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PROPERTIES OF THE INSPIRATION-RELATED ACTIVITY OF SYMPATHETIC PREGANGLIONIC NEURONES OF THE CERVICAL TRUNK IN THE CAT

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SUMMARY

1. The experiments reported here have examined some temporal characteristics of the inspiration-related sympathetic discharge of the cat in control conditions and during forcing of the respiratory oscillator into marked deviations from its natural frequency. The purpose of these experiments was to establish whether or not the relation of sympathetic to phrenic nerve activity shows properties consistent with the hypothesis that the inspiration-related sympathetic discharge is driven by a neural oscillator, independent of, but coupled and stably entrained to, the brain-stem respiratory oscillator.

2. The electrical activity of the whole cervical sympathetic trunk ($n = 26$) or of small strands of the cervical trunk containing single units ($n = 20$) and of the phrenic nerve was recorded in pentobarbitone-anaesthetized, paralysed, artificially ventilated, sino-aortic denervated cats. Most of the cats were bilaterally vagotomized.

3. The onset of the inspiratory burst of the sympathetic preganglionic neurones had a fixed delay from the onset of the phrenic nerve burst. The level of activity within the burst, in whole cervical trunk recording, reached a maximum in early inspiration and then was maintained at approximately this level for the rest of inspiration (twenty-two out of twenty-six cats). In four cats the activity level increased throughout the burst. Individual sympathetic preganglionic neurones displaying inspiration-related burst firing were characteristically recruited in early inspiration and thereafter maintained an approximately constant firing frequency for the rest of inspiration.

4. Electrical stimulation of afferents in the superior laryngeal nerve during various phases of the respiratory cycle caused equivalent, phase-dependent, resetting patterns of both phrenic nerve and inspiration-related sympathetic discharge.

5. In cats with intact vagus nerves, entrainment of the brain-stem respiratory oscillator to the frequency of the respiratory pump was used to change the frequency of the former, within limits, by changing the frequency of the latter. Over the range of frequencies tested, the pump-to-phrenic delay varied as a function of frequency, while the delay between phrenic and sympathetic burst onset was essentially independent of frequency.

6. In hyperthermic, hypocapnic cats phrenic nerve burst frequency increased up to about 300 bursts/min from a value of 15 bursts/min in normothermia–

normocapnia. At all frequencies within this range the sympathetic burst maintained a delay, with respect to the phrenic burst, which was essentially independent of frequency.

7. The fact that phrenic nerve and sympathetic burst maintained a 1:1 relation with essentially constant delay over all frequencies tested is inconsistent with the known behaviour of coupled neural oscillators. Therefore, the equality of period of phrenic nerve burst and inspiration-related sympathetic discharge is unlikely to result from the activity of an autonomous sympathetic oscillator coupled to the brain-stem respiratory oscillator. Instead, these results are compatible with the hypothesis of a common oscillator which drives both the phrenic and the sympathetic discharge.

8. In some experimental conditions phrenic nerve activity without a corresponding sympathetic burst was observed. This dissociation was seen (i) during baroreceptor activation caused by the increased systemic arterial pressure resulting from injection of a pressor drug, and (ii) when, during abnormal respiratory rhythms, a phrenic nerve burst occurred at a short interval after a preceding burst. This dissociation is probably due to depression of the sympathetic neurone and/or to depression of the input from the brain-stem respiratory oscillator. Hence these observations are not inconsistent with the hypothesis above.

INTRODUCTION

In normal experimental conditions a large fraction of sympathetic preganglionic and post-ganglionic neurones of the cat fire in rhythmic bursts, synchronous with inspiration (e.g. Cohen & Gootman, 1970; Preiss, Kirchner & Polosa, 1975; Barman & Gebber, 1976). The rhythmic bursting persists in the absence of respiratory movements and following sino-aortic denervation and vagotomy. Therefore this rhythm is thought to be generated by central mechanisms (Tang, Maire & Amassian, 1957). Two hypotheses have been proposed to explain the mechanism of generation of this inspiration-related component of sympathetic discharge. One attributes this firing pattern to facilitatory input from brain-stem inspiratory neurones to sympathetic preganglionic, or to antecedent, neurones. This hypothesis receives support from observations of analogies in the responses to a variety of stimuli of inspiratory motoneurones and of sympathetic preganglionic neurones with inspiration-related firing pattern (Preiss *et al.* 1975; Preiss & Polosa, 1977; Gerber & Polosa, 1978, 1979; Connelly & Wurster, 1985). The other hypothesis proposes that the inspiration-related activity of sympathetic neurones is driven by a hypothetical neural oscillator, independent of, but coupled to, the brain-stem respiratory oscillator, and entrained to the latter at normal respiratory frequencies (Koepchen, 1962; Barman & Gebber, 1976).

In the present study an analysis was made of the temporal structure of the inspiration-related sympathetic burst and experiments were performed to clarify its generation mechanism. Specifically, these experiments were aimed at the question of whether or not the relation of sympathetic to phrenic nerve activity showed behaviour expected of a system of two coupled oscillators (Pavlidis, 1973). Therefore, the delay between the two bursting rhythms was examined under conditions in which the respiratory oscillator was forced to assume frequencies different from its natural

frequency, both in the transient and in the steady state. To this end respiratory frequency was modified by various means, which included changes in body temperature and stimulation of vagal or superior laryngeal nerve afferents.

METHODS

Cats of either sex were used (2·5–4·5 kg). Anaesthesia was effected with pentobarbitone (35 mg/kg I.P., followed by I.V. supplements of 9 mg/kg every 3 h). With this dosage the withdrawal reflex on pinching forepaw or hind paw was suppressed for the duration of the experiment. After cannulation of the trachea all animals were artificially ventilated, while continuously monitoring tidal CO_2 concentration with an infra-red gas analyser and tracheal pressure with a strain gauge, and paralysed with pancuronium bromide (initial dose 200 μg/kg followed by maintenance doses of 100 μg/kg given every 2–3 h, when the effect of the previous dose had worn off, as evidenced by the appearance of spontaneous breathing movements, and after testing for adequacy of the level of anaesthesia). Artificial ventilation was adjusted to obtain, in control conditions and during ventilation with room air or with 100% O_2, end-tidal P_{CO_2} of 30–45 mmHg. A respiratory pump rate of 15–18 cycles/min and peak tracheal pressure of 4–6 cmH_2O were used in control conditions. In some experiments, in which high respiratory pump frequencies were used which resulted in hyperventilation, CO_2 mixtures in O_2 were used to maintain end-tidal P_{CO_2} close to control values. In these cases in control conditions ventilation was with 100% O_2. An artery and vein were cannulated for continuous recording of systemic arterial pressure and for injection of drugs, respectively. Rectal temperature was maintained at 37 °C by means of a servo-controlled infra-red heat lamp.

The electrical activity of the whole phrenic nerve and cervical sympathetic trunk was recorded monophasically, after desheathing, with silver hook electrodes, amplified (bandpass 30 Hz–10 kHz), displayed on an oscilloscope and stored on magnetic tape. After half-wave rectification and low-pass filtering (R–C circuit with 100 ms time constant), the electrical activity was also displayed on a pen recorder. These rectified, low-pass filtered, records of neural activity are usually referred to in the literature as 'integrated' activity. The level of 'zero' activity for the recording of the cervical sympathetic trunk was obtained by applying procaine to the nerve or by crushing the nerve proximal to the recording electrode. Thin filaments were dissected from the cervical sympathetic trunk, under a dissection microscope, for single-unit recording. All units studied were spontaneously firing in control conditions. In seven cats the central end of the cut internal branch of the superior laryngeal nerve was desheathed and mounted on a pair of silver hook electrodes for stimulation. Monophasic square-wave pulses (0·2 ms duration) from a stimulator were delivered through a stimulus isolation unit. Trains of stimuli were delivered at selected times during the respiratory cycle, by triggering the stimulator at variable delays from phrenic burst onset with a square-wave pulse obtained at the onset of the burst. All nerves were kept under mineral oil in a pool made with the skin flaps.

In all animals the aortic nerves were identified bilaterally, separated from the vagus nerves and cut. The vagus nerves were also cut bilaterally in the neck except in the experiments of entrainment of the respiratory oscillator to the respiratory pump. The carotid sinus nerves were also cut bilaterally in all experiments, except in those in which the effects of baroreceptor activation on the inspiration-related sympathetic discharge were studied. In the text, the term inspiration is used to define the phase of phrenic nerve activity from onset to beginning of rapid decline, while the interval between the end of one inspiration and the onset of the next is defined as expiration. Lung inflation is indicated by the increase in tracheal pressure caused by the respiratory pump. The phase relation of the respiratory pump cycle to the central respiratory cycle is defined by the delay (in ms) between onset of phrenic nerve burst and onset of rise in tracheal pressure. Similarly, the phase relation of the sympathetic burst cycle to the central respiratory cycle is defined as the delay (in ms) between phrenic burst onset and sympathetic cervical burst onset.

Entrainment of the phrenic nerve burst cycle to the respiratory pump cycle refers to the condition in which the periodic changes in lung volume, caused by the respiratory pump, affect the rhythm of phrenic nerve activity in such a way that lung inflation occurs with a fixed delay, which is a function of pump frequency, following phrenic burst onset. Intrinsic frequency of an oscillator is defined as the frequency in the absence of input.

RESULTS

Pattern of sympathetic discharge in inspiration

When the electrical activity of the whole cervical sympathetic trunk was recorded under normal experimental conditions, a burst of spikes was observed in close temporal relation to the phrenic nerve burst (Fig. 1*A* and *B*). The peak amplitude of the sympathetic burst increased or decreased when phrenic burst amplitude

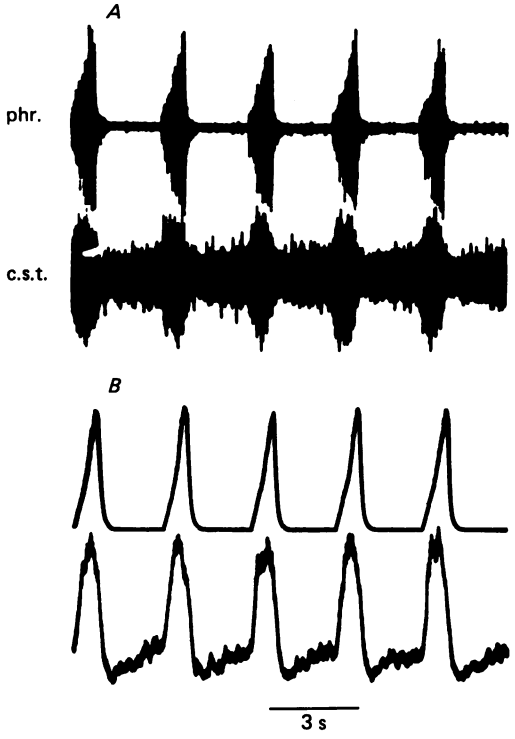


Fig. 1. The inspiration-related sympathetic discharge. *A*, recording of the electrical activity of the phrenic nerve (phr. upper trace) and cervical sympathetic trunk (c.s.t.). *B*, same records as in *A* after half-wave rectification and low-pass filtering (time constant 100 ms).

increased or decreased with changes in CO_2 or anaesthetic level (Fig. 2). Onset and termination of the sympathetic burst occurred in a fixed time relation to onset and termination of the phrenic nerve burst, for a given set of experimental conditions, and were delayed with respect to the latter. A measure of the constancy of the time relation, the standard deviation of the phrenic to sympathetic burst onset delay, is shown in Figs. 3 and 9. The onset delay of the sympathetic burst varied inversely with end-tidal P_{CO_2} (Fig. 3), as would be expected if the output of brain-stem inspiratory neurones, which is also known to vary inversely with end-tidal P_{CO_2} (Cohen, 1968), was providing the synaptic drive for the burst. The delay varied also with the level of anaesthesia and was longest at the highest anaesthetic doses (not shown). The values for onset delay shown in Fig. 3 are typical for the group of animals studied. After the onset, the contour of the low-pass filtered inspiration-related

sympathetic wave showed one of two trajectories. The most frequent trajectory (twenty-two cases out of twenty-six) was characterized by an initial progressive increase in amplitude, reaching its maximum during the initial part of the phrenic nerve burst (approximately during the first 30–40% of the phrenic nerve burst duration); afterwards amplitude stayed approximately constant for the remainder

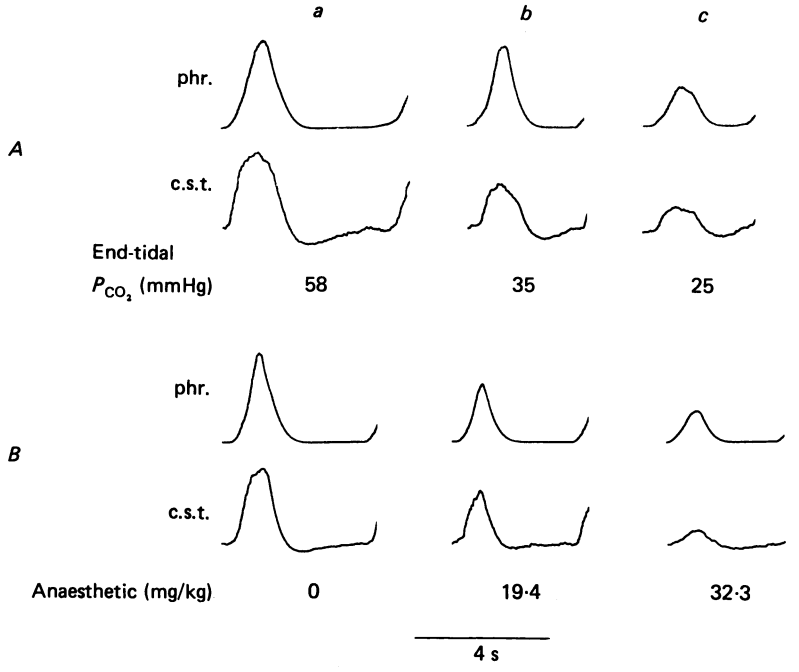


Fig. 2. Relation between phrenic (phr.) and cervical sympathetic trunk (c.s.t.) burst discharge at various levels of central respiratory drive. Each trace is the average of 20 sweeps. *A*, decrease in central chemical respiratory drive by hyperventilation in air has equivalent effects on the phrenic and sympathetic bursts. Even at the lowest levels of P_{CO_2} which produced rhythmic phrenic nerve activity (*c*) there was an associated sympathetic discharge. *B*, increasing levels of I.V. pentobarbitone anaesthesia cause an equivalent depression of phrenic and sympathetic bursts.

of the duration of the phrenic nerve burst (Fig. 4*A*). This wave shape will be referred to as square-wave-like. In the remaining four cases the trajectory was a replica of the trajectory of the low-pass filtered phrenic nerve burst, i.e. amplitude increased progressively, reaching its peak in approximate coincidence with the peak of the phrenic nerve wave (Fig. 4*B*). This wave shape will be referred to as ramp-like. After the occurrence of the peak of the phrenic wave, both types of trajectories decayed to the pre-burst activity level (three out of twenty-six), or to below it (twenty-three out of twenty-six), at a rate similar to, or slower than, that at which phrenic nerve activity decayed. In control conditions, inspiration-related sympathetic discharge was present in all the animals studied. The discharge persisted without attenuation after I.V. administration of up to 20 mg hexamethonium bromide/kg, which suggests that it was recorded from preganglionic axons.

The temporal characteristics of the inspiration-synchronous sympathetic discharge

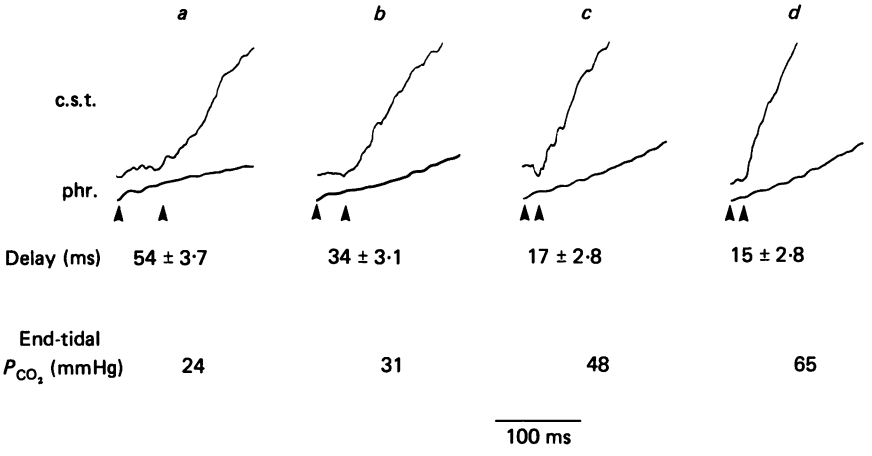


Fig. 3. Effects on the phrenic (phr.) to sympathetic (c.s.t.) burst onset delay of various levels of end-tidal P_{CO_2}. Each trace is the average of 25 sweeps; times (ms) indicate the delay at the respective end-tidal P_{CO_2}. Increasing P_{CO_2} from a relatively hypocapnic (*a*) to a normocapnic (*b*) level decreases the delay by 20 ms. Hypercapnia (*c* and *d*) produces further decrease in delay. Arrows indicate onset times. End-tidal P_{CO_2} values in *b*, *c* and *d* were obtained by ventilation with CO_2-containing gas mixtures.

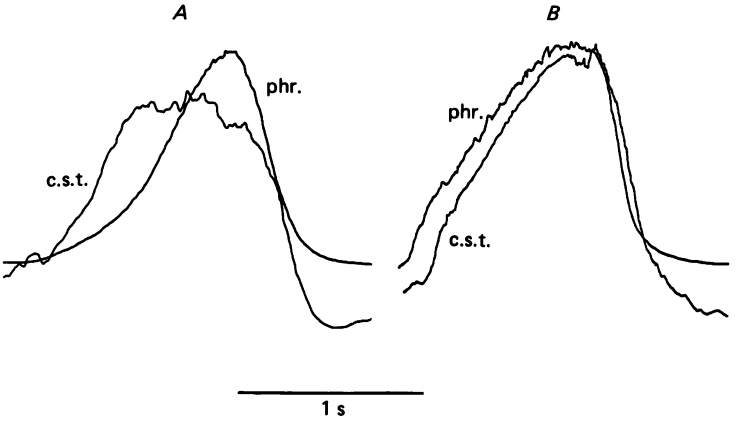


Fig. 4. Comparison of phrenic bursts (phr.) and inspiration-related bursts (recorded in the cervical sympathetic trunk (c.s.t.)). Rectified, low-pass filtered whole nerve recordings. *A* and *B* are from two different cats. Each trace is the average of 25 sweeps. *A*, the most commonly observed sympathetic wave form has a square-wave shape. *B*, exceptionally, the sympathetic wave form has the same ramp-shape as the phrenic wave form.

were examined in twenty units of the cervical sympathetic trunk. These units were silent during expiration and discharged a burst of spikes during inspiration. The inspiratory burst, in various units, was made of from three to thirty-two spikes (Fig. 5). For a given unit and for a given set of experimental conditions the number of spikes in the burst was remarkably constant. The times, during the phrenic nerve burst, at which these units were first recruited are shown in Fig. 6. The majority (sixteen out of twenty) was recruited during the first 30% of inspiration. Once recruited, the majority of units fired at a frequency similar to the onset frequency.

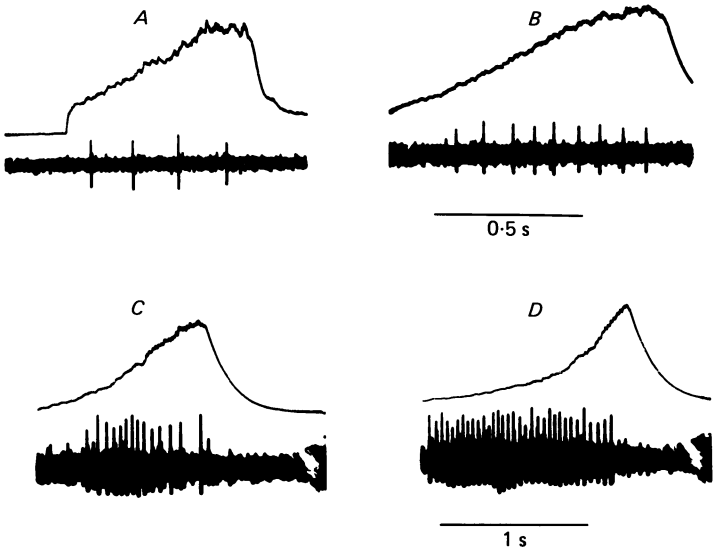


Fig. 5. Firing patterns of sympathetic preganglionic units during their inspiratory burst. Four different units are shown, generating bursts with different numbers of spikes. Top trace in each panel is low-pass filtered phrenic nerve burst. Calibrations under *B* and *D* apply also to *A* and *C*, respectively.

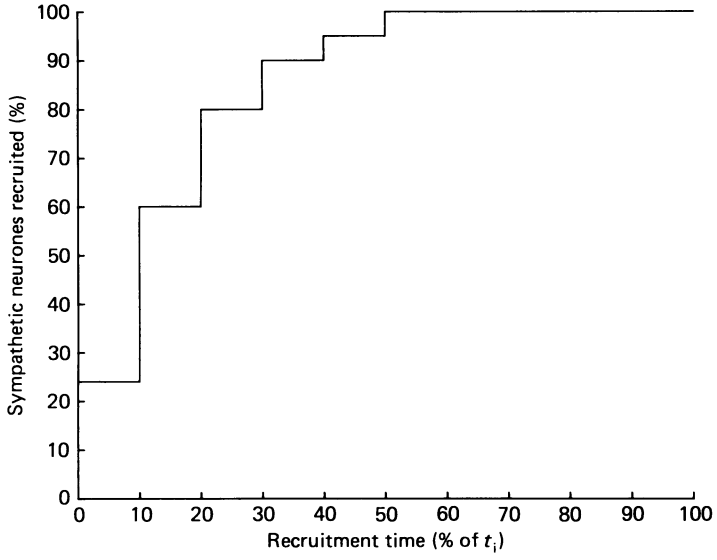


Fig. 6. Cumulative histogram of recruitment times of sympathetic units during inspiration. Recruitment time is time between phrenic burst and sympathetic unit burst onset. This time is expressed as a percentage of inspiration duration (t_i). $n = 20$.

Thus, the ratio of the last to the first interspike interval of the burst was, on average, $1{\cdot}15 \pm 0{\cdot}42$ ($n = 20$, $P < 0{\cdot}01$). For the same twenty units, 65% of the intraburst interspike intervals following the first were within $\pm 20\%$ of the first interval. This is in contrast with the intraburst firing pattern of phrenic motoneurones which is characterized by a progressive acceleration of frequency during the burst (Iscoe, Dankoff, Migicovsky & Polosa, 1976).

Experimental tests of the coupled oscillator hypothesis

Superior laryngeal nerve stimulation: phase–response curve. The properties of a biological oscillator can be characterized by the effect of a stimulus, presented at different times (or phases) of the cycle, on the duration of the same cycle, i.e. its phase–response curve (Pavlidis, 1973). It is known that superior laryngeal nerve afferents can reset the rhythm of the phrenic motoneurone burst (Larrabee & Hodes, 1948). On the basis of the coupled oscillator hypothesis, mentioned in the Introduction, the premise for this experiment (performed on seven cats) is that any phase advance or phase delay, caused in the cycle of the respiratory oscillator by the stimulus, would result in the output of this oscillator reaching the hypothetical sympathetic oscillator during a different phase of its cycle. Hence, it is predicted that the delay between the outputs of the two oscillators should be different from that observed in the unstimulated condition (Pavlidis, 1973; Jalife, 1984; Delmar, Jalife & Michaels, 1985).

Electrical stimuli (trains of 4 stimuli at 200 Hz, 0·2 ms, 0·2 mV) were delivered to the central cut end of the superior laryngeal nerve every fifth respiratory cycle, at various delays from the onset of the phrenic nerve burst. Fig. 7 shows the resulting phase–response curve for phrenic nerve and inspiration-related sympathetic discharge, i.e. the changes in cycle duration (or phase shift, expressed as a percentage of control) as a function of the time of the cycle (expressed as a fraction of the normalized cycle duration) at which the stimulus was delivered. Except for the shortening of the cycle caused by stimuli delivered during the last part of inspiration (Fig. 7*b*), at all other times of the cycle the effect of stimulation was a prolongation, which varied in magnitude in a characteristic manner with stimulus timing. The significant finding, in relation to the hypothesis being tested, was that the phase shift caused by the stimulus was the same for both phrenic and inspiration-related sympathetic discharge, i.e. the two wave forms maintained the same temporal relation to each other during the curtailed or prolonged cycles as they did in the unstimulated cycles. An additional observation was that stimuli delivered during inspiration caused characteristic changes in the phrenic wave form which were always associated with equivalent changes in the wave form of the inspiration-related sympathetic discharge (e.g. *a* in Fig. 7) as described earlier (Bachoo & Polosa, 1985).

Entrainment of the phrenic nerve burst to changing respiratory pump rates. In another experiment (performed on six cats) the periodic stimulus provided by phasic lung inflation was used to modify in a stable manner, i.e. entrain, the cycle duration of the brain-stem respiratory oscillator. For a system of two coupled oscillators, entrainment is produced by the periodic input from the driving oscillator modifying the period of the driven oscillator on a cycle by cycle basis (von Holst, 1939). Stable entrainment in a 1:1 ratio between driving and driven oscillator frequencies is

limited, in the case of described neural oscillators, to a narrow range of frequencies bordering the intrinsic frequency of the driven oscillator (Pinsker, 1977*b*; Ayers & Selverston, 1979). Within the limits of stable 1 : 1 entrainment, a change in frequency of the driving input will shift the phase at which the input reaches the driven oscillator, accordingly modifying the period of the driven oscillator in such a way that

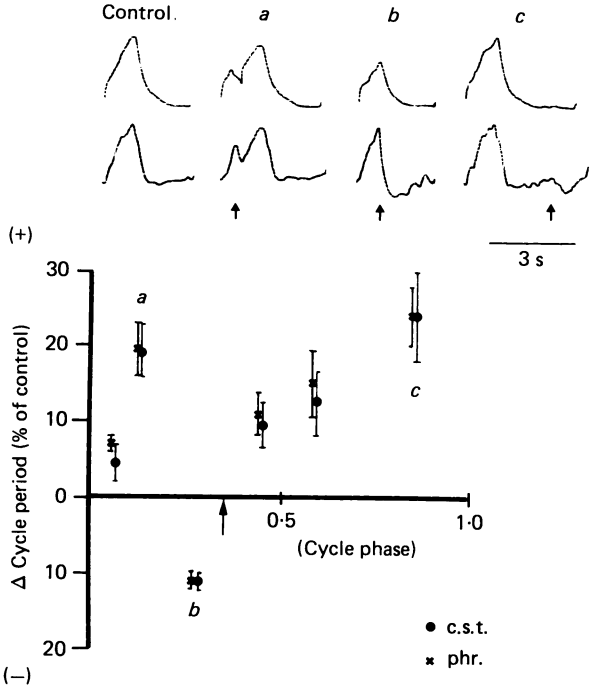


Fig. 7. Identity of the resetting effect of a train of stimuli to the superior laryngeal nerve (4 stimuli at 200 Hz, 0·2 ms, 0·2 mV) on the rhythm of the phrenic (phr.) and of the inspiration-related sympathetic (c.s.t.) discharge. This effect is displayed in the graph as the change in cycle duration on the ordinate (expressed as a percentage of control) caused by stimuli presented at various times of the cycle (shown on the abscissa and expressed as cycle phase, i.e. fraction of cycle duration). Plus and minus signs indicate prolongation and shortening, respectively. Arrow on abscissa indicates the end of the inspiration phase of the cycle. Inspiration onset is at the origin. Superior laryngeal nerve stimulation prolongs the period of the phrenic and of the inspiration-related sympathetic discharge except when delivered during the last part of inspiration. Insets show phrenic nerve (top) and sympathetic wave form in control conditions and for stimulation (arrow) in middle (*a*) and late (*b*) inspiration and late expiration (*c*). Notice that the two wave forms have similar shapes and maintain the same temporal relation to each other during curtailed or prolonged cycles as in the control, unstimulated, cycles. Each point in the graph is average ±S.D. of 20 cycles. Each wave form in insets is average of 10 sweeps. Notice similarity of phrenic and sympathetic wave forms.

equality with the period of the rhythmic input is achieved. When the period of the driving oscillator is too different from that of the driven oscillator, entrainment ratios different from 1 will occur (Pavlidis, 1973). If the bursting rhythms of the phrenic nerve and of the cervical sympathetic trunk were the result of the activity of such coupled oscillators, a varying delay between the respective wave forms at different

frequencies would be expected. In addition, the frequencies at which stable 1:1 entrainment of the two wave forms could be maintained would be limited to a range bordering the intrinsic frequency of the hypothetical, driven, sympathetic oscillator. Alternatively, if the inspiration-related sympathetic wave form is the result of the sympathetic preganglionic neurones sharing with phrenic motoneurones input from the respiratory oscillator, the two wave forms may be expected to maintain a constant delay at all frequencies.

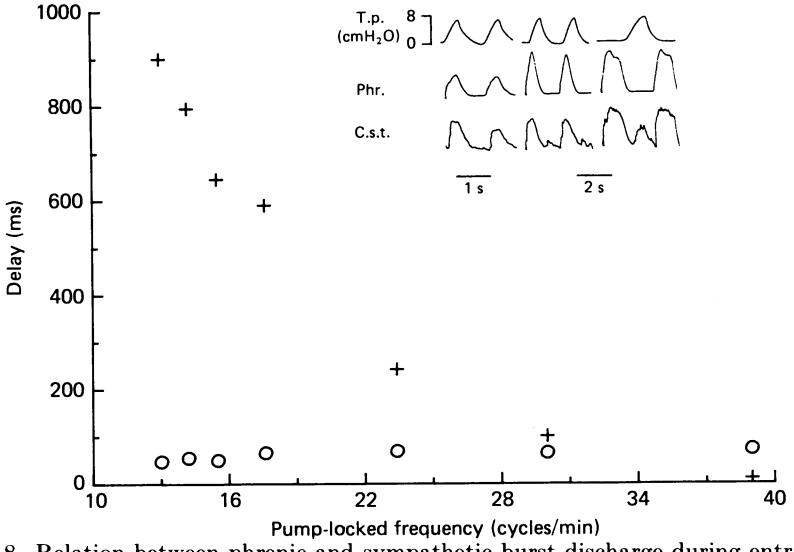


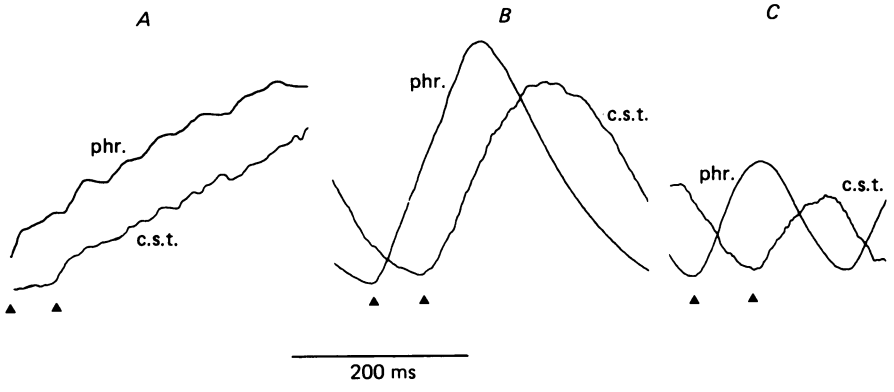
Fig. 8. Relation between phrenic and sympathetic burst discharge during entrainment of the phrenic nerve burst to changing respiratory pump rates. Cat with intact vagus nerves. The plot shows on the ordinate the delay (ms) between phrenic and sympathetic burst onsets (○) at various phrenic burst frequencies (abscissa) obtained by changing respiratory pump frequency in a range within which there is a 1:1 ratio of pump to phrenic burst frequency. +, delays (ms) between onsets of phrenic burst and of lung inflation (measured as tracheal pressure) at the various phrenic burst frequencies shown on the abscissa. Notice relative constancy of the delay between phrenic and sympathetic burst. In contrast, notice large variation in the delay between phrenic burst and lung inflation, which is typical of biological oscillators driven by a periodic synaptic input of varying frequency, as may be generated by another oscillator (Pavlidis, 1973; Pinsker, 1977; Ayers & Selverston, 1979). Insets show sample records of tracheal pressure (t.p.), phrenic (phr.) and sympathetic (c.s.t.) discharge from the same experiment plotted in the graph. At the lowest respiratory pump frequency (right-most records) inflation occurs in expiration and is associated with a burst of sympathetic discharge as described previously (Bachoo & Polosa, 1986). Each point on the graph is average of 20 cycles. Each wave form in insets is average of 10 sweeps.

Sensory input, presumably from pulmonary stretch receptor afferents, generated by lung inflation at various frequencies, was used to entrain the respiratory oscillator to the respiration pump (Cohen, 1969; Vibert, Caille & Segundo, 1981; Petrillo, Glass & Trippenbach, 1983) and thereby change the respiratory oscillator frequency, within limits, by changing ventilation pump frequency. The results of such an experiment are shown in Fig. 8. Within the ventilation pump frequencies of 13 and 39 cycles/min, at constant tidal volume, the ratio of phrenic nerve burst rate to pump rate was 1. The delay between the phrenic nerve burst and the tracheal pressure trace (the latter

giving an indication of the time course and amplitude of the input) changed in a characteristically smooth manner over this range of frequencies (Fig. 8, +) from 900 ms to near zero. This is a behaviour typical of biological oscillators driven by periodic synaptic input of variable frequency, as may be generated by another oscillator (Pavlidis, 1973; Pinsker, 1977*a*; Ayers & Selverston, 1979). In contrast, over the same range of frequencies the delay between phrenic nerve burst and inspiration-related sympathetic discharge remained approximately constant (Fig. 8, ○). A small increase (by 8 ms) at the higher frequencies was within the magnitude of change that can be observed as a result of changes in excitability of the sympathetic preganglionic neurone or in the magnitude of the input. This trend can be accounted for by the changes in end-tidal P_{CO_2} which occurred with changes in respiratory pump frequency between 13 and 39 cycles/min. In a typical experiment, end-tidal P_{CO_2} varied from 48 mmHg at the lowest frequency to 35 mmHg at the highest frequency. At frequencies greater than 18 cycles/min, CO_2 was administered to the animal to prevent marked hypocapnia. At constant, high respiratory pump frequency, an increase in delay of comparable magnitude was observed when end-tidal P_{CO_2} was changed within this range (see Results, first section) by switching from ventilation with various CO_2-containing gas mixtures to ventilation with room air. Thus, Fig. 8 shows an absence of changes in the delay between phrenic and inspiration-related sympathetic discharge in the frequency range in which a 1:1 relation between respiratory pump and phrenic nerve burst frequencies exists. Analogous results were obtained in the other five cats in which a similar experiment was performed. It should be mentioned that outside the range of frequencies in which 1:1 phase locking between phrenic burst and respiratory pump frequency occurs, entrainment ratios of phrenic to pump frequency greater or smaller than 1 occurred, as described in Petrillo *et al.* (1983). The inspiration-related sympathetic discharge always duplicated the phrenic behaviour and maintained a constant delay relative to the phrenic nerve burst. Thus, constancy of the delay between the inspiration-related sympathetic discharge and the phrenic nerve burst was observed under all relations of phrenic frequency to pump frequency studied.

Hyperthermic hypocapnic polypnoea. The phase delay of the inspiration-related sympathetic discharge to phrenic nerve burst was studied under conditions in which changes in phrenic nerve burst frequency greater than those caused by superior laryngeal nerve stimulation or by lung inflation were produced. In two vagotomized, sino-aortic denervated, paralysed, artificially ventilated cats hyperthermia (42 °C rectal temperature) produced by radiant heat was used to induce polypnoea. In one cat, in normocapnia, the frequency of the phrenic nerve burst increased from 15 bursts/min at 37 °C to 36 bursts/min at 42 °C. A further increase in frequency, to 324 bursts/min, was obtained when, at a temperature of 42 °C, the animals were made hypocapnic by hyperventilation in air (end-tidal P_{CO_2} 10 mmHg; Cohen 1964; Monteau, Hilaire & Ouedraogo, 1974). Similar results were obtained in the other cat. The inspiration-related sympathetic discharge frequency maintained a 1:1 relation to the phrenic burst frequency over the whole range of frequencies. Fig. 9 shows that the delay between the two wave forms varied relatively little in spite of a 20-fold change in frequency, being 43 ms in control conditions and 60 ms at the highest respiratory frequency. The increased delay at the highest frequencies (an increase of less than 1 % of control cycle period) can be attributed to changes in the excitability

of the preganglionic neurone as well as in the properties of the synaptic drive, resulting from factors such as hypocapnia (see Fig. 3), hyperthermia and high bursting rates. An additional feature to be noted in Fig. 9 is that the relative constancy of the phrenic burst onset–sympathetic burst onset delay over the whole range of frequencies causes the greatest part of the sympathetic burst to occur during the expiratory phase of the respiratory cycle at the highest respiratory frequencies.



Phrenic to sympathetic burst delay at various respiratory cycle durations

Cycle duration (ms)	3800	1060	760	300	185
Delay (ms) mean ± S.D.	43 ± 2·7	41 ± 2·6	42 ± 2·7	47 ± 3·8	60 ± 4·3

Fig. 9. Phase relation between phrenic and sympathetic burst discharge during hyperthermic, hypocapnic polypnoea in a vagotomized cat. The Table shows that the delay between phrenic and sympathetic wave forms varies little within the wide range of respiratory cycle durations obtained in this experiment. Each value is the mean of forty measurements. See text for possible explanations of the increase in delay at the highest frequency. *A*, *B* and *C* show the average wave form (40 sweeps) recorded at cycle duration values of 3800, 450 and 185 ms respectively. Arrow heads indicate delay. Abbreviations: phrenic, phr.; sympathetic cervical trunk, c.s.t.

Cases of absence of inspiration-related sympathetic discharge in the presence of central inspiratory activity.

The picture emerging from the experiments described in the previous section is one of a tight relationship between phrenic and sympathetic burst activity. This picture is consistent with results of previous experiments demonstrating parallel changes of the two bursts in a number of different experimental situations (changes in systemic P_{CO_2}: Preiss & Polosa, 1977; Connelly & Wurster, 1985; lung inflation: Gerber & Polosa, 1978; superior laryngeal nerve stimulation: Gerber & Polosa, 1979; Bachoo & Polosa, 1985). However, in some experimental conditions the inspiration-related sympathetic discharge was seen to disappear although the phrenic nerve burst was present. One such condition is during the depression of sympathetic preganglionic neurone activity, presumably of baroreceptor origin, associated with the pressor response caused by I.V. injection of noradrenaline (Iggo & Vogt, 1962). In these

conditions (Fig. 10) the peak amplitude of the phrenic nerve burst was markedly attenuated (by approximately one-half with respect to control) and the inspiration-related sympathetic discharge disappeared. The reduction in the level of central inspiratory activity, caused by the baroreceptor reflex (Nishino & Honda, 1982), is unlikely to be the entire cause of the disappearance of the inspiration-related sympathetic discharge because reductions of comparable magnitude in the level of central inspiratory activity, caused by lowering end-tidal P_{CO_2} or by increasing the level of anaesthesia, resulted in a roughly proportional reduction in this discharge, as in the amplitude of phrenic nerve activity (Fig. 2). Therefore, the absence of the

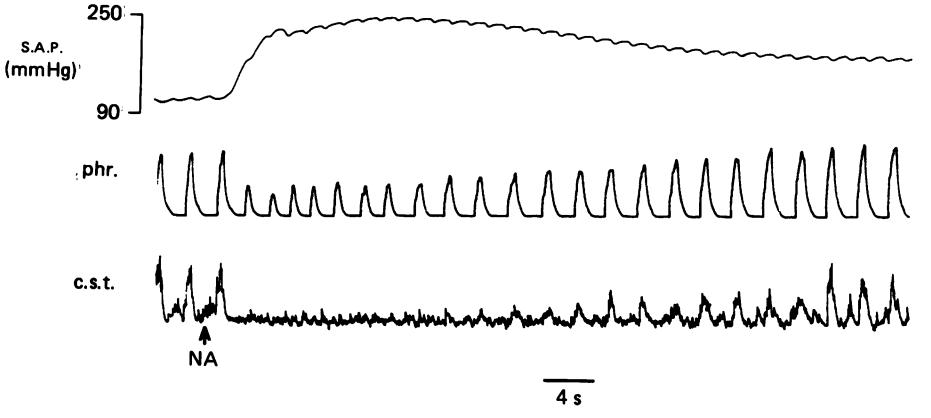


Fig. 10. Disappearance of inspiration-related sympathetic discharge during the arterial pressure increase caused by injection of noradrenaline (NA; 5 μg/kg I.V.). From top: mean systemic arterial pressure (S.A.P.), low-pass filtered phrenic and cervical trunk recording. The disappearance is presumably due to baroreceptor-evoked depression of (i) sympathetic neurone excitability and (ii) input from brain-stem inspiratory neurones (as suggested by the depression in peak phrenic amplitude). After sino-aortic denervation, the same increase in arterial pressure had no effect on either sympathetic or phrenic nerve activity (not shown). Time of NA injection indicated by arrow.

inspiration-related sympathetic discharge is likely due to a block of the input from brain-stem inspiratory neurones along its transmission path to the sympathetic preganglionic neurone by the powerful inhibitory action of the arterial baroreceptors.

Another condition in which the sympathetic discharge corresponding to a phrenic burst was absent was the occurrence of central inspiratory activity (in rather unusual experimental situations) during the phase of the respiratory cycle immediately following the end of inspiration. An example is shown in Fig. 11. This record was obtained in an animal with intact vagus nerves in which entrainment between phrenic nerve burst and respiratory pump frequency occurred in a 2:1 ratio. There was, for each pump cycle, a phrenic nerve burst of normal amplitude and duration, leading the inflation by 150 ms and followed, at 310 ms from the peak, by another burst of much smaller amplitude and shorter duration. The smaller burst coincided with the deflation phase of the respiratory pump cycle. This burst occurred at the time of the respiratory cycle at which the level of sympathetic discharge was at its minimum. As Fig. 11 shows, there was no sympathetic burst corresponding to the small phrenic burst. Another example is shown in Fig. 12. These records are from a cat in which

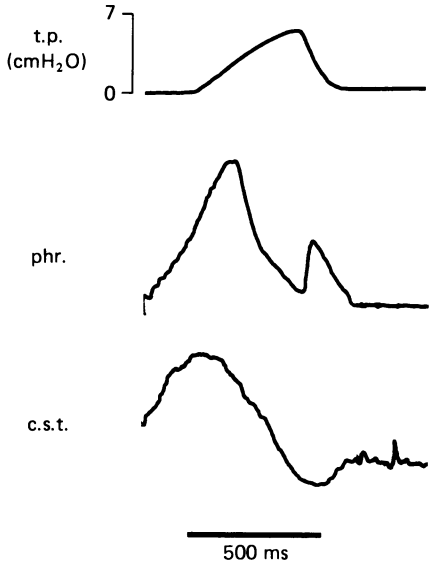


Fig. 11. Absence of inspiration-related sympathetic discharge when a phrenic burst occurs with a short delay after the end of the preceding phrenic burst. This condition occurred during phase locking of the phrenic nerve burst to the ventilation pump frequency, with two phrenic bursts occurring for each pump cycle. Notice presence of sympathetic burst when large phrenic burst occurs, absence when small phrenic burst occurs. Tracheal pressure, t.p.; phrenic nerve activity, phr.; cervical sympathetic trunk activity, c.s.t. Averages of 2–4 sweeps.

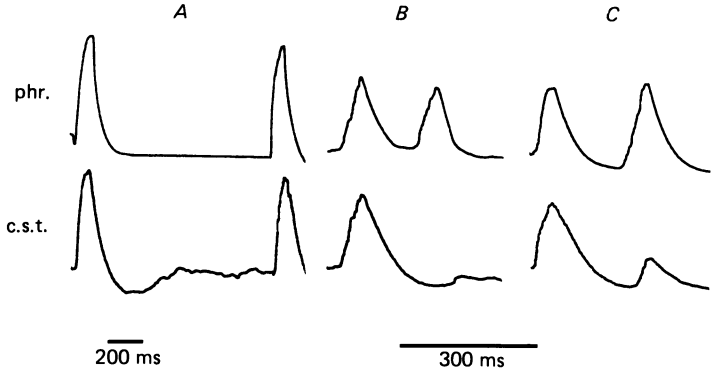


Fig. 12. Absence of inspiration-related sympathetic discharge when a phrenic burst occurs with a short delay after the end of the preceding phrenic burst. Phrenic nerve activity, phr.; cervical sympathetic trunk activity, c.s.t. *A*, *B* and *C*, during spontaneous 'gasping' respiratory cycle duration is very irregular. Notice absence of second sympathetic burst in *B* and its presence, but with great attenuation, in *C*.

phrenic nerve activity spontaneously became of the gasping type (St. John & Knuth, 1981), i.e. was characterized by bursts of short duration, fast rise time and irregular occurrence (Fig. 12*A*, *B* and *C*). Some of the phrenic bursts occurred in close succession. When the onset of a burst occurred at an interval of 130 ms or less after the peak of the preceding one, no corresponding sympathetic discharge occurred (Fig. 12*B*). At an interval of 170 ms (Fig. 12*C*) the inspiration-related sympathetic

discharge was present but was much attenuated in amplitude (compare with Fig. 12*A*). The observations shown in Figs. 11 and 12 could be explained with the hypothesis of a post-inspiratory phase of sympathetic preganglionic neurone depression, during which the input from brain-stem inspiratory neurones could become less effective in producing a discharge. Results consistent with this hypothesis were

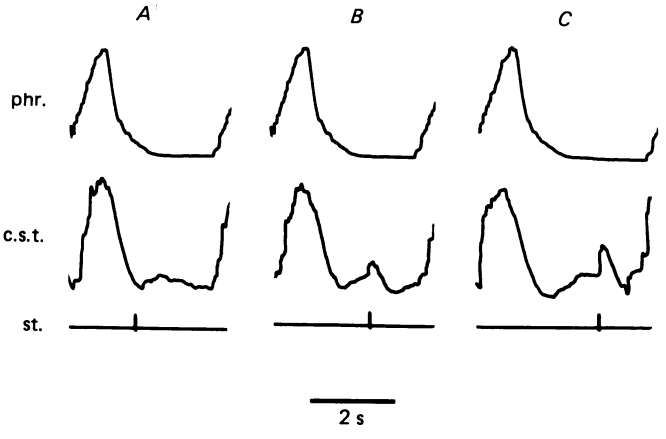


Fig. 13. The sympatho-excitatory effect of a train of stimuli to the superior laryngeal nerve (8 pulses at 40 Hz, 2V, 0·2 ms) varies with time in expiration. The excitatory effect is absent in early expiration (*A*). It appears later in expiration and increases with time in expiration (*B* and *C*). Phrenic nerve activity, phr.; cervical sympathetic trunk activity, c.s.t.; stimulus, st. Each trace is average of 10 sweeps.

obtained by stimulation of superior laryngeal nerve afferents, which have been shown to have an excitatory action on the sympathetic preganglionic neurone, independent of the central respiratory activity (Bachoo & Polosa, 1985). A train of stimuli was given at various times of the expiratory phase (Fig. 13). A stimulus train, given 640 ms after the end of inspiration, did not cause a discharge (Fig. 13*A*) of sympathetic preganglionic neurones. A discharge first appeared when the stimulus train was given 1·6 s after the end of inspiration (Fig. 13*B*) and the discharge increased in amplitude as the stimulus was given later in expiration 2 s after inspiration end (Fig. 13*C*). Since in hypocapnia, resulting in abolition of rhythmic phrenic nerve activity, the response was similar in amplitude to that recorded in Fig. 13*C*, these data are interpreted as showing that after the end of inspiration the sympathetic preganglionic neurone undergoes a period of depression, which wanes with time in expiration.

DISCUSSION

The experiments just reported have provided a description of several properties of the inspiration-related sympathetic discharge. Some of the experiments have studied the delay between phrenic and inspiration-related sympathetic wave forms either in response to a discrete stimulus (to the superior laryngeal nerve) presented at various phases of the respiratory cycle or, in the steady state, when the phrenic nerve frequency was changed over a wide range of values by an external rhythmic

input (the periodic lung inflation by the respiratory pump) or by altering physiological variables (temperature, end-tidal P_{CO_2}). These observations are relevant to the question of whether or not the inspiration-related sympathetic discharge results from the activity of a hypothetical neural oscillator, synaptically coupled and entrained to the respiratory oscillator (Koepchen, 1962; Barman & Gebber, 1976).

The most obvious feature of the pattern of resetting of the phrenic and sympathetic activity cycle by superior laryngeal nerve stimulation is their close similarity, resulting in the absence of relative phase shifts. On the assumption that the superior laryngeal nerve afferent input acts independently on the respiratory oscillator and on the postulated sympathetic oscillator, the observed similarity implies (Pavlidis, 1973) that the two neurone systems undergo an identical cycle of varying sensitivity to this input. Although the details of the physiological mechanisms which generate the phrenic and inspiration-related sympathetic wave forms are incompletely understood, it seems unlikely that the properties of the two systems should be identical. Hence, the above assumption of an action of the laryngeal afferents independently on two oscillators can also be considered unlikely. In view of the well-known, apnoea-promoting, superior laryngeal nerve reflex (Larrabee & Hodes, 1948) a more likely assumption is that the superior laryngeal nerve input acts primarily to reset the central respiratory cycle and that the cycle of the putative sympathetic oscillator is perturbed secondarily by synaptic coupling between the two. However, this hypothesis is also unlikely, since for a system of coupled oscillators it is predicted that during perturbation of either oscillator the delay between the two output wave forms will change (Pinsker, 1977*b*), and yet no change in the delay between phrenic burst and inspiration-related sympathetic discharge was found during the perturbed cycle or the succeeding cycles. The similarity of the pattern of response of phrenic and inspiration-related sympathetic wave forms to superior laryngeal nerve stimulation, together with the absence of response features expected from a system of coupled oscillators, suggests that the superior laryngeal nerve input acts on a common oscillator which drives both the phrenic and the sympathetic discharges.

It is well known (Vibert *et al.* 1981; Petrillo *et al.* 1983) that in cats with intact vagus nerves the phrenic nerve discharge becomes time-locked to lung inflation by the respiratory pump, via the Hering–Breuer reflex circuit, in a manner which complies with theoretical predictions (Pham Dinh, Demongest, Baconnier & Benchetrit, 1983; Petrillo & Glass, 1984) and experimental observations (Pinsker, 1977*a*) of the behaviour of a neural oscillator driven by a periodic input. Namely, (i) the range of frequencies over which stable 1:1 entrainment can be maintained is limited to a set which borders the intrinsic frequency of the respiratory oscillator (i.e. the frequency in the absence of pulmonary stretch receptor input) and (ii) at each respiratory pump frequency, within this set, the forcing input (inflation) assumes a unique position in the phrenic activity cycle. For frequencies higher than the intrinsic frequency of the respiratory oscillator, inflation occurs with a short delay from the phrenic burst onset, resulting in a shortened phrenic nerve discharge and subsequent expiratory duration (Clark & von Euler, 1972). For frequencies lower than the intrinsic frequency of the respiratory oscillator, inflation occurs in expiration and results in a prolongation of expiration and of the subsequent phrenic nerve discharge (Zuperku & Hopp, 1985). Thus, within the range of stable 1:1 entrainment of the

respiratory oscillator frequency to the respiratory pump frequency there is a continuous change in the delay between inflation and onset of phrenic nerve discharge as a function of frequency (Fig. 8; see also Petrillo *et al.* 1983, Fig. 10a). In marked contrast, the absence of any comparable change in the delay between phrenic and sympathetic wave forms (Fig. 8) over the same range of frequencies suggests that the equality of period of phrenic and inspiration-related sympathetic discharge is not due to mechanisms comparable to those which maintain equality of respiratory pump and phrenic period. Thus, these findings make it unlikely that the frequency of the inspiration-related sympathetic discharge results from the activity of an autonomous neural oscillator coupled to the respiratory oscillator. Instead, these results are compatible with the hypothesis of a common neural oscillator which drives both the phrenic and the sympathetic discharges.

Although, as stated above, experimental observations (Wendler, 1974; Pinsker, 1977*a*; Ayers & Selverston, 1979; Peterson & Calabrese, 1982) uphold the theoretical predictions (Pavlidis, 1973; Winfree, 1980) that a stable 1:1 entrainment of coupled oscillators is limited to within a narrow range of frequencies bordering the free-run frequency for the coupling conditions prevailing in neural systems, the possibility may be considered that the absence of a frequency-dependent change in the delay between inspiration-related sympathetic discharge and phrenic discharge in the pump-locking experiments was due to the too limited range of frequencies tested. In the experiments of hyperthermic, hypocapnic polypnoea there was an approximately 20-fold increase in phrenic burst and inspiration-related sympathetic discharge frequency, again without apparent frequency-dependent phase shifts between the two wave forms.

The entrainment of oscillators is a phenomenon which is ubiquitous through the animal kingdom (von Holst, 1939), extending from oscillators with free-run periods of milliseconds (Winfree, 1980) to circadian oscillators (Moore-Ede, Sulzman & Fuller, 1982). The present experiments have focused on two testable features of the phrenic and inspiration-related sympathetic discharge which would help to identify the existence of the postulated sympathetic oscillator, namely the range of frequencies over which a stable 1:1 relation is maintained and the delay between the respective wave forms. The general applicability of these testable features is demonstrated by the published examples of known synaptically coupled neural oscillators, or of known biological oscillators which can be entrained by rhythmic synaptic inputs (Perkel, Schulman, Bullock, Moore & Segundo, 1964; Levy, Iano & Zieske, 1972; Stein, 1976; Pinsker, 1977*a*; Ayres & Selverston, 1979; Peterson & Calabrese, 1982; for comprehensive reviews see: Winfree, 1980; Pinsker & Ayers, 1983; Selverston & Moulins, 1985).

Although the mechanisms underlying entrainment in the examples cited above are likely to be different, all these studies illustrate the generality of the features of coupled oscillators, upon which the present experiments have focused. The qualifying limitation must be made, in extrapolating from the data just reviewed to the experimental situation of the present experiments, that the dynamic behaviour of entrained oscillators in invertebrate pace-maker cells or in non-neural mammalian systems may not be applicable to complex neural oscillators. However, the experimental evidence appears to be to the contrary (Pinsker & Ayers, 1983).

Since the hypothesis of coupled oscillators has been shown to be unlikely by the results discussed previously, the fixed temporal relation between phrenic nerve discharge and the inspiration-related sympathetic discharge may be explained by the hypothesis that the phrenic motoneurones and the sympathetic preganglionic neurones are driven by a common rhythmic input. Although onset and offset of the inspiration-related sympathetic discharge have a fixed temporal relation to the onset and offset of phrenic nerve activity, in the majority of cases the shapes of the two wave forms were different. The difference may result from differences in the properties of the pathways delivering the oscillator output to the two neurones. For instance, the sympathetic preganglionic neurones may be receiving the same periodic, ramp-shaped excitatory post-synaptic potential as the phrenic motoneurones (Berger, 1979), and the recruitment and firing properties of the two neurones may account for the difference in wave shape. In normocapnia, phrenic motoneurones are recruited throughout inspiration (St. John & Bartlett, 1979) and their firing frequency increases with time during inspiration, reaching a maximum at end-inspiration (Iscoe *et al.* 1976). The shape of the inspiration-related sympathetic discharge could be explained with the hypothesis that the majority of sympathetic preganglionic neurones are recruited early in inspiration and thereafter their firing frequency stays more or less constant. The data presented on the temporal structure of the inspiration-synchronous bursts of single sympathetic preganglionic neurones are consistent with this hypothesis. Interestingly, the inspiration-synchronous discharge of the whole hypoglossal nerve has a square-wave-like shape, similar to that of sympathetic nerves (Hwang, Bartlett & St. John, 1983). Hypoglossal motoneurones are recruited mostly at inspiration onset and, once recruited, fire at a relatively constant rate during inspiration (Hwang *et al.* 1983). A property of the sympathetic preganglionic neurone which may be contributing to the maintenance of a relatively constant firing frequency during inspiration is a large-amplitude, long-duration, summating, after-hyperpolarization (Yoshimura & Nishi, 1982).

Finally, the hypothesis of a common oscillator driving both phrenic motoneurones and sympathetic preganglionic neurones does not require that both populations of driven neurones be always simultaneously active. Differences in excitability of the neurones themselves or of the connecting pathways to the driving oscillator may be expected to result in the possible absence of rhythmic activity in one and persistence in the other, as shown by some of the present observations (Figs. 10–13).

The conclusions reached in the present study may appear to be in contrast with some of the conclusions reached by Barman & Gebber (1976). It must be realized, however, that the data on which these two studies are based are different. The observations of Barman & Gebber (1976) concern a slow amplitude modulation (with period similar to that of the phrenic bursting rhythm) of a 3–4 Hz repetitive wave form recorded in sympathetic post-ganglionic nerves. In several of their records this slow modulation appears to be independent of, i.e. not phase-locked to, the phrenic nerve burst. In contrast, the present study concerns the periodic burst of sympathetic preganglionic neurone firing, which previous work has defined as inspiration-related on the basis of constant phase-relation to, and similarity of properties to, the phrenic nerve burst (Preiss *et al.* 1975; Gerber & Polosa, 1979). The data presented in the study by Barman & Gebber (1976) are mostly non-stationary, and therefore

information concerning the statistical properties of the slow modulating signal and its phase relation to the phrenic nerve cycle is not available for some of their most intriguing observations (e.g. their Figs. 4 and 5). Thus, in the absence of insight into the relation, if any, between the periodic, inspiration-related, sympathetic burst studied here and the slow modulation studied by Barman & Gebber (1976), it cannot be stated whether the discrepancy between these two sets of conclusions is real or is the result of different phenomena being studied in the two investigations.

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