

Open-flow plethysmography with pressure-decay compensation

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Accepted 29 October 2002

Abstract

Whole-body plethysmography is widely used to measure ventilation in awake, unrestrained animals. However, the explicit solution for volumetric analysis of the plethysmograph signal depends upon a closed system, which limits experimental design. Although often used, open-flow plethysmography is complicated by the time-decay of pressure signals generated in the open chamber (e.g. equivalent volume displacements will yield different pressure pulse magnitudes depending upon the rate of application, dP/dt). This problem may be alleviated by first characterizing the time rate of pressure-decay, dP_k/dt , as a function of pressure magnitude, P , in the plethysmograph, $dP_{k(P)}/dt$. Then for each point $P(t)$ in the original signal, subtract the corresponding $dP_{k(P)}(t)/dt$ from each $dP(t)/dt$ of the original signal to determine the decay-compensated derivative for that point, $dP^*(t)/dt$, and then numerically integrate $dP^*(t)/dt$ to generate a pressure-decay compensated signal. The result is a ‘virtual closed plethysmograph’ trace that enables confident quantitative determination of ventilatory events and volumes with the full advantage of an open-flow plethysmograph.

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Keywords: Methods; Plethysmography; Plethysmograph; Virtually closed; Tidal volume; Measurement of ventilation

1. Introduction

Whole-body plethysmography enjoys wide use because it enables non-invasive measurement of ventilation in awake, unrestrained animals. The basis for this method derives from pressure changes detectable within a rigid chamber, i.e.

the plethysmograph, as the animal breathes. Converting this pressure signal to actual ventilatory volumes is routinely done using the principle described by [Drorbaugh and Fenn \(1955\)](#) with variations and refinements by others (e.g. [Malan, 1973](#); [Jacky, 1978](#); [Chauvi-Berlinck and Bicudo, 1998](#) for an overview, see [Mortola and Frappell, 1998](#)). However, the explicit solution for this volumetric calibration depends upon a closed chamber, although this limitation has not prevented its application to open-flow, or flow-through plethysmography (e.g. [Malan, 1973](#); [Pap-](#)

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penheimer, 1977; Jacky, 1978; Frappell and Daniels, 1991; Szewczak and Jackson, 1992; Seifert et al., 2000). Open-flow plethysmography offers a distinct advantage over a closed chamber arrangement because experiments can continue for indefinite, undisturbed periods without variation in the gas composition as occurs in a closed chamber. In addition, metabolism can be continuously monitored using an open-flow system (Frappell and Daniels, 1991; Sullivan and Szewczak, 1998). We present here an approach to improve the accuracy of open-flow plethysmography by numerically recovering the original signal from the time-decayed signal of an open-flow plethysmograph. This approach does not supplant any of the existing theoretical basis for whole-body plethysmography, however it does enable that basis to be extended to an open-flow arrangement. Furthermore, the method we present here could be applied generally to other signals with a complex time-decay attenuation, e.g. improving the resolution of instantaneous oxygen consumption (Bartholomew et al., 1981).

Determination of ventilatory volumes using this barometric method depends upon five parameters:

- 1) pressure changes within the chamber
- 2) chamber water vapor pressure
- 3) chamber internal temperature
- 4) animal body temperature
- 5) the pressure/volume response of a known volume displacement

The latter measurement, that of the calibration volume, is straightforward in a closed chamber; however, the time-decay of pressures generated in the open chamber complicates volume calibration. The chamber openings, whatever their respective resistances or impedances, will attenuate the pressure signal to some degree. As a result equivalent volume displacements can yield dramatically different pressure pulses, depending upon the time rate of application, dP/dt . A calibration pulse delivered at a greater magnitude of dP/dt will elicit a higher peak pressure than one delivered more slowly which allows more of the applied pressure to leak out of the chamber before completion of the pulse. A system in which the

time constant of the pressure-decay is much greater than the ventilatory frequency minimizes but does not eliminate the uncertainty (Pappenheimer, 1977; Jacky, 1978). Efforts to match the dP/dt of the calibration pulses to that of the animal's ventilation (Malan, 1973; Bucher, 1981) also result in incomplete compensation because ventilatory efforts can vary from breath to breath, and even individual breaths vary in dP/dt from beginning to end. Thus a single dP/dt -matched calibration pulse can provide only a compromised average, particularly with changing metabolic demand or ventilatory stimulation.

Frappell and Daniels (1991) observed that an open-flow system should behave as simple first order high-pass filter in which the pressure-decay of the signal can be described by a single exponential relationship. This would theoretically enable a numeric recovery of the signal character prior to any pressure-decay. However, in practice this solution does not fully recover the signal because other confounding variables depart the system's dP/dt response from that of an ideal exponential decay, for example:

- 1) the arrangement of the pressure transducer connection
- 2) transitional effects from adiabatic to isothermal expansion
- 3) analog signal conditioning, i.e. high pass, low pass, or band pass filters
- 4) dynamic response of the transducer element
- 5) inertance of the gas in the chamber and tubing
- 6) system capacitance, e.g. from tubing compliance

The complex interaction of these variables creates an intractable system that hinders a practical theoretical solution, if only because of the prohibitive effort of tracking each variable. As an alternate approach, we have developed a method to characterize the pressure/volume and pressure-decay characteristics of the working plethysmograph and then apply that information to numerically compensate the raw pressure signal. The resultant compensated signal behaves as a virtual closed system, and thus renders it nearly insensitive to the time rate of pressure changes

within it. This eliminates the pressure/volume calibration problem inherent with open-flow plethysmography and permits a confident application of volumetric calculations using the theoretical basis from a closed system. As an additional benefit, our method removes the blunting of ventilation traces that can occur in an open-flow plethysmograph, restoring the resolution of the ventilatory trace to a condition more suitable for assessing ventilatory event timing.

2. Methods

2.1. Animals

Adult male C57BL/6 mice provided the ventilation data used in this study. The mice were part of a colony used for another study. These mice were maintained at 24 °C in a 12/12 h light/dark cycle with food and water provided ad libitum.

2.2. Description of the apparatus

The plethysmograph used in this study was constructed of 1.3 cm thick acrylic with internal dimensions of $3 \times 3 \times 9$ cm³. Incurrent and excurrent air flowed through via ports at either end. A reference chamber of similar volume isolated the plethysmograph signal from ambient pressure noise. Side ports on the plethysmograph and reference chamber were connected to opposite sides of a differential pressure transducer (model DP103-18, Validyne). Airflow through the plethysmograph and reference chamber originated from a single source and then branched through the separate plethysmograph and reference circuits.

Incurrent and excurrent air through both chambers passed through flow resistors. These flow resistors were constructed by packing pieces of monofilament nylon line (8-pound test) into 3 mm i.d. polyvinyl tubing (Tygon, #3603). We adjusted the lengths of monofilament line (range 1–3 cm) in each resistor to balance the total resistance in each circuit, and between the chambers, by minimizing the pressure differential with gas flowing as in the experiment but without an animal in the plethysmograph. A fine needle valve was used in the

reference circuit distal to the reference chamber for final pressure balancing. The incurrent and excurrent flow resistors served as low-pass filters to enable detection of transient ventilatory-induced pressure, yet allowed continuous gas flow through the chambers. They also reduced ambient pressure noise from affecting the plethysmograph signal. In closed-plethysmograph mode, the flow resistors on the plethysmograph were replaced with stopcocks in a closed position.

The pressure transducer signal was demodulated (Validyne model CD-15) and sent through a simple RC high pass filter with a time constant of 33 sec to maintain the signal around a zero baseline. The signal was then sent to a chart recorder (Gould model 2400S) for amplification and analog low-pass filtering (15 Hz setting) to remove electrical noise spikes, and finally sampled with 12 bit resolution at 250 Hz for real-time monitoring and data storage by computer (Apple Macintosh Power PC; National Instruments Lab-NB A/D board and LabVIEW software). Humidity data were acquired with a humidity sensor (model IH-3602, Hy-Cal Engineering, El Monte, CA) fitted just downstream of the chamber. The data analysis method we describe in this paper was implemented using routines we created with LabVIEW software.

We used a 0.33 ml syringe attached to the top of the plethysmograph with a female Leur-lok port as a calibration plunger. A registration mark on the plunger shaft facilitated positioning the plunger to achieve a 50 microlitre volume. Fifty microlitres were selected as the calibration volume because it produced a pressure peak about twice that of ventilatory movements produced by mice in the plethysmograph. We measured the ambient pressure within the plethysmograph by temporarily fitting a tube to the calibration syringe port attached to a pressure sensor within a glass jar.

2.3. Terminology and basic principle

The decay-compensation method described herein follows from the closed plethysmograph principle described by [Drorbaugh and Fenn \(1955\)](#):

$$V = V_{\text{cal}} \times \left(\frac{P}{P_{\text{cal}}} \right) \left(\frac{T_p(P_a - P_{w_a})}{T_p(P_a - P_{w_p})} \right) \quad (1)$$

where V is the ventilatory volume, V_{cal} is the applied calibration volume, P is the pressure signal in the plethysmograph, P_{cal} is the pressure elicited by V_{cal} , T_p is the pulmonary temperature of the animal (in absolute degrees), which may be assumed to be the same as the body temperature, P_a is the ambient pressure within the plethysmograph at equilibrium, P_{w_a} is the ambient water vapor pressure within the plethysmograph, T_a is the ambient air temperature within the plethysmograph (in absolute degrees), and P_{w_p} is the animal's pulmonary water vapor pressure.

Most investigators apply Eq. (1) to calculate just the tidal volume (V_t) from local peaks in the pressure trace. However, we will use Eq. (1) to determine any volume V from the pressure P along a pressure signal time series generated in the plethysmograph. Thus, we will consider P as a continuous function of time t (and therefore differentiable), and V to be dependent upon P as defined by Eq. (1), i.e.

$$V(t) = f(P(t)) \quad (2)$$

In a closed plethysmograph, applying an incremental volume, as with a syringe plunger, will elicit a corresponding pressure increment within the

plethysmograph that remains until the volume is withdrawn (Fig. 1). However, in an open-flow plethysmograph, a similarly applied incremental volume will elicit a pressure pulse that returns to baseline (Fig. 2) as the applied pressure-decays from airflow out of the open plethysmograph. Obviously, for any detectable pressure pulse to occur, the time rate of the pressure application, dP/dt , must exceed the magnitude of the time rate of the pressure-decay, dP_k/dt . The plethysmograph's incurrent and excurrent flow resistors determine the dP_k/dt . The greater the resistance, the nearer the open-flow plethysmograph approaches the behavior of a closed system. However, in practice these resistances must be limited to avoid excessive pressure within the plethysmograph. Alternatively, to compensate for high resistances, a vacuum can be applied to the excurrent port to achieve a suitable ambient pressure within the plethysmograph.

Because of the pressure-decay, the $P(t)$ observed at any point (other than equilibrium) in an open-flow plethysmograph will always be less than the $P(t)$ that would be observed if the plethysmograph were closed, $P^*(t)$. Similarly, the dP/dt observed at any point will always be less than the dP/dt that would be observed if the plethysmograph were closed, dP^*/dt . (We define

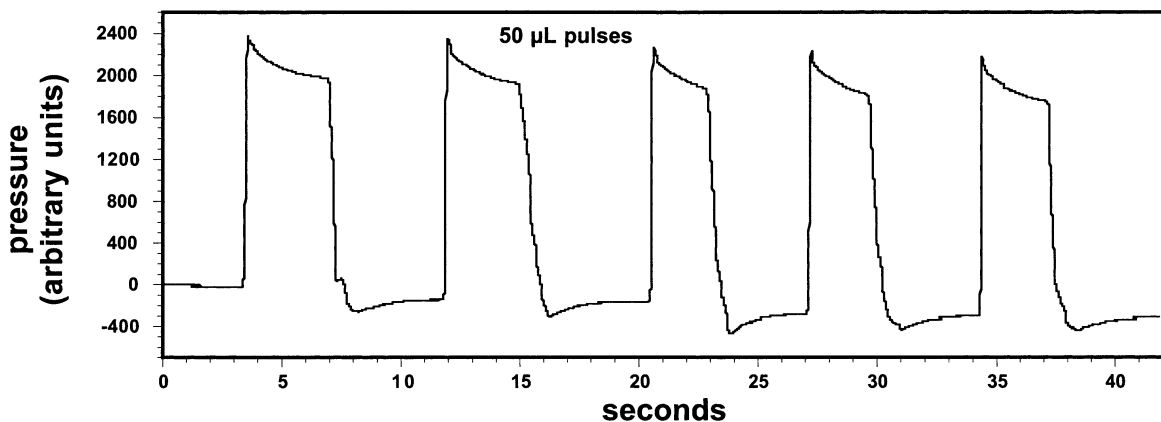


Fig. 1. Example time series of 50 μL calibration pulses in a closed plethysmograph. The upward deflections indicate pressurization of the plethysmograph with the calibration volume, while the steep downward deflections indicate when the calibration volume was withdrawn. Changes in the pressure trace between these calibration maneuvers result from non-steady state adiabatic-induced pressures and the consequent movement toward an isothermal equilibrium (Malan, 1973; Chaui-Berlinck and Bicudo, 1998).

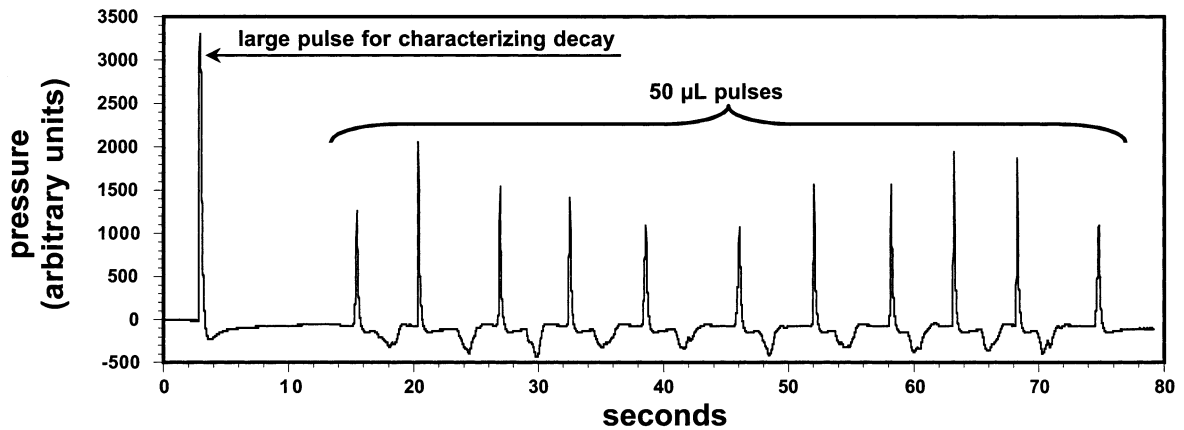


Fig. 2. Example calibration file from an open-flow plethysmograph. The first large pulse was used for characterizing the pressure-decay of the plethysmograph (see text for details). The 50 μL calibration pulses were produced in the same manner as those displayed in Fig. 1. Different pulse heights reflect different rates at which the calibration pulses were injected, despite having equivalent volumes.

parameters with an asterisk notation to be the closed system analogs of the initial, uncompensated parameters.)

Because a continuous function is equal to the integral of its derivative, it then follows that for a pressure time series $P(t)$ from $t=0$ to $t=n$ that:

$$P(t) = \int_0^n (dp/dt)dt \quad (3)$$

and similarly that

$$P^*(t) = \int_0^n (dp^*/dt)dt \quad (4)$$

Thus, if the dP^*/dt can be determined for each time t , then a virtual pressure trace $P^*(t)$ may be numerically reconstructed from the original $P(t)$ of the open-flow plethysmograph, and that forms the basis for the method we present.

The observed dP/dt results from the opposing action of the ‘push’ of the applied pressure dP^*/dt and the ‘pull’ of the pressure-decay dP_k/dt , i.e.:

$$dP/dt = dP^*/dt + dP_k/dt \quad (5)$$

(For positive pressure pulses, the dP_k/dt will have a negative slope. Thus, adding it to dP^*/dt will decrease the resulting value of dP/dt , and account for its pull relative to the push of the pressure pulse.) Rearranging yields:

$$dP^*/dt = dP/dt - dP_k/dt \quad (6)$$

The dP/dt may be determined directly from the acquired pressure signal. The dP_k/dt describes the rate at which the pressure signal, unaffected by a pushing dP^*/dt , returns to equilibrium, and is a function of P . The pressure-decay responds to any pressure deflection, positive or negative, by returning it to equilibrium, and behaves somewhat as an overdamped spring analog. However, for the open-flow plethysmograph, the dP_k/dt relates to P in a more complex manner because of the multiple factors described in Section 1. Although that hinders the derivation of a predictive relation for dP_k/dt as a function of P , it does not preclude deriving an empirical relationship for dP_k/dt as a function of P based upon an unimpeded relaxation curve of a pressure pulse in the plethysmograph under use.

2.4. Decay-compensation procedure

Our method determines dP_k/dt as a function of P using a prescribed calibration routine, then applies that function to generate a pressure-decay compensated signal, $P^*(t)$, as per Eq. (6) and Eq. (4). The pressure-decay compensated calibration data is then used to define the plethysmograph pressure/volume calibration, P_{cal}^* .

It is imperative to acquire the calibration data file under the same operating conditions of the

plethysmograph as those used for the animal data collection. We typically perform a calibration routine immediately after an animal data session in the plethysmograph, or after a series of runs using the system at the same conditions. A calibration file consists of one or more large pressure pulses followed by a series of calibration pulses applied at a variety of rates, intended to overlap the dP/dt typical of the ventilatory data to be analyzed (Fig. 2). To ensure a system response representative of that with the animal, we place a proxy object within the plethysmograph that displaces a comparable volume as that of the animal under investigation. To fully characterize the system, the calibration volume should be selected to elicit a pulse height at least as large as the greatest ventilatory pulse height. An applied volume of about one and a half times to twice the calibration volume serves for the decay characterization pulse(s) (Fig. 3), while the series of calibration volume pulses (V_{cal}) are used to determine P_{cal}^* .

We have found that a third order polynomial regression provides a suitable representation of dP_k/dt vs. P in our system. However, other systems may require a different evaluation. The range over which to regress the pressure-decay response curve should begin from a value P greater than any of the calibration pulses and end at the bottom of the curve where it approaches or reaches a zero slope. (Note that the desired

polynomial regression is not of the pressure P vs. time (Fig. 3), but rather is of the *derivative* of this curve vs. its value P .) In practice, the plethysmograph response to a large pressure pulse does not always yield a result in which the curve subsides to a zero slope at zero P . However, we find that shifting the decay data to define P to be zero at the point of zero slope provides a favorable decay-compensation result.

With our system, the decay-compensation result was sensitive to the exact range over which the decay relationship is characterized (Fig. 3). Additionally, some pulses did not provide a favorable result, possibly because of vibration or other signal noise. It is therefore helpful to have several decay characterization pulses in the calibration file. To insure a favorable selection for characterizing the decay relationship, we use a routine that displays the decay-compensated calibration file based on the selection. A result that recovers the signal to an appearance as close to that as the pulses would appear in a closed system (Fig. 1) is the goal (Fig. 4).

To complete the calibration, we generate a pressure-decay compensated transform of the calibration pulse series. For each point in the time series, $P(t)$, determine its derivative $dP(t)/dt$, evaluate its corresponding rate of decay $dP(t)_k/dt$ using its absolute value $|P(t)|$ in the polynomial regression, and sum according to Eq. (6) to yield $dP(t)^*/dt$. The absolute value is specified because

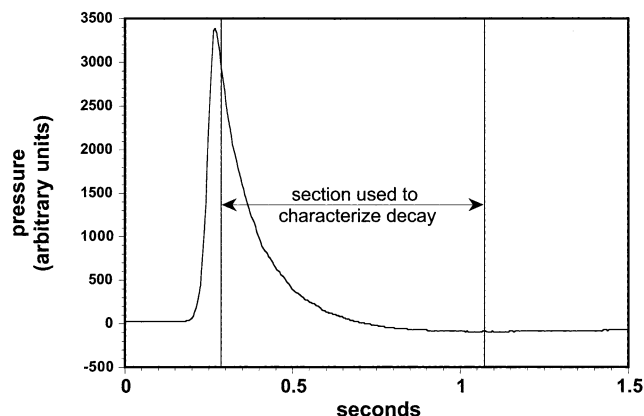


Fig. 3. Detail of the decay characterization pulse shown in Fig. 2. To fully characterize the pressure-decay dynamics of the plethysmograph system, the magnitude of decay characterization pulses should be sufficient to provide a selection exhibiting a steady decay in which the upper limit encompasses the magnitude of the largest calibration pulses.

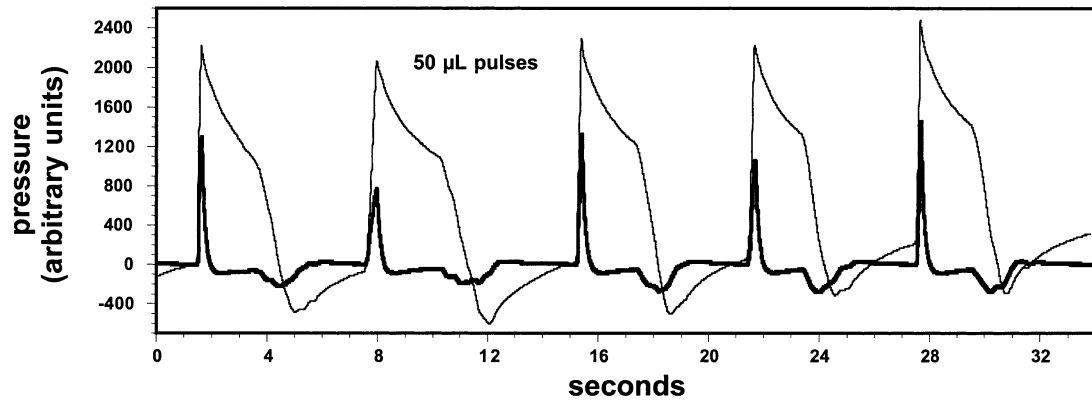


Fig. 4. Detailed portion of the calibration pulse series shown in Fig. 2 (bold line trace) superimposed with the same data following pressure-decay compensation (fine line trace) from an open-flow plethysmograph. Note that the pressure-decay compensated trace has nearly recovered the appearance of calibration pulses generated in the closed plethysmograph (Fig. 1).

some excursions of the time series will fall below the equilibrium pressure of the plethysmograph. However, we found that the pressure-decay response of our system was symmetric within the range of ventilatory pressures we measured. Other systems may require separate regressions to evaluate positive and negative pressure excursions if a nonsymmetrical response is found. The resulting $dP(t)^*/dt$ time series is then numerically integrated according to Eq. (4) to generate the pressure-decay compensated time series $P^*(t)$. The transformed calibration data (Fig. 4) is nearly restored to the appearance of calibration pulses in the closed plethysmograph (Fig. 1). We then take the mean calibration pulse height to determine P_{cal}^* .

We then apply the same procedure to transform the associated ventilatory data to a pressure-decay compensated time series and translate that to a volume time series using Eq. (1). V_t and other ventilatory variables are then determined from the resultant time series (Fig. 5).

2.5. Ventilation trials

For each experiment, a mouse was removed from its cage, weighed, and then placed into the plethysmograph. We adjusted the gas mixture flowing through the plethysmograph to achieve an inspired P_{O_2} of 159 Torr. We allowed the mouse to acclimate to the plethysmograph for at

least 30 min while monitoring its ventilation and metabolism. Experimental data was acquired only when the mouse appeared to have settled into a calm state of regular ventilation and metabolism. We then collected at least 30 min of ventilatory data.

The ventilatory data exhibited short (1 min or less) bouts of regular rhythmic breathing, punctuated by periods of irregular pressure activity in the pressure trace from the mouse moving within the plethysmograph. As a standard for measuring tidal volume, we selected sequences from the bouts of regular rhythmic breathing. These selected sequences ranged from 19 to 127 breaths and 0.12–0.83 min from which we determined V_t according to the principles described by Drorbaugh and Fenn (1955) and adapted to a flow-through system (Malan, 1973; Bucher, 1981). For comparison, we applied the above-described decay-compensation algorithm to the same selected sequences.

3. Results

3.1. Calibration pulses

For the example series of eleven 50 μ L calibration pulses shown in the figures, the mean of raw, uncompensated pulse heights was 1496 ± 327 (standard deviation), range: 1098–2058 in arbitrary units.

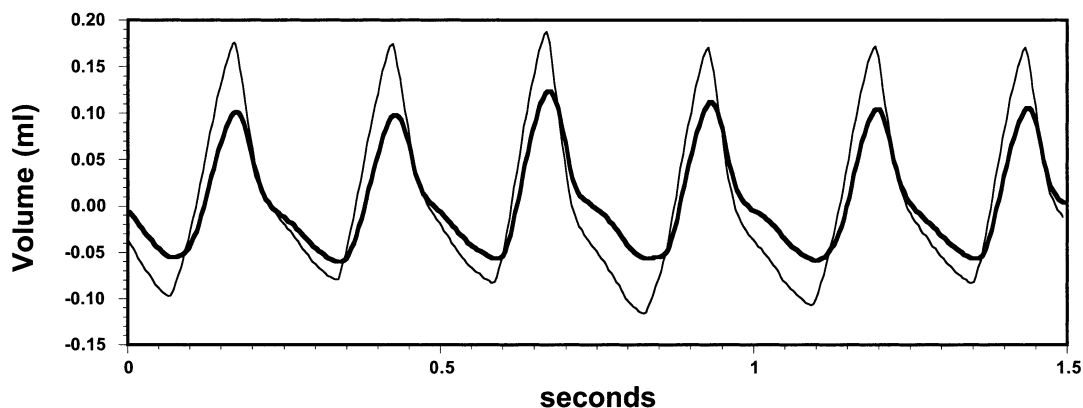


Fig. 5. Detailed portion from a mouse ventilation series in the open-flow plethysmograph. The raw, uncompensated time series (bold line trace) is superimposed with the same data following pressure-decay compensation (fine line trace). Note that the pressure-decay compensated trace has recovered from the blunting caused by the pressure-decay of the open-flow plethysmograph. In addition to more accurate ventilatory volume analysis, the 'sharpening' of the ventilatory signal by this method can facilitate revealing ventilatory structure and the timing of ventilatory events.

trary pressure units. The same calibration series after decay-compensation treatment was 2407 ± 54 (Figs. 2 and 6). A series of ten 50 μ l calibration pulses made at the same time in the same plethysmograph, but closed to flow, averaged

2434 ± 55 (Fig. 1). The decay-compensated pulses did not significantly differ from the closed plethysmograph calibration pulses ($P = 0.29$, for the means; $P = 0.47$ for the standard deviations, two-tailed Student t -test).

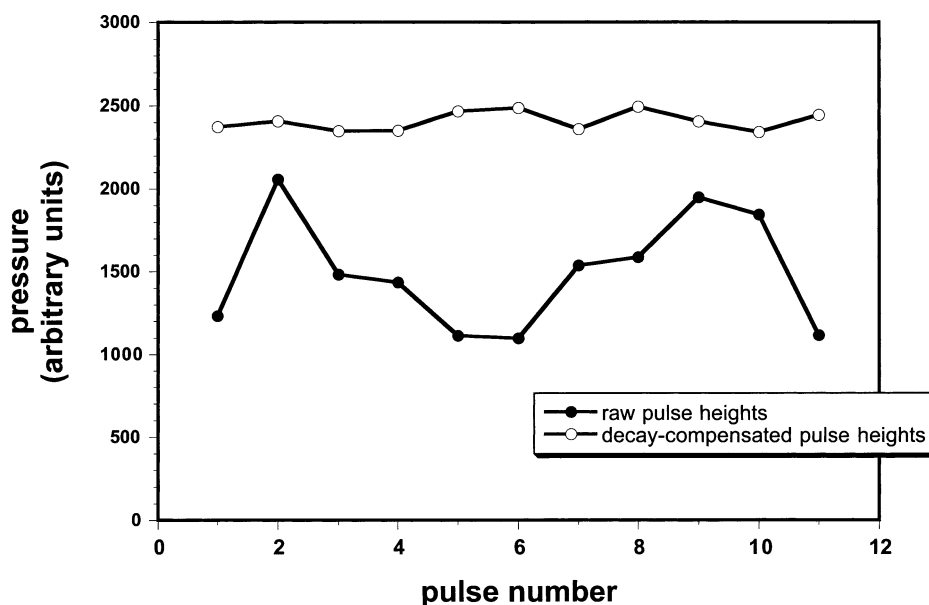


Fig. 6. Comparison of raw, uncompensated calibration pulse heights from the data shown in Fig. 2 with that of the pressure-decay compensated pulses of the same data set. Although generated from the 50 μ l calibration volume, the raw pulse heights vary considerably as a result of differential rates of injection. The pressure-decay compensation procedure restored them to reflect their equivalent volume.

3.2. Ventilation trials

From 22 selected sequences of rhythmic breathing from 8 mice, the V_t was determined to be 11.5 ± 0.5 ml/kg (\pm standard error) using the uncompensated data and applying the standard method of [Drorbaugh and Fenn \(1955\)](#) adapted to a flow-through system ([Malan, 1973](#); [Bucher, 1981](#)). Applying the decay-compensation algorithm to the same sequences the V_t was determined to be 8.64 ± 0.50 ml/kg.

4. Discussion

We use an analog high pass filter in our system to maintain the ventilatory signal near baseline. Without the filter, baseline drift and shifts that occur from animal movement would necessitate reduced amplification and consequent reduction in signal resolution to keep the signal in range. The filter facilitates good resolution of the signal with the 12-bit analog-to-digital conversion of our system. The decay-compensated signal recovers the drifting baseline that was present in the original data before the high pass filter. This demonstrated that the numerical algorithm compensated for the electrical decay of the signal, in addition to the physical pressure-decay of the signal. (The recovered drifting is not a problem in post-processing because the transformed signal can then be described with greater bit resolution.)

Accurate analysis of ventilatory data depends upon recognizing the inception, end-tidal, and end-expiration of ventilatory maneuvers. Identification of the end-tidal point involves a straightforward peak detection routine. However, the inception and end-expiration do not necessarily correspond to a minimum point in the way that end-tidal corresponds to a maximum point, particularly when there are ventilatory pauses. This occurs because the pressure within an open-flow plethysmograph will move toward the chamber's equilibrium if left in disequilibrium at the end of the breath. In such a case, ventilatory inception may not occur at a local minimum, but rather at an inflection in the trace ([Fig. 7](#)). We recommend some logic applied to the analysis to recognize

these events. For our work, we have found that a local maximum in either the second derivative or the curvature of the ventilation signal dependably recognizes the inception of ventilation. Whichever method is selected, the logic should be sufficiently robust to avoid false recognitions from signal noise. To accomplish that we apply a smoothing filter (LabVIEW FIR windowed filter, low pass, Blackman window) sufficient to eliminate minor noise-generated peaks and render a continuous curve, then execute a logic routine to recognize local peaks within a prescribed window of data. When creating the logic routine, we used a plot that overlaid the selected points with the ventilatory trace to provide feedback for optimizing the filter settings and algorithm.

[Tankersley et al. \(1994\)](#) documented that the V_t of mice vary with strain. Thus our results from C57BL/6 mice are best compared with reported values from this strain. [Huey et al. \(2000\)](#) measured the V_t of C57BL/6 mice to be 8.7 and 8.5 ml/kg using a head-out dual chamber and open-flow plethysmograph, respectively. [Tankersley et al. \(1994\)](#) reported C57BL/6 V_t s of 6.8 and 8.1 ml/kg in two separate trials using a closed plethysmograph. Our result of 8.6 ml/kg using the decay-compensation algorithm compares well with these reported values, whereas the V_t result of 11.5 ml/kg using the uncompensated data seems high. The volumetric calibration of the uncompensated pressure trace depended upon the selection of a suitable calibration pressure pulse. We selected a calibration pressure pulse with a mean dP/dt closest to the mean dP/dt of the mouse's own ventilation. However, even that criterion can lead to error because, as previously mentioned, breaths vary in dP/dt from beginning to end, and from breath to breath. Thus a single dP/dt -matched calibration pulse can provide only a compromised average. Furthermore, the mean dP/dt criterion will lead to further error if the calibration peak height is substantially different than the ventilatory peaks because the drive to equilibrium varies with P and this affects the mean dP/dt .

As demonstrated by this ventilation trial, our motivation for developing this method was borne out of our need for dependable and repeatable volumetric results in an open-flow plethysmo-

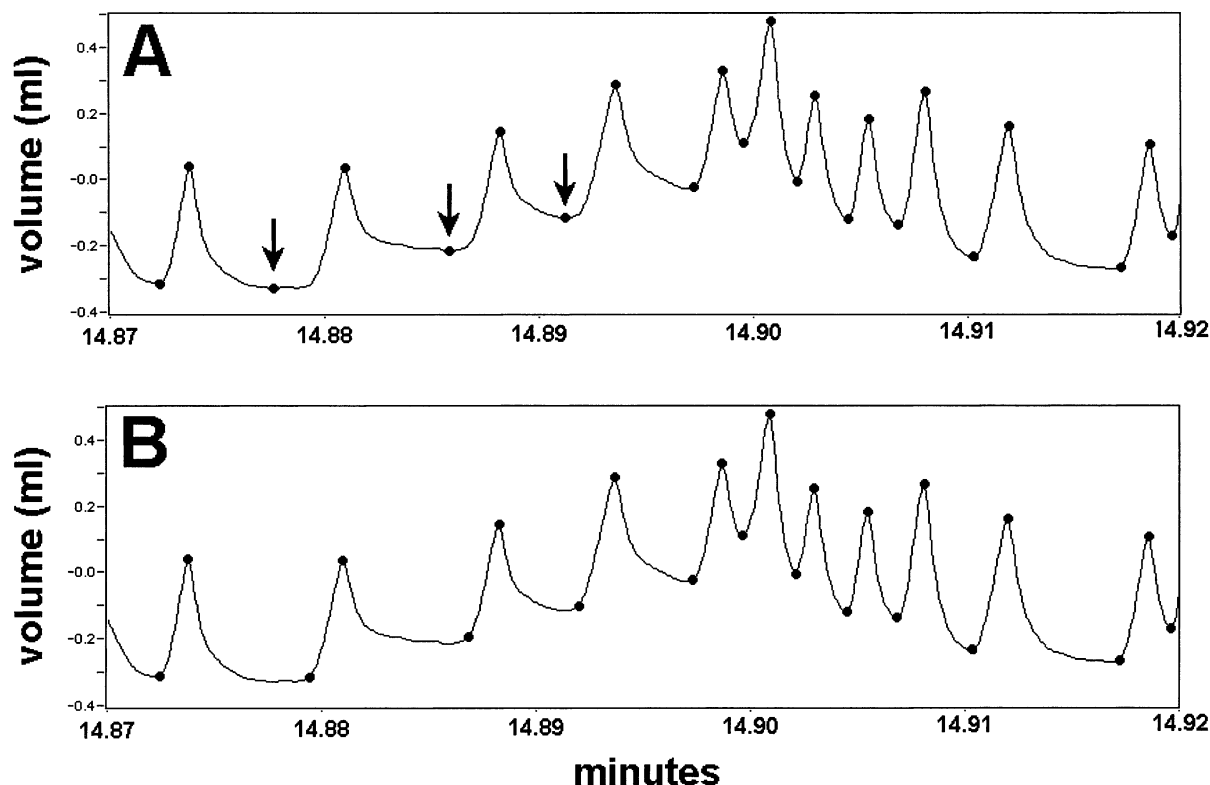


Fig. 7. Detail of pressure-decay compensated ventilation trace from an open-flow plethysmograph showing that minimum points preceding ventilations do not always correspond to ventilatory inception (arrows on panel A). The actual points of ventilatory inception may be recognized from local maxima in the second derivative or curvature of the trace (panel B).

graph. Previously described calibration methods incompletely compensate the open-flow system, and depend upon operator technique (Malan, 1973; Jacky, 1978). The pressure-decay compensation method essentially eliminates the troublesome rate-sensitivity of the calibration volume procedure inherent to the open-flow plethysmograph. Accurate determination of ventilatory volumes depends upon the accurate determination of the calibration pulse height, P_{cal} in Eq. (1). Despite the substantial variability of the pulse heights of the raw calibration signal (Fig. 2), the pressure-decay compensated pulse heights of the same data exhibited essentially the same variability as that of pulse heights generated in the plethysmograph when closed. In the example data presented here, the uncompensated P_{cal} values ranged by nearly a factor of two, which would similarly, and unac-

ceptably, affect the resulting ventilatory volume determination. The most appropriate P_{cal} value might possibly be selected from such a range; however, such a selection always leaves some measure of doubt. The method we present here eliminates uncertainty over the selection of P_{cal} and enables confident quantitative ventilatory volume determination with the full advantages of an open-flow plethysmograph.

Acknowledgements

Grants from the National Science Foundation (SGER-0086268) and The National Institutes for Health (NIH-HL 17731) supported the research used to support the work presented in this paper.

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