Selective loss of high-frequency oscillations in phrenic and hypoglossal activity in the decerebrate rat during gasping

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Marchenko, Vitaliy, and Robert F. Rogers. Selective loss of high-frequency oscillations in phrenic and hypoglossal activity in the decerebrate rat during gasping. Am J Physiol Regul Integr Comp Physiol 291: R1414–R1429, 2006.—Respiratory motor outputs contain medium- (MFO) and high-frequency oscillations (HFO) that are much faster than the fundamental breathing rhythm. However, the associated changes in power spectral characteristics of the major respiratory outputs in unanesthetized animals during the transition from normal eupneic breathing to hypoxic gasping have not been well characterized. Experiments were performed on nine unanesthetized, chemodenservated, and barodenervated, decerebrate adult rats, in which asphyxia elicited hyperpnea, followed by apnea and gasping. A gated fast Fourier transform (FFT) analysis and a novel time-frequency representation (TFR) analysis were developed and applied to whole phrenic and to medial branch hypoglossal nerve recordings. Our results revealed one MFO and one HFO peak in the phrenic output during eupnea, where HFO was prominent in the first two-thirds of the burst and MFO was prominent in the latter two-thirds of the burst. The hypoglossal activity contained broadband power distribution with several distinct peaks. During gasping, two high-amplitude MFO peaks were present in phrenic activity, and this state was characterized by a conspicuous loss in HFO power. Hypoglossal activity showed a significant reduction in power and a shift in its distribution toward lower frequencies during gasping. TFR analysis of hypoglossal activity revealed the increasing importance of an initial low-frequency “start-up” burst that grew in relative intensity as hypoxic conditions persisted. Significant changes in MFO and HFO rhythm generation during the transition from eupnea to gasping presumably reflect a reconfiguration of the respiratory network and/or alterations in signal processing by the circuitry associated with the two motor pools.

FAST RHYTHMS OCCUR IN A VARIETY of neural systems at various levels including cortex, thalamus, brain stem, and spinal cord (14). The study of such rhythms is expected to provide insight into neural interactions that produce physiologically important output patterns. Fast oscillatory rhythms are present in all respiratory muscles, nerves, and central neurons in mammals, including humans (1, 11, 13, 40, 45). These and many other studies have shown the presence of at least two general fast respiratory output rhythms present in cat or rabbit phrenic nerve records: medium frequency oscillations (10–50 Hz, MFO) and high-frequency oscillations (50–100 Hz, HFO).

Although much of the early work in fast oscillations in respiratory motor nerves was conducted in cats, rodents have become a popular experimental model for respiratory rhythmogenesis. However, a limited number of experiments have examined HFO in rodents (24, 29). These in vivo studies reported that MFO and HFO in rodents are at frequencies twice those in cats and rabbits (1, 11, 44), and they are significantly higher than those in in situ perfused juvenile rats (47, 51) and in vitro neonatal cat preparations (23).

MFO, and especially HFO, are very sensitive to anesthesia, as evidenced by prominent spectral peaks in unanesthetized, decerebrated animals (10). Despite the depressive effects of anesthesia and popularity of the rodent model, to date only one study has used decerebrate rats (29) during normal eupnea, and no studies have been published regarding fast respiratory motor output rhythms in decerebrate adult rats during anoxia-induced behaviors in vivo. Therefore, in the present study we used precociously-decerebrated, unanesthetized adult male rats to characterize the changes in fast respiratory motor outputs rhythms between eupneic and mild to severe hypoxic/hypercapnic states in adult rats.

Respiratory responses to hypoxia in mammals typically progress through four phases: hyperpnea, primary apnea, gasping, and terminal (or secondary in case of successful resuscitation) apnea (16). Gasing, as a distinct motor behavior or functional state, is a very important mechanism of auto-resuscitation in infant and adult mammals, including human (35), rats (15, 18), and mice (22).

Despite its physiological importance, the frequency composition of phrenic nerve activity during gasping is not resolved. On the basis of results using fast Fourier transform (FFT)-derived parametric analysis in decerebrate cats, St-John (49) suggested that two hallmarks of gasping (compared to eupnea) are the shift of HFO to even higher frequencies and a marked decrease in MFO power. However, other studies (e.g., 2), using autoregressive nonparametric spectral and wavelet analyses, reported a shift in HFO toward lower frequencies, as well as high power in MFO, during gasping in anesthetized adult cats and young piglets. This discrepancy may be explained by preparation (decerebrate vs. anesthetized), analytical methods (parametric vs. nonparametric), or even recording method (mono- vs. biphasic). Because biological signals are often nonstationary in character, other nonparametric methods have been applied for spectra estimation of respiratory output frequencies, including time-variant wavelet analyses (e.g., 3) and time-frequency representation (TFR) smoothed pseudo-Wigner-Ville distribution (SPWVD; see Ref. 32). The latter study only analyzed diaphragmatic electromyogram (EMG) and hypoglossal nerve recordings in the anesthetized mouse, and time-frequency components of phrenic discharge were not described. Therefore, the time-frequency representations of the physiological significance of these oscillations during gasping need to be revealed.

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phrenic nerve activity in unanesthetized adult rats during eupnea and gasping have not been characterized.

In light of these unknowns, the two major goals of the following study are 1) to definitively characterize the dynamics of the frequency components of the phrenic and hypoglossal nerves in the adult rat, without the effects of peripheral feedback and anesthesia; and 2) to resolve the discrepancies regarding the frequency components in the phrenic nerve during severe hypoxia/hypercapnia-induced behaviors, particularly gasping. To achieve these goals, we applied classic time-invariant FFT, and a new FFT-based TFR analysis, developed in our laboratory. Using these methods, we tested the hypothesis that gasping is characterized by a shift in HFO toward higher frequencies and a reduction in MFO in the phrenic discharge. In addition, we applied these methods to the hypoglossal nerve, as this is a prominent tool used for respiratory rhythm detection in in vitro (slice or en bloc) studies to monitor network state.

MATERIALS AND METHODS

Animal Preparation

General surgical preparation. All procedures were approved by the University of Delaware Institutional Animal Care and Use Committee. Experiments were performed on nine adult, male Sprague-Dawley rats (340–380 g). Spontaneously breathing animals were anesthetized with isoflurane vaporized in O2 (MDS Matrix; 4–5% induction, 1–2% maintenance). A tracheotomy was performed via a ventral approach, and animals were intubated with a tracheal glass tube, after which they were artificially ventilated (60 cycles/min, 2.5–3.0 ml tidal volume; Columbus Apparatus) with the same gas mixture. The femoral artery and saphenous vein were then cannulated for measurement of arterial pressure and infusion of drugs, respectively. Arterial and tracheal cannulae were connected to pressure transducers (CDXII, Argon Medical) for monitoring blood pressure (BP) and inflation pressure using conventional amplifiers (Gould Statham). Signals from three small subcutaneous electrodes were amplified and filtered (Neurolog, Digitimer, Hertfordshire, England) and used to monitor ECGs via an audio amplifier (model AM10; Grass Instruments). The level of anesthesia was determined by the absence of flexor reflexes and changes in heart rate and blood pressure in response to pinches of the distal hind limbs. End-tidal CO2 was maintained between 4.5 and 5.5% (Capstar CWE) by adjusting minute volume, and rectal temperature was maintained at 37.0 ± 0.4°C via a servo-controlled heating blanket coupled to a rectal thermometer (Harvard Apparatus), during all surgical procedures. Left and right phrenic (Ph) and the medial branches of the XII nerves were placed on bipolar silver electrodes, and a positive end expiratory load of 1.5 cm H2O applied. With the rat in the supine position, the Ph nerves and the medial branches of the XII nerves were placed on bipolar silver electrodes and immersed in a mineral oil pool formed by skin flaps. Monophasic recordings (0.5–5.000 Hz; Neurolog, Digitimer) of the different activity were obtained after crushing the peripheral end of each nerve between the recording electrodes. The four neurograms, expiratory CO2 level, arterial blood pressure, ECG, and intratracheal pressure were all recorded on the hard disk of a PC at a sampling rate of 10,000 Hz/channel using a 16-bit analog-to-digital converter system (AD Instruments).

Gasp induction. Asphyxia is the most common means of inducing progressive hypoxia/hypercapnia in laboratory animals (38) and was used in the present study. Asphyxia was produced by shutting off the ventilator, and responses occurred in three phases. The first response to asphyxia (Ph and XII nerve hyperactivity) was observed just after cessation of ventilation. Hyperpneic bursts with interburst intervals longer than control (i.e., eupnea) were omitted from analysis. The next response to asphyxia was complete silence of any nerve activity. Following this silent phase, gasps were observed (see Fig. 2). Asphyxia was induced after 15 min of control nerve recording during eupnea.

Data Analysis

Spectral analysis. Ph and XII inspiratory (I) and expiratory (E) tags, indicating the start and the end of inspiratory activity (I-phases) for each nerve, were obtained from digitally integrated signals generated from the raw neurograms (τ = 50 ms). I- and E-tag demarcations were obtained using level discrimination and verified manually. All nerve signals were digitally filtered by a low-pass (300 Hz) and high-pass (10 Hz) filters with a stop band at 450 Hz (40-dB attenuation at 345 Hz). Carefully avoiding ground loops and eliminating electrical line noise obviated the need to use a 60-Hz notch filter.

Custom-written software, based on the FFT algorithm, was created in the Spike2 software environment (Cambridge Electronic Design). Script languages were developed in our laboratory for estimating “gated” (only epochs during inspiration) power spectra for all nerves. Each I-phase started at the onset of I-phase (I-tag) and ended at the offset of I-phase (E-tag). This procedure allowed us to analyze inspiratory and expiration data separately.

To calculate the power spectra from the neurograms of the four different nerves, we used equal-duration epochs (FFT window) and FFT resolution. The FFT-window width was less than the longest mean XII inspiratory duration during eupnea (Table 1), which corresponded to 4.096 data points (i.e., 409.6 ms, at a frequency resolution of 2.44 Hz/bin). All data were analyzed with a 50% overlap of the FFT window. In cases in which a 50% overlap extended past the end of the inspiratory burst, we used two windows; one starting at the beginning of the burst and one ending at the end of the burst. This prevented loss of spectral information from the part of nerve activity in excess of the FFT window. Inspiratory bursts shorter than 409.6 ms were padded with zeros. Results from overlapping regions (FFT window positions) of the burst were averaged. No standard window filtering functions (e.g., Hanning or Hamming) were applied.

Mechanical artifact suppression. To exclude the influence of cardiovascular mechanical artifacts on the nerve recordings, we used “partial autospectral” analysis (e.g., Ref. 27). Partial autospectral

infused with 5.0% dextrose in 0.9% saline (1.0–1.5% body weight or 3.5–5.0 ml/h) to maintain a mean BP of at least 100 mmHg.

Recording. To prevent peripheral influences on motor nerve outputs, all animals were baro- and chemoreceptor denervated via bilateral transection of the vagus (just below the nodose ganglion) and carotid sinus nerves. This ensured that responses to hypercapnia and hypoxia would be via central chemosensation (4). Bilateral pneumothorax was performed before recording to eliminate lung inflation-related movement artifacts, with a positive end expiratory load of 1.5 cm H2O applied. With the rat in the supine position, the Ph nerves and the medial branches of the XII nerves were placed on bipolar silver electrodes and immersed in a mineral oil pool formed by skin flaps.
Phrenic, ms 263 ± 22
XII, ms 581 ± 57
XII-pre-Phrenic, ms 279 ± 26
RR, cycles/min⁻¹ 59.15 ± 0.03
Anoxic phases, s N/A
F Phrenic, % control N/A
F XII, % control N/A

Table 1. Differences in respiratory parameters of phrenic and hypoglossal (XII) nerves during eupnea and hypoxia/hypercapnia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Eupnea</th>
<th>Hyperpnea</th>
<th>Silent Phase</th>
<th>Gasping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phrenic, ms</td>
<td>263 ± 22</td>
<td>288 ± 31</td>
<td>N/A</td>
<td>224 ± 28</td>
</tr>
<tr>
<td>XII, ms</td>
<td>581 ± 57</td>
<td>874 ± 94</td>
<td>N/A</td>
<td>233 ± 32</td>
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<tr>
<td>XII-pre-Phrenic, ms</td>
<td>279 ± 26</td>
<td>581 ± 43</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>RR, cycles/min⁻¹</td>
<td>59.15 ± 0.03</td>
<td>71.16 ± 5.11</td>
<td>N/A</td>
<td>48.69 ± 7.26</td>
</tr>
<tr>
<td>Anoxic phases, s</td>
<td>N/A</td>
<td>81.83 ± 11.62</td>
<td>55.25 ± 9.74</td>
<td>N/A</td>
</tr>
<tr>
<td>F Phrenic, % control</td>
<td>N/A</td>
<td>+ 20.34 ± 2.54</td>
<td>N/A</td>
<td>− 16.86 ± 1.81</td>
</tr>
<tr>
<td>F XII, % control</td>
<td>N/A</td>
<td>+ 19.52 ± 2.81</td>
<td>N/A</td>
<td>− 34.35 ± 2.55</td>
</tr>
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</table>

Value are presented as means ± SE. Phrenic, XII-pre-Phrenic, and XII: averaged duration of inspiratory discharge for phrenic, pre-Phrenic hypoglossal burst, and entire hypoglossal burst. RR: respiratory rate. Anoxic phases: duration of different anoxic phases (hyperpnea, silent phase, and gasping). f, changes in amplitude of integrated nerve activity (+, percent increase; −, percent decrease) compared to control. N/A, not applicable.

Time-frequency analysis: sliding zero-interval subtraction method.

To address nonstationarities associated with changes in state, we developed time-frequency methods for power spectra estimation. Different software applications generate TFR using either a parametric FFT-based method (e.g., spectrogram.m by MATLAB) or non-parametric methods (e.g., SPWVD). Given our application, the various time-frequency analysis programs that are currently available proved suboptimal. First, the various SPWVD-based algorithms are very slow. Second, some methods (e.g., spectrogram.m) are not valid for computing low-frequency bands. Third, all methods yield results with varying degrees of band widening. Because we needed to analyze a large amount of data (derived from thousands of phrenic and hypoglossal inspiratory bursts), we required a fast, accurate method.

To achieve this, we used an FFT-based MATLAB function (pwelch.m) for estimating power spectra density, and then created a novel TFR algorithm, which we term the zero-interval subtraction (ZIS) method. As depicted in Fig. 1C, ZIS calculates the difference between spectrum S2 (calculated from an epoch of data, including a zeroed segment) and spectrum S1 (calculated from the same epoch, but without a zeroed segment). The number of segments (N) depended on the length of the signal (S, data points), sampling rate (Hz), on the desired low-frequency limit for analysis \( f_{low} \) (set to 10 Hz), and on a constant, \( k \), which allows for scaling of the zeroed segment:

\[
N = \text{round}(k \times f_{low} \times S/(2 \times SR))
\]

(2)

The width of the zeroed sliding segment, which was kept approximately constant \( k = 10 \), resulting in 190–210 data points for the zeroed segment; varied due to rounding to nearest integer), allowed for accurate and consistent low-frequency estimates down to 10 Hz. The length of the segment can be derived by dividing \( S \) by \( N \). The zeroed segment was advanced in steps one-half of its width (i.e., 50% overlap). This enabled us to analyze adjacent, overlapping data segments. At each temporal position of the zeroed window, spectra S1 and S2 were calculated, and the appropriate subtraction was performed. The “difference spectra” \( \Delta S \) were stored in two-dimensional matrices containing the values of the amplitudes at each frequency vs. temporal position. Because we slid the zeroed segment with 50% overlap, 2N-1 power spectral vectors (time points) per inspiratory burst were created in positions normalized relative to the inspiratory period. The individual vectors at the same time position were averaged over all the analyzed bursts, producing a 2N-1 \( \times 164 \) array \((0–400.16 \text{ Hz at } 2.44 \text{ Hz/bin frequency resolution})\). Thus all ZIS data TFR is presented in normalized time axes ranging from burst onset \((t = 0)\) to the end of the burst \((t = 1)\), although we typically extended past this point to analyze postinspiratory activity. These matrices were then graphed as standard isocountour plots, with smooth pseudocolored interpolation. TFR results were grouped and averaged according to state (eupnea, hyperpnea, and gasping) both within individual animals and across all animals.

Evaluation of TFR method. We tested all available TFR-related software applications, including ZIS, using a 600-ms-long sinusoidal signal, created with 0.1-ms resolution and containing three pure frequencies: 10 Hz (start to 200 ms), 10 and 80 Hz (200–600 ms) and 10, 80, and 160 Hz (400–600 ms), all with the equal amplitudes (set to 1.0 as default in arbitrary units, AU), as shown in Fig. 1A1. The results of the parametric FFT-base power spectral estimation are given in Fig. 1A2 where the relative amplitude of each spectral peak reflects the running time for each frequency. The results of the ZIS TFR method are provided in Fig. 1B1 and match well to the original signal components. In addition, ZIS works ~250 times faster (5.041 vs. 1,260.25 s) than SPWVD [Time-Frequency Toolbox for MATLAB developed by Centre National de la Recherche Scientifique (France) and by Rice University, Digital Signal Processing Group, http://gdr-isis.org/Applications/tfb/iutsn.univ-nantes.fr/auget/tfbftp.html] and 5.4 times faster (5.041 vs. 27.2214 s) than WaveMetrics’ “wigner” function. This slower performance is due to algorithm implementation. The reconstructed (i.e., averaging at each frequency in the time dimension) ZIS power spectral estimate is provided in Fig. 1B2 (2.44 Hz/bin resolution). This result compares favorably with the FFT-based method (Fig. 1A2) in that the ZIS-derived peaks are narrower at the three frequencies of interest. Another very important advantage of the ZIS method (compared to SPWVD and wavelet analysis) is its ability to perform gated TFR on whole bursts of different durations.

Our analysis of the ZIS algorithm performance shows excellent TFR resolution at zero segment lengths greater than 1/32 (128 points or 3.125%) of the FFT window width (4,098 data points). To keep time resolution constant, the number of zeroed segments varied depending on inspiratory burst length. For example, zeroed segments were between \( 1/111 \)th (gasp) and \( 1/15 \)th (hyperpnea) the length of the FFT window for Ph bursts. Expanding the zeroed segments increases the accuracy of low-frequency estimates at the expense of temporal resolution. For this reason, the ZIS method is more applicable at faster sampling rates (10 kHz in our case). The ZIS produced results qualitatively similar to those produced by SPVWD methods (not shown).
The ratio of the amplitude spectra $S_1$, $S_2$, and $\Delta S$ are shown on Fig. 1D. The relative ratio between the result spectra ($\Delta S$) and the control spectra $S_1$ supports the validity of the ZIS method, as the ratios for all the subtracted frequencies are almost identical (Fig. 1E). This provides strong evidence of the linearity of the ZIS algorithm.

Statistical analysis. The data were obtained from an ensemble of $n$-phases ($n > 20$ for eupneic breathing and $n > 10$ for hyperpnea and for gasping). To demonstrate a consistent trend within a given state, the power spectra were averaged over the number of breaths analyzed within that state for a given animal. To identify consistent global trends within the three states, the spectra were grouped into eupneic, hyperpneic, or gasping categories and averaged over all animals. Power spectra were reconstructed from the ZIS results and were compared qualitatively to FFT-calculated spectra. We also calculated the power spectrum during the expiratory phase of nerve activity and used this as the “background” value. Data are presented as means ± SE, and a $P$ value < 0.05 obtained from any statistical tests was interpreted as significant, as was a 95% confidence level. To evaluate the statistical significance, the lower confidence value was computed for each frequency bin and was compared with the upper confidence.
RESULTS

Bilateral recordings of Ph and XII (medial branch) electrical activity were made in nine peripheral baro- and chemodenerated, precocillarily decerebrated, paralyzed rats that were maintained under artificial ventilation without anesthesia. The results reported are based 928 eupneic, 386 hyperpneic, and 186 gasping bursts produced by nine rats. The general characteristics such as respiratory rate, burst amplitude and duration, length of hyperpnea, primary apnea, and gasping are described in Table 1. After cessation of ventilation, three prominent phases of anoxic response were observed in seven animals: initial hyperpnea (H, Fig. 2), silent phase (S, Fig. 2) and gasps (G, Fig. 2). One animal did not produce any gasps, and the final one produced fewer than 10 gasps. In the remaining seven rats, the above sequence of changes in nerve activity was noted, a typical example of which is shown in Fig. 2 (expanded traces for details).

Changes in Phrenic Power Spectra from Eupnea to Gasping

Power spectral analysis was used to characterize the changes in fast rhythms within the Ph activity during different behavioral states. We considered both parametric (FFT) and nonparametric (ZIS) analyses of Ph bursts in the frequency domain (see MATERIALS AND METHODS). All statistics regarding maximal Ph spectral peaks and band ranges during eupnea, hyperpnea, and gasping are provided in Table 2. Collapsing the ZIS plots in the time dimension provides an average power over the entire breath (Fig. 3A1–A3; Fig. 4A1–A3, dashed lines), and this result is superimposed on the original FFT-estimated autospectrum (Fig. 4A, thick lines; error bars not shown for clarity) to direct comparison between the two methods.

Eupnea. Figure 3A1 shows the Ph power spectra for individual animals during eupnea. From these overlaid spectra, two major features are consistent across animals: one peak each in the MFO and HFO bands. Across animals, MFO was larger, but not statistically significantly so. During eupnea, the most prominent ZIS-reconstructed averaged power spectra peaks are located at the 78.1 and 175.8 Hz (see Table 2 for frequency bands). The lower band was categorized as MFO, and the higher band as HFO. Across all animals, the time-averaged ZIS results fit the FFT-estimated spectra well (Fig. 4A1, dashed vs. solid line), with the former displaying more distinct separation between the peaks. The ZIS TFR results, shown in Fig. 4B1, indicate that HFO begins just after the onset of Ph activity, reaching maximal values by 1/5 of the way through the burst, and maintaining these high levels until ~2/3 of the way through the inspiratory cycle. MFO activity begins later, steadily increases in intensity from 1/4 through 1/2 of the burst, and reaches sustained maximal values from 1/2 to 3/4 of the way through the cycle. Although MFO had greater maximum value than HFO, the difference in their amplitudes averaged over the whole cycle was not statistically significant. Taken together, these results demonstrate that the incrementing discharge pattern in the Ph neurogram during eupnea (Fig. 2, bottom, E) results from a dramatic increase in MFO overlaid upon a steady, early-starting and frequency-broadening HFO. In addition, it emphasizes the rather broad frequency power contributions. All frequencies above 40 Hz were significantly greater than expiratory control (Fig. 4A1, bottom).

In addition, the onset of inspiration was typically characterized by a very brief (i.e., single cycle) discharge, with duration corresponding to low frequencies (25–70 Hz; low MFO) that is evident in the Ph burst shown in Fig. 4C1 (*). This brief feature is barely detected by the TFR analysis over all animals (Fig. 4B1), because it is short lived and not present in every eupneic breath. However, this feature is obvious under digital integration (Fig. 4C1, histogram).

Hyperpnea. Figure 3A2 illustrates the variability in ZIS-derived power spectra between animals during hyperpnea, which display relative consistency in that all contain at least one MFO (usually MFO2), one HFO, and one upper-HFO peak (UHFO). The average power spectra (7 animals, 386 inspiratory bursts) estimated by both FFT- and ZIS-based methods (Figs. 4A2) reveal more complexity in their spectral structures than during eupnea, as does the TFR analysis (Fig. 4B2). This is, in part, due to interanimal variability, but also to the presence of more peaks per spectra in individual animals (Fig. 3A2) than during eupnea (Fig. 3A1). The lowest major frequency band (53.7–151.4 Hz) in the power spectrum was categorized as MFO with two peaks, at 85.5 and 122.1 Hz (see Table 2 for summary). When calculated using FFT-based analysis, only one MFO-related maximal peak was detected (Fig. 4A2, solid line). This FFT-estimated MFO peak was not significantly different in frequency than the single MFO peak during eupnea using either FFT or ZIS analysis. The HFO peak (187 Hz) is slightly (but not statistically significantly) larger than the MFO peak. Another conspicuous difference between the hyperpnea and eupnea states is the presence of a higher, less powerful band (224.6–283.2 Hz) detected by both methods, but more clearly shown by the ZIS reconstruction. This was classified as UHFO (see Ref. 32).

Unlike the case during incrementing eupnea, the ZIS analysis shows that the characteristic “bell-shaped” hyperpnea burst (Fig. 2, bottom, H) consists of a temporally complex combination of power at various frequencies. A lower MFO band (50–90 Hz, Fig. 4B2), otherwise hidden in time-averaged ZIS and FFT representations (Fig. 4A2 and 4C2, shading and *), dominates the earliest portion of the Ph activity. The startup complex now consists of 2–3 cycles of low-frequency oscillations, as labeled in the Ph neurogram (Fig. 4C2, *). The power in this band is short-lived, mostly dissipating by ~1/5th of the way through the burst. Beginning just after the start of the burst, a band of HFO (180–220 Hz) increases and sustains the activity through more than 2/3 of the burst length. The two prominent MFO bands make significant contributions starting
Fig. 2. Archetypical respiratory motor responses to asphyxia. Upper seven traces, from top: BP, blood pressure (mmHg); ITP, intratracheal pressure (mmHg); RR, respiratory rate; Ph, phrenic nerve (mV); XII, hypoglossal nerve (mV); $f_r$, integrated nerve activity (time constant = 50 ms). Arrow indicates cessation of ventilation. Expanded traces: nerve activity during eupnea (E), hyperpnea (H), and gasping (G) bursts. Bottom: across-animal averaged and normalized integrated Ph (black) and XII (gray) activity in the three states examined.

Table 2. Average ZIS-reconstructed phrenic power spectral peaks under three conditions

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<thead>
<tr>
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<th>E</th>
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<tr>
<td><strong>MFO</strong></td>
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<td>Maximal peak, Hz</td>
<td>78.1</td>
<td>85.5</td>
<td>122.1</td>
<td>41.5</td>
<td>87.9</td>
<td>175.8</td>
<td>187.0</td>
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<tr>
<td>Amplitude, AU $\times 10^{-1}$</td>
<td>7.97 ± 0.81</td>
<td>10.56 ± 1.15</td>
<td>9.34 ± 1.23</td>
<td>2.31 ± 0.29</td>
<td>2.46 ± 0.32</td>
<td>6.96 ± 0.74</td>
<td>11.44 ± 1.26</td>
</tr>
<tr>
<td>Band, Hz</td>
<td>36.6–109.7</td>
<td>53.7–102.5</td>
<td>109.9–151.4</td>
<td>17.1–58.6</td>
<td>65.9–129.4</td>
<td>156.3–229.5</td>
<td>158.7–214.8</td>
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<td><strong>HFO</strong></td>
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<td><strong>UHFO</strong></td>
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Values are presented as means ± SE. E, eupnea; H, anoxic hyperpnea; G, gasping; AU, arbitrary units; MFO, medium-frequency oscillations; HFO, high-frequency oscillations; UHFO, upper HFO.
~1/3 of the way through the cycle and continuing through to the end, resulting in lengthening of the plateau of the “bell-shaped” activity pattern, and abruptly ending (Fig. 4B2).

Gasping. The most conspicuous changes in the power spectra of Ph activity occurred during gasping. Figure 3A3 demonstrates the qualitative consistency in the power spectra across all seven animals that produced >10 gasps. In particular, the majority of the power is contained within two bands (MFO1 and MFO2), although there is variability in the relative distribution between the two. When averaged across all animals, the FFT and ZIS-based analyses (Fig. 4A3) show that almost all of the power was delivered via the bimodally distributed MFO band, with a marked reduction or elimination of higher MFO and HFO power. All power above 120 Hz, including the HFO-related band (161.7–219.3 Hz), was statistically indistinguishable from expiratory background levels (Fig. 4A3, bot-
Again, the ZIS-reconstructed spectrum shows more pronounced separation of the peaks (41.5 and 87.9 Hz) than does the FFT-derived average power spectrum, indicating the benefits of a nonparametric analysis. The TFR highlights the temporal structure of the power carried by these two distinctive MFO sub-bands, with the higher-frequency, broader sub-band beginning later in the breath, and carrying more sustained power through the first half of the burst (Fig. 4B3). Thus the rapidly rising shape of the gasp (Fig. 2, bottom, G) is initiated by the even lower MFO band (compared to eupnea and hyperpnea), and this is evident in the “start-up” component of the neurogram (Fig. 4C3, *), which shows 2–4 low-frequency oscillations in its activity. This is supplemented and eventually dominated by the higher-MFO band, and the majority of the power in both is quenched by 55% of the burst, with a modest resurgence of the low-MFO band from 70 to 100% of the way through the burst (Fig. 4B3).

Both MFO and HFO activity is readily apparent in the raw data during eupnea and gasping, shown in Fig. 5.

Changes in Hypoglossal Power Spectra from Eupnea to Asphyxia

XII Power Spectra were quantified using the same methodological approach applied to Ph data. Figure 6 is organized in exactly the same manner as Fig. 4B and 4C, except that we

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Fig. 4. Averaged Ph power spectra during three different states, estimated by FTT and ZIS methods. A1–A3: averaged power spectra for eupnea (A1), hyperpnea (A2) and gasping (A3) estimated by FFT (solid line, left y-axis, V^2) and ZIS time-averaged power spectrum reconstruction (dashed line, right y-axis). Dashed gray line at the bottom of each graph shows the 95% confidence level during background (expiratory) activity. Lower plot shows bin-by-bin P values (P = 0.05, dashed line) between spectra and expiratory activity. B1–B3: across-animal averaged and smoothed isocontour plot of TFR, estimated by ZIS, for eupnea (B1), hyperpnea (B2), and gasping (B3). Time duration is normalized to breath lengths within each category. Color bar in B1–B3 and right y-axis in A1–A3 in arbitrary units (AU). C1–C3: examples of single Ph bursts during eupnea, hyperpnea, and gasping, respectively, highlighting early MFO “start-up” component (*) and complexes (marked above trace). Traces include discrete digital integration (histogram), leaky integrator (τ = 50 ms; smooth line), and raw neurogram (bottom). Shading and vertical lines in A denote frequency bands and peaks estimated from time-averaged ZIS.
include a marker in the TFR results (black vertical lines, Fig. 6B1 and 6B2) indicating the onset of the phrenic burst. The end of XII burst coincided very closely to that of the Ph in all breaths analyzed.

**Eupnea.** Figure 3B1 shows the XII power spectra for individual animals during eupnea. These overlaid spectra demonstrate the consistency of the major features (multiple peaks at specific locations) across all animals. As shown on Fig. 6A1, FFT and ZIS-based averaged XII power spectra during eupnea were broadband and pyramidal-shaped, with constituent frequencies ranging from 50 to 300 Hz. The maximal power spectral value is located at 187 Hz (Table 3). There are other smaller peaks with maxima at 148.5 and 229.5 Hz, respectively (see Tables 3 and 4). The time-averaged ZIS results (dashed line, Fig. 6A1) coincide well with the FFT estimates (solid line).

The results of TFR analysis reveal broad, medium, and higher frequency components in the portions of the XII bursts preceding the onset of Ph activity (left of black vertical line in Fig. 6B1). These frequencies increase nearly uniformly as the burst proceeds from pre-Ph to Ph-related activity, with the majority of power contained in 150–250 Hz frequencies. There was one uniquely Ph-related band (120–140 Hz) in XII activity (Fig. 6B1).

**Hyperpnea.** Figure 3B2 illustrates the variability in ZIS-derived XII power spectra between animals during hyperpnea, which are relatively consistent in the low and medium frequency features and more variable in the high frequencies. Figure 6A2 illustrates the results of FFT-based (solid line) and ZIS-reconstructed (dashed line) XII analyses, demonstrating that, with Ph activity, there is a marked increase in overall power (note y-axis scale in Fig. 6A1 vs. 6A2). The XII activity during hyperpnea is distributed in a bell-shaped manner. Under this condition, there are three major XII bands (each partially split between pre-Ph and Ph-related parts, Fig. 6B2), with increasing peak magnitudes located at 92.3, 136.7, and 185.5 Hz (see Tables 3 and 4).

TFR analysis reinforces the dramatic increase in pre-Ph duration of XII activity during hyperpnea (Fig. 2, bottom, H, and 6B2). In addition to duration, two qualitative differences are evident when comparing the pre-Ph XII activity in hyperpnea with eupnea: 1) the appearance of an early component at 70–110 Hz; and 2) the progression of frequency recruitment from lower to higher frequencies as the pre-Ph burst advances in time. Together, these create the ramping and plateau-like pre-Ph portion of the XII burst. During Ph-related periods (i.e., to the right of the vertical line in Fig. 6B2), active bands continue to make contributions, and appear to quench in reverse order from recruitment, with progressively lower frequencies ceasing later (70–110 Hz bands excepted).

**Gasping.** During gasping, the averaged XII power spectrum is also asymmetrical and broadband, skewed even more toward lower frequencies (Fig. 6A3). Power in multiple frequency bands were detected, with the largest peak at 109.9 Hz and two smaller peaks at 158.7 Hz and 187.0 Hz. Figure 3B3 displays the XII power spectral patterns across all seven animals that produced gasps. Compared with the two other states, there is much more interanimal variability in magnitude of the power spectra contained within the two major bands (25–150 and 150–225 Hz).

During gasping, the XII shows no pre-Ph activity (Fig. 2, bottom, G), and the power appears scattered among various frequency bands (Fig. 6B3). Unlike the case of Ph activity during
gasping (Fig. 4B3), at least some power is maintained in medium and higher-frequency regions. Interestingly, it appears that activity in most frequency regimes is not stable throughout the entire burst, and there is a marked diminution in frequencies above 240 Hz, compared with eupnea and hyperpnea.

Table 3. Distribution of averaged ZIS-reconstructed XII power spectra peaks for three frequency band widths under three different conditions

<table>
<thead>
<tr>
<th></th>
<th>50–125</th>
<th>125–200</th>
<th>200–300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal Peak, Hz</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>92.3</td>
<td>148.5</td>
<td>187.0</td>
</tr>
<tr>
<td>G</td>
<td>109.9</td>
<td>187.0</td>
<td>158.7</td>
</tr>
<tr>
<td>E</td>
<td>136.7</td>
<td>185.5</td>
<td>187.0</td>
</tr>
<tr>
<td>Amplitude, AU $\times 10^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>30.90 ± 4.11</td>
<td>18.88 ± 2.01</td>
<td>42.58 ± 5.33</td>
</tr>
<tr>
<td>G</td>
<td>4.44 ± 0.52</td>
<td>23.78 ± 2.55</td>
<td>3.67 ± 0.41</td>
</tr>
<tr>
<td>E</td>
<td>39.82 ± 4.17</td>
<td>39.82 ± 4.17</td>
<td>3.89 ± 0.43</td>
</tr>
<tr>
<td>Band, Hz</td>
<td>112.3–156.3</td>
<td>166.0–212.4</td>
<td>144.1–170.0</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Abbreviations are the same as in Table 2.
HFO, and UHFO bands increase significantly when comparing eupnea (solid line) to hyperpnea (dashed line; gray area $P$ values $<0.05$ in E-H), whereas there is widespread, significant decrease in power at all frequencies $>50$ Hz (E and G, H and G) during gasping (dotted line). Thus, during the initial phase of hypoxia/hypercapnia, there is exaggeration of eupneic-related bands (Fig. 7A1).

A similar pattern is illustrated in XII activity (Fig. 7A2). Unlike the band-selective Ph differences, initial responses to hypoxia/hypercapnia show significant increases in all frequency bands above 40 Hz that are present during eupnea (E-H) in XII discharge. Analogous to the Ph data, gasping (E-G) produces a selective loss at frequencies only $>60$ Hz (ZIS) in XII activity.

Figure 7, B1 and B2, provides a comparison between prephrenic (pre-Ph; dashed) and phrenic-related (Ph-r; solid) spectral characteristics of XII activity during eupnea and hyperpnea, respectively. During eupnea (Fig. 7B1), the pre-Ph and Ph-r spectra of XII are nearly identical and mimic the shape of the entire nerve spectrum (Fig. 7A2). During hyperpnea (Fig. 7B2), the pre-Ph part of the XII spectrum (dashed line) is shifted to lower-frequency values, in the form of increased power in the same frequencies as those present in the Ph-r portion (solid line). The $P$ value function (Fig. 7, B1 and B2, bottom) shows many more significant differences between pre-Ph and Ph-r spectra in frequencies below 150 Hz than in those above this level. The results are summarized in Table 4.

**DISCUSSION**

Our overall findings were that 1) oscillations comprise dynamically changing frequency bands in the unanesthetized “deafferented” adult rat model; 2) the data presented here clarify to a large degree the controversy regarding changes in oscillatory behavior during severe hypoxia/hypercapnia-evoked gasping; 3) an early, low-frequency MFO component emerges in response to progressive hypoxia/hypercapnia; and 4) a good deal of the controversy surrounding these issues is due to the various animal preparations, analytical methods, and definitions of gasping.

**Clinical Relevance**

Although a lower mammalian species with reduced neuraxis, our experimental model shows all four classic phases of the anoxic response: initial hyperpnea, primary apnea, gasping, and secondary (or terminal) apnea (Fig. 2; Refs. 15 and 19). These classic phases of respiratory response to hypoxia or anoxia have been described in clinical observations: “In response to asphyxia, there is an initial period of arousal and hyperpnea, then primary apnea lasting seconds or minutes, and then a gasping stage. The gasps become progressively weaker and finally result in terminal apnea unless external support is provided. Gasping respirations are easily recognized as the presence of a rapid inspiratory rise accompanied by a retarded
expiratory phase preceded and followed by a cessation of breathing movements.” (Ref. 34, p. 167; see also Ref. 37).

**Principal Findings**

Our data suggest that the HFO in both nerves (but particularly the Ph), are more sensitive to hypoxic/hypercapnic conditions than are MFO-generating mechanisms, as the former are greatly exaggerated during hyperpnea and almost abolished during gasping. Hyperpnea produces significant increases in power at almost all frequencies in XII, and gasping reduces power almost uniformly in all frequencies (Fig. 7). The mechanisms underlying these shifts remain unclear and will require careful studies of neuronal behavior in respiratory networks to uncover.

**Phrenic characterization.** Schmid et al. (44) described the changes in Ph MFO and HFO in response to CO₂ stimulation in (anesthetized or decerebrated) vagotomized rabbits, in which they found that HFO power steadily rose with increasing respiratory drive, and our results in the rat agree well with their finding. In contrast to HFO, characterized by a spectral peak of relatively narrow bandwidth, the MFO spectrum usually consisted of a broad complex, in some cases composed of two distinct peaks (i.e., MFO was heterogeneous), and the low- and high-frequency sub-bands of the MFO complex were related predominantly to the first and last third of inspiration, respectively (44). Our data showed similar results during hyperpnea (Fig. 4, A2 and 4B2). The high sensitivity of Ph HFO power to chemoreceptor stimulation has been noted repeatedly in other species (see Ref. 17 for a review) and is consistent with our findings. It is possible that Ph MFO observed during gasping results from reconfiguration of central networks, and the initial hyperpnea may provide an early or transitional glimpse of this reconfiguration. Our results demonstrate that this occurs in the absence of peripheral cardiorespiratory sensory inputs.

Another unique finding of this study, revealed by ZIS TFR analysis, included the presence of an early, short-lasting, MFO burst under all states examined (Fig. 4), similar to that shown by Cohen et al. (10) in cats and by Marchenko et al. (29) in rats. The power and duration of this early start-up component (Fig. 4, C1–C3) grow steadily compared with other frequency components as anoxia progresses (Fig. 4, B1–B3). It is worth noting that this feature would be masked in a parametric analysis but is clear in the nonparametric TFR analysis. It is not clear whether the same mechanism that produces MFO during this start-up period also produces MFO later in the burst. During eupnea and hyperpnea, it exists at lower frequencies than any others present during the rest of the inspiratory cycle, but during gasping, it represents one of the dominant frequencies in the burst. This finding suggests that the start-up components and the lower MFOs, in general, may share a common mechanism (in all states), which is activated with increasing vigor as hypoxia/hypercapnia becomes more severe.

Our general finding is that Ph power is dynamically distributed over the course of inspiration, but the details of these dynamics and their constituent frequencies differ from other published accounts. For example, our ZIS TFR results are in stark discord with SPWVD-based TFR performed on diaphragmatic EMG records reported by O’Neal et al. (32) in anesthetized mice. These investigators reported the absence of any significant power (at any frequency) during the entire first half of eupneic inspiration. Unfortunately, O’Neal et al. (32) did not report a definitive data-sampling rate, so their SPWVD window of 360 data points represents an unspecified time window. This lack of specificity translates into an unknown fraction of the inspiratory period analyzed. Thus the TFR data shown in their figures, normalized between 0 and 1, end at some unknown point into the respiratory cycle, perhaps cutting off the latter portions of inspiration, and this may account for some discrepancies between our results and theirs. By contrast, we analyzed (and represented in our ZIS TFR plots) entire bursts, regardless of their length. Moreover, O’Neal et al. (32) used biphasic recordings in anesthetized mice, and both factors may be sources of differences between our results and theirs. Biphasic recording methods are deemed inappropriate for spectral analysis (9, 39), which is why we used monophasic recording techniques for both Ph and XII. On the other hand, our results support the general findings of early HFO and late MFO predominance in decerebrate animals, described in cats by Christakos et al. (7, 8), and in (a subset of) the rats described in Marchenko et al. (29).

**Hypoglossal characterization.** To our knowledge, this is the first study to examine the frequency components of the activity of the medial branch of XII in decerebrate, unanesthetized, peripherally denervated rats in vivo. In addition, this is the only study to analyze time-frequency dynamics of XII activity in any rat preparation, with particular reference to the onset of Ph activity. Our results demonstrate that during eupneic breathing, the pre-Ph and Ph-related activity are composed of different combinations of frequencies, with a dramatic power increase in the 130–160 Hz band during the beginning of the Ph-related epochs (Fig. 6B1) compared with any time during the pre-Ph epochs. It is important to note that this band-specific feature is

| Table 4. Distribution of time-averaged ZIS-reconstructed XII power spectra peaks for three frequency band widths during different phases of inspiration |
|-----------------|-----------------|-----------------|-----------------|
|                 | 30–125           | 125–200         | 200–300         |
|                 | H               | H               | H               |
| pre-Ph_P        | 36.2            | 87.9            | 144.1           |
| Ph_r_P          | N/A             | N/A             | 190.4           |
| pre-Ph_A        | 14.7±1.52       | 23.54±2.45      | 14.23±1.48      |
| Ph_r_A          | N/A             | N/A             | 17.83±1.91      |
| pre-Ph_B        | 9.8–56.2        | 65.9–102.5      | 117.2–158.7     |
| Ph_r_B          | N/A             | N/A             | 168.5–212.4     |

Values are presented as means ± SE × 10⁻²; pre-Ph_P, Ph_r_P, prephrenic and phrenic-related maximal peaks mean (Hz); pre-Ph_A, Ph_r_A, prephrenic and phrenic-related amplitude of maximal peaks in arbitrary units; pre-Ph_B, Ph_r_B, prephrenic and phrenic-related bandwidth range (Hz).
lost when one averages over the entire pre-Ph- and Ph-related epochs because the power in this bandwidth is relatively modest, but constant, during pre-Ph and has both dramatic increases and decreases during the Ph-r activity (Fig. 6B1). Furthermore, under hyperpneic conditions, there is a shift in temporal pattern of the 180–210 Hz band, particularly with regard to the timing of the peak (Fig. 6, B1 vs. B2). In addition, during hyperpnea, the 130–160 Hz band shows increased relative discharge during pre-Ph activation, compared with eupnea. This may indicate recruitment of additional pools of premotor neurons that are activated during hyperpnea or may be a reflection of the prominent depolarizing effects of hypoxia on XII motor neurons (20, 36). Interestingly, almost all major XII frequency bandwidths during hyperpnea (especially ~120–160 Hz) are temporally split between pre-Ph- and Ph-related parts of the burst, a finding that supports the possibility of two independent mechanisms producing oscillations during different periods of the burst (42, 52).

The only other study to perform TFR analysis on XII activity was done by O’Neal et al. (32) in the anesthetized mouse (no particular XII branch was identified). These investigators reported very little power during early inspiration in XII activity. This result differs dramatically from our findings but does not belie some of the raw nerve records presented (e.g., Fig. 7 in that study), which appear qualitatively different from those we recorded. Although this may be a species difference, it is possibly due to other factors, including the course frequency resolution used (7.8 Hz per bin), or other factors in animal conditions (e.g., anesthesia).

Anoxic Responses in Other Models

There are considerable difficulties in comparing our data with others that address gasping mechanisms. Unfortunately, many papers do not show continuous Ph recordings (Fig. 2) during control periods through gasping during hypoxic, anoxic, ischemic, or asphyxic tests in decerebrate, perfused juvenile rats (e.g., 33, 53), anesthetized cats (2, 38, 46), or decerebrate cats (54). Because only selected epochs of records were shown, it is difficult to compare their results with ours because it is not clear what specific gasping characteristics were present. Under normal anesthesia, initial hyperpnea is followed by depression if the hypoxia becomes severe, but this response can rarely be demonstrated in deeply anesthetized animals at any level of hypoxia (31). Subsequent recovery of eupnic Ph pattern is much more successful during reoxygenation before the gasping phase (31). Therefore, it is possible that late hyperpneic bursts have been reported as gasping (Fig. 3 of Ref. 33; Fig. 1 of Ref. 53), even though primary apnea never occurred. Without (a description of) the entire experimental record, it is very difficult to evaluate these behaviors definitively.

Using α-chloralose-anesthetized cats, Solomon (46) did describe the full time course of the response to hypoxia (94% N₂-6% O₂), and it consisted of primary apnea, gasps, and secondary apnea, without any initial hyperpnea. Richter et al. (41) ventilated nembutal-anesthetized cats with 93% N₂-7% O₂ for 5 min to produce hypoxic responses. In contrast to Solomon (46), they reported only an initial hyperpnea and primary apnea with no gasps. Therefore, the anesthetic agent may profoundly influence the responses to hypoxia in the same species. This is likely due to the varying effects on neurotransmitter systems that are affected by commonly used anesthetics (12, 41, 48).

A controversy exists regarding the Ph spectral characteristics during gasping. In a recent review, St-John (49) postulated that one distinction between eupnea and gasping is a shift in HFO to higher frequencies (39, 54). By contrast, Akay et al. (2) reported that Ph HFO power was diminished relative to MFO, with a shift in both toward lower frequencies during gasping in response to progressive hypoxia. Our data are in general accord with the latter view, and we observed that Ph HFO is all but eliminated during gasping (Fig. 4, A3 and B3, and 7A). We note that in the case of the study by Tomori et al. (54), these differences may arise from species differences (cats vs. rats) or from differences in the preparation (sino-aortic intact vs. denervated). The integrated Ph activity presented as prototypical (Fig. 1 of Ref 54) appears more akin to our hyperpnea response in that it is bell-shaped rather than decrementing (Fig. 2). On the other hand, the burst is shorter in duration than during eupnea, and there is no pre-Phrenic portion of XII activity, both of which characterize our gasping behavior (Fig. 2). Our data suggest that the most prominent feature of gasping is the systemic shift in Ph power to lower frequencies, which directly contradicts the characterization made by St-John (49). This difference is probably due to differences in gasping models.

Concerning these differences, the most interesting and relevant data regarding the evolution of gasping during anoxia were obtained from freely behaving rats at postnatal days 2, 5, 10, and 15 (e.g., 15, 18). Respiratory responses to anoxia through all of these ages have prototypical patterns, which begin with initial hyperpnea followed by primary apnea. After primary apnea, three stages of gasps (stage I, II, and III) were observed, separated by apneas. The first two stages are characterized by the existence of an extended decrementing ramp ("tail") that follows the initial rapid rise in Ph activity. Stage III has only a rapidly rising peak without any ramplike tail. All of these stages are shown in Fig. 5 of Fewell et al. (15). Our results resemble stage III gasping, where only the initial start-up portion of the Ph discharge was observed with a very brief (if any) decrementing tail.

Taking into account the results obtained from developing rats, from adult anesthetized and decerebrate cats and from the present data, we summarize the Ph response to anoxia or severe hypoxia in Fig. 8. All gasping stages have a very fast and high-amplitude increase in Ph activity (Fig. 8, shaded area). Our data demonstrate the G pattern without a significant decrementing ramp tail ("-dr" in Fig. 8). We suggest that the gasping described herein reflects the core or kernel of oscillatory behavior of the respiratory rhythm generator in this state and is essentially one of pure MFO.

In contrast to our results, all studies (see above) performed on in vivo anesthetized or decerebrate cats, or in situ artificially perfused decerebrate juvenile rats, reported Ph gasping discharges containing a considerable decrementing tail-like ramp after the initial increase in amplitude (G+dr in Fig. 8). This may account for the differences in results of spectral analyses, in which the ramp is produced by the brain stem HFO-generating system, while the initial discharge (start-up) is produced by an MFO-generating circuit. The gasps in the present study show virtually no Ph HFO during gasping and have no significant decrementing tail in the Ph activity, whereas other studies that produced a decrementing tail do...
contain HFO. For example, the preferentially increasing MFO band observed in anesthetized cats during gasping elicited by severe hypoxia (2) could be due to the relative absence of a significant decrementing tail. Considering the studies conducted in freely behaving rats (15, 18), our results suggest that the principal difference between eupnea and gasping is that during eupnea the incrementing ramp is responsible for smooth lung expansion, whereas during gasping the start-up discharge is responsible for rapid lung expansion, and the tail-like decrementing ramp (if present) is responsible for maintaining lung inflation, thereby prolonging the time for gas exchange.

**Consideration of Analytical Methods**

In addition to different experimental models, differences in data analysis methods can profoundly affect results. The study most relevant to the present one is that of Marchenko et al. (29), performed in decerebrate rats. These investigators estimated HFO power (via parametric FFT analysis) at a lower frequency (106–160 Hz, mean 132 Hz) during eupnea than our present results. One reason for this discrepancy can be attributed to the use of “bin combining/summing” (8), which results in an artificial reduction in frequency. In addition, they reported a higher variability, with regard to which half of inspiration (first vs. second) contained MFO vs. HFO bandwidths. In some animals, their results were very similar to ours, whereas in others, they were not. This may have reflected variability in the condition of the animals, particularly with regard to the method of decerebration.

Regardless of method, another source of variability resides in our assumption that the system is stationary (within a given state), particularly during anoxia. We grouped bursts that fit our general shape criteria together, but it is likely that the system changes between, for example, the first and last hyperpneic bursts. These concerns can only be addressed by more detailed analyses than we offer here.

**Neural System Implications**

The available data from anesthetized rats suggest that HFO is not a feature of individual rat phrenic motor neurons. Kong and Berger (25) recorded from individual Ph motor neuron fibers, but rarely observed interspike intervals <10 ms (i.e., 100 Hz), even during hypercapnic conditions. Hayashi and Fukuda (21) reported that the highest Ph motor neuron firing rates in anesthetized rats were 55 ± 14 Hz, providing little basis to claim that some neurons may fire at 180 Hz. One study, performed in anesthetized cats (8) did seem to show that HFO (65 Hz) was present in the autospectrum of individual cat Ph motor neuron firing (Fig. 3 of that paper). However, interspike interval analysis of the same unit revealed a peak only at ~33 ms (Fig. 4 of that paper), corresponding to ~30 Hz (i.e., MFO), and almost no intervals at or below the 15.4 ms required for 65-Hz HFO oscillations. Other cat studies (e.g., 30, 50) also failed to demonstrate HFO in Ph motor neuron discharges. These studies suggest that HFO is a population-based phenomenon, rather than a property of individual Ph motor neurons. Nonetheless, the lack of data derived from unanesthetized decerebrate rats demands that we reserve judgment regarding the ability of individual motor neurons/units to produce HFO. It is likely that MFO produced later in the burst results from increased recruitment and firing rate of individual motor neurons, whereas early HFO is due to activity of multiple neurons with lower individual rates but constant phase shifts.

**Neuromuscular Implications**

Fast respiratory rhythmic output in the early phase of Ph discharge may promote efficiency in muscle contraction (17). Thus, HFO, at least in activation of diaphragm motor units, may create a “catchlike effect” described by van Lunteren and Sankey (55). These authors used rat diaphragm muscle strips, stimulating them with 2–4 shocks at 100–200 Hz “bursts” at the onset of 10- to 50-Hz subtetanic trains. Their results revealed that a high-frequency burst of pulses at the onset of a subtetanic train of stimulation promotes the diaphragm to hold its contractile force at a higher level than expected from the subtetanic trains alone, because of the catch-like property of muscle. This property has been well documented in other skeletal muscles (6) and plays an important role in prevention of muscle fatigue in humans (5). Our general finding of the domination of HFO during the early portion and MFO during the latter portion of Ph discharge (Fig. 4B1) supports the
findings of studies in rats (29) and cats (7, 8) and may represent the underlying neural mechanism of this phenomenon in vivo.

In conclusion, we characterized the dynamic spectral components contained within the bursts of Ph and XII activity under three different conditions. Our primary conclusion is that, compared with eupnea, there exists a clear shift in power distribution toward lower frequencies during gasping. This may reflect the activation of a “core” gasping system that may or may not be a subset of the central pattern and rhythm generator within the central nervous system. When pushed into this state, it appears that there is some conservation of effort in attempts to recover from (what would be in the natural state) ventilatory insufficiency or severe hypoxia. Alternatively, we may view this behavioral output as the remnant of the original system that is less susceptible to anoxic shock or acute metabolic stress.

Having defined the changes in power spectra in the XII and Ph activity under three conditions, it remains unclear as to how these are synchronized or coordinated among the four motor nerves considered. Examining the coordination and potential shared oscillatory drive can be achieved by analyzing dynamic coherence levels between the nerves during the bursts. In another study, we will present novel TFR analysis of Ph-Ph, XII-XII, and Ph-XII coherence, thereby providing a dynamic representation of coherence during inspiration under the same three behavioral states examined herein.

GRANTS
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