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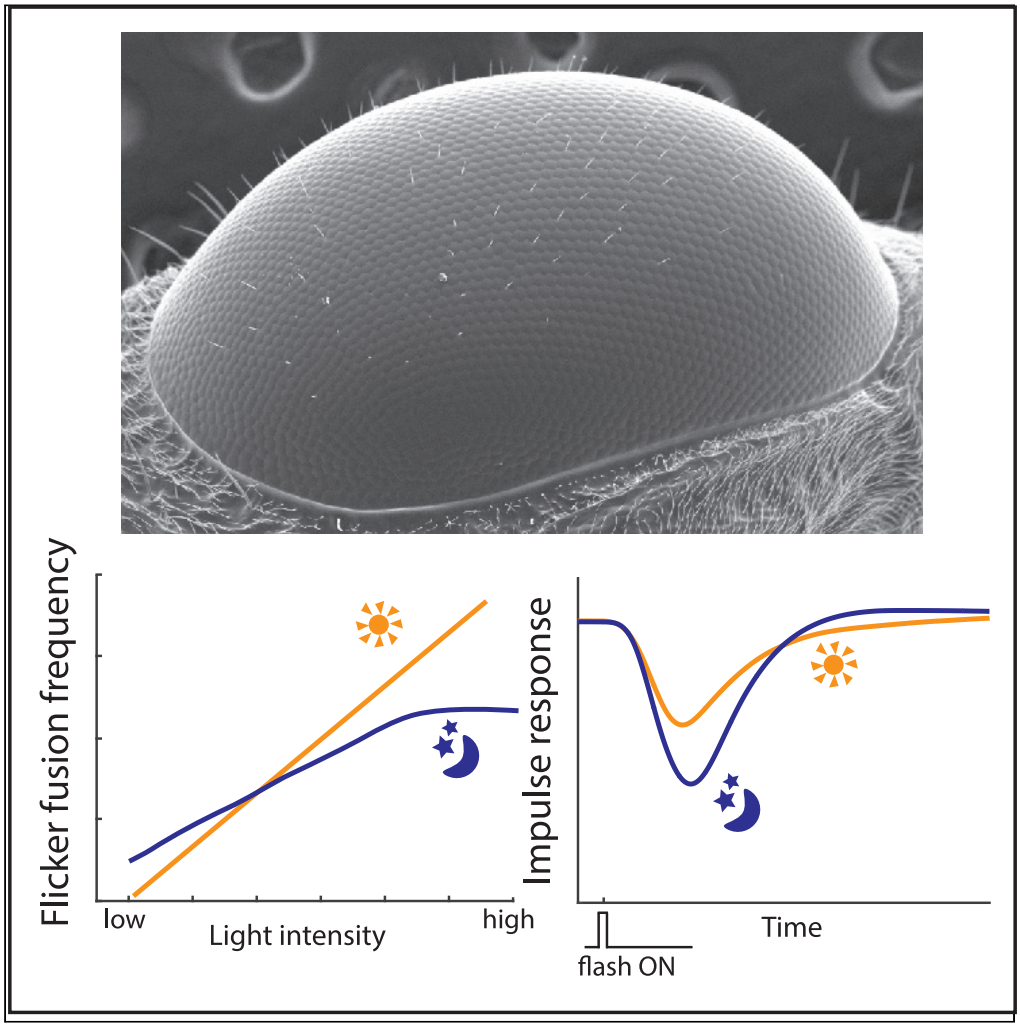

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Highlights

Flicker fusion frequency is lower in nocturnal ants compared to diurnal ants

Latency and duration of the impulse response shorten at night in nocturnal ants

In ants, the FFF is not predicted by the measured impulse response characteristics

Ogawa et al., iScience *25*, 104134
April 15, 2022
https://doi.org/10.1016/j.isci.2022.104134

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Nocturnal *Myrmecia* ants have faster temporal resolution at low light levels but lower adaptability compared to diurnal relatives

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SUMMARY

Nocturnal insects likely have evolved distinct physiological adaptations to enhance sensitivity for tasks, such as catching moving prey, where the signal-noise ratio of visual information is typically low. Using electroretinogram recordings, we measured the impulse response and the flicker fusion frequency (FFF) in six congeneric species of *Myrmecia* ants with different diurnal rhythms. The FFF, which measures the ability of an eye to respond to a flickering light, is significantly lower in nocturnal ants (~125 Hz) compared to diurnal ants (~189 Hz). However, the nocturnal ants have faster eyes at very low light intensities than the diurnal species. During the day, nocturnal ants had slower impulse responses than their diurnal counterparts. However, at night, both latency and duration significantly shortened in nocturnal species. The characteristics of the impulse responses varied substantially across all six species and did not correlate well with the measured flicker fusion frequency.

INTRODUCTION

At night, the ambient light intensity can be over 100 million times lower than on a bright sunny day, which severely limits the signal to noise ratio of visual information (Land and Nilsson, 2012; O'Carroll and Warrant, 2017). Animals active at night thus must develop strategies to improve visual sensitivity. Eyes of nocturnal animals have indeed evolved to match the absolute sensitivity and spatial resolving power to the ambient light intensities they typically experience and to the tasks they carry out (e.g., Narendra et al., 2011; Narendra et al., 2017; Snyder et al., 1977; Warrant, 1999).

One strategy to enhance the eye's sensitivity is through anatomical adaptations by developing larger lenses, wider and longer rhabdoms, and a reflective tapetum that doubles the light path to improve light capture (Greiner, 2006; Narendra et al., 2011; Somanathan et al., 2009; Warrant, 1999; Warrant and McIntyre, 1993). These anatomical adaptations, however, may come at the expense of spatial resolving power. This can be problematic because animals require adequate spatial resolving power to discriminate small objects to capture prey or to pinpoint specific locations. Another strategy is to modify the physiological properties of the eye. Increasing the integration time of photoreceptors, for instance, improves photon capture, signal to noise ratio, and contrast discrimination (Frederiksen et al., 2008). This is analogous to having a longer shutter speed in a camera: a longer visual integration time makes the world brighter and improves the reliability of images in dim light (Narendra et al., 2013c; Nørgaard et al., 2008). But this comes at the expense of temporal resolution, which makes it difficult to perceive spatial detail while moving (Srinivasan and Bernard, 1975), and fast-moving objects will appear blurry (Warrant, 1999). With these strategies, animals trade-off spatial and temporal resolving power for increased light sensitivity (Warrant, 1999; Warrant and McIntyre, 1993).

The temporal characteristics of ant's eyes have evolved in accordance with their lifestyles (de Souza and Ventura, 1989). For example, the fast-moving *Pseudomyrmex phyllophilus* has a photoreceptor response duration of approximately 15 ms compared to the slow-moving *Camponotus rufipes* and *Atta sexdens rubropilosa* (40–46 ms) when recorded in a dark-adapted state during the day (de Souza and Ventura, 1989). Although *P. phyllophilus* displays visual avoidance behavior when confronted with an obstacle while moving, *C. rufipes* and *A. sexdens rubropilosa* seem incapable of visually perceiving objects placed in their

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https://doi.org/10.1016/j.isci.2022.104134

path (de Souza and Ventura, 1989). Similar to other insects, ants that move faster and are active at a bright light are likely to have faster photoreceptors and high temporal resolving power (de Souza and Ventura, 1989; Howard et al., 1984).

The anatomical and physiological characteristics of eyes are often modulated by circadian rhythms as well (Arikawa et al., 1987; Brodrick et al., 2021; Horridge et al., 1981; Menzi, 1987; Meyer-Rochow, 1999). Common diurnal changes in eyes include the migration of retinal screening pigments (Arechiga et al., 1990; Bennitt, 1932; Bruin and Crisp, 1957; Hariyama et al., 1986), changes in size of the light-sensitive structure (Chamberlain and Barlow1987; Dearry and Barlow, 1987; Williams, 1982a), and increasing the acceptance angle of photoreceptors for improving light capture at night (Hariyama et al., 2001; Leggett and Stavenga, 1981). Such diurnal changes typically result in a daily modulation of photoreceptor response amplitudes, i.e., larger amplitude at night compared to the day, which enhances sensitivity in many nocturnal animals (e.g., *Camponotus* ants (Menzi, 1987), beetle (Jahn and Wulff, 1943), cockroach (Wills et al., 1985), crickets (Tomioka and Chiba, 1982), horseshoe crab (Barlow, 1983), isopod (Hariyama et al., 1986), and crayfish (Larimer and Smith, 1980)).

Ants of the genus *Myrmecia* are an ideal system to study the temporal characteristics of photoreceptors because closely related and sympatric species are active at different times of the day. Specifically, different species range from being strictly diurnal to diurnal-crepuscular to being exclusively nocturnal (Greiner et al., 2007; Narendra et al., 2011, 2017). Both diurnal and nocturnal ants forage individually and rely on vision to hunt large prey (Narendra et al., 2013a; Reid et al., 2013). Diurnal *Myrmecia* ants tend to have a smaller body size, with some species being monomorphic, whereas nocturnal species are relatively larger and usually polymorphic (Sheehan et al., 2019). Diurnal *Myrmecia croslandi* tend to walk faster (5–9 cm s^{-1}) (Zeil et al., 2014) compared to nocturnal *Myrmecia pyriformis* (0.9–8 cm s^{-1}) (Narendra et al., 2013c). All *Myrmecia* ants, regardless of their preferred activity time, possess a pair of apposition compound eyes. Nocturnal ants have larger lenses (38μm diameter) and wider rhabdoms (5.9μm) than diurnal ants (~22 and ~1.3μm respectively) (see Table 1 in Narendra et al., 2017). These differences lead to a 27-fold increase in optical sensitivity (Greiner et al., 2007; Narendra et al., 2011, 2017). Behavioral and anatomical investigations suggest that workers of the diurnal *Myrmecia gulosa* have smaller interommatidial angles of 1.7° (Via, 1977) than the nocturnal *M. pyriformis* which has an estimated interommatidial angle of 2.1° in the medio frontal eye region (Reid, 2010). Electrophysiological measurements revealed that spatial resolving power was 0.57 cycles per degree (cpd) in the nocturnal *Myrmecia midas*, whereas it was 0.60 cpd in the diurnal *Myrmecia tarsata*. The spatial resolving power is associated with ommatidial facet diameters, which were larger in the nocturnal *M. midas*. Interestingly, the contrast sensitivity functions do not differ between diurnal and nocturnal ants, which are determined by a combination of spatial resolution and sensitivity, the amount of light absorbed by each photoreceptor (Ogawa et al., 2019).

In addition to the aforementioned optical adaptations, *Myrmecia* ants possess a range of pupillary mechanisms that control photon capture (Narendra et al., 2016). These ants have a variable primary pigment cell pupil that constricts the crystalline cone to control light flux (Narendra et al., 2016). In the nocturnal *M. pyriformis,* the closing of this aperture is light-dependent but also undergoes a circadian rhythm and only fully opens at night. This suggests the improved eyes' sensitivity to maintain reliable vision for foraging and navigation at night (Freas et al., 2018; Jayatilaka et al., 2018; Narendra et al., 2013b, 2013c, 2017; Raderschall et al., 2016). The strictly diurnal *M. croslandi* lacks this primary pigment cell pupil (Narendra et al., 2016). In the day-active ants, bright light conditions force migration of the retinular cell pigments toward the rhabdom. It is unknown at this stage whether the increased contrast sensitivity in the nocturnal species is fully explained by the anatomical adaptation alone as well as the diurnal physiological modulation that might affect their temporal resolution.

Here, we aim to identify whether nocturnal *Myrmecia* ants employ physiological adaptations to increase their sensitivity in dim light conditions. For this, we compared the temporal response properties of diurnal and nocturnal *Myrmecia* species both at day and night. We used electroretinography to measure the impulse response, the response to a brief flash of light, at six different intensities to evaluate the diurnal modulation of sensitivity and estimate the temporal characteristic of the photoreceptor response. We also investigated their critical flicker fusion frequency (cFFF) to understand the ability of these different species to see fast motion. The FFF was the maximum frequency of a flickering light at which a retinal response

could be recorded with an electroretinogram (ERG) and was determined by taking measurements at eleven different light intensities.

RESULTS

We studied workers of six species of *Myrmecia* ants, ranging from being predominantly diurnal (*M. croslandi*, *M. tarsata*, and *M. gulosa*) to strictly nocturnal (*M. pyriformis*, *M. midas*, and *Myrmecia vindex*) (Greiner et al., 2007; Narendra et al., 2010; Ogawa et al., 2015; Sheehan et al., 2019). The temporal response characteristics of photoreceptors of each ant species were determined by measuring the impulse response and the flicker fusion frequency (FFF) using Electroretinograms (ERGs) at different times of the day and at different light intensities.

We recorded stable and repeatable ERGs from both diurnal and nocturnal *Myrmecia* species at different times of the day. The response amplitude to a 1ms flash of light increased strongly with increasing light intensity for all species (Figure 1; Table 1A). There was a clear interaction between preferred activity time and recording time (Table 1D).

First, we compared the four characteristics of the impulse response: response amplitude, response latency, time to peak, and response duration, change at times of the day in diurnal and nocturnal *Myrmecia* species (Table 2).

Response amplitude

We recorded the impulse responses to the brightest light intensity (5.81×10^{-5} W/cm^{-2}; Figure 2) and to a flash three log units dimmer (Figure 3). Measuring impulse response at the brightest light intensity revealed a striking difference between day and night in two nocturnal *Myrmecia* species. We found a significant interaction between recording time and activity time on the response amplitude to the bright flash (Figure 2; Table 3C). This effect is driven by the larger responses at night compared to responses in the daytime in two nocturnal species: *M. midas* and *M. vindex* (Figures 2E, 2F and 4A, post-hoc test, *M. midas*: $z = 5.1$, $p < 0.001$; *M. vindex*: $z = 5.9$, $p < 0.001$). In contrast, there was no significant difference in response amplitudes between during the day and at night in the diurnal species (Figures 2A–2C and 4A, post-hoc test, $z = 0.91$, $p = 0.76$) and the nocturnal *M. pyriformis* (Figures 2D and 4A, post-hoc test, $z = 1.56$, $p = 0.53$).

In addition, the response amplitudes did not differ between diurnal and nocturnal species, both during the day (post-hoc test, $z = -0.27$, $p = 0.99$) and at night (post-hoc test, $z = 2.17$, $p = 0.11$). However, the response amplitudes measured during the species' preferred activity time — during the day in diurnal species and during the night for nocturnal species — was significantly larger in the nocturnal species

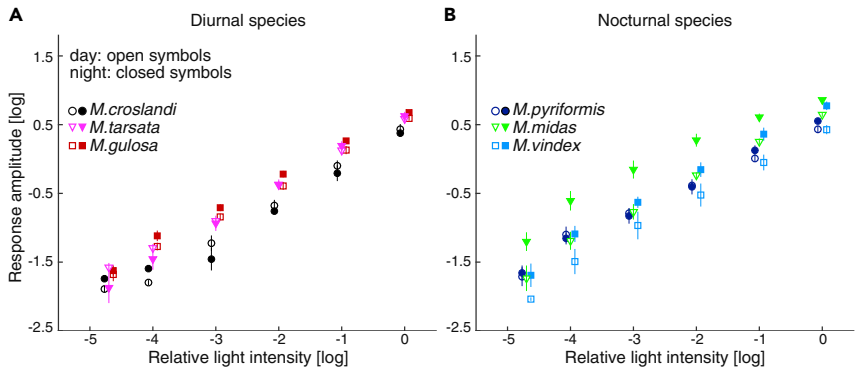

Figure 1. The impulse response amplitude in six *Myrmecia* ant species

The response amplitude to a 1ms flash of light increases with increasing light intensity in diurnal (A) and nocturnal (B) *Myrmecia* ant species. Data points show mean ± SEM ERG response amplitudes to 1ms flashes of light were measured during day (open symbols) and at night (closed symbols) across six different light intensities. Only in *M. midas* and *M. vindex* the curves are significantly different between day and night. Data points for each species are slightly shifted from actual light intensities for clarity. Maximum light intensity was 5.81×10^{-5} W/cm^{-2}.

Table 1. Results of the linear mixed-effects model analysis for testing the relationship between the amplitude of impulse response, stimulus intensity, recording time, and the species preferred activity time

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) **Light intensity of stimuli**	1	−452.45	470.68	<0.001
B) Activity time (diurnal or nocturnal)	1	−451.74	1.43	0.23
C) **Recording time (day or night)**	1	−444.42	16.07	<0.001
D) **Activity time: Recording time**	1	−443.76	11.74	<0.001

[a]Final Model: Peak response amplitude ~ Light intensity of stimuli + Recording time + Activity time: Recording time + (1|species/animal ID). Bold terms had a significant effect and were part of the final model.

(Figure 4A magenta boxes in diurnal species vs. gray boxes in nocturnal species, post hoc test, z = 2.61, p = 0.04).

Differences in response amplitudes to dim flashes mirrored those for bright flashes (Figure 5A; Table 4). Notably, the response amplitude was significantly larger in *M. midas* at night (post hoc test, z = 6.43, p < 0.001).

Response latency

There was a clear interaction between recording time and preferred activity time on the response latency to the bright flash (Figure 4B; Table 5C), which was driven by two nocturnal species *M. pyriformis* and *M. vindex* (Figure 4B, post hoc test, *M. pyriformis*: z = 2.64, p = 0.05; *M. vindex*: z = 5.07, p < 0.001), whose response latency was much shorter at night. Response latency did not vary between day and night in diurnal species (post hoc test, z = −0.86, p = 0.79) and in the nocturnal *M. midas* (post hoc test, z = −0.86, p = 0.79).

In addition, response latency neither did differ between diurnal and nocturnal species at night (post hoc test, z = 0.72, p = 0.88) nor between the species' preferred activity times (post hoc test, z = −0.12, p = 0.99), as it became shorter in nocturnal species at night. However, during the day, response latency was significantly shorter in diurnal species (1.7–2.5 ms) compared to nocturnal species (post hoc test, 6.1 to 9.1 ms, z = −5.19, p < 0.001).

Differences in response latency to dim flashes mirrored those for bright flashes (Figure 4B; Table 6).

Time to peak

There was a significant interaction between recording time and preferred activity time on the timing of the peak response to the bright flash (Figure 4C, Table 7C). The difference was mostly driven by the nocturnal ant *M. vindex* during the day and the slow response of the diurnal ant *M. gulosa* at night (Figure 4C, post hoc test, *M. vindex*: z = 6.16, p < 0.001; *M. gulosa*: z = −3.32, p < 0.01).

Table 2. Summary characteristics of the impulse response at the brightest intensity of flash light in six species of *Myrmecia* ants

	peak amplitude (mV)		latency (mS)		time to peak (mS)		duration (mS)	
	Day	night	day	night	day	night	day	night
M. croslandi	−2.7 ± 0.4	−2.4 ± 0.2	2.5 ± 0.5	6.5 ± 1.3	28.7 ± 1.3	30.3 ± 1.7	40.2 ± 5.9	28.3 ± 1.1
M. tarsata	−3.8 ± 0.5	−4.2 ± 0.5	1.7 ± 0.5	0.9 ± 0.5	31.2 ± 1.9	29.0 ± 1.5	33.5 ± 2.2	28.6 ± 1.3
M. gulosa	−3.9 ± 0.3	−4.7 ± 0.3	2.0 ± 1.0	2.4 ± 0.7	31.5 ± 0.8	39.7 ± 0.7	33.6 ± 1.6	39.6 ± 1.1
M. pyriformis	−2.7 ± 0.3	−3.6 ± 0.4	8.3 ± 0.8	3.7 ± 1.9	46.8 ± 2.9	46.2 ± 0.9	49.8 ± 3.2	44.9 ± 1.4
M. midas	−4.4 ± 0.3	−7.3 ± 0.4	6.1 ± 1.2	2.7 ± 1.8	39.0 ± 1.5	35.6 ± 2.0	42.2 ± 2.4	40.3 ± 1.8
M. vindex	−2.7 ± 0.4	−5.9 ± 0.8	9.1 ± 3.0	0.2 ± 1.5	49.7 ± 4.6	33.8 ± 1.6	48.6 ± 4.7	33.0 ± 0.2

All values are mean ± SE

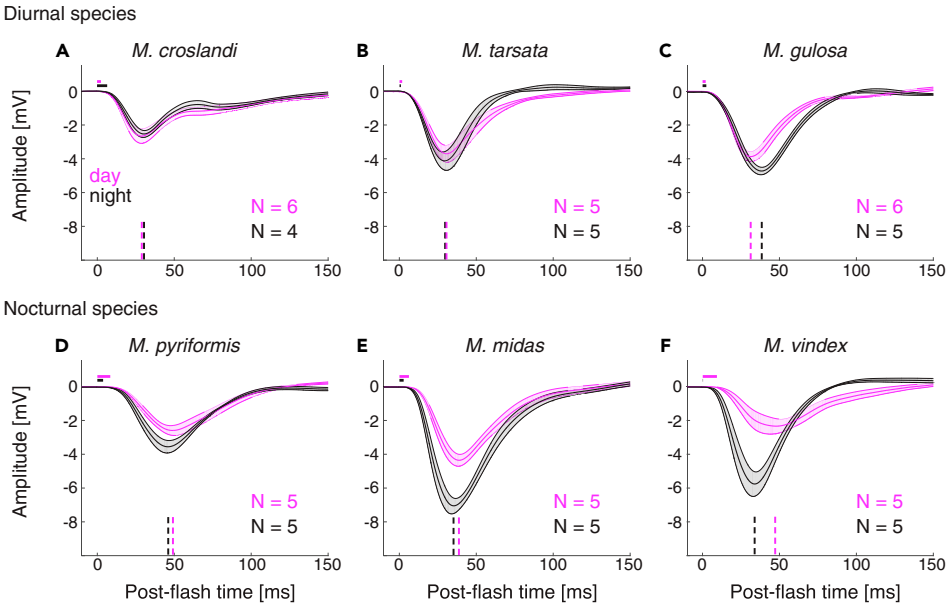

Figure 2. Circadian rhythm modulations in the four characteristics of the impulse response to a bright light in six species of *Myrmecia* ants

Diurnal species are shown in (A-C) and nocturnal are in (D-F). Shaded areas show (mean ± SEM; N, sample number). Impulse responses are shown during the day (magenta) or at night (black). At night, response amplitudes in nocturnal species are typically larger compared with their diurnal relatives. Horizontal solid lines above the curves indicate response latency. Dash vertical lines indicate the time to peak amplitude. Flash intensity: 5.81×10^{-5} W/cm^{-2}.

There was a clear overall effect of slower times to peak in nocturnal species, during the day (post-hoc test, $z = -5.06$, $p < 0.001$) and between the species' preferred activity times (post-hoc test, $z = -2.78$, $p = 0.02$). However, the time to peak was not significantly different between nocturnal and diurnal species at night (post hoc test, $z = -1.81$, $p = 0.24$).

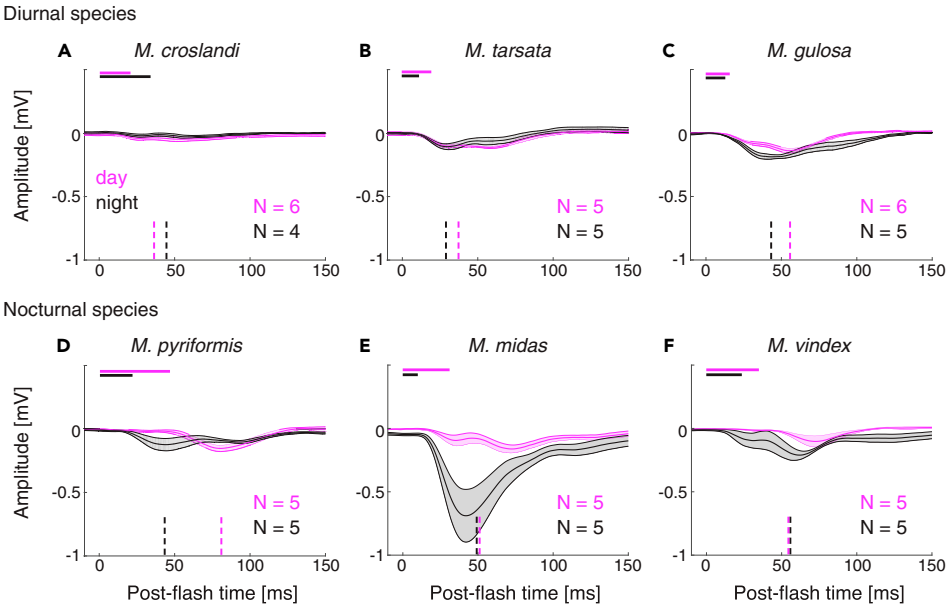

Figure 3. Circadian rhythm modulations of the impulse response to a dim light are detectable in nocturnal species

3-log dimmer light intensity than in Figure 2 is used. Details are as outlined in the legend of Figure 2. The response amplitudes to dimmer stimuli are typically larger in nocturnal species compared with diurnal relatives.

Table 3. Results of the linear mixed-effects model analysis for testing the effects of recording time and activity time on the response amplitude of the impulse response for bright lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) Activity time (diurnal or nocturnal)	1	−109.08	0.97	0.32
B) **Recording time (day or night)**	1	−100.88	17.37	<0.001
C) **Activity time: Recording time**	1	−94.05	12.79	<0.001

[a]Final Model: Peak response amplitude to bright lights ~ Recording time + Activity time: Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

Measurements of time to peak were too noisy to be analyzed for the dim flashes, especially in diurnal species (Figure 3). However, there was a clear delayed response in the nocturnal *M. pyriformis* and in *M. midas* during the day (Table 8; Figure 5C, post hoc test, *M. pyriformis*: $z = 4.64$, $p < 0.001$; *M. midas*: $z = 3.31$, $p < 0.01$).

Response duration

On an average, the duration of the impulse response was longer in nocturnal species (Figure 4D; Table 9A) and longer during the day for all species (Table 9B; Figure 4D). This effect was driven mostly by the diurnal ant *M. croslandi* (post-hoc test, $z = 3.1$, $p = 0.01$) and the nocturnal *M. vindex* (post hoc test, $z = 4.12$, $p < 0.001$, Figure 4D). There was no interaction between recording time and preferred activity time of species on the response duration (Table 9C). The response duration was not significantly different from the species' preferred activity times (post hoc test, $z = -1.27$, $p = 0.55$).

Measurements for response duration were too noisy to be analyzed meaningfully for the dim flashes (Figure 3).

Temporal resolution

The temporal resolution was determined by measuring the flicker fusion frequency (FFF) at various light intensities. The FFFs increased with light intensity for all species at all times of the day (Figure 6). The maximum FFFs for the brightest stimulus were significantly higher in diurnal species (142.5–188.7 Hz) compared to nocturnal species (72.1–125.2 Hz; Table 10; Table 11B). There was no difference in FFFs between daytime and nighttime recordings in any species (Table 11C). The FFFs for the three log unit dimmer stimuli were not significantly different between diurnal and nocturnal species (Table 12). The slopes of FFFs were steep in diurnal species compared to nocturnal species at the three log unit dimmer stimuli (Figure 6).

DISCUSSION

When stimulated with a brief flash of light, the response properties of photoreceptors in *Myrmecia* ants vary between diurnal and nocturnal species. Circadian modulations in the four characteristics of the impulse response are significant predominantly in the nocturnal species. Notably, the latency and duration of response in the nocturnal species were longer during the day but shorter during the night. The shorter responses in the nocturnal species at night resulted in the similar characteristics of the impulse response between diurnal and nocturnal species at their preferred activity times. There was a trend of faster temporal characteristics in the diurnal species during the day compared with their nocturnal relatives, similar to that seen in some Hymenoptera (Frederiksen et al., 2008) and Lepidoptera (Chatterjee et al., 2020). This has also been found in mesopelagic crustaceans that exhibit a distinct correlation between eye's temporal resolution and relative intensity differences in their light environments (Frank, 1999). However, although there is an overall trend across the species, a striking result of our experiment is the high variability with which each species modifies their response characteristics with time of day and adaptation state. Each species seems to have a different set of characteristics which suggests that different selective pressures act on different aspects of their impulse response properties. Importantly, the effects of all these changes lead to consistent differences in the FFF across both nocturnal and diurnal species.

Flicker fusion frequency (FFF) of six *Myrmecia* ants

Flicker fusion frequency reflects an integration of all measures seen in the impulse response and measures the speed of the visual system. Typically, visual systems of animals active in brightly lit conditions have faster phototransduction and higher temporal resolution compared to slowly moving animals or those

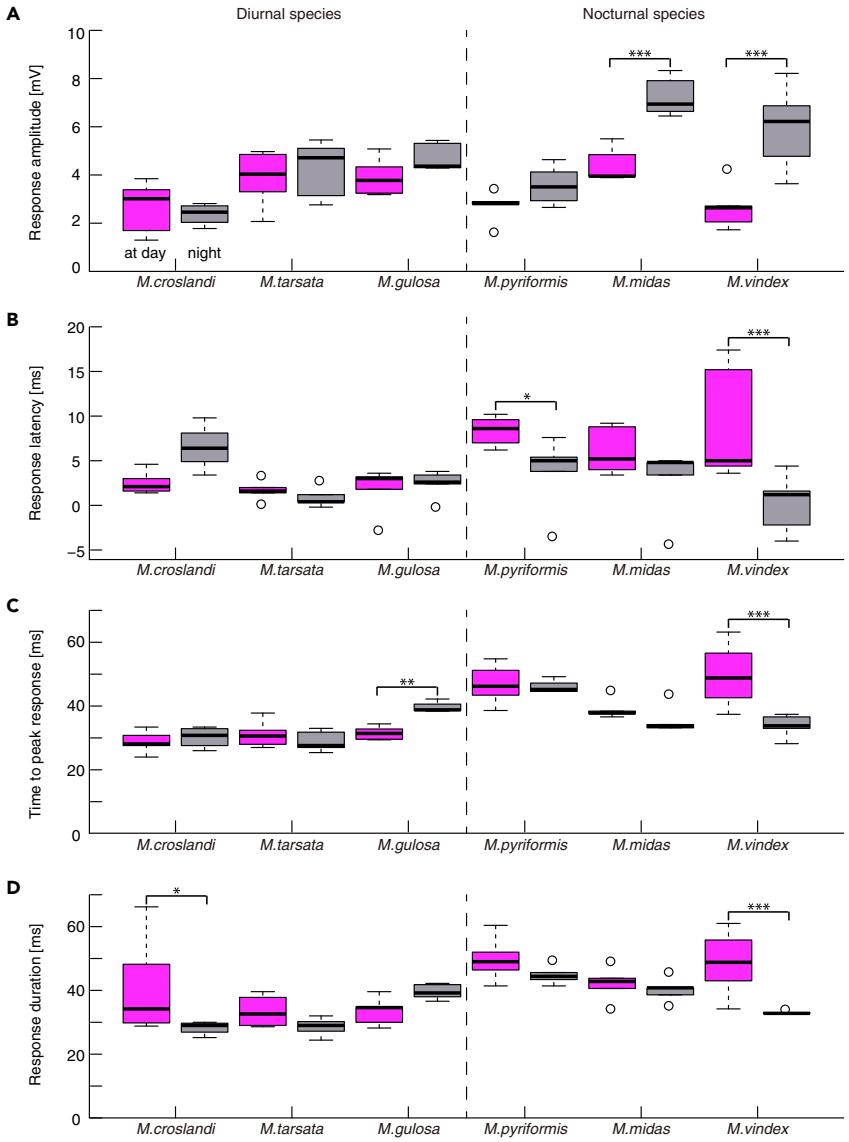

Figure 4. Temporal characteristics of impulse responses to bright lights during the day (magenta) or at night (gray) in three diurnal and three nocturnal *Myrmecia* ants

(A–D). This summary figure is based on the data shown in Figure 2. Variation in (A) response amplitude, (B) response latency, (C) time to peak, and (D) response duration of impulse responses. Boxplots show medians (thick lines), 25th and 75th percentile, whiskers (90th and 10th percentiles), and outliers (circles). Asterisks (*) indicate statistically significant differences: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

active in dimly lit conditions (Boström et al., 2017; Frank, 1999; Fritsches et al., 2005; Healy et al., 2013; Jensen and Swenson, 1974; Ryan et al., 2017; Warrington et al., 2017). Therefore, fast responses with an FFF up to 300 Hz are found in bees and flies and slow responses with an FFF of about 20 Hz are found in locusts and crickets (Autrum, 1958). In the pedestrian *Myrmecia* ants, the cFFF, which is the FFF to the highest light intensity, was lower in nocturnal animals (~125.2 Hz) compared to their diurnal relatives (~188.7 Hz).

Nocturnal *Myrmecia* ants appear to have tuned their FFFs for very low light intensities. At the dimmest light intensities, they achieve a faster FFF than the diurnal species (31.6–48.2 Hz in nocturnal species at night versus 10–31.3 Hz in diurnal species at day, Figure 6). However, they are less able to adjust their FFF with increasing light, indicated by the shallower slope in the relationship between light intensity and FFF. Therefore, the diurnal species reach higher FFF at brighter light intensities (Figure 6). This suggests

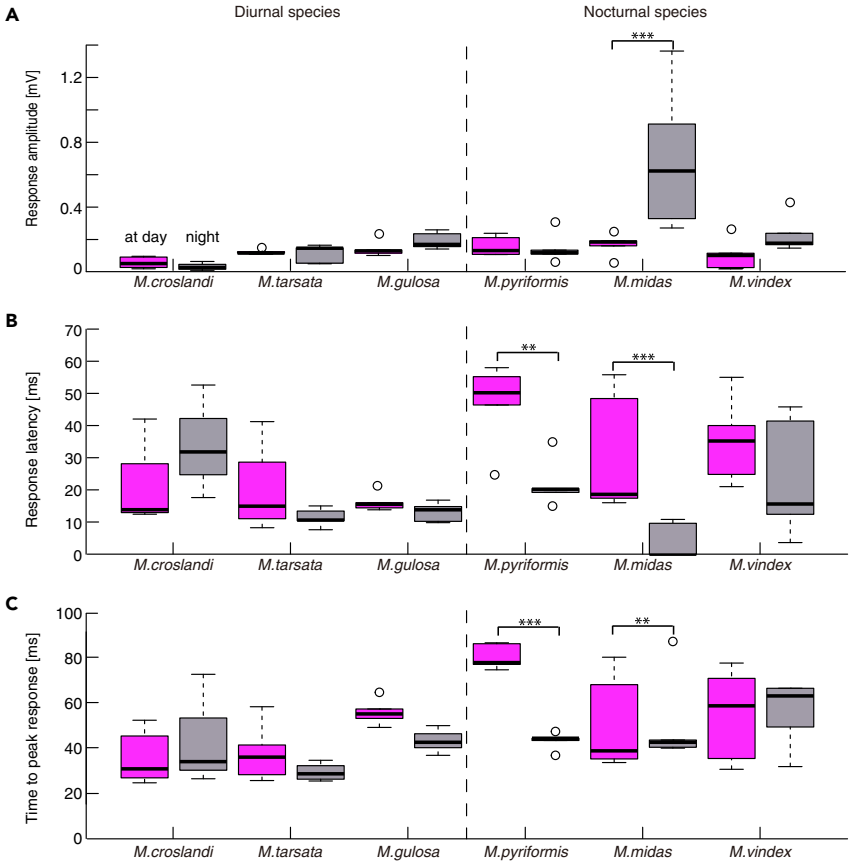

Figure 5. Temporal characteristics of impulse responses to dim lights during the day (magenta) or at night (gray) in three diurnal and three nocturnal *Myrmecia* ants

(A–C). The summary figure is based on the data shown in Figure 3. Variation in (A) response amplitude, (B) response latency, and (C) time to peak. Details as outlined in legend of Figure 4. No quantitative analysis was possible of response duration due to small signal sizes. Asterisks (*) indicate statistically significant differences: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

that there is a constraint on how adaptable the FFF is for different light intensities and there is a trade-off between the adaptability of the FFF and its speed at low light intensities.

For relative comparisons on FFFs across species, we used the same procedure and threshold in the present study. The absolute numbers for the FFFs may need to be calibrated against behavioral experiments.

Comparison of the time to peak with other insects

The speed of the photoresponse in *Myrmecia* ants is relatively fast even though they are relatively slow moving (Howard et al., 1984). For example, the diurnal *Myrmecia* had relatively faster photoreceptors

Table 4. Results of the linear mixed-effects model analysis for testing the effects of recording time and activity time on the response amplitude of the impulse response for dim lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) Activity time (diurnal or nocturnal)	1	13.52	2.45	0.12
B) **Recording time (day or night)**	1	15.51	6.42	0.01
C) **Activity time: Recording time**	1	19.32	5.32	0.02

[a]Final Model: Peak response amplitude to dim lights ~ Recording time + Activity time: Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

Table 5. Results of the linear mixed-effects model analysis for testing effects of recording time and activity time on the latency of impulse response for bright lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) **Activity time (diurnal or nocturnal)**	1	−166.908	5.593	0.02
B) **Recording time (day or night)**	1	−167.052	5.304	0.02
C) **Activity time: Recording time**	1	−156.441	14.873	<0.001

[a]Final Model: Latency of impulse response to bright lights ~ Activity time + Recording time + Activity time: Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

with time to peak of *ca*. 30 ms compared to hoverfly *Eristalis tenax* (38 ms), locust *Locusta migratoria* (55 ms), and housefly *Musca domestica* (41 ms) in the dark-adapted state (Howard et al., 1984). In addition, it is known that sit and wait hunters such as the nocturnal spider *Cupiennius salei* have slow photoreceptors with time to peak exceeding 100 ms (Pirhofer-Walzl et al., 2007). The interspecies variation of time to peak amplitudes is small in *Myrmecia* ants and was comparable between diurnal and nocturnal *Myrmecia* ants. It is perhaps because diurnal and nocturnal *Myrmecia* species have similar foraging behavior where they both capture fast flying or walking insects and spiders.

Impulse response characteristics and FFFs

The temporal resolution of an eye is thought to be determined by how fast photoreceptors respond to the flickering light. Therefore, we expected FFFs to have a strong link with the temporal characteristics of impulse response. For example, the short latency, time to peak, and duration should result in a fast FFF. Although we found shortened latency and slower time to peak of the impulse response in nocturnal *Myrmecia* ants during the night (Figure 2, Figures 4B and 4C), the FFFs in nocturnal species were not different between day and night (Figure 6). This suggests that the initial speed of the impulse response and time to peak do not reflect the FFFs. This is clearly evidenced in the diurnal *M gulosa*, which had a longer response duration and a longer time to peak during the night but a higher FFF.

Variability between species

Myrmecia ants appear to combine different anatomical and physiological mechanisms to adjust their eye's sensitivity according to species specific demands on top of their preferred activity time. There are strong and significant variations in the four characteristics of impulse response among both diurnal and nocturnal species. Interestingly, those characteristics become more similar at each species' preferred activity time. However, the observed variation and the fact that the impulse characteristics change between day and night but the FFFs don't, suggest that there are other factors that drive the FFF we have not currently considered.

Integration time

Increasing the integration time of photoreceptors can enhance the visual sensitivity by increasing the photon capture, the signal to noise ratio and contrast discrimination (Warrant, 1999). We thus expected that nocturnal *Myrmecia* would have longer integration times to adapt to the dim light condition compared to diurnal relatives and that longer integration times come at the expense for temporal resolution (Howard and Snyder, 1983; Srinivasan and Bernard, 1975; Warrant, 1999). This agrees with our findings that the duration of responses was significantly slower in nocturnal ants compared to diurnal species (Figure 2; Figure 4D). However, it was not different between nocturnal and diurnal *Myrmecia* if we compare them at

Table 6. Results of the linear mixed-effects model analysis for testing effects of recording time and activity time on the latency of impulse response for dim lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) Activity time (diurnal or nocturnal)	1	−244.055	3.529	0.06
B) **Recording time (day or night)**	1	−242.355	6.930	0.01
C) **Activity time: Recording time**	1	−234.974	10.809	0.001

[a]Final Model: Latency of impulse response to dim lights ~ Recording time + Activity time: Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

Table 7. Results of the linear mixed-effects model analysis for testing effects of recording time and activity time on the time to peak response amplitude of impulse response for bright lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) **Activity time (diurnal or nocturnal)**	1	−197.78	7.55	0.01
B) Recording time (day or night)	1	−200.74	1.62	0.20
C) **Activity time:Recording time**	1	−191.35	11.18	<0.001

[a]Final Model: Time to peak amplitude of impulse response to bright lights ~ Activity time + Activity time: Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

each species' own preferred activity time (Figure 4D). This result is mainly driven by two species, *M. gulosa*, a diurnal species that significantly increased response duration at night and *M. vindex*, a nocturnal species that strongly decreased its response duration at night. The daily changes in response duration could be one of the ways to maintain similar FFFs in diurnal and nocturnal species at their respective activity schedules.

It is well-known that the action of voltage-gated potassium channels correlates with lifestyle and habitat in flies (Laughlin and Weckström, 1993; Weckström and Laughlin, 1995). Diurnal changes in the temporal characteristics of the impulse response in nocturnal *Myrmecia* might be modulated by the voltage-gated potassium channels to avoid costly and unnecessary ion channel fluxes by inactivating the potassium conductance when they are not required.

Larger impulse response amplitude in the nocturnal species at night

The nocturnal *Myrmecia* showed significant daily changes in their response amplitude compared to the diurnal species (Figures 1–3). The diurnal modulation in the amplitude suggests that the nocturnal *Myrmecia* use an increased response amplitude as a strategy to enhance the absolute sensitivity of their eyes and to adjust their response properties to the dim light conditions they experience (Figure 1B).

Both anatomical and physiological mechanisms potentially modulate the ERG amplitude over the course of a day. Both diurnal and nocturnal *Myrmecia* ants have a circadian rhythm modulated radial migration of retinular screening cell pigment granules in compound eyes (Narendra et al., 2016). In addition, all *Myrmecia* ants, except the strictly diurnal *M. croslandi*, have a variable primary pigment cell pupil that constricts the crystalline cone to form a narrow aperture to regulate the amount of light entering the retina (Narendra et al., 2016). The closing of the aperture is dependent on light intensity, whereas the opening of the aperture is modulated by the circadian rhythm. The maximal opening of the aperture occurs only at night and in dark conditions in *M. pyriformis*. Surprisingly, in our results, we found no evidence for diurnal modulation in the response amplitude for *M. pyriformis, M. tarsata,* or *M. croslandi* (Figures 2 and 4A). The circadian modulation in structural changes can have a profound effect on visual sensitivity and produce greater amplitude of ERGs at night in nocturnal animals, e.g., horseshoe crab (Barlow et al., 1977), crayfish (Rodríguez-Sosa and Aréchiga, 1982). Future anatomical investigation in *M. vindex* and *M. midas* may inform us of the role circadian rhythms play in the migration of retinular screening pigment granules and how this regulates amplitude modulation.

Table 8. Results of the linear mixed-effects model analysis for testing effects of recording time and activity time on the time to peak response amplitude of impulse response for dim lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) Activity time (diurnal or nocturnal)	1	−254.12	1.88	0.17
B) **Recording time (day or night)**	1	−250.14	9.83	<0.01
C) Activity time:Recording time	1	−247.24	3.67	0.06

[a]Final Model: Time to peak amplitude of impulse response to dim lights ~ Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

Table 9. Results of the linear mixed-effects model analysis for testing effects of recording time and activity time on the duration of impulse response for bright lights

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) **Activity time (diurnal or nocturnal)**	1	−212.34	7.93	<0.01
B) **Recording time (day or night)**	1	−212.58	7.44	0.01
C) Activity time: Recording time	1	−207.79	1.17	0.28

[a]Final Model: Duration of impulse response ~ Activity time + Recording time + (1|species). Bold terms had a significant effect and were part of the final model.

Although membrane turnover of photoreceptors alter rhabdom size in many arthropods such as spiders (Blest, 1978), crabs (Brodrick et al., 2021; Nässel and Waterman, 1979), and blowflies (Williams, 1982b), resulting in the diurnal changes in response amplitude, this is not known to occur in ants (Menzi, 1987; Narendra et al., 2016). In addition, amplification of photoreceptors varies throughout a day and the quantal responses to individual photons (i.e., bumps) often become larger during the night as shown in the scarabaeid beetle *Anoplognathm* (Meyer-Rochow and Horridge, 1975) and in locusts (Horridge et al., 1981). Moreover, in the horseshoe crab, noise is decreased by reducing the rate of spontaneous bumps by up to 100% at night. Besides, the response is increased by elevating photon catch as much as 30 times and increasing gain as much as 40% (Barlow et al., 1987). However, it is still not clear what the underlying mechanisms of higher response amplitude in the nocturnal *Myrmecia* ants at night are.

Temporal characteristics of different photoreceptors

The nocturnal *M. vindex* and the diurnal *M. croslandi* possess three spectrally distinct photoreceptors, with spectral sensitivities in the UV, blue, and green parts of the spectrum (Ogawa et al., 2015). Spectral measurements at different temporal frequencies revealed that UV receptors are slower, compared with blue and green receptors. Because our light source does not emit the short wavelength range of the spectrum, these results show the temporal characteristics of faster photoreceptors during the day, indicating the maximum limits of temporal resolution in the blue and green region of the spectrum. Understanding temporal characteristics of the slower UV photoreceptors is important to quantify in future studies, because UV contrast is highly effective for sky/ground segmentation which supports navigation in ants (Stone et al., 2014; Möller, 2002).

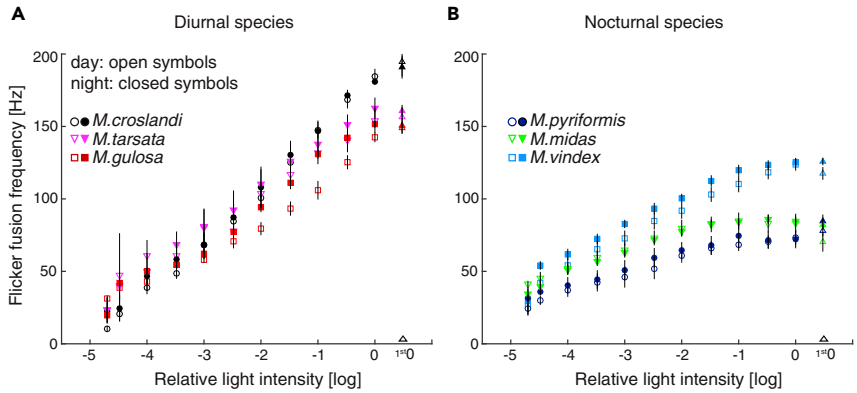

Figure 6. Flicker fusion frequency (FFF) of *Myrmecia* ants

Flicker fusion frequency (FFF) of *Myrmecia* ants increase with light intensity in diurnal (A) and nocturnal (B) *Myrmecia* ant species. Data points show mean ± SEM FFFs were measured during day (open symbols) and at night (closed symbols) across 11 different light intensities. The measurements are taken in ascending order of light intensity to avoid dark adaptation issues. To check for degradations in response amplitude over time, the FFFs at the highest light intensity are measured before the 20 min dark adaptation period (shown as upright triangles at first0) and again at the end of the intensity series (shown at 0). The FFFs do not vary between day and night in any species. However, FFF differs depending on light intensity, species, and preferred activity time (Table 10).

Table 10. The critical flicker fusion frequency in *Myrmecia* ants

	at day (Hz)	at night (Hz)
M. croslandi	188.7 ± 6.4	180.6 ± 2.2
M. tarsata	154.2 ± 6.6	162.6 ± 7.3
M. gulosa	142.5 ± 3.4	151.6 ± 2.5
M. pyriformis	73.3 ± 5.1	72.1 ± 6.2
M. midas	83.3 ± 6.5	84.6 ± 3.2
M. vindex	123.7 ± 4.2	125.2 ± 3.1

All values are mean ± SEM.

In conclusion, we found that the nocturnal *Myrmecia* species have lower FFFs compared to diurnal relatives at bright light intensities, but their eyes are faster at very low light intensities. The results suggest that the nocturnal *Myrmecia* species appear to push their vision to be functionally better under dim light conditions rather than in bright light conditions. We anticipated that changes in the impulse response would correlate well with changes in flicker fusion frequency. If eyes were linear filters, we would indeed expect the impulse response to predict the flicker fusion frequencies perfectly. However, the FFFs did not significantly change when measured during day or night, even though the circadian modulations in the four characteristics of the impulse response were significant, particularly in the nocturnal species. The observed lack of a strong correlation between impulse response characteristics and FFFs suggests that the system contains significant nonlinearities, and it will need further research to test how the impulse response characteristics translate to changes in the FFFs. FFFs as measured here, should be considered as a more robust estimate of the speed of vision compared to predictions based on the characteristics of the impulse response.

Limitations of the study

Our study nicely shows the maximum limits of temporal resolution in the blue and green region of the light spectrum, but it does not account for the UV region. It would be important to take this into account in future studies because UV contrast is useful for ants that navigate using terrestrial cues.

ERG measurements are influenced by multiple cell types, not just photoreceptors; besides, although correlated, they do not accurately measure the behavioral cut-offs. It would be important to use behavioral and intracellular recording techniques to reveal the physiological mechanisms causing the modulation throughout the day.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact

Table 11. Results of the linear mixed-effects model analysis on the flicker fusion frequency

Terms (added or subtracted from final model[a])	df	logLik	L. Ratio	p-value
A) **Light intensity**	10	−2898.47	927.04	<0.001
B) **Activity time (diurnal or nocturnal)**	1	−3352.05	19.87	<0.001
C) Recording time (day or night)	1	−3361.51	0.96	0.33
D) Body length	16	−3349.78	24.41	0.08
E) **Light intensities: Activity time**	10	−2658.89	459.39	<0.001

[a]Final Model: Flicker fusion frequency ~ Light intensity of stimuli + Activity time + light intensity of stimuli: Activity time + (1| animal ID). Bold terms had a significant effect and were part of the final model.

Table 12. Results of the linear mixed-effects model analysis on the flicker fusion frequency for the three log unit dimmer stimuli

Terms (added or subtracted from final model)	df	logLik	L. Ratio	p-value
Activity time (diurnal or nocturnal)	1	−252.47	0.71	0.40
Recording time (day or night)	1	−252.45	0.75	0.39

- Materials availability
- Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Study species
- METHOD DETAILS
  - Electrophysiology
- QUANTIFICATION AND STATISTICAL ANALYSIS

ACKNOWLEDGMENTS

This study was supported by an Australian Research Council grant to A.N. (FT140100221, DP150101172) and J.M.H. (DP180100491).

AUTHOR CONTRIBUTIONS

Y.O., A.N., and J.M.H. designed the study. Y.O. collected the data. Y.O. and J.M.H analyzed the data. J.M.H. built the equipment and wrote the software. Y.o. wrote the first draft of the manuscript. Y.O., A.N., and J.M.H. revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing or financial interests.

Received: September 15, 2021
Revised: February 10, 2022
Accepted: March 17, 2022
Published: April 15, 2022

REFERENCES

Arechiga, B.Y.H., Banuelos, E., Frixione, E., Picones, A., and Rodrtguez-sosaf, L. (1990). Modulation of crayfish retinal sensitivity by 5-hydroxytryptamine. J. Exp. Biol. *150*, 123–143.

Arikawa, K., Kawamata, K., Suzuki, T., and Eguchi, E. (1987). Daily changes of structure, function and rhodopsin content in the compound eye of the crab *Hemigrapsus sanguineus*. J. Comp. Physiol. A. *161*, 161–174.

Autrum, H. (1958). The electrophysiological analysis of the visual system in insects. Exp. Cell Res. *14*, 426–439.

Barlow, R.B., Bolanowski, S.J., and Brachman, M.L. (1977). Efferent optic nerve fibers mediate circadian rhythms in the *Limulus* eye. Science *197*, 86–89.

Barlow, R.B., Jr. (1983). Circadian rhythms in the *Limulus* visual system. J. Neurosci. *3*, 856–870.

Barlow, R.B., Jr., Kaplan, E., Renninger, G.H., and Saito, T. (1987). Circadian rhythms in *Limulus* photoreceptors. J. Gen. Physiol. *89*, 353–378.

Bennitt, R. (1932). Diurnal rhythm in the proximal pigment cells of the crayfish retina. Physiol. Zool. *5*, 65–69.

Blest, A.D. (1978). The rapid synthesis and destruction of photoreceptor membrane by a dinopid spider: a daily cycle. Proc. R. Soc. B *200*, 463–483.

Boström, J.E., Haller, N.K., Dimitrova, M., Ödeen, A., and Kelber, A. (2017). The flicker fusion frequency of budgerigars (*Melopsittacus undulatus*) revisited. J. Comp. Physiol. A. *203*, 15–22.

Brodrick, E.A., Roberts, N.W., Sumner-Rooney, L., Schlepütz, C.M., and How, M.J. (2021). Light adaptation mechanisms in the eye of the fiddler crab *Afruca tangeri*. J. Comp. Neurol. *529*, 616–634.

Bruin, G.D., and Crisp, D. (1957). The influence of pigment migration on vision of higher Crustacea. J. Exp. Biol. *34*, 447–463.

Chamberlain, S.C., and Barlow, R.B., Jr. (1987). Control of structural rhythms in the lateral eye of *Limulus*: interactions of natural lighting and circadian efferent activity. J. Neurosci. *7*, 2135–2144.

Chatterjee, P., Mohan, U., Krishnan, A., and Sane, S.P. (2020). Evolutionary constraints on flicker fusion frequency in Lepidoptera. J. Comp. Physiol. A. Neuroethol. Sensory, Neural Behav. Physiol. *206*, 671–681.

de Souza, J.M., and Ventura, D.F. (1989). Comparative study of temporal summation and rsesponse form in hymenopteran photoreceptors. J. Comp. Physiol. A. *165*, 237–245.

Dearry, A., and Barlow, R.B. (1987). Circadian rhythms in the green sunfish retina. J. Gen. Physiol. *89*, 745–770.

Frank, T.M. (1999). Comparative study of temporal resolution in the visual systems of mesopelagic crustaceans. Biol. Bull *196*, 137–144.

Freas, C.A., Wystrach, A., Narendra, A., and Cheng, K. (2018). The view from the trees: nocturnal bull ants, *Myrmecia midas*, use the surrounding panorama while descending from trees. Front. Psychol. *9*, 1–15.

Frederiksen, R., Wcislo, W.T., and Warrant, E.J. (2008). Visual reliability and information rate in the retina of a nocturnal bee. Curr. Biol. *18*, 349–353.

Fritsches, K.A., Brill, R.W., and Warrant, E.J. (2005). Warm eyes provide superior vision in swordfishes. Curr. Biol. *15*, 55–58.

Greiner, B. (2006). Adaptations for nocturnal vision in insect apposition eyes. Int. Rev. Cytol. *250*, 1–46.

Greiner, B., Narendra, A., Reid, S.F., Dacke, M., Ribi, W.A., and Zeil, J. (2007). Eye structure correlates with distinct foraging-bout timing in primitive ants. Curr. Biol. *17*, R879–R880.

Hariyama, T., Meyer-Rochow, V.B., and Eguchi, E. (1986). Diurnal changes in structure and function of the compound eye of *Ligia Exotica* (Crustacea, Isopoda). J. Exp. Biol. *123*, 1–26.

Hariyama, T., Meyer-Rochow, V.B., Kawauchi, T., Takaku, Y., and Tsukahara, Y. (2001). Diurnal changes in retinula cell sensitivities and receptive fields (two-dimensional angular sensitivity functions) in the apposition eyes of *Ligia exotica* (Crustacea, Isopoda). J. Exp. Biol. *204*, 239–248.

Healy, K., McNally, L., Ruxton, G.D., Cooper, N., and Jackson, A.L. (2013). Metabolic rate and body size are linked with perception of temporal information. Anim. Behav. *86*, 685–696.

Horridge, G., Duniec, J., and Marčelja, L. (1981). A 24-hour cycle in single locust and mantis photoreceptors. J. Exp. Biol. *91*, 307–322.

Howard, J., and Snyder, a.W. (1983). Transduction as a limitation on compound eye function and design. Proc. R. Soc. B Biol. Sci. *217*, 287–307.

Howard, J., Dubs, A., and Payne, R. (1984). The dynamics of phototransduction in insects - a comparative study. J. Comp. Physiol. A. *154*, 707–718.

Jahn, T.L., and Wulff, V.J. (1943). Electrical aspects of a diurnal rhythm in the eye of *Dytiscuc fasciventris*. Physiol. Zool. *16*, 101–109.

Jayatilaka, P., Murray, T., Narendra, A., and Zeil, J. (2018). The choreography of learning walks in the Australian jack jumper ant *Myrmecia croslandi*. J. Exp. Biol. *221*, jeb185306.

Jenssen, T.A., and Swenson, B. (1974). An ecological correlate of critical flicker-fusion frequencies for some *Anolis* lizards. Vis. Res. *14*, 965–970.

Land, M.F., and Nilsson, D.-E. (2012). Animal Eyes, Second edition (Oxford University Press).

Larimer, J.L., and Smith, J.T.F. (1980). Circadian rhythm of retinal sensitivity in crayfish: modulation by the cerebral and optic ganglia. J. Comp. Physiol. A *136*, 313–326.

Laughlin, S.B., and Weckström, M. (1993). Fast and slow photoreceptors a comparative study of the functional diversity of coding and conductances in the Diptera. J. Comp. Physiol. A *172*, 593–609.

Leggett, L.M.W., and Stavenga, D.G. (1981). Diurnal changes in angular sensitivity of crab photoreceptors. J. Comp. Physiol. A *144*, 99–109.

Maddess, T., James, A.C., Goldberg, I., Wine, S., and Dobinson, J. (2000). Comparing a parallel PERG, automated perimetry, and frequency-doubling thresholds. Investig. Ophthalmol. Vis. Sci. *41*, 3827–3832.

Menzi, U. (1987). Visual adaptation in nocturnal and diurnal ants. J. Comp. Physiol. A *160*, 11–21.

Meyer-Rochow, V.B. (1999). Compound eye: circadian rhythmicity, illumination, and obscurity. In Atlas of Arthropod Sensory Receptors, E. Eguchi and Y. Tominaga, eds. (Springer Verlag), pp. 97–125.

Meyer-Rochow, V.B., and Horridge, G.A. (1975). The eye of *anoplognathus* (Coleoptera, scarabaeidae). Proc. R. Soc. B Biol. Sci. *188*, 1–30.

Möller, R. (2002). Insects could exploit UV–green contrast for landmark navigation. J. Theor. Biol. *214*, 619–631. https://doi.org/10.1006/jtbi.2001.2484.

Narendra, A., Reid, S.F., and Hemmi, J.M. (2010). The twilight zone: ambient light levels trigger activity in primitive ants. Proc. R. Soc. B *277*, 1531–1538.

Narendra, A., Reid, S.F., Greiner, B., Peters, R.A., Hemmi, J.M., Ribi, W.A., and Zeil, J. (2011). Caste-specific visual adaptations to distinct daily activity schedules in Australian *Myrmecia* ants. Proc. R. Soc. B *278*, 1141–1149.

Narendra, A., Alkaladi, A., Raderschall, C.A., Robson, S.K.A., and Ribi, W.A. (2013a). Compound eye adaptations for diurnal and nocturnal lifestyle in the intertidal ant, *Polyrhachis sokolova*. PLoS One *8*, e76015.

Narendra, A., Gourmaud, S., and Zeil, J. (2013b). Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. Proc. R. Soc. B Biol. Sci. *280*, 20130683.

Narendra, A., Reid, S.F., and Raderschall, C.A. (2013c). Navigational efficiency of nocturnal *Myrmecia* ants suffers at low light levels. PLoS One *8*, e58801.

Narendra, A., Greiner, B., Ribi, W.A., and Zeil, J. (2016). Light and dark adaptation mechanisms in the compound eyes of *Myrmecia* ants that occupy discrete temporal niches. J. Exp. Biol. *219*, 2435–2442.

Narendra, A., Kamhi, J.F., and Ogawa, Y. (2017). Moving in dim light: behavioral and visual adaptations in nocturnal ants. Integr. Comp. Biol. *57*, 1104–1116.

Nässel, D.R., and Waterman, T.H. (1979). Massive diurnally modulated photoreceptor membrane turnover in crab light and dark adaptation. J. Comp. Physiol. A *131*, 205–216.

Nørgaard, T., Nilsson, D.-E., Henschel, J.R., Garm, A., and Wehner, R. (2008). Vision in the nocturnal wandering spider *Leucorchestris arenicola* (Araneae: sparassidae). J. Exp. Biol. *211*, 816–823.

O'Carroll, D.C., and Warrant, E.J. (2017). Vision in dim light: highlights and challenges. Philos. Trans. R. Soc. B Biol. Sci. *372*. https://doi.org/10.1098/rstb.2016.0062.

Ogawa, Y., Falkowski, M., Narendra, A., Zeil, J., and Hemmi, J.M. (2015). Three spectrally distinct photoreceptors in diurnal and nocturnal Australian ants. Proc. R. Soc. B Biol. Sci. *282*, 20150673.

Ogawa, Y., Ryan, L.A., Palavalli-Nettimi, R., Seeger, O., Hart, N.S., and Narendra, A. (2019). Spatial resolving power and contrast sensitivity are adapted for ambient light conditions in Australian *Myrmecia* ants. Front. Ecol. Evol. *7*, 1–10.

Pirhofer-Walzl, K., Warrant, E., and Barth, F.G. (2007). Adaptations for vision in dim light: impulse responses and bumps in nocturnal spider photoreceptor cells (*Cupiennius salei* Keys). J. Comp. Physiol. A *193*, 1081–1087.

Raderschall, C.A., Narendra, A., and Zeil, J. (2016). Head roll stabilisation in the nocturnal bull ant *Myrmecia pyriformis* : implications for visual navigation. J. Exp. Biol. *219*, 1449–1457.

Reid, S.F. (2010). Life in the dark: Vision and navigation in a nocturnal bull ant (The Australian National University, PhD thesis).

Reid, S.F., Narendra, A., Taylor, R.W., and Zeil, J. (2013). Foraging ecology of the night-active bull ant *Myrmecia pyriformis*. Aust. J. Zool. *61*, 170–177.

Rodríguez-Sosa, L., and Aréchiga, H. (1982). Range of modulation of light sensitivity by accessory pigments in the crayfish compound eye. Vis. Res. *22*, 1515–1524.

Ryan, L.A., Hemmi, J.M., Collin, S.P., and Hart, N.S. (2017). Electrophysiological measures of temporal resolution, contrast sensitivity and spatial resolving power in sharks. J. Comp. Physiol. A *203*, 197–210.

Sheehan, Z., Kamhi, J.F., Seid, M.A., and Narendra, A. (2019). Differential investment in brain regions for a diurnal and nocturnal lifestyle in Australian *Myrmecia* ants. J. Comp. Neurol. *527*, 1261–1277.

Snyder, A.W., Laughlin, S.B., and Stavenga, D.G. (1977). Information capacity of eyes. Vis. Res. *17*, 1163–1175.

Somanathan, H., Kelber, A., Borges, R.M., Wallén, R., and Warrant, E.J. (2009). Visual ecology of Indian carpenter bees II: adaptations of eyes and ocelli to nocturnal and diurnal lifestyles. J. Comp. Physiol. A *195*, 571–583.

Srinivasan, M.V., and Bernard, G.D. (1975). The effect of motion on visual acuity of the compound eye: a theoretical analysis. Vis. Res. *15*, 515–525.

Stone, T., Mangan, M., Ardin, P., and Webb, B. (2014). Sky segmentation with ultraviolet images can be used for navigation. Robot. Sci. Syst. *10*, 47.

Tomioka, K., and Chiba, Y. (1982). Persistence of circadian ERG rhythm in the cricket with optic tract severed. Naturwissenschaften *69*, 395–396.

Via, S.E. (1977). Visually mediated snapping in the bulldog ant: a perceptual ambiguity between size and distance. J. Comp. Physiol. A *121*, 33–51.

Warrant, E.J. (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. Vis. Res. *39*, 1611–1630.

Warrant, E.J., and McIntyre, P.D. (1993). Arthropod eye design and the physical limits to spatial resolving power. Prog. Neurobiol. *40*, 413–461.

Warrington, R.E., Hart, N.S., Potter, I.C., Collin, S.P., and Hemmi, J.M. (2017). Retinal temporal resolution and contrast sensitivity in the parasitic lamprey *Mordacia mordax* and its non-parasitic derivative *Mordacia praecox*. J. Exp. Biol. *220*, 1245–1255.

Weckström, M., and Laughlin, S.B. (1995). Visual ecology and voltage-gated ion channels in insect photoreceptors. Trends Neurosci. *18*, 17–21.

Williams, D.S. (1982a). Ommatidial structure in relation to turnover of photoreceptor membrane in the locust. Cell Tissue Res. *225*, 595–617.

Williams, D.S. (1982b). Rhabdom size and photoreceptor membrane turnover in a muscoid fly. Cell Tissue Res. *226*, 629–639.

Wills, A.S., Page, T.L., and Colwell, C.S. (1985). Circadian rhythms in the electroretinogram of the cockroach. J. Biol. Rhythms *1*, 25–37.

Zeil, J., Narendra, A., and Sturzl, W. (2014). Looking and homing: how displaced ants decide where to go. Philos. Trans. R. Soc. B Biol. Sci. *369*, 20130034.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw data files	This paper	Cloudstor: https://cloudstor.aarnet.edu.au/plus/s/1xyTxevo5QGUmVq
Experimental models: Organisms/strains		
Myrmecia croslandi	Wild caught, Canberra, Australia	N/A
Myrmecia tarsata	Wild caught, Sydney, Australia	N/A
Myrmecia gulosa	Wild caught, Sydney, Australia	N/A
Myrmecia pyriformis	Wild caught, Canberra, Australia	N/A
Myrmecia midas	Wild caught, Sydney, Australia	N/A
Myrmecia vindex	Wild caught, Perth, Australia	N/A
Software and algorithms		
RStudio Version 1.1.419	RStudio, Inc. Boston, MA, US	https://www.rstudio.com/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yuri Ogawa (yuri.ogawa@flinders.edu.au).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- FFF and impulse response data have been deposited at Cloudstor and are publicly available as of the date of publication. DOI is listed in the key resources table.
- All original code is available from the lead contact upon request.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study species

We studied workers of six species of *Myrmecia* ants, whose daily activity patterns were previously identified (Greiner et al., 2007; Narendra et al., 2010; Ogawa et al., 2015; Sheehan et al., 2019). These six species can be classified as predominantly diurnal (*M. croslandi*, *M. tarsata* and *M. gulosa*), or predominantly nocturnal (*M. pyriformis*, *M. midas* and *M. vindex*). *M. croslandi* and *M. pyriformis* were collected from Canberra (35.1650° S, 149.750° E), *M. gulosa*, *M. midas* and *M. tarsata* from Sydney (33.3746° S, 150.4604° E; 33.4608° S, 151.0640° E; 33.4611° S, 151.0640° E, respectively) and *M. vindex* from Perth (31.5905° S, 115.4918° E). To minimise disruption of circadian rhythms, diurnal species were exposed to sunlight during the day. Nocturnal species were kept in the dark throughout the day, to match the light conditions they encounter inside the nest during the day and in low ambient light condition at night. All ants were provided access to sugar water.

METHOD DETAILS

Electrophysiology

The temporal response characteristics of photoreceptors of each ant species were determined by measuring the impulse response and the flicker fusion frequency (FFF) using Electroretinograms (ERGs)

at different times of the day and at different light intensities. ERGs were recorded through a differential amplifier (DAM50, World Precision Instruments Inc., FL, USA) connected to a computer via a 16-bit data acquisition board (USB-6353, National Instruments, Austin, TX, USA or Micro1401-3, Cambridge Electronic Design Ltd., Cambridge, England).

Animals were kept on ice for five minutes before removing their legs and gaster. Each individual ant was fixed with their dorsal side up, to a plastic stage with bees' wax before being mounted in a Faraday cage. A silver/ silver-chloride wire of 0.1 mm diameter was inserted into the mesosoma and served as the indifferent electrode. As an active electrode, a platinum wire of 0.254 mm diameter was attached to the lateral surface of the compound eye with conductive gel (Livingstone Inter- national Pty Ltd., New South Wales, Australia).

All experiments were carried out at room temperature (21–25°C) in the dark. Animals were dark-adapted for 20 min before each experiment. To investigate the effect of circadian rhythms on eyes, the experiments were performed both during the day (3–11 h post-sunrise) and at night (1–9 h post-sunset).

A cool white light emitting diode (LED) with 5 mm diameter was used as a light source (C503C-WAS-CBADA151, Cree Inc, Durham, NC, USA). The LED was set at 10° elevation at 14 cm from the animal resulting in an angular size of 2° degrees.

The impulse response was measured as the voltage response to a 1 ms flash of light followed by 2 s of darkness. The response was averaged over 100 repetitions. To measure the diurnal changes in eye sensitivity, six different intensities over a 5-log unit range (relative intensities; 0.00002, 0.0001, 0.001, 0.01, 0.1 and 1) were used in ascending order both during the day and at night. We controlled the intensity by PulseWidthModulation (PWM) flickering at 1kHz. The light source produced a maximum irradiance of (5.81×10^{-5} W/cm^{-2}) at the surface of the eye (ILT1700, International Light Technologies). The response amplitude at each light intensity was used to generate the response intensity function (V-log I).

To identify the temporal characteristics of the impulse response we measured the following four parameters at the brightest flash intensity: 1) Response amplitude (mV), measured as the minimum amplitude of the hyperpolarizing response; 2) Response latency (ms), defined as the time for the response to exceed three standard deviation of noise after stimulus onset. The standard deviation of the noise was calculated from all voltage changes in the last 500 ms before stimulus onset; 3) Time to peak (ms), measured as the time from stimulus onset to peak amplitude, and 4) Response duration (ms), measured as the full-width of the response at half the maximum amplitude.

The FFF was estimated as the highest temporal frequency at which the ERG reached a criterion threshold. The experimental design has been described in detail in a previous study (Warrington et al., 2017). Briefly, the visual stimulus followed a square-wave flicker over a range of stimulation frequencies from 2 to 200 Hz. Each frequency was presented for 20 s and the average response amplitude calculated using a Fast Fourier Transform (Maddess et al., 2000). FFF were measured at 11 different light levels over a ~5 log unit intensity range (1.33×10^{-9} to 5.81×10^{-5} W/cm^{-2}). To evaluate any degradation of the response over time, the FFF at the highest intensity was tested as a control before starting the series of FFF measures, increasing in 0.5 log unit steps apart from the lowest stimulus intensity (relative intensity at 0.00002) with 20 min dark adaptation in between. At high light intensities, the LED generated a measurable electrical artefact that looked like the response of the eye. The largest possible artefact was measured as the maximum signal amplitude recorded at the highest light intensity by covering the LED with a black cloth and then used as the response threshold. FFF was defined as the frequency at which the response power (log10 of the response amplitude power) crossed the threshold for each animal (see Figure 1 in Warrington et al., 2017 for details).

QUANTIFICATION AND STATISTICAL ANALYSIS

To test whether the response intensity function differs between diurnal and nocturnal species, we implemented a linear mixed-effects model using the maximum likelihood (ML) estimation method in the nlme package in RStudio (Version 1.1.419, RStudio, Inc. Boston, MA, US). Light intensities of stimuli, recording time (day or night), preferred activity time of species (diurnal or nocturnal) and the interaction between recording time and preferred activity time were considered as fixed effects in the model.

Animal identity nested within species was used as a random effect. Model assumptions were checked graphically.

We used linear mixed-effects models in RStudio to assess which characteristics of the impulse response were affected by the recording time (day or night), activity time (diurnal or nocturnal, depending on species) and their interaction. Species was used as the random effect because each individual was used for one measurement. To identify which species contributed strongly to the overall effect, we performed post-hoc analyses using general linear hypotheses with the multcomp package in RStudio.

The post hoc test was performed to compare each characteristic of the impulse response at different recording times between diurnal and nocturnal species. This allowed us to compare impulse responses in diurnal and nocturnal species at their activity times, i.e., comparing measurements in diurnal species during the day to those in nocturnal animals at night.

A linear mixed-effects model was used for testing whether the FFFs differed according to recording time and preferred activity time of species. Light intensities of stimuli, recording time (day or night), preferred activity time of species (diurnal or nocturnal) and their interaction were used as fixed effects. Animal identity was used as the random effect.