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RESEARCH ARTICLE

Arctic reindeer extend their visual range into the ultraviolet

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SUMMARY

The Arctic has extreme seasonal changes in light levels and is proportionally UV-rich because of scattering of the shorter wavelengths and their reflection from snow and ice. Here we show that the cornea and lens in Arctic reindeer do not block all UV and that the retina responds electrophysiologically to these wavelengths. Both rod and cone photoreceptors respond to UV at low-intensity stimulation. Retinal RNA extraction and *in vitro* opsin expression show that the response to UV is not mediated by a specific UV photoreceptor mechanism. Reindeer thus extend their visual range into the short wavelengths characteristic of the winter environment and periods of extended twilight present in spring and autumn. A specific advantage of this short-wavelength vision is the use of potential information caused by differential UV reflections known to occur in both Arctic vegetation and different types of snow. UV is normally highly damaging to the retina, resulting in photoreceptor degeneration. Because such damage appears not to occur in these animals, they may have evolved retinal mechanisms protecting against extreme UV exposure present in the daylight found in the snow-covered late winter environment.

Key words: ultraviolet vision, Arctic reindeer, light scatter.

INTRODUCTION

The visual system of most mammals spans a spectral range of approximately 400–700 nm (Kelber et al., 2003; Bowmaker, 2008). Arctic mammals such as reindeer (*Rangifer*) experience extreme photic conditions with long periods of permanent light in summer and darkness in winter. In addition, polar regions have proportionally high levels of environmental UV light because of a high degree of atmosphere (Rayleigh) scatter and reflections from snow and ice (Weatherhead et al., 2007). Thus, diffuse radiation becomes the dominant element in the global radiation pattern in the blue–violet part of the spectrum, contributing to the characteristic blue colouration of the Arctic twilight environment, in mid-day in midwinter and during dusk and dawn in spring and autumn.

If significant amounts of UV light enter the reindeer eye, it is possible that the retina uses this information, although it carries the risk of retinal damage. One reason for thinking that UV vision might be of use to this Arctic mammal is that objects that absorb UV would have high contrast against the highly reflective snow surface. In light of this it is interesting to note that lichens (e.g. Cladinae) that form a key part of the reindeer winter diet do not reflect UV light (Petzold and Godward, 1988).

The aim of this study was to determine the sensitivity of Arctic reindeer to this part of the spectrum. We therefore measured the spectral transmission through their cornea and lens at a range of wavelengths down into the UV. We also recorded the electrophysiological responses from their retina to such stimulation. The results showed that UV light was transmitted through the anterior eye and that the retina responded electrophysiologically to this transmission. We also performed a molecular genetic analysis to determine the type of receptors that are responsible for the UV response.

MATERIALS AND METHODS

Animals

Male Arctic reindeer [*Rangifer tarandus tarandus* (Linnaeus 1758)] 15–20 months old were used in this study. They were purchased from semi-domesticated herds belonging to Sámi pastoralists in Troms and Finnmark counties, Norway (69–70°N). Experiments were conducted at the University of Tromsø (69°46′N) where the animals had *ad libitum* access to concentrate feed and water or snow. They moved freely in large outdoor pens subjected to natural photoperiod and ambient temperature. Experiments were performed in June and December and, because there were no significant differences in results generated at these two time points, the data have been pooled. All electrophysiology experiments took place between 09:00 and 18:00 h. Each trial lasted 3–4 h, including the dark adaptation time, and was limited in duration and therefore in the number of interventions by how long we could keep each animal safely anaesthetised. After completion of experiments, animals were killed by bleeding following a blow to the head using a retractable bolt pistol. Permission to conduct experiments on reindeer was granted by the National Animal Research Authority of Norway.

Measurements of lens and corneal spectral transmission

One eye was removed from five separate animals immediately following death and the cornea and lens were dissected and frozen at –20°C. Subsequently, they were thawed and individually mounted in air and scanned in front of an integrating sphere using a Shimadzu

2101 UVPC spectrophotometer (Shimadzu, Milton Keynes, UK). Transmission at 800 nm was set at 100%. In experiments undertaken for different reasons, cornea and lenses from other ungulates and fish were compared pre- and post-freezing and it was shown that freezing had no significant impact on spectral transmission.

Electrophysiological recordings

Prior to electroretinogram (ERG) recordings, animals (N=18) were captured and given a single intramuscular (i.m.) injection of medetomedine (0.15–0.2 mg kg^{-1}; Domitor®, Orion Corporation, Espoo, Finland), which induced a sustained, light anaesthesia (Tyler et al., 1990). Vital parameters including respiratory rate, body temperature, heart rate, blood oxygenation and eye movement were monitored continuously throughout each trial. Additional intramuscular medetomidine injections (0.05–0.1 mg kg^{-1}) were administered as necessary to maintain adequate anaesthesia. Anaesthesia was terminated by an i.m. injection (scaled dosage) of atipamezol-HCl (0.5–1.0 mg kg^{-1}; Antisedan®, Orion Corporation). All animals regained consciousness and were standing and/or walking within 5–20 min of antidote injection.

The stimulus equipment used for the ERG recordings was specifically built for these experiments as a portable unit and transported from London, UK, and set up in an *ad hoc* laboratory in Tromsø, Norway. The ERG results presented here are part of a wider study designed to investigate seasonal changes in visual sensitivity in reindeer. Anaesthetised animals were dark adapted for a minimum of 30 min and placed on a table on their right side. The left eye was dilated with tropicamide (1%) and phenylephrine (2.5%). Eye position was stabilised with a scleral suture, and a gold foil corneal ERG electrode was placed under the lower lid. A Ganzfeld dome 15 cm in diameter, illuminated by LED arrays, was placed over the left eye. ERGs were recorded to Ganzfeld stimulation at various peak wavelengths [white (420–620 nm), red (625 mn), green (525 nm), blue (450 nm) and UV (372 nm)] within a range of 0.00001–165 µW intensities. The LEDs were driven by a combination of current and pulse width modulation to generate the wide range of intensity control, which was further extended by the use of neutral density filters. The eye was periodically irrigated with a solution of proxymetacaine, methylcellulose and saline. Recordings were made using a computer-based data-acquisition system (Turney et al., 2007). For each stimulus, recordings were made sub-threshold and an intensity series was recorded. The initial response detected in all cases was the scotopic threshold response (STR). This response originates in the retinal ganglion cells and, although driven by the photoreceptors, this is the first normally detectable scotopic response caused by amplification within the neural retina (Seiving et al., 1986). The amplitude and peak time of the STR and the b-wave component (elicited in inner retina/post-receptor) (Dowling, 1970) were measured at each intensity level. Responses to white stimuli were recorded over the full range of intensities (~nine log units) under scotopic conditions, followed by light adaptation and photopic testing. The amplitudes and peak times of both the ERG a-wave (elicited in the photoreceptors) and b-wave components (elicited in the inner retina) were measured at each intensity level to determine the dynamic range of the ERG response (Dowling, 1970). Different photoreceptor types have different temporal characteristics (Hecht and Shlaer, 1936); therefore, the UV response was recorded at various stimulus frequencies (ranging from 1 to 25 Hz) to assess the temporal characteristics of the response to UV stimulation and determine whether this was mediated by rods or cones.

Molecular genetics

Total RNA was isolated from the retina/retinal pigment epithelium of one reindeer, using Tri Reagent (Sigma-Aldrich, Milton Keynes, UK), and mRNA was reverse transcribed into cDNA using Superscript III (Invitrogen, Paisley, UK) with oligo d(T) primer (Invitrogen). Opsin sequences were PCR-amplified using primers designed to the bovine SWS1 opsin sequence. Opsin sequences were expressed *in vitro* using established methods (Carvalho et al., 2006; Cottrill et al., 2009). The full-length coding region of the SWS1 opsin was isolated by PCR using the bovine primers and cloned into the eukaryotic expression plasmid pMT4. The resulting plasmid was used to transiently transfect HEK-293T cells. The recombinant visual pigment was extracted and column purified with the Rho1D4 antibody. Pigment was regenerated by incubation with 11-cis-retinal and analysed using a Spectronic Unicam UV500 dual-beam spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). After three independent recordings, the pigment was bleached by exposure to bright fluorescent light for 30 min, denatured with hydroxylamine and re-analysed. The bleached pigment spectra were subtracted from the dark spectra to produce a difference spectrum and a peak absorbance (λ_{max}) value using standard computer programs. The resulting spectrum was overlaid with visual pigment templates and best-fit spectral curves were obtained.

RESULTS

Spectral transmission of the cornea and lens

The cornea and lens transmitted near UV, with the wavelength of 50% transmission at 322 and 385 nm, respectively (Fig. 1). There was little variation in the transmission characteristics between individual animals. Hence, near UV passes into the reindeer eye.

Electrophysiological recordings from the retina

To determine whether the reindeer retina detects UV and, if so, by what mechanism, ERG responses to UV, blue, green and white stimuli from threshold were recorded. The LEDs chosen were selected with the aim of differentiating between rods (human rod λ_{max}≈498 nm) (Dowling, 1987; Brown and Wald, 1963) and short-wavelength cones (human short-wavelength cones λ_{max}≈420 nm) (Brown and Wald, 1963; Bowmaker, 2008). Although the LED matches for these were not perfect, the stimulator was designed with a view to examine the spectral balance of the retina. The experiment was limited by commercial availability and/or cost and the need to use an LED-based system because of portability. ERGs were established for white light (420–620 nm) as a reference.

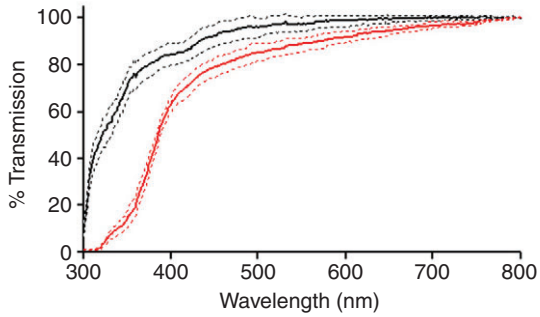

Fig. 1. Spectral transmission profiles for reindeer cornea (black, N=5) and lens (red, N=5). The dotted lines represent ±1 s.d. Both structures transmitted down into the UV to approximately 300–320 nm. The difference between the corneal and lens transmission, with the cornea transmitting more light at nearly all points including UV, is due to its relative thinness in relation to the lens.

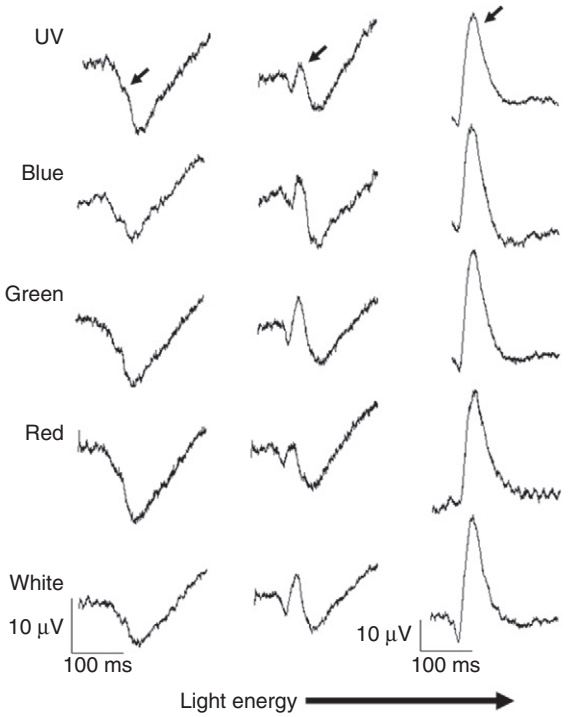

Fig. 2. Electroretinogram (ERG) responses to increasing energy levels of UV, blue, green, red and white light stimulation in a representative reindeer. The b-wave (arrows) develops and increases in amplitude (positivity) with increasing energy. The intensity level required to generate a similar response using white light is approximately six times greater than that for the other stimuli. For each stimulus wavelength, the initial response is a marked negativity followed by an increasingly pronounced positivity (arrow). The response patterns were similar for each stimulus wavelength examined and this pattern was observed in the 18 reindeer tested. The responses shown for each stimulus is predominately within the mammalian rod range.

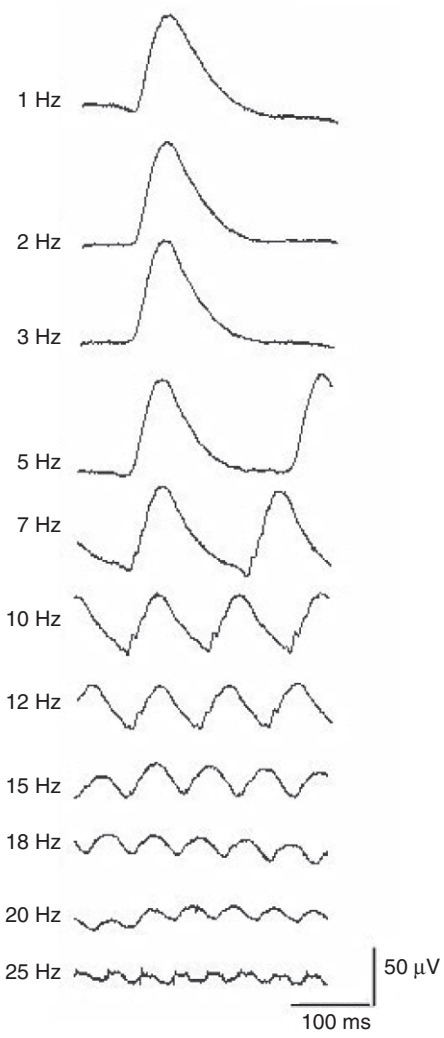

Fig. 3. ERG responses to UV stimulation at different temporal frequencies in reindeer. To reveal which receptor type (i.e. rod or cone) was responding, the temporal response to the UV stimulus was recorded. The temporal frequency characteristics of UV response at low intensity stimulation were traced. Stimulus frequencies range from 1 to 25 Hz. There is no change in b-wave (positive component) amplitude from 1 to 5 Hz. Between 5 and 18 Hz, the amplitude of the b-wave decreases with increasing frequency. Responses can be traced up to 25 Hz (Hogg et al., 2007). The temporal responses of human rods do not go above 18 Hz (Hogg et al., 2007), hence responses at 20 and 25 Hz are those of cone photoreceptors.

The first physiological response detected by the retina at low intensities is the STR, which is the main negative deflection seen in Fig. 2 (column 1). As intensity is increased, a small positive wave appears on the leading edge of the STR; this is the developing b-wave (arrow, upper row, Fig. 2). This increases in prominence and reduces in time to peak until the STR (Fig. 2, column 2) is obliterated by the b-wave (Fig. 2, column 3). The overall response profiles to each of the stimuli were similar. At the low energy levels used to establish threshold, it is usually only the rods that should respond without cone input (Rushton and Powell, 1972; Norby et al., 1984). However, as the green stimulus used was close to the maximum sensitivity of rods, it should elicit a greater response for a given stimulus intensity than that found to blue stimuli at similar low energy levels, but this was not the case. Hence, it is possible that both rod and cone photoreceptors are responding at very low light levels in the UV–blue range.

Human rods and cones have different temporal response characteristics (Hecht and Shlaer, 1936; Kelly, 1974; Hogg et al., 2007). To determine whether UV was detected by rod and/or cone photoreceptors, the temporal characteristics of the UV response at low luminance levels (1.8 µW) were investigated (Fig. 3). These showed no change in b-wave amplitude between 1 and 5 Hz. Beyond 5 Hz there was a steady reduction in b-wave amplitude with increasing stimulus frequency. Human rods do not respond above 18 Hz, but the short wavelength sensitive cones responded up to 25 Hz (Hogg et al., 2007); therefore, it is likely that there is a cone input at these low luminance levels, which at lower frequencies is masked by the rod input. Hence, the UV stimulus is rod mediated at low luminance levels, but short wavelength cones appear to also respond to this stimulus even though it is below their normal threshold (Auerbach and Wald, 1955).

Molecular genetics: cloning and sequencing of the SWS1 opsin gene and spectral analysis of the encoded pigment

A full-length coding sequence for reindeer SWS1 opsin, which encodes the pigment present in short-wavelength cones, was

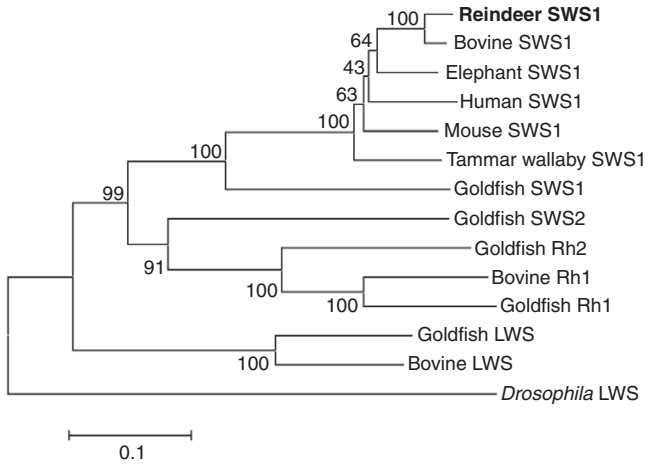

Fig. 4. Phylogenetic tree of rod and cone opsins showing the positioning of the reindeer sequence in the SWS1 lineage. The tree was generated from amino acid sequences by neighbour joining (Saitou and Nei, 1987) using the MEGA phylogenetics package (Kumar et al., 2001). The robustness of each branch point is indicated by the bootstrap values. The scale bar indicates the number of amino acids substitutions per site. The tree was rooted using *Drosophila* Rh1 opsin as an outgroup. GenBank accession numbers: bovine SWS1, NM_174567; bovine LWS, NM_174566; bovine Rh1 (rod), NM_001014890; elephant SWS1, AY686753; goldfish SWS1, D85863; goldfish SWS2, L11864; goldfish Rh2, L11866; goldfish LWS, L11867; goldfish Rh1 (rod), L11863; human SWS1, NM_001708; mouse SWS1, NM_007538; Tammar wallaby, AY286017; *Drosophila* Rh1, NM_079683.

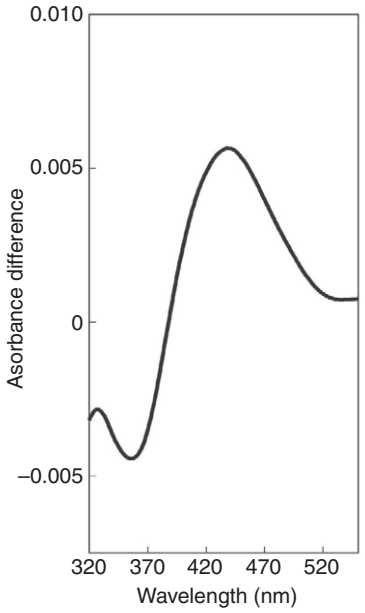

Fig. 5. Difference spectra for the regenerated recombinant reindeer SWS1 pigment. Expression of the reindeer SWS1 opsin protein gave a calculated λ_{max} of 439 nm, showing that the reindeer SWS1 sequence encodes a pigment that absorbs maximally in the violet range of the spectrum.

obtained by PCR amplification using retinal cDNA as a template and primers designed to the sequence of bovine SWS1 opsin. Phylogenetic analysis (Fig. 4) confirmed the identity of this sequence as the reindeer SWS1 orthologue (GenBank accession no. FN808318). The sequence encodes Tyr at site 86 (Cowing et al., 2002; Fasick et al., 2002), which indicates that it will generate a violet-sensitive rather than a UV-sensitive pigment. UV pigments have Phe at site 86. *In vitro* expression (Carvalho et al., 2006; Cottrill et al., 2009) confirmed the absence of a specific UV-absorbing pigment; the resulting pigment, when regenerated with 11-cis-retinal, gave a peak at 439 nm (Fig. 5), which is similar to the peak sensitivity of bovine SWS1 cones at 435 nm (Cowing et al., 2002) and somewhat long-shifted compared with Old World primates at 430 nm.

DISCUSSION

These results reveal that near UV enters the Arctic reindeer eye and that their retinae respond to this electrophysiologically. Genetic analysis reveals that sensitivity to such short wavelengths is not mediated by a separate UV receptor. At low levels it is rod mediated and at higher levels it is probably mediated by short-wavelength cones.

There are two main photoreceptor types in the retina, rods and cones, with the former mediating achromatic vision at low luminance levels and the latter mediating chromatic vision at higher luminance levels. The peak spectral wavelength of the blue stimulus used here (450 nm) is comparable with that of human short-wavelength cones (420 nm) (Brown and Wald, 1963; Dowling, 1987; Bowmaker, 2008). The peak spectral wavelength of the green stimulus (525 nm) is comparable with the peak sensitivity of human rods (498 nm) (Brown and Wald, 1963; Dowling, 1987). LEDs with more specific tuning frequencies were not available at the time of experimentation. Experiments to determine the energy levels required to generate scotopic responses showed similar responses to blue, UV and green stimuli. However, as the level of illumination was in the scotopic range, much smaller energy levels should have been required for the green as this favours rods.

Rods and cones have different temporal characteristics. Human rods do not respond to frequencies above 18 Hz (Hecht and Shlaer, 1936; Kelly, 1974) and short-wavelength-sensitive cones do not respond to frequencies above 25 Hz (Hogg et al., 2007), but long/medium-wavelength-sensitive cones respond to frequencies exceeding 60 Hz (Hecht and Shlaer, 1936; Kelly, 1974). Here, a 1.8 μW UV stimulus was temporally modulated, and the electrophysiogical results suggest that this stimulated rod photoreceptors at these low luminance levels. Had this energy level been in white rather than UV light, it would have been firmly in the lower human rod range. However, it is important to stress that these are human data and not for UV light. As light levels increase and there is a switch to cone function, the role is probably taken over by short-wavelength cones as responses were found up to 25 Hz, which is the response limit of human cones and beyond that of human rods. However, again it is important to stress that these comparisons are between species and white light and UV (Hecht and Shlaer, 1936; Kelly, 1974; Hogg et al., 2007).

Ideally, these experiments would have been extended to include those that generated comprehensive spectral sensitivity functions and measurements of chromatic adaptation. However, the experimenters were limited by the local availability of equipment, the *ad hoc* nature of the experimental conditions and the length of time deemed safe to keep reindeer anaesthetised.

It is known that some rodents (mice and rats), bats and marsupials respond to UV stimulation (Calderone and Jacobs, 1995; Deeb et al., 2003; Winter et al., 2003; Hunt et al., 2009). However, the mechanisms responsible for UV vision and the potential function of this ability are likely to be different between reindeer and rodents for two reasons. First, rats and mice are nocturnal and photophobic, hence their UV exposure is likely to be minimal. Second, mice have

retained the ancestral UV-sensitive form of the SWS1 pigment (Hunt et al., 2001) whereas reindeer, like most mammals, possess an SWS1 pigment that is long-wavelength shifted to be maximally sensitive in the violet region of the spectrum. However, they may share one feature. Although the mouse lens transmits more UV than the reindeer lens (Henriksson et al., 2010), the difference between the two may simply be down to lens size, as the reindeer lens is much larger than that of the mouse. When the lenses of the two animals are compared in terms of their UV transmission per unit volume, differences between the two animals will be much smaller than for the whole lens. Scaling is an issue in image formation, because the light scatter associated with larger eyes, such as in reindeer, may be incompatible with UV sensitivity (Winter et al., 2003). Our result contradicts this, although image-forming difficulties are acknowledged.

The characteristics of the electrophysiological responses to increasing intensities of white light revealed that ERG waveforms in the reindeer were similar to those commonly found in mammals (Turney et al., 2007). However, unlike in most mammals, clear responses to UV stimulation were obtained at a range of intensities. There are few studies of potential ERG responses to UV light in mammals comparable in size and lifestyle to the reindeer. Support for the notion that the responses reported here are related to the UV-rich environment of Arctic reindeer comes from Jacobs et al. (Jacobs et al., 1994), who failed to find any UV response to such stimulation in white-tailed deer (*Odocoileus virginianus*) or fallow deer (*Dama dama*).

The λ_{max} of the reindeer is expected to be similar to that of other mammals, with an α-band around 500 nm, implying that rod receptors would be very insensitive in the UV region of the spectrum. The β-band, however, peaks in the UV (Govardovskii et al., 2000), and so may account for UV sensitivity under scotopic conditions. Such a mechanism has previously been suggested to account for UV sensitivity in a colour-blind phyllostomid flower bat (Winter et al., 2003). At photopic levels, the α-band of the violet-sensitive cone pigment might be expected to confer sensitivity to UV, although the β-band may again be important. An alternative explanation is that reindeer possess two copies of the *SWS1* gene that encode a violet-sensitive and a UV-sensitive pigment, respectively. UV-sensitive pigments are certainly found in mammals (Hunt et al., 2009) but, with just one exception (Tada et al., 2009), duplications of the *SWS1* gene are non-existent amongst vertebrates and there was certainly no evidence for a second *SWS1* transcript in reindeer retinal mRNA. A final possibility is that UV sensitivity is conferred by a sensitising pigment, as was found in a deep-sea dragon fish (Douglas et al., 1999). Such pigments have not been reported outside dragon fish and the fish pigment operates in the far-red not the UV region of the spectrum.

UV light is relatively abundant at high latitudes because of its high atmospheric (Rayleigh) scatter resulting from the low position of the sun on the horizon. Because of this wavelength-dependent scatter, diffuse radiation is greater in the UV than in the human visible spectrum and is the dominant component of global Arctic irradiance (Henriksen et al., 1989). Further, snow and ice surfaces may reflect as much as 80% of atmospheric UV. This is particularly marked in late winter and spring when the ground is snow covered and day length is rapidly increasing (Weatherhead et al., 2007). During the mid-winter day when the sun remains below the horizon, and during spring and autumn hours of twilight, the available light is exclusively Rayleigh scattered and the higher energy shorter wavelengths predominate. Under these circumstances, near UV forms a significant proportion of the available illumination.

There are two obvious potential advantages for reindeer extending their visual range into the UV. First, one key food item, lichens of the genus *Cladonia*, displays strong absorption in the UV relative to its absorption in other visible wavelengths (Petzold and Goward, 1988). Furthermore, it has also been shown that wolf and white fur generally exhibits low UV reflectance (Reynold and Lavigne, 1981; Lavigne and Øritsland, 1974). Thus, for the reindeer, both their preferred feed and their main predators appear with enhanced contrast against general UV-reflecting backgrounds. An additional potential benefit of UV sensitivity may be related to the fact that the UV reflectance of snow changes with the quality of its surface (Meinander et al., 2008), which could be of importance for the reindeer both in foraging and local movement on what may otherwise appear to be a bland surface.

Exposure to UV can produce a photokeratitis known as snow blindness where the cornea suffers a form of sunburn (Hemmingsen and Douglas, 1970; Collier and Zigman, 1987). There is no evidence for snow blindness in Arctic mammals, and the explanation for this is unknown. The presence of scavenging components such as ascorbic acid (Ringvold, 1980) and seasonal changes in the cornea (Ringvold et al., 2003) have been suggested as ameliorating factors. That the anterior eye of this animal is permissive to UV also raises the question of why this radiation does not damage the neural retina. In relation to this, it is interesting to note that no Arctic mammal appears to display photophobia or attempt to avoid light exposure, behaviours that are typically found in UV-sensitive rodents.

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