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Semi-automated assessment of transdiaphragmatic pressure variability across motor behaviors

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ABSTRACT

We developed and tested a semi-automated algorithm to generate large data sets of ventilatory information (amplitude, premotor drive and timing) across a range of motor behaviors. Adult spontaneously breathing, anesthetized mice ($n=27$) underwent measurements of transdiaphragmatic pressure (Pdi) during eupnea, hypoxia-hypercapnia, and tracheal occlusion with values ranging from 8 ± 1 to 9 ± 2 to 44 ± 3 cmH_2O, respectively. Premotor drive to phrenic motor neurons (estimated by the rate of rise during initial 60 ms) was ~5-fold greater during tracheal occlusion compared to other behaviors. Variability in Pdi amplitude (normalized to spontaneously occurring sighs for each animal) displayed minimal evidence of complex temporal structure or dynamic clustering across the entire period of examination. Using a deterministic model to evaluate predictor variables for Pdi amplitude between successive inspiratory events, there was a large correlation for premotor drive and preceding Pdi amplitude vs. Pdi amplitude ($r=0.52$). These findings highlight substantial variability in Pdi amplitude that primarily reflects linear components rather than complex, dynamic effects over time.

1. Introduction

Breathing is a life sustaining behavior accomplished by activation of ventilatory muscles such as the diaphragm (DIAm) in mammals. In fact, the DIAm is the primary muscle responsible for generating forces necessary for inspiration and contributes in coordinated fashion with other muscle groups to accomplish higher force non-ventilatory behaviors such as coughing or sneezing (Mantilla et al., 2010; Mantilla and Sieck, 2011; Sieck, 1989, 1991, 1994). Clinically, transdiaphragmatic pressure (Pdi) measurements are commonly used in evaluating a patient's ability to sustain ventilation for diagnostic purposes, cf. the American Thoracic Society and the European Respiratory Society Statement on Respiratory Muscle Testing (2002). Across a range of species, Pdi approximates DIAm force, having been validated in sheep (Bazzy and Haddad, 1984), dogs (Hubmayr et al., 1990), cats (Sieck and Fournier, 1989), piglets (Watchko et al., 1986), hamsters (Sieck, 1991, 1994), rats (Gill et al., 2015; Mantilla et al., 2010; Mantilla and Sieck, 2011) and mice (Greising et al., 2013b). Thus, data acquired from Pdi measurements is used to yield clinically and experimentally important information regarding the ability to generate DIAm force. Analyses of Pdi amplitude across motor behaviors may provide important information regarding premotor drive to respiratory muscles such as the DIAm.

Rhythmic activation of respiratory muscles, such as the DIAm, reflects timing characteristics that are likely imposed by central pattern generators (Feldman et al., 2013). The amplitude of a single breath, or inspiratory event, in turn relates to the specific premotor drive, and may be determined at the level of the motor unit pool for the different ventilatory muscles (Holstege, 2014; Mantilla et al., 2014). Analyses of variability in Pdi amplitude (i.e., variance and temporal structure of differences between successive inspiratory events) can provide useful insight into the neuromotor control of respiratory muscles, and may also inform conditions of injury, disease or aging. Recent studies using data acquired via DIAm electromyography (EMG) provide important insight into the timing of motor unit recruitment and the level of premotor drive associated with DIAm activation across a range of ventilatory and higher force non-ventilatory behaviors (Gill et al., 2015; Mantilla et al., 2014; Seven et al., 2014, 2013). By developing a semi-automated method to analyze Pdi it is possible to obtain and evaluate large data sets containing information about the amplitude and premotor drive of successive inspiratory events across motor behaviors. We hypothesize that the premotor drive to phrenic motor neurons is not related to the timing characteristics of each inspiratory event, indicating an

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independence of Pdi amplitude and timing. In the present study, deterministic models of variance in Pdi amplitude were generated to evaluate the relationship between the premotor drive, timing and Pdi amplitude characteristics for successive inspiratory events.

2. Methods

2.1. Animals

Adult mice (6–24 months of age, 36.3 ± 1.3 g body weight) of two different commonly used strains (C57BL/6J, $n = 7$; C57 × 129, $n = 20$) were studied. All experimental procedures were approved by the Institutional Animal Care and Use committee at the Mayo Clinic, in accordance with the National Institutes of Health Guidelines. Mice were anesthetized by an intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) prior to determining Pdi.

2.2. Pdi measurements

As previously described, Pdi measurements were conducted in spontaneously breathing, anesthetized animals (Gill et al., 2015; Greising et al., 2013b; Mantilla et al., 2010; Mantilla and Sieck, 2011; Sieck, 1991, 1994; Sieck and Fournier, 1989). The abdomen was tightly constrained and the trachea was instrumented using a metal 19G cannula (Fishman #2512119). Following calibration as recommended by the manufacturer, a pair of Millar Mikro-Tip pressure transducer catheters (3.5 F, SPR-524; Millar Instrumentation, Houston TX) were inserted into the mid-esophagus and stomach. Data were digitized (2000 Hz) using a PowerLab 8/35 data acquisition system.

Measurements of Pdi were conducted during eupnea and following exposure to hypoxia (10% O_2) and hypercapnia (5% CO_2); each for 5 min. In addition, Pdi measurements were obtained during a 15 s sustained tracheal occlusion. Measurements were also analyzed separately for any naturally occurring deep breaths ("sighs"), as previously described (Gill et al., 2015; Greising et al., 2013b; Mantilla et al., 2010). Adequate recovery time was given between motor behaviors (usually ~5 min).

2.3. Analytical procedures

For the purposes of this study, each instance of DIAm activation measured as a peak in Pdi was considered an inspiratory event regardless of the motor behavior. Consistent with previous studies (Gill et al., 2015; Greising et al., 2013b; Mantilla et al., 2010), Pdi amplitude for a single inspiratory event was calculated manually as the difference between the maximum pressure and an observer-decided baseline in LabChart (Millar Instrumentation). Such measurements were conducted in inspiratory events from 4 mice (usually ~40 inspiratory events during eupnea and hypoxia-hypercapnia and the maximum 5 inspiratory events during tracheal occlusion). Respiratory frequency (min^{-1}) was calculated by counting the number of inspiratory events over a 15 s period during eupnea and hypoxia-hypercapnia.

2.3.1. Semi-automated Pdi measurements

In order to generate a large data set of Pdi measurements, a semi-automated technique was developed involving 3 steps: *(1) Band-pass filtering:* (0.3–30 Hz; in LabChart) to center the power of the signal around zero, remove any offset and high frequency noise components related to cardiac activity. The segment to be analyzed was selected and exported into a data file for further data analysis; *(2) Threshold selection:* Using MATLAB (The MathWorks Inc., Natick, MA, 2012) a threshold σ was arbitrarily set at 30% of the median value of all positive peaks (identified by the findpeaks function in MATLAB) in the segment of the Pdi recording being measured (usually ~3 min). In this fashion *baseline cycles* were below σ differentiating individual *peak cycles* above the threshold σ (Fig. 1). A single peak in each *peak cycle* was identified as the maximum (if in rare cases more than one peak was present); *(3) Baseline definition:* The baseline used in determining the amplitude of a peak was selected automatically by the average of all inflection points within the *baseline cycle* (i.e., upward and downward deflections were identified by a two-step application of the findpeaks function for both the original and inverted *baseline cycle* segment). The Pdi amplitude for a single inspiratory event (n) was calculated as the difference between the maximum peak and the preceding interpeak baseline. Average respiratory frequency was obtained from the instantaneous respiratory frequencies measured from the inverse of the peak-to-peak period. The executable analytical program is available upon request.

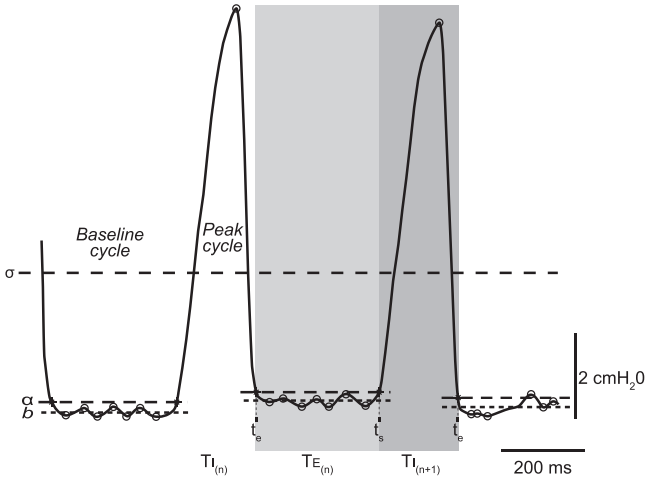

Fig. 1. Representative tracing of transdiaphragmatic pressure (Pdi) measurements in an adult spontaneously breathing mouse, highlighting the automated algorithm analysis of individual inspiratory events. The threshold σ differentiates *baseline* and *peak cycles* based on a running time average of the signal. Within the *baseline cycle*, the mean value of all inflexion points was used to determine the baseline value (b; dashed horizontal lines). Peak Pdi amplitude was calculated as the difference between the maximum point during the peak cycle and the baseline during the preceding baseline cycle. A threshold α was determined by adding 3% of the successive peak amplitude to the baseline within the preceding baseline cycle. This threshold α was used to determine the end-time (t_e) for the preceding peak and the start-time (t_s) for the successive peak. These time points were used to determine the inspiratory time (T_I; shaded dark gray region) and expiratory time (T_E; shaded light gray region).

Statistical analyses comparing manual and semi-automated methods of Pdi analyses were conducted with JMP (JMP version 10.0; SAS Institute Inc.) using two main outcomes. *Breath by breath comparisons* were conducted for the same inspiratory events (20 during eupnea and 20 during hypoxia-hypercapnia for each spontaneously breathing mouse) in a total of 4 mice. *Intra-animal comparisons* (using representative samples for each animal but without ensuring identical sampling for the two methods) were conducted in a total of 18 mice. Data for comparisons of both Pdi amplitude and respiratory frequency were examined by linear regression and Bland–Altman analysis (Bland and Altman, 1986) with a 95% confidence interval, as appropriate. Coefficient of variation (CV) for each parameter analyzed was determined for each animal and data are summarized across animals.

2.3.2. Analyses of Pdi amplitude

Seven mice (male, 12 months of age; 39.4 ± 2.8 g body weight) were used for semi-automated analysis of premotor drive, timing and Pdi amplitude characteristics for successive inspiratory events across various motor behaviors ranging from eupnea to higher force