Chronobiology International, 2013; 30(6): 828–836

ISSN: 0742-0528 print / 1525-6073 online
DOI: 10.3109/07420528.2013.773439

informa
healthcare

Effects of circadian phase and melatonin injection on anxiety-like behavior in nocturnal and diurnal rodents

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Animals show daily rhythms in most bodily functions, resulting from the integration of information from an endogenous circadian clock and external stimuli. These rhythms are adaptive and are expected to be related to activity patterns, i.e., to be opposite in diurnal and nocturnal species. Melatonin is secreted during the night in all mammalian species, regardless of their activity patterns. Consequently, in diurnal species the nocturnal secretion of melatonin is concurrent with the resting phase, whereas in nocturnal species it is related to an increase in activity. In this research, we examined in three diurnal and three nocturnal rodent species whether a daily rhythm in anxiety-like behavior exists; whether it differs between nocturnal and diurnal species; and how melatonin affects anxiety-like behavior in species with different activity patterns. Anxiety-like behavior levels were analyzed using the elevated plus-maze. We found a daily rhythm in anxiety-like behavior and a significant response to daytime melatonin administration in all three nocturnal species, which showed significantly lower levels of anxiety during the dark phase, and after melatonin administration. The diurnal species showed either an inverse pattern to that of the nocturnal species in anxiety-like behavior rhythm and in response to daytime melatonin injection, or no rhythm and, accordingly, no response to melatonin.

Keywords: Activity, degu, elevated plus-maze, fat sand rat, laboratory rat, spiny mouse, Tristram's jird

INTRODUCTION

Daily rhythms in animals are manifested in most bodily functions, ranging from gene expression to behavior, and include changes in activity patterns, reaction time, and memory. These rhythms result from the integration of information derived from the endogenous circadian clock and external stimuli. They are adaptive and are expected to be related to activity patterns, i.e., to be opposite in diurnal and nocturnal species.

The exact mechanisms defining the circadian system as nocturnal or diurnal are still unknown. There are no conspicuous differences distinguishing the suprachiasmatic nuclei (SCN) of diurnal species from those of nocturnal species (Lambert et al., 2005; Smale et al., 2003), and therefore many researchers suggested that the differences in the circadian function of diurnal and nocturnal species are not found in the SCN itself, but rather in the interpretation of the SCN signals (e.g., Hagenauer & Lee, 2008; Lee, 2004; Smale et al., 2003, 2008). The hormone most typically associated with the SCN is melatonin. It is secreted at night in all mammalian species, regardless of their activity patterns (Armstrong, 1989; Vivanco et al., 2007). Consequently, in diurnal species the nocturnal secretion of melatonin is concurrent with the quiescence phase (sleepiness and decreased locomotor activity and body temperature). In nocturnal species, in contrast, the nightly regular endogenous signal of melatonin is related to vigilance and to an increase in body temperature and locomotor activity (Aparicio et al., 2006; Arendt, 2000; Aschoff, 1960; Cagnacci et al., 1992; Hagenauer & Lee, 2008; Huber et al., 1998; Lee, 2004; Zhdanova et al., 2002; Zisapel et al., 1998). One hypothesis that arises from these observations is that melatonin has different and even opposite physiological and behavioral effects on nocturnal and diurnal species. Supporting this hypothesis are studies showing that melatonin treatment promotes sleep and reduces activity levels and body temperature in diurnal species, whereas in nocturnal species it increases alertness, locomotor activity, and body temperature (Arendt, 2000; Dollins et al., 1994; Hastings et al., 1992; Huber et al., 1998; Mendelson, 2002; Mendelson et al., 1980; Roseboom et al., 1996; Zisapel et al., 1998).

Several studies have demonstrated the effects of the circadian phase on multiple measures of

Submitted November 13, 2012, Returned for revision January 16, 2013, Accepted January 30, 2013

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depressive/anxiety-like behaviors and hypothalamic-pituitary-adrenal (HPA) reactivity in a variety of mammals and primates (Kronfeld-Schor & Einat, 2012; Verma et al., 2010). Many of these studies, however, did not eliminate other possible effects on behavior, such as lighting conditions during tests, and therefore the link between the circadian phase and the SCN to these measures remains unknown. Furthermore, the short-term effects of melatonin on different measures of anxiety were tested mostly on nocturnal laboratory rats and mice (Datta & King, 1979, 1980; Golombek et al., 1996; Golus & King, 1981; Papp et al., 2006, 2010), which are the most commonly used model animals in biomedical studies, whereas information from diurnal species is scarce.

The earliest evolved mammals were nocturnal insectivores (Crompton, 1980). Even though nocturnality remained the most common activity pattern for mammals (Park, 1940), independent evolutionary transitions into diurnality occurred among both closely and remotely related species (Hut et al., 2012; Roll et al., 2006). Hence the neurological mechanisms underlying nocturnal activity patterns should be more similar than those underlying diurnal activity patterns, which developed independently as different evolutionary events, and therefore are expected to be more diverse (Cohen & Kronfeld-Schor, 2006; Cohen et al., 2009; Smale et al., 2003, 2008). An understanding of the circadian system of several different diurnal species is fundamental to our comprehension of the mechanisms underlying activity patterns. A comparative approach is expected to distinguish those features that evolved during specific evolutionary events from adaptations that are common to all diurnal species.

Most studies comparing different functions in diurnal and nocturnal species have focused on a small group of phylogenetically related nocturnal rodents (such as mice, hamsters, and rats, all myomorpha), whereas the diurnal mammals belong to different evolutionary lineages (such as myomorpha, hystricomorpha, sciuromorpha, primates, etc.), in which diurnality most probably developed independently (Smale et al., 2003). Moreover, comparisons were made between diurnal and nocturnal species of different families: laboratory rats and Siberian hamsters, with squirrels (Meijer & Rietveld, 1989), with guinea pigs (Kurumiya & Kawamura, 1988), with degu and Nile rats (Nixon & Smale, 2007), and even with pigeons (Demas et al., 2004). Phylogenetically related species are more similar than species that belong to different evolutionary lineages. Hence, differences in the neurological pathways underlying circadian rhythms in these species are more likely to be the ones responsible for the differences in the circadian rhythms, because they are unrelated to the wider sequences of major adaptive changes that occurred alongside the division into suborders and families of rodents.

The goal of our research was to determine whether there is a daily rhythm in anxiety-like behavior; whether it differs between nocturnal and diurnal species; and how melatonin affects the expression of anxiety in species with different activity patterns. To answer these questions, we compared diurnal and nocturnal members of the same genus: the common spiny mouse (*Acomys cahirinus*) and the golden spiny mouse (*Acomys russatus*); or members of the same subfamily (Gerbillinae): Tristram's jird (*Meriones tristrami)* and the fat sand rat *(Psammomys obesus*). We also included in the study two species that are commonly used in circadian rhythm studies: the nocturnal laboratory hooded rat and the diurnal degu (*Octodon degus*).

MATERIALS AND METHODS

Animals

Common and Golden Spiny Mice

For each of the experiments, 12 males of each species (approximately 5 mos old; average weight: common spiny mice: 53.6 ± 2.6 g, golden spiny mice: 59.6 ± 4.2 g) from our breeding colony at the I. Meir Segals Garden for Zoological Research at Tel Aviv University were individually housed in $33 \times 18 \times 13$-cm plastic cages and kept at $28 \pm 1\,^{\circ}$C (approximate lower critical temperature of their thermal neutral zone) on a 12:12 light-dark (LD) cycle (lights on at 10:00 h = ZT [zeitgeber time] 0, approximately 500 lux) with standard rodent chow (product 19510; Koffolk, Petach-Tikva, Israel) and water available ad libitum during a 3-wk acclimation period and during the days of the experiments.

Tristram's Jirds, Laboratory Rats, and Degu

For each of the experiments, 12 males of each species (jirds: approximately 6 mos old, average weight: 86.4 ± 6.3 g; rats: approximately 6 mos old, average weight: 378.8 ± 31.1 g; and degu: approximately 1 yr old, average weight: 203.4 ± 15.7 g) from our breeding colony at the I. Meir Segals Garden for Zoological Research at Tel Aviv University were individually housed in $21 \times 31 \times 13$-cm plastic cages on a 12:12 LD cycle (lights on at 10:00 h = ZT 0, approximately 500 lux) with standard rodent chow (product 19510; Koffolk) and water available ad libitum during a 3-wk acclimation period and during the days of the experiments. Jirds and rats were kept at $25 \pm 1\,^{\circ}$C (common captivity conditions; Briese, 1985; Gordon, 1987; Kagan et al., 1983; Moran, 1993; Nava & Carta, 2001) and degu at $20 \pm 1\,^{\circ}$C (temperature at which degu are reported to be strictly diurnal; Hagenauer & Lee, 2008; Lee, 2004).

Fat Sand Rats

For each of the experiments, 12 males (approximately 5 mos old; average weight: 192 ± 12.5 g) from Harlen Laboratories (Jerusalem, Israel) were individually housed in $21 \times 31 \times 13$-cm plastic cages and kept at

$26 \pm 1\,^{\circ}C$ (commonly used captivity conditions; Ashkenazy et al., 2009; Palgi et al., 2005) on a 12:12 LD cycle (lights on at 10:00 h = ZT 0, approximately 500 lux). Due to their susceptibility to develop diabetes, the fat sand rats were given special low-carbohydrate rodent chow (product 19560; Koffolk) and water available ad libitum during a 3-wk acclimation period and during the days of the experiments.

All procedures were conducted in accordance with and approved by the Institutional Animal Ethics Committee (L-11-033, L-11-050) and meet the ethical standards of the journal as outlined in Portaluppi et al. (2010). All efforts were made to minimize the number of animals used and their discomfort.

Experimental Design

Experiment 1: Effect of the Circadian Phase on Expressions of Anxiety-like Behavior

Following a 3-wk acclimation period, anxiety-like behavior was assessed at ZT 2, ZT 10, ZT 14, and ZT 22, using the elevated plus-maze test. Twelve animals of each species were divided into four groups. Each group underwent the first experiment at a different ZT, as shown in Table 1, and completed the experiment in all three other ZTs, with 3 d separating one experiment from the next.

The elevated plus-maze is an extensively validated test of anxiety in rodents of different sizes and activity patterns (Ashkenazy et al., 2009; Hogg, 1996; Lister, 1987; Rex et al., 1994). The black aluminum apparatus was elevated 50 cm above ground and consisted of two opposing open arms (50 cm long × 10 cm wide) separated by a central square, and two arms of the same dimensions, but enclosed by 15-cm-high walls.

The test was conducted under red dim light (20 lux, during both the dark and the light phase) in order to avoid having two variables (time and light). Moreover, red dim light does not affect the circadian rhythm nor the endogenous secretion of melatonin (Brainard et al., 1984; Haim & Zisapel, 1999; Morris & Tate, 2007; Nava & Carta, 2001; Poeggeler et al., 1995; Quay, 1963), and light (or the lack of it) has different masking effects on activity levels in nocturnal and diurnal species (Cohen et al., 2010; Rotics et al., 2011), which is expected to confound the results. Animals were placed in the central square of the maze, free to explore the apparatus for 6 min. Behavior in the maze was filmed.

Experiment 2: Effect of Melatonin on Anxiety-like Behavior

After a 3-wk acclimation period, anxiety-like behavior was assessed following daytime melatonin administration, using the elevated plus-maze test. The experiment was performed during the light phase, at ZT 10 (the ZT at which levels of anxiety-like behavior were highest in the nocturnal species, and lowest in the diurnal degu, in the first experiment) under red dim light (20 lux). Twelve animals of each species were divided into two groups: one group ($n = 6$) received an intraperitoneal (i.p.) injection containing melatonin (product M5250; Sigma, St. Louis, MO, USA) dissolved in ethanol 100% 50 mg/1 mL. We did not find information on short-term effects of melatonin on anxiety in spiny mice, jirds, sand rats, and degu. The dosage used for short-term effect in mice is 15 mg/kg) Golombek et al., 1993; Papp et al., 2006, 2010; Sugden, 1983), and for rats it is 25 mg/kg (Golombek et al., 1993; Papp et al., 2006, 2010; Sugden, 1983). Therefore, to compensate for body mass effect we used a slightly higher for spiny mice, which are bigger than laboratory mice (15 mg/kg) and a slightly higher dosage for jirds (20 mg/kg), which are even heavier. For laboratory rats, sand rats, and degu, which are approximately the same size, we used 25 mg/kg, as known to be effective in laboratory rats. Double-distilled water was added to attain an injection volume of 0.1 mL for spiny mice and jirds and of 0.2 mL for sand rats, laboratory rats, and degu. The control group ($n = 6$) received an i.p. injection containing the same quantity of 100% ethanol and double-distilled water per body weight of each species, as calculated for the first group.

Following the injection, all animals remained for 30 min in complete darkness and were then individually placed in the central square of the maze and were free to explore the apparatus for 6 min. Behavior in the maze was filmed.

Data Analysis

Data were analyzed using Statistica 7.0 (Statsoft, 1984–2005; Tulsa, OK, USA). Anxiety-like behavior was quantified as the ratio between the measured time spent in the open arms and the total time spent in both open and closed arms. Activity levels were assessed by

TABLE 1. Order of participation in the first experiment.

	ZT 2	ZT 10	ZT 14	ZT 22
Day 1	Animals 10, 11, 12	Animals 7, 8, 9	Animals 4, 5, 6	Animals 1, 2, 3
Day 5	Animals 7, 8, 9	Animals 4, 5, 6	Animals 1, 2, 3	Animals 10, 11, 12
Day 9	Animals 4, 5, 6	Animals 1, 2, 3	Animals 10, 11, 12	Animals 7, 8, 9
Day 13	Animals 1, 2, 3	Animals 10, 11, 12	Animals 7, 8, 9	Animals 4, 5, 6

The order is repeated for each species. Some subjects have their first exposure to the elevated plus-maze at each circadian time point. The order of participation in the test had no significant effect on the results for all species ($p > 0.1$; $n = 12$).

calculating the number of entries to all arms of the maze. Differences were considered significant when $p<0.05$

The effect of the circadian phase on the relative time spent in the open arms and on the number of entries to all arms of the maze (Experiment 1) was examined using repeated-measures analysis of variance (ANOVA). The order of participation in the test (Table 1) was added as a categorical variable, to ensure that it had no significant effect. Bonferroni adjustment post hoc tests were used for post hoc comparisons.

The effect of melatonin administration on the relative time spent in the open arms and on the number of entries to all arms of the maze (Experiment 2) was examined using Student's t test, comparing the group treated with melatonin with the control group.

RESULTS

Experiment 1

The order of participation in the test (Table 1) had no significant effect on the results for all species ($p>0.1$; $n=12$). Therefore, it was excluded from the analysis.

ANOVA revealed a significant circadian rhythm in anxiety in all three nocturnal species: the common spiny mouse showed significantly higher levels of anxiety ($F(3, 33)=20.11$, $p<0.001$) during the light phase, compared with the dark phase (post hoc: ZT 2 and ZT 10 are significantly different from ZT 14 and ZT 22, $p<0.01$; Figure 1a). Activity levels of the common spiny mouse did not change significantly along the different ZTs ($F(3, 33)=1.24$, $p=0.310$), implying that the differences in anxiety levels along the ZTs are not simply the result of circadian changes in activity levels.

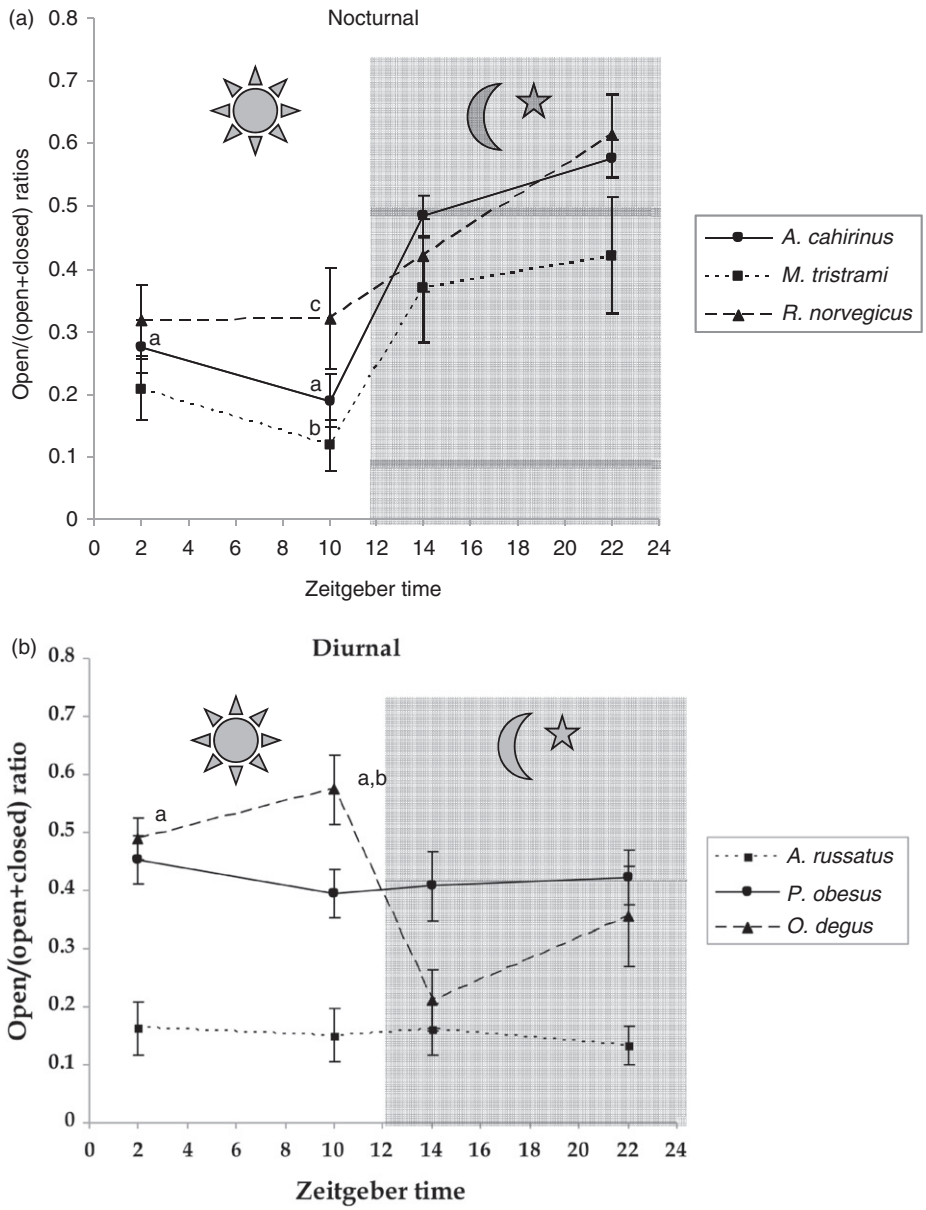

FIGURE 1. The ratio between time spent in the open arms and total time spent in both open and closed arms (mean ± SE) at different ZTs throughout the dial cycle in (a) nocturnal and (b) diurnal species. There was a significant daily rhythm in anxiety in all three nocturnal species and in the diurnal degu: the golden spiny mouse and the fat sand rat showed no significant changes in expressions of anxiety during different hours of the dial cycle. (a) a denotes significant differences ($p<0.01$) from ZT 14 and ZT 22; b denotes significant differences ($p<0.05$) from ZT 14 and ZT 22; c denotes a significant difference ($p=0.06$) from ZT 22. (b) a denotes a significant difference ($p<0.005$) from ZT 14; b denotes a significant difference from ZT 22.

Similar to the common spiny mouse, the Tristram's jird showed significantly higher levels of anxiety ($F(3, 33) = 6.77$, $p < 0.005$) during the light phase, compared with the dark phase (post hoc: ZT 10 is significantly different from ZT 14 and ZT 22, $p < 0.05$; Figure 1a). Activity levels of the Tristram's jird did not change significantly along the different ZTs ($F(3, 33) = 1.65$, $p = 0.202$).

The laboratory rat also showed significant daily rhythm in levels of anxiety ($F(3, 33) = 3.16$, $p = 0.03$). However, Bonferroni post hoc test found only marginally significant difference between ZT 2 and ZT 22 ($p = 0.06$) and between ZT 10 and ZT 22 ($p = 0.07$). Using Fisher least significant difference (LSD) test, the differences between the light phase (ZT 2 and ZT 10) and the dark phase (ZT 14 and ZT 22) were significant ($p < 0.05$; Figure 1a). Activity levels of the laboratory rat were significantly lower ($F(3, 33) = 4.07$, $p = 0.014$) at ZT 10 than at other ZTs. In spite of the differences in activity levels between ZT 10 and ZT 2, no significant differences in anxiety levels were found comparing ZT 10 and ZT 2, and therefore it is reasonable to assume that the differences in anxiety along ZTs are not simply the result of circadian changes in activity levels.

The diurnal degu showed an inverse pattern to that of the nocturnal species: its levels of anxiety were significantly lower ($F(3, 33) = 9.29$, $p < 0.001$) during the light phase (ZT 2 and ZT 10) compared with the dark phase (post hoc: ZT 10 and ZT 2 are significantly different from ZT 14, $p < 0.005$, and ZT 2 is significantly different from ZT 14, $p < 0.05$; Figure 1b). Activity levels of the degu were significantly lower at ZT 14 compared with ZT 2 ($F(3, 33) = 3.13$, $p = 0.038$). Since no significant differences in anxiety levels were found between ZT 14 and ZT 2, it is again reasonable to assume that the differences in anxiety along ZTs are not simply the result of circadian changes in activity levels.

The golden spiny mouse and the fat sand rat showed no significant changes in expressions of anxiety during the different hours of the diel cycle (golden spiny mouse: $F(3, 33) = 0.18$, $p = 0.905$; fat sand rat: $F(3, 33) = 0.76$, $p = 0.523$) (Figure 1b), nor in activity levels (golden spiny mouse: $F(3, 33) = 1.92$, $p = 0.152$; fat sand rat: $F(3, 33) = 2.776$, $p = 0.0566$).

Experiment 2

Melatonin administration had a significant effect on all three nocturnal species as well as on the diurnal degu: the common spiny mouse, the Tristram's jird, and the laboratory rat showed a significant decrease in anxiety in response to melatonin injection ($n = 6$) compared with control ($n = 6$) (common spiny mouse: $t = 12.44$, $p < 0.005$; Tristram's jird: $t = 2.55$, $p = 0.029$; laboratory rat: $t = 4.15$, $p < 0.005$) (Figure 2a). No significant differences in activity were found between groups (common spiny mouse: $t = 9.29$, $p = 0.14$; Tristram's jird: $t = -0.19$, $p = 0.849$; laboratory rat: $t = 1.29$, $p = 0.225$).

The diurnal degu showed an inverse response pattern to that of the nocturnal species, with a significant increase in anxiety levels in response to melatonin injection ($n = 6$) compared with control ($n = 6$; $t = -4.59$, $p < 0.005$) (Figure 2b). No significant differences in activity were found between groups ($t = -0.31$, $p = 0.76$).

Melatonin administration had no effect on anxiety-like behavior levels in the golden spiny mouse ($t = 0.42$, $p = 0.352$) and fat sand rat ($t = 1.89$, $p = 0.088$) (Figure 2b), nor on their activity levels (golden spiny mouse: $t = 0.95$, $p = 0.364$; fat sand rat: $t = -0.33$, $p = 0.751$).

DISCUSSION

We found a pronounced daily rhythm of anxiety-like behavior in all three nocturnal species (Figure 1), which exhibited significantly lower levels of anxiety-like behavior during the dark phase than during the light phase. The diurnal degu showed an inverse pattern to that of the nocturnal species, with higher levels of anxiety-like behavior during the dark phase. The diurnal golden spiny mouse and fat sand rat, however, did not show significant changes in anxiety-like behavior level throughout the diel cycle (Figure 1). Exogenous melatonin caused a significant decrease in anxiety-like behavior levels in the three nocturnal species, and an increase in anxiety-like behavior levels in the diurnal degu, whereas the golden spiny mouse and the fat sand rat showed no significant changes in anxiety-like behavior levels (Figure 2).

The daily rhythm of anxiety-like behavior that we found is consistent with the reported effect of melatonin on anxiety-like behavior in laboratory mice (Karakaş et al., 2011), and with the known circadian rhythms of different physiological and behavioral functions. Previous studies have shown that diurnal and nocturnal animals exhibit differences in circadian rhythm in activity levels, body temperature, hormone secretion, etc. (Aschoff, 1960; Challet, 2007; Hagenauer & Lee, 2008). We found that species with different activity patterns differed in the daily rhythmicity of anxiety-like behavior levels: those levels in the nocturnal species were high during the light phase, and low during the dark phase; whereas in the diurnal species, the degu showed an inverse pattern of anxiety-like behavior, while the golden spiny mouse and fat sand rat showed no rhythmicity in anxiety-like behavior whatsoever. These findings may reflect a possible role of the diel changes in anxiety levels in the regulation of behavior and activity throughout the diel cycle: whereas the nocturnal species all presented a rather similar anxiety-like behavior pattern and response to melatonin administration, the diurnal species showed larger variation. This variation possibly reflects the evolution of diurnal activity patterns: the earliest evolved mammals were nocturnal insectivores (Park, 1940), hence diurnality, which evolved in many different evolutionary lineages, is expected to be more diverse (Smale et al., 2003). This

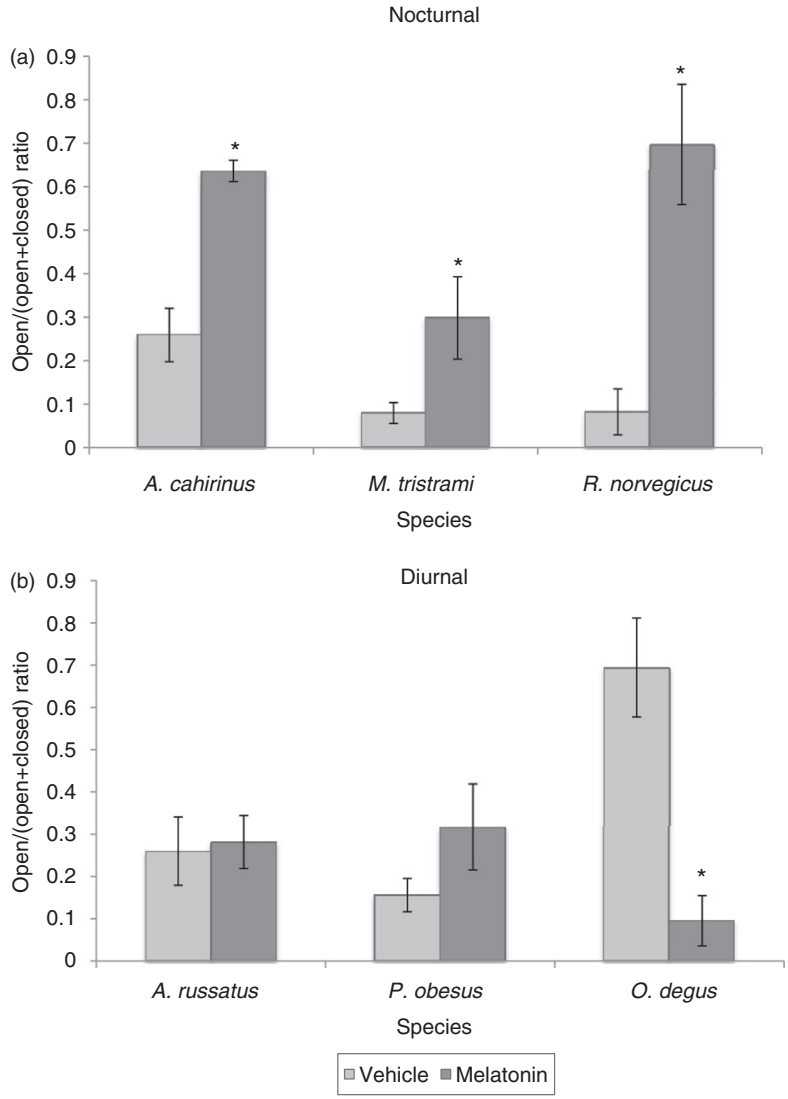

FIGURE 2. The ratio between time spent in the open arms and total time spent in both open and closed arms (mean ± SE) following melatonin administration to (a) nocturnal and (b) diurnal species. The asterisks denote significant differences ($p < 0.05$) compared with vehicle.

diversity might also be expressed in behavioral and mental rhythms such as anxiety.

Two of the diurnal species, the golden spiny mouse and fat sand rat, expressed no daily rhythm in anxiety-like behavior and no response to melatonin administration. The fat sand rat has never been captured in the field at night (Ilan, 1985), and shows a diurnal pattern of body temperature and melatonin secretion in the laboratory (Schwimmer et al., 2010). However, in a recent study we conducted under laboratory conditions (Barak & Kronfeld-Schor, in press), the sand rat showed diverse activity patterns. The golden spiny mouse is also a diurnal rodent that shows a lack of circadian rhythm in anxiety-like behavior. It presents a variety of adaptations, some of which support diurnality, such as dark skin and pigmentation and a high concentration of ascorbic acid in its eyes (Koskela et al., 1989), whereas others support nocturnality, such as different characteristics of the cornea (Kronfeld-Schor et al., 2001), its potential for nonshivering thermogenesis (Kronfeld-Schor et al., 2000), and its response to the lunar cycle (Gutman et al., 2011). This variety of adaptations has led to the hypothesis that the golden spiny mouse is at an evolutionary transitional phase between a diurnal and a nocturnal activity pattern (Cohen & Kronfeld-Schor, 2006; Cohen et al., 2009; Cohen et al., 2010; Kronfeld-Schor & Dayan, 2008). The lack of a circadian rhythm in anxiety-like behavior in the above two species may reflect an adaptive mechanism that enables adjustment of activity pattern to changes in the environment, which could provide an advantage in extreme environments such as the desert. Alternatively, it may reflect an evolutionary transitional phase from nocturnality to diurnality, which would necessitate elimination of increased anxiety during the light phase. A similar finding has been reported for common and golden spiny mice (Cohen et al., 2010; Rotics et al., 2011), where the masking effect of light on activity is very pronounced in common spiny mice, but is absent in golden spiny mice.

In four out of the six species tested in this study, we found a significant daily rhythm in anxiety-like behavior and a significant response to melatonin, which were in accord with their activity rhythm. It was previously

suggested that masking may help to confine activity to the ''correct'' part of the diel cycle (Redlin et al., 2005). Here we suggest that circadian changes in anxiety levels might play a similar role in regulating the time and type of the animal's activity throughout the diel cycle. Anxiety-like behavior in rodents is also expressed in a tendency to hide and in reduced foraging and exploration behavior (Lister, 1987; Pellow & File, 1986; Pellow et al., 1985). Therefore, whereas in strictly nocturnal species a circadian pattern of anxiety could contribute to the regulation of activity according to the optimal hours, in species that switch from nocturnal to diurnal activity, or species that are required to show circadian plasticity and regulate their activity according to environmental conditions, this pattern might be a hindrance.

The findings from the second experiment reveal a significant decrease in anxiety in the nocturnal species in response to melatonin administration. If we consider the decrease in anxiety as promoting foraging and exploration, the results of the second experiment are consistent with the known effects of melatonin on nocturnal species, e.g., increased alertness, locomotor activity, and body temperature (Hastings et al., 1992; Huber et al., 1998; Mendelson, 2002; Mendelson et al., 1980). Like the effect of the circadian phase on their anxiety levels, the effect of melatonin differed between the diurnal and nocturnal species, according to the circadian rhythm in anxiety that they had exhibited in the first experiment. In contrast to the nocturnal species, the diurnal degu showed increased anxiety levels following melatonin administration. This result is consistent with known differences in physiology and behavior of nocturnal and diurnal species in response to exposure to light and to melatonin administration (Aschoff, 1960; Hagenauer & Lee, 2008).

It is interesting to note that chronic melatonin injections (100 μg melatonin for 3 wks 5 and 8.5 h after light onset) to fat sand rats maintained at a similar photoperiod resulted in increased anxiety-like behavior (Ashkenazy et al., 2009). On the daily scale, the pattern of melatonin secretion contains information regarding the phase of the circadian clock as well as about the photic environment. On the chronic or seasonal scale, it contains information regarding changes in day lengths across the seasons and animals use this information to anticipate and prepare for seasonal changes in the environment (Bartness et al., 1993). It was previously shown that photoperiod affects anxiety-like behavior in different rodent species; for example, long daylight conditions appear to induce an anxiety-like response in voles (Ossenkopp et al., 2005), but were reported to be anxiolytic in hamsters (Prendergast & Nelson, 2005), and resulted in an increased anxiety-like behavior in mice that was reversed by a melatonin antagonist (Kopp et al., 1999). However, these results relate to the chronic effect of photoperiod and melatonin, and not to the daily cycle. We suggest that a daily cycle in anxiety-like behavior exists at least in some species year round, but may fluctuate around a different level, which by itself may show seasonal fluctuations. These two scales, daily and seasonal, contain very different information, and therefore it is not surprising that the responses to acute and chronic melatonin injections are very different in this species.

The daily rhythm in anxiety-like behavior levels in the six species tested here is in agreement with the effect of melatonin on these measures. This agreement supports the hypothesis that melatonin acts as a link between the circadian rhythm and cognitive and affective changes. The nocturnal species showed significantly lower levels of anxiety during the dark phase, in which melatonin is endogenously secreted. Accordingly, melatonin administration during the light phase (when no endogenous melatonin is secreted) caused a significant decrease in anxiety. The diurnal degu, however, showed significantly higher levels of anxiety during the dark phase. Hence, the effect of the circadian phase on anxiety is also consistent with the effect of melatonin on the degu.

Our findings support the hypothesis that the difference between diurnal and nocturnal species is located ''downstream'' to the SCN, i.e., in opposing interpretations of the melatonin signal. They also demonstrate that the time of day/night when behavioral tests are conducted is crucial because it may affect the results and sensitivity of the test.

Our results offer one more step towards understanding the complex mechanisms underlying activity patterns and behavior. For deeper characterization of the foundations of circadian rhythms and disorders, more research is needed in the form of comparisons of different species of different genera, identification of the ideal timing for experiments, and the design of tests for comparing among species of various sizes, evolutionary stages, behaviors, and functioning abilities.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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