The effect of varying laboratory conditions on the locomotor activity of the nocturnal Namaqua rock mouse (*Micaelamys namaquensis*) and the diurnal Four-striped grass mouse (*Rhabdomys dilectus*)

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Highlights

•Cage enrichment affects diurnal and nocturnal rodent species differently.

•Twilight extends diurnal rodent activity time and truncates it in nocturnal species.

•Ambient temperature cycles did not change activity profiles in either species.

•Diurnal species show more flexible activity patterns than nocturnal species.

Running title: Lab activity in wild nocturnal and diurnal rodents

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Abstract

Rodents are the most common laboratory animals all over the world, however, most studies on the effects of laboratory conditions on the behavior and physiology of the study animals have been performed on traditional laboratory animals. We investigated the effects of environmental enrichment, lighting conditions and ambient temperature cycles on the locomotor activity of wild trapped, nocturnal Namaqua rock mice and diurnal Four-striped grass mice. When considering the general activity of the two species, the diurnal species showed more variability in locomotor activity than the nocturnal species. Cage enrichment differentially affected the intensity of the locomotor activity in the two species. Despite a decrease in activity, the diurnal species showed more structured rhythms in an enriched cage. Twilight conditions changed the behavior of both species, the active time of the nocturnal animal was contracted to the completely dark hours of the light cycle, while the active time of the diurnal species was extended in the longer daylight hours. The natural light appears to stabilize the entrainment of the diurnal species. The natural ambient temperature cycle caused changes in intensity of activity, but reinforced entrainment in both species. These results show that changes in laboratory housing conditions can affect the activity of captive wild animals and that these effects are species specific. By increasing our understanding of the effects of different environmental factors on the outcomes of experiments, both the results obtained, and the welfare of the captive animals may be improved.

Keywords: Twilight, enrichment, locomotor activity, *Micaelamys namaquensis*, *Rhabdomys dilectus*, temperature cycle

1. Introduction

Locomotor activity rhythms are a fundamental element of all animals and are determined by both internal and external factors (Barak and Kronfeld-Schor., 2013; Sassi et al., 2015). Depending on their time of activity, animals are frequently classified as nocturnal or diurnal. However, the temporal niches of mammals are distributed over a gradient of diurnality, between and within species (Refinetti 2008). The daily light cycles are the most prominent environmental cue where to animals entrain their activity rhythms, but there are numerous other environmental factors that can modulate and refine the entrainment of activity rhythms (Boulos et al., 1996; Augustsson et al., 2003; Boulos et al., 2002; Comas and Hut, 2009; Lahmam et al., 2008; Bailoo et al., 2018; Van Jaarsveld et al., 2019). The activity rhythms that are displayed by animals in their natural habitat are therefore the combined effect of many different influencing factors. In the laboratory, environmental conditions can be controlled and simplified to investigate the influence of factors individually.

Animals in captivity are usually limited in the environmental enrichment provided during laboratory experiments, the enrichment presented is also standardised along ethical guidelines depending on the species used (Lewejohann et al., 2006). These standardised conditions allow different factors to be monitored independently without interaction between two or more factors at the same time. However, when animals are housed in impoverished conditions that do not allow them to express their natural repertoire of behaviors, they may develop stereotypic behaviors (Mason, 1991). A frequent argument against cage enrichment for laboratory animals is increased variability in data, however a growing body of evidence indicates the contrary (Augustsson et al., 2003; Wolfer et al., 2004; Baumans et al., 2010; Bailoo et al., 2018).

Standard conditions for many laboratory studies include a square wave light-dark (LD) cycle with no temporal variation in light intensities. It is known that changes in light

intensity and color at dawn and dusk are important for the entrainment of activity in some animals, thus natural lighting cycles may have a profound effect on the onset and offset of activity in rodents (Boulos et al., 2002; Zubidat et al., 2009; Walmsley et al., 2015). Studies are also frequently conducted at constant temperatures, mostly around room temperature (between 20 - 25°C). Even when temperature cycles are presented, it is frequently at a constant higher ambient temperature for a set period of time, followed by a constant lower ambient temperature for a set time (Francis and Coleman, 1988; Ellis et al., 2009; Refinetti, 2010). To date, very few circadian studies have been performed simulating a natural ambient temperature cycle in the laboratory to assess the effect on daily activity rhythms (van Jaarsveld et al., 2019).

In this study, we investigated two wild trapped rodent species from South Africa. Both the Four-striped grass mouse (*Rhabdomys dilectus*) and the Namaqua rock mouse (*Micaelamys namaquensis*) are widely distributed throughout South Africa. Both of these species have been investigated in the laboratory before, therefore the general activity patterns in the laboratory is known. *Rhabdomys dilectus* is a small solitary species that inhabits mesic and humid grasslands in the eastern parts of southern Africa (Rambau et al., 2003; Meynard et al., 2012). It has been described as primarily diurnal (Schradin and Pillay, 2003; Rymer et al., 2013). Conversely, *M. namaquensis* is a polygynous, communally nesting species, that prefers rocky habitats with tall grass (Russo et al., 2010). Previous studies have described it as strictly nocturnal (Muteka et al., 2006; van der Merwe et al., 2014). These two species differ in their active time and type of habitat, both factors may affect the response of animals to changes in laboratory conditions.

Standard laboratory conditions are sometimes very different from what animals experience in the field, and the behaviour observed in the laboratory may not reflect the behaviour of animals in their natural habitat. Hence, it may not always be appropriate to extrapolate

laboratory findings to predict behaviour of animals in the field. Our aims were to investigate the effects of variations in environmental conditions in the laboratory on the locomotor activity of a nocturnal and a diurnal rodent species. Firstly, we examined the effect of cage enrichment on the locomotor activity rhythms in the two species. Secondly, locomotor activity under a square wave LD cycle was compared to that under a simulated dawn-dusk light cycle. Lastly, we investigated the impact of constant ambient temperature versus a simulated natural temperature cycle. Since sex differences are frequently observed in the behaviour of animals, we also considered this possibility. We predicted that all of the environmental factors tested would alter the locomotor activity of the study species, and that it may affect the nocturnal and diurnal species differently since they occur in different habitats and they differ in their fundamental temporal activity distributions.

2. Materials and methods

2.1 Animal capture and housing

Twelve (six males and six females; mean body mass = 48.4 ± 3.2g) *M. namaquensis* and twelve (six males and six females; mean body mass =37.6 ± 3.7g) *R. dilectus* were used in this study. The *R. dilectus* were captured from a high rainfall grassland region within Rietvlei Nature Reserve in Pretoria (GPS: -25.882125° S, 28.263915 °E), South Africa, whereas *M. namaquensis* were collected on rocky outcrops in Telperion private game reserve in Gauteng province along the border with Mpumalanga province of South Africa (-25.703790 °S, 28.981446 °E). Trapping permits were granted from Gauteng Nature Conservation (CPF6-0126) and the respective nature reserves.

Animals were captured using Sherman live traps, baited with a mixture of peanut butter and oats rolled into small balls. During the trapping period for *M. namaquensis*, traps were opened before dusk and closed at dawn, as *M. namaquensis* is known to be strictly

nocturnal. However, during the trapping effort for *R. dilectus*, entry into the Game reserve was restricted to between 07:00 and 17:00, therefore the traps remained open for 24-hour periods to maximise the trapping success. Traps were checked at 07:00, between 11:00 and 13:00, and again at 16:30 to prevent animals from overheating. Individuals captured were inspected to determine relative age, only adult males and non-gravid females were retained and transported to the Small Animal Physiological Research Facility on the experimental farm at the University of Pretoria, where they were maintained for the duration of the experiment. All other animals, including non-target species were released.

Animals were housed individually in cages in climate-controlled rooms where the light and temperature conditions could be manipulated. Animal cages measured 58 × 38 × 36 cm in size and were lined with wood shavings. Animals were provided with empty plastic containers to use for shelter as standard items throughout all the experiments with other items added during the course of the experiments. The mice were provided with *ad libitum* access to water and maintained on a mixed diet of sliced fresh food (such as banana, carrot and apples), and commercially available mixed seeds and specialised rodent pellets (protein 14%, crude fibre 4%, fat 4%; Supreme Petfoods Ltd, Suffolk, UK) to meet their dietary requirements. Animals were allowed to acclimate for one month in the laboratory before experiments commenced. Cages were cleaned at the end of each experimental light and temperature cycle, prior to the next one being initiated.

2.2 Experimental Set up

To capture the locomotor activity of the mice, a passive infrared captor (Quest PIR internal passive infrared detector; Elite Security Products [ESP], Electronic Lines, London, United Kingdom) was placed at the top of each cage. Sensors were placed into custom made holders that maintained the correct angle of the captor to cover the entire floor of each cage. The sensors require displacement of the animal to record activity but are not triggered by

stationary activities such as grooming and eating. Activity counts were recorded per minute by the program VitalView (Minimitter Co. Inc., Sunriver, Oregon; http://minimitter.com) on a computer system. Activity data were downloaded from the computer at the end of each light cycle and then imported into Microsoft Excel and prepared for data analysis.

2.3 Light Cycles

The experiment consisted of four consecutive light-dark (LD) and temperature cycles that were performed on both species in the following order LD_{NE} LD_{E}, LD_{N} and LD_{NT}. Under the LD_{NE} cycle, animals were subjected to a 12L:12D (06:00-18:00 light) square wave light cycle, a constant ambient temperature (T_a) of 25°C and no additional environmental enrichment apart from an empty plastic container. During the LD_{E} cycle, mice were presented with similar environmental conditions as during the LD_{NE} cycle, but with the addition of tissue paper, three toilet rolls and a rock as environmental enrichment. During the LD_{N} cycle, all conditions remained similar apart from the square wave light cycle that was replaced with a 14L:10D light cycle (05:00-19:00 light) with light decreasing and increasing along a ramp for the first two and last two light hours of the day to simulate dawn and dusk. For the last cycle (LD_{NT}), the parameters from the previous cycle were retained, but the ambient temperature cycled between 16°C (at 04h00) and 30°C (at 15h00) to simulate temperatures of an average summer day. Light and temperature cycles were presented for 21-23 days, with one or two days between the different cycles to allow for cleaning of the cages and resettlement of the animals. Experimental procedures were approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (EC018-16).

2.4 Data Analysis

The data collected from the animals during the different light cycles were downloaded from the computer at the research facility. The last 14 days of each light and temperature cycle were analysed to avoid carryover effects from the previous cycle. All statistical comparisons

were performed using IBM SPSS v25 (SPSS Inc., Chicago, Illinois). Hourly activity counts were compared between different light and temperature cycles, and different light phases (light / crepuscular / dark). Data were not normally distributed therefore non-parametric generalized linear mixed models were used. Light cycle, light phase and gender were considered as fixed factors in the analyses. All two way and three-way interactions of factors were investigated, and least significant difference was used in the *post hoc* tests. The average onset and offset times of activity, as well as chi-square periodograms for each light cycle of each animal were calculated using Clocklab software (ClockLab™; Actimetrics, Evanston, Illinois, USA).

3. Results

3.1 12L:12D without enrichment

Micaelamys namaquensis displayed strictly nocturnal activity, animals were significantly more active during the dark phase of LD_{NE} compared to the light phase ($F_{1, 23.31} = 674.84$, $P < 0.001$; Table 1; Fig 1a, Fig 2, see Fig S1 for individual animal plots). No differences were observed between male and female activity during LD_{NE} ($F_{1, 23.31} = 2.13$, $P = 0.14$). The average onset time for *M. namaquensis* was 17h49 ± 00h11 and offset occurred around 05h58 ± 00h10.

Overall, *R. dilectus* displayed similar amounts of average activity counts during the light and dark phases of LD_{NE} ($F_{1,22.70} = 0.50$, $P=0.50$; Table 1; Fig 3a, Fig 4, see Fig S2 for individual animal plots), with peaks around dawn and dusk. All animals showed daily activity rhythms very close to 24h when a periodogram analysis was performed. During LD_{NE} three individuals showed a clear diurnal activity pattern, with the remaining nine individuals displaying more variable activity (Fig 2a). Females were significantly more active than males during the LD_{NE} cycle ($F_{1,22.70} = 72.81$, $P< 0.001$). On and offsets could not be determined for

Table 1. The average locomotor activity counts for *Micaelamys namaquensis* and *Rhabdomys dilectus* when exposed to different enrichment, lighting and temperature regimes. LD_{NE} = non-enriched light-dark cycle, LD_E = enriched light-dark cycle; LD_N = natural light-dark cycle; LD_{NT} = natural temperature light-dark cycle.

Species	**Light Cycle**	**Mean ± SE activity counts**	
		Light phase	**Dark phase**
Micaelamys namaquensis	LD_{NE}	1.47 ± 0.04	24.1 ± 0.73
	LD_E	1.93 ± 0.06	39.03 ± 1.1
	LD_N	2.13 ± 0.06	38.91 ± 1.25
	LD_{NT}	2.68 ± 0.08	42.69 ± 1.37
Rhabdomys dilectus	LD_{NE}	28.24 ± 0.85	20.61 ± 0.62
	LD_E	21.06 ± 0.63	13.49 ± 0.4
	LD_N	24.04 ± 0.68	5.42 ± 0.18
	LD_{NT}	17.94 ± 0.51	4.27 ± 0.14

Figure 1. The average locomotor activity counts per hour (± SEM) of *Micaelamys namaquensis* during the 24 hours of the day during the four different light cycles. Greyed out areas show hours of darkness. (A) Non-enriched cycle (LD_{NE}) with a square wave light cycle (6-18L) and a constant T_a of 25°C; (B) Enriched cycle (LD_E) with the same environmental conditions than $LD_{NE,}$ but with added enrichment items; (C) natural light cycle (LD_N) with a simulated dawn and dusk as indicated by the faded grey areas (5-7am and pm); (D) natural temperature cycle (LD_{NT}) where ambient temperature cycled between 16 (04h00) and 30°C (15h00), indicated by the dotted line.

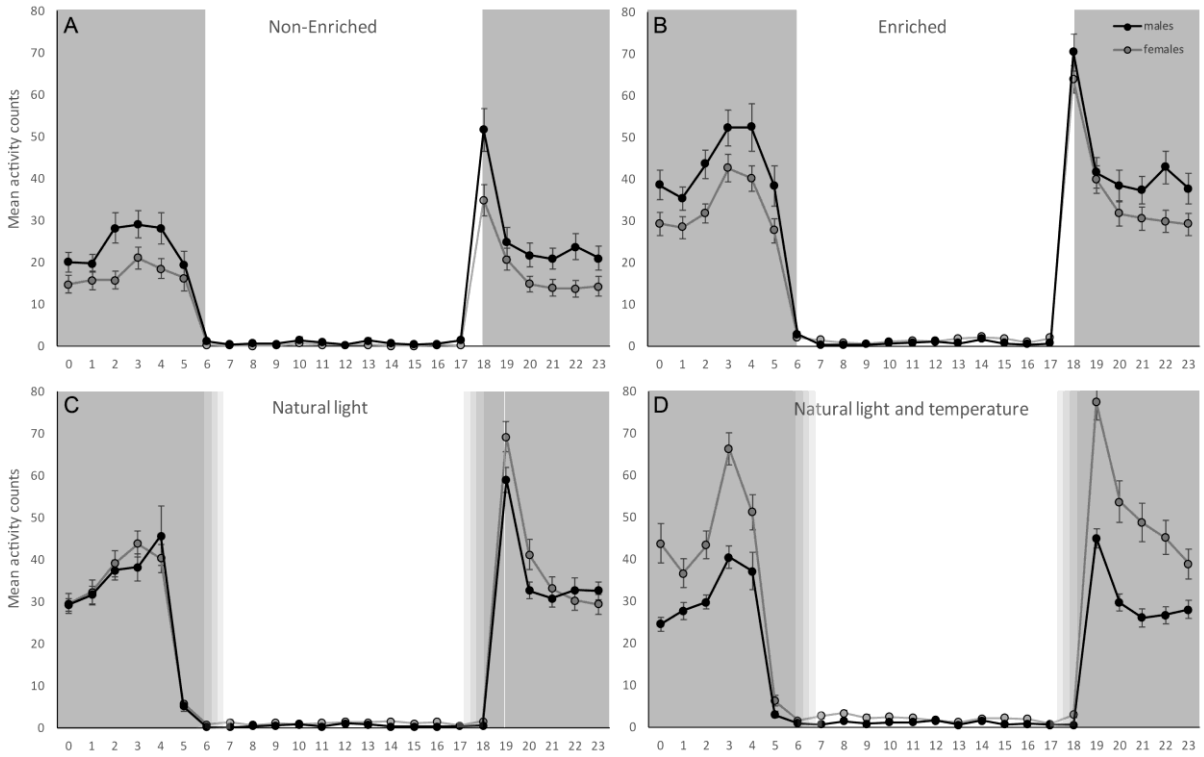


Figure 2. A double plotted actogram of a *Micaelamys namaquensis* showing robust nocturnal entrainment during all four light and temperature cycles. *M. namaquensis* was very responsive to changes in light cycles that results in the contraction of activity at the start of LD_N. White and black bars on top of the actograms indicate light and dark periods, with consecutive days on the y-axis. Ambient temperature was constant for the first three cycles, and temperature cycled between 16 (04h00) and 30°C (15h00) in the last cycle.

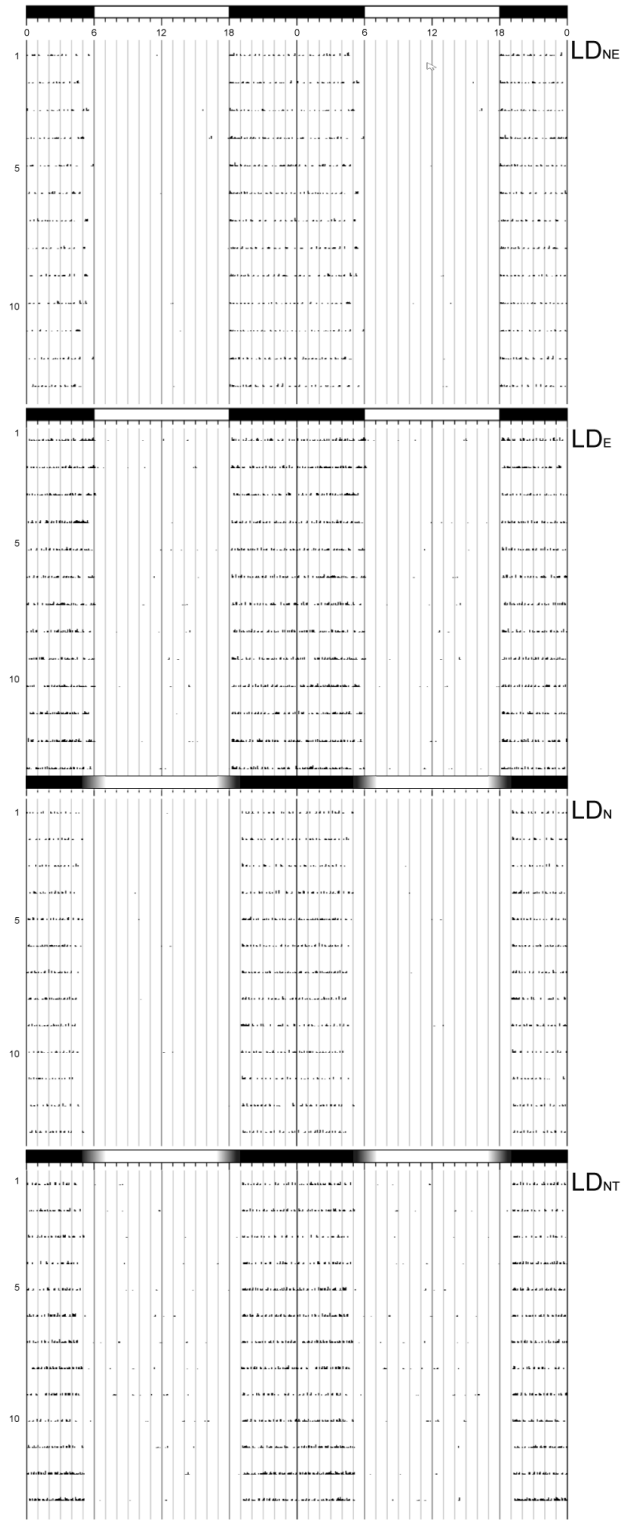


Figure 3. The average locomotor activity counts per hour (± SEM) of *Rhabdomys dilectus* during the 24 hours of the day during the four different light cycles. Greyed out areas show hours of darkness. (A) Non-enriched cycle (LD_{NE}) with a square wave light cycle (6-18L) and a constant T_a of 25°C; (B) Enriched cycle (LD_E) with the same environmental conditions than LD_{NE} and added enrichment items; (C) natural light cycle (LD_N) with a simulated dawn and dusk as indicated by the faded grey areas (5-7am and pm); (D) natural temperature cycle (LD_{NT}) where ambient temperature cycled between 16 (04h00) and 30°C (15h00).

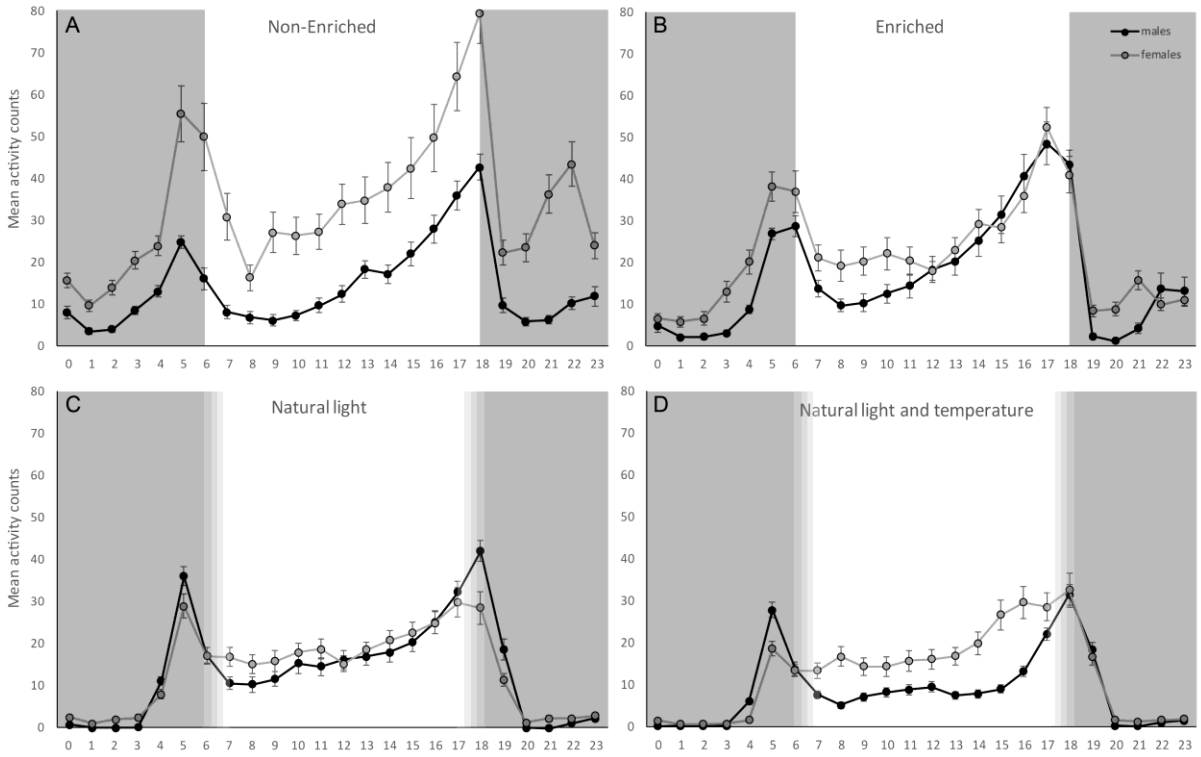
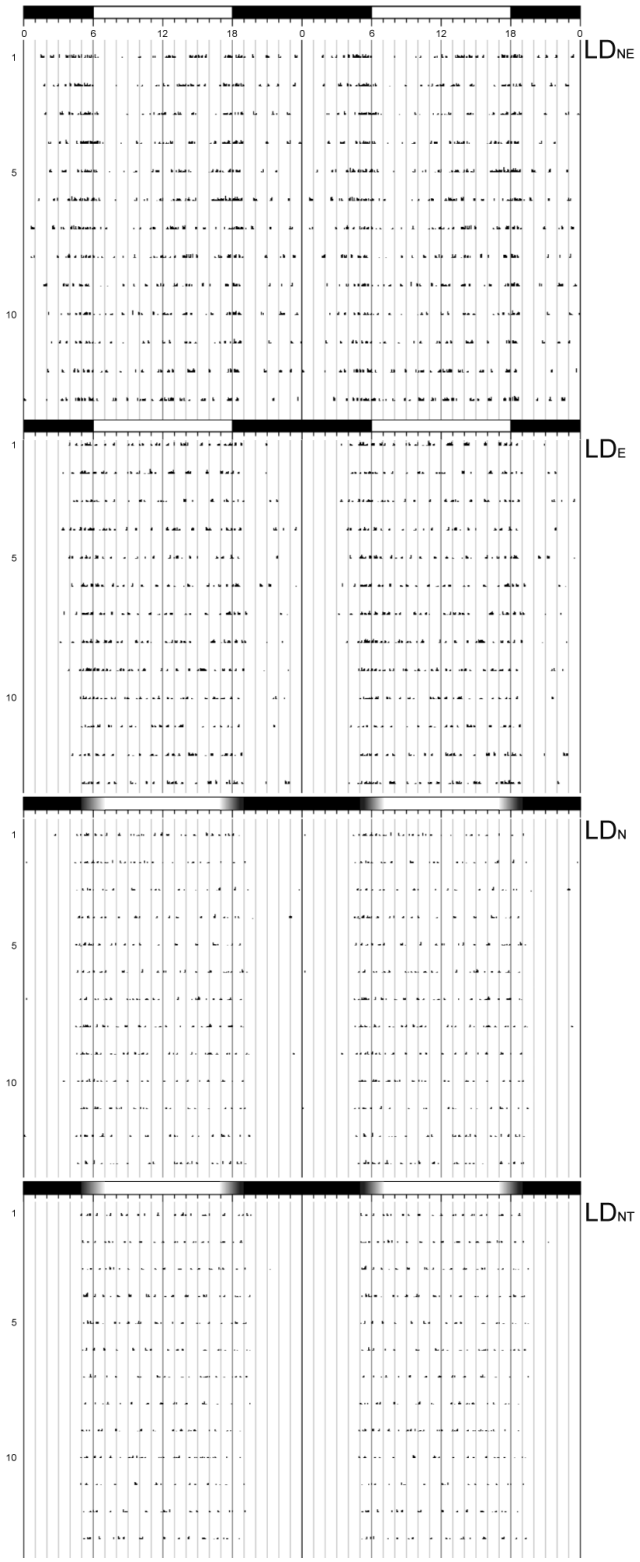


Figure 4. A double plotted actogram showing the activity of a *R.dilectus* over the different light and temperature cycles. The activity rhythm becomes more robust when introducing enrichment. White and black bars on top of the actograms indicate light and dark periods, with consecutive days on the y-axis. Ambient temperature was constant for the first three cycles, and temperature cycled between 16 (04h00) and 30°C (15h00) in the last cycle, indicated by the dotted line.



all individuals due to the activity distribution over the 24h period. For those where it could be determined, average time of activity onset was 05h24 ± 01h01 and offset of activity occurred around 19h22 ± 00h17.

3.2 12L:12D with environmental enrichment

The activity of *M. namaquensis* remained strictly nocturnal with average activity counts per hour during the dark phase of LD_E significantly higher than during the light phase ($F_{1, 23.32}$ = 2447.53, P < 0.001; Fig 1b). Activity significantly increased following the introduction of enrichment when compared to LD_{NE} ($F_{5,23.32}$ = 91.08, P<0.001; Table 1; Fig 1b, Fig 2). Female *M. namaquensis* were more active than males during LD_E ($F_{1,23.32}$ = 25.72, P < 0.001). The mean time of activity onset was 17h52 ± 00h12, whereas offset occurred at 06h07 ± 00h07.

During this experimental cycle, the average activity counts per hour during the light phase were significantly higher than during dark phase ($F_{1,22.70}$ = 90.76, P < 0.001; Table 1; Fig 3b, Fig 4). Seven animals showed clear diurnal activity, and three became crepuscular with clear bimodal peaks of activity during the transition periods between light and dark, whereas the remaining three mice still expressed some nocturnal activity. The activity counts of *R. dilectus* was significantly decreased in comparison to LD_{NE} ($F_{5,22.70}$ = 64.15, P < 0.001; Table 1; Fig 2b). Female *R. dilectus* were again significantly more active than the males ($F_{1,22.70}$ = 22.92, P < 0.001). Average onset of activity for *R. dilectus* was 04h12 ± 00h48 and offset occurred at 19h10 ±00h16.

3.3 Simulated dawn-dusk light cycle

The introduction of a simulated natural dawn-dusk period in the light cycle effectively increased the hours of light to 14 and reduced the completely dark hours to 10. During this cycle, *M. namaquensis* significantly decreased its overall activity counts per hour when

compared to LD_E ($F_{5, 23.31}$ = 91.0.8, P < 0.001; Table 1; Fig 1c, Fig 2). The average activity count per hour in the dark phase of LD_N was significantly higher than activity both in the light phase and twilight phase ($F_{2,23.31}$ = 1098.10, P < 0.001), while there was no significant difference between the average activity count per hour of the twilight and light phases ($F_{2,23.31}$ = 1098.10, P = 0.39). Males and females displayed similar average activity counts per hour during this light cycle ($F_{1,23.31}$ = 0.13, P = 0.72). The onset of activity was delayed by about one hour to 18:51 ± 10:32 and the offset was advanced by almost an hour to 05:15 ± 14:32.

Locomotor activity of *R. dilectus* decreased significantly ($F_{5,22.70}$ = 64.15, P < 0.001; Table 1; Fig 3c, Fig 4) during LD_N when compared to LD_E. Entrainment patterns for *R. dilectus* became more defined during LD_N with 10 animals showing distinct diurnal entrainment and two animals displayed crepuscular activity. Average activity counts per hour during the twilight phase were significantly higher than activity counts for both the light phase and the dark phase ($F_{2,22.70}$ = 186.89, P < 0.001, Fig 2c). *R. dilectus* females displayed similar levels of activity to males during this cycle ($F_{1,22.70}$ = 1.68, P = 0.12). The activity onset of *R. dilectus* was similar to the previous cycle at 04h16 ± 00h17 and the offset was delayed until 19:56 ± 00h20.

3.4 Dawn-dusk light cycle with natural temperature cycle

During the LD_{NT} cycle, where both a natural dawn-dusk cycle and a temperature cycle were present, *M. namaquensis* significantly increased its activity compared to LD_N ($F_{5,23.31}$ = 91.08, P < 0.05). All animals showed strictly nocturnal activity for the duration of LD_{NT}, nocturnal activity levels were significantly higher than activity levels during the twilight or light phase ($F_{2,23.31}$ = 1279.24, P < 0.001, Table 1; Fig 1d, Fig 2). There was no significant difference in the level of activity between the light and twilight phase ($F_{2,23.31}$ = 1279.24, P = 0.76). During $LD_{NT,}$ females were significantly more active than males ($F_{1,23.31}$ = 34.45, P < 0.001). The

average time of activity onset and offset were similar compared to the previous cycle, activity onset occurred at 18h56 ± 00h09 and the offset at 05:14 ± 00h10.

The activity of *R. dilectus* was further decreased from the previous light cycle ($F_{5,22.70}$ = 64.15, P < 0.001; Table 1; Fig 3d, fig 4). The mice showed significantly higher levels of activity during twilight hours when compared to light ($F_{2,22.70}$ = 161.66, P < 0.001) and dark hours ($F_{2,22.70}$ = 161.66, P < 0.001, Fig 2d). All individuals showed rhythmic activity during LD_{NT}, four individuals showed crepuscular rhythms and the remaining eight displayed diurnal activity patterns. Females were significantly more active than males ($F_{1,22.70}$ = 5.14, P < 0.05). The activity onset of *R. dilectus* was at 04h32 ± 00h11 and the offset at 19h43 ± 00h16.

Discussion

Environmental factors such as enrichment, photoperiod and ambient temperature are usually controlled for in laboratory studies when investigating the circadian rhythms of animals. Since all of these factors are known to affect the behavior of animals in captivity (Refinetti, 2004, 2010; Abramov et al., 2008; Van Jaarsveld et al., 2019), we investigated these factors by sequentially introducing each factor to elucidate its effect on the activity rhythms of the two study species in the laboratory.

4.1 General locomotor activity

Locomotor activity is frequently used as a proxy for the entrainment of activity rhythms (Ishii et al., 1996; Benstaali et al., 2001; Ackermann et al., 2016). In this study, *M. namaquensis* showed robust and consistent nocturnal activity rhythms throughout all experimental conditions, confirming the strict nocturnality of the species (Van der Merwe et al., 2014; Van der Merwe et al., 2017). In contrast, *R. dilectus* showed substantial inter-individual variation, not only in their patterns of locomotor activity, but also in their levels of

activity. Some individuals were highly active, whereas others were relatively inactive throughout the experiment. A closely related species, *R. pumilio*, was found to have substantial intra-specific variation (Van der Merwe et al., 2017). The ancestral state of mammals is believed to be nocturnality with diurnality evolving as a secondary trait in rodents (Roll et al., 2006). Many diurnal rodents still have nocturnal adaptations such as rod dominated retinae and heightened olfactory sensitivity (Peichl, 2005; Hayden et al., 2010). Thus, diurnal species frequently have more variable and flexible activity patterns (Kas and Edgar, 1999; Blanchong and Smale, 2000; Cohen and Kronfeld-Schor, 2006; Barak and Kronfeld-Schor, 2013).

4.2 Effect of environmental enrichment

The nocturnal *M. namaquensis* remained strictly nocturnal for the duration of our study, however, the locomotor activity rhythms of *R. dilectus* showed increased robustness with the addition of environmental enrichment. The nocturnal *M. namaquensis* increased its activity in the presence of cage enrichment, whereas the diurnal species decreased its activity. This result is puzzling, and without reversing the housing conditions to determine if the activity patterns also revert to the previous levels, it cannot be said with certainty whether this effect is as a result of the enrichment or habituation to the laboratory. In our study, the nocturnal and diurnal species received similar enrichment items that rendered different effects. Species specific changes in activity patterns suggest that the natural habitat of the species should be taken into account when enrichment is considered. It also emphasises the difficulty in recommending enrichment for animals and the fact that it potentially cannot be standardised across species with vastly different natural histories.

4.3 Exposure to dawn and dusk

In our study, we simulated dawn and dusk by changing the intensity of the light, while the light spectrum remained constant. The activity of the nocturnal *M. namaquensis* appears to be strongly masked by light as active periods were instantly compressed to the complete dark periods of the light cycle. Van der Merwe et al. (2014) also showed animals shifted their activity immediately to remain in dark phase when light cycles were altered. The locomotor activity of *R. dilectus* became robustly entrained to the light cycle under the natural twilight conditions, with all individuals exhibiting rhythmic activity patterns. The diurnal *R. dilectus* extended its active time by almost an hour during both dawn and dusk. Similarly, Schumann et al. (2005) found that the closely related *R. pumilio* increased its activity time by almost an hour during a natural light cycle compared to a square wave light cycle.

Exposure to twilight is known to change the on- and offsets of rodent activity and the duration thereof (Boulos et al., 2002; Schumann et al., 2005; Lahmam et al., 2008; Comas and Hut, 2009). It can also influence the robustness of the entrainment in some species, such as hamsters (Boulos et al., 2002), but not in others, for instance house mice and squirrel monkeys (Boulos et al., 1996; Comas and Hut, 2009).

4.4 Natural ambient temperature cycle

We simulated a natural temperature cycle that gradually increases and decreases over a 24-hour period, corresponding to summer ambient temperatures in the habitats of the two study species (South African Weather Service 2019). *Micaelamys namaquensis* showed the highest level of activity during this cycle. A nocturnal species such as *M. namaquensis* is by default active during the cooler parts of a 24-hour day as temperatures can drop considerably at night. The increase in activity levels exhibited by *M. namaquensis* may be a form of behavioral thermoregulation to maintain their body temperatures during cooler conditions as the activity shows a peak just before dawn, overlapping with the coldest part of the cycle.

Rodents appear to show variable responses to low ambient temperatures, some species show a reduction in locomotor activity at cooler temperatures (Vaanholt et al., 2006; Sears et al., 2009; Wróbel and Bogdziewicz, 2015), whereas others increase their activity (Ishii et al., 1996).

Conversely, *R. dilectus* exhibited the lowest activity during the duration of the experiment. The warmer daytime temperatures may have suppressed the activity of *R. dilectus*. It is a diurnal species, thus most of its activity falls in the warmest part of the day. The largest proportion of its activity was exhibited during the light phase of the light cycle, but the intensity of the activity was higher during the twilight hours. Activity during the twilight hours were reduced during LD_{NT} when compared to LD_{N}, which may be because the twilight hours in our experiment overlapped with the hottest and coldest parts of the light cycle. Several rodent species show reduced locomotor activity at ambient temperatures that approach their thermoneutral zones (Finger, 1976; Gordon, 1993; Pálková et al., 1999; Gaskill et al., 2009; Oosthuizen and Bennett, 2015). The ambient temperature cycle appears to reinforce the entrainment to the light cycle, it causes changes in the intensity of the activity, but no changes were observed in the activity onsets and offsets in either of the species. Nonetheless, the response of animals to ambient temperatures appears to be dependent on the actual temperatures. A laboratory study subjecting *M. namaquensis* and *R. dilectus* to extreme daytime temperatures showed that both species became more active during the day, related to an increase in water drinking to assist with thermoregulation (Jacobs et al., 2020).

4. Conclusions

In conclusion, we found our diurnal species to have more variable activity patterns than the nocturnal species. This is consistent with previous research (Barak and Kronfeld-Schor,

2013; Blanchong and Smale, 2000; Cohen and Kronfeld-Schor, 2006; Kas and Edgar, 1999). This study also demonstrates that the environmental conditions under which laboratory animals are maintained can have a large effect on their behavior. Environmental enrichment and the addition of more natural conditions in terms of lighting and temperature differentially affected the profiles and levels of activity in the two study species. The differences may be related to the natural activity times and habitats of the animals.

Laboratory studies are often implemented in larger field studies or used for largescale conservation management decisions. Thus, it is imperative to improve our understanding of the effects that individual factors have on the outcomes of experiments. Further laboratory investigations are warranted, not only on the activity of animals, but also other behaviors and physiological traits to determine whether factors such as enrichment and temperature variations could be a source of physiological stress.

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Declaration of interest statement

The authors do not declare any conflict of interest.

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