Effects of the colour of photophase light on locomotor activity in a nocturnal and a diurnal South African rodent

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Running head: Effect of light colour on rhythms

ABSTRACT

Many physiological and behavioural responses to varying qualities of light, particularly during the night (scotophase) have been well documented in rodents. We used varying wavelengths of daytime (photophase) lighting to assess daily responses in locomotor activity in the nocturnal Namaqua rock mouse (*Micaelamys namaquensis*) and diurnal four-striped field mouse (*Rhabdomys pumilio*). Animals were exposed to three light-dark cycle regimes: a short-wavelength- (SWLC, blue), a medium-wavelength- (MWLC, green) and a long-wavelength light-dark cycle (LWLC, red). Overall, daily locomotor activity of both species changed according to different wavelengths of light, the diurnal species displayed most activity under the SWLC and the nocturnal species exhibited the highest levels of activity under the LWLC. Both species showed an increase in diurnal activity and a decrease in nocturnal activity under the LWLC. These results indicate an attenuated responsiveness to long wavelength light in the nocturnal species, but this does not appear to be true for the diurnal species. These results emphasize that the effect of light on the locomotor activity of animals depends on both the properties of the light and the temporal organization of activity of a species.

Key words: diurnal, locomotor activity, *Micaelamys namaquensis*, nocturnal, photophase wavelength, *Rhabdomys pumilio*

INTRODUCTION

In most mammals, biological functions are adjusted to the light-dark cycle through an endogenous time-keeping system coordinated by a master clock, the suprachiasmatic nucleus (SCN; Doyle and Menaker, 2007). The SCN receives photic information from melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina (Aggelopoulos and Meissl, 2000; Hattar et al., 2006), but more recent evidence suggests that the rods and cones also contribute to non-visual SCN-related processes via indirect pathways (Dkhissi-Benyahya et al., 2007; Altimus et al., 2010; Gooley et al., 2010; van Diepen et al., 2013; Weng et al., 2013). Clock function appears to be similar in diurnal and nocturnal animals, indicating that differences in active times are determined by mechanisms downstream from the SCN (Ramanathan et al., 2010).

Nocturnal and diurnal animals differ in their temporal activity patterns and their circadian systems show anatomical and physiological adaptations to their temporal niches. In the retina, the ratio of cones to rods typically reflects the activity pattern of a species, such that diurnal species tend to have considerably more cones than nocturnal species (Jacobs, 1993). In addition, nocturnal and diurnal species may have different sensitivity thresholds to varying qualities of lighting (Kumar and Rani, 1999; Peichl, 2005; Zubidat et al., 2009, 2010a, 2010b). Variations in light intensity, spectral wavelength, duration and timing of the light exposure can all affect the circadian timing system differentially (Gorman et al., 2003; Duffy and Wright, 2005; Aral et al., 2006; Zubidat et al., 2009, 2010b).

Dawn and dusk are times of the day when both the intensity and spectral composition of light changes significantly (Roenneberg and Foster, 1997). At positive solar angles, colours are more yellow whereas there is a reduction in medium wavelengths at negative solar angles (Brown, 2016). Previously it was believed that changes in light intensity is a more potent zeitgeber for entrainment than light colour in rodents (Geetha et al., 1995), however, more recent studies showed that changes in light colour also contribute to entrainment (Bonmati-Carrion et al., 2017; Walmsley et al., 2015). Light at night disrupts circadian rhythms, but the extent of the disruption depends on the wavelength and whether the species is nocturnal or diurnal (Bonmati-Carrion et al., 2017).

It has been hypothesized that both nocturnal and diurnal species may interpret light with the same spectral composition differently as a result of differences in their circadian systems

(Bonmati-Carrion et al., 2017). Most studies that have investigated the daily response to light with different wavelengths, presented light stimuli during scotophase (Bonmati-Carrion et al., 2017), overlapping with the active phase of nocturnal species. Although light at night alters the activity of both nocturnal and diurnal species, nocturnal species show a much more pronounced response. In this study, we evaluated the locomotor activity of a diurnal and a nocturnal species in response to light with different wavelengths during photophase, which overlaps with the active phase of the diurnal species.

Our study species, the Namaqua rock mouse (*Micaelamys namaquensis*) and the four-striped field mouse (*Rhabdomys pumilio*) are two terrestrial African rodent species that inhabit opposing temporal niches. *M. namaquensis* is nocturnal, whereas *R. pumilio* is diurnal with marked peaks around dawn and dusk (Skinner and Chimimba, 2005). The general locomotor activity rhythms have been characterized in the laboratory for both *R. pumilio* and *M. namaquensis* (Schumann et al., 2005; Van der Merwe et al., 2014). Furthermore, the retinal photoreceptor compositions of both species appear to be complementary to their temporal niches (van der Merwe et al., 2018).

We evaluated locomotor activity in the two species in response to three light-dark cycles of short-, medium-, and long-wavelengths of near-monochromatic light during photophase since diurnal lighting is more natural for both nocturnal and diurnal species. We aimed to determine whether the wavelength dependency of activity entrainment observed with scotophase light exposure, is also present with photophase light and whether this would differ for diurnal and nocturnal species. We anticipated that photophase lighting overall would have a smaller effect on the nocturnal species, and that the longer wavelengths of light would have the largest effect on the entrainment of the diurnal species since these wavelengths change considerably during twilight hours when *R. pumilio* shows peaks in their natural activity.

MATERIAL AND METHODS

Animal housing

Eight male *M. namaquensis* were captured at Goro Game Reserve (Limpopo Province, South Africa, 22°58'S, 29°25'E), and 8 male *R. pumilio* were collected from Birha farms (Eastern Cape Province, South Africa, 33°22'S, 27°19'E). Individual animals were maintained in semi-transparent plastic cages (58 x 38 x 36 cm) in a temperature-controlled room (25 °C ± 1°C, ~60% relative humidity). Animals received a plastic shelter and tissue paper for nesting material. The mice had *ad libitum* access to water and food, the latter comprised of parrot seed mix (Marlton's, Durban, South Africa) with apple and carrot. Animals were acclimatized to the laboratory conditions on a 12L:12D light schedule with a fluorescent overhead light between one and two months before commencement of experiments.

Experimental setup

Double sets of Light Emitting Diodes (LED) strip lights (RGB, DC12V, 14.4 W, IP55, SMD 5050, 30 LED/m) were installed over the adjoining animal cages, approximately 50 cm above cage floor, the irradiance was 0.2-0.3 W/m^2 for all light colours (See Appendix 1 for details about photophase light exposure). Animals were given one week to acclimatize to the light conditions and locomotor activity was recorded during the second and third weeks.

Cages were fitted with infrared motion captors (Quest PIR internal passive infrared detector; Elite Security Products (ESP), Electronic Lines, London, UK) positioned to record activity across the entire cage floor area. The activity counts were summed per minute with VitalView software (VitalView^{TM}, Minimitter Co., Sunriver, OR, USA). Double-plotted actograms were constructed with the program ActiView (ActiView^{TM}, Minimitter Co., Sunriver, OR, USA).

Analysis

IBM SPSS Statistics v21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. For each species, mean hourly activity counts were compared between the three WLCs and within each WLC (photophase vs. scotophase). Data were not normally distributed thus generalized linear mixed models were used, with a gamma distribution and a log link function. Spectral wavelength and phase of the day were used as fixed factors, all interactions were investigated and the *post hoc* least significant difference test was used where significant differences (P<0.05) were detected. All values are expressed as mean ± SEM.

RESULTS

Micaelamys

All *M. namaquensis* exhibited distinctly nocturnal locomotor activity under all light cycles ($F_{1,16116}$=1124.5, p<0.001) (Table 1, Fig. 1A, 2A). Overall levels of activity differed significantly between the different light spectra ($F_{1,8058}$=101.82, p<0.001). The highest level of activity occurred under red light and the lowest under blue light. During the day, all light spectra rendered different amounts of activity with the least amount during blue light, and the highest amount during red light (p<0.001 for all combinations). During the night, animals were less active when exposed to red photophase light compared to blue light (p=0.013), activity under red and green light were similar (p=0.250), as well as under green and blue light (p=0.180).

Table 1. Mean hourly activity counts during photophase and scotophase for *M. namaquensis* and *R. pumilio* under different wavelength light-dark cycles (WLCs). Data are means ± SEM. Significant values are in bold.

M. namaquensis	SWLC	MWLC	LWLC	GLMM ($F_{1,8058}$; *P*)
Photophase	1.95 ± 0.14	2.9 ± 0.18	9.64 ± 0.52	120.19; **<0.001**
Scotophase	60.28 ± 0.98	55.32 ± 1.1	51.28 ± 0.83	3.07; **0.046**
GLMM ($F_{1,665}$; *P*)	434.7; **<0.001**	415.3; **<0.001**	296.7; **<0.001**	
R. pumilio				
Photophase	10.32 ± 0.47	9.65 ± 0.49	14.62 ± 0.79	16.8; **<0.001**
Scotophase	5.46 ± 0.29	5.39 ± 0.42	4.36 ± 0.28	13.3; **<0.001**
GLMM ($F_{1,8058}$; *P*)	52.3; <**0.001**	45.9; <**0.001**	130.3; <**0.001**	

Figure 1. Actograms showing typical activity patterns of a diurnal *R. pumilio* and a nocturnal *M. namaquensis*; across the three wavelength light-dark cycles (WLCs). Consecutive days are on the y-axis, and black and white bars at the top indicate scotophase and photophase, respectively. LWLC – long wavelength cycle, MWLC – medium wavelength cycle, SWLC – short wavelength cycle.

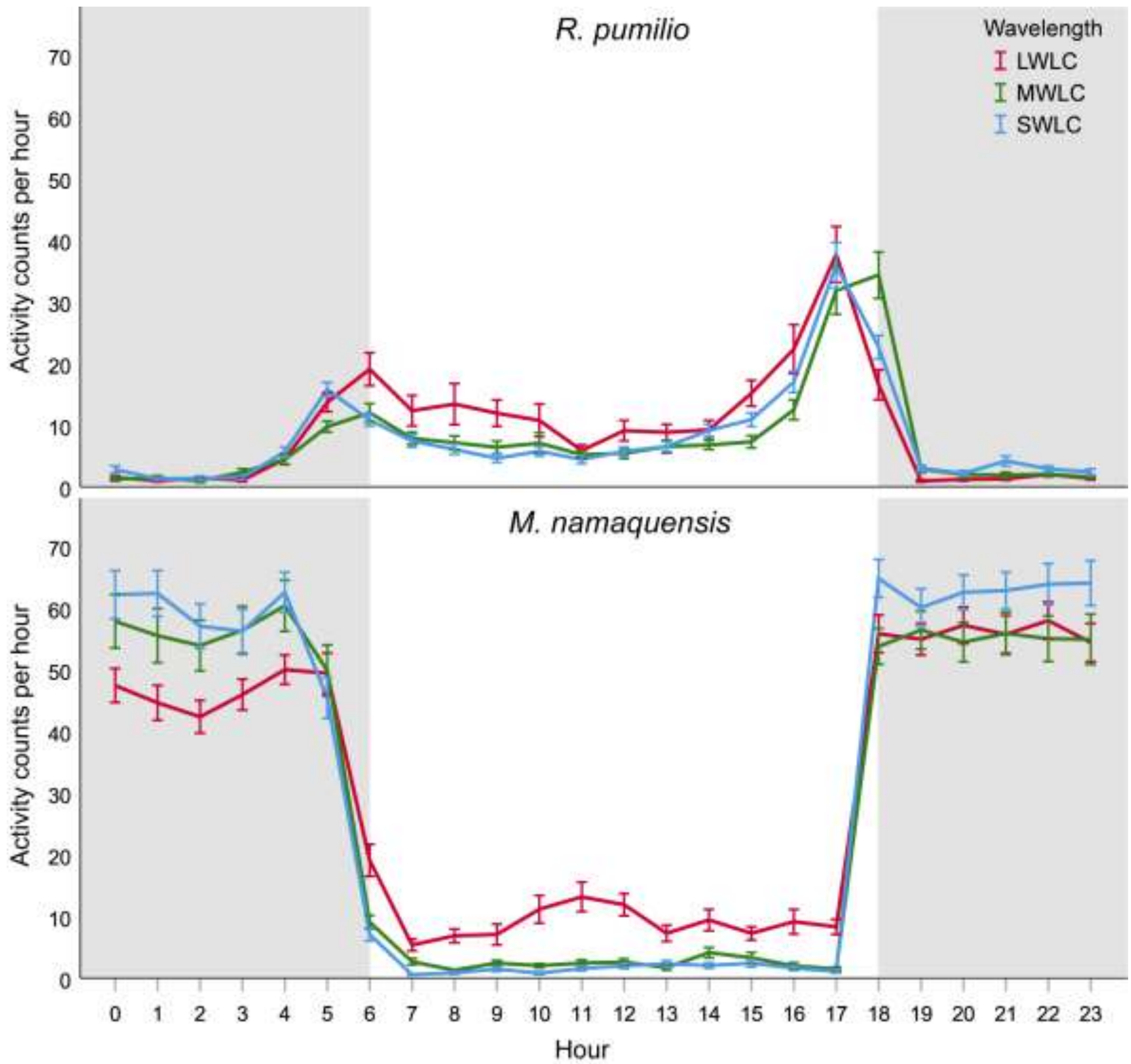

Figure 2. Mean hourly activity counts (means ± SEM) over the 24-hour day in *M. namaquensis* and *R. pumilio* during three successive wavelength light-dark cycles (WLCs). Grey areas indicate dark phases. LWLC – long wavelength cycle, MWLC – medium wavelength cycle, SWLC – short wavelength cycle.

Rhabdomys

In *R. pumilio*, activity showed peaks around dawn and dusk, but animals were consistently more active during the day compared to the night for all WLCs ($F_{1,16116}$ = 328.58, p<0.001) (Table 1, Fig. 1B, 2B). Overall levels of activity were different when animals were exposed to different spectra of photophase light ($F_{1,8058}$=8.09, p<0.001), activity was higher under blue light compared to red (p<0.006) and green light (p<0.001), but under red and green light it was similar ($p=0.203$). The distributions between photophase and scotophase activity were significantly different under the various photophase light spectra (Table 1). During the day, animals were most active when presented with red light (red-green; $p<0.001$; red-blue: $p=0.043$), and least active under green lighting (green-blue: $p=0.002$). During the night, animals were significantly less active under red photophase lighting compared to green ($p=0.001$) and blue ($p<0.001$), whilst they were most active during blue light exposure (green-blue: $p=0.011$).

DISCUSSION

Light is the primary *zeitgeber* for circadian entrainment in both nocturnal and diurnal animals (Sharma and Chandrashekaran, 2005). The rapid changes in the intensity and wavelength of light during the twilight hours serves as important cues for both nocturnal and diurnal species (Roenneberg and Foster, 1997) signaling the beginning and end of activity in nocturnal animals whereas many diurnal animals have increased activity at dawn and dusk. The effect of light intensity on activity and other physiological processes has been studied extensively (Ikeno et al., 2016; Leach et al., 2013; Lonstein et al., 2019; van der Merwe et al., 2017). The influence of the wavelength of light is comparatively much less explored and is mostly investigated with light exposure during the night, when nocturnal animals presumably will be more affected (Bonmati-Carrion et al., 2017; Spoelstra et al., 2015). In this study, we exposed a nocturnal and a diurnal species to different wavelengths of light during the photophase to assess the effects on locomotor activity during a time when animals are naturally exposed to light.

Our results show that exposure to different photophase light spectra affects locomotor activity in both the nocturnal and diurnal species, with red light having the largest effect on the temporal activity expression of the species. The diurnal *R. pumilio* showed diurnal activity with peaks around twilight under all three lighting conditions, the same activity profile occurred under normal lighting conditions (Schumann et al., 2005). Blue photophase light exposure renders the highest level of activity, similar levels were observed under green and red light, yet the highest day-time activity and the lowest night-time activity was expressed under red light. The nocturnal *M. namaquensis* maintained a nocturnal activity rhythm under all wavelengths, but the overall activity increased with an increase in the wavelength of the light. As in *R. pumilio,* activity under red lighting increased during the day and reduced during the night. Under 'normal' lighting conditions, locomotor activity of *M. namaquensis* appears to be strongly masked by light, restricting locomotor activity to complete darkness, but under constant darkness (DD), although the activity rhythm is endogenous, activity is also displayed during the subjective day (van der Merwe et al., 2014).

The effect of light with varying spectral compositions seems dependent on the timing of presentation (photophase or scotophase) and the temporal active phase of the particular species. Exposure to blue light suppresses melatonin maximally (Thapan et al., 2001), which is normal during the day, but not the night. The temporal activity pattern of a species would affect its actual exposure to light administrated during a certain phase of the day. Blue light at night completely disrupts the spontaneous activity patterns in nocturnal rats, whereas the diurnal degu is relatively unaffected by it (Bonmati-Carrion et al., 2017).

The light spectrum also affects the activity of non-mammalian vertebrates. Similar to *R. pumilio,* diurnal zebra fish larvae show more overall activity under blue photophase light than red light (Di Rosa et al., 2015). Broad spectrum white light of different temperatures (cool/warm) does not affect spontaneous behaviour, activity levels or entrainment of nocturnal rodents (Geetha et al., 1995; Kapogiannatou et al., 2016),

Red light at night seems to have an attenuated effect compared to other colours (Bonmati-Carrion et al., 2017; Peirson et al., 2018; Spoelstra et al., 2015). Due to their photoreceptor complement, rodents may show a reduced sensitivity to long wavelengths of light and therefore experience it as less intense (Jacobs et al., 2004; van der Merwe et al., 2018). This certainly seems to be true for the nocturnal species in our study. Activity of *M. namaquensis* is strongly masked by broad spectrum white light (van der Merwe et al., 2014), also under blue and green light, but under red light there is a marked increase in daytime activity, indicating a reduced responsiveness to this light spectrum. The diurnal *R. pumilio* also shows an increase in day-time activity under red light. Under both laboratory and natural lighting conditions, *R. pumilio* exhibit activity peaks around dawn and dusk (Schumann et al., 2005), marking the time of the day during which long wavelength light is prevalent in the natural environment. Longer wavelengths of light could serve as an indicator of twilight and these animals may perceive the prolonged red light exposure as an extended twilight, thus increasing their activity.

CONCLUSION

We showed that entrainment of activity in both the nocturnal and diurnal species is dependent on the wavelength of photophase light. Overall, daily activity changes with different wavelengths of light, red light seems to affect the circadian systems of the nocturnal and diurnal species differentially. Both species exhibit more activity during the day and less during the night under red light exposure, indicating a reduced sensitivity to longer wavelengths of light in nocturnal species and potentially an increased responsiveness in the diurnal species. These results emphasize that the effect of light on the locomotor activity of animals depends on both the properties of the light and the temporal organization of activity of a species. Both light intensity and spectrum is important for entrainment in rodents, and species with different temporal activity patterns may use light differentially.

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