Influence of Chemoreflexes on Respiratory Variability in Healthy Subjects

Joost G. Van den Aardweg and John M. Karemaker

Department of Physiology, Academic Medical Center, Amsterdam; and Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands

The background of this study was the hypothesis that respiratory variability is influenced by chemoreflex regulation. In search for periodicities in the variability due to instability of the respiratory control system, spectral analysis was applied to breath-to-breath variables in 19 healthy subjects at rest. During room-air breathing, coherent oscillations in end-tidal CO_2 ($P_{ET_{CO_2}}$) and mean inspiratory flow (V_I/T_I) were found in 15 subjects with frequencies mostly below 0.15 cycles per breath. Coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I were expressed by gain (0.13 to 0.34 L/second · kPa) and phase (−170° to +8°). The oscillations in V_I/T_I were in phase with inspiratory volume (V_I). A model that describes the effects of chemoreflex feedback to noise in the system could explain these gains and phases, whereas a model without chemoreflex could not. During 100% O_2 breathing, only eight subjects had coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I. The coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I were interpreted as a manifestation of chemoreflex activity. We conclude that respiratory variability is not a random process but contains information on chemoreflex properties, such as the chemoreflex gain. The analysis of respiratory variability therefore provides a new tool to study the action of the chemoreflexes without application of external stimuli.

Keywords: chemoreceptors; respiratory variability; spectral analysis

Is the normal variability of respiratory parameters from breath-to-breath a random process? This would imply that, for example, inspiratory volume (V_I) or inspiratory and expiratory time (T_I and T_E, respectively) are independent of previous breaths. However, due to the circulatory delay from the lungs to the systemic arteries, an "accidental" change in V_I causes a change in arterial P_{O_2} and P_{CO_2} that only becomes manifest during the following breaths at a normal breathing frequency (1, 2). An adaptation of V_I to such a change therefore inevitably leads to a dependency between successive breaths. Conversely, purely random variability of V_I implies that V_I does not take part in the feedback control of Pa_{O_2} and Pa_{CO_2} through the chemoreflexes.

Several authors have found evidence for a nonrandom breath-to-breath variability of respiratory parameters in the normal steady state (3–5). Significant (auto)correlations have been found between successive values of V_I, T_I, and T_E (4, 6). Specific variability patterns have also been found, mainly in the form of subtle oscillations with a cycle time of approximately 25 seconds to more than 3 minutes (6–10). Clear periodic breathing is seldom observed in healthy subjects during wakefulness (3, 7, 11, 12), but often occurs during sleep or at high altitude (13, 14). The cause of frank periodic breathing like Cheyne-Stokes breathing in cardiac failure is probably an instability of the chemoreflex-feedback control system (15–17). It has been supposed that spontaneous changes in breathing pattern can induce (dampened) oscillations due to chemoreflex feedback in healthy subjects as well (8, 18, 19).

The aim of the present study was to derive information on respiratory regulation from the normal breathing pattern in the steady state. The hypothesis was that because of the delays and time constants of the chemoreflexes, continuous regulation tends to induce oscillations in ventilatory drive (represented by mean inspiratory flow, V_I/T_I) with a certain coherency with oscillations in end-tidal P_{CO_2} ($P_{ET_{CO_2}}$). To identify such oscillatory components and their mutual relationships, power and cross-spectral analysis was applied to breath-to-breath respiratory variables in 19 healthy subjects at rest. To test the hypothesis that the features of coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I are compatible with the characteristics of chemoreflex-feedback regulation, experimental spectra were compared with theoretical spectra derived from a chemoreflex model. The breathing pattern was also analyzed during 100% O_2 breathing to estimate the contribution of the peripheral chemoreflex (20).

METHODS

Subjects and Measurements

Nineteen healthy nonsmoking medical students were studied with a history free of cardiopulmonary disease and normal physical examination (9 male and 10 female, aged 23 ± 3 years, body mass index 22.2 ± 3.1 kg/m^2, mean ± SD). The hospital ethical committee approved the protocol. Informed consent was obtained. The subjects knew which measurements were performed. To prevent "conscious" breathing, they were told that the study involved blood pressure regulation. They sat in a comfortable chair in a quiet room and breathed through a cushion-sealed face mask fitted with elastic bands around the head (dead space ~ 70 ml).

A Lilly type pneumotachograph (Siemens pressure transducer, Munich, Germany) was connected to the mask and hung with an elastic cord to the ceiling. A two-way nonrebreathing valve (S and W, Copenhagen, Denmark) was connected to the pneumotachograph. The inspiratory limb was connected to a stopcock (through a 1-m spirometer tube) which could be switched from room air to 100% O_2 from a 100 L bag. The stopcock was hidden behind a curtain so that the subject did not know which gas was inspired. The experiments began between 9:00 and 10:00 A.M. Recordings started after a 5-minute acclimatizion period. There were two episodes of 30 minutes with more than 5 minutes in between, performed in a random order, during which the subjects breathed either air or 100% O_2 (starting when end-tidal P_{O_2} exceeded 85 kPa). Also measured were P_{O_2} and P_{CO_2} in the facemask (partial pressures in dry air, Centronic 200 MGA mass spectrometer, Croydon, United Kingdom), arterial O_2 saturation (Sa_{O_2}, Ohmeda Biox ear pulse oximeter, Madison, WI), finger arterial pressure (Finapres BMI-TNO, Amsterdam, Netherlands) and a single-channel chest-lead ECG. All signals were recorded on a Bell and Howell T4 recorder (Durham, NC) with airflow, ECG and blood pressure on FM channels and the other signals on a direct record channel using a Kayser Threde K 1180 pulse code modulator (Munich, Germany). The frequency response was 0–625 Hz for FM channels and 0–105 Hz for pulse code modulated channels.

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Correspondence and requests for reprints should be addressed to J. G. van den Aardweg, M.D. Ph.D., Afdeling Longziekten 117, Medisch Centrum Alkmaar, P.O. Box 501, 1800 AM Alkmaar, The Netherlands. E-mail: j.g.vanden.aardweg@mca.nl

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After digitizing at 200 Hz, breath-to-breath variables were derived from the pneumotachogram. To reduce a disproportionate influence of isolated deep breaths, the values of breaths with V_I greater than 1.5 times the mean of adjoining breaths were linearly interpolated. $P_{ET_{CO_2}}$ and end-tidal P_{O_2} ($P_{ET_{O_2}}$) were derived as the maximal P_{CO_2} (and minimal P_{O_2}) during the last second of expiratory flow. Beat-to-beat mean blood pressure and R-R interval were derived from the finger pressure and ECG. Means and SDs of each variable were compared between air and O_2 breathing (paired *t* test) with a prior log-transformation of SDs of ventilatory variables (10). Group values are means ± SEM.

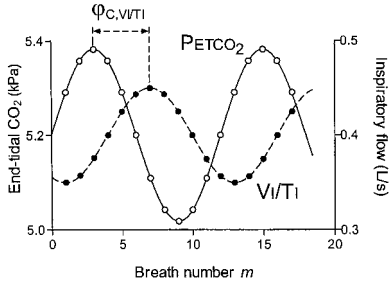

Figure 1. Example of oscillations in breath-to-breath end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) and mean inspiratory flow (V_I/T_I) with a cycle duration of 12 breaths. The phase from $P_{ET_{CO_2}}$ to V_I/T_I ($\varphi_{C,VI/TI}$) is −120°.

Spectral Analysis

Power spectra. In search of oscillatory components in respiratory variability, power spectra were derived for each variable. The underlying concept of spectral analysis is that each variability pattern can be written as the sum of a number of oscillations with a certain frequency, amplitude and phase (21). The power is proportional to the squared amplitude of such an oscillation. The reason to use power instead of amplitude is that the mean power, as it is defined here, equals the variance. Thus, the power spectrum shows the extent to which oscillation at each frequency contributes to the overall variance. For a variable that changes from breath to breath (with breath number M), the oscillation frequencies (f_m) are in cycles per breath. For example, when f_m is 0.25 cycles per breath, the oscillation has a cycle duration of four breaths. In a series of M breaths, only a limited number of frequencies can be discerned. The slowest oscillation has a frequency of 1/M cycles per breath. The other frequencies are all multiples of this basic frequency. The highest possible frequency is 0.5 cycles per breath.

Cross spectra. With spectral analysis it is also possible to "dissect" two variables into oscillatory components and to analyze the relationship between these oscillations. Figure 1 shows an example of two oscillations that occur in the breath-to-breath variability of $P_{ET_{CO_2}}$ and V_I/T_I. The cycle duration is 12 breaths (f_m is 0.083 cycles per breath). Both oscillations have a constant amplitude here, 0.36 kPa for $P_{ET_{CO_2}}$ and 0.10 L/second for V_I/T_I. The relationship between the oscillations is expressed by the gain and phase. The gain from $P_{ET_{CO_2}}$ to V_I/T_I ($G_{C,VI/TI}$) is the ratio of the amplitude of V_I/T_I to the amplitude of $P_{ET_{CO_2}}$. In the example, $G_{C,VI/TI} = 0.10/0.36 = 0.28$ L/second · kPa. The phase from $P_{ET_{CO_2}}$ to V_I/T_I ($\varphi_{C,VI/TI}$) is defined as negative when a maximum in $P_{ET_{CO_2}}$ occurs less than half a cycle before a maximum in V_I/T_I. In the example, a maximum in $P_{ET_{CO_2}}$ occurs four breaths before a maximum in V_I/T_I, so that $\varphi_{C,VI/TI} = -(4/12) \cdot 360° = -120°$. When the relationship between $P_{ET_{CO_2}}$ and V_I/T_I is linear, an increase in the amplitude of $P_{ET_{CO_2}}$ is accompanied by a proportional increase in the amplitude of V_I/T_I. A perfectly linear relationship between two oscillations is completely described by gain and phase. In reality, some "noise" always occurs so that an *exact* determination of gain and phase is never possible. The gain and phase can, however, be estimated with a certain level of confidence, depending on the number of degrees of freedom of the data and the degree of linearity of the relationship. For the relationship between $P_{ET_{CO_2}}$ and V_I/T_I, the degree of linearity is described by the squared coherency $k^2_{C,VI/TI}$ which ranges from zero (no linear relationship) to one (perfectly linear relationship). The (estimated) squared coherency can be interpreted as a squared correlation coefficient for variations with a specific frequency. Similarly, the estimated gain can be seen as a frequency-specific linear regression coefficient. The squared coherency, gain, and phase spectra describe these estimates for all frequencies that occur within the variability of the two variables. Together they are derived from the "cross spectrum" (21). Cross-spectral analysis thus amounts to a linear regression in the frequency domain.

Cross spectra were determined for $P_{ET_{CO_2}}$ and V_I/T_I, V_I/T_I and V_I, V_I and T_E, and for $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$. We were particularly interested in the relationship between $P_{ET_{CO_2}}$ and V_I/T_I as these variables are related to the input and output of the chemoreflexes (1, 22, 23). "Coherent oscillations" in $P_{ET_{CO_2}}$ and V_I/T_I were defined as oscillations with (*1*) a significant coherency between $P_{ET_{CO_2}}$ and V_I/T_I and (*2*) a power of both $P_{ET_{CO_2}}$ and V_I/T_I that is significantly higher than the mean power.

Details of spectral analysis. A trend was removed from the data to reduce the influence of very low frequencies. The data were multiplied by a Tukey window and transformed from the "breath number" (m) domain to the "frequency" (f_m) domain with the discrete Fourier transform (21). The spectra were smoothed by a triangular running window (width ~ 0.01 cycles per breath) to increase the number of degrees of freedom of each spectral estimate (24). The power spectral estimate was considered as significantly higher than the mean power when the lower limit of the 90% confidence interval was higher than the mean. The centroid frequency, defined as the frequency below which 50% of the power occurs, was determined for each variable (6). The expected centroid frequency for white noise is 0.25 cycles per breath (21). The centroid frequencies were tested for deviation from white noise with the Kolmogoroff-Smirnov test (21). Cross-spectral estimates were considered significant if the lower limit of the 90% confidence interval of the squared coherency was larger than 0.3 (21).

To calculate group averages for cross-spectral estimates, the frequencies between zero and 0.5 cycles per breath were divided into bins of 0.01 cycles per breath. The reason was that the frequencies (multiples of 1/M) differed between the subjects because of differing M. For each subject, the mean cross-spectral estimate for each frequency bin was derived, only including cross-spectral estimates with a significant coherency. These mean values were used to calculate the group means. Coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I were compared between air and 100% O_2 breathing as to occurrence (25) and oscillation frequency (Wilcoxon rank sum test). $p < 0.05$ was taken as significant.

RESULTS

Air Breathing

The subjects breathed regularly during the experiments without falling asleep. Only in Subject 19 the last 120 breaths were discarded because of relative hyperventilation. Group averages for breath-to-breath means and SDs are given in Table 1. The steady state was confirmed by the relatively small SDs of blood pressure and R-R interval. Sa_{O_2} was above 94% during air breathing. The number of breaths was 442 ± 25, of which 1.3 ± 0.3% were interpolated as sighs.

In the example of Figure 2, periodicities in the breath-to-breath variability are not obvious. Neither is there a clear relationship between changes in $P_{ET_{CO_2}}$ and V_I/T_I. Only the variations in $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ seem to be related and appear out-of-phase. The corresponding power spectra of $P_{ET_{CO_2}}$ and V_I/T_I are shown in Figure 3. The power of both variables was concentrated at low frequencies (below 0.20 cycles per breath). This also appears from the low centroid frequencies in most subjects (Table 2).

The *bold lines* in Figure 3 represent the part of the power spectra that meet the criteria for coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I. This occurs at a small peak in both spectra at approximately 0.09 cycles per breath, corresponding to a cycle of about 11 breaths or roughly 50 seconds, as the mean breath duration ($\overline{T}_{TOT}$) was 4.4 seconds for this subject. Coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I were found in 15 of the 19 subjects. Figure 4 shows the averaged gain and phase for coherent oscillations, most of which occurred below 0.15 cycles per breath. The phase $\varphi_{C,VI/TI}$ was mostly negative, on the average about −90°. This means that a maximum in the oscillation in $P_{ET_{CO_2}}$ occurred less than half a cycle before a maximum in V_I/T_I (as in Figure 1). Table 3 gives a summary of the

TABLE 1. MEANS AND STANDARD DEVIATIONS OF RESPIRATORY AND CIRCULATORY VARIABLES

	Air ($n = 19$)		100% O_2 ($n = 18$)	
	Mean	SD	Mean	SD
V_I/T_I, L/second	0.403 ± 0.011	0.036 (0.023, 0.075)	0.459* ± 0.019	0.043 (0.022, 0.67)
V_I, L	0.611 ± 0.025	0.075 (0.040, 0.201)	0.700* ± 0.037	0.091 (0.029, 1.03)
T_I, seconds	1.52 ± 0.06	0.16 (0.082, 0.40)	1.53 ± 0.06	0.15 (0.058, 0.42)
T_{TOT}, seconds	4.34 ± 0.31	0.63 (0.18, 1.85)	4.09 ± 0.22	0.52 (0.16, 2.31)
$P_{ET_{CO_2}}$, kPa	5.59 ± 0.09	0.16 (0.10, 0.37)	5.38* ± 0.11	0.18 (0.12, 0.42)
$P_{ET_{O_2}}$, kPa	15.12 ± 0.20	0.40 (0.20, 0.95)	> 85	
BP, mm Hg	78.3 ± 1.0	4.6 (3.2, 7.2)	80.6 ± 2.0	4.5 (3.1, 6.9)
RRI, ms	0.881 ± 0.026	0.066 (0.045, 0.089)	0.934* ± 0.028	0.078* (0.041, 0.151)

Definition of abbreviations: BP = blood pressure (mm Hg); $P_{ET_{CO_2}}$, $P_{ET_{O_2}}$ = end-tidal P_{CO_2} and P_{O_2} (kPa); RRI = R-R interval from the ECG; T_{TOT} = total breath duration (seconds); V_I, T_I = inspiratory volume (L) and time (seconds); V_I/T_I = mean inspiratory flow (L/second).
Values are group means ± SEM (for breath-to-breath means) or geometric means and range in parentheses (for breath-to-breath SD).
* Significant difference between air and 100% O_2 breathing ($p < 0.05$).

other cross-spectral estimates at the frequencies of coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I. It shows that in 14 of the 15 subjects, the oscillation in V_I/T_I was tightly coupled to an in-phase oscillation in V_I. Almost every coherent oscillation in $P_{ET_{CO_2}}$ and V_I/T_I was accompanied with an out-of-phase oscillation in $P_{ET_{O_2}}$.

100% O_2 Breathing

Means and SDs of breath-to-breath variables during air breathing are compared with 100% O_2 breathing in Table 1. The number of breaths was 425 ± 12 with 1.5 ± 0.4% sighs. The smaller number of breaths compared with air breathing was due to the criterion that $P_{ET_{O_2}}$ had to exceed 85 kPa. In Subject 1 a reliable pneumotachogram was not obtained. The mean V_I was significantly higher than during air breathing (difference 0.089 ± 0.028 L), while the mean $P_{ET_{CO_2}}$ was significantly lower (difference −0.21 ± 0.04 kPa). None of the respiratory variables showed a significant difference in breath-to-breath SD between air and O_2 breathing (Table 1). The centroid frequencies were not significantly different from air breathing. Coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I were only found in eight subjects, significantly less often than during air breathing ($p < 0.01$, Table 3). The coherency occurred at a mean frequency of 0.055 cycles per breath (range 0.023–0.096, not significantly different from air breathing).

Model Simulation

The spectra can be interpreted by showing that they are compatible with a supposed mechanism. This approach is strengthened when the spectra cannot be explained by alternative hypotheses. It is, however, difficult to deduce, even from a simple hypothesis, what the spectra would precisely look like. We therefore expressed our hypotheses in mathematical equations and algebraically derived the spectra from these equations. This also improves the insight into the way the various physiologic mechanisms can influence the spectra.

The main hypothesis was that the variability of $P_{ET_{CO_2}}$ and V_I/T_I is influenced by chemoreflex feedback. Suppose that these variables would, instead, vary at random from breath to breath according to a normal distribution with a given mean and variance. Then the variability would contain all possible oscillations to the same extent (if the series of breaths is infinitely long). The power would thus be constant as a function of frequency, equal to the variance ("white noise") (21). If, however, chemoreflexes continuously react to such noise, the noise becomes filtered so that the power becomes significantly higher or lower than the mean power at particular frequencies. Such a filter mechanism can also influence the relationship between otherwise independent variables, resulting in a significant coherency.

Because breath-to-breath variability is a discontinuous process in the course of time, we used "discontinuous" equations (difference equations). The input to the model is white noise, which is mainly filtered by the chemoreflex. Provided that the difference equations are linear, the resulting spectra can be derived in a straightforward manner (21). We therefore applied linearized equations for the chemoreflex response to changes in $P_{ET_{CO_2}}$ and for the effects of ventilation on $P_{ET_{CO_2}}$. This is supported by the relatively high coherencies between $P_{ET_{CO_2}}$ and V_I/T_I (Table 3). The model is analogous to earlier models of hemodynamic variability (24, 26).

Description of the Chemoreflex-Feedback Model

A schematic of the model is shown in Figure 5. The variability is "driven" by two sources of noise, ε_1 and ε_2. The noise ε_1 is noise that directly affects $P_{ET_{CO_2}}$, such as changes in breathing frequency, cardiac output, or mixed venous P_{CO_2}. The noise ε_2

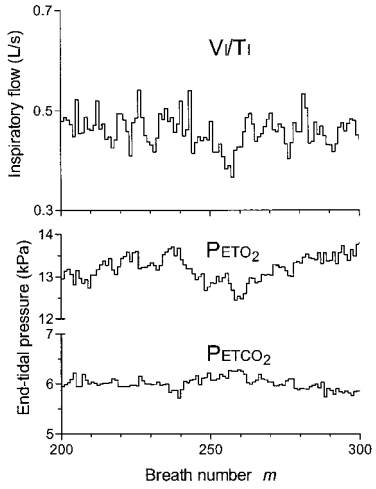

Figure 2. Breath-to-breath variability in Subject 4 during air breathing. V_I/T_I, mean inspiratory flow; $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$, end-tidal P_{CO_2} and P_{O_2}.

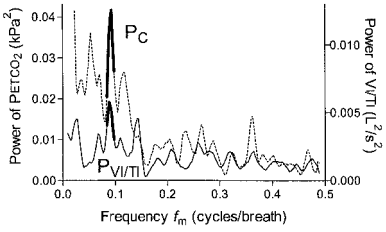

Figure 3. Power spectra of end-tidal P_{CO_2} (P_C, *dotted line*) and mean inspiratory flow (P_{V_I/T_I}, *continuous line*) as a function of frequency in cycles per breath (from Subject 4). *Bold lines* indicate the part of the spectra where the relationship between oscillations in $P_{ET_{CO_2}}$ and V_I/T_I is coherent.

TABLE 2. CENTROID FREQUENCIES FOR BREATH-TO-BREATH VARIABLES

Variable	Centroid frequency (*cycles per breath*)	n
V_I/T_I	0.160 ± 0.011	16
V_I	0.105 ± 0.009	19
T_I	0.133 ± 0.012	10
T_E	0.122 ± 0.011	18
$P_{ET_{CO_2}}$	0.097 ± 0.012	13

Definition of abbreviations: $P_{ET_{CO_2}}$ = end-tidal P_{CO_2} (kPa); T_E = expiratory time; T_I = inspiratory time (seconds); V_I = inspiratory volume (L); V_I/T_I = mean inspiratory flow (L/seconds).

Centroid frequencies are group means ± SEM. n, number of subjects in whom the centroid frequency implied a significant difference from white noise (from 19 subjects).

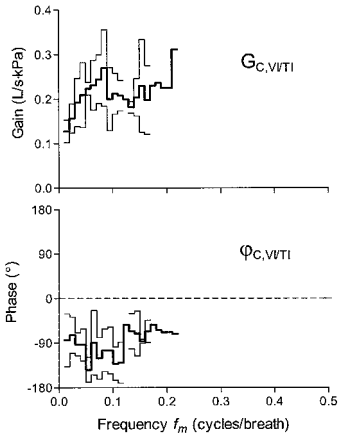

Figure 4. Gain ($G_{C,VI/TI}$) and phase ($\varphi_{C,VI/TI}$) for coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I during air breathing. *Bold lines* are group means and *thin lines* are means ± SD for each frequency bin of 0.01 cycles per breath. Only significant gain and phase estimates are included (from 15 subjects with coherent oscillations).

has a direct influence on V_I/T_I and can be due to any changes in respiratory drive that are not caused by chemoreflex feedback (22, 27). It is assumed that the chemoreflex response only consists of an increase in V_I/T_I after an increase in $P_{ET_{CO_2}}$ (1, 22). It is assumed that a change in V_I/T_I leads to a proportional change in V_I. The variability of T_I is neglected, so that T_I is considered equal to the mean value ($\overline{T}_I$). In the online data supplement (Section A) it is shown that this implies that the ratio of a change in V_I to a change in V_I/T_I equals $\overline{T}_I$. The constant c_2 in Figure 5 expresses the ventilatory influence of V_I on $P_{ET_{CO_2}}$. The minus sign for c_2 indicates that an increase in V_I leads to a decrease in $P_{ET_{CO_2}}$. The constant c_1 expresses the dependency of $P_{ET_{CO_2}}$ on the previous breath, mainly because of the buffer of the FRC.

Development of respiratory oscillations by chemoreflex feedback. The supposed principle of the development of respiratory oscillations by chemoreflex feedback has been described by a number of authors (e.g., 14, 15, 16, 17, 18, 28). Essential is the delay of the response. Figure 6 shows the simplest version of the present model that can explain the occurrence of respiratory oscillations. The dependency of $P_{ET_{CO_2}}$ on the previous breath is neglected here. It is assumed that the chemoreflex response to an "accidental" increase in $P_{ET_{CO_2}}$ (*arrow* in Figure 6) leads to an increase in V_I/T_I after a delay of two breaths. The correction (a decrease in $P_{ET_{CO_2}}$) thus occurs two breaths after the initial increase in $P_{ET_{CO_2}}$. This decrease in $P_{ET_{CO_2}}$ in turn leads to a corrective increase in $P_{ET_{CO_2}}$ after two breaths, and so on. The result is a dampened oscillation in $P_{ET_{CO_2}}$ and V_I/T_I with a cycle duration of four breaths, or a frequency of 0.25 cycles per breath. The cycle duration increases if the respiratory drive persists during following breaths ("short-term potentiation") (1, 29) or the dependency of $P_{ET_{CO_2}}$ on the previous breath increases.

Power and Cross-Spectra according to the Model

When the situation of Figure 6 is extended to repetitive accidental disturbances in $P_{ET_{CO_2}}$, multiple dampened oscillations are generated in $P_{ET_{CO_2}}$ and V_I/T_I. This implies a filter mechanism that selectively amplifies random disturbances in $P_{ET_{CO_2}}$ around a "resonance" frequency. Figure 7 shows an example of a purely peripheral chemoreflex model. Without chemoreflex, the two sources of noise would initially result in white noise in $P_{ET_{CO_2}}$ with a constant power (*see* Figure 7A for c_1 = 0). Due to the dependency of $P_{ET_{CO_2}}$ on the previous breath, the power is amplified below ~ 0.20 cycles per breath and suppressed at higher frequencies ("low-pass filter", *see* Figure 7A for c_1 = 0.5). Chemoreflex feedback further amplifies the power around 0.12 cycles per breath and suppresses the rest of the spectrum (resonance or band-pass filter phenomenon). The power of V_I/T_I also shows a resonance around this frequency (*open circles* in Figure 7). For lower frequencies, the power of V_I/T_I is also amplified, as opposed to $P_{ET_{CO_2}}$. These opposing effects of chemoreflex activity reflect an adequate action of the reflex, which tends to suppress spontaneous changes in $P_{ET_{CO_2}}$ by increasing the variability of V_I/T_I. Without chemoreflex, the total power of V_I/T_I would be considerably lower.

The squared coherency ($k^2_{C,VI/TI}$) is maximal at the resonance frequency (Figure 7C). Without chemoreflex, the coherency would be low for all frequencies. The relationship would then exclusively be due to the ventilatory influence of V_I/T_I on $P_{ET_{CO_2}}$. The coherency would thus be determined by the contribution of noise in V_I/T_I to the noise in $P_{ET_{CO_2}}$.

The gain ($G_{C,VI/TI}$) in Figure 7D refers to the "closed-loop" situation (as in Figure 6).The chemoreflex gain (G_χ) is the "open-loop" gain from $P_{ET_{CO_2}}$ to V_I/T_I (neglecting the ventilatory influence of V_I/T_I on $P_{ET_{CO_2}}$). When the frequency is zero, G_χ equals the peripheral chemoreflex sensitivity (S_p). At the resonance frequency, $G_{C,VI/TI}$ is almost equal to G_χ (only slightly higher). Without chemoreflex, the gain would be low (G_0).

Figure 7E shows that the closed-loop phase $\varphi_{C,VI/TI}$ is closely related to the open-loop chemoreflex phase φ_χ at the resonance frequency. The phase φ_χ is almost linearly related to the frequency, which is mainly determined by the chemoreflex delay. Without chemoreflex, $\varphi_{C,VI/TI}$ would lie approximately in the range between −150° and −170° (φ_0). A more extensive analysis is given in Section D of the online data supplement. An alternative model is also analyzed where short-term potentiation of respiratory drive occurs after other stimuli (27, 30).

Interpretation of Experimental Data with the Model

1. The variability of $P_{ET_{CO_2}}$, V_I/T_I, and V_I is not random, but is subject to low-pass filter mechanisms. Possible filter mechanisms are the dependency of $P_{ET_{CO_2}}$ on the previous breath, chemoreflex feedback, and short-term potentiation of respiratory drive.
2. Chemoreflex feedback can explain coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I. The reflex delay causes resonance at a frequency where the squared coherency is high. This frequency is mainly determined by the reflex delay, but shifts to lower frequencies when the dependency of $P_{ET_{CO_2}}$ on the previous breath becomes more important. Experimentally observed frequencies are compatible with peripheral chemoreflex feedback, with an unknown contribution of the central response. Gains and phases are compatible with the chemoreflex model.
3. Chemoreflex feedback through central chemoreceptors can explain coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I during 100% O_2 breathing.
4. Without chemoreflex, coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I are unlikely as there is no resonance at a specific fre-

TABLE 3. CROSS-SPECTRAL ESTIMATES FOR COHERENT OSCILLATIONS IN END-TIDAL CO_2 AND MEAN INSPIRATORY FLOW

Relation	n	Squared Coherency	Gain	Phase
Air				
$P_{ET_{CO_2}} \rightarrow V_I/T_I$	15	0.76 (0.53, 0.90)	0.24 (0.13, 0.34)	−85° (−170°, +8°)
$V_I/T_I \rightarrow V_I$	14	0.72 (0.52, 0.90)	1.88 (0.92, 3.87)	−1° (−57°, +38°)
$V_I \rightarrow T_E$	8	0.69 (0.55, 0.88)	6.0 (1.6, 15)	−5° (−86°, +120°)
$P_{ET_{CO_2}} \rightarrow P_{ET_{O_2}}$	14	0.90 (0.81, 0.99)	2.09 (1.33, 2.77)	−179° (−200°, −150°)*
100% O_2				
$P_{ET_{CO_2}} \rightarrow V_I/T_I$	8	0.79 (0.65, 0.93)	0.26 (0.12, 0.51)	−105° (−146°, −34°)
$V_I/T_I \rightarrow V_I$	5	0.85 (0.67, 0.92)	2.86 (0.86, 5.86)	+4° (−48°, +47°)
$V_I \rightarrow T_E$	4	0.77 (0.69, 0.84)	4.0 (2.1, 9.3)	−24° (−63°, +17°)

Definition of abbreviations: $P_{ET_{CO_2}}$, $P_{ET_{O_2}}$ = end-tidal P_{CO_2} and P_{O_2} (kPa); T_E = expiratory time; T_{TOT} = total breath duration (seconds); V_I, T_I = inspiratory volume (L) and time (seconds); V_I/T_I = mean inspiratory flow (L/second).

All values are group means from individual spectral estimates at the frequency of maximal coherency between $P_{ET_{CO_2}}$ and V_I/T_I. Ranges are in parentheses. $P_{ET_{CO_2}} \rightarrow V_I/T_I$ refers to cross-spectra with $P_{ET_{CO_2}}$ as input and V_I/T_I as output variable, etc. n, number of subjects with significant coherency.

* To obtain the smallest phase range, 360° was subtracted from the lower limit of the range (the estimated phase cannot be distinguished from the phase plus or minus a multiple of 360°).

quency, and the expected coherency is low. The corresponding gain is low compared with experimental data. The phase is confined to a range from about −150° to −170° and cannot explain most experimental values.

5. Short-term potentiation of V_I/T_I without chemoreflex-feedback can explain the relatively high coherencies and gains between $P_{ET_{CO_2}}$ and V_I/T_I below approximately 0.08 cycles per breath. The phase is, however, equal to the situation of conclusion 4.

DISCUSSION

Interpretation of Coherent Oscillations in End-Tidal CO_2 and Mean Inspiratory Flow

The coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I that were found in 15 subjects were interpreted as the result of spontaneous "noise" in the respiratory system, which is filtered by the chemoreflex. Sighs, coughs, and changes in CO_2 production or cardiac output may all add to such noise. This mechanism was proposed by Modarreszadeh and colleagues (8), who found that the highest power of $P_{ET_{CO_2}}$ and minute volume occurred below 0.10 cycles per breath in quietly breathing subjects. They applied an end-tidal CO_2 buffering technique which allowed an artificial reduction of the power of $P_{ET_{CO_2}}$ below 0.10 cycles per breath (under hyperoxic conditions). The consequence was a significant reduction in the power of minute volume. This is the most convincing evidence that a considerable part of the ventilatory variability can result from a response to fluctuations in $P_{ET_{CO_2}}$. Another indication that part of the respiratory variability is due to chemoreflex activity comes from a study in rats where the variability of phrenic nerve activity was studied (31).

The frequencies of coherent oscillations were mostly below 0.20 cycles per breath. Most of these frequencies could be explained by the delays of the peripheral chemoreflex, with a shift to lower frequencies due to the dependency of $P_{ET_{CO_2}}$ on the previous breath. The cycle durations were 5 to 12 breaths (~ 20–60 seconds). This contrasts with the cycle time of frank periodic breathing during high-altitude hypoxia, which is mostly 18 to 25 seconds (13, 32). A similar difference in cycle time has been observed between normoxia and isocapnic hypoxia (10). The "fast" periodic breathing during hypoxia is probably caused by peripheral chemoreflex activity as well (20). This does not preclude, however, a role for peripheral chemoreceptors in the development of the relatively "slow" oscillations in the present study. The model predicts a difference in cycle time between normoxia and hypoxia if hypoxia is considered as an "amplifier" of the peripheral chemoreflex sensitivity to Pa_{CO_2} (33). As explained in Section D.2.3 of the online data supplement (Figure E8), a shift to a shorter cycle time due to an increased chemoreflex sensitivity can be expected if there is a certain dependency of $P_{ET_{CO_2}}$ on the previous breath. In the model, it is actually this dependency that adds to the "slowness" of the respiratory system during normoxia. The contribution of the peripheral feedback loop in our study is further supported by the reduction of coherent oscillations by 100% O_2. A contribution of the central feedback loop to the variability can also be expected below approximately 0.10 cycles per breath. However, because such slow oscillations can occur less often during a recording time of 30 minutes, the chance of finding them is smaller than for faster oscillations. The inhalation of 100% O_2 has probably also altered the central drive, as suggested by the increased mean ventilation. This may be secondary to a reduction of the Haldane effect or a suppression of central hypoxic inhibition (34).

The squared coherency between $P_{ET_{CO_2}}$ and V_I/T_I has a double meaning here. First, it is a measure of linearity of the relationship between $P_{ET_{CO_2}}$ and V_I/T_I at a specific frequency, regardless of its physiologic interpretation. Second, according to the model, it is a direct estimate of the "loop gain" of the feedback system. The loop gain reflects the amplification in

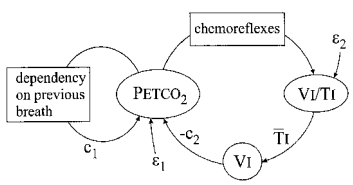

Figure 5. Schematic of the chemoreflex-feedback model. The variability of $P_{ET_{CO_2}}$ and V_I/T_I is driven by two sources of noise, ε_1 and ε_2. The noise is filtered by the chemoreflex-feedback loop and the dependency of $P_{ET_{CO_2}}$ on the previous breath. c_1, coefficient of the dependency of $P_{ET_{CO_2}}$ on the previous breath; c_2, coefficient of the influence of V_I on $P_{ET_{CO_2}}$; $\overline{T}_I$, mean T_I.

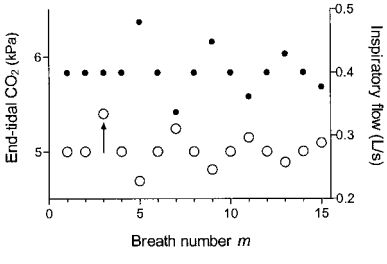

Figure 6. Dampened oscillation in $P_{ET_{CO_2}}$ and V_I/T_I caused by delayed chemoreflex feedback after an accidental increase in $P_{ET_{CO_2}}$. *Open circles,* breath-to-breath $P_{ET_{CO_2}}$ (*left axis*), *arrow* indicates accidental increase in $P_{ET_{CO_2}}$; *closed circles,* V_I/T_I (*right axis*).

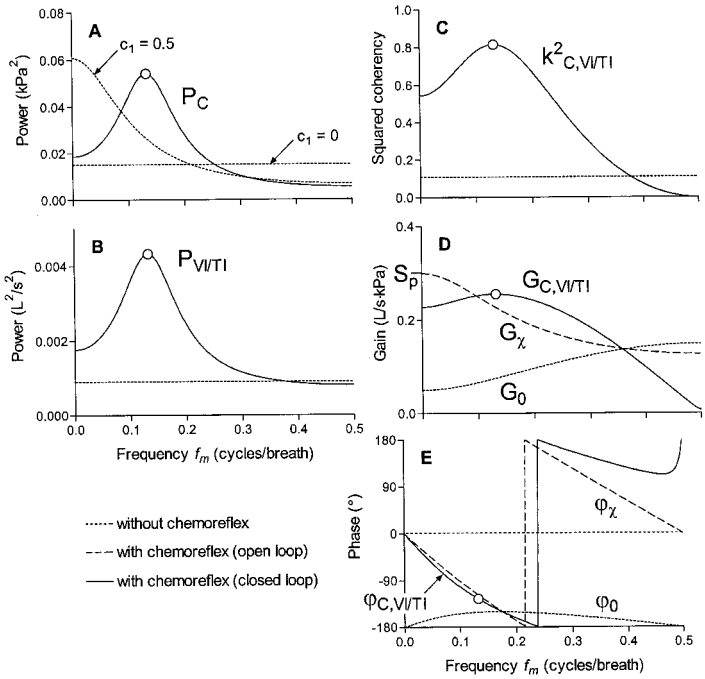

Figure 7. Hypothetical spectra for the model without chemoreflex (*dotted lines*) or with only a peripheral chemoreflex (*continuous lines*). P_C, power of $P_{ET_{CO_2}}$; c_1, coefficient of the dependency of $P_{ET_{CO_2}}$ on the previous breath; $P_{VI/TI}$, power of V_I/T_I; $k^2_{C,VI/TI}$, squared coherency between $P_{ET_{CO_2}}$ and V_I/T_I; $G_{C,VI/TI}$ and $\varphi_{C,VI/TI}$, closed-loop gain and phase from $P_{ET_{CO_2}}$ to V_I/T_I; G_χ and φ_χ, open-loop chemoreflex gain and phase from $P_{ET_{CO_2}}$ to V_I/T_I; G_0 and φ_0, gain and phase from $P_{ET_{CO_2}}$ to V_I/T_I for the situation without chemoreflex; S_p, peripheral chemoreflex sensitivity (in L/second · kPa). *Open circles* refer to the resonance frequency.

the feedback loop and is a major determinant of the tendency to oscillate (15). Other more indirect estimation procedures for loop gain have been described for pseudorandom CO_2 inhalation (35) or transient changes in $P_{ET_{CO_2}}$ (16).

The model analysis revealed a close relationship between the closed-loop gain from $P_{ET_{CO_2}}$ to V_I/T_I and the open-loop chemoreflex gain for coherent oscillations. This makes an estimation of the chemoreflex gain possible during the steady state without additional stimulation (e.g., in sleep studies or during continuous monitoring in the ICU). We did not, however, compare this method with a standard technique. The approach is not essentially different from the analysis of dampened respiratory oscillations induced by spontaneous sighs (18), acute changes in inspiratory P_{CO_2} (16), or pseudorandom CO_2 stimulation (35). A drawback is that the facemask could have influenced the breathing pattern (12), although we did not find coherent oscillations in every subject. This probably depends on the ratio between the different sources of noise. The variability of arterial P_{O_2} may also play a role because $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ oscillated out-of-phase, so that an additive effect on peripheral chemoreceptors is possible (36).

The finding that the phase between V_I/T_I and V_I was about zero for coherent oscillations (Table 3) indicates that V_I/T_I during a given breath was positively related to V_I of the same breath. Because the gain of this relationship was about equal to the mean T_I (*see* also Table 1), this could be explained by the model as a direct influence of changes in V_I/T_I on V_I at a relatively constant T_I. Apparently, there was no strong mechanism that counteracted the evoked changes in respiratory drive (V_I/T_I) by an adaptation of T_I (5). The relationship between V_I and T_E was relatively weak, with a rough clustering of the phase between V_I and T_E around zero (Table 3). This may be related to a mechanism that lengthens T_E in response to high V_I, although the Hering-Breuer reflex is probably only operating for V_I above 1.2 L (37). Anyway, a zero phase between V_I and T_E argues against a strong influence of chemoreflexes on T_E, because chemoreflexes would simultaneously increase V_I/T_I (and thus increase V_I) and shorten T_E (23).

Modeling of Chemoreflex Feedback

A number of mathematical models have already been implemented to explain periodic breathing from chemoreflex characteristics in heart failure or during hypoxia (e.g., 15, 16, 17). Several models also give a good description of dampened oscillations after sudden changes in the system such as a sigh or an increase in inspiratory CO_2 (16, 18, 35). The present model is actually a simplification of existing models where the relationship between respiratory variables is described from breath to breath (18, 35, 38, 39). What is new is the analytical derivation of power and cross-spectra from such simplified linear difference equations. The reason to explore respiratory variability in the frequency domain is that the supposed linear interactions constitute a time-invariant filter to noise in the system. We tried to explain the spectral features by a model as simple as possible. The major advantage is that this gives insight into the main mechanisms of the variability. A drawback is that nonlinear interactions can occur that are not always well approximated by a linear model. For example, a highly variable T_{TOT} would change the chemoreflex response in terms of number of breaths, so that the response function becomes nonlinear. The same holds true for variations in chemoreflex sensitivity or cardiac output. The ventilatory influence of V_I/T_I on $P_{ET_{CO_2}}$ is probably more nonlinear for larger variations (40).

In conclusion, we found that coherent oscillations in $P_{ET_{CO_2}}$ and V_I/T_I occur in the normal respiratory variability. These oscillations could be explained as a chemoreflex response to spontaneous changes in the respiratory system, indicating that respiration is continuously adjusted by the chemoreflexes so that the respiratory variability is not random. Previously, the function of the chemoreflexes was mostly studied with external stimuli (e.g., inhaled CO_2). While the response to such stimuli shows the capability of the reflexes to respond, the analysis of respiratory variability makes it possible to derive information on the actual performance of the chemoreflexes.

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References

1. Bellville JW, Whipp BJ, Kaufman RD, Swanson GD, Aqleh KA, Wiberg DM. Central and peripheral loop gain in normal and carotid-body resected subjects. *J Appl Physiol* 1979;46:843–853.
2. Cunningham DJC. A model illustrating the importance of timing in the regulation of breathing. *Nature* 1975;253:440–442.
3. Priban IP. An analysis of some short-term patterns of breathing in man at rest. *J Physiol (Lond)* 1963;166:425–434.
4. Tobin MJ, Yang KL, Jubran A, Lodato RF. Interrelationship of breath components in neighboring breaths of normal eupneic subjects. *Am J Respir Crit Care Med* 1995;152:1967–1976.
5. Newsom Davis J, Stagg D. Interrelationships of the volume and time components of individual breaths in resting man. *J Physiol (Lond)* 1975;245:481–498.
6. Jubran A, Grant BJB, Tobin MJ. Effect of hyperoxic hypercapnia on variational activity of breathing. *Am J Respir Crit Care Med* 1997;156: 1129–1139.
7. Lenfant C. Time-dependent variations of pulmonary gas exchange in normal man at rest. *J Appl Physiol* 1975;22:675–684.
8. Modarreszadeh M, Bruce EN. Ventilatory variability induced by spontaneous variations of Pa_{CO_2} in humans. *J Appl Physiol* 1994;76:2765–2775.
9. Hlastala MP, Wranne B, Lenfant CJ. Cyclical variations in FRC and other respiratory variables in resting man. *J Appl Physiol* 1973;34:670–676.
10. Jubran A, Tobin MJ. Effect of isocapnic hypoxia on variational activity of breathing. *Am J Respir Crit Care Med* 2000;162:1202–1209.
11. Gillam PMS. Patterns of respiration in human beings at rest and during sleep. *Bull Eur Physiopath Resp* 1972;8:1059–1070.
12. Van den Aardweg JG, Karemaker JM. Respiratory variability and associated cardiovascular changes in adults at rest. *Clin Physiol* 1991;11: 95–118.
13. Brusil PJ, Waggener TB, Kronauer RE, Gulesian P. Methods for identifying respiratory oscillations disclose altitude effects. *J Appl Physiol* 1980;48:545–556.
14. Chapman KR, Bruce EN, Gothe B, Cherniack NS. Possible mechanisms of periodic breathing during sleep. *J Appl Physiol* 1988;64:1000–1008.
15. Khoo MCK, Kronauer RE, Strohl KP, Slutsky AS. Factors inducing periodic breathing in humans: a general model. *J Appl Physiol* 1982;53: 644–659.
16. Carley DW, Shannon DC. A minimal mathematical model of human periodic breathing. *J Appl Physiol* 1988;65:1400–1409.
17. Longobardo GS, Cherniack NS, Fishman AP. Cheyne-Stokes breathing produced by a model of the human respiratory system. *J Appl Physiol* 1966;21:1839–1846.
18. Khoo MCK, Marmarelis VZ. Estimation of peripheral chemoreflex gain from spontaneous sigh responses. *Ann Biomed Eng* 1989;17:557–570.
19. Jensen JI, Mosekilde E, Ward DS. A system dynamics model of human respiratory regulation. In: Whipp BJ, Wiberg DM, editors. Modelling and control of breathing. New York: Elsevier; 1983. p. 316–321.
20. Lahiri S, Hsiao C, Zhang R, Mokashi A, Nishino T. Peripheral chemoreceptors in respiratory oscillations. *J Appl Physiol* 1985;58:1901–1908.
21. Jenkins GM, Watts DG. Spectral analysis and its applications. San Fransisco: Holden-Day; 1968.
22. Whitelaw WA, Derenne JP, Milic-Emili J. Occlusion pressure as a measure of respiratory center output in conscious man. *Respir Physiol* 1975;23:181–199.
23. Ledlie JF, Nielsen SG, Cherniack NS, Fishman AP. Effects of hypercapnia and hypoxia on phrenic nerve activity and respiratory timing. *J Appl Physiol* 1981;51:732–738.
24. DeBoer RW, Karemaker JM, Strackee J. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. *Am J Physiol* 1985;253 (*Heart Circ Physiol* 22):H680–H689.
25. Armitage P, Berry G. Statistical methods in medical research. Oxford: Blackwell; 1987. 123–125.
26. Van den Aardweg JG, Van Steenwijk RP, Karemaker JM. A chemoreflex model of relation between blood pressure and heart rate in sleep apnea syndrome. *Am J Physiol* 1995;268 (*Heart Circ Physiol* 37): H2145–H2156.
27. Swanson GD, Ward DS, Bellville JW. Posthyperventilation isocapnic hyperpnea. *J Appl Physiol* 1974;37:723–735.
28. Carley DW, Shannon DC. Relative stability of human respiration during progressive hypoxia. *J Appl Physiol* 1988;65:1389–1399.
29. Georgopoulos D, Bshouty Z, Younes M, Anthonisen NR. Hypoxic exposure and activation of the afterdischarge mechanism in conscious humans. *J Appl Physiol* 1990;69:1159–1164.
30. Benchetrit G, Bertrand F. A short-term memory in the respiratory centres: statistical analysis. *Respir Physiol* 1975;23:147–158.
31. Khatib MF, Oku Y, Bruce EN. Contribution of chemical feedback loops to breath-to-breath variability of tidal volume. *Respir Physiol* 1991;83: 115–128.
32. Waggener TB, Brusil PJ, Kronauer RE, Gabel RA, Inbar GF. Strength and cycle time of high-altitude ventilatory patterns in unacclimatized humans. *J Appl Physiol* 1984;56:576–581.
33. Honda Y, Tani H. Chemical control of breathing. In: Altose MD, Kawakami Y, editors. Control of breathing in health and disease. In: Lenfant C, editor. Lung biology in health and disease, Vol. 135. New York: Marcel Dekker; 2000. p. 41–88.
34. Holtby SG, Berezanski DJ, Anthonisen NR. Effect of 100% O_2 on hypoxic eucapnic ventilation. *J Appl Physiol* 1988;65:1157–1162.
35. Ghazanshahi SD, Khoo MCK. Estimation of chemoreflex loop gain using pseudorandom binary CO_2 stimulation. *IEEE Trans Biomed Eng* 1997;44:357–366.
36. Akiyama Y, Kawakami Y. Clinical assessment of the respiratory control system. In: Altose MD, Kawakami Y, editors. Control of breathing in health and disease. In: Lenfant C, editor. Lung biology in health and disease, Vol. 135. New York: Marcel Dekker; 2000. p. 251–287.
37. Iber C, Simon S, Skatrud JB, Halowald MW, Dempsey JE. The Breuer-Hering reflex in humans. *Am J Respir Crit Care Med* 1995;152:217–224.
38. Busso T, Liang PJ, Robbins PA. Breath-to-breath relationships between respiratory cycle variables in humans at fixed end-tidal P_{CO_2} and P_{O_2}. *J Appl Physiol* 1996;81:2287–2296.
39. Modarrheszadeh M, Kump KS, Chizeck HJ, Hudgel DW, Bruce EN. Adaptive buffering of breath-by-breath variations of end-tidal CO_2 of humans. *J Appl Physiol* 1993;75:2003–2012.
40. Chilton AB, Stacy RW. A mathematical analysis of carbon dioxide respiration in man. *Bull Math Biophys* 1952;14:1–18.