

**Temporal flexibility in activity rhythms of a diurnal rodent,
the Ice rat (*Otomys sloggetti*)**

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Abstract

Diurnality in rodents is relatively rare and occurs primarily in areas with low night-time temperatures such as at high altitudes and desert areas. However, many factors can influence temporal activity rhythms of animals, both in the field and the laboratory. The temporal activity patterns of the diurnal ice rat were investigated in the laboratory with, and without, access to running wheels, and in constant conditions with running wheels. Ice rats appeared to be fundamentally diurnal but used their running wheels during the night. In constant conditions, general activity remained predominantly diurnal while wheel running was either nocturnal or diurnal. In some animals, entrainment of the wheel running rhythm was evident, as

demonstrated by free-running periods that were different from 24h. In other animals, the wheel running activity abruptly switched from nocturnal to subjective day as soon as the animals entered DD, and reverted back to nocturnal once returned to LD, suggesting the rhythms were masked by light. Wheel running rhythms appears to be less robust and more affected by light compared to general activity rhythms. In view of present and future environmental changes, the existence of more unstable activity rhythms that can readily switch between temporal niches might be crucial for the survival of the species.

Key words

activity rhythms, circadian, Ice rat, infrared captor, light cycles, *Otomys sloggetti*, wheel running

Introduction

In any functional ecosystem, many different species co-exist, each with its own unique niche (Kronfeld-Schor and Dayan 2008). The niche of a species is shaped by interactions with biotic and abiotic variables in its environment and is defined both in terms of space and time. Time is a critically important element of an ecological niche, and animals arrange their temporal activity to optimise their survival and reproductive potential (Campi and Krubitzer 2010). Although the temporal niches of mammals are frequently categorised as diurnal or nocturnal, there exists a gradient of diurnality and nocturnality, both between and within species where strictly nocturnal and diurnal are the opposite ends of the spectrum (Refinetti 2008).

Many species have evolved specific adaptations to their particular temporal niches (Kronfeld-Schor and Dayan 2003). These adaptations may differ significantly for light (L) and dark (D), and when a species is well adapted for a particular temporal niche, it may be ill

adapted for the other (Levy et al. 2019). However, although adaptations impose a constraint on the active time of a species, it does not necessarily completely exclude activity from a specific temporal niche. Despite morphological, physiological and behavioural adaptations to temporal niches, diurnality and nocturnality does not seem to be determined by the circadian pacemaker located in the suprachiasmatic nucleus (SCN). The rhythms generated by the endogenous pacemaker rely on a number of clock genes that has specific temporal expression patterns, which are largely similar in nocturnal and diurnal animals (Challet 2007). The SCN is not equally sensitive to light over the 24 hours, and in both nocturnal and diurnal animals it is most sensitive to light during the night (Mahoney et al. 2001; Smale et al. 2001). This suggests that the distinction between diurnal and nocturnal is specified by mechanisms downstream from the circadian clock (Ramanathan et al. 2010).

Light is the most prominent environmental factor that influences the timing of activity rhythms. Light can affect activity patterns of animals either by entrainment of the circadian clock or the more direct process of masking (Barak and Kronfeld-Schor 2013). Entrainment is a gradual process whereby the circadian clock synchronises to environmental time cues, and in the absence of these cues, the rhythm is maintained by the internal clock (Golombek and Rosenstein 2010). Masking on the other hand is an immediate process where activity is either enhanced (positive masking) or suppressed (negative masking) by light (Redlin et al. 2005; Barak and Kronfeld-Schor 2013).

Besides light, other biotic and abiotic environmental factors can also shape activity patterns of animals (Shkolnik 1971; Shuai et al. 2014; Ikeda et al. 2016). The temporal distribution of activity can shift or even invert with changes in environmental conditions (Hut et al. 2012). The activity patterns of many rodent species are investigated under controlled conditions in the laboratory; however, the laboratory setting differs vastly from what animals experience in their natural environment. The natural habitat of animals is much more complex

in almost every aspect; their behavioural repertoires are wider, more complex and probably more plastic (Hut et al. 2012). Activity patterns can differ significantly under laboratory and natural conditions (Hut et al. 2012; Barak and Kronfeld-Schor 2013).

Several rodents that display diurnal activity in the field invert their activity patterns to nocturnal when introduced into the laboratory (Cohen and Kronfeld-Schor 2006; Tomotani et al. 2012; Barak and Kronfeld-Schor 2013). In addition, the methodology of activity measurement in the laboratory, such as with an infrared motion detector and a running wheel, can present substantially different activity profiles for the same animal (Hut et al. 2012). Access to a running wheel, itself, can alter the temporal activity pattern of rodents dramatically (Novak et al. 2012). In some species, access to wheels can induce activity switches from diurnal to nocturnal in some of the animals; in others activity remain primarily diurnal (Katona and Smale 1997; Kas and Edgar 1998; 1999; Nixon and Smale 2004; Cohen and Kronfeld-Schor 2006; Cohen et al. 2009).

African vlei rats or laminate-toothed rats from the genus *Otomys* are widespread in sub-Saharan Africa. From a few field studies and anecdotal field observations, it appears that many of the vlei rats display diurnal or crepuscular activity in their natural environments (Packer 1980; Haim and Fairall 1987; Vermeulen and Nel 1988; Hinze and Pillay 2006). With the exception of the Karoo bush rat (*O. unisulcatus*), members of the genus *Otomys* are usually associated with alpine, montane and sub-montane habitats (Monadjem et al. 2015), which are generally cooler areas that would promote diurnal activity (Willmer et al. 2000). Taxonomic groupings of rodents tend to show similar temporal activity patterns, which seems to be related to habitat type (Roll et al. 2006). The timing of activity is an important adaptation for the survival of species, especially for species that live in more challenging habitats, such as mountains or deserts (Hut et al. 2012). Currently 31 *Otomys* species are recognised (Monadjem et al. 2015), and their taxonomy and morphology have been described comprehensively

(Monadjem et al. 2015). The genus *Otomys* is grouped with Old World rats and mice, classified in the family Muridae and subfamily Murinae. Fossil and DNA evidence suggests Otomyinae is a sister group to *Arvicanthis*, another group of diurnal African rodents (Monadjem et al. 2015).

The genus *Otomys* is of interest since, although the activity and behaviour of these animals are not particularly well studied, many of the species appear to be diurnal. Since diurnality has evolved multiple times over evolutionary times, it is important to study multiple diurnal species to further our understanding of diurnal circadian systems (Roll et al. 2006; Hagenauer and Lee 2008). In addition, the species in this genus inhabit a wide range of habitat types and altitudes, providing the ideal opportunity for comparative studies both in the laboratory and in the field.

The ice rat (*Otomys sloggetti*) resides at high altitudes (higher than ~2700 m above sea-level) in the mountains of South Africa (Monadjem et al. 2015). Like most other rodents in colder habitats, they breed during the summer (Hinze 2005), when most males have descended testes, and juvenile animals are captured; whereas in autumn, no young animals are present and male testes are retracted (*pers. obs.*). Although ice rats are colonial, their social relationships are complex. They huddle together below ground but once aboveground, they avoid and chase one another (Hinze et al. 2013). Thus, the social interactions belowground may be an obligatory thermoregulatory behaviour. In the laboratory, mothers and offspring can be housed together for ~3 months before they start fighting (*pers. obs.*). The activity pattern of the ice rat has been described as diurnal in the field (Hinze and Pillay 2006), but it has not been investigated in the laboratory. The aim of this study was therefore to investigate the daily activity rhythms of the ice rat under laboratory conditions, with and without access to running wheels. It was predicted that the animals would show diurnal activity when they did not have access to wheels. Since some other diurnally active rodents, such as *Octodon degus* and *Arvicanthis niloticus*, become

more nocturnal with the introduction of running wheels (Katona and Smale 1997; Blanchong et al. 1999; Kas and Edgar 1999), it was anticipated that the phase of the activity may switch to nocturnal when the animals did have access to running wheels. In addition, to assess whether the animals have free-running rhythms of general and wheel running activity, animals were subjected to a square wave 12L:12D light cycle and subsequently to constant darkness (DD). Most diurnal animals do show free-running rhythms in constant darkness; therefore, this was also expected in the ice rats.

Material and Methods

Animal collection and housing

Experimental animals were collected from Tiffindell Ski resort, Eastern Cape in South Africa (30°39'13"S, 27°55'34"E) under an Eastern Cape Nature Conservation permit (CRO213/17CR and CRO14/17CR). Animals were trapped with Sherman live traps baited with peanut butter and oats, and thereafter they were transported to the Small Animal Physiological Research Facility on the experimental farm at the University of Pretoria. In the laboratory, animals were housed individually in semi-transparent plastic containers (58 x 38 x 36cm) lined with wood shavings in a climate controlled experimental room. All animals were provided with a plastic shelter, tissue paper for bedding and a pipe, and one group also had access to a running wheel. Water and food were provided *ad libitum*. Animals were fed daily at random times, on a selection of fresh fruit, vegetables, seeds and other dry food (Burgess nuggets; Burgess Pet Care, UK).

Experimental procedures

Experimental procedures were approved by the ethical committee of the University of Pretoria (EC080-15) and conform to international standards (Portaluppi et al. 2010). Animals were introduced into the experiment 3 months after arrival in the laboratory. During the first

part of the experiment, animals were maintained under a natural light cycle with a simulated dawn and dusk from 05h00-07h00 and 17h00-19h00 and a constant ambient temperature of $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The light intensity was 330 lux at full strength. Dawn and dusk conditions were simulated as increasing and decreasing percentages of full-strength light that was adjusted every 10 min (Table S1). Experimental animals were divided into two groups, 9 animals (5♂, 4♀) did not have running wheels, while the other 10 (5♂, 5♀) did have access to running wheels, in their cages. In both groups, general locomotor activity was recorded with infrared motion detectors. Running wheel revolutions were also recorded in animals with access to wheels. Body weights of individual animals are in Table S2.

Subsequently, 8 animals (6♂, 2♀) with access to running wheels were first subjected to a 12L:12D square wave light cycle for 14 days, and thereafter they were maintained in DD for 14 days, followed by another 7 days on a 12L:12D square wave light cycle. During this time, general locomotor activity and wheel running revolutions were recorded in the animals. Body weights of individual animals are in Table S3.

Recording and analysis

An infrared motion detector (Quest PIR internal passive infrared detector, Elite Security Products (ESP), Electronic Lines, London, UK) was mounted over each cage to cover the entire floor space. The activity counts were summed per minute and were recorded on a computer outside the experimental room using VitalView software (VitalView™, Minimitter Co., Sunriver, OR, USA). The running wheels were fitted with reed switches to record the wheel revolutions; this was also summed per minute and recorded with the VitalView software. Double-plotted actograms were produced with the program ActiView (ActiView™, Minimitter Co., Sunriver, OR, USA) to visualise the data. Data from the last 14 days of each cycle were used for analysis. For general activity and wheel running in the natural daylight, activity counts

and wheel revolutions were summed per hour. The hourly raw data were analysed to provide an estimate of the proportion of activity occurring during light, twilight and darkness. Generalised linear mixed models were used to compare the locomotor and wheel running activity of the different cycles, with cycle, sex and light/dark cycle as fixed factors. For the comparison of LD/DD activity counts and wheel revolutions, the periods of the constant darkness (DD) activity were obtained for individual animals, and daily light phase/subjective day and dark phase/subjective night values were calculated with Clocklab (ClockLab™; Actimetrics, Evanston, Illinois, USA), since the periods in DD were different from 24h. Here, generalised linear mixed models were used to compare the locomotor and wheel running activity of the different cycles, with experiment and light/dark cycle as fixed factors. Males and females were not separated, since the female sample was too small. All interactions were considered. Least significant differences were used to assess *post hoc* differences and $P = 0.05$ was considered as significant.

Results

General activity

In the laboratory, ice rats were active throughout the day and the night but showed progressively less general activity from light to darkness (Table 1; Figure 1A, 2A,B; Table S2). Animals were more active during the light hours compared to twilight ($P = 0.007$) and dark hours ($P < 0.001$), and more active during twilight than dark hours ($P < 0.001$). All animals showed significant nycthemeral rhythms as determined by Cosinor analysis (all $P < 0.001$). Onsets and offsets of general activity could, for the most part, not be determined, as there were no distinct breaks in the activity.

Table 1. Statistical analysis of the general activity and wheel running of animals with and without wheels on a natural light cycle, and a comparison between a 12L:12D square wave light cycle and subsequent constant darkness (DD) with access to wheels. Light phase = light, twilight, dark.

Natural light cycle (wheel vs no wheel)

<i>General activity</i>	$F_{(2,6372)}$	P
Wheel vs no wheel	87.9	< 0.001
Light phase		
Overall	55.07	< 0.001
No wheel	93.11	< 0.001
Wheel	9.57	< 0.001
Males vs females	0.627	0.429
<i>Wheel running activity</i>		
	$F_{(1,3360)}$	P
Light phase	70.76	< 0.001
Males vs females	0.0077	0.781

12L:12D – DD (wheel access)

<i>General activity</i>	$F_{(1,308)}$	P
LD vs DD	0.397	0.529
LD light/dark	10.32	0.001
DD subj day/ subj night	19.21	< 0.001
<i>Wheel running activity</i>		
	$F_{(1,308)}$	P
LD vs DD	2.32	0.129
LD light/dark	45.85	< 0.001
DD subj day/ subj night	6.51	= 0.011

Animals that did not have access to wheels showed overall more general activity than animals that did have access to running wheels (Table 1; Figure 1A; Table S2). This is true for the light ($P < 0.001$) and twilight ($P < 0.001$) hours, but similar activity was exhibited during the dark ($P = 0.735$) hours. The 24h activity profiles of animals that did not have access to wheels differed from those that had access to wheels.

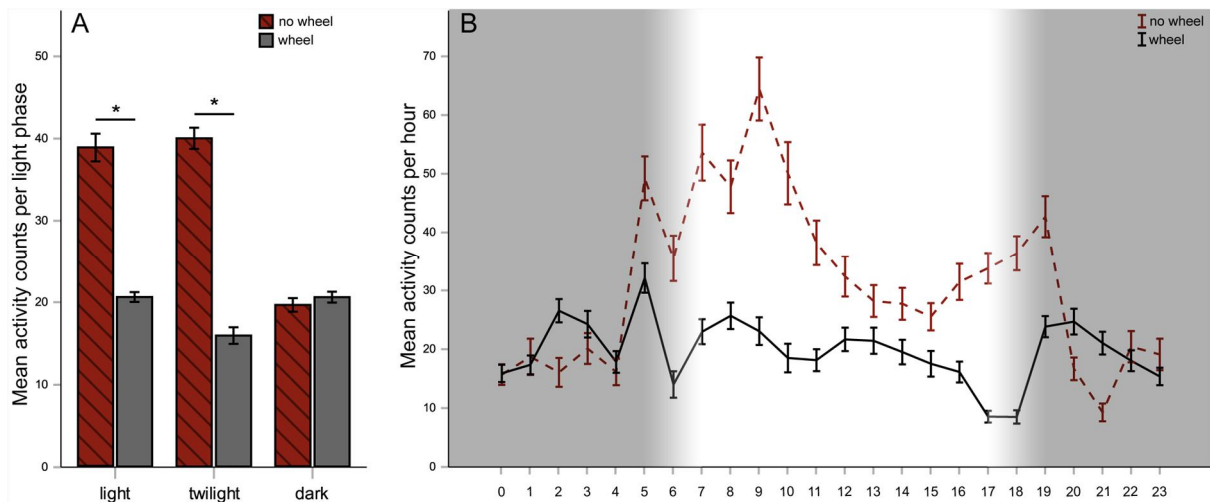
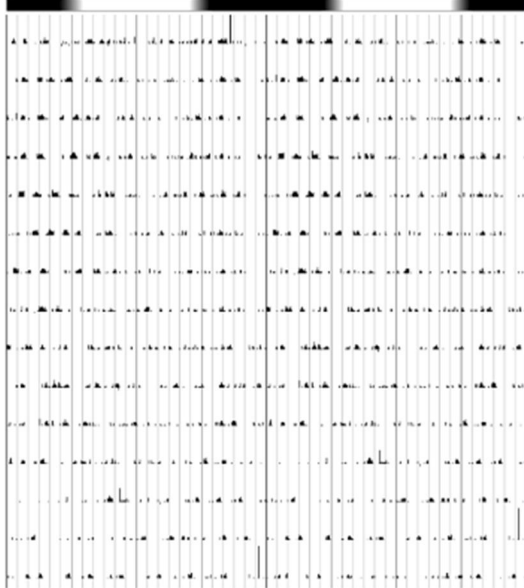


Figure 1. General activity of ice rats with and without access to running wheels as measured by an infrared captor (A) per light cycle and (B) per hour. Dashed bar / line – animals without wheels, solid bar / line – animals with wheels.

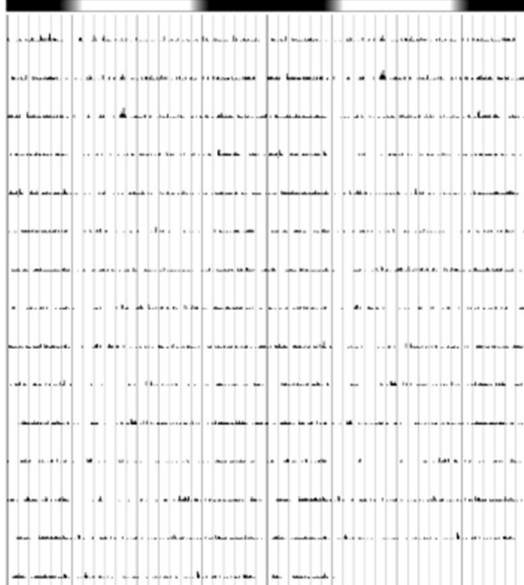
The phase of the light contributed significantly to the activity profile of animals that did not have wheels (Table 1; Figure 1B). Animals were equally active during the light hours and twilight hours ($P = 0.664$), and significantly less active during the dark hours compared to light ($P < 0.001$) and twilight ($P < 0.001$) hours. The activity profile of animals with wheels was also affected by the phase of the light (Table 1; Figure 1B). Compared to twilight hours, animals were more active during the light ($P < 0.001$) and darkness ($P < 0.001$), while similar activity levels were shown during light and dark hours ($P = 0.338$). The general activity of animals without wheels were not correlated with their body weight ($R^2 = 0.244$, $P = 0.526$), but in animals that had access to wheels, larger animals were less active ($R^2 = -0.643$, $P = 0.045$).

No overall difference was apparent in the general activity of males and females (Table 1). Similar levels of general activity were apparent during the twilight ($P = 0.195$) and dark ($P = 0.229$) hours, but males were more active during the day compared to the females ($P = 0.013$).

A General activity - without wheel



B General activity - with wheel



C Wheel running activity

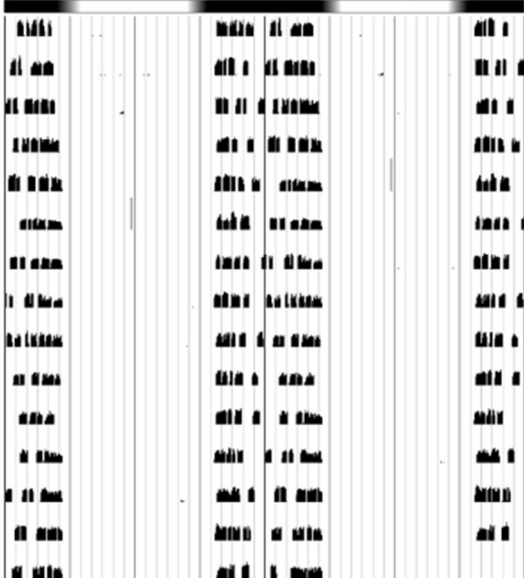


Figure 2. Actograms of representative animals subjected to natural light cycles (A) general activity of an animal without a running wheel (B) general activity of an animal with a running wheel (C) running wheel activity of the same animal as in (B). The light phase is indicated on top of the actogram.

Wheel running

Wheel running activity of ice rats showed a very different profile compared to the general activity. Light phase affected the wheel running activity of animals significantly; animals ran on the wheels almost exclusively during the night (Table 1; Figure 2C, 3A; Table S2). Animals ran on their wheels significantly more during the night compared to twilight ($P < 0.001$) and the day ($P < 0.001$), and more during twilight compared to the day ($P < 0.001$; Figure 3B). Wheel running activity had a mean onset of 36.43 ± 10.96 min after lights off, and a mean offset of 21.15 ± 13.96 min before lights on, with onsets more precise than the offsets. Animals ran an average of 6.701 ± 1.491 km per day (range: 0.322 – 17.745 km). Wheel running activity of animals was not correlated with body weight ($R^2 = -0.203$, $P = 0.881$).

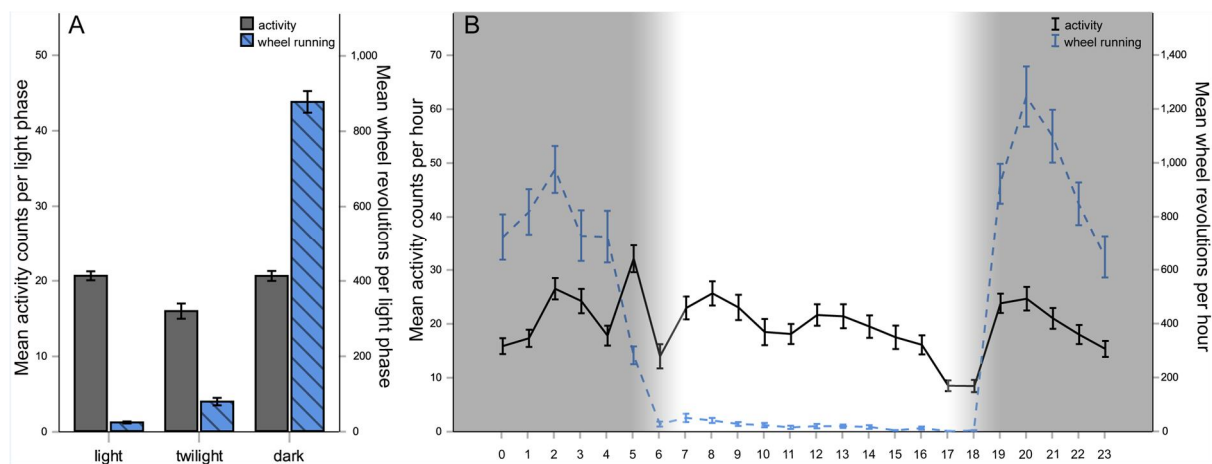


Figure 3. General activity and wheel running of the same animals (A) per light cycle and (B) per hour. Solid bar / line – general activity as measured by infrared captor, dashed bar / line – wheel revolutions.

Overall wheel running activity did not differ between males and females (Table 1); however, differences were apparent during the different phases of the light cycle. Males showed more wheel running than females during the twilight ($F_{1,3360} = 3.97$, $P = 0.047$), and females ran more during the night ($F_{1,3360} = 5.61$, $P = 0.018$). During the light hours, running activity did not differ between males and females ($F_{1,3360} = 0.06$, $P = 0.805$).

Constant conditions (with wheel access)

General activity

Both during the 12L:12D light cycle (LD) and subsequent constant darkness (DD), ice rats on average displayed significantly more general activity during the light or subjective light periods (Table S3, Figure 4, 5); although, 2 animals displayed more nocturnal activity. Total activity counts increased from LD to DD, but not significantly so (Table 1). The nocturnal and subjective night activity counts remained similar between LD and DD ($F_{1,308} = 0.712$, $P = 0.399$); likewise, the activity counts during subjective day in DD and the light phase activity counts in LD did not change ($F_{1,308} = 2.92$, $P = 0.088$). The period of the circadian activity rhythm during DD was close to 24h (mean: 24.1 ± 0.13 h).

Wheel running activity

Ice-rats ran more on their wheels during DD compared to LD, but not significantly so (Table 1). During both LD and DD, animals ran more during the night and subjective night (Table S3, Figure 4, 5). During LD, animals ran on their wheels significantly more during the night compared to the subjective night in DD ($F_{1,308} = 10.71$, $P = 0.001$), and significantly less during the day compared to subjective day in DD ($F_{1,308} = 20.79$, $P < 0.001$). The period of the wheel running rhythm during DD was also close to 24h (mean 24.01 ± 0.13 h).

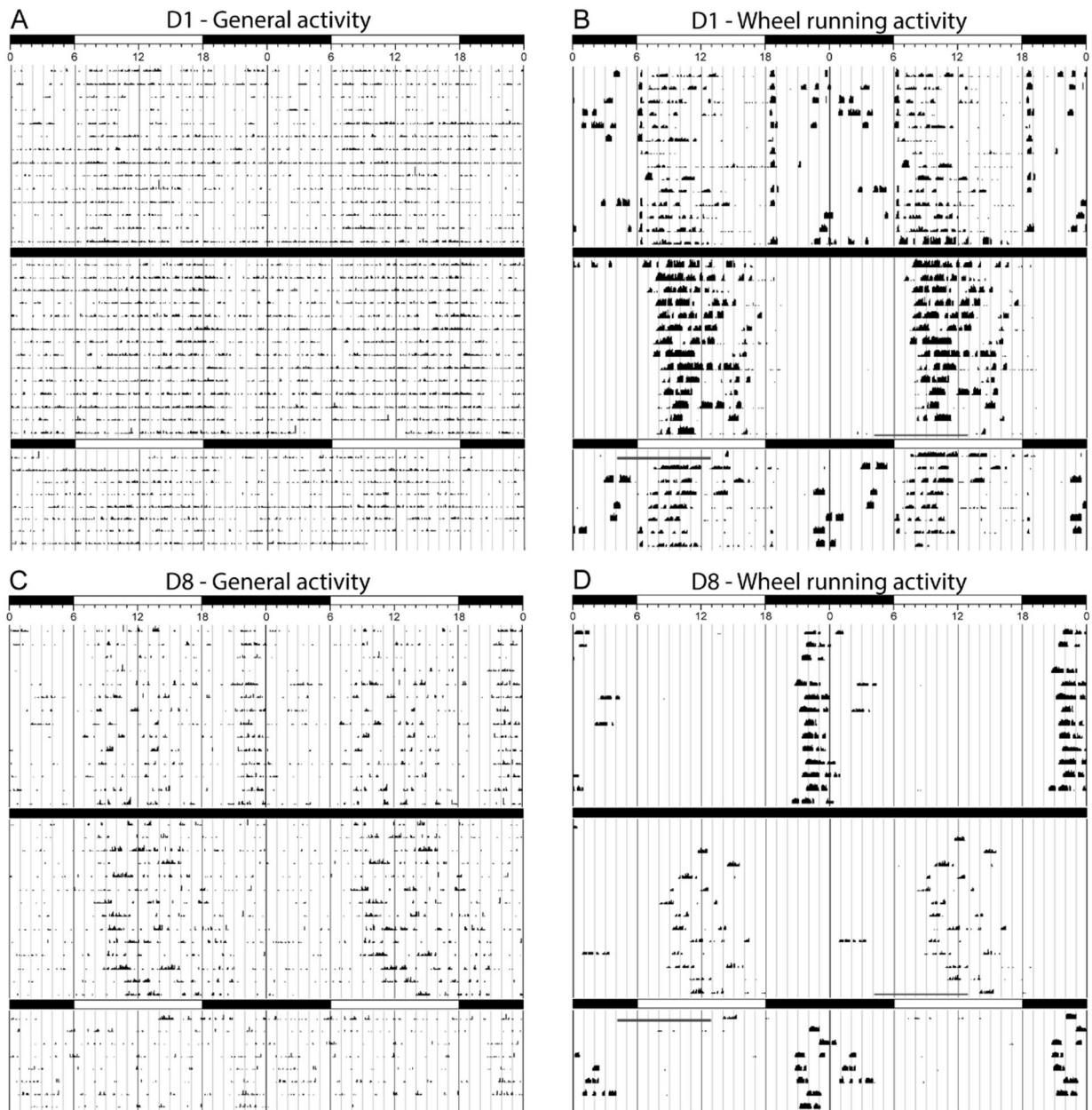


Figure 4. Actograms of general activity and wheel running activity of representative animals that show diurnal wheel running under DD. The general activity of animal D1 is presented in (A), and its wheel running activity in (B). The nocturnal wheel running bout disappears in DD. The general activity of animal D8 is presented in (C) and its wheel running activity in (D). Wheel running activity switches abruptly from nocturnal to diurnal under DD.

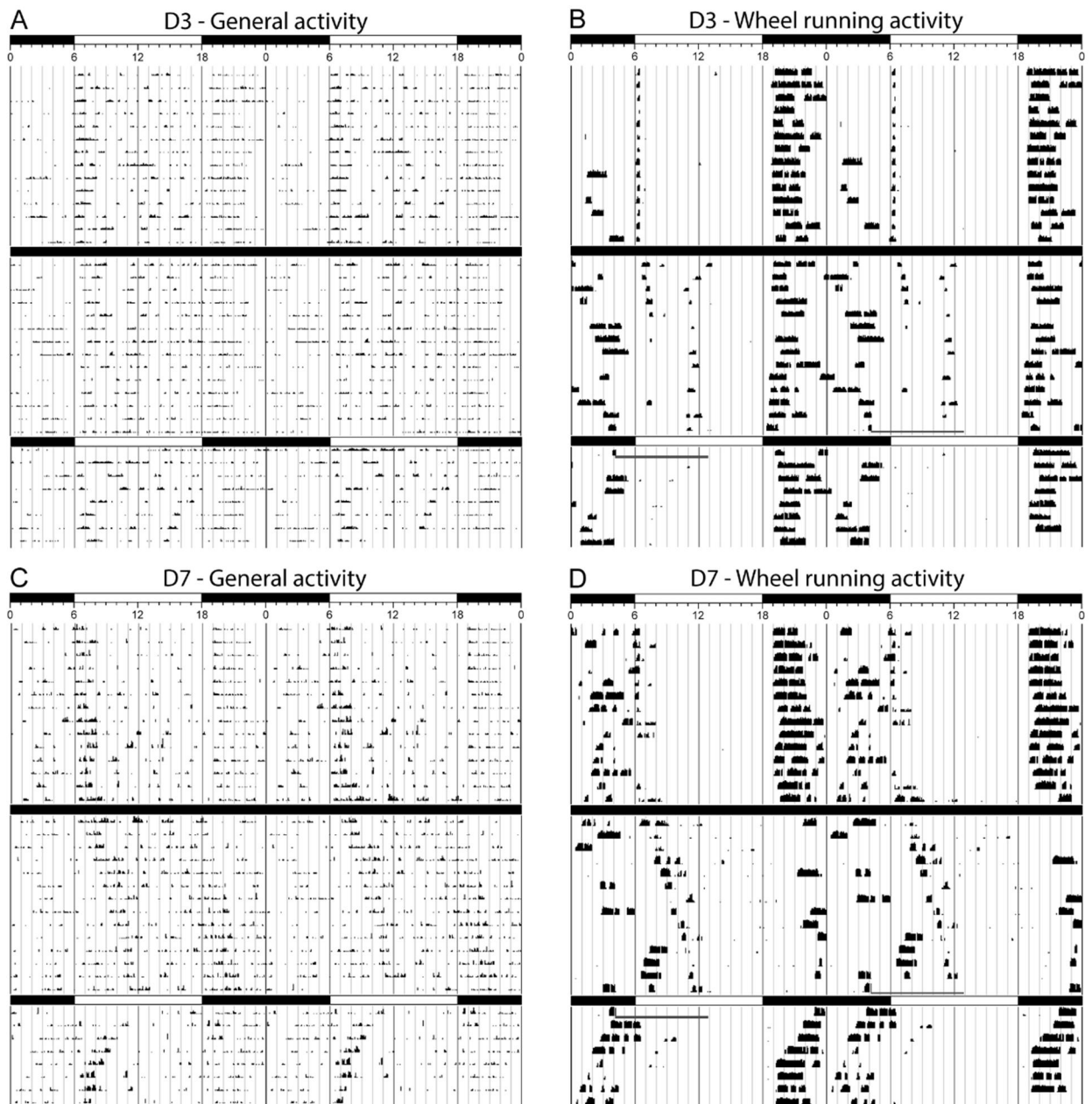


Figure 5. Actograms of general activity and wheel running activity of representative animals that show nocturnal wheel running under DD. The general activity of animal D3 is presented in (A), and its wheel running activity in (B). Wheel running activity remains nocturnal with a small amount of diurnal running added under DD. The general activity of animal D7 is presented in (C) and its wheel running activity in (D). Wheel running activity remains nocturnal, but free-runs with a period longer than 24h, entrainment is restored 5 days after the light is switched on again.

Wheel running activity under DD showed variable results. Even though the sample size was 8 animals, four different patterns could be identified. One animal showed diurnal wheel running with small bouts of nocturnal running during LD, while under DD wheel running became more pronounced during the subjective day and the nocturnal running bouts disappeared (Figure 4B). Two animals exhibited strictly nocturnal wheel running under LD and switched their running activity immediately to subjective day under DD (Figure 4D). Three animals displayed nocturnal wheel running during LD, and while wheel running activity remained during the subjective night during DD, there was increased wheel running during the subjective day (Figure 5B). The last two animals showed nocturnal wheel running during LD, which seemed to free-run under DD (Figure 5D).

Discussion

General activity in the laboratory

Ice rats are strictly diurnal in the field (Hinze and Pillay 2006), but in the laboratory, all ice rats were active during the day and the night. Animals without wheels were more diurnal, while animals with access to wheels displayed similar amounts of general activity during the day and the night. Activity rhythms of several other diurnal species have been investigated in the laboratory, and they show variable activity patterns when introduced to captive conditions. Some species such as the Nile grass rat (*Arvicanthis niloticus*) and degu (*Octodon degus*) continue to display diurnal general activity when they do not have access to wheels (Kas and Edgar 1998; Blanchong and Smale 2000), whereas others, such as the fat sand rat (*Psammomys obesus*), golden spiny mice (*Acomys russatus*) and tuco-tucos (*Ctenomys* aff. *knighti*), switch to nocturnal activity immediately when they are introduced to the laboratory irrespective of the presence of wheels (Cohen and Kronfeld-Schor 2006; Tomotani et al. 2012; Barak and Kronfeld-Schor 2013). Patterns of general activity in diurnal species that still display diurnal

activity in the laboratory seem to become considerably more nocturnal with the introduction of running wheels (Katona and Smale 1997; Blanchong et al. 1999; Kas and Edgar 1999). In ice rats, this appears to be the case as well. When animals had access to wheels, general activity was suppressed during the day compared to animals without wheels, while nocturnal activity counts were similar to that of animals without running wheels. The infrared motion detectors may not have picked up all the movement of animals in the wheels; therefore, the night-time general activity of ice rats with running wheels is probably slightly underestimated in this study. The dampened general activity rhythm observed in animals with running wheels requires further investigation to determine if it is a true reflection of the rhythm or an artifact of the method of measurement.

General activity of diurnal rodent species seems rather varied under constant conditions of DD. Six of the eight ice rats showed more diurnal activity in LD, and this diurnal trend was amplified in DD. The period of the activity rhythm of all animals was close to 24h (3 shorter, 5 longer than 24h). This findings is consistent with the activity shown by Nile grass rats, animals showed diurnal activity in LD, and in DD some animals had free-running rhythms longer, and some shorter than 24h (Challet et al. 2002). Fat sand rats, golden spiny mice and tuco-tucos are diurnal in the field but switch to nocturnal in the laboratory, regardless of wheel access. They retained this nocturnality in DD; the former two species displayed free-running rhythms shorter than 24h, and the latter around 24h (Cohen and Kronfeld-Schor 2006; Tomotani et al. 2012; Barak and Kronfeld-Schor 2013). Degus switch their activity to nocturnal with wheel access. They remain nocturnal in DD with wheels, but if the wheels are removed under DD, their activity reverts to diurnal. They free-ran with a period shorter than 24h (Kas and Edgar 1999). This supports the notion that some species that display diurnal activity in the field are inherently nocturnal, and the activity is masked by some environmental factor in their natural habitat (Cohen and Kronfeld-Schor 2006). Species that are truly diurnal retain this

temporal phase of activity in constant conditions, albeit with large inter- and intraspecific variation, and it seems that ice rats fall in this latter group.

Diurnality in the field may be driven by a number of environmental factors, such as thermoregulation and competitive exclusion (Shkolnik 1971; Gutman and Dayan 2005; Bennie et al. 2014). This is supported by studies where animals that are diurnally active in the field show a nocturnal phase preference immediately after introduction to standard laboratory conditions without access to running wheels (Cohen and Kronfeld-Schor 2006; Tomotani et al. 2012; Barak and Kronfeld-Schor 2013). In this regard, ice rats may be evolutionary better adapted to the diurnal niche to make use of behavioural thermoregulation, since physiologically and morphologically they are not particularly well adapted to inhabit cold environments. Their thermal biology is similar to that of *Otomys* species found in warmer areas (Richter et al. 1997), and morphologically, the only difference is shorter tails and slightly smaller ears than other species (Richter 1997). Their fur is denser than that of other species but also shorter than expected (Rymer et al. 2007).

Wheel running

Wheel running is distinct from general activity, and access to a running wheel can significantly change the activity patterns of animals (Novak et al. 2012). Most animals, even in the wild, will run spontaneously if they have access to wheels (Meijer and Robbers 2014; Tachinardi et al. 2014; Joshi and Pillay 2018; Manzanares et al. 2018). In this study, ice rats used their wheels readily and almost exclusively at night. Several other diurnal species show nocturnal wheel running in the laboratory (Kas and Edgar 1999; Barak and Kronfeld-Schor 2013). It has been suggested that the increase in body temperature caused by wheel running could prompt animals to shift this activity to what would be cooler times of the day in nature (Kas and Edgar 1999; Hagenauer and Lee 2008; Cohen et al. 2009). Several studies have shown

that animals prefer cooler ambient temperatures as their body temperatures and locomotor activity increase (Gordon 1993; Refinetti 1996; Gaskill et al. 2009). Light may be associated with warmer daytime temperatures, and in the laboratory cause an amplified masking response to wheel running (Hagenauer and Lee 2008). In diurnal Nile grass rats and Mongolian gerbils (*Meriones unguiculatus*), wheel running is suppressed by light and enhanced by darkness, while animals without wheels show the opposite response (Redlin and Mrosovsky 2004; Weinert et al. 2007).

A large amount of individual variability was noticeable in wheel running activity of ice rats. In DD, diurnal and nocturnal patterns of wheel running could be identified. In some animals drifting of the nocturnal free-running rhythm was visible, supported by the re-entrainment period when animals were subjected to LD again. In other animals, nocturnal wheel running continued into subjective night unchanged, which could be a free-running rhythm close to 24h. In the remainder of the animals, wheel running occurred during the subjective day in DD, either by losing the nocturnal wheel running component and increasing the already existing subjective day running component, or by shifting the exclusively nocturnal component abruptly to entirely diurnal. The abrupt shift from nocturnal to diurnal wheel running could indicate negative masking of a diurnal rhythm, since these diurnal patterns under DD reverted to the previous nocturnal pattern immediately after the animals were returned to a LD light cycle again. Most diurnal animals that have been investigated display nocturnal wheel running rhythms under DD (Kas and Edgar 2001; Cohen and Kronfeld-Schor 2006; Valentinuzzi et al. 2009; Barak and Kronfeld-Schor 2013). Only the Nile grass rat displays predominantly diurnal wheel running rhythms in LD and maintains this phase preference under DD, with some increased activity during the subjective night (Katona and Smale 1997). Some ice rats switched their wheel running activity from nocturnal in LD to diurnal in DD, and it

reverted back to nocturnal immediately once an LD cycle was reinstated. This would imply that wheel running activity in these animals is masked by light, but not the general activity.

The free-running periods of the ice rats were very close to 24h, with 2 at 24h, 2 shorter and 4 longer than 24h. The periods of the wheel running rhythms were close to, but not always exactly, the same as that of the general activity periods. This could potentially indicate separate rhythms for general activity and wheel running, with the general activity being more stable and robust; whereas, the wheel running rhythm is more likely to switch temporally.

Access to wheels has been shown to influence activity patterns of diurnal rodents. In fat sand rats, access to a wheel strengthens the activity rhythms, and activity onsets and offsets become clearer and more precise (Tal-Krivisky et al. 2015). This does not seem to be the case in the ice rat; precise wheel running activity was observed, but onsets and offsets could still not be determined in the general activity. In golden spiny mice, wheel access increased their activity (Cohen et al. 2009). In ice rats, animals with wheels showed less general activity compared to animals without wheels, but with the wheel running activity added, they were probably also more active, especially considering the relative distances they covered in the wheel. On average, ice rats covered between 6 - 8 km per day, but with large intraspecific variability. Total distances that animals run per day vary considerably, both between and within species (Richter et al. 2014). The amount of wheel running depends on several factors, such as sex, and in laboratory animals, also the strain (Manzanares et al. 2018). No sex differences were observed in running wheel activity, but differences may have been obscured by the small sample size. In laboratory rats and mice, females consistently run more than males (Jones et al. 1990; Konhilas et al. 2015; Manzanares et al. 2018).

It has been suggested that wheel running is a stereotypic behaviour, and although wheel running does share some characteristics with stereotypic behaviour, it also has properties that

is different from stereotypic behaviour (Richter et al. 2014). Besides exercise, voluntary wheel running has been shown to have physiological and cognitive benefits (Van Praag et al. 1999; Johnson and Mitchell 2003). Because animals are confined in the laboratory and their natural behavioural repertoire severely restricted, wheel running may represent some other behaviour that usually occurs in their natural habitat, for instance, predator avoidance, searching for refuge or escaping behaviour (Hut et al. 2012). In ice rats, at least daytime wheel running seemed to be replacement activities; some animals would run on their wheels when startled. This raises the question of whether this could also be the case for night-time wheel running, for instance, since the animals do not have holes to retreat to during the night, and they may run on the wheels as a proxy to seek shelter. Wheels can also be used for climbing (Richter et al. 2014). Most ice rats climb on top of their wheels, balancing themselves against the cage wall and can sit there for extended periods of time. This ‘wheel-sitting’ was only seen during the day and could substitute for sun basking that animals would do in the field during the day. Alternatively, it could be used as a vantage point, since the wheel is the highest point in the cage and allows the animals to see outside their cages to some extent.

Conclusions

This study provides the first report of the activity patterns of the ice rat in laboratory conditions. This species appears to be to be intrinsically diurnal, and like other diurnal animals, ice rats exhibit large individual differences in activity. They display predominantly diurnal general activity without wheels, whereas wheel running occurs almost exclusively during the night. The wheel running rhythm appears to be less robust and stable, and more strongly influenced by light compared to the general activity rhythm. Although access to running wheels changes the activity of the animals, this cannot simply be attributed to masking effects, since some animals clearly show free-running rhythms of wheel running.

The results of this study highlighted a number of areas where more targeted studies can elucidate the interactions of light, general activity and wheel running activity in this species. For example, simultaneous measurement of activity by filming it and using IR motion detectors would provide clarification on how representative IR captor activity measurements are in the presence of a running wheel. The measurement of general activity of animals prior to having access to wheels would show whether and how wheels alter the behaviour of animals. Comparisons between activity patterns generated with and without a simulated natural light cycle will emphasise the relative importance of twilight in the entrainment process. To elucidate the masking and entrainment relationship of wheel running and light, it will be useful to measure body temperature simultaneously with activity, while subjecting animals with and without wheels to constant conditions and other masking light regimes. Since some species do show male-female differences in activity, sex differences in activity responses can also be investigated. Finally, studying brain regions that are involved in circadian rhythmicity, such as the SCN, and extra-SCN regions that may be involved in the mediation of wheel running activity and the masking process, such as the lateral habenula, would further add to the current understanding of the circadian mechanism of diurnal rodents and animals in general.

To conclude, in light of present and future environmental changes, a thorough understanding of diurnal circadian systems and how these systems interact with different environmental factors is necessary to predict how animals would respond to such changes and how it would affect their survival.

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