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Design and Nonlinear Modeling of a Sensitive Sensor for the Measurement of Flow in Mice

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Abstract

Objective—While many studies rely on flow and pressure measurements in small animal models of respiratory disease, such measurements can however be inaccurate and difficult to obtain. Thus, the goal of this study was to design and implement an easy to manufacture and accurate sensor capable of monitoring flow.

Approach—We designed and 3-D printed a flowmeter and utilized parametric (resistance and inertance) and nonparametric (polynomial and Volterra series) system identification to characterize the device. The sensor was tested in a closed system for apparent flow using the common mode rejection ratio (CMRR). The sensor properly measured tidal volumes and respiratory rates in spontaneously breathing mice. The device was used to evaluate a ventilator's ability to deliver a prescribed volume before and after lung injury.

Main Results—The parametric and polynomial models provided a reasonable prediction of the independently measured flow (Coefficient of determination (C_v)=0.9591 and 0.9147 respectively), but the Volterra series of the 1st, 2nd, and 3rd order with a memory of six time points provided better fits (C_v =0.9775, 0.9787, and 0.9954, respectively). At and below the mouse breathing frequency (1–5 Hz), CMRR was higher than 40 dB. Following lung injury, the sensor revealed a significant drop in delivered tidal volume.

Significance—We demonstrate that the application of nonparametric nonlinear Volterra series modeling in combination with 3-D printing technology allows the inexpensive and rapid fabrication of an accurate flow sensor for continuously measuring small flows in various physiological conditions.

Key Terms

Mouse flow sensor; nonlinear system identification; Volterra series; parametric fitting; nonparametric fitting

Introduction

Measuring lung mechanical function in small animals is central to many investigations into the pathophysiology of pulmonary disease, yet it remains a challenge due to the very small

gas flows involved (Bates and Irvin 2003, Irvin and Bates 2003). For example, a key problem associated with the measurement of small flows using a pneumotachograph is the finite input impedance of any differential pressure transducer that is used to measure the resistive pressure drop across the device; this can give rise to a limited dynamic common mode rejection ratio that causes a flow to be registered purely as a result of uniform pressure oscillations within the lumen of the pneumotachograph (Schuessler *et al* 1993). One approach to dealing with the problem is to use a different method for determining flow, such as from the rate of change of position of a piston within a cylinder which, with appropriate calibration for gas compression, leads to a knowledge of flow on purely geometric grounds (Schuessler and Bates 1995). The problem with this is that due to gas compression and pressure losses in the valves, the delivered flow into the mouse is different from the flow computed from piston motion. To estimate the delivered flow, a complex calibration procedure is required which however does not guarantee that the delivered flow is the same as the desired flow (Thammanomai *et al* 2007).

Nevertheless, the measurement of small flows with a pneumotachograph is still possible, in principle, provided any confounding dynamic and nonlinear characteristics of the device are accounted for with sufficient accuracy. Indeed, the design and implementation of flow sensors for rodents has already been shown to be feasible. For instance, Giannella-Neto *et al.* (Giannella-Neto *et al* 1998) designed and calibrated an effective rat pneumotachograph based on rigorous design criteria considering laminar flow, measurable pressure difference, and minimal gas exchange and interference with the rodent's respiratory mechanics. In the characterization of the rat pneumotachograph, the nonlinear pressure-flow characteristics of the device were accounted for via a nonparametric polynomial model, and an important conclusion was the importance of nonlinear calibration for accurate flow measurements. To account for nonlinearities, Korenberg and Hunter (Korenberg and Hunter 1996) have shown that a Volterra series approach can be used to represent and identify a wide range of nonlinear systems. Importantly, this nonparametric modeling technique does not assume any model structure implying that nonparametric fitting does not limit the sensor model to any particular form. Furthermore, the Volterra series can account for both nonlinear as well as dynamic aspects of a small device such as a mouse pneumotachograph, providing even more precision. These properties, as well as the ability to use system memory, allows the Volterra series to provide system identification with high accuracy across a required dynamic range. Accordingly, we sought to develop a general approach to the empirical characterization of pressure-flow characteristics based on the Volterra series (Korenberg and Hunter 1996). Furthermore, with the maturation of 3-D printing technologies, inexpensive and rapid implementation of arbitrarily detailed internal sensor structure can now be realized. This allows exploration of complex internal geometries so that sensor dead space, sensitivity, and flow resistance can be optimized. In the present study we therefore applied the Volterra series to a 3-D printed custom-designed pneumotachograph and validated its use in measuring tracheal airflow in mice.

Methods

Sensor Design

Figure 1 shows the flow sensor design, which is based on the common pneumotachograph concept where the pressure drop along a conduit is related to the axial flow within it. The conduit of our novel sensor is divided into several sections. The majority of the pressure drop along the conduit occurs across an inner core of diameter 0.5 mm and length of 5.8 mm. Outer sections having 2 mm internal diameter (ID) and 3.5 mm length allow the device to be conveniently connected into a ventilation circuit. The inner and outer sections are connected by conical sections with a diameter that increases linearly between the inner and outer sections over a length of 4.6 mm, creating an inclination angle of 9.26° . Two lateral ports (ID = 0.5 mm, outer diameter OD = 4.6mm, length = 3mm) are placed symmetrically near the wide end of each conical section to permit the measurement of differential pressure across the major resistive portion of the conduit. A single port on the outlet side of the conduit is placed for measurement of gauge pressure. The design shown in Figure 1 was implemented using a 3-D printer (Objet30 Scholar, resolution: 0.1mm, VeroBlack Matte, EPIC, Boston University, Boston).

The design criteria included a resistance that is large enough to produce a measurable pressure drop with the tiny flows typically encountered in mice, but not too large to significantly affect the animal's breathing pattern.

The inner volume (dead space) of the sensor was approximately 0.035 ml. The Functional Residual Capacity of mice ranges from 0.2 to 0.5 ml (Mitzner *et al* 2001, Vanoirbeek *et al* 2010) and the Total Lung Capacity (TLC) from 0.8 to 1.5 ml (Vanoirbeek *et al* 2010). The dead space volume is thus only about 2 to 4% of TLC. More importantly, assuming a 25 g mouse and a V_T of 8ml/kg, the dead space volume is 17.5% of the delivered tidal volume (0.2 ml). With this information, the ventilator settings can be adjusted (in this case to 9.7 ml/kg) to make sure that sufficient volume is delivered to the mouse for proper gas exchange.

Based on the outlined design, the differential pressure transducer only measures pressure across the inner core and the transitional regions and not the entire flow sensor. However, as later detailed, the pressure drop used to characterize the sensor is measured across its total length. Thus, for system identification to be representative and flow measurements to be accurate, it is both convenient and important that the total resistance of the sensor be due mostly to the core and the transitional region such that the pressure used to characterize the sensor and the pressure used to calculate flow from experiments closely match. Using Poiseuille's law, equivalent resistances were obtained for every region across the sensor (inlet and outlet, inner core, and the transitional region in between). For the transitional region which had varying radii, an integration of Poiseuille equation was carried out. We found that the core and the transitional region contributed to an estimated 99.5% of the sensor's overall flow resistance (Table A, Supplementary material). It should be noted here that we assumed no flow to occur in the side ports.

The total respiratory system resistance of the mouse can vary between 0.5 and 0.8 cmH₂O.s/ml (Vanoirbeek *et al* 2010, McGovern *et al* 2013). The resistance of the flow sensor is approximately 0.9 cmH₂O.s/ml and so it is of the same order as the mouse's resistance. Since the majority of the experiments use a ventilator to deliver tidal breath to the mice, the current design did not consider reducing this value by an order of magnitude. In case this is required, by simply modifying the design to have an inner diameter core of 1.1 mm reduces the resistance to around 0.0538 cmH₂O.s/ml which composes 91.6% of the overall sensor resistance. The modified sensor's resistance will only be 11% and 7% of the mouse resistance respectively while the dead space will increase to 22.7% of the delivered tidal volume. Tables B and C in the supplementary material include the measurements and characteristics of the alternative design respectively.

Data Acquisition

To test the device, we connected the inlet port (outer diameter, OD = 4.4 mm) via plastic tubing to a flexiVent small animal ventilator (flexiVent Legacy, Module 1, Scireq Inc., Montreal, CA), while the outlet port (OD = 4.4mm) was connected to a 20 gauge tracheal cannula. A differential pressure transducer (Biopac Systems, Model TSD160A) for recording airway flow was connected to the two symmetrical side ports by 12 mm of plastic tubing. A gauge pressure transducer (WPI, 07B PNEU05) was connected to the single port for airway pressure measurement. The signals from both transducers were amplified (World Precision Instruments (WPI), Model TBM4-F), digitized (WPI, DataTrax) at 500Hz sampling frequency and saved on a computer (WPI, Quad 16-EFA-400). The transducers were calibrated in units of cmH₂O using a water manometer.

Dynamic Response

To identify the dynamic response of the sensor to a time-varying flow, a 16 seconds band-limited white noise volume signal comprised of sinusoidal components with frequencies between 0.5 and 20 Hz at 0.5 Hz increments, equal amplitudes, and random phases was applied to the inlet of the device using the ventilator. The signal was scaled so that the peak-to-peak amplitude in flow corresponded to a physiologically representative range for a mouse (± 8 ml/s). During the volume perturbation that was delivered to the device, the pressure (P) and volume (V) signals at the inlet were also measured by the ventilator and the flow (F) was obtained as the time-derivative of V using a differential filter of order 50, 22 Hz bandpass frequency, 25 Hz stop band frequency, and 256 Hz sampling frequency (f_s) (MATLAB, R2011a).

We studied the sensor in an open system configuration (no load on the outlet). To characterize the sensor parametrically, F and P were fit in MATLAB with the time-domain model

$$P(n) = RF(n) + I \frac{dF(n)}{dt} \quad (1)$$

where R is resistance, and I is inertance, and n is the data point index running from 1 to N ($N=4095$). To characterize the sensor nonparametrically, we first applied a nonparametric

polynomial fit previously described and implemented by Giannella-Neto et al. (Giannella-Neto *et al* 1998) and Tang et al. (Tang *et al* 2003). Briefly, V can be written as the integral of F :

$$V(n) = \frac{1}{f_s} \sum_{i=1}^n F(i) \quad (2)$$

If F is assumed to be a polynomial function of P with order m then

$$F(n) = c_1 P(n) + c_2 P(n)^2 + \dots + c_m P(n)^m \quad (3)$$

And

$$V(n) = \frac{1}{f_s} \sum_{i=1}^n F(n) = \frac{c_1}{f_s} \sum_{i=1}^n P(i) + \frac{c_2}{f_s} \sum_{i=1}^n P(i)^2 + \dots + \frac{c_m}{f_s} \sum_{i=1}^n P(i)^m \quad (4)$$

In vector form this is written as

$$V = \frac{1}{f_s} X C + E \quad (5)$$

Where C is an $m \times 1$ column vector with the polynomial coefficients c_1 to c_m , X is an $N \times m$ matrix whose columns are increasing powers of P evaluated at time points $i=1 \dots n$, and E , a column vector of length N , is the error. C can be estimated as

$$C = (X'X)^{-1} X'V f_s \quad (6)$$

The last fitting technique included the nonparametric Volterra series up to 3rd-order. A 3rd-order series is written as

$$\begin{aligned} F(n) = & h_0 + \sum_{i=1}^M h_1(i) P(n-i+1) + \sum_{i_1=1}^M \sum_{i_2=1}^M h_2(i_1, i_2) P(n-i_1+1) P(n-i_2+1) \\ & + \sum_{i_1=1}^M \sum_{i_2=1}^M \sum_{i_3=1}^M h_3(i_1, i_2, i_3) P(n-i_1+1) P(n-i_2+1) P(n-i_3+1) \end{aligned} \quad (7)$$

where h_j is the Volterra kernel of order j . These kernels were identified using the approach of Korenberg and Hunter (Korenberg and Hunter 1990, 1996). M represents the system's memory, and n goes from M to N . For example, if $M=6$, then $n=6, 7 \dots N$ and F is calculated

using the current and 5 previous P values; for the 1st F value calculated ($n=6$), $h_1(1)$ will be multiplied with the current P value, $P(6)$, while $h_1(6)$ will be multiplied by the 1st P value, $P(1)$.

To briefly summarize the method, Eq. 7 was written in the following form

$$y(n) = \sum_{m=0}^{M-1} a_m p_m(n) + e(n) \quad (8)$$

where $y(n)$ are the measured flow values $F(n)$, the parameters a_m correspond to the individual Volterra kernels, p_m is related to the input $P(n)$ including higher order and cross-term multiplications, and e is the error. First, p_m was computed from $P(n)$ and was used to construct orthogonal functions via a modified Gram-Schmidt procedure (Rice 1966). These orthogonal functions were then used in a least square fit and the a_m values were calculated through a recursive procedure. The 1-D matrix a_m contains all kernel values arranged from the lowest to the highest order (i.e. a_0 is the zero-order kernel) (Korenberg and Hunter 1990, 1996). For a 3rd-order Volterra series, the zero-order kernel h_0 is a single value, the 1st-order kernel h_1 is a 1-D matrix of size M , the 2nd-order kernel h_2 is a 2-D symmetric matrix of size $M \times M$, and the 3rd-order kernel h_3 is a 3-D matrix of size $M \times M \times M$. Any non-zero elements from h_2 and h_3 signify nonlinearity due to interactions between P terms.

The main difference between the system identification techniques embodied in Eq. 1, Eq. 4, and Eq. 7 is that the first is a linear parametric model without memory and with parameters that can be related to physical quantities such as radius and length. The second approach is nonlinear and nonparametric but without memory while the last one also has memory, is nonlinear and nonparametric. Thus, the parameters in Eqs. 4 and 7 have no direct physical interpretation.

We used the fitting parameters identified in Eqs. 1, 4, and 7 to predict flow (F_{fit}) from P for comparison to F recorded by the ventilator. This comparison was quantified by the coefficient of determination (C_V) given by

$$C_V = 1 - \frac{N-1}{N-c-1} \frac{\sum_i^N (F_{fit}(i) - F(i))^2}{\sum_i^N (F(i) - \bar{F})^2} \quad (9)$$

where c is the number of coefficients (equal to the number of parameters in the parametric and polynomial fits or the number of unique kernels in the Volterra series) and the bar above F denotes the average. Finally, to confirm that the improved Volterra series performance is due to their enhanced ability to describe dynamic systems rather than noise, we applied a simple cross-validation exercise for all 3 techniques in which we fitted half the data and used the other half for validation and compared the C_V values as well.

Common Mode Rejection Ratio (CMRR)

The CMRR of the differential pressure transducer connected to the flow sensor was determined using composite volume perturbations containing 5 frequencies (2, 5, 11, 19 and 31 Hz) based on the non-sum non-difference criterion (Suki and Lutchen 1992) with peak-to-peak amplitudes of 2 and 4ml/kg. The two ports of the differential pressure transducer in each configuration were exposed to the same dynamic pressure excursions while a separate pressure transducer measured these pressure excursions relative to atmosphere (P_1). Using P_1 together with P_2 , the measured differential pressure across the sensor, CMRR, was calculated as a function of frequency (f):

$$CMRR(f) = 20 \log_{10} \left[\frac{P_1(f)}{P_2(f)} \right] \quad (10)$$

where $P_1(f)$ and $P_2(f)$ are the Fourier transforms of P_1 and P_2 respectively.

Mouse Experiments

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Boston University. The flow sensor was used in two sets of experiments to evaluate its performance. Before each experiment, the ventilator together with the flow sensor and tracheal cannula attached were calibrated for their combined internal airflow resistance, inertance and gas compressibility. Mice (C57BL/6) were anaesthetized with an intraperitoneal injection of Nembutal (80 mg/kg). Depth of anesthesia was confirmed by absence of response to toe pinch. Mice were then tracheostomized and attached to the ventilator.

In one set of experiments, and after a period of ventilation, the spontaneous breathing patterns of two anesthetized mice (both weighing 23 g) were recorded for up to 2 minutes. Tidal volume (V_T), respiratory rate (RR), and their product, minute ventilation (MV), was calculated for every breath from the recorded signals. In a second set of experiments, mice ($n=5$, 29.8 ± 2.0 g) were ventilated at 8 ml/kg using variable ventilation (VV) (Thammanomai *et al* 2008). VV maintains the same average tidal volume as conventional ventilation (CV), but has an inter-breath variability such that each breath delivers a different V_T with its RR adjusted so that MV remains the same for every breath. V_T was selected randomly from a power-law distribution as described previously (Thammanomai *et al* 2008). After delivery of VV under baseline conditions the lungs were lavaged by injecting 0.1 ml of warm phosphate-buffered saline into the tracheal cannula. VV was then resumed with the same settings. In order to assess the ventilator's ability to deliver the prescribed tidal volume per weight (8 ml/kg), the mean value of $V_T(\overline{V_T})$ computed by the sensor during VV, both before and after lavage, was compared to the prescribed V_T (V_P).

Data Analysis

All data analyses were performed using MATLAB. A paired t-test was used to assess the effect of lavage treatment on $\overline{V_T}$. Individual V_T measurements before and after lavage were compared using the rank-sum test. Statistical significance was accepted at $p < 0.05$.

Results

Dynamic Response

The pressure-flow characteristics of the flow sensor were well characterized both parametrically ($C_V=0.9591$) and nonparametrically ($C_V=0.975$, 0.977 , and 0.994 for the 1st, 2nd, and 3rd order respectively and 0.9147 for the polynomial fit) as illustrated in Figure 2. For the parametric fit, the values of R and I were $0.5758 \text{ cmH}_2\text{O.s.ml}^{-1}$ and $0.0015 \text{ cmH}_2\text{O.s}^2.\text{ml}^{-1}$, respectively. For, the 3rd order nonparametric polynomial fit the values of c_1 , c_2 and c_3 were $1.7920 \text{ ml.s.cmH}_2\text{O}^{-1}$, $0.00224 \text{ ml.s.cmH}_2\text{O}^{-2}$, and $-0.0193 \text{ ml.s.cmH}_2\text{O}^{-3}$ respectively. For the 3rd order Volterra fit, h_0 was equal to 0.4914 ml.s^{-1} while h_1 was given by the vector of values $(0.654, 1.390, -0.598, 1.601, 1.273, 0.2832) \text{ ml.s}^{-1}\text{cmH}_2\text{O}^{-1}$ where the 1st component in this vector is $h_1(1)$. The fact that $h_1(1)$ is smaller in magnitude than several other components signifies that flow depends much more on some previous P values than on the current one. The h_2 values are shown in Figure 3(a), and a selected slice for $h_3(i_1, i_2, 6)$, where i_1 and i_2 vary from 1 to 6, are shown in Figure 3(b). The contour plots in Figure 3 demonstrate two main features. First, there are high absolute kernel values along the main diagonal that reflect a nonlinear dependence of flow on the current and previous pressure values individually (i.e., without cross-interactions). Second, there are also high absolute kernel values off the main diagonal with values comparable to those on the diagonal, showing that flow has a nonlinear dependence on cross-interactions between both current and past values of pressure. The values of these kernels are presented as supplementary data in Table D.

When cross-validation was applied using the 1st, 2nd, and 3rd order Volterra series, recall that the C_V on the first half of the data were equal to 0.9771 , 0.9786 , and 0.9953 , respectively. The same respective C_V values during the prediction on the second half of the data were 0.9717 , 0.9723 , and 0.9886 . As expected the latter values are slightly smaller than the former; however, these C_V values are still close to 1. More importantly, they exceed the C_V of the other fitting techniques following cross-validation: for the parametric fit, the fitting C_V was 0.9597 while the validation C_V decreased to 0.9586 whereas for the polynomial the C_V slightly decreased from 0.9147 to 0.9145 .

Figure 4 demonstrates that the CMRR of the differential pressure transducer combined with the flow sensor was above 40 dB at and below the mouse's breathing frequency (1–5Hz) (Zehendner *et al* 2013). Furthermore, the results for 2ml/kg and 4ml/kg are virtually identical throughout the frequency range indicating that CMRR is independent of the delivered tidal volume.

Mouse Experiments

Figure 5 shows examples of volume recorded from two spontaneously breathing anaesthetized mice following a period of ventilation. The total number of cycles were divided into groups consisting of 13 consecutive breaths and for each group the average values for V_T , RR, and MV are plotted as function of time in Figure 6. Both mice showed increasing V_T over time; however, the RR in mouse 2 was fairly steady whereas RR in mouse 1 displayed a sudden decreasing trend after 1 min. This resulted in a plateau in MV in mouse 2 whereas RR continuously increased in mouse 1.

In the mechanically ventilated mouse experiments, the natural breathing pattern of the mouse was overridden by the ventilator. Figure 7 shows an example of the measured VV pattern in a mouse highlighting breath-to-breath variability of both V_T and RR. Table 1 shows the statistics on $\overline{V_T}$ primarily indicating its deviation from V_P and the ventilator's inability to deliver the prescribed volume in both healthy and injured lungs. Thus, generally, the delivered V_T was considerably lower than the prescribed in all mice both before and after lavage. Furthermore, there was an additional discrepancy in comparing $\overline{V_T}$ before and after lavage. Four out of the five mice showed a decreased V_T after lavage compared to before lavage and this was statistically significant in three mice.

At the end of the experiments, the mice responded to the toe pinch test and spontaneous breathing was observed due to chest wall movement suggesting that the sensor was tolerated and the data collected is viable for analysis.

Discussion

The aim of this study was to design, model, and test a simple 3-D printed flow sensor. The design is easily adjustable, scalable, and rapidly printable through the use of 3-D printing technology allowing researchers to optimize the design based on their needs, available tools, and the specifications of their equipment (i.e., transducer sensitivity). More importantly, the Volterra approach provides an accurate and general characterization of the behavior of the sensor, allowing tracheal airflow to be measured in living mice.

Dynamic Response

The sensor displayed dynamic nonlinear behavior (Figure 3) which explains why the selected nonparametric Volterra series outperformed the parametric and the polynomial models as demonstrated by the higher C_V values. Despite the fact that the C_V values are close to each other, the inset in Figure 2 reveals that the 3rd-order Volterra series fit best recapitulates the actual ventilator data and thus provides the most accurate flow measurements. The improved accuracy justifies the addition of extra parameters in the model. Furthermore, the application of cross-validation also yielded higher C_V values for the Volterra series demonstrating that the added accuracy is attributed to the enhanced description of system dynamics.

The nonparametric fitting with memory is a significantly more complicated procedure; however once implemented, it can improve the quality of the measurements through two

mechanisms. We can use the kernels to try and improve the fit by using longer memory for the same order or using a higher order of the same memory. These decisions can be based on the kernel values. For instance, $h_2(5,5)$ is equal to -11.95 but it drops down an order of magnitude to -1.88 at $h_2(6,6)$ which represents the highest 2nd order lag. On the other hand, the value of $h_3(4,4,6)$ is two orders of magnitude higher than that of $h_3(6,6,6)$. This suggests that increasing memory may not increase in accuracy significantly. Alternatively, the relatively large absolute 3rd order kernel values at low lags suggest that increasing the order has a larger effect than memory. Another advantage of the nonparametric fitting is that it requires no assumption for an a priori system structure. The kernels can account for many factors without having to consider the many possible model parameter structures. In short, for nonphysiological system identification, there is no need to interpret the parameters and/or assume any structure and thus nonparametric identification is a feasible option to obtain a more accurate system characterization than using parametric fitting.

Concerning the flow sensor's calibration and since the volume delivered is load dependent, our data suggest that the volume delivered to the flow sensor is not the same as the prescribed one. This, however, should not affect the calibration of the flow sensor since it was done in an open system and any load dependence mostly influenced by the compliant loads rather than resistive ones. More importantly though, the actual fitting used the calculated volume rather than the theoretical one and thus load dependence of the ventilator should be accounted for.

The general trend regarding the delivered V_T is in agreement with Thammanomai et al. (Thammanomai *et al* 2007) who showed that the ventilator's delivered volume is less than the prescribed one due to load dependence. This indicates that the actual delivered V_T depends on the animal's response to a given treatment based on a change in its mechanical impedance. However, there was only a slight drop following lavage, the largest being -4.43% in mouse 4. To unravel this discrepancy, we calculated the respiratory system resistance (Rrs) and elastance (Ers) values from which we obtained the magnitude of the complex impedance ($|Zrs|$) at the breathing frequency ($\sim 4\text{Hz}$). Before lavage, the mean and standard deviation were Rrs: $2.45 \pm 0.83 \text{ cmH}_2\text{O.s.ml}^{-1}$, Ers: $40.50 \pm 6.57 \text{ cmH}_2\text{O/mL}$, and $|Zrs|$: $2.92 \pm 0.83 \text{ cmH}_2\text{O.s.ml}^{-1}$. Following lavage, these values were Rrs: $2.36 \pm 0.49 \text{ cmH}_2\text{O.s.ml}^{-1}$, Ers: $52.44 \pm 1.42 \text{ cmH}_2\text{O/mL}$, and $|Zrs|$: $3.08 \pm 0.38 \text{ cmH}_2\text{O.s.ml}^{-1}$. Thus, even though Ers increased by 29.5% due to lavage, $|Zrs|$ increased by only 5.3% since it is more dependent on the changes in Rrs (-3.8%) at around 4Hz . Furthermore, to briefly reiterate the findings by Thammanomai et al. (Thammanomai *et al* 2007), we used the same composite signal perturbation to calculate CMRR with different loads (no load, 9, 18, and 27 ml syringe). With increasing load, there was an evident dependence of reduced volume delivery. (Fig. A, Supplementary material)

CMRR arises from asymmetries in the flow sensor and/or the differential pressure transducer, amplifier, and the magnitude of flow sensor resistance (Farré *et al* 1989, Peslin *et al* 1984). CMRR is highest when the resistance of the flow sensor is comparable to or higher than the impedance of the subject and when asymmetry is most pronounced. In our application, due to 1) the low resolution of 3-D printing (0.1 mm) compared to the tubing dimensions connecting the sensor outlets with the transducer ports, 2) the comparable values

between the flow sensor and mouse resistance, and 3) the large pressure range of the pressure transducer (requiring an amplifier and making the system more susceptible to noise), the CMRR is likely more dependent on the transducer (asymmetrical structure and range), tubing and the amplifier characteristics. Thus, in general, any improvements to CMRR would require selecting an amplifier, transducers with proper range and high CMRR and minimizing the length and asymmetry of the flow sensor outlets and the associated tubing.

The current CMRR of our sensor is within acceptable limits. Peslin et al. (Peslin *et al* 1984) addressed the issue of minimizing human impedance measurement errors obtained by the forced oscillatory technique (DuBois *et al* 1956) waveform with a maximum frequency range of 30 Hz. For impedance measurements, the best available transducer had a CMRR of 70 dB at a frequency of 30 Hz, and they recommended a minimum CMRR of 40 dB at 30 Hz or the highest frequency of interest (Peslin *et al* 1984). The calculated CMRR values from our setup were less by around 10 dB than the minimum threshold. However, the flexiVent system is generally used to probe impedance only up to 20 Hz at which our CMRR is 35 dB and for the purposes of tracking flow during ventilation at the ventilation frequencies between 1 and 5 Hz (Zehendner *et al* 2013), the CMRR is above 40 dB (Fig. 4). If further accuracy is required particularly for impedance measurements, Farré et al. (Farré *et al* 1989) proposed a general technique to correct for transducer asymmetry for linearly behaving transducers which resulted in a maximum error in measured respiratory mechanics (resistance, inertance, and compliance) of 4%. Even without the correction, for a CMRR of 40 dB and a respiratory to pneumotachograph ratio of 5 (close to our design), the percent error in measured impedance is less than 5% (Farré *et al* 1989). However, more interestingly, it is actually possible to use the Volterra series to account for the transducer's asymmetry and thus correct for flow regardless of the CMRR. This technique is also independent of any linearity assumption. To exemplify, we corrected the transducer's measured pressure by fitting/matching it to P_{through} using Volterra series. Then the corrected pressure was used to measure a corrected flow. With a Volterra series of order 1 and memory 6, the corrected flow has a C_v of 0.9335 in comparison to the unadjusted flow ($C_v=0.685$). (Fig. B, Supplementary material). Thus, the Volterra series can account for all system nonlinearities regardless of transducer characteristics. However, this calibration should be carried out every time the setup is changed (i.e., transducer, sensor, or tubing).

One limitation of the current sensor design which affects its practicality is the resolution of the 3-D printer. Since in our case the resolution was 0.1 mm and the inner core diameter was 0.5 mm, it was necessary to calibrate every sensor individually. The best currently available 3-D printers have resolutions as low as 0.02 mm which would give a 15% error in the theoretical value of the sensor's resistance.

Another limitation related to the ventilation procedures in our experiments. Since a ventilator was used in the experiments, any effect of the sensor on the mouse's breathing pattern was overridden. Thus, the physiological effects of the flow sensor on breathing need to be examined without using the ventilator. As a preliminary test, expired carbon dioxide levels were recorded for around a minute in a mouse following tracheostomy and cannulation. A T-tube was secured to the cannula with the side port attached to a capnometer

(MicroCapStar, CWE Inc, Pennsylvania) and measurements of CO_2 was carried out with and without the flow sensor connected to the other end the T-tube. In the absence of the flow meter $\%\text{CO}_2$ varied between 3.1 and 3.8% whereas in the presence of the flow meter, $\%\text{CO}_2$ was between 2.7 and 3.3% (Fig. C, Supplementary material). These values are somewhat under the normal range of 4.0–5.5% (Thal and Plesnila 2007) possibly due to the anesthesia and/or the resistance of the endotracheal tube, which we estimated to be $0.4 \text{ cmH}_2\text{O.s.ml}^{-1}$, a value surprisingly close to R_{rs} . Thus, while our $\%\text{CO}_2$ values can be considered acceptable, further tests may be warranted if the flow sensor is to be used without ventilator support.

Mouse Experiments

The flow sensor was used to properly predict flows continuously, whether for VV or spontaneous breathing in the mouse experiments, for the total experimental time, and across the range of collected physiological mouse flow values.

However, more importantly, this ability to monitor continuously, revealed a considerable discrepancy between the V_P and $\overline{V}_T$ which has important implications for ventilation studies.

It reveals that in general experimental settings, $\overline{V}_T$ could be significantly different than V_P and the control and treatment groups may in fact receive different V_T 's. The former could be expected since ventilator never delivers the prescribed volume exactly due to gas compression in the cylinder. Corrections for gas compression can be made, in principle, but the flow sensor is able to measure the delivered volume directly, which has the advantage of avoiding errors and nonlinearities in the ventilator's calibration. This is further justified since most ventilators assume linear behavior when they calibrate for system properties (gas compliance, resistance and inertance); however, these could have some degree of nonlinearity especially when the flow sensor itself and the cannula are nonlinear.

More problematic though is the difference in $\overline{V}_T$ between groups. This can adversely alter the experimental outcome either by reaching a wrong conclusion or missing a treatment's beneficiary effect. For example, unnoticed reduction in V_T due to the load dependence of the ventilator was shown to severely compromise lung function leading poor survival of mice with acute lung injury (Thammanomai *et al* 2007). Our flow sensor can provide assistance to avoid such pitfalls by reducing both inter- and intra-group variabilities. First, it can be used post experimentally to verify that groups received the same or the desired V_T further strengthening any observation. Second, it can help researchers characterize a flow-load characteristic curve for their ventilators (along with any external connections) to correct for ventilator-load dependence. Third, it can also be a helpful tool when used as an online feedback mechanism to adjust the ventilator settings such that the delivered and prescribed settings match as the experiment is being conducted.

An additional benefit is the ability to monitor expiratory flow rates and determine if the subject is fully exhaling. If that is not the case, auto positive end-expiratory pressure (PEEP) might buildup altering the delivered flow and final outcome. This is critical in ventilator-induced lung injury models where unknowingly adding PEEP misleads to interpretations of the injury mechanism.

Conclusions

We have shown that nonparametric nonlinear system identification with memory (Volterra series) can be effectively utilized in a rapidly fabricated 3-D printed pneumotachograph-type flow sensor that provides accurate measurements of air flow in small experimental animals allowing researchers to monitor flow continuously, correct for any nonlinear system dynamics, and provide better experimental control. The current device is designed specifically for and well tolerated by ventilated mice, but the design can be readily adjusted to accommodate other rodents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bates JHT, Irvin CG. Measuring lung function in mice: the phenotyping uncertainty principle. *J. Appl. Physiol.* Bethesda Md 1985. 2003; 94:1297–306.
2. DuBois AB, Brody AW, Lewis DH, Burgess BF. Oscillation Mechanics of Lungs and Chest in Man. *J. Appl. Physiol.* 1956; 8:587–94. [PubMed: 13331841]
3. Farré R, Navajas D, Peslin R, Rotger M, Duvivier C. A correction procedure for the asymmetry of differential pressure transducers in respiratory impedance measurements. *IEEE Trans. Biomed. Eng.* 1989; 36:1137–40. [PubMed: 2807323]
4. Giannella-Neto A, Bellido C, Barbosa RB, Melo MFV. Design and calibration of unicapillary pneumotachographs. *J. Appl. Physiol.* 1998; 84:335–43. [PubMed: 9451654]
5. Irvin CG, Bates JHT. Measuring the lung function in the mouse: the challenge of size. *Respir. Res.* 2003; 4:4. [PubMed: 12783622]
6. Korenberg MJ, Hunter IW. The identification of nonlinear biological systems: Volterra kernel approaches. *Ann. Biomed. Eng.* 1996; 24:250–68.
7. Korenberg MJ, Hunter IW. The identification of nonlinear biological systems: Wiener kernel approaches. *Ann. Biomed. Eng.* 1990; 18:629–54. [PubMed: 2281885]
8. McGovern TK, Robichaud A, Fereydoonzad L, Schuessler TF, Martin JG. Evaluation of Respiratory System Mechanics in Mice using the Forced Oscillation Technique. *JoVE J. Vis. Exp.* 2013:e50172–e50172. [PubMed: 23711876]
9. Mitzner W, Brown R, Lee W. In vivo measurement of lung volumes in mice. *Physiol. Genomics.* 2001; 4:215–21. [PubMed: 11161000]
10. Peslin R, Jardin P, Duvivier C, Begin P. In-phase rejection requirements for measuring respiratory input impedance. *J. Appl. Physiol.* 1984; 56:804–9. [PubMed: 6706784]
11. Rice J. A Theory of Condition. *SIAM J. Numer. Anal.* 1966; 3:287–310.
12. Schuessler TF, Bates JH. A computer-controlled research ventilator for small animals: design and evaluation. *IEEE Trans. Biomed. Eng.* 1995; 42:860–6. [PubMed: 7558060]
13. SchuesslerTF, , BatesJHT, , MaksymGN. Estimating tracheal flow in small animals. Proceedings of the 15th Annual International Conference of the IEEE Engineering in Medicine and Biology Societ; Proceedings of the 15th Annual International Conference of the IEEE Engineering in Medicine and Biology Societ; 19935601

14. Suki B, Lutchen KR. Pseudorandom signals to estimate apparent transfer and coherence functions of nonlinear systems: applications to respiratory mechanics. *IEEE Trans. Biomed. Eng.* 1992; 39:1142–51. [PubMed: 1487277]
15. Tang Y, Turner MJ, Yem JS, Baker AB. Calibration of pneumotachographs using a calibrated syringe. *J. Appl. Physiol.* 2003; 95:571–6. [PubMed: 12704091]
16. Thal SC, Plesnila N. Non-invasive intraoperative monitoring of blood pressure and arterial pCO₂ during surgical anesthesia in mice. *J. Neurosci. Methods.* 2007; 159:261–7. [PubMed: 16945419]
17. Thammanomai A, Hueser LE, Majumdar A, Bartolák-Suki E, Suki B. Design of a new variable-ventilation method optimized for lung recruitment in mice. *J. Appl. Physiol.* 2008; 104:1329–40. [PubMed: 18339891]
18. Thammanomai A, Majumdar A, Bartolák-Suki E, Suki B. Effects of reduced tidal volume ventilation on pulmonary function in mice before and after acute lung injury. *J. Appl. Physiol. Bethesda Md* 1985. 2007; 103:1551–9.
19. Vanoirbeek J AJ, Rinaldi M, De Vooght V, Haenen S, Bobic S, Gayan-Ramirez G, Hoet PHM, Verbeken E, Decramer M, Nemery B, Janssens W. Noninvasive and invasive pulmonary function in mouse models of obstructive and restrictive respiratory diseases. *Am. J. Respir. Cell Mol. Biol.* 2010; 42:96–104. [PubMed: 19346316]
20. Zehendner CM, Luhmann HJ, Yang J-W. A Simple and Novel Method to Monitor Breathing and Heart Rate in Awake and Urethane-Anesthetized Newborn Rodents. *PLOS ONE.* 2013; 8:e62628. [PubMed: 23658756]

Fig. 1.

Sensor design. The sensor's front view, side view and axial cross-section view. The sensor can be connected to a ventilator on one side (1) and a mouse tracheal cannula on the other (2). The two adjacent side ports are connected to a differential pressure transducer for flow measurements, and the third side port is connected to a pressure transducer for absolute pressure measurements. The inner core (3) has a diameter of 0.5 mm spanning a length of 5.8 mm. Dimensions are in mm.

Fig. 2.

Ventilator (circles) and fitted flow (lines). The parametric fit (dashed light gray), polynomial fit (dashed dark gray) and the nonparametric fits (medium gray, dark gray and black solid lines) captured the prescribed flow well. The fits of the Volterra nonparametric model were slightly better with higher adjusted C_v values (>0.97) compared to 0.9591 for the parametric model and 0.9147 for the polynomial model. The parametric and polynomial model fits tended to overshoot or undershoot the actual data at the extremities, and observing the insets, the 3rd order Volterra series (black) was the most accurate in following the actual data. One second of the total 16 second signal is shown.

Fig. 3.

Contour plots for 2nd order kernels (h_2) (Panel A) and a selected slice of the 3rd order kernels ($h_3(i_1, i_2, 6)$) (Panel B). The Volterra kernels are symmetric ($h_2(i_1, i_2) = h_2(i_2, i_1)$ and $h_3(i_1, i_2, k = 1:6) = h_3(i_2, i_1, k = 1:6)$ as can be seen using identity line (black dashed line). The contour plots show the extent of nonlinear behavior within the system. For instance, the current flow value highly depends on the square of the 3rd and 4th previous P values since the contour shows high kernel values at that location $h_2(4,4)$ and $h_2(5,5)$ respectively. The absolute values of $h_2(5,4)$ and $h_2(4,5)$ are also high and the current flow value has high nonlinear dependence on the kernels and thus the interaction of the 3rd and the 4th previous P values. Similarly, $h_3(3,3,6)$ and $h_3(4,4,6)$ show a cubic flow dependence on the 2nd and 3rd previous P values, respectively, while $h_3(3,4,6)$ and $h_3(4,3,6)$ show a nonlinear dependence of flow on the interaction of the 2nd and 3rd previous P values with the previous 5th P value (since $k=6$). Kernel values are presented as supplementary data in Table D.

Fig. 4. CMRR as a function of frequency at 2 (black) and 4 (gray) ml/kg tidal volume using a composite volume perturbation containing 5 frequencies between 2 and 31 Hz. The curves represent the CMRR of the differential transducer connected to the sensor and the amplifier.

Fig. 5.
Measured tidal excursions in two spontaneously breathing mice. The mice were left to breathe by themselves without any mechanical intervention as the flow was being recorded. The volume was obtained by integration of the estimated flow.

Fig 6.

Breath by breath analysis of spontaneous breathing for two anaesthetized and tracheostomized mice. Every time point represents the mean and standard error of 13 consecutive breaths for A) tidal volume, B) respiratory rate, and C) minute ventilation.

Fig 7.

Measured lung volume changes during mechanical ventilation. The flow sensor was able to continuously record the flow waveform data. Shown is a 2 second demonstration of a measured variable ventilation pattern in which the tidal volume delivered changes with every breath. The tidal volume as well as the breath duration of two cycles are shown (gray). A larger tidal volume (0.14 ml) is delivered during a longer time (0.234 s) compared to a smaller tidal breath (0.11 ml) delivered in a shorter period (0.186 s). However, the minute ventilation (Tidal Volume/Breath Duration) for the two breaths is the same (0.59 ml.s^{-1}). The volume was obtained by integration of the estimated flow.

Table 1Statistical Comparison on V_T before and after Lavage

Mouse	Weight (gm)	V_P (ml)	V_T Before Lavage					V_T After Lavage					p-value (rank-sum)
			$\overline{V_T}$	$\overline{V_T}/V_P$ (%)	Median	25%	75%	$\overline{V_T}$	$\overline{V_T}/V_P$ (%)	Median	25%	75%	
1	33.6	0.27	0.12	44	0.109	0.094	0.130	0.11	42	0.107	0.090	0.127	<0.001
2	27.8	0.22	0.16	71	0.149	0.130	0.172	0.16	70	0.147	0.127	0.172	0.1094
3	28.9	0.23	0.14	60	0.127	0.112	0.149	0.14	61	0.132	0.114	0.155	0.0017
4	29.7	0.24	0.15	65	0.144	0.128	0.167	0.15	62	0.138	0.119	0.164	<0.001
5	28.7	0.23	0.15	67	0.143	0.124	0.165	0.15	65	0.140	0.120	0.166	0.0141