Long-Term Modulation of Respiratory Network Activity Following Anoxia In Vitro

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INTRODUCTION

Neural networks underlying the production of rhythmic behaviors are functionally flexible and capable of producing many different patterns of activity (Marder and Calabrese 1996; Stein et al. 1997). This plasticity is essential for adapting neural activity to changes in the internal and external environment of the organism. In many vertebrate and invertebrate neural networks, activation of sensory and/or modulatory inputs results in immediate alterations in network activity (Stein et al. 1997). More recent work has begun to examine modulatory processes resulting in changes in network activity that outlast a perturbation or modulatory influence for minutes to hours or even days to weeks (Marder et al. 1996; Parker et al. 1998; Thoby-Brisson and Simmers 1998).

The mammalian respiratory system is modulated over a wide range of time scales, ranging from seconds to years (Gozal and Gozal 2001; Powell et al. 1998; Turner et al. 1997). One important modulator of respiration demonstrating transient and persistent changes is oxygen level. Changes in oxygen level alter both the frequency and pattern of respiration (Powell et al. 1998). In some cases, these changes elicit alternations in the respiratory rhythm after termination of the change. For example, brief (3–5 min) repetitive episodes of hypoxia elicit increases in respiratory frequency as well as increases in the amplitude of integrated motor neuronal bursts in vivo (Bach and Mitchell 1996; Baker and Mitchell 2000; Fregosi and Mitchell 1994; Hayashi et al. 1993; Millhorn et al. 1980; Turner and Mitchell 1997). These changes persist for 30–90 min and are collectively referred to as long-term facilitation (LTF) (Fuller et al. 2000; Powell et al. 1998). Although the degree of influence varies with preparation (Bach and Mitchell 1996; Turner and Mitchell 1997), animal strain (Fuller et al. 2000), age (Zabka et al. 2001), and experimental conditions (Baker and Mitchell 2000), it appears that changes in motor neuron burst amplitude are due to a direct modulation at the level of the motor nucleus (Fuller et al. 2000; Kinkladze et al. 1998). The site of the frequency modulation is still unknown, but the most likely site is the neuronal network responsible for respiratory rhythm generation. The pre-Bötzinger complex, located in the ventrolateral medulla, has been proposed to be the site of respiratory rhythm generation (Gray et al. 2001; Smith et al. 1991). Isolation of the pre-Bötzinger complex (PBC) in a transverse slice preparation preserves rhythmicity in the respiratory network (Funk et al. 1994; Ramirez et al. 1996; Smith et al. 1991). Recent work has shown that the in vitro respiratory response to lowered oxygen resembles the whole animal respiratory response (Haddad and Jiang 1993; Hwang et al. 1983; Lieske et al. 2000; Neubauer et al. 1990; Telgkamp and Ramirez 1999). That is, anoxia elicits a biphasic response that includes an initial frequency augmentation followed by a depression often leading to apnea (Ramirez et al. 1997b, 1998; Richter et al. 1991, 1993; Telgkamp and Ramirez 1999).

In this study, we used the rhythmic transverse slice preparation to determine how the respiratory network recovers from brief episodes of central anoxia. We find there is a transient frequency increase after a single anoxic episode, whereas there is a long-term increase after multiple episodes. This frequency modulation occurs in the absence of any persistent change in network population activity. Some of this work has appeared in abstract form (Blitz et al. 1999).

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METHODS
Preparation
Neonatal (P0–7) male and female mice (CD-1; Charles River Laboratories, see www.criver.com/1999rm/htdocs/cdmice_swiss.html) were used in this study. Mice were deeply anesthetized with ether and decapitated at spinal level C5–L5 in accordance with a protocol approved by the University of Chicago Animal Care and Use Committee. As described previously (Ramirez et al. 1996; Telgkamp and Ramirez 1999; Thoby-Brisson and Ramirez 2000), the brain was removed from the skull and transferred immediately into ice-cold artificial cerebrospinal fluid (ACSF). The brain stem was isolated from the remainder of the brain and glued rostral side up to an agar block with cyanoacrylate. Thin slices were taken until the region of the brain stem containing the pre-Bötzinger complex was reached. A 600 to 700 μM thick slice was cut at this point and transferred immediately to a recording chamber. The slice was continuously perfused with ACSF (29–31°C) bubbled with 95% O2–5% CO2. The preparation was allowed to stabilize for 30–60 min. The potassium concentration was then raised to 8 mM over a 30-min period to maintain rhythmic activity (Ramirez et al. 1996; Smith et al. 1991). Anoxia was induced by switching from bubbling the ACSF with 95% O2–5% CO2 to bubbling with 95% N2–5% CO2 for a period of 3 min. Using 95% N2–5% CO2 results in anoxic conditions throughout the slice within 30–50 s (Ramirez et al. 1997a). The 3-min duration chosen for this study consistently elicited the biphasic response and the termination of anoxia occurred during the end of augmentation or the beginning of the depression.

Solutions
ACSF contained (in mM) 118 NaCl, 3 KCl, 1.5 CaCl2, 1 MgCl2·6 H2O, 25 NaHCO3, 1 NaH2PO4, and 30 D-glucose (pH = 7.4 bubbled with 95% O2–5% CO2).

Recording and data analysis
Extracellular recordings of population activity in the PBC were performed using glass microelectrodes (120 to 150 kΩ resistance) placed on the surface of the slice. Each burst of population activity reflects fictive eupnic inspiratory activity (Lieske et al. 2000; Telgkamp and Ramirez 1999). Larger-amplitude bursts reflect fictive sigh bursts (Lieske et al. 2000) Signals were amplified, filtered, and integrated (Fig. 1). Data were digitized using a Digidata board (Axon Instruments, Foster City, CA), printed on chart paper, and stored on computer. Data were analyzed off-line with customized software routines for Igor Pro (Wavemetrics, Lake Oswego, OR). Inspiratory bursts were detected using a manually set threshold. Period was measured as the time from threshold crossing of one inspiratory burst to threshold crossing of the next burst. Sigh bursts are triggered by normal inspiratory bursts (Lieske et al. 2000) and were therefore included in the measurements of period. Data are reported as frequency, the inverse of period. Burst amplitude was measured from baseline to peak of the burst, whereas burst width was measured as time across the burst at half-maximal amplitude.

For the purposes of this study, we have designated three time periods following the end of anoxia. We define early recovery as the time beginning after the respiratory cycle with the longest cycle period (peak depression) following termination of anoxia. Late recovery is defined as beginning 10 min post anoxia and long-term recovery as beginning 30 min post anoxia. To determine fictive respiratory frequency at discontinuous time points, the average of 20 consecutive cycles was taken. To quantify the continuous recovery, instantaneous frequency or amplitude was measured and binned in consecutive 10-s intervals.

Figures were made using Prism version 3.0 (GraphPad Software, San Diego, CA) and CorelDraw version 9 (Corel Corporation, Ontario, Canada). Data are reported as means ± SE. Statistical significance was determined with Prism using paired t-test, one-way, one-way repeated-measures, and two-way repeated-measures ANOVA followed by Tukey t-test for post hoc analysis as indicated. Statistical significance was considered to be P < 0.05.

RESULTS
Respiratory activity during recovery from a single brief anoxia
Representative integrated recordings from the PBC before, during, and at several time points after anoxia during a single experiment illustrate a typical PBC population response (Fig. 2). In agreement with previous data, during anoxia, there was an increase in frequency (Telgkamp and Ramirez 1999) (Fig. 2) followed by a depression. During early recovery (see METHODS for terminology), the frequency was slower than control (i.e., below baseline frequency) but increased above control...
levels during late recovery. By long-term recovery, fictive respiratory frequency was no longer increased above pre-anoxia levels.

Although these qualitative changes always occurred, the exact time course of the anoxic augmentation and subsequent depression varied slightly between different preparations, resulting in a variable time at which the recovery began. Thus to examine the onset of recovery across preparations, we designated the longest cycle length post anoxia as the peak depression and then designated the subsequent cycle in each preparation as the start of early recovery. The percent change in frequency of the first several cycles during this early recovery was then averaged for a number of preparations. When the early recovery is aligned in this manner, it is evident that after the peak depression, the frequency of respiratory activity remains below baseline level for several cycles. There is a progressive return to baseline frequency within ~10 respiratory cycles (Fig. 3A) (n = 12). The integrated burst amplitude was not consistently altered during the first 10 cycles (Fig. 3B).

To assess recovery beyond the first several cycles, we used the time after the end of the anoxic episode as the reference. Instantaneous frequency from one experiment is plotted in Fig. 4A1. Prior to anoxia, the mean frequency was 0.24 Hz. In this experiment, the anoxic augmentation had terminated by the end of the anoxia and a frequency depression is evident immediately after anoxia. The frequency gradually increased and was 0.34 Hz at 10 min post anoxia (Fig. 4A1). In other experiments, the frequency augmentation continued after termination of the anoxia (Fig. 4A2). The fictive respiratory frequency in control conditions varied from 0.05 to 0.47 Hz. Therefore we normalized the data as percent frequency change relative to pre-anoxia when compiling the data. These data demonstrate that after anoxia the frequency augmentation terminated by ~1.5 min. This was followed by a depression for ~2 min (Fig. 4A2). The frequency then increased beyond control values and reached a peak by 10 min post anoxia (Fig. 4A2; n = 12). The mean frequency increased from 0.23 ± 0.14 to 0.30 ± 0.15 Hz at 10 min after anoxia (P < 0.001, paired t-test; n = 15).

The increased fictive respiratory frequency could be due to a decrease in either the expiratory or inspiratory phase duration or both components. Measurements of the integrated inspiratory burst duration revealed no significant change postanoxia, indicating that the frequency increase was due solely to a decrease in the expiratory phase duration. The burst width prior to anoxia was 0.44 ± 0.08 and 0.47 ± 0.11 s at 10 min after anoxia (n = 7; not significant, paired t-test).

We also extended our analysis of PBC burst amplitude through late recovery. In the experiment shown in Fig. 4B1 (same experiment as Fig. 4A1), burst amplitude during the 10 min prior to anoxia was relatively constant. After anoxia there was no persistent change in amplitude over the 10 min measured. In the averaged data, there was no progressive change in burst amplitude during this 10-min period as there was for frequency, although there was an increased amplitude for about 20 s at ~3 min post anoxia (Fig. 4B2; n = 12). The mean relative PBC burst amplitude was 0.32 ± 0.14 units immediately prior to anoxia and 0.31 ± 0.12 units at 10 min after the end of the anoxic episode (n = 7).

To determine the duration of the frequency increase, we measured the mean frequency of 20 cycles at 5-min intervals following anoxia. Over this longer time scale, it was important to control for possible nonspecific long-term effects. Therefore the fictive respiratory frequency was also measured in preparations that were not exposed to anoxia. The frequency in these unperturbed preparations tended to decrease over time, although no time point was significantly different from the initial frequency (Fig. 5; 2-way ANOVA). The mean frequency in these preparations was 0.22 ± 0.06 Hz at 0 min and 0.20 ± 0.08 Hz at 90 min (n = 6). As demonstrated in the preceding text, the frequency post-anoxia reached a maximal value at 10 min after anoxia (n = 5). The frequency then began to decrease. The frequency in anoxia-exposed and unperturbed preparations was only significantly different at 10 and 35 min after anoxia (P < 0.05, two-way ANOVA).

Recovery of respiratory activity following brief repetitive anoxia

In vivo, there is often little change after a single hypoxic episode. However, there is a progressive increase with repetitive episodes of hypoxia that can last ~90 min (Baker and Mitchell 2000). We determined whether repetitive episodes of

**Fig. 3.** Fictive respiratory frequency, but not PBC burst amplitude, was depressed after a single anoxic exposure and gradually returned to control values. Percent change in frequency (A) and PBC burst amplitude (B) after anoxia compared with preanoxia is plotted against cycle number. Peak depression refers to the cycle with the longest period following return to normoxia. Early recovery begins with the following cycle.
anoxia had similar progressive effects on fictive respiratory frequency in the slice preparation. In 13 preparations, we induced three episodes of 3-min exposures to anoxia, each separated by 10 min of normoxia. The frequency at 10 min after the end of each anoxic episode was faster than preanoxia (Fig. 6B; repeated measures one-way ANOVA, \(P < 0.05\)). However, there was no statistical difference among the frequencies 10 min after the first, second, and third anoxic episodes (Fig. 6; repeated-measures one-way ANOVA). Three successive 3-min episodes with 5-min intervals also elicited a significant increase in frequency at 10 min after the third anoxic episode compared with pre-anoxia (paired \(t\)-test, \(P < 0.001\); \(n = 11\); Fig. 6B). The mean respiratory frequencies 10 min after a single anoxia or 10 min after three anoxic episodes with either 10- or 5-min intervals were not significantly different (one-way ANOVA).

We next determined the duration of the frequency increase following repetitive anoxic episodes. To assess possible time-dependent effects, we monitored unperturbed respiratory activity for longer time intervals to match the longer time necessary to perform three consecutive anoxic episodes. After repeated anoxic episodes with 10-min intervals, the percent change was significantly different from unperturbed preparations at each time point from 10 to 75 min after the termination of the anoxia (Fig. 7A; \(n = 6\) unperturbed, \(n = 5\) anoxia exposed; two-way ANOVA). After three 5-min interval anoxic episodes, the percent change was significantly different from unperturbed preparations at all time points from 10 to 90 min (Fig. 7B; \(n = 6\) unperturbed, \(n = 5\) anoxia exposed; two-way ANOVA).

**DISCUSSION**

We demonstrate that anoxic exposure was followed by a brief period of frequency depression. After a single anoxic exposure, this short frequency depression was followed by a short frequency facilitation before a return to baseline fre-
frequency. In contrast, repetitive brief anoxic exposures (3 × 3 min) elicited a long-term frequency facilitation that lasted for ≈90 min in the in vitro respiratory network of mice.

Previous work demonstrated that the in vitro fictive respiratory response during exposure to lowered oxygen resembles the whole animal respiratory response (Haddad and Jiang 1993; Hwang et al. 1983; Lieske et al. 2000; Neubauer et al. 1990; Telgkamp and Ramirez 1999). Here, we show that brief anoxic exposures elicited a response from the in vitro respiratory network that resembles the recovery of respiratory activity in vivo following repeated hypoxic exposures. The initial recovery from anoxia consists of a short-term depression in frequency similar to the in vivo transient decrease in respiratory frequency known as short-term depression (STD) or post hypoxia frequency decline (PHFD) (Bach et al. 1999, Coles and Dick 1996; Dick and Coles 2000). We find after this depression, the frequency increases until reaching a relatively stable frequency above baseline levels that persists for ≈90 min after repetitive anoxic episodes. Long-term changes in respiratory frequency and motor neuron burst amplitude following repetitive hypoxia occur in vivo and collectively are termed long-term facilitation (LTF) (Bach and Mitchell 1996; Baker and Mitchell 2000; Fregosi and Mitchell 1994; Millhorn et al. 1980). In vivo, LTF occurs as a progressive increase with repeated hypoxic exposure, while there is no long-lasting response after a single exposure (Baker and Mitchell 2000).

Similarly, we found no long-term change after a single anoxic exposure. Furthermore, although there was no significant increase with additional episodes of anoxia in our study, there was a tendency for the frequency to be greater following the second and third anoxic exposures.

LTF in vivo consists of increases in frequency (Bach and Mitchell 1996; Baker and Mitchell 2000; Cao et al. 1992; Turner and Mitchell 1997) and increases in motor neuron burst amplitude in several respiratory-related motor nerves (Baker and Mitchell 2000; Bach and Mitchell 1996; Fregosi and Mitchell 1994; Hayashi et al. 1993). However, the extent of the modulatory effects varied. For example, in several in vivo studies, a long-term change in motor neuron burst amplitude is elicited in the absence of any change in respiratory frequency (Fregosi and Mitchell 1994; Hayashi et al. 1993), while in some studies intermittent hypoxia evoked neither a frequency nor motor neuron burst amplitude modulation (Dwinell et al. 1997; Janssen and Fregosi 2000). This is not unexpected because in a variety of model systems the modulatory effects

![FIG. 6. Fictive respiratory frequency tended to increase with repetitive anoxia. A: integrated PBC population recordings 10 min after the first and 10 min after the 3rd episode of anoxia with 10-min intervals. Each anoxic exposure was 3 min in duration. B: plot of mean frequency before and 10 min after 3-min anoxic exposures with 10-min intervals (A) or 5-min intervals (C). *P < 0.05, **P < 0.001.](image)

![FIG. 7. Increase in fictive respiratory frequency after repetitive anoxia persisted for ≈90 min. Mean percent change in frequency is plotted against time for preparations exposed to 3 min of anoxia 3 times with 10-min intervals (A) or with 5-min intervals (B). In both graphs, ■, preparations exposed to anoxia; □, preparations that were left unperturbed for comparable amounts of time. *P < 0.05.](image)
depend critically on the type of preparations, the experimental conditions, the species, and even strains of animals (Fuller et al. 2000; Harris-Warrick 2000; Katz and Harris-Warrick 1999; Meyrand et al. 2000; Tankersley 2001; Tankersley et al. 1994; Teshiba et al. 2001; Zabka et al. 2001). Our studies were conducted in P0–7 neonatal mice. Although the response of the developing respiratory system during repetitive episodes of hypoxia is similar to the adult response, little is known regarding the recovery of respiratory activity following repetitive hypoxia during neonatal life (Gozal and Gozal 2001). To our knowledge, this is the first examination of LTF in a mouse preparation and it suggests the immature mouse respiratory network is capable of such long-term modulation.

In vivo studies demonstrate long-term changes at both the network and motor levels (Bach and Mitchell 1996; Baker and Mitchell 2000; Cao et al. 1992; Fregosi and Mitchell 1994; Hayashi et al. 1993; Turner and Mitchell 1997). Here, we examined recovery of the respiratory network (PBC) from anoxia. While it is safe to assume that changes in respiratory frequency will be transmitted to the motor output, our study does not address additional modulatory changes that may occur at the motor level. Although during anoxic exposure of the slice preparation there is no significant modulation in the amplitude of integrated population activity in the PBC, there is a significant amplitude modulation of hypoglossal motor neuron bursts (Telgkamp and Ramirez 1999). Thus in addition to the network effects we measured, it will be interesting to investigate whether there also are centrally elicited long-term changes at the motor level that can be elicited in this slice preparation by repetitive anoxic exposure.

The hypoxia-induced LTF is only dependent on the pattern of hypoxic exposure and not on the duration of hypoxia as even a prolonged hypoxic exposure does not evoke LTF (Baker and Mitchell 2000). One possible explanation for this finding is that the modulatory neurons responsible for mediating LTF are only transiently activated by hypoxia and cease to discharge during prolonged hypoxia. If this was the case, several brief hypoxic episodes would elicit increased release of a neuromodulator, while a prolonged hypoxia would not be different from a single short hypoxic exposure. Possible candidates for this role are serotonergic neurons located within the Raphe nucleus. It has been demonstrated that blockade of serotonin receptors can abolish both the motor amplitude and frequency component of LTF in vivo (Bach and Mitchell 1996; Kinkead and Mitchell 1999). It has been hypothesized (Bach and Mitchell 1996; Fuller et al. 2000), that LTF is induced by chemoreceptor activation of serotonergic raphe neurons. Several lines of evidence support this hypothesis: carotid nerve stimulation elicits LTF for minutes to hours (McCrimmon et al. 1995; Powell et al. 1998); direct carotid sinus nerve or hypoxic stimulation results in c-fos expression in the raphe pallidus of anesthetized rats (Erickson and Millhorn 1994); Raphe activity is persistently elevated during LTF in cats (Morris et al. 2000); electrical stimulation of raphe obscurus can elicit LTF (Millhorn 1986). Raphe neurons have projections to the phrenic (Holton et al. 1990; Manaker et al. 1992; Pilowsky et al. 1990) and hypoglossal (Jiang et al. 1991) motor nuclei. It is not yet known whether there is prolonged release of serotonin that maintains LTF. A model of LTF suggests a long-lasting change in intracellular signaling molecules may be responsible for LTF maintenance (Fuller et al. 2000). It is also possible this change might be due to changes in gene expression that can play a role in long term modulation in other systems (Parker et al. 1998).

Despite similarities between in vivo LTF and our findings, there can be no contribution from peripheral chemoreceptor activation in the present in vitro slice preparation. However, it is possible that the in vitro long-term change we have described here may still be the same phenomenon that is activated further downstream in the slice preparation. For example, this in vitro change may also be mediated by the Raphe nuclei, as the transverse slice preparation contains portions of the Raphe nuclei. Furthermore, it has been demonstrated that Raphe stimulation alters respiratory frequency in a slice preparation (Al-Zubaidy et al. 1996). Thus one possibility is that hypoxia activates Raphe neurons centrally that in turn influence pacemaker neurons in the pre-Bötzinger complex. Pacemaker neurons are implicated in the generation of the respiratory rhythm (Koshiya and Smith 1999; McCrimmon et al. 2000; Thoby-Brisson and Ramirez 2000), and the in vitro respiratory network anoxic response appears to be due to a subset of pacemaker neurons (Thoby-Brisson and Ramirez 2000). When isolated from the remainder of the network, these neurons show an increase followed by a decrease in bursting frequency on the same time scale as the network anoxic response. This biphasic response was only observed in pacemaker neurons and not follower neurons suggesting that these neurons have specific oxygen-sensing properties (Thoby-Brisson and Ramirez 2000). It will be interesting to determine if this same population of neurons demonstrates a long-lasting increase in frequency following anoxia when synapses within the network are blocked and whether this modulation is serotonin dependent. Although pacemaker neurons are likely candidates, other sites may also contribute to the O2 sensitivity in this slice preparation. It could also be that changes in oxygen are not sensed directly but perhaps indirect changes are sensed, such as elevations in lactic acid. In support of the possibility that central changes in O2 act directly within the network, focal hypoxia within the pre-Bötzinger complex elicits increases in burst amplitude and frequency of phrenic nerve bursts (Solomon et al. 2000). However, this does not necessarily rule out a role of serotonin, as PBC neurons might require serotonin to be in a state in which they are capable of responding to changes in O2 level. Long-lasting changes in network activity and configuration are characteristic of rhythm-generating networks and can be induced by endogenous neuromodulators or the activation of sensory inputs. In the swim system of lamprey, for example, substance P elicits a protein-synthesis-dependent increase in the frequency of fictive swimming that persists for hours (Parker et al. 1998). Additionally, in the crustacean stomatogastric nervous system, activation of sensory pathways with modulatory actions elicits long-lasting changes in neural network activity (Hooper et al. 1990; Katz and Harris-Warrick 1989).

While the observed long-term changes in fictive respiratory activity are not unusual for in vitro neuronal networks, they were unexpected in light of results obtained from lesion experiments. Given that many ideas in respiratory physiology are derived from lesion experiments, it is important to discuss our in vitro finding in the context of these results. It is generally thought that LTF depends on carotid body chemoreceptor activation. Yet the slice preparation exhibits a long-term fre-
quency facilitation in the absence of this chemoreceptor input. Similarly, it is generally thought that the central response to hypoxia is a respiratory depression, yet the slice preparation exhibits a frequency augmentation. Many of these ideas are based on experiments involving chemoreceptor denervation. However, chemoreceptor deafferentation is difficult to interpret. Chemoreceptor afferents are tonically active in room air (Marchal et al. 1992). Thus following deafferentation, the CNS receives decreased chemoreceptor input, which typically signals hypoxic conditions (Fukuda et al. 1987; Lahiri et al. 1980). A subsequent hypoxic exposure will therefore result in mixed messages: the missing chemoreceptor drive signals hypoxia, while the brain signals hypoxic conditions. Perhaps as a result of these mixed messages, the excitatory component of the hypoxic response is missing. Indeed, if the blood supply to the chemoreceptors is kept normoxic while the animal is exposed to hypoxia, there is an excitatory respiratory response, indicating that the CNS response is excitatory (Curran et al. 2000). It will be interesting to examine whether under these conditions, LTF is elicited in vivo despite the lack of a hypoxic signal from peripheral chemoreceptors.

LTF has also been shown to be absent following cerebellotomy in vivo (Hayashi et al. 1993). However, cerebellar lesions are difficult to interpret as the cerebellum has major influences on respiratory control (Cotter et al. 2001, Mori et al. 2001, Yates et al. 1993) and, consequently a cerebellotomy can have effects on various aspects of respiratory activity, including altering the modulatory milieu within the medulla. The reported dependency of LTF on the release of neuromodulators (Fuller et al. 2000) may explain the absence of LTF following cerebellotomy. Similarly, pontine lesions are difficult to interpret. It has been observed that lesioning the pons abolishes the short-term depression or posthypoxic frequency decline (Cole and Dick 1996; Dick and Cole 2000). In the in vitro slice, however, short-term depression (STD) is observed despite the absence of the pons, cerebellum, or peripheral chemoreceptors (see Fig. 3). Although, the observed posthypoxic changes (LTF and STD) have striking similarities to the posthypoxic response in vivo, it must be cautioned that the underlying mechanisms in vitro and in vivo may not be identical, as discussed in the preceding text. However, similar caution is necessary when interpreting lesion experiments as lesions may also alter the modulatory milieu and the network characteristics of the respiratory network within the medulla. Thus while lesion experiments contribute to our understanding of respiratory control, abolition of portions of the hypoxic response are not necessarily indications that the lesioned region is responsible for generating or mediating that portion of the hypoxic response. Examination of such phenomena as LTF in the more isolated conditions of the in vitro slice preparation may provide new insights into respiratory function.

The in vitro slice preparation will serve as a useful model system in which to continue to probe general questions of network recovery following perturbation. In addition, studying these issues in the mammalian respiratory system may have important clinical implications. For example, brief periodic episodes of decreased oxygen intake occur due to the respiratory disorder obstructive sleep apnea. It has been suggested that a mechanism such as LTF may play a role in stabilizing breathing in response to these hypoxic events (McCracken et al. 1995; Powell et al. 1998).

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