

Validation of Volume–Pressure Recording Tail-Cuff Blood Pressure Measurements

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BACKGROUND

The American Heart Association has recommended tail-cuff blood pressure measurement for high-throughput experimental designs, including mutagenesis screens and genetic crosses. However, some tail-cuff methods show good agreement with radiotelemetry and others do not, indicating that each tail-cuff method requires independent validation.

METHODS

We validated the volume–pressure recording (VPR) tail-cuff method by comparison to simultaneous radiotelemetry measurements.

RESULTS

Bland–Altman analysis of 560 cycles from 26 independent measurement sessions showed good agreement between

VPR and radiotelemetry measurements, with tail-cuff measurements being 0.25 mm Hg lower than telemetry measurements on average. However, the VPR method was less accurate, compared to radiotelemetry, at extreme high and low (i.e., <110 or >180 mm Hg) systolic blood pressures (SBPs).

CONCLUSIONS

We conclude that the VPR tail-cuff method provides accurate blood pressure measurements over the physiological range of blood pressure in mice.

Am J Hypertens 2008; **xx**:xxx-xxx © 2008 American Journal of Hypertension, Ltd.

The American Heart Association recently published recommendations for blood pressure measurement in experimental animals.¹ This scientific statement recommended direct blood pressure measurement (i.e., radiotelemetry, fluid-filled catheters) for many blood pressure research applications. Telemetry enables chronic blood pressure measurements in conscious, unrestrained, untethered mice (for review, see ref. 2). Moreover, telemetry provides very accurate blood pressure measurements compared to simultaneous measurements from a fluid-filled catheter.³ Telemetry has been used in genetically modified mice to examine the roles of various genes, including genes encoding the adenosine A1 receptor,⁴ endothelial nitric oxide synthase,⁵ and angiotensin 1 receptor,⁶ in blood pressure control and has become the gold standard for direct blood pressure measurement.⁷

The American Heart Association recommendations for blood pressure measurement in experimental animals¹ advocated indirect blood pressure measurement (i.e., tail-cuff) for high-throughput experimental designs, including mutagenesis screens and genetic crosses. However, the accuracy of indirect

blood pressure methods can vary widely. Pulse-based tail-cuff measurements have been proven accurate by comparison to simultaneous telemetry measurements of blood pressure, whereas flow-based measurements showed poor agreement with direct telemetry.⁸ Thus, it is important to evaluate and validate each tail-cuff method independently.

Volume–pressure recording (VPR) is an additional tail-cuff method for measuring systolic (SBP) and diastolic blood pressure (DBP) in experimental animals. VPR technology is applicable to high-throughput experiments as recommended by the American Heart Association, but this technology has not been validated. Therefore, we sought to evaluate the accuracy of the VPR method by comparison with simultaneous telemetry measurements.

METHODS

Radiotelemetry vs. VPR blood pressure measurement. To verify the accuracy of the VPR noninvasive blood pressure monitoring system (CODA-6, Kent Scientific, Torrington, CT), we compared SBP and DBP measured simultaneously by radiotelemetry (Data Sciences International, St Paul, MN) and non-invasively during 26 measurement sessions, on 2 days, using seven individual mice. The measurements were taken at the University of Michigan using male C57BL/6 or CD-1 mice purchased from The Jackson Laboratory (Bar Harbor, ME) or Charles River (Wilmington, MA), respectively. The protocol was approved by the University of Michigan Animal Care and Use Committee.

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Received 18 April 2008; first decision 26 May 2008; accepted 8 September 2008; advance online publication 9 October 2008. doi:10.1038/ajh.2008.301

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The protocol for the comparison study was similar to that used for the published comparison of telemetry vs. other noninvasive blood pressure measurement systems.⁸ Briefly, the catheter of a telemetric blood pressure transducer (model TAP20-C20; Data Sciences International, St Paul, MN) was passed into the left common carotid artery and advanced so that the tip just entered (<1 mm) the arch of the thoracic aorta, and the body of the transducer was secured in the abdominal cavity. If needed, the mice were treated with postoperative fluids, ampicillin (100 mg/kg SC), or buprenorphine (0.1–0.5 mg/kg SC) as described previously.⁸ All mice were allowed to recover from surgery for at least 1 week before and were acclimated to the restrainer for 10–20 min per day for at least 3 days before starting the comparison study. Following this acclimation period, mice typically remained relatively calm and still in the restrainer on the day of testing. To facilitate the acclimation process, the mice were handled gently and not forced to enter the restrainer and the ambient temperature was maintained at warm room temperature (25–30°C). Special care was taken to not overheat the mice or excessively restrict movement. Telemetry blood pressure measurements were acquired simultaneously by placing the receiver pad adjacent to the tail-cuff device and synchronizing instrument clocks such that 10-s telemetry data segments were collected throughout the duration of each noninvasive measurement session (10 acclimation cycles followed by 20 measurement cycles). DBP, SBP, and mean arterial pressures were collected and the corresponding telemetry blood pressures were used for comparison to SBPs and DBPs measured noninvasively.

Limits of agreement (Bland–Altman) analysis. Seven individual mice underwent 26 total measurement sessions as described earlier; we considered each measurement session as an independent sample and the measurement cycles within each session for each mouse as replicates. We used Bland–Altman analysis^{9,10} to assess the agreement between the tail-cuff and radiotelemetry methods. The bias (i.e., mean difference between the two methods) was estimated as the mean of all individual differences. Standard deviations of the limits of agreement were estimated by two-way ANOVA with mouse, session, and their interaction term fitted on the differences. The total variance was obtained by aggregating the variance within the same mouse/session and the variance between mouse, session, their interaction. The standard deviation was calculated subsequently as the square root of the variance. The 95% limits of agreement are estimated as the mean difference \pm 2 s.d. The differences vs. the averages of the paired measurements along with the 95% limits of agreement were plotted for visual inspection. A similar analysis was also performed using linear mixed model with mouse, session, and their interaction all as random effects.

RESULTS

The purpose of our study was to validate the noninvasive VPR method of measuring blood pressure by comparison with radiotelemetry. The final dataset included 560 pairs of VPR and telemetry blood pressure values from 26 measurement sessions. Bland–Altman analysis showed that the VPR method underestimated SBP by 0.25 mm Hg on average (Figure 1a),

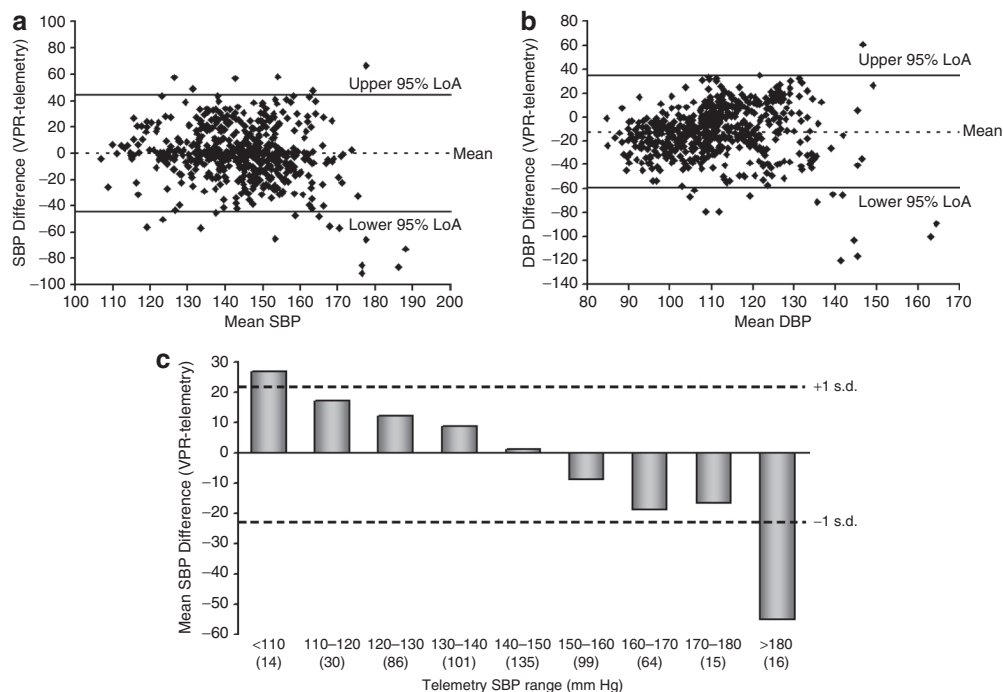


Figure 1 | Comparison of blood pressure measurements made by telemetry and volume–pressure recording (VPR) methods. Bland–Altman analyses of (a) systolic (SBP) and (b) diastolic blood pressures (DBP). The dotted lines represent the average difference between telemetry and VPR measurements (i.e., bias) and the solid lines represent the upper and lower 95% confidence limits of agreement. Each point represents 1 of 560 measurements taken during 26 independent measurement sessions. (c) Mean difference between telemetry and VPR measurements of systolic blood pressure grouped by 10-mm Hg increments of systolic blood pressure measured by telemetry. The number of individual measurements per group is shown in parentheses below each blood pressure range.

with a standard deviation of 22.7 mm Hg. This limit of agreement is consistent with that for a pulse-based tail-cuff method compared to telemetry.⁸ The VPR method underestimated DBP by 12.2 mm Hg, with a standard deviation of 24.0 mm Hg (Figure 1b). These standard deviations were calculated from the traditional linear model ANOVA results according to the limits of agreement analysis method with multiple observations per individual described by Bland and Altman.¹⁰ Standard deviations calculated using a linear mixed model ANOVA for SBP (22.3 mm Hg) and DBP (23.5 mm Hg) were slightly less than those calculated using the fixed linear model, indicating that the unbalanced groups did not affect the fixed model analysis.

To further examine whether the accuracy of the VPR method varied at extreme low or high blood pressures, we calculated the mean difference between the methods for a series of telemetry SBP intervals (10 mm Hg). We found that method bias between the VPR and telemetry values was >1 standard deviation from the method bias calculated from the total dataset when the telemetry measurements were either <110 or >180 mm Hg (Figure 1c). This finding suggests that the VPR method is most accurate between 110 and 180 mm Hg.

We also evaluated whether tail volume affected the accuracy of the VPR method. To do this, we clustered the 560 pairs of blood pressure values into seven groups according to tail volume and calculated the mean difference between the methods for each cluster. The estimated method bias was not consistently different between the groups, indicating that changes in tail volume do not decrease the accuracy of the VPR method (data not shown).

DISCUSSION

The purpose of the study was to validate the VPR tail-cuff system by comparing continuous intravascular blood pressures measured by telemetry with noninvasive blood pressures measured by tail-cuff. Because the pressure comparisons were made on simultaneous measurements in individual cardiac cycles, we have eliminated possible differences in heart rate as a confounding factor in this protocol. These methods do not, and should not be expected to, agree completely because they are different measures of blood pressure. However, the techniques should agree generally, particularly when individual measurements of systolic and diastolic pressure are made simultaneously. The data presented demonstrate that VPR measurements of SBP generally agree with simultaneous telemetry measurements across the physiological blood pressure range in mice. In contrast, VPR measurements of DBP were slightly but consistently lower than simultaneous telemetry measurements.

The difference between the SBP and DBP measurement accuracy with the VPR method may be due to the detection of these blood pressure points. Although the method for measuring both SBP and DBP is based on tail volume, the changes in tail volume that reflect the SBP and DBP are different. At the start of the measurement cycle, blood is pushed from the tail by the VPR cuff and then the occlusion cuff inflates to prevent

blood flow back into the tail. When the occlusion cuff deflates to the SBP, blood begins to flow back into the tail, increasing the tail volume. The occlusion cuff pressure at which the tail volume increases is the SBP. The tail volume will continue to increase as the occlusion cuff deflates until blood flow into and out of the tail equalizes; the occlusion cuff pressure at this point is the DBP. Because the initial increase in tail volume is more pronounced than the tail volume equalization point, it can be more readily identified to provide an accurate SBP measurement.

There are limitations to measuring blood pressure by either tail-cuff or telemetry, and careful consideration should be given to which method to use for a specific experimental design. Radiotelemetry measurement of intravascular blood pressure requires an invasive surgical procedure associated with operative and postoperative morbidity and mortality. Intra-abdominal or subcutaneous placement of the transmitter and isolation in an individual cage (required by the telemetry receiver) can contribute to behavioral stress. Additionally, limited battery life, initial investment and ongoing refurbishment cost, and level of surgical skill need to be considered before choosing radiotelemetry. Nevertheless, the exceptional temporal resolution and continuous, detailed record of cardiovascular performance may be justified or even required for some experimental designs, particularly when studying circadian patterns or transient treatment responses.

As an alternative to radiotelemetry, tail-cuff measurement of blood pressure avoids general anesthesia and surgery, postoperative stress, and behavioral stress of isolation required by radiotelemetry. However, changes in time of day, ambient conditions, operator handling of each animal, or subtle behavioral differences between groups, strains, or individual animals can introduce experimental variability to the measurement. Because individual tail-cuff measurements are taken at a discrete moment in time, a single measurement could be greatly affected by these confounding factors. Even when multiple measurements are automatically recorded in rapid succession during a measurement session, these confounding variables may contribute experimental variability to a measurement session.

On the basis of the data presented, we conclude that the VPR method accurately measured SBP, but underestimated DBP. Because we have used this system to measure blood pressure in >2,500 mice, the VPR method appears suitable for high-throughput genetic screens as suggested by the American Heart Association.¹ Previous mouse crosses investigating the genetic basis of blood pressure control used a training week to acclimate the mice to the measurement procedure and environment followed by a measurement week to collect the blood pressure data for analysis.^{11–13} In our studies using the VPR method to phenotype offspring from mouse intercross populations, we found that the mean and standard deviation of SBP were nearly identical between the training and measurement weeks, suggesting that the training week is not needed for tail-cuff measurement of blood pressure using VPR technology. However, we cannot determine whether the training week can be eliminated for other tail-cuff methods.

Disclosure: The authors declared no conflict of interest.

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