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Vestibular loss disrupts daily rhythm in rats

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Martin T, Mauvieux B, Bulla J, Quarck G, Davenne D, Denise P, Philoxène B, Besnard S. Vestibular loss disrupts daily rhythm in rats. *J Appl Physiol* 118: 310–318, 2015. First published December 11, 2014; doi:10.1152/japplphysiol.00811.2014.—Hypergravity disrupts the circadian regulation of temperature (Temp) and locomotor activity (Act) mediated through the vestibular otolithic system in mice. In contrast, we do not know whether the anatomical structures associated with vestibular input are crucial for circadian rhythm regulation at 1 G on Earth. In the present study we observed the effects of bilateral vestibular loss (BVL) on the daily rhythms of Temp and Act in semipigmented rats. Our model of vestibular lesion allowed for selective peripheral hair cell degeneration without any other damage. Rats with BVL exhibited a disruption in their daily rhythms (Temp and Act), which were replaced by a main ultradian period (τ <20 h) for 115.8 ± 68.6 h after vestibular lesion compared with rats in the control group. Daily rhythms of Temp and Act in rats with BVL recovered within 1 wk, probably counterbalanced by photic and other nonphotic time cues. No correlation was found between Temp and Act daily rhythms after vestibular lesion in rats with BVL, suggesting a direct influence of vestibular input on the suprachiasmatic nucleus. Our findings support the hypothesis that the vestibular system has an influence on daily rhythm homeostasis in semipigmented rats on Earth, and raise the question of whether daily rhythms might be altered due to vestibular pathology in humans.

vestibular system; biological rhythms; temperature; locomotor activity; rats

LOCATED IN THE TEMPORAL BONES, the vestibular system is composed of three semicircular canals that encode head rotation velocity and two otolithic macula that encode linear acceleration and gravity force. The vestibular system is primarily involved in gaze stabilization and postural control (23), but its influence has been expanded to autonomic and bone regulation (11, 61, 63), spatial learning and memory (5, 6, 56), and spatial orientation and perception of gravitational verticality (37). Studies carried out in environments in which gravity is altered have shown evidence of a possible influence of the vestibular system on hypothalamic circadian function in rats (27) and mice (18, 46); disruption of circadian rhythms of temperature (Temp) and locomotor activity (Act) have been similarly observed in both species for about 7 days in a 2 G environment generated by a centrifuge. Knockout mice devoid of otolithic vestibular sensors [het(−/−) mice] remained unaffected by hypergravity at 2 G (18). However, when placed in constant darkness (DD) or bright light conditions (LL), they showed an altered period-lengthening response compared with wild-type mice (the latter exhibiting a free-running activity rhythm) (16a).

Circadian rhythms are driven by a biological clock located within the suprachiasmatic nucleus (SCN) (31, 57), and different external time cues (zeitgebers) synchronize the endogenous activity of the SCN to environmental demands. The day/night cycle caused by Earth's rotation acts as the main external cue and is encoded by the retinal sensor whose information is transmitted to the biological clock primarily through a retino-hypothalamic pathway (14, 43, 45). From a phylogenetic standpoint, the visual and vestibular sense organs developed within the same time window more than 500 million years ago, well before the hearing system (21). Both types of sensory information are always combined at numerous cerebral levels. The brainstem generates postural reflexes (vestibular nucleus), vestibulo-ocular reflexes, and optocinetic reflexes (oculomotor nucleus). The subcortical level (thalamus, hippocampus) is involved in spatial learning and memory (5, 38). The cortex (associative area and vestibular cortex) is needed for verticality perception and spatial perception of the body (12, 36). However, the hypothesis that the vestibular system might influence circadian and visual information has arisen only recently, highlighted by vestibular stimulation studies in an artificial hypergravity environment. Consequently, circadian rhythm disturbances observed in microgravity during space missions might reveal a role for the vestibular system in hypothalamic circadian function. Circadian impairment in space might be caused either by the loss of the abnormal light/dark cycle or by inhibition of the otolithic signal due to loss of gravity (13, 60). On Earth, circadian rhythms have never been explored in the numerous vestibular-deficiency rodent models (26, 35, 62) mimicking a switch to a zero G level.

Therefore, the aim of this work was to study whether the loss of vestibular input on Earth disrupts daily rhythmicity in semipigmented rats. We used continuous measurement of core Temp and Act while keeping visual information functional.

METHODS

Animals

Male Long-Evans rats (n = 18, 400–500 g, Janvier, France) were individually housed under constant temperature (23 ± 1°C) with a 12:12-h light:dark cycle (lights on from 8:00 A.M. to 8:00 P.M.). Food and water were available ad libitum. Rats were then randomized into two groups. The first group underwent a bilateral vestibular loss (BVL group, n = 9); the second group consisted of sham-operated rats (SO group, n = 9) and was used as the control group. Experiments were carried out in accordance with the European Communities Council Directive 2010/63/UE and French law. The protocol was approved by the Regional Animal Ethical Committee (CENOXEMA) in April 2012 (registration 0412–02).

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Protocol

Under anesthesia, each rat was implanted intraperitoneally with a telemetric device to continuously record core Temp and Act by actimetry. Animals were then kept in a convalescence period of 15 days under 12:12-h light/dark environmental conditions. Starting on the 16th day, Temp and Act of each rat were recorded continuously for 4 days to establish the daily rhythmicity without any lesion under control conditions. Rats then underwent a second brief anesthesia to inject either arsalinate (BVL group) or saline (SO group). After the lesion, two periods of continuous recordings of Temp and Act were set up; the first period started immediately after recovery from anesthesia and lasted for 7 days, and the second period started 1 mo after the injections and also lasted for 7 days. This second period was established to check the long-term effects of vestibular loss.

General Procedures

Surgical procedure to record temperature and activity. A C50-PXT device (Data Sciences International, St. Paul, MN) was used to measure core Temp and Act, the latter being defined by all horizontal linear motions. The device is a cylindrical implant with a 2-mo lifetime (duration of battery operation). Implantation of the sensor was carried out as described by the Data Sciences International video "Surgical procedures Aorta-BP, ECG w C50-PXT." The sensor was implanted intra-abdominally after midline laparotomy under isoflurane anesthesia. Animals were kept in convalescence for 15 days after implantation of a telemetric sensor. An injection of 2 ml (ip) of antibiotic (amoxicillin-clavulanic acid; Augmentin) and 2 ml of analgesic (paracetamol) was given once a day for 3 days to prevent nociception.

Vestibular lesion procedure. A chemical model of a vestibular lesion was chosen to explore the effect of a sudden disruption of all vestibular inputs. Transtympanic arsanilate injection leads to selective lesion of vestibular hair cells without any damage to the external ear tract, eustachian tubes, oropharynx, VIIIth nerve, or Scarpa's ganglion, indicating a lack of diffusion of arsanilate through the peripheral tissues and blood and through the sheath of the VIIIth cranial nerve up to the brainstem (62).

Each rat in the BVL group received a single bilateral dose (0.1 ml/30 mg) of sodium arsanilate (Sigma-Aldrich) dissolved in 0.9% saline solution under volatile anesthesia (2% isoflurane) in oxygen (flow rate 2 liters/min). The injection was carried out through the anterior part of the tympanum using a 1-ml syringe (needle diameter 0.8 mm), and arsanilate was deposited into the middle ear cavity [(see details in 62)]. The SO group received a single bilateral injection of 0.9% saline solution (0.1 ml) by the same route. Each rat then received one dose of paracetamol (prodafalgan; Merck) (1 ml/25 mg ip) once a day for 2 days to decrease nociception due to the tympanic lesion.

Analysis of Daily Rhythms

Both Temp and Act data obtained from each rat during control conditions and following transtympanic injection were used to calculate the different parameters of the daily rhythms (period, amplitude, acrophase, and mesor) using Times Series Analysis Serial Cosinor 6.3 (www.euroestech.net) software (TSA) following the procedure established by Gouthiere et al. (20).

As the daily parameters of Temp and Act evolved throughout the 7 days following the lesion, a 60-h sliding window (2.5 × the 24-h period) was moved in increments of 4 h to calculate the speed of adjustment, which is the time required for resynchronization with the external synchronizers. The periods were calculated using Lomb & Scargle, and Jenkins & Watts periodograms. Other parameters (acrophase, amplitude, and mesor) were then determined by a classic cosinor analysis, which allowed determination of the best fit of a combined 24-h period cosine function of the form: $Y(ti) = M + A\ cos(\omega ti + \phi)$, where M, A, and ϕ represent the mesor (mean level), the peak to trough amplitude, and the daily acrophase (peak time) respectively; and ωti corresponds to the fixed 24-h period of the rhythm. Each period was validated at $P < 0.05$.

The parameters obtained for each rat in both BVL and SO groups were then compared before injection, 5 to 7 days after injection, and 1 mo later using a repeated-measures ANOVA (Sigmastat) and the nonparametric Mann-Whitney test.

Periodogram analyses cannot be performed during the 10 to 50 h just after vestibular lesion because of the increasing mesor of Temp. In an effort to complete the analysis of the early and strongly decreased Temp observed in rats in the BVL group just after injection and to discriminate between short-term hypothermia related to anesthesia and the vestibular loss effect in the data recorded, we chose to use nonlinear modeling focusing on the first 4 days after lesion/placebo injection (see results in the *Decreased Core Temperature* section). This modeling was carried out using R 2.15.1 (www.r-project.org) software.

We observed a nonlinear pattern of Temp stabilization after vestibular lesion-induced temperature drop. Figure 1 presents the data together with short-term and long-term trends determined using a smoothing procedure. To capture this effect, we selected a generalized nonlinear least squares estimation (package nlme) as a modeling approach.

We fitted three simple models to the data, accounting for different temperature changes after lesion:

$$\mathrm{m0}: temp = fin + fin^{BVL}\,\mathrm{II}(BVL)$$

$$\mathrm{m1}: temp = fin - p_1 \cdot e^{-p_2 \cdot \mathrm{t}}$$

$$\mathrm{m2}: temp = fin - \left[p_1 + p_1^{BVL} \cdot \mathrm{II}(BVL)\right] \cdot e^{-\left[p_2 + p_2^{BVL} \cdot II(BVL)\right] \cdot \mathrm{t}}$$

Here, *temp* and *t* are the temperature and the time variable, respectively, II(*BVL*) represents a dummy variable (value 1 for those observations belonging to the BVL group and zero for the other group). Moreover, fin, fin^{BVL}, $p1$, $p2$, p_1^{BVL}, and p_2^{BVL} correspond to the parameters to be estimated. That is, model m0 corresponds to the simple situation of two different constant temperatures for BVL and control rats. Model m1 allows for an exponential temperature recovery in time. The constant intercept *fin* represents the final temperature from which an exponentially decreasing difference is subtracted (for

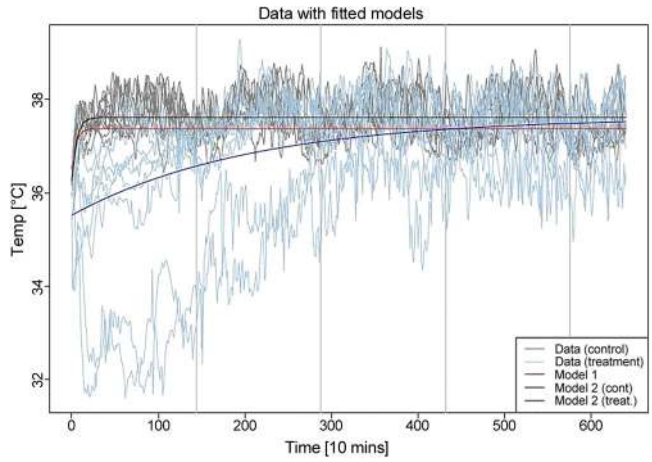

Fig. 1. Traces of all recordings of temperature data fitted with m1 and m2 models immediately after transtympanic injection in the sham-operated (SO, control) group and in the bilateral vestibular loss (BVL, treatment) group. The solid red line represents the inferior model, m1. The solid black and blue lines result from the preferred model, m2. The first 4 days are represented by gray vertical lines. After transtympanic injection, we observed a decrease of 2.09 ± 0.3°C in BVL rats (t = 2.43, $P = 0.015$) with a differential decrease of −0.74 ± 0.3°C in BVL rats (t = 2.43, $P = 0.015$) compared with control.

Table 1. *Mean circadian parameters of temperature rhythm in rats before lesion, 5–7 days after injection, and 1 mo after*

	Period, h	Amplitude, °C	Acrophase, h	Mesor, °C
BVL rats				
Control condition	24.1 ± 0.33	0.82 ± 0.28	1:32 ± 1:44	37.1 ± 0.69
5–7 Days after BVL	16.9* ± 10.22	0.66* ± 0.16		37.45 ± 0.42
31 Days after BVL	23.9 ± 0.11	0.96 ± 0.12	2:15 ± 0:33	37.52 ± 0.19
SO rats				
Control condition	24.1 ± 0.16	0.80 ± 0.18	1:55 ± 1:30	37.52 ± 0.19
5–7 Days after BVL	24 ± 0.15	0.69 ± 0.13	1:52 ± 2:00	37.6 ± 0.17
31 Days after BVL	23.9 ± 0.14	0.98 ± 0.18	2:37 ± 0:50	37.56 ± 0.23

BVL, bilateral vestibular lesion; SO, sham-operated. ANOVA revealed that rats in the BVL group presented a significant (*) main ultradian component ($F_{3,34} = 4.3$, $P < 0.05$) compared with the mean period of rats in the sham-operated group 5 days after lesion ($P < 0.01$) and the period recorded before the vestibular lesion in rats in the BVL group ($P < 0.05$). Amplitude remained significantly decreased (0.66 ± 0.16°C) at days 5–7 ($P < 0.02$).

$t = 0$, the difference is exactly $p1$). The parameter p_2 determines how fast the recovery takes place. The most complex model, m2, allows both the difference and recovery speed for rats in the BVL group to differ from that of the control group. As an example, the first parameter, p_1, is the coefficient of the control group in the equation above, and $p_1 + p_1^{BVL}$ corresponds to the coefficient of the treatment group.

Linear correlation analysis. A linear correlation analysis was performed between the two variables in both groups during the first 4 days following the injections to test the possible effect of Act (mobility and abnormal walking) on Temp daily rhythm in rats in the BVL group. Special attention was paid to spurious relationships (22). Therefore, all series (9 for Temp and 9 for Act, a total of 18 per group) were tested for stationarity with a Kwiatkowski-Phillips-Schmidt-Shin (KPSS) test, and differentiated if necessary.

To explore the relationship of Temp and Act more deeply, the analysis of a correlation patterns between temperature and activity was split from *day 0* (transtympanic injection) into four time periods: *day 1*, *day 2*, *day 3*, and *day 4+* (the last day was slightly extended to *day 5*). The estimated correlation coefficients for each day were tested using a nonparametric Wilcoxon-Mann-Whitney test to evaluate the hypothesis of a location (median) different to zero in each group.

RESULTS

Decreased Core Temperature

All rats in the BVL group showed a sharp albeit brief decrease in Temp after bilateral lesion followed by a progressive rise for 10–50 h, with variation between individual rats. Preliminary analysis using two simple linear mixed effects models, one with and one without group effect, allowed us to confirm that central body temperatures were similar in rats in the BVL (37.0 ± 0.96°C) and SO (37.4± 0.55°C) groups before lesion (L.-ratio 1.76, $P = 0.184$; t = 1.33, $P = 0.202$).

Model comparison showed that m1 was clearly superior to m0 (L.-ratio = 31.3, $P < 0.0001$), and that m2 was also superior to m1 (L.-ratio = 81.5, $P < 0.0001$). The estimated model was as follows: $temp = 37.61 - [1.35 + 0.74 \cdot \mathrm{II}(BVL)] \cdot e^{-[5.46 + 200.94 \cdot \mathrm{II}(BVL)] \cdot t}$.

Estimated parameters of the m2 model highlight two findings. First, after transtympanic injection, Temp showed a significant decrease of 2.09 ± 0.49°C in rats in the BVL group. The control group showed a slight anesthesia-induced fall in temperature (1.35 ± 0.18°C; t = 7.14, $P < 0.0001$) with a differential decrease of −0.74°C ± 0.3°C compared with rats in the BVL group (t = 2.43, $P = 0.015$). Second, the temperature then progressively rose in an asymptotic curve in the BVL group (Fig. 1).

Daily Rhythms

Periodogram analysis of recordings before the lesion demonstrated that each rat exhibited a normal daily rhythm for Temp and Act without any difference between the two groups for period, acrophase, amplitude, and mesor (Tables 1 and 2). These daily rhythms were synchronized with the light/dark cycle.

Core temperature. Daily rhythms of Temp were strongly disrupted in the first days after arsanilate injection in the BVL group (Fig. 2). Following the decrease in Temp, periodogram analysis demonstrated a transient inhibition or switch to an ultradian rhythm (τ <20 h, $P < 0.01$) until the 24-h period recovery (24.1 ± 0.33 h) in the BVL group within 2 to 8 days (115.8 ± 68.6 h), showing variability ($4 < \tau < 8$ h) in individual rats (see examples in Fig. 3). Daily rhythmicity was therefore recovered. In comparison, most rats in the SO group did not show any change in daily period (24.2 ± 1.5 h, $P = 0.85$) just after placebo injection compared with the control condition. Amplitude was still strongly decreased throughout the ultradian episode in the BVL group and was still significantly affected at *days 5* to *7* (0.66 ± 0.16°C) compared with the control condition (0.82 ± 0.28°C; $P < 0.02$).

The mean period remained significantly altered in the BVL group at *days 5* to *7* compared with the SO group (24 ± 0.15 h; $P < 0.02$). Four rats in the BVL group recovered a daily rhythm near to 24 h (at *days 5* to *7*) faster than the other rats leading to a mean period of 16.9 ± 10.22 h at that time point (Table 1). However, the amplitude was not significantly different between the two groups, probably due to the slight but not significant damping observed in the SO group (see Table 1).

A daily period of Temp (23.9 ± 0.11 h) was observed 1 mo later in the BVL group, similar to the results observed before the lesion (24.1 ± 0.33 h) and similar to results in the SO group (23.9 ± 0.14 h) (TSA, $P < 0.05$). Amplitude, mesor, and acrophase were also similar to those recorded before the lesion (Table 1).

Locomotor activity. No significant differences were observed in the mean level of Act from lesion to *day 7* (ANOVA, $P = 0.6$) between the BVL (2.40 ± 0.55 count/min) and SO (2.55 ± 0.69 count/min) groups. However, the BVL group did not exhibit rhythmicity until 7 days after injection, switching instead to an ultradian (τ <20 h) or nonrhythmic component (Fig. 2). No significant acrophase could be detected after lesion in the BVL group (Table 2). Conversely to Temp, Act amplitude only seemed to be

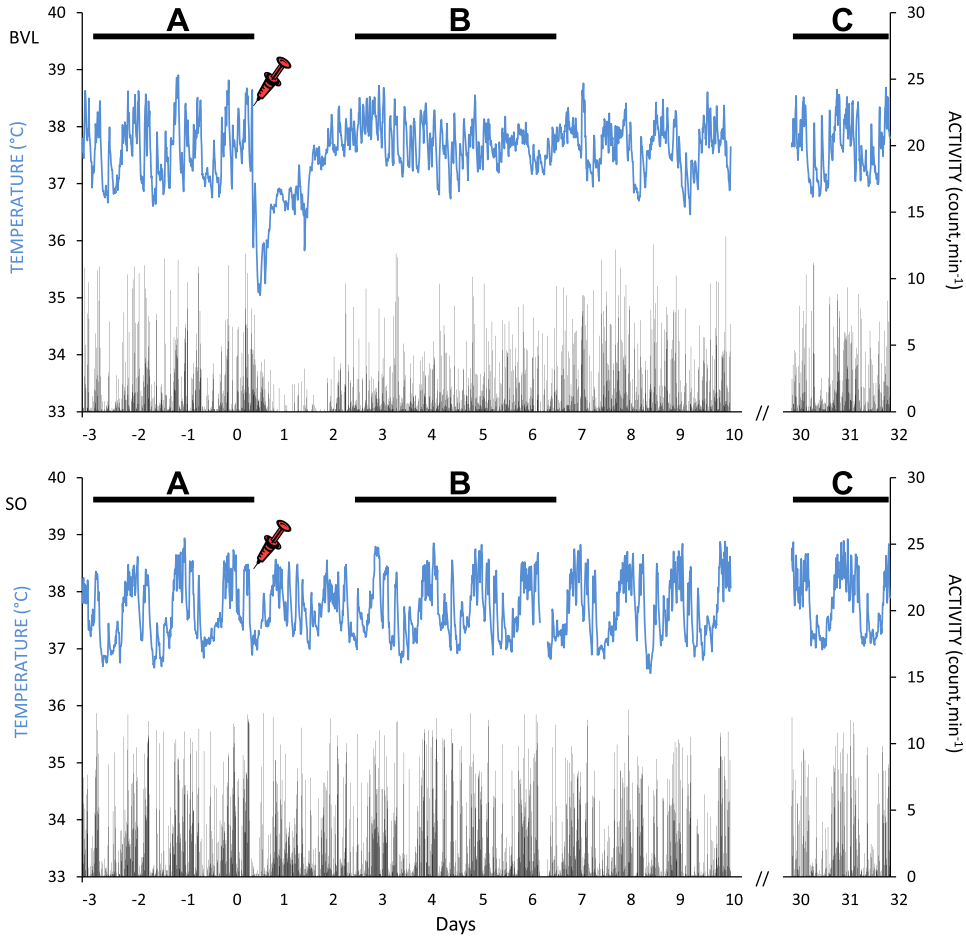

Fig. 2. Traces of recordings of temperature (curves) and locomotor activity (histograms) rhythms in one rat in the BVL group (*top*) and one rat in the SO group (*bottom*). *A*: before transtympanic injection, rats in both the BVL and SO groups exhibited a similar daily rhythm of both temperature and activity (*day −3* to *day 0*). Immediately after arsanilate injection at *day 0* (syringe symbol) a large drop in temperature followed by progressive recovery was observed in the BVL rat. *B*: following recovery, periodogram and COSINOR analysis revealed that the temperature and activity daily rhythms were inhibited or changed to a main ultradian period (τ) (τ <20 h, $P < 0.01$) until *day 7* as shown by the oscillations in temperature and activity values in the BVL rat compared with the SO rat. *C*: daily rhythms of both temperature and locomotor activity were recovered at *day 30* in the BVL rat compared with the SO rat.

decreased in the BVL group at *days 5* to *7* compared with the control condition (1.92 ± 0.56 vs. 3.54 ± 2.22 count/min, $P = 0.06$). The SO group exhibited a daily period of locomotor activity rhythm soon after saline injection (24.03 ± 0.3 h). There was a relative but not significant change (21:22 ± 8:03; $P = 0.09$) in the acrophase at *days 5* to *7* compared with the control condition (02:53 ± 1:34 h) with no modification of the amplitude (2.66 ± 0.82 count/min) or mesor (2.58 ± 0.68 count/min).

The period remained altered in the BVL group at *days 5* to *7* compared with the SO group (24.03 ± 0.3; $P < 0.02$). Two rats in the BVL group recovered a daily rhythm nearly 24 h faster (from *day 5* to *day 7*) than the other ones, leading to a mean period of 12.33 ± 10.33 h at that time point (Table 2). The amplitude tended to be decreased only in the BVL group (1.92 ± 0.56 count/min) compared with the SO group (2.66 ± 1.82 count/min), probably due to the slight but not significant damping observed in the SO group (see Table 2). The mesor (2.44 ± 0.42 count/min) did not change significantly in the BVL group compared with the SO group (2.58 ± 0.68 count/min) or the rhythm recorded before transtympanic injection in the control condition (2.87 ± 1.33 count/min) (Table 2).

One month after the vestibular lesion, all daily rhythm parameters for Act (Table 2) were recovered in rats in the BVL group compared with those obtained in control conditions or in the SO group.

Linear Correlations for Activity and Temperature

The preliminary KPSS test revealed that the stationarity hypothesis was rejected for each of the nine data series of Temp and Act in the SO group. Moreover, in the BVL group, the stationarity hypothesis was rejected for all 18 series. Therefore, we applied a first-order differencing to all series before testing for linear correlation. In the various series the stationarity hypothesis was not rejected in the SO or BVL groups. Consequently, disruption of Temp daily rhythm does not seem to be related to modulation of locomotor activity induced by a vestibular syndrome.

No significant correlation between Temp and Act was present in the BVL group after vestibular lesion in contrast to the SO group ($P < 0.01$). In the SO group, the average estimated correlation coefficient was 0.20 ± 0.07. In contrast, the average estimated correlation coefficient was 0.013 ± 0.18 in the BVL group, which was not significantly different from zero.

Figure 4 displays box plots of the correlation coefficients in both SO (*left*) and BVL (*right*) groups for *days 1* to *4+*. In the BVL group, the average correlation coefficient oscillated more or less around 0 for the first 4 days even if we noticed an increase up to 0.06 at *day 4+*. Carrying out a Wilcoxon-Mann-Whitney test resulted in the following *P* values (corrected by the Holm's method): 1.0 at *day 1*, 0.3 at *day 2*, 1.0 at *day 3*, and 1.0 at *day 4*. Therefore, the hypothesis of a location different

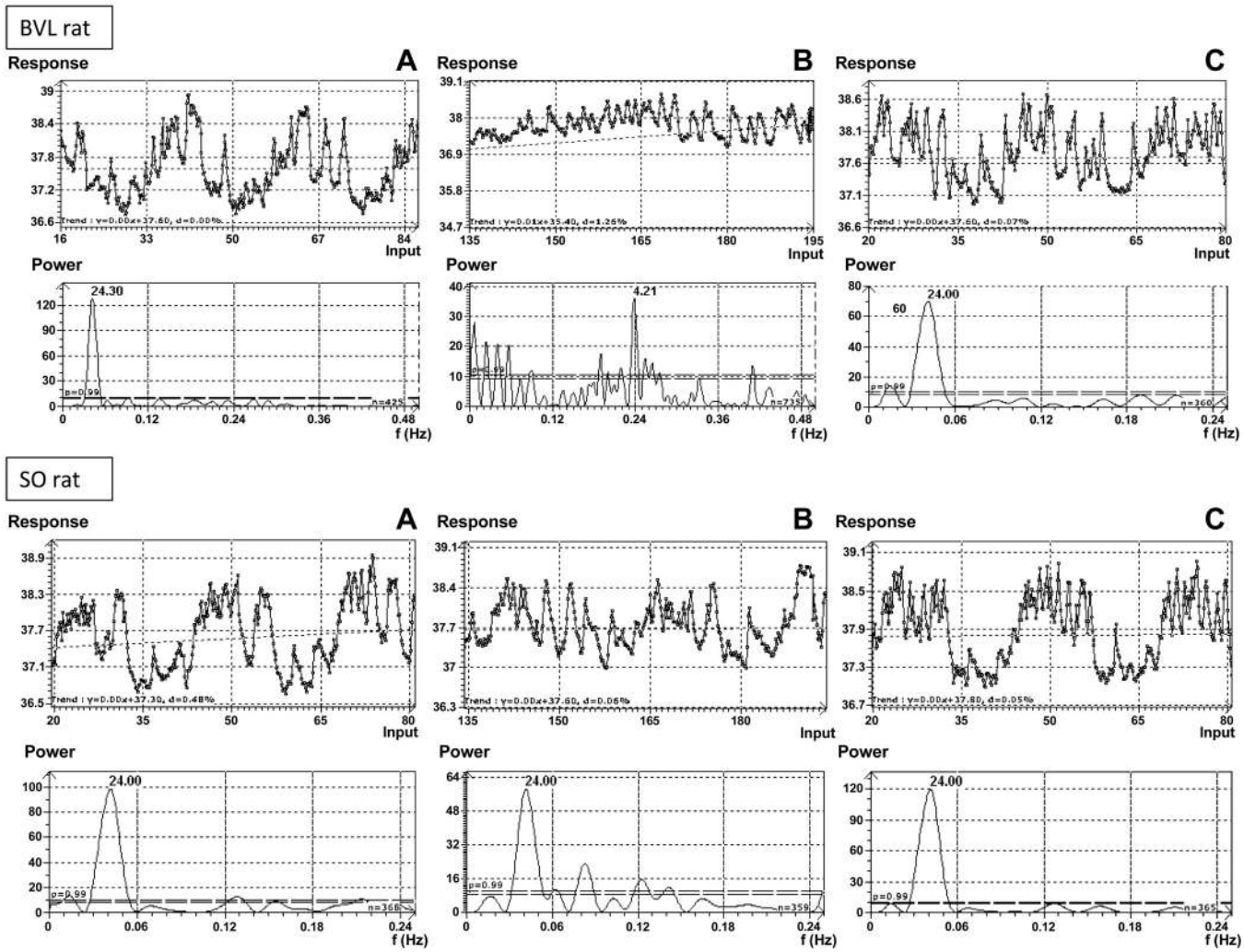

Fig. 3. Results of the Lomb and Scargle periodogram analysis. Each frame represents an example of a BVL (*top*) and an SO (*bottom*) rat. As the daily parameters of Temp and Act evolved throughout the 7 days following the lesion, a 60-h sliding window (2.5 × the 24-h period) was moved in increments of 4 h to calculate the speed of adjustment, which is the time required for resynchronization with the external synchronizers. The analysis of the rat response to transtympanic injection (top graphs for each BVL and SO rat) are expressed in power where each peak represents the period of the daily rhythm in hours. *A*: the 24-h period was detected before lesion in both rats. *B*: after lesion the BVL rat showed a main ultradian rhythm of temperature (4.21 h in this case), whereas the period remained unchanged in control rats. *C*: the period of the daily rhythm of temperature was then returned to the same period observed before transtympanic injection in the BVL rat and compared with the SO rat.

from zero was not rejected for any time period in the BVL group, demonstrating the lack of Temp/Act correlation for each day in the BVL group. However, we may suppose that the correlation started to recover from *day 4*.

On the other hand, the average correlation coefficients in the control group were significantly different from zero for each day (see Figure 4). The Wilcoxon tests rejected the null hypothesis (location to zero) for each time period (all $P =$

Table 2. *Mean circadian parameters of activity rhythm in rats before lesion, 5–7 days after injection, and 1 mo after*

Group activity, count/min	Period, h	Amplitude, °C	Acrophase, h	Mesor, °C
BVL group				
Control condition	24.3 ± 0.4	3.54 ± 2.22	3:03 ± 0:43	2.87 ± 1.33
5–7 Days after BVL	12.33* ± 10.33	1.92 ± 0.56		2.44 ± 0.42
31 Days after BVL	23.95 ± 0.21	2.78 ± 0.5	2:39 ± 1:12	2.73 ± 0.57
SO group				
Control condition	24.1 ± 0.21	2.84 ± 1.24	2:53 ± 1:34	2.71 ± 0.84
5–7 Days after BVL	24.03 ± 0.3	2.66 ± 1.82	21:22 ± 8:03	2.58 ± 0.68
31 Days after BVL	23.94 ± 0.46	2.82 ± 1.60	3:12 ± 1:15	2.49 ± 0.84

ANOVA revealed a significance difference ($P < 0.001$) between the mean period of both groups 5 days after lesion ($P < 0.001$) and in the BVL group in the period before the vestibular lesion ($P < 0.001$), with the BVL group exhibiting a main ultradian component.

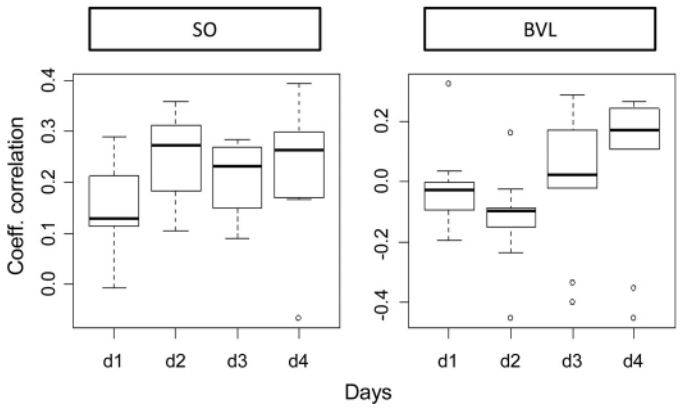

Fig. 4. Box plots of the estimated correlation coefficients in control (*left*) and BVL (*right*) groups from *day 1* to *day 4+*. Temperature and activity correlation was not significantly different from 0 from *day 1* to *day 4+* in the BVL group, whereas a correlation occurred in the control/SO group.

0.0156), demonstrating that a Temp/Act correlation occurred during each of the first 4 days in the SO group.

DISCUSSION

Our study showed that the loss of vestibular inputs impaired daily rhythmicity of core temperature and locomotor activity in semipigmented rats. Vestibular lesion induced a switch to an ultradian rhythm during the days following the vestibular lesion, similar to that observed after manipulation of the light/dark cycle (such as LL) through the visual system in healthy rats (9, 28). These findings strongly suggest that the vestibular system constantly influences hypothalamic regulation of daily rhythms on Earth, as previously highlighted under artificial hypergravity conditions in control mice compared with mice devoid of vestibular gravisensors (18, 46).

Unexpectedly, both vestibular loss in our study and vestibular stimulation by centrifugation at 2 G in previous studies (18, 27, 46, 49, 51) similarly inhibited daily rhythms of Temp in rodents.

The vestibular organ sends incessant tonic or transitory phasic action potentials, conveyed via the vestibulo-cochlear VIIIth cranial nerve and generated by hair cells located within the otolithic sensors or semicircular canals (21, 53). The signals are integrated and relayed to the supramesencephalic projections by the vestibular nucleus (10). Head movements and gravitational force changes are then encoded and integrated within the vestibular nuclei, which are formed from birth to adulthood in mammals (32). Despite the paucity of evidence of a monosynaptic connection, one main indirect neuronal pathway has been shown to exist from the vestibular nuclei to the SCN. Vestibular inputs have been shown to be relayed by the intergeniculate leafet (IGL) nucleus through the geniculohypothalamic tract, where both photic and nonphotic stimuli influence circadian rhythmicity (29).

As expected, c-Fos expression is increased in the SCN during 2 G stimulation for 1 wk compared with het(−/−) mice devoid of otolithic inputs (17). Moreover, c-Fos expression is not observed in the SCN of rats after a 1-h light pulse at 2 G, whereas it is robust when rats are exposed to a similar 1-h light pulse at the same circadian time at 1 G (47). Altogether, these data support the possibility of a direct effect of vestibular inputs on the circadian clock. The loss of vestibular inputs induced by lesion here or hypergravity stimulation might exceed the range of physiological vestibular input value encountered at 1 G, which would explain the disruption of daily rhythms in both cases. In agreement with this suggestion was the acute drop in the temperature set point immediately after vestibular lesion, as already reported in previous studies in rodents (18, 27, 46, 49, 51), squirrel monkeys (15, 16), and dogs (50) exposed to hypergravity. The findings also speculatively suggest the potential influence of vestibular input on thermoregulation mechanisms (18). The vestibular nucleus might indirectly influence thermoregulation mechanisms through the imbalance between heat production and heat loss equilibrium encountered in altered gravity (19). However, thermoregulatory mechanisms in hypergravity or weightlessness did not reach a consensus (55). The role of gravity in temperature regulation through the vestibular system requires further study. Changes in body temperature regulation may be due to gravity itself, circadian disturbance, or changes in thermoregulatory effectors (heat production and heat loss) due to altered gravitational load and convective changes.

Circadian rhythms could be subjected to alteration that distorts the original output from the SCN, also called the masking effect (41), which directly alters circadian rhythms in a positive or negative way (1). Acute unilateral loss or long-term bilateral loss in humans and rodents is known to cause daily locomotor impairment, expressed as a vestibular syndrome such as gait ataxia and walking deviation in humans, and head bobbing and cycling in rodents (5, 54, 62).

Consequently, disruption of daily rhythms of Temp in our study might be masked by the daily increases or decreases in global motion due to a vestibular syndrome. However, the level of locomotion remained similar between the lesioned and control groups after transtympanic injection as previously reported in het(−/−) vs. control mice during 2 G centrifugation (19). This suggests that vestibular loss has only a qualitative and not a quantitative effect on locomotor activity.

Here we confirm that global locomotor activity in the days after transtympanic injection was similar in BVL and SO rats according to one of our recent papers using a similar vestibular lesion model by arsanilate (61). In that previous work we showed that diurnal and nocturnal locomotor activity independently remained at the same level in both groups, whereas rats with BVL suffered from a vestibular syndrome maximal between *day 2* and *day 7* after lesion compared with unharmed control rats (60). Consequently, if rats with BVL suffer from a vestibular syndrome, their diurnal/nocturnal global locomotor activity remained similar to that of rats in the SO group, but it was more divided within the first days after lesion. This suggests that the sleep/wake state was probably more fragmented; however, the total sleep time might not have changed, and thus would not influence the daily rhythms of Temp and Act (60, 61).

Although daily rhythms of Temp and Act in rats in the control group recovered within about 1 wk, rats in the BVL group still showed a high level of vestibular symptoms at 1 mo due to the lack of compensation inherent in a one-step bilateral lesion (5, 62). Consequently, walking and postural parameters between rats in the SO and BVL groups remained similar up to 1 mo after vestibular lesion, in contrast to daily rhythms that independently recovered in the BVL group after 1 wk. This

suggests that unbalance or locomotor vestibular disorders induced by vestibular syndrome cannot influence the disruption of daily rhythms. Second, no correlation between Temp and Act in the BVL group was observed, demonstrating that the decrease in the circadian rhythm of Temp and the vestibular locomotor syndrome were dissociated within the first days following the lesion in rats in the BVL group. Moreover, the daily rhythm of Temp was recovered shortly before those of Act in some rats in the BVL group, suggesting that Act does not influence Temp during the recovery process.

In mammals, information related to the light/dark cycle is considered to be the main exogenous time cue (i.e., zeitgeber) allowing synchronization of the endogenous clock located in the SCN with the environment in which the organism has evolved. It is mainly and directly conveyed by the visual system from the retina to the SCN via a monosynaptic pathway, the retinohypothalamic tract (33, 44), and indirectly by the intergeniculate leafet via the geniculohypothalamic tract (24).

Intensity of the stimulation of the vestibular system would then be dependent on the light/dark cycle, the period of the species, and the level of locomotor activity, including body and head motion. In rats, onset and end of nocturnal activity could be a signal that helps to enhance the stability and precision of the entrainment to the light/dark cycle (42). Vestibular input could thus be complementary and in synergy with the visual system and thus modulate biological rhythms and sleep/wake cycle regulation. In fact, visual and vestibular afferences to the IGL projecting to the SCN are strongly entangled anatomically (29). Consequently, the effect of activity onset on the sleep/wake cycle in rats (42) and physical activity on circadian rhythm amplitude and phase in humans (2, 8, 39), which were previously explained by endogenous changes (3), might be partially conveyed by the vestibular sense organ. This in turn might reinforce synchronization of the circadian rhythms via the IGL.

This effect is specific to the vestibular system because the visual and proprioceptive systems were still functional in rats in both the BVL and control groups, with the latter showing no circadian changes. Whereas the otolithic signal encoding gravity and linear acceleration is probably involved in vestibulo-SCN modulation, as suggested by het(−/−) mice (18), the role of semicircular canal inputs in circadian rhythm modulation remains to be explored. Het(−/−) mice are not only deficient in otolithic signals, but probably also in canal-mediated vestibulo-ocular reflexes (25), so het(−/−) mice also suffer from central integration impairment of semicircular canal inputs. Additionally, the IGL integrates afferences from the medial vestibular nucleus, with the latter receiving input from both semicircular canals and saccular receptors (29). These neuroanatomical data thus suggest that semicircular information encoding head motion during the active period, whether nocturnal or diurnal, varying with species, cannot compensate for otolithic deficiency. This suggests that semicircular canals could be involved in vestibulo-SCN interactions.

Our spectral analysis, which has not been previously reported in studies inducing vestibular stimulation by centrifugation in rodents, showed that Temp switched to an ultradian rhythm after the loss of vestibular information. These subharmonic rhythms became the main rhythmic component within 4 to 8 h after lesion in vestibulo-deficient semipigmented rats. An ultradian rhythm has been reported using visual information manipulation in rodents exposed to constant bright light (28), probably caused by a desynchronization of neuronal electrical activity in the SCN (48). Ultradian rhythms >4 h are completely suppressed in SCN-lesioned rats (30) but are still expressed with a 4- to 8-h period in non-SCN-lesioned rats kept in LL (9), suggesting a key role of multiple oscillators in the SCN in the generation of ultradian rhythms. However, this persistence of ultradian rhythms in LL may also signify that mechanisms distinct from the generation of circadian rhythms in the SCN are involved (58). Hypothetically, suppression of vestibular input could *1*) disrupt the circadian mechanisms of the SCN for a few days or *2*) trigger non-SCN mechanisms for a few days before compensation by the light/dark cycle.

The recovery of daily rhythmicity of Temp and Act is probably counterbalanced by the predominant influence of the light/dark cycle in our semipigmented rats that were highly sensitive to light compared with albino species. Another possibility is the spontaneous progressive recovery of the firing rate of vestibular nucleus neurons. This recovery is observed within hours to days following a bilateral labyrinthectomy, which is probably compensated for by the integration of nonlabyrinthic information at the vestibular nucleus level through visual and proprioceptive inputs at the vestibular nucleus [see review in (40)]. Neurogenesis in vestibular nuclei has been demonstrated after unilateral lesion in cats, which could contribute to vestibular and postural function recovery (34, 59). The behavioral/temperature changes could also result from an acute response to inflammation or tissue degeneration. Further explorations in which unknown zeitgeber (DD, LL) could conceal the lesion effect are required to confirm the vestibular contribution to circadian rhythm regulation.

In conclusion, the loss of vestibular sense-organ input induces an early disruption of daily rhythms of Temp and Act, and generation of ultradian rhythms for about 1 wk after the lesion and before progressive recovery. Vestibular inputs on Earth seem to be involved in daily rhythm regulation by influencing the SCN independently of direct proprioceptive and muscular inputs, as well as other nonphotic stimuli such as physical activity influencing circadian amplitude and/or entrainment of Temp (2, 39) and melatonin rhythms (4, 7, 8). Although we do not know whether the direction of the modulation (disruption) is related to our model potentially mimicking a 0 G exposure or to a global nonspecific input, the vestibular system might be a synchronizer of the biological clock. Our findings thus raise the question of whether circadian rhythms might be altered in the context of vestibular pathology in humans, and whether this could be explored as a marker of disease severity.

APPENDIX

Supplemental Method

We observed that vestibular lesion induced a nonlinear pattern with a stabilization of Temp after the surgery-induced temperature drop. Figure 1 presents the data together with short-term and long-term trends determined by a smoothing procedure. To capture this effect, we selected generalized nonlinear least squares estimation (package nlme) as a modeling approach. To account for serial within-subject correlation of our longitudinal data, we imposed an error structure that was typical for a time-series AR (1). Heteroscedasticity between the treatment and control groups was integrated into the residual variance function.

To evaluate whether a variable has a significant effect, we followed the approach described in (52) and compared models with and without

the respective variable of interest using a likelihood ratio test, and additionally considered the model selection Akaike information criterion (AIC) and Bayesian information criterion (BIC). For comparison of nonnested models, only AIC and BIC tests were taken into consideration.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

T.M., G.Q., D.D., P.D., and S.B. conception and design of research; T.M., B.P., and S.B. performed experiments; T.M., B.M., and J.B. analyzed data; T.M., B.M., G.Q., D.D., P.D., and S.B. interpreted results of experiments; T.M., J.B., G.Q., D.D., and S.B. prepared figures; T.M., B.M., J.B., G.Q., D.D., P.D., and S.B. drafted manuscript; T.M., B.M., J.B., G.Q., D.D., P.D., and S.B. edited and revised manuscript; T.M., B.M., J.B., G.Q., D.D., P.D., B.P., and S.B. approved final version of manuscript.

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