Phase resetting of the respiratory cycle before and after unilateral pontine lesion in cat

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OKU, YOSHITAKA, AND THOMAS E. DICK. *Phase resetting of the respiratory cycle before and after unilateral pontine lesion in cat.* J. Appl. Physiol. 72(2): 721–730, 1992.—The pontine respiratory group (PRG) facilitates the mechanism for terminating the inspiratory phase but may influence other phases in the respiratory cycle as well. We determined the effects of PRG lesions on the response of the respiratory cycle to superior laryngeal nerve stimulation delivered in each phase of the cycle in decerebrate, vagotomized, paralyzed, and ventilated cats ($n = 6$). We measured the duration of inspiration (TI) and expiration (TE) for three breaths before and in the perturbed breath and TI for three breaths after the perturbation. The delay to next inspiration was plotted against the phase at which the stimulus was delivered. Before lesioning, premature inspiratory termination was followed by phase-dependent shortening of TE. After lesioning, premature inspiratory termination did not systematically change the following TE. Breath-by-breath variability (measured 50 breaths) increased and stimulus aftereffects (prolonged TI in the subsequent cycle) were augmented following lesions. These data indicate that the PRG plays an important role in the control of TE after perturbation and in the stability of the respiratory central pattern generator.

central pattern generator; control of respiration; Kölliker-Fuse nucleus; parabrachial nuclei; pontine respiratory group; superior laryngeal nerve; breathing stability

THE CENTRAL RESPIRATORY pattern generator (CPG) is located in the brain stem. The structure of this CPG is undefined, but in a neurophysiological model of this generator, medullary neurons determine timing and amplitude of the cycle with pontine and vagal neurons exciting the cells that terminate inspiration (24). This model explains the following observations made after separation of the rostral pons from the medulla: *1*) rhythmic breathing (21), *2*) an increase in the lung volume that terminates inspiration (8), and *3*) apneustic breathing after transection of the vagi or when lung inflation is withheld (21). Classically, these phenomena have been explained by the off-switch theory in which inspiration is terminated when the inspiratory off-switch reaches threshold (2, 5). Apneusis results because inspiratory activity is saturated before the off-switch neurons reach threshold. This inspiratory off-switch has been modeled to receive excitatory input from both the pontine respiratory group (PRG) and vagal afferent nerves innervating pulmonary stretch receptors (2, 24). Therefore the PRG facilitates phase transition between inspiration and expiration (I-E transition), and elimination of this input would delay I-E transition by disfacilitating the off-switch.

In addition to the specific role in I-E transition, the PRG may affect other phases in the respiratory cycle, e.g., transition between expiration and inspiration (E-I transition) for the adjustment of the duration of expiration according to the duration and magnitude of the preceding inspiratory discharge (7, 12). We hypothesize that the PRG is a part of an integrated dynamic oscillator in the sense that it has effects throughout the respiratory cycle. To test this hypothesis, we examined the effects of lesions in the PRG on the response of the respiratory cycle to perturbation stimuli throughout the cycle.

Resetting is defined by the shift in phases between that predicted from unperturbed cycles and that occurring after the perturbation stimulus. The shift in phases depends on the strength of the perturbing stimulus and the phase of the cycle when the stimulus is delivered. Phase-resetting curves (PRCs) plot the shift in phases against the phase of the cycle when the perturbing stimulus was delivered. Cophase plots display the delay to next inspiration instead of the shift in phases. Both of these curves show the phase-dependency of the effect of perturbation that characterizes the oscillator. A transition period may be necessary for the cycle to return to its steady state. Stimulus aftereffects are recorded during this period. The duration of this period, the relaxation time, also characterizes the oscillator.

The purpose of the present study was twofold: to examine these phase-resetting characteristics before and after unilateral pontine lesion and to assess the role of the PRG in respiratory pattern generation using this analytic approach. If the PRG is a part of a dynamic oscillator and does more than provide facilitatory input, then qualitative changes should appear in the overall breathing pattern, stimulus aftereffects, and PRCs after PRG lesioning.

METHODS

Surgical procedures. Adult cats (2.2–3.5 kg, $n = 6$) of either sex were sedated with ketamine (10 mg/kg im) and anesthetized with methoxyflurane (2.8–3.0% in O_2 to induce anesthesia, 0.5–1.5% in O_2 to maintain anesthesia). A femoral artery and vein were cannulated to record blood pressure and to infuse fluids and drugs, respectively. The urethra was cannulated to collect urine and to monitor urine formation. Body temperature was re-

corded with an esophageal thermistor and was kept within the range of 37.0–38.0°C by means of a heating blanket during surgery and infrared heat lamps during the experiment. The trachea was cannulated for ventilation.

Animals were decerebrated using the technique of Kirsten and St. John (16). External carotid arteries were ligated caudal to the lingual arteries bilaterally. A craniotomy was formed in parietal bones. The brain stem was transected at midcollicular ($n = 4$) or precollicular level ($n = 2$), and nervous tissue rostal to the transection was aspirated. The cerebellum remained intact.

Animals were placed in a stereotaxic apparatus. The head and the dorsal spinal processes of the seventh cervical and third or fourth lumbar vertebrae were fixed. Lower extremities were elevated during experiments if needed to maintain mean blood pressure above 80 mmHg. End-tidal CO_2 was measured with an infrared analyzer (Beckman LB-2) and was maintained between 4.5 and 5.0%. Exposed nervous and muscle tissues were covered with 3.0% agar solution to prevent evaporation. Right cervical phrenic (C_5 root) and right or left superior laryngeal nerve (SLN) were isolated, partially desheathed, placed on bipolar electrodes (Ag), and covered with petroleum jelly. Vagi were transected bilaterally. Animals were paralyzed with gallamine triethiodide (flaxedil, 5 mg/kg iv, with supplemental doses as required) and were ventilated with a volume pump.

Experimental protocol. Phrenic nerve activity was recorded (100–10,000 Hz) to monitor the respiratory cycle. The proximal end of the SLN was stimulated at various phases during the respiratory cycle (≥10% of the cycle, more at E-I transition). The following stimulus parameters were kept constant: pulse duration, 0.2 ms; train duration, 500 ms; and frequency, 100 Hz. Results obtained from a long train duration (500 ms) were compared with those obtained from a short train duration (50 ms) in two animals. Three stimulus currents were used. Initially, we used the minimal stimulus strength that terminated inspiration in early inspiration (old phase =0.1). Then we decreased the current below the threshold for terminating inspiration in late inspiration. Finally, current was increased for terminating inspiration at the onset of inspiration. Each stimulus was preceded by five control breaths. Stimulus pulse train and phrenic nerve signal were amplified, and resistance-capacitance was integrated (time constant 200 ms), sampled with analog-to-digital converter (Data Translation, DT-2801-A), and stored on a Bernoulli cartridge for the subsequent off-line data analysis.

The stimulus protocol was repeated before and after lesioning the PRG, medial parabrachial-Kölliker-Fuse nuclear complex. We targeted the Kölliker-Fuse nucleus with Horsley and Clarke stereotaxic coordinates: P 4.0, L 5.0, H −3.0 mm. A tungsten microelectrode was placed at those coordinates through an occipital craniotomy at a 45° angle to avoid the bony tentorium. The target area was mapped by delivering cathodal stimulus pulses (1-ms pulse duration, 10-Hz frequency) until we found a location where a short-latency inhibition followed by an excitation of phrenic nerve activity was observed with a stimulus <50 μA. An electrolytic lesion (DC 10 mA, 10-s duration) was made at this location. If inspiratory time was not lengthened, we repeated the lesions at this location and at 1 mm dorsal and ventral to the original site. We stopped lesioning when we observed an inspiratory plateau on integrated phrenic nerve activity, and then we repeated the stimulation protocol if the pattern stabilized and had a near periodic behavior.

Data analysis (*Fig. 1*). The duration of inspiration (TI) was measured from the onset of phrenic nerve activity to its offset, and the duration of expiration (TE) was measured from offset to onset of its next burst of activity. The variability of these measurements was calculated for 50 breaths before and after PRG lesions.

We determined changes in stimulus aftereffects, i.e., effects of the stimulus on succeeding breaths before and after lesioning the PRG. TI was used as an index of stimulus aftereffects. We measured TI in three breaths before the perturbation stimulus, in the perturbed breath, and in three breaths after the perturbed breath. These variables were measured before and after PRG lesions.

We used the same terminology to describe phase-resetting properties of the oscillator as previous investigators (19, 22) used. As shown in Fig. 1, old phase was the time from the onset of inspiration to the onset of the stimulus, whereas cophase was the time from the offset of the stimulus to the onset of each rescheduled breath. Both old phase and cophase were normalized by dividing these phases by the average period of control breaths ($n = 3$) preceding the stimulus. Cophase plots were constructed by plotting cophase against old phase. We constructed PRCs in which phase difference between the perturbed and unperturbed breaths was plotted against old phase. By definition, if the onset of the first rescheduled breath occurred during the stimulus, then this event was missed in cophase plots but was identified in PRC plots. These plots represent the phase-dependent resetting characteristics throughout the cycle.

Two types of resetting have been observed from a cophase plot. *Type 1* resetting (weak perturbation) occurs when cophase has a net change of one cycle of old phase. *Type 0* resetting (strong perturbation) occurs when cophase has a net change of zero as old phase is varied through one full cycle. If the perturbation is sufficiently small, then the time at which the next inspiration starts is barely different from that predicted without perturbation. Then, cophase ≈1 − old phase, and the average slope of cophase plots would be −1. This is *type 1* resetting. If the stimulus is strong enough that the rhythm is reset by a certain degree independent of when the stimulus is delivered, then the average slope of cophase plots would be 0. This is *type 0* resetting. This classification represents the global or average effect of perturbation on the oscillator throughout the cycle and thus can be used as an index of the effectiveness of a specific perturbation on the oscillator regardless of phase-dependent resetting characteristics.

Statistical analysis was performed with paired or unpaired t tests and analysis of variance depending on whether the data of the two groups compared were related or not and whether population variances were equal or not. The equality of population distribution and vari-

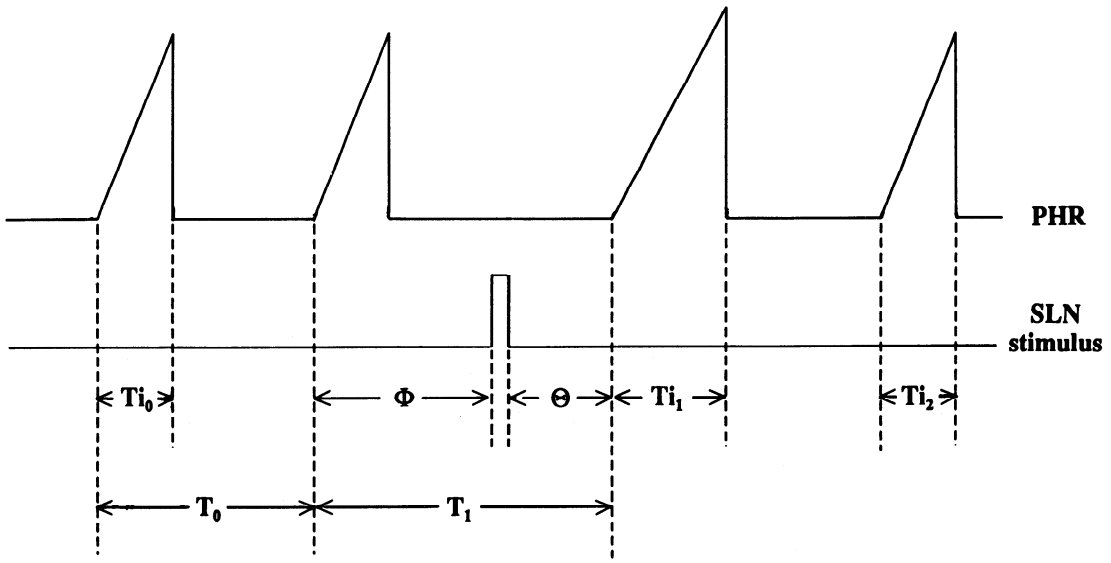

FIG. 1. Schematic of terms used in text. *Top*: continuous tracing, integrated phrenic nerve activity; *bottom*: continuous tracing, stimulus signal. Tracing shows control breath, perturbed breath with perturbation stimulus delivered in late expiration, transition period, and cycle returning to control pattern. Stimulus aftereffects are estimated from changes in duration of inspiration (TI) which was measured from onset of phrenic nerve activity to its offset. We compared TI in 1st (Ti_1) and 2nd (Ti_2) breath after perturbation to average of 3 control breaths (T_0). To generate cophase plots, Θ was normalized by control breath T_0 and plotted against the old phase, Φ.

ances was assessed by χ^2 test and F test, respectively. Probabilities <0.05 were taken as significant.

RESULTS

Breathing pattern and perturbation (electrical stimulation of SLN) of that pattern are shown for an animal with an intact pons in Fig. 2. In these three examples, TI and TE are regular and the breathing pattern appears stable. Consequently, the variability of TI and TE was low (Table 1). The perturbing stimulus had phase-dependent effects on the respiratory cycle (Fig. 2). The strength of this stimulus was less than the minimal stimulus current that terminated inspiration at the onset of inspiration. The onset of the next cycle was delayed when the stimulus was delivered during expiration (Fig. 2*A*). Inspiratory activity was inhibited transiently when the stimulus was delivered at early inspiration (Fig. 2*B*, reversible I inhibition). TI was prolonged in the perturbed breath but returned to the steady-state value in the subsequent breath. Inspiration was terminated immediately when the stimulus was delivered slightly later during inspiration, and the following TE could be altered depending on the timing and strength of the stimulus (Fig. 2*C*, premature I termination or irreversible I inhibition). Again, there was no apparent effect on TI in the subsequent breath. As stimulus current increased, the transition phase between reversible and irreversible responses shifted toward the E-I transition, and eventually, in five of six animals, only I termination was observed. However, in one animal reversible I inhibition remained at the E-I transition even with stimulus current increased maximally (10 mA).

Unilateral and partial lesions of the PRG affected both the breathing pattern and the response to perturbations of the respiratory cycle (Fig. 3). Lesions prolonged TI but had small variable effects on TE (Fig. 3, Table 1). Increases in TI (n = 6) were associated with a plateau at peak inspiratory activity. Variability in TI also increased in five of six animals (F test, Fig. 3, Table 1). However, effects of the lesion on TE were inconsistent because TE shortened (n = 4), lengthened (n = 1), or did not change (n = 1). Variability of TE was not significantly different from that with intact PRG in five of six animals (F test, Table 1).

Figure 3 shows the effects of SLN stimulation with an intermediate stimulus strength on the respiratory cycle after partial PRG lesion. When the stimulus was deliv-

TABLE 1. *Changes in respiratory parameters before and after PRG lesion*

	Before Lesion			After Lesion		
Cat No.	TI	TE	TT	TI	TE	TT
1	1.74±0.04	2.33±0.06	4.07±0.07	2.20±0.06	2.27±0.08	5.47±0.13
2	1.24±0.06	2.89±0.08	4.13±0.13	2.72±0.28	2.46±0.12	5.18±0.35
3	0.91±0.02	2.65±0.05	3.57±0.05	2.08±0.13	1.87±0.06	3.96±0.17
4	1.91±0.06	2.03±0.06	3.94±0.10	2.74±0.27	1.82±0.08	4.55±0.26
5	1.12±0.03	3.21±0.06	4.33±0.07	3.82±0.35	3.07±0.06	6.89±0.40
6	1.28±0.03	2.32±0.03	3.60±0.03	2.50±0.12	3.30±0.18	5.80±0.21

Values are means ± SD in s. PRG, pontine respiratory group; TI and TE, inspiratory and expiratory durations, respectively; TT, total cycle duration.

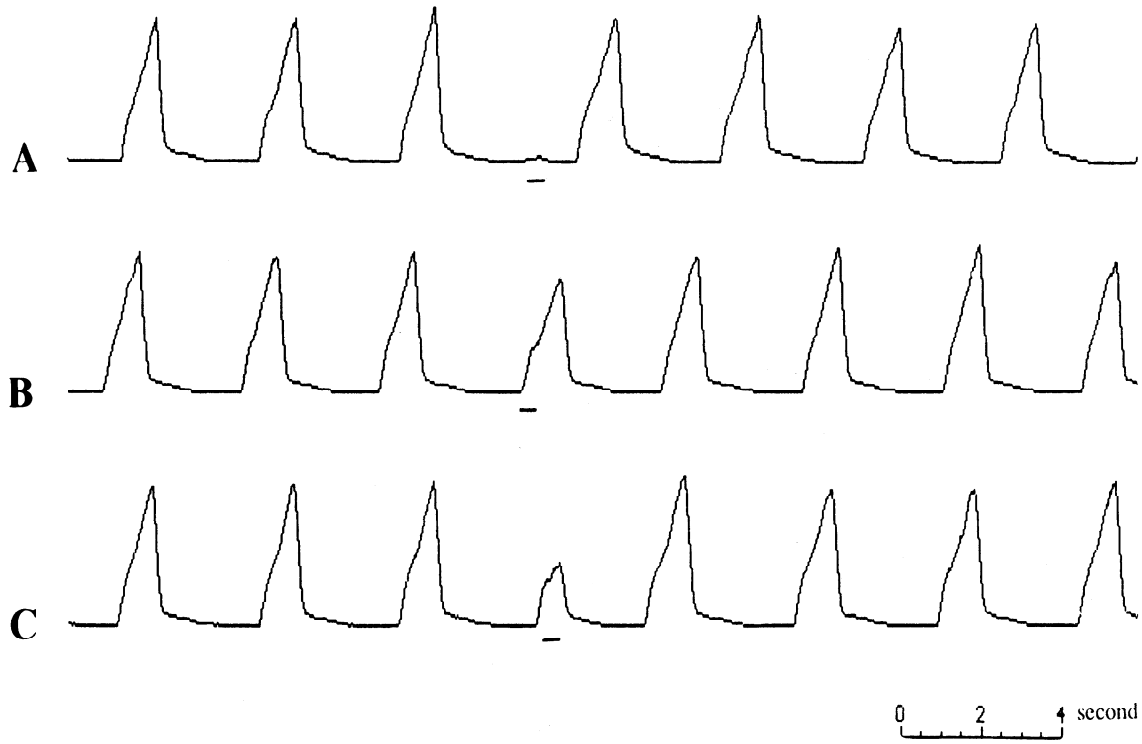

FIG. 2. Effect of electrical stimulation of superior laryngeal nerve (SLN) on respiratory cycle in animal with intact pons. In each panel, trace is integrated phrenic nerve activity, and stimulus trains are indicated by solid line below this trace. Stimulus strength (500-ms train duration, 0.2-ms pulse duration, 100 Hz, and 10 μA) was just below threshold that prematurely terminated inspiration at onset of I phase. *A*: expiratory duration (TE) prolongation. *B*: reversible I inhibition. *C*: premature I termination. Stimuli were delivered at 0.94 (*A*), 0.02 (*B*), and 0.05 (*C*) of old phase; I-E transition is 0.35 of old phase, and normalized pulse train duration is 0.13.

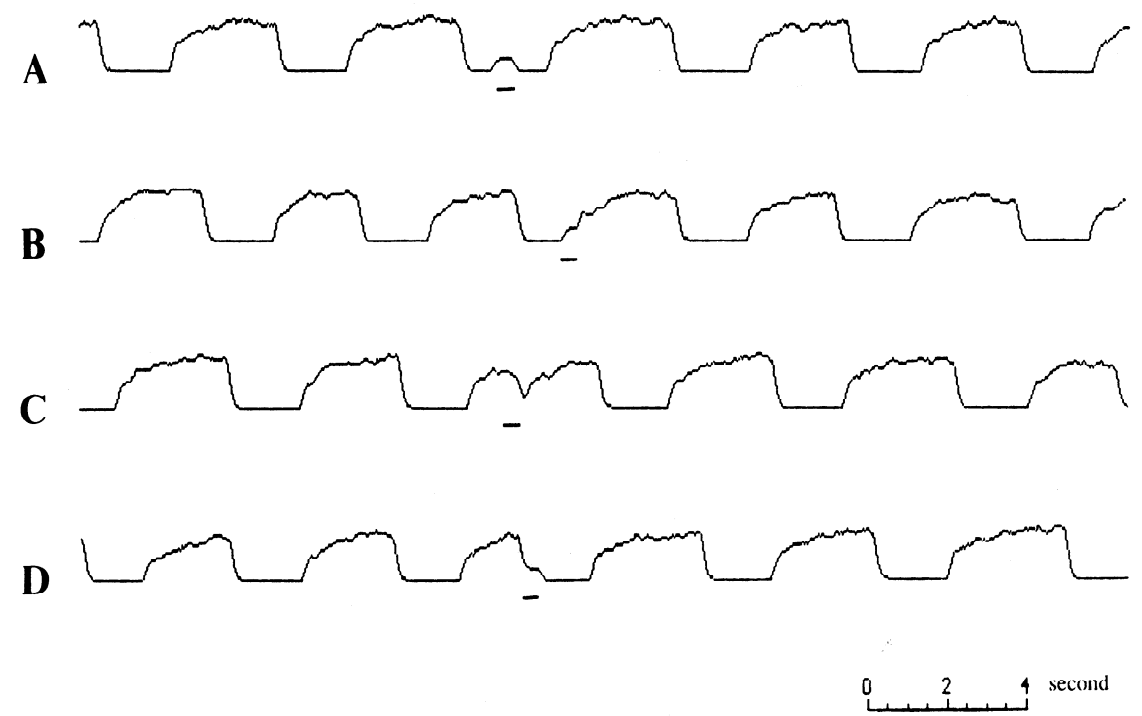

FIG. 3. Effect of electrical stimulation of SLN on respiratory cycle in same animal as Fig. 2 but with unilaterally and partially lesioned PRG. Traces are same as Fig. 2, and stimulus (500-ms train duration, 0.2-ms pulse duration, 100 Hz, and 1 mA) was increased to be just below threshold that prematurely terminated inspiration at onset of I phase. *A*: TE prolongation followed by TI prolongation. *B*: I facilitation followed by TI prolongation. This occurred in two of six animals after lesioning. *C*: reversible I inhibition. *D*: premature I termination. First small humps corresponding to stimuli on phrenic nerve activity are stimulus artifacts. Stimuli were delivered at 0.69 (*A*), 0.80 (*B*), 0.17 (*C*), and 0.24 (*D*) of old phase; I-E transition is 0.60 of old phase, and normalized pulse train duration is 0.14.

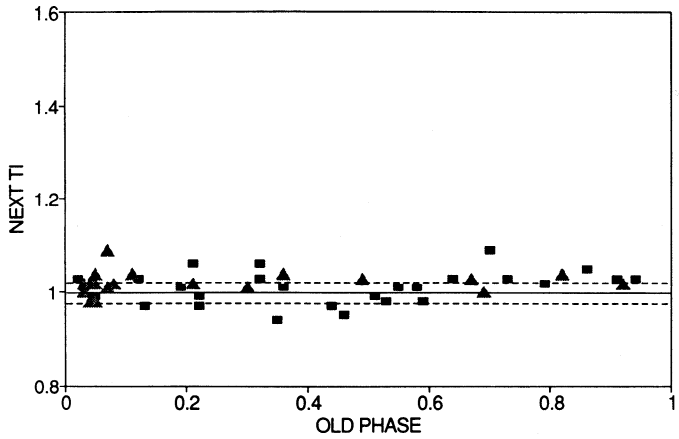

FIG. 4. Effects of SLN stimulation [50-ms train duration, 0.2-ms pulse duration, 100 Hz, and 0.1 mA (△) and 0.4 mA (■)] at different phases of cycle on TI in breath immediately after perturbed breath in animal with intact pons. TI is normalized to 3 previous control breaths. Dotted lines show 99% confidence levels. Perturbation stimuli had significant but small effect (<10%) on TI of breath after perturbation.

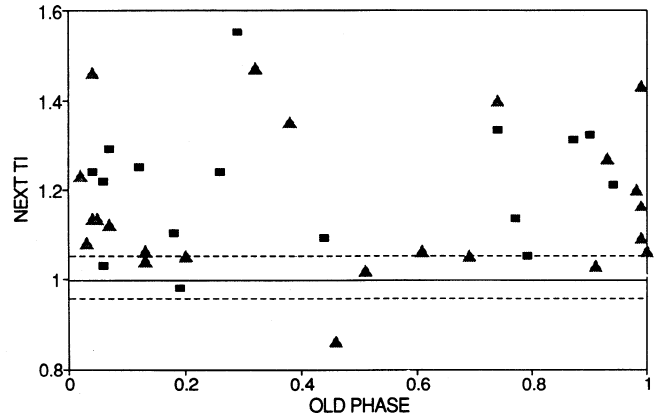

FIG. 5. Effects of SLN stimulation [50-ms train duration, 0.2-ms pulse duration, 100 Hz, and 0.2 mA (△) and 0.4 mA (■)] at different phases of cycle on TI in breath immediately after perturbed breath in same animal as Fig. 4 with unilateral and partial PRG lesion. TI is normalized to 3 previous control breaths. Dotted lines show 99% confidence levels. TI was prolonged phase dependent.

TABLE 2. *Changes in off-switch threshold before and after PRG lesion*

	Superior Laryngeal Nerve, mA	
Cat No.	Before lesion	After lesion
1	0.015	0.5
2	0.002	0.007
3	0.02	6
4	0.02	1
5	0.012	0.2
6	0.02	0.06

Values are means ± SD.

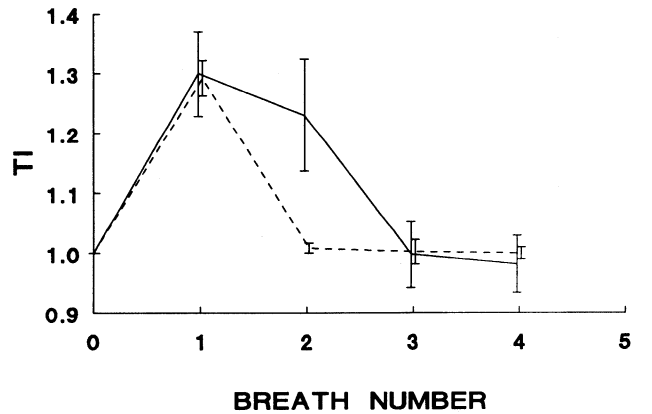

FIG. 6. Transient changes in TI after perturbation stimulus at early I (old phase <0.1) before and after lesioning PRG. TI is plotted against breaths. TI is normalized to 3 previous control breaths (*breath 0*). Perturbation stimulus, strength of which is below threshold for premature I termination (0.4 mA, 50-ms train duration), is delivered in *breath 1*. Perturbation stimulus current decreased phrenic nerve activity transiently, prolonging *breath 1* both before (dashed line) and after (solid line) lesioning PRG. Stimulus aftereffects are evident by increase in TI in *breath 2* after lesioning. Values are means ± SD of TI for 5 trials.

ered during early or midexpiration, the onset of the next cycle was delayed and the TI after the perturbed cycle was prolonged (Fig. 3*A*), a result similar to the one obtained in animals with intact pons. In two of six cats, inspiration was initiated when a stimulus was delivered at late expiration (Fig. 3*B*). This phenomenon was not observed in animals with intact pons. Inspiratory activity was inhibited transiently and TI was prolonged when a stimulus was delivered during early inspiration (Fig. 3*C*, reversible I inhibition). When a stimulus was delivered later during inspiration, inspiratory activity was terminated (Fig. 3*D*, premature I termination). Stimulus aftereffects were evident in subsequent breaths. Both irreversible and reversible I termination were followed by a prolonged inspiratory period in the next cycle (Fig. 3, *C* and *D*). As stimulus current increased, the transition phase between these two responses shifted toward the E-I transition, again a result similar to animals with intact pons. However, the minimal stimulus strength required to terminate inspiration in early I phase (old phase 0.01) increased after PRG lesions (Table 2). This increase in threshold was highly variable (range from 3 to 300 times).

We assessed stimulus aftereffects to perturbation stimuli that were short (train duration of 50 instead of 500 ms) and at the same current (0.4 mA) before and after lesioning. We compared TI from the breath immediately after the perturbed breath with that before the perturbation (Figs. 4 and 5) and plotted TI for the breaths after a perturbation stimulus that caused reversible I inhibition when delivered in early I phase (Fig. 6). In an animal with an intact pons, perturbation stimuli had a significant ($P < 0.01$, χ^2 test) but small effect (<10%) on the TI of the breath after the perturbation (Figs. 4 and 6). However, after PRG lesions, the TI was prolonged in the breath immediately after the perturbation stimulus (Figs. 5 and 6). This TI prolongation occurred even with a short train (50 ms) of pulses (Figs. 5 and 6) and was dependent on the phase at which the perturbing stimulus was delivered (Fig. 5). Prolongation of the subsequent TI was greatest if stimuli were delivered in mid-TI (Fig. 5). TI returned to the control level within two cycles after perturbations (Fig. 6). With a longer stimulus-pulse train (500 ms), the effects of the perturbation lasted additional cycles.

We constructed cophase plots to examine the phase dependency of the response to the perturbing stimulus

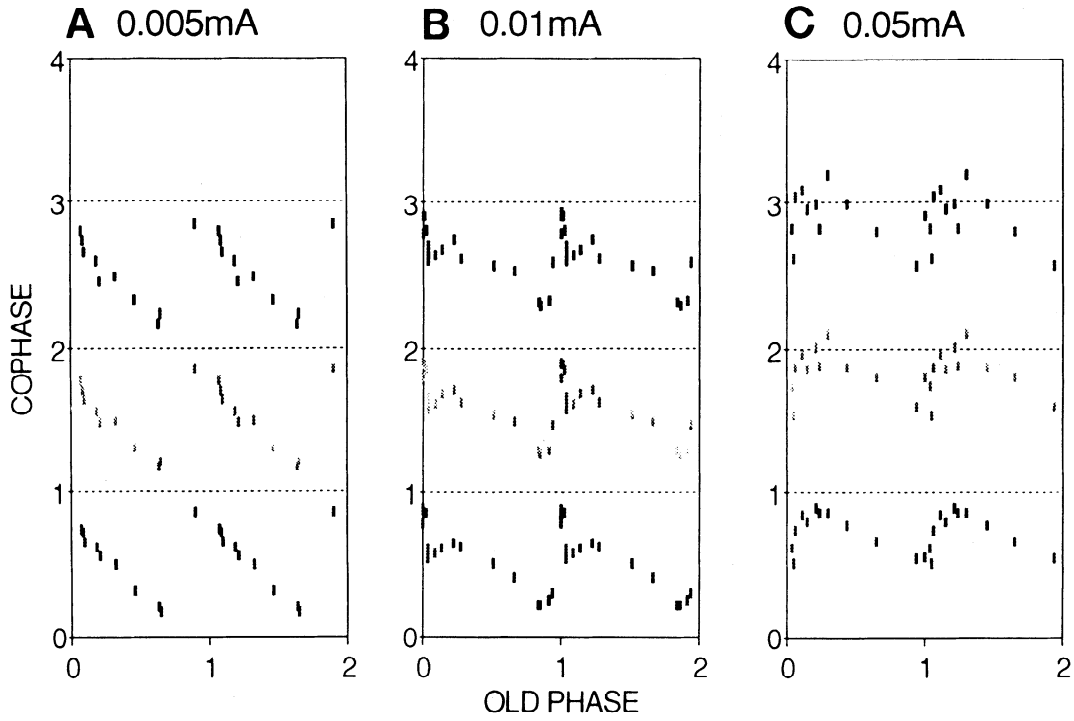

FIG. 7. Effect of SLN stimulation (500-ms train duration, 0.2-ms pulse duration, and 100 Hz), with stimulus current increasing from *A* to *C*, delivered at various phases on respiratory cycle in animal with intact pons. First, second, and third cophases are plotted twice against old phase. I-E transition is 0.35 of old phase.

before (Fig. 7) and after (Fig. 8) PRG lesion. First, second, and third cophases were plotted twice against old phase to form first, second, and third cophase plots (Figs. 7 and 8). If we assume that cophase plots in Fig. 7, *A* and *C*, are continuous, the resetting with weak current (Fig. 7*A*) is classified as *type 1* and the resetting with strong current (Fig. 7*C*) is classified as *type 0*. Figure 7*B* shows a resetting pattern obtained with a current of an intermediate strength. Because of a discontinuity at an E-I transition (1.0 in old phase), cophase plots cannot be classified as either *type 0* or *type 1* resetting. In the second and third cophase plots, points span this discontinuity. With strong perturbation stimuli (*type 0* resetting), perturbations adjacent to the E-I transition produced the greatest

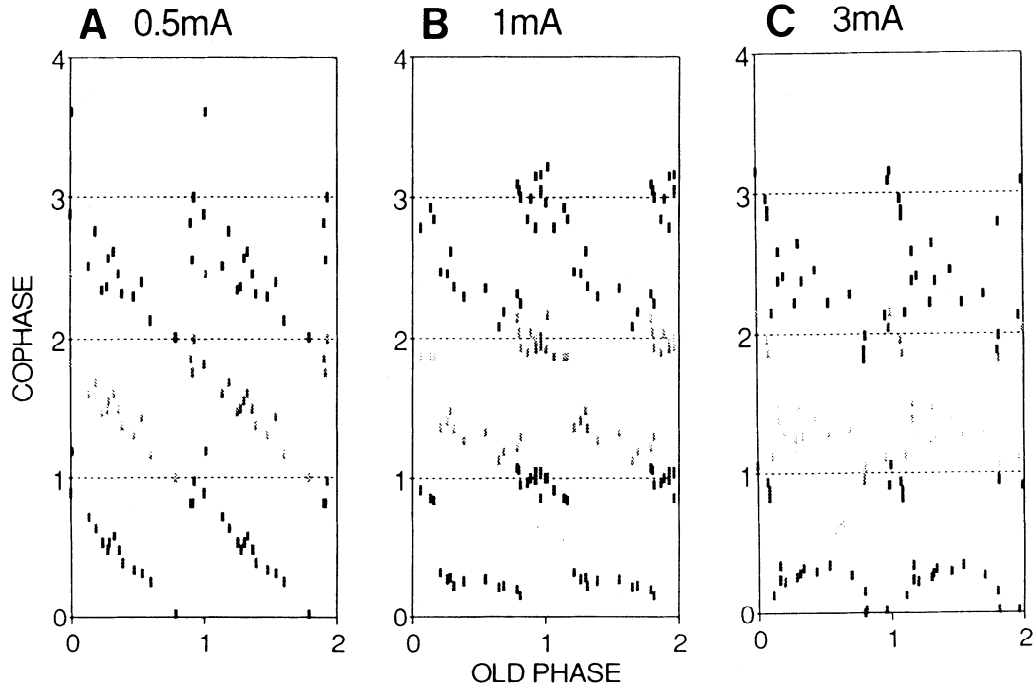

FIG. 8. Cophase plots for SLN stimulation (500-ms train duration, 0.2-ms pulse duration, and 100 Hz), with stimulus current increasing from *A* to *C*, delivered at various phases on respiratory cycle in same animal as in Fig. 4 but with unilaterally and partially lesioned PRG. I-E transition is 0.6 of old phase.

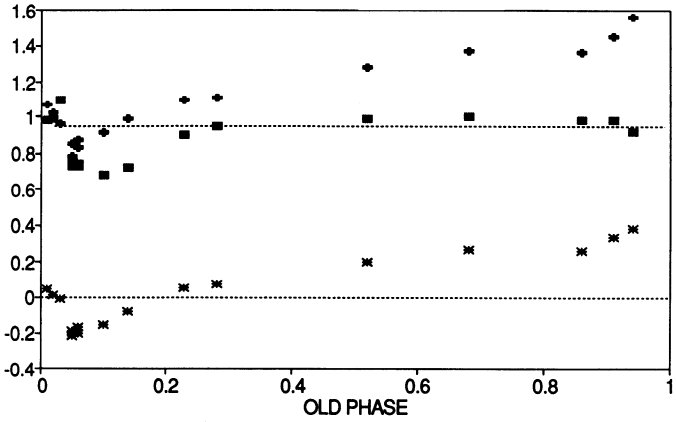

FIG. 9. Phase-aligned plots of phase shift (*), TI (■), and TE (+) in perturbed breath for SLN stimulation (500-ms train duration, 0.2-ms pulse duration, 100 Hz, and 10 μA) that was just below threshold that prematurely terminated inspiration at outset of I phase in animal with intact pons. Discontinuity was apparent in early inspiration (0.04 of old phase). There was no discontinuity at I-E transition (0.35 of old phase).

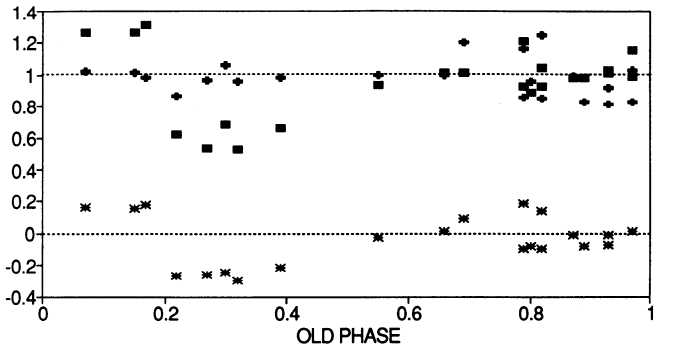

FIG. 10. Phase-aligned plots of phase shift (*), TI (■), and TE (+) in perturbed breath for SLN stimulation (500-ms train duration, 0.2-ms pulse duration, 100 Hz, and 1 mA) that was just below hreshold that prematurely terminated inspiration at onset of I phase in same animal as Fig. 9 but with unilaterally and partially lesioned PRG. Discontinuity that occurred in early inspiration remained (0.2 of old phase, shifted to right because of prolongation of TI). An additional discontinuity was observed at late E phase because of facilitation of I phase. I-E transition is 0.6 of old phase.

phase shift, whereas perturbations adjacent to the I-E transition showed the least phase shift. In other words, E-I transition was most and I-E transition was least sensitive to perturbation. The E-I transition (late expiration to early inspiration) became insensitive with weak perturbation stimuli.

Cophase plots in Fig. 8 are from the same animal as in Fig. 7 but after unilaterally and partially lesioning the PRG. *Type 1* resetting was obtained with a weak current (Fig. 8*A*). Figure 8*C* appears to be almost *type 0* resetting in the first cophase plot, but reversible I termination remained at the E-I transition and caused a discontinuity at this region. Furthermore, increased variability in the respiratory cycle obfuscated the interpretation of the cophase plots. However, compared with Fig. 7*C*, cophase plots in Fig. 8*C* shifted down and flattened between old phase values of 0.2 and 1 and between 1.2 and 2. Figure 8*B* shows resetting pattern obtained with a current of an intermediate strength. Because a large discontinuity occurred at early I phase in cophase plots, it is not classified as either *type 0* or *type 1* resetting. In the second and third cophase plots, points appear to bridge this discontinuity. All cophase plots in Fig. 8*B* showed a similar downward shift during the same period in old phase as Fig. 8*C*.

The first PRC was plotted together with TI and TE in the perturbed breath to determine transient resetting characteristics (Figs. 9 and 10). By definition, PRC is phase shift plotted against old phase. Thus, PRC is affected by both TI and TE in the perturbed breath. In an animal with an intact pons, premature I termination was followed by shortened TE, which resulted in strong phase-dependent resetting (steep slope) in the PRC. A discontinuity was observed in the PRC at early inspiratory phase for a stimulation between those that showed *type 0* and *type 1* resetting (Fig. 9). A discontinuity was not apparent at the I-E transition (0.35 of old phase). After unilateral and partial PRG lesion, premature I termination did not alter systematically the following TE (paired *t* test). Consequently, PRC was less steep and cophase plots flattened during inspiration. A larger discontinuity in the PRC was seen at early I phase, and in two out of six cats, an additional discontinuity was observed at late E phase due to I facilitation (Fig. 10).

The most discrete lesion that provided these results is shown in Fig. 11. The lesion was restricted to the ventrolateral parabrachial nuclei including Kölliker-Fuse subnucleus. In the other animals, lesions included this subnucleus but were more extensive.

DISCUSSION

Integrated phrenic nerve activity was highly regular in decerebrate, vagotomized, and ventilated animals with an intact pons. Stimulus aftereffects were not evident as a prolongation of TI. TE was shorter after premature I termination. Variability of the breathing pattern, in particular, TI, increased after unilateral and partial lesion of PRG. This variability was associated with an apparent stimulus aftereffect, i.e., TI prolongation in the succeeding cycle. TE after premature I termination was not significantly different from the control value, which produced a flat cophase plot during inspiratory phase. The data from the intact animals support the work of Paydarfar et al. (22) and of Lewis et al. (19). Data from the lesioned animals indicate that the medullary rhythm generator remains intact but the characteristics of the respiratory pattern generator are altered. The results support the notion that the PRG is a part of a dynamic oscillator with influences throughout the respiratory cycle.

Two theoretical models have been proposed for the respiratory oscillation, the integrate-and-fire (IF) model and the limit-cycle oscillator (see Ref. 11 for review). These two models have emerged from completely different concepts. In IF models, a quantity rises to a threshold leading to an event (e.g., I-E switching). The off-switch theory is an example of an IF model. A limit-cycle oscillator was derived originally from the mathematical term representing a nonlinear dynamic behavior generated by a set of derivative equations. A limit cycle is defined as an oscillation that is reestablished after a small perturbation delivered at any phase of the oscillation (11). Only the simplest forms of these models are distinguishable by

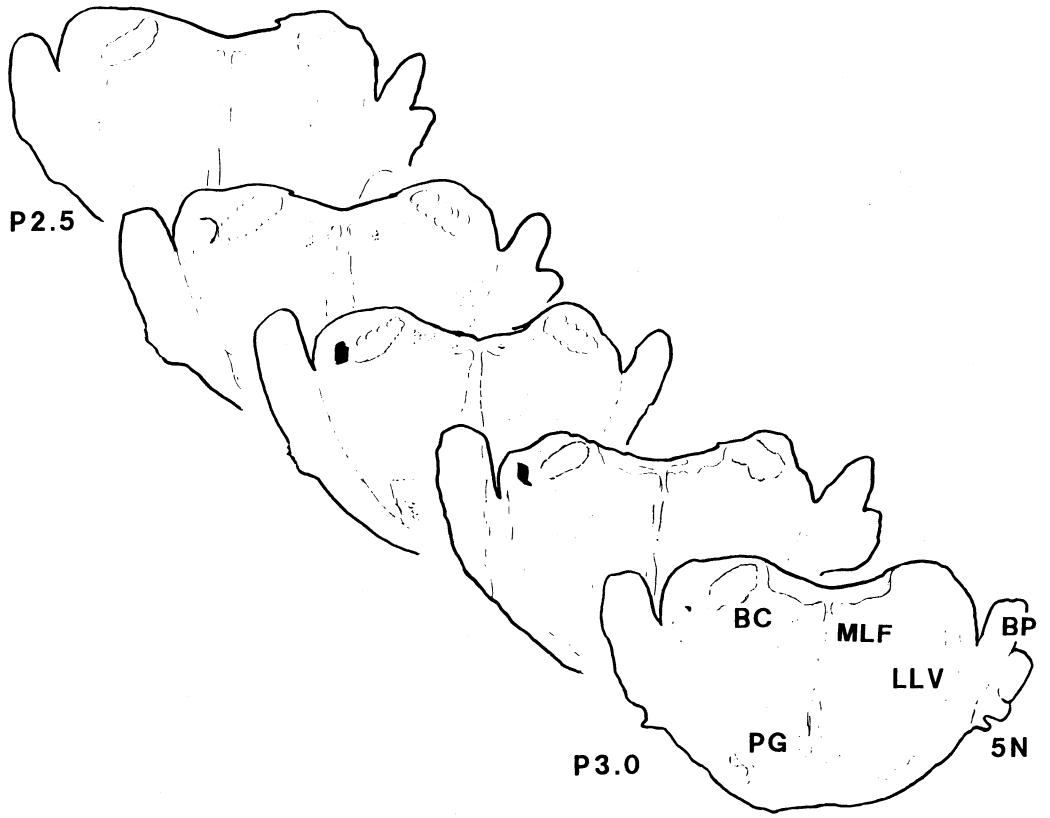

FIG. 11. Representative coronal sections (A/P levels: P2.5–P3.0) showing unilateral and partial lesion in PRG. This lesion (solid black area) generated data for Figs. 3, 5, and 8. BC, brachium conjunctivum; MLF, medial longitudinal fasciculus; LLV, ventral nucleus of lateral lemniscus; BP, brachium pontis; 5N, trigeminal nerve root; PG, pontine gray.

their phase-resetting characteristics, and it may be impossible to distinguish these two models in an experimental context. Indeed, IF models can be approximated by limit-cycle models in some cases (23). In this paper, we do not distinguish between these models. Rather, both modeling concepts can be used to interpret our results.

In a phase-resetting experiment, the perturbation produces a condition different from the steady state. The transient behavior of the oscillator returning to the steady state provides insights regarding the dynamics of the oscillator. But the influence of the perturbation may last longer than the duration of the stimulus if the perturbation is processed by an integrative mechanism. In this circumstance, intrinsic properties of the oscillator would be masked. Indeed, Younes and Polacheck (30, 31) reported that pontine and vagal inputs undergo a leaky integrative processing. In light of this conclusion, our results would be interpreted as the consequence of two factors: intrinsic properties of the oscillator and leaky integrator-type processing mechanisms.

Variability of the respiratory cycle. The measurable properties of an oscillatory system that can index the stability of a system are its cycle-to-cycle variability and its relaxation time, which is the time required for an oscillator to return to the limit cycle or to the steady state after a perturbation (27). Deterministic variability and stochastic noise are the two components in breath-by-breath variability. Deterministic variability could result from the interaction between central respiratory oscillator and various feedback loops, e.g., central and peripheral chemical feedbacks (3, 14) as well as vagal feedback (26). In modeling studies, irregular breathing has been attributed to unstable feedback controls (15, 26). In this study, these feedback controls were open, the vagi were cut, and animals were ventilated. Thus increased breath-to-breath variability after a lesion in the PRG is attributable to the instability of the respiratory oscillator itself. In our experiments, an enhanced stimulus aftereffect could be an increase in relaxation time. The association of an increase in relaxation time and an increase in variability is consistent with the behavior of other biological oscillatory systems (27).

An interpretation employing an IF model is that an increase in breath-by-breath variability and enhanced stimulus aftereffects is the result of changes in afferent mechanisms. For example, the following effects would be predicted if the time constant of the leaky integration of afferent inputs (29, 30) were prolonged after lesioning the PRG. First, the rate of rise in activity to reach the off-switch threshold would decrease, prolonging TI. Second, the rate of decline in activity would decrease, enhancing stimulus aftereffects. Third, the sensitivity of changes in phase duration to small changes in threshold or activity would increase as the rate of rise in activity near I-E transition decreases. Thus all our results, i.e., increased TI, enhanced stimulus aftereffects, and increased variability, can be explained by either a limit-cycle oscillator or an IF model.

The role of PRG in determining the stability of the breathing pattern is distinct from the role of the vagus

nerve. Transection of the vagi produces a more regular rhythm than that in vagi-intact animals (26). In addition, stimulation of either SLN or PRG elicits short-latency phrenic responses as well as I-E transitions, which differs from effects of vagal nerve stimulation (1, 6, 13). Therefore the neurophysiological models showing a common mode of action for the PRG and vagus nerve may be an oversimplification. However, as opposed to this speculation, Younes et al. (29) provided evidence that phase-timing responses to pontine and vagal inputs display both integrative and adaptive characteristics with similar time constants but different gains, suggesting that these two inputs share common processing pathways.

Stimulus aftereffects. A stimulus aftereffect expressed by changes in TI was reported but not apparent in animals with intact pons with stronger perturbation stimuli (19, 22). Lewis et al. (19) reported that the response to a second perturbation stimulus was affected by the preceding perturbation stimulus for as many as nine cycles in intact animals (19). This stimulus aftereffect could be related to changes in the oscillator, such as a disfacilitation of the off-switch, or related to changes in the preprocessing of afferent information, such as a persistent decrease in synaptic efficacy conveying afferent signals. Similarly, we found that a stimulus aftereffect before lesioning was significant but small (Fig. 4). In 10 of 19 trials before lesioning with stimuli at the threshold level (0.4 mA in Fig. 4), the TI of the following breath increased beyond the 99% confidence limit (>1.025), but in these cases the changes were <10% of TI. However, after lesioning, there was an apparent prolongation in TI (Figs. 5 and 6).

The additional effects after lesioning may be related to the greater stimulus current rather than the lesion itself. The threshold of the perturbation stimulus increased variably after lesioning. The definition of our threshold was the minimal stimulus strength required to terminate I prematurely at early inspiration (old phase 0.01). However, in two cases the increases in stimulus current were minimal, but all animals showed stimulus aftereffects at currents below threshold. Indeed, in the examples of stimulus aftereffects (Figs. 4–6), the stimulus current was the same before and after lesioning. We tested the effects of high stimulus currents (>2 mA) in two animals before lesioning. The only effect that we observed with an increase in stimulus current rather than with lesion was the advancement of the next inspiration with stimuli delivered in late E. Even this effect was episodic, with the most frequent observation being a prolongation of TE. Because we were able to dissociate stimulus aftereffects from absolute current ranges and because stimulus aftereffects were not apparent in animals with intact pons even with high stimulus currents, the change in stimulus threshold itself does not affect the interpretation of these data.

Phase-resetting characteristics. Cophase plots with intact PRG in our experiments are comparable with previous reports (17, 19, 22). Cophase plots showing *type 0* resetting seem to be shifted up with respect to other plots. This could be the result of using a relatively long stimulus. Kitano and Komatsu (17) suggested that a long stimulus train could terminate I and continue to act during the following E and thus prolong TE. This effect might shift up cophase plots of strong perturbation. Indeed, our cophase plots are most comparable with those of Paydarfar et al. (22), who also used a long stimulus (up to 2,000 ms). Cophase plots with lesioned PRG shifted down and flattened compared with those with intact PRG between old phase values of 0.2 and 1 and between 1.2 and 2. This is partially the consequence of prolonged TI and relatively unchanged TE.

In animals with intact PRG, TE after premature I termination shortened, which caused strong phase-dependent resetting characteristics during inspiration. After lesioning the PRG, TE after premature I termination was not significantly different from the control value, regardless of the phase during which the stimulus was delivered. This resulted in flat cophase plots (less phase-dependent resetting) during the phase of premature I termination. The results suggest that lesioning the PRG may alter the configuration of the respiratory pattern generator in controlling TE. This is consistent with the concepts previously proposed, i.e., that the PRG play an important role in the adjustment of TE according to the duration and magnitude of the preceding inspiratory discharge (7, 12).

Discontinuity. In our experiment, the first cophase plot and PRC were discontinuous for stimulations with intermediate strength (Figs. 7–10). In animals with intact pons, a discontinuity was observed at early I (between old phases 0 and 0.05) (Figs. 7 and 9). This was consistent with previous results (17, 19). In lesioned animals, the first cophase plot and the PRC showed large discontinuities (Figs. 8 and 10). In two of six animals with lesioned PRG, we observed in the PRC another discontinuity at late E when inspiration was initiated rather than delayed by the stimulus (Figs. 3 and 10). This discontinuity is not apparent in cophase plots (Fig. 8) because of TI prolongation after I facilitation.

The I-facilitatory effect of SLN stimulation was apparent only in those animals where the threshold for resetting by SLN stimulation increased >100-fold. This facilitation may be related to changes in the perturbation stimulus due to the types of afferent fibers activated and therefore indirectly related to changes occurring after the lesion. However, a similar phenomenon has been described by Fadiga et al. (9) and Romaniuk and Budzinska (25). Fadiga et al. reported that vagal inspiratory inhibition switched to excitation after rostropontine transection in the cat. Romaniuk and Budzinska found that an I-inhibiting vagal reflex changed to I-facilitatory after brain stem midline transection in rabbits. The underlying neurophysiological mechanism for this resetting is unknown.

Previous anatomic and physiological experiments indicate that partial lesions of the PRG could affect the response to SLN stimulation directly and preferentially at the E-I transition. First, SLN afferent fibers may project to the parabrachial area (4), although this is not supported by recent anatomic data (20). The PRG, especially the Kölliker-Fuse nucleus, has extensive projections to the medullary respiratory groups, including those regions that have predominantly expiratory activ-

ity, such as the Bötzinger cell complex and caudal ventral respiratory group in cats (28). Second, physiological data have indicated effects on TE by PRG lesions. Lung inflation during expiration prolongs the expiratory phase. This response is even greater after lesioning the PRG (18). Although the PRG has been known to affect TI and I-E transition (8, 10), there is support for our findings regarding the primary difference in the respiratory response to SLN stimulation occurring at the E-I transition.

In conclusion, we have demonstrated that lesioning the PRG increased variability in the respiratory cycle, enhanced stimulus aftereffects, and changed the phase-resetting characteristics. These results indicate that the PRG is part of a dynamic oscillator with influences throughout the cycle. In particular, the lateral PRG functions to incorporate afferent information in the generation of the breathing pattern. This finding is consistent with previous results (9). In addition to the recognized role of the PRG at the I-E transition, our data indicate that the lateral PRG has dramatic effects on the stability of the respiratory CPG and the control of TE after perturbation.

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