that presenting the stimulus conditions in a randomized order (which is a well-established way of carrying out such experiments) is not sufficient to correct for variations in time.

We have also conducted experiments to check the influence of short periods of dark adaptation. These experiments show

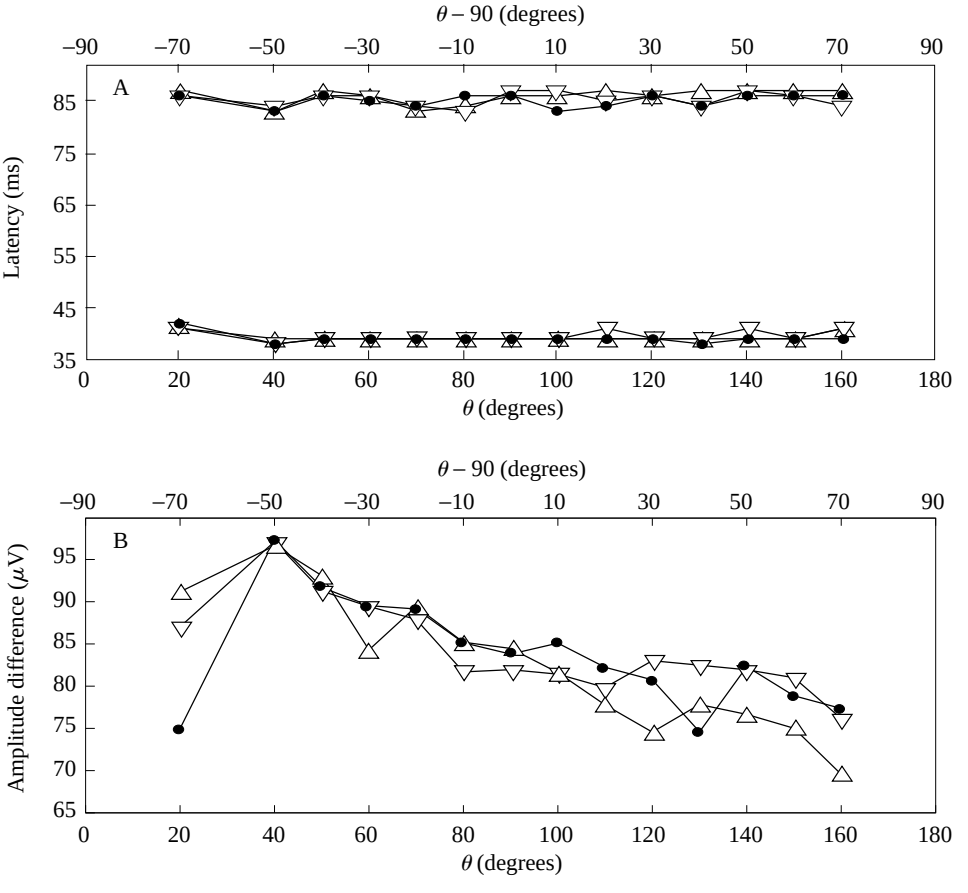

Fig. 2. (A) Latencies of the a-wave (lower traces) and the b-wave (upper traces). (B) Amplitudes of the ERGs (from the same experiment) taken as the difference between the b-wave maximum and the a-wave minimum. The ERGs were obtained in sets of three: the first one in response to polarized flashes, with the ***E***-vector at an angle θ (lower horizontal axis; ▽), the second one in response to an unpolarized (reference) stimulus (●) and the third one in response to a polarized stimulus with an angle of $\theta-90^\circ$ (upper horizontal axis; △). This was repeated for increasing values of θ, so that higher values of θ coincide with an increase in time. White flashes of light, with an intensity of 20 μW (6.4×10^{12} photons per flash), were given each second, with a duration of 123 ms. The latencies are very stable and do not depend on whether the light stimulus is polarized. The amplitudes also do not depend on the stimulus being polarized; the observed decline in amplitude is probably caused by physiological changes.

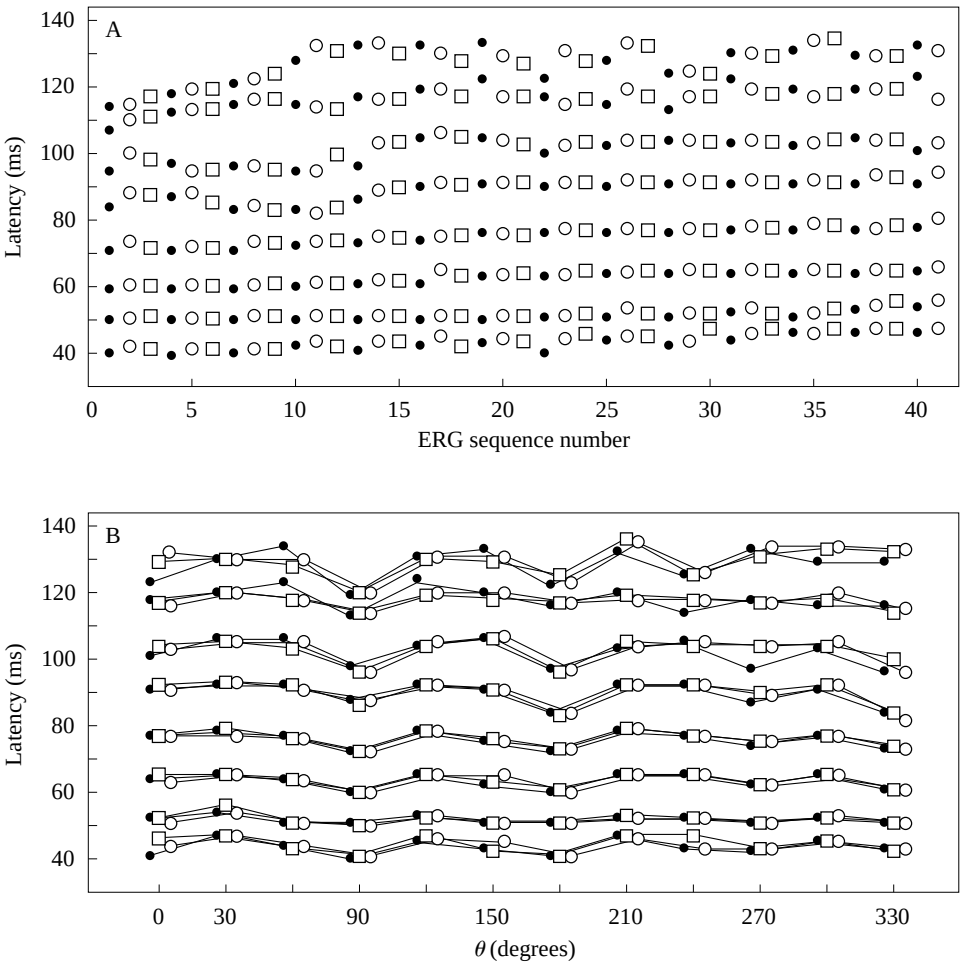

Fig. 3. Latencies of the oscillatory potentials, with the lowest trace depicting the latencies of the peak of the first oscillatory potential, the next highest trace those of the next peak, etc. In A, the latencies are shown in the order in which the ERGs were measured (denoted by the sequence number of the ERG). The squares indicate the responses to polarized stimuli with varying ***E***-vector orientations (θ), the open circles denote the responses to stimuli polarized at a fixed angle of 60°, and the filled circles show the responses to the reference stimuli. The same latency data are replotted in B, but as a function of the angle of the ***E***-vector. The stimuli were monochromatic light flashes (123 ms; centre wavelength 575 nm; radiant power 20 μW; number of photons per flash 7.1×10^{12}), which were given continuously throughout the experiment, at a rate of 1 s^{-1}. The area 20° below the fovea was stimulated. ERGs were recorded without additional illumination; between the recording periods (each lasting 10 min), an adaptation lamp (100 lx at the eye of the pigeon) was turned on for 5 min. When the responses to the polarized test stimuli were plotted alone, a dependency on the orientation of the ***E***-vector could be inferred. However, because the responses to the unpolarized and to the polarized stimuli, whose ***E***-vector orientation was fixed, vary in the same manner, such a conclusion is not warranted.

that the periods of darkness during which we recorded the responses were too short to account for changes in the ERG that could be explained as a result of the state of polarization of the stimulus (Fig. 4).

In summary, neither a qualitative nor a quantitative evaluation of the processed ERG variables obtained in these pilot studies revealed a link between the state of polarization of the stimulus (orientation of the ***E***-vector, unpolarized or polarized) and the ERG response. Other features of the stimulus, such as colour or intensity, did, of course, influence the shape and characteristics of the ERG, but under all these conditions the state of polarization did not matter. These results, however, did indicate to us that experiments such as these must be set up carefully: changes in ERG characteristics in response to various states of polarization are apparently small in comparison with the variations caused by changes in the physiological conditions. We therefore started a series of experiments designed in such a manner that small differences in response to polarized stimuli could be detected better and, in addition, that would enable us to verify these differences statistically.

Flashing stimuli

Initially, we wanted to analyze our results using the method described by Delius *et al.* (1976), so we evaluated the ERGs by visual inspection. In contrast to what they reported, however, we did not find that the shape of the b-wave depended on the orientation of the ***E***-vector.

Because we recorded responses to only four standard orientations of the ***E***-vector, we did not try to establish a relationship between the ERG features mentioned above and the stimulus angle based on periodicity. Instead, the ERG variables were determined for these angles and compared mutually by means of paired *t*-tests. For each session, we also compared the pooled responses to the polarized stimuli for each wavelength with the responses to the unpolarized stimuli.

The ERG variables were measured under different conditions, yielding many combinations to be compared statistically to check the effect of polarization. The above-mentioned *t*-tests for paired comparisons yielded some significant differences (at the level of P=0.05), but statistical theory predicts that, if samples from two identical distributions are compared, the probability of cases in which the samples

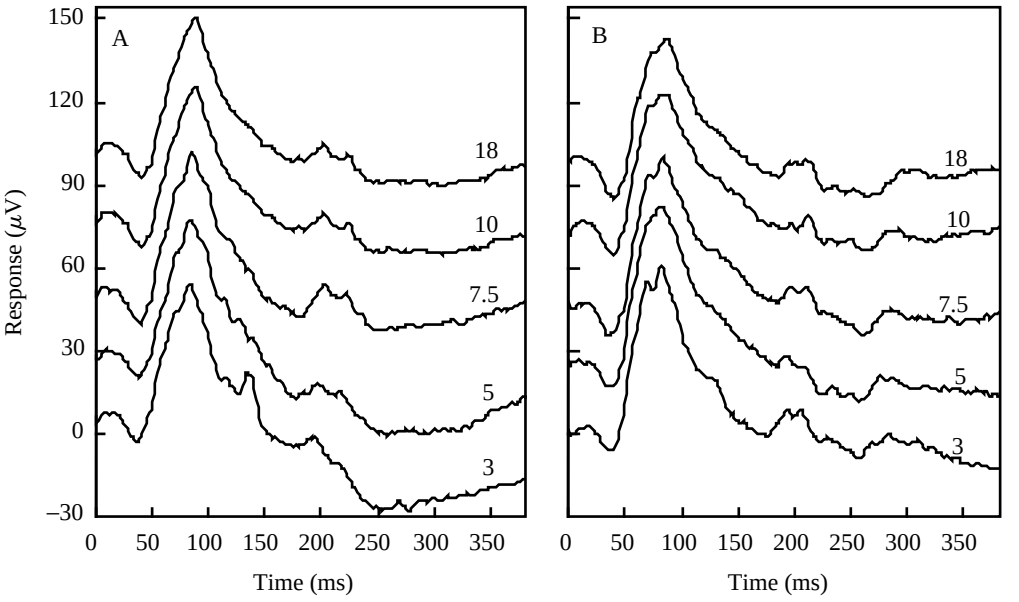

Fig. 4. Responses to identical stimuli after dark and light adaptation. Flashes of white light (N=32; duration 130 ms; radiant power 20 μW; number of photons per flash 6.8×10^{12}) were used. The ERGs obtained after increasing periods of darkness are depicted in A, whereas in B the matching control ERGs are shown (for the sake of clarity the traces have been shifted vertically). The stimulation regimen was as follows: after 5 min of light adaptation (100 lx at the eye level of the pigeon), a control response was obtained. Then followed a period of darkness (given beside each trace in minutes), at the end of which an ERG was again measured. This was repeated a number of times with longer dark adaptation times. This type of measurement was often carried out prior to examining the effects of polarized light. It is obvious that the effect of dark adaptation under these circumstances is negligible, even for the longer periods of darkness. Although this procedure is not suited for gauging the rate of dark adaptation (the flashes were so intense that light adaptation was induced during the measurement of the ERG), it appears that dark adaptation is a slow process.

differ significantly is equal to the level of significance of the test involved. However, when the results that were apparently significant were compared with the results of other, identical experiments, it appeared that these occurrences were not systematic. Sometimes, the differences could be traced to abrupt changes in the physiological condition of the animal.

Analysis of the latencies of the individual wavelets did not reveal differences between the responses to polarized and unpolarized stimuli (Fig. 5).

Rotating stimuli

We recorded responses to light with a rotating ***E***-vector and to light whose intensity was modulated (with a stationary ***E***-vector). The ERGs and their autocorrelograms evoked by the latter type of stimulus all displayed a marked periodicity. In contrast, autocorrelograms of the responses to polarized rotating stimuli did not contain components with the stimulus frequency (Fig. 6). This was always so, whether white or coloured stimuli were used and irrespective of the speed of rotation (0.5–4 Hz).

Discussion

In many arthropod species, our understanding of the

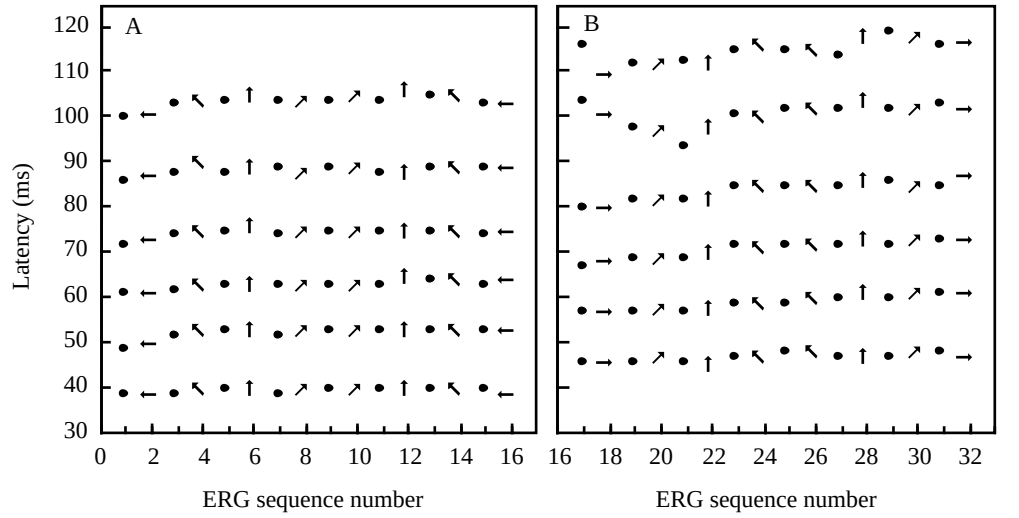

Fig. 5. Latencies of oscillatory potentials elicited by flashes (140 ms) of white light (A) and by monochromatic light of 393 nm (B). Filled circles show the responses to reference stimuli, whereas the arrows show responses to polarized light, with the angle of the symbol denoting the orientation of the ***E***-vector (see Fig. 1). The centre part of the retina, near the fovea, was stimulated; the radiant power was 6 μW for both types of stimuli (2.2×10^{12} and 1.7×10^{12} photons per flash, respectively). The latencies following 393 nm flashes are longer than those following stimulation with white light. There is no relationship between the latencies of the oscillatory potentials and the orientation of the ***E***-vector.

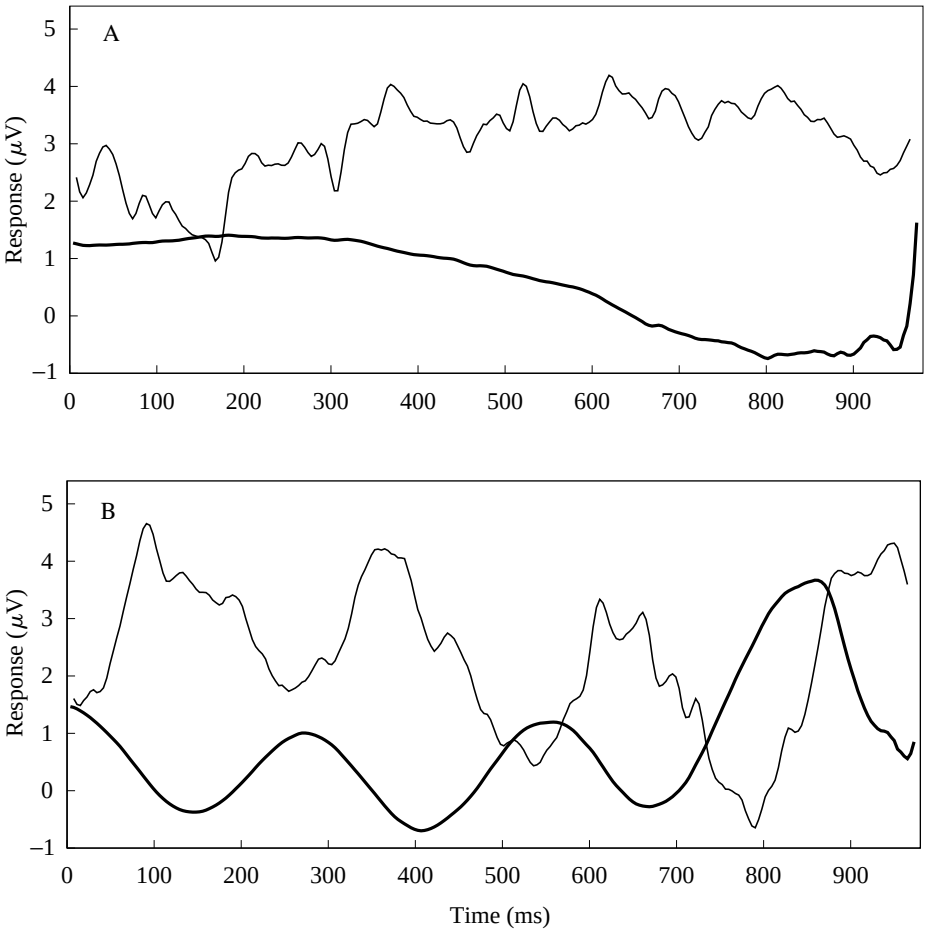

Fig. 6. (A) Responses to monochromatic light stimuli (494 nm; 4.7×10^{13} photons s^{-1}) with a rotating ***E***-vector (at a constant intensity level). The ERG following stimulation with the polaroid rotating at 3.9 Hz is plotted as a thin trace and the autocorrelogram of this ERG is plotted as a thick trace. There is no periodicity. The orientation of an ***E***-vector at a given angle θ cannot physically be distinguished from one having an angle θ+180°, so the frequency of stimulation proper is 7.8 Hz rather than 3.9 Hz. In B, the response to intensity modulation (with a static ***E***-vector) and the autocorellogram are shown: a marked periodicity is present, as would be expected.

detection of polarized light is well established (Waterman, 1981). This is particularly true for insects (Wehner, 1989), where the generally accepted model of the underlying mechanism is supported by a coherent and consistent compilation of evidence from disciplines as diverse as ultrastructural research, neurophysiology and psychophysics.

This contrasts with the situation in vertebrates, where several structures and mechanisms have been proposed to explain polarization sensitivity. The outer segments of vertebrate visual cells are made up of stacks of disc-like lamellar membranes containing rhodopsin. These discs are oriented with their plane normal to the light entering the eye. The rhodopsin can translate and rotate freely in the membrane, except that the longitudinal axis of the chromophore (or pigment) of the rhodopsin is oriented (almost) parallel to the disc membrane in which it resides (Rodieck, 1973). Light is absorbed strongly when the ***E***-vector is parallel to the longitudinal axis of the chromophore. Thus, chromophores are optimally oriented for absorbing light that enters the outer segment along the principal axis, regardless of the orientation of the ***E***-vector. This is in contrast to the effect of illuminating the outer segment from the side. Under these circumstances, linearly polarized light whose ***E***-vector parallels the main axis of the outer segment will be absorbed far less efficiently than light whose ***E***-vector is perpendicular to this axis. Because the latter manner of illumination is unnatural, this mechanism does not provide a basis for sensitivity to polarized light (cf. Liebman, 1975).

In the salamander, it has been proposed that the pineal gland mediates the reported sensitivity to polarized light (Adler and Taylor, 1973). The same mechanism is also assumed to operate in reptiles, one species of which (a desert lizard, *Uma notata*) has been shown to display time-compensated polarotactic escape behaviour (Adler and Phillips, 1985).

At least four different mechanisms have been proposed for fish. For anchovies, a peculiar, fork-shaped structure of the twin cone (with the lamellae parallel to the incident light) is suggested to be the basis of sensitivity to polarized light (Waterman, 1981). Hawryshyn *et al.* (1990) have shown that juvenile trout display polarotactic behaviour with a 180° ambiguity. They proved that the ultraviolet-sensitive cone plays a role in this polarization sensitivity (when they grow older, trout lose their sensitivity to ultraviolet light and, therefore, also their ability to detect polarized light). Cameron and Pugh (1991), however, advance the hypothesis that the twin cone is responsible for polarization sensitivity. In a behavioural study, they showed that the green sunfish is maximally sensitive to polarized light when the axis of vibration is either horizontal or vertical. To explain these results, they proposed a model based on the waveguide

properties of the twin cones, which are organized in an orthogonal pattern in this species. They also support this model experimentally by showing that this sensitivity coincides with the spectral sensitivity of the twin cone pigment (which does not extend down to the ultraviolet). Their model predicts a 90° ambiguity, so that it could only be of limited use in analyzing complex polarization patterns. The results described above contrast with the findings of Waterman and Aoki (1974) and Waterman and Hashimoto (1974), who were unable to find a relationship between the polarization sensitivity they recorded from the optic tectum and the retinal receptors of the goldfish.

Various theories have been formulated to explain the behaviour of pigeons in experiments that examined their sensitivity to polarized light. As a possible explanation for their results, Kreithen and Keeton (1974) proposed that the foveal depression acts as a radial analyzer. They argued that light would strike the photoreceptor cells in this part of the retina at oblique angles so that these cells would respond differently to polarized light with different orientations of the ***E***-vector. They suggested two mechanisms that would cause light to enter these photoreceptors obliquely: either the non-axial alignment of the cones near the sloping walls of the fovea, or reflection from the opposite slope of the fovea. The first suggestion is ill-founded because the foveal depression is confined to the cell layers vitreal to the visual cells. In birds, the outer segments of the foveal cones are aligned towards the incoming light, a fact known since the last century (Van Genderen Stort, 1887; Cajal, 1893; see also Lockhart, 1979). The second suggestion is highly unlikely to play a part because the foveal depression is known to be shallow in the pigeon (Van Genderen Stort, 1887; Galifret, 1968; Lockhart, 1979) and the difference in refractive index between the vitreous humour and the neural layers is small, so only small amounts of light can be reflected. This can easily be verified by using the Fresnel formulae that describe the amount of light reflected (Born and Wolf, 1983). We have calculated this with the following assumptions: that the incident light is maximally polarized, that the slope of the wall of the foveal depression is 30°, and that the indices of refraction of the vitreal neural layer and of the vitreous humour are 1.85 and 1.35, respectively. If the incident linearly polarized light has an intensity equal to 1, than the amount of light reflected, when the ***E***-vector is perpendicular to the plane of incidence, is 0.018, whereas for the parallel direction this value is 0.008. Because the receptors receive this reflected light in addition to direct light, the intensity difference will cause a contrast modulation as low as 0.0005. Taking into account that the above-mentioned values are overestimates, this mechanism is unlikely to be important. A third argument against this theory is that the cones in the light-adapted retina of the pigeon are surrounded by epithelial pigments, which effectively screen them from light entering at oblique angles. This is particularly true for foveal cones and can be inferred from the results of Waelchli (1883), who (in a footnote) reported difficulty in separating the retina from the choroid in the pigeon, because the epithelial cells remained attached to the choroid. The processes of the epithelial cells, which contain the melanin pigment granules, are relatively firmly coupled to the outer segments of the cones. To circumvent this problem, Waelchli adapted the pigeons to the dark for about 30 min to induce the epithelial processes to retract. He reported that this approach was successful, except in the fovea. It must be concluded that, in the photopic state, foveal cone outer segments are very well protected from stray light. A fourth counter argument is that light reflecting from the foveal walls would impair visual acuity.

It is unlikely that the results can be explained on basis of the breeds of pigeon that have been examined (we used racing homers, whereas Kreithen and Keeton, 1974, used White Carneaux pigeons), because Carneaux pigeons have been bred for meat production, whilst homing pigeons have explicitly been selected over several centuries for their navigational performance. In other words, the reverse situation would have been more understandable. There is a far more plausible reason: in the experimental apparatus of Kreithen and Keeton (1974), the light that was passed through a polaroid sheet was not depolarized in advance, so that rotating this sheet may have led to intensity modulations (Coemans *et al.* 1990).

Delius *et al.* (1976) suggested that the double cones are responsible for the polarization sensitivity they found in their pigeons. From the results of Montgomery and Heinemann (1952), who reported that the pigeon could not discriminate between polarized light stimuli (projected on a pecking key) with differently oriented ***E***-vectors, Delius *et al.* (1976) inferred that the red field is apparently insensitive to polarized light stimulation. They claimed that this was caused by the sparsity of double cones in this area. However, Hodos *et al.* (1991) found that the double cones outnumber all other types of cone in the red field of the pigeon (except for one pigeon that was over 16 years old), so the latter argument does not make sense.

Young and Martin (1984) proposed a model that predicts that the avian double cone could be involved in polarization sensitivity. Their model is based on seven assumptions, the first of which is that the inner segment, in combination with the oil droplet, acts as a receiving antenna. This is in contrast to the observations of Enoch and Tobey (1981) in the goldfish retina, who found waveguide behaviour in the twin cone outer segment. Because of this, and the simplifications that were assumed, Young and Martin's (1984) model has (as expressed by the authors themselves) a more theoretical than practical value.

There were a number of reasons for testing polarized stimuli whose ***E***-vector was rotating. First, it is known that stimulation with a stationary ***E***-vector can lead to adaptation. Humans can detect polarized light because entopic images (Haidinger's brushes and the analogous Boehm's brushes) become visible. The optimal condition to see these brushes is when the ***E***-vector is rotated because, otherwise, adaptation causes these images to fade away (Vos, 1963). Electrophysiologically, a similar effect has been shown by Leggett (1976) in a crab: he found a response to a rotating ***E***-vector that disappeared when a non-rotating stimulus was given. It could also be argued that

flashes of polarized light constitute a highly unnatural stimulus and that a rotating ***E***-vector does not. The rationale for the latter assertion is the behaviour often observed when homing pigeons are released, for example at the start of a racing contest. Under cloudy conditions, the birds fly around in circles for a while before they apparently decide on the homing direction. Because this behaviour is more pronounced under more heavy overcast skies, we initially hypothesized that, in the absence of a visible sun, this circling is used to scan the celestial polarization pattern (but this reasoning, of course, can also be applied to other environmental cues, such as olfactory information or the earth's magnetic field). Therefore, we conducted a series of experiments using a stimulus whose ***E***-vector orientation rotated at a relatively low speed. The results of these experiments were completely negative, so this hypothesis is not supported.

The ERG is a mass response, consisting of contributions from various elements of the retina, so it is conceivable that a small response, specific to the angle of polarized light, is overlooked. If the double cones were involved in the detection of polarized light, this would have a profound effect on the ERG because they are the most numerous type of cone (Hodos *et al.* 1991). Since we found no such obvious change, two possibilities emerge: either the double cones are not involved in the detection of polarized light, or their contribution is much more complex and refined than that predicted by the model of Young and Martin (1984) or by the model of Cameron and Pugh (1991) and is such that it does not manifest itself in the ERG. In that case, more probing measurements would be called for. The latter case is reminiscent of the above-mentioned experiments of Waterman and collaborators, who were able to demonstrate sensitivity to polarized light in the optic tectal units of goldfish, whereas this was not found in the ERG (Waterman and Aoki, 1974; Waterman and Hashimoto, 1974).

It has been suggested (Phillips and Waldvogel, 1988) that pigeons observe the celestial polarization pattern after sunset and use this information to define the absolute compass directions with reference to their sun compass. Similarly, passerine nocturnal migrants are believed to employ the polarization patterns of the sky that are present at dusk (Able, 1990) or at dawn (Moore, 1986) as a means of orientation.

From these observations, it might be inferred that rods are involved in perceiving polarized light. To verify this idea, we investigated polarization sensitivity in pigeons that had been dark-adapted for at least 40 min. Flashes (0.02 or 0.07 μW; 477 nm; 20 $^{\circ}$ below the area centralis) with a duration of 130 ms were given continuously throughout the experiment. The results of these two experiments provided no evidence for the presence of a rod-based sensitivity to polarized light.

With hindsight, this is not surprising, since all visual cells tend to orient themselves actively so that they point towards the centre of the pupil, thus enabling optimal light catch (Enoch, 1981). This means that the only way scotopic polarization sensitivity could be brought about is excluded, because rods are known to display a dichroic behaviour only when light impinges non-axially (see above).

Behavioural evidence also militates against the possible involvement of rods in polarization sensitivity, because pigeons have a highly diurnal lifestyle and avoid flying at night. If they do fly at night, they adhere to their initial course and appear to avoid landing. Nevertheless, the nocturnal celestial dome is known to contain sources of polarization (for an overview, see Können, 1980). For example, the mesospheric night clouds (80 km above sea level) can emit light that has an exceptionally high degree of polarization (96 %). The ***E***-vector orientation is orthogonal to the position of the sun and, owing to the extreme height of these clouds, this pattern remains visible long after the sun has set. There are limitations to the use of this polarization information, because these clouds are visible only between May and August, provided the sky is clear. Another drawback is that the intensity of the light radiated by these clouds is very low (Können, 1980).

In general, our results indicate that there are no aspects of the pigeon ERG that depend on the polarization state of the stimulus. Our observations are completely opposed to the findings of Delius *et al.* (1976). Because of this, we conducted a series of additional experiments that were set up to mimic the experimental conditions of Delius *et al.* (1976). We exchanged our wedge polarizer for the same type of dichroic sheet (HN38) and used the same order of stimulation (but a series of eight ERGs in response to polarized flashes was always followed by an identical series of eight reference stimuli, whereas Delius and his co-workers did not record responses to unpolarized light flashes). Part of this work is described in Coemans *et al.* (1990). These results did not support the findings of Delius *et al.* (1976). It is difficult to explain this discrepancy. Differences between pigeons may be involved, but since we used 19 pigeons, it is unlikely that they would all have been insensitive to polarized light, whereas Delius *et al.* (1976) reported that four out of five pigeons showed differential ERGs in response to polarized stimulation.

The ***E***-vector-dependency of the shape of the ERG reported by Delius *et al.* (1976) stems from a shift in the latencies of oscillatory potentials. Oscillatory potentials were also present in our recordings. If perception of polarized light were reflected in the ERG in the way suggested by Delius *et al.* (1976), only a very peculiar process, one that locks the latencies of the peaks of the oscillatory potentials, must be assumed to have been operating during our experiments.

We have carried out many experiments (more than 1000 ERGs have been evaluated) and quantified our results, most of which have been tested statistically. We conclude that the electrophysiological observations reported by Delius *et al.* (1976) must have been erroneous.

Because the laboratory behavioural experiments that were alleged to have proved the pigeon's ability to perceive the angle of the ***E***-vector (Kreithen and Keeton, 1974; Delius *et al.* 1976) have not been void of parasitic cues (caused by selective reflection, see Coemans and Vos, 1992), and field experiments do not yield direct proof (Phillips and Waldvogel, 1988), there is no evidence that pigeons are sensitive to polarized light.

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