Vagal feedback in the entrainment of respiration to mechanical ventilation in sleeping humans

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Simon, Peggy M., Alfred M. Habel, J. Andrew Daubenspeck, and J. C. Leiter. Vagal feedback in the entrainment of respiration to mechanical ventilation in sleeping humans. J Appl Physiol 89: 760–769, 2000.—We studied the capacity of four “normal” and six lung transplant subjects to entrain neural respiratory activity to mechanical ventilation. Two transplant subjects were studied during wakefulness and demonstrated entrainment indistinguishable from that of normal awake subjects. We studied four normal subjects and four lung transplant subjects during non-rapid eye movement (NREM) sleep. Normal subjects entrained to mechanical ventilation over a range of ventilatory frequencies that were within ±3–5 breaths of the spontaneous respiratory rate of each subject. After lung transplantation, during which the vagi were cut, subjects did demonstrate entrainment during NREM sleep; however, entrainment only occurred at ventilator frequencies at or above each subject’s spontaneous respiratory rate, and entrainment was less effective. We conclude that there is no absolute requirement for vagal feedback to induce entrainment in subjects, which is in striking contrast to anesthetized animals in which vagotomy uniformly abolishes entrainment. On the other hand, vagal feedback clearly enhances the fidelity of entrainment and extends the range of mechanical frequencies over which entrainment can occur.

vagal afferents; state; non-rapid eye movement sleep; Hering-Breuer reflex

WHEN RESPIRATORY ENTRAINMENT is present, a fixed and repetitive coupling exists between mechanical inflation and neural inspiratory activity. Entrainment may occur at a 1-to-1 ratio (one mechanical inflation to one neural respiratory effort), but other integral ratios may be seen, as well as aperiodic, chaotic behavior in the transition between different integral ratio entrainment patterns (16). Most respiratory entrainment studies have been performed in anesthetized animals (3, 12, 16). The respiratory system will not entrain to mechanical ventilation after bilateral vagotomy in anesthetized animals (11, 16, 22), which, in addition to other findings, has lead to the conclusion that the Hering-Breuer reflex plays an essential role in entrainment. Evidence of the Hering-Breuer reflex during respiratory entrainment may be either 1) a shortening of neural inspiratory time (TI) when machine inflations precede neural TI or 2) expiratory time (TE) prolongation when machine inflations begin late in neural TI or early in TE. Entrainment has also been studied in anesthetized humans (8), and the Hering-Breuer reflex seemed important for entrainment in that study as well. TE was prolonged in these subjects when mechanical inflations occurred during the inspiratory-expiratory transition (late TE). Finally, mathematical models that incorporated the inspiratory and expiratory effects of the Hering-Breuer reflex have successfully reproduced the integral entrainment ratios and aperiodic patterns seen in anesthetized cats (16).

Simon et al. (21) recently investigated entrainment in awake and sleeping “normal” humans during normocapnia and mild hypercapnia. They found that 1:1 entrainment at a constant mechanical ventilator volume (1.5 times each subject’s spontaneous volume) was maintained over a much wider range in awake humans than in anesthetized animals and humans. TI was shortened in sleeping normal subjects when mechanical inflation occurred slightly before or early in neural inspiration and entrainment persisted during sleep, but at 1-to-2 and 1-to-1 entrainment ratios, more typical of previous studies in anesthetized animals. Furthermore, the range of machine frequencies in which entrainment occurred was smaller during sleep compared with wakefulness. The greater mechanical ventilator frequency range of entrainment in conscious subjects suggests that there were either additional entraining stimuli present during wakefulness or cortical influences modified the respiratory control system to enhance and expand the range of 1:1 entrainment.

In the present study, we tested the hypothesis that vagally mediated afferent information is required for entrainment in awake and sleeping humans. We pursued this hypothesis because debate continues about the importance of the Hering-Breuer reflex in the control of ventilation in humans. We studied normal subjects and subjects that had undergone either heart-lung or double-lung transplantation in which the lungs

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were denervated. In this way, we were able to re-examine the roles of the vagus and the Hering-Breuer reflex in entrainment responses in the absence of anasthesia. In previous studies of entrainment after vagotomny in animals, the animals were anesthetized as well as vagotomized. Anesthesia may reduce mechano-receptor activity (14), reduce central sensitivity to a variety of respiratory stimuli, and diminish the mechanical output of the respiratory system, thereby blunting the strength of the Hering-Breuer reflex and any other stimuli that may provide entraining cues.

METHODS

Subjects. We recruited 11 lung transplant patients that had undergone either a double-lung or heart-lung transplant within the past 10 years. The patients were all in stable, good health at the time of the studies. One woman and five men, aged 39–59 yr, were able to complete the study. Two of the patients were studied during wakefulness, and four were studied during non-rapid eye movement (NREM) sleep. Seven of the eleven transplant recipients were unable to sleep under the conditions of the experiment. We recruited 11 normal adult volunteers with no history of cardiopulmonary disease. We obtained data from four normal, healthy volunteers (three women and one man), aged 18–35 yr. The remaining seven subjects were unable to sleep under the conditions of the experiment. None of the subjects had a background in respiratory physiology nor did they know the objectives of the study or train for the study. The Institutional Review Board of the Mayo Clinic approved the study, and informed consent was obtained from all subjects.

Measurements. Subjects were ventilated through a nasal continuous positive airway pressure mask attached to a Puritan-Bennett 7200 ventilator (Carlsbad, CA) that was modified for research purposes. The auditory alarm functions on the ventilator were disabled. In addition, when needed, 12% CO₂ in O₂ was added to the inspired gas via the O₂ inlet to adjust the inspired CO₂ fraction to a target end-tidal CO₂ (PETCO₂).

After calibration, measurements of airway pressure and flow were obtained from the analog output of the ventilator. Tidal volume (VT) was obtained by integrating flow. End-tidal gas was sampled from a port attached to the mask. The CO₂ concentration was measured with a calibrated infrared capnograph (model 1260, Novametrix, Wallingford, CT). Diaphragmatic electromyographic (EMG) activity on the right side of the chest was monitored with surface electrodes (Red Dot, 3M, St. Paul, MN) placed in the anterior axillary line over the sixth and seventh intercostal spaces. Inspiratory activity and respiratory timing were measured from the diaphragm EMG recordings. Electroencephalographic (EEG) activity (monitored from the C4-A1 and CZ-OZ leads), the submental EMG, and the electrooculographic activity were used to document sleep stages. EMG and EEG activities were processed using a TECA-42 EMG instrument (Pleasantville, NY). All signals were displayed and recorded using an AstroMed, MT 8000-strip chart recorder (West Warwick, RI) and recorded on magnetic media using a computer acquisition program (LabVIEW, National Instruments, Austin, TX).

Experimental protocol. The same protocol was used in normal and transplant subjects. All studies were performed on supine subjects in beds. Subjects participating in the sleep protocols were asked to deprive themselves of sleep (<2 h) the night before the study and told to avoid caffeinated beverages for 12 h before the study began. The protocols had three periods in which we measured 1) spontaneous eucapnic ventilation, 2) spontaneous respiratory rate during mechanical ventilation, and 3) entrainment at respiratory rates above and below the spontaneous mechanical ventilation rate. The awake protocols began after the subject acclimated to the lab and was relaxed or, if it was a sleep study, once stable stage II or III–IV NREM sleep was established. The spontaneous, isocapnic ventilation trial consisted of a 5-min observation period during which the subject breathed unassisted in the flow-by mode. The ventilator settings were as follows: continuous positive airway pressure = 0 cmH₂O, baseline flow = 20 l/min, and flow sensitivity = 3 l/min. Average eupneic tidal volume (VT) and PETCO₂ were measured during the final 3 min of this period. In the second phase of the protocol, preset volume ventilation was administered for 5 min. The subject triggered each breath during preset volume ventilation, but VT was fixed and equal to 130% of the spontaneous VT. The inspiratory flow rate was 25–35 l/min in a square waveform. The machine backup rate was 2 breaths/min (bpm), with a flow-by threshold of 5 l/min that allowed each subject to choose his or her own respiratory rate. The rate of subject-triggered ventilator breaths at constant VT and flow rate was labeled the “spontaneous respiratory rate.” The average spontaneous respiratory rate was measured during the last 3 min of this period. CO₂ was added to the ventilator to maintain PETCO₂ equal to the level present in spontaneous breathing during NREM sleep, but PETCO₂ was allowed to vary in the studies performed during wakefulness. In the third period of the protocol, ventilator trigger mechanisms were disabled, and machine rates were initially set equal to or 1 bpm above the spontaneous respiratory rate for each subject. Every 3 min, the machine rate was varied 1 bpm below or above the spontaneous respiratory rate. Phase angles (θ) were calculated (see below) from data obtained in the last 1.5 min of each 3-min trial. In waking subjects, the trial was terminated at low machine rates when the subject complained of respiratory discomfort and at high machine rates when the expiratory phase of the machine cycle was not long enough to allow inspiratory flow to return to zero or when inspiratory EMG activity was undetectable. In sleeping studies, trials were terminated at low ventilator frequencies by arousal of the subject and at high ventilator frequencies when the EMG signals were lost or inspiratory flow failed to return to zero. Neuromechanical inhibition of surface EMG activity occurred in all subjects at a ventilator frequency above the spontaneous rate. No data were taken from the ventilator frequencies at or above the frequency that surface EMG activity first started to drop out, because we could not reliably track breath-by-breath changes in θ when the EMG signal was erratic.

Data analysis and statistics. The onset of the subject’s neural respiratory activity was determined from the onset of surface EMG activity of the diaphragm. We determined the phase relationships between the onset of surface EMG activity and the machine cycle with methods described previously (21). The phase delay is the time in seconds from the onset of spontaneous inspiration to the onset of machine inflation. The θ, which describes the relationship between machine onset and surface EMG onset, was determined by calculating the phase delay, dividing by the cycle time of the ventilator, and multiplying by 360°.

\[ \theta = \left[ \text{[EMG onset time} - \text{ventilator onset time]} / \text{ventilator cycle duration} \right] \times 360 \]

Onset of machine inflation was assigned a θ of 0° when machine inflation and surface EMG onset occurred at the
same time. When surface EMG activity preceded machine inflation, \( \theta \) was between \(-180°\) and \(0°\), and, when surface EMG activity occurred during or after machine inflation, \( \theta \) was between \(0°\) and \(+180°\).

In analyses of the Hering-Breuer reflex, neural \( T_i \) was obtained from the duration of inspiratory activity measured from surface EMG activity and compared at different \( \theta \) in each subject studied during NREM sleep. The diaphragm EMG was often contaminated by the EKG signal, and the termination of respiratory muscle EMG activity was difficult to measure exactly. We selected only the breaths that had a clearly defined termination of EMG activity. We selected the measurable \( T_i \) values from all ventilator frequencies during sleep to obtain \( T_i \) values over the entire range of \( \theta \).

We calculated the average and standard deviation of \( \theta \) at each machine frequency for each subject using methods appropriate for angles (10). We took a mathematical approach to the definition of entrainment and defined entrainment as a statistically significant (\( P < 0.05 \)) and unique concentration of \( \theta \) around a mean \( \theta \) value. If the distribution of \( \theta \) was homogenous from \(-180°\) to \(+180°\) (i.e., no significant single \( \theta \)), then we determined whether there were significant concentrations of \( \theta \) from \(-180°\) to \(0°\) and from \(0°\) to \(+180°\). Occasionally, a single cluster of \( \theta \) was arrayed around \(0°\), but we identified these as two significant clusters because the ranges we examined for significant concentrations were arbitrarily separated at \(0°\). To avoid defining two clusters when only one might exist, we tested that \( \theta \) concentrations were unique by shifting the range of a mean \( \theta \) \(\pm 60°\) and then recalculating that mean \( \theta \). If significant concentrations of \( \theta \) could be identified in multiple ranges of phase angles, then the \( \theta \) were not unique, and we concluded that entrainment was not present. If significant and unique concentrations of \( \theta \) were found in both of these ranges, then a 1:2 entrainment existed. No other stable entrainment ratios were seen. We chose the simplest entrainment ratio that identified significant and unique mean \( \theta \). For example, if we identified significant 1:1 entrainment, we did not look further for other entrainment ratios. When entrainment occurred, the standard deviation provided a measure of the tightness of phase locking.

This statistical approach to entrainment is more stringent than visual inspection, but something may be lost. In the transitional zone between 1:2 and 1:1 entrainment, subjects often had brief periods of 1:2 entrainment that were followed by longer periods of 1:1 entrainment. Our statistical analysis defined this as 1:1 entrainment with greater variability than a consistent pattern of 1:1 entrainment throughout the test period. Thus our analysis may underestimate the true extent of entrainment in favor of a stricter, more rigorous definition. We are unlikely to have overestimated the occurrence of entrainment in the transplant subjects, but, by the same token, brief periods of entrainment simply did not meet our threshold criterion.

Angles are periodic and, therefore, are not normally distributed. For this reason, statistical inferences were made using the von Mises distribution, which is analogous to a normal distribution but appropriate for periodic functions. Probability distribution functions for \( \theta \) at each machine frequency were calculated from the mean angle and a concentration parameter that is inversely related to the variance (10). The individual probability curves were summed across subjects as a function of machine rate expressed relative to the spontaneous rate. The summed probabilities at each machine frequency were normalized to keep the area under each probability curve constant among the machine frequencies; the probability distribution across machine frequencies was plotted in three dimensions (breath order, \( \theta \), and relative probability) using Matlab (Math Works, Natick, MA).

### RESULTS

**Patient characteristics and respiratory variables during wakefulness and sleep.** The clinical characteristics of the transplant subjects are summarized in Table 1. The individual and average \( V_t \) and spontaneous respiratory frequencies during mechanical ventilation and the range of frequencies in each subject for all conditions studied are shown in Table 2.

**Effect of wakefulness on entrainment responses.** Figure 1 shows a phase scatter plot for one of the two lung transplant subjects studied during wakefulness. A 1:1 entrainment was apparent over a wide range of machine frequencies. The standard deviations around each \( \theta \) were also small. This subject had larger standard deviations around each \( \theta \) than the other lung transplant subject studied during wakefulness. We compared the \( \theta \) and the standard deviations in these two lung transplant subjects to the range of \( \theta \) seen in normal, waking subjects in the study of Simon et al. (21). In Fig. 2, the mean \( \theta \) and the 95% confidence intervals for normal subjects are plotted as functions of the mechanical ventilator rate expressed in each subject, relative to the spontaneous respiratory rate, which was set equal to zero. The \( \theta \) of transplant subjects fell close to or within the 95% confidence intervals of the normal subjects. A one-way ANOVA appropriate for periodic functions (10) was performed on the mean \( \theta \), which was determined by combining all mechanical ventilator frequencies and subjects within each group (normal vs. transplant subjects). This analysis did not

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**Table 1. Characteristics of lung transplant recipients**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Disease</th>
<th>Transplant type, date</th>
<th>FEV(_1), liters (%predicted)</th>
<th>FVC, liters (%predicted)</th>
<th>FEV(_1)/FVC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>58</td>
<td>α1-Antitrypsin</td>
<td>DLT 1992</td>
<td>3.75 (102%)</td>
<td>4.45 (97%)</td>
<td>84%</td>
</tr>
<tr>
<td>T2</td>
<td>42</td>
<td>CF</td>
<td>HLT 1994</td>
<td>2.69 (72%)</td>
<td>3.13 (69%)</td>
<td>86%</td>
</tr>
<tr>
<td>T3</td>
<td>42</td>
<td>α1-Antitrypsin</td>
<td>DLT 1993</td>
<td>2.45 (61%)</td>
<td>4.25 (86%)</td>
<td>57%</td>
</tr>
<tr>
<td>T4</td>
<td>39</td>
<td>PPH</td>
<td>HLT 1994</td>
<td>3.05 (85%)</td>
<td>4.10 (94%)</td>
<td>76%</td>
</tr>
<tr>
<td>T5</td>
<td>59</td>
<td>α1-Antitrypsin</td>
<td>HLT 1986</td>
<td>2.90 (85%)</td>
<td>3.55 (83%)</td>
<td>82%</td>
</tr>
<tr>
<td>T6</td>
<td>46</td>
<td>PPH</td>
<td>HLT 1986</td>
<td>2.70 (77%)</td>
<td>4.26 (100%)</td>
<td>63%</td>
</tr>
</tbody>
</table>

Clinical characteristics of 6 lung transplant (T) patients. FEV\(_1\), forced expiratory volume in 1 s; FVC, forced vital capacity; PPH, primary pulmonary hypertension; CF, cystic fibrosis; HLT, heart-lung transplant; DLT, double lung transplant.
respiratory variables in normal and transplant subjects

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous VT, ml</th>
<th>Spontaneous Frequency, breaths/min</th>
<th>End-Tidal CO₂, Torr</th>
<th>Entrainment Range, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wakefulness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>420</td>
<td>10</td>
<td>34</td>
<td>7–14</td>
</tr>
<tr>
<td>T6</td>
<td>500</td>
<td>10</td>
<td>36</td>
<td>7–13</td>
</tr>
<tr>
<td>NREM sleep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplantation subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>550</td>
<td>15</td>
<td>40</td>
<td>15–20</td>
</tr>
<tr>
<td>T2</td>
<td>500</td>
<td>13</td>
<td>49</td>
<td>10–17</td>
</tr>
<tr>
<td>T3</td>
<td>750</td>
<td>13</td>
<td>40</td>
<td>12–17</td>
</tr>
<tr>
<td>T4</td>
<td>600</td>
<td>14</td>
<td>40</td>
<td>9–17</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>600 ± 108</td>
<td>14 ± 1</td>
<td>42 ± 2</td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>460</td>
<td>15</td>
<td>45</td>
<td>12–18</td>
</tr>
<tr>
<td>N2</td>
<td>600</td>
<td>12</td>
<td>40</td>
<td>10–13</td>
</tr>
<tr>
<td>N3</td>
<td>510</td>
<td>14</td>
<td>40</td>
<td>10–18</td>
</tr>
<tr>
<td>N4</td>
<td>400</td>
<td>16</td>
<td>43</td>
<td>12–18</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>493 ± 85</td>
<td>14 ± 2</td>
<td>42 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Tidal volume (VT) spontaneous frequency and end-tidal CO₂ during mechanical ventilation and the range of entrainment were tabulated for all subjects in all studied conditions. NREM, non-rapid eye movement; N, normal subjects.

reveal any difference in θ between transplant patients and normal subjects during wakefulness. Furthermore, θ did not consistently change as a function of ventilator frequency in either normal subjects or lung transplant patients. Just as in normal subjects, the θ in lung transplant subjects hovered around 0° as the machine rate increased or decreased relative to the spontaneous rate. It was our hypothesis that lung transplant subjects could not entrain to the ventilator during wakefulness or sleep. We did not feel compelled to study additional awake lung transplant subjects, because the ready occurrence of entrainment in the two lung transplant subjects disproved the first half of our hypothesis.

Effect of NREM sleep on entrainment responses. Figure 3 shows phase scatter plots from three representative sleeping subjects during mechanical ventilation. The data were taken from one normal subject (A) and from two lung transplant subjects (B and C). The normal subjects exhibited 1:1 entrainment around and above the spontaneous rate. As the ventilator rate was lowered below the spontaneous rate, the entrainment pattern bifurcated into 1:2 entrainment (one mechanical inflation for two neural efforts). This pattern was typical of the other normal subjects during NREM sleep in this study and that of Simon et al. (21). At a machine frequency of 12, there was only one significant concentration of θ, with a mean value of –63°. There were intermittent episodes of 1:2 entrainment, but these did not occur with sufficient frequency to reach statistical significance. This pattern of intermittent 1:2 entrainment and less well-focused entrainment phase angles is typical in the region of the bifurcation from 1:1 to 1:2 entrainment in normal subjects. The neural effort lagged the mechanical inflation (positive θ) at machine frequencies above the spontaneous rate and led mechanical inflation below the spontaneous rate (negative θ). There was a smooth transition from high ventilator rates and positive θ to low ventilator rates and negative θ in normal subjects, and θ was often close to zero at the spontaneous rate.

All lung transplant subjects demonstrated 1:1 entrainment at one or more mechanical ventilator frequencies but remained less able to entrain to the ventilator. The lung transplant subject whose θ data
Among the four transplant subjects, spontaneous rate without causing arousal from sleep. Entrainment at all frequencies except the spontaneous rate was significantly less in transplant subjects (P < 0.001), and, if entrainment did occur in transplant subjects, it was more likely to occur at ventilator rates below the spontaneous rate.

We constructed three-dimensional composite probability distribution curves that reflect the relative likelihood (z axis) of $\theta$ from $-180$ to $180^\circ$ (y axis) at each ventilator rate (x axis) expressed relative to the spontaneous rate for normal and lung transplant subjects (Fig. 4, A and B, respectively) during NREM sleep. In normal subjects, the $\theta$ were tightly concentrated (large concentration parameters and large relative probabilities) at each ventilator frequency. The trajectory from positive $\theta$ above the spontaneous rate to negative $\theta$ below the spontaneous rate was clear. There was also a clear bifurcation of probabilities $\sim 2$–3 breaths below the spontaneous rate, which reflected 1:2 entrainment. As a result, the distribution of probabilities resembled a discrete mountain ridge rising from a low plain, until low mechanical ventilator frequencies were reached, when a new ridge rose $\sim 180^\circ$ out of phase with the dominant ridge. In striking contrast, the composite relative probability distribution for lung transplant subjects started with a single well-formed ridge at mechanical frequencies above the spontaneous rate but degenerated into a low-lying ridge with outlying hills off the main ridge near the spontaneous rate and a flat plateau at ventilator frequencies below the spontaneous rate. These composite figures demonstrate that entrainment was less common in transplant subjects, especially at ventilator rates below the spontaneous rate. We analyzed the probability of entrainment using Fisher’s exact test and compared the frequency of entrainment across all ventilator rates examined in normal and transplant subjects. The probability of entrainment at any particular mechanical ventilator rate was significantly less in transplant subjects (P < 0.001), and, if entrainment did occur in transplant subjects, it was more likely to occur at ventilator frequencies above the spontaneous rate.

Fig. 3. Phase scatter plots from 3 representative sleeping subjects, 1 normal subject (A), and 2 lung transplant subjects (B and C), plotted as a function of the ventilator frequency. Subjects T2 (B) and T1 (C) demonstrated the least and most entrainment, respectively, among all transplant subjects. A 1-to-1 ratio of subject efforts to machine breaths often occurred at frequencies within 20% of the spontaneous rate, and a bifurcation to a 2-to-1 coupling ratio appeared at slower ventilator frequencies in normal subjects. Transplant patients never demonstrated 2:1 coupling and demonstrated 1:1 coupling only at machine frequencies above the spontaneous respiratory rate during sleep.

during NREM sleep are shown in Fig. 3B represents one extreme among the responses of the lung transplant subjects; this subject was the least able of any subject studied to establish stable entrainment to the ventilator. One-to-one entrainment was seen at only two machine rates above the spontaneous rate. In contrast to normal subjects, we saw no stable 1:2 entrainment in this or any other lung transplant subject. Figure 3C shows the other extreme of responses among transplant subjects. This subject established stable 1:1 entrainment at all frequencies except the spontaneous rate. We could not study any frequencies below the spontaneous rate without causing arousal from sleep. Among the four transplant subjects, $\theta$ tended to be positive at ventilator rates above the spontaneous rates and negative at ventilator rates below the spontaneous rates. However, the pattern was less consistent, and the trajectory of $\theta$ from positive to negative was less distinct than in normal subjects.

In respect to our method of defining entrainment, one may legitimately ask two questions. 1) Have we imposed entrainment order on data that were actually aperiodic? And, if no entrainment was present, were the data truly aperiodic? In Fig. 5, we plotted individual $\theta$ from sequential breaths at a single ventilator
frequency in the normal subject and for two ventilator frequencies in the transplant subject shown in Fig. 3, A and B, respectively. In the normal subject, we found 1:2 entrainment at this frequency (11 bpm), and inspection of the $\theta$-breath order plot reveals stable 1:2 entrainment that is consonant with the statistical analysis. A constant $\theta$ pattern across sequences of breaths was typical of stable entrainment in both normal and transplant subjects, and we do not believe that we imposed entrainment order where there was none. In respect to the second question, we studied only one VT, making our study a more limited exploration of VT and frequency effects on entrainment than that performed on anesthetized animals. We never saw aperiodic behavior in the normal subjects we studies, although Simon et al. (21) did see aperiodic behavior in a previous study of normal sleeping subjects. In the transplant subjects, we could not identify entrainment by statistical criteria at some ventilator frequencies, but the lack of stable periodic coupling between the ventilator and the subject’s inspiratory activity alone is not proof of aperiodic behavior in the sense used by previous investigators. Aperiodic behavior is described as breathing patterns in the transitional zones between stable entrainment ratios in vagally intact anesthetized animals in which inflation modifies neural activity but in irregular and unpredictable ways, i.e., the neural re-

Fig. 4. Composite probability distribution of phase angles at each ventilatory frequency plotted for normal (A) and transplant subjects (B). Ventilator frequencies are expressed relative to the spontaneous rate (equal to 0 for each subject), and, at each frequency, the total area under the probability curve is 1. Frequencies at which phase angles were densely packed about some mean angle show high probabilities at the mean phase angle, and probabilities that quickly drop to 0 moving away from the mean phase angle. Dips in the probabilities between breaths, moving along the respiratory frequency axis, are artifacts of the surface-fitting routine in Matlab. Note that the probability distribution is tight around the mean phase angle and bifurcates into two clear peaks at lower machine frequencies in the normal subjects (A). In contrast, the probability distribution is narrowly focused only at ventilator frequencies above the spontaneous rate. Below the spontaneous rate, the probability distribution of the transplant subjects (B) is widely dispersed, as opposed to the well-defined range of peaks in normal subjects.

Fig. 5. Individual phase plots, drawn as a function of breath order, plotted for a normal subject entrained to a ventilator in a 1-to-2 ratio (A) and a transplant subject showing no entrainment at 2 different ventilator rates (B and C). , Actual data; ..., predicted phase angles if respiratory rate was constant and completely independent of the ventilator rate.
spiratory rate and the ventilator rate were coupled but only weakly (15, 16).

In Fig. 5, B and C, the measured θ and the θ that would occur if the subject had a fixed respiratory frequency independent of and unaffected by mechanical inflations have been plotted together as a function of ventilator breath order. In Fig. 5B, for example, the ventilator rate was 11 bpm. If the subject’s respiratory rate were 13.7 bpm, then every breath taken by the subject would differ by a θ of 53°, and the subject would lag the ventilator by a consistent time and θ on each breath. The results of a similar calculation comparing predicted θ differences at a ventilator rate of 16 bpm and a neural ventilatory rate of 14.2 bpm is shown in Fig. 5C. The neural respiratory rates were chosen with malice aforesight to emphasize the similarity of the predicted and actual θ. Nonetheless, this modeling demonstrates that the θ relationship between two independent neural and mechanical ventilatory rates may fit the data reasonably well, particularly at the lower ventilator rate (Fig. 5B). Applying a similar analysis to other ventilator frequencies at which phase angles were not entrained often revealed patterns of θ relationships that imply the presence of two independent oscillators (as opposed to the weakly coupled oscillators that are present in aperiodic patterns). The neural frequency tended to be closer to the spontaneous rate, regardless of the ventilator rate when ventilator and neural events were not coupled. Hence, the θ duration of neural activity was consistently shorter than the ventilator cycle at low ventilator rates (Fig. 5B) and longer than the ventilator cycle at high ventilator rates (Fig. 5C). This analysis indicates that the lack of entrainment need not imply aperiodic behavior in the neural activity; the neural oscillator may be perfectly periodic but completely independent of the equally periodic mechanical inflation. Distinguishing between deterministic chaos (coupled oscillators with nonlinear dynamics) and stochastic noise (independent oscillators) requires an appallingly large amount of data (2). Unfortunately, we do not have enough data at each ventilator frequency to make these distinctions. We can only raise the possibility that the behavior was not aperiodic but a manifestation of two independent oscillators.

Assessing the quality of entrainment. As described above, entrainment was less likely to occur in transplant subjects, but we also tested the hypothesis that, when entrainment occurred, it was less well focused. To analyze the fidelity of entrainment, we pooled the standard deviations from each mean θ in which entrainment was present from all subjects within the normal and transplant groups. We compared four groups: lung transplant subjects during wakefulness and NREM sleep and normal subjects during wakefulness and NREM sleep. The data from the normal, waking subjects were taken from five subjects studied previously during wakefulness (21). The results of this comparison are shown in Fig. 6. A one-way ANOVA appropriate for periodic data revealed that significant differences existed among the groups, and unpaired tests between groups, using P values adjusted for multiple comparisons, indicated that the standard deviations in lung transplant subjects during sleep were significantly greater than in any other condition. Furthermore, the standard deviations were not different between normal and transplant subjects during wakefulness. Thus, when entrainment was present during NREM sleep, it was less accurately fixed to particular θ in the lung transplant subjects.

Effect of neural TI on timing between machine and respiratory cycles. The role of the vagus has been implicit in our description of entrainment in normal and transplant subjects. We explicitly examined the effect of mechanical inflation of the lungs on neural TI in all sleeping subjects. In Fig. 7, neural TI that was determined from the diaphragm EMG has been expressed as a function of the θ of muscle activity. When θ was positive, the mechanical inflations preceded neural activity, lung volume increased early in neural TI, inspiratory activity was terminated prematurely, and neural TI was shortened. This response is a manifestation of the inspiration-inhibiting Hering-Breuer reflex and requires vagal feedback. Shortening of TI at positive θ was seen in all four normal subjects. In contrast, TI remained constant at all θ in the lung transplant group. The capacity to entrain to mechanical ventilation varied slightly among the transplant subjects, but there was no evidence, based on the changes in TI shown in Fig. 7, that the lungs were more or less effectively denervated in particular subjects.

DISCUSSION

In multiple studies of anesthetized animals, vagotomy abolished entrainment to mechanical ventilation. This led to the hypothesis that vagal feedback was required for entrainment. The responses of the transplant subjects observed in the present study demonstrate that there is no absolute requirement for vagal feedback to produce entrainment either during wakefulness or sleep. The transplant subjects were clearly
less able to establish entrainment as the ventilator frequency deviated from each subject’s spontaneous frequency, and entrainment of neural respiratory activity, when present, was less tightly phase locked to the ventilator.

Entrainment during wakefulness. Entrainment in vagotomized patients during wakefulness was indistinguishable from entrainment seen in normal awake subjects by Simon et al. (21). Furthermore, the ranges of ventilator frequencies in which entrainment occurred were broader during wakefulness than during sleep in both normal and vagotomized lung transplant subjects. Hering-Breuer reflexes, which are thought to play an important role in entrainment (13, 15), are not readily demonstrable in awake humans (7, 9, 19, 20). Thus the ready entrainment of lung transplant patients to mechanical ventilation and the paucity of Hering-Breuer reflex control of ventilation in awake normal subjects lead us to conclude that other stimuli and reflexes promote respiratory entrainment in awake humans, regardless of the state of the vagi. Possible entraining stimuli range from simple auditory

![Fig. 7. Inspiratory time (TI) plotted as a function of phase angles for 4 normal subjects (A) and 4 transplant subjects (B) during sleep. Phase angles and TI from all ventilator frequencies for each subject were included. In normal subjects, TI shortened consistently as the phase angle moved from negative to positive values, which is consistent with the action of the Hering-Breuer reflex; however, there was no relationship between TI and the phase angle in transplant subjects, implying that the Hering-Breuer reflex was not present in the lung transplant subjects.](image-url)
cues from the ventilator to forebrain influences on respiratory control (5). Conscious or unconscious efforts to optimize the comfort of the ventilatory pattern may also contribute to each subject’s efforts to match neural respiratory timing to the mechanical events controlled by the ventilator. The power of entraining cues and the sense of comfort during wakefulness cannot be underestimated; the range of entrainment around the spontaneous respiratory rate was consistently greater during wakefulness than during sleep.

**Entrainment during sleep.** Conscious factors promoting entrainment are lost during sleep, but vagal reflexes are probably more active. In normal subjects during sleep, 1:1 entrainment was easily established and could be maintained at ventilator frequencies within ~15% of each subject’s spontaneous rate (compare Figs. 4A and 5A). Moreover, 1:2 entrainment was demonstrable in approximately one-half of the normal subjects at frequencies ~15–35% below the spontaneous rate (21). The pattern of 1:1 entrainment that bifurcated into 1:2 entrainment as the ventilator frequency was reduced progressively below each subject’s spontaneous rate resembles the entrainment responses of vagally intact anesthetized humans and animals. The influence of the Hering-Breuer reflex was apparent in normal subjects when we examined T1 as a function of θ (Fig. 7A). T1 was longer when neural inspiration led mechanical inspiration, and volume-related feedback increased late in T1 and in the T1-T2 transition (this occurs when the spontaneous rate is greater than the mechanical rate). T1 was shorter when mechanical inflation led neural inflation, resulting in increased volume feedback early in T1. Finally, a pattern of integral entrainment ratios identical to that seen in anesthetized animals was predicted from mathematical models that explicitly included the Hering-Breuer reflex as a volume-dependent, time-varying inspiratory off-switch and expiratory on-switch (15). For all these reasons, we believe that entrainment in normal sleeping subjects reflected a strong influence of the Hering-Breuer reflex. Therefore, we were surprised to see any evidence of entrainment in the lung transplant subjects. However, statistically significant entrainment occurred in all lung transplant subjects at one or more ventilator frequencies. Entrainment occurred at far fewer frequencies in the transplant subjects compared with the normal subjects. First, we never saw 1:2 entrainment in transplant subjects. Second, 1:1 entrainment in normal subjects generally occurred symmetrically, within ±2–3 bpm around the spontaneous rate, but entrainment was never seen below the spontaneous rate in the lung transplant subjects. In other words, the transplant subjects could increase their neural respiratory rate to match the mechanical ventilator, but they could not slow their neural respiratory rate when the ventilator rate was reduced. Entrainment at or close to the spontaneous frequency requires modulations of neural T1 and T2 that are probably too small for us to detect. We detected T1 modulation in normal subjects at entrainment rates 1–3 bpm different from the spontaneous rate and should have seen T1 modulation in the lung transplant subjects that entrained to machine frequencies 1–3 breaths above the spontaneous rate. However, there was no evidence of θ modulation of T1 in any of the transplant subjects, regardless of whether neural T1 preceded or followed the onset of mechanical ventilation. Because the transplant subjects did not change T1, they must have modulated T2. When entrainment occurred at ventilator frequencies greater than the spontaneous rate, it implied that the transplant subjects were able to shorten neural T2 because we saw no θ-related changes in T1. However, they were not able to lengthen T2 to establish entrainment at ventilator frequencies below the spontaneous rate. The entraining stimulus seemed to elicit a response that preferentially shortened T2 when the neural respiratory rate was slower than the mechanical ventilator frequency, but entraining stimuli were unable to sufficiently prolong T2 to establish entrainment at mechanical ventilatory frequencies slower than the spontaneous rate. Unfortunately, T2 data were not available to us to permit confirmation of this supposition.

Respiratory entrainment to mechanical ventilation may have occurred in the transplant subjects if the lungs were incompletely denervated. The anastomosis in a double-lung transplantation is in the distal one-third of the trachea, and the upper two-thirds of the trachea, the larynx, and upper airway are normally innervated. The trachea is richly innervated by the vagus; however, the trachea does not seem to provide a sensitive index of volume-related feedback (4). Furthermore, if vagal afferents arising from the trachea were important zeitgebers in the lung transplant subjects, we could have expected some changes in TV as a function of θ, but we found no evidence that vagal afferents remaining after transplantation modified TV. The relationship between the observed changes in TV and θ may be a relatively insensitive index of vagal afferent activity (although our data in normal subjects suggest the contrary), and regrowth of the vagus may have partially re-inervated the lungs. In a previous study, the Hering-Breuer reflex exerted a more powerful effect on T2 than on T1 (1), but the Hering-Breuer reflex was more effective in prolonging rather than in shortening T2. This response is not consistent with the asymmetrical T2 shortening that is necessary to explain the occurrence of entrainment only at ventilator frequencies greater than the spontaneous respiratory rate. Thus, as best we could tell, we found no evidence of Hering-Breuer reflex mechanisms in the lung transplant subjects.

There are other possible reflex mechanisms responsible for our findings. Upper airway afferents provide information related to airflow, temperature, pressure, and airway CO2 (23). Whereas these stimuli might provide entraining cues, none of the reflex responses to upper airway stimulation provides the asymmetrical control of T2 that is required to explain the pattern of entrainment in the transplant subjects. Chest wall afferents may also provide entraining stimuli, although chest wall reflexes usually inhibit phrenic activity and shorten T1 (17) and do not account for the
pattern of timing changes we observed in the lung transplant subjects. However, the respiratory rhythm was entrained to intercostal afferent information by repetitive electrical stimulation of intercostal nerves in anesthetized and vagally intact cats (18). Phrenic afferents may provide an entraining signal, but no specific reflex effects on respiratory timing have been described that fit the responses of the transplant subjects (6). Finally, arterial Po2 and Po2 fluctuate with each ventilatory cycle and may provide periodic cardioc hemosensory stimulation that is capable of entraining ventilation, but we know of no identified reflex arising from any of these stimuli that affects T1 and T2 in the way predicted to enhance entrainment in transplant subjects at ventilator frequencies above the spontaneous rate. However, we do recognize that afferent information from a variety of sources persists after lung transplantation and may provide effective cues to entrainment.

We saw entrainment, in an admittedly attenuated form, in sleeping lung transplant subjects. In contrast, vagotomy in anesthetized cats abolishes entrainment. Decerebrate, unanesthetized cats readily entrain to the ventilator, and vagotomy abolishes entrainment in this model as well. One might argue that sleeping humans are simply more sensitive to entraining stimuli than vagotomized animals that are anesthetized or decerebrate. However, it seems more likely that sleep does not reduce central nervous system sensitivity to afferent stimuli as completely as anesthesia. The lack of entrainment in decerebrate cats argues that the brain stem alone cannot support entrainment in the brain stem alone cannot support entrainment in the absence of vagal feedback. Therefore, entrainment during NREM sleep in the lung transplant subjects may originate from some suprapontine, but subconscious integration of respiratory-related afferent information.

In summary, we studied entrainment to mechanical ventilation during sleep in normal subjects and vagotomized lung transplant patients. The transplant subjects entrained well during wakefulness. Furthermore, they demonstrated significant entrainment during sleep at ventilatory frequencies equal to or greater than the spontaneous respiratory rate. However, entrainment in transplant subjects occurred over a narrower range of mechanical ventilator frequencies than in normal subjects, and, when entrainment did occur, the standard deviations around each entrained δ were larger in transplant subjects. We conclude that there is no absolute requirement for vagal feedback to induce entrainment in sleeping subjects; this is in striking contrast to anesthetized animals in which vagotomy uniformly abolishes entrainment. On the other hand, vagal feedback clearly enhances the fidelity of entrainment and extends the range of mechanical frequencies in which entrainment can occur.

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