Cover Page

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8

THE AMPLITUDE OF THE SCN ELECTRICAL ACTIVITY RHYTHM IS ENHANCED BY EXERCISE

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ABSTRACT

Circadian rhythms are important for the daily timing of physiology and behavior. A growing body of evidence suggests that exercise improves circadian rhythmicity; however, the underlying mechanism remains unknown. Here, we investigated the effect of exercise on the amplitude of the SCN's daily rhythm in electrical activity. The SCN's rhythm was measured by performing *in vivo* electrophysiological recordings of SCN neurons in freely moving mice. Voluntary exercise was then encouraged by placing a running wheel in the animal's cage. We found that voluntary exercise induced a significant increase in the amplitude of electrical activity in the SCN (from 168 Hz to 192 Hz; $P<0.05$). These data indicate that the beneficial effects of exercise result from an increase in the amplitude of the SCN's electrical activity rhythm. Therefore, because electrical activity is the primary output of the SCN, exercise can strengthen the role of the SCN as a circadian pacemaker.

INTRODUCTION

Robust rhythmic changes in the environment have existed since the start of lift on Earth, and these rhythms have led to a 24-hour rhythm in the behavior and physiology of many organisms. In mammals, these rhythms are driven by an endogenous clock located in the suprachiasmatic nucleus (SCN) at the base of the hypothalamus. The SCN contains approximately 10,000 neurons on each side, which form a bilaterally coupled neuronal network [1]. Individual neurons within the SCN generate circadian rhythms in molecular and cellular events and in their electrical firing frequency.

Light is the principal external signal that synchronizes the SCN with the external day/night cycle. In the retina, incoming light is detected by three classes of photoreceptors: rods, cones, and intrinsically photosensitive retinal ganglion cells (pRGCs), which contain the photopigment melanopsin. Upon activation, these photoreceptors transmit light information via direct projections from the pRGCs to the SCN [2]. Although light is the most important cue for synchronizing the SCN with the environment, it has become evident that the SCN is also sensitive to other factors, including behavioral activity [3]. For example, behavioral activity can induce changes in the period of the rhythm, and behavioral activity can inhibit light-induced phase shifts in the circadian rhythm [4-7]. Moreover, scheduled exercise restores rhythmicity in mice lacking the neurotransmitter vasoactive intestinal peptide (VIP) or its receptor, VPAC2 [8, 9]. In humans, increased physical activity accelerates re-entrainment to a new light-dark cycle [10, 11]. Additionally, an increase in behavioral activity can improve the rest-activity rhythm of elderly persons and patients with Huntington's disease or dementia [12-14]. In aged mice, voluntary exercise improves the rhythmicity of electrical activity in the SCN [15]. Despite these compelling results, the mechanism that underlies the beneficial effect of exercise on the circadian system has not been elucidated fully. Identifying this mechanism may serve as a basis for developing therapeutic strategies designed to strengthen the circadian system and/or restore disease-associated deficits in the circadian system.

In vivo electrophysiological recordings of SCN neurons revealed high levels of electrical activity during the day and low levels of electrical activity during the night. Thus, the SCN's level of electrical activity is in anti-phase with the animal's behavior (i.e., the animal's level of physical activity). The SCN's level of electrical activity is acutely increased when the animal is exposed to external light [16]. In contrast, electrical activity is acutely suppressed when the animal is spontaneously active [17-19]. The magnitude of this suppression is correlated with the intensity of behavioral activity; thus, a large suppression occurs when the mouse's behavioral activity is intense, whereas relatively smaller suppression occurs when the animal's activity level is less intense (for example, when eating or grooming) [18]. The behavior-induced suppression in the SCN's electrical activity is not measured in

all SCN neurons [17, 18], suggesting the presence of regional specificity in the organization of input pathways. Because nocturnal animals are behaviorally active predominantly during the night (i.e., during the trough of the SCN's rhythm), we hypothesized that the amplitude of the SCN's rhythm will increase if the animal increases its activity level during the night [18]. To test this hypothesis, we measured SCN electrical activity rhythms in the absence and presence of a running wheel in the cage; in both conditions, we measured >3 consecutive cycles. In support of our hypothesis, we found that the amplitude of SCN activity increased significantly when the animal's activity level was increased.

METHODS

Animals

Adult (3-6 months old) male CB57BL/6 wild-type mice (Harlan, Horst, the Netherlands) were housed in a 12:12-hour light:dark cycle. The mice were housed individually in transparent plastic cages with access to standard food and water *ad libitum*. All animal experiments were approved by the Animal Experiment Ethics Committee of Leiden University Medical Center.

Electrode implantation

The mice were anesthetized using a combination of ketamine (100 mg/kg body weight), atropine (1 mg/kg b.w.), and xylazine (20 mg/kg b.w.). Under deep anesthesia, a stainless steel tripolar electrode was inserted into the SCN using a stereotactic frame with a digital readout (Stoelting Co., Wood Dale, IL). Two twisted polyimide insulated electrodes for differential recording were implanted in the SCN, and a third, uncoated electrode was implanted in the cerebral cortex as a ground. The electrodes were implanted at a 5-degree angle relative to the vertical axis, at the following coordinates: 0.61 mm lateral to Bregma and at a depth of 5.38 mm below the dura. Three additional screws were placed in the skull and fixed to the electrodes using dental cement to keep the electrodes in a fixed position.

In vivo electrophysiological recordings of the SCN

After a minimum of one week following the implantation surgery, the mice were connected to a custom-built recording setup in order to measure the extracellular multi-unit activity (MUA) of SCN neurons. In the recording setup, a counterbalanced swivel system was used to give the animal a full range of unrestricted movement. The electrical signal was amplified and bandwidth filtered at 0.5-5 kHz. Window discriminators were used to convert the action potentials to digital pulses, which were stored as 10-second epochs for off-line analysis (using CircaV1.9 custom-made software). Passive infrared (PIR) motion detectors were used to simultaneously measure the animal's level of behavioral activity.

Analysis of in vivo electrophysiology data

The *in vivo* electrophysiology data were smoothed using a penalized least-squares algorithm (Eilers, 2003). The peaks and troughs were determined by calculating the maximum and minimum values, respectively, of the smoothed data. These values were then used to calculate the amplitude of the waveform (i.e., the absolute difference between the peak and trough values, in Hz).

Wheel-running activity

After recording one week of baseline extracellular neuronal activity in the SCN, we placed a running wheel in the recording setup in order to promote voluntary behavioral activity. The running wheel was placed at a near-horizontal angle (i.e., it functioned as a tilted disk) in order to avoid interference with the recording cable from above. The animal's behavior was monitored via the video recording to monitor when the animal ran in the wheel. Activity was measured as passive infrared detections per 10-second epoch.

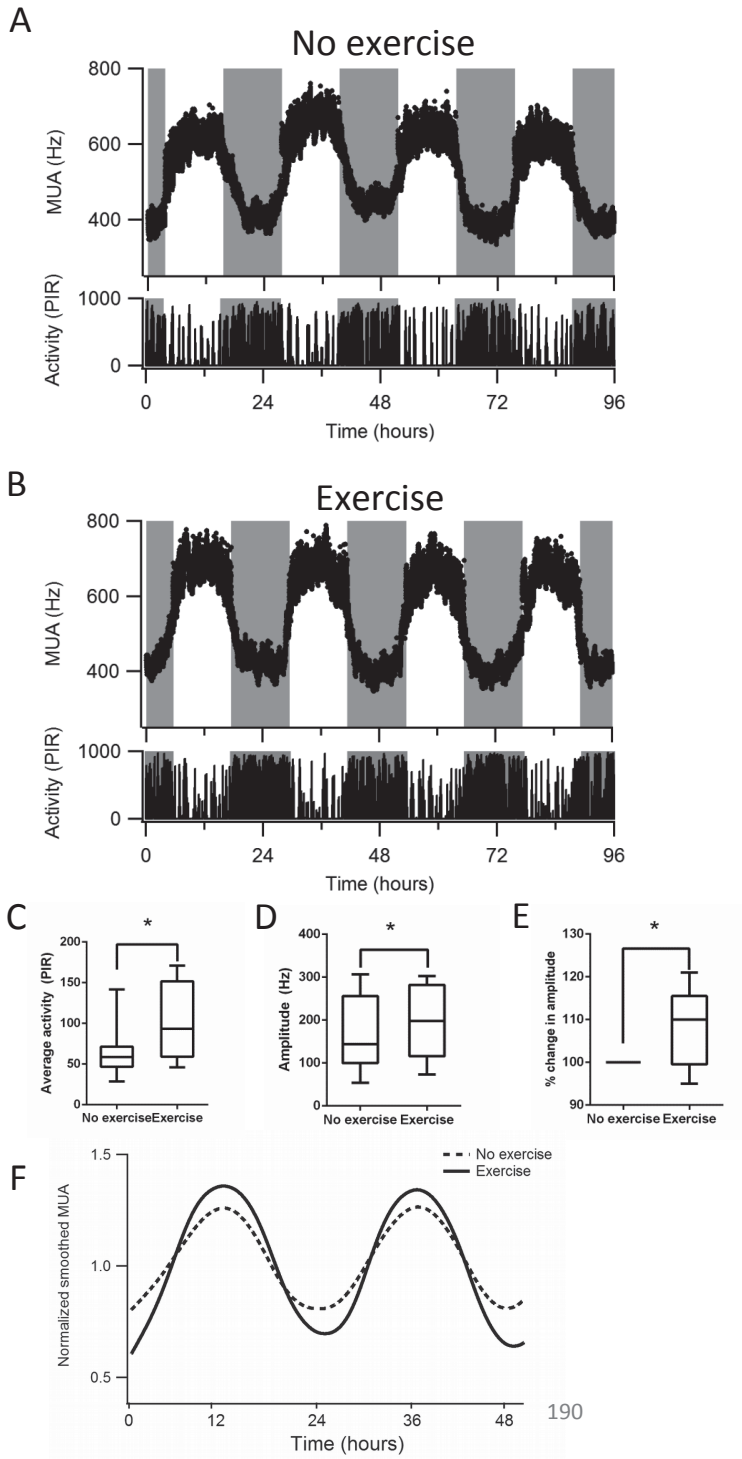
Statistical analysis

The data were analyzed using GraphPad Prism software (GraphPad, La Jolla, CA). The paired Student's *t*-test was used to analyze the differences between the two recording conditions (i.e., wheel vs. no wheel). *P*-values are reported for each statistical test performed, and differences with a *P*-value <0.05 were considered to be significant.

RESULTS

We successfully recorded the extracellular electrical activity in the SCN from ten mice housed under a 12:12-hour light:dark cycle. As expected, these recordings revealed high electrical activity during the day (i.e., activity peaked during the light phase) and low electrical activity during the night (Fig. 1A, upper plot). A completely opposite pattern was observed with respect to behavioral activity, with a low activity level during the day and high activity during the night (Fig. 1A, lower plot). Thus, low and high electrical activity levels in the SCN correspond to high and low behavioral activity, respectively, on a circadian time scale.

After one week of baseline recording, a running wheel was placed in the recording setup to increase the animal's behavioral activity level (Fig 1B). Behavioral activity increased significantly when the mice had access to a running wheel (from 64.4±10.0 PIR/10 sec to 99.1±14.6 PIR/10 sec; *P*=0.006, paired Student's *t*-test, n=10 mice; Fig 1C). The *in vivo* electrophysiology data were smoothed, and the peak, trough, and slope at half-maximum value were used to calculate the amplitude and peak width of the waveform of the SCN rhythm. We calculated the amplitude of the waveform of the SCN rhythm before and after a running wheel was placed in the


A
No exercise
800
600
400
MUA (Hz)
1000
0
Activity (PIR)
0
24
48
72
96
Time (hours)
B
Exercise
800
600
400
MUA (Hz)
1000
0
Activity (PIR)
0
24
48
72
96
Time (hours)
C
*
200
150
100
50
0
Average activity (PIR)
No exercise
Exercise
D
*
400
300
200
100
0
Amplitude (Hz)
No exercise
Exercise
E
*
130
120
110
100
90
% change in amplitude
No exercise
Exercise
F
No exercise
Exercise
1.5
1.0
0.5
Normalized smoothed MUA
0
12
24
36
48
Time (hours)

190

recording setup. Access to the running wheel significantly increased the amplitude of the daily rhythm in SCN activity (13.6%: from 168±27 Hz to 192±26 Hz; *P*=0.04, paired Student's *t*-test, n=10 mice; Fig. 1D and E). To determine the acute effect of increased behavioral activity on electrical activity in the SCN, we calculated the change in the difference between the first peak and trough following the introduction of the running wheel. After introducing the running wheel, the mean difference between the first peak and trough was 206±25 Hz, which is significantly larger than the baseline amplitude (168±27 Hz)(*P*=0.02, paired Student's *t*-test, n=10 mice). The smoothed data were then normalized in order to better visualize the change in amplitude after the running wheel was introduced (Fig. 1F). Despite increasing the amplitude of the SCN activity, introducing the running wheel had no effect on the width of the cycle (11.8±1.1 hours vs. 12.4±0.6 hours in the absence and presence of a running wheel, respectively; *P*=0.31, paired Student's *t*-test, n=10 mice).

F-periodogram analysis on SCN electrical levels were performed to calculate the changes in rhythm strength of the SCN electrical activity rhythm after placement of a running wheel in the recording setup. Exercise induced by running wheel activity lead to a significant increase in rhythm strength of 12.6% compared to the control condition without exercise (*P*<0.01, paired Student's *t*-test, n=10 mice)(Fig. 2),

Acute suppressions in SCN neuronal activity in response to behavioral activity were observed in 3 out of 10 mice, while in the other 7 mice they were not observed. No difference in change of amplitude between the groups with and without suppressions in the presence versus the absence of a wheel was detected (*P*=0.78, unpaired Student's *t*-test).

◀ **Figure 1.** A. Example *in vivo* recording of SCN electrical activity (upper panel) and physical activity (lower panel) measured for four consecutive days in a freely moving mouse housed in a 12:12-hour light:dark cycle. The gray shading indicates the dark phase, and the white background indicates the light phase. Electrical activity in the SCN was measured as multi-unit activity (MUA). Behavioral activity was measured using a passive infrared (PIR) detector; the black bars indicate when the mouse is active, and the bin size is 10 seconds. B. An example *in vivo* recording of electrical activity and physical activity in the same mouse shown in A after the introduction of a running wheel. C. Mean (±SEM) behavioral activity levels measured over four days in the absence (black bar) and presence (gray bar) of a running wheel. Behavioral activity levels were recorded by passive infrared detectors in 10-second blocks. D. Mean (±SEM) amplitude of the electrical activity recordings in the absence and presence of a running wheel. Amplitude was calculated at the difference between the peak and trough of activity (see panels A and B). E. Mean (±SEM) percent change in amplitude relative to baseline. F. Normalized smoothed electrical activity recordings for two consecutive days. The data were normalized by setting the average value of each recording to 1.0. The dashed line represents the smoothed data recorded in the absence of a running wheel (baseline), and the solid line represents the smoothed data recorded in the presence of a running wheel. *, *P*<0.05.

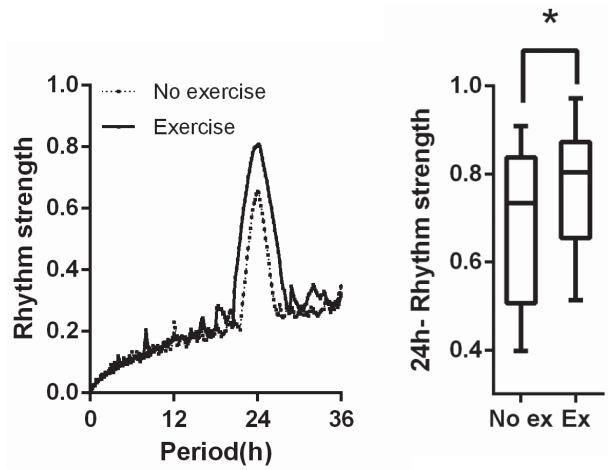


Figure 2. Rhythm strength calculated by F-periodogram analysis in the presence and absence of a running wheel in the recording setup. The left graph represents an individual example of the F-periodogram analysis of the SCN electrical activity levels with and without exercise. The right graph shows the mean (±SEM) 24-h rhythm strength of SCN electrical activity recordings in the absence and presence of a running wheel (i.e. exercise). *, $P<0.01$.

DISCUSSION

In this study, we performed *in vivo* extracellular recordings of SCN neurons in freely moving mice while simultaneously measuring behavioral activity in the absence and presence of a running wheel. These experiments were designed to test the hypothesis that the amplitude in electrical activity of the SCN rhythm is increased by voluntary exercise. This hypothesis was based on previous studies that reported behavioral activity–induced suppression of SCN electrical activity [17-19]. In support of our hypothesis, we found that the amplitude of rhythm in SCN electrical activity was increased by 13.6% when the animals had access to a running wheel. The rhythm strength of the 24h-period in SCN electrical activity was increased by 12.6% during running wheel access. The amplitude increase in the rhythm was detected in individual recordings, despite differences in recording location, indicating that activity influences the SCN as a whole.

Suppressions were detected in response to behavioral activity in three out of 10 recordings, whereas no suppressions were observed in the other seven mice. The absence of behavioral activity–induced suppression of SCN electrical activity may be attributed to heterogeneity within the SCN. Each *in vivo* recording of SCN electrical activity measures a unique subpopulation of neurons within the SCN, and some subpopulations may receive input from brain areas that are activated upon behavioral activity, whereas other SCN populations may not receive this input. Previous studies reported behavioral activity–induced suppression in 18%

of recordings in rats [17], 64% of recordings in mice [18], and 100% of recordings in hamsters [19]. These differences might reflect the relative importance of behavioral input in various species. Notably, suppressed SCN neuronal activity occurs exclusively in response to *spontaneous* (i.e., non-evoked) behavioral activity. Evoking behavioral activity by mildly disturbing the animal does not lead to suppressions in SCN electrical activity; on the contrary, electrical activity in the SCN increases with *evoked* behavioral activity [18].

It is not immediately evident how the increase in SCN rhythm amplitude and rhythm strength are achieved at the neuronal level. Of note, the two measures reflect different aspects of a rhythmic processes, as amplitude is related to the magnitude of the day-night difference, while rhythm strength is closely related to rhythm precision. Behavioral activity may induce acute effects on the waveform of the SCN rhythm by suppressing electrical activity in the SCN. Behavioral activity as well as running wheel activity induces suppressions in the SCN mainly during the active phase [17-19], the phase that coincides with low levels of electrical activity in the SCN. We therefore speculate that voluntary exercise exerts its effect primarily at night by deepening the trough of the rhythm in SCN electrical activity, thereby increasing the rhythm's absolute amplitude. We measured an acute effect of increased behavioral activity induced by running wheel activity on the amplitude of the SCN rhythm; this effect was detectable as early as the first cycle after the running wheel was introduced. Thus, acute suppressions in electrical activity directly explains the amplitude increase and may indirectly contribute to rhythm strength.

Additionally, the beneficial effects of exercise on the SCN may be due to an increased synchronization of SCN neurons. Such an increase in the synchronization presumably causes a narrower peak in the waveform of the SCN rhythm [20]. In support of this mechanism, behavioral activity increases neurotransmitter levels in the SCN [21], which may in turn increase the synchronization among SCN neurons. However, this process is not likely to occur acutely, requiring at least a few days to develop. Because we found no effect of enhanced behavioral activity on the width of the peak in SCN neuronal activity—even when measured after at least four days in the presence of running wheel activity— we have no evidence for this mechanism and favor the hypothesis that the increase in amplitude is due to acute suppressed SCN electrical activity.

The neuronal pathway that underlies the suppressive effect of behavioral activity on SCN activity is unclear. Two afferent pathways relay information regarding behavioral activity to the SCN—the geniculohypothalamic tract projects from the intergeniculate leaflet (IGL) to the SCN, and a serotonergic pathway projects from the raphe nuclei to the SCN. The IGL expresses high levels of neuropeptide Y (NPY), and behavioral activity increases the levels of both serotonin [22] and NPY [23]. Injecting NPY into the third ventricle or electrically stimulating the IGL induces

non-photic phase shifts [24, 25]. Similarly, the application of serotonin produces non-photic phase shifts in both *in vivo* [26] and *in vitro* systems [27]. Electrically stimulating the raphe nuclei drives the release of serotonin in the SCN [28, 29], leading to suppressed *c-fos* expression and suppression of electrical activity of SCN neurons both *in vivo* and *in vitro* [29-32]. Thus, both of these efferent pathways may play a role in mediating the suppressive effect of behavioral activity on SCN neuronal activity.

In aged mice, the amplitude of the *in vivo* electrical activity rhythm in SCN neurons decreases by approximately 50% [33]. Our findings indicate that increased voluntary physical exercise may be beneficial for circadian rhythmicity in elderly humans and in patients with dementia. Here, we report that running wheel activity also acutely increases the amplitude of SCN electrical activity and enhances rhythm strength in young mice. This finding is consistent with a recent study that found that exercise has an acute beneficial effect on the temporal activity profile of young and aged mice (Gu et al., under revision). Moreover, the benefit of voluntary behavioral activity lasts beyond the duration of the behavioral activity itself, as the increased SCN amplitude is preserved in *in vitro* recordings *[15]*.

Physical exercise also affects the circadian system in humans. For example, in the elderly, fragmented sleep-wake cycles can be reduced by exercise [12]. Exercise can also accelerate re-entrainment to a new light-dark cycle [10], and physical activity can help the circadian system adapt to a new work shift [34]. Thus, exercise is a promising non-invasive intervention for improving the circadian system. Such an intervention may be valuable to the elderly, shift workers, travelers who cross time zones, and blind persons who lack external light cues.

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