Synchronized Fast Rhythms in Inspiratory and Expiratory Nerve Discharges During Fictive Vocalization

KEN NAKAZAWA, ANTONIO R. GRANATA, AND MORTON I. COHEN
Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461

Nakazawa, Ken, Antonio R. Granata, and Morton I. Cohen. Synchronized fast rhythms in inspiratory and expiratory nerve discharges during fictive vocalization. J. Neurophysiol. 83: 1415–1425, 2000. In precollicular decerebrate and paralyzed cats, respiratory nerve activities were recorded during fictive vocalization (FV), which consisted of a distinctive pattern of I decreased inspiratory (I) and expiratory (E) phase durations, 2 marked increase of phrenic activity and moderate changes of recurrent laryngeal (RL) and superior laryngeal (SL) I activities, and 3 massive recruitment of laryngeal and abdominal (ABD: lumbar) E activities. FV was produced by electrical stimulation (100 Hz) in the midbrain periaqueductal gray (PAG) or its putative descending pathways in the ventrolateral pons (VLP). Spectral and correlation analyses revealed three types of effect on fast rhythms during FV. 1) **I activities**: the coherent high-frequency oscillations in I (I-HFOs, 60–90 Hz) present in phrenic and RL discharges during the control state did not change qualitatively, but there was an increase of power and a moderate increase (4–10 Hz) of frequency. Sometimes a distinct relatively weak stimulus-locked rhythm appeared. 2) **RL and SL activities during E**: in recruited discharges, a prominent intrinsic rhythm (coherent E-HFOs at 50–70 Hz) appeared; sometimes a distinct relatively strong stimulus-locked rhythm appeared. 3) **ABD activities during E**: this recruited activity had no intrinsic rhythm but had an evoked oscillation locked to the stimulus frequency. Thus FV is characterized by 1) appearance of prominent coherent intrinsic rhythms in RL and SL E discharges, which presumably arise as a result of excitation and increased interactions in laryngeal networks; 2) modification of intrinsic rhythmic interactions in inspiratory networks; and 3) evoked rhythms in augmenting-E neuron networks without occurrence of intrinsic rhythms.

**INTRODUCTION**

The respiratory musculature, including the diaphragm and the upper airway and abdominal muscles, functions not only during respiration but also during other motor activities, such as swallowing, vomiting, coughing, sneezing, and vocalization. Each of these behaviors is based on a coordinated pattern of respiratory muscle activities, and this pattern is also present in respiratory nerve activities recorded during the “fictive” behavior elicited in paralyzed animals (Satoh et al. 1998; Shiba et al. 1996; Umezaki et al. 1998). Moreover, because these fictive behaviors are observed in decerebrated animals, we can conclude that they are generated at brain stem levels.

Vocalization can be elicited by electrical or chemical stimulation at sites in the midbrain periaqueductal gray (PAG) (Jürgens 1998; Larson 1991; Nonaka et al. 1999; Shiba et al. 1999; Zhang et al. 1994) and by electrical stimulation in a region of the ventrolateral pons (VLP), designated as the pontine call site (PCS), which is thought to contain descending pathways from the PAG to the lower brain stem (De Lanerolle 1990; Kanai and Wang 1962; Sakamoto et al. 1997). The vocalization pattern is characterized by 1) increase of respiratory rate by reduction of inspiratory (I) and expiratory (E) phase durations, 2) increase of the I activities of the vocal fold abductor and the diaphragm, and 3) marked increase of the E activities of the vocal fold adductor and tensor muscles and of the abdominal muscles (Satoh et al. 1998; Yamanaka et al. 1993). Fictive vocalization (FV) elicited in paralyzed animals exhibits similar patterns in the activities of respiratory nerves that innervate those muscles: phrenic (PHR), recurrent laryngeal (RL), external branch of superior laryngeal (SL), and lumbar abdominal (ABD) (Nakazawa et al. 1997; Shiba et al. 1996).

In earlier studies, it was found that in normal respiration there is widespread occurrence of high-frequency oscillations (I-HFOs, usual range 50–100 Hz) in neural I activities, such as PHR and RL (Christakos et al. 1991, 1994; Cohen et al. 1987, 1997). Because I-HFOs in different nerve activities are correlated (coherent), it was suggested that they originate from a common source, such as a subpopulation of the central I pattern generator (Cohen et al. 1997). In contrast, similar coherent fast rhythms in RL activities during the E phase (E-HFOs) are rare but have been observed in a fraction (11%) of decerebrate cat preparations (Huang et al. 1993a). The presence of HFOs may depend on physiological state, as suggested for example by their disappearance from PHR activity during fictive vomiting, even though there is strong overall PHR activity (Cohen et al. 1992).

Therefore we used spectral and coherence analysis to study properties of I-HFOs and E-HFOs in various respiratory nerve discharges during FV. The most striking effect observed was the appearance during FV of coherent intrinsic E-HFOs in RL and SL activities. This effect suggests the existence of widespread interactions within the network(s) that generate RL and SL E activity. Preliminary reports have been published as abstracts (Nakazawa et al. 1999a,b).

**METHODS**

**Experimental preparation**

Experiments were done on cats (2.5–3.5 kg) that were decerebrated at the precollicular level, using standard methods (Kirsten and St. John 1978). Surgical preparation was performed under 3–5% halothane, the adequacy of anesthesia being attested by absence of movements and blood pressure changes. After decerebration and completion of surgery, the halothane was removed, and
the animals were paralyzed by infusion of gallamine triethiodide (5 mg·kg⁻¹·h⁻¹). Recordings were not started till at least 2 h after removal of halothane. A pneumothorax was done, and artificial ventilation was applied via a mechanical ventilator (usually at 30 cycles/min) connected to a 1- to 2-cm expiratory load. End-tidal CO₂ level (monitored by an infrared analyzer) was maintained at 4–5% by varying ventilation or the composition of the input gas mixture (0–5% CO₂ in 100–95% oxygen). To maintain fluid balance and keep systolic blood pressure ≥100 mmHg, the animals were perfused with 0.9% saline containing 5% glucose (4 mg·kg⁻¹·h⁻¹). Rectal temperature was kept at 37–39°C by use of a heating pad. At the end of the experiment, the animals were given an overdose of pentobarbital sodium.

**Recordings**

Bilateral recordings were taken from PHR and RL nerves; and unilateral recordings were taken from the external branch of the SL nerve and from an ABD nerve (at lumbar segment 1). With the cat in a supine position, the severed nerves were mounted on bipolar electrodes and immersed in separate mineral oil pools at cervical and lumbar levels. The nerve activities were recorded monophonically (band-pass 1–5,000 Hz) after crushing the end of the nerve on the distal electrode wire.

Digitized recordings were made by signal entry directly into a PC-based A-D converter (RC Electronics), using a sampling rate of 2,500 Hz (0.4-ms bin duration). Analogue signals recorded were 1) nerve discharges, 2) intratracheal pressure, and 3) femoral arterial pressure. Pulse signals recorded were 1) pulses marking the onset of the inspiratory (I) and expiratory (E) phases, derived from PHR discharge by specialized circuitry (Cohen 1968) and 2) stimulus-related pulses (i.e., the logic pulses that triggered the current pulses used for stimulation).

The nerve signals, after filtering (40-Hz high-pass), full-wave rectification, and integration (time constant 100 ms), were monitored on a polygraph, together with arterial blood pressure, intratracheal pressure, and stimulus marker.

**Electrical stimulation**

Electrical stimulation was delivered in two regions: 1) in and around the PAG at the level of the mesencephalic trigeminal nucleus, at 1.0–1.5 mm from the midline, just ventrolateral to the ventral border of the aqueduct (Horsley-Clarke coordinates A 1.0 to 2.5, L 1.0 to 2.0, H +2.0 to 0), and 2) in the VLP, at the level of the decussation of the brachium conjunctivum, ventral to the central tegmental field and lateral to the pontine gray (Horsley-Clarke coordinates P 2.0 to A 1.0, L 3.0 to 5.0, H −4.5 to −6.0).

Concentric bipolar electrodes (Rhodes SNEX-100, 0.25 mm diam, impedances 20–50 KΩ) were inserted through the ventral brain stem surface and moved in 0.5-mm steps while applying stimulus trains. When a site was found where stimulation produced the specific pattern of fictive vocalization (Fig. 2), the stimulus current was set at 1.5 times threshold, and the site was used for the remainder of the experiment. In each recording run, usually four to six FV episodes were produced, each containing four to six respiratory cycles. Adequate time for recovery was allowed between stimulus trains.

Stimulus parameters were as follows: 0.2-ms duration, 80–300 µA, frequency 50–150/s. Stimuli were applied via a stimulus isolation unit, and the applied current was measured by a current probe. To verify that current did not change during repeated stimulus trains, the output of the current probe was monitored on an oscilloscope. At the end of the experiment, a lesion was made by passing radio-frequency current through the electrode, and the brain was processed to obtain histological sections with Nissl staining for identification of the stimulation site.

**STIMULUS ARTIFACT REMOVAL.** In some cases, short-duration stimulus artifacts appeared in the nerve recordings; these could contribute a component that was seen in the stimulus-nerve cross-correlation histogram (Fig. 1A). The artifacts were eliminated from the digitized data by means of an algorithm that 1) used a stimulus tag array to locate the stimulus pulse position, 2) located the corresponding bin in the analogue data array, and 3) replaced the data value in that bin by the mean of the values in the preceding and the succeeding bin. The consequent disappearance of the artifact is shown in the example of Fig. 1B.

**Data analysis**

**DATA WINDOWS.** Using an I and E pulse tag array derived from the phrenic potential recording (Cohen 1968), portions of each nerve signal data array corresponding to the I phase or the E phase were identified. Four ensembles of data windows were created: control I and E phases (those occurring during spontaneous respiration), which were selected from the time portion immediately preceding applica-

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**FIG. 1.** Effect of removal of stimulus artifacts from digitized records of right recurrent laryngeal (RRL) discharge. The cross-correlation histograms (CCH) of stimulus pulses (α = 2.512) vs. RRL discharge were derived from activity in 20 expiratory (E) phases during fictive vocalization (FV) produced by ventrolateral pons (VLP) stimulation at 100 Hz. CCH bin duration: 0.4 ms. A: before artifact removal. B: after artifact removal.
tion of a stimulus train, and test I and E phases (those occurring during an episode of fictive vocalization produced by stimulation). Correlation and spectral computations were done for 20–30 phases in each ensemble, these having been selected from 4–6 FV episodes or corresponding control periods.

CROSS-CORRELATION HISTOGRAMS (CCH). The CCH between the stimulus pulse signal and each nerve signal was computed for each type of gate. The CCHs were normalized according to the formula given in Cohen and Feldman (1984).

FREQUENCY-DOMAIN ANALYSIS. Autospectra of the signals and coherence spectra for pairs of signals (including the coherence between the stimulus-related pulse signal and each nerve signal) were computed using methods previously described (Christakos et al. 1991). The analogue signals were sampled in 2.0-ms bins, the data value in each bin being derived by summation of data values in the original 0.4-ms sampling bins. This procedure acted as a low-pass filter with effective sampling rate of 500 Hz. The pulse signal was low-pass filtered using the sinc function (Christakos et al. 1984).

For each I or E phase of an ensemble, the original time windows were truncated: each window started at the onset of the phase and terminated at a time corresponding to the end of the shortest phase in the ensemble. The purpose of this procedure was to ensure that the individual data windows were of equal length. Then the data array in a window was augmented by zeros to make its length equal to a power of 2 (fast Fourier transform requirement). Thus the spectral bin resolution was set by the number of data points in the windows and depended on the duration of the shortest phase in the ensemble.

For each data window, the autospectra of signals and the cross-spectra (real and imaginary parts) of pairs of signals were computed. The final spectra were obtained as averages over the ensemble, and after smoothing with a three-point moving average the spectra were used to compute the coherence function. Autospectral values are presented here as relative amplitudes, i.e., the value in each frequency bin is the fraction of the total energy that is present in the narrow band around that frequency. In addition, for each signal the average energy per unit time (RMS: root-mean-square power), in arbitrary units, was calculated. This variable was used for comparing activity levels between different states.

As explained in the Appendix of Christakos et al. (1991), the coherence function of two signals is a measure of the linear correlation between the signals at various frequencies. It can vary between 0 and a theoretical maximum of 1.0, which represents a perfect correlation at that frequency. To evaluate the statistical significance of coherence estimates, the upper 95% confidence value, which depends on the number of data windows used, was calculated (Jenkins and Watts 1968). Coherence values above that value were deemed to be significantly different from zero at the 95% confidence level ($P < 0.05$).
RESULTS

In seven precollicular decerebrate, gallamine-paralyzed, artificially ventilated cats, FV was produced by electrical stimulation in either the PAG or in the region of its putative descending pathways in the VLP. FV is characterized as a specific pattern of discharge in I and E nerves that corresponds to the pattern of actual vocalization (Shiba et al. 1996). In confirmation of earlier reports (De Lanerolle 1990; Sakamoto et al. 1997), we found no difference between response patterns elicited from the two regions (PAG vs. VLP). In this paper, we present only the results obtained by stimulation at 100 Hz.

Properties of nerve discharges during FV

An example of the typical FV pattern is seen in Fig. 2, which shows recordings (after rectification and integration) from several respiratory nerves before and during an episode of FV produced by VLP stimulation. (The ventilation cycle, with fixed volume and rate of 30/min, remained unchanged during stimulation.) The stimulus train, which was started during an E phase and produced an immediate termination of the I phase, lasted for 8.8 s and produced a decrease of both I and E phase durations by about one-half (mean decrease: I, 54%; E, 52%).

I ACTIVITIES. In FV there occurred 1) an increase of slope and peak amplitude of both left and right PHR discharges, with RMS increase of 112 and 64%, respectively; 2) similar effects on the ramp pattern of both left and right RL discharges, with moderate increase of RMS power (57 and 58%, respectively); and 3) disappearance of the phasic augmenting I pattern of SL discharge.

E ACTIVITIES. In FV there occurred a marked increase of discharges: 1) in RL, the decrementing discharge in early E was replaced by discharge with a plateau pattern lasting through all of E; 2) in SL, discharge silence was replaced by discharge with a plateau pattern; and 3) in ABD, discharge silence was replaced by discharge with an augmenting pattern.

The responses described in the present paper were obtained in vagally intact cats with the ventilator kept at fixed volume and rate throughout the recording. In this situation, during the control state the central respiratory cycle was usually synchronized in a 1:2 relation to the ventilatory cycle (cf. Cohen 1969); and during stimulation the synchronization changed to a 1:1 pattern (cf. Fig. 2). Although the FV pattern was qualitatively similar in vagotomized and vagally intact preparations, we do not present here a detailed statistical analysis of the differences.

For the seven preparations of the study, the changes of the variables (durations and discharge amplitudes) produced during FV were generally in the range of those shown in Fig. 2. The mean changes from the control values were as follows: I phase duration, −49%; E phase duration, −54%; PHR power during I, +137%; RL power during I, +36%; SL power during I, 0%. The lack of mean change in power of SL was due to cancellation of negative and positive

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TABLE 1. Incidence of different rhythm types during fictive vocalization

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Number of Cases</th>
<th>Rhythm Types in I</th>
<th>Rhythm Types in E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of Cases</td>
<td>Number of Cases</td>
</tr>
<tr>
<td>PHR</td>
<td>1</td>
<td>6 0 0 0 7</td>
<td>NA</td>
</tr>
<tr>
<td>RL</td>
<td>2</td>
<td>3 2 0 7</td>
<td>1 6 0 0 7</td>
</tr>
<tr>
<td>SL</td>
<td>1</td>
<td>0 0 6 7</td>
<td>1 4 2 0 7</td>
</tr>
<tr>
<td>ABD</td>
<td>NA</td>
<td>0 0 4 0 4</td>
<td>0 0 4 0 4</td>
</tr>
</tbody>
</table>

I, inspiratory; E, expiratory; A, intrinsic rhythm only; B, intrinsic and stimulus-locked rhythms; C, stimulus-locked rhythm only; D, no fast rhythm; NA, not applicable; PHR, phrenic; RL, recurrent laryngeal; SL, superior laryngeal; ABD, abdominal.

FIG. 3. Discharges of respiratory nerves in time windows indicated by rectangles in Fig. 2: during control (left, spontaneous respiration) and during test (right, FV) I phase (top) and E phase (bottom). Bin duration 0.4 ms. For the display, the original signals were filtered (1–250 Hz) by a digital algorithm. For each phase and each signal, discharge in the control phase and the test phase is normalized to the same amplitude scale.
changes: decrease in three of seven cases and increase in four of seven cases. During E, in all preparations there was increase or appearance (in previously silent portions of E) of RL, SL, and ABD activities.

In the original recordings of Fig. 3, we show the patterns of nerve discharges during portions of an I phase (top) and an E phase (bottom) during spontaneous (spont.) respiration (left) and during stimulus-produced FV (right). These are shown on an expanded time scale for the time segments marked in Fig. 2B. Ic (spont. I phase): PHR and RL activities have high-frequency oscillations (HFOs), whereas SL activity shows no obvious rhythm. It (I phase during FV, with window starting before the onset of I): PHR and RL activities are increased and have stronger HFOs, whereas SL activity is reduced. Ec (spont. E phase): there is appreciable nonrhythmic activity in RL, and low-level activity (possibly noise) in SL and ABD. Et (E phase during FV): there is a large increase of RL, SL, and ABD activities, with strong rhythmicity in RL but no obvious rhythmicity in SL and ABD.

The properties of fast rhythms (HFOs, range 60–90 Hz) in the nerve activities during FV, as well as during the control state of spontaneous respiration, were ascertained by spectral and correlation analysis. Four types of rhythmic pattern were observed during FV: 1) intrinsic rhythm, which was independent of, i.e., not locked to, the stimulus pulses; 2) stimulus-locked rhythm; 3) coexistence of types 1 and 2; and 4) absence of fast rhythm. Table 1 shows the incidence of these types of rhythm for the nerve activities in the seven cats of the study.

I PHASE OF FV (TABLE 1, LEFT). In PHR discharge, HFOs were always present, with admixture in six of seven cases of stimulus-locked rhythm of variable strength. A similar admixture (3/7 cases) was found in RL discharge, but in two of seven cases only intrinsic rhythm, and in two of seven cases only stimulus-locked rhythm, occurred. In contrast, HFOs were rare in SL discharge during I (1/7 cases), the incidence of these HFOs being reduced from the three of seven cases in the control state.

E PHASE OF FV (TABLE 1, RIGHT). Both RL and SL discharges had admixtures of intrinsic rhythm (E-HFO) and stimulus-locked rhythm. In contrast, ABD discharge (4 cases) showed only stimulus-locked rhythm. It should be emphasized that during the control (spont.) E phase, intrinsic rhythms were absent even when there was considerable activity (as in RL E activity of Fig. 2).
Rhythms in inspiratory nerve discharges

It is well-known that HFOs (frequency range 50–100 Hz) are ubiquitous in I discharges (Christakos et al. 1991, 1994; Cohen et al. 1987, 1997). In the present study, we have used spectral analysis to ascertain the changes in this rhythmic activity during FV. During the control state (spontaneous respiration), in all seven cases I-HFOs were present in PHR and RL discharges, but these were present in SL discharge for only three of seven cases.

For the preparation of Fig. 2, the autospectra of discharges during I (PHR, RL, and SL nerves) in Fig. 4 show changes between spont. respiration (left) and FV (right): 1) All activities had a prominent HFO peak at 67.4 Hz during spontaneous I phases (A1–D1). 2) During FV, the HFO peak frequency increased to 74.2 Hz (A2–D2). 3) The relative power of the PHR signal at the frequency peak was much greater during FV than during spont. respiration (A2 vs. A1, values of 0.078 and 0.027, respectively). This indicates a greater concentration of power around the peak frequency, i.e., increased rhythmicity. Moreover the increase in total power (RMS) was very large (T/C ratio: LPH, 2.12; RPH, 1.64). 4) For RL activity, there was little change of relative power at the peak frequency (B2 vs. B1, C2 vs. C1) and only a moderate increase of RMS power with FV (T/C ratio: LRL, 1.57; RRL, 1.58). 5) For SL, the relative power of the autospectral peak was small, but its value was about doubled as a result of FV (D2 vs. D1, values of 0.015 and 0.008, respectively).

The coherences between pairs of I activities (Fig. 5) show the common HFO rhythm of the activities during each state. It can be seen that there is little change in the value of the main coherence peak between spont. respiration (left) and FV (right). This suggests that, although HFO autospectral power increased for each member of a pair, the coupling between the signals did not change markedly.

In all seven preparations, the peak frequency of the HFO rhythm increased during FV over the control value: 1) spont. respiration, mean 74.1 ± 8.8 (SD) Hz, range 64.5–89.8 Hz; 2) FV, mean 80.3 ± 9.1 Hz, range 73.0–99.6 Hz. The mean increase of frequency was 6.2 Hz, which was significant at P < 0.01 by the one-tailed t-test.

In addition to the increase of power of the HFO component in I activities during FV, there usually appeared an additional component at the stimulus frequency. This phenomenon is shown in Fig. 6 for PHR discharge in another preparation. During FV, the peak frequency of the HFO component increased to 70.3 Hz (B) from the control value of 64.5 Hz (A).
In addition, during FV there appeared a distinct peak at 100 Hz, the stimulus frequency. This component was locked to the stimulus pulses, as indicated in the stimulus-phrenic (STIM-LPH) coherence spectrum (C) by a peak at 100 Hz with coherence value of 0.77, and as also indicated by the rhythm (10-ms period) in the stimulus-phrenic CCH (D). (The CCH also indicates the absence of stimulus artifacts, which if present would have led to a spurious correlation.)

The magnitude of the stimulus-locked component during the I phase of FV varied with the preparation. Such locking occurred in six of seven PHR recordings (STIM-PHR coherence range 0.13–0.77); in three of six cases the magnitude of the stimulus-locked peak was greater than that of the HFO peak (as in Fig. 6B). A stimulus-locked component appeared in five of seven RL recordings (STIM-RL coherence range 0.11–0.61), and both intrinsic and stimulus rhythms were present in three cases. Stimulus locking did not occur in SL recordings during I.

Rhythms in expiratory nerve discharges

During the E phase of FV, RL E discharges always exhibited an intrinsic fast rhythm. Such rhythms in spontaneous RL E discharges had occasionally been found in earlier studies and were designated as E-HFOs (Huang et al. 1993a). An example...
of this rhythm produced during FV by VLP stimulation is shown in Fig. 7, where the autospectra of both left (A) and right (B) RL discharges have a narrow peak around 62.5 Hz. No such peak was found in the autospectra of LSL (C) and LABD (D) discharges; but those discharges had prominent autospectral peaks at the stimulus frequency (100 Hz). However, the small peaks at 100 Hz in the RL autospectra (A and B) indicate the presence of a stimulus-locked component.

In this case, the stimulus-locked component is apparent in the coherences of LRL activity to RRL, LSL, and LABD activities (Fig. 8). The left-right RL coherence (A) has a prominent peak at 62.5 Hz (value = 0.946) at 62.5 Hz and its absence at this frequency in the other coherences. Note also the prominent peaks at 100 Hz (stimulus frequency) in all coherences.

In all seven preparations, E-HFOs were present in RL activity during FV, with mean 63.8 ± 5.0 Hz and range 57.6–71.3 Hz. In the three preparations with recordings from both RL nerves, the left-right coherence peaks had values >0.9. For the six of seven cases where RL had a stimulus-locked component, the range of the stimulus-RL coherence peak values was 0.17–0.67.

The incidence of E-HFO rhythms was less in SL discharges: only five of seven cases showed an E-HFO component, as these nerve activities are also apparent in the stimulus-nerve CCHs (Fig. 9). The periodicity of the waveforms (10 ms) indicates the locking of activity to individual stimulus pulses. Also, the absence of sharp stimulus-locked deflections (e.g., as in Fig. 1A) indicates that the values of the coherences and cross-correlations did not arise from stimulus artifacts. The differing strengths of the correlations for different signals are indicated by the vertical markers. However, a more exact estimate of the degree of correlation is provided by the values of the stimulus-nerve coherences at the stimulus frequency: LRL, 0.635; RRL, 0.555; LSL, 0.855; LABD, 0.469.

In all seven preparations, E-HFOs were present in RL activity during FV, with mean 63.8 ± 5.0 Hz and range 57.6–71.3 Hz. In the three preparations with recordings from both RL nerves, the left-right coherence peaks had values >0.9. For the six of seven cases where RL had a stimulus-locked component, the range of the stimulus-RL coherence peak values was 0.17–0.67.

The incidence of E-HFO rhythms was less in SL discharges: only five of seven cases showed an E-HFO component, as
but does not show a peak at the stimulus frequency, thus verifying the absence of this component in RL activity.

Finally, in the four preparations where ABD activity was recorded, intrinsic E-HFOs were not observed during the E phase of FV, but in all cases a stimulus-locked rhythm was observed (cf. example in CCH of Fig. 9D). The range of stimulus-nerve coherence values at 100 Hz was 0.19–0.82.

Thus there was a gradation in the strength of the intrinsic E-HFOs: they were most prominent in RL activity, less prominent in SL activity, and absent in ABD activity.

**Discussion**

In the present study, we have used spectral and correlation analysis to examine the fast rhythms that are present in various respiratory nerve activities during FV. These are of interest because they may indicate interactions between neurons within a given network as well as interactions between neurons of different networks.

### Rhythms in I activities during FV

During the control I phase (spontaneous respiration), PHR and RL activities had the typical HFO rhythms that have been reported in earlier studies (Christakos et al. 1991, 1994; Cohen et al. 1987, 1997). During FV, I-HFO peak frequency was always larger than the control value; the mean increase for the seven preparations was 6.2 Hz (from 74.1 to 80.3 Hz). The degree of change of power (RMS) between control values and values during FV differed between signals. The mean power of PHR discharge more than doubled (137% increase), the mean RL power increased only moderately (36%), and the SL power on the average did not change, with some preparations showing an increase and others a decrease. These differences might be related to differential change of pattern between control and FV: PHR discharge retained its augmenting pattern, but with an increase of slope and peak amplitude; the RL plateau pattern did not change markedly; and the SL augmenting pattern disappeared together with reduction of discharge.

In addition, during FV there appeared an additional component that was locked to the stimulus frequency (100 Hz); it was present for PHR (6/7 cases) and for RL (5/7 cases) but not for SL. The stimulus-locked component was indicated in autospectra and coherences by the presence of a peak at this frequency that was distinct from the I-HFO peaks (Fig. 6, A–C) and in stimulus-nerve CCHs by presence of a periodic waveform with period of 10 ms (Fig. 6D). (Examination of the CCHs verified that this component did not arise from stimulus artifacts.)

The relative strength of the intrinsic versus the stimulus-locked component differed between preparations. Therefore one must consider the possibility that differences in the locus of stimulation might have activated pathways with different targets. This question could only be approached by a detailed anatomico-functional study. An alternative hypothesis is that one pathway was being activated, but that there was then a divergence of output to different targets.

Thus the general effect of FV on PHR and RL discharges was excitatory, and the effect on SL discharge was depressant. But FV did not produce a drastic (qualitative) change in I-HFO rhythms; rather it produced graded changes in frequency and amplitude. This effect might be due to the strength of the
medullary I-HFO generating system connections, which would be resistant to outside inputs. However, it appears that FV input also produces an independent stimulus-locked excitation. Possibly there is interaction between the intrinsic and stimulus-locked components, as suggested by the broadening of the HFO peak in some cases (Fig. 6B); this possibility cannot be evaluated by linear spectral analysis but could perhaps be examined with bispectral analysis.

Rhythms in E activities during FV

During control cycles, RL activity either had no discharge in E or had an early-E decrementing discharge, whereas usually SL activity was absent in E (cf. Fig. 2). During FV, both RL and SL activities were greatly increased and assumed a plateau pattern.

The most striking effect we observed during FV was the appearance during the E phases of an intrinsic fast rhythm (range 57.6–71.3 Hz) in RL discharges and to a lesser extent in SL discharges. The autospectral and coherence peaks at the frequency of this rhythm were distinct from peaks of stimulus-locked components (Figs. 7 and 8). In the three cases where recordings were taken from both left and right RL nerves, strong coherence peaks (values >0.9) were seen in left-right coherence spectra (Fig. 8A). Thus this phenomenon seems identical to the spontaneously occurring E-HFOs in RL nerve discharges that were observed in a small fraction (11%) of preparations in other experiments (Huang et al. 1993a).

In those cases, the E-HFO synchronization appeared in conditions where there was a massive increase of overall RL E activity, such as occurs after an I phase where inflation is withheld (Sica et al. 1985).

A similar but weaker coherence relation was found between the E-HFOs of RL and SL activities during the E phases of FV (Fig. 10C). Significant RL-SL coherence peaks at the E-HFO frequency were found in five of seven cases, with coherence peaks (range 0.11–0.62) that were smaller than those of left-right RL coherence.

To analyze the unitary origin of the E-HFOs, in an earlier study (Huang et al. 1993b) we took recordings of individual RLE fibers dissected from an RL nerve that had prominent E-HFOs. The individual RLE units had plateau or bell-shaped discharge patterns similar to those of RL nerve activity during FV in the present study (Fig. 2). The unit-nerve coherences had peaks of high value (>0.7) at the E-HFO frequency, indicating the existence of widespread correlations between units at that frequency (cf. discussion of unit and population synchrony relations in Christakos 1997).

The presence of strong correlations between RLE unit and population (nerve) activities (Huang et al. 1993b) suggests that the motoneurons are driven by common rhythmic inputs. Further, the presence of narrow E-HFO coherence peaks in left-right RL coherences (Fig. 8A) and of somewhat weaker coherence peaks in RL-SL coherences (Fig. 10C) suggests that there are widespread network interactions between neurons of different bilateral networks as well as between neurons within particular networks.

These observations also suggest that there is a specific network or set of networks that is responsible for regulation and recruitment of RL E motoneuron activity. Under conditions of quiet breathing, interactions within the network are likely to be small, as indicated by the absence of E-HFOs. When a large excitatory input arrives during FV, the increased firing of neurons involved in RLE activity results in activation of excitatory and inhibitory loops and thereby produces the intrinsic E-HFO rhythmic firing. The relative weakness of this rhythm in SL E activity compared with RL E activity suggests that the RLE-related system may be driving the SL-related system.

In addition to their intrinsic rhythms, RL and SL E activities also had a stimulus-locked component (Fig. 9). The E-HFO intrinsic component and the stimulus-locked component were manifested as distinct spectral peaks of unrelated frequency. The relative magnitude of the two components differed between preparations.

ABDOMINAL E ACTIVITIES. During FV, although there was a massive recruitment of ABD activity (which was absent during the control state), there was no appearance of intrinsic fast rhythms like those in RLE activity. Instead, ABD discharge became synchronized to individual stimuli, as indicated by a high stimulus-nerve coherence at the stimulus frequency. Thus the ABD motoneurons and their premotor neurons were capable of following stimulus inputs, but seemingly the associated medullary network was not capable of rhythm-generating interactions. This weakness or lack of intrinsic population rhythm has been reported in earlier studies of spinal and medullary augmenting expiratory neurons (Cohen et al. 1985, 1992).

Thus during FV the group of I activities and the group of E activities each had two distinct components, an intrinsic rhythm (I-HFO or E-HFO, respectively) and a stimulus-locked component. However, the relative strength of the two components differed between I and E activities: the intrinsic-rhythm component is stronger in I than in E activities, and conversely for the stimulus-locked component. This difference explains the presence of I-HFOs and the absence of E-HFOs in spontaneous respiration. Moreover, the system that generates abdominal augmenting E neuron activity seems to act primarily as a “follower” system with limited interactive capability.

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Address for reprint requests: M. I. Cohen, Dept. of Physiology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461.

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REFERENCES


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