Voluntary exercise enhances activity rhythms and ameliorates anxiety- and depression-like behaviors in the sand rat model of circadian rhythm-related mood changes

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HIGHLIGHTS

• Diurnal fat sand rats are an advantageous model for circadian effects on mood.
• In short photoperiods (SP), sand rats develop depression- and anxiety-like behaviors.
• Voluntary exercise in sand rats strengthens circadian activity rhythms.
• Voluntary exercise in sand rats ameliorates SP-induced pathological behavior.

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ABSTRACT

Physical exercise is a non-pharmaceutical treatment for affective disorders. The mechanisms of its effects are unknown although some suggest a relationship to synchronization of circadian rhythms. One way to explore mechanisms is to utilize animal models. We previously demonstrated that the diurnal fat sand rat is an advantageous model for studying the interactions between photoperiods and mood. The current study was designed to evaluate the effects of voluntary exercise on activity rhythms and anxiety and depression-like behaviors in sand rats as a step towards better understanding of the underlying mechanisms. Male sand rats were housed in short photoperiod (SP; 5 h light/19 h dark) or neutral light (NP; 12 h light/12 h dark) regimens for 3 weeks and divided into subgroups with or without running wheels. Activity was monitored for 3 additional weeks and then animals were tested in the elevated plus-maze, the forced swim test and the social interaction test. Activity rhythms were enhanced by the running wheels. As hypothesized, voluntary exercise had significant effects on SP animals’ anxiety- and depression-like behaviors but not on NP animals. Results are discussed in the context of interactions between physical exercise, circadian rhythms and mood. We suggest that the sand rat model can be used to explore the underlying mechanism of the effects of physical exercise for mood disorders.

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1. Introduction

Abnormalities of the internal clock are hypothesized to be a critical component of affective disorder pathophysiology [1–3]. Mood disorders such as major depressive disorder (MDD), bipolar disorder (BDP) and seasonal affective disorder (SAD) are strongly associated with disruption in biological rhythms including the sleep/wake cycle, social rhythms, appetite, hormone levels and body temperature [2,4,5].

In mammals, the primary endogenous clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and its neurons have an internal rhythm of about 24 h which can be synchronized by external time givers (zeitgebers) in an adaptive process called entrainment. The entrainment process leads to synchronization of the internal clock to a 24 h rhythm. The most important zeitgeber of the SCN is light, but other cues such as social interactions and physical activity are also
able to entrain the clock [6]. Other than the central clock, peripheral clocks were identified in almost all cells in the body [7,8]. These clocks are mostly dependent on the master clock of the SCN but are also dependent on other cues such as temperature, glucose levels and glucocorticoids. Under certain conditions (e.g., shift work and jet-lag) these oscillators may desynchronize from the SCN control, leading to circadian rhythms [9,10], which in animal models was shown to lead to depressive-like behavior [11,12] and may lead to depression in humans [12–16]. The complex nature of interactions between internal desynchronization and affective disorders is not fully understood. While dysregulation of clocks may have an etiological role in certain types of depression, such as bipolar disorder (BDP) or seasonal affective disorder (SAD) it may also be the consequences of affective pathology in other disorders [2].

Antidepressants and mood stabilizers are the leading treatments for affective disorders and were demonstrated to have significant effects on circadian rhythms [17,18]. Other interventions that are used in the context of affective disorders and are clearly related to circadian rhythms include sleep deprivation, light treatment and physical exercise [1,19,20]. Exercise is an emerging non-pharmacological treatment that may benefit individuals afflicted with affective disorders [21,22], but the mechanisms involved in the antidepressant effects of physical exercise are unknown [20,22]. Yet, physical exercise was found to act as a zeitgeber, strengthen the circadian system and accelerate re-entrainment in human and rodent sleep–wake cycles, supporting its application as a treatment for circadian rhythm misalignment resulting from jet-lag or shift-work [20,23–26].

One affective disorder that is strongly related to biological rhythms is seasonal affective disorder (SAD), a disorder in which patients experience depressive episodes starting in the fall or winter when days get shorter and lasting until spring, when they experience remission [27]. To the best of our knowledge, physical exercise is yet to be thoroughly investigated as a possible beneficial intervention for SAD, but at least in one study, aerobic exercise was associated with significant reductions in depression severity, which was comparable to the effects of bright light treatment [28].

One way to explore treatment effects and mechanisms of therapeutic action is to utilize appropriate animal models [29]. In that context, we previously demonstrated that the fat sand rat, a diurnal rodent, is an advantageous model animal for seasonal affective disorder despite the fact that in laboratory conditions these animals do not maintain a classical diurnal activity pattern [30]. When maintained in short photoperiod conditions (19 h dark/5 h light, SPs), sand rats develop an anxiety- and depression-like phenotype that is relieved after treatment with bright light or with antidepressant drugs [31–35]. The current study was therefore designed to evaluate the effects of voluntary exercise (wheel running) on activity rhythms and anxiety- and depression-like behaviors in sand rats acclimated to short photoperiod as a step towards later exploration of possible mechanisms.

2. Materials and methods

2.1. Animals

Sixty four male fat sand rats (Psammomys obesus; ~6 months old; Harlan, Jerusalem) were used for the experiment. Animals were individually housed in standard plastic cages (42 cm × 26 cm × 15 cm) positioned in temperature-controlled rooms (25 °C). Animals were provided with ad-lib water tap and special low-energy pellets (product 19560, Koplock, Israel). The special food is needed because sand rats develop diabetes when fed with regular rodent chow [36].

All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and the Israeli Ministry of Health guide for the care and use of laboratory animals and were approved by the Tel-Aviv University IACUC (protocol # L-12-050).

2.2. Photoperiod conditions

Sand rats were acclimated to short photoperiod (SP; 5 h light/19 h dark, N = 30) with lights on at 08:00 and off at 13:00 or to neutral photoperiod (NP; 12 h light/12 h dark) with lights on at 08:00 and off at 20:00. Light intensity was 800 lx. The photoperiod regimen was chosen based on previous results demonstrating that this SP regimen results in the development of depression- and anxiety-like behaviors compared with the neutral photoperiod regimen (12L/12D) [31–33] and based on the activity pattern of sand rats in nature, where they are active for about 5 h around midday during winter [37,38]. Animals were maintained in these photoperiod conditions for 3 weeks before the start of any manipulation or testing. This time period was found to be sufficient for physiological acclimation [39] and synchronization of circadian rhythms [40].

It is important to note that the study was run as two separate experiments: the first evaluated the effects of running wheels in animals acclimated to neutral photoperiods and the second evaluated the effects of running wheels in sand rats acclimated to short photoperiods. The separation into two experiments was done because of technical limitations.

2.3. Treatment

After 3 weeks of acclimatization, each photoperiod condition group of sand rats was further divided into two groups (N = 16/subgroup): One subgroup was transferred to new cages containing running wheels while the other group was transferred to new cages without running wheels. General activity was recorded for 3 weeks using infrared detectors (general activity detector: Intrusion detector model MH10; Crow group, Kiriat-Teufa, Israel) and wheel running activity was recorded using inductive sensors (S18-C, Aeco sensors, Italy) connected to a computer. Data were collected at 6-min intervals via designated software (ICPC, Netanya, Israel). Three weeks later, the animals underwent a series of behavioral experiments to evaluate their anxiety- and depression-like behaviors as described below.

2.4. Behavioral tests

Following 3 weeks of in-cage recording of animals’ activity and wheel running, sand rats were subjected to 3 standard behavioral tests for anxiety- and depression-like behaviors. The tests were performed in succession, one per day and included the elevated plus maze (EPM), forced swim test (FST) and social interaction (SI) test.

2.4.1. Elevated plus-maze

The EPM is frequently used to evaluate anxiety-like behavior in rodents including mice [64], voles [65], hamsters [62], rats [63] and sand rats [31,32,33,35]. The test presents the rodent with a conflict between the tendency to remain in a safe enclosed area and the need to explore new environments that could hold an adaptive value [66]. For the present study, a black aluminum EPM was used. The maze 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 was elevated 50 cm above the floor and light levels at the open arms were 200 lx. The test started at least an hour after light onset in the rooms (09:00) and animals were tested only during the next 3.5 h, well within the light hours in the colony rooms of the SP group. Sand rats were individually placed in the center of the maze, and their behavior was digitally recorded for a 5 min session. Recordings were used for later manual scoring of behaviors. At the end of the session animals were returned to their cages and the maze was wiped clean with 70% ethanol before the start of the next session. Scoring of the EPM included the time and the number of entries into each arm and was done by an investigator blind to treatment.
2.4.2. Forced swim test

The FST is a commonly used test for evaluation of depression-like behavior and assessment of antidepressant effects in rats and mice. With several methodological alterations, this test was also used in sand rats [31–34]. For the present study we followed the established sand rat protocol. Each animal was subjected to the FST apparatus twice over two consecutive days, and the second exposure was considered the test session. Testing started an hour after the onset of light and ended within the light period in the colony rooms. Each animal was placed individually into a white opaque cylinder, 30 cm in diameter and 45 cm high, filled with water (22–23 °C) to a depth of 25 cm. The test was digitally recorded from above for later manual scoring of behavior. As noted in previous work with sand rats, their ability to float in the water is lower than the ability of rats or mice and therefore, the standard measure of floating time in the FST was replaced with measures of “time to sink” [31–35]. When a sand rat is placed in the cylinder, it first attempts to actively swim and struggle, but at some point they stop and then they sink into the water. They immediately start swimming again and go above water but at some point they stop again and sink again. Uninterrupted, this cycle will continue until the animal is unable to sustain itself above water. Based on previous work, the established FST measures for the sand rats’ FST, reflecting depression-like behavior are “time to 1st sink” and “time to 2nd sink” where a sink event is defined by the animal going entirely under water for approximately 2 s. Accordingly, immediately following the second sink, the sand rat is taken out of the water by the experimenter and placed in its home cage and the test is terminated. Water is replaced in the cylinder every test. Recordings are used to time the sink events by an experimenter blind to treatments.

2.4.3. Social interaction

A social interaction test for rodents is commonly used to evaluate aggression anxiety-like and depression-like behaviors after a variety of manipulations. This test was previously utilized with different species such as rats, mice [41] and gerbils [42]. For the present study, the social interactions test was performed by placing together two animals from the same group in an unfamiliar testing arena. The main measures for the test were the number and duration of social interactions and of aggressive interactions. Because the result of a single test is codependent

Fig. 1. Representative actograms with corresponding chi-square periodograms of the same individual (produced using Clocklab software) of two individuals from each treatment. Black and white bars at the top of the actograms represent dark and light phases respectively. In the actograms, days are presented below each other.
on the behavior of two subject animals, the couple tested is referred to as one tested unit.

The testing arena was 57 cm × 36 cm with 30 cm walls. Animals were placed in the arena for a 10 min session and behavior was digitally recorded from above for later manual scoring. Social behavior was scored for the number of events and time interacting and included all events of non-aggressive touch between the animals. Aggressive behavior was defined as all events of fighting, attempts to bite or biting and pinning down. At the end of the session the animals were returned to their home cages and the arena was wiped clean with 70% ethanol before the next trial started.

2.5. Statistical analysis

Data for the continuous home cage activity and the running wheel activity were analyzed with the Clocklab software for circadian biology (Coulbourn Instruments, USA), which was used to generate actograms and for chi-square analysis.

As mentioned above, for technical reasons, the behavioral tests for the neutral and short photoperiod groups were run as separate experiments and therefore the effects of exercise wheels on behavior in each of the photoperiod regimens were analyzed separately. Data for the EPM and the social interaction test were analyzed using Student's t-test and data for the FST were analyzed using a repeated measures analysis of variance (ANOVA) with wheels as the main factor and sink event (sink 1 and sink 2) as the repeated measure factor.

3. Results

3.1. Home cage activity patterns (general activity and wheel running)

As demonstrated in Fig. 1, the average pattern of general activity and wheel running differed between the four groups: Chi square analysis (Clocklab) revealed that in the NP group with no wheel, 9/12 animals had a significant activity rhythm, while in the SP group with no wheel 7/12 animals had a significant 24 h activity rhythm. In the presence of a running wheel, all animals in both groups had significant activity rhythms both for general activity and for wheel running. Moreover, the amplitude of the general activity in the SP groups was tripled in the group which had access to the running wheel (Fig. 2). Animals acclimated to short photoperiods ran more on average than animals acclimated to neutral photoperiods (614 ± 87 and 206 ± 65 revolutions/h respectively).

3.2. Elevated plus maze

The availability of running wheels did not affect the open/closed time ratio in animals maintained in neutral photoperiods [Fig. 3A; t(22) = 0.05, p = 0.96] but significantly increased the ratio in animals maintained under short photoperiod conditions [Fig. 3B; t(28) = 3.05, p = 0.005] suggesting an anxiolytic-like effect of voluntary exercise in these sand rats.

3.3. Forced swim test

As expected, the exposure to running wheels had no effects on the behavior of sand rats in the training day (data not shown). However, in the test day, voluntary exercise had no effects on animals maintained in neutral photoperiods [Fig. 4A; ANOVA wheel effects: F(1, 21) = 1.59, p = 0.22] but significantly increased the time to sink in animals maintained under short photoperiods [Fig. 4B; ANOVA wheel effect: F(1, 28) = 7.61, p = 0.01].

3.4. Social interactions

There was no effect of wheels on the time or number of social interactions of animals maintained in neutral photoperiods [Fig. 5A; for
Wheels group.

Fig. 5. Yet, we suggest that these animals still maintain for almost all diurnal rodents when maintained in laboratory conditions a more nocturnal than diurnal activity pattern, as previously described this diurnal rodent model of depression.

Fig. 5. Anxiety-like phenotype induced by the short photoperiod regimen in that exposure to running wheels ameliorated the depression- and anxiety-like effects as detailed in the Materials and methods section. * represents significant difference from the No Wheels group.

number: t(10) = 0.44, p = 0.67; for duration: t(10) = 0.51, p = 0.62]. However, for animals maintained in the short photoperiod conditions, the presence of running wheels significantly increased the number and duration of social interactions [Fig. 5B, for number: t(12) = 2.87, p = 0.014; for duration: t(12) = 2.58, p = 0.024]. The presence of wheels did not significantly affect the number or duration of aggressive interactions in any of the photoperiod groups (data not shown).

4. Discussion

The results of the present study clearly demonstrate that 3 weeks of voluntary exercise had a major effect on the activity rhythms of sand rats maintained under short photoperiods and a much more limited effect on animals maintained in neutral photoperiods. These data suggest that exposure to running wheels ameliorated the depression- and anxiety-like phenotype induced by the short photoperiod regimen in this diurnal rodent model of depression.

It is important to note that the sand rats in the present study showed a more nocturnal than diurnal activity pattern, as previously described for almost all diurnal rodents when maintained in laboratory conditions (e.g., [30]). Yet, we suggest that these animals still maintain “diurnal traits”. The switch from the ancestral nocturnal activity to a diurnal activity pattern requires a set of anatomical, physiological and behavioral adaptations which occur over the course of evolution (reviewed by [43, 44]). For example, the fat sand rat has a remarkably cone-rich retina (41% of total photoreceptor numbers in both central retina and peripheral retina) which adapted to day light vision [45]. Whereas the differences between the circadian systems of diurnal and nocturnal mammals are still not fully elucidated, it is reasonable to assume that the circadian system of diurnal species is more homologous to the human system than that of nocturnal species, and therefore diurnal models may serve as better models for circadian system related diseases.

Activity patterns (including both general activity and wheel running) of sand rats were more robust and less fragmented when they had free access to a running wheel, and the effect was more pronounced in animals acclimated to short photoperiod, which had weaker activity rhythms without a running wheel. This effect was demonstrated despite the fact that as previously shown, sand rats lose their classical diurnal activity pattern when maintained in laboratory conditions [30]. The animals in these conditions do not become nocturnal but adopt a unique pattern that its biological underpinnings are still unclear. Yet, in the context of our model, the effects of photoperiod manipulations in the sand rat and their relevance to affective disorders had been repeatedly demonstrated [31–35]. Studies in mice show that regularly scheduled wheel running can synchronize the circadian clock [46], and free access to a running wheel is able to alleviate most changes in the circadian system associated with aging; aged mice housed with a running wheel had stronger circadian rhythms in locomotor activity, faster recovery of internal synchrony following light/dark shift, and increased amplitude of SCN firing rates compared to aged mice housed without a running wheel [26]. A decreased amplitude in different physiological and behavioral rhythms was also described in depressed patients (e.g., [47]), including a decrease in amplitude of gene expression in different brain areas [48].

Voluntary exercise in running wheels is known to have significant effects on a variety of measures including enhancement of locomotor activity in rodents [49] and influencing the phase of circadian rhythms via the intergeniculate leaflet and its connections with the SCN [50]. In the current study, voluntary exercise also ameliorated the depression- and anxiety-like phenotype induced by short photoperiod in the fat sand rats, as evident from the increased open/closed number of entries ratio in the elevated plus maze, longer time to sink in the forced swim test, and more social interactions as measured by the number of events or by the total interaction time compared with animals maintained without wheels. Whereas it is possible to suggest that at least in part, the positive effects of running wheels in the FST could be the consequences of better physical fitness, this change cannot explain the entire profile of effects. Moreover, similar effects of exercise on depression- and anxiety-like behaviors were previously described in mice, as well as humans [51–54].

Relatively little is known about the underlying mechanisms that mediate the effects of non-photic stimuli on the circadian system, but it was shown that two afferent pathways, the geniculohypothalamic tract, fibers emanating from the intergeniculate leaflet (IGL) containing neuropeptide Y (NPY) and gamma-aminobutyric acid (GABA), and the
serotonergic input from the midbrain raphe nuclei are necessary for the entraining effect of exercise on circadian rhythms [55,56]. The underlying mechanisms of the therapeutic effects of exercise are also unclear, although some theories had been raised including its effect on the circadian system [20,23], energy regulation [57], and brain 5-HT levels [54], and effects on the activation of neuroplasticity signaling factors such as BDNF and VGF [58–60], which may be mediated by its effect on mitochondrial activity and gene expression [54]. Direct exploration of these mechanisms can be significantly supported by good animal models and the current study was designed to evaluate the utility of the sand rat model of SAD to investigate the effects of exercise. The effects of exercise in SAD were evaluated only in a few studies, showing that in humans exercise may have beneficial effects [28,61]. However, to the best of our knowledge the effects in an animal model for SAD were never tested.

In conclusion, our results clearly show that voluntary exercise ameliorates pathological-like activity patterns as well as depression- and anxiety-like behaviors in the model supporting the important role of physical activity in mood modulation. Additionally, our results further validate the fat sand rat as a preferable animal model for investigating the underlying pathophysiology of the interactions between circadian rhythms and mood and for exploring potential new treatments for affective disorders.

References


