1   Dim light at night affects the locomotor activity of nocturnal African pygmy mice (*Mus*

2   *minutoides*) in an intensity dependent manner

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28   Abstract

29   Rodents are integral components of ecosystems as they provide several important ecosystem

30   services. Despite their importance as prey, pollinators and seed distributors, African rodents

31   are largely understudied. The effect of anthropogenic changes such as artificial light at night

32   extend past urban areas to peri-urban and rural habitats and can have profound effects on entire

33   ecosystems. We investigated the effect of dim light at night (dLAN) on the locomotor activity

34   rhythms of the African pygmy mouse (*Mus minutoides*). Pygmy mice showed a dramatic,

35   intensity dependent reduction in their locomotor activity when subjected to dLAN, which was

36   accompanied by a delay in the activity onset. We also considered masking responses with a

37   dark pulse during the day and a light pulse at night. All animals became inactive in response to

38   a light pulse during the night, whereas approximately half of the animals showed activity during

39   a dark pulse in the day. Our results suggest that the African pygmy mouse is highly sensitive

40   to light and that their activity is strongly masked by light. In their natural environment,

41   vegetation could shield pygmy mice against high light levels, however other anthropogenic

42   disturbances can affect the behaviour of these animals and could affect their survival.

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1. Introduction

Biological rhythms drive physiological and behavioural processes in organisms to occur rhythmically, at the right moment in time. They occur within all three domains of life (Archaea, Eubacteria, and Eukarya) and can be either exogenous or endogenous (1, 2). Exogenous rhythms only occur as a response to periodic input from the external environment and cease once the periodic external input disappears. In contrast, endogenous rhythms are produced by biological clocks within an organism and are independent of external cues (1). Circadian rhythms are biological rhythms with periods of around 24 hours (3). In mammals, they are generated by a master clock located in the suprachiasmatic nucleus (SCN) in the basal hypothalamus (4). The master clock ensures that the endogenous circadian rhythms throughout the body remain in synchronisation with each other (4, 5).

To be biologically relevant, internal biological rhythms must be synchronised to external environmental rhythms, and for many organisms, their survival depends on this (6). Synchronisation occurs through the process of entrainment, which is the adjustment of the endogenous clock according to input from external cues (7). Light is the most important entraining cue for most animals (1, 4). Light can affect animals in two ways, indirectly through the entrainment of the master circadian oscillator, or directly through masking (8-10). Masking can be negative, when light decreases activity, or positive, when it increases activity (11). Light affects nocturnal and diurnal animals in different ways, in nocturnal animals, it tends to reduce activity whereas it increases activity in diurnal animals (8, 12). Masking is thought to complement entrainment to achieve appropriate timing of physiology and behaviour (13). Since masking responses are acute and direct, it can help animals to have fast, adaptive responses to harmful light stimuli (14).

Anthropogenic activities can change the ecological landscape significantly. Urban growth and urbanisation occur at an alarming pace all over the world, especially in developing nations (15, 16). The growth of urban populations often accompanies urban spatial expansion, but rates of expansion have surpassed urban population growth in some regions, such as West Africa (17). Urban expanse (the spatial extension of built-up areas) poses a major ecological threat, not only to the ecosystems and biodiversity of rural areas, but peri-urban areas as well (17, 18). Peri-urban areas lie just outside or between urban areas, where the major human land-use is usually residential and agricultural (15, 19). Peri-urban areas hold great ecological importance, as they provide ecosystem services to urban areas and act as refugia and corridors for many species (18, 20).

84   Along with habitat loss, the most prominent disturbances are light, sound and air pollution.
85   Ecological light pollution refers to artificial light that disrupts the daily light-dark patterns in
86   ecosystems (21). Sources of ecological light pollution include direct sources such as motor
87   vehicles, streetlights, residential areas, and skyglow, which is scattered light in the atmosphere
88   (21, 22). Artificial light at night (ALAN) impacts a multitude of behaviours such as activity,
89   sleep, foraging and vigilance, species interactions, navigation, mate acquisition and
90   reproduction of animals, and the effects may be immediate and severe (21-27).

91   The effects of light at night have been studied extensively in birds, both in the field and in the
92   laboratory (28-34), and the mechanism of light disruption is well understood. Although fewer
93   studies focus on the effect of artificial light at night on mammals, classic laboratory rodent
94   models such as mice, rats and hamsters are well studied (35-43). However, laboratory rodents
95   are typically bred in captivity for many generations, and may not be representative of wild
96   populations and/or species. Furthermore, the levels of light at night provided in laboratory
97   studies vary widely (35, 37, 38, 42, 44), and the brightness of the light presented may not
98   always be comparable to ecological levels of light at night. Since about 70% of mammals are
99   nocturnal (45), and their active times directly overlap with periods when artificial light at night
100 is present, it becomes increasingly important to determine the effects of light at night on this
101 group of animals.

102 Rodent responses to artificial light at night are primarily shaped by the temporal niches that
103 they occupy but are also influenced by their respective habitats and habits. Many nocturnal
104 rodents become less active in the presence of illumination at night, both in the lab and in the
105 field (21, 39, 46-50), whereas diurnal animals either extend their active periods into the night
106 or show no response (51-55). While it is clear that light at night can modify the behaviour of
107 animals both in the laboratory and in the field (56), it can elicit either a masking effect or disrupt
108 the circadian timing depending on the nature of the light and the habits of the animal (41).

109 Many of the rodents in Africa are poorly studied, and there is a paucity of studies regarding the
110 effect of light at night. In fact, to the best of our knowledge, there are no published literature
111 available on Southern African rodents to date. It is therefore critical to firstly gain a better
112 understanding of the general biology of the animals, and secondly, to determine how urban
113 expanse and the usage of artificial light at night will affect animals, in particular within cities
114 and peri-urban areas. This will be integral for the persistence and survival of wildlife, and the
115 preservation of biodiversity.

116 The African pygmy mouse is the smallest rodent in Africa, and is strictly nocturnal (57). They
117 have a wide distribution in sub-Saharan Arica and usually occur in savanna and grassland

habitats, although they can live in a wide variety of habitats (58, 59). The diet of the pygmy mouse mainly comprises seeds and insects (58), thus, they play a role in maintaining their habitats (59). Like many other rodents, pygmy mice also serve as essential prey to a number of predators (60). The African pygmy mouse contributes greatly to the functioning of food webs and ecosystem services (such as seed dispersal) in peri-urban areas; however, their survival in these areas is potentially threatened by artificial light at night. Pygmy mice are exclusively active during the night both in the laboratory and the field (57). When these mice were subjected to a shorter dark phase, their active phase was reduced but resulted in increased activity in order to meet energy requirements (57). This suggests that the presence of artificial light at night can have serious consequences for the health of the mice. Pygmy mice typically do not occur in urban areas but remain on the fringes in peri-urban areas.

The aims of this study were twofold, first to evaluate masking responses, and second, to assess the effect of artificial light at night in the African pygmy mouse. We investigated masking responses to determine the flexibility of the pygmy mouse activity rhythms. We tested masking responses with a one-hour dark pulse during the day (inactive period of the mice) and a one-hour light pulse during the night (active period of the mice). We expected that the strictly nocturnal activity of the pygmy mouse would be entrained to the natural light cycles, such that the animals would not show an increase in activity during a dark pulse during the day (inactive period) but would show a distinct suppression of activity during the night (active period). We also considered the effect of dim artificial light at night (dLAN) on the locomotor activity of the mice by presenting them with increasingly brighter intensities of light at night. We evaluated the temporal distribution and activity onset of the activity, as well as the overall level of activity. We predicted that animals would remain active during the night but that the activity onset would be later when dLAN is present, and that overall activity levels would decrease during dLAN.

2. Material and methods

2.1 Animal capture and housing

Animals were collected in the Kyalami area, South Africa (25°55'06"S, 28°04'09"E) using Sherman traps baited with peanut butter and oats. Subsequently the animals were transported to the Small Animal Physiological Research Facility on the Experimental farm at the University of Pretoria. We used twelve males (5.40 ± 0.09g) and eight females (3.34 ± 0.08g) in this experiment, males were adult, and females were sub-adult at the start of the experiment to ensure that we were not introduce pregnant animals into the experiment. Animals were housed individually in glass containers (23cm x 46cm x 30 cm) lined with a layer of soil and were

152 provided with a toilet roll and egg carton shelter, ample dried grass for nesting material and a

153 rock and a stick for enrichment. Animals were fed on budgie seed (Marltons Pet Care (Pty)

154 Ltd, Durban, RSA) and a small piece of fresh fruit or vegetables per day, feeding times were

155 randomised during the light phase of the 24h cycle since it was less likely to cause disturbance

156 to the activity recordings, and times were recorded. Water was provided *ad libitum*. The animal

157 room was maintained at 23 ± 0.5 °C on a 14L:10D light cycle that included simulated dawn

158 and dusk periods from 05h00 to 07h00 in the morning and 17h00 to 19h00 in the evening. Day-

159 time illumination was provided by overhead fluorescent lights with an intensity of 400 lux at

160 floor level, whereas night time was in complete darkness (0 lux). Experimental procedures

161 were approved by the Animal Ethics committee of the University of Pretoria (NAS311/2020).

162 *2.2 Experimental setup*

163 Medusa passive infrared motion detectors (Texecom Ltd., UK) were fitted above each housing

164 container and positioned in such a way that locomotion could be detected across the entire floor

165 surface. Since animals were housed in glass containers, cardboard dividers were placed

166 between containers to prevent individual passive infrared detectors from recording activity in

167 adjacent housing containers. The locomotor activity recorded by the infrared detectors was

168 captured and relayed to a computer using the program Vitalview (Minimitter Co. Inc., Sunriver,

169 Oregon; http://minimitter.com). During LAN cycles, night-time illumination was produced by

170 warm white LED strip lights (12V/DC 3528) attached to single-channel adjustable dimmers

171 (Communica, RSA) to produce very low intensity light. Light intensity was measured with a

172 Mastech digital light meter (MS6612 series, Florida, United States).

173 *2.3 Experimental procedures*

174 Locomotor activity was recorded under different lighting conditions to assess masking

175 responses and the effect of dLAN in the laboratory. Animals were subjected to each light cycle

176 for 3 weeks, the first week served as acclimation and data for the last two weeks were analysed.

177 The same animals were used for both experiments, although the sample sizes varied slightly

178 for each section, two animals died during the experiment, and we encountered a problem with

179 an IR detector that rendered the data for that animal unsuitable to use.

180 *Masking* (n=19) – Study animals were subjected to a 14L:10D light cycle, which included a

181 simulated dawn and dusk period (05:00-07:00 and 17:00-19:00) to create a semi-natural

182 lighting scenario. Ambient temperature was maintained at a constant 23 ± 0.5 °C. A one-hour

183 dark pulse during the day (12:00-13:00) and a one-hour light pulse during the night (00:00-

184 01:00) was introduced by switching the overhead lights on for these one-hour periods (300 lux

185 at floor level). Both the light and dark pulses were introduced simultaneously, i.e. during the

186 same 24h cycle. Although masking responses are immediate, activity was recorded for 3 weeks.

187 *LAN* - To investigate the effect of dLAN, animals were maintained on the 14L:10D light cycle

188 at a constant 23 ± 0.5 °C. Locomotor activity of the animals was first recorded under a dark

189 night to serve as a control (n = 20). Subsequently, animals were exposed to light at night at

190 three different light intensities, 0.5 lux (n = 20), 1 lux (n = 19) and 2 lux (n = 17). To ensure

191 that animals did not habituate to the experimental conditions and to detect potential changes in

192 behavioural responses, one week of dark nights were introduced between the dLAN cycles.

193 During this week, animals could re-entrain their activity to the dark night before the next LAN

194 cycle was initiated. The activity from the control period before the dLAN light cycles did not

195 differ from that during the control period after the dLAN cycles (t-test, P = 0.183). We weighed

196 animals before each light cycle, and after the final light cycle.

197 *2.4 Statistical analyses*

198 Activity counts were summed per minute and per hour using Microsoft Excel (Microsoft Corp.,

199 Redmond, WA, USA). To visualise results, double-plotted actograms were generated using the

200 program ActiView (Minimitter Co., Sunriver, Oregon, USA). Statistical analysis of locomotor

201 activity data was conducted using IBM SPSS Statistics for Windows, Version 27.0 (SPSS Inc.,

202 Chicago, IL, USA).

203 Masking data was analysed using a generalised linear mixed model to compare the activity

204 counts during an hour-long dark pulse and an hour-long light pulse to the two hours before and

205 two hours after it. We used a gamma distribution with a log link function, ID and day as random

206 factors, and sex and hour as fixed factors. Least significant difference pairwise post hoc

207 comparisons were used to determine individual differences.

208 A generalised linear mixed model with a gamma distribution and identity link was constructed

209 to compare the mean activity of animals between the control and different dLAN light cycles.

210 Light cycle (control, 0.5 lux, 1 lux and 2 lux), the phase of the day (dark, twilight or light) and

211 the sex of the animals were used as fixed factors, experimental day and ID were used as

212 repeated measures, and least significance difference pairwise post hoc comparisons were

213 included for individual differences. Activity onsets were determined manually for each day,

214 activity starts rather abruptly and usually at high intensity, therefore it was not challenging to

215 determine onsets. A generalised linear mixed model was used to analyse the activity onsets,

216 with id and experimental day as random factors, and sex and light cycle as fixed factors. Least

217 significant difference pairwise post hoc comparisons were used to determine differences

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218 between light cycles. Body weights of the animals were assessed using a one-way ANOVA.

219 The significance level was maintained at $P < 0.05$.

220 ## 3. Results

221 *3.1 Masking effects during the day and night*

222 Activity counts of five hours were compared during the day, two hours prior to the dark pulse

223 (L1 and L2), the hour-long dark pulse (DP) and two hours after the dark pulse (L4 and L5).

224 About half of the animals (9/19) showed no response to a dark pulse presented during the day,

225 whereas the other half (10/19) increased their activity during this time. The mice did not

226 increase their activity every day and showed considerable variation in the number of days that

227 they responded to the dark pulse (2-18 days out of the 21-day experimental period) (Figure 1C,

228 D, E). Overall, animals showed significantly different levels of activity during these hours

229 (mean activity counts per hour - L1: 0.62±0.24; L2: 0.28±0.08; DP: 14.5±1.86; L4: 2.61±0.46;

230 L5: 0.29± 0.07) ($F_{4,1320} = 40.35$, $P < 0.001$). Mean activity was significantly higher during the

231 dark pulse compared to the two hours prior to, and the two hours after the pulse (all

232 comparisons to DP: $P < 0.001$; Figure 1A). Females also showed a larger response to the dark

233 pulse compared to the males ($F_{1,1320} = 10.41$, $P = 0.001$). They were more active than the males

234 two hours before the dark pulse ($F_{1,1320} = 9.65$, $P = 0.002$), during the dark pulse ($F_{1,1320} = 4.32$,

235 $P = 0.038$), and the first hour after the dark pulse ($F_{1,1320} = 5.66$, $P = 0.018$).

236 Five hours of activity were assessed during the night, two hours prior to a light pulse (D1 and

237 D2), a light pulse (LP) and two hours after the light pulse (D4 and D5). Again, animals

238 displayed significantly different levels of activity during the five hours (mean activity counts

239 per hour - D1: 46.04±2.61; D2: 42.43±2.47; LP: 6.48±0.53; L4: 48.04±2.16; L5: 54.5± 2.48)

240 ($F_{4,1320} = 92.13$, $P < 0.001$). Activity was significantly suppressed during the light pulse

241 compared to the dark hours prior and after it (all comparisons to LP: $P < 0.001$; Figure 1B). All

242 animals showed a complete suppression of activity during the light pulse presented during the

243 night, for all nights presented (Figure 1C, D, E). Males and females showed a similar

244 suppressive response during the light pulse ($F_{1,1320} = 0.59$, $P = 0.443$) but were significantly

245 more active one hour before ($F_{1,1320} = 7.31$, $P = 0.007$) and one hour after ($F_{1,1320} = 4.58$, $P =$

246 0.032) the light pulse.

247 *3.2 Temporal distribution of activity*

248 The phase of the day significantly affected the amount of activity the animals displayed ($F_{2,}$

249 $_{25848} = 1235.07$, $P < 0.001$, Tables S1, S2). Animals were primarily active during the night

250 (mean activity count - dark: 61.27±0.83), significantly less so during the twilight hours (mean

activity count - twilight: 2.19±0.22) (night vs twilight: P < 0.001) and showed even less activity during the daylight hours (mean activity count - light: 0.15±0.04) (twilight vs day: P < 0.001; Figure 2A). This was the case for all light cycles (all P < 0.001 for night > twilight > light; Figure 2B).

3.3 Effect of LAN on locomotor activity

The presence of light at night significantly reduced the locomotor activity of pygmy mice ($F_{3,25848}$ = 288.61, P < 0.001, Figure 2, Figure 3). Animals were significantly more active during the control cycle (mean counts/h: 25.96 ± 0.50) compared to 0.5 lux (mean counts/h: 5.03 ± 0.14), 1 lux (mean counts/h: 3.66 ± 0.11) and 2 lux (mean counts/h: 3.06 ± 0.11) LAN at night (all comparisons P < 0.001). Animals also displayed more activity at 0.5 lux LAN compared to 1 lux and 2 lux LAN (both P < 0.001). Animals showed the lowest levels of activity at 2 lux LAN (1 lux vs 2 lux: P = 0.002, Table S1).

During the light hours, activity was reduced from the control cycle to 0.5 lux LAN (P = 0.003), whereafter it increased again during the 1 lux LAN cycle (0.5 lux-1 lux: P < 0.001), whereafter daytime activity remained similar during the 2 lux LAN cycle (1 lux-2 lux: P = 0.265). Animals were less active during the twilight hours during the 0.5 lux LAN cycle compared to the control cycle (P < 0.001), whereafter activity showed an increase during the 1 lux cycle (0.5 lux-1 lux: P < 0.001) and thereafter remained stable during the 2 lux LAN cycle (1 lux-2 lux: P = 0.678). At night, the activity of pygmy mice consistently reduced with higher light intensities (Control > 0.5 lux > 1 lux > 2 lux, all combinations P < 0.001). Overall, no sex difference was apparent in the activity of animals ($F_{1, 25\,848}$ = 0.015, P = 0.904, Table S1).

Once the body weight of the mice stabilised, the overall body weight of the animals did not change with the presence of increasing ALAN ($F_{3,74}$ = 0.072, P = 0.975).

3.4 Activity onsets

The presence of LAN altered the onset of the pygmy mouse activity significantly (control – 18:55 ± 01:05; 0.5 lux - 19:14 ± 02:43; 1 lux - 19:07 ± 01:56; 2 lux – 19:07 ±00:43) ($F_{3,551}$ = 18.93, P < 0.001; Figure 4). The onset of activity was significantly delayed during all LAN cycles compared to the control cycle when animals were exposed to a dark night (P < 0.001 compared to all LAN cycles). The activity onset during 0.5 lux LAN was delayed the most, significantly more compared to 1 lux (P = 0.038) and 2 lux (P = 0.036). There was no difference in the activity onset time between 1 lux and 2 lux LAN (P = 0.969). Activity onsets were determined for the first 8 days after a switch in lighting conditions. The onset of activity differed between the different days ($F_{7,551}$ = 0.18, P = 0.034) but not in a logical pattern and

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was therefore not explored further. Overall, males had a later onset time of activity compared to females ($F_{1,551}$ = 13.58, P < 0.001), although the interaction between sex and cycle was only significant for 0.5 lux (Ctrl: P = 0.181; 0.5 lux: P < 0.001; 1 lux: P = 0.351; P = 0.628).

4. Discussion

4.1 Masking

Time can be defined as an ecological niche (61), and animals show morphological, physiological and behavioural adaptations to their specific temporal niches. Pygmy mice are known to be strictly nocturnal, both in the laboratory and in the field (57). We aimed to investigate the flexibility of the activity rhythms in pygmy mice. In our study, we observed that pygmy mice that were subjected to a simulated dawn and dusk period become active only during complete darkness, indicating that the animals are strictly nocturnal.

We investigated the masking responses of pygmy mice following a dark pulse during the day and a light pulse during the night. Masking responses are dependent on the natural temporal niche of a species and the chronotype of the individual animal, which will determine whether the animal is awake when the masking pulse is administrated. All pygmy mice showed a sharp reduction in locomotor activity when a light pulse was presented in the middle of the dark phase. This is expected for nocturnal animals and suggests that their activity is masked by light. Similarly, nocturnal common spiny mice (*Acomys cahirinus*) reduced both their general activity and wheel running following a light pulse at night, whereas diurnally active golden spiny mice (*A. russatus*) showed no response to light pulses at night (62). Nocturnal chronotypes of a diurnal species, the Nile grass rat (*Arvicanthis niloticus*), displayed reduced wheel running but their general activity was not affected by a light pulse during the night, but diurnal chronotypes increased both wheel running and general activity (63). Diurnal fat sand rats (*Psammomys obesus*) showed no response to light pulses (64).

Dark pulses during the day appear to have a more unpredictable effect on the animals. Only nine of the pygmy mice showed a positive masking response during the day, and increased activity was not necessarily evident every day. The remainder of the animals did not show any response to the dark pulse. This indicates that the animals that do show a masking response to a dark pulse during the day were awake to become active and suggests that the strictly nocturnal activity pattern of the pygmy mouse may at least partly be a masking response and not completely resulting from entrainment. It is however evident that the amplitude of the activity in response to a dark pulse is much lower than that of the activity during the active phase of the animals, not all animals become active during the dark pulse, and the animals that respond

to a dark pulse, do not respond every day. Some nocturnal animals, such as the house mouse (*Mus musculus*) do not respond to dark pulses at all (65), whereas nocturnal chronotypes of the Nile grass rat increased their wheel running activity but not their general activity (63). Since dark pulses would be presented during the inactive phases of nocturnal animals, a reduced, or absent response is to be expected. Diurnal Nile grass rats reduced both their activity and wheel running during a dark pulse (63), but fat sand rats do not respond to dark pulses (64). We therefore conclude that the activity of pygmy mice is strongly masked by light, and they will become active opportunistically, when it is dark enough for them to deem the predation risk low enough.

4.2 Light at night and activity onset

The presence of light at night suppressed the locomotor activity of African pygmy mice. Dim light at night with an intensity of 0.5 lux resulted in an 80% reduction in overall activity, with even further reductions in activity at 1 lux and 2 lux light at night. The activity reductions were accompanied by a delay in the onset of activity. Several other nocturnal rodents also show reduced activity in the presence of light at night, both in the laboratory and the field (37, 39, 49, 50), and Patagonian leaf eared mice also show an increased reduction in activity under higher light intensities (49). In nature, animals must manage the trade-off between predation risk and foraging success. A brighter night sky is perceived as a greater predation risk, which prompts the reduction in foraging and locomotor activity (21, 57, 66).

To meet the energetic demands for survival, animals can also shift their active times towards times of the day that present lower risks (66). Most rodents are nocturnal (67), but in response to energetic or other challenges, activity shifts towards the day are not uncommon (66, 68, 69). While some rodents modify their temporal niches to optimise survival, others completely switch their activity from nocturnal to diurnal to avoid competition or other adverse conditions (66, 69, 70). Some rodents are also known to switch from diurnal activity in the field to nocturnal activity in the laboratory (71-75). When subjected to light at night, pygmy mice showed a slight increase in day-time activity compared to when they were exposed to a dark night, but daytime activity was always significantly less than night time activity.

Activity onsets were shifted later when dLAN was present. When presented with a dark night, activity commenced a few minutes before compete darkness, whereas when animals were exposed to 0.5 lux light during the night, the activity was delayed by approximately 20 minutes, and by about 12 minutes for 1 lux and 2 lux light at night. The delay in activity onset was expected, however the shorter delay in the commencement of activity at higher light levels during the night was not. This indicates that the animals show some type of habituation to the

light at night, although this was independent from the level of activity. Nevertheless, despite the massive reduction in nocturnal locomotor activity when exposed to LAN, we did not observe temporal niche switching in the pygmy mice, and they also did not lose weight when subjected to increasing amounts of ALAN. Our animals were fed *ad libitum*, and pygmy mice are known to cache food in their nests in captivity (57). Animals were also observed stuffing their cheeks with small seeds in the laboratory. Since we did not anticipate the magnitude of the response to ALAN in the pygmy mice, we did not specifically monitor the food intake throughout the experimental procedures other than weighing the animals before and after each cycle. It is possible that ALAN could have a bigger effect on the food intake of the animals should the food be presented further from their shelters. The small size of the animals precluded the assessment of any endocrine factors; therefore we cannot comment on the stress levels of the animals with increasing ALAN. Our experimental conditions probably did not alter the circadian rhythms of pygmy mice given that upon removal of dLAN, activity onsets shifted back to the onset of darkness immediately after was removed, activity levels increased instantly, and animals did not shift their active periods towards the day. Nevertheless, it is clear that ALAN has the strong masking effect on the locomotor activity of the pygmy mice.

4.3 Biological implications for pygmy mice

Laboratory studies can be a useful starting point to assess the effect of artificial light at night on small rodents. Experimental variables can be closely regulated and separated (76), and lately, can recreate individual natural conditions much more closely. In such a way, elusive and obscure species can be contained and closely monitored to obtain solid initial information regarding the physiology and behaviour of such species. An initial impression of the flexibility of the circadian system and the amount of diversity in a species can be gained and based on that, more refined and informed field studies can be designed for targeted questions.

In contrast to the laboratory environment, many biotic and abiotic factors interact and influence behaviours of animals in the field. Microhabitats can affect predation risk and also modify the animal's perception of risk. Desert dwelling rodents were found to be most active when they were under vegetation with low light levels (new moon) and least active with full moon in open habitats (77). The experimental light intensities the pygmy mice were exposed to in the lab were comparable to that of a full moon in a clear sky (78), and the cage setup was equivalent to a relatively open habitat. In effect, we recreated a very risky environment, or a worst-case scenario, which the animals clearly perceived as such given the severe reduction in their activity. African pygmy mice live in shallow burrows or existing shelters such as holes, fallen logs and under rocks in the field (79). When foraging, pygmy mice are likely restricted to

vegetation that shields them from the majority of light at night, whether it be the illumination of the moon or artificial light at night (60). A previous study indicated that the presence of light at night affects the home range sizes of small rodents negatively and have implications for their movement patterns (80). Current and future habitat transformation and degradation associated with urban expansion potentially pose a larger threat to the persistence of African pygmy mice in sub-urban and peri-urban areas. The transformation of their habitat would likely expose pygmy mice to higher levels of light at night that would hinder them from carrying out normal behaviours such as foraging, caching, and finding conspecifics (21, 22). We therefore cautiously predict that pygmy mice will be able to persist in sub-urban areas with increasing light pollution provided that there is sufficient vegetation coverage, however this will have to be verified with a dedicated investigation.

5. Conclusions

In conclusion, our results revealed that artificial light at night has a significant, intensity dependent effect on the locomotor activity of African pygmy mice. Activity levels are lower at higher dLAN light intensities and were shifted later. Pygmy mice are rigid in their temporal niche selection and show little variation in their activity patterns. Some of the pygmy mice display positive masking when subjected to a dark pulse during the day, implying that the animals are awake during the day, just not active. The ability to become active when a dark pulse was presented during the day, suggests that their activity is strongly masked by light, but that the animals will become active opportunistically. The activity of the pygmy mouse appears to depend upon the level of risk perceived by the animals, which from a light perspective, may be alleviated by vegetation cover in their natural habitat. However, given the anxious nature of the pygmy mice, other anthropogenic changes such as noise and habitat destruction also have the potential to disrupt the behaviour of these small animals, and the combined effects of these disturbances could have a devastating effect on the survival of these small mammals.

Data accessibility statement

Data used for analysis is available on Dryad: https://doi.org/10.5061/dryad.9w0vt4bm4

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624   Figure legends:

625   **Figure 1**. (A) Mean activity counts ($\pm$ SE) of pygmy mice during a one-hour dark pulse during

626   the day (black bar) and two hours prior and two hours after the pulse (white bars). (B) Mean

627   activity counts ($\pm$ SE) of pygmy mice during a one-hour light pulse during the night (white bar)

628   and two hours prior and two hours after the pulse (black bars). (C) An actogram of a pygmy

629   mouse (MM13♂) that displays positive masking during the day and negative masking during

630   the night. In (D), animal (MM15♀) shows negative masking during the night and positive

631   masking on some days during the day, and (E) shows an animal (MM2♂) that did not show

632   positive masking during the day. Animals were subjected to a 14L:10D light cycle, including

633   a dawn and dusk period, animals commence activity when it is completely dark, and ceases

634   activity before the light comes on again. Black bars on top of the graph indicates light phases,

635   and consecutive days are on the y-axis.

636   **Figure 2**. (A) Mean activity hourly counts ($\pm$ SE) during the dark, twilight and light phases of

637   each of the light cycles pygmy mice were subjected to. (B) Mean hourly activity counts for

638   each of the different light cycles over the 24-hour period of the day.

639   **Figure 3**. Double plotted actograms of a representative mouse (MM1♂) for each of the

640   different LAN cycles (A) control, (B) 0.5 lux light at night, (C) 1 lux light at night, (D) 2 lux

641   light at night. Consecutive days are depicted on the y-axis, greyed-out areas are the twilight

642   and dark phases.

643   **Figure 4**. Mean time of activity onset for 8 days after the light cycles changed, for each light

644   cycle. The onset was later when light at night was present.
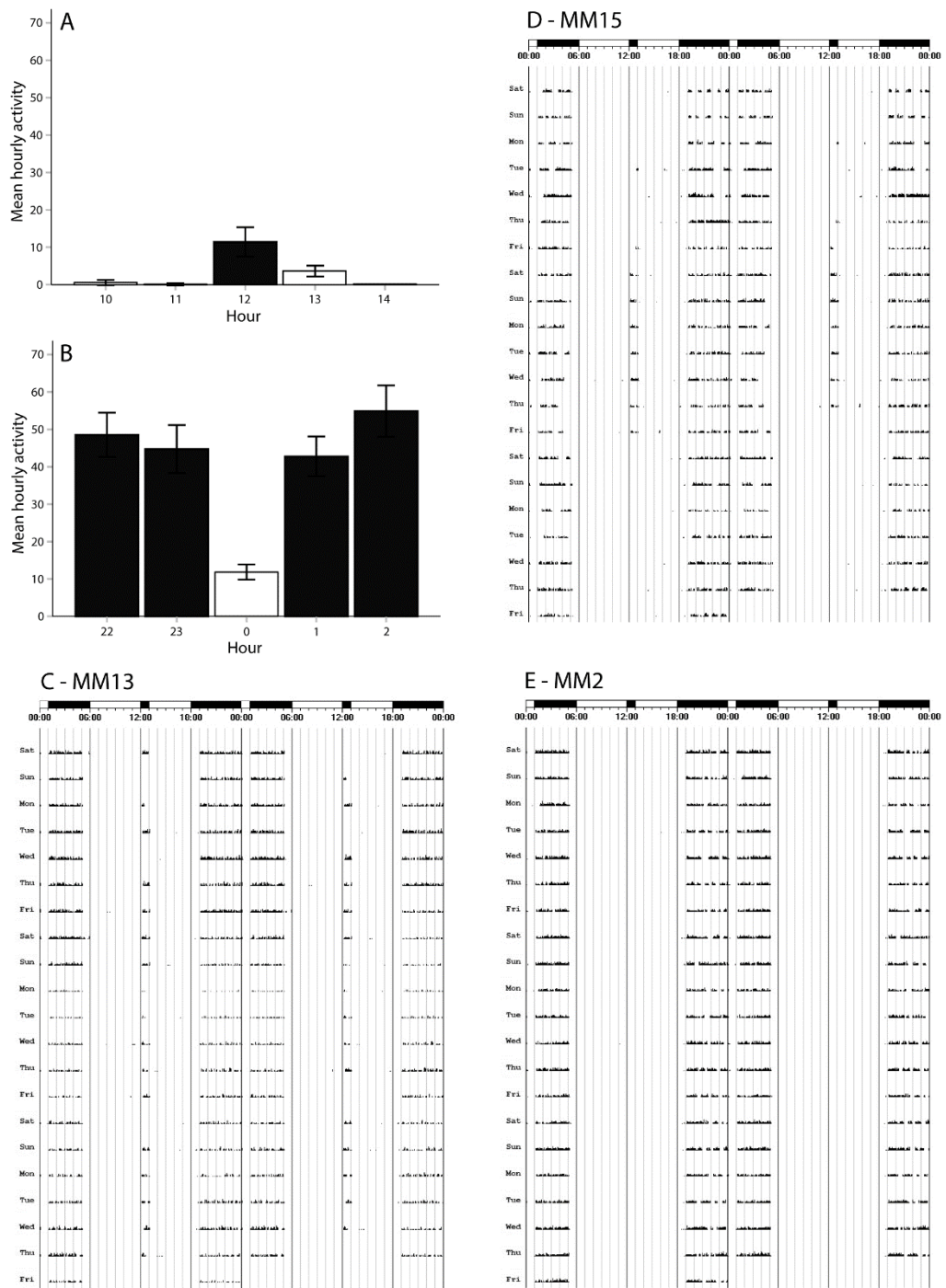
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Figure 1. (A) Mean activity counts (± SE) of pygmy mice during a one-hour dark pulse during the day (black bar) and two hours prior and two hours after the pulse (white bars). (B) Mean activity counts (± SE) of pygmy mice during a one-hour light pulse during the night (white bar) and two hours prior and two hours after the pulse (black bars). (C) An actogram of a pygmy mouse (MM13♂) that displays positive masking during the day and negative masking during the night. In (D), animal (MM15♀) shows negative masking during the night and positive masking on some days during the day, and (E) shows an animal (MM2♂) that did not show positive masking during the day. Animals were subjected to a 14L:10D light cycle, including a dawn and dusk period, animals commence activity when it is completely dark, and ceases activity before the light comes on again. Black bars on top of the graph indicates light phases, and consecutive days are on the y-axis.
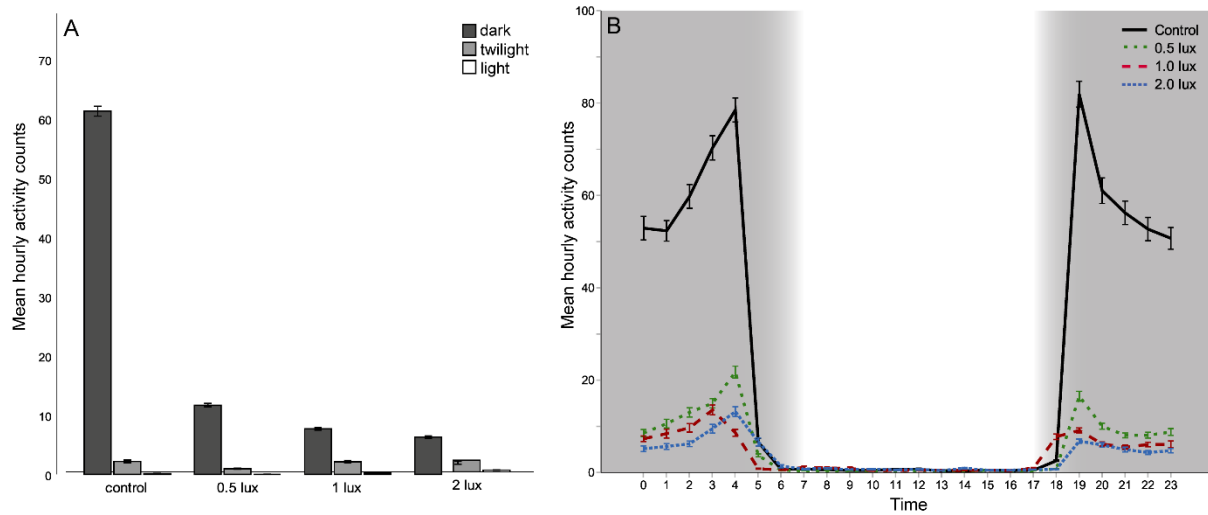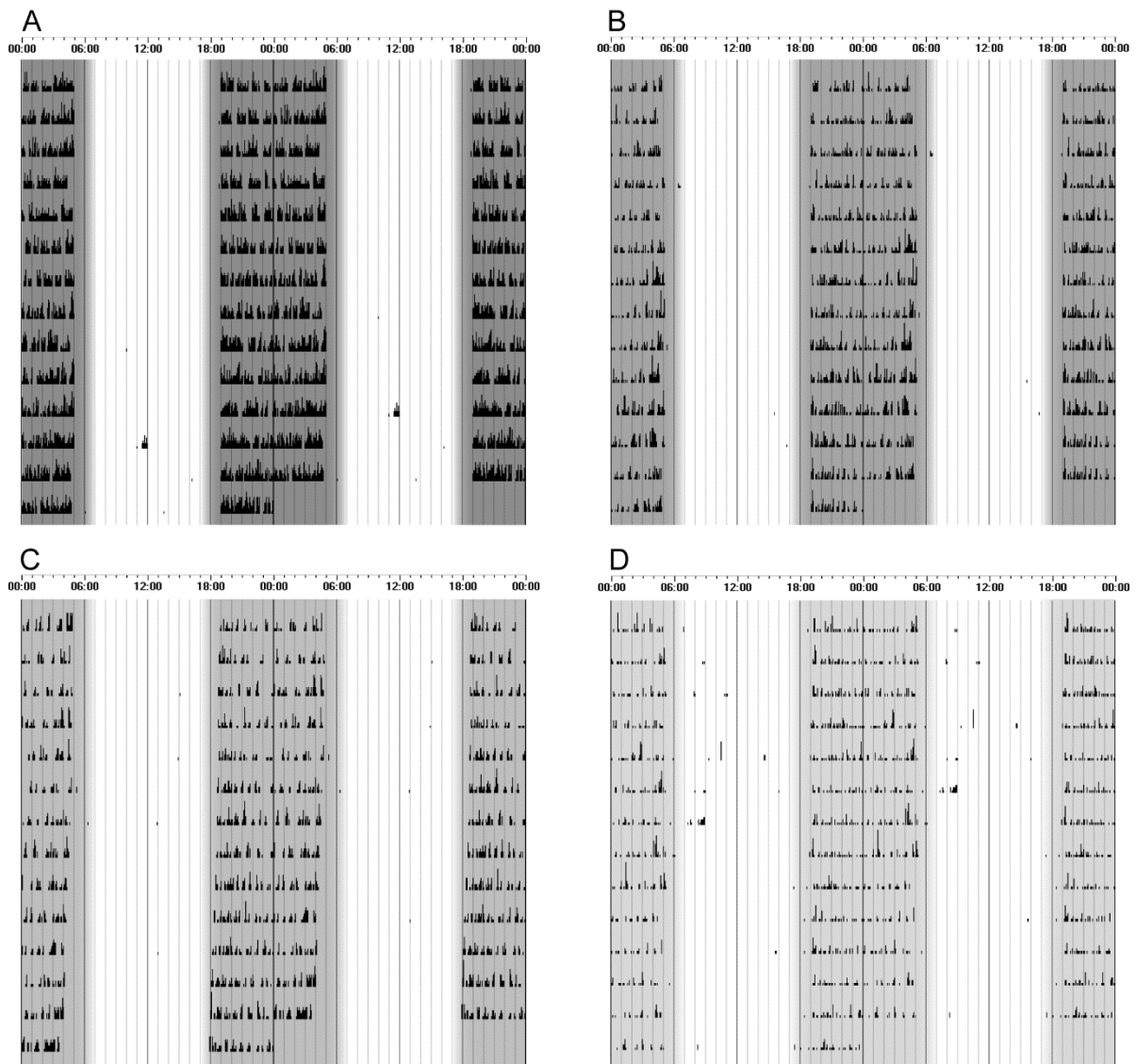
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Figure 2. (A) Mean activity hourly counts (± SE) during the dark, twilight and light phases of each of the light cycles pygmy mice were subjected to. (B) Mean hourly activity counts for each of the different light cycles over the 24-hour period of the day.
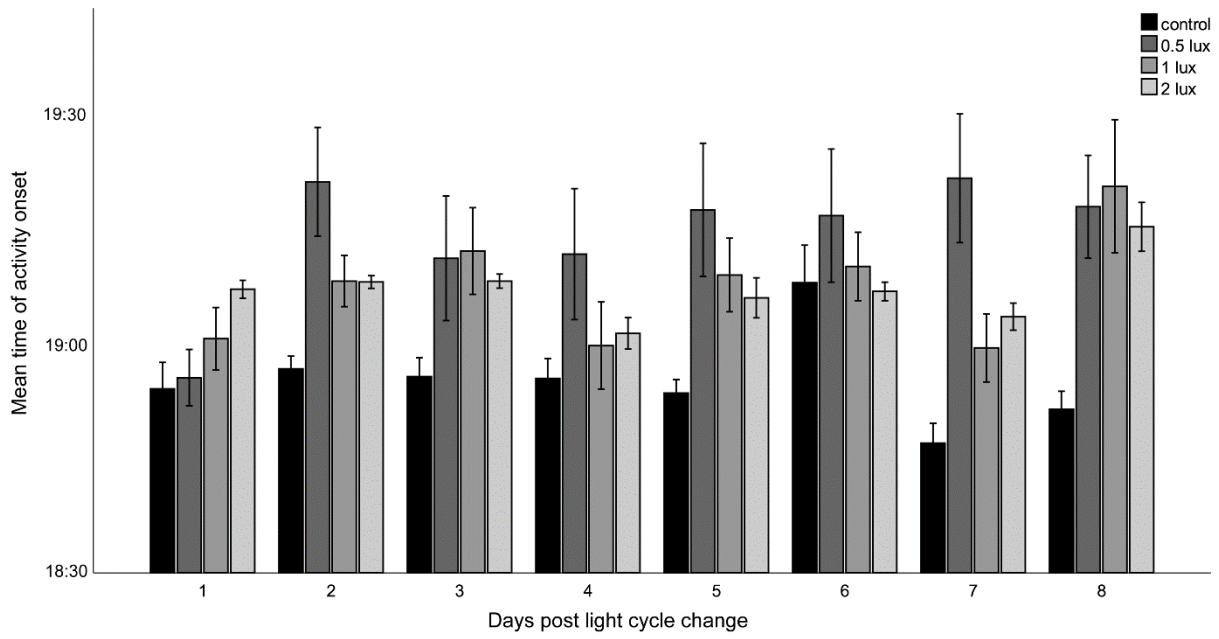
Figure 3. Double plotted actograms of a representative mouse (MM1♂) for each of the different LAN cycles (A) control, (B) 0.5 lux light at night, (C) 1 lux light at night, (D) 2 lux light at night. Consecutive days are depicted on the y-axis, greyed-out areas are the twilight and dark phases.

675

676 **Figure 4**. Mean time of activity onset for 8 days after the light cycles changed, for each light cycle.

677 The onset was later when light at night was present.