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# Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool

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**Butz, Genelle M., and Robin L. Davisson.** Long-term telemetric measurement of cardiovascular parameters in awake mice: a tool for physiological genomics. *Physiol Genomics* 5: 89–97, 2001.—The recent miniaturization of implantable radiotelemetric devices offers the possibility of an accurate, reliable, and simple phenotyping tool for long-term, hands-off measurement of blood pressure in unrestrained, untethered mice; however, use has been limited because of high morbidity and mortality in all but larger-than-average mice. Also, because the device was developed for abdominal aorta implantation at the renal artery level, its use has not been feasible in studies where infrarenal blood flow is critical, i.e., in pregnant mice. We provide details of a very successful alternative approach for implanting radiotelemeters in mice, whereby thoracic aortic implantation of the pressure-sensing catheter is combined with subcutaneous placement of the transmitter body along the right flank. We used female C57/BL6 (C57) or BPH/5 mice, a strain derived from the cross of inbred hypertensive and hypotensive mouse strains. We show that this is a reliable procedure for providing high-fidelity mean arterial pressure (MAP) and heart rate (HR) recordings for 50–60 days in mice weighing 22 g on average but as small as 17 g. No morbidity or mortality was observed in either strain using this procedure. Importantly, neither strain fully recovered from anesthesia and surgery, as indicated by a return of normal circadian rhythms, until 5–7 days postsurgery. This was also reflected in significantly elevated baseline MAP and HR levels in both strains during this recovery period. Moreover, strain-related differences in relative increases in MAP during the first 5 days of recovery masked the significant elevation in BPH/5 baseline MAP (vs. C57) observed in fully recovered mice. This suggests that methods must allow at least 5–7 days recovery from surgery to provide accurate cardiovascular (CV) phenotyping in mice. Finally, we show that CV parameters can be monitored continuously before, during, and after pregnancy in mice using this alternative implantation approach. The device did not interfere with conception, gestation, delivery, or postnatal care of pups. These results demonstrate the feasibility of stress-free, long-term monitoring of CV parameters in pregnant or nonpregnant mice of typical size and offer exciting possibilities for application in CV functional genomic research.

blood pressure; heart rate; telemetry; circadian; pregnant; transgenic mice; gene targeting; gestation

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WITH SEQUENCING OF THE ENTIRE human genome near completion, the challenge of linking genes to function has just begun. Development of model systems for testing the role of known and newly discovered genes in normal and pathophysiological states is already underway. Indeed, there is an ever-growing list of genetically engineered mouse strains, with a large number of them designed to study cardiovascular regulation and disease (2, 4, 7, 8). Technologies that allow accurate, reliable, and high-throughput cardiovascular phenotyping in mice have become critical in this functional genomics era.

Until recently, arterial blood pressure (BP) in mice has been measured either indirectly using tail cuff plethysmography (8, 16) or directly using indwelling fluid-filled catheters (4, 9, 10). Plethysmography measures only systolic pressure and requires extensive animal training, heating, and restraint. The fluid-filled catheter method requires that mice are tethered, and catheter patency limitations prevent long-term measurements (more than 1–2 wk). The recent development of miniature implantable radiotelemetric devices offers the possibility for long-term, hands-off BP measurement in untethered mice living in their home cages (12). However, limitations associated with abdominal aortic implantation of the transmitter, the surgical approach originally developed for this technology (10), have prevented broad implementation of this technology so far. First, the size of the pressure-sensing catheter prevents placement of it in the abdominal aorta without obstruction of blood flow to the lower extremities except in larger-than-average adult mice (must be at least 30 g) (1, 12). Second, perhaps due to lengthy anesthesia periods and trauma associated with abdominal surgery, there is high morbidity and mortality using the conventional abdominal aorta method (1, 12). Third, since catheter placement using this approach potentially compromises uterine blood flow, it is not feasible in pregnant mice.

Since we are interested in studying cardiovascular regulation during pregnancy, we wanted to develop an alternative method for reliable and continuous measurement of BP before, during, and after pregnancy in mice using radiotelemetry. Since female mice of reproductive age weigh on average 22 g, the secondary goal was to develop the radiotelemetric technique for application in smaller mice. Carlson and Wyss (1) recently reported a protocol for implantation of radiotelemeters

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in mice weighing on average 28 g. With carotid placement of the catheter tip and midscapular region placement of the transmitter body, they report a much higher success rate compared with the conventional abdominal aorta technique. Here we describe an alternate approach that we have developed in which thoracic aortic (via the left carotid artery) implantation of the pressure-sensing catheter is combined with subcutaneous placement of the transmitter body along the right flank. Our results document that this is an extremely reliable procedure for accurately measuring basal mean arterial pressure (MAP) and heart rate (HR) in mice weighing 22 g on average, but as small as 17 g. Additionally, we show that continuous radiotelemetric recording of cardiovascular parameters using this technique does not interfere with pregnancy.

## METHODS

### *Animals and Housing*

Experiments were performed on 3-mo-old female C57/BL6 (C57) or BPH/5 mice (kind gift of G. Schlager, University of Kansas), a strain derived from the cross of the inbred hypertensive strain BPH/2 and hypotensive strain BPL/1 (15). Mice were housed in standard polypropylene cages placed in a temperature- and humidity-controlled facility, were maintained in a 12:12-h light-dark cycle (6 AM to 6 PM lights on), and were fed standard mouse chow (Teklad LM-485) with water available ad libitum. For the pregnancy studies, 3-mo-old male C57 mice were bred to female C57 mice at the times indicated below. Care of the mice used in the experiments met or exceeded the standards set forth by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

### *Telemetry Probe Implantation Procedure*

*Pressure-sensing catheter implantation.* The components of the radiotelemetry system (Data Sciences International, St. Paul, MN), including the mouse BP telemetry device, have been described in detail previously (12). Female C57 or BPH/5 mice were anesthetized with ketamine (90 mg/kg ip) and ace promazine (1.8 mg/kg ip), and the neck was shaved and disinfected. Animals were placed on their backs on a heated surgical surface with forelimbs secured using tape and the head held parallel to the body by a small piece of tubing looped around the upper incisors and anchored to the surface. This allows easier access to the region to be dissected. A ventral midline skin incision was made from the lower mandible posteriorly to the sternum (~3 cm), and the submaxillary glands were gently separated using sterile cotton swabs. The left common carotid artery was isolated using fine forceps, with care being taken to avoid disturbing nerve fibers running parallel to the artery. The left carotid is preferable, since the right brachiocephalic artery branches off of the right carotid artery, potentially compromising blood flow to this region. Two silk ligatures (7–0, Braintree) were passed under the vessel and used for both ligation and retraction (Fig. 1*A*). The anterior silk suture (Fig. 1*A*, *a*), placed just caudal to the bifurcation of the interior and exterior carotid arteries (see Fig. 1*E*, *inset* $E^1$), was used to ligate the artery. The posterior suture (Fig. 1*A*, *b*), placed loosely about 0.5 cm from the anterior tie, was used for temporary occlusion of the artery during catheter insertion. Gentle tension was applied to both ligatures to slightly retract and lift the vessel (Fig. 1*A*) before making a tiny incision in the carotid artery just posterior to *suture a* using Vannas spring micro-scissors (Fine Science Tools, catalog no. 15610-08). After carefully removing the protective cover from the tip of the catheter, the catheter was gently grasped using vessel cannulation forceps (Fine Science Tools, catalog no. 00608-11) and inserted into the carotid artery with the aid of fine forceps used to hold the vessel incision open. The occlusive posterior *suture b* was released, and the catheter was advanced to the point where the small notch on the tubing (the transition between the thin-walled tubing and remaining outer tubing, 10 mm from the tip) resided at the vessel opening (even with *suture a*, see Fig. 1, *B* and *E*, *inset* $E^1$). Inserting the catheter up to this landmark notch assures the critical placement of the pressure-sensing tip just inside of the thoracic aorta (see Fig. 1*E*, *inset* $E^2$). The notch also serves as a useful tool for anchoring the catheter into the vessel; the loose ends of *suture a* were tied securely around the catheter at the notch. The loose ends of the occlusive posterior *suture b* were also brought up and tied securely around the vessel/catheter (Fig. 1, *B* and $E^1$). A single microdrop of Nexaband veterinary adhesive was applied to the site of catheter insertion into the vessel (Fig. 1*B*, arrow). It should be emphasized that that only a very small amount of adhesive is required, and that care should be taken to avoid undue application of adhesive to surrounding tissues and nerve fibers.

*Implantation of transmitter subcutaneously along the right flank.* Through the same ventral neck incision, a subcutaneous pouch was formed for placement of the transmitter body along the animal's right flank (C57, $n = 12$; BPH/5, $n = 6$). Using a pair of blunt dissecting scissors (Fine Science Tools, catalog no. 14018-14), the skin was gently dissected free from underlying tissue starting at the right neck region and proceeding posteriorly to form a "pocket" along the right flank (Fig. 1*C*). It is important that the pocket be made sufficiently large to house the transmitter without unduly stretching the skin, because pressure necrosis could result. The transmitter was slipped under the skin and down into the pocket along the flank as close to the right hindlimb as possible (see Fig. 2*A*). A small drop of Nexaband was placed on the catheter in the right neck region to further secure the device (Fig. 1*D*, arrow). The neck incision was closed using 6–0 silk and further sealed with tissue adhesive (Fig. 2*A*). Mice were kept warm on a heating pad and monitored closely until fully recovered from anesthesia.

*Implantation of transmitter on the back.* In some C57 mice ($n = 7$), we installed the transmitter body on the back at the midscapular region according to the procedure described in detail by Carlson and Wyss (1). Briefly, the upper back was shaved, and a horizontal incision was made. The transmitter was placed in the opening and sutured to both the muscle and the skin (with the suture loop around the probe) via the suture rib on the probe as described. A separate ventral neck incision was then made, the catheter was tunneled subcutaneously to the neck, and the catheterization procedure was carried out as described. Mice were observed postoperatively as above.

### *Fluid-Filled Catheter Implantation Procedure*

In a separate cross-validation experiment, female C57 mice ($n = 7$) were surgically instrumented with fluid-filled carotid arterial catheters for direct measurement of MAP and HR according to methods described by us previously (4). Briefly, animals were prepared as above, and sterile hepa-

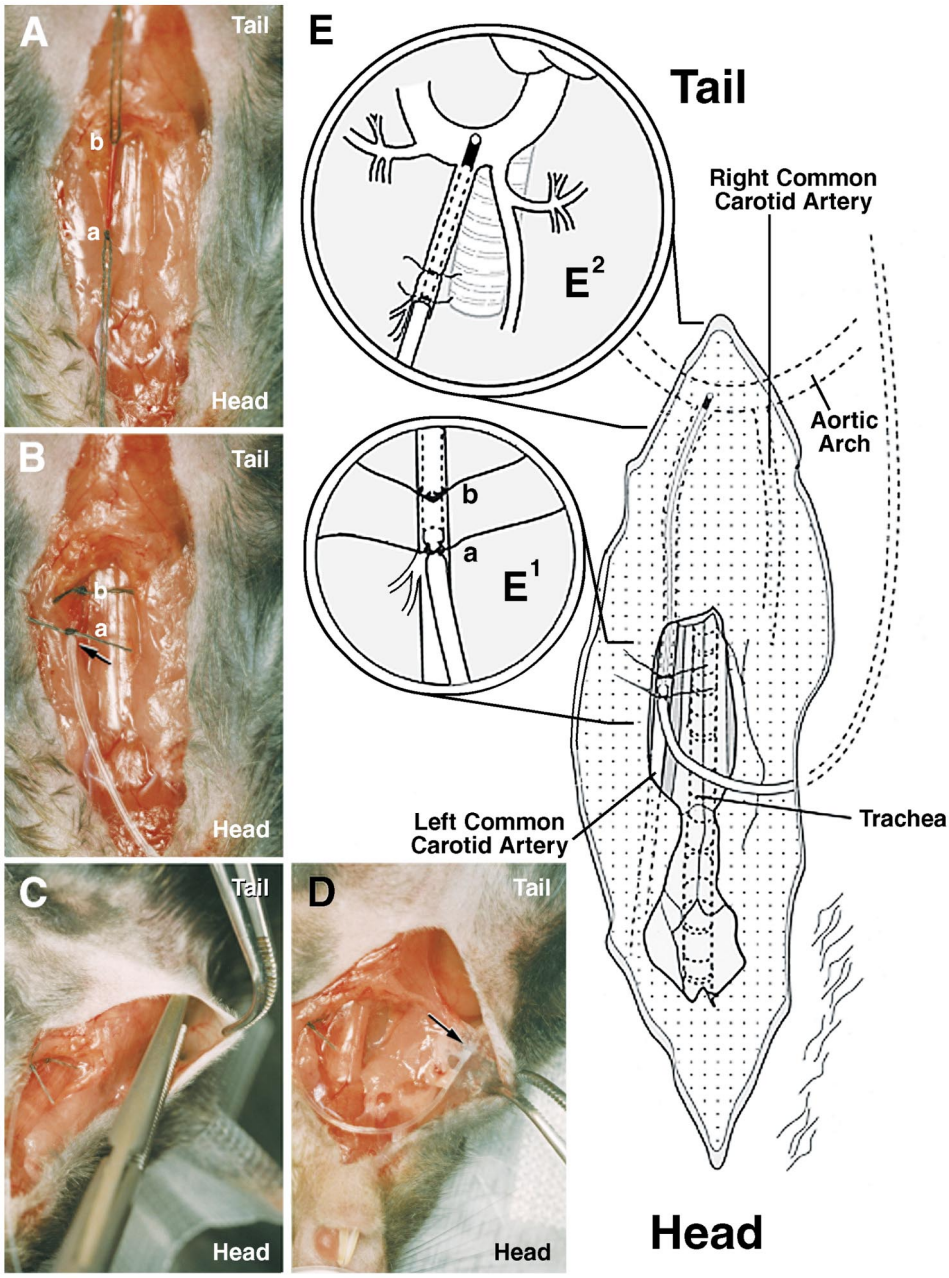


Fig. 1. Surgical procedure for implantation of the telemetry device in the thoracic aorta via the left common carotid artery. See text for detailed explanation. Note the head/tail orientation of the mouse. *Insets* $E^1$ and $E^2$ are enlargements illustrating the catheter insertion site and tip placement (in the thoracic aorta), respectively. In *A*, *B*, and *inset* $E^1$, *a* and *b* refer to the anterior and posterior silk ligatures, respectively. Arrows in *B* and *D* indicate placement of the microdrop of Nexaband veterinary adhesive.

rinized saline-filled catheters were inserted into the left common carotid artery. Catheters were tunneled subcutaneously, exteriorized, and sutured in place as described before (4). Lines were flushed daily with dilute heparinized saline.

*Experimental Protocol*

*Telemetry experiments.* Following full recovery from anesthesia, mice were returned to their home cages (placed atop telemetry receivers), where they continued to be monitored daily throughout the study for general condition, body weight, food and water intake, state of surgical wound healing, and any signs of morbidity. Radiotelemeters were magnetically activated immediately upon return to the home cage, and BP (pulsatile waveforms and MAP), HR, and locomotor activity were recorded continuously for 12 days.

The pregnancy study, carried out only in C57 mice, began on *day 13* postsurgery. At this time, the telemeters were magnetically switched off, and male C57 breeders were placed with females. Each morning females were examined for the presence of a vaginal plug. Upon detection of a plug, males were removed, radiotelemeters were turned on again, and female mice were weighed daily. If a significant weight gain was not observed by 7 days postplug, then a male was returned to the cage and the process repeated. MAP and HR were recorded continuously during pregnancy and then intermittently (to conserve transmitter battery life) every 3 days (for 24-h intervals) for 2–3 wk postpartum. Mice were weighed daily throughout the experiments, and litter sizes and pup weights were recorded during the postnatal period. Female C57 mice ($n$ = 7) that had not been implanted with radiotelemeters served as controls. They were similarly bred, weighed, and observed during and after pregnancy.

*Fluid-filled catheter experiments.* MAP and HR were recorded in conscious freely moving female C57 mice both at 3

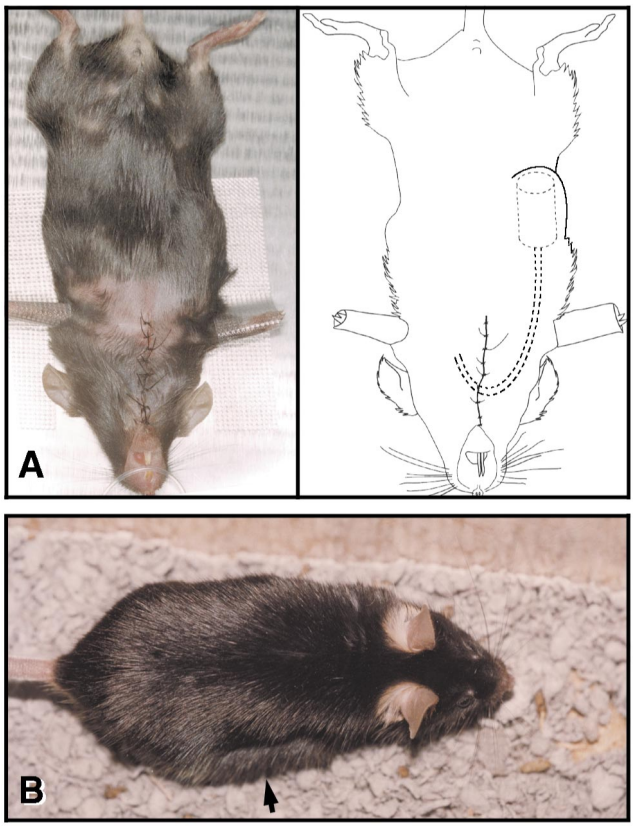


Fig. 2. *A*: ventral view of an anesthetized C57/BL6 (C57) mouse immediately following surgical implantation of the telemetry device in the thoracic aorta (via the left carotid artery). Positioning of the transmitter body along the animal's right flank is shown at *right*. *B*: a typical C57 mouse 55 days following implantation of the radiotelemetry device. The transmitter body, placed subcutaneously along the animal's right flank, is indicated by the arrow. This particular mouse weighed 22 g, and delivered a litter of 8 pups 2 wk prior to these photographs.

days and again 7 days following implantation of fluid-filled carotid catheters as described (4). After connection of the arterial line to a PowerLab (ADInstruments, PowerLab/4S)-coupled pressure transducer, mice were allowed 60 min stabilization period before obtaining baseline MAP and HR recordings for 60 min.

### *Data Acquisition and Analysis*

Radiotelemetry data were collected continuously (sampling every 5 min for 10-s intervals) as indicated and stored using the Dataquest ART data acquisition system (Data Sciences International) as described in detail previously (12). MAP, HR, and locomotor activity data collected from the first 12 consecutive days following surgery were plotted as mean values over each hour. All other data are expressed as means ± SE calculated for the day postsurgery or over the stage of pregnancy indicated [pre, early (6–8 days), middle (11–13 days), late (18–20 days), post (2–3 wk postdelivery)]. Differences in MAP and HR at the different time points, between methods for recording (radiotelemetric vs. fluid-filled catheters) and/or between strains were analyzed by repeated measures ANOVA followed by the Student's modified *t*-test with Bonferroni correction for multiple comparisons between means using the modified error mean square term from the ANOVA. Student's *t*-test was used to compare litter sizes, average pup sizes, and weight gain during pregnancy between mothers with and without radiotelemetry implants.

## RESULTS

### *General Condition of the Mice*

A total of 25 female mice ranging in weight from 17 to 24 g (21.8 ± 3 g) were implanted with radiotelemeters. All 18 mice (C57, $n = 12$; BPH/5, $n = 6$) in which the transmitter body was placed along the right flank exhibited rapid recovery, with resumption of normal food and water intake within 24 h of surgery. They returned to presurgical body weight (subtracting the weight of the implant) on average by 4 days (4.1 ± 0.5 days) and did not exhibit any signs of morbidity during recovery or during the remainder of the experiment (50–60 days total) (see Fig. 2*B*). In contrast, 6 of the 7 mice in which the transmitter body was placed on the back had complications. In general, the mice appeared to have restricted head movement and showed low levels of activity (data not shown). Three mice exhibited excessive scratching of the back, thereby perturbing the wound and exteriorizing the probe within the first week following surgery. The remaining 3 mice showed signs of morbidity during the breeding phase of the experiment, eventually exteriorizing the transmitter before becoming pregnant. None of these 6 mice returned to presurgical body weights before being euthanized due to complications. One mouse appeared healthy, returned to its presurgical weight by 10 days postsurgery, and did become pregnant. However, it was found dead in the cage during the second week of pregnancy. The necropsy report was not conclusive with regard to reason for death, but did note inflammation and necrosis at the site of transmitter body implantation on the back.

### *MAP and HR Measurements During Recovery from Surgery*

All reported telemetry data were collected from C57 and BPH/5 mice with right flank-implanted transmitters. We began recording MAP, HR, and locomotor activity immediately following surgical implantation of the device to establish the time point at which daily circadian rhythms returned. Data from the first 12 consecutive days following surgery were plotted for each animal and inspected visually. A typical 24-h rhythm in a C57 mouse over 4 consecutive days starting a few hours after recovery from anesthesia, and again ~1 wk (6.5 days) after surgery, is shown in Fig. 3. For the first several days following surgery, the circadian rhythms appeared disorganized with regard to the light cycle. It was not until 5–7 days after surgery (5.7 ± 0.8 days, $n = 17$) that circadian organization of MAP, HR, and activity, defined previously in mice as a diurnal rhythm with peak values at the onset of the active dark period and minimum values during the quiescent light period (9), returned. The time course of recovery of circadian patterns was similar for both strains.

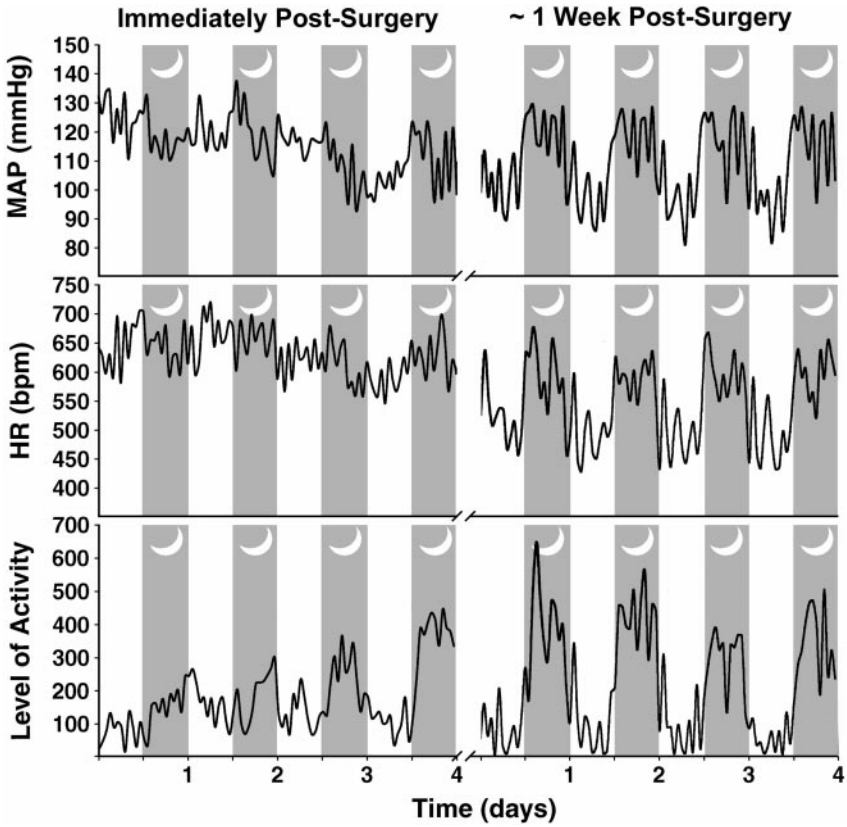


Fig. 3. Typical 24-h circadian rhythms of mean arterial pressure (MAP, *top*), heart rate (HR, *middle*), and locomotor activity (*bottom*) recorded by radiotelemetry in the same C57 mouse for 4 consecutive days starting after recovery from anesthesia (at 6 AM the morning following surgery) and ~1 wk following implantation of the telemetry device. Circadian organization of cardiovascular and activity parameters does not occur until 5–7 days postsurgery. Plots represent mean values over each hour in a 12:12-h light-dark cycle. Gray segments with moons indicate the dark period (6 PM to 6 AM).

In addition to affecting circadian rhythms, anesthesia/surgery also significantly affected baseline MAP and HR in both strains of mice for the first several days following surgery. Table 1 summarizes 24-h averages of basal MAP and HR in C57 and BPH/5 mice obtained from the time immediately following recovery from anesthesia until 7 days postsurgery. HR was significantly elevated for 2 and 4 days, respectively, in C57 and BPH/5 mice compared with values obtained a week after surgery. Baseline MAP was also significantly elevated for the first several days following implantation of the telemetry device in both strains. It remained increased for a full 5 days following surgery in C57 mice, and interestingly, it was not until this time point that a significant difference between baseline MAP in C57 and BPH/5 strains emerged. Starting 5 days postsurgery (and throughout the remainder of the 60-day experiment, data not shown), BPH/5 mice had significantly elevated baseline MAP compared with that of C57 mice. Prior to 5 days of recovery, although baseline MAP was significantly elevated in both strains relative to that obtained a week after surgery, a relatively greater increase in baseline MAP in C57 during this time contributed to the inability to detect a significant difference between the two strains. Strain-related differences in HR were not detected at any of the time points.

Next we wanted to cross-validate results obtained by telemetry with another method. We compared telemetry MAP and HR recordings with those obtained by

Table 1. *Twenty-four-hour averages of basal MAP and HR in C57/BL6 and BPH/5 mice obtained by radiotelemetry starting immediately following recovery from anesthesia until 1 wk after implantation of the device*

| | Day Postsurgery | | | | | | |
|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* | *6* | *7* |
| | | | | *C57/BL6* | | | |
| MAP, mmHg | 128 ± 6* | 125 ± 4* | 122 ± 5* | 121 ± 7* | 119 ± 4* | 111 ± 5 | 103 ± 6 |
| HR, beats/min | 668 ± 12* | 617 ± 9* | 562 ± 11 | 569 ± 13 | 559 ± 14 | 571 ± 12 | 553 ± 10 |
| | | | | *BPH/5* | | | |
| MAP, mmHg | 139 ± 6* | 136 ± 7* | 134 ± 8 | 134 ± 7 | 133 ± 6† | 132 ± 4† | 127 ± 1† |
| HR, beats/min | 661 ± 9* | 635 ± 18* | 607 ± 13* | 613 ± 18* | 577 ± 16 | 554 ± 10 | 540 ± 13 |

Values are means ± SE; C57, $n = 11$ mice; BPH/5, $n = 6$ mice. MAP, mean arterial pressure; HR, heart rate. *$P < 0.05$, vs. 7 days postsurgery. †$P < 0.05$, C57 vs. BPH/5 for day postsurgery.

indwelling fluid-filled catheters in separate C57 animals ($n$ = 7). Since continuous recordings were not possible with the fluid-filled catheters, we chose 3 and 7 days postsurgery as time points for comparison. At 3 days after implantation of the fluid-filled catheters, a typical time point used for determining baseline BP in a number of mouse models (4, 9), MAP was significantly elevated (126 ± 7) relative to that recorded 7 days after surgery (106 ± 5, $P < 0.05$, Fig. 4, *right*), and these values were nearly identical to those obtained by telemetry at these time points (Fig. 4, *left*). HR was not significantly different at 3 vs. 7 days postoperatively (582 ± 12 vs. 573 ± 14 bpm, respectively, $P > 0.05$), which is also corroborative of the telemetry data.

With radiotelemetry, excellent pulsatile pressure profiles, including a visible dichrotic notch, were obtained in 17 of 18 mice studied using the carotid artery/right flank implantation procedure (Fig. 5, *top*). The integrity of the signal remained constant throughout the 50- to 60-day experimental period in these mice, with only a slight diminution of pulse pressure (Δ10 ± 4 mmHg, $n$ = 17; Fig. 5, *bottom*). One C57 mouse exhibited a sudden loss of the signal during the second week following surgery. Inspection of the device upon explant revealed a thrombus at the catheter tip.

*MAP and HR Before, During, and After Pregnancy*

We wanted to determine whether radiotelemetry would provide a stress-free means for recording cardiovascular parameters before, during, and after pregnancy. These studies were performed in the C57 mice only. After allowing 7 days recovery from implantation of the device (see above), we recorded baseline MAP and HR for 5 consecutive days prior to breeding the mice, throughout pregnancy, and for 2–3 wk following delivery. Compared with prepregnancy baseline values, the only significant change in BP was observed in midpregnancy (11–13 days gestation), with a moderate fall in MAP (pre, 104 ± 5 vs. mid, 92 ± 5, $P < 0.05$; Fig. 6*A*). HR, on the other hand, was significantly elevated throughout pregnancy (pre, 556 ± 10; early, 632 ± 12; middle, 656 ± 14; and late, 649 ± 20; $P < 0.05$) but returned to pregestational levels following delivery (Fig. 6*B*).

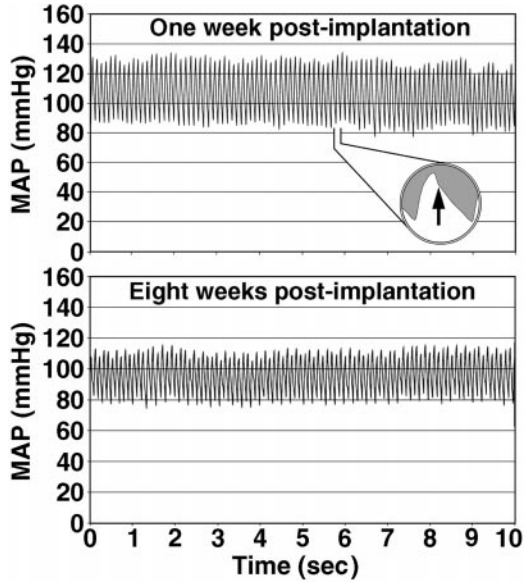


Fig. 5. Typical pressure waveforms of the same C57 mouse at 1 wk (*top*) and 8 wk (*bottom*) postimplantation of the radiotelemeter in the thoracic aorta (via the left carotid artery). Excellent pulsatile profiles were obtained, with a visible dichrotic notch (see arrow, *inset*).

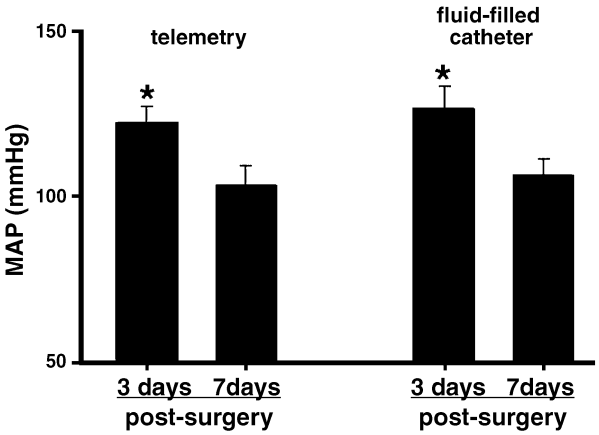


Fig. 4. Summary of baseline MAP in C57 mice obtained by either telemetry ($n$ = 11) or fluid-filled catheters ($n$ = 7). MAP was recorded both at 3 and 7 days postsurgery for both methods. *$P < 0.05$, 3 vs. 7 days postsurgery.

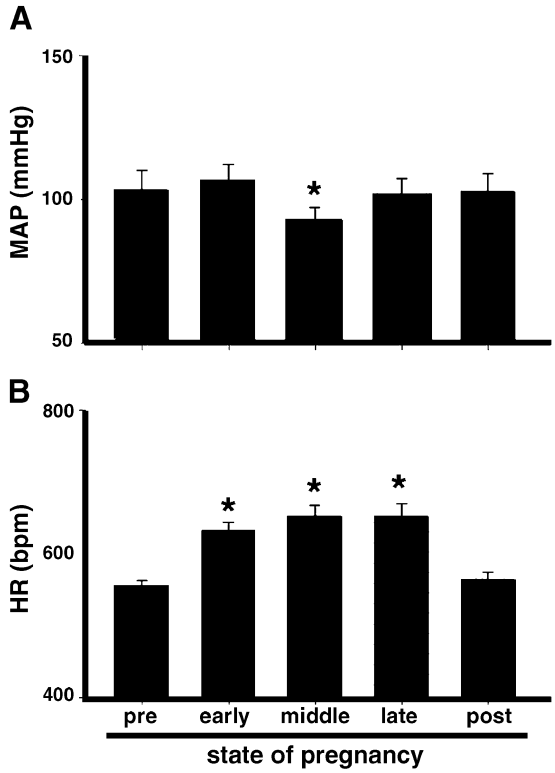


Fig. 6. Summary of MAP (*A*) and HR (*B*) in C57 mice before (pre), during different stages of pregnancy (early, 6–8 days; middle, 11–13 days; and late, 18–20 days), and during the postpartum period (post) by radiotelemetry. *$P < 0.05$, compared with "pre" values.

*Effects of Radiotelemetry Implants on Pregnancy*

To determine whether the telemetry devices interfered with the ability of mice to conceive or to deliver normal litters, we compared the pregnancies of C57 mice with and without implants. Although it took on average 2 days longer to detect a vaginal plug in mice with implants (4.3 ± 0.4 days) vs. those without (2.1 ± 0.3 days, $P < 0.05$), there was no difference in the overall rate of conception. Of the 11 mice with telemeters, only 1 did not exhibit weight gain by 7 days after plug detection; however, this mouse did become pregnant upon introduction of the male breeder a second time. Of the 7 mice without implants, 1 exhibited a similar pattern. Mice with and without telemetry implants gained similar amounts of weight over the course of pregnancy (Fig. 7*A*) and delivered similar size litters (Fig. 7*B*). The average pup size (postnatal *day 10*) was also not different in litters from mothers with and without telemetry implants (Fig. 7*C*). Finally, all pups, regardless of whether their mother was implanted with a transmitter, developed normally and were weaned on postnatal *day 21*.

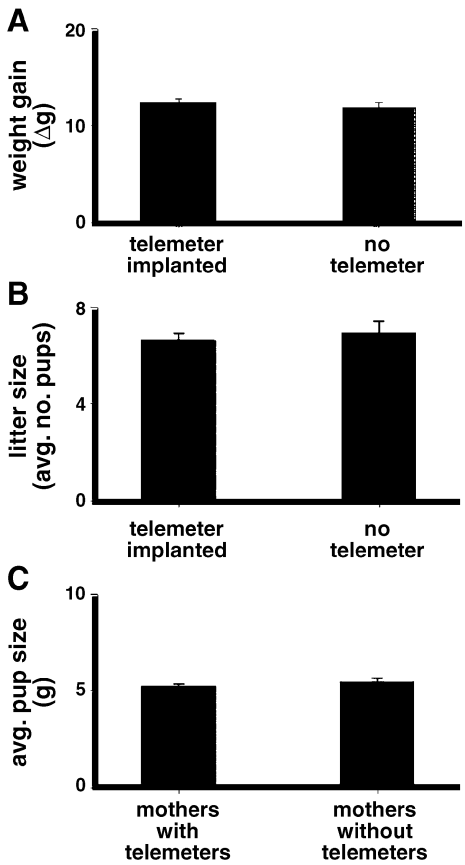


Fig. 7. Summary of weight gain during pregnancy (*A*), average number of pups per litter (*B*), and average pup size in litters (*C*) of C57 mothers with and without telemeters implanted. No significant differences in any of these parameters were observed between groups.

## DISCUSSION

Using a new approach for placement of the transmitter device, we demonstrate that radiotelemetry provides an excellent method for long-term BP phenotyping in mice. Implantation of the pressure-sensing catheter in the thoracic aorta via the left carotid artery and subcutaneous placement of the transmitter body along the right flank is an extremely reliable procedure for providing high-fidelity BP and HR recordings over several months. There was nearly a 100% success rate using this procedure in two different strains of mice weighing an average of 22 g, and as small as 17 g. Our results also demonstrate the feasibility of radiotelemetric monitoring of cardiovascular parameters throughout pregnancy in mice. With practice, the implantation procedure can be completed within about 20 min, making it a high-throughput approach to BP phenotyping as well.

Our findings are consistent with those of Carlson and Wyss (1) in that we also found the carotid artery placement to be tremendously more reliable and successful than the abdominal aorta method for mice smaller than 30 g. Although not presented here, we initially struggled with the abdominal placement as well, and our low success rate with it in part led to our development of the approach in the current study. In contrast to the report of Carlson and Wyss (1), however, we did not have success with placement of the transmitter body on the back at the midscapular region. In addition to limiting head movement and general locomotor activity, the mice had access to the back incision with their hind legs and scratched it excessively. This, perhaps combined with activity during breeding, led to wound opening and exteriorization of the probes in most cases. We had much better success with placement of the transmitter body subcutaneously along the right flank. Not only is it more convenient in that the entire implantation procedure can be carried out through a single small incision, also, the animals cannot easily access the inner right flank region through scratching. The implants remained relatively undisturbed over the months of implantation. The smaller size of mice in our study, along with the added stress of the breeding protocol, may account for differences in success with the midscapular placement between our study and that of Carlson and Wyss (1).

In addition to being reliable, our results indicate that radiotelemetry is also a highly accurate method for baseline BP phenotyping. Cross-validation of telemetric recordings with those obtained by fluid-filled catheters in separate mice demonstrated nearly identical MAP values by these two techniques. Similar findings were reported for validation of telemeters against Millar transducer-tipped catheters (12). An additional important finding from this part of our study that we believe has broad implications for BP phenotyping in general is that mice do not fully recover from anesthesia and surgery, as indicated by the return of normal circadian patterns, for 5–7 days. In both C57 and BPH/5 strains, circadian patterns remained disorga-

nized with regard to the light cycle until about 6 days following implantation of the telemetry device. The effects of surgery/anesthesia seem to also be reflected in altered baseline cardiovascular values in both strains. Compared with values obtained a week after surgery, both MAP and HR were significantly elevated for several days following surgery in C57 and BPH/5 mice. Although the time course for recovery of the circadian rhythms was similar in both strains, the relative effect of anesthesia/surgery on baseline MAP and HR values differed between C57 and BPH/5. A relatively greater increase in MAP during the first several days following surgery in C57 than in BPH/5 mice (compared to values 7 days after surgery) appeared to have masked the significant difference observed in this parameter in animals that are fully recovered. In fact, a significant difference in baseline MAP between these two strains was not detected until 5 days following surgery. This is also the amount of time it took for baseline MAP in C57 mice to reach a stable level. Taken together, these findings suggest that recordings taken prior to 5 days after surgery (i.e., a typical time for so-called conscious recordings in mice) may not provide accurate cardiovascular phenotyping and that a 5- to 7-day recovery period is critical. The strain-related differences in recovery revealed in this study underscore the importance of this waiting period, especially in studies of genetically altered or inbred strains of mice. Our basal MAP and HR data recorded in C57 mice at 7 days postsurgery by both telemetry and fluid-filled catheters are in close agreement with other reports using either method (1, 9). Thus we believe that the fluid-filled catheter method does provide an accurate short-term BP phenotyping method, as long as catheters can be kept patent to allow for adequate time for recovery from surgery. If this is not possible or when experimental paradigms require continuous direct recording over weeks or months, then radiotelemetry is a crucial tool.

This is not to say that implementation of radiotelemetry is without caveats. Certainly, one consideration is cost. The initial investment in equipment is relatively substantial. Additionally, there are recurrent costs for refurbishment of transmitter batteries and pressure-sensing catheters. However, attention to conservation of battery life and efforts to maximize transmitter reuse can reduce these recurrent costs considerably. Battery life with continuous use is about 1.5 mo, but the time course of a study and/or the life of the transmitter can be extended by magnetically switching the transmitters off and on repeatedly over time to conserve the battery. For example, we have analyzed MAP and HR over a 6-mo period in mice by recording for 24-h intervals every 4 days. Extension of battery life by this method even in studies with shorter time courses (typically using shorter "off" intervals), in combination with transmitter explant and reuse, has also helped us reduce refurbishment costs. We have been relatively successful with reusing transmitters one or two times, but have had limited success beyond that. An inability to completely remove all adhesive debris and difficulties with the catheter gel have been the primary limitations.

At least two concerns might reasonably be raised about implantation of the pressure-sensing catheter in the thoracic aorta via the carotid artery. First, this procedure prevents the left carotid sinus from directly seeing arterial pressure, raising the possibility that baroreflex function might be comprised. While this is a concern, previous studies in dogs have shown that even after total denervation of both left and right carotid sinus baroreceptors, baroreflex function returns to normal within ~1 wk as a result of compensatory effects of the remaining baroreceptors (12). Although we have not directly evaluated baroreflex function in mice with telemeters, our preliminary baroreflex studies in mice with fluid-filled catheters implanted in the left carotid artery appear to support these findings. Investigations are ongoing.

The other concern associated with carotid placement of the telemeter is that of inducing cerebral ischemia due to ligation of the carotid artery. Although our studies did not assess this possibility directly, we did not observe changes in behavior attributed to ischemia such as twisting, body rotations (in a direction contralateral to the damaged hemisphere), or diminished locomotor activity (3). Indeed, behaviorally, mice with carotid catheters (either transmitters or fluid-filled) were indistinguishable from unoperated controls. It should be noted, however, that some studies have identified strain-related differences in susceptibility to ischemia induced by bilateral carotid artery occlusion in mice, perhaps related to differences in cerebrovascular anatomy (5). Future studies investigating the effects of unilateral carotid occlusion and the potentially confounding role of genetic background would be useful.

Finally, an additional goal of this study was to establish a method for continuous stress-free recording of cardiovascular parameters in mice during pregnancy. Previous studies in pregnant rats or mice have utilized indwelling fluid-filled catheters in anesthetized animals (6, 13) or the tail-cuff method with preheating and restraint (16, 17), but we were concerned about the potentially confounding influences such manipulations could have. We reasoned that the long-term, hands-off recording potential of radiotelemetry would allow us to implant the transmitters before the mice were bred, allow adequate recovery time, and then record MAP and HR continuously before, during, and after pregnancy without interruptions such as restraint, handling, or anesthesia/surgery. Indeed, our results indicate that the transmitter device did not interfere with conception, gestation, delivery, or postnatal care of the pups. Furthermore, we were able to continuously track changes in MAP and HR throughout pregnancy and during the postpartum period in C57 mice. Interestingly, the significant drop in MAP at midgestation followed by a return to normal during late pregnancy and the marked increase in HR throughout pregnancy are consistent with what occurs in women during pregnancy (11). To our knowledge, this is the first report of normal baseline cardiovascular data collected over the

entire course of pregnancy and postpartum period under stress-free conditions in rodents. This approach may have important applications in future studies designed to understand the pathogenesis of pregnancy-related cardiovascular diseases such as preeclampsia.

The emerging field of functional genomics has a goal to link the delineation of the genome to the function of the whole organism and organ systems in health and disease. One prerequisite for the successful achievement of this critical goal is the development and optimal deployment of tools that allow accurate, reliable, and high-throughput phenotyping. We believe that radiotelemetry, as described in detail and utilized in this study, is an important such tool and represents a key step in cardiovascular phenotyping in mice. The ubiquitousness of murine models in genetic research and of cardiovascular disease in the developed world indicates the potential significance of this particular tool in a broad range of future cardiovascular functional genomic research.

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