

A new method for measurement of blood pressure, heart rate, and activity in the mouse by radiotelemetry

PERRY A. MILLS,¹ DANIEL A. HUETTEMAN,¹ BRIAN P. BROCKWAY,¹ LYNN M. ZWIERS,¹
A. J. MICK GELSEMA,² ROBERT S. SCHWARTZ,³ AND KLAAS KRAMER⁴

¹Data Sciences International, St. Paul, Minnesota 55126; ²IBIS Instrumentation Canada, Ottawa, Ontario, Canada K1Z 7Z3; ³Mayo Clinic, Rochester, Minnesota 55905; and ⁴Department of Pharmacochimistry, Division of Molecular Pharmacology, Leiden/Amsterdam Center for Drug Research, Free University, 1081 HV Amsterdam, The Netherlands

Mills, Perry A., Daniel A. Huetteman, Brian P. Brockway, Lynn M. Zwiers, A. J. Mick Gelsema, Robert S. Schwartz, and Klaas Kramer. A new method for measurement of blood pressure, heart rate, and activity in the mouse by radiotelemetry. *J Appl Physiol* 88: 1537–1544, 2000.—A simple and reliable means for accurate, chronic measurement of pulsatile blood pressure (BP) from conscious, freely moving laboratory mice was developed and validated. The newly developed device consists of a small (1.9 ml, 3.4 g), fully implantable radiotelemetry transmitter. Initial frequency response tests showed an adequate dynamic response; the average –3-dB point found in five transmitters was 145 ± 14 (SD) Hz. BP, heart rate, and locomotor activity were recorded from 16 chronically (30–150 days) implanted mice. Mean arterial and pulse pressure, checked at regular intervals, ranged from 90–140 mmHg and from 30–50 mmHg, respectively, throughout the study. Transmitter BP measurements were validated against a Millar 1.4-Fr. transducer-tipped catheter. The mean error of the transmitters for diastolic pressures was $+1.1 \pm 6.9$ mmHg ($n = 7$). The error for systolic pressures was, on average, 2.7 ± 3.9 mmHg larger. This new device accurately monitors BP, heart rate, and locomotor activity in conscious, untethered, freely moving mice living in their home cages for periods of at least 150 days.

conscious animals; chronic implant; frequency response; biocompatibility

THE MOST COMMON TECHNIQUES currently employed for monitoring blood pressure (BP) in conscious mice are the use of a tail-cuff device (11) or the use of an exteriorized, fluid-filled catheter that refers pressure to a transducer located nearby (12).

The tail-cuff method has the advantage of being noninvasive. However, the accuracy of BP measurements with this method in rodents is known to be greatly affected by environmental factors, as well as by any physiological or pharmacological factor that influences blood flow in the tail (6, 7, 11, 12). Continuous measurement of pulsatile arterial pressure cannot be accomplished with the tail-cuff method but can be obtained with exteriorized catheters. When used with

caution, particularly with respect to catheter size, length, and compliance (all of which influence the dynamic response of the system), reasonably accurate measurements of systolic, diastolic, and pulse pressure can be made for several days to up to 1–2 wk. Decreasing catheter patency is the usual problem that limits the duration of reliable recordings (12).

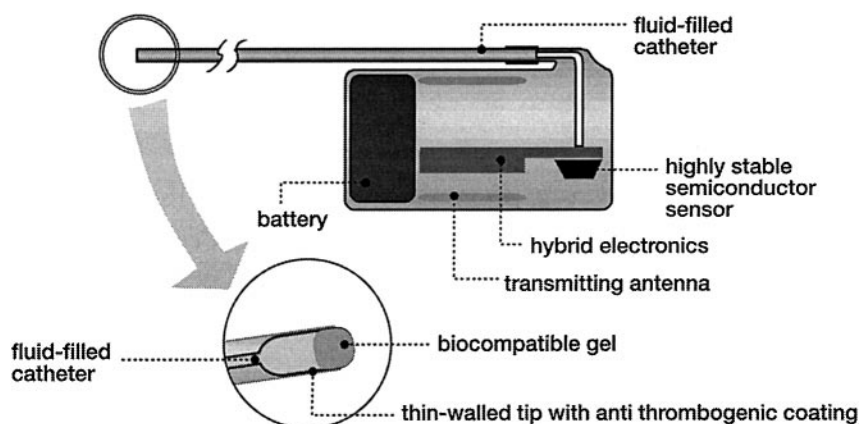
This article describes a radiotelemetry device that can circumvent many of the problems associated with conventional methods of BP monitoring in mice. This implantable device provides accurate and reliable measurements of systolic, diastolic, and mean BP as well as heart rate (HR) and locomotor activity from freely moving mice housed in their home cages. Continuous or semicontinuous recordings can be made from mice for periods of weeks up to several months. The system allows for the storage and automated analysis of recorded BP data as waveforms, as beat-to-beat systolic, mean, and diastolic pressures and HR data, or as any combination of these options. The frequent sampling and recording of highly accurate data by this system lead to a decrease in intra- and interindividual variability, allowing statistically significant conclusions to be drawn from studies using considerably smaller animal populations than with conventional BP monitoring techniques. The longevity of the implants allows, in addition, for within-group crossover studies or participation of individual animals in multiple sequential studies, potentially further reducing animal use.

MATERIALS AND METHODS

The majority of components of the radiotelemetry system used in this study existed previously and have been described in more detail elsewhere (4). In brief, the new, implantable mouse BP transmitter (described below) provides a direct measurement of arterial pressure and telemeters it digitally from within the animal. The receiver detects the radiofrequency signal from the transmitter and converts it into a serial bit stream. Ambient barometric pressure is also measured and subtracted from the telemetered pressure by data collection software (Dataquest A.R.T., Data Sciences International, St Paul, MN) to compensate for changes in atmospheric pressure. During the various protocols of this study, data were collected and stored to disk using the Dataquest A.R.T. data acquisition system.

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.

Fig. 1. Schematic drawing of mouse blood pressure (BP) transmitter. A 5-cm-long fluid-filled catheter with a thin-walled tip (*inset*) refers intra-arterial pressure to a sensor located in the body of the intraperitoneal implant, which also contains the electronics for signal handling and radio transmission, powered by a silveroxide alkaline zinc battery.



Mouse BP Transmitter

The implantable mouse BP transmitter used in this study is shown schematically in Fig. 1. It contains a semiconductor strain gauge sensor measuring 1.5×1.5 mm. This sensor is mounted on Pyrex to isolate it from thermal and mechanical stresses, thereby increasing stability of the pressure measurements. Micropower electronics (to amplify the sensor signal, modulate the radio frequency, and transmit the information) are located on a 6.3×13.7 -mm multilayer ceramic hybrid and are powered by a silver cell.

Arterial pressure is referred to the sensor via a 0.4-mm-diameter (OD), 5-cm-long fluid-filled catheter. The catheter is composed of two distinct sections. The distal 4 mm is composed of thin-walled, highly compliant urethane with an antithrombogenic surface preparation (patent pending). The function of this thin-walled tip is to efficiently transmit the high-frequency components of the pressure signal into the lumen of the catheter. The entire compliant tip section must therefore be located within the vessel. The remainder of the catheter (referred to as the stem) is specially constructed of urethane with a 0.1-mm wall and a 0.2-mm lumen to provide a combination of low compliance and kink resistance. The total compliance of the stem and pressure sensor is 0.024 nl/mmHg.

The lumen of the catheter is filled with a low-viscosity fluid, whereas the distal 2 mm of the thin-walled tip is filled with a blood-compatible gel that prevents blood from entering the catheter lumen (5). An antithrombogenic film is applied to the distal 1 cm of the catheter. The electronics, sensor, and battery are packaged in a thermoplastic housing, coated with silicone elastomer to provide biocompatibility. A suture tab molded into the housing provides three sites for securing the device *in vivo* during implantation. Total weight of the implant is 3.4 g, and its volume displacement is 1.9 ml. The device has a continuous-use battery life of 8 wk. Transmitter power can be toggled off and on with the module *in situ* by passing a magnet near the animal, thus extending the useful battery life of the implant.

Study Protocols

The present study is composed of three separate protocols, as described below.

Protocol 1: Testing the mouse BP transmitter frequency response. A major concern during the development of the mouse BP transmitter was the adequacy of its frequency response. This was determined by analyzing continuous BP waveforms obtained from the ascending aorta of two anesthetized CD-1 mice (30 g body wt; Charles River, Wilmington, MA), using a Millar SPR-671 transducer-tipped catheter

(TTC; Millar Instruments, Houston, TX). Frequencies were artificially elevated by increasing HR to ~550–600 beats per minute (bpm) with injections of isoproterenol ($0.75 \mu\text{g/kg ip}$). Data recorded and stored by Dataquest A.R.T. were analyzed for calculation of the power spectrum using MatLab (The Math Works, Natick, MA). These power spectra were used to visualize the significant harmonics of the BP signal in the mouse aorta.

The frequency responses of five mouse BP transmitters were subsequently quantified by using a Bio-Tek model 601A blood pressure system calibrator (Bio-Tek Instruments, Winooski, VT). The pressure-sensing catheter of a mouse BP transmitter and a Millar SPR-671 TTC were inserted into the pressure measurement chamber, which was carefully flushed and filled with distilled water to remove all air bubbles. The chamber was then excited with sinusoidal frequencies ranging from 0.1 to 200 Hz. Samples of the responses of the TTC and the mouse BP transmitter were collected and stored by custom-made software and analyzed by a Hewlett Packard vector signal analyzer (HP 89410A).

Protocol 2: Testing the mouse BP transmitters' long-term performance and biocompatibility. Long-term performance and biocompatibility of the mouse BP transmitter were studied in 16 adult, male outbred Swiss Webster (SW) mice (31.5 ± 4.0 g body wt, range of 24.5–41.5 g; Harlan ND4, Harlan Sprague Dawley, Indianapolis, IN). Offset of each transmitter was checked before implantation. A maximum implant offset of ± 2 mmHg at ambient pressure and room temperature was deemed acceptable.

Immediately before implantation, each transmitter was soaked in sterile saline at room temperature for a minimum of 10 min to dimensionally stabilize the catheter material.

In preparation for surgical implantation of the device, the animal was placed in a sealed chamber and anesthetized with 3% isoflurane (IsoVet, Shering Plough, Union, PA) in oxygen, using a calibrated vaporizer (CDS 2000, Anesco, Georgetown, KY). During the surgical procedure, the isoflurane concentration was reduced (1.5–2.0%) to maintain a respiration rate between 60 and 90 breaths/min. Aseptic conditions were maintained throughout the surgical procedure. The animal was then placed on a warm operating surface, and the ventral abdomen was shaved. The peritoneal cavity was accessed via a ventral midline incision (~3 cm), and the intestines were gently retracted with saline-soaked gauze, permitting access to the abdominal aorta from the renal arteries to the iliac bifurcation. Under a binocular operation microscope, an occlusion suture (5-0 silk) was inserted between the aorta and the vena cava just caudal to the left renal artery. With gentle tension placed on the occlusion suture to temporarily occlude blood flow, the aorta was punctured ~2 mm cranial to the iliac

bifurcation with a 25-gauge hypodermic needle bent at a 90° angle. The catheter was grasped with a pair of vessel cannulation forceps (S&T 00608–11, Fine Science Tools, North Vancouver, BC, Canada), and, with the use of the bent needle as a catheter introducer, the catheter was inserted upstream into the aorta to a length of 5–6 mm. The area was then cleaned with sterile cotton applicators, and the catheter insertion site was sealed with a minimal application of tissue adhesive (Vetbond, 3M, St. Paul, MN). While the tension on the occlusion suture was slowly released, the catheter entry site was observed for leakage. The cannulated aorta was irrigated with 2% lidocaine to prevent vessel spasm and covered with a 3 × 5-mm cellulose fiber patch. The patch was secured to the surrounding tissues with additional tissue adhesive, thus both anchoring the catheter and fostering connective tissue growth. The peritoneal cavity was flooded with warm saline, and the intestines were then gently massaged back into place. The device body was positioned in the abdomen, and the suture rib on the transmitter housing was incorporated into the abdominal wall closure by using a 4-0 nonabsorbable suture. The skin was closed with 9-mm surgical staples.

Animals were returned to their home cage and closely monitored for 4–6 h after surgery; during this time, they were provided with supplemental warmth by a 40° warming pad placed under half of the cage bottom. The animals were closely monitored for 4–6 h. When fully recovered, the mice were housed individually in standard polycarbonate cages (21 × 32 × 18 cm) under 12:12-h light-dark conditions. Food and water were available ad libitum. The mice were examined daily for any abnormal clinical signs during the first 7 days postsurgery and three times per week thereafter. The quality of the BP signal was checked at similar intervals. BP signals showing mean arterial pressures (MAP) ranging from 90 to 140 mmHg and pulse pressures ranging from 30 to 50 mmHg were considered satisfactory.

Blood pressure transmitter data were validated on the last day of the implantation period in 11 of the 16 mice. For this, the animals were anesthetized with isoflurane and positioned in supine position on a warm operation table. The left carotid artery was then prepared for catheterization with a 1.4-Fr. (480 µm OD) Millar model SPR-671 TTC. Before catheterization, the Millar TTC was carefully zero balanced and calibrated to accurately represent 0, 100, and 200 mmHg. The TTC was advanced ≥12 mm into the vessel until a strong systemic BP signal with MAP ranging between 65 and 100 mmHg was observed, indicating that the tip had advanced ~2 mm into the thoracic aorta. The TTC was fixed into position with a ligating suture. Systemic BP waveforms were then recorded simultaneously from the BP transmitter and the TTC by the Dataquest A.R.T. data acquisition system. A 1-min portion of these recordings was used for analysis. These periods were subdivided into 30 sections of 2 s, during which the Dataquest A.R.T. analysis module calculated the average systolic, diastolic, and mean BP values as measured by the transmitter and the TTC. Thus 30 pairs of validation data were obtained per mouse for each of these three features. The validation pairs were analyzed for their bias, estimated by the mean difference and the standard deviation of the differences between pairs after subtraction of zero offset from the transmitter, according to the methods described by Bland and Altman (3). Zero offsets were determined after explantation by measuring the transmitters' response to exposure to ambient pressure.

Three chronically implanted mice were, at the end of their chronic implantation period (50–100 days), anesthetized and perfused with heparinized saline, followed by a 2.5% solution of glutaraldehyde in phosphate buffer (14). Perfused aortas

were excised with the pressure-sensing catheters in situ. The samples were coated with 1 nm of platinum by ion beam sputtering at 9.4 kV and subsequently imaged using a Hitachi S4700 field-emission scanning electron microscope.

At completion of the study, the abdomen of each mouse was examined after transmitter explantation for unusual growths, discoloration of organs, or other gross pathological signs.

Protocol 3: Studying the effect of implantation on locomotor activity. To measure locomotor activity, the effect of the telemetry device on running wheel activity before and after implantation was studied in four SW mice. The animals weighed 27.7–28.7 g at the time of surgery. Implantation was performed as described for *protocol 2*. Before and after the operation, animal body weights were measured three times per week.

Running wheels, 11 cm in diameter, were purchased at a local pet shop. They were customized with optical sensors such that a light beam was interrupted on each complete revolution of the wheel. The total number of revolutions was recorded by the Dataquest A.R.T. collection system.

Before implantation surgery, the mice were allowed to acclimate to the running wheels for 10 days. After surgery, the running wheels were immobilized during a 10-day period to facilitate full recovery and then the wheels were freed and the subjects were allowed to resume their running wheel activity for another 20 days until termination of the study.

Data Analysis and Statistical Methods

Data are expressed as means ± SD. Differences between transmitter and Millar TTC in BP measurements observed during the validation studies are expressed as transmitter values minus TTC values. Differences in running wheel activity before and after implantation were analyzed by means of a repeated-measures two-way ANOVA, followed by a paired *t*-test; *P* < 0.05 was regarded as significant.

RESULTS

Frequency Response

A typical power spectrum of mouse BP, calculated from a pressure recording using a Millar TTC in the aorta of an anesthetized mouse while HR was elevated by an intraperitoneal injection of isoproterenol, is shown in Fig. 2. The highest peak, representing HR, is found at ~9 Hz. Harmonics with diminishing power can be seen up to the 12th harmonic. Because maximum HR in conscious mice has been estimated as 800 bpm (Ref. 10; fundamental frequency = 13.33 Hz), a linear frequency response (±3 dB) of the mouse BP transmitter up to the sixth harmonic, or 80 Hz, is considered adequate for an accurate reproduction of the original waveform (8, 15).

The frequency response of a typical mouse BP transmitter is shown in Fig. 3. Increasing the driving frequency from 0.5 to 70 Hz resulted in a flat response curve (within ±0.5 dB). At still higher frequencies, attenuation started gradually; the –3-dB point shown in Fig. 3 was reached at 172 Hz. The average for the –3-dB point found in the frequency response curves of five transmitters was 145 ± 14 Hz.

Long-Term Performance and Biocompatibility

Sixteen mice were studied after implantation with a BP transmitter. Two of these were found dead 3.5 and 5

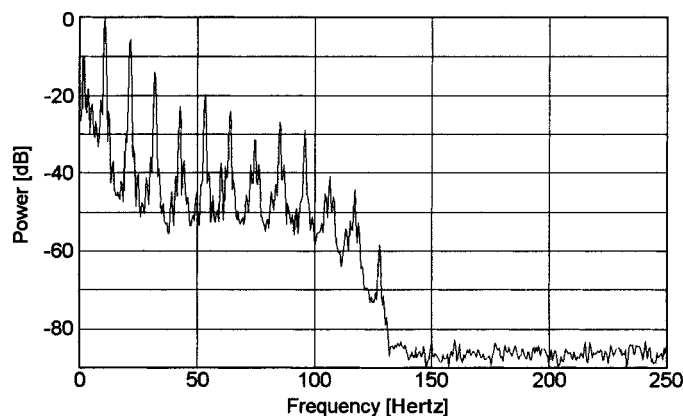


Fig. 2. Power spectrum of mouse BP, derived by means of an aortic Millar SPR-671 transducer-tipped catheter (TTC). Note the relatively low heart rate as represented by the first peak at ~ 8.9 Hz (534 beats/min). Significant but progressively attenuated components occur up to the 9th harmonic; smaller components up to the 12th harmonic can still be discerned.

days after implantation, respectively. The death of one of these mice was probably due to blood clot formation at the tip of the catheter, found during necropsy. In the second case, fecal blockade was found during necropsy, likely due to bowel injury during surgical implantation. Both deaths were considered to be a direct consequence of surgical errors.

A third mouse was found dead in his cage 119 days after implantation. The BP signals recorded from this mouse on *days 30, 60, and 90* were satisfactory. Necropsy showed excessive encapsulation of the transmitter, thought to be due to bacterial contamination as a result of inadequate sterile techniques during implantation.

The 13 successfully completed studies lasted on average 77.3 ± 44.8 days (range of 30–150 days). The absolute levels of BP and pulse pressure were satisfactory for each transmitter at all intervals postimplantation. Validation of the BP measurements by simultaneously recording aortic pressure via the implanted transmitter and a Millar TTC inserted via the carotid

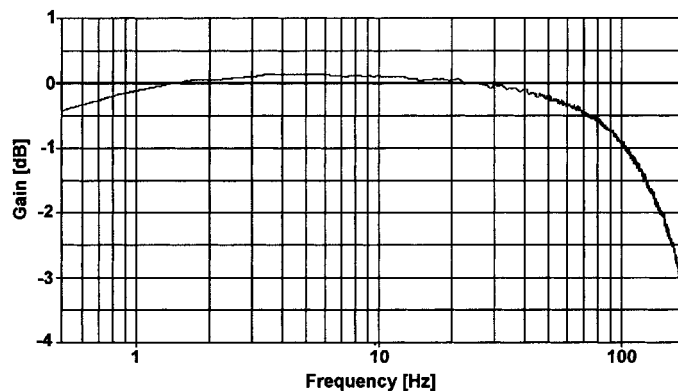


Fig. 3. Frequency response of a typical PA-C20 mouse BP transducer relative to the response of a Millar SPR-671 TTC. Both instruments respond essentially similarly at frequencies ranging from 0 to ~ 50 Hz. Output of the PA-C20 becomes attenuated at higher frequencies; the -3 dB point is reached at 172 Hz.

artery into the thoracic aorta was attempted at the end of each study. Because of technical and surgical difficulties, this validation failed in six cases. Two of the six failures were due to technical difficulties with the TTC probe, from which no valid BP values could be obtained. In the four remaining cases, highly unstable values were recorded from the BP sensors after introduction of the TTC probe, even though reliable BP registrations had been made from the transmitter data until shortly before the induction of anesthesia for the validation procedure.

We had successful validations in seven mice, with an average implant duration of 74 ± 45.1 (range of 33–150) days. A typical example of a validation record from one of these mice is shown in Fig. 4. Figure 4A shows a 2-s record from both sensors. The pressure readings of the transmitter include a $+2.7$ mmHg zero-offset error found after explantation. Clearly, the transmitter values are consistently ~ 8 mmHg higher than the TTC values. Note the ~ 10 -ms time lag between the two waveforms, due to the placement of the TTC in the thoracic aorta and the downstream position of the catheter tip of the transmitter in the abdominal aorta. From a 1-min BP record from this mouse, 30 pairs of systolic, mean, and diastolic pressure values were extracted for further analysis. In Fig. 4B, the offset-adjusted transmitter values (y-axis) are plotted against the TTC values, along with the line of equality. Figure 4C shows the offset-adjusted difference between the two BP measurement methods plotted against the mean for the measurements. The solid line in Fig. 4C indicates the mean difference ($+4.93$ mmHg), and the dashed lines indicate the limits of agreement (means ± 2 SD; $+2.94$ to $+6.93$ mmHg). Thus 95% of the transmitter measurements are between 3 and 7 mmHg higher than those of the TTC. Inspection of Fig. 4, B and C, shows that the differences tended to be larger with increasing pressures. This trend was found in five of the seven validations; on average, the differences between transmitter and TTC values for systolic pressures were 2.65 mmHg larger than the differences for diastolic pressures. Because this effect was considered to be from a kinetic energy-derived pressure component in the measurements of the transmitters (15), which is minimal at the end of the diastole, further estimates of the agreement between the two methods of BP measurement were derived from the sets of diastolic pressures only. An example is shown in Fig. 4D for the values of the diastolic pressures recorded from the same mouse as in Fig. 4, A–C. The mean difference between the transmitter and TTC measurements (transmitter – TTC) was $+3.76 \pm 0.25$ mmHg; thus we estimate that this particular transmitter overestimated pressures by between $+3.27$ and $+4.25$ mmHg compared with the TTC. Bias estimates for all seven individual transmitters are given in Table 1. It should be noted that the values in Table 1 were adjusted for zero-offset drift. The possible cause(s) for these remaining differences is reviewed in the DISCUSSION.

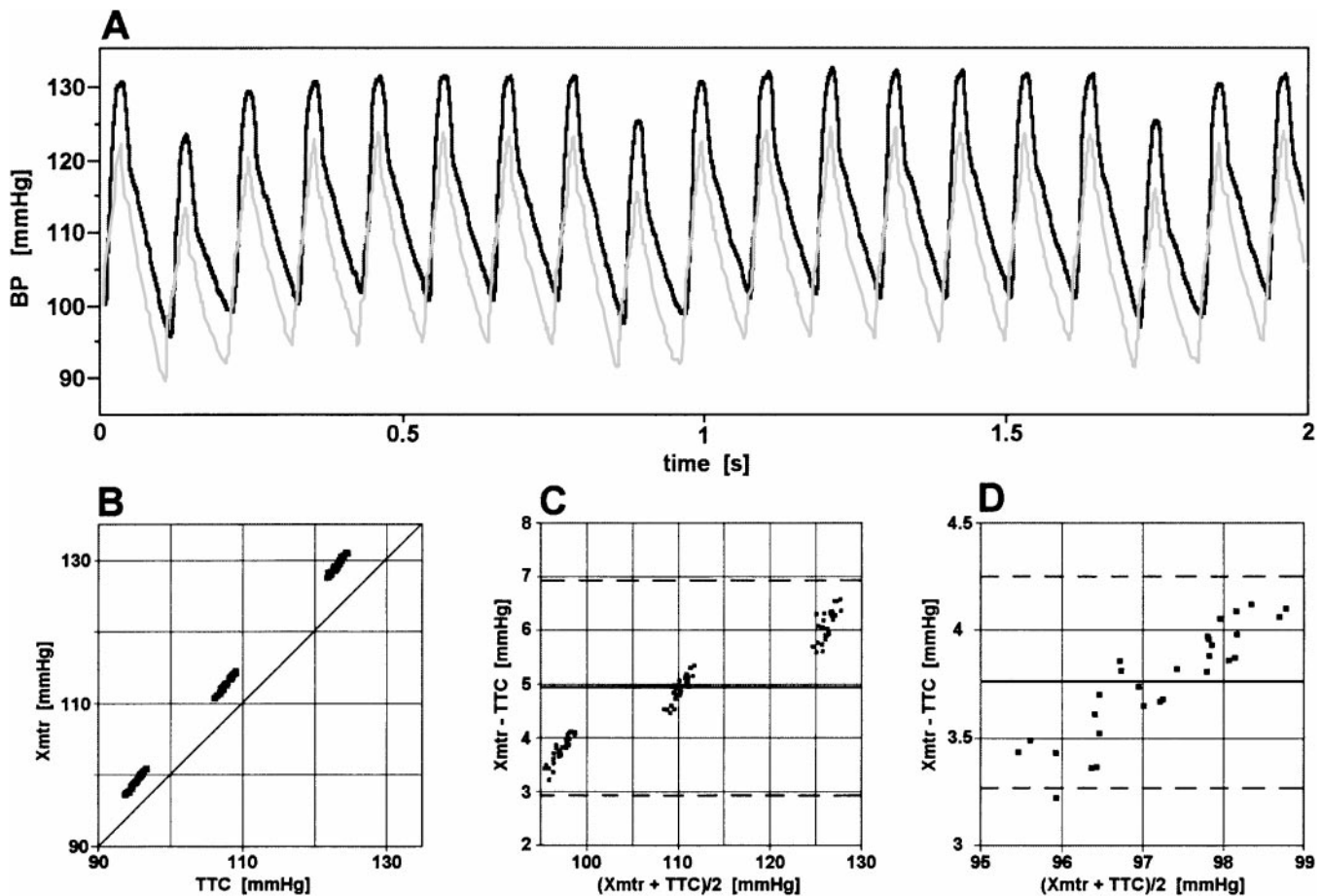


Fig. 4. *A*: simultaneous BP waveforms from a Millar TTC (gray trace) and PA-C20 transducer (solid black trace) in a mouse 33 days after implantation. Transmitter values in this record have not been corrected for a +2.7 mmHg zero-offset error found at explantation. *B*: relationship of simultaneous PA-C20 (Xmnt) vs. Millar TTC BP measurements in the mouse shown in *A*. Note that offset-corrected transmitter values are slightly higher than TTC values and more so for systolic than for diastolic pressures. *C*: differences between the PA-C20 and Millar TTC (Xmnt - TTC) measurements vs. average measurements from the same set of values as shown in *B*. Mean of differences (bias) is indicated by solid horizontal line; limits of agreement (means \pm 2 SD) are indicated by interrupted lines. *D*: same as *C* but for diastolic pressures only.

On explantation of the transmitters from 15 mice (the transmitter of the mouse that was found dead was not tested), average zero offset was 1.39 ± 2.49 mmHg (range of -2.9 to $+5.1$ mmHg). There was no obvious (linear) relationship between postimplantation offset and implant duration ($r^2 = 0.002$; $F = 0.029$).

No gross pathological signs were noticed postexplantation in the abdomen of 13 mice. In one mouse, the transmitter was found encapsulated, thought to be due to introduction of fecal bacteria during implantation 33 days earlier. In a second mouse, minor fibrous adhe-

sions between adjacent intestinal segments were found 35 days after implantation.

Minor plaque formation on the intravascular surface of the catheter was found at autopsy in 6 of the 16 mice. In an additional two, evidence of aorta plaque was found. Scanning electron microscopy revealed various stages of organizing thrombus as well as platelet adhesion on the covering surface of the explanted catheters. In many cases, however, well-delineated endothelium was found on the catheter surface (Fig. 5) with very little evidence of inflammatory responses.

Table 1. Zero-offset-adjusted differences between measurements (transmitter - TTC) in the 7 successful validations

	Transmitter						
	1	2	3	4	5	6	7
Means \pm SD	6.52 ± 0.32	3.76 ± 0.25	5.54 ± 1.65	7.26 ± 0.61	-11.88 ± 0.27	-0.05 ± 0.93	-3.16 ± 0.50
Limits of agreement	5.88 to 7.17	3.27 to 4.25	2.24 to 8.84	6.03 to 8.49	-12.41 to -11.35	-1.91 to +1.81	-4.17 to -2.16

Values are in mmHg. TTC, transducer-tipped catheter.

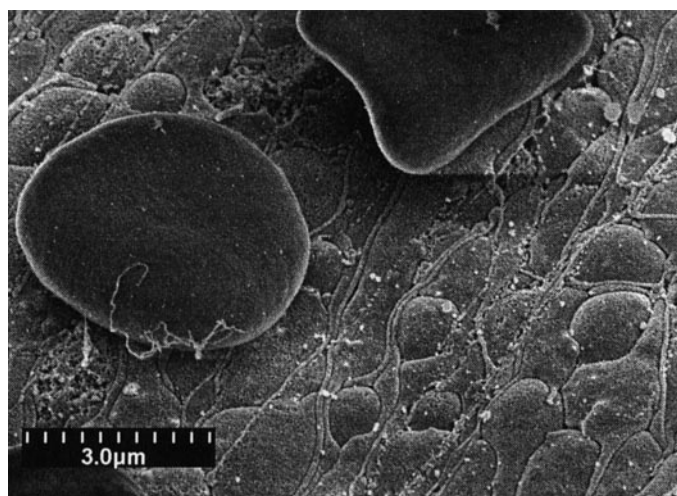


Fig. 5. Scanning electron photomicrograph of the covering of the intravascular section of a mouse aorta catheter, harvested after 126 days of implantation. Note the well-delineated endothelium, organized as a continuous, closed layer. Magnification: $\times 10,000$; bar = 3 μm .

Occasional macrophages were seen, but no other cells typifying inflammation, such as giant cells, lymphocytes, or eosinophils, were seen.

Effect on Running Wheel Activity

Figure 6 shows body weight changes (\pm SD) in four SW mice in the 10 days before implantation and during the 4 wk after implantation. Mean body weight on day 10 before surgery was 28.1 ± 0.5 g. On average, a 10% loss in body weight (after subtraction of transmitter weight) was noted in the first 24 h after surgery, followed by a further 2.5% decrease in the subsequent 2–3 days. Body weight was restored to preimplantation levels ~ 2 wk after surgery.

These four mice were trained to running wheel activity during the 10 days before implantation and were allowed to resume this activity after a 10-day recovery period after implantation.

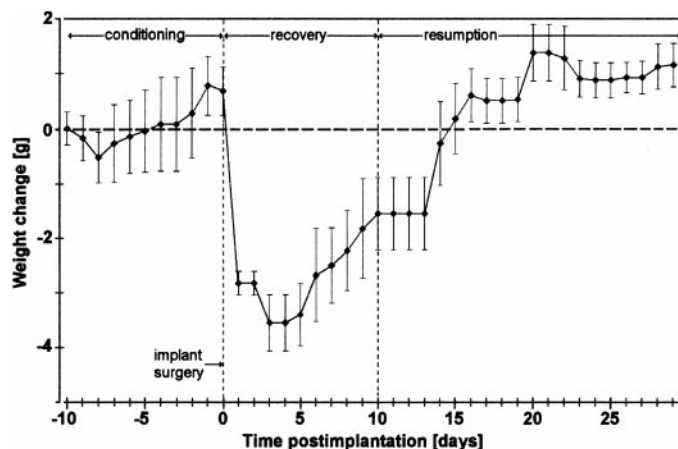


Fig. 6. Average change in body weights of 4 mice during 10 days before implantation (during conditioning to running wheels), during 10 days of recovery (with running wheels immobilized), and during subsequent 20 days (running wheel activity resumed). Average body weight on day 10 was 28.1 ± 0.5 g.

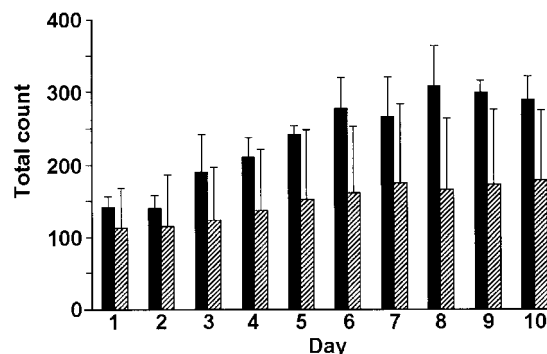


Fig. 7. Average running wheel activity (\pm SD) of 4 mice during 10 days before implantation (solid bars) and on days 10–20 after implantation (hatched bars). Mice ran significantly ($P < 0.001$) less after implantation.

Average running wheel activity of these 4 mice on days 10–20 postimplantation was 35% less (range of -46% to $+3\%$) than that measured during the 10 days before implantation (Fig. 7). These differences were statistically significant ($P < 0.001$). Running wheel activity on days 21–30 postimplantation (after which the study was terminated) did not further improve (data not shown).

DISCUSSION

The present studies evaluate the properties of a new device for chronic measurement of pulsatile BP and HR in conscious, untethered, freely moving mice, consisting of a fully implantable pressure transducer and radiotransmitter and related hardware and software. Dynamic calibrations showed a relatively flat (± 3 dB) frequency response of the mouse BP transmitters up to 145 ± 14 Hz, more than adequate to represent aortic BP waves in the mouse accurately, even at the highest HRs. Long-term implantations have shown no detrimental effects on health or gross behavior of the mice, and no gross pathological signs were recorded at autopsy. After 30–150 days of implantation, the absolute offset error of the devices ranged from -2.9 to $+5.1$ mmHg, apparently independent of implant duration. The mean bias of the devices (after adjustment for individual offset error) was $+1.1 \pm 6.9$ mmHg.

Two major technical difficulties were encountered during the present studies: the challenge of implantation surgery and unexpected problems encountered during validations. The small size and delicate nature of the mouse arteries proved to be demanding on hand-eye coordination and required the steady hand of the operator to successfully catheterize the vessel. Correct temperature control during implantation surgery and throughout recovery from anesthesia proved to be essential, as was the strict maintenance of a sterile environment. The use of an inhalation anesthetic was also considered an important factor in achieving a high success rate. It allowed the surgeon to finely control the depth of anesthesia and provided supplemental oxygen in the face of depressed respiration rates induced by anesthesia. On completion of the procedure, the anesthesia can be discontinued and the

animal can be promptly returned to normal cardiac and respiratory function, which is essential for prevention of hindlimb ischemia, one of the major complications of aorta catheterization in rodents, particularly in mice (12).

Other problems were encountered during the transmitter validations at the end of chronic implantation periods. Although apparently reliable and stable recordings had been made throughout the implantation periods, the introduction of the Millar TTC probe via a carotid artery into the thoracic aorta commonly resulted in unstable and sometimes rather variable signals recorded by the transmitter. It seems highly unlikely that disturbances of laminar blood flow, caused by the Millar TTC probe, would have influenced measurements by the transmitter located downstream, as the two were separated by the length of most of the thoracic and upper abdominal aorta. We cannot offer a satisfactory explanation for the instability in pressure measurements by the transmitter accompanying introduction of the Millar TTC.

A potential source of disagreement between the transmitter-recorded pressures and those recorded by the Millar TTC is the variability of pressures recorded by the latter. The combined variability due to hysteresis and changes in temperature and linearity amounts to ± 6 mmHg (13).

The differences in pressure measurements between the two methods found in the present validation studies could have arisen from changes in offset and/or gain of the new mouse BP transmitter. Changes in zero offset (at ambient pressure) have been recorded at the end of the validation procedures and ranged from -2.9 to $+5.1$ mmHg, which is within the manufacturer's specifications. Five transmitters in the present study were implanted for over 100 days. Zero-offset drift in these five transmitters amounted to less than ± 1 mmHg/mo. This agrees well with chronic (6–12 mo) bench tests with water-submerged transmitters, which suggest a random offset drift of ~ 1 mmHg/mo.

Although offset errors were taken into account, differences in diastolic pressures varying from -11.9 to $+7.3$ mmHg between transmitter and Millar TTC pressures remained (Table 1). One might speculate that these remaining differences were due to changes in the transmitters' gain. This seems highly unlikely, however, as the individual differences were not reflected by similar but smaller differences in the amplitude of pulse pressure. It seems plausible that the remaining differences in measurement, as listed in Table 1, are merely due to (various combinations of) measurement errors.

The differences between BP measurements from the transmitter and the Millar TTC probe per individual animal proved not to be the same for systolic and diastolic pressures; differences between the two methods in systolic pressures were usually slightly ($+2.4$ to $+6.1$ mmHg) higher than those in diastolic pressures (see Fig. 4C). This was thought to be a reflection of the principal differences in shape between the two BP probes and their positions relative to the bloodstream.

The pressure sensor of the Millar probe is mounted close to the tip of the probe and is positioned so that the blood flows along the sensor; i.e., it measures lateral pressure but not "end-on pressure" (15). In contrast, the sensor of the transmitter is located in the body of the implant, and pressure is referred to the sensor through a fluid-filled catheter, the tip of which is exposed to lateral pressure as well as end-on pressure, as the catheter points upstream, facing the bloodstream in the abdominal aorta. Peak (systolic) blood flow in the aorta of an anesthetized mouse has been reported as 80–90 cm/s (9, 16). The kinetic pressure equivalent resulting from a flow of this magnitude impacting on the catheter tip (equal to $\rho v^2/2$) is therefore $80^2/2$ to $90^2/2$ dyn/cm² (assuming $\rho = 1$), which amounts to 2.4–3.0 mmHg (15). This approximates the amount by which the differences in systolic pressures between the two methods are larger than those in diastolic pressures. Because of this, and in view of the fact that blood flow during diastole is negligible, we based the calculation of the true differences in measurement between the two methods, or bias, on the values of diastolic pressures only (Table 1).

The implant was well tolerated by the mice in this study. After an initial 12.5% decline in body weight in the days following the operation, body weight was restored to preimplantation levels ~ 2 wk later. Importantly, no mice showed overt signs of decreased facility of their hind legs, which is a known risk of interruption of blood flow through the aorta in small rodents. Two mice had to be euthanized within 5 days following implantation, and one mouse was found dead 17 wk after the operation. These incidents were regarded as a result of initial inadequate surgical precautions and procedures to counter the considerably higher sensitivity of mice vs. larger rodents to the risks of anesthesia and surgery. One year after these initial studies with the new mouse BP transmitter, the rate of implantation survival achieved by one of the authors (Huetteman) is now consistently over 90%.

Locomotor activity was significantly affected by the implants. Ten to twenty days after implantation, an average 35% decrease in running wheel activity was found in the four mice tested. This effect was mainly due to the lack of activity of one of the four mice, whose overall activity after implantation was only 12% compared with before implantation. In the other three mice, this number varied from 69 to 104%. Autopsy revealed encapsulation of the transmitter, due to surgical introduction of fecal bacteria in the low-activity mouse but not in the other three running-wheel mice, suggesting a causal relationship. One should realize that, although the implants weigh only 3.4 g, this added weight represented a sudden 12% increase in average body weights of these mice. However, studies have shown that, after a recovery time of 7–10 days after implantation of intraperitoneal transmitters with a weight and volume displacement similar to those of the present BP transmitters, mice coped very well with the weight of these transmitters. No differences in behavioral parameters, such as eating, drinking, climb-

ing, locomotion, grooming, and resting, were found 7–10 days after implantation compared with nonimplanted animals (1).

Minor plaque formation on the surface of the catheter was found at autopsy in 6 of the 16 mice. In an additional two mice, evidence of aorta plaque was found. The origin of this unexpected and apparent species-specific consequence of chronic catheter implantation was summarily investigated. However, no correlation was found between the incidence of plaque formation and any of the following single variables: implant duration, chronological order of implantation, differences between TTC- and transmitter-measured pressures, or transmitter offset. Scanning electron microscopy revealed various stages of organizing thrombus as well as platelet adhesion on the covering surface of the explanted catheters. In many cases, however, well-delineated endothelium was found on the catheter surface (Fig. 5). Thus catheters showed excellent endothelial coverage and the development of neointima, consistent with other devices that are highly biocompatible. The paucity of inflammatory responses or rejection at the intravascular tip of the catheter, as judged from only occasional macrophages and total lack of giant cells, lymphocytes, and eosinophils, also suggests high biocompatibility of the device.

Further studies on the properties and tolerability of the new mouse BP transmitter and on associated catheter plaque formation will be needed. One objective should be to identify methods of implantation that will simplify the surgical procedure, thus allowing the use of this promising new device among a broader base of laboratories.

The present findings indicate that, with the use of the new mouse BP transmitter in freely moving mice, a more efficient, reliable, and less labor-intensive method than the measurement techniques (tail-cuff, exteriorized catheters) described in the literature thus far can be obtained.

This work was supported in part by National Heart, Lung, and Blood Institute Grants 1R43-HL-55823-01 and 2R44-HL-55823-02.

Address for reprint requests and other correspondence: B. P. Brockway, Data Sciences International, 4211 Lexington Ave.

North, Suite 2244, St. Paul, MN 55126-6164 (E-mail: bbrockway@datasci.com).

Received 10 September 1999; accepted in final form 6 December 1999.

REFERENCES

1. **Baumans V, Bouwknecht JA, Boere H, Kramer K, van Lith HA, van de Weerd HA, and van Herck H.** Intraperitoneal transmitter implantation in mice: effects on behavioural parameters and body weight (Abstract). Available from: *Noldus Information Technology*, 1998. <http://www.noldus.com/events/mb98/abstracts/baumans.htm> [2000, Feb 24].
2. **Bazil MK, Krulan C, and Webb RL.** Telemetric monitoring of cardiovascular parameters in conscious spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 22: 897–905, 1993.
3. **Bland JM and Altman DG.** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*: 307–310, 1986.
4. **Brockway BP and Hassler CR.** Application of radiotelemetry to cardiovascular measurements in pharmacology and toxicology. In: *New Technologies and Concepts for Reducing Drug Toxicities*, edited by Salem H and Baskin SI. Boca Raton, FL: CRC, 1993, p. 109–132.
5. **Brockway BP, Mills PA, and Miller JT** (Inventors). *Device for Chronic Measurement of Internal Body Pressure*. US Patent 4846191. 11 July 1989.
6. **Buñag RD.** Facts and fallacies about measuring blood pressure in rats. *Clin Exp Hypertens [A]* 5: 1659–1681, 1983.
7. **Buñag RD, McCubbin JW, Page IH.** Lack of correlation between direct and indirect measurements of arterial pressure in unanaesthetized rats. *Cardiovasc Res* 5: 24–31, 1971.
8. **Geddes LA.** *Handbook of Blood Pressure Measurement*. Clifton, NJ: Humana, 1991.
9. **Hartley CJ, Michael LH, Entman ML.** Noninvasive measurement of ascending aortic blood velocity in mice. *Am J Physiol Heart Circ Physiol* 268: H499–H505, 1995.
10. **Kramer K, van Acker SA, Voss H-P, Grimbergen JA, van der Vijgh WJ, and Bast A.** Use of telemetry to record electrocardiogram and heart rate in freely moving mice. *J Pharmacol Toxicol Methods* 30: 209–215, 1993.
11. **Krege JH, Hodgin JB, Hagemann JR, and Smithies O.** A noninvasive computerized tail-cuff system for measuring blood pressure in mice. *Hypertension* 25: 1111–1115, 1995.
12. **Mattson DL.** Long-term measurement of arterial blood pressure in conscious mice. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R564–R570, 1998.
13. **Millar Instruments, Inc.** *Product Specifications Model SPR-671 Pressure Transducer*. Houston, TX: Millar Instruments.
14. **Millonig G.** Advantages of a phosphate buffer for OsO₄ solutions in fixation. *J Appl Phys* 32: 1637, 1961.
15. **Nichols WW and O'Rourke MF.** *McDonald's Blood Flow in Arteries*. Philadelphia, PA: Lea & Febiger, 1990.
16. **Taffet GE, Hartley CJ, Wen X, Pham T, Michael LH, and Entman ML.** Noninvasive indexes of cardiac systolic and diastolic function in hyperthyroid and senescent mouse. *Am J Physiol Heart Circ Physiol* 270: H2204–H2209, 1996.