

# Layers of exercise hyperpnea: Modulation and plasticity<sup>☆</sup>

Gordon S. Mitchell<sup>a,\*</sup>, Tony G. Babb<sup>b</sup>

<sup>a</sup> *Department of Comparative Biosciences, University of Wisconsin, 2015 Linden Drive, Madison, WI 53706, USA*

<sup>b</sup> *Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas and University of Texas Southwestern Medical Center, Dallas, TX 75231, USA*

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## Abstract

Despite the fundamental biological significance of the ventilatory response to mild or moderate physical activity (the exercise hyperpnea), we still know remarkably little concerning its underlying mechanisms. Part of the difficulty in revealing those mechanisms may arise due to confusion between multiple mechanistic layers, each contributing to the impressive degree of regulation achieved. The primary, feedforward exercise stimulus or stimuli increase ventilation in approximate proportion to changes in metabolic rate. Chemoreceptor feedback then minimizes deviations from optimal blood gas regulation, most often preventing excessive hypocapnia in non-human mammals. Recent evidence has accumulated, suggesting that adaptive control strategies including modulation and plasticity may adjust the feedforward and/or feedback contributions when blood gas homeostasis proves inadequate. In this review, we present evidence from a goat model of exercise hyperpnea concerning the existence of modulation and plasticity, and specifically mechanisms known as short-term and long-term modulation of the exercise ventilatory response. Throughout the review, we consider available evidence concerning the relevance of these mechanisms to humans. Plasticity is a property only recently recognized in the neural system subserving respiratory control, and the application of these concepts to the exercise ventilatory response in humans is in its infancy. Modulation and plasticity may confer an ability of individuals to adapt their exercise ventilatory response so that it remains appropriate in the face of life-long changes in endogenous (e.g. development, aging, onset of disease) or exogenous (e.g. altitude, wearing a breathing apparatus during physical exertion) physiological conditions.

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\* Corresponding author. Tel.: +1 608 263 9826; fax: +1 608 263 3926.

E-mail address: [mitchell@svm.vetmed.wisc.edu](mailto:mitchell@svm.vetmed.wisc.edu) (G.S. Mitchell).

## 1. Introduction

The ventilatory response to mild or moderate exercise (the “exercise hyperpnea”) is the largest ventilatory adjustment during a normal human life, and is critical for even the most mundane of daily activities (e.g. walking). Despite its fundamental importance, our

understanding of the mechanisms underlying exercise hyperpnea remains poor at best, and has advanced little during the past 55 years (Grodins, 1950; Dempsey et al., 1985; Haouzi, 2006). Investigators may have been discouraged by the collective weight of either negative results, or correlative results without demonstration of causality, from experiments to test several interesting and reasonable hypotheses between the 1960s and 1980s. Because of the lack of clear progress, it seems that few laboratories currently focus their research efforts on the mechanisms of exercise hyperpnea. Despite the relative paucity of new conceptual insights, the exercise hyperpnea remains a fascinating phenomenon of fundamental importance to life.

Perhaps because of the relative lack of research in recent years, studies of exercise hyperpnea, ultimately a neural process, have scarcely benefited from the rapidly evolving techniques and perspectives of modern neurobiology. Indeed, exceptional progress in basic neurobiology during the past two decades may give reason for optimism that an understanding of exercise hyperpnea and its mechanisms is attainable. It may be timely to revisit the question: how does ventilation increase during mild or moderate physical activity?

Critical questions necessary to understand exercise hyperpnea concern: (1) the primary, feedforward stimulus to increased breathing during mild or moderate exercise, (2) sensory feedback mechanisms that correct errors in the primary response, and (3) adaptive control strategies that enable the system to adjust when confronted with changing physiological (e.g. disease or aging) or environmental conditions (e.g. altitude). Although the former topics are of great importance, and have framed the discussion for decades, the focus of this review will concern less well-known properties of the respiratory control system, including the adaptive control strategies of modulation and plasticity in the exercise ventilatory response (Mitchell and Johnson, 2003).

Although neural mechanisms controlling breathing during exercise have traditionally been regarded as fixed and immutable, compelling evidence has accumulated in recent years, demonstrating that the neural system subserving respiratory control exhibits considerable capacity for modulation and plasticity (Feldman et al., 2003; Mitchell and Johnson, 2003; Forster, 2003; Goshgarian, 2003; Carroll, 2003; Morris et al., 2003). However, these properties cannot be understood with-

out an appreciation for the fundamental feedforward and feedback mechanisms of exercise hyperpnea, since these neuronal mechanisms are the substrate for modulation and plasticity. Conversely, it may be very difficult to study the feedforward and/or feedback mechanisms of exercise hyperpnea without an appreciation for modulation and plasticity, since these features may confuse attempts to assess the feedforward stimulus—it may be a “moving target.” Thus, we view the exercise hyperpnea as a complex integration or “layering” of mechanisms, each operating in unique time domains, governed by different cellular, synaptic or network neural mechanisms, and triggered in different physiological or environmental circumstances to varied degrees. The different mechanisms may contribute to blood gas homeostasis by successive approximation: a primary stimulus that is adjusted by feedback, with both tailored over longer time domains by mechanisms of plasticity.

## 2. Multiple mechanisms (the “layers”) of exercise hyperpnea

A general schema representing feedforward, feedback and adaptive control mechanisms, and their relationship to other important aspects of the respiratory system are illustrated in Fig. 1A. There are at least five identifiable mechanisms contributing to the ventilatory response during mild to moderate exercise, each revealed under different experimental circumstances (Mitchell et al., 1993). Three of these mechanisms are forms of adaptive control.

### 2.1. Feedforward exercise stimulus

The primary exercise stimulus operates in a feedforward manner with respect to arterial PCO<sub>2</sub> regulation (Houk, 1988; Bennett and Fordyce, 1985, 1988; Mitchell, 1990) and remains poorly understood despite decades of study. Many investigations have focused on the hypothesis that the primary stimulus results from a neurogenic component, arising in the central nervous system or periphery, and most likely represents a combination of factors (Bennett and Fordyce, 1985, 1988; Dempsey et al., 1984, 1985; Whipp and Ward, 1982; Eldridge, 1994; Forster, 2000; for recent update, see Haouzi, 2006). For this discussion, the mechanisms contributing to the primary or feedforward exercise

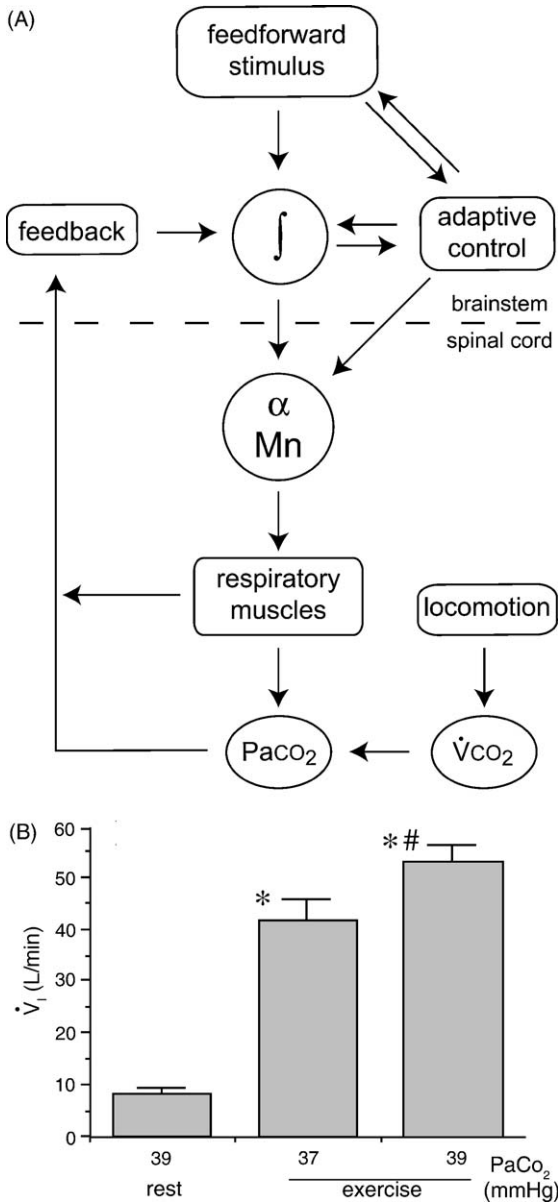


Fig. 1. (A) Schema of mechanisms (“layers”) contributing to the exercise ventilatory response during mild or moderate exercise. The primary stimulus or stimuli acts in a feedforward manner with respect to the regulation of arterial PCO<sub>2</sub>, providing an approximate match between the increase in ventilation and metabolic rate induced by exercise (i.e. locomotion). Any mismatch between increased metabolic rate and the feedforward ventilatory stimulus will be attenuated by sensory feedback (primarily CO<sub>2</sub>-chemoreceptors), restraining ventilation if the feedforward stimulus is excessive, and boosting it if the feedforward stimulus is inadequate. Feedforward and feedback mechanisms are integrated in the CNS by brainstem

stimulus will remain an interesting and important mystery. The essential feature of the stimulus (or stimuli) that constitute the primary drive to breathe during exercise is that it operates in a feedforward manner with respect to arterial PCO<sub>2</sub> regulation, and tracks changes in the metabolic carbon dioxide production induced by exercise. In most terrestrial vertebrates, the magnitude of the feedforward exercise stimulus is slightly in excess of that needed to maintain homeostasis of arterial PCO<sub>2</sub>; thus, the level of PaCO<sub>2</sub> declines slightly from rest to exercise (Dempsey et al., 1985). In humans, on the other hand, the feedforward stimulus is a variable function of exercise, and appears to be slightly underdetermined during mild exercise, precisely matched to metabolic rate during moderate exercise, and then slightly excessive during heavier exercise, even before the lactic acid threshold is reached (Clark et al., 1980; Dempsey et al., 1984, 1985; Bennett and Fordyce, 1985, 1988).

## 2.2. Chemoreceptor feedback

If arterial PCO<sub>2</sub> increases or decreases due to any mismatch between the primary, feedforward exercise stimulus, and the increase in metabolic carbon dioxide production, CO<sub>2</sub>-chemoreceptor feedback adjusts the exercise ventilatory response, thereby minimizing changes in arterial PCO<sub>2</sub> from rest to exercise (Babb, 1997a,b; Babb et al., 2003; Dempsey et al.,

respiratory neurons (integral sign), and at the level of the spinal cord via the respiratory motoneurons (αMn), ultimately activating the respiratory muscles. If the regulation of PaCO<sub>2</sub> is disrupted by changes in physiological or environmental conditions, adaptive control strategies are evoked. Such adaptive control strategies include adjustments in short (i.e. modulation) and long time scales (i.e. plasticity). (B) Data from goats (Mitchell, 1990) illustrating the interplay between feedforward and chemofeedback mechanisms in regulating exercise hyperpnea. When the animals exercise on a treadmill, ventilation increases to slight excess in this species due to an overdetermined feedforward stimulus. As a result, PaCO<sub>2</sub> decreases from 39 to 37 mmHg. When PaCO<sub>2</sub> is experimentally restored to 39 mmHg, ventilation increases further indicating that chemofeedback had restrained ventilation, preventing excessive hyperventilation due to the feedforward stimulus. With successive approximations due to feedforward combined with feedback mechanisms, PaCO<sub>2</sub> is regulated with good (not perfect) precision from rest to exercise in this species. Adaptive control strategies are not revealed unless the conditions change, altering the need for respiratory motor output to assure adequate PaCO<sub>2</sub> regulation.

1985; Bennett and Fordyce, 1988; Forster et al., 1993; Mitchell, 1990). Without chemoreceptor feedback in non-human mammals, the normal feedforward exercise ventilatory response, if acting alone, is expected to decrease arterial  $\text{PCO}_2$  by more than 10 mmHg (Mitchell, 1990; Dempsey et al., 1985). However, collectively, the primary exercise stimulus and chemoreceptor feedback maintain  $\text{PCO}_2$  with minimal decrease in  $\text{PaCO}_2$  (e.g. 1–3 mmHg) during mild-to-moderate exercise (Bennett and Fordyce, 1985; Mitchell, 1990). The role of chemoreceptor feedback during mild to moderate exercise in goats is illustrated in Fig. 1B. When goats exercise, increasing metabolic rate approximately four-fold, minute ventilation increases to the extent that  $\text{PaCO}_2$  decreases from 39 to 37 mmHg. However, when the level of  $\text{PaCO}_2$  is experimentally restored to 39 mmHg during exercise, minute ventilation significantly increases from poikilocapnic conditions. This increase represents the “braking” action of chemoreceptor feedback on exercise hyperpnea. Oxygen-sensitive chemoreceptors are not expected to play a significant role in these conditions since the prevailing level of  $\text{PaO}_2$  was experimentally elevated, and did not enter a range expected to elicit chemoreflexes in goats (Mitchell, 1990).

In humans, a similar qualitative picture can be drawn, but with the more complex pattern of matching between the feedforward stimulus and changes in metabolic rate; ventilation may be accentuated, unaffected or diminished by chemoreceptor feedback depending the exercise intensity (Bennett and Fordyce, 1985, 1988; Dempsey et al., 1984, 1985; Clark et al., 1980). Thus, in mammalian species, the interaction of an imperfectly matched feedforward stimulus with chemoreceptor feedback maintains arterial  $\text{PCO}_2$  with only minimal disruption of homeostasis, an error signal sufficiently small to make experimental detection of that signal a challenge (Bennett and Fordyce, 1985, 1988; Dempsey et al., 1985).

### 2.3. Modulation and plasticity

If physiological conditions change, for example increasing physiological dead space or increasing/decreasing resting ventilatory drive, an adjustment in the ventilatory response is required to maintain similar regulation of arterial blood gases from rest to exercise (Oren et al., 1981; Mitchell et al., 1984;

Mitchell, 1990; Schaefer and Mitchell, 1989). Without such adjustments, less precise arterial  $\text{PCO}_2$  regulation would result (Mitchell, 1990). Adjustments are made by multiple, potentially overlapping mechanisms that represent forms of modulation and/or plasticity (defined in Mitchell and Johnson, 2003). In the context of this discussion, modulation represents a within-trial alteration of the feedforward or feedback components of exercise hyperpnea due to the actions of neurochemicals that induce neuromodulation. Typical examples of candidate neurochemicals that induce neuromodulation include monoamines (e.g. serotonin and norepinephrine) and many neuroactive peptides (e.g. thyroid releasing hormone, substance-P, etc.). A characteristic feature of neuromodulation is that when the proximate stimulus to neuromodulator release is gone (and changes in the neuromodulator are no longer present in the neural system), the behavior reverts to normal in a relatively short time scale (many seconds). The major differentiating feature between modulation and plasticity is that when the proximate stimulus is removed, and the physiological impact remains for an extended period (minutes to life-long); the persistent effect represents plasticity. Plasticity may arise due to repetitive activation of a synaptic pathway along the neural substrate of exercise hyperpnea (i.e. activity-dependent plasticity), or may be initiated by a neuromodulator (i.e. neuromodulator-induced plasticity; Mitchell and Johnson, 2003). Neuromodulator-induced plasticity appears to make a greater contribution to the neuronal system controlling breathing versus other well-studied systems, such as the mammalian hippocampus, a critical site for cognitive learning (Mitchell and Johnson, 2003). Although little is known concerning the existence and mechanisms of plasticity in the exercise ventilatory response, the mechanisms described to date appear to reflect the general bias of the respiratory control system towards neuromodulator-induced plasticity (e.g.. long-term modulation, see below). Three forms of modulation and plasticity in the exercise ventilatory response are discussed below.

### 2.4. Short-term modulation (STM)

STM is revealed with experimental perturbations that alter resting ventilatory drive and blood gases (Mitchell et al., 1984; Mitchell and Johnson, 2003).

For example, with acute increases in respiratory dead space, the subsequent exercise ventilatory response is increased in goats (Mitchell, 1990) and humans (Poon, 1992), thereby maintaining arterial  $\text{PCO}_2$  regulation with the same precision from rest to exercise (Fig. 2). The neural mechanism increasing the exercise ventilatory response with increased dead space in goats, referred to as *short-term modulation*, results from complex, central neural integration of the feedforward exercise stimulus under the influence of an important neuromodulator, serotonin (Bach et al., 1993; Mitchell et al., 1993). After a single exercise trial with increased dead space, STM is no longer observed after the dead space has been removed (Mitchell, 1990), indicating that it is readily reversible and is therefore a form of modulation versus plasticity (Mitchell and Johnson, 2003). Although STM has been demonstrated in normal human subjects (Poon, 1992), the serotonin-dependence of STM in humans has not been confirmed.

### 2.5. Long-term modulation (LTM)

When goats (Martin and Mitchell, 1993; Turner et al., 1997) and humans (Wood et al., 2003) are exposed to repeated exercise trials (20–70) with increased external dead space (i.e. hypercapnic exercise), a persistent augmentation of the exercise ventilatory response is observed in subsequent exercise trials without dead space (Fig. 6). This persistent augmentation has been referred to as *long-term modulation* (LTM) (Martin and Mitchell, 1993; Turner et al., 1997; Wood et al., 2003) and can be regarded as a form of motor learning or neuroplasticity resulting from enduring changes in system characteristics (Mitchell and Johnson, 2003). Similar to STM, LTM is a serotonin-dependent mechanism in goats (Fig. 7) and may, in fact, result from repeated activation of STM (Johnson and Mitchell, 2001). LTM is a potentially important contributor to exercise hyperpnea in humans (Helbling et al., 1997; Turner and Summers, 2002; Wood et al., 2003), although some investigators have been unable to confirm its existence (Cathcart et al., 2005; Moosavi et al., 2002). More recent, comprehensive studies of LTM suggest that variability in reports of LTM in humans may be due to the limited number of training trials in the earlier, negative studies (Wood et al., 2003).

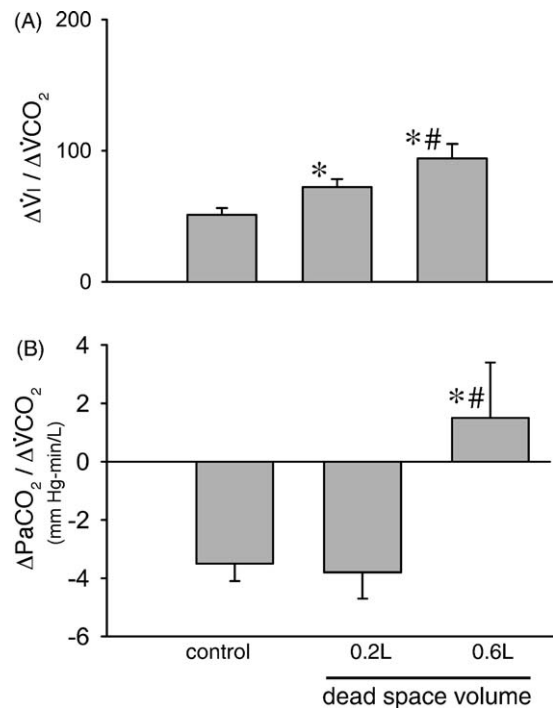


Fig. 2. Short-term modulation (STM) of the exercise ventilatory response with acute increases in respiratory dead space in goats (data from Mitchell, 1990). (A) With “normal” (i.e. wearing a mask) exercise on a treadmill at 4.8 km/h, 5% grade, the exercise ventilatory response can be characterized as the slope of the relationship between ventilation and metabolic carbon dioxide production. (B) The result is a decreasing level of  $\text{PaCO}_2$  that can be characterized by the slope of its relationship with metabolic carbon dioxide production. When 0.2 L of dead space are added to the mask, the exercise ventilatory response significantly increases, thus enabling similar  $\text{PaCO}_2$  regulation from rest to exercise as in control conditions. This augmentation of the exercise ventilatory response is the manifestation of STM, and results from an augmentation of the feedforward exercise stimulus (Mitchell, 1990). With 0.6 L dead space, the exercise ventilatory response increases further, but not sufficiently to regulate  $\text{PaCO}_2$  which now increases from rest to exercise. Thus, the increased exercise ventilatory response may be due to differential chemoreceptor feedback from rest to exercise among experimental conditions. When these same goats were exercised with 0.6 L dead space at a slower speed (2.4 km/h), they increased ventilation sufficiently to regulate  $\text{PaCO}_2$  with the same precision as in control conditions (not shown). Thus, the inherent range of STM is limited in terms of exercise intensity and/or dead space volume. (\*) Significantly different from control and; (#) Significantly different from control and 0.2 L.

## 2.6. Multiplicative hypoxia-exercise interaction

Another unique mechanism contributing to the exercise ventilatory response is observed during hypoxic exercise. During hypoxia, the exercise ventilatory response increases in excess of that required to maintain constant relative PaCO<sub>2</sub> regulation from rest to exercise, resulting in hypocapnia relative to normoxic exercise (Dempsey et al., 1972; Schaefer and Mitchell, 1989). The specific stimulus responsible for the hypoxia-exercise interaction remains unknown, but it most likely represents a combination of serotonin-dependent STM with an additional stimulus to breathe beyond STM (Schaefer and Mitchell, 1989). Further consideration of the hypoxic exercise ventilatory response is beyond the scope of this paper.

## 3. Mechanisms of short-term and long-term modulation

As in other established models of respiratory plasticity (Mitchell et al., 2001; Feldman et al., 2003), modulation and plasticity of the exercise ventilatory response appear to be serotonin-dependent (McCrimmon et al., 1995; Mitchell and Johnson, 2003), including both short-term and long-term modulation (Bach et al., 1993; Mitchell et al., 1993; Henderson and Mitchell, 2000; Johnson and Mitchell, 2001). Although *short-term modulation* represents a within trial, readily reversible response, *long-term modulation* is a longer lasting alteration that constitutes a form of plasticity. The remaining goal of this review is to present the scarce available evidence concerning the mechanisms underlying STM and LTM in non-human animal models, and to consider their relevance to humans. We will speculate concerning the implications of these mechanisms for aging, sex differences and the onset of obesity or disease (neurodegenerative, traumatic brain or spinal cord injury, or lung diseases).

### 3.1. Short-term modulation

STM was initially demonstrated in goats (Fig. 2), and has been verified in humans, although the authors did not call it by that name (Clark et al., 1980; Poon, 1992). STM is revealed when a small external dead space (200–250 ml) is imposed at rest and during mild

and moderate exercise (Mitchell, 1990; Turner et al., 1997). At rest, the dead space marginally decreases the alveolar ventilation, thereby increasing resting ventilatory drive via classical CO<sub>2</sub>-chemoreceptor feedback. STM is manifested as an increased slope of the relationship between ventilation and metabolic carbon dioxide production ( $\dot{V}_{\text{CO}_2}$ ) during exercise (i.e. an increased exercise ventilatory response) without change in the slope of the relationship between PaCO<sub>2</sub> and  $\dot{V}_{\text{CO}_2}$ . Thus, STM with increased dead space cannot be explained by changes in chemoreceptor feedback from rest to exercise (Mitchell, 1990). However, with a larger dead space of 600 ml, the goat still increases the exercise ventilatory response, but not adequately to prevent PaCO<sub>2</sub> increase from rest to exercise at 4.8 km/h with a 5% grade. Thus, at least part of the increased exercise ventilatory response may be attributable to extra chemoreceptor stimulation during exercise (relative to rest). However, when the exercise intensity was limited to 2.4 km/h, 5% grade, the goats with 600 ml dead space regulated PaCO<sub>2</sub> effectively (Mitchell, 1990). Because goats are able to regulate PaCO<sub>2</sub> with a 250 ml dead space, but not with a 600 ml dead space, except at lower work rates, the capacity for STM is limited by the size of the dead space imposed and/or exercise intensity. Although these inherent limits may restrict the potential significance of STM to only modest exercise challenges, such modest exercise challenges are the most common challenges faced during a normal life and may have substantial biological significance.

In humans, STM has been reported in only a small number of younger male subjects. When confronted with increased dead space (Poon, 1992) or increased inspired CO<sub>2</sub> through a limited range of hypercapnia and exercise intensity (Clark et al., 1980), young male human subjects increase their exercise ventilatory response. To date, no studies have systematically addressed the robustness of STM in young men over a range of dead space volumes and exercise intensities. Preliminary data from a single young adult male are presented as the percent change in the exercise ventilatory response with 0.4 L external dead space at 30 and 60 W of cycling exercise (Fig. 3). As a result of STM, the exercise ventilatory response increased 40% without significant change in end-tidal CO<sub>2</sub> from rest to exercise (not shown) at 30 W, but the capacity for STM was reduced at 60 W, similar to goats at higher levels of dead space and/or exercise intensity. Further studies

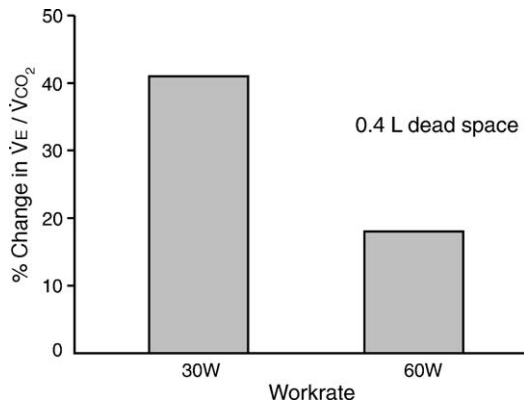


Fig. 3. Preliminary data from a single 27-year-old male human subject demonstrating an increased exercise ventilatory response with 0.4 L dead space at a cycle work rate of 30 W, but less so at 60 W. These data tend to confirm previous reports of STM in young male human subjects, but suggest that the range of this mechanism may be limited to mild exercise, at least under the experimental conditions studied.

concerning the operational range of STM in humans seem warranted.

There is little evidence concerning differences in the capacity for STM in women versus men, or in older subjects of either sex. Some suggestive evidence is available that STM is impaired in middle-aged men relative to younger men (Clark et al., 1980; Poon, 1992). Abundant evidence has accumulated in other models of respiratory neuromodulation and neuroplasticity indicating prominent age-dependent sexual dimorphisms (Behan et al., 2002, 2003). Thus, systematic studies of STM in older humans (>60 year.) as well as sex differences seem warranted.

STM is a central neural mechanism that requires spinal serotonin receptor activation (Bach et al., 1993; Mitchell et al., 1993; McCrimmon et al., 1995). Our working hypothesis is that STM requires the combined activation of neural pathways leading from medullary respiratory pre-motor neurons that convey respiratory drive in accordance with the feedforward exercise stimulus, and descending raphe serotonergic neurons activated uniquely (or more robustly) during hypercapnic exercise (Mitchell et al., 1993; Fig. 8A). It is further proposed that STM requires the convergence of these pathways onto spinal inspiratory motoneurons, such as phrenic and external intercostals motoneurons. Several lines of evidence support this idea. First, in cats, locomotion and hypercapnia both activate caudal

raphe neurons (Veasey et al., 1995). Thus, the caudal raphe neurons, which project to spinal respiratory motoneurons (for review, see McCrimmon et al., 1995), are activated by the very same conditions that induce STM: hypercapnic exercise. Further, STM requires serotonin receptor activation (Bach et al., 1993), and the relevant serotonin receptors are in the spinal cord (Mitchell et al., 1993, 1995; Fig. 4). This conclusion is based on observations that: (1) systemic administration of the broad-spectrum serotonin receptor antagonist, methysergide (Bach et al., 1993), (2) global serotonin depletion with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Bach et al., 1993), (3) raphe neuronal inhibition with intravenous serotonin 1A receptor activation (Henderson and Mitchell, 2000), and (4) intra-spinal injections of methysergide (Mitchell et al., 1993, 1995) or ketanserin (unpublished observations) impair STM with increased respiratory dead space in goats (Fig. 4). Collectively, such evidence presents a strong case that serotonin plays a critical role in the mechanism of STM in goats, and that the necessary actions of serotonin are within spinal regions associated with respiratory motoneurons. We postulated that serotonin modulates the excitability of respiratory motoneurons, increasing their response to the same descending respiratory drive associated with exercise (Bach et al., 1993; McCrimmon et al., 1995; Mitchell and Johnson, 2003; Fig. 8A). Although details concerning the cellular/synaptic actions of serotonin on spinal respiratory motoneurons are virtually unknown, the reversible nature of STM from exercise trial-to-trial suggest that serotonin modifies the excitability of respiratory motoneurons, or strengthens their synaptic inputs, by covalent modification of existing proteins such as the phosphorylation and closure of potassium channels (for review see McCrimmon et al., 1995; Fig. 8B) and/or phosphorylation of glutamate receptors, increasing their current for the same ligand binding (see Fuller et al., 2000; Fig. 8B). Whatever the mechanism underlying STM, it must be rapidly reversible (within 20 min) since the exercise ventilatory response is restored to normal 20 min after the dead space tube has been removed.

It has not yet been determined if STM in young male humans is serotonin-dependent as it is in female goats (see above). One experimental tool commonly used to investigate serotonergic mechanisms in humans is to deplete brain serotonin levels by dietary trypt-

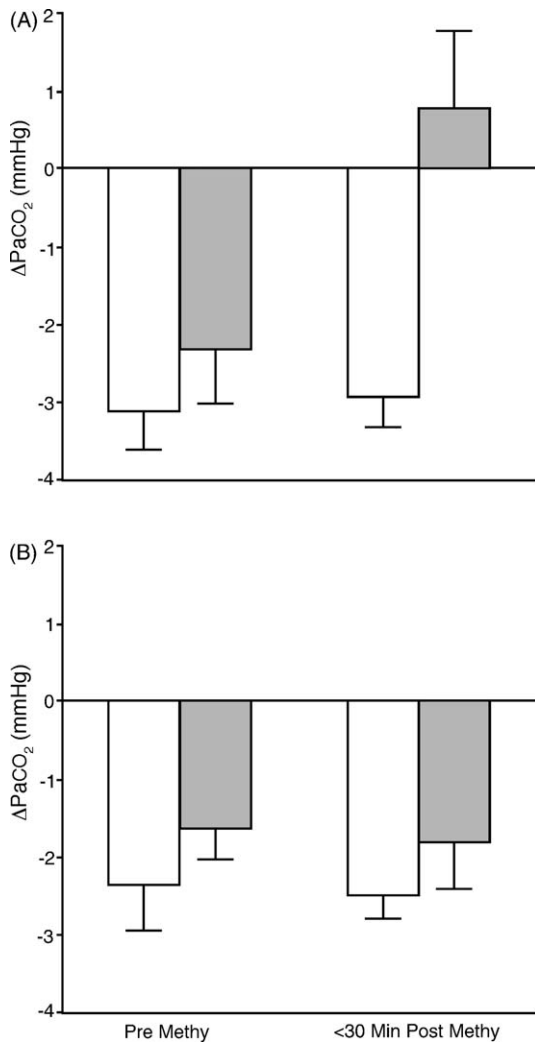


Fig. 4. STM is attenuated in goats with intraspinal injections of methysergide, a serotonin receptor antagonist (data from Mitchell et al., 1993). (A) (upper panel) After vehicle injection (artificial CSF), the decrease from rest to exercise was similar with (shaded bar) and without (open bar) 0.25 L dead space. When 1 mg methysergide was injected into the spinal CSF, PaCO<sub>2</sub> regulation was impaired with increased dead space (shaded bar, right pair) relative to the control conditions (open bar, right pair). Thus, STM requires spinal serotonin receptor activation. (B) (lower panel) The experiments were repeated after intravenous injections of vehicle and the same small dose of methysergide as a control for systemic drug distribution. At this dose, intravenous methysergide was without effect.

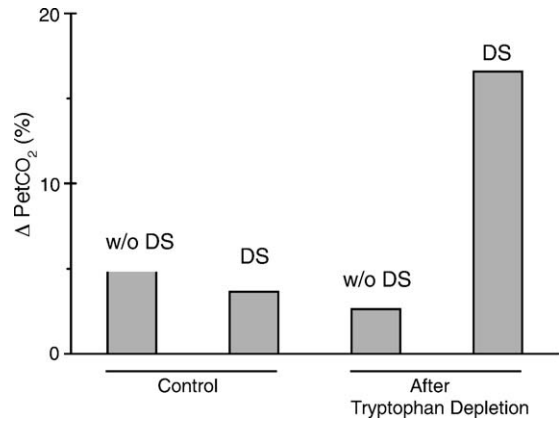


Fig. 5. Preliminary data from one 49-year-old male human subject demonstrating that STM in humans may also be serotonin-dependent. In control conditions, the increase in end-tidal PCO<sub>2</sub> from rest to exercise was similar with (w/o DS) and with increased dead space (DS, 0.4 L). The same subject was then subjected to tryptophan depletion (see text), presumably depleting whole body serotonin levels. After tryptophan depletion, the increase in end-tidal PCO<sub>2</sub> was similar to control conditions without dead space. However, with the added dead space, regulation of end-tidal PCO<sub>2</sub> was impaired, suggesting a loss of STM.

trypothan restriction (Young et al., 1985; Carpenter et al., 1998; Hrboticky et al., 1989; Kirwin et al., 1997; Park et al., 1994; Struzik et al., 2002; Williams et al., 1999). Here, we report preliminary evidence that tryptophan depletion impaired STM with increased respiratory dead space in one middle-aged (49 year) man (Fig. 5). Following a 24 h low tryptophan diet, and after an overnight fast, this middle-aged man drank an amino acid mixture without tryptophan. Five hours later, data were collected at rest and during exercise, both with and without an imposed external dead space. The percentage change in end-tidal PCO<sub>2</sub> during exercise with external dead space is shown in Fig. 5 for both control conditions (before depletion) and after tryptophan depletion in the same subject. The capacity for STM with increased dead space was impaired by tryptophan depletion as indicated by a greater increase in end-tidal PCO<sub>2</sub> from rest to exercise with 0.4 L dead space. These results are strikingly similar to findings in goats that had been given *p*-chlorophenylalanine; *p*-chlorophenylalanine is a tryptophan hydroxylase inhibitor that reduces whole-body serotonin concentrations (Bach et al., 1993; Mitchell et al., 1983). Both *p*-chlorophenylalanine in goats, and tryptophan depletion in humans, lead to hyperventilation without impact

on the hypercapnic ventilatory response (Mitchell et al., 1983; Struzik et al., 2002). Furthermore, both impair the capacity for STM with increased dead space. Since both treatments are known to deplete serotonin in the CNS, these preliminary data support the hypothesis that STM in humans is serotonin-dependent, just as it is in goats. However, further experimental verification of this hypothesis is necessary.

### 3.2. Long-term modulation

#### 3.2.1. Chronic spinal sensory denervation

The first evidence of long-term modulation of the exercise ventilatory response was provided by experiments on goats with a chronic sensory denervation of the thoracic spinal cord (thoracic dorsal rhizotomy from T2 to T12; Mitchell et al., 1990). After this surgery, which eliminates important classes of sensory fibers coursing from inspiratory and expiratory intercostal muscles to the central nervous system, goats no longer tolerate even mild exercise when wearing a respiratory mask and its associated dead space/resistance. Although the goats were able to walk, increased metabolic rate was not matched with a well-coordinated ventilatory response, leading to hypoventilation and progressive hypercapnia during the exercise trial (Mitchell et al., 1990). However, with each subsequent exercise trial, ventilatory responses improved, indicating progressive, experience-dependent functional recovery (Mitchell et al., 1990). Functional recovery was not dependent on regrowth of the severed dorsal roots, as evidence for sensory regeneration was lacking even one year post-surgery (Mitchell et al., 2000). However, functional recovery was associated with increased density of monoaminergic nerve terminals (particularly serotonin) and increased monoamine concentrations within the surgically affected area, as well in cervical spinal segments associated with the phrenic motor nucleus, an important inspiratory motor nucleus distant from the surgically affected area (Mitchell et al., 2000). Although it is unclear if changes in spinal monoamine concentrations played a causal role in functional recovery of the exercise ventilatory response following thoracic dorsal rhizotomy, the observation that serotonergic innervation was increased within the phrenic motor nucleus suggests functional compensatory mechanisms. Indeed, in a non-exercise related

model of respiratory plasticity, chronic cervical sensory denervation increased the capacity for spinal, serotonin-dependent phrenic long-term facilitation following intermittent hypoxia (Kinkead et al., 1998). In specific, chronic cervical dorsal rhizotomy (C3–C5) increased serotonin terminal density within the phrenic motor nucleus, increased the size of phrenic motoneurons, and enhanced serotonin-dependent phrenic long-term facilitation following acute intermittent hypoxia (Kinkead et al., 1998). Chronic cervical dorsal rhizotomy increased the concentrations of neurotrophins, such as brain derived neurotrophic factor (BDNF) (Johnson et al., 2000), a key element in many forms of neuroplasticity, including phrenic long-term facilitation (Baker-Herman et al., 2004). Further, chronic cervical dorsal rhizotomy increases synaptic strength in silent synaptic pathways to phrenic motoneurons in the cervical spinal cord (Fuller et al., 2002). Collectively these observations suggest that chronic sensory denervation alters the spinal milieu in a manner that increases the inherent capacity for plasticity (e.g. following intermittent hypoxia or repetitive exercise). With this background, we hypothesized that repeated associations between hypercapnia (due to ventilatory failure) and exercise initiated mechanisms of neuroplasticity that enhanced ventilatory responses during future exercise trials (Mitchell et al., 1990; Martin and Mitchell, 1993; McCrimmon et al., 1995), thereby accounting for functional recovery following chronic thoracic dorsal rhizotomy. Experiments utilizing chronic sensory denervation indicate an impressive degree of adaptive control, including plasticity (functional recovery) in the exercise ventilatory response (Mitchell et al., 1990).

#### 3.2.2. LTM following repeated hypercapnic exercise in normal goats

Repeated hypercapnic exercise induced by explicit pairing of exercise and dead space for 14–20 exercise trials elicits a longer lasting modulation of the ventilatory response to mild or moderate exercise—long-term modulation (LTM; Martin and Mitchell, 1993; Johnson and Mitchell, 2001; Mitchell and Johnson, 2003). LTM is expressed as an augmentation of the exercise ventilatory response, and greater hypocapnia during exercise under control conditions (i.e. without dead space) for several hours after the final hypercapnic exercise trial (Fig. 6). However, with successive exercise trials, the exercise ventilatory response slowly returns to

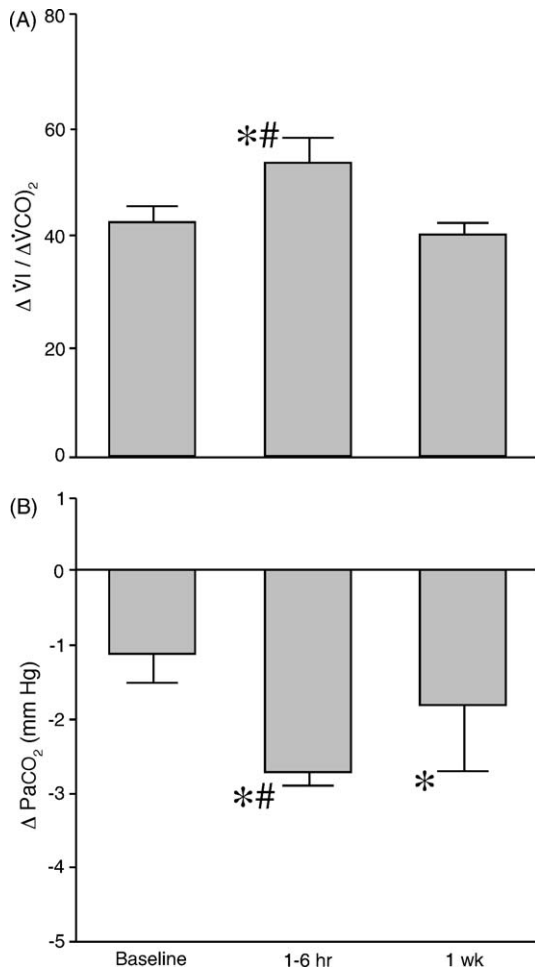


Fig. 6. Data from goats demonstrating the capacity for long-term modulation (LTM) of the exercise ventilatory response following repeated hypercapnic exercise (data from [Martin and Mitchell, 1993](#)). Relative to control, baseline conditions, the exercise ventilatory response (upper panel) and the change in PaCO<sub>2</sub> from rest to exercise (lower panel) were accentuated following training with hypercapnic exercise (14–20 trials over a 2-day period; 1.0L dead space paired explicitly with exercise). This augmentation lasted hours following the final training trial and represents LTM. With successive exercise trials, the exercise ventilatory response returned towards normal. One week post-training, there still appeared to be some residual effect of training, but this was not repeated in another trial on the same goats months later ([Martin and Mitchell, 1993](#)).

normal over a period of hours ([Martin and Mitchell, 1993](#)). LTM appears to result from an enhancement of the feedforward exercise stimulus without change in the resting CO<sub>2</sub>-chemoreflex sensitivity ([Martin and Mitchell, 1993](#)). In similar experiments, goats were

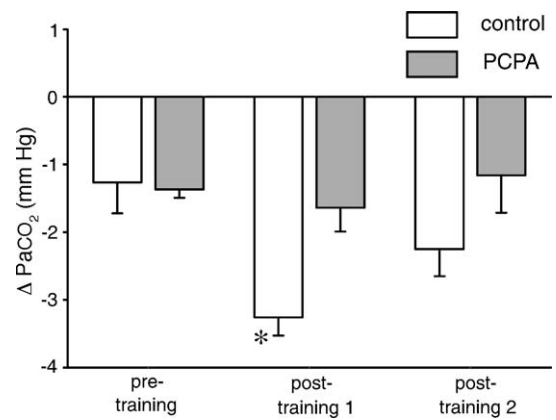


Fig. 7. LTM is serotonin-dependent (data from [Johnson and Mitchell, 2001](#)). Goats were subjected to the LTM training trial before and after pre-treatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine, which depletes whole body serotonin levels ([Mitchell et al., 1983](#)). Following hypercapnic training (>1 h), the goats exhibited an accentuated decrease in PaCO<sub>2</sub> from rest to exercise, a hallmark of LTM. However, after serotonin depletion, LTM was no longer observed.

subjected to repeated hypoxic exercise, but no evidence for the establishment of LTM was observed ([Turner et al., 1995](#)). Thus, there is at least some inherent capacity for adaptive control strategies in the exercise ventilatory response of normal female goats, although the effect appears to be restricted to hypercapnic (versus hypoxic) exercise with the protocols tested. As yet, there is no evidence that this effect is as enduring as functional recovery of the exercise ventilatory response following chronic thoracic dorsal rhizotomy ([Fig. 7](#)).

Similar to STM, LTM induced by repeated hypercapnic exercise is a serotonin-dependent mechanism. For example, long-lasting serotonin depletion with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine abolishes LTM following repeated hypercapnic exercise ([Johnson and Mitchell, 2001](#)). Similar experiments were not performed with using a serotonin receptor antagonist such as methysergide since it would be difficult to assure adequate receptor antagonism in a time frame necessary to cover the training period. Based on similarities in the proximate stimuli (i.e. dead space induced hypercapnia with exercise), and their mutual serotonin-dependence, we hypothesized that repeated activation of STM initiates the mechanisms leading to LTM and that they are, thus, mechanistically linked in a manner analogous to the

consolidation of short-term into long-term memories (Johnson and Mitchell, 2001; Mitchell and Johnson, 2003).

Virtually nothing is known concerning the cellular/synaptic mechanisms that give rise to LTM of the exercise ventilatory response in goats, or in any other model. However, since both LTM of the exercise ventilatory response and the best studied form of respiratory plasticity, phrenic long-term facilitation following intermittent hypoxia (Mitchell et al., 2001; Feldman et al., 2003), are serotonin-dependent and have a similar time course, it is tempting to speculate that they share common mechanistic features. Thus, whereas it is most likely that STM of the exercise ventilatory response results from covalent modification of existing proteins within respiratory motoneurons (Fig. 8B), the establishment of LTM, like LTF, is more likely to require the synthesis of new proteins (Fig. 8B; Baker and Mitchell, 2002). One requirement to estab-

lish phrenic long-term facilitation following intermittent hypoxia is serotonin-dependent synthesis of brain derived neurotrophic factor (BDNF; Baker-Herman et al., 2004). Since repetitive exercise increases BDNF expression at multiple sites in the central nervous system (Cotman and Berchtold, 2002), including the spinal cord (Gomez-Pinilla et al., 2002), increased BDNF synthesis may also be a required step in the establishment of LTM following repeated hypercapnic exercise. However, at this time, there is no direct experimental verification of this hypothesis. One observation may suggest that unique mechanisms differentiate LTM and phrenic long-term facilitation: LTM is predominantly expressed as an augmented frequency response to exercise (Martin and Mitchell, 1993) whereas long-term facilitation is generally expressed as an increase in the amplitude of respiratory motor output (Mitchell et al., 2001). Thus, LTM may involve cellular/synaptic changes at neurons more directly involved in respira-

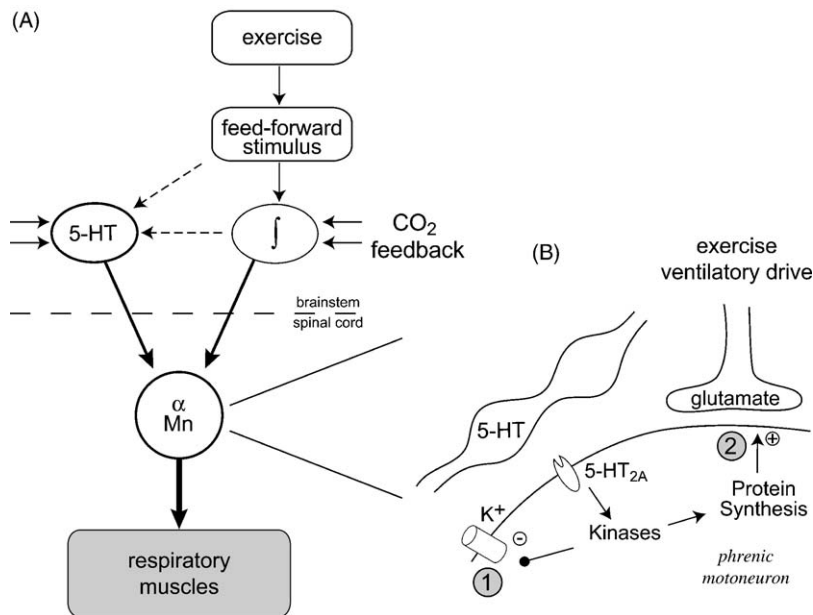


Fig. 8. Hypothetical mechanisms of STM and LTM: (A) network model of STM, drawn after (Bach et al., 1993). It is postulated that an exercise linked feedforward stimulus activates brainstem respiratory pre-motor neurons (integral sign), thereby increasing descending drive to spinal alpha-motoneurons and increasing respiratory muscle contraction. With an increased dead space, classical chemoreceptor feedback will stimulate breathing at rest, but will also directly or indirectly stimulate raphe serotonergic neurons, which are known to project to brainstem respiratory neurons as well as spinal respiratory motoneurons. (B) Since it appears that STM results from spinal serotonin receptor activation (see Fig. 3), we postulate that receptor activation on the respiratory (e.g. phrenic) motoneurons activates kinases, which then act to increase motoneuron excitability by, for example, closing a potassium channel (1), or by increasing synaptic strength of the bulbospinal input to respiratory motoneurons by increasing post-synaptic glutamate receptor current.

tory rhythm generation (versus motoneurons), although the detailed cellular/synaptic mechanisms may still share common features at that distinct site.

If LTM of the exercise ventilatory response shares common mechanistic features with phrenic long-term facilitation following intermittent hypoxia, then there are several predictions that would follow. First, if this mechanistic similarity exists, then it is reasonable to speculate that LTM will decrease with advancing age in males, but will increase with age in females until reproductive cycling ceases (Zabka et al., 2001a,b; Behan et al., 2002, 2003). Furthermore, genetic background may influence the expression of LTM as it does with phrenic long-term facilitation (Fuller et al., 2000, 2001; Bavis et al., 2003). Finally, it is expected that the pattern of stimulus presentation will matter since phrenic long-term facilitation is a classic example of pattern-sensitive neuroplasticity: intermittent stimuli are more effective than sustained stimulus presentations (Baker and Mitchell, 2000; Mitchell et al., 2001). All of these speculations concerning age, sex, genetics or the detailed training protocols that elicit LTM of the exercise ventilatory response form the rationale for testable hypotheses, and each may be an interesting area for future investigations.

There are several observations that are difficult to explain in making a case that LTM of the exercise ventilatory response and phrenic long-term facilitation following intermittent hypoxia share common mechanisms. First, repeated hypoxic exercise does not cause detectable LTM (Turner et al., 1995), despite the fact that repeated hypoxia is the proximate stimulus to respiratory long-term facilitation. However, there may be important details that differentiate these mechanisms despite fundamental similarities. Crucial variables may include the state of the animal and the pattern of stimulus presentation. For example, 5 min intervals between hypoxic episodes elicit respiratory long-term facilitation, whereas 30 min intervals do not (Bach et al., 1999). Thus, the 15–30 min intervals between repeated hypoxic exercise trials may be too long for the establishment of LTM by the same cellular processes that underlie long-term facilitation. Another apparent contradiction is that intermittent hypercapnia in anesthetized rats elicits long-term depression versus long-term facilitation of phrenic nerve activity (Bach and Mitchell, 1998; Baker et al., 2001). However, this depression may arise from additional, inhibitory

mechanisms that override an underlying facilitation (Baker et al., 2001) and may in fact be unique to the anesthetized rodent preparation. Details of the LTM training protocol, such as the coincidence of exercise and hypercapnia, may sway the mechanism towards an enhancement (i.e. LTM) versus hypercapnia-induced depression. Clearly, a great deal of research needs to be done before we will understand mechanisms that underlie LTM following repeated hypercapnic exercise in goats, or any other species.

### 3.2.3. Evidence for LTM in humans

Evidence for LTM in humans has been conflicting. Several studies indicate a degree of LTM during the early onset of arm (Helbling et al., 1997; Summers and Turner, 2003) or leg (Turner and Summers, 2002) exercise after repeated hypercapnic exercise training. However, none of these studies observed LTM during steady-state exercise as reported in goats following hypercapnic treadmill training (Martin and Mitchell, 1993). Other studies did not find LTM after a small number of hypercapnic exercise training trials, either during the onset or during steady-state exercise (Cathcart et al., 2005; Moosavi et al., 2002). Only one study of the exercise ventilatory response during steady-state leg exercise produced a robust LTM, but only after a substantially greater number of hypercapnic exercise training trials (Wood et al., 2003). Thus, the apparent controversy between these reports may result from the study design. In general, studies with a small number of hypercapnic exercise trials (<10) did not observe steady-state LTM, whereas the lone human study to observe a robust steady-state LTM used a substantially greater number of training trials (70 trials; Wood et al., 2003). There may also be variability in results due to differences in the level of dead space (i.e. hypercapnia) used in the training protocols. Turner and Stewart (2004) suggested that LTM of ‘early’ and ‘steady-state’ exercise ventilation may be related to the number/intensity of conditioning trials, and may represent different time-domains of the motor learning process. Other models of spinal plasticity in a motor system, such as associative conditioning of the H reflex in primates or rodents (Wolpaw, 1997), require thousands of training trials versus the few trials necessary for many forms of cognitive learning. LTM may be similar in its requirement for a larger number of training trials, an idea consistent with the available literature.

In a recent study, [Turner and Stewart \(2004\)](#) demonstrated that LTM of the early onset exercise ventilatory response could be evoked by repeated exercise trials with inspiratory resistive loading—without hypercapnia. Thus, stimuli other than hypercapnia per se, such as increased tidal volume or ventilatory drive, may be the relevant factors in the initiation of LTM ([Turner and Stewart, 2004](#)). This novel observation is significant because it extends the concept of plasticity in the exercise ventilatory response to multiple physiological conditions associated with impaired pulmonary mechanics, but with minimal disruption of gas exchange. Thus, LTM could play a role in accommodating mechanical challenges, ensuring adequate ventilatory responses during exercise in conditions as diverse as normal aging, weight gain, the onset of lung disease, or other changes of biological and medical concern.

#### 4. Potential significance

STM and LTM represent a capacity of the respiratory control system to accommodate changes in prevailing endogenous (e.g. respiratory mechanics, pulmonary gas exchange) and exogenous (e.g. imposed breathing apparatus used by various professionals during exertion, altitude) conditions. For example, the elderly must adapt their breathing at rest and during exercise to age-related changes in pulmonary gas exchange and respiratory mechanics if they are to maintain an optimal ventilatory response during exercise ([Babb and Rodarte, 2000](#); [DeLorey and Babb, 1999](#)). A failure to do so may limit their capacity to exercise, thereby limiting normal daily activities. Indeed, the normal ventilatory response during mild-to-moderate exercise is greater in old versus young human subjects ([Brischetto et al., 1984](#); [DeLorey and Babb, 1999](#); [Poulin et al., 1994](#); [Babb et al., 2003](#)), potentially reflecting the utilization of adaptive mechanisms for the deteriorating efficiency in pulmonary gas exchange. On the opposite end of the age spectrum, modulation and plasticity may be necessary to guide normal development of the exercise ventilatory response, or to at least adjust and modify that response as conditions change during the transition from neonatal life to adulthood. However, the existence of STM and LTM require greater verification in humans, where

neither of these mechanisms nor even longer lasting forms of plasticity have been studied adequately.

Although apparent limitations in the operational range of STM and LTM may raise questions concerning the physiological significance of these mechanisms, most physical activity throughout life is within the mild to moderate range, and preservation of blood gas homeostasis for these greater periods of time may have disproportionate impact on the life of an individual. Perhaps even more importantly, we should not let the limits of our current experimental protocols dominate our thinking. It is possible that the true significance of LTM will be known until trials have been designed where literally hundreds or thousands of experiences are imposed. This may be a system that resists the change imposed by experience dependent plasticity unless that experience is a recurrent feature of exercise. Thus, there is an unexplored potential for neuroplasticity to play a long-lasting role in sculpting the exercise ventilatory response, in a time-domain ranging from days to weeks to even years. Of course, such prolonged “training” protocols become logistically difficult when using human experimental subjects; appropriate animal models will be critical to advance our understanding in this regard. Further, we will not have a full appreciation for the potential significance of modulation and plasticity in the exercise ventilatory response until individual differences based on age, sex, genetics, and previous history (i.e. “metaplasticity;” [Mitchell et al., 2001](#); [Mitchell and Johnson, 2003](#)) have been evaluated. Only then will we have some appreciation for the pertinence of plasticity in respiratory motor control to disease or injury (e.g. lung disease, obesity, congestive heart failure, neurodegenerative diseases, spinal cord injury, etc.).

#### 5. Summary

Despite its fundamental biological and medical importance, our understanding of exercise hyperpnea and its underlying mechanisms remains rudimentary at best. Rather than viewing this complex and fascinating homeostatic response as a single mechanism, acting alone, it may be more appropriate to consider the layers that go into such a complicated regulatory task. These layers may provide precise regulation by way of successive approximation: gross regulation, followed

by successively more refined adjustments, ultimately leading to a response that appears nearly ideal. Concepts of modulation and plasticity are still relatively new within the context of studies on exercise hyperpnea. Considerable research will be necessary before we have a full appreciation of their true significance.

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