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Lateral parabrachial nucleus mediates shortening of expiration during hypoxia

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Abstract

Acute hypoxia elicits complex time-dependent responses including rapid augmentation of inspiratory drive, shortening of inspiratory and expiratory durations (T_I , T_E), and short-term potentiation and depression. The central pathways mediating these varied effects are largely unknown. Here, we show that the lateral parabrachial nucleus (LPBN) of the dorsolateral pons specifically mediates T_E -shortening during hypoxia and not other hypoxic response components. Twelve urethane-anesthetized and vagotomized adult Sprague-Dawley rats were exposed to 1-min poikilocapnic hypoxia before and after unilateral kainic acid or bilateral electrolytic lesioning of the LPBN. Bilateral lesions resulted in a significant increase in baseline T_E under hyperoxia. After unilateral or bilateral lesions, the decrease in T_E during hypoxia was markedly attenuated without appreciable changes in all other hypoxic response components. These findings add to the mounting evidence that the central processing of peripheral chemoafferent inputs is segregated into parallel integrator and differentiator (low-pass and high-pass filter) pathways that separately modulate inspiratory drive, T_I , T_E and resultant short-term potentiation and depression.

Keywords

Lateral parabrachial nucleus; hypoxia; respiratory control

1. Introduction

Abrupt exposure to low oxygen elicits a fast (<1 min) peripheral chemoreceptors-mediated increase in respiratory ventilation to a higher plateau for ~5 min (phase I) followed by a slow (5–20 min) hypoxic ventilatory decline or “roll-off” of the initial response (phase II) that is mediated by central (and perhaps also peripheral) mechanisms (Bisgard et al. 1995). This biphasic pattern has been extensively documented in humans (Cross et al. 1952; Weil et al. 1976; Easton et al. 1986; Duffin 2007; Steinback et al. 2007) and in many mammalian species (Woodrum et al. 1981; Long et al. 1984; Melton et al. 1988; Gershan et al. 1996; Vizek et al. 1998). Upon return to normoxia or hyperoxia (phase III), ventilation decreases rapidly then more slowly (~2 min) toward baseline levels, in what is known as respiratory afterdischarge

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or decay of short-term potentiation (STP) in humans and animal models (Eldridge et al. 1986; Georgopoulos et al. 1990).

During hypoxic ventilatory decline, all breathing pattern components (tidal volume, V_T ; respiratory frequency, f ; inspiratory time, T_I ; expiratory time, T_E) exhibit similar time-dependent changes that collectively decreases ventilation regardless of whether the hypoxia is maintained isocapnic or poikilocapnic (Steinback et al. 2007). Also, these depression effects are not mirrored in phase III as post-hypoxia short-term memory (Fig. 1A) suggesting that they are gated to the hypoxic input. Both these observations support the notion (Fig. 1B) that hypoxic ventilatory decline in all ventilatory components are mediated by common mechanisms that habituate the hypoxic stimulus (Poon et al. 2000a; Poon et al. 2006) – such as sensory hypoxic decline in the carotid body (Cummings et al. 2005) or synaptic depression in the medial and commissural nucleus tractus solitarius (NTS) (Zhou et al. 1997; Poon et al. 2000c; Bantikyan et al. 2009), a brainstem region which provides the first central synaptic relay to carotid chemoafferent inputs (Gozal et al. 2000).

Changes in breathing pattern during phase I and phase III of acute hypoxia are much more complex (Fig. 1A), part of which have been expounded in previous reviews (Powell et al. 1998; Poon et al. 2000a). In anesthetized animals, STP elicited by carotid sinus nerve stimulation (as a time-resolved proxy for the hypoxic stimulus) is manifested as an exponential (~seconds) augmentation of inspiratory drive in phase I comprised of both an increase in phrenic discharge amplitude (Wagner et al. 1991) and decrease of T_I (Poon et al. 1999), with subsequent afterdischarge (off-transients of STP) for both variables in phase III. In contrast, hypoxia or carotid sinus nerve stimulation elicits a rapid increase in f followed by short-term depression (STD, ~1 min) in phase I that is mirrored by subsequent poststimulus rebound decrease and slow recovery (~minutes) of f in phase III (Hayashi et al. 1993; Dick et al. 2000). The latter (off-transients of STD) is known as posthypoxia frequency decline (PHFD) and is due to an increase in T_E (Dick et al. 2000). Indeed, both the on- and off-transients of STD of respiratory frequency are ascribable to corresponding time-dependent changes in T_E (Poon et al. 2000b). Similar STD of expiration (as measured by increases in T_E) and STP of inspiration (as measured by increases in V_T and decreases in T_I) on timescales of minutes are also found in awake humans (Gardner 1980; Steinback et al. 2007).

Interestingly, it has been pointed out that the temporal patterns of respiratory STP and STD resemble those of a biphasic neural integrator and differentiator (in time domain), or equivalently, low-pass and high-pass filters (in frequency domain) that dynamically transform carotid chemoafferent inputs (Poon et al. 1999; Poon et al. 2000a; Poon et al. 2000b; Poon et al. 2006). In support of this notion, recent studies using time-resolved decomposition of the respiratory response to carotid sinus nerve stimulation revealed that the central relay of carotid chemoafferent traffic beyond the NTS is comprised of parallel integrator-differentiator pathways that separately modulate inspiratory drive, T_I and T_E (Young et al. 2003). Specifically, four parallel pathways projecting from NTS to the respiratory rhythm generator have been postulated to mediate the hypoxic respiratory response (Fig. 1B): 1) fast-slow integrator pathways that mediate the rapid and STP augmentation of inspiratory drive; 2) fast-slow integrator pathways that mediate the rapid and STP shortening of T_I ; 3) a slow differentiator pathway that mediates the STD prolongation of T_E ; and 4) a fast integrator pathway (time constant ~2 sec) that mediates the rapid shortening of T_E . Similar integrator-differentiator pathways also account for the off-transients of all these response components (Fig. 1b).

There is evidence that the STD pathway (pathway 3) is mediated by certain expiratory neurons in the A5 region of the ventrolateral pons (Coles et al. 1996; Dick et al. 2000). Apart from this, the neural correlates of the remaining pathways are presently unclear. Here, we show that

pathway 4 (mediating the rapid shortening of T_E during hypoxia) involves the lateral parabrachial nucleus (LPBN), a subnucleus of the dorsolateral pons 'pneumotaxic' integrative region in the rat (Song et al. 2006). Specifically, we show that chemical or electrolytic lesions of the LPBN markedly attenuate the hypoxia-elicited rapid shortening of T_E without appreciably affecting all other hypoxic response components in anesthetized, vagotomized rats. Our results lend further support for the proposed carotid chemoafferent parallel integrator-differentiator model (Fig. 1B) and suggest a new role for the LPBN in respiratory control.

2. Methods

Animal preparation

Experiments were done on 12 male adult Sprague-Dawley rats (330–380 g, Charles River Laboratories). All experimental protocols had been reviewed and approved by the M.I.T. Committee on Animal Care in accordance with published guidelines. After injecting atropine sulphate (0.025 mg, s.c.), the rat was anesthetized with urethane (Sigma, 1.5 g/kg, i.p.). Trachea was intubated for artificial ventilation. The femoral vein and artery were cannulated for infusing solution (Lactated Ringer's solution, 0.05–0.1 ml/min) or monitoring arterial blood pressure, respectively.

Rats were paralyzed with pancuronium bromide (Sigma, initial dose 0.5 mg, i.v., supplemented every hour at 0.1 mg, i.v.) and ventilated with hyperoxic medical air (40% O_2 balance N_2) by using a CWE AVS-1 ventilator. A respiratory gas analyzer (CWE Gemini) was used to monitor end-tidal O_2 and CO_2 levels ($P_{ET}O_2$ and $P_{ET}CO_2$). The latter was maintained at $5.0 \pm 0.2\%$ (38 ± 1.5 mmHg) or $5.5 \pm 0.2\%$ (41.8 ± 1.5 mmHg), which was the CO_2 -recruitment threshold (Boden et al. 1998) plus 1.0% or 1.5%. Heart rate was monitored by recording ECG with subcutaneous needle electrodes. Body temperature was kept at 36.5 ± 0.2 °C with a temperature controller (CWE, TC-831). During the experiment, the depth of anesthetization was checked regularly. Whenever a noxious stimulus (clamping the hind paw) caused changes in pupil size, respiration and heart rate or elicited a withdrawal reflex, a supplementary dose of urethane (1/10 original dosage) was given intravenously to maintain adequate anesthesia.

The right phrenic nerve and both vagus nerves were isolated and severed at the cervical level from ventral approach. The head of the rat was then fixed in a stereotaxic frame (KOPF 1430, David Kopf Instruments, Tujunga, CA) in a tilted position (with Bregma 1.5 mm higher than Lambda) with the dorsolateral pons being readily accessible from a vertical dorsal approach. A craniotomy (diameter 0.5 cm) was performed at interaural level. Dura and pia were carefully removed. The exposed brain surface was covered with petroleum jelly.

Recording of phrenic discharge

To record phrenic discharge, the separated right phrenic nerve was exposed from dorsal approach and mounted on a bipolar platinum wire electrode (FHC). The raw phrenic discharge signal (Phr) was amplified (CyberAmp 380, Axon Instruments, Union City) and sampled (at 10 KHz) into a Dell PC with LabView (National Instruments, Austin, TX). In most experiments, the Phr signal was integrated with a Paynter filter (time constant 15 ms).

Pontine lesions

The LPBN was lesioned either chemically or electrolytically. The stereotaxic coordinates of the LPBN were -0.20 (caudal) $+0.2$ mm (rostral) to the level of lambda, $2.3 - 2.4$ mm lateral to midline, and $7.7 - 8.2$ mm below lambda surface. Chemical lesion was made unilaterally to one side using a glass micropipette (tip diameter $15 - 30$ μ m) filled with kainic acid solution (KA, from Sigma; concentration at 1μ g/ μ l in ACSF). The pipette was connected to a BH2 microinjector (Harvard Apparatus, Holliston, MA). A total volume of 50 nl was injected by

giving multiple pressure pulses to the micropipette. At the end of the experiment, the injection site was marked by passing anodal D.C. (100 μ A for 60 sec) through the injection pipette. Electrolytic lesions were made bilaterally to both sides with a tungsten microelectrode (tip diameter 1–2 μ m, impedance 0.5–1 M Ω ; Micro Probes, Gaithersburg, MD). The lesion current was 100 μ A, anodal D.C., lasting 30 sec.

Hypoxia test

Only brief poikilocapnic hypoxia (1 min) was used in this study in order to minimize the effects of hypoxic ventilatory decline and decreases in $P_{ET}CO_2$ (as a result of decreased body metabolism) and in arterial blood pressure during hypoxia. Each hypoxia test lasted 7–10 min: 1 min of pre-hypoxia baseline, 1 min of hypoxia, and 5–8 min of posthypoxia recovery. Hypoxia was applied by switching the hyperoxic ventilation gas to 8% O_2 (balance N_2). A control hypoxia test was performed right before KA or electrolytic lesions of the LPBN. After lesions, the animals were allowed to stabilize for 30–45 min before another hypoxia test was given.

In animals that received unilateral KA lesion, separate hypercapnia tests were also performed (see companion report (Song et al. 2009)). In this event the hypoxia tests were performed at 15 min after each hypercapnia test, when the animal's $P_{ET}O_2$, $P_{ET}CO_2$ and blood pressure had returned to corresponding pre-test baseline levels.

Data analysis

T_I , T_E and f were measured for each respiratory cycle from Phr. Amplitude of inspiratory motor output (J_{Phr}) was measured as the peak of the integrated Phr signal. Inspiratory drive was calculated as the ratio J_{Phr}/T_I . These values were also normalized against their corresponding average (1 min) pre-hypoxia baseline values in control or lesioned conditions. Each animal served as its own control for all statistical analyses.

Histology

At the end of the experiment, the animal was killed with an overdose of urethane (2 g/kg, i.v.) and immediately perfused transcardially with 300 ml of heparinized saline followed by another 300 ml of chilled paraformaldehyde solution (4% in 0.05 M PBS). The brain was removed, post-fixed and cut into 100- μ m coronal sections on a vibratome. Sections were then stained with cresyl violet and checked microscopically for the loci of lesions or injections.

3. Results

Loci of chemical or electrolytic lesions in the LPBN

In the six animals that received unilateral KA injection, the injection sites as marked by post-injection electrolytic lesions were verified to lie at the central or external-lateral subnucleus of the LPBN (Fig. 2). In the other six animals that received bilateral electrolytic lesions, the lesioned area comprised a hole (400–800 μ m in diameter) surrounded by a layer of dead neurons. The epicenters of such lesions were at the central or external-lateral subnucleus (Fig. 2). In both cases, surrounding structures such as the Kölliker-Fuse nucleus, superior cerebellum peduncle, and even medial parabrachial nucleus were occasionally invaded. We did not see any changes in respiratory pattern when the superior cerebellum peduncle was partially lesioned. However, apneusis was always observed when the Kölliker-Fuse or medial parabrachial nucleus was encroached upon as with previous studies (Denavit-Saubie et al. 1980); such animals were excluded from analysis.

Expiratory phase is prolonged after bilateral lesions at LPBN

In animals with unilateral KA injection, all respiratory rhythm variables (f , T_E , T_I) at 30 min post-injection were not significantly different from the corresponding pre-injection baseline values under hyperoxia ($P>0.1$, two-tailed paired t test; Table 1). In animals with bilateral electrolytic lesions, T_I after lesions was also not significantly different from control ($P>0.1$) but f was significantly decreased from a baseline value of 44.2 ± 1.5 to 35.8 ± 1.7 min^{-1} ($P<0.01$), primarily due to a prolongation of T_E (from 0.80 ± 0.05 to 1.14 ± 0.06 sec, $P<0.01$; Table 1).

Lesions at LPBN markedly attenuate the shortening of expiration during hypoxia

Before LPBN lesions, hypoxia elicited rapid increases in f that peaked within 30 sec before declining toward baseline (STD). Upon resumption of hyperoxia, f first dropped abruptly below baseline and then recovered gradually, evidencing PHFD in the off-transients of STD. (Fig. 3)

After lesions, the increase in f during hypoxia became much smaller in both absolute and relative magnitudes (Fig. 4A). In the unilateral lesion group, the peak increase in f (over pre-hypoxia baseline) was $40.7\pm3.4\%$ before and $10.9\pm1.5\%$ after lesion (73% attenuation). The blunting of the respiratory frequency response to hypoxia was caused mainly by a weakening of the hypoxic response in T_E . Before lesion, the maximal shortening of T_E was $-37.3\pm2.0\%$ (below pre-hypoxia baseline), coincident with the maximal increase in f . After lesion, the maximal T_E shortening was only $-13.0\pm1.7\%$ (65% attenuation). In the bilateral lesions group (Fig. 4B), the peak increase in f was $28.1\pm3.7\%$ before and $5.8\pm3.6\%$ after lesions (79% attenuation). Again, this was largely due to a much smaller shortening of T_E (maximal shortening $-25.7\pm3.3\%$ vs. $-1.0\pm6.4\%$; 96% attenuation).

Posthypoxia frequency decline is not affected by LPBN lesions

In the unilateral lesion group, the average PHFD at 20 sec post-hypoxia was $-17.9\pm5.7\%$ before and $-13.6\pm4.3\%$ after lesion. In the bilateral lesions group, the corresponding values were $-29.9\pm4.4\%$ and $-28.8\pm5.3\%$. In both cases, the differences in PHFD between the control and lesioned conditions were not statistically significant ($P>0.1$, two-tailed paired t test) (Fig. 4).

Inspiratory components of the hypoxic response are not affected by LPBN lesions

In either control or lesioned conditions, hypoxia caused similar progressive shortening of T_I (Fig. 5) and progressive increases of $\int\text{Phr}$ and $\int\text{Phr}/T_I$ (Fig. 6). All these changes reached peak values at the end of the 1-min hypoxic challenge indicating gradual development of STP in these variables. Upon resumption of hyperoxia, all these variables returned gradually to the corresponding baseline levels, evidencing afterdischarge. The response in T_I during and after hypoxia were not significantly different between the control and both lesioned conditions ($P>0.1$, two-way ANOVA with repeated measures), whether in normalized or absolute values. The normalized values of $\int\text{Phr}$ and $\int\text{Phr}/T_I$ during and after hypoxia were also not significantly different between the control and both lesioned conditions ($P>0.1$, two-way ANOVA with repeated measures).

4. Discussion

The most important finding of this study is that lesions at the LPBN markedly and selectively attenuate the rapid shortening of T_E (and resultant increase in f) during hypoxia but have no appreciable effects on other response components in anesthetized, vagotomized rats. Early studies of STP and STD in anesthetized and vagotomized rats simulated isocapnic hypoxia by keeping $P_{ET}\text{CO}_2$ constant (Hayashi et al. 1993). However, changes in $P_{ET}\text{CO}_2$ may not necessarily indicate similar changes in arterial PCO_2 since alveolar dead space [hence arterial-alveolar PCO_2 difference (Severinghaus et al. 1957)] may increase during hypoxia (Olson

1994). Subsequent studies show that poikilocapnic hypoxia elicits similar (albeit smaller) STP and STD effects in anesthetized or awake rodents (Coles et al. 1996; Kline et al. 2002) and awake humans (Steinback et al. 2007). In the present study, we employed brief poikilocapnic hypoxia in a consistent manner (with similar corresponding decreases in $P_{ET}CO_2$) before and after LPBN lesioning to elicit similar STP and STD without causing significant hypoxic ventilatory decline in both conditions. We found that the rapid shortening of T_E and corresponding increase in f were the only hypoxic response component that was significantly affected after unilateral or bilateral LPBN lesions.

In this study, lesions were made with either KA or electrolysis and were histologically identified to be at the central or external-lateral subnucleus of the LPBN. Electrolysis destroys neurons, glia, and axons of passage at the lesioned area, whereas KA selectively kills neurons that express glutamate kainate receptors at the injection site (Coyle et al. 1978). Considering the abundant expression of glutamate receptors in the dorsolateral pons (Takayama et al. 1993; Chamberlin et al. 1994), KA would cause significant neuronal death when injected into this region. In an early study performed in adult rats, injection of 0.1 μ l of KA solution (concentration at 0.36 μ g/ μ l) into dorsolateral pons resulted in neuronal loss in an area with diameter of $\sim$ 0.5 mm (Mizusawa et al. 1995). Accordingly, we estimate that the diameter of affected area for the present dosage (0.05 μ l at concentration of 1 μ g/ μ l) should be between 0.5–1.0 mm, thus comparable to the size of electrolytic lesions both of which covered a major part of the LPBN but not all. The consistency between the effects of KA and electrolytic lesions made either unilaterally or bilaterally confirms that the LPBN was the specific site that mediated the rapid shortening of T_E during hypoxia.

Another important finding of this study is that bilateral LPBN lesions resulted in a significant increase in baseline T_E (and corresponding decrease in f) under hyperoxia without appreciable changes in T_I . This observation corroborates the notion that the LPBN also mediates T_E -shortening by tonic factors beside peripheral chemoreceptor inputs, such as central chemoreceptor inputs (Song et al. 2009). The relative stability of T_E and f after unilateral lesion compared to bilateral lesions indicate possible compensatory (plasticity) effects in the contralateral (intact) pathway after unilateral lesion. We did not compare the changes in $\dot{V}_{Phr}$ and $\dot{V}_{Phr}/T_I$ before and after lesions because the absolute values of phrenic recordings (in arbitrary units) might drift in the course of a lengthy experimental procedure (lesioning and subsequent stabilization). Thus, only the relative changes in $\dot{V}_{Phr}$ and $\dot{V}_{Phr}/T_I$ during and after brief hypoxia are presented.

The LPBN constitutes a subdivision of the dorsolateral pons ‘pneumotaxic center’ in the rat (Song et al. 2006). Chemical or electrical stimulations at this structure in cats or rats evoke predominantly respiratory facilitation, as opposed to respiratory inhibition for the more ventrally located Kölliker-Fuse nucleus or medial parabrachial nucleus (Cohen 1971; Takayama et al. 1993; Chamberlin et al. 1994; Lara et al. 1994; Dawid Milner et al. 2003). It has been suggested that the LPBN may play an important role in integrating respiratory-related afferents with other afferents such as nociceptive, thermosensitive, somatic proprioceptive and cardiovascular-related afferents (Jiang et al. 2004; Potts et al. 2005; Song et al. 2006; Morrison et al. 2008). In addition, several lines of evidence point to a possible involvement of the LPBN in mediating hypoxic respiratory response. For example, chronic lesion of a dorsolateral pontine region that included the LPBN resulted in decreased ventilatory response to hypoxia and hypercapnia in awake rats (Mizusawa et al. 1995). Anatomical data show that the LPBN has rich projections from chemoafferent- or chemoreception-related medullary structures that receive peripheral chemoreceptor inputs, such as commissural NTS and retrotrapezoid nucleus (Song et al. 2004; Rosin et al. 2006; Takakura et al. 2006). Neurons in LPBN express c-Fos protein in response to hypoxia challenge or carotid sinus nerve stimulation (Berquin et al.

2000; Bodineau et al. 2001) and their discharges are excited by carotid body stimulation (Hayward et al. 1995).

Extending these previous findings, the present study revealed that the LPBN specifically mediated the rapid shortening of T_E (hence rapid increase in f) during hypoxia and not any other hypoxic response components. Furthermore, the present study provided evidence indicating that the LPBN also mediated T_E -shortening by certain tonic inputs, such as central chemoreceptor inputs (Song et al. 2009). Taken together, these findings raise the possibility that the LPBN may be the site of the putative fast integrator whereby central chemoreceptor inputs are potentiated by peripheral chemoreceptor inputs to shorten T_E (Fig. 1B). This observation, together with earlier suggestion that PHFD is mediated by a subdivision of the pneumotaxic center in the ventrolateral pons (Coles et al. 1996), corroborate the notion (Fig. 1B) that the central processing of peripheral chemoafferent inputs is segregated into parallel integrator-differentiator pathways that separately modulate inspiratory drive, T_I , T_E and resultant STP and STD (Young et al. 2003).

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Fig. 1. Components of the hypoxic respiratory response and corresponding central modulatory pathways

A: Idealized curves showing the time-dependent responses in respiratory frequency (f), expiratory duration (T_E), inspiratory duration (T_I), inspiratory amplitude ($I_{Phr.}$), and minute ventilation ($\dot{V}$) to acute hypoxia. STP, short-term potentiation; STD, short-term depression. **B:** Hypothetical model showing parallel central pathways that mediate various components of the hypoxic respiratory response. CSN, carotid sinus nerve; NTS, nucleus tractus solitarius; $\int_L$, leaky neural integrator; vl-pons and dl-pons, ventrolateral and dorsolateral pons; central chemoaff., inputs from central chemoreceptors; E and I, expiratory and inspiratory rhythm generators; Phr.m/pre-m phrenic motoneuron/premotoneuron. Open and filled triangles indicate excitatory and inhibitory effects. Adapted from Young et al. (2003).

Fig. 2. Histological sections showing LPBN lesions in two representative animals

Left column: Montage photomicrographs showing the kainic acid injection site. The center of injection (arrowhead) was at the external-lateral LPBN. *Right column:* Photomicrographs and camera-lucida drawings on standard plates (Plate 51–53 of (Paxinos et al. 1986)) showing bilateral electrolytic lesions. The lesioned area (shaded) had dimensions of about $700 \times 700 \times 700 \mu\text{m}$ in the left side, and about $500 \times 500 \times 500 \mu\text{m}$ in the right side. The centers of lesions (*) on both sides were at the central LPBN. In both sides, Kölliker-Fuse nucleus (KF) was not affected. 5, trigeminal motor nucleus; scp, superior cerebellum peduncle. Numbers indicate the distances (in μm) of each section from the centers of microinjection (left column) or lesions (right column). See also (Song et al. 2009).

Fig. 3. Examples of poikilocapnic hypoxia test before and after LPBN lesion

Representative recordings of raw (Phr.) and integrated phrenic discharge ($\int$ Phr.); expired CO₂ level (ECO_2 , expiration upward); and femoral artery blood pressure (BP). Shaded section indicates hypoxia. A: Control hypoxia test performed before lesion. B: Hypoxia test performed at 30 min after microinjection of KA. Note the almost identical changes in $P_{ET}CO_2$ and blood pressure during poikilocapnic hypoxia before and after lesion.

Fig. 4. Unilateral or bilateral lesions at the LPBN attenuated the increase in respiratory frequency and shortening of expiration during hypoxia without affecting PHFD or STD

Left column: Hypoxic responses in normalized respiratory frequency (f) and expiratory duration (T_E). Data are means $\pm$ SE ($n=6$). The increases of f and shortenings of T_E during hypoxia (shaded areas) became much smaller after unilateral (A) or bilateral lesions (B) at the LPBN. However, at time 80 sec (20 sec post-hypoxia), the two curves again converged and differences were insignificant at this point ($P>0.1$, two-tailed paired t test). *Right column:* Responses in f (cycles/min.) and T_E (sec) shown in absolute values.

Fig. 5. Shortening of inspiration and STP during and after hypoxia were not affected by unilateral or bilateral lesions at LPBN

Response curves are expressed in normalized (left panels) or absolute values (right panels) for unilateral lesion group and bilateral lesions group. None of the response curves are significantly different between control (Ctr.) and lesioned conditions ($P > 0.1$, two-way ANOVA with repeated measures).

Fig. 6. Increases of phrenic discharge amplitude and inspiratory drive and their STP during and after hypoxia were not affected by unilateral or bilateral lesions at LPBN
 $\dot{V}_{Phr}$, phrenic discharge amplitude; $\dot{V}_{Phr} / \dot{V}_I$, inspiratory drive. None of the response curves are significantly different between control (Ctr.) and lesioned conditions ($P > 0.1$, two-way ANOVA with repeated measures).

Table 1

Effects of LPBN lesions on baseline respiratory patterns.

		Before	After
Unilateral Lesion (n=6)	f (min ⁻¹)	39.2±1.8	43.0±3.3
	T _E (sec)	1.15±0.06	1.05±0.12
	T _I (sec)	0.40±0.03	0.42±0.03
Bilateral Lesions (n=6)	f (min ⁻¹)	44.2±1.5	35.8±1.7 [*]
	T _E (sec)	0.80±0.05	1.14±0.06 [*]
	T _I (sec)	0.52±0.02	0.55±0.03

Data are means±SE of 1-min baseline recordings before or after LPBN lesions. f , respiratory frequency; T_E and T_I, expiratory and inspiratory durations.

^{*} P<0.01 (two-tailed paired t test).