

Circadian blood pressure and heart rate rhythms in mice

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Li, Ping, Steven H. Sur, Ralph E. Mistlberger, and Mariana Morris. Circadian blood pressure and heart rate rhythms in mice. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R500–R504, 1999.—The circadian pattern of mean arterial pressure (MAP) and heart rate (HR) was measured in C57BL mice with carotid arterial catheters. Cardiovascular parameters were recorded continuously with a computerized monitoring system at a sampling rate of 100 Hz. The tethered animals were healthy, showing stabilized drinking and eating patterns within 2 days of surgery and little loss of body weight. Analysis of the 24-h pattern of MAP and HR was conducted using data from 3–6 consecutive days of recording. A daily rhythm of MAP was evident in all mice, with group mean dark and light values of 101.4 ± 7.3 and 93.1 ± 2.9 mmHg, respectively. The group mean waveform was bimodal, with peak values evident early and late in the dark period, and a trough during the middle of the light period. The phase of maximum and minimum values showed low within-group variance. Mean heart rate was greater at night than during the day (561.9 ± 22.7 vs. 530.3 ± 22.3 beats/min). Peak values generally occurred at dark onset, and minimum values during the middle of both the dark and the light periods. We conclude that it is possible to perform measurements of circadian cardiovascular parameters in the mouse, providing new avenues for the investigation of genetic models.

water intake; computerized systems; cardiovascular system

CIRCADIAN CLOCKS HAVE powerful influences on almost all bodily functions, including temperature, activity, drinking, eating, blood pressure, and heart rate. Cyclical changes are seen in the frequency and timing of cardiovascular pathologies in humans, with increases seen most often in the morning when blood pressure is rising (3, 17). Alterations in the circadian blood pressure cycle are also observed in hypertensive humans, in particular those characterized as salt sensitive (4, 19, 20). Likewise, in an animal model of hypertension and salt sensitivity, the mRen-2 transgenic rat, there are changes in the circadian blood pressure pattern that are corrected by converting enzyme therapy (9, 15).

New genetic methods have greatly expanded our tools for the study of blood pressure control. It is possible to selectively remove and replace genes, to add extra genes, and to control expression via conditional constructs. Mice are the most frequently used animals for the development of genetically manipulated models. One factor that has been limiting in the study of cardiovascular function in mice is the difficulty in

measuring blood pressure in the conscious, nonstressed animal. Initial studies were conducted using tail cuff plethysmography or direct measurement in restrained animals (2, 5, 11). However, neither method is effective in obtaining basal levels, studying stimulus-induced changes, or measuring circadian patterns. Recent advances in instrumentation have resulted in the development of cannulation methods that allow for long-term recording. For example, a femoral arterial catheter and tether system was used successfully for chronic monitoring in mice (10). However, there is no information on the circadian pattern of blood pressure in mice, only heart rate as evaluated with electrocardiograms (12, 18). Using our experience in chronic blood pressure measurement in rats (1, 8), we applied these techniques to mice (16). Blood pressure was measured continuously using tethered animals with carotid arterial catheters infused with heparinized saline (16).

The objective of the present study was to use chronic monitoring methods to study the circadian pattern of blood pressure and heart rate in normal mice. The ultimate goal is to apply this approach to the study of genetically engineered models of cardiovascular dysfunction.

METHODS

The study used male C57BL mice (24–28 g; Charles River Laboratories, Boston, MA) housed at 22°C, under a 12:12-h light-dark cycle (0500–1700 light period), with ad libitum access to water and standard mouse chow. Daily consumption of food and water was monitored. All experimental protocols were approved by the Laboratory Animal Care and Use Committee of Wright State University.

Arterial catheters were prepared from Micro-Renathane tubing (0.025 mm OD $\times$ 0.012 mm ID; Braintree Laboratories, Braintree, MA), with the end heat-stretched (approximately one-half the original diameter) and beveled. A small piece of polyethylene tubing (PE-60, 1 mm) was attached with superglue at ~ 10 mm from the tip of the catheter. Mice were anesthetized with a ketamine-xylazine mixture (70:6 mg/kg im) and placed on a water-circulating heating pad (37°C). Utilizing aseptic techniques and with the aid of a dissecting microscope, a catheter was inserted into the right common carotid artery. Briefly, the neck region was dissected using blunt dissection to locate and isolate the right common carotid artery. The artery was ligated with a suture (5–0 silk), placed ~ 3 mm below the bifurcation of the carotid arteries (internal/external from the common). The artery was occluded with a microclip (no. 18055–03; Fine Science Tool, Foster City, CA) ~ 8 mm distal from the ligation, and a small cut was made in the vessel wall with microscissors (no. 15000–08, Fine Science Tool). The catheter was inserted into the artery, the clip was removed, and the catheter was advanced until it reached the PE-60 collar, where it was secured in place with two sutures (5–0 silk). Figure 1 illustrates the surgical procedure and the catheter design.

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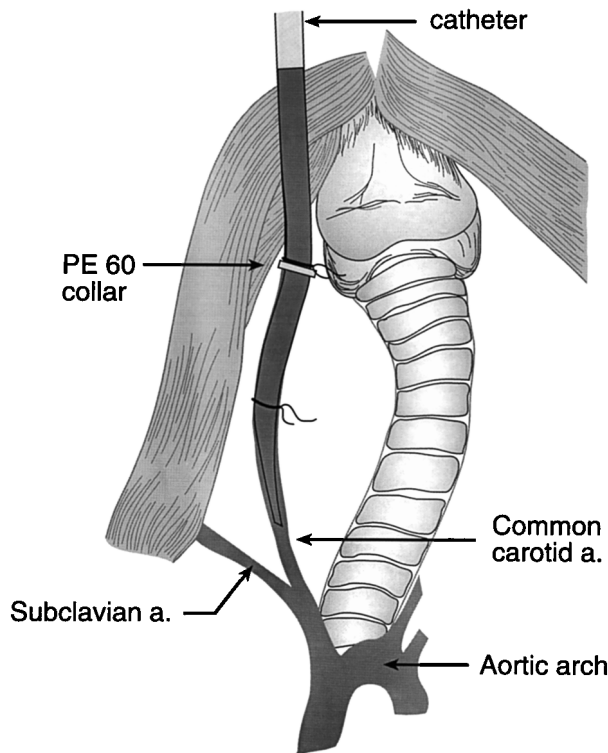


Fig. 1. Schematic diagram of the surgical field and arterial catheter. The catheter is 10 mm in length from collar to catheter tip. a, Artery.

The catheter was tunneled subcutaneously to pass through a polysulfone button tether (model LW62; Instech Laboratories, Plymouth Meeting, PA), which was surgically attached to the back muscles. The catheter was passed through a stainless steel spring attached to a swivel (model 375/25, Instech Laboratories) located on top of the cage. The tether and swivel system allowed the animal to move freely, while protecting the arterial catheter. Heparinized saline (80 U/ml) was continuously infused at 10 μ l/h using a syringe pump (model 220; Kd Scientific, Boston, MA) and 3-ml disposable syringes.

A flow-through blood pressure transducer (model 041-500503A; Argon, Athens, TX) was calibrated and connected to a computerized data-acquisition system (model MP100WSW; BIOPAC Systems, Santa Barbara, CA) specifically designed for cardiovascular measurements. Systolic (SBP) and dia-

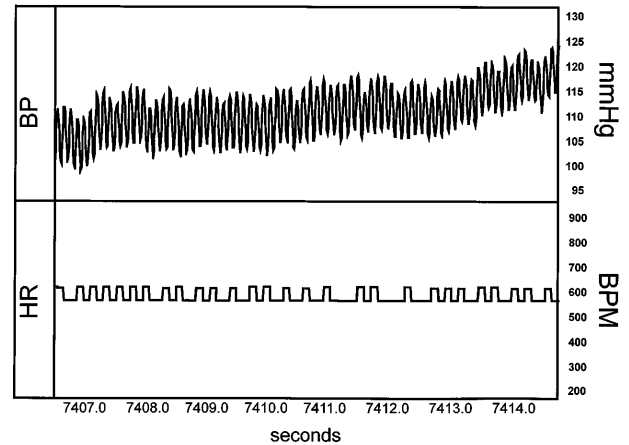


Fig. 2. Typical blood pressure (BP) and heart rate (HR) tracing obtained during chronic monitoring using the BIOPAC computerized data-acquisition system. BPM, beats/min.

stolic arterial blood pressure (DBP) and heart rate were sampled at 100 Hz and recorded online using a computer (XPS-D300; Dell Computer Corporation, Austin, TX) and a removable disk storage system (Jazz Drive; Iomega, Roy, UT). Mean arterial pressure (MAP) was calculated using the formula $MAP = DBP + (SBP - DBP)/3$. After collection, the data were processed by obtaining 10-min means of MAP and heart rate using a subroutine of the BIOPAC system (a cosine wave function with a period of 600 s). The animals were allowed to recover from surgery for 2–3 days before data collection. For the circadian analysis, cardiovascular parameters were recorded continuously for 3–6 days. MAP and heart rate data were converted to text files for plotting and analysis using Circadia, a program for the analysis of biological rhythms (Behavioral Cybernetics, Boston, MA). Daily rhythms of MAP and heart rate were quantified by calculating ratios of mean values in the dark and light periods. Data were then smoothed once using a 30-min moving average in which each 10-min data point was summed with the immediately preceding and following 10-min data points. A 1:4:1 weighting ratio was used (the center point in each 30-min average was multiplied by four, and the sum of the three points was divided by six). Average waveforms were calculated for each animal and the zeitgeber time (where zeitgeber time 0 is light onset and zeitgeber time 12 dark onset, by convention) of the maximum and minimum values was recorded. Mean values are reported $\pm$ SE. A paired Student's

Table 1. Food and water intake

	Day Postsurgery						
	1	2	3	4	5	6	7
Water, ml/24 h	1.3 $\pm$ 0.5	3.9 $\pm$ 1.0	4.3 $\pm$ 0.6	5.7 $\pm$ 0.2	6 $\pm$ 0.5	4.5 $\pm$ 0.8	5.8 $\pm$ 0.3
Food, g/24 h	1.0 $\pm$ 0.4	2.8 $\pm$ 0.6	4.2 $\pm$ 0.4	5.4 $\pm$ 0.1	5.2 $\pm$ 0.4	4.1 $\pm$ 0.9	4.5 $\pm$ 0.3

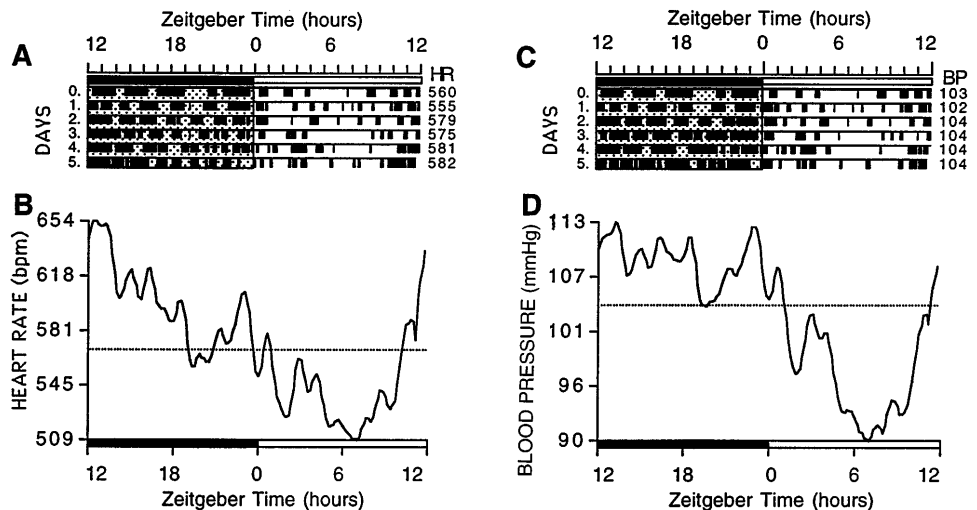
Data are presented as means $\pm$ SE; $n = 5$.

Table 2. Circadian analyses of mean arterial pressure and heart rate

	Dark Period	Light Period	Ratio (Dark/Light)	Mean	Zeitgeber Time	
					Maximum	Minimum
Blood pressure, mmHg	101.4 $\pm$ 3.2	93.1 $\pm$ 2.9†	1.1 $\pm$.02	97.0 $\pm$ 3.1	14.9 $\pm$ 0.8	6.7 $\pm$ 0.2
Heart rate, beats/min	562.0 $\pm$ 22.7	530.3 $\pm$ 22.2*	1.1 $\pm$.02	550.2 $\pm$ 18.0	12.1 $\pm$ 0.5	19.7 $\pm$ 0.3

Data are presented as means $\pm$ SE; $n = 5$ animals. * $P < 0.05$, † $P < 0.01$ dark vs. light.

Fig. 3. A: raster plot illustrating daily variations of HR in a representative mouse recorded for 6 consecutive days in a 12:12-h light-dark cycle. Each line represents 24 h and consists of 144 consecutive 10-min time bins plotted from left to right. Mean HR is indicated to right of each line. Dark period is represented by stippling. B: waveform of the daily rhythm in HR obtained by averaging the 6 days of recording represented in A. Dark period is represented by shaded bar along the *abscissa*. Daily mean HR is indicated by dotted line. C: raster plot illustrating daily variations in mean arterial pressure (BP) in the same representative mouse as described in A. D: average waveform of BP.



t-test was used to compare the mean light and dark values, with significance set at $P < 0.05$.

RESULTS

By day 3 postsurgery, the mice showed a stabilized pattern of food and water intake, suggesting that they had recovered from the surgical stress (Table 1). Body weight averaged 24.8 ± 0.4 g before surgery compared with 24.4 ± 1.0 g at the end of the experiment. Figure 2 shows a typical blood pressure and heart rate tracing exactly as seen on the computer monitoring screen. Daily means, dark-to-light ratios, and the phase of maximum and minimum values for blood pressure and heart rate are summarized in Table 2. Blood pressure was greater at night than during the day in all mice (Table 2, $P < 0.01$). This nocturnality was clearly evident in the raster plots and the average waveform for blood pressure and heart rate of a single animal measured for 6 days (Fig. 3, A–D). The group average waveforms (Fig. 4) further revealed a tendency toward bimodality, with peak values occurring usually early and late in the dark period and a trough during the middle of the light period. The timing of both the maximum and the minimum values showed low within-group variance (Table 2).

Mean heart rate was greater at night ($P < 0.05$) and, by comparison with arterial pressure, showed more ultradian periodicities, in the 1.5- to 2.0-h range, in the raster plots and average waveforms (Table 2, Figs. 3 and 4). However, maximum daily values were consistently evident near dark onset, and minimum values were evident during the middle of both the light and dark periods.

DISCUSSION

Using chronically catheterized mice with a computerized data acquisition system, it was possible to continuously monitor blood pressure and heart rate for more than 5 days. This data was used to determine the 24-h pattern of cardiovascular parameters for the first time in mice. Statistical analysis revealed a diurnal rhythm of blood pressure and heart rate, with peak values near

the onset of the dark period and minimum values during the middle of the light period.

A detailed description of the surgical and recording methods is included because there are few reports of chronic cardiovascular measurements in mice. The method is a modification of one developed in our laboratory for use in rats (1, 8). The cage system, designed for microdialysis studies, is ideal for chronic

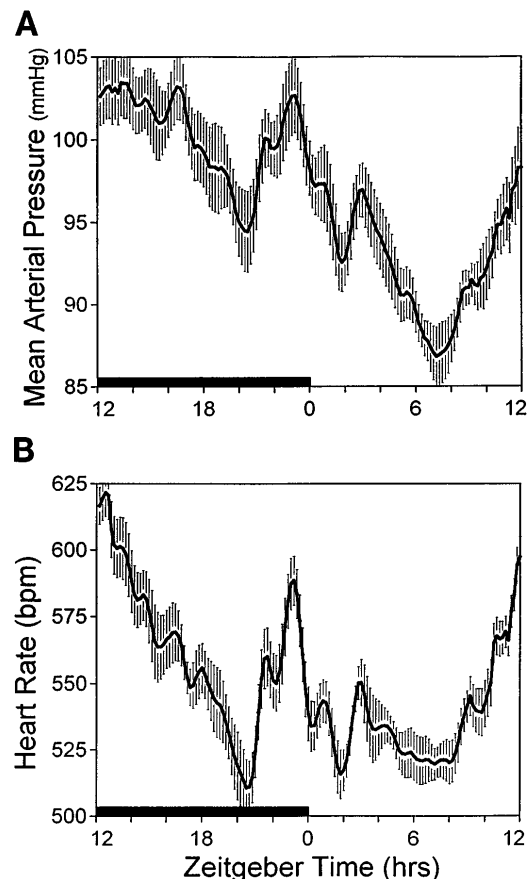


Fig. 4. Group mean waveforms of BP (A) and HR (B) from 5 mice recorded in a 12:12-h light-dark cycle. Dark period is indicated by shaded bar along the *abscissa*. Data show group means $\pm$ SE ($n = 5$).

blood pressure monitoring in mice. The swivel is attached to a counterbalanced pole, allowing for free mobility and a rapid recovery from surgery. Indeed, within 2 days the mice were active and showed a stable pattern of water and food intake. Two factors helped in maintaining catheter patency, the slow heparin infusion and the catheter design, formed from Micro-Renathane tubing, a plastic suggested to reduce proliferative overgrowth. A similar catheter design was used by another investigator to measure blood pressure in mice (10). Their protocol used femoral arterial catheters that were flushed with heparinized saline to prevent clotting. The downside of this method is that femoral catheters often cause tissue necrosis by reducing blood flow. In addition, periodic flushing of the catheter is tedious and not always effective. A computerized data acquisition system is a requirement for such studies in which parameters are monitored continuously, resulting in the collection of large amounts of data. With a sampling rate of 100 Hz, ~500 MB of disk space is required for monitoring 2 mice for 24 h.

An additional advantage of the chronic cannulation method is the ability to collect blood samples or administer injections as well as measure blood pressure. Telemetric recording systems can be used to measure cardiovascular parameters in the unperturbed state; however, they do not permit blood sampling (12, 18). Collection of nonstress blood samples in mice has proved difficult. Most studies in mice use decapitation with or without anesthesia for the collection of blood. The data suggest that, depending on the method, plasma hormone levels can be extremely variable (2, 7, 13). Preliminary experiments using the chronic catheter system indicate that it is possible to collect blood without disturbing the animal as seen by behavior and plasma vasopressin levels.

It is not surprising that the circadian rhythm in mice was similar to that seen in rats. As nocturnally active animals, they show peaks of activity, intake, and cardiovascular patterns during the dark period. The functional significance of the increase in blood pressure and heart rate is probably to allow the animals to meet the metabolic demands of increased activity. There was a diurnal pattern with the maximum MAP at 3 h after lights off compared with 14 h in rats in a similar experimental setup (9). The pattern in mice was very consistent, especially for MAP, as seen by the low within-group variability for a variety of parameters. With regard to heart rate, the maximal value was evident during the early dark phase. This is consistent with two recent reports which employed telemetric methods to study 24-h patterns of heart rate (12, 18). As with our data, the 24-h pattern showed peaks and troughs in heart rate (every 1–2 h) similar to that seen for MAP. These rhythms with a periodicity shorter than the circadian are loosely classified as ultradian. Visual comparison of the results from mice and rats suggests more prominent ultradian MAP and HR rhythms in mice. It is likely that these are associated with rhythms in locomotor and gustatory behavior.

The role of circadian rhythms in the control of physiological function has received increasing attention. In clinical medicine, a specialty has evolved that focuses on the interrelationships between circadian phase and treatment regimens, designated as chronotherapeutics. It is known that drugs have a better effect when provided at the time of most need. For example, antihypertensive medication, when given with a controlled onset delivery system, was most effective when the drug and the blood pressure peaks were synchronized (14). In hypertensive rats, the time of administration of antihypertensive drugs such as converting enzyme inhibitors, blockers, and sympatholytics affected the magnitude of the hypotensive response (6, 15).

Studies of cardiovascular function have moved toward the use of genetically engineered models in which key genes are removed or added. The results show that there is a correlation between blood pressure and the genetic trait. For example, removal of the angiotensin AT_{1a} receptor gene produced an animal with lower blood pressure (5), whereas the addition of the angiotensinogen gene produced hypertension (11). The latter study used chronically cannulated mice; however, the mice were restrained for the measurement, resulting in high variability. There are no reported studies that analyze genetic influences on the circadian blood pressure rhythms in these models.

In conclusion, a method has been demonstrated for the long-term measurement of blood pressure and heart rate in the conscious mouse, allowing for the evaluation of the pattern of the circadian changes. The next stage will be the transfer of this technology for use in models with genetic disruption of the cardiovascular system.

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