Physiological and Genomic Consequences of Intermittent Hypoxia
Invited Review: Intermittent hypoxia and respiratory plasticity

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Mitchell, Gordon S., Tracy L. Baker, Steven A. Nanda, David D. Fuller, Andrea G. Zabka, Brad A. Hodgeman, Ryan W. Bavis, Kenneth J. Mack, and E. B. Olson, Jr. Invited Review: Intermittent hypoxia and respiratory plasticity. J Appl Physiol 90: 2466–2475, 2001.—Intermittent hypoxia elicits long-term facilitation (LTF), a persistent augmentation (hours) of respiratory motor output. Considerable recent progress has been made toward an understanding of the mechanisms and manifestations of this potentially important model of respiratory plasticity. LTF is elicited by intermittent but not sustained hypoxia, indicating profound pattern sensitivity in its underlying mechanism. During intermittent hypoxia, episodic spinal serotonin receptor activation initiates cell signaling events, increasing spinal protein synthesis. One associated protein is brain-derived neurotrophic factor, a neurotrophin implicated in several forms of synaptic plasticity. Our working hypothesis is that increased brain-derived neurotrophic factor enhances glutamatergic synaptic currents in phrenic motoneurons, increasing their responsiveness to bulbospinal inspiratory inputs. LTF is heterogeneous among respiratory outputs, differs among experimental preparations, and is influenced by age, gender, and genetics. Furthermore, LTF is enhanced following chronic intermittent hypoxia (CIH), indicating a degree of metaplasticity. Although the physiological relevance of LTF remains unclear, it may reflect a general mechanism whereby intermittent serotonin receptor activation elicits respiratory plasticity, adapting system performance to the ever-changing requirements of life.

IN THIS REVIEW, EVIDENCE WILL be presented that intermittent hypoxia elicits unique, serotonin-dependent mechanisms of plasticity in the central neural control of breathing. These forms of central nervous system (CNS) plasticity are unique because they are elicited by intermittent hypoxia but not by an equivalent duration of sustained hypoxia. Recent literature will be reviewed concerning two specific forms of plasticity elicited in time domains of many minutes to days by intermittent hypoxia: 1) long-term facilitation (LTF) of phrenic motor output as a result of 3–10 hypoxic episodes and 2) enhanced phrenic LTF as a result of chronic intermittent hypoxia (CIH). A working model has been developed suggesting that, although these forms of plasticity differ in time course and in their requirement for gene transcription, they are initiated by the same sequence of events. The model may have global implications, representing a mechanism of plasticity common to other neural systems.

RESPIRATORY RESPONSES TO HYPOXIA

Recent reviews have emphasized the complex, time-dependent mechanisms observed during and after hyp-
oxia (16, 88). For example, the response during short-term hypoxia (minutes) consists of at least three distinct mechanisms in rats: 1) the acute response, 2) short-term potentiation, and 3) short-term depression (42, 88). Collectively, these responses constitute the short-term hypoxic ventilatory (or phrenic) response. After a single, brief hypoxic exposure (5 min), respiratory motor activity returns to prehypoxic levels within 10–15 min in anesthetized rats (6). On the other hand, successive episodes at 5-min intervals lead to a long-lasting (>1 h) posthypoxia facilitation of respiratory motor output referred to as LTF (32, 42, 69, 88). Numerous other mechanisms contributing to ventilation during or after hypoxia of different durations or exposure patterns can be discerned (88), but an extensive discussion of these mechanisms is beyond the scope of this review.

**LTF IN DIFFERENT EXPERIMENTAL PREPARATIONS**

LTF was first described in anesthetized cats that received episodic electrical stimulation of the carotid sinus nerve (75), thus demonstrating that LTF is a central (vs. peripheral) neural mechanism. Since that time, a number of reports utilized episodic carotid sinus nerve stimulation, chemoreceptor activation, or episodic hypoxia to elicit LTF in a range of experimental preparations. In anesthetized, vagotomized cats, LTF was observed after electrical stimulation of the carotid sinus nerve in phrenic (30, 75, 76) and parasternal inspiratory intercostal nerve activity (30), as well as hypoglossal and nasal dilator activity and tidal volume (66). In each of these studies, LTF was observed for at least 1 h after stimulation. The protocol used to elicit LTF was high-frequency electrical stimulation of the carotid sinus nerve (25 Hz), a rate exceeding the range of discharge frequencies in carotid chemoefferent neurons during hypoxia (cf. Ref. 29). Phrenic LTF has been reported to last at least 10 min after episodic activation of carotid chemoefferent neurons with close, intra-arterial injections of CO2-saturated solutions (79, 80), but there have been no complete studies using episodic hypoxia in anesthetized cats. An additional potential confound in these studies is that the CO2-apneic threshold differs widely among respiratory motor outputs in anesthetized cats. Thus, to generate rhythmic, respiratory activity in the inspiratory intercostal (30) or hypoglossal motor output (48, 66), baseline CO2 levels were established well above the apneic threshold for phrenic activity. Thus phrenic LTF was minimized or obscured in studies focused on other respiratory motor outputs (30, 48, 66). There is also evidence that an intact vagus nerve minimizes or delays the manifestation of hypoglossal LTF, although not LTF of tidal volume, in anesthetized cats (66). Thus a number of experimental details potentially influence the expression of LTF in anesthetized cats, even when the same stimulation protocol is used.

LTF has been investigated extensively in rats. In anesthetized rats, electrical stimulation of the carotid sinus nerve elicits a relatively short and small phrenic LTF (42, 62). Most studies used episodic hypoxia in anesthetized rats as an experimental model and generally revealed substantial phrenic LTF lasting for at least 1 h after hypoxia (7–9, 33, 34, 42, 54, 55). Hypoxia-induced LTF has also been reported for hypoglossal motor output in anesthetized rats (7–9, 33), although there is considerable variation among rat substrains (32, 33). Again, there are important confounding influences in different experimental preparations, as exemplified by the inability to demonstrate phrenic LTF in anesthetized, spontaneously breathing rats (47) or in anesthetized, ventilated rats with cerebellectomy (42). Nevertheless, we have recently demonstrated ventilatory LTF in unanesthetized, spontaneously breathing rats (83; see Fig. 1), indicating that LTF is expressed in rats under physiological conditions. Regardless, the manifestations of LTF in rats are varied, and these differences may reflect different mechanisms (see WORKING MODEL).

LTF tends to be smaller, shorter, and more difficult to elicit in unanesthetized animals but has been demonstrated following episodic hypoxia in awake dogs (21), goats (104), ducks (78), and rats (83). On the other hand, ventilatory LTF was not observed in normal awake or sleeping humans (3, 70), although humans with inspiratory flow limitation exhibit ventilatory LTF after episodic hypoxia during non-rapid eye movement sleep (3). Furthermore, a preliminary report sug-

![Fig. 1. Expression of long-term facilitation (LTF) following episodic stimulation in 4 experimental rat preparations. All data for respiratory amplitude (peak integrated phrenic activity or tidal volume) have been normalized as a percent change from baseline, allowing for direct comparisons of poststimulation responses. Central nervous system (CNS) stimulation indicates the phrenic nerve response after episodic electrical stimulation of the carotid sinus nerve in anesthetized, vagotomized and ventilated rats (composite of Refs. 42 and 62). Anesth. artificial vent. (H), phrenic nerve responses following three 5-min hypoxic episodes in anesthetized, vagotomized and pump-ventilated rats (composite of Refs. 9 and 33). Unanesthetized (H), tidal volume response of unanesthetized rats following five 5-min hypoxic episodes (data from Ref. 83). Anesth. spontaneous vent. (H), esophageal pressure response in anesthetized, vagotomized, spontaneously breathing rats (data from Ref. 47). Arterial PCO2 was held isocapnic with respect to baseline levels in each case with the exception of the unanesthetized, spontaneously breathing rats, which were poikilocapnic. These comparative results indicate that LTF varies with the experimental protocol in its magnitude and time course, even within the same species and strain.
gested that patients with obstructive sleep apnea exhibit hypoxia-induced LTF when awake (71). This latter observation, if confirmed, is consistent with concepts of “metaplasticity” in LTF (see Metaplasticity of LTF: Chronic Intermittent vs. Sustained Hypoxia) and suggests that caution should be exercised before ruling out the existence of LTF in any species or experimental preparation. The absence of LTF in one circumstance does not rule out its expression in another.

Although most species and preparations exhibit LTF to some degree, there are quantitative differences in magnitude and time course that raise questions as to whether these reports are in fact manifestations of the same underlying mechanism. For comparison, the magnitude and time course of phrenic or ventilatory LTF reported in rats under several experimental conditions or with different stimulation protocols are illustrated in Fig. 1. After episodic stimulation of the carotid sinus nerve (42, 62), phrenic LTF is of modest size and exhibits a progressively decrementing pattern, suggesting a duration of <1 h. Similar patterns have been observed in awake dogs (21), goats (104), and ducks (78) after episodic hypoxia but not in anesthetized cats with carotid sinus nerve stimulation (75, 76). In contrast, phrenic LTF reveals a progressively augmenting pattern after episodic hypoxia in anesthetized rats (8, 9, 32). Although a similar pattern has been observed in awake rats exposed to episodic hypoxia (83), the apparent magnitude of ventilatory LTF was less in these experiments. The magnitude difference between phrenic LTF in anesthetized rats and ventilatory LTF in unanesthetized rats may arise from the nonisocapnic conditions in the latter group. Thus hypocapnia and the associated CO2-chemoreceptor feedback may have reduced the apparent ventilatory LTF. When arterial blood gases were restored to normal 60 min after episodic hypoxia, the magnitude of LTF in anesthetized and unanesthetized rats was similar. Another contrast is the report of Janssen and Fregosi (47), which indicated minimal phrenic LTF in anesthetized but spontaneously breathing rats. The most likely explanation for the minimal phrenic LTF in these experiments was the relatively high level of arterial CO2 in relation to the CO2-apneic threshold for rhythmic phrenic motor output (47).

SUSTAINED VS. INTERMITTENT HYPOXIA

There are fundamental differences between sustained and intermittent hypoxia in terms of their influence on respiratory control. For example, although LTF is elicited by episodic hypoxia in anesthetized rats, it is not observed after equivalent durations of sustained hypoxia (9). Similarly, acute intermittent isocapnic hypoxia elicits ventilatory LTF in awake goats (104), but sustained hypoxia does not, even with longer cumulative exposures (25, 28).

GENETIC INFLUENCES ON LTF

The manifestation of LTF varies widely among genetic populations of rats, even within the same strain (32, 33). For example, the magnitude of phrenic LTF varies between Sprague-Dawley rats from the same supplier but from different colonies (Harlan Sprague Dawley; colony 236 vs. 205), even when the same experimental protocol was used (32). In a blinded experiment, we compared hypoglossal LTF between Sprague-Dawley rats from two different suppliers (Harlan Sprague Dawley colony 236 vs. Charles River/Sasco colony K62) and found that it was present only in rats from Charles River/Sasco (33). Despite their common origins, years of genetic drift in these isolated populations of Sprague-Dawley rats apparently caused significant differences in the anatomy (22) and physiology of monoaminergic systems. Thus, in studies of LTF or any form of plasticity, it is essential to consider genetic influences. There may be overt differences between seemingly similar genetic strains. Considerable variation is also to be expected in genetically diverse populations (such as humans). Regardless, the absence of LTF in an individual does not ensure that it cannot be expressed under different physiological circumstances (see discussion of metaplasticity below).

AGE AND GENDER INFLUENCES ON LTF

Age and gender affect the expression of phrenic and hypoglossal LTF in complex ways. Virtually all of the studies on rats reported above were conducted on young adult (3–4 mo) male rats of the Sprague-Dawley strain. However, we have recently found that phrenic LTF is greatly reduced and hypoglossal LTF is virtually eliminated in middle-aged (>12 mo) male rats when using the same experimental protocol (109). In female rats, LTF is affected by the estrus cycle, being larger in diestrus than in estrus (110). Furthermore, regardless of the estrus cycle, phrenic and hypoglossal LTF actually increase middle-aged (>12 mo) female rats relative to young adults (110). Thus advancing age, gender, and estrus cycle are important considerations in any study of LTF, its manifestations, or its significance. The striking age–gender interaction, particularly in the control of LTF in hypoglossal motor output, bears a strong similarity to the incidence of obstructive sleep apnea in the human population (17), although the lack of menopause in female rats must be accounted for. Nevertheless, pending specific investigations concerning possible links between age, gender, LTF (or a lack of LTF), and the incidence of obstructive sleep apnea in males and females, firm conclusions cannot be made.

There are no reports concerning the existence of LTF in developing mammals. However, intermittent hypoxia alters the subsequent short-term hypoxic response in piglets (81, 107) and neonatal rats (40, 41). In piglets, episodic hypoxia decreases the short-term hypoxic ventilatory response relative to that observed during continuous hypoxia of equal cumulative duration (81, 107). In neonatal rats, intermittent hypoxia diminishes ventilatory roll off during the hypoxic episodes by a mechanism associated with nitric oxide (40, 41). In neither case was the ventilatory response after
intermittent hypoxia reported. Such posthypoxic measurements would be of considerable interest given the profound influence of age on the expression of LTF (see above) and the high incidence of intermittent hypoxia in the newborn (see Refs. 40, 81).

**METAPLASTICITY OF LTF: CHRONIC INTERMITTENT VS. SUSTAINED HYPOXIA**

CIH augments the short-term hypoxic ventilatory response in humans (36, 51, 94, 96, 97) and the short-term hypoxic phrenic response in rats (60, 61). We recently demonstrated that enhanced CNS integration of carotid chemosensitive inputs is sufficient to explain this increase in the short-term hypoxic phrenic response (59), although an additional effect at the carotid body chemoreceptors cannot be ruled out. CIH also elicits a form of metaplasticity (1, 19, 53), manifested as enhanced phrenic LTF following three subsequent hypoxic episodes (61). Another interesting effect of CIH is the induction of hypoglossal LTF in a rat substrain that does not ordinarily express significant hypoglossal LTF (Ref. 61 and Baker, Zabka, and Mitchell, unpublished observations). Additional evidence for metaplasticity in LTF is observed following cervical dorsal rhizotomy, a procedure that enhances both phrenic and hypoglossal LTF following episodic hypoxia (4, 55). The existence of metaplasticity in respiratory motor control indicates that an absence of LTF in any given respiratory motor output or experimental circumstance does not constitute evidence that LTF cannot be induced in that same motor output after suitable preconditioning (e.g., CIH and dorsal rhizotomy).

There are interesting reports that suggest that metaplasticity may play a role in circumstances such as disordered breathing during sleep. For example, as mentioned above, patients with obstructive sleep apnea expressed ventilatory LTF when awake, whereas normal awake subjects did not (71). Similarly, when obstructive sleep apnea patients are treated with continuous positive airway pressure to reduce the incidence of apneas, the awake hypoxic ventilatory response decreases (103), an effect one would predict when CIH (apneic episodes) are discontinued. Regardless of its significance to disordered breathing during sleep, further investigations are warranted to elucidate the mechanisms and manifestations of metaplasticity elicited by CIH in the neural control of breathing.

Chronic sustained hypoxia, in contrast to CIH, has been extensively studied and elicits ventilatory acclimatization, characterized by a progressive augmentation of ventilation at rest and an increased short-term hypoxic ventilatory response (15, 16, 87). In some models, carotid body hypoxia is both necessary and sufficient to elicit ventilatory acclimatization (16). Because a progressive rise in carotid chemosensitive discharge frequency is observed during sustained hypoxia (14, 16), it appears that chronic sustained hypoxia predominantly elicits plasticity within the carotid body (vs. CNS). However, additional effects within the CNS cannot be ruled out, particularly with more severe or prolonged hypoxia (87). Indeed, the central integration of carotid chemosensitive neurons appears to be increased in rats exposed to 7 days of sustained hypoxia (26). On the other hand, a preliminary report suggests that phrenic LTF induced by carotid sinus nerve stimulation is unchanged after 7 days of sustained hypoxia (27), a clear difference from the enhanced phrenic LTF following CIH. We suggest that CIH is more effective at eliciting (serotonin dependent) CNS plasticity, whereas sustained hypoxia has greater effects on the carotid body, acting via distinct cellular mechanisms.

**MECHANISM(S) OF LTF**

Since the first demonstrations of LTF by Millhorn and colleagues (74–76) in anesthetized or decerebrated cats, most studies concerning its mechanism(s) have been conducted on young adult male Sprague-Dawley rats that were anesthetized, paralyzed, vagotomized, and artificially ventilated. Thus the following discussion must be considered largely in the context of hypoxia-induced LTF in anesthetized rats pending verification in other model systems.

**LTF is a central neural mechanism.** Because LTF can be elicited by episodic activation of chemosensitive neurons in the carotid sinus nerve of anesthetized, vagotomized, and ventilated cats or rats (42, 62, 75, 76), it is a central neural mechanism that does not require hypoxia per se. On the other hand, a degree of LTF is still observed following episodic hypoxia in carotid-denervated cats (35) and rats (Bavis and Mitchell, unpublished observations). Thus LTF may consist of at least two distinct mechanisms, one associated with the activation of synaptic pathways by chemosensitive neurons and another attributable to hypoxic effects on CNS neurons.

**LTF requires serotonin receptor activation.** Millhorn et al. (76) demonstrated that LTF is serotonin dependent because it could be blocked by a serotonergic neurotoxin (5,7-dihydroxytryptamine), a serotonin depleter (p-chlorophenylalanine), and a broad-spectrum serotonin receptor antagonist (methysergide). Furthermore, electrical activation of raphe pallidus or raphe obscurus elicits a degree of LTF (74). LTF elicited by episodic hypoxia in rats also requires the activation of serotonin receptors (8), most likely of the 5-HT2 receptor subtype (54). Recent studies demonstrated that serotonin receptor activation is necessary during but not after hypoxia, indicating that serotonin receptor activation is necessary for the induction but not maintenance of LTF (34). Furthermore, the relevant receptors for phrenic LTF have now been localized to the spinal cord because intrathecal methysergide administration blocks phrenic but not hypoglossal (i.e., cranial) LTF (11). Thus we suggest that the relevant serotonin receptors are located within the phrenic motor nucleus, either on or in the immediate vicinity of phrenic motoneurons. By inference, LTF in other respiratory motor pools, including hypoglossal motoneurons (8) and inspiratory intercostal motoneurons (30), most likely...
requires serotonin receptor activation within these respective motor nuclei.

Because ketanserin pretreatment blocks LTF (34, 54) and ketanserin has a 30- to 100-fold greater affinity for 5-HT$_{2A}$ vs. 5-HT$_{2C}$ receptors (38, 44, 45, 112), we suggest that 5-HT$_{2A}$ receptors initiate LTF. This suggestion is supported by the observation that labeled phrenic motoneurons express 5-HT$_{2A}$ but not 5-HT$_{2C}$ receptors (G. J. Basura and H. G. Goshgarian, personal communication).

The involvement of serotonin in the mechanism of LTF does not rule out contributions from other neurotransmitter systems. For example, there are indications that genetically manipulated mice deficient in nitric oxide synthase are unable to express LTF, suggesting a role for nitric oxide (56).

**LTF requires spinal protein synthesis.** We recently demonstrated that spinal administration of protein synthesis inhibitors abolishes phrenic LTF (10). Specifically, intrathecal administration of emetine and cyclohexamide blocked phrenic LTF but had no effect on hypoglossal LTF in the same animals. The continued LTF of hypoglossal output suggests that effective concentrations of the protein synthesis inhibitors were restricted to the spinal cord. Indeed, if larger doses of emetine were delivered, both phrenic and hypoglossal LTF were abolished, consistent with systemic distribution of the higher drug dose (Baker and Mitchell, unpublished observations).

**LTF is associated with increased ventral spinal brain-derived neurotrophic factor synthesis.** Although the relevant spinal proteins in the mechanism of LTF are unclear, we hypothesize that the neurotrophin brain-derived neurotrophic factor (BDNF) plays a pivotal role. BDNF is a member of the neurotrophin family (nerve growth factor, neurotrophin-3, and neurotrophin-4/5). Neurotrophins mediate their effects via receptor tyrosine kinases (TrK), and the relevant BDNF receptor is TrK-B. BDNF is necessary and sufficient for several important models of synaptic plasticity (cf. Refs. 18, 58, 86, 93, 101), such as hippocampal long-term potentiation.

Complex interactions exist between serotonin and BDNF. For example, BDNF is a potent neurotrophic factor for serotonergic neurons, promoting their phenotypic elaboration (63, 64) and increasing serotonin levels and turnover (2, 98) by increasing tryptophan hydroxylase expression (99). Conversely, pharmacological manipulations of brain serotonin levels affect BDNF mRNA expression. Increased serotonin availability increases BDNF mRNA in some brain regions (frontal cortex), whereas it decreases it in other regions (dentate gyrus) (111), quite possibly by the activation of 5-HT$_{2A}$ receptors (105). Collectively, there is considerable evidence that BDNF has the potential to play a key role in serotonin-dependent plasticity.

In support of the hypothesis that BDNF plays a key role in serotonin-dependent LTF, we demonstrated that episodic hypoxia (3 episodes of 5-min duration) increases ventral spinal BDNF protein levels in the cervical segments associated with the phrenic motor nucleus (Baker and Mitchell, unpublished observations). Increased BDNF concentrations were observed 60 min after the final hypoxic episode, suggesting that it has an appropriate time course for involvement in LTF. The rapid time course further suggests the possibility of increased translation from existing BDNF mRNA vs. transcriptional regulation (37, 102). Increased ventral spinal BDNF concentrations were abolished by intrathecal administration of a serotonin receptor antagonist (methysergide) or protein synthesis inhibitor (emetine), indicating a strong correlation with the incidence of LTF. Although these results are strictly correlative, they do suggest that BDNF has the relevant characteristics to play a causal role in the mechanism of LTF.

**MECHANISM OF CIH-INDUCED PLASTICITY**

Systemic administration of either methysergide (60) or ketanserin (5) abolished the CIH-induced increase in the short-term hypoxic phrenic response in anesthetized rats, suggesting that serotonin receptor activation is necessary in the underlying mechanism. On the other hand, although systemic methysergide abolished enhanced phrenic LTF following CIH (60), the selective 5-HT$_{2}$ receptor antagonist ketanserin only partially reversed LTF enhancement (5). Thus, although serotonin receptor activation is necessary for enhanced phrenic LTF following CIH, 5-HT$_{2}$ receptors no longer account for the entire effect. We suspect that the injection of novel serotonin receptors and increased BDNF levels via transcriptional regulation play key roles in the mechanism of enhanced phrenic LTF following CIH.

An additional distinction between the mechanisms of CIH and ventilatory acclimatization to sustained hypoxia is revealed by the fact that rats exhibited normal ventilatory acclimatization to sustained hypoxia after serotonin depletion with p-chlorophenylalanine (84). In contrast, methysergide completely blocked the CIH-induced augmentation of the short-term hypoxic phrenic response and LTF following CIH. Thus it appears that the effects of CIH require an augmentation of serotonergic modulation, whereas the effects of chronic sustained hypoxia do not.

**OTHER MODELS OF PATTERN-SENSITIVE PLASTICITY**

The concept that intermittent (spaced) vs. sustained (massed) stimulation is more effective at eliciting certain forms of neuroplasticity has been established in a number of experimental paradigms, including serotonin-dependent synaptic plasticity in *Aplysia* (67), conditioned foot contractions in *Hermesenda* (82), olfactory conditioning in *Drosophila* (13) and honeybees (92), habituation to danger stimuli in crabs (31), contextual fear conditioning, spatial learning, and socially transmitted food preferences in mice (57), and some forms of hippocampal synaptic plasticity (46). The concept that some forms of plasticity require prior experience with the same stimulus has also been established in a number of experimental models, including stress-
induced plasticity in the catecholaminergic nervous system (91). Conversely, there appear to be other distinct forms of plasticity that are elicited preferentially by sustained stimuli. For example, increased carotid body sensitivity to hypoxia is more effectively elicited by sustained vs. intermittent hypoxia (14, 16). In association, sustained hypoxia appears to be a more powerful activator of the transcription factor cAMP response element binding protein in the carotid body than intermittent hypoxia (106).

Although the mechanisms underlying profound differences between intermittent and sustained stimuli in initiating plasticity are not clear, they may arise from unique cellular properties elicited by cytosolic calcium oscillations. Calcium oscillations reduce the effective threshold for the activation of transcription factors and thus of gene expression (24, 72). Furthermore, calcium-sensitive kinases can become autophosphorylated (89), leading to elevated and sustained activation following rapid calcium spikes. Thus intermittent calcium spikes (triggered by 5-HT2A receptor activation) may be more efficient than steady levels at inducing plasticity. In addition, the induction of gene transcription associated with some forms of neuroplasticity is elicited most effectively when the same stimulus has been presented at an earlier time (e.g., Ref. 91). Recent evidence indicates that the transcriptional regulation of BDNF exhibits just such pattern-sensitive behavior (see Fig. 3).

**WORKING MODEL**

In Fig. 2, a working model is presented that suggests that common events in phrenic motoneurons initiate LTF and enhance LTF after CIH. This model may help to explain some of the apparent differences in LTF observed in different experimental paradigms (Fig. 1). In our model, LTF and enhanced LTF are initiated by repeated serotonergic 5-HT2A receptor activation on phrenic motoneurons, thereby increasing intracellular kinase activity (largely protein kinase C). As a result, glutamatergic receptors associated with descending respiratory drive are phosphorylated (or upregulated), increasing glutamate-induced currents and phrenic motor output for the same descending respiratory drive (i.e., LTF). Direct interactions between kinases and glutamate receptors could underlie shorter versions of LTF, as exhibited with intermittent carotid sinus nerve stimulation (Fig. 1). Direct kinase actions on glutamate receptors are expected to be of a shorter duration, decrementing, and similar in many respects to short-term facilitation in Aplysia, a model of synaptic facilitation (minutes) that requires protein kinase C activation (20).

Direct interactions between the kinases and glutamate receptors cannot account for longer-lasting forms of LTF, since spinal application of protein synthesis inhibitors blocks LTF following intermittent hypoxia (10). The more robust protein synthesis-dependent form(s) of LTF that occurs as a result of intermittent hypoxia in anesthetized rats (Fig. 1) is associated with increased ventral spinal BDNF synthesis. We postulate that increased BDNF synthesis results from increased translation of preexisting BDNF mRNA because of its relatively rapid time course (i.e., 60 min after hypoxia). In its time course and dependence on protein synthesis via translational regulation, phrenic LTF is similar to serotonin-dependent intermediate-term facilitation in Aplysia (39, 68).

If intermittent hypoxia and the associated 5-HT2A receptor activation continue, additional mechanisms are initiated, leading to enhanced phrenic LTF. We postulate that enhanced phrenic LTF following CIH requires increased gene transcription and that the resulting CIH-induced gene transcription increases the expression of at least two proteins necessary for enhanced phrenic LTF: 1) BDNF and 2) a novel serotonin receptor(s) that accounts for the inability of ket-
BDNF plays a key role in respiratory plasticity following intermittent hypoxia (via protein kinase A and Trk-B, respectively), thereby enhancing phrenic LTF.

Recent evidence suggests that BDNF gene transcription exhibits the pattern sensitivity necessary to play a key role in LTF and enhanced LTF following CIH. In Fig. 3, pattern-sensitive transcriptional activation of BDNF genes is illustrated for cultured cortical neurons transfected with the first promoter region of the BDNF gene and a luciferase reporter gene (Nanda and Mack, unpublished observations). In these neurons, activity-dependent transcriptional activation was investigated 24 h after episodic exposure to glutamate (three 5-min episodes, 15-min interval) and continuous glutamate exposures of similar cumulative duration (15 min and 60 min). In each case, data are presented as a percentage of control, 24 h after the beginning of glutamate exposure. Continuous glutamate exposures scarcely increased transcription, whereas episodic glutamate increased BDNF gene transcription severalfold. Thus BDNF is a neuroactive molecule exhibiting the pattern-sensitive transcriptional regulation necessary for a prominent role in neuroplasticity following CIH. Activity-dependent BDNF release from vagal and petrosal sensory neurons is also more effectively triggered by episodic vs. continuous stimulation (12). Collectively, these data establish a powerful precedent for pattern-sensitive gene expression and release of a neuroactive substance that plays a major role in several forms of synaptic plasticity (18, 58, 86, 93, 101).

In the final analysis, each time domain of LTF predicted in this discussion is orchestrated by kinases that were activated by serotonin receptors on phrenic motoneurons. If intermittent hypoxia and subsequent serotonin release persists, these kinases initiate mechanisms leading to increased protein synthesis necessary to consolidate longer-lasting forms of respiratory plasticity. Although we focused on relatively few proteins in this model, it is likely that other proteins will be necessary in the mechanism(s) underlying LTF and/or enhanced phrenic LTF following CIH. This model is under continual refinement (e.g., see Ref. 32) and will, we hope, ultimately lead to a better understanding of these potentially important mechanisms of respiratory plasticity.

**SIGNIFICANCE**

Respiratory plasticity is now recognized in a number of circumstances, including the effects of hypoxia, exercise, neural injury, and developmental experience. By comparing and contrasting diverse models of plasticity in respiratory motor control, we hope that fundamental principles will emerge, providing a more comprehensive picture of respiratory plasticity, its mechanisms, and its importance in the control of breathing. We have already started to suspect common elements in different models of plasticity in adults because serotonin and (we suspect) BDNF appear to play key roles in respiratory plasticity after intermittent hypoxia, dorsal rhizotomy (50, 55), and may be critical in long-term modulation of the exercise ventilatory response (Refs. 49, 65, 77; R. A. Johnson and G. S. Mitchell, unpublished observations). Common features in these diverse models of plasticity may suggest that a fundamental mechanism underlies many forms of plasticity in the CNS, particularly the ventral spinal cord in which few examples of plasticity are known. We hypothesize that an important but unrecognized mechanism of CNS plasticity is a change in the capacity for (serotonergic and BDNF) neuromodulation. To our knowledge, such a mechanism has not been proposed in any vertebrate neural system, although there is precedent in the invertebrate literature (52).

By understanding these mechanisms, we may gain insights into natural compensatory mechanisms during disease and the rationale for therapeutic intervention (e.g., pharmacological or physical therapy). Diseases of relevance to respiratory motor control that may involve (or be treated by utilizing) the capacity for respiratory plasticity in adult animals include breathing disorders during sleep (100), the onset of lung disease, sudden infant death syndrome (90), congenital central hypoventilation syndrome (73, 108), respiratory impairment after spinal cord injury, parkinsonism (95), and anxiety hyperventilation disorders (23, 85).

Although we do not yet know the specific relevance of phrenic LTF or enhanced phrenic LTF following CIH in

![Fig. 3](http://jap.physiology.org/)

**Exposure Pattern**

Fig. 3. In dissociated cortical neurons, the BDNF promoter 1 region was transfected along with a luciferase reporter gene. The cultures were exposed to glutamate in different patterns, presumably increasing neuronal activity. Glutamate exposures were 15 and 60 min continuous and episodic (three 5-min exposures with a 15-min washout interval). All data represent the response 24 h after the onset of glutamate exposures, expressed as a percentage of controls. Neither 15 nor 60 min continuous exposure significantly increased BDNF gene transcription. In contrast, episodic glutamate exposure increased BDNF transcriptional activity severalfold. These data (Nanda and Mack, unpublished observations) establish a precedent for pattern-sensitive gene expression in the CNS and demonstrate that the BDNF gene exhibits the pattern sensitivity necessary to play a key role in respiratory plasticity following intermittent hypoxia.
any physiological or pathophysiological state, we nevertheless view intermittent hypoxia as a suitable model to study the capacity for and mechanisms of respiration-related plasticity in the CNS. Only by thorough investigations of their mechanisms and manifestations will an appreciation of their biological significance be gained.

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