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ENHANCED ELECTRICAL OUTPUT OF THE SCN CLOCK BY BEHAVIORAL ACTIVITY FEEDBACK IN THE DAY-ACTIVE GRASS RAT *ARVICANTHIS ANSORGEI*

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Abstract

Mammalian circadian rhythms are regulated by the suprachiasmatic nucleus (SCN). The SCN generates high levels of electrical activity during the day and low levels during the night, and transmits these to other brain areas. In nocturnal rodents, behavioral feedback to the SCN induces suppression of electrical firing rate. To examine the effects of behavioral activity on the SCN in a diurnal mammal we performed *in vivo* and *ex vivo* electrophysiological recordings in the SCN of diurnal grass rats (*Arvicanthis ansorgei*). To determine the characteristics of the system *in vivo* and *in vitro*, we performed a detrended fluctuation analysis (DFA). *In vivo* electrophysiological recordings revealed that episodes of behavioral activity of the animal coincided with epochs of enhanced SCN firing rates. These increments were acute and sustained for the full duration of behavioral activity. Consequently, the amplitude of the SCN activity rhythm is enhanced by behavioral activity of the grass rat when it occurs during the day. *Ex vivo* measurements of SCN firing rate from grass rats revealed circadian rhythms that were devoid from acute excitations, indicating that they are causally driven by extra-SCN areas. DFA analysis showed that the complexity of the signal in the SCN *in vivo* is scale invariant, whereas *in vitro* scale-invariance is not present. The results indicate that in diurnal and nocturnal species behavioral activity has opposite effects on the SCN discharge rate, leading to an enhancement of the amplitude of the SCN electrical activity rhythm, in both nocturnal and diurnal species through opposing mechanisms.

Introduction

Mammals have evolved an internal clock that enables anticipation and adaptation to 24-h changes in the environment on earth. The master clock of the body resides in the suprachiasmactic nucleus (SCN) of the ventral hypothalamus (1). Single neurons in the SCN generate a circadian rhythm based on the cyclic expression of clock genes and protein products (2,3). The rhythmic expression of clock proteins results in a rhythm in SCN electrical activity (i.e. high during the day and low during the night), and the rhythmic release of humoral factors, which results in circadian rhythms in physiology and behavior (4,5). Synchronization of the SCN clock to the ambient light-dark cycle is primarily accomplished by light information, which is perceived by the retina and projected to the SCN via the retinohypothalamic tract.

While light is the main synchronizer of the SCN, SCN's rhythmicity is also influenced by non-photic factors such as the animal's behavior. Behavioral activity (6) and substances that are involved in regulating behavioral activity, such as neuropeptide Y (NPY) (7) and serotonin agonists (8), affect the phase of the SCN (9). The application of NPY (10-13) or serotonin receptor agonist 8-OH-DPAT (14,15) to SCN neurons *in vitro* decreases the neuronal firing rate. *In vivo* measurements of the SCN's electrical activity in freely-moving night-active rodents such as hamsters (16), rats (17) and mice (18) revealed that behavioral activity induces acute suppression of the SCN's firing rate. In mice, suppressions of the SCN electrical activity induced by spontaneous behavior are superimposed on the SCN's circadian modulation, and the magnitude and duration of the suppressions are respectively dependent on the intensity and duration of the behavioral event (18). Because mice are night-active animals, they show elevated levels of activity during the night, which suppresses the SCN firing even more. Consequently, SCN rhythm amplitude increases, thereby improving rhythmicity at the periphery.

In humans, physical exercise accelerates the synchronization of sleep-wake rhythms to the external light-dark cycle (19-22) and improves health and well-being (23-29). We hypothesize that in day-actives species behavioral activity during the day will increase the amplitude of the SCN electrical output, as a result of behaviorally induced enhancements of SCN electrical firing. To test this, *in vivo* electrophysiological measurements were performed in the SCN of freely-moving day-active grass rats (*Arvicanthis ansorgei*). SCN discharge levels were overall higher during day time and lower during the night. Analysis of the *in vivo* recordings revealed that episodes of behavioral activity coincided with enhanced firing rates. Consequently, the amplitude of the SCN activity rhythm was increased by day-time activity. *Ex vivo* ("brain slice") recordings, in which the SCN is isolated from afferent and efferent connections, were performed to examine the causal direction of these effects and showed a complete lack of the short term increments in electrical

activity. The complexity of the signal was determined by a detrended fluctuation analysis (DFA). While *in vivo* the signal showed scale invariance, indicating a network of feedback interactions (30-32), *in vitro* this scale invariance was absent. The results show that the effect of behavioral activity on the SCN of the diurnal *Arvicanthis*, is opposite to the effect seen in nocturnal species, leading to an enhancement of the amplitude of the SCN electrical activity rhythm, in both diurnal and nocturnal species, via opposing mechanisms.

Methods

Animals and housing

Male grass rats (*Arvicanthis ansorgei*, breeding colony Chronobiotron, UMS 3415, CNRS University of Strasbourg, France) were subjected to12 hours light (200 lux) and 12 hours red dim light (<5 lux) cycles. All experiments were performed under the approval of the French Ministry of Higher Education and Research.

Micro-electrode implantations

Procedures for *in vivo* measurements of multiunit activity from SCN neurons have been previously described for rats (33) and mice (18). At a minimum of 8 weeks (150g-300g), tripolar stainless steel micro-electrodes (Plastic One MS333-3-BIU-SPC, Roanoke VA, United States) were implanted in anesthetized (0.13 ml Zoletil (Virbac, Carros, France) and 0.2 Rompun 0.5% (Bayer Pharma, Puteaux, France) per 100 g bodyweight) grass rats with the use of a stereotactic instrument. Two twisted electrodes (Polyamide-insulated; bare electrode diameter 125 µm) for differential recordings were aimed at the SCN (coordinates: 0.8 mm posterior to bregma, 0.9 mm lateral to the midline, 7.8 mm ventral to the dura mater, under a 5° angle in the coronal plane), and a third uncoated electrode (reference electrode) was placed in the cortex. The differential amplifier was based on the design of Yamazaki and coworkers (16).

In vivo SCN electrical recordings

After a recovery period of at least 5 days, the grass rat was individually housed in a temperature controlled recording chamber (22 °C), where water and standard laboratory food (SAFE, Augy, France) were available *ad libitum*. The grass rat was able to move freely during the time of recording. The electrical signal was amplified, bandwidth filtered (500 Hz – 5 kHz), and window discriminators were used to convert action potentials into digital pulses that were counted in 10 second bins by Circa V1.9 software (OriginLab)) and MATLAB (Mathworks Inc.).

Behavioral activity recordings

In vivo SCN electrical recording set-ups were equipped with a drinking sensor and a Passive Infra-Red (PIR) motion detector positioned 30 cm above the floor of the cage. Behavioral activity and SCN electrical activity were recorded simultaneously in 10 second time bins.

Data Analysis *in vivo* electrophysiology

Electrical activity date were smoothed in MATLAB (Mathworks Inc.). The magnitude of the behaviorally induced change in neuronal firing rate was defined as the difference between baseline of the electrical activity and the average electrical discharge during the episode of behavioral activity. The baseline level was defined as the mean neuronal firing rate during 2 minutes before the episode of behavioral activity.

Histological verification micro-electrode

After the *in vivo* measurements, the location of the micro-electrode was histologically checked. To sacrifice the grass rats, the animals received intraperitonally an overdose of sodium pentobarbital (CEVA, Libourna, France, 0.3 ml/100g bodyweight). An electrical current of 40µA was passed through the recording electrode to deposit iron at the electrode tip. The brains were collected and immersed in 4% paraformaldehyde with 0.5 g of C6FeK4N6·3H20 (Sigma Aldrich, Lyon, France) for 2 days. Brains were sectioned coronally in 40µm slices and stained with cresyl violet for microscopic verification of the recording site.

Ex vivo SCN electrical recordings

Grass rats were entrained to 12 h light : 12 h dark cycle for at least 30 days. The animals were scarified at ZT 21 (± 0.5 h) under dim red light., and the brains were removed within 1 minute of decapitation. Brain slices (~450 microns thick) were prepared using a tissue chopper, and the slice containing the SCN was transferred to a laminar flow chamber within six minutes after decapitation (31). The tissue was bathed in bicarbonate-buffered ACSF that was gassed by continuously blowing a warmed, humidified mixture of O2 (95%) and CO2 (5%) over the solution. The slice was submerged in the solution and stabilized using an insulated tungsten fork, and settled in the recording chamber for ~1 h before the electrodes were placed in the center of the SCN. Action potentials were recorded using 50-µm 90% platinum/10% iridium electrodes. The signals were amplified 10 k time and bandpass-filtered (0.3 Hz low-pass, 3 kHz high-pass). The action potentials that exceeded a predetermined threshold well above noise (~5 µV) were counted in 10-second bins using a custom-made automated computer program.

Data Analysis *in vitro* electrophysiology

The electrophysiological data were analyzed using a custom-made program in MATLAB as described previously (32). The time of maximum activity was used as a marker of the phase of the SCN and was determined as the first peak in multiunit activity. Multiunit recordings of at least 24 hours in duration that expressed a clear peak in multiunit activity were moderately smoothed using a least-squares algorithm (34). Subsequently, the SCN peak time, the peak width, and the relative

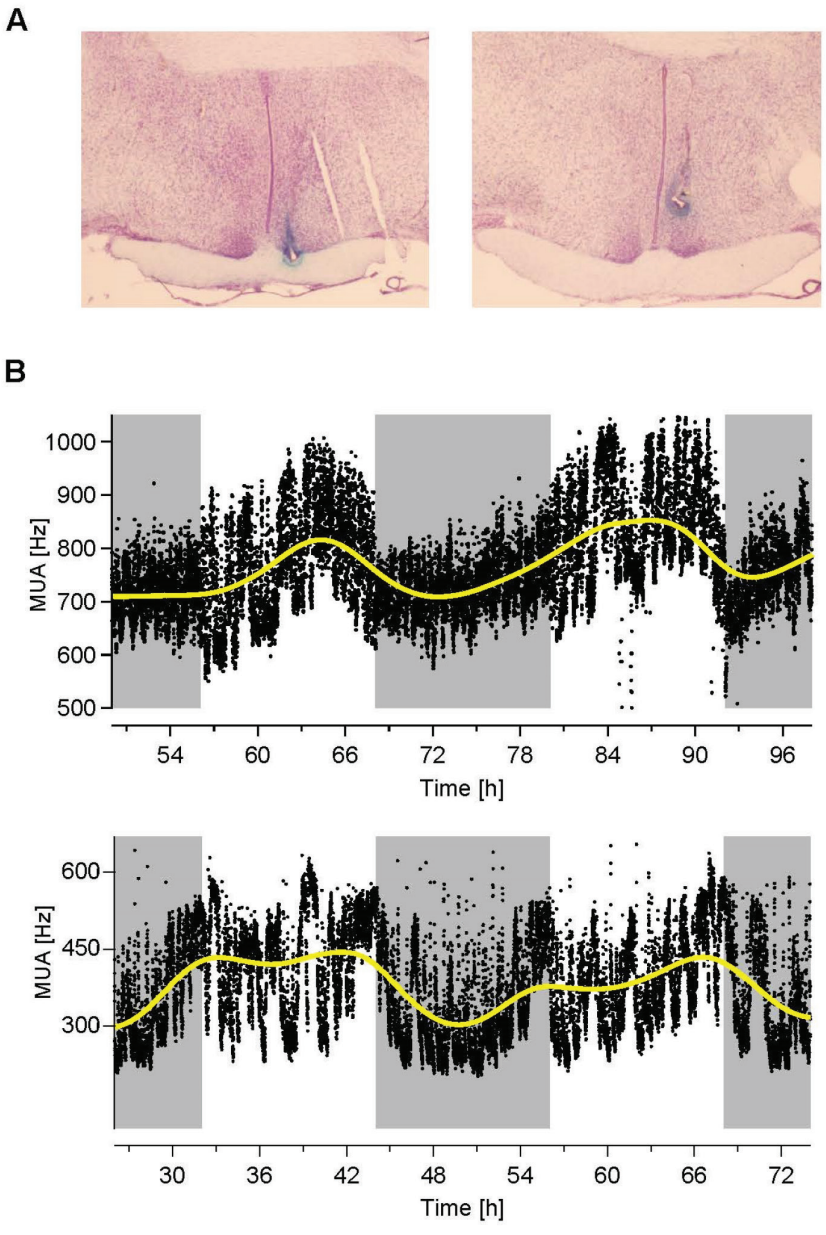

Figure 1. SCN multiunit electrical activity (MUA) *in vivo*. A. Two examples of a coronal slice of the *A. ansorgei* brain with the SCN right above the optic chiasm at the base of the hypothalamus. The location of the electrode was verified by the blue spout which is marked using an electrolytic current. **B**. Two examples of a raw trace of SCN electrical activity *A. ansorgei* kept in LD 12:12. The x-axis shows actual clock time in hours and the y-axis shows SCN MUA in Hz. The grey background represents periods of darkness. The smoothed fitted line of the MUA is plotted in yellow.

peak amplitude (peak-to-trough ratio) of the first cycle in vitro were determined. Statistical analyses were performed using SPSS. All summary data are reported as the mean ± the standard error of the mean (SD). P-values were calculated using the two-tailed Student's t-test, and differences with $p<0.05$ were considered to be statistically significant.

Assessment of scale invariance using detrended fluctuation analysis

In order to measure the scale invariance behavior in the behavior activity fluctuation, the detrended fluctuation analysis (DFA) was performed (32,35,36). The DFA was implemented as follows: (i) The integrated time series was divided into m non-overlapping "boxes". The number of data points in each box was n = N/m, where n represents the timescale. (ii) In each box, the n data points were fitted by a second-order polynomial function which represented the "local trends". (iii) In each box, we defined the "residuals" as the n data points subtracting the local trends. (iv) The root-mean-square of the residuals for each box was calculated. For the integrated time series, the mean of the root-mean-square from each of the m boxes stands for the fluctuation amplitude F(n). (v) We changed the timescale n satisfying $4 \leqslant n \leqslant 8640$, and repeated (i-iv). (vi) The fluctuation amplitude F(n) as a function of the timescale n was plotted in double-logarithmic coordination.

The activity fluctuation function can be presented by a power-law form $F(n) \sim n^{\alpha}$, if the fluctuations maintain the scale-invariant behavior. The scaling exponent α describes the scale-invariant correlation of the activity fluctuations. If α is equal to 0.5, the correlation is absent in activity fluctuation, which corresponds to white noise. If α is smaller than 0.5, there are negative correlations in the activity fluctuations, i.e. larger recording data values have more probability of being followed by smaller recording data values and vice versa; If α is larger than 0.5, there are positive correlations in the activity fluctuations, i.e. larger recording data values are more likely to be followed by larger recording data values and vice versa.

Results

In vivo SCN firing pattern in diurnal grass rats

In vivo SCN electrical activity measurements were performed successfully in 4 day-active grass rats (*Arvicanthis ansorgei*) and showed high levels during the day and low levels during the night (585 ± 220 Hz vs 562 ± 228 Hz, respectively). These rhythms were in phase with the animal's behavioral activity rhythm.

Acute excitations of the SCN firing rate by behavioral activity

Examination of the recordings of SCN electrical activity revealed in 3 out of 4 animals enhancements of the SCN firing frequency that were superimposed on the circadian rhythm in SCN discharge rate. Detailed analysis of the locomotor activity data showed that episodes of spontaneous behavioral activity were consistently present at times of enhanced SCN discharge rates (Figure 2a). Typically, at the start of the behavioral activity, an acute increase in firing rate was displayed, and the SCN firing frequency remained elevated for the complete duration of behavioral activity (Figure 2b).

SCN firing pattern *ex vivo*

To test whether increments in SCN electrical activity found *in vivo* are caused by behavioral activity or vice versa, we performed *ex vivo* electrophysiological recordings on the isolated SCN. *In vitro* electrophysiological recordings in SCN from grass rats revealed unimodal sinusoidal waveform patterns in the isolated SCN. SCN electrical activity was maximally high around midday (ExT 12.4 ± 1.6 h), and the elevated electrical activity interval was 10.6 ± 2.2 h. Increments in SCN electrical activity superimposed on the circadian modulation, as found *in vivo*, were absent *ex vivo* (Figure 3).

Fractal patterns of SCN discharge rate *in vivo* and *ex vivo*

A detrended fluctuation analysis (DFA) was performed over a recording period of 3 days (LD 12h: 12h) *in vivo*. The analysis showed that the fluctuation function *F(n)* of the electrical activity possessed a power-law form (a straight line in the log-log plot: $F(n) \sim n^{\alpha}$) at time scales from ~10 seconds up to 10 hours (Figure 4a). The power-law form reflects a fractal temporal structure and the scaling exponent α of 0.93 ± 0.09 indicates strong fractal correlations in SCN electrical activity fluctuations. DFA showed that the fluctuation function *F(n)* of *in vitro* SCN electrical activity possessed a power-law form with a breakpoint around 10 minutes (Figure 4b). At time scales from ~ 10 seconds up to 10 minutes the scaling exponent α was similar to that found *in vivo* (0.93 ± 0.07), and at time scales from 10 minutes to 10 hours the scaling exponent was 1.5 ± 0.09 (Figure 4c).

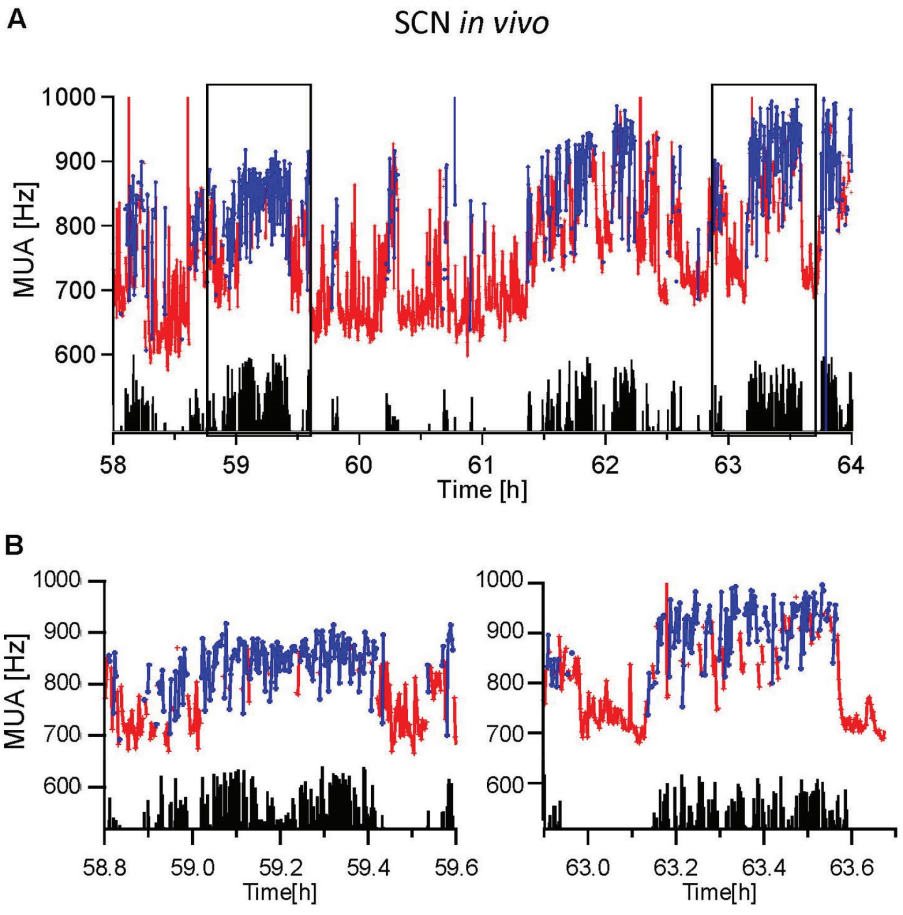

Figure 2. SCN multiunit electrical activity (MUA) *in vivo* during active (blue) and inactive (red) episodes. A. To visualize the effect of behavioral activity on SCN firing rate, SCN MUA recorded while the animal displayed behavioral activity (measured by passive infrared detectors) are plotted in blue, and MUA traces recorded while the animal was inactive are shown in red. Behavioral activity is plotted below the SCN electrical activity traces. The x-axis shows actual clock time in hours and the y-axis shows SCN MUA in Hz. Behaviorally-induced enhancements of SCN MUA are present during the day (upper graph) and during the night (lower graph). **B.** Expanded plots of SCN MUA during episodes of behavioral activity during the day as indicated by the boxes in A.

Enhancement of the amplitude of the SCN electrical output rhythm by behavioral activity

The effect of behavioral activity on the SCN firing rate was investigated by determining behavioral-induced enhancement of the normalized SCN discharge rates. During the dark period (night), epochs of behavioral activity caused a 17% increase of SCN

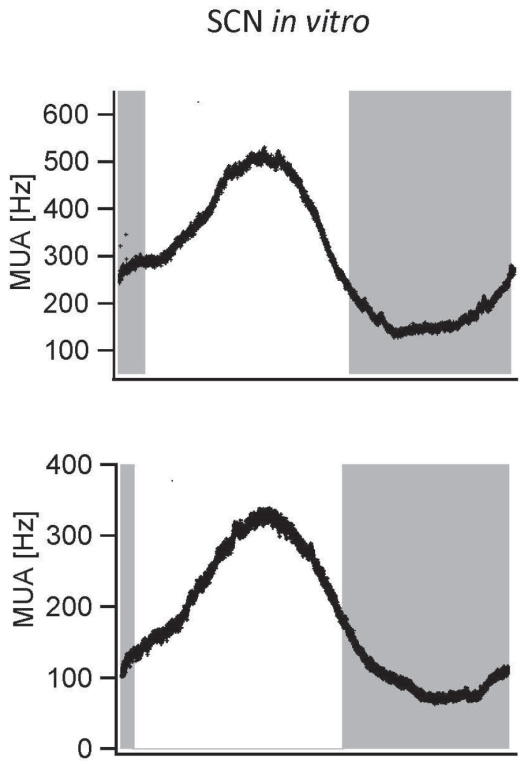

Figure 3. SCN multiunit electrical activity (MUA) *ex vivo*. Two raw traces of SCN electrical activity measured *ex vivo*. The white-grey background represent the light-dark regime to which the grass rats were entrained and the y-axis shows MUA in Hz. SCN discharge rate of *A. ansorgei* follow a unimodal circadian pattern, with maximal electrical activity at midday (ExT 12.4 ± 1.6 h, n=8). MUA in diurnal grass rats were elevated (measured by the peak width of the waveform) for a duration of 10.6 ± 2.2 h.

firing rates, and during the light period, behavioral-induced enhancements led to a 20% increase of the SCN discharge rate. We plotted the number of action potentials as a function of the time, and distinguished between behaviorally active and inactive episodes of the grass rats. Clear circadian rhythms exist both in absence and in the presence of behavioral activity (Figure 5a). The smoothed curves through the SCN neuronal activity rhythms revealed that the level of the rhythm was enhanced in the presence of activity. When the day-active grass rat only displays behavioral activity during day-time and behavioral in-activity during night time, the amplitude of the SCN electrical activity rhythm will be maximally high (Figure 5b). On the other hand, if the animal is active during night-time and rests during day-time, the rhythm of the SCN will be dampened or even reversed (Figure 5c).

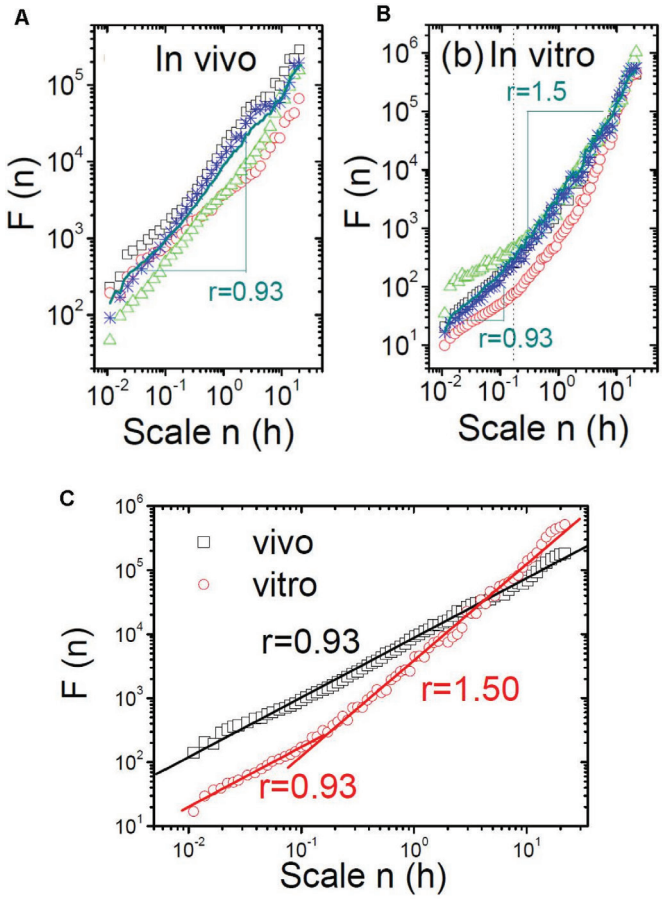

Figure 4. Fractal patterns of MUA fluctuations *in vivo* and *ex vivo*. A,B Results of individual A. ansorgei: four for *in vivo* recordings during 3 days of LD 12:12 and four for *ex vivo* recordings during at least 24 h. Examples of raw data are shown in Figure 1 and 3. **C.** Group averages of *in vivo* and *ex vivo* recordings. Data are shown in log-log plots. At time scales from ~10 seconds up to ~10 hours, the function *in vivo* shows a power-law form (straight line in the log-log plot) with the scaling exponent α=0.93, indicating fractal correlations in raw data. At time scales < ~6 minutes, the function *ex vivo* shows a power-law form with a virtually identical scaling exponent as *in vivo*. At time scale > ~6 minutes, the function *ex vivo* shows a power-law with the scaling exponent α=1.5.

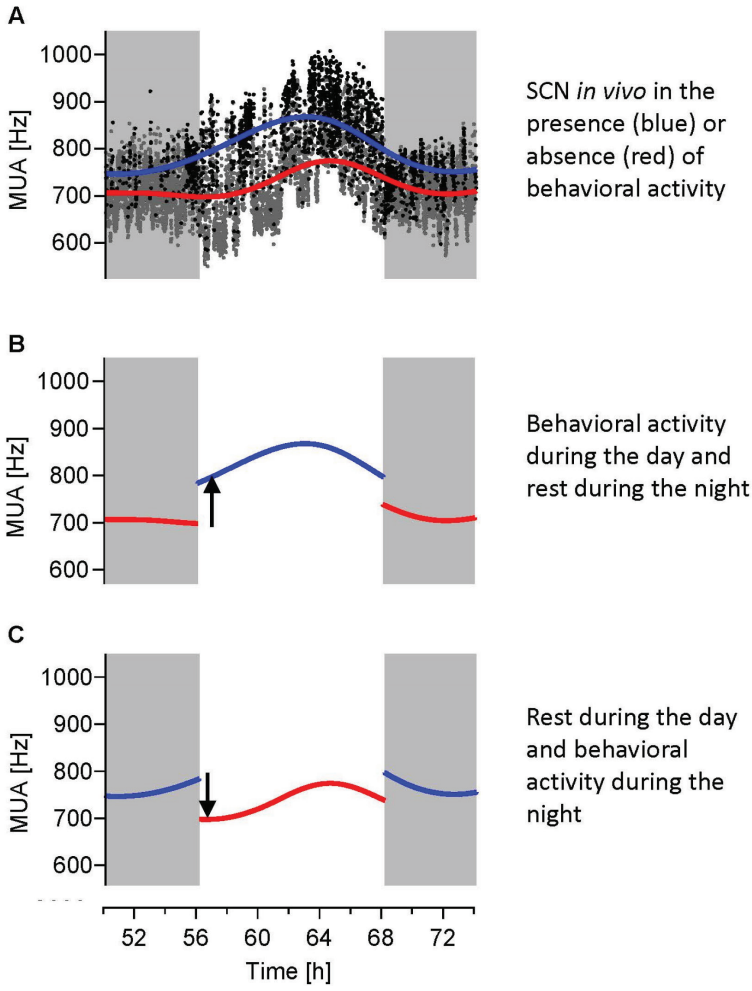

Figure 5. Circadian profile of MUA in the presence or absence of PIR-recorded behavioral activity. A. To visualize the effect of behavioral activity on SCN rhythm amplitude, the passive infrared data are integrated in the MUA data: whenever passive infrared movement was detected within a 10 s recording bin, the MUA measured in that bin is displayed with a black dot. Grey dots show MUA points from bins when no movement was detected. Smoothed lines were drawn through the black dot (blue line) and grey dots (red line) to illustrate the presence of a circadian rhythm in either profile. **B.** To visualize the effect of a proper behavioral activity rhythm on SCN rhythm amplitude, the fitted line of MUA measured during behavioral activity is plotted for the day and the fitted line of the MUA measured during inactivity is plotted for the night. **C.** To visualize the effect of a completely reversed behavioral activity rhythm on SCN rhythm amplitude, the fitted line of the MUA measured during inactivity is plotted for the day and the fitted line of the MUA measured during activity is plotted for the night. The x-axis shows actual clock time in hours and the y-axis shows SCN MUA in Hz. The grey background represents periods of darkness.

Discussion

In this present study we performed *in vivo* measurements of the SCN's electrical firing pattern in day-active grass rats (*Arvicanthis ansorgei*). The results show that in grass rats SCN discharge is overall higher during the day and lower during the night. Analysis of the *in vivo* electrical activity recordings revealed that electrical discharges were acutely enhanced during the full duration of behavioral activity. A DFA analysis showed the presence of long-range fractal regulation in the SCN, indicating a healthy physiological state. *In vitro*, the SCN's electrical activity followed a unimodal waveform and was free from acute enhancements of SCN discharge rate. Moreover, the patterns of long range fractal regulation completely broke down, reflecting a lack of feedback network interactions. Together the results indicate that other than in nocturnal rodents, behavioral activity leads to increments in SCN electrical activity, consequently leading to an enhancement of the amplitude of the circadian pacemaker in day-active rodents.

SCN electrical activity pattern *in vivo*

By the use of implanted microelectrodes we were able to measure the electrical activity of the SCN in freely-moving *A. ansorgei*, while monitoring their behavioral activity with passive infrared detectors. The recordings show that the SCN firing pattern from diurnal grass rats follow a circadian pattern that is higher during daytime and low during night time. This finding is similar to previous *in vivo* measurements of the SCN's discharge rate in the diurnal chipmunk (Eutamias sibiricus) (45) and nocturnal rodents such as hamsters (16), rats (17,37) and mice (18,46). Furthermore, our data is in agreement with *in vitro* recordings performed on the SCN of several nocturnal rodents (37,37-41) and indicates that the SCN's electrical activity waveform *in vitro* is similar in diurnal and nocturnal rodents. This finding is supported by previous studies showing similarities in daily variation of clock gene expression (42-44) and in the circadian expression of Fos in the SCN of nocturnal and diurnal rodents (45,46).

Behavioral induced-enhancements of SCN discharge rate

We observed epochs of increased SCN discharge rate superimposed on the circadian electrical activity rhythm of the SCN in day-active grass rats. Detailed investigation of the SCN electrical activity recordings revealed that in 3 out of 4 animals acute enhancements of the SCN firing rate correlated to bouts of behavioral activity. Accordingly, the mean firing rate of the SCN measured during behavioral activity was increased relative to SCN firing rates measured during inactive behavioral states. As behavioral activity of day-active animals is concentrated during the day, enhancements of SCN electrical activity is especially present during the day. As a result, the peak in SCN electrical activity is elevated and thus the SCN rhythm amplitude can be

boosted by behavioral activity during the animal's active phase, i.e. the phase of the cycle where SCN electrical activity is high. Previous studies performed in nocturnal hamster (16), rat (17) and mice (18) revealed behaviorally-induced suppressions of SCN firing rate. As nocturnal animals show high levels of behavioral activity during the night, the suppression of SCN firing rate is predominantly present during the night. Because of this, the SCN electrical activity levels are lowered even more (18), and the amplitude of the SCN rhythm is boosted. Our results indicate that the amplitude of the SCN rhythm of both diurnal and nocturnal animals can be enhanced by behavioral activity, but via opposite behaviorally-induced alterations of the SCN firing rate.

Unimodal SCN electrical activity pattern *ex vivo*

To investigate the direction of the relationship between enhancements SCN electrical and behavioral activity, we performed *ex vivo* measurements of SCN electrical activity from diurnal grass rats, to characterize the endogenous firing pattern of the SCN without feedback from other brain areas. Our data reveal that the firing rate of the SCN displays a circadian pattern, showing highest electrical activity during day time and lowest electrical activity during night time. Importantly, the circadian firing pattern of the isolated SCN of day-active grass rats are unimodal, and absent from acute enhancements of SCN discharge rate. The absence of variability *ex vivo* indicates that the variability arises from communication between the SCN and extra-SCN areas.

Fractal patterns of SCN discharge rate *in vivo* and *ex vivo*

To test whether fractal patterns of SCN electrical activity in grass rats are determined by network interactions between the SCN and extra-SCN areas, we performed detrended fluctuation analysis (DFA). The analysis showed that the fluctuation function *F(n)* of the *in vivo* SCN electrical activity recorded during 3 days (LD 12h :12h) possessed a power-law form (a straight line in the log-log plot: $F(n) \sim n^{\alpha}$) at time scales from ~ 10 seconds up to 10 hours (Fig 4). The power-law form indicates a fractal temporal structure and the scaling exponent α of 0.93 ± 0.09 indicates strong fractal correlations in SCN electrical activity fluctuations. DFA showed that the fluctuation function *F(n)* of *in vitro* SCN electrical activity possessed a power-law form with a breakpoint around 10 minutes. At time scales from ~ 10 seconds up to 10 minutes the scaling exponent α was practically the same as found *in vivo* (0.93 ± 0.07), and at time scales from ~ 10 minutes to 10 hours the scaling exponent was 1.5 ± 0.09. Thus, it seems that fractal patterns at time scales > 10 minutes emerge from the interaction between the SCN and extra-SCN areas.

Neuronal pathways involved in behavioral feedback to the SCN

In vivo, the SCN receives non-photic information via major inputs from the raphe nuclei, which contains serotonin (5-HT) (47), and from the intergeniculate leaflet (IGL), which contains among others neuropeptide Y (NPY) and GABA (48). In nocturnal rodents, increased levels of behavioral activity increase the levels of 5-HT (49) and NPY in the SCN (50). Interestingly, 5-HT receptor agonist 5-HT1A (8) and NPY (7) are able to induce phase shifts in the SCN's rhythm. Accordingly, the ablation of serotonergic afferent SCN pathways (9,51,52) or the administration of NPY antibodies (53) to the SCN attenuates the phase shifting effects of non-photic stimuli on the SCN. The application of NPY (10-13) or 5-HT receptor agonist 8-OH-DPAT (14,15) to SCN neurons *in vitro* decreases the neurons' firing rate, which suggest the potential involvement of these factors in behaviorally-induced suppression of SCN firing rate found in nocturnal rodents (16-18).

Our *in vivo* recordings of SCN electrical activity in the day-active *A. ansorgei* revealed behaviorally-induced enhancements of the SCN discharge rate, opposed to behaviorally-induced suppression found in nocturnal rodents (16-18). We hypothesize that neurotransmitters involved in behavioral-feedback to the SCN clock effect the SCN firing rate adversely in diurnal and nocturnal species. This hypothesis is supported by research on the effect of serotonergic activation on light resetting of the SCN in the day-active *A. ansorgei* (54). The authors showed that injections of 5-HT receptor agonists induce small phase advances during the subjective night (54), while in nocturnal species 5-HT receptor agonists cause large phase advances only during the subjective midday (55-57). Also GABA is able to induce different responses in the SCN of nocturnal and diurnal rodents; activating GABAA receptors mice during the subjective day induces phase advances in nocturnal rodents, whereas the SCN of diurnal grass rats display a phase delay (58). Another potent factor projecting behavioral information to the SCN is neuropeptide Y (NPY). In the *Arvicanthis nicloticus*, behavioral activity increase the levels of NPY in the IGL of nocturnal rodents (59), and behavioral activity is associated with Fos expression in NPY-containing neurons in the IGL of the *A. niloticus* (60). Moreover, in the *A. nicoloticus*, the elimination of the IGL leads to an absence of NPY fibers within the SCN, suggesting the presence of functional NPY projections from the IGL to the SCN (60). Our study together with existing literature indicate differences in SCN's responsiveness to neurotransmitters between nocturnal and diurnal animals. These differences should be investigated more elaborate in order to make recommendations to improve the well-being of humans based largely on results from studying nocturnal species.

Conclusions

We show that behaviorally-induced enhancements of SCN firing rate in the day-active grass rat significantly increases the amplitude of the SCN rhythm. In diurnal animals, when maximal behavioral activity occurs in phase with maximal SCN electrical activity (i.e. during day time), the electrical activity increases as a result of behaviorally-induced enhancements. Previous studies have revealed that in humans behavioral activity during the popper time of day can synchronize the circadian system (24,25,61-63). For example, in elderly and people suffering from Alzheimer's disease increased daytime exercise leads to improvements of sleep-wake cycles, mood and performance (23,26-29,64,65). Our results indicate that behavioral activity can boost the amplitude of the circadian clock, and contribute to the understanding of the interplay between the SCN and the periphery in humans.

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