Differential effects of photoperiod length on depression- and anxiety-like behavior in female and male diurnal spiny mice

Miriam Ben-Hamo a,1, Katy Tal a,1, Rotem Paz-Cohen a, Noga Kronfeld-Schor a, Haim Einat b,*

a Department of Zoology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel
b School of Behavioral Sciences, Tel-Aviv Yaffo Academic College, Tel-Aviv, Israel

HIGHLIGHTS

• Short photoperiod induces depression- and anxiety-like behavior in male diurnal rodents.
• We compared the response to short photoperiod of diurnal female and male spiny mice.
• Males and females differed in their behavioral responses with less sensitivity of females.
• Sex hormones are not sufficient to explain the difference between sexes.

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ABSTRACT

The relationships between biological rhythms and affective disorders are known but their underlying biology not clear. There is difficulty in studying circadian rhythms in humans and appropriate animal models are hard to identify or develop. Some studies show that diurnal rodents can be advantageous model animals for the study of interactions between biological rhythms and affective disorders but previous studies did not include females whereas in humans there are sex differences in affective disorders. The present study tested the effects of short photoperiods in both males and females of the diurnal golden spiny mouse (Acomys rusatus). Adult, female and male spiny mice were housed in either neutral photoperiod (12:12 light/dark; NP), or short photoperiod (5:19 light/dark; SP) conditions. After 3 weeks acclimatization, animals were tested for spontaneous activity in an open field, elevated plus maze (EPM), sweet solution preference (SSP) and the forced swim test (FST).

Both sexes responded to the SP, but while SP males showed increased anxiety-like behavior in the EPM and depression-like behavior in the FST, females showed increased activity, reduced anxiety-like behavior in the EPM, depression-like response in the SSP and no effect in the FST.

Differences between sexes were previously demonstrated in behavioral tests that followed a variety of manipulations, and were usually explained in the context of sex hormones. Yet, the current results cannot be compared with previous data from diurnal rodents and further testing of females from other diurnal rodents are needed to explore whether these differences are a general phenomenon or possibly unique to golden spiny mice.

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1. Introduction

Multiple lines of evidence clearly demonstrate a tight relationship between biological rhythms and affective disorders including both depression and bipolar disorder. These evidence include attenuated physiological rhythms, including cortisol release and motor activity [1,2]; disruption of the rhythmic expression of circadian clock genes in patients diagnosed with major depressive disorder [3], and changes to sleep architecture and the sleep-wake cycle in both depression and mania [4–6]. Moreover, a number of treatments used in affective disorders have also been shown to affect circadian rhythms [7], and sleep deprivation is an effective treatment of depression [8]. Overall, these data strongly support the connection between circadian rhythms and affective disorders [for review see [9]].

One specific affective disorder that probably best demonstrates a relationship between biological rhythms and affect is seasonal affective disorder (SAD). SAD was first described by Rosenthal [10] and is specified as a major depressive disorder with a seasonal pattern [11]. SAD is characterized by symptoms such as depressed mood, anhedonia, and fatigue, and is manifested when days get shorter and goes to remission when days get longer [10] (and more rarely during summer with remission during winter). Understanding the neurobiological mechanisms
underlying the affective responses to photoperiod is crucial for understanding the pathogenesis of SAD; however, there is difficulty in studying circadian rhythms in humans due to the intervening nature of these studies and the need to isolate the subjects from their normal environment and daily routines for long periods of time. Yet, appropriate animal models for SAD are not easy to identify or develop [12], and we suggested it is because most of the model animals used in research are nocturnal [13].

In this context, efforts in the last decade were dedicated to develop a diurnal rodent model that might be advantageous for the study of SAD, as well as for understanding the relationship between biological rhythms and affect. This work demonstrated that several diurnal rodent species, including the fat sand rat (Psammomys obesus), grass Nile rat (Arvicanthis niloticus), Mongolian gerbils (Meriones unguiculatus) and the degu (Octodon degus) indeed change their behavior and show depressive- and anxiety-like phenotypes in response to changes in day length [14–18]. Moreover, the behavioral changes displayed by these diurnal rodents respond to similar treatment used to alleviate symptoms of SAD in humans including antidepressant drugs, bright light exposure and voluntary exercise [19–22].

These previous studies offered strong support to the notion that diurnal rodents can be advantageous in the study of the interactions between biological rhythms and affective disorders. However, all these experiments were conducted on males. Affective disorders in humans are found in both sexes, and sex differences exists in most affective disorders, with both depression and anxiety disorders being more prevalent in women than in men [23]. The use of female model animals is frequently neglected in biomedical research for many reasons, but recently there is growing understanding that studies should be done on both sexes in order to be able to make more comprehensive conclusions [24,25]. Hence, the present study was designed to test the effects of chronic exposure to short photoperiods in males and females of a diurnal rodent species, the diurnal golden spiny mouse (Acomys russatus).

2. Materials and methods

2.1. Animals

A total of 30 adult males and 55 adult females (over 3 months old) golden spiny mice (A. russatus) from the breeding colony at the I. Meir Segals Garden for Zoological Research at Tel-Aviv University were used for this experiment. Animals were transferred from the breeding colony and singly housed in plastic cages (33 × 18 × 13 cm) in temperature controlled rooms set to 28 °C [26,27]. Animals were provided with free access to food (standard rodent chow, 19510, Koffolk, Petach-Tikva, Israel) and water. All experimental procedures followed the Israel Ministry of Health regulations on the use of laboratory animals and were approved by the Tel-Aviv University IACUC (protocol # L-14-002).

2.2. Experimental design

The experiments described below were performed with three different cohorts of animals; 30 males were run in one experimental design, 23 females in another experimental design and 32 females in a third experimental design. The need to run the study in smaller cohorts and unequal numbers is because spiny mice are not commercially available, and the animals in the study were derived from our own small breeding colony. In each of these cohorts, animals were assigned to one of two experimental groups: 1) neutral photoperiod with 12:12 light/dark cycle (NP); and 2) short photoperiod with 5:19 light/dark cycle (SP). For both treatment groups room lights were switched on at 08:00 h = ZT 0 with an intensity of 500 lx. In the NP room lights were switched off at 20:00 h = ZT 12 whereas in the SP room, lights were switched off at 13:00 h = ZT 5. Spiny mice were allowed three weeks of acclimation to these experimental conditions as this time period was found to be sufficient for physiological acclimation [28] and synchronization of circadian rhythms [26]. Following the acclimation period, animals were evaluated in a number of tests for affective-like changes. All behavioral tests were conducted between ZT 0 to ZT 5 (light phase for both groups).

2.3. Cohorts and order of tests

Cohort 1: 30 male spiny mice were tested in the elevated plus maze and the forced swim test. Half of the animals from each group (randomly selected) were also tested for spontaneous activity in an open field.

Cohort 2: 23 female spiny mice were tested in the sweet solution preference test, the open field and the forced swim test.

Cohort 3: 32 female spiny mice were tested in the sweet solution preference test and in the elevated plus maze.

2.4. Open field test

To assess spontaneous activity levels, each animal was tested in a large open arena composed of a 200 × 200 cm floor with 50 cm high walls and made of white PVC [29]. Infra-red illumination (830 nm filter, Tracksys, IR LED illuminator, UK) was used to allow recording individual activity with a video camera (B/W ICCD-47E, Ikegami Tsushinki, Japan) placed 250 cm above the center of the arena for a period of 10 min.

2.5. Elevated plus-maze

The EPM is frequently used to evaluate anxiety-like behavior in rodents [30]. For the present study the maze was constructed from black aluminum and consisted of two open arms (50 cm long and 10 cm wide) and two closed arms (same dimensions with 15 cm high walls). The plus maze is elevated 50 cm above the floor and light levels at the open arms are 200 lx. Animals are individually placed in the center of the maze, and their behavior is digitally recorded for a 5 min session. Recordings are used for later manual scoring of behavior. At the end of each session, animals are returned to their cages and the maze is wiped clean with 10% ethanol before the start of the next session. Scoring of the EPM included the time and the number of entries into each arm and is done by an investigator blind to treatment.

2.6. Sweet solution preference

To assess unconditioned reward-seeking (hedonic) behavior, we used the sweet solution preference test [31]. Animals were provided with two identical plastic bottles, one with tap water and the other with 1% saccharin solution for a period of one week. Bottles were weighed before they were introduced to the cages and then 48, 96 and 144 h later when the test was terminated and the saccharin bottles removed. Bottle position was switched during the 48 and 96 weighing to prevent possible effects of side preference in drinking. The ratio of saccharin solution/total liquids (saccharin solution + water) consumption was calculated, termed “sweet solution preference ratio” and served as the main measure for the test. This test with different variations is widely accepted as a measure for reward seeking behavior and is frequently used in studies of affective disorders [31–35].

2.7. Forced swimming test

The forced swim test (FST) is a standard test for depression-like behavior and antidepressants effects [36]. As the golden spiny mice are not good swimmers like rats and mice, we used a variation of the test that was previously validated in our labs where despair-like behavior is defined by sinking in the water rather than floating [21,22,31,37,38]. Animals were individually placed into 10 L plastic buckets (27 cm height; 30 cm diameter) containing 8 L of water at 25 °C. Animals were recorded using a camera placed above the bucket and closely watched by the experimenter. Animals usually swim for a while and then stop.
swimming and sink underwater for a short while after which they resume swimming with head above water. A “sink” was defined as head under water for 2 s and when an animal sank for the second time it was taken out of the water by the experimenter. If an animal did not show sinking, it was taken out of the water after 5 min. The test was performed twice in two following days where the first day was defined as the training session and the second day was defined as the test session [21,22]. Water in the bucket was changed in between every other animal.

2.8. Statistical analysis

Data for the sweet solution preference were analyzed using mixed analysis of variance (ANOVA) with photoperiod and cohort (two female cohorts) as main factors and time of weighing as repeated measures factor. Data for the FST (test session) were analyzed using repeated measures ANOVA with photoperiod as main factor and sinking (Sink 1 and Sink 2) as the repeated measures factor. For activity in the open field and for the measures of the EPM data were analyzed using a student’s t-test with photoperiod as independent variable.

3. Results

3.1. Open field spontaneous activity

Spontaneous activity in an open field was only tested in some of the animals. No differences were found between male golden spiny mice maintained in the neutral and short photoperiods [NP: 11,333 ± 863 cm, SP: 12,650 ± 1853 cm, t(12) = 0.70, p = 0.5]. The females showed a trend for increased activity when kept under SP conditions, but the trend did not reach statistical significance [Females - Neutral: 11,605 ± 921 cm, Short: 14,971 ± 1414 cm, t(21) = 2.03, p = 0.056]. Power calculations (GraphPad Statmate 2.0) show that the experiment was only at approximately 50% power and it is therefore possible that with a higher number of animals per group this effect would have been significant.

3.2. Elevated plus maze

Interestingly, short photoperiods had contrasting effects on the behavior of male and female golden spiny mice in the EPM. Whereas in males shortening the photoperiod resulted in increased anxiety-like behavior, in females it induced a reduction in anxiety-like behavior. In males, short photoperiod resulted in reduced time in the open arms [Fig. 1A, t(26) = 2.4, p = 0.024] and a similar trend for increased time in the open/closed arms [Fig. 1B, t(26) = 1.88, p = 0.07] and a significant reduction in activity levels represented by the total number of entries to all (open and closed) arms [Fig. 1C, t(28) = 2.4, p = 0.023]. In females, short photoperiods resulted in a significant increase in time spent in the open arms [Fig. 1D, t(29) = 2.46, p = 0.02] and in the open/closed arms time ratio [Fig. 1E, t(29) = 2.12, p = 0.042] and a trend for increased activity reflected in the total number of arms entries [Fig. 1F, t(30) = 1.69, p = 0.1].

3.3. Forced swim test

Short photoperiod conditions resulted in faster sinking of males in the FST suggesting a depressive-like behavior [Fig. 2A, repeated measures ANOVA across Sink 1 and Sink 2 events, photoperiod effect: F(1,25) = 11.6, p = 0.002; sink effect: F(1,25) = 12.67, p = 0.001; interaction: F(1,25) = 0.1, p = 0.79]. However, the same regimen of light had no effect on the females [Fig. 2B, repeated measures ANOVA across Sink 1 and Sink 2, photoperiod effect: F(1,20) = 0.08, p = 0.78; sink effect: F(1,20) = 7.53, p = 0.013; interaction: F(1,20) = 0.14, p = 0.71].

3.4. Sweet solution preference

The sweet solution preference test was performed in both female cohorts but not in the males. A mixed statistical analysis across cohorts, photoperiod conditions and day of measuring showed that SP animals consistently had lower preference ratio for the sweet solution suggesting a depressive-like behavior [Fig. 3, photoperiod effect: F(1,68) = 5.11, p = 0.03; cohort effect: F(1,68) = 1.35, p = 0.25; weighing day: F(2,68) = 6.11, p = 0.004]. Please note that as there is no cohort effect, for presentation purposes the two cohorts were pooled together in the figure.

4. Discussion

Previous studies clearly demonstrated that when diurnal rodents are maintained in short photoperiod conditions, they develop a phenotype that can be explained by depressive- and anxiety-like behavioral changes [15,31,37,39–41]. However, all of these studies used only males. Here, we show that the diurnal golden spiny mouse, A. russatus, shows alterations in its behavior in response to a short photoperiod, similar to those observed in other diurnal rodents previously studied. Moreover, we report sexual differences in the affective responses of these animals to the short photoperiod. Our results concur with previous studies showing depressive- and anxiety-like behavior in male diurnal rodents kept under a short photoperiod, but point to sexual differences in this species with females displaying different behavior than that observed in the males.

Male golden spiny mice maintained under short photoperiods show a similar phenotype to what was previously demonstrated in fat sand rats, grass Nile rats, Mongolian gerbils, and degu [18,35,37,40,42,43] including no change in spontaneous activity, but an increase in anxiety-like behavior in the EPM and depressive-like behavior in the FST. In contrast, females show a significantly different phenotype that includes a depressive-like behavior in the sweet solution preference test but no effects in the FST, while showing increased activity in the open field test and increased preference for the open arm in the EPM, both suggestive of reduced anxiety-like behavior. Sexual differences in affective responses were previously reported in animal studies, and they concur with the difference in prevalence of affective disorders in men and women [25,44].

One behavioral difference that is demonstrated in a number of studies is that as a general rule, female rodents are more active than males [45,46]. Some studies indicated differences in the same tests that were used in the current study and in response to a variety of interventions. For example a recent study compared female and male Sprague Dawley rats in a number of behavioral tests with and without drug interventions [45]. This study found that administration of the anxiolytic drug diazepam resulted in the anxiolytic-like effect of increased time in the open arms of the EPM in males but had no effects in females [45]. Moreover, administration of the prototypic antidepressant drug imipramine resulted in reduced immobility in the FST in males, but not in females [45]. Another interesting difference reported in that same study is that across tests and states, female rats were more active than male rats [45].

Another study that tested the behavioral effects of short or long photoperiods in hamsters also demonstrated reversed effects on females and males, however the trends were opposite of those we found in the current study: SP female hamsters showed less time in the open arms of the EPM and SP male hamsters showed increased time in the open arms [47].

Regardless of the differences between females and males in the current study, short photoperiod conditions resulted in some indications of depressive-like behavior in both sexes. Males demonstrated depression-like behavior in the FST whereas females showed depression-like behavior in the sweet solution preference test but not in the FST and unfortunately due to technical problems, the males were not tested in the sweet solution preference test. The lack of effect of short photoperiods
FST stands in contrast with not only the male data in the current study but also with previous studies in males of other diurnal rodents [31,35,37,40,43]. Yet, female rodents are demonstrated in some studies to be more active than males in the FST [48,49] and moreover, the sensitivity of male and female behavior in the FST to interventions that induce depression-like behavior in males and to antidepressant drugs is different [48,50–52]. Hence it is possible that the lack of effect in the FST in females is related to lower susceptibility to stressful interventions. Moreover, in humans, the prevalence of SAD increases in areas that are further from the equator [53] demonstrating that when photoperiod stress increases (shorter days), a larger percentage of people respond to the stress. It is therefore plausible that if the photoperiod stress was more significant (shorter days), the females would have also responded.

The effects seen in the females sweet solution preference test may appear to be in contrast with SAD in people where carbohydrate craving is a common symptom [54]. However, it is important to remember that the test as it is used here is not a simple measure for “sugar craving” but in fact a model that represents broader aspects of reward seeking behavior [31,55]. In this context, it is important to remember that

**Fig. 1.** Effects of short photoperiods on behavior in the EPM: in males, time in the open arms [Fig. 1A, t(26) = 2.4, p = 0.024]; open/closed time ratio [Fig. 1B, t(26) = 1.88, p = 0.07]; total number of entries to all (open and closed) arms [Fig. 1C, t(28) = 2.4, p = 0.023]. In females, time in the open arms [Fig. 1D, t(29) = 2.46, p = 0.02]; open/closed arms time ratio [Fig. 1E, t(29) = 2.12, p = 0.042]; total number of arms entries [Fig. 1F, t(30) = 1.69, p = 0.1].

**Fig. 2.** Effects of short photoperiods on behavior in the FST: in males [Fig. 2A, repeated measures ANOVA across Sink 1 and Sink 2 events, photoperiod effect: F(1.25) = 11.6, p = 0.002; sink effect: F(1.25) = 12.87, p = 0.001; interaction: F(1.25) = 0.1, p = 0.79] and in females [Fig. 2B, repeated measures ANOVA across Sink 1 and Sink 2, photoperiod effect: F(1.20) = 0.08, p = 0.78; sink effect: F(1.20) = 7.53, p = 0.013; interaction: F(1.20) = 0.14, p = 0.71].
modeling a disorder or a domain of a disorder is usually not a precise duplication of the disorder but an attempt to find some levels of homology [9]. It is however unfortunate that we do not have data for males in the sweet solution preference test and to the best of our knowledge there is no such previous data in spiny mice. Yet, we have previously demonstrated that short photoperiod conditions reduce sweet solution preference in male sand rats [31] and male grass Nile rats [35]. Moreover, severe disruption of circadian rhythms using constant light in nocturnal male rats [56] or male mice [57] also resulted in reduced sweet solution preference. It is therefore reasonable to suggest that this effect would have also appeared in male spiny mice.

The EPM is generally used to model anxiety-like behavior, and test the effects of anxiogenic and anxiolytic interventions [58,59]. Because depression and anxiety are frequently co-morbid, it is a common practice in animal models work to evaluate anxiety-like behavior when evaluating depressive-like behavior. Indeed, previous studies with male diurnal rodents show that when maintained in short photoperiods, animals develop both depressive-like behavior and anxiety-like behavior [31,43]. However, the effects of short photoperiods are constant and replicable in inducing depressive-like behavior in a variety of tests, the effects on anxiety-like behavior are rarely consistent, even in males. For example, in a study of the grass Nile rat, short photoperiods resulted in depressive-like behaviors in the FST and the sweet solution preference test but had no effects on anxiety-like behavior in the light/dark box [35]. Similarly, in a recent study of the Degu, short photoperiods induced depressive-like behavior in the FST and the sweet solution preference test as well an anxiety-like behavior in the open field but not in the light/dark box [43]. It is therefore possible that the effects of shortening the photoperiod on anxiety are not as clear as the effects on depression and this is similar to the clinical manifestation of SAD, which is primarily characterized by a phenotype of depression and not necessarily increased anxiety [54]. Yet, the current results of the behavior in the EPM are even more counterintuitive because the effects of short photoperiod on females suggest an anxiolytic-like effect (see Figs. 1D and 1E). Activity level can greatly vary the response in tests such as the EPM, which is considered as a measure of exploratory behavior [60,61,62]. The results show that female spiny mice maintained in short photoperiods appeared to be more active in the EPM compared with the neutral photoperiod controls and it is possible that the increased activity might have influenced the other measures as well. It would have been interesting to also examine both males and females in tests for anxiety-like behavior that are not based on exploration such as the novelty suppressed feeding test or the marble burying test [61] but these tests were not used in the study.

It is beyond the scope of this paper to try and dissect all possible underlying mechanisms that could explain the differences between the females and the males. A seemingly simple explanation might attribute these differences to hormonal states as suggested before for other models and situations [e.g. [63]]. Golden spiny mice are seasonal breeders: in a field study we found that males were reproductively active (with developed testes) beginning in January and ending in October, with a peak in January–February, while females appeared reproductively active (pregnant or lactating) starting in February and ending in November, with a peak in March–June [64]. It is possible that under natural photoperiod, when both sexes are reproductively active, reproductive hormones affect males and females differently. However, it is also possible that such explanation would be overly simplistic because [1] many studies show minimal differences between male and female rodents in similar tests [e.g. [65–67]]. [2] The hormonal state of females depends on their stage in the estrous cycle and it could therefore be expected that the variability in response in females would be higher than in males but this does not seem to be the case as the homogeneity of variance for males and females in the EPM is not different (Levene test for homogeneity of variance, data not shown). [3] The estrous cycle in spiny mice is unstable and irregular (12–26 days cycle; [68] Wube et al. 2008, Katz et al. submitted) and therefore the changes of synchronization between the females, which may result in low variance and significant differences are low and were not observed in a previous study under similar conditions [69] (Katz et al., submitted).

In summary, the present study helps to generalize the observed effects of short photoperiods to induce depressive- and anxiety-like behavior in male diurnal rodents. The results with females are equivocal and cannot be compared with previous data in other diurnal rodents as no other studies using this paradigm were performed with females. It is suggested to continue to examine females from other diurnal rodent species in order to explore whether the sex differences are a general phenomenon or possibly unique to golden spiny mice.

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