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THE IMPORTANCE OF TIMING ON THE RESPIRATORY EFFECTS OF INTERMITTENT CAROTID SINUS NERVE STIMULATION

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SUMMARY

1. The respiratory response, measured directly as tidal volume or indirectly by using integrated peak phrenic activity, to intermittent electrical stimulation of the carotid sinus nerve was determined in anaesthetized cats.

2. Stimulation at rates of 20–25 Hz for 0·5 sec had a rapid effect, increasing inspiratory airflow and phrenic discharge, but only if applied during inspiration. An increase in tidal volume or peak level of integrated phrenic discharge occurred only if the stimulus was exhibited during the second half of inspiration. Continuous stimulation had no greater effect on size or frequency of breathing than did intermittent inspiratory stimuli alone. Stimulation during expiration had no effect on the form or magnitude of subsequent breaths.

3. Stimuli in expiration led to a prolongation of expiration. Stimuli in late inspiration caused a prolongation of both inspiration and expiration. Because of these effects, the respiratory rate could be changed by stimulation; in some instances entrainment of respiration by the intermittent carotid sinus nerve stimuli occurred.

4. The findings are attributable to modulation of incoming carotid sinus nerve information by the central respiratory neurones, which use primarily that which arrives during inspiration. They show a possible mechanism by which oscillating signals may have a different effect than their mean level would indicate.

INTRODUCTION

Oscillation of the alveolar CO_2 and O_2 tensions occurs with each respiratory cycle (Dubois, Britt & Fenn, 1952; Chilton, Barth & Stacey, 1954; Yamamoto, 1960). Transmission of these oscillations to the blood reaching

the carotid artery occurs with both O_2 (Purves, 1966) and with CO_2, the latter being reflected by pH oscillation having the same period as respiration. The magnitude of the fluctuation varies directly with tidal volume and inversely with respiratory rate up to rates as high as 30–35/min (Honda & Ueda, 1961; Band, Cameron & Semple, 1969).

A decade ago Yamamoto (1960) suggested that the oscillations in arterial P_{CO_2} might provide a stimulus to respiration different from that indicated by the mean level of P_{α,CO_2}, and Yamamoto & Edward's (1960) experimental study supported that concept. Since that time there have been a number of investigations whose results contradict the hypothesis (Fenn & Craig, 1963; Lamb, Anthonisen & Tenney, 1965; Hornbein, 1965; Cunningham, Elliott, Lloyd, Miller & Young, 1965; Lamb, 1967; Fenner & Berndt, 1970); but other studies utilizing a variety of experimental approaches including intermittent inspiration of CO_2 (Dutton, Chernick, Moses, Bromberger-Barnea, Permutt & Riley, 1964), infusion of CO_2 into the carotid arteries (Dutton, Fitzgerald & Gross, 1968), tube breathing (Fenner, Jansson & Avery, 1968; Goode, Brown, Howson & Cunningham, 1969) and hypoxic exercise (Bhattacharyya, Cunningham, Goode, Howson & Lloyd, 1970) have lent their support to its importance.

Since it is generally assumed that the delay in arrival of the stimulus at the central CO_2-H^+ receptors and their response time are too great for these receptors to be responsible for the effects of oscillations, these effects would have to be mediated primarily by the peripheral chemoreceptors. That their speed of response is sufficient to follow oscillating chemical stimuli has been shown by the fact that variations in carotid sinus nerve activity exhibit the same period as respiration (Hornbein, Griffo & Roos, 1961; Hornbein, 1965; Biscoe & Purves, 1967; Leitner & Dejours, 1968). Furthermore, Fitzgerald, Leitner & Liaubet (1969) have shown in the cat that the carotid body output is able to follow intermittent arterial CO_2 stimuli at rates up to about 1/sec. It is less certain that hypoxic responses are fast enough to be of much importance (Black, McCloskey & Torrance, 1966; McCloskey, 1968; Fitzgerald *et al.* 1969).

It has been suggested that the increased effect of oscillating signals might originate at the level of the carotid body itself; that the arterial P_{CO_2} crosses a threshold for CO_2 stimulation leading to rectification of the signal (Bhattacharyya *et al.* 1970). Another suggestion has been that the rate of rise of the carotid arterial CO_2 stimulus acting either directly via the carotid bodies or during processing of the neural information in the medulla is an important factor (Dutton, Hodson, Davies & Fenner, 1967). Recently, however, several studies have suggested that the timing of the stimulation and neural discharge of the carotid body with relationship to the respiratory cycle is a significant variable. Black & Torrance (1967) and

Band, Cameron & Semple (1970) have demonstrated in cats that stimulation of the carotid body by CO_2 and pH changes at various times in the respiratory cycle had markedly different effects, stimulation in expiration lengthening expiratory duration but having no effect on inspiration, stimulation in inspiration causing a significant increase in tidal volume. Bernards & Sistermans (1969) have reported comparable findings in the dog.

Electrical stimulation of the carotid sinus nerve appears to have a similar effect. Black & Torrance (1967) reported the same results as with chemical stimulation, and Howard, Bromberger-Barnea, Fitzgerald & Bane (1969) showed not only that the ventilatory response varied markedly with inspiration and expiration but that it could vary depending on the time the stimulus was given within the inspiratory period itself.

The present study defines in more detail the relationship between the timing of carotid sinus nerve (CSN) stimulation and the phrenic nerve and ventilatory responses. It shows that the CSN-medullary-phrenic reflex is of short latency and that the phrenic response does not long outlast the stimulus. It not only confirms the importance of the time of stimulation, with relation to the phase of the respiratory cycle, on tidal volume, but also demonstrates the ability of intermittent CSN signals to affect durations of inspiration and expiration and to influence respiratory rate.

METHODS

Cats weighing between 3·0 and 5·1 kg were anaesthetized with ether and then given chloralose (40 mg/kg) and urethane (250 mg/kg) via a foreleg vein. The animal was placed supine on a table with a rigid head mount. The trachea was intubated through a neck incision and a femoral artery cannulated for blood pressure recording using a strain gauge transducer (Statham P 23D). Temperature was continuously monitored with a rectal thermistor (Yellow Springs Inst. Co.) and maintained at 37–38° C by means of a heating mat or lamp. Continuous sampling (250 ml./min) and recording of airway P_{CO_2} was accomplished by means of a small catheter inserted in the airway and analysis by an infra-red CO_2 analyser (Beckman LB 1) with linearized output (Beckman LB 1 linearizer). Unless otherwise noted, all experiments were carried out with end-tidal P_{CO_2} in the normal range for the cat (30–35 mm Hg). Both vagus nerves were isolated but left intact with loose ligatures around them for later sectioning. Further dissection exposed the region of the carotid artery bifurcation and the CSN which was freed from surrounding tissue and placed on bipolar stimulating electrodes in pool of mineral oil.

Experiments were carried out in two groups of animals:

Group 1. Ten experimental runs were made in three animals during spontaneous breathing. The CSN was stimulated (Ortec, Inc. dual-channel modular stimulator) with short trains of pulses, given at various times in the respiratory cycle over a large number of breaths. Because of the increased tidal volume and drop in P_{CO_2} resulting from some stimulations, four to five unstimulated breaths were allowed to occur between stimulations. Air flow and tidal volume were recorded along with arterial pressure, end-tidal P_{CO_2} and the stimulus marker. A small pneumotacho-

graph and differential strain-gauge pressure transducer (Statham P-97) were used to determine air flow and, after integration, tidal volume. The system was calibrated for volume with a syringe of known volume. In these animals the catheter tip for CO_2 analysis was located distally to the pneumotachograph screen so volume measurements were not affected by the flow to the analyser.

Group 2. Twenty-four experimental runs were carried out in seven animals (one of them was also a member of group 1). They were prepared in the same way as above with the addition that a phrenic nerve root (usually C5 on the right) was isolated, cut, the sheath of the proximal end retracted and the nerve placed in a pool of mineral oil on platinum bipolar electrodes for recording. Paralysis was induced with gallamine triethiodide (Flaxedil, American Cyanamid Co.), approximately 3 mg/kg I.V., the animals were maintained at constant end-tidal P_{CO_2} by means of a volume respirator (Phipps and Bird Model 7088–700), and phrenic nerve activity used to follow respiratory changes. Stimulation of the carotid sinus nerve was carried out as in group 1, but on alternate or every third cycles.

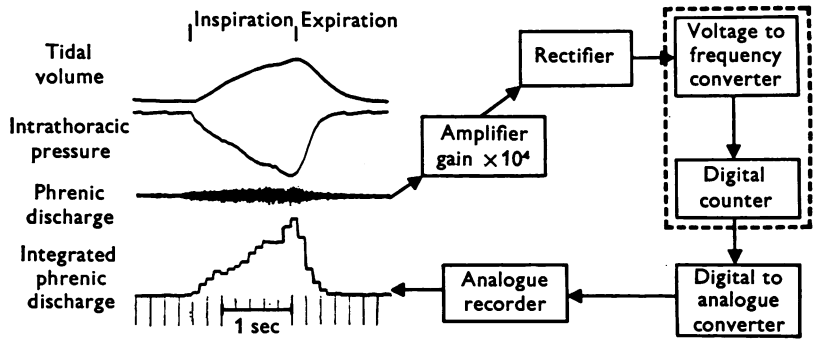

Fig. 1. Diagram of the method of processing phrenic discharge used to give integrated phrenic activity. The cat in this example was breathing spontaneously and was not part of the present study. Each step of the tracing represents the phrenic activity of the preceding 0·1 sec. In this and subsequent Figures the steps have been connected by retouching for better legibility. The two units within the dotted lines are the equivalent of an integrating digital voltmeter.

In both groups, trains of square-wave pulses at frequencies of 20–25 or 100 Hz with a pulse duration of 0·5 msec were used to stimulate the CSN. The train duration was usually 0·5 sec or 0·1 sec but in some cases the train was manually timed to coincide with the period of inspiration or that of expiration and compared to continuous stimulation throughout the respiratory cycle. The stimulus voltage chosen was sufficient to cause a demonstrable effect on the size of the breath when applied at the appropriate time but was otherwise kept as low as possible and no attempt was made to obtain a maximal response. The actual values used were between 0·6 and 1·5 volts and were not changed during the course of an experimental run.

In those experiments in which phrenic activity was used to follow respiration, the phrenic discharge was amplified by an AC amplifier (Grass Instrument Co., Model P-15) with 100 and 1000 Hz low and high frequency cut-offs and was processed as shown in Fig. 1 to give integrated phrenic activity. A linear relationship between the peak 0·1 sec level of the integrated phrenic activity and most of the range of tidal volume has been reported elsewhere (Eldridge, 1971). If one accepts the premise that

tidal volume is a good criterion for determining the output of the respiratory control system, then these findings indicate that the peak level of phrenic activity should be equally good. For this reason changes in peak phrenic activity will be interpreted, in the results to follow, to mean the same as a change in tidal volume. Since the electronic counter used in integrating phrenic activity always lags one counting period, 0·1 sec in this case, all measurements reported herein were corrected to real time.

In all experiments, events were viewed on a storage oscilloscope (Tektronix, Inc. R564B), recorded by means of a multichannel oscillographic recorder (Sanborn Co. 550M) and recorded on magnetic tape (Honeywell 8100 7-channel tape recorder) for subsequent playback and analysis.

RESULTS

Since the findings were similar in all animals, whether they were breathing spontaneously or were paralysed, they will be considered together. However, the use of peak phrenic activity as a neural analogue of respiratory activity, with open loop control conditions in which ventilation, blood P_{O_2}, P_{CO_2} and pH remained constant, permitted a more complete analysis of the effects of CSN stimulation. Although baroreceptor as well as chemoreceptor fibres in the CSN might be expected to be stimulated in these experiments, the effects on blood pressure when present at all were always small and pressor in type and were always delayed in relation to the stimulus and its effects on ventilation or phrenic discharge.

Effects on tidal volume and peak phrenic activity

Brief stimulation of the CSN during inspiration had an almost immediate effect on inspiration, regardless of its relationship to the time of inspiration. There was always an immediate increase in the rate of air flow, lasting only as long as the stimulus. This is shown by an increased slope of the tidal volume tracing and the changed shape of the inspiratory curve in spontaneously breathing cats (Figs. 2 and 3) and an increased slope of the integrated phrenic activity (Fig. 4). However, only those stimulations which occurred in the last half of inspiration had an effect on the magnitude of tidal volume (Figs. 2 and 3) or the height of the integrated phrenic activity (Fig. 4). The later in inspiration the stimulus occurred, the greater was the increase in tidal volume and peak neural activity. The maximum tidal volumes and phrenic activity with late inspiratory stimuli ranged from 146 to 233 % of unstimulated breaths. Since no attempt was made to use either maximum voltages or stimulus frequencies, the true maximum was probably higher. In the few animals tested, increased frequency (100 Hz) at the same voltage led to a greater maximum volume than the frequency (20–25 Hz) used in most of the experiments.

When given in expiration, brief CSN stimuli had no effect on the form or

magnitude of the subsequent breath. Usually, a stimulation in expiration caused no obvious immediate inspiratory movement (Fig. 2); but in some cats studied with the higher frequency stimulus, there was a small inspiratory movement at the time of the stimulus (Fig. 3) and often a tiny burst of phrenic action potentials could be discerned on the phrenic nerve recording even with the 20–25 Hz stimulus (cf. Figs. 14*A*, *H* and 15*A*). These had no effect on the size of the subsequent breath.

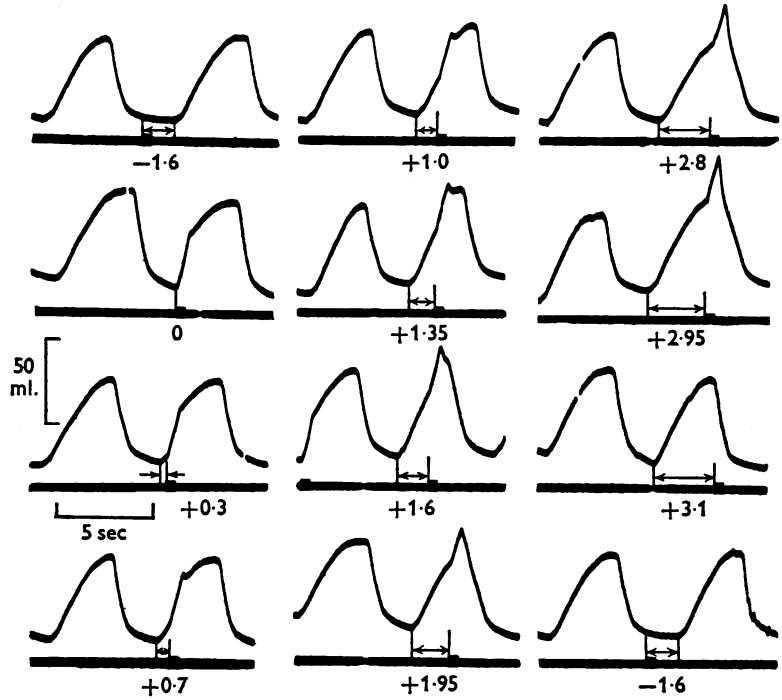

Fig. 2. Effect of carotid sinus nerve stimulation (0·5 sec duration, 22 Hz) on tidal volume (inspiration upwards) in one cat. The stimulus during expiration in the upper left tracing begins 1·6 sec before the next inspiration and has no effect. Downwards and then to the right, the stimulus onset is progressively later, the numbers showing the time in seconds from onset of inspiration to onset of stimulus. Note that all inspiratory stimuli promptly affect air flow, but that only late inspiratory stimuli cause an increase in tidal volume. A stimulus which falls just after the end of inspiration (+3·1 sec) has no effect. Cat A 25, vagi cut, P_{CO_2} = 33 mm Hg; non-stimulated tidal volumes = 50 ml.

The relationships between the timing of onset of an 0·5 sec stimulus and the effect on tidal volume or peak phrenic activity for three animals are shown in Fig. 5. Whether one looks at the stimulus time in absolute terms or as a percentage of inspiratory duration, it is apparent that stimulations

in the first half of inspiration have little effect on the size of the breath; whereas those in the latter half have an increasing effect, the maximum occurring when the stimulus is applied after 70% of inspiration has been completed. Similar results are seen in Fig. 6 where the findings of all cats studied, representing over 800 individual stimulations in spontaneously breathing animals with cut vagi and paralysed animals with vagi intact or cut, are considered together.

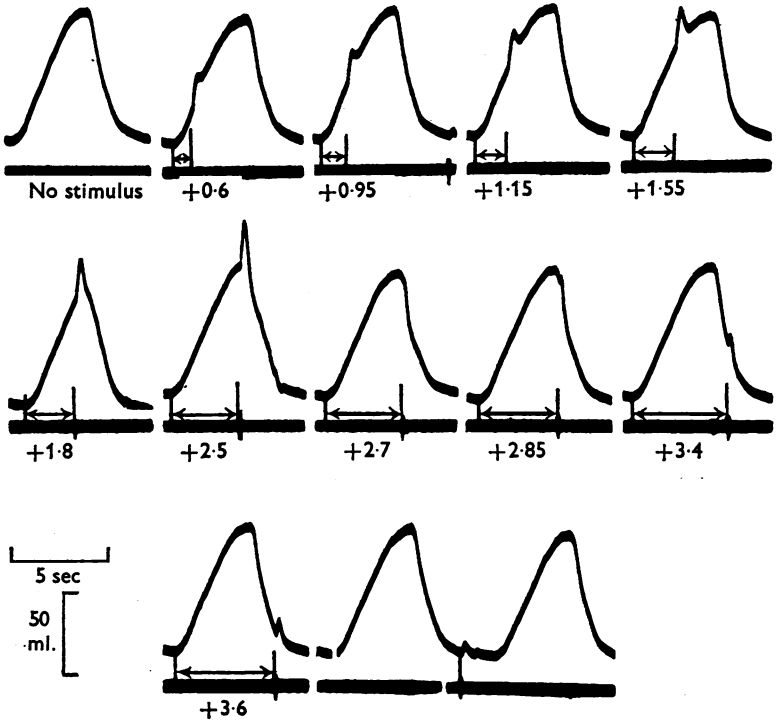

Fig. 3. Same cat and conditions as shown in Fig. 2, but CSN stimulation was at 100 Hz, 0·1 sec in duration. Times from onset of inspiration to onset of stimulus are shown in seconds and become progressively longer from left to right. Note that the duration of the effect on air flow or tidal volume is much briefer than in the previous figure. In this cat, there was a small inspiratory movement after CSN stimulation in expiration (e.g. +3·4 sec, +3·6 sec, and in mid-expiration at lower right).

Although the presence or absence of vagal feed-back did not affect the basic relationship between stimulus time and effect on inspiration, the findings were more clear cut when vagal influences were eliminated. In one of the spontaneously breathing animals, a CSN stimulus early in inspiration led to an inhibition of inspiration. This effect disappeared after vagal

sectioning and was presumably the result of vagal inhibition. In the paralysed animals whose respirator-produced ventilation was out of phase with phrenic discharge some variation in phrenic discharge and respiratory rate occurred even without CSN stimulation. This again largely disappeared when the vagi were cut.

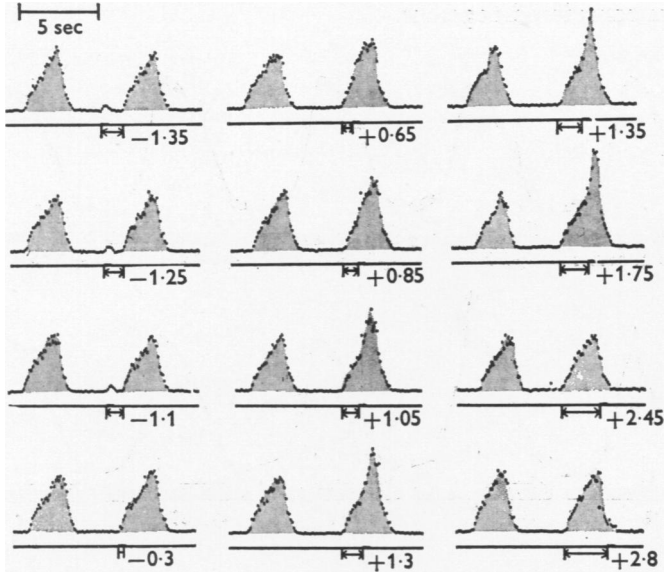

Fig. 4. Effect of carotid sinus nerve stimulation (0·5 sec duration, 25 Hz) on integrated phrenic activity. The left of each pair of breaths is not stimulated, the right has a stimulus preceding (left column) or following (centre and right) the onset of inspiration. Numbers represent stimulus onset time in seconds from onset of inspiration. Expiratory stimuli have no effect on phrenic discharge, whereas inspiratory stimuli affect its slope if early and its height if late. Cat A 21, paralysed, P_{CO_2} = 32 mm Hg.

Effect of intermittent versus continuous CSN stimulation

Stimulation of the CSN throughout expiration had no effect on the magnitude of phrenic activity whereas stimulation throughout inspiration had a significant effect (Fig. 7). In this same Figure, it can be seen that continuous stimulation had no greater effect on the size and frequency of breathing than did the intermittent inspiration stimuli alone.

Effects on durations of inspiration and expiration

Because of the breath to breath variation in phrenic discharge and respiratory rate when the vagi were intact, only those studies with vagal influences removed will be considered here. The duration of inspiration was

not affected by a CSN stimulus given in expiration. Stimuli during inspiration, however, changed the length of inspiration in opposite ways depending upon the time in the respiratory cycle the stimulation was given. Stimulation in the first half of inspiration tended to shorten the total duration, sometimes markedly so; but the duration was progressively increased if stimuli were given in the second half, the later the timing of the stimulus the greater the extension (Fig. 8).

The duration of expiration was affected by CSN stimuli given in both

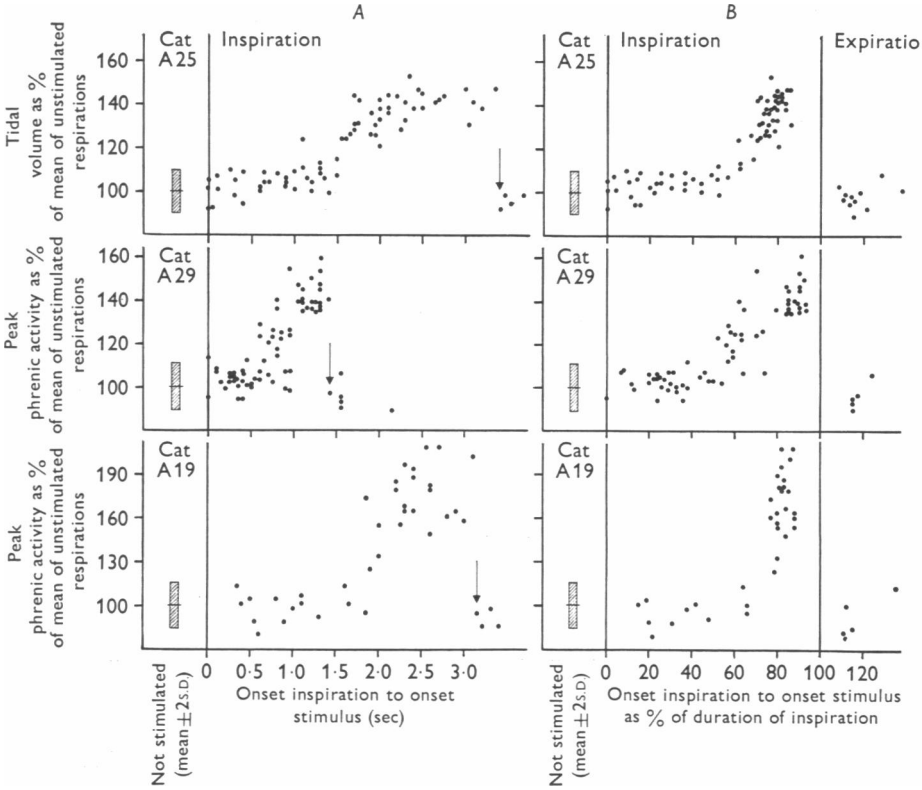

Fig. 5. The increase in tidal volume or peak phrenic activity resulting from CSN stimuli (20–25 Hz, 0·5 sec duration) given at various times in the respiratory cycle. Changes occurring with stimulation are shown as a percentage of the mean of unstimulated control breaths.

In *A* (left column) the time from onset of inspiration to the onset of stimulation is shown in seconds. Vertical arrows mark the beginning of expiration. In *B* (right column) the time from onset of inspiration to stimulation is shown as a percentage of the duration of inspiration.

Little effect on breath size is noted until over 50 % of inspiration has been completed. Stimulation during expiration has no significant effect on the subsequent inspiration.

late inspiration and in expiration. Stimuli in early inspiration did not affect expiratory duration, but those late inspiratory stimuli which led to increased breath size and phrenic activity were associated with longer expiratory durations. Stimuli given in the early part of expiration had no marked effect, but those given in the second half of expiration caused a

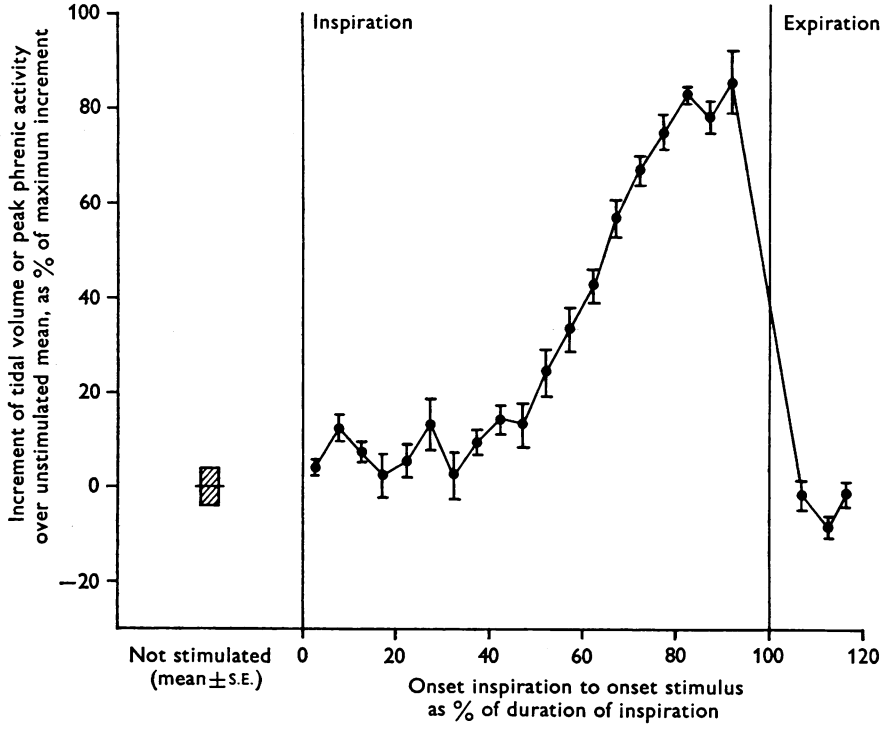

Fig. 6. Changes in tidal volume or peak phrenic activity with CSN stimuli given at various times during inspiration. Composite results of all cats studied, arranged in bins for each 5% of inspiration. All stimuli were 0·5 sec in duration at 20–25 Hz. In order to normalize the data obtained in different cats the change from the mean of unstimulated breaths has been used, and for a given cat has been calculated as a % of the maximum increment found on stimulation. Zero % increment is the unstimulated mean.

clear prolongation (Fig. 9). Only if the stimulus came very late in expiration was there again a shortening due to the fact that the next inspiration was initiated early by the stimulus. This is seen to occur in two of the cats in Fig. 9 and is shown in the recording in Fig. 10.

Effect on respiratory rate

Because of the ability of brief CSN stimuli to cause prolonged expiration, early onset of inspiration and shortened or prolonged inspiration, all depending upon their location in the respiratory cycle, the respiratory rate can easily be affected by these stimuli. Fig. 11 shows this phenomenon in a

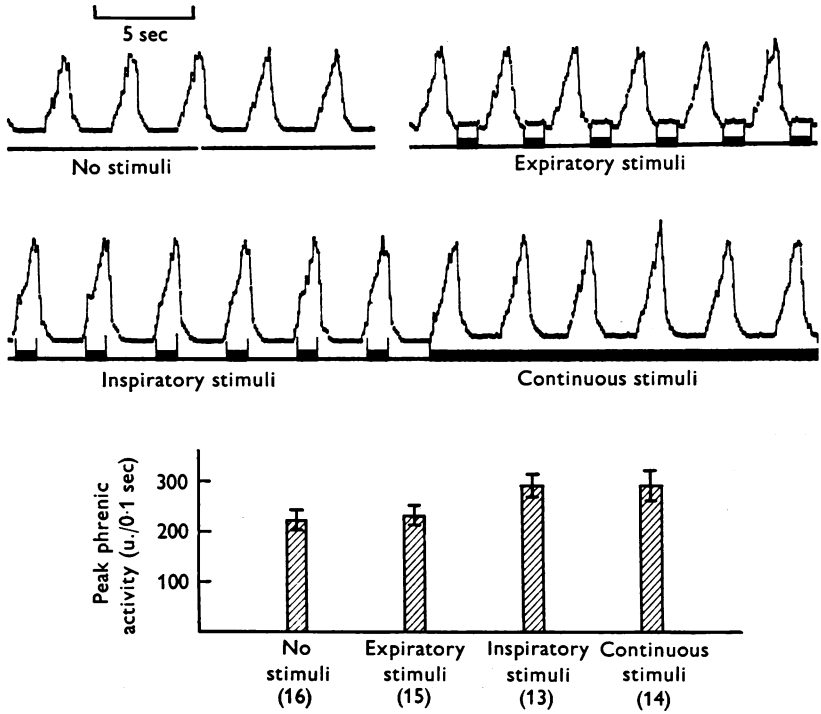

Fig. 7. Showing that repeated expiratory stimuli (22 Hz) produce no increase in peak phrenic activity over control, that inspiratory stimuli produce a significant increase and that continuous stimulation causes no further increase over inspiratory stimulation alone. Lower panel shows mean ± 2 S.D. for each condition. The figures in brackets represent the numbers of breaths in each group. Cat A 29, paralysed, vagi cut, P_{CO_2} = 33 mm Hg.

cat whose end-tidal P_{CO_2} was in the normal range. A more dramatic example is seen in an animal (Fig. 12) whose end-tidal P_{CO_2} had been lowered sufficiently to reduce phrenic activity considerably below normal. In this case, CSN stimulation led to entrainment of the bursts of respiratory activity at rates varying from 11·1/min to 17·6/min, both a decrease and increase from the natural unstimulated rate in this cat of 16·2/min. At faster stimulation rates (Panel *A*, Fig. 12), the stimuli affected only some of the breaths and caused a varying rate and size on a regular pattern, with

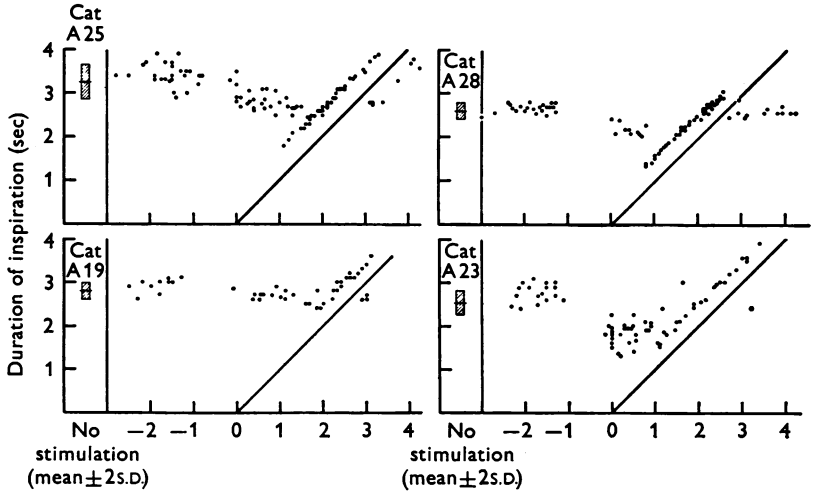

Fig. 8. Effect of CSN stimulation (0·5 sec duration, 20–25 Hz) on inspiratory durations in four cats. Stimuli in late expiration have no effect. Early inspiratory stimuli shorten inspiration, whereas late inspiratory stimuli prolong it. Stimuli to right of diagonal line are in early expiration and have no significant effect. Cats A 25 and A 28 are breathing spontaneously, cats A 19 and A 23 are paralysed.

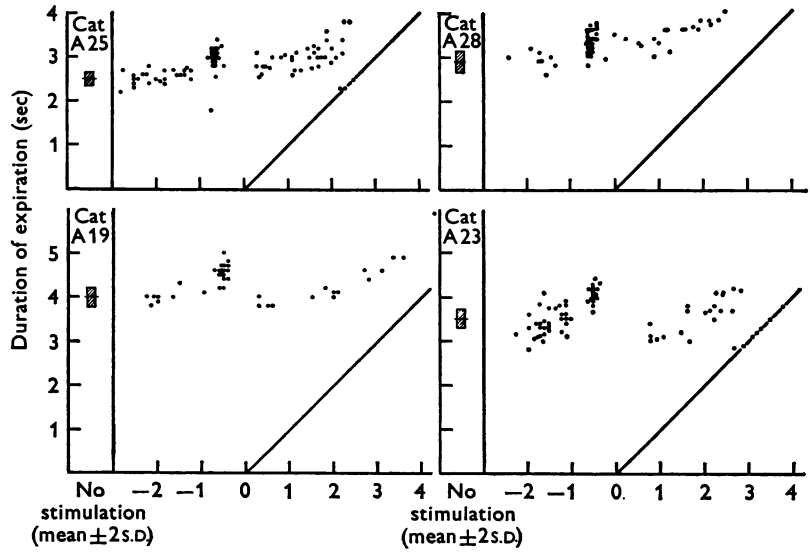

Fig. 9. Effect of CSN stimulation on expiratory durations in the same cats as Fig. 8. Both late inspiratory stimuli and late expiratory stimuli prolong expiration. In cats A 25 and A 23 it can be seen that the latest stimuli may actually cause a shortening of expiration by early initiation of the next inspiration (points lying on the diagonal lines).

Fig. 10. Tracing of phrenic activity in a paralysed cat showing the early onset of inspiration resulting from a CSN stimulation (25 Hz, 0·5 sec duration). The dashed brackets show where an unstimulated inspiration would have started.

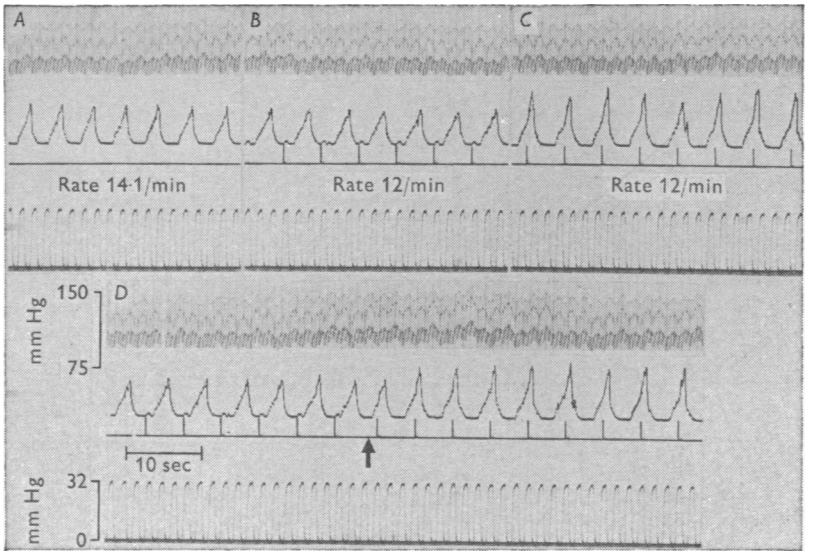

Fig. 11. Effect of CSN stimuli (22 Hz, 0·5 sec duration, onset indicated by small vertical bars in *B*, *C* and *D*) given at a rate of 12/min on respiratory rate in a paralysed cat with constant P_{CO_2}. Top tracing, arterial pressure; middle tracing, integrated phrenic activity; bottom tracing, airway P_{CO_2} (end tidal = 32 mm Hg). *A*, no CSN stimulation. *B*, stimulation during the last half of expiration. *C*, stimulation during the last half of inspiration. *D*, continuous record showing shift (accomplished manually at arrow) from expiratory to inspiratory stimulation. Respiratory rate remains constant at the stimulation rate of 12/min.

Because late inspiratory and late expiratory stimuli prolong the duration of the stimulated half-cycle, respiration is entrained and the rate slowed by either, but with different effects on the peak level of phrenic activity.

the appearance of a varying block of the stimulus. At still faster stimulus rates (not shown in the Figure) the respiration again became regular, alternate stimuli affecting inspiration when given in inspiration and having no effect when given in expiration, with the appearance of a 2 to 1 block.

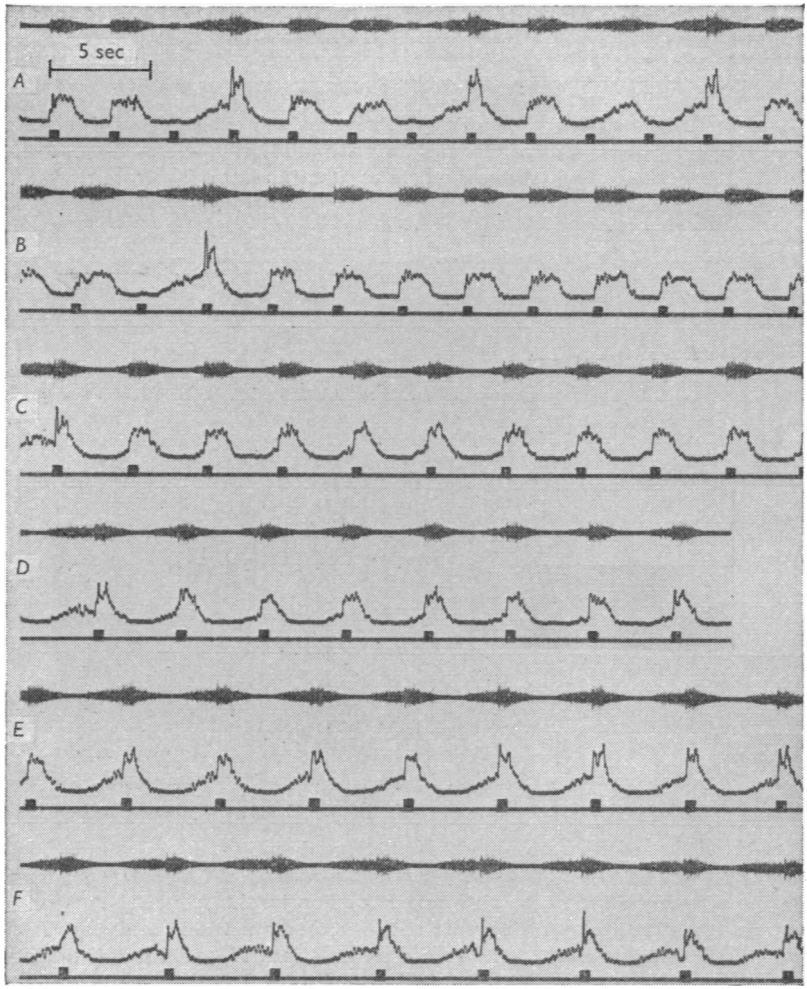

Fig. 12. Paralysed cat (A 29), vagi cut, hyperventilated to end-tidal P_{CO_2} of 24 mm Hg, showing entrainment of respiration by CSN stimulation (22 Hz, 0·5 sec duration). Top tracing, phrenic discharge; middle tracing, integrated phrenic activity; bottom tracing, stimulus marker. Unstimulated respiratory rate was 16·2/min. *A*, stimulus rate 19·3/min, every fourth stimulus blocked. In the remaining sections, stimulus and respiratory rates are the same: *B*, 17·6/min, *C*, 15·4/min, *D*, 13·9/min, *E*, 12·2/min, *F*, 11·1/min.

Effect on phrenic discharge

Stimulation of the CSN during inspiration always led, after a brief latent period, to increased phrenic nerve discharge characterized by an increased spike frequency and larger spikes (Figs. 13*C–G*, *I* and 14B_2, C_2). Stimulation during expiration was often followed by a burst of smaller

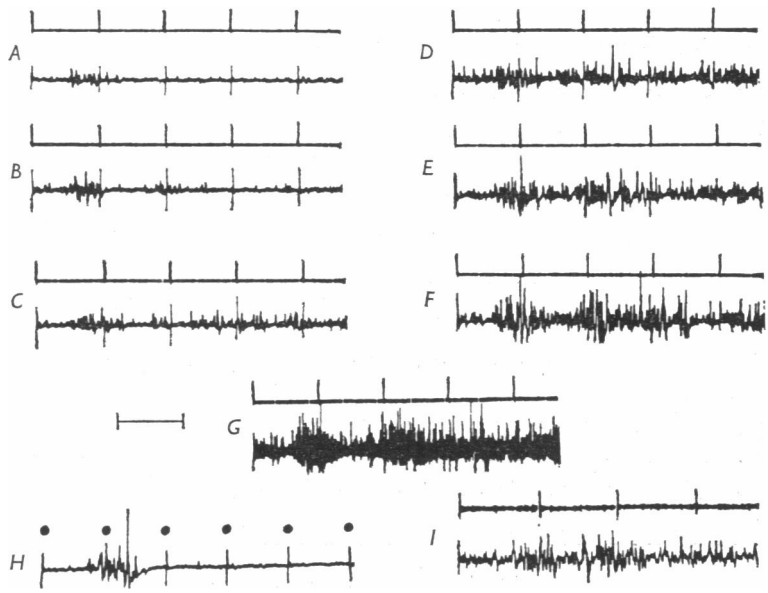

Fig. 13. Recordings of phrenic nerve discharge after initiation of CNS stimulation showing latency of response in three cats (*A–G*, Cat A23; *H*, Cat A43; *I*, Cat (A29). Upper tracings mark the stimuli (circles in *H*), lower tracings show phrenic discharge (stimulus artifacts are also seen in some). The oscilloscope sweep was triggered by the first of the series of CSN shocks. Time bar = 50 msec.

The stimulus was delivered in expiration in *A*, *B* and *H*, and during inspiration in *C–F* and *I*. Panel *G* has five superimposed stimulated sweeps during inspiration. Latencies range from 25 to 33 msec.

phrenic action potentials (Fig. 13*A*, *B*, *H* and 14*A*) which occasionally were enough to cause a measurable inspiratory movement in a spontaneously breathing cat (cf. Fig. 3). The latency of the phrenic response, after the first stimulus of the usual series of shocks, ranged from 25 to 36 msec among the various cats studied (Figs. 13 and 14). There was some variation in the latency in a single cat but no clear relation could be made between it and the time of the stimulus in the respiratory cycle.

A brief phrenic inhibition, which occurs after single CSN shocks and during the above noted latent period for excitatory effects, has been reported (Biscoe & Sampson, 1970). This was looked for but only occasionally found. The effect may be present in Fig. 13*F*, *G*, but other first stimulations in the same cats failed to show such depression of phrenic activity (Figs. 13*D*, *I* and 14 B_2, C_2).

The phrenic excitation resulting from the first shock of a series of eleven or twelve usually lasted approximately 30 msec whether given in an expiration or inspiration. In expiration, subsequent shocks did not have

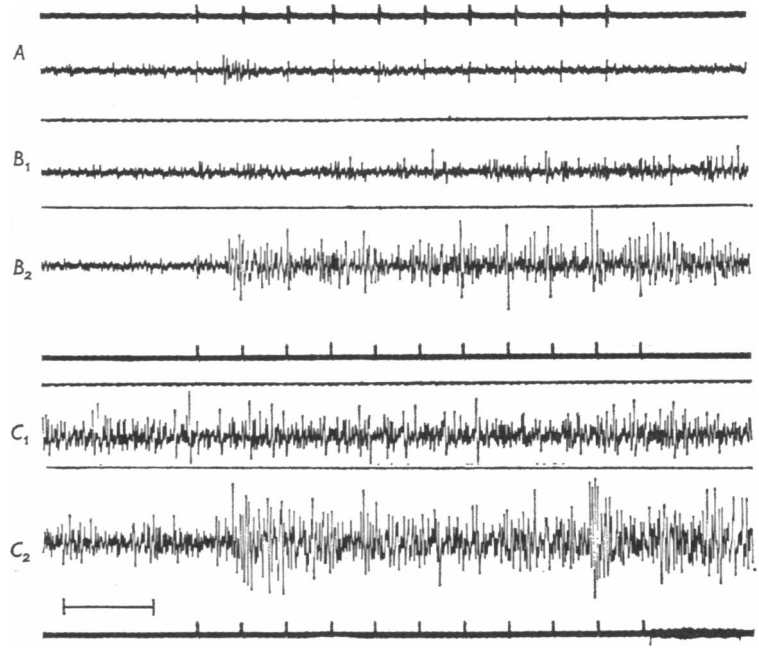

Fig. 14. Effect on phrenic discharge of CSN stimulation (0·5 sec duration at 22 Hz) given at different times in respiratory cycle in a paralysed, vagotomized cat. P_{CO_2} = 33 mm Hg. Time bar = 100 msec.

A, stimulus begun half way through expiration. A burst of phrenic action potentials follows the first shock after a latency of about 30 msec, but subsequent shocks cause no phrenic activity. Upper tracing shows stimulus markers.

B_1, phrenic discharge during first 25% of an unstimulated inspiration. B_2, same portion of inspiration as in B_1, but CSN stimulus begun early in inspiration. After a latency of about 30 msec phrenic activity increases and persists as long as the stimulation continues. Lower tracing shows stimulus markers.

C_1, phrenic discharge during last 25% of an unstimulated inspiration. C_2, same portion of inspiration as in C_1, but stimulus begun after 75% of inspiration had been completed. There is a marked increase in frequency and size of action potentials. Lower tracing shows stimulus markers.

any further effect. In contrast, additional shocks during inspiration continued to cause a phrenic response, but after the second or third stimulus the individual responses merged with the general increase in phrenic activity so that the effect of a specific shock could not be discerned. Nevertheless, the effect of stimulation, while having a significant effect during any part of inspiration, did not long outlast the duration of stimulation (Fig. 15). Another example of this is found in a paralysed cat which had been hyperventilated almost to the point of extinction of phrenic activity

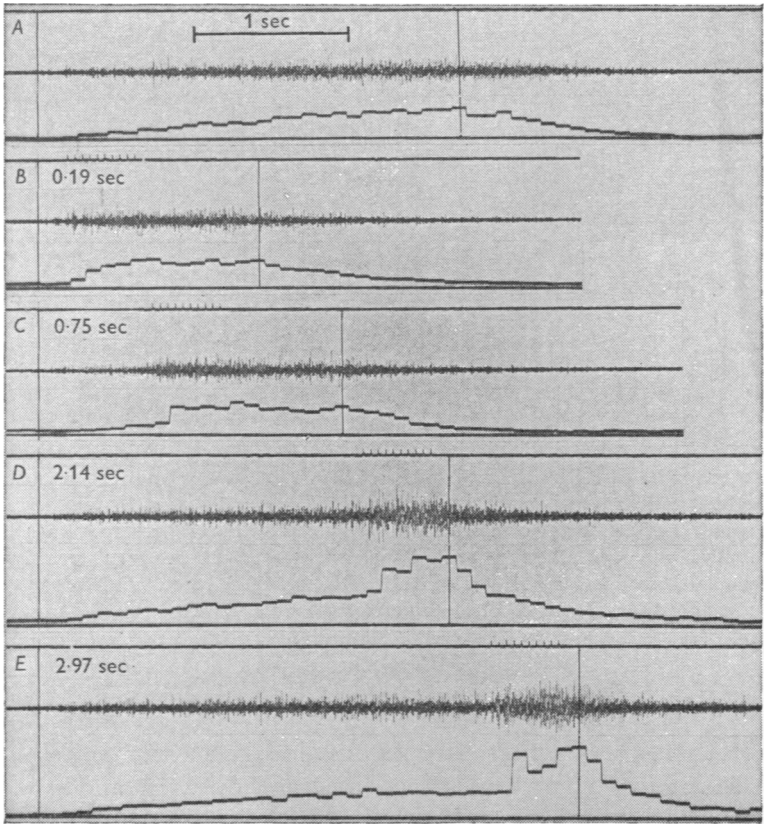

Fig. 15. Effect on phrenic discharge (middle tracing) and integrated phrenic activity (lower tracing) of CSN stimulation (0·5 sec duration, 22 Hz) given at various times in inspiration. Upper tracing shows stimulus markers. Vertical lines on left indicate start of inspiration, lines on right indicate end of inspiration. The cat is paralysed and vagotomized. P_{CO_2} = 33 mm Hg.

An unstimulated respiration is shown in *A*. In *B–E* progressively later stimulations are shown. The effects on inspiratory duration and phrenic discharge described in the text are clearly seen. The increased phrenic activity resulting from stimulation does not persist long after the stimulation ends.

(Fig. 16). CSN stimulation had the usual rapid effect of increasing phrenic activity. There was a progressive enhancement of activity with progressively longer periods of stimulation, but in each case the activity began to fade soon after the stimulation ceased. The rapid onset and offset of the phrenic response to CSN stimulation would thus appear to be the explanation for the failure of early inspiratory stimuli to have an effect on late inspiration, represented by peak phrenic discharge or magnitude of tidal volume.

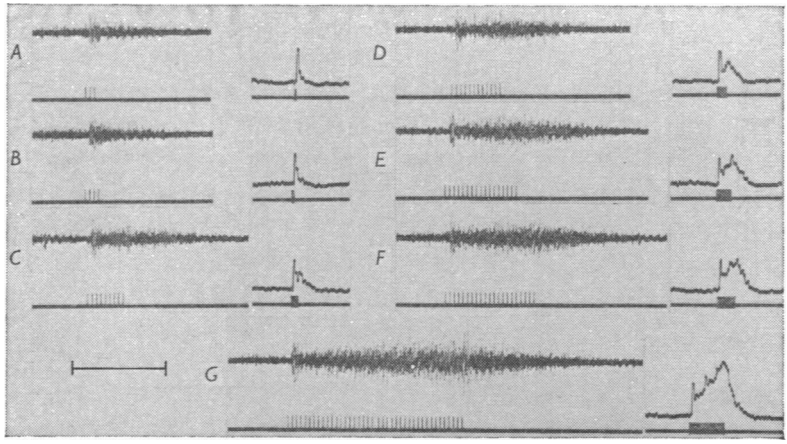

Fig. 16. Effect on phrenic discharge (left panel) and its integrated activity (right panel) of CSN stimuli of various durations in a paralysed, vagotomized cat which had been hyperventilated (P_{CO_2} = 24 mm Hg) almost to the point of extinction of phrenic activity. Time bar represents 1 sec in phrenic discharge tracings and 5 sec in integrated activity tracings. Stimulus durations are *A*, 0·1 sec; *B*, 0·2 sec; *C*, 0·4 sec; *D*, 0·6 sec; *E*, 0·8 sec; *F*, 1 sec; *G*, 2 sec. Phrenic activity increases as long as the stimulation persists but begins to decrease soon after it ends.

DISCUSSION

This study has shown that electrically induced afferent impulses in the carotid sinus nerve have a rapid effect on phrenic discharge and may cause a significant increase of tidal volume or peak phrenic discharge rate. However, the timing of the impulses with relation to the phase of the respiratory cycle is critical to their effect on respiration, for brief stimulation significantly affected flow, volume and phrenic discharge only if given in inspiration and caused an increase in breath size or peak phrenic discharge only if given in the last half of inspiration. On the other hand, stimulation affected respiratory rate, whether given in inspiration or expiration, the effect depending on the location in the respiratory cycle.

The main outlines of the present findings are in agreement with those

of the brief report of Black & Torrance (1967) who showed that inspiratory stimulation increased the depth of that inspiration and expiratory stimulation increased the duration of expiration; and to those of Howard, Bromberger-Barnea, Fitzgerald & Bane (1969) relating to CSN stimulation in the cat. There are, however, some differences between the latter author's findings and the present study in that they found that stimulation early in inspiration decreased the tidal volume. Such an inhibition of inspiration could have been due to baroceptor inhibition (see discussion below), but probably more important is that fact that their animals were breathing spontaneously with intact vagi. Since inhibition of inspiration was found in the present study only in the spontaneously breathing cats with intact vagi and was abolished by vagal section, it would appear to be a vagal effect and not an intrinsic part of the CSN-medullary-phrenic reflex.

Electrical stimulation of the carotid sinus nerve inevitably activated baroreceptor fibres along with those from chemoreceptors. The blood pressure changes, however, were small when present at all and were quite delayed in relation to the effects on respiration. Since it has been shown that the anaesthetic used in this study, chloralose, inhibits the vasomotor centre response to baroceptor stimulation, the small pressor response seen probably resulted from the activation of chemoreceptor afferents (Neil, Redwood & Schweitzer, 1949). With this consideration and since stimulation at the appropriate time always led to increased phrenic discharge, it is unlikely that baroceptor inhibitory effects on respiration were of great importance. In this connexion, Biscoe & Sampson (1970) reported that single shocks to the carotid sinus nerve caused, after a latency of 5–10 msec, a brief (20–40 msec) depression of phrenic activity that they attributed to baroceptor stimulation. Higher intensity stimuli or a brief tetanus evoked bursts of phrenic action potentials, which were attributed to chemoreceptor fibre stimulation. In the present study such phrenic inhibition was occasionally seen after the first or second shock of the usual train of 12, but excitatory effects were always more prominent. One possible explanation is that the chloralose anaesthesia reduced the effectiveness of baroreceptor stimulation. Another is the possibility that the stimulus intensities were high enough to obscure baroreceptor inhibition, a finding noted by Biscoe & Sampson in some of their experiments.

Although it might be argued that electrical stimulation of the CSN is not physiological, it would appear to be a valid way of determining the effect of chemoreceptor input on respiration. Studies in which chemical stimulation of the chemoreceptors has been used show similar results (Black & Torrance, 1967; Bernards & Sistermans, 1969; Band *et al.* 1970). Similarly, Dutton, Hodson, Davies & Chernick (1967) noted that a step change of

P_{CO_2} at the carotid body in the dog was more likely to be accompanied by a prompt ventilatory response if it arrived during inspiration rather than expiration. Finally, an associated study (Eldridge, 1972) in which precise measurements of chemoreceptor discharge and phrenic activity were made following brief chemical injections into the ascending aorta, demonstrates phase relationships between stimulus and phrenic response which are almost the same as with electrical stimulation.

Although it has been suggested that some of the effects of a step change in chemical stimulation of the carotid body with CO_2 are local and related to an overshoot in neural discharge following sudden stimulation (Black, McCloskey & Torrance, 1966), this and the other studies in which electrical stimulation has been used indicate that the findings are due largely to the handling of CSN impulses in the medullary control system. Since the phrenic response lasts only about as long as the stimulus and an excitatory response occurs even in expiration, it is probable that the CSN impulses have largely a depolarizing effect on the inspiratory neurones regardless of the phase of the respiratory cycle. They appear to add to depolarizing influences already acting on the neurones and thus have a significant effect during inspiration when the neurones are already depolarized and firing, but little effect during expiration when they are more polarized and are quiescent. The differences in response at various times in the respiratory cycle would thus appear to be related not to the carotid body chemoreceptors but to the excitatory state of the central inspiratory neurone pool. In respiratory terms the response of ventilation or phrenic activity to the CSN stimulus is quite rapid. In neurophysiological terms, however, the latency of 30 msec is relatively long. This would suggest that the CSN impulses pass through a multisynaptic neuronal pathway before they reach the final medullary inspiratory neurones which in turn activate the phrenic motoneurones.

While the study does not directly prove that oscillating signals from the carotid body affect respiration differently from non-oscillating signals, it does demonstrate a mechanism whereby this might occur. Since the central control neurones apparently have the property of rectifying afferent information and using primarily that which is arriving during inspiration, only part of that information will affect their discharge. If the level of afferent information is itself fluctuating, it is apparent then that the mean level of the information coming from the carotid body will be different from that which is used in respiratory control. In light of this consideration, the ability of CSN signals to influence durations of both halves of the respiratory cycle and therefore rate becomes more significant. It may be that the peak of an oscillating signal tends to entrain respiratory rate thereby assuring an appropriate relationship between the peak signal and

inspiration. It is of interest that there is often a whole number relationship between heart rate and respiratory rate that is intensified by manoeuvres which increase carotid body chemoreceptor output and eliminated by bilateral carotid sinus nerve section (Weiss & Salzano, 1970). The timing of the arrival at the carotid body of the chemical stimulus, whose value is oscillating because of breathing, is a function of lung-to-carotid body circulation time. That time, in turn, is a function not only of velocity of blood flow but, since the heart is ejecting intermittently, also of heart rate. It therefore seems likely that the association of heart and respiratory rates is at least partly related to entrainment of respiration by the changing carotid body stimulation whose precise timing is affected by heart rate.

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