# SYNCHRONIZATION OF DISCHARGE, SPONTANEOUS AND EVOKED, BETWEEN INSPIRATORY NEURONS

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*Abstract.* (i) In midcollicular decerebrate, unanaesthetized, vagotomized, paralyzed cats, efferent phrenic discharge was recorded simultaneously with inspiratory unit and inspiratory wave activity in the rostral lateral medulla. Phrenic motoneurons tend to fire in synchronous bursts, as indicated by the occurrence of high-frequency oscillation (60–110/sec; median oscillation period 13 msec) in the whole-nerve recording. (ii) Crosscorrelation analysis of medullary inspiratory unit vs. phrenic activity showed that most units in the rostral medulla had oscillation of discharge which was locked to phrenic oscillation; peak unit activity usually occurred 3–5 msec before peak phrenic activity. Since this delay is comparable to the latency of a phrenic response evoked by an electric stimulus in the inspiratory regions, it is suggested that these medullary inspiratory neurons are part of the efferent inspiratory pathway from medulla to spinal cord. (iii) In the region near the nucleus ambiguus, wave activity occurring during the inspiratory phase was recorded; its phase relation to phrenic oscillation was similar to the unit→ phrenic phase relation. It is suggested that the wave activity reflects synchronized synaptic potentials in neighbouring structures. (iv) By electrical stimulation of the rostral pontine pneumotaxic system (nucleus parabrachialis), it was possible to force the oscillation frequency as well as to change the phase relations of activity. (v) Thus, medullary inspiratory activity has a short-term synchrony. It is suggested that this synchrony arises from: (a) reexcitant connections between inspiratory neurons; (b) similar time courses of post-spike inexcitability in different inspiratory neurons. The phenomenon of high-frequency oscillation in inspiratory discharge furnishes an electrical sign of inspiratory activity, which should prove useful in analysis of the mechanisms producing inspiratory discharge.

In the analysis of respiratory periodicity, two major questions arise: (i) How does the relatively slow oscillation of respiratory discharge develop from rapid neural changes (pre- and post-synaptic events occurr-

ing on a time scale of milliseconds)? (ii) How is activity synchronized between the large numbers of neurons which discharge in a particular portion of the cycle, e.g., between different neurons of the inspiratory network? These questions are also of more general neurophysiological import, since similar problems arise in the analysis of other neural networks.

In the present study, I have approached these questions by exploring the phenomenon of short-term synchronization between inspiratory neurons, which is manifested in the final common pathway by high-frequency oscillation (60–110/sec) of phrenic discharge. The phrenic oscillations were reported in the earliest oscillographic studies (Gasser and Newcomer 1921, Gasser 1928) as well as by subsequent investigators (Rijlant 1937, Wyss 1939, 1955-56). In my own experiments, I have studied both the spontaneous phrenic oscillations and their modification by brain stem electrical stimulation; further, I have recorded similar oscillations of inspiratory unit and wave activity, locked to phrenic oscillation, in the medulla oblongata. The data were analyzed by the methods of auto- and cross-correlation. The aim was to use these oscillations as an "electrical signature" of inspiratory activity, in order to explore some of the mechanisms by which inspiratory centre connections give rise to the patterns of inspiratory discharge.

## METHODS

Experiments were performed on midcollicular decerebrate cats (2.7–3.5 kg) with vagotomy, neuromuscular blockade (by intravenous infusion of gallamine triethiodide), and thoracotomy. Surgery was performed under ether or fluothane anaesthesia, which was discontinued at least 2 hr prior to recording. The animals were artificially ventilated with 100% $O_2$ by a positive-pressure ventilator, whose rate and volume could be adjusted to maintain any desired end-tidal $CO_2$ level (monitored with a rapid infrared analyzer). Blood pressure was monitored with a Statham pressure transducer, and where necessary was maintained by intravenous infusion of 5% glucose in physiologic saline. Rectal temperature was maintained at 36–38°C by use of a heater.

All data were recorded on magnetic tape and later played back for photography and computer analysis.

### Phrenic nerve recording

The phrenic nerve (usually the left) was exposed in the neck by a dorsal approach, ligated and cut; the central end was mounted on a bipolar electrode, with the cut end crushed over the peripheral hook to obtain a monophasic recording. The monophasic signal was recorded with

bandpass 0.8–1,250 cycle/sec. In addition, a high-pass recording of the phrenic potentials (preamplifier bandpass 80–10,000 cycle/sec) was used to obtain a signal with a stable baseline for derivation of pulses marking the phases of the respiratory cycle. This signal, after suitable processing, yielded standard pulses marking the onset of the inspiratory (I) and expiratory (E) phases (Cohen 1968).

### Microelectrode recording

The dorsal surface of the brain stem was exposed by removal of muscle, bone, and a portion of the cerebellum (by suction); to prevent drying, the surface was covered with a warmed mixture of vaseline and mineral oil. Microelectrodes were inserted into the medulla and pons by means of a hydraulic manipulator, the insertion being guided by surface landmarks and manipulator co-ordinates. The microelectrodes (commercially obtained from Transidyne General Corp.) consisted of thin insulated tungsten rods (tips coated with platinum black) having tip exposure of 1 $\mu$. The microelectrode signal was passed through a source follower and then into two preamplifiers of different bandpass to obtain: (i) a high-pass signal (for spike activity), bandpass 80–10,000 cycle/sec; (ii) a low-pass signal (for spike activity), bandpass 8–1,0000 cycle/sec. Most usually, unit activity with negative-positive spikes was recorded; but in some cases activity with positive-negative spikes was stable enough to allow prolonged recording. The unit signal, after further amplification, was passed through an amplitude discriminator, to obtain a standard pulse for each spike.

The locations of recorded activity were determined from manipulator readings and from histological sections stained with cresyl violet and luxol blue. To aid in localization, in some cases small lesions were made by passage of current (100 $\mu$a for 15–20 sec) through the microelectrode.

### Electrical stimulation

Electric stimuli (100 $\mu$sec duration, 0.2–0.7 ma) were applied in the dorsolateral pontine regions of the "pneumotaxic centre" (Bertrand and Hugelin 1971, Cohen 1971). The bipolar stimulating electrode consisted of a pair of twisted, enamel-coated nichrome wires (total tip size about 0.3 mm). Stimuli were usually applied at predetermined times in the respiratory cycle, by means of delayed triggers derived from the I and E pulses (for further details, see Cohen 1971).

*Average response computation*

An averaging computer (CAT 1000) was used to obtain (i) summed spontaneous and (ii) summed evoked activity: (i) The patterns of summed spontaneous discharge were obtained by using pulses derived from the I or E pulses to trigger the computer analysis sweeps. In some cases, in order to compute average activity immediately preceding the I pulse (in the late expiratory and very early inspiratory phases), the method of "time reversal" was used: Pulses delayed by a fixed time from the I pulses were recorded on tape, together with the original signals; the tape was played in a backward direction; and the delayed pulses were used to trigger the computer analysis sweeps, the duration of which was set so that activity immediately preceding and following the original I pulses occurred during the sweeps (*see* Fig. 1). (ii) The average evoked responses to electric stimuli were obtained by using analysis sweeps triggered by pulses associated with the stimulus trains.

*Correlation computation*

Auto- and cross- correlation functions were obtained with a correlation computer (Mnemotron 258).

1. *Filtering.* In order to improve signal-to-noise ratio and to eliminate unwanted frequency components, the recorded signals were passed through RC filters before entry into the correlator. The usual bandpass was: (i) for phrenic activity, 0.8–250 cycle/sec; (ii) for medullary wave activity, 16–250 cycle/sec (the low-frequency cutoff point being chosen to eliminate artifacts due to cardiac pulsation). It was verified that this filtering did not affect the frequency components of interest (60–110/sec) or introduce appreciable phase shift: (i) Similar correlograms (particularly with respect to phase relations) were obtained from the filtered and unfiltered signals (the latter, however, furnished noisier correlograms). (ii) When a 100/sec sine wave was passed through the RC filters, the observed phase shift was less than 5%, which was less than the uncertainty introduced by the correlator bin resolution (7–10%).

2. *Correlation functions.* The following were computed (usually for 50–100 respiratory cycles): autocorrelograms (ACG) of phrenic, medullary wave, and medullary unit (spike) activity; crosscorrelograms (CCG) of phrenic vs. medullary wave activity; crosscorrelograms of phrenic vs. unit activity. The usual bin duration was 1 msec and the duration of the analysis sweep (consisting of 32 bins) was 32 msec, so that 2–3 cycles of oscillation were obtained in the correlograms.

3. *Unit correlograms.* Since the correlator operated on analog inputs,

in order to process unit activity it was necessary to provide a signal in analog form; this usually consisted of 1 msec pulses ($\pm 2.5$ v), each derived from the original standard unit pulse. The analog pulse duration of 1 msec was chosen to provide adequate sampling during the 1 msec bins.

4. *Gating.* In some cases, only that portion of a signal occurring during the inspiratory phase was allowed to enter the correlator, by means of a gate derived from the I and E pulses. This gating procedure was necessary to separate activity in the two phases, since: (i) medullary slow potentials sometimes had different frequency components in each phase, and (ii) units which fired during both phases had different discharge frequencies in each phase.

The computer memory contents were read out in analog form to an inkwriter or oscilloscope, and in digital form to a printer. The oscilloscope display was photographed with a Polaroid camera. In the case of activity summed with "time reversal" (*see* above), the correct real time direction in the photographic display was obtained by: (i) using the invert mode of the oscilloscope amplifier, and (ii) turning the photograph through 180°.

## RESULTS

### High-frequency oscillation in phrenic discharge

The occurrence of oscillatory waves of activity (frequency range 60–110/sec) in efferent phrenic discharge, which was reported by earlier investigators (Gasser 1928, Rijlant 1937, Wyss 1939), indicates that phrenic motoneurons tend to fire in short synchronized bursts of activity, which are interspersed with relative inactivity. In the present study, this phenomenon was intensively investigated. It was consistently found in the preparation used: the unanaesthetized, decerebrate, vagotomized and paralyzed cat. However, both the degree of oscillation (amplitude of the waves) and its period varied with the individual preparation. In 12 cats, the oscillation period ranged from 9 to 17 msec; the median period was 13 msec (frequency 77/sec).

Among the conditions which influenced the occurrence and magnitude of the phenomenon were (i) anaesthesia and (ii) $CO_2$ tension: (i) It was consistently observed that as the animal recovered from fluothane anaesthesia, the phrenic oscillation gradually increased. Further, in two animals where sodium methohexital (Brevital) was used for anaesthesia, the phrenic oscillation did not appear for many hours after the administration of the last dose. (ii) Increase of $CO_2$ tension augmented the phrenic oscillation (*see* Fig. 10). Therefore, in order to obtain large waves and a high signal-to-noise ratio (which was useful for crosscorrelation with oscillatory

medullary activity), recordings were routinely taken with the animals at elevated end-tidal $CO_2$ levels (6–8%). However, the existence of oscillation was by no means dependent on hypercapnia, since it also occurred during normocapnia (ca. 4% end-tidal $CO_2$).

A typical example of oscillatory activity in monophasically recorded efferent phrenic discharge is shown in Fig. 1. The top trace shows phrenic activity occurring during a single inspiratory phase. The discharge starts



Phr

100 msec

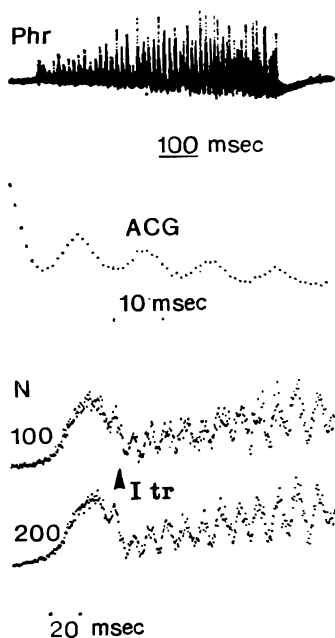ACG

10 msec

N
100

I tr

200

20 msec

Fig. 1. Oscillation of phrenic discharge and its analysis by autocorrelation and summing computation. Top: monophasic recording of efferent phrenic potentials during one inspiratory phase (negativity of proximal electrode upward). Middle: autocorrelogram (ACG) of phrenic potentials in 50 inspiratory phases (from recording which furnished top trace); bin duration 1 msec; sweep duration 64 msec. Bottom pair of traces: summed phrenic activity (from same recording) near the start of the inspiratory phase, using two different sample sizes. The smaller sample (top, $N = 100$) is included in the larger sample (bottom, $N = 200$). Analog readout gain for top trace is twice that for bottom. Analysis sweeps (0.5 msec bins) were triggered by pulses delayed 150 msec from the I triggers (I tr), with tape played backwards (method of "time reversal").

sharply with an initial burst, which is then followed by a series of waves of apparently regular period, but with some variability of amplitude. However, the amplitude of the waves tends to increase as the inspiratory phase progresses. Finally, near the end of the phase, there is some tendency towards desynchronization of activity.

To establish that such activity was truly oscillatory rather than randomly fluctuating, autocorrelation functions were computed from the phrenic potentials. The autocorrelograms, such as that for the case of Fig. 1 (ACG, middle trace), had the form typically obtained from a mixture of a noise signal and a periodic signal (Lee 1960, ch. 12): an exponentially decaying waveform with superimposed oscillations. Thus, the existence of oscillation was statistically validated by the autocorrelation computation. In the case of Fig. 1, the period of oscillation, as read from the autocorrelogram, was 13 msec.

An interesting finding is that the phrenic oscillation tends to be time-locked to the start of the inspiratory phase. This was demonstrated by using the average response computer to sum phrenic potentials in analysis sweeps started by the I trigger (or by a pulse related to the I trigger). The resulting traces of summed activity (Fig. 1, bottom traces) contain periodically recurring waves, which shows that on the average the oscillation was locked to the initiating trigger (start of inspiratory phase). The possibility that the apparent locking was a statistical artifact due to insufficient averaging was precluded by the observation that the summed waveform was doubled in amplitude when the sample size was doubled (from $N = 100$ to $N = 200$), as shown in Fig. 1, bottom (the readout gain of the bottom trace of the pair being half that of the top trace). Therefore, since summed activity increased proportionately to $N$, the summing computation was extracting a component truly time-locked to the trigger, according to signal theory (Lee 1960).

Therefore, it can be asserted that the initial phrenic burst at the start of the inspiratory phase is associated with events setting off a "ringing" type of oscillation. The locking of oscillation to the start of the phase was observed in all cats. In many cats, following the initial burst there was a depression of activity, which then gave way to recurring waves of discharge, gradually growing in amplitude. These waves were locked to the initial burst and the succeeding depression, as demonstrated by the averaging method illustrated in Fig. 1.

By application of electrical stimulation to appropriate brain stem regions, phrenic responses can be evoked which are locked to individual stimuli. In the present study, stimulation was applied to the region of the nucleus parabrachialis (dorsolateral rostral pons), which is believed to be the site of the pneumotaxic mechanism. Such stimulation produces two types of inspiratory-facilitatory effect: a) a stimulus train applied during the inspiratory phase produces increased phrenic discharge (evoked responses); b) a stimulus train applied during the expiratory phase produces switching to the inspiratory phase, i.e., premature termination of the expiratory phase (Bertrand and Hugelin 1971, Cohen 1971).

The effect of stimulus trains delivered in this region during the inspiratory phase was to replace the spontaneous phrenic oscillation with an imposed oscillation at the stimulus frequency. As shown in the traces of summed activity in Fig. 2 (analysis sweeps triggered from the start of the inspiratory phase), the spontaneous or control (C) activity had an oscillation locked to the start of the I phase. When a train of 15 stimuli at 100/sec was applied (the first shock occurring 20 msec after the I trigger), after 3–4 stimuli the periodicity of discharge was shifted to that of the stimulus train. This change from the spontaneous to the imposed frequen-
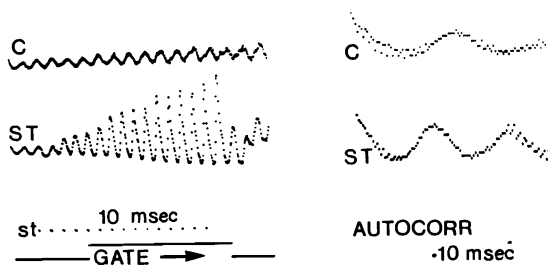
Fig. 2. Spontaneous phrenic oscillation (locked to start of inspiratory phase) and its modification by stimulus trains delivered at rostral pontine inspiratory-facilitatory point (nucleus parabrachialis). Same recording as in Fig. 1. Left traces: summed phrenic potentials in analysis sweeps ($N = 200$ each, 0.5 msec bins) triggered by I pulses. C: control activity (no stimuli delivered). ST: activity during I phases in which stimuli were delivered. Stimulus trains (st) consisted of 15 stimuli (dots) at 100/sec, with first stimulus occurring 20 msec after I pulse. Control and stimulation phases were alternated. GATE: phrenic signal was allowed to pass into correlation computer during gate occurring 60–180 msec after onset of I phase. Right traces: autocorrelograms (AUTOCORR) of activity during gates in 200 control (C) and 200 stimulation (ST) phases.

cy can be seen in the autocorrelograms (right), computed from control (C) activity and from activity during the stimulus trains (ST); they show that stimulation shifted the period from 13 msec (spontaneous) to 10 msec (which was the interstimulus interval). Finally, as seen in the left trace (ST), after the end of the stimulus train the period of oscillation returned to the control value (13 msec).

The ease of forcing of phrenic oscillation by stimulation was dependent on stimulus frequency; the forcing was greatest when the stimulus frequency was near the spontaneous frequency. This phenomenon is illustrated in Fig. 3, which shows the summed responses to trains of five stimuli, at three different frequencies (interstimulus intervals 5, 10, and 15 msec). It can be seen that the largest evoked responses occurred when the interstimulus interval was 10 msec (B), a value close to the period of spontaneous oscillation, 9 msec, which was measured by autocorrelation of spontaneous activity (lowest trace). At higher (A) and lower (C) stimulus frequencies, the evoked waves were considerably smaller. Thus, stimulus forcing is most effective when the stimulus frequency is close to the spontaneous frequency.

Two other effects of stimulation are also seen in Fig. 2 and 3: (i) During a stimulus train, there is gradual increase of amplitude of successive evoked waves. Further, this progressive augmentation is greatest at the optimal stimulus frequency (Fig. 3B). (ii) Following the wave evoked by the last stimulus of a train, there occur one or more cycles of

post-stimulus oscillation, which are apparent in the traces of summed activity. As also shown in an earlier paper (Fig. 1 in Cohen and Gootman 1969), the period of the post-stimulus oscillation is the same as that of the spontaneous oscillation characteristic of the individual preparation.

Another phenomenon of interest is that the amplitude of the evoked phrenic response is dependent on time of stimulus delivery during the
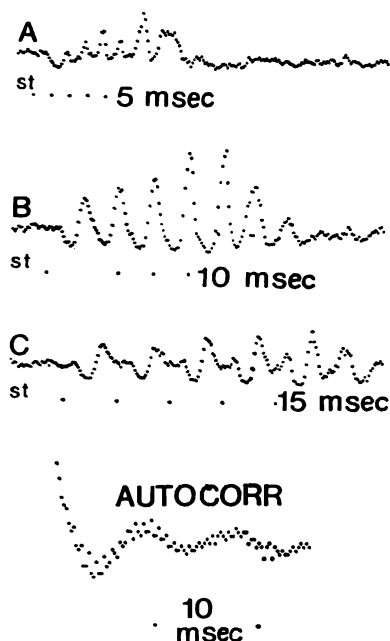


Fig. 3. Dependence of size of evoked phrenic oscillations on stimulus frequency. For each frequency, stimulus trains were delivered to nucleus parabrachialis in the middle portion of alternate inspiratory phases. Traces *A–C*: analog readouts of summed phrenic potentials in analysis sweeps (N = 50 each, 0.5 msec bins) triggered by pulses occurring 500 msec after start of I phase. Stimulus trains (st) consisted of five stimuli (indicated by dots), with the following interstimulus intervals (msec): *A*, 5; *B*, 10; *C*, 15. The first stimulus of each train was delayed from the sweep trigger by the interstimulus interval. Bottom trace: autocorrelogram (AUTOCORR) of spontaneous phrenic activity in 50 control phases (which alternated with stimulation phases of *C*); phrenic signal was allowed to enter correlator during gate started 500 msec after I pulse and ended by E pulse.

inspiratory phase. Thus, as seen in the traces of summed responses in Fig. 4, the later in the inspiratory phase that a stimulus train is applied, the larger is the evoked increase of phrenic discharge, as shown by the increased amplitude of the individual stimulus-evoked waves. Thus, as inspiration progresses it becomes easier to excite inspiratory neurons.
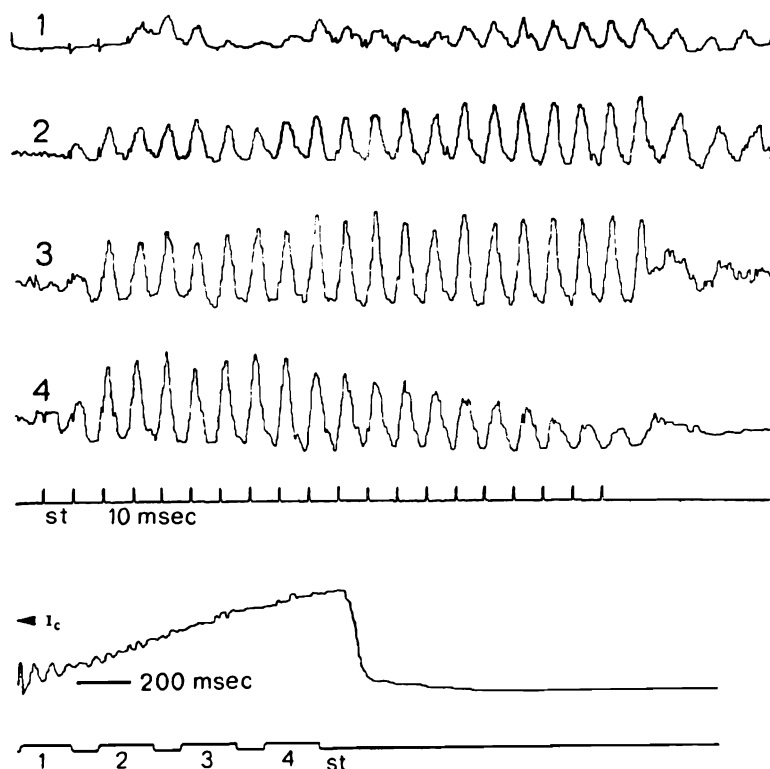
Fig. 4. Dependence of amplitude of evoked phrenic responses on delivery time of stimulus trains during inspiratory phase (stimulation site: nucleus parabrachialis). Each of traces *1–4* is the analog readout of computer-summed phrenic potentials (80 analysis sweeps, 0.5 msec bins) related to stimulus trains delivered at a given time in the I phase. Sweep triggers (start of each trace) were pulses delayed from onset of the I phase by: *1*, 10 msec; *2*, 300 msec; *3*, 600 msec; *4*, 900 msec. Timing of individual stimuli (st) shown under trace *4*: each train consisted of 20 stimuli at 100/sec, with the first stimulus occurring 10 msec after the sweep trigger. Bottommost traces: time relation of stimulus trains (*st* markers *1, 2, 3, 4*) to control I phase; spontaneous phrenic activity pattern is shown by the trace of summed phrenic potentials (filtered, half-wave rectified signal) in analysis sweeps ($N = 20$, 10 msec bins) triggered by start of control I phase ($I_c$, arrow).

## High-frequency oscillation in medullary activity

In a search for oscillatory activity locked to phrenic oscillation, micro-electrodes were inserted into those regions of the rostral medulla where inspiratory activity predominates, i.e., the regions 1–3 mm rostral to the obex and 2–4 mm lateral to the midline (Baumgarten et al. 1957, 1960, Haber et al. 1957, Batsel 1964, Merrill 1970, Bianchi 1971). Recordings were taken firing during both phases of the cycle which had higher

discharge from all inspiratory neurons encountered, as well as from those neurons frequency during the inspiratory phase. In addition, wave activity in these regions was recorded. By crosscorrelation of medullary and phrenic activity, it was possible to ascertain whether the former had an oscillation locked to the phrenic oscillation.

Three types of inspiratory activity were found, each in a different portion of the lateral rostral medulla: 1) In the region ventral to the nucleus and tractus solitarius (2.5–3.0 mm lateral to the midline, 1.8–2.5 mm ventral to the medullary surface), dense aggregates of inspiratory neurons were found; in many cases it was not possible in such recordings to separate spikes from different units. This is the region, shown in Fig. 5 as *A*, originally described by Baumgarten, Baumgarten
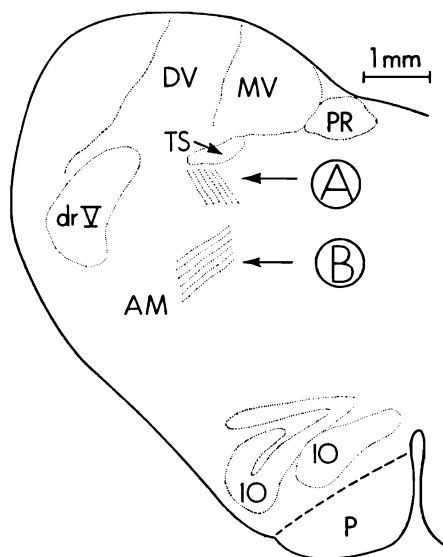


Fig. 5. Cross section of rostral medulla (plane P 10), showing regions where oscillatory inspiratory activity was recorded: *A*, dorsal respiratory nucleus, which is the site of dense inspiratory unit activity; *B*, region where inspiratory wave activity was found. Drawing constructed from histological sections with aid of Fig. 39 in Verhaart 1964. Abbreviations: AM, nucleus ambiguus; dr V, descending root of Vth nerve; DV, descending vestibular nucleus; IO, inferior olive; MV, medial vestibular nucleus; P, pyramid; PR, nucleus praepositus hypoglossi; TS, nucleus tractus solitarius.

and Schaefer (1957) as an inspiratory centre and named the "dorsal respiratory nucleus" by Bianchi (1971). 2) In a more ventrolateral region (3.0–4.0 mm lateral to the midline, 3.0–4.0 mm ventral to the medullary surface), many inspiratory neurons were found, intermixed with expira-

tory neurons; here it was usually easier to record from isolated units than in region (1). This ventrolateral region corresponds to that described by Merrill (1970) and named the "ventral respiratory nucleus" by Bianchi (1971). 3) In a region medial to region (2), located near the nucleus ambiguus and shown in Fig. 5 as B (2.5–3.5 mm lateral to the midline, 3.0–4.0 mm ventral to the medullary surface), oscillatory waves of activity, locked to the phrenic waves, were found. Similar wave activity was observed in this region of the medulla of the rabbit by Achard and Bucher (1954).

### Medullary inspiratory wave activity

The inspiratory wave activity in the ventrolateral medulla had the appearance of "slow potentials", consisting of waves of approximately triangular form, on which no superimposed spike activity was discernible. (Occasionally, expiratory unit activity was recorded by the same electrode.) A typical example of inspiratory wave activity is seen in Fig. 6, which shows both the phrenic (Phr) and the medullary (Med) activity simultaneously recorded during one inspiratory phase (top left). Examination of the recordings on an expanded time scale (B, bottom left) suggests that the oscillation period of both signals was the same, and that there was a definite phase relation between the two signals. The validity of these impressions was established by correlation analysis. As shown in the autocorrelograms (top right), the two signals had the same period of oscillation, about 11 msec. Further, the crosscorrelogram (CCG) of the two signals (bottom right) shows oscillation with this period, indicating that on the average the two oscillations were locked. The CCG also indicates the phase relation of the locking: since medullary activity was correlated with succeeding phrenic activity (M→P), the occurrence of a peak at the fourth bin of the correlogram (i.e., at a 3 msec delay) means that the peak of phrenic activity tended to follow the peak negativity of medullary activity by 3 msec.

In eight cats, 13 different recordings of such wave activity were made, each at a different point located in the general region of Fig. 5B. In 11 of these recordings, the phase relation between phrenic and medullary activity was similar to that in the CCG of Fig. 6: peak phrenic activity lagged peak medullary negativity by 3–5 msec in different cases, with the oscillation periods ranging from 9 to 13 msec. In two other cases, the lag of phrenic activity was greater (8 and 10 msec, with oscillation periods of 13 msec); the phase relation in one of these cases is shown in the CCG of Fig. 11.

*Expiratory centre wave activity.* In two cases, recordings in the caudal
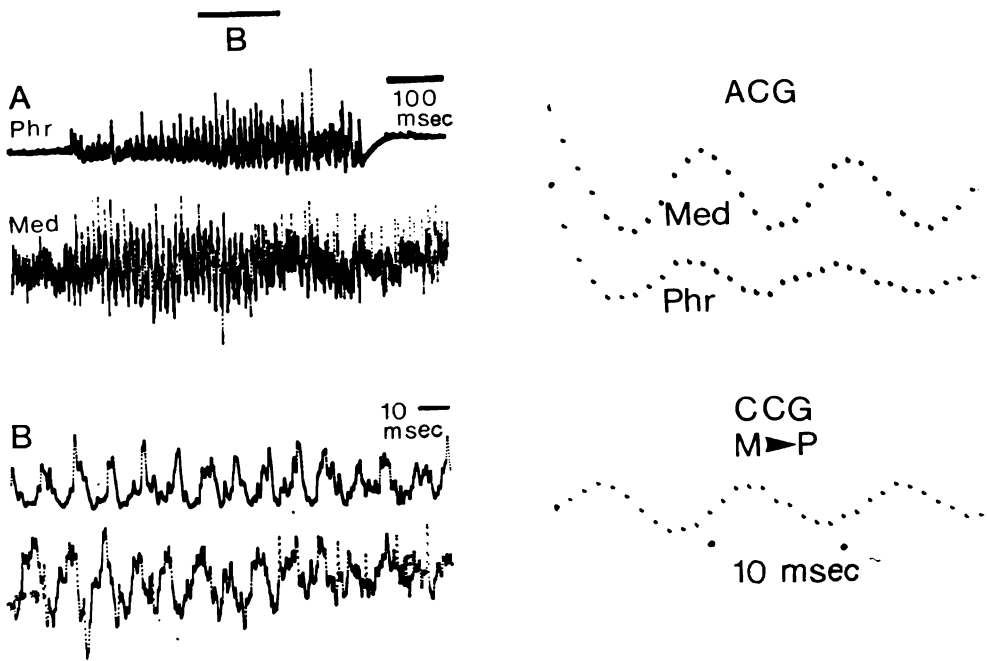
Fig. 6. Relation between phrenic oscillation and oscillation of medullary inspiratory waves, analyzed by correlation computation. Left traces: *A*: Simultaneous recording of phrenic and medullary activity during one inspiratory phase (negativity upward for both signals); medullary recording site similar to that in Fig. 5 (B). *B*: Portion of trace set *A* (marked by horizontal line *B* above) displayed on expanded time scale. Right traces: autocorrelograms (ACG) of medullary (Med) and phrenic (Phr) activity; crosscorrelogram (CCG) of medullary vs. phrenic (M→P) activity. Computations done on sample of 100 inspiratory phases (using recording which furnished left traces); signals passed through I gates before entry into correlator. Dot marking first bin of each correlogram is retouched. In this and subsequent Figures, correlograms are analog readouts of computer memory contents; 32 1-msec bins. The designation *A*→*B* means that signal *A* was correlated with subsequently occurring signal *B* (i.e., past values of signal *A* were stored in correlator memory bins and correlated with real-time value of signal *B*).

medullary expiratory centre (Baumgarten et al. 1957, Haber et al. 1957) revealed the presence of oscillatory waves locked to phrenic oscillation. (In fact, by serendipity, medullary wave activity was first noticed here.) In this region, located 2–3 mm caudal to the obex, 3–4 mm lateral to the midline, and 3–4 mm ventral to the medullary surface, dense expiratory unit activity was recorded; and during the inspiratory phase, large oscillatory waves occurred. Crosscorrelation revealed that the phase relation between these waves and phrenic activity was opposite to the usual relation in the inspiratory regions, i.e., phrenic activity lagged peak *positivity* by 4 msec in each case.

## Medullary inspiratory unit activity

An example of an inspiratory unit whose activity had the same oscil-
lation as phrenic discharge is shown in Fig. 7. This unit was found in
the ventral respiratory nucleus. As shown in the oscilloscope traces of
phrenic and unit activity during one inspiratory phase (top left), the
unit started firing about 150 msec after the start of phrenic discharge,
its frequency increased throughout the inspiratory phase (reaching
a maximum of ca. 300/sec), and its discharge ceased abruptly at the
end of the phase. Some further details of the activity pattern are shown
in traces on an expanded time scale (right). During the early part of the
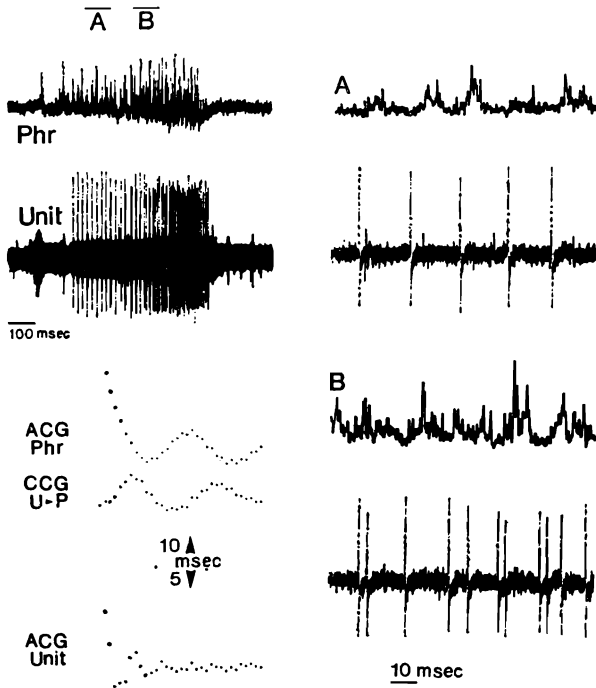discharge burst (A) each phrenic wave was associated with a single



Fig. 7. Example of activity of a medullary inspiratory unit (found in ventral
respiratory nucleus) which had oscillation locked to phrenic oscillation, as shown
by correlation analysis. Left traces, top: phrenic and unit activity during one
inspiratory phase; unit spikes are positive-negative. A, B (right): portions of left
traces (marked by horizontal lines A and B above) displayed on expanded time
scale. Correlograms (lower left), computed from sample of 65 inspiratory phases.
Phrenic autocorrelogram (Phr ACG) and unit→phrenic crosscorrelogram (U→P
CCG): 32 1-msec bins. Unit ACG: 32 0.5-msec bins (obtained by tape playback
at half speed). Unit signal input to correlator: 1-msec pulses, ±2.5 v.

spike; while later in the discharge burst (*B*) there was a tendency for each phrenic wave to be associated with a doublet (two spikes separated by about 3 msec).

The relation between phrenic oscillation and this unit's oscillation is seen in the phrenic ACG and the unit→phrenic CCG (Fig. 7, middle traces, left). Both of these correlograms showed the same oscillation period (16–17 msec), indicating that the unit's discharge had an oscillatory component with the same period as phrenic oscillation. This oscillation was also seen in the unit ACG with 1 msec bin duration (not shown), which had a small peak at the 17th bin. The phase relation of phrenic and unit activity is shown in the CCG: peak phrenic activity tended to follow peak unit activity by about 5 msec (as indicated by the peak at the sixth bin). Finally, another component of unit oscillatory activity is seen in the unit ACG with 0.5 msec bin duration (lowest trace, bottom left); here, the occurrence of a peak at 3.0–3.5 msec delay indicates the existence of oscillation with this period, which corresponds to the doublet interval.

The high discharge frequency of the unit whose activity is shown in Fig. 7 was typical of many medullary inspiratory neurons, which had maximum discharge frequencies in the range 100–300/sec.

*Multiple inspiratory unit activity.* As already mentioned, in the dorsal
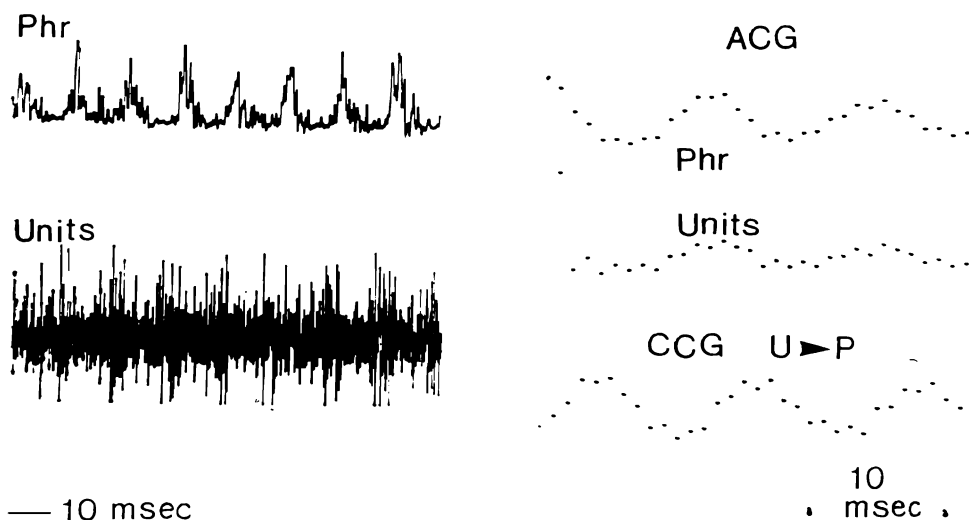


Fig. 8. Multiple unit activity (recorded in dorsal respiratory nucleus) which had oscillation locked to phrenic oscillation, as shown by correlation analysis. Left traces: phrenic and unit activity (negativity upward for both) in the middle of an inspiratory phase (sweep starts 310 msec after I trigger). Correlograms (right) computed from sample of 45 inspiratory phases. For abbreviations *see* Fig. 7.

respiratory nucleus dense aggregates of inspiratory units were found, making it difficult to separate spikes of individual units. An example of a recording taken in this region is shown in Fig. 8. The oscilloscope traces (left) show activity in the middle of an inspiratory phase; it can be seen that each phrenic wave is associated with a burst of several inspiratory units. To handle such recordings, an arbitrary triggering level of the amplitude discriminator was set, so as to allow spikes of comparable amplitudes fired by 3–4 individual units to produce standard pulses; and correlation analysis was then applied to these derived pulses. As seen in the phrenic and multi-unit ACG's (right), both types of activity had the same oscillation period, 12 msec. The unit→phrenic CCG had a peak át the fifth bin, indicating that peak phrenic activity tended to lag peak multi-unit activity by 4 msec. Analyses similar to that shown in Fig. 8 were performed in four different cats on multi-unit recordings obtained from the dorsal respiratory nucleus, and furnished similar results: the unit→phrenic lags ranged from 3 to 5 msec, with oscillation periods ranging from 10 to 16 msec.
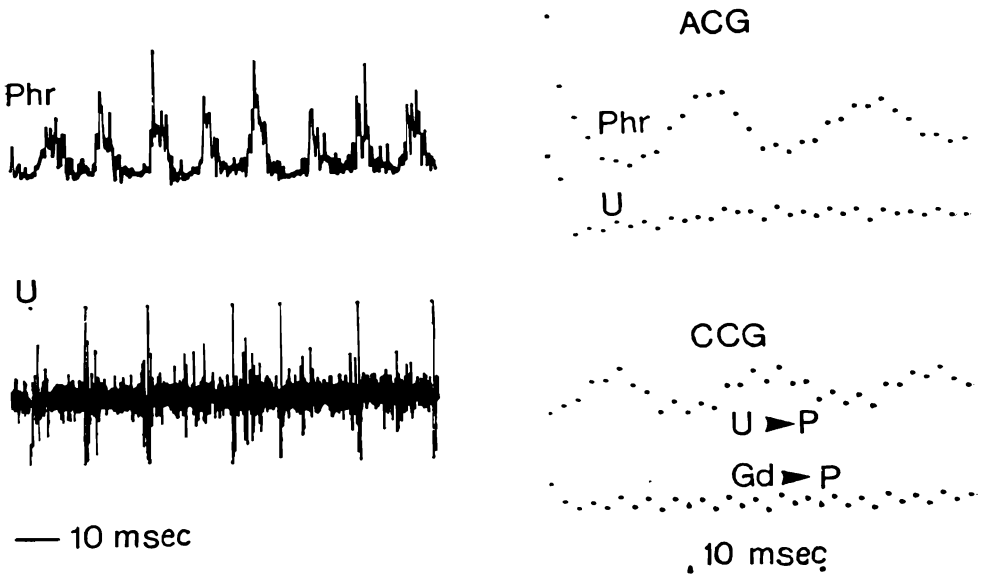


Fig. 9. Activity of a medullary inspiratory unit (found in dorsal respiratory nucleus) which had oscillation locked to phrenic oscillation, as shown by correlation analysis. Left traces: phrenic and unit activity (negativity upward for both) in the middle of an inspiratory phase (sweep starts 290 msec after I trigger); tops of spikes clipped due to amplifier overload. Correlograms (right) computed from sample of 100 inspiratory phases; abbreviations as in Fig. 7 and 8. Gd→P CCG: crosscorrelogram of a ground signal with phrenic activity in 100 inspiratory phases. Dot marking first bin of Phr ACG retouched.

Finally, another variant of inspiratory unit activity which had oscilla-
tion locked to phrenic oscillation is illustrated in Fig. 9, which shows
activity in the middle of an inspiratory phase. This unit was found 90 $\mu$
lower, in the same electrode track, than the units shown in Fig. 8. It can
be seen in the oscilloscope traces (left) that most phrenic waves were
associated with a single unit spike, but that an occasional phrenic wave
had no such associated spike. The phrenic ACG (right) has an oscillation
period of 12 msec, but no such oscillation appears in the unit ACG. How-
ever, the existence of unit oscillation locked to phrenic oscillation is
demonstrated in the unit→phrenic CCG, which has a period of 12 msec;
this CCG also shows that peak phrenic activity lagged peak unit activity
by 5 msec. The ability to detect the oscillation of unit activity by cross-
correlation, and the failure of detection by autocorrelation, illustrate the
theorem (Lee 1960, ch. 12) that crosscorrelation is a more efficient detector
of oscillation which is mixed with noise than is autocorrelation. Finally,
the last CCG shown in Fig. 9 (right, bottom) illustrates a control procedure
used to check for spurious correlation. A ground signal was crosscorre-
lated with the phrenic signal (from the same recording which furnished
the other correlograms); and as might be expected the resultant corre-
logram was flat, since no correlation between the ground signal and the
oscillatory phrenic signal existed.

An observation which may be of considerable importance is that in
the regions where inspiratory unit activity was plentiful (dorsal and
ventral respiratory nuclei), oscillatory inspiratory wave activity was not
discernible. Conversely, as already stated, inspiratory unit activity was
not discernible in the recordings of inspiratory wave activity.

I will now summarize the oscillatory properties of the total inspira-
tory unit population sampled in the medulla (19 units in 10 cats), which
consisted of two subgroups: 1) purely inspiratory units ($n = 13$); 2) units
which fired in both phases of the cycle, but with higher discharge
frequency during the inspiratory phase ($n = 6$). Crosscorrelograms
between unit and phrenic activity were computed only when appreciable
phrenic oscillation existed, as shown by autocorrelation. Further, the
number of cycles (75–100) used for each computation was more than
sufficient to reveal correlation, if it existed. In the purely inspiratory
group (1), 9 of the 13 units had CCG's showing oscillation locked to
phrenic oscillation; while in the group with both inspiratory and ex-
piratory discharge (2), only 2 of the 6 units had such oscillation (the
CCG's being computed using only those spikes occurring during the
inspiratory phase). For 10 of the 11 units which had oscillation locked to
phrenic discharge, the phase relations were similar to those already
shown (Fig. 7, 8 and 9): phrenic activity lagged unit activity by delays

ranging from 3 to 5 msec, for oscillation periods ranging from 9 to 17 msec. In the remaining unit, which had an early-inspiratory pattern, there was no lag between unit and phrenic activity (see Fig. 12). With the exception of the distinction between subgroups (1) and (2), it was not possible, because of the limited sampling, to distinguish between units which did or did not have oscillation, on the basis of other properties of their discharge.

### Pontine unit activity

Recordings were taken from three pontine units: one midpontine unit located at the level of the trapezoid body, and two rostral pontine units located in the region of the nucleus parabrachialis. All three units had similar patterns: tonic discharge with somewhat higher frequency in the inspiratory than in the expiratory phase. Crosscorrelation analysis showed that the midpontine unit had a weak oscillation in the inspiratory portion of its discharge; this oscillation was similar in its phase relation with phrenic activity to that observed in medullary inspiratory units (unit→ phrenic lag of 5 msec). The two rostral pontine units (recorded in the same cat) showed no trace of oscillation locked to phrenic discharge, even though in the same cat medullary inspiratory units were found which showed prominent phase locking to phrenic discharge.

### Effect of $CO_2$ tension

As previously mentioned, increase of $CO_2$ tension tended to increase the degree of phrenic synchronization (oscillation). An example of how raised $CO_2$ tension increases oscillation in both phrenic and inspiratory unit activity is shown in Fig. 10. The traces of summed phrenic and unit activity (left) show the average discharge patterns ($N = 75$ for each trace) at two different end-tidal $CO_2$ levels: 5.4% (A) and 7.0% (B). It can be seen that the inspiratory unit had an augmenting activity pattern which resembled very closely that of phrenic discharge. The effect of raised $CO_2$ was to increase both phrenic and unit discharge; the number of unit spikes/inspiration went from 49 to 55, and the average discharge frequency during the inspiratory phase from 62 to 73/sec. At the same time, as seen in the phrenic ACG and the unit→phrenic CCG, the magnitude of oscillation in both phrenic and unit activity increased, as indicated by the larger peak-to-peak amplitudes of the periodic component in each correlogram. However, changing the $CO_2$ tension did not alter the oscillation period (13 msec).
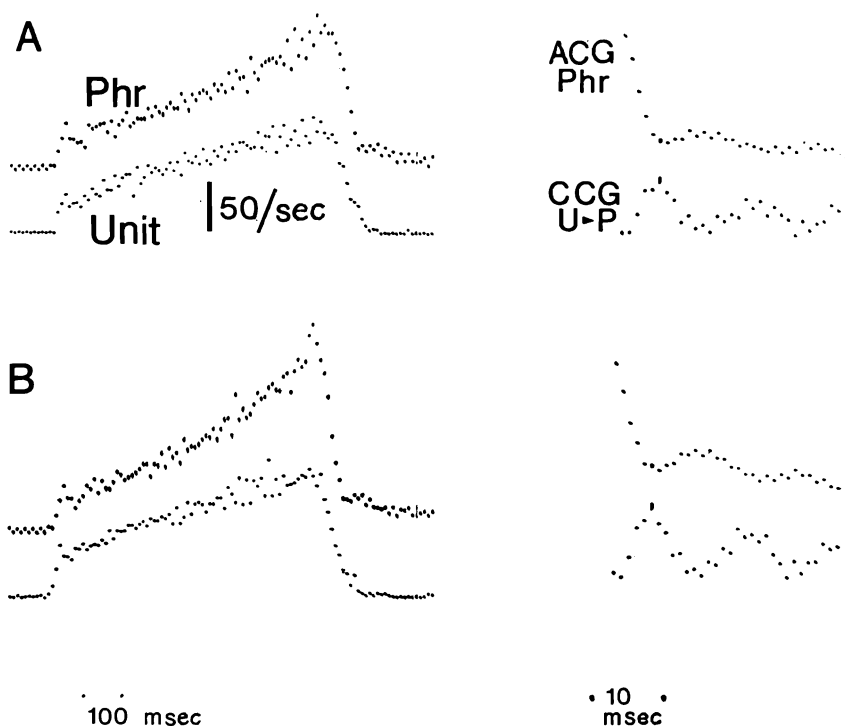
Fig. 10. Effect of $CO_2$ tension on oscillation in phrenic discharge and in activity of a medullary inspiratory neuron. End-tidal $CO_2$: *A*, 5.4%; *B*, 7.0%. Left traces: patterns of phrenic and unit discharge displayed in traces of summed phrenic potentials (after processing through a 5-msec time constant high-pass RC filter, followed by half-wave rectification) and summed unit activity (using 0.5-msec, $\pm2.5$ v pulse input to averaging computer). Analysis sweeps (10 msec bins) initiated by triggers ($N = 75$) delayed 1 sec from I triggers, with tape played backwards. For each signal, gains of the two summed waveforms are the same (note vertical line of unit frequency calibration). Right traces: phrenic autocorrelograms (identical gains) and unit→phrenic crosscorrelograms (identical gains) computed from same samples (75 inspiratory phases at each $CO_2$ level); unit input: 1-msec, $\pm2.5$ v pulses. Readout gain of CCG's is twice that of ACG's.

*Modification of medullary oscillatory activity*
*by pontine electrical stimulation*

The changes in medullary oscillatory wave activity produced by electrical stimulation in the nucleus parabrachialis are seen in Fig. 11, which shows stimulus-related activity recorded at a point where prominent spontaneous oscillations occurred (CCG, bottom right). In order to modify activity, trains of 20 stimuli at 100/sec were delivered in alternate respiratory cycles, each train starting 1.8 sec after the start of the expiratory
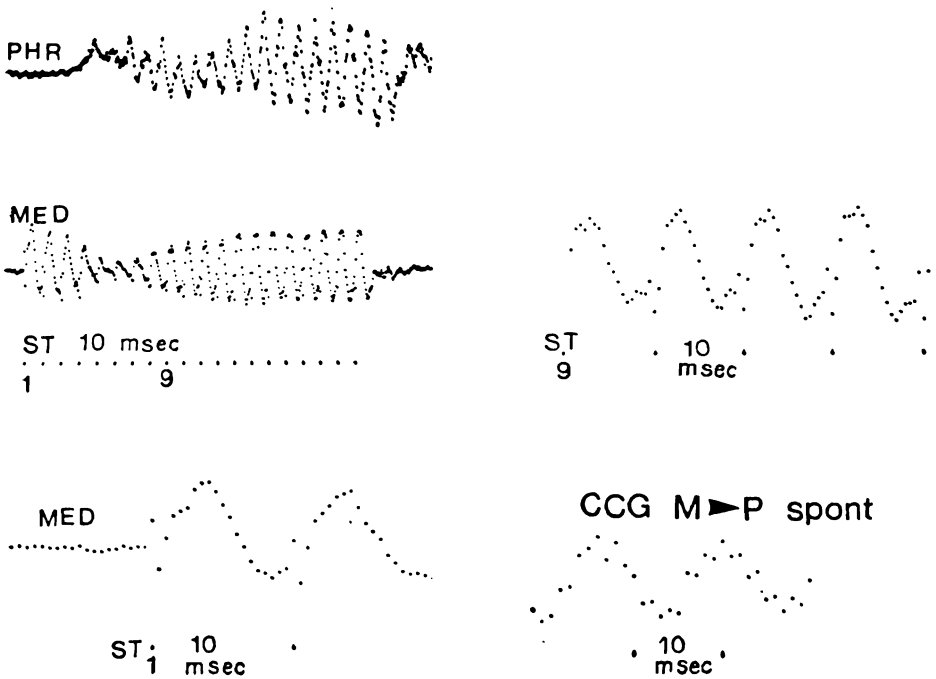
Fig. 11. Effects of rostral pontine electrical stimulation (in nucleus parabrachialis) on summed phrenic (PHR) and medullary (MED) inspiratory wave activity. Summed waveforms derived from activity associated with 50 stimulus trains, each having 20 stimuli at 100/sec. Analysis sweeps (0.5 msec bins) triggered by pulses occurring 1.8 sec after start of expiratory phase, each pulse preceding the first stimulus of a train by 10 msec. Left, top: traces on slow time scale; stimulus (ST) timing indicated by dots. Left, bottom: summed medullary activity on expanded time scale, showing activity at beginning of stimulus train (dots indicate stimuli *1* and *2*). Right, upper traces: phrenic and medullary summed activity (expanded time scale) during middle of stimulus train (dots indicate stimuli *9–13*). Right, bottom trace: medullary→phrenic crosscorrelogram of spontaneous (spont) activity, based on sample of 50 inspiratory phases in run immediately preceding stimulation run; signals passed through I gates before entry into correlator. Dot marking first bin retouched.

phase. As shown in the trace of summed phrenic activity (top left), the stimulus trains triggered the initiation of an inspiratory phase, the expiratory phase duration being shortened from the control value of 2.2 to 1.8 sec. The latency for triggering of the inspiratory phase was 35–40 msec. Further, the stimuli continued to be delivered during the early part of this inspiratory phase, and they produced phrenic and medullary waves which were locked to individual stimuli.

The most striking effect shown in Fig. 11 is the short latency of the medullary evoked response. As seen in the trace with expanded time scale

(bottom left), the latency of the response to the first stimulus was about 1 msec. Further (traces, top left), three of these medullary responses were evoked before the initiation of the triggered inspiratory phase (as indicated by the initial phrenic burst). Thus, there is a rather short pathway from the nucleus parabrachialis to the region of medullary inspiratory wave activity; and this activity can be greatly increased well before the triggering of a premature inspiratory phase.

The effects of stimulation on the phase relation between phrenic and medullary activity is seen in expanded traces (Fig. 11, top right) showing activity in the middle of the stimulus train. The spontaneous oscillation (13 msec period) had an unusual phase relation between the two signals; as shown in the CCG (bottom right) peak phrenic activity lagged peak medullary negativity by about 8 msec. During stimulation, the period of both signals was changed to 10 msec (the interstimulus interval); and the two signals were opposite in phase, i.e., the peak of the phrenic wave occurred about 5.5 msec after the peak of the medullary wave, this delay being about one half of the imposed oscillation period. The same relative phase shift existed in spontaneous activity (delay of 8 msec in a 13 msec period). Thus, though electrical stimulation imposed an oscillation having higher frequency than the spontaneous oscillation (10 msec vs. 13 msec period), the timing of both phrenic and medullary signals shifted to the same degree.

In another stimulation test, performed in another cat, stimulus trains were delivered to the nucleus parabrachialis during the middle of the inspiratory phase. The results were similar to those shown in Fig. 11 with respect to: (i) latency of the medullary evoked waves; (ii) phase relation of medullary and phrenic evoked waves. In this case, however, since the interstimulus interval was the same as the spontaneous oscillation period (10 msec), stimulation produced no change in the absolute timing of the waves, but rather only locking of the waves to the individual stimuli.

In a few cases, the method of electrical stimulation was used to produce concomitant changes in inspiratory unit and in phrenic activity. The example of an inspiratory unit presented in Fig. 12 is mainly of interest as an illustration of the method. The spontaneous pattern of summed unit activity (CONTR, top left) was early-inspiratory: the unit started firing at the start of the inspiratory phase, its maximum discharge frequency (ca. 200/sec) was reached in 50 msec, and then the discharge gradually decreased till it reached zero level well before the end of the inspiratory phase. This decrementing pattern contrasts with the augmenting pattern of phrenic discharge, which is also shown. The phase relation of this unit's oscillation (13 msec period) to the phrenic oscillation was unusual: there was no phase shift, i.e., the peaks of phrenic and of unit activity
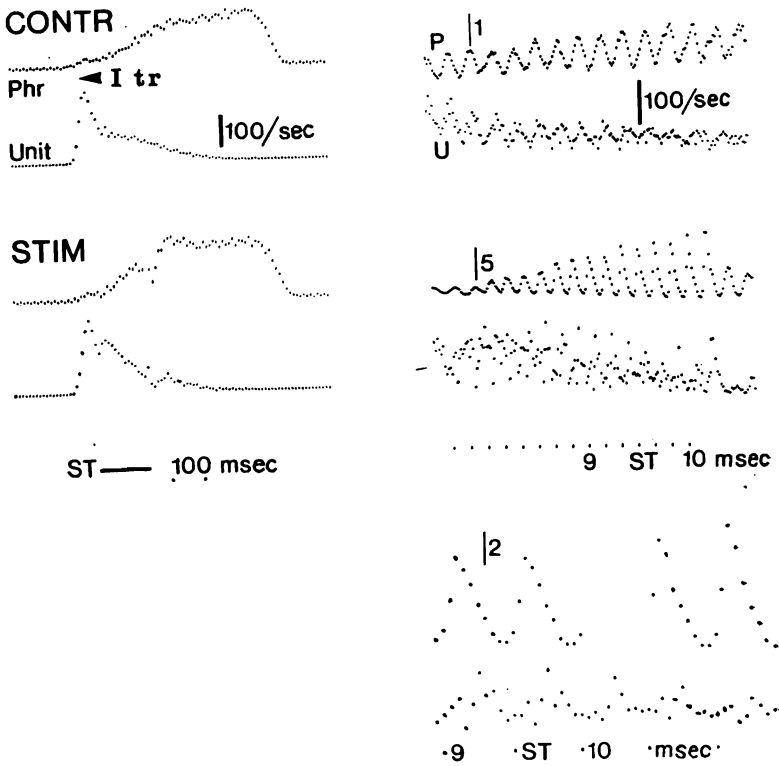
Fig. 12. Effects of rostral pontine electrical stimulation (in nucleus parabrachialis) on phrenic discharge and on activity of an early-inspiratory unit. Same recordings as used for Fig. 1 and 2. Control (CONTR) inspiratory phases (no stimulation) and inspiratory phases in which stimuli were applied (STIM) were alternated. 200 stimulus trains of 15 stimuli at 100/sec were used. Left traces: summed phrenic and unit activity during CONTR and STIM phases. Phrenic input: potentials were sent through a 5-msec time constant high-pass RC filter and then half-wave rectified. Unit input: 1-msec, ±2.5 v pulses. Analysis sweeps (10 msec bins) initiated by triggers ($N = 200$) delayed by 740 msec from I triggers (I tr), with tape played backwards. Timing of stimulus train indicated by horizontal line (ST). Right traces: summed phrenic (P) and unit (U) activity on faster time scales. Phrenic input: original (unfiltered) potentials; unit input: 1-msec, ±2.5 v pulses. Analysis sweeps (1 msec bins) initiated by triggers ($N = 200$) occurring 10 msec after I triggers; for STIM trace set, initiating trigger preceded first stimulus of train by 10 msec. Stimulus timing (ST) indicated by dots. Right, bottommost traces: display of STIM traces on expanded time scale, showing activity near middle of stimulus train (dots indicate stimuli 9–13). Note that readout gain of phrenic signal in each of the right traces is different (vertical lines indicate 1, 5, and 2 voltage units).

coincided within 1 msec (as verified from the computer digital readout). This phase relation can be seen in the traces of summed phrenic and unit activity on a faster time scale (top right), in which it is also apparent that both phrenic and unit oscillation were locked to the initiating trigger (pulse delayed 10 msec from the I trigger).

The effects of trains of 15 stimuli at 100/sec (the first stimulus occurring 20 msec after the I trigger), applied in the nucleus parabrachialis, are shown in the traces of summed stimulus-related activity (STIM). As seen in the traces on a slow time scale (left), stimulation resulted in increased phrenic discharge and increased unit discharge frequency; the latter effect is apparent in the trace as a slowing of the rate of decrement of discharge. The effects of stimulation on the phase relation between unit and phrenic activity are shown in the traces on faster time scales (middle and bottom sets of traces, right). During the early part of the stimulus train there was a disruption of the unit's periodicity; but later unit activity again became periodic and was locked to the period of the stimuli (10 msec). As seen in the trace pair on the fastest time scale (bottom right), which shows activity in the latter part of the stimulus train, the phrenic peaks tended to occur 2 msec, and the peaks of unit activity 5 msec, after each shock; so that during this part of the stimulus train the phrenic peaks lagged the unit peaks by 7 msec. Thus, stimulation changed the unit→phrenic phase relation, since during spontaneous activity there was no delay between the two signals.

Because of the unusual characteristics of the unit activity illustrated in Fig. 12, no general conclusions about the inspiratory population can be drawn from this example. However, the method illustrated should prove to be a powerful tool in further analyses of relations between phrenic and medullary inspiratory unit activity.

DISCUSSION

*Synchronization (high-frequency oscillation) of inspiratory discharge*

The existence of high-frequency oscillations (60–110/sec) in efferent phrenic discharge means that during the inspiratory phase the phrenic motoneurons tend to fire in synchronized bursts of activity, separated by times of relative inactivity. The consistent appearance of synchronization under the conditions of the present study, and the fact that it occurs in several different species (cat, rabbit, dog; Rijlant 1937) indicate that the phenomenon reflects fundamental properties of the organization of the inspiratory centres.

However, the fact that the phenomenon is often absent under other conditions, e.g., in the spontaneously breathing decerebrate cat (Cohen, unpublished observations) and in the anaesthetized cat (*see* traces in Bianchi 1971, and Fig. 1 in Cohen 1968), indicates that it is not essential for the generation of inspiratory discharge. Among the conditions which favoured the appearance of the phenomenon in the present study were: (i) lack of anaesthesia; and (ii) moderate hypercapnia. But contrary to earlier observations (Wyss 1955-56), extreme hypercapnia and hypoxia are not necessary for its occurrence. Other contributory factors may have been: (i) diminution of ventilated-related afferent input (by vagotomy and neuromuscular blockade), and (ii) removal of forebrain influences (by decerebration).

The common feature of the several conditions which seem to favour synchronization is that there is an increase in the average level of inspiratory activity. If the synchronization phenomenon is caused by reexcitant connections between inspiratory neurons (as hypothesized below), it seems reasonable that increased discharge of some inspiratory neurons would cause greater excitation as well as synchronization of firing in many other members of the inspiratory network.

In spite of its lack of universality, the synchronization effect is useful in the analysis of the mechanisms of inspiratory discharge, since it is an eletrical sing of inspiratory activity which may indicate temporal relations between different members of the inspiratory network.

Several observations relevant to the nature of synchronization were provided by experiments in which pontine electrical stimulation (in the region of nucleus parabrachialis) was used to modify the spontaneous oscillation: (i) Stimulation could force oscillation of inspiratory activity at frequencies different from the spontaneous frequency. (ii) However, the efficacy of the forced synchronization was a function of stimulus frequency: the closer that the stimulus forcing frequency was to the spontaneous frequency, the greater was the degree of evoked synchronization. (iii) Following a stimulus train, there occurred several cycles of post-stimulus oscillation at the same frequency as spontaneous oscillation.

Thus, in each preparation there is an intrinsic frequency of synchronized inspiratory activity. When the stimulus forcing frequency is close to this intrinsic frequency, the activity of the population is easily synchronized to the stimuli, presumably because the shocks tend to fall in the most efficacious phase relation to the excitability cycle of the neurons. Further, the increase of synchronization (as compared to spontaneous synchronization) persists following stimulation, in the form of post-stimulus oscillation; its subsequent decay is presumably due to the gradually increasing temporal dispersion of discharge of individual neurons.

## Medullary inspiratory unit activity

The observation in medullary recordings of both inspiratory unit and inspiratory wave activity, which had high-frequency oscillation locked to phrenic oscillation, means that this synchronization phenomenon originates at the brain stem level. The method of crosscorrelation between medullary and phrenic activity proved to be a sensitive detector of such oscillation, since the computation extracted that portion of the medullary signal which had the same frequency as phrenic oscillation (see Lee 1960, ch. 12).

The nature of the phase relations between the phrenic oscillations and the oscillations in medullary inspiratory unit activity is relevant to the question of how the oscillation originates and is transmitted from medulla to spinal cord. Crosscorrelation analysis showed that there was a relatively narrow range of delays (3–5 msec) between phrenic and medullary inspiratory unit activity: peak phrenic activity tended to follow peak unit activity by delays of 3–5 msec in different cases (for oscillation periods ranging from 9–17 msec). These delays are comparable in duration to the latencies (ca. 3 msec) of evoked phrenic responses produced by electrical stimulation of medullary inspiratory regions (Rijlant 1942, Pitts 1943).

The similarity of duration between (i) the unit→phrenic delay in spontaneous activity and (ii) the medullary→phrenic latency of the evoked response suggests the hypothesis that these medullary inspiratory neurons send impulses down the spinal cord via their projecting axons to excite phrenic motoneurons. Further, the shortness of both the delays and latencies suggests that there are few synapses between the medullary neurons and the phrenic motoneurons. The hypothesis is supported by observations in experiments involving antidromic stimulation, which showed that an appreciable fraction of the inspiratory neurons in the dorsal and ventral respiratory nuclei send their axons down the spinal cord to cervical levels (Ñakayama and Baumgarten 1964, Merrill 1970, Bianchi 1971). Thus, those medullary inspiratory neurons having oscillation locked to phrenic oscillation seem to be part of the efferent inspiratory pathway from medulla to spinal cord.

The observation that some inspiratory neurons did not have oscillatory discharge locked to phrenic oscillation, even though the latter was prominent, suggests that there are differential connections and functions for different subsets of the inspiratory population. Thus, oscillation locked to phrenic oscillation was less common among neurons which fired during both phases, but with higher frequency in inspiration: only 2 of 6 such units showed the phenomenon. With the exception of this observation, however, the population sampling is too limited to allow general con-

clusions about possible relations between lack of synchronization and other properties of a neuron's discharge.

It is of interest that the two rostral pontine units observed (which had tonic discharge with higher frequency during inspiration) did not have oscillation locked to phrenic oscillation. This observation suggests that feedback from the medullary inspiratory centres to the rostral pontine pneumotaxic centre is relatively weak, and tends to support the idea of Bertrand and Hugelin (1971) that the pneumotaxic system is to some extent an independent self-reexciting oscillator.

### Medullary inspiratory wave activity

The occurrence in the medulla of oscillatory wave activity, locked to phrenic oscillation, indicates that there was considerable synchronization of activity in certain medullary regions, since sizable summated extracellular potentials were produced. The usual phase relations between such medullary waves and phrenic activity were similar to the unit→phrenic phase relations, i.e., phrenic activity lagged medullary wave negativity by 3–5 msec. However, by analogy with the arguments against the spike origin of the cortical alpha rhythm, it seems unlikely that the medullary waves arise from summation of spikes. A further argument against the spike origin of the waves is that the latter were not found in regions where inspiratory unit activity was plentiful.

A probable explanation for the origin of the medullary inspiratory waves is that they are the extracellular reflection of synchronized synaptic potentials in many inspiratory cells, whose processes are perhaps oriented so as to produce considerable summation. Further observations supporting this hypothesis are: (i) Oscillatory wave activity, locked to phrenic oscillation, was found in expiratory centre recordings, but it was opposite in phase to activity in the inspiratory regions. This phase inversion suggests that the expiratory centre waves are the reflection of hyperpolarization in expiratory neurons. Indeed, such oscillations have been observed to occur during the inspiratory phase in intracellular recordings of expiratory neurons (Salmoiraghi and Baumgarten 1961, Fig. 2; Mitchell and Herbert 1971). (ii) Rostral pontine electrical stimulation evokes wave responses at points where spontaneous waves occur, but there is no sign of evoked spike activity. The fact that evoked waves occur well before (35–40 msec) any sign of evoked phrenic activity (Fig. 11) suggests that subthreshold synaptic excitation is taking place in a population of cells.

Since the medullary oscillatory waves were recorded in the region near the nucleus ambiguus (as also reported by Achard and Bucher 1954),

it is possible that they reflect synchronized potentials in vagal inspiratory motoneurons. However, synchronization (high-frequency oscillation) is rare in efferent vagal discharge, and when it does occur is weak compared to the simultaneously occurring phrenic synchronization (Wyss 1955-56). These facts cast doubt on the vagal motoneuron origin of the synchronized waves. The question can only be resolved by simultaneous recording of medullary, vagal, and phrenic activity.

### Origin of synchronized inspiratory oscillations

Among the factors which might contribute to the origin of oscillation are: (i) negative feedback connections between different subgroups of inspiratory neurons, with some neurons inhibiting others; (ii) positive feedback (reexcitant) connections between inspiratory neurons; (iii) the existence of similar time courses of post-spike inexcitability (hyperpolarization) in different inspiratory neurons; (iv) slowly changing or "tonic" input (varying with the period of the respiratory cycle) to inspiratory neurons.

The existence of reexcitant connections between inspiratory neurons would seem to be essential for synchronization. Such a positive feedback mechanism would work as follows: When the lowest threshold members of the inspiratory network start to discharge, they send impulses to other members which result in the latter's excitation, and as a result a large number of inspiratory neurons fire together within a short time (e.g., within the 3–5 msec preceding a peak of phrenic activity). This synchronized output, which can be seen in recordings from the dorsal respiratory nucleus (Fig. 8), projects to the level of the phrenic motor nucleus, and causes synchronous excitation of many phrenic motoneurons. When the synchronized medullary burst ends, there follows a reduction of phrenic motoneuron excitation, and one cycle of oscillation is completed.

In order to explain the cessation of each synchronized burst of the medullary inspiratory neurons projecting to cervical levels, one might invoke the hypothesis that those neurons also excite a second group of neurons, which feed back inhibitory impulses to the members of the former group. However, an argument against this hypothesis is that the activity of almost all the medullary inspiratory neurons which were observed had approximately the same phase relation to phrenic activity (3–5 msec delay between unit and phrenic signals). If inhibitory cells which were activated in an oscillatory manner existed, one might expect their discharge to have a different phase relation with phrenic discharge than that of the excitatory cells, e.g., the spikes would tend to occur later than the spikes of excitatory cells and therefore nearer to the peaks of

phrenic activity. This argument is not conclusive, since if such inhibitory cells produced long lasting inhibition they could fire relatively early in each cycle of high-frequency oscillation and still produce an inhibition which was maximal at a somewhat later time in the cycle. In order to resolve this question, a more detailed analysis of phase relations would be useful, especially through simultaneous recordings from two or more medullary inspiratory neurons.

The origin of the oscillation could be explained without invoking inhibition. It could arise from the combined effect of two mechanisms: (i) reexcitant connections between different inspiratory neurons; (ii) similar time courses of post-spike inexcitability in different inspiratory neurons. Thus, following synchronized firing in the inspiratory population, the neurons would become inexcitable at about the same time and discharge would stop; if they had similar recovery times, they would become susceptible to excitatory input together and a new cycle of oscillation could occur. The fact that many medullary inspiratory neurons fire at high frequencies (100–300/sec) might make post-spike depression an important factor.

What remains to be explained is how the initial burst of inspiratory activity (at the start of the inspiratory phase), as well as each individual synchronized burst, are started. It may be hypothesized that slowly changing inputs to medullary inspiratory neurons produce changes of excitability level on a slower time scale than that of the synchronized bursts. For example, it has been suggested in earlier papers (Cohen and Wang 1959, Cohen 1970) that the transition from the expiratory to the inspiratory phase is produced by the gradually increasing excitatory input of pontine expiratory-inspiratory neurons to medullary inspiratory neurons. When threshold is reached for a critical subpopulation of inspiratory neurons, the reexcitant mechanisms already mentioned come into play, and a massive burst of inspiratory activity initiates the inspiratory phase (Fig. 1). This is followed by depression of activity, and after recovery another synchronous burst occurs, et seq. Thus, the high-frequency oscillation near the start of the inspiratory phase tends to be locked to the burst initiating the phase (Fig. 1, bottom).

A further explanation is required to account for the gradually increasing excitability level of inspiratory neurons as the inspiratory phase progresses. The excitability augmentation is manifested by: (i) increasing amplitude of phrenic oscillations (Fig. 1, top); (ii) increasing amplitude of evoked phrenic responses as stimuli are applied later in the inspiratory phase (Fig. 4). This progressive excitability change cannot be due to self-reexcitant feedback of inspiratory neurons which have oscillatory discharge, since the cessation of an individual burst would result in loss

of the excitatory input for succeeding activity. Rather, one has to invoke the possible mechanism of a slowly changing or "tonic" excitatory input to inspiratory neurons. A possible source of such input might be the rostral pontine neurons which fire tonically with higher frequency in inspiration (Cohen and Wang 1959, Bertrand and Hugelin 1971), and which do not have high-frequency oscillation (as in the limited sample of two neurons in this study).

Thus, to arrive at an overall view of the generation of inspiratory discharge patterns, it is necessary to consider both: (i) short-term interactions between inspiratory neurons, which help produce the high-frequency oscillations of inspiratory discharge; and (ii) more slowly changing interactions (between medullary inspiratory neurons and between medullary and pontine neurons), which produce the slower changes of discharge pattern throughout the inspiratory phase.

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Page 155, line 11 of Abstract:
   *instead of* $(V/V_T)$ *should be* $(\dot{V}/V_T)$
Page 173 first line from bottom should read:
   use $\dot{V}$-$PCO_2$ curves.
Page 191, line 19 from top:
   *instead of* bandpass 8–1,0000 cycle/sec *should be* bandpass 8–1,000 cycle/sec

# ERRATA

Page 191, lines 18–19 should read:

     (ii) a low-pass (for wave activity), bandpass 8–1,000 cycle/sec. Most usually,

Page 198, last line and page 199, first two lines should be replaced by:

     were taken from all inspiratory neurons encountered, as well as from those neurons firing during both phases of the cycle which had higher discharge frequency during the inspiratory phase. In addition, wave

Page 471, line 11 should read:

     of the bar. Then this platform was changed to a smaller 25×12.5 cm one

Page 490, line 6 should read:

     of the bar-pressing avoidance responses in cats. Bull. Acad. Pol. Sci. Sér. Sci. Biol. 20: