Circadian rhythms and depression: effects of exercise in an animal model

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Solberg, Leah C., Teresa H. Horton, and Fred W. Turek. Circadian rhythms and depression: effects of exercise in an animal model. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R152–R161, 1999.—There is a clear link between altered circadian rhythms and depressive disorders, although the nature of this relationship is unknown. In addition, exercise affects both mood and alters clock function. To investigate the relationship between circadian rhythms, depression, and exercise, 3-wk-old mice housed on a 12:12-h light-dark cycle were exposed to chronic stress (CS) for 6 wk before being placed into constant darkness (DD). One-half of both the control and stressed mice were given access to a running wheel. Stressed mice consumed significantly less of a 2% sucrose solution during CS and exhibited a significant increase in immobility in the forced swim test 3 wk after the termination of stress relative to control mice. These effects were more pronounced in mice without running wheels. Stressed mice also exhibited altered percent distribution of total activity and increased fragmentation of daily activity rhythms during CS relative to control mice. Alterations in percent distribution were more pronounced in animals without running wheels. No activity rhythm changes were seen in DD, and there were no differences in light-induced phase shifts between stressed and control mice. These results suggest that CS causes long-term depressive-like symptoms but does not have long-lasting effects on activity rhythms. These changes were more pronounced in mice without running wheels, suggesting that exercise may protect against the harmful effects of stress.

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In our studies, we adapted the CMS protocol for mice. We exposed C57Bl/6J mice to 6 wk of chronic stress while continually monitoring their daily rhythm of locomotor activity. To study the role of activity/exercise on the effects of chronic stress, one-half of the mice were placed in cages with running wheels and one-half of the mice were in cages without wheels.

This study had three main objectives: 1) to determine if C57Bl/6J mice would develop depressive-like symptoms after exposure to chronic stress, similar to those found in the rat, 2) to investigate long-term changes in the daily pattern of activity associated with chronic stress, both in DD and in response to a light pulse, and 3) to investigate protective properties that exercise may have against the effects of stress in this model of depression.

MATERIALS AND METHODS

Subjects. Twenty-four C57Bl/6J mice, weighing 7–11 g, were purchased from Jackson Laboratories (Bar Harbor, ME) at 3 wk of age. Mice were kept under constant ambient temperature (21 ± 1°C) with food and water available ad libitum, unless otherwise specified. Animals were placed on a 12:12-h LD cycle (lights on at 0100, off at 1300, Central Standard Time) during the stress procedure (6 wk). After this time, to test the effects of chronic stress on free-running rhythms, all animals were transferred to DD.

Experimental protocol. Immediately on arrival mice were weighed and placed into individual cages in light-tight boxes (6 cages per box). To introduce the mice to a sucrose solution and to obtain baseline data on sucrose consumption, mice were given bottles of both water and 2% sucrose. Twenty-four hours later, bottles were removed and weighed to measure liquid intake. The water bottles were then replaced. Sucrose intake was measured again for a 1-h period, 6 h after lights off during the second 24-h period. On the basis of body weight and sucrose intake (during both the 24- and 1-h periods), mice were assigned to experimental or control groups (n = 12 in each group). Body weight, in addition, was used to separate animals in an effort to minimize future changes in sucrose intake caused by differences in body size. One-half of the animals in each group were placed in cages with running wheels, and one-half were placed in cages without wheels. Control animals were placed into separate light-tight boxes from experimental animals. Experimental animals were exposed to 6 wk of chronic stress, whereas control animals were left undisturbed during this 6-wk period, except for scheduled cage cleaning, feeding, and weighing.

The stress procedure began 4 days after arrival (at 25 days of age) and persisted for 6 wk. During this time, control and experimental animals were weighed weekly. A 1-h sucrose test was given to all animals twice a week (see below). At the end of the 6 wk, 2 days before being placed into DD, animals were given an open-field test (OFT). This procedure was performed within 2 h of the time of lights on. One final stress (paired housing) was given the following day, 24 h before release into DD. Animals were kept in DD for the remainder of the experiment. By placing the animals into DD, we were able to analyze their free-running rhythms, an indicator of the status of the internal clock. To test for possible long-term effects of the stress procedure on photic resetting of the circadian clock, each mouse was given a 15-min light pulse (300 lx of white light) 3.5 h after the onset of activity, 2 wk after being placed into DD. Previous work with nonstressed C57Bl/6J mice demonstrated that light pulses delivered at this time produce phase delays of ~40 min (33). That same day, mice were given a 1-h sucrose test 6 h after the onset of activity. One week later, a forced-swim test (FST) was administered 2.5 h after activity onset, again followed by a 1-h sucrose test. The FST was administered to assess long-term behavioral changes of the chronic stress protocol. Time after the onset of activity is used as a marker of the phase of each animal’s internal timing system (20). For example, for a nocturnal animal in DD, the onset of activity coincides with what would be the time of lights off if on an LD cycle. See Fig. 1 for a timeline of the experiment.

Chronic stress procedure. The chronic stress procedure was adopted from Willner et al. (45) and consisted of the following stressors: 1) 16-h water deprivation (water bottles were removed from cage during this time), 2) 5-min tail suspension (animals were held upside down by their tail with metal tongs; mice were shaken periodically during these 5 min), 3) 1- to 2-h restraint (animals were placed in a 50-ml conical tube with breathing holes), 4) 30–45 min paired housing (animals were placed in the cage of another mouse also in the stress group; each week the home cage mouse alternated), 5) soiled cage (100 ml 16–18°C water was poured into cage), and 6) 5 min forced swim in cool water (16–18°C). Each week, the stressors were presented in a different order and given at different times of the day (both during the dark and light periods). When the stress was given during the dark period, animals were brought out into the light during the time of the stress. This resulted in light pulses ranging from 2 to 60 min occurring two or three times a week. Although this may have affected the rhythms of the animals, the benefit of being able to observe the animals during the stress outweighed the disadvantages of affecting the animals’ rhythms with the light.

Sucrose test. Preliminary data have shown that C57Bl/6J mice prefer a 2% sucrose solution over regular, unsweetened water (L. Solberg, unpublished). Two times each week, 6 h after lights out, animals were given bottles of both water and 2% sucrose for a 1-h period. The test was administered 6 h after lights out, because preliminary studies demonstrated that mice consume more water during their active period (L. Solberg, unpublished), thereby enhancing our chance of seeing a difference in sucrose consumption. After 1 h, both bottles were removed and again weighed. Bottles were administered and removed in the dark using an infrared (IR) viewer (Find-R-Scope, Optical Systems). Both total sucrose consumption and percent preference for sucrose over water are reported. Percent preference was calculated according to the

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<th>Time Line of Experiment</th>
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<td>CHRONIC STRESS / 12:12 LD</td>
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<td>Daily Activity</td>
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Fig. 1. Time line of experiment. Animals were exposed to chronic stress for 6 wk while on a 12:12-h light-dark (LD) cycle. Stress was terminated and animals were placed into constant darkness (DD) for 3 wk. An open-field test (OFT) was given to both experimental and control animals after the stress and before being placed into DD. A 15-min light pulse was administered to all groups after 2 wk in DD and a forced-swim test (FST) was given after the full 3 wk in DD. Throughout the experiment, sucrose tests were administered to all groups twice a week and activity rhythms were monitored daily.
following formula: percent preference = \[\frac{\text{[sucrose intake/sucrose + water intake]}}{100}\].

OFT. Each mouse was placed in the open field (a wooden box 22 x 26 in. with its floor divided into nine equal squares by red adhesive tape) for a 5-min period. The experimenter left the room during the testing, and activity of the mice was videotaped. Videotapes were watched the following day by two observers blind to the experimental condition of the mice. Total number of line crosses was scored.

FST. This test is used frequently to test behavioral despair in rodents (27). The test was administered under IR light and videotaped using an IR camera (RCA, Lancaster, PA). Mice were removed from their cages and placed into a 1.5-liter beaker filled with 18°C water for a 5-min period. Activity of the swimming mice was scored the following day by two blind observers. The behavior of the mice was placed into one of the following two categories: 1) immobile, defined as making only the necessary movements to stay afloat, or 2) active swimming, defined as active struggling in an effort to escape the water. The total amount of time the mice exhibited in each category of behavior was recorded. In the FST, the largest effects are often seen during the first minute (L. Solberg, unpublished). Therefore, we analyzed results of the test during the first minute as well as for the full 5 min of the test.

Activity data acquisition and analysis. Microswitches activated by a revolving axle were used to monitor daily activity of animals with running wheels. IR sensors (Petite 101, Newark, NJ) were used to monitor daily activity of animals without running wheels. Both wheel revolutions and number of bar crossings of the IR sensors were continuously recorded with an online data acquisition system (Chronobiology Kit, Stanford Software Systems).

Total daily activity (defined as the total number of wheel revolutions or IR bar crossings per day), along with percentage of activity in both the light and dark periods (while on an LD cycle), was analyzed using the Activity Count Program of the Chronobiology Kit. Analyses of the activity count data were done during two time periods: 1) during stress, a 3-wk period beginning 2 wk after the start of the stress procedure while mice were on a 12:12-h LD cycle, and 2) after stress, a 10-day period while mice were in DD, beginning immediately after termination of the stress.

The number of bouts of activity, along with the duration of the first daily bout (in minutes), and the amount of activity (in wheel revolutions or bar crossings) during this first daily bout were analyzed using a bout analysis computer program, PATTERN (25). Analysis of the first daily bout is an important parameter because this is when C57B1/6 mice show the largest amount of consolidated activity and therefore where we would expect to see the greatest difference in chronically stressed animals (33). A bout of activity was defined as a minimum of 5 min of continuous wheel running or bar crossing activity. The interbout interval was set at 20 min, such that the number of bouts defined by the computer program were similar to those identified by eye. The first daily bout encompassed, or occurred immediately after, lights out. Analyses of the bout analysis data were done during two time periods: 1) during stress, a 10-day analysis in LD starting 2 wk after stress began, and 2) after stress, a 10-day analysis while in DD, beginning immediately after termination of the stress. A 10-day period was chosen because the PATTERN program is able to effectively identify significant differences in this timeframe (25). In addition, 7–14 days is a common time frame for analysis of circadian rhythm parameters (e.g., Ref. 24). Data for each animal were averaged over the specified time periods, and the average for each animal was then used in the appropriate statistical analysis (see below).

RESULTS

Body weight. When a repeated-measures two-way ANOVA was used, we found a significant stress \times wheel interaction \([F_{1,19} = 4.97, P < 0.05]\). During the course of the study, the stress, no-wheel group gained less weight than the no-stress, no-wheel controls, whereas the stress, wheel group gained more weight than the no-stress, wheel controls (see Fig. 2). There was no main effect of stress or wheel on body weight. The significant interaction indicates that the effect of stress is dependent on the presence or absence of a running wheel.

Sucrose consumption. During the chronic stress, stressed animals drank significantly less sucrose than nonstressed controls, with a more pronounced effect seen in animals without running wheels (see Fig. 3). Stress significantly reduced both sucrose consumption \([F_{1,19} = 22.28, P < 0.001]\) and percent preference \([F_{1,19} = 12.11, P < 0.01]\). Post hoc analysis revealed that the significant effect of the stress resulted from the suppression of sucrose consumption in mice without running wheels (Newman-Keuls, \(P < 0.05\)). This is most likely due to the fact that sucrose consumption for the no-wheel group had significantly declined by 3 wk after stress began, whereas a decrease was not seen in the wheel group until 5 wk after stress began. There was no significant stress \times wheel interaction.

OFT. Performance on the OFT was not significantly affected by the stress or wheel conditions.

FST. Performance on the FST was significantly affected by stress and the presence of a running wheel. As expected, the largest difference was seen during the first minute of the test. During the first minute of the test, stressed animals were more immobile than non-
stressed controls, with a more pronounced effect seen in mice without running wheels (see Fig. 4A). For the first minute, there was a significant effect of stress ($F_{1,19} = 21.04, P < 0.001$) and of the wheel ($F_{1,19} = 13.41, P < 0.01$). The effect of stress was statistically significant in the no-wheel group only (Newman-Keuls, $P < 0.01$). During the full 5 min of the test, stressed animals were significantly more immobile than nonstressed animals regardless of the presence or absence of a running wheel (see Fig. 4B). There was a significant effect of the stress ($F_{1,19} = 13.24, P < 0.01$) and the presence of a wheel ($F_{1,19} = 33.87, P < 0.001$). The stress effect was seen in both the wheel and no-wheel conditions (Newman-Keuls, $P < 0.05$). There was no stress $\times$ wheel interaction for either the first minute or the full 5 min of the FST.

Daily activity rhythm analysis. Distribution of activity in the dark and light periods was significantly affected by stress and the presence of a running wheel. Stressed animals showed an increase in the percentage of total activity spent during the light period, with a decrease in the percentage of total activity during the dark period. This tendency was much more pronounced in animals without running wheels (see Fig. 5A). Significant main effects of stress ($F_{1,19} = 22.50, P < 0.001$), wheel ($F_{1,19} = 50.30, P < 0.001$), and a stress $\times$ wheel interaction ($F_{1,19} = 8.31, P < 0.01$) were seen for the percentage of time spent active during both the light and dark periods. This effect was only found in the no-wheel group (Newman-Keuls, $P < 0.05$). No significant differences were seen in activity after termination of stress when the animals were in DD ($P > 0.1$).

To compare total activity between both wheel and no-wheel groups, data for total daily activity in each condition were normalized (see MATERIALS AND METHODS). Stressed animals exhibited significantly less daily activity than nonstressed controls, with a more pronounced effect seen in animals with access to a running wheel (see Fig. 5B). A significant main effect of both stress ($F_{1,19} = 33.89, P < 0.001$) and wheel ($F_{1,19} = 19.11, P < 0.001$), along with a stress $\times$ wheel interaction ($F_{1,19} = 19.11, P < 0.001$) was found for the normalized total activity during the stress. The effect of stress was only significant in the wheel group (Newman-Keuls, $P < 0.05$). No significant effect was seen after termination of the stress under DD conditions.

Daily bout analysis. The actograms in Fig. 6 illustrate that during the stress procedure the activity patterns of the stressed mice are less precise and more fragmented than those of the control mice. On more objective analysis, stressed mice exhibited an increase in the number of daily bouts, a decrease in the duration of the first daily bout, and a decrease in the number of wheel rotations or bar crossings during the first daily bout compared with the nonstressed control mice (see Fig. 7). A significant main effect of stress ($F_{1,19} = 20.35, P < 0.001$) was found for the number of bouts during the time of stress (see Fig. 7A). This effect was seen in both the wheel and no-wheel groups (Newman-Keuls, $P < 0.05$). There was also both a significant main effect of stress ($F_{1,19} = 22.0, P < 0.001$) and wheel ($F_{1,19} = 5.28, P < 0.05$) for the duration of the first daily bout (see Fig. 7B). Again, the difference of the stress was seen for both the wheel and no-wheel groups (Newman-Keuls, $P < 0.05$). There was no stress $\times$ wheel interaction for either the number of bouts or the duration of the first daily bout. Because of inherent differences in data acquisition between the wheel and no-wheel groups, the number of wheel rotations or bar crossings during the first daily bout were normalized (see MATERIALS AND METHODS). A significant main effect of stress ($F_{1,19} = 11.81, P < 0.01$) was found (see Fig. 7C). The stress effect was only seen in the no-wheel group (Newman-Keuls, $P < 0.05$). No significant changes were seen in any of the above parameters after termination of the stress.

Phase shifting. There were no significant differences seen in the phase-shifting properties of the clock to a light pulse between the stress and control groups (see Fig. 6). There was also no effect of the presence of a wheel on the response to a phase-shifting light pulse.

DISCUSSION

Chronic stress caused depressive-like symptoms in C57Bl/6J mice. Many of these effects were greatly diminished in animals given access to a running wheel, suggesting that exercise alleviates the effects of chronic stress in mice.

During chronic stress, stressed mice consumed significantly less of a 2% sucrose solution than control mice. Three weeks after termination of stress, during the FST,
there was also a significant increase in immobility in the stressed mice compared with controls, suggesting a higher level of despair in these animals. Stressed mice also exhibited disruptions in their daily activity rhythms during the chronic stress period (altered percent distribution of total activity, increased fragmentation). These results demonstrate that chronic stress in C57Bl/6J mice causes behavioral changes similar to those described in the CMS model of depression in rats (44).

We used C57Bl/6J mice in this study because of the wealth of genetic information that is available on the mouse. Future studies using the chronic stress model in the mouse may enable investigators to determine how specific genes and mutant alleles influence the incidence and severity of depressive-like symptoms in this model. For example, the Clock mutation, which has recently been isolated in C57Bl/6J mice (41), is necessary for normal functioning of the circadian pacemaker. When the Clock gene is mutated, heterozygote mice exhibit a lengthened period and homozygotes become arrhythmic after being placed into DD. It is not known if these mice are more prone to developing depressive-like behavioral symptoms after chronic stress. Use of these mice may be especially useful in future studies examining the relationship between circadian rhythm disturbances and depression.

The depressive-like symptoms that we observed in this study were much more pronounced in mice without access to a running wheel. The decrease in sucrose consumption was seen in the stressed mice without running wheels 1 wk sooner than in stressed mice that had access to running wheels. Chronic stress also had a

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**Fig. 3.** A: sucrose intake during the course of the study. B: percent preference for sucrose during the course of the study (see MATERIALS AND METHODS for calculation). *There is a decrease in sucrose consumption and percent preference for sucrose significantly sooner in mice without running wheels (P < 0.05). Arrows indicate start and end of stress procedure. Last time point was taken after the FST.
much greater effect on immobility in the first minute of the FST for animals without wheels. An increase in immobility was also seen in control (nontreated) mice without wheels relative to control mice with and without access to a running wheel (P < 0.05).

Changes in the cardiovascular and neuroendocrine systems have been purported to be involved in the beneficial effects of exercise against the harmful effects of stress (for reviews, see Refs. 14 and 34). The exact nature of these changes, however, is not clear. A decreased cardiovascular response to stress has been seen in both exercise-trained humans and rats (13, 14). However, whereas some human studies have demonstrated a lower rise in cortisol to a psychological stress, others have found no change in the activity of the hypothalamic–pituitary–adrenal axis.
In addition, exercise training in rats results in a decreased ACTH response to a running stress, whereas the ACTH response is increased after both immobilization and foot shock stressors (42, 43). Human studies have also shown that exercise-trained individuals exhibit decreased levels of norepinephrine after stress relative to sedentary controls (34). In contrast, Dishman et al. (7) have shown that rats with access to a running wheel exhibit increased levels of norepinephrine in both the locus ceruleus and the dorsal raphe relative to control animals without a running wheel. Although the neuroendocrine mechanisms of exercise training remain controversial, several studies converge to show that exercise training does lead to a decrease in anxiety in exercise-trained animals. For example, access to a running wheel in rats leads to decreased latency after a foot shock and decreased anxiety in the OFT (6, 7). Four weeks of swimming exercise in rats also leads to decreased anxiety in the OFT as well as an enhancement of postsynaptic 5-hydroxytryptamine (5-HT)\textsubscript{2} receptor responsiveness to quipazine along with a decrease in responsiveness of 5-HT\textsubscript{3A} receptors to 8-hydroxy-2-(di-n-propylamino)tetrahydrolinal (5).

Some hypothesize that the above mentioned behavioral changes may be a result of enhanced central serotonin synthesis and turnover in exercise-trained animals (for a review, see Ref. 2). Serotonin is one of the main neurotransmitters that is often altered in depression and provides an important link back to the circadian clock located in the SCN (22, 28). In addition, serotonergic pathways, which provide both direct and indirect inputs into the clock, are believed to play a major role on the feedback effects of activity on the central circadian clock (10, 17). Additionally, light can alter the response of the circadian system to a serotonin agonist (26), and serotonin can alter the response of the circadian clock to light (30). Specifically, a serotonin agonist has been shown to potentiate the phase-shifting response to light in the hamster (29).
Thus one possible route by which exercise has the ability to improve mood may involve the serotonergic pathways to the circadian clock system.

In the present study, no differences were seen in the phase-shifting properties of light between the wheel and no-wheel groups. However, mice with access to running wheels did exhibit less depressive-like behavior in the FST relative to animals without running wheels, irrespective of exposure to stress. Whether there were changes in the serotonergic system in mice with access to running wheels is not known. Our findings suggest that the serotonergic system to the clock may not be altered in mice given access to running wheels. Alternatively, the presumed enhanced serotonergic activity from wheel running may not play a major role in altering the phase-shifting properties of light in mice.

In the present study, several circadian rhythm parameters were altered in mice exposed to chronic stress during the chronic stress paradigm. However, there were no long-term changes in activity rhythms after stress termination. During chronic stress, stressed animals exhibited a decreased percentage of total activity during the dark period (when mice are normally active) and an increased percentage of total activity during the light period (when mice are normally inactive). In addition, stressed mice showed decreased total activity and increased fragmentation of daily activity rhythms during the chronic stress procedure. No changes were seen in circadian phase during the chronic stress procedure. In addition, the above mentioned changes did not persist after termination of the stress. It is therefore possible that the changes in daily activity rhythms seen during the chronic stress procedure may have been a result of the chronic stress per se, rather than an effect on the master circadian clock. In support of this hypothesis, there were also no differences in the phase-shifting properties of the SCN to light between the stressed and control mice after termination of the stress.

Other studies investigating the effects of stress or of stress-induced depression on circadian rhythm parameters in rodents have led to conflicting results. Social defeat and inescapable shock in rats, two stress paradigms that lead to several depressive-like behaviors, result in a decreased amplitude in the daily rhythms of temperature, heart rate, and activity (12, 18, 36). However, changes in free-running period or phase-shifting responses to light are rarely seen after stress in animal models. Although uncontrollable shock results in a lengthening of the free-running period (35), chronic immobilization, novelty, and handling stressors have little effect on the free-running period or phase angle of entrainment in rats (1). Meerlo et al. (19) also showed that a defeat stress in rats does not affect the free-running period nor the phase-shifting properties of the clock to light. These studies, in accordance with ours, suggest that the functioning of the internal pacemaker, or SCN, is not affected by stress. Whereas the chronic stress procedure used in the present study effectively induced depressive-like symptoms, it did not produce...
long-lasting effects on circadian rhythms. These results allude to the idea that circadian rhythm disorders may not be directly caused by stress or depression, but instead may be due to a third factor (e.g., a neurochemical or hormonal imbalance) that results in both the depression and the rhythm disruptions. However, to more clearly understand the relationship between depression and circadian rhythm disruptions, it will be important to use an animal model in which long-lasting alterations in both behavior and activity rhythms are found.

In conclusion, it appears that there is an intricate and complex network linking circadian rhythms, depression, and exercise. Stress hormones (glucocorticoids) and the monoamines (serotonin and norepinephrine), most likely play a large role in this network. Alterations in these important neural and humoral messengers by stress have the ability to affect both mood and clock function. Exercise has also been shown to alter these messengers, such that the damaging effects of stress are reduced. In the present study, no long-term changes in daily activity rhythms were seen, suggesting that stress does not directly affect the master pacemaker (SCN). However, chronic stress in mice did lead to depressive-like behavioral changes, which were reduced by access to a running wheel. Evidence is thus provided that exercise does protect against the damaging effects of chronic stress. This model could therefore be used to further investigate the mechanisms involved in the protective properties of exercise against stress.

Perspectives

We have demonstrated that access to a running wheel in mice alleviates the deleterious effects of chronic stress. This supports human studies showing that exercise has both antidepressant properties and can protect against the harmful effects of stress. Although this has been demonstrated in humans, the physiological mechanisms underlying the positive effects of exercise against stress are not known. As such, the present model (chronic stress in mice) could be used to dissect such mechanisms. This knowledge may help to design both pharmacological and nonpharmacological treatments for patients with hyperreactivity to stress and/or depressive disorders.

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