Circadian periodicity of cerebral blood flow revealed by laser-Doppler flowmetry in awake rats: relation to blood pressure and activity

C. A. Wauschkuhn, K. Witte, S. Gorhey, B. Lemmer, and L. Schilling

Division of Neurosurgical Research, Department of Neurosurgery, and Institute of Pharmacology and Toxicology, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany

Submitted 8 December 2004; accepted in final form 6 May 2005

Wauschkuhn, C. A., K. Witte, S. Gorhey, B. Lemmer, and L. Schilling. Circadian periodicity of cerebral blood flow revealed by laser-Doppler flowmetry in awake rats: relation to blood pressure and activity. Am J Physiol Heart Circ Physiol 289: H1662–H1668, 2005. First published May 13, 2005; doi:10.1152/ajpheart.01242.2004.—Cardiovascular parameters such as arterial blood pressure (ABP) and heart rate display pronounced circadian variation. The present study was performed to detect whether there is a circadian periodicity in the regulation of cerebral perfusion. Normotensive Sprague-Dawley rats (SDR, ~15 wk old) and hypertensive (mRen2)27 transgenic rats (TGR, ~12 wk old) were instrumented in the abdominal aorta with a blood pressure sensor coupled to a telemetry system for continuous recording of ABP, heart rate, and locomotor activity. After 5–12 days, a laser-Doppler flow (LDF) probe was attached to the skull by means of a guiding device to measure changes in brain cortical blood flow (CBF). After the animals recovered from anesthesia, measurements were taken for 3–4 days. The time series were analyzed with respect to the midline estimating statistic of rhythm (i.e., mean value of a periodic event after fit to a cosine function), amplitude, and acrophase (i.e., phase angle that corresponds to the peak of a given period) of the 24-h period. The LDF signal displayed a significant circadian rhythm, with the peak occurring at around midnight in SDR and TGR, despite inverse periodicity of ABP in TGR. This finding suggests independence of LDF periodicity from ABP regulation. Furthermore, the acrophase of the LDF was consistently found before the acrophase of the activity. From the present data, it is concluded that there is a circadian periodicity in the regulation of cerebral perfusion that is independent of circadian changes in ABP and probably is also independent of locomotor activity. The presence of a circadian periodicity in CBF may have implications for the occurrence of diurnal alterations in cerebrovascular events in humans.

circadian rhythm; cerebral perfusion; arterial blood pressure; locomotor activity; radiotelemetry

CIRCADIAN PERIODICITY is an innate feature of many body functions, most obvious in physical activity and the sleep-wake cycle, and it is also well documented for regulation of arterial blood pressure (ABP) and heart rate. In addition, cardiovascular functions such as heart stroke volume, cardiac output, and total peripheral resistance may also display significant day-night changes (1, 6, 41). Whether the distribution of cardiac output and perfusion of individual organ beds also show circadian periodicity is not clear. In animal experiments, evidence in favor of circadian variation has been presented for cardiac perfusion (13) and, similarly, for renal plasma flow (36) and skin and muscle perfusion, the latter being attributed to the activity cycle (7). However, even under strict controlled conditions, circadian changes in muscle and skin perfusion were found in young healthy subjects (4, 21, 48) and, although somewhat attenuated, in the skin of patients with arterial occlusive disease (4).

Brain cortical blood flow (CBF) is also subject to regularly occurring fluctuations of different periodicities. Such changes are well established for the transition from wakefulness to sleep and also in relation to different sleep states (3, 16, 27, 39, 44). The presence of a circadian periodicity may be extracted from continuously monitored data covering ≥24 h, i.e., the period length to be detected, but a discontinuous regimen of data sampling may also yield suitable data sets. However, a sufficiently long period has been observed in only a small number of animal studies (2, 3, 11, 16, 33, 39). The results were contradictory, because, some of the studies presented mostly indirect evidence in favor of diurnal changes (11, 16, 33), whereas others failed to present such evidence (2, 3, 39). Furthermore, Diamant and coworkers (8) did not observe circadian changes in blood flow velocity in the middle cerebral artery of humans. We have, therefore, measured alterations of CBF in freely moving rats continuously over several days by means of laser-Doppler flowmetry (LDF). Monitoring was performed in parallel with telemetric recordings of locomotor activity, systolic and diastolic ABP, and heart rate. To relate 24-h variations of CBF to cardiovascular parameters, we used normotensive Sprague-Dawley rats (SDR) and compared the results with those obtained from hypertensive (mRen2)27 transgenic rats (TGR), which display an inverse ABP profile (24, 26). The time series were analyzed by established algorithms, and the results suggest a circadian periodicity in CBF that is independent of the regulation of blood pressure.

MATERIALS AND METHODS

Adult male SDR (Janvier, Le Genest St. Isle, France) and TGR (Taconic M & B, Ry, Denmark) were kept under a 12:12-h light-dark cycle and had free access to food and tap water. The experiments were approved by the federal ethical committee (Regierungspraesidium Karlsruhe, no. 122/00) and were performed in accordance with institutional and state guidelines.

Surgical procedures were performed under deep enflurane anesthesia (Abbott, Wiesbaden, Germany) with the animals breathing spontaneously. In the first operation, a telemetry-coupled ABP sensor (model TA11PA, Data Sciences, St. Paul, MN) was implanted as described previously (24). Briefly, the abdomen was cut longitudinally along the midline, and the catheter of the sensor was inserted into the aorta distal to the renal arteries. The transmitter was fixed to the peritoneum, and the abdomen was closed in layers by sutures and

Address for reprint requests and other correspondence: L. Schilling, Div. of Neurosurgical Research, Dept. of Neurosurgery, Univ. Hospital Mannheim, Theodor Kutzer-Ufer 1-3, D-68135 Mannheim, Germany (e-mail: lothar.schilling@nch.ma.uni-heidelberg.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
suture clips. After the surgery was completed, the animal was transferred to a plastic cage and allowed to recover while individually housed.

At 5–12 days after insertion of the telemetric ABP sensor, the animals were again anesthetized, and the skin over the skull was incised along the midline. A small burr hole was drilled in the right parietal skull bone, with care taken to leave the inner layer intact. The position in the parietal cortex differed slightly from animal to animal. A purpose-built mounting system to guide and protect an LDF probe was glued to the skull over the burr hole and secured by a layer of dental cement, with the attachment improved by three screws inserted around the burr hole. A drawing of the guide device is shown in Fig. 1. A small glass fiber (0.43 mm outer diameter) was introduced into the guiding device and advanced until the tip touched the inner bone layer. Great care was taken not to break this inner layer. The recording by this fiber (LDF_total) includes the signal generated by the cortical perfusion. The fiber was carefully tightened, and a second glass fiber was attached to the first so that its tip was positioned directly above the guiding device. The recording by this fiber (LDF_artifact) presents the signal generated by the animal roaming or moving its head (e.g., during feeding, drinking, and grooming). The skin was sutured, and the animal was transferred to its cage and allowed to recover.

Data acquisition. The telemetric monitoring system for recording systolic and diastolic ABP, heart rate, and locomotor activity consisted of a receiver plate (model RLA1020) positioned under the cage, a multiplexer (model BCM-100), and an interface card (model DQ-1088, each from Data Sciences) inserted into a personal computer. Data were monitored via Labpro software (Data Sciences). Blood pressure curves of 5-s duration were recorded every 5 min at a signal-sampling rate of 500 Hz, and the mean values for systolic and diastolic ABP, along with heart rate and locomotor activity, were stored on hard disk.

For measurement of alterations of cortical perfusion, glass fibers were connected to a dual-channel LDF monitor (model DRT 4, Moor, Axminster, UK). The fibers were supported by a counterbalanced lever arm (Harvard, Holliston, MA), which was appropriately counterbalanced. The output of the LDF monitor was delivered to a personal computer equipped with an analog-to-digital converter and IOX software (EMKA Technologies, Paris, France). The primary sampling rate was 150 Hz. Data were downsampled to 1 Hz, and the mean values averaged over 60 s were stored on hard disk. The clocks of the monitoring systems were synchronized and started concomitantly for continuous registration.

Data analysis. The telemetric data were analyzed by the WIN-ABPM-FIT program (49), which employs the cosinor analysis method for calculating the following values: 1) the midline estimating statistic of rhythm (MESOR, i.e., the mean value of the rhythm analyzed), 2) the amplitude (i.e., the difference between the mean value and the maximum deflection of the fitted curve), and 3) the acrophase (i.e., the time at which the maximum deflection of the fitted curve occurs). The fitting procedure was based on the following equation

\[ f(t) = \text{MESOR} + \sum_{i=1}^{n} \left( \text{amplitude}_i \cdot \cos \left( (t - \text{acrophase}_i) \frac{2\pi}{\tau} \right) \right) \]  (1)

where \( i \) is the index for the number of harmonics included (\( n = 6 \) for 24, 12, 8, 6, 4.8, and 4 h, respectively).

In addition, the actual length of the circadian period contained in each original curve was determined by using the Lomb-Scargle method, which is incorporated in the WIN-ABPM-FIT program. Briefly, the Lomb-Scargle method is a least-squares power spectrum analysis procedure that can be used to analyze unequally spaced data. When applied to equally spaced data (as in the present study), it is superior to classical power spectrum analysis, inasmuch as it allows for a higher-frequency solution (45).

The data acquired by the LDF fibers were subtracted from each other (LDF_total - LDF_artifact) to extract the signal generated by the cortical perfusion, i.e., the LDF_CBV signal. Because the originally recorded LDF signals greatly varied between animals at the level of arbitrary units, we determined the mean LDF_CBV value for each 24-h period and calculated the actually measured values as percentage of the mean day value to improve interindividual comparability. These relative data were averaged over 5-min periods to give the same data format that was provided for the telemetrically recorded signals and fed into the WIN-ABPM-FIT program for analysis.

The data obtained from the SDR and TGR were averaged and presented as means ± SD. Mean values were compared by \( t \)-test, and statistical significance was set at \( P < 0.05 \).

RESULTS

Data acquisition started immediately after recovery of the animals from anesthesia, but data from the 1st day were

Fig. 1. Schematic drawing of the mounting system for permanent attachment of the laser-Doppler flowmetry (LDF) fiber to the rat brain. The system consists of a support plate (1) with a hole in the center, allowing light to pass through it to the brain surface. The support plate is equipped with a flange to house a socket (2) guiding the LDF fiber (4, LDF_total) molded. At the upper end of the socket, a compression seal (5) and a sleeve (6) are embedded to fix the glass fiber by means of a cap nut (7). The support plate and the ball bearing are glued to the skull bone and further fixed by a layer of dental cement, with the attachment improved by 3 screws inserted into the skull around the burr hole. After fixation of the support plate and the ball bearing coupled with the socket, a hollow shaft (8) is imposed on the mount to prevent contact of blood or tissue fluid with the ball bearing. A second glass fiber (9, LDF_artifact) is tightly attached to the first (4), so that its tip is above the guiding device, to monitor movement artifacts.
discarded from analysis. In one TGR, we could not detect a significant circadian period in the locomotor activity, and no significant circadian rhythm was found in the LDFCBF signal in one SDR and one TGR. Original traces showing 3 days of continuous registration of data recorded in the two LDF channels (LDFtotal and LDFartifact) are shown for a SDR and a TGR in Fig. 2A. In the SDR and TGR, the mean signal intensities were significantly higher during the lights-off (i.e., activity phases) than during the lights-on (i.e., subjective rest) phases ($P < 0.01$ for LDFtotal, LDFartifact, and LDFCBF). Accordingly, the 24-h period was the most significant one in each trace. Comparable results were obtained for all animals included in the analysis. In an individual experiment, the LDFtotal fiber was advanced backward so that it no longer measured in the cortex. In this experiment (Fig. 2B), the mean differential signal (LDFtotal − LDFartifact) was $-3.9 \pm 10.0$ arbitrary units during a 36-h monitoring period. Thus LDFCBF can conveniently be extracted by subtracting LDFartifact from LDFtotal.

The averaged hourly values for all parameters recorded are shown in Fig. 3 for SDR and TGR. The exact period lengths of the averaged hourly values as calculated by the Lomb-Scargle method are given in Table 1, along with the 95% confidence intervals, which covered the 24-h period in each case. In the cosinor analysis applied to the individual time series, the 24-h oscillation was the major rhythm, although additional periods of 12, 8, 6, or 4 h sometimes also reached statistical significance. The mean values for the MESOR and the amplitude of the fitted 24-h rhythm are listed in Table 1. The absolute levels and the amplitudes of the systolic and diastolic ABP differed significantly between SDR and TGR, the latter clearly being in the phase of developing hypertension (26).

The acrophases of the parameters are shown in Fig. 4. Although ABP in the TGR showed the expected inverse periodicity, there was no difference in the circadian pattern with respect to LDFCBF between SDR and TGR, suggesting that this circadian rhythm did not change with the blood pressure rhythm in TGR. Similar observations have been reported for renal plasma flow (36). The acrophases of the LDFCBF curves preceded those of the locomotor activity in the SDR ($P < 0.10$) and the TGR ($P < 0.05$; Fig. 4). Furthermore, there was no correlation between the acrophases of the LDFCBF and the activity curves in animals of either strain ($r = 0.119$ and $r = 0.107$ for SDR and TGR, respectively). Interestingly, we found one SDR in which the circadian rhythm in the LDFCBF value was inverse, showing the acrophase at 2 PM. This animal was excluded from calculation of the mean acrophase position.

**DISCUSSION**

In the present study, continuous LDF recordings of the cerebral microcirculation were performed concomitantly with recordings of blood pressure, heart rate, and locomotor activity in conscious freely moving rats to study circadian changes in these parameters. The results suggest 1) the presence of a circadian rhythm in the regulation of CBF, 2) independence of the LDFCBF signal from circadian pressure regulation, and 3) no apparent dependence of LDFCBF on periodicity in heart rate.
and locomotor activity on the basis of differences in the respective acrophase positions.

Systolic and diastolic blood pressure, heart rate, and locomotor activity were determined by radiotelemetry with the sensor chronically implanted in the abdominal aorta. This technique is considered the state of the art for monitoring physiological functions in freely moving laboratory animals, including rats and mice (for review see Ref. 23). In contrast, LDF methodology for studying cerebral circulation has mainly been employed in anesthetized animals, and only a few investigators have used it to measure changes in cerebral perfusion in awake rats (17, 18, 33). The LDF signal is characterized by a high temporal resolution, but the data from LDF monitoring systems are provided in arbitrary units only. However, it has been shown that these data significantly correlate with measurements of blood flow in absolute values (i.e., in ml/100 g/100 min) (14, 20, 22, 31, 40). Therefore, LDF methodology is well suited for experimental studies of periodic changes in organ blood flow, even during prolonged periods of monitoring.

To allow the awake animals to move freely within the cage, we designed a new mounting device for guiding and fixing the glass fibers to the head. This device contains a ball bearing, which enables the animal to rotate clockwise and counterclockwise and also to turn its head during all behavioral activity, including sleeping, feeding, and grooming. The mounting device and the fibers did not impose any apparent restriction on the animals’ daily activity, and accordingly, the animals showed no signs of discomfort during the monitoring periods.

The glass fiber was inserted into the skull bone, with care taken to keep the thin inner bone layer intact. This approach protects the cortex from involuntary injury by the fiber during the surgical procedure, as well as during the subsequent monitoring period, but it does not interfere with the light entering or

Fig. 3. Circadian rhythms of heart rate (HR), systolic and diastolic arterial blood pressure (sBP and dBP), activity (Act) (all measured by telemetry), and calculated LDFCBF signal (CBF) in SDR (n = 8) and TGR (n = 7). Data are percentages of each 24-h mean value to improve comparison between animals. Open bars, lights on (7 AM–7 PM); solid bars, lights off (7 PM–7 AM).

Table 1. Analysis of 24-h periodicity of telemetric data and LDFCBF signal in conscious freely moving SDR and TGR

<table>
<thead>
<tr>
<th></th>
<th>Period length</th>
<th>MESOR</th>
<th>Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, min⁻¹</td>
<td>SDR</td>
<td>24.2 (23.8–24.6)</td>
<td>351.6 ± 3.26</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>24.2 (23.6–24.8)</td>
<td>353.0 ± 15.3</td>
</tr>
<tr>
<td>Systolic ABP, mmHg</td>
<td>SDR</td>
<td>23.1 (22.2–24.0)</td>
<td>114.8 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>23.8 (23.5–24.1)</td>
<td>186.1 ± 15.3*</td>
</tr>
<tr>
<td>Diastolic ABP, mmHg</td>
<td>SDR</td>
<td>23.6 (22.8–24.6)</td>
<td>85.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>24.0 (23.5–24.5)</td>
<td>134.2 ± 14.4*</td>
</tr>
<tr>
<td>Locomotor activity, min⁻¹</td>
<td>SDR</td>
<td>24.9 (23.7–26.1)</td>
<td>14.0 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>23.9 (23.2–24.6)</td>
<td>10.1 ± 0.6*</td>
</tr>
<tr>
<td>LDFCBF, % daily mean value</td>
<td>SDR</td>
<td>23.5 (22.0–25.0)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TGR</td>
<td>24.2 (22.7–25.7)</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are means ± SD, except for period lengths (in hours) as obtained by the Lomb-Scargle method, where mean and 95% confidence intervals are given. Midline estimating statistic of rhythm (MESOR) and amplitude of the 24-h oscillation fitted to recorded data were obtained by cosinor analysis. Laser-Doppler Flow (LDF)-derived values are expressed as percentage of daily mean values to account for interindividual variation in the originally monitored signal. SDR, Sprague-Dawley rats; TGR, m(Ren2)27 transgenic rats; ABP, arterial blood pressure; CBF, cerebral blood flow. *P < 0.05 vs. respective value in SDR.
leaving the brain tissue. Osborne (33) implanted the glass fiber into the brain parenchyma for long-term measurements. However, because the trauma associated with intraparenchymal positioning may interfere with the normal tissue function for an extended period of time after insertion, this approach was not adopted in the present study.

A major problem inherent in LDF monitoring is generation of artifacts by movements of the fiber. Artifacts are generated when the animal roams the cage or moves its head during feeding, drinking, and grooming. These artifacts arise from the speckle movement, because the light emerging from the tip fiber is not homogenous but has a speckled appearance. However, this speckle movement does not affect the Doppler shift induced by the moving erythrocytes, as pointed out by Gush and King (19). The speckle-movement artifacts are a well-known phenomenon in the pertinent literature, and Newson and coworkers (30) suggested the use of fibers in parallel to allow an index of fiber movement to be obtained that is independent of blood flow changes. Although Gu and coworkers (17, 18), in their measurement of cortical perfusion in awake rats, mentioned no problem with artifacts, Osborne (33) used simultaneous behavioral registration to identify artifactual peaks in the LDF signal. In a first series of experiments, we found a clear circadian periodicity in the LDF signal when the light transmission from the tip of the LDF fiber to the cortex was blocked (results not shown). These artifacts must be eliminated before the data are analyzed. To eliminate the artifacts, we developed a new approach using two fibers arranged in parallel. One fiber ending above the guiding device on the skull monitored LDF\textsubscript{artifact} exclusively, while the second fiber actually resting in the skull picked up the microcirculatory signal, yet superimposed on the artifact (LDF\textsubscript{total}). This two-fiber approach enabled us to derive the signal generated by the cortical perfusion (i.e., LDF\textsubscript{CBF}) by simply subtracting LDF\textsubscript{artifact} from LDF\textsubscript{total}, according to Newson and coworkers (30). The resulting time series could easily be analyzed by established algorithms.

Ample evidence has been presented in favor of a circadian rhythm in blood pressure and heart rate and its relation to clinical medicine, as recently reviewed elsewhere (25, 42). However, additional cardiovascular parameters, including cardiac output and total peripheral resistance, may also display such a periodicity (6). Furthermore, evidence in favor of 24-h changes in cardiac perfusion (13), renal plasma flow (36), and skin and muscle perfusion (7) has been derived from animal studies. In human subjects, microcirculation measurements have revealed circadian changes in skin perfusion (4, 21, 48), endothelial-dependent dilatation (9), and vascular tone and flow-mediated responses (34, 35, 38). We previously detected day-night differences in vascular reactivity in rat arteries isolated from different organ beds (47). Thus alterations in local vascular function, including sympathetic innervation and endothelial function, may be implicated in the generation of circadian periodicity of organ blood flow. The results of the present study provide, for the first time, evidence in favor of a circadian periodicity in the regulation of CBF, with the peak flow detected during the night. The finding of inverse periodicity of blood pressure in TGR strongly suggests that the regulation of circadian rhythm in CBF is not coupled to blood pressure periodicity. Alternatively, if they were coupled, one might speculate about a different mechanism or a shift of the coupling in TGR. Because the circadian rhythm of activity is maintained in TGR (i.e., SDR and TGR are nocturnal animal strains), one might speculate about an increase in neuronal activity and, thus, metabolism in regions involved in sensorimotor control, contributing to the 24-h changes of the LDF\textsubscript{CBF} signal. However, some evidence argues against this speculation: 1) the position of the LDF probes was not constant but differed slightly from animal to animal, and 2) the acrophase of the LDF\textsubscript{CBF} signal occurred consistently before that of the locomotor activity. Thus the circadian periodicity of CBF appears to be generated independently from the activity cycle. However, because this assumption is based on measurements in rats only, further evidence is needed from different species displaying a nocturnal or a diurnal pattern of activity.

Previously, it was recognized that the onset of cardiovascular events, including cardiac infarction (29, 43), and cerebrovascular events, such as subarachnoid hemorrhage (12, 15, 32) and stroke (5, 28, 37, 46), displays a 24-h pattern. The risk of a stroke is 49% higher between 6 AM and 12 PM than would be expected at random distribution over the day (10). The circadian changes of CBF shown in the present study may be an important factor in determining the morning peak of cerebrovascular events. However, the pathophysiological link remains to be clarified.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge the assistance of A. Kwapis and H. Scheffel in inserting the telemetry device and LDF system. J. Sprakl (CAD Engineering) for kindly providing the drawing of the LDF guide system and D.
REFERENCES


