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Temporal phasing of locomotor activity, heart rate rhythmicity, and core body temperature is disrupted in VIP receptor 2-deficient mice

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Hannibal J, Hsiung HM, Fahrenkrug J. Temporal phasing of locomotor activity, heart rate rhythmicity, and core body temperature is disrupted in VIP receptor 2-deficient mice. Am J Physiol Regul Integr Comp Physiol 300: R519–R530, 2011. First published December 22, 2010; doi:10.1152/ajpregu.00599.2010.—Neurons of the brain’s biological clock located in the hypothalamic suprachiasmatic nucleus (SCN) generate circadian rhythms of physiology (core body temperature, hormone secretion, locomotor activity, sleep/wake, and heart rate) with distinct temporal phasing when entrained by the light/dark (LD) cycle. The neuropeptide vasoactive intestinal polypeptide (VIP) and its receptor (VPAC2) are highly expressed in the SCN. Recent studies indicate that VIPergic signaling plays an essential role in the maintenance of ongoing circadian rhythmicity by synchronizing SCN cells and by maintaining rhythmicity within individual neurons. To further increase the understanding of the role of VPAC2 signaling in circadian regulation, we implanted telemetric devices and simultaneously measured core body temperature, spontaneous activity, and heart rate in a strain of VPAC2-deficient mice and compared these observations with observations made from mice examined by wheel-running activity. The study demonstrates that VPAC2 signaling is necessary for a functional circadian clock driving locomotor activity, core body temperature, and heart rate rhythmicity, since VPAC2-deficient mice lose the rhythms in all three parameters when placed under constant conditions (of either light or darkness). Furthermore, although 24-h rhythms for three parameters are retained in VPAC2-deficient mice during the LD cycle, the temperature rhythm displays markedly altered time course and profile, rising earlier and peaking 4–6 h prior to that of wild-type mice. The use of telemetric devices to measure circadian locomotor activity, temperature, and heart rate, together with the classical determination of circadian rhythms of wheel-running activity, raises questions about how representative wheel-running activity may be of other behavioral parameters, especially when animals have altered circadian phenotype.

neurotransmitter; circadian rhythms; entrainment; jetlag; running wheels; telemetry

THE CIRCADIAN SYSTEM consists, besides the master clock in the hypothalamic suprachiasmatic nucleus (SCN), of a food-entrainable oscillator most likely located in the brain, although of unknown localization (5), and peripheral clocks in most peripheral organs (9). The biological clock in the SCN is synchronized (entrained) by the light/dark (LD) cycle and generates distinct temporal phasing of the core body temperature rhythm, hormone secretion, activity (sleep/wake cycle), and heart rate to ensure maximal adaptation for survival and reproduction. The light-entrained rhythmicity can be influenced by nonphotic stimulation (activity, food, sleep deprivation, etc.) (9) or directly by light, which may lead to light-induced phase shift of the circadian rhythm and/or masking [light-inhibiting activity at night (negative masking), whereas darkness presented during the day leads to increased activity (positive masking)] (20). The basic elements of the SCN clock are a group of so-called clock genes expressed within most of the SCN neurons. The clock genes drive the molecular clock via complex autoregulatory feedback loops regulating their own transcription (25). Importantly, the individual SCN neurons need to be synchronized to a functional clock driving circadian rhythm (19, 25). The neuropeptide vasoactive intestinal polypeptide (VIP) and its receptor VPAC2, are highly expressed in the SCN (16), and the VIP-containing neurons project to cells within the entire SCN and other brain areas outside the nucleus (1). Recent studies indicate that VIPergic signaling plays an essential role in maintenance of ongoing circadian rhythmicity by synchronizing SCN cells and by maintaining rhythmicity within individual neurons (3, 11, 18, 19). The mutant mice lacking VIP (3, 6) or the VPAC2 receptor (3, 11, 18, 19) do not display robust circadian rhythm of physiology and behavior, although tissue outside the SCN sustains circadian rhythm in clock gene expression (31). Neither the mechanisms nor the phenotype of the neurons by which VIP/VPAC2 signaling exerts its function, leading to this dysfunctional clock, are well known. In VPAC2 receptor-deficient mice (VPAC2−/−) SCN neurons continue to oscillate, but due to different period length in each cell, desynchronization of SCN output signaling occurs (13, 19). Lack of synchronization most likely leads to the aberrant gating of photic inputs in these animals (13, 19) and disturbed metabolic rhythm and feeding behavior (4, 31). The VPAC2−/− animals can, however, be entrained on a restricted feeding schedule probably via a food-entrainable circadian oscillator operating independently of the SCN (31). Thus, VIP/VPAC2 knockout mice represent a unique model in which the circadian information from the SCN is not transmitted, a model different from mice lacking core clock genes or SCN lesions leading to neuronal loss and/or damaged neuronal connectivity of SCN neuronal pathways. The core body temperature displays circadian rhythmicity, but the temperature rhythm in VIP/VPAC2-deficient mice and simultaneous measurements of activity rhythm have so far not been examined in these animals. Consequently, we implanted telemetric devices to simultaneously measure core body temperature, spontaneous activity, and heart rate in a strain of VPAC2-deficient mice (2)
and compared these observations with observations made from mice examined by wheel-running activity.

**MATERIAL AND METHODS**

**Animals**

Details on the generation of VPAC2 knockout mice on a C57/6 background have been described (2). Wild-type and VPAC2−/− mice were bred from heterozygote animals over more than five generations before being included in the study. All animals (equal number of each gender) were included in the study at 10–12 wk of age and maintained in a 12:12-h light-dark (LD) cycle and housed in individual cages with food (Altromin 1324; Altromin Spezialfutter, Germany) and water ad libitum unless otherwise stated. Animals were treated according to the principles of Laboratory Animal Care (Law on Animal Experiments in Denmark, publication 382, June 10, 1987) and under Danish Veterinary Authorities (Dyreforsoegstilsynet) license no. 2008/561-1445.

**Methods**

**Measurements of wheel-running activity rhythms.** A total of 16 wild-type and 16 VPAC2−/− mice (8 males and 8 females in each group) were transferred to individual cages equipped with a running wheel (diameter: 23 cm, 4 magnets/wheel) in ventilated, light-tight chambers with controlled white lighting (300 lux). Wheel-running activity was monitored by an online personal computer connected via a magnetic switch to the Running Wheel Activity System (consisting of QA-4 activity input modules, DP-24 dataports, and Vital View data acquisition system version 4.1; Mini Mitter, Sunriver, OR) (10). Wheel revolutions were collected continuously in 10-min bins. Animals were entrained to a LD cycle [lights on at 7:00 AM designated Zeitgeber time (ZT) = 0, off at 7:00 PM = ZT12] for at least 14 days before start of experiments.

**Measurements of gross locomotor activity, core body temperature and heart rate.** In a total of 12 animals (3 wild-type males and 3 female animals, 3 homozygous males and 3 female animals) a radio transmitter device (PDT-4000 HR E-mitter; Respironics, Mini-Mitter) was used to measure heart rate, core body temperature, and gross motor activity under general anesthesia [a subcutaneous injection of a mixture of fentanyl (0.20 mg/kg body wt), fluanisone (6.25 mg/kg body wt), and midazolam (3.13 mg/kg body wt)] implanted in the abdominal cavity by sterile techniques with cardiac electrodes placed in the chest muscles (see E-Mitter Implantation Procedure, part no. 910–0014-05 Rec C; Respironics, Mini-Mitter). After the operation, the mice received antibiotic treatment by a single dose of ampicillin. Postoperative pain was reduced by a single subcutaneous injection of 5 mg/kg body wt of carprofen. Radio signals for all three physiological parameters were recorded by a receiver board (ER-4000 energizer receiver) underneath the cage housing each animal and stored via Vital View Data Acquisition System (version 4.1; Mini Mitter) on a personal computer in 6-min bins. The mice were allowed to recover from surgery for at least 2 wk before onset of the experiments. The ambient temperature was 21–22°C within the cabin that stored 12 cages that were not equipped with running wheels.

**Light source and light-intensity measurements.** White lighting was delivered from fluorescent tubes placed on top of each cage. The light intensity could be adjusted from 10 to 900 lux via resistance. Light intensity was measured using an Advantest Optical Power meter TQ8210 (MetricTest, Hayward, CA), and measurements were determined at settings of 514 nm, 300 lux (115.0 μW/cm²), 70 lux (19.1 μW/cm²), and 10 lux (4.3 μW/cm²).

**Experimental Design**

Endogenous period evaluated in running wheels. A free-running period (τ) was assessed during days 4–18 in constant darkness (DD) using ClockLab (ActiMetric Software; Coulbourn Instruments, Wilmette, IL).

Eight-hour phase delays/advances (jetlag) at different light intensities evaluated using wheel-running activity. To examine whether the changed sensitivity to light influenced the time of reentrainment during large shifts of the external LD cycle, animals were exposed to 8-h phase delays of the external LD cycle followed by an 8-h phase advance after reentrainment to the new LD cycle. Reentrainment was defined as the first day of consecutive days in which the onset occurred within 30 min in phase with the new LD cycle. These experiments were performed at light intensities of 300, 70, and 10 lux.

**Data Analysis**

Data obtained from the Mini Mitter Running Wheel activity system and the ER-4000 energizer receiver system were analyzed in ClockLab (ActiMetric Software, Coulbourn Instruments) running under Matlab (version 14, service pack 2 for Windows; MathWorks, Natick, MA) environment. Onset was determined using the onset determination function in ClockLab applied on the actograms.

**Statistics**

Statistics were performed using GraphPad Prism version 4.0. For comparison of two independent groups the Mann-Whitney U-test was used. P < 0.05 was considered statistically significant.

**RESULTS**

**Activity**

Disrupted locomotor activity (wheel running) in VPAC2−/− mice during DD. To characterize the circadian behavior in our VPAC2−/− mice, we first placed the animals in cages with standard running wheels during a 12:12-h LD cycle. Wild-type mice entrained to the LD cycle and, like the wild-type mice, VPAC2−/− mice also had their main activity during the dark phase with onset at the transition zone from light to dark. Compared with wild-type mice, however, VPAC2−/− mice ran significantly less during the night, while daytime activity was similar in the two genotypes (Table 1). When transferred into DD, wild-type mice continued to have a stable free-running rhythmic activity with a predictable onset determined by their τ of 23.50 ± 0.04 h (Fig. 1A, left, top, Table 1). All VPAC2−/− mice, on the other hand, showed already in the first cycle of DD a phase advance of the activity onset of ~6–8 h (Fig. 1A, top, right). The rhythmic activity continued during the next cycles in these mice, but when compared with wild-type mice, the onset became ill defined, indicating lack of clock control. Evidence for the lack of clock-controlled locomotor activity was substantiated by a batch analysis using the χ² periodogram and Fast Fourier transformation, which demonstrated that our VPAC2−/− mouse strain displays weak rhythmicity in DD
with several period lengths of which the most significant \( \tau \) is 23.01 ± 0.28 h. (Fig. 1, B–C, bottom, Table 1).

Prominent masking behavior during large phase shift of the external LD cycle (jetlag) in VPAC2\(^{-/-}\) mice. Previous studies in mouse strains lacking the VPAC2 receptor or VIP have indicated that activity in these mice primarily is driven by the LD cycle and not by the circadian clock (3, 6, 11, 14). To evaluate the ability to readjust behavior in our VPAC2\(^{-/-}\) mice, an 8-h shift of the LD cycle (8-h delay followed by 8-h advance) was initiated at light intensities of 300, 70, and 10 lux (Fig. 2). When evaluated by the activity onset, wild-type mice were able to phase delay their rhythmic behavior within the first or second cycle after the 8-h delay. However, when examining the activity at the offset, wild-type mice used 5–6 cycles to be fully phase shifted (Fig. 2, top, blue). When wild-type animals were 8-h phase advanced, they used 6–8 cycles before being entrained to the new LD cycle (Fig. 2, top, blue). This pattern was identical at all three light intensities (Fig. 2, top, blue).

VPAC2\(^{-/-}\) mice showed a different activity pattern (Fig. 2, middle red and merged bottom). When the LD phase was delayed by 8 h, VPAC2\(^{-/-}\) mice adjusted their activity rhythm to the new dark phase within 1–2 cycles, evaluated at both on- and offset (Fig. 2, middle red and merged bottom). Similarly, when the animals were advanced by an 8-h shift of the LD cycle, VPAC2\(^{-/-}\) mice adjusted the phase of onset and offset within 1–2 cycles (Fig. 2, middle red and merged bottom). This pattern of activity, which was found at all three light intensities, strongly indicates that the behavior in VPAC2\(^{-/-}\) mice is determined by negative masking. The role of masking behavior was further evaluated in VPAC2\(^{-/-}\) mice by placing mice in an ultradian cycle of 3.5:3.5-h LD cycle. This regimen is useful for assessing masking, because it is difficult to entrain circadian rhythms to days of 7 h or multiples of 7 h (23). As a result, the dark and light portions of the cycle move across the circadian cycle, coming to their initial positions after 1 wk, which ensures that all phases of a circadian cycle are tested (23). We found by analysis of 7 days of wheel-running activity during the 3.5:3.5-h LD cycle that wild-type mice spend 77.54 ± 13% of their activity in the dark phase, whereas the VPAC2\(^{-/-}\) mice spend 87.23 ± 1.9% of their activity in the dark phase (\( P = 0.0011 \), Mann-Whitney U-test; see also Fig. 3). This is illustrated in batch actograms in Fig. 3C and by a \( \chi^2 \) periodogram analysis of the 7-h period, which revealed that VPAC2\(^{-/-}\) mice are completely synchronized to the 3.5:3.5-h LD cycle. The wild-type mice, on the other hand, demonstrated two patterns, one robust with a period length of 7-h cycle (red line in Fig. 3A) and one with a period length close to 25 h (blue line in Fig. 3A, see also 3C, left).

Altered wheel-running activity during skeleton photoperiods in VPAC2\(^{-/-}\) mice. To further characterize the ability of light to drive the activity pattern in VPAC2\(^{-/-}\) mice, we next examined the effects of a 11:11/11 LD cycle photoskeleton regimen. All VPAC2\(^{-/-}\) mice showed similar changes in activity when placed in photoskeleton conditions (Fig. 4). During the normal LD regimen, wheel-running activity was confined to the dark phase in wild-type and VPAC2\(^{-/-}\) animals. When photoskeleton was initiated, wild-type mice consolidated an average of 83% of their total activity to the subjective night, whereas the VPAC2\(^{-/-}\) mice showed two activity periods corresponding to both their subjective day and subjective night periods with an average of 44% of their

Table 1. Summary of activity data

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>VPAC2(^{-/-})</th>
<th>P Values</th>
<th>Wild Type</th>
<th>VPAC2(^{-/-})</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>No.</td>
<td>8 (4 M, 4F)</td>
<td>8 (4 M, 4F)</td>
<td>NS</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>( \tau ), DD, h</td>
<td>23.50 ± 0.04</td>
<td>(23.01 ± 0.28)</td>
<td>NS</td>
<td>23.53 ± 0.13</td>
<td>23.53 ± 0.03</td>
</tr>
<tr>
<td>Daytime activity, 300 lux</td>
<td>1.773 ± 0.20</td>
<td>1.877 ± 0.24</td>
<td>NS</td>
<td>1.761 ± 0.24</td>
<td>1.784 ± 0.37</td>
</tr>
<tr>
<td>Nighttime activity, 300 lux</td>
<td>23.308 ± 2.642</td>
<td>13.194 ± 2.088</td>
<td>0.014</td>
<td>21.517 ± 2.079</td>
<td>25.098 ± 5.110</td>
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<tr>
<td>Total activity, LD, 300 lux</td>
<td>25.080 ± 2.770</td>
<td>15.071 ± 2.171</td>
<td>0.004</td>
<td>23.278 ± 2.317</td>
<td>26.883 ± 5.318</td>
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<td></td>
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</tr>
<tr>
<td>No.</td>
<td>6 (3 M, 3F)</td>
<td>6 (3 M, 3F)</td>
<td>NS</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>( \tau ) Activity, DD, h</td>
<td>23.90 ± 0.05</td>
<td>23.48 ± 0.32</td>
<td>NS</td>
<td>23.80 ± 0.01</td>
<td>24.04 ± 0.06</td>
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<tr>
<td>Activity, ( \tau ), LL, h</td>
<td>24.87 ± 0.19</td>
<td>(25.32 ± 2.23)</td>
<td>NS</td>
<td>24.40 ± 0.20</td>
<td>25.38 ± 0.25</td>
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<tr>
<td>( \tau ) Heart rate, DD, h</td>
<td>23.88 ± 0.04</td>
<td>23.24 ± 1.80</td>
<td>NS</td>
<td>23.83 ± 0.03</td>
<td>23.93 ± 0.07</td>
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<tr>
<td>( \tau ) Heart rate, LL, h</td>
<td>24.87 ± 0.19</td>
<td>25.15 ± 1.90</td>
<td>NS</td>
<td>24.47 ± 0.07</td>
<td>25.27 ± 0.07</td>
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<tr>
<td>( \tau ) Temp, DD, h</td>
<td>23.84 ± 0.02</td>
<td>23.57 ± 0.37</td>
<td>NS</td>
<td>23.80 ± 0.01</td>
<td>23.90 ± 0.00</td>
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<tr>
<td>( \tau ) Temp, LL, h</td>
<td>25.20 ± 0.14</td>
<td>(29.05 ± 0.37)</td>
<td>NS</td>
<td>24.85 ± 0.05</td>
<td>25.23 ± 0.19</td>
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<tr>
<td>Daytime activity, 300 lux</td>
<td>4.744 ± 0.41</td>
<td>4.752 ± 0.87</td>
<td>NS</td>
<td>4.430 ± 0.24</td>
<td>5.058 ± 0.39</td>
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<tr>
<td>Nighttime activity, 300 lux</td>
<td>10.096 ± 1.050</td>
<td>6.932 ± 1.000</td>
<td>0.054</td>
<td>9.393 ± 0.42</td>
<td>10.798 ± 2.201</td>
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<tr>
<td>Total activity, LD, 300 lux</td>
<td>14.840 ± 1.147</td>
<td>11.684 ± 1.674</td>
<td>NS</td>
<td>13.823 ± 1.74</td>
<td>15.856 ± 2.349</td>
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<tr>
<td>Daytime heart rate</td>
<td>5.504 ± 0.181</td>
<td>5.607 ± 0.279</td>
<td>NS</td>
<td>5.258 ± 0.293</td>
<td>5.750 ± 0.134</td>
</tr>
<tr>
<td>Nighttime heart rate</td>
<td>7.524 ± 0.786</td>
<td>6.806 ± 0.328</td>
<td>NS</td>
<td>7.255 ± 0.239</td>
<td>7.793 ± 0.167</td>
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<tr>
<td>Heart rate, DD, 11 days in DD</td>
<td>13.028 ± 0.347</td>
<td>12.412 ± 0.594</td>
<td>NS</td>
<td>12.513 ± 0.328</td>
<td>13.543 ± 0.239</td>
</tr>
<tr>
<td>Total heart rate</td>
<td>15.106 ± 0.482*</td>
<td>14.448 ± 0.510</td>
<td>NS</td>
<td>14.069 ± 0.290</td>
<td>16.110 ± 0.78</td>
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<tr>
<td>Total heart rate, 11 days in DD</td>
<td>11.076 ± 0.304</td>
<td>10.816 ± 0.472</td>
<td>NS</td>
<td>10.709 ± 0.501</td>
<td>11.443 ± 0.275</td>
</tr>
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</table>

Data are presented as means ± SE. VPAC2, vasoactive intestinal polypeptide receptor-2; M, male; F, female; DD, constant darkness; LL, constant light; Temp, body temperature; NS, not significant. P values represent the analysis by Mann-Whitney U-test. Tau values from VPAC2\(^{-/-}\) are indicated in parentheses due to low power. *Wild type: \( P < 0.002 \) DD vs. LL; †VPAC2\(^{-/-}\): \( P < 0.004 \) DD vs. LL.
Fig. 1. Locomotor activity in vasoactive intestinal polypeptide 2+/− (VPAC2+/−) and VPAC2−/− mice. A: representative double-plotted wheel-running actograms of VPAC2+/− (left) and VPAC2−/− (right) mice during a 24-h light-dark (LD) cycle followed by free-running in constant darkness (DD: indicated by shading). B: free-running endogenous period \( \tau \) is determined for both genotypes during 15 days of DD. C: Fast Fourier transformation (FFT) confirms that VPAC2−/− mice lose the strong endogenous period during DD. Note that VPAC2−/−, when released into DD during the first cycle (arrow in A), immediately advances activity onset by ~6 h.
activity during the subjective night (P < 0.05, Mann-Whitney U-test) (Fig. 4).

Gross locomotor activity (telemetric measurement) in VPAC2−/− mice during DD and LL. Gross locomotor activity measured by telemetry demonstrated that wild-type mice entrained to the LD cycle, and when released into DD, the free-running \( \tau \) was significantly longer, compared with \( \tau \) determined in running wheels (23.50 ± 0.04 vs. 23.90 ± 0.05, \( P < 0.001 \), Mann-Whitney U-test, Table 1). Wild-type mice also displayed more daytime activity when determined by telemetry (32% of total activity) compared with activity measured by running wheels (7% of total activity). During telemetric recording, VPAC2−/− animals synchronized their activity weakly to the LD cycle and displayed ~41% of the total activity during the day compared with ~12% of the total activity during daytime when placed in running wheels (Figs. 5 and 7, Table 1). When released into DD activity they became completely arrhythmic from the first day (Figs. 5 and 6). We found no significant difference in period length between males and females (both genotypes) in a sample size of three animals of each sex in each group (Figs. 5 and 6). When light conditions were shifted from LD to LL wild-type animals continued their rhythmic activity with a prolonged \( \tau \), which did not differ significantly between sexes (Figs. 5 and 6). In contrast, the VPAC2−/− mice became arrhythmic when conditions were shifted from LD to LL (Figs. 5 and 6).

Heart Rate

Heart rate rhythmicity in VPAC2−/− mice during DD and LL. Heart rate measurement determined by telemetric devices demonstrated that wild-type and VPAC2−/− mice synchronized heart rate activity to the 12:12-h LD cycle, and heart rate was found to be significantly higher during the night in both groups (\( n = 6 \) in each group, means ± SE; VPAC2+/+ mice: 5,504 ± 181 vs. 7,524 ± 178, \( P = 0.002 \); VPAC2−/− mice: 5,607 ± 279 vs. 6,806 ± 328, \( P = 0.02 \), Mann-Whitney U-test; Table 1). The heart rate of VPAC2−/− mice at subjective night seemed to be lower than that of wild-type animals, although the difference was not statistically significant (Table 1). When placed in DD, circadian rhythmicity of heart rate in wild-type animals persisted with a phase and with a \( \tau \) identical to that found for gross locomotor activity (Table 1, Figs. 5–7). On the contrary, VPAC2−/− mice lost the 24-h rhythmicity in heart rate when placed in DD (Figs. 5 and 6). When light conditions were shifted to LL, wild-type animals continued their rhythmic heart rate activity with a prolonged \( \tau \), whereas the VPAC2−/− mice displayed no rhythmicity (Figs. 5 and 6). Overall, the light conditions seem to have a strong influence (positive and negative masking) on total heart rate in both groups. During darkness (night in LD and DD) heart rate was higher in both groups with no significant differences between the groups. Light (day in LD and LL) on the other hand, decreased heart rate in both groups with no significant differences between the groups (Table 1).

Core Body Temperature

Disruption of temporal phasing of core body temperature in VPAC2−/− mice during the LD-cycle. Core body temperature was measured in wild-type and VPAC2−/− mice during various conditions of light and darkness. Wild-type mice entrained...
to the LD cycle with a rise in body temperature starting ∼4 h before lights off (Fig. 7, right) peaking at the early subjective night (ZT12–13), after which it slowly declined during the following hours, reaching the daytime level at ZT0. Compared with the simultaneous telemetric determination of rhythms in HR and locomotor activity, the phase and period length were found to be identical in all three physiological parameters (Figs. 5–7). VPAC2−/− mice also showed a robust rhythmic change of body temperature during a 12:12-h LD cycle, but the time course and profile were different. Compared with the wild-type animals, body temperature rhythm in VPAC2−/− mice was advanced in phase. Thus, VPAC2−/− mice exhibited a peak in body temperature ∼4 h before lights off (ZT8), which is 5–6 h earlier than wild-type animals (Fig. 7, right). The temperature profile in VPAC2−/− mice started with a rise at lights on (ZT0) peaking 8 h later, while in wild-type mice the onset of rise in body temperature was at ZT8, reaching maximum after 5 h (Fig. 7, right). Compared with the phase of the telemetrically determined rhythms of heart rate and locomotor activity, the phase of body temperature is advanced in VPAC2−/− mice, indicating a disruption of the temporal organization regulating these physiological parameters (Figs. 5 and 7). When conditions were shifted to DD, the VPAC2−/− mice showed very weak body temperature rhythmicity in the periodogram with two period lengths ∼22–23 h (Fig. 6, right, top). In LL VPAC2−/− mice also displayed very weak body
Fig. 4. VPAC2−/− mice show altered locomotor activity during photoskeleton of the 1/11:1/11-h LD cycle. A: representative actograms of wheel-running locomotor activity are shown for one VPAC2+/+ and one VPAC2−/− mouse. Mice were kept in a 12:12-h LD cycle for 4 wk, followed by a photoskeleton period of 1/11:1/11-h LD cycle for 10 days followed by DD. B: batch actograms of VPAC2+/+ and VPAC2−/− mice (averages of 8 animals of each genotype) are shown. Yellow bars represent the light phase of the skeleton photoperiods in A and B. Note that VPAC2−/− switch locomotor activity during the photoskeleton regimen as evidenced by increased daytime activity. This is summarized in C, which shows the average activity of 8 animals in each genotype plotted as a repeat of one 24-h cycle.
temperature rhythmicity in the periodogram with several different period lengths (Fig. 6, right, bottom). In contrast to the period length of \( \frac{1}{25} \) h in the wild-type mice, VPAC2 mutant mice displayed various periods shorter than 24 h in LL (Figs. 5 and 6).

**DISCUSSION**

The present study demonstrates that VPAC2 signaling is necessary for a functional circadian clock-driving activity, core body temperature, and heart rate rhythmicity. Furthermore, although a 24-h rhythm for all three parameters is sustained in VPAC2-deficient mice during the LD cycle, most likely as a result of masking, our results reveal that VPAC2 signaling is necessary for the alignment of the circadian phases of the various physiological rhythms. Finally, the use of telemetric devices to measure circadian locomotor activity, temperature, and heart rate together with the classical determination of circadian rhythms of wheel-running activity raises questions about how representative wheel-running activity may be of other behavioral parameters, especially when animals have altered circadian phenotype.

**Locomotor Activity in VPAC2-Deficient Mice**

Previous studies in mice lacking either VIP/peptide histidine isoleucine (PHI) or the VPAC2 receptor (other strains) have demonstrated an almost identical phenotype of locomotor activity in which mutant mice become less rhythmic or completely arrhythmic in DD conditions (3, 6, 11, 14). The locomotor activity of our strain of VPAC2-deficient mice was evaluated by wheel-running activity and telemetry, and the results from wheel-running experiments demonstrate that our VPAC2-deficient mice behave very similar to the previously described mice lacking VIP/PHI and the VPAC2 receptor (3, 6, 11, 14). The nocturnal behavior seen in VPAC2-deficient mice during the LD cycle seems to be a result of the suppressing effects of light (i.e., negative masking) revealed by jetlag experiments, by placing animals in ultra-short photoperiods or in a skeleton photoperiod. During the latter regimen, wild-type mice entrained to the skeleton photoperiod, whereas mutant mice showed no entrainment. A striking finding also found in VIP/PHI and in another strain of VPAC2-deficient mice (3, 6, 11, 14) was an abnormal phasing of the activity onset starting 4–8 h before predicted, when the mice entered into DD. The advanced activity phase observed in the first cycle of DD in VPAC2-deficient mice may be related to the advanced phase in core body temperature, which we observed in these animals (see below). Compared with wheel-running, the telemetrically determined activity revealed a slightly different pattern of activity in VPAC2-deficient mice. Most remarkable was the relatively high activity in the light phase with prominent short activity/rest periods.
Fig. 6. VPAC2−/− mice become arrhythmic from the first day in DD or LL. Batch analysis of Cnts, HR, and TP in VPAC2+/+ and VPAC2−/− mice (3 males and 3 females of each genotype) was performed when the animals were kept in DD or LL (see also Fig. 5). When VPAC2+/+ mice were released into DD, the animals continued their rhythmic behavior with identical \( \sigma \) for locomotor activity, HR, and TP (top, left). In LL, \( \tau \) was prolonged for all 3 parameters being close to 25 h (bottom, left). In contrast, VPAC2−/− mice were unable to generate rhythmic behavior in activity, HR, and TP under constant conditions (right). The y-axis is average amplitude.
periods throughout the LD cycle and the complete lack of rhythmicity in constant conditions of DD or LL. A similar pattern of activity was recently reported in another strain of VPAC2-deficient mice also using telemetry (32). Both we and Sheward et al. (32) found that wild-type activity was under circadian control, whereas VPAC2-deficient mice demonstrated activity bouts more randomly over the 24-h cycle and loss of rhythmicity in DD. All together, the results from both studies indicate that VPAC2-deficient mice lack a functional clock. Thus, use of the classical determination of circadian rhythms by wheel-running activity in these mice seems not to disclose the disturbed control of gross locomotor activity as revealed by telemetry. Along this line, we also observed that \( \tau \) activity determined by wheel running was different from \( \tau \) determined by telemetry in both wild-type and VPAC2 knockout animals. The \( \tau \) measured by running wheel was found to be \( \sim 0.3 \sim 0.4 \) h shorter, compared with \( \tau \) determined by telemetry for both genotypes, although the \( \tau \) determination in VPAC2-deficient mice had low power. It might be argued that this difference could represent a less precise determination of activity onset due to background noise using telemetry. This seems, however, less likely since \( \tau \) determined for both heart rate and body temperature was almost identical to that of gross motor activity (Table 1 and Fig. 5). More likely, the presence of a running wheel may be responsible for the shortening of \( \tau \), and, accordingly, increased activity and changes of period length in rodents has been demonstrated in a number of studies (7, 17). In the common mole (Microtus arvalis), introduction to running wheel has been shown to alter ultradian (feeding) activity toward being primarily nocturnal (33), and a similar pattern of increased night activity is seen in the VPAC2-deficient mice in the presence of a running wheel. In the common mole, feeding behavior is leading the ultradian behavior in the absence of a running wheel. Interestingly, when examining feeding behavior in VPAC2 knockout mice, Bechtold et al. (4) demonstrated that the animals had altered timing of food consumption with a distribution corresponding to the gross locomotor activity observed in VPAC2-deficient mice in the present study. Recent studies indicate that VIP signaling may influence period length, since in hamsters chronically injected intracerebroventricularly with a VIP agonist, the \( \tau \) becomes longer (21). On the other hand, \( \tau \) measured during

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**Fig. 7.** Batch analysis (6 animals of each genotype, 3 of each gender) of Cnts, HR, and TP in VPAC2\(^{+/+}\) (blue) and VPAC2\(^{-/-}\) (red) mice kept during an LD cycle of 7 days (top). In VPAC2\(^{+/+}\) mice activity, HR and TP varied rhythmically within the 24-h LD cycle, displaying a significant increase during the dark period, which is clearly illustrated (middle) that represents batch analysis; average of 3 days. The VPAC2\(^{-/-}\) mice also showed rhythmic changes in all 3 parameters in LD. However, whereas phasing of the activity and HR was identical in the 2 genotypes, the TP phase in VPAC2\(^{-/-}\) mice was significantly advanced by 6–8 h compared with the VPAC2\(^{+/+}\) mice (top, left). As seen (middle), the temperature rise in VPAC2\(^{-/-}\) mice is already at dawn, whereas in wild-type mice the TP starts rising at ZT8–9. Bottom: identical to the middle just including the variation of the values (SE). No statistical difference in amplitude of the three parameters was found between the 2 genotypes.
wheel running is found to be shorter in mice overexpressing the VPAC2 receptor (30), indicating that the role of VIP signaling on period length is rather complex. In a recent study, it was demonstrated that scheduled exercise has a powerful effect on the behavior of VPAC2-deficient mice, making them able to be rhythmic for a longer time in DD (22). This observation could explain why our VPAC2-deficient mice were able to keep the rhythm for a longer period when equipped with running wheels compared with telemetric recordings of the animals.

Heart Rate in VPAC2-Deficient Mice

Cardiovascular regulation is closely associated with locomotor activity, and the SCN plays an important role in the circadian control of the cardiovascular system (15, 26, 27). In VPAC2-deficient mice, heart rate regulation is under strong influence of the LD cycle as seen for gross locomotor activity control, but compared with wild-type mice, no circadian regulation exists under constant DD or LL conditions. In wild-type mice, on the other hand, a circadian influence is obvious, and wild-type mice placed in constant DD or LL demonstrate a period length in heart rate that was identical to and in phase with locomotor activity. Similar changes in heart rate were reported in another strain of VPAC2 mutant mice (32), and thus there is increasing evidence that lack of VPAC2 signaling leads to disruption of circadian cardiovascular control. This notion is further supported by a newly published study in VIP-deficient mice. In accordance with the present study, VIP-deficient mice demonstrated a lack of circadian control of heart rhythmicity (28). Also, changes in heart rate when exposed to an acute light pulse at subjective night were altered and delayed in VIP-deficient mice, indicating that VIP/VPAC2 signaling is important for circadian and light-regulated heart rate control (28). Our study also demonstrates the 24-h oscillation in heart rate seen in VPAC2-deficient mice is strongly associated with gross motor activity, rather than rhythmic change, in core body temperature and metabolism (4), which is phase advanced compared with the heart rate and activity rhythm. Previous studies have revealed that regulation of heart rate activity is under clock control (15, 26, 27). This was recently illustrated in genetically modified mice lacking the clock gene Per2. This mouse is characterized by altered period length and progressive loss of circadian heart rate rhythmicity in DD (34). Mice lacking another clock gene, Clock, display damped heart rate compared with wild-type mice in DD (29). Recent data from VPAC2-deficient mice in which blood pressure, heart rate, gross locomotor activity, and sleep patterns were measured, demonstrated that there was a robust diurnal rhythm in heart rate and blood pressure in VPAC2-deficient mice, which seems to be depending upon the activity rhythm. These findings indicate a central role of SCN regulation via VPAC2 signaling on cardiovascular and sleep physiology (32). These data and our results indicate that VPAC2-deficient mice behave like “clockless” mice, which make an interesting model for studying circadian cardiovascular regulation different from mice lacking core clock genes or SCN lesion animals (which may have neuronal loss and/or damaged neuronal connectivity of SCN neuronal pathways). Whether lack of VPAC2 signaling alters the risk for cardiovascular disease in these mutant mice remains, however, to be investigated.

Effects on Temperature Regulation in VPAC2-Deficient Mice

The most striking finding in the present study was the misalignment between body temperature on one hand and gross locomotor activity and heart rate on the other hand in VPAC2-deficient mice kept under LD conditions. We and others have shown that light has a strong repressing effect on locomotor activity (3, 6, 11, 14) and heart rate in both wild-type and VPAC2+/− mice (32), but the advanced body temperature rhythm in VPAC2+/− mice during LD suggests that light is unable to suppress body temperature in these animals. Consequently, an involvement of VIP/VPAC2 signaling in light-regulated control of body temperature is likely. Schroeder et al. (28) recently demonstrated that mice lacking VIP display a similar advanced phase of core body temperature compared with wild-type mice, supporting the importance of VIP/VPAC2 receptor signaling in circadian phasing of temperature. We are unable to determine whether this misalignment of body temperature phase is a result of scrambled VIP/VPAC2 signaling in the SCN, in other areas of the brain, or in the retina.

VIP-containing neurons are known to project within the SCN and to extra-SCN areas, and, of these, VPAC2 mRNA have been demonstrated in the subparaventricular zone, the parvocellular hypothalamic paraventricular nucleus, the dorsomedial hypothalamic nucleus, and the anterior thalamic paraventricular and the paratenial nuclei (16). It is possible that neurons in the paraventricular nucleus convey VIPergic SCN-efferent signals to downstream autonomic neurons involved in cardiac control and/or neurons in the subparaventricular zone involved in temperature regulation. VIP is found in retinal amacrine cells as is the VPAC2 receptor (12). Recent studies have revealed that retinal wiring and photoreceptor signaling can alter nocturnal animals to become diurnal in phase (8), and it is possible that lack of VPAC2 expression in the retina may change the effects of light on body temperature regulation. Interestingly, the results of misalignment of the body temperature phase in the present study are in agreement with a recent study examining the role of VIP/PHI and VPAC2 signaling in circadian metabolic control. The study from Bechtold et al. (4) reported on altered metabolic (oxygen consumption and carbon dioxide production) and feeding behavior in VIP/PHI and VPAC2-deficient mice. By examining food consumption and metabolic rate during a normal LD cycle, it was shown that both VIP and VPAC2-deficient mice had a phase-advanced metabolic rate compared with wild-type mice peaking at subjective day, ~4 h earlier than wild-type mice. VIP- and VPAC2-deficient mice had also reduced metabolic rate in LD (4). In both strains, food consumption was reduced and was significantly higher during the light period (4). The findings that VPAC2 and VIP-deficient mice have abnormal phasing of body temperature regulation (28) add new information to the role of VIP/VPAC2 signaling in body temperature regulation. Circadian temperature regulation is an important part of the homeostatic mechanism that controls temperature levels in the body. It is believed that body temperature is regulated from a termoregulatory set point and circadian oscillation of body temperature is opposed by the termoregulatory system controlling heat production and heat loss (24). Heat production is a result of metabolic processes, and our results on body temperature regulation in VPAC2-deficient mice seem to agree with the advanced metabolic rate reported by Bechtold et al. (4). Circadian oscillation in
body temperature has previously been shown to persist in the absence of daily oscillation in food consumption (24). In VPAC2-deficient mice altered food consumption (4) coincides with altered body temperature regulation showing that VIP receptor signaling is important for central metabolic control.

Perspectives and Significance

The present study demonstrates that the 24-h rhythm for three physiological parameters, gross locomotor activity, heart rate, and core body temperature are retained in VPAC2-deficient mice during the LD cycle. However, the phase of core body temperature was advanced compared with the phase of heart rate and activity, indicating that VPAC2 signaling seems to be necessary for the alignment of the circadian phases of these three physiological parameters. Furthermore, the use of telemetric devices to measure circadian locomotor activity, temperature, and heart rate, together with the classical determination of circadian rhythms of wheel-running activity, raises questions about how representative wheel-running activity may be to other behavioral parameters, especially when animals have altered circadian phenotype.

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