Hypoxia-induced phrenic long-term facilitation: emergent properties

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Abstract

Just as in other neural systems, plasticity is a hallmark of the neural system controlling breathing. One spinal mechanism of respiratory plasticity is phrenic long-term facilitation (pLTF) following acute intermittent hypoxia. Although cellular mechanisms giving rise to pLTF occur within the phrenic motor nucleus, different signaling cascades elicit pLTF in different conditions. These cascades, referred to as "Q" and "S" pathways to phrenic motor facilitation (pMF), interact via cross-talk inhibition. Whereas the Q pathway dominates pLTF after mild to moderate hypoxic episodes, the S pathway dominates after severe hypoxic episodes. The biological significance of multiple pathways to pMF is not known. We discuss the possibility that interactions between pathways confer emergent properties to pLTF, including: 1) pattern sensitivity and 2) metaplasticity. Understanding these mechanisms and their interactions may enable us to optimize intermittent hypoxia induced plasticity as a treatment for patients that suffer from ventilatory impairment or other motor deficits.

Keywords

plasticity; motor neuron; intermittent hypoxia; phrenic nerve; pattern sensitivity; metaplasticity

1 Introduction

The respiratory control system has been historically viewed as fixed and immutable, controlled primarily via negative feedback from sensory receptors.[1] This view was held in large part because breathing is an automatic, often subconscious motor behavior. However, control systems governed by negative feedback alone are frequently unstable due to inappropriate reflex gain.[2] Systems that preserve homeostasis through strong negative feedback loops are vulnerable to insults; robust control systems optimize for worst-case scenarios (ie. ventilatory failure), and incorporate mechanisms to prevent such failure when the system is challenged. Plasticity is one key property of neural systems, such as the respiratory control system, that promote robust and effective homeostatic regulation.[1] In this brief review, we use a general definition of respiratory plasticity, namely: a change in future system performance (ie. breathing or blood gas regulation) based on experience.[1]

Plasticity in the neural system controlling breathing has only been widely appreciated only for the past few decades .[1,3–5] Recently, the field of respiratory neuroplasticity has grown considerably; plasticity has been discovered at the neuromuscular,[6] chemoreceptor,[7] spinal,[8]

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and brainstem,[9] levels of respiratory control, and our knowledge concerning these forms of plasticity is increasing at a rapid pace. Here we focus on a single, widely studied model of spinal respiratory plasticity, phrenic long-term facilitation (pLTF). pLTF is a persistent increase in phrenic motor output lasting hours after a few brief episodes of low oxygen, or acute intermittent hypoxia (AIH; see refs 10–13). Considerable progress has been made towards an understanding of cellular and network mechanisms giving rise to pLTF.[10–11] One recent realization is that multiple distinct cellular cascades give rise to similar phenotypic plasticity.[10] An important question is: why do these multiple pathways exist? In this brief review, we will consider the potential advantages conferred by this complexity. In specific, we develop the hypotheses that the existence of multiple interacting pathways confers two emergent properties of pLTF: pattern sensitivity and metaplasticity.

2 Phrenic long-term facilitation (pLTF)

Millhorn and colleagues originally demonstrated that episodic carotid sinus nerve stimulation elicits a long lasting increase in phrenic motor output in anesthetized cats.[3,4] We subsequently demonstrated a similar phenomenon in anesthetized rats following three brief hypoxic episodes, a phenomenon termed phrenic long-term facilitation (pLTF).[12,13] Following acute intermittent hypoxia (AIH), pLTF is expressed as a prolonged increase in phrenic nerve burst amplitude lasting several hours after the final hypoxic episode (Figure 1A). pLTF is a form of serotonin- and protein synthesis-dependent spinal plasticity.[12,14,15] pLTF induction is independent of increased phrenic nerve activity[16–17] and represents a form of neuromodulator induced plasticity[1] distinct from conventional forms of activity-dependent synaptic plasticity such as hippocampal long-term potentiation (LTP).[18]

On hallmark of pLTF is pattern sensitivity; pLTF is elicited by intermittent, but not a single period of acute sustained hypoxia (ASH) with the same cumulative duration (Figure 1B).[19] Although similar pattern sensitivity is shared by many forms of neuroplasticity,[20–24] we have little understanding of how such pattern sensitivity arises in any system. Another property of pLTF is its ability to express metaplasticity;[1] for example, following cervical dorsal rhizotomy[25] or chronic intermittent hypoxia,[26] subsequent responses to AIH (ie. pLTF) are amplified. We still do not fully understand mechanisms that confer metaplasticity.

Although our understanding of mechanisms giving rise to respiratory plasticity remains incomplete, considerable progress has been made.[10,11,27,28] One realization is that multiple, distinct cellular cascades exist, each capable of eliciting long-lasting phrenic motor facilitation (pMF; a general term describing augmented phrenic burst amplitude that includes pLTF).[10] These pathways interact in interesting and complex ways, possibly increasing flexibility as the respiratory control system responds to diverse challenges throughout life.

In this review, we make the argument that interactions between distinct cellular cascades to phrenic motor facilitation confer emergent properties to respiratory plasticity, including pLTF pattern sensitivity and metaplasticity. An understanding of the diverse mechanisms giving rise to pMF and their implications will help to understand pattern sensitivity and metaplasticity in other forms of neuroplasticity, and will be essential as we begin to harness the potential of AIH-induced spinal plasticity to treat severe clinical disorders that impair breathing, such as spinal cord injury[29–31] or ALS.[32]

3 Multiple cellular mechanisms of pLTF: the Q and S pathways

pLTF is frequently studied in anesthetized, paralyzed and ventilated rats administered a standardized AIH protocol (3, 5 min hypoxic episodes; PaO_2 35–45 mmHg; 5 min intervals;

see ref 25). We refer to this protocol as moderate AIH. Following moderate AIH, pLTF requires spinal serotonin type 2 (5-HT_2) receptor activation,[12,14,15] new synthesis of brain-derived neurotrophic factor (BDNF),[33] activation of its high-affinity receptor (TrkB),[33] and ERK MAP kinase signaling.[34] Because 5-HT_2 receptors are G_q protein-coupled receptors,[35] we refer to this as the "Q pathway" to pMF (Figure 2A). Pharmacological activation of other G_q coupled receptors (such as α_1 receptors) elicit similar pMF.[10]

A distinct pathway to pMF relies on activation of G_s protein coupled metabotropic receptors, such as adenosine 2A (A_{2A})[36] and 5-HT_7 receptors.[37] This form of pMF requires new synthesis of an immature TrkB isoform (not BDNF) and PI3 kinase/Akt signaling (not ERK).[36] We refer to this as the "S pathway" to pMF since multiple G_s protein-coupled metabotropic receptors elicit the same mechanism (Figure 2B).[10] Contrary to our initial expectation, the S pathway does not contribute to pLTF following moderate AIH,[38] but dominates pLTF following severe AIH.[39] In fact, the S pathway negatively regulates pLTF with moderate AIH, demonstrating inhibitory interactions between pathways;[38] based on these findings, we proposed that the predominant interaction between pathways is "cross-talk inhibition." Although the mechanistic basis of this cross-talk inhibition is not fully understood, S to Q inhibition may require PKA activity (Hoffman and Mitchell, unpublished).

During severe AIH, the S pathway is activated to a greater extent, and dominates pLTF.[39] Rats exposed to a severe AIH exhibit pLTF phenotypically similar to moderate AIH, but via an A_{2A} receptor-dependent (serotonin-independent) mechanism (i.e. S pathway).[39] This finding suggests that cross-talk inhibition between pathways assures dominance of one or the other; the switch appears to be precipitous since PaO_2 levels above 35 mmHg elicit pLTF via the Q pathway[35] whereas PaO_2 levels of 30 mmHg or below elicit pLTF via the S pathway.[39] We suggest that this transition occurs because of relatively greater accumulation of extracellular adenosine during severe hypoxic episodes, shifting the balance towards the S pathway. Once the tipping-point is reached, we hypothesize that the now dominant S pathway suppresses the Q pathway. Although the subordinate pathway does not positively contribute to pLTF, it nevertheless modulates (inhibits) the dominant pathway.[38]

4 More pathways to phrenic motor facilitation

Three other stimuli elicit unique forms of pMF in anesthetized rats. Spinal injections of the growth/trophic factors vascular endothelial growth factor (VEGF) or erythropoietin (EPO) near the phrenic motor nucleus cause pMF via mechanisms that require both ERK and Akt activation for full expression.[40,41] We suspect that these hypoxia-sensitive genes enable phrenic motor plasticity in longer time domains. For example, VEGF or EPO induced pMF might play a role in longer-term adjustments of phrenic motor activity during chronic intermittent or sustained hypoxia.[40,41]

A completely different cellular cascade gives rise to pMF after brief periods of phrenic inactivity (iPMF) induced by hypocapnia, vagal stimulation and/or isoflurane.[42] Unlike pLTF, iPMF requires atypical PKC activation.[43,44] iPMF might ensure that this critical motor pool is constantly active. For example, iPMF might contribute to the preservation of adequate phrenic activity when synaptic inputs are disrupted, such as by spinal injury.

5 Emergent properties of pLTF

Some properties of pLTF, such as pattern sensitivity and metaplasticity, may be determined by interactions between competing pathways to pMF.[26,45] These emergent properties may determine whether pLTF is expressed or not after a given stimulus (eg. different patterns or severity of hypoxia), or its magnitude (eg. greater magnitude in response to the same AIH).

5.1 Pattern sensitivity

Pattern sensitivity is a common feature in many models of neuroplasticity, including models of serotonin-dependent synaptic facilitation.[20,21] pLTF is pattern-sensitive since it is induced by moderate AIH, but not acute sustained hypoxia (ASH) of similar severity and cumulative duration (9–25 min; Figure 1B).[12] Pattern sensitivity of respiratory plasticity was initially recognized in studies of ventilatory LTF (vLTF) in goats,[46,47] and subsequently observed in rat[48,49] and human vLTF.[50–55] Interestingly, vLTF in humans requires slightly elevated CO_2 during AIH.[56] It was recently reported that elevated CO_2 reveals vLTF in humans exposed to 32 minutes of ASH,[57] although the investigators found that about half of this vLTF was due to drift in ventilation caused by sustained hypercapnia alone. Griffin et al.[57] observed a persistent increase in ventilation for 20 min after AIH or ASH, but they did not measure ventilation at later time points necessary to clearly demonstrate LTF. Thus, further studies are needed to confirm that vLTF is pattern-sensitive in awake humans.

Although significant progress has been made, the mechanism of pLTF pattern sensitivity remains unknown. The optimal spacing interval has not been characterized, but is somewhere between 1 and 30 minutes.[58] pLTF is relatively insensitive to other characteristics of AIH, such as the severity or duration of hypoxic episodes. For example, pLTF is unaffected by the level of PaO_2 from 35 to 60 mmHg,[59,60] or by episode durations between 15 sec to 5 min.[61] Similar to AIH-induced pLTF, pMF induced by intraspinal 5-HT requires episodic injections, suggesting that pattern sensitivity occurs at or downstream from 5-HT receptor activation.[16,17,62] Here, we propose three possible mechanisms that could contribute to pLTF pattern-sensitivity.

5.1a Serotonin receptor desensitization (Figure 3A)—Serotonin receptors are diverse, with complex signaling and activation requirements; the $5\text{-}HT_2$ class alone includes multiple isoforms with unconventional properties, such as g-protein independent signaling[63] or constitutive activity.[64] One pertinent feature of $5\text{-}HT_2$ receptors is desensitization, which is a decrease ligand response after prolonged ligand exposure. In the brain, $5\text{-}HT_2$ receptors desensitize rapidly with persistent elevations in extracellular serotonin via mechanisms that involve receptor internalization and/or functional uncoupling.[65] Receptor internalization occurs when agonist bound receptors are internalized by clathrin coated pits into endosomes, where they are sequestered until reinserted or degraded. Functional uncoupling disrupts G-protein signaling and occurs via receptor phosphorylation, usually by G protein-coupled receptor kinases (GRKs); this phosphorylation causes the protein arrestin to bind the receptor, preventing further G protein activation.[66] *In vitro* studies demonstrate $5\text{-}HT_2$ receptor desensitization after 5–10 min of agonist exposure,[67,68] well within the time frame of a 25 minute ASH exposure. Although no studies have assessed $5\text{-}HT_2$ receptor desensitization in the spinal cord, it is a candidate to undermine pLTF during ASH.

5.1b Regulation of protein phosphatase activity (Figure 3B)—Protein phosphatases are inhibitory to many forms of CNS synaptic plasticity;[69,70] the expression of plasticity is often regulated by protein phosphatase activity during the induction phase. pLTF is constrained by constitutive okadaic acid-sensitive protein phosphatases during ASH,[45] Thus, in rats pretreated with spinal okadaic acid, ASH elicits pLTF.[45] Reactive oxygen species (ROS) inhibit protein phosphatases involved in plasticity.[71] Indeed, ROS produced by NADPH oxidase are necessary for pLTF expression,[72] and this requirement is offset by spinal protein phosphatase inhibition with okadaic acid.[73] Thus, NADPH oxidase and okadaic acid sensitive protein phosphatases (most likely PP2A) appear to constitute a "regulatory cassette" that determines if and how much pLTF will be expressed (Fig. 3B).[37,64] One likely difference between ASH and AIH in their ability to elicit pLTF could be relative ROS production (and phosphatase inhibition). While no published studies have

directly compared ROS production in response to AIH vs. ASH, there is evidence that hypoxia-induced superoxide production occurs predominantly during reoxygenation[73–75] suggesting that AIH could generate greater ROS (vs. ASH) because of multiple reoxygenations. Further studies are needed to determine how AIH (vs. ASH) stimulates greater ROS production in the cervical spinal cord.

5.1c Cross-talk inhibition between Q and S pathways (Figure 3C)—Although pLTF shifts to S pathway-dependence with severe AIH,[39] it is not known how this shift occurs. We proposed that "cross-talk inhibition" between pathways (Figure 3C) enables one pathway to gain an "upper hand" and, thus, dominate pMF. A key factor is the strength of the initiating stimulus (5-HT$_2$ versus A$_{2A}$ receptor activation). For example, during severe AIH, greater adenosine formation/accumulation may increase A$_{2A}$ receptor activation, creating stronger S pathway activation and, subsequently, Q pathway suppression. The prevailing mechanism of pLTF may result from competition between pathways for dominance (Figure 3C).

We speculate that, at some levels of hypoxia (severity, duration, etc), inhibitory interactions could become balanced, creating an impasse where the S or Q pathways offset one another (ie. no pLTF). If so, this state represents an emergent property of competing pathways to pMF, and could underlie pLTF pattern-sensitivity. During longer hypoxic exposures, such as moderate ASH, greater adenosine formation/accumulation[76] may cause balanced activation of the Q and S pathways (Figure 3C), thereby obscuring pLTF. This hypothesis is consistent with the study by Griffin et al., (2012) since hypercapnia could cause greater serotonin release, shifting the balance towards the Q pathway and vLTF expression. Further studies are needed to determine the role of pathway interactions in pLTF pattern sensitivity.

5.1d Are other forms of pMF pattern-sensitive?—While many studies have focused on pLTF pattern sensitivity following moderate AIH, no studies have examined pattern sensitivity in the other forms of pMF described above (ie. S pathway, VEGF- and EPO-induced pMF, or iPMF). Studies are needed to determine if these forms of pMF are also pattern-sensitive.

5.2 Metaplasticity

Metaplasticity is loosely defined as "plastic plasticity", but more specifically as the ability of prior experience to alter subsequent plasticity.[77] Metaplasticity can be expressed as enhanced pLTF triggered by pre-conditioning of adults with repetitive intermittent hypoxia[26,78,79] or cervical sensory denervation.[25] On the other hand, developmental exposure to chronic intermittent hypoxia suppresses pLTF in adult rats.[80] pLTF enhancement from pre-conditioning is particularly interesting because it could increase the success of AIH protocols applied to improve breathing capacity or other motor functions after spinal injury.[8,27–30] Thus, metaplasticity (and its mechanisms) are important considerations for clinical utilization of AIH-induced motor plasticity.

5.2a Enhanced pLTF after pre-conditioning with repetitive intermittent hypoxia—Rats exposed to chronic intermittent hypoxia (CIH; 10–12% O$_2$/air, 2–5 min intervals, 8–12 hrs/night) exhibit enhanced serotonin-dependent pLTF.[26] Other investigators have reproduced this effect with shorter hypoxic episodes (5–12% O$_2$, 15s episodes with 5 min intervals, 8h/day) to more closely simulate episodic hypoxia during sleep disordered breathing.[81] In this study, pLTF enhancement was blocked by antioxidant administration[81] demonstrating ROS dependence. Thus, CIH enhances pLTF, presumably by enhanced Q pathway signaling (ie. serotonin and ROS dependent). Although CIH is a potent stimulus to metaplasticity, it also elicits morbidity, including hypertension, hippocampal apoptosis and

cognitive deficits, among others.[82–84] Thus, we developed more subtle protocols of repetitive intermittent hypoxia that elicit pLTF metaplasticity without detrimental effects elicited by CIH.[30,85,86]

Rats exposed to modest protocols of repetitive acute intermittent hypoxia (rAIH) exhibit enhanced pLTF[78,79] and increased respiratory and non-respiratory somatic motor recovery following cervical spinal injury.[30] Two different rAIH protocols were used in these studies. Daily AIH (10, 5 min episodes of 10.5% O_2, 5 min intervals, 7 days) improved respiratory and forelimb function and increased phrenic burst amplitude in rats with C2 spinal hemisections.[30] In Brown-Norway rats, a strain with low constitutive pLTF and no hypoglossal LTF, daily AIH enabled hypoglossal LTF, but an apparent doubling of pLTF was only marginally significant.[85] These results suggest that daily AIH is a modest inducer of metaplasticity in the phrenic motor pool of otherwise normal rats. Rats exposed to AIH 3 times per week (3xwAIH; 10, 5min episodes/day, 3 days per week for 4 weeks) exhibit enhanced pLTF,[78,79] and profound neurochemical plasticity in the phrenic motor nucleus,[86] suggesting that longer, less frequent exposures are more effective at eliciting pLTF metaplasticity. Neither protocol caused hippocampal apoptosis, astrogliosis, or hypertension,[30,85,86] suggesting the ability to elicit respiratory metaplasticity without detectable pathology. Further studies are needed to determine optimal protocols to elicit respiratory metaplasticity, so that AIH-induced plasticity can be harnessed as a means of restoring respiratory and non-respiratory motor function in clinical disorders that challenge ventilatory control.

What mechanism underlies pLTF metaplasticity following rAIH? In animals exposed to daily AIH, increased spinal BDNF was observed,[85] suggesting Q pathway enhancement. If the Q pathway is enhanced following rAIH, what mechanism underlies this enhancement? One possibility is decreased cross-talk inhibition. For example, decreased S pathway activation (Figure 4A) could increase Q pathway dependent pLTF and BDNF synthesis. Alternatively, decreased inhibitory interactions between pathways would enable direct S pathway contributions to pLTF, even with moderate AIH, since the pathways would be uncoupled (Figure 4B). Indeed, greater phospho-Akt levels were observed after 3xwAIH for 10 weeks,[86] consistent with increased S pathway activation. Finally, pLTF metaplasticity may arise directly from a more robust Q pathway (Figure 4C); following 10 weeks of 3xwAIH, 5-HT$_{2A}$ receptor, BDNF, TrkB and phospho-ERK expression are all increased,[86] suggesting "hypertrophy" of this cellular cascade. Further studies are needed to determine how rAIH causes pLTF metaplasticity.

5.2b Other forms of metaplasticity in the phrenic motor system—This brief review was intended to pose rather than answer questions. It is not expansive enough to cover all potential forms of metaplasticity in respiratory motor pools. However, factors known to induce metaplasticity in other models of plasticity, such as hippocampal long-term potentiation include prior activation[77,87,88] and stress.[90] Hippocampal LTP is impaired by stress or glucocorticoids.[90] In the phrenic motor pool, we postulate that stress has two separate effects. Initially, stress might induce pMF, due to norepinephrine release from locus coeruleus followed by activation of α_1 receptors on phrenic motor neurons.[10] On the other hand, if pLTF is similar to other models of plasticity, chronic exposure to stressful stimuli may impair pLTF, possibly through a glucocorticoid-dependent mechanism. Anecdotally, rats subject to stressful stimuli often fail to express pLTF; however, further studies are needed to determine the effects of stress on phrenic motor plasticity, and to confirm or refute these speculations.

6 Conclusion and Significance

Distinct pathways (ie. Q and S pathways) give rise to phenotypically similar phrenic motor facilitation. However, since the Q and S pathways interact via cross-talk inhibition, either pathway can dominate and produce pLTF; at a specific balance point, it is possible that these pathways neutralize one another, with equal and opposing inhibition of the other. Thus, cross-talk inhibition may have the ability to confer key emergent properties of pLTF, such as pattern-sensitivity. Another emergent property, enhanced pLTF (ie. metaplasticity) may arise from diminished inhibitory coupling of these pathways, so that both combine to produce a larger (enhanced) pLTF following AIH. The presence of multiple, competing pathways to pLTF confers flexibility, enabling different manifestations of plasticity as an animal responds to diverse conditions that vary in severity, pattern and/or duration of hypoxia. Greater understanding of multiple pathways giving rise to respiratory motor plasticity and their interactions could increase our understanding of physiological responses to environmental or pathological changes.

As one example, human subjects at high altitude experience chronic sustained hypoxia, giving rise to homeostatic increases in ventilation; this form of respiratory plasticity is often referred to as ventilatory acclimatization to high altitude.[90,91] What role, if any, do the Q and S pathways and their interactions play in this process? Further studies are needed to determine how pathways interact to produce changes in respiratory control appropriate for the prevailing conditions. As another example, humans most frequently experience intermittent hypoxia during sleep, particularly in individuals suffering from obstructive sleep apnea. In such cases, does the severity and pattern of hypoxic episodes determine the type and extent of compensatory respiratory plasticity? These differences may also be of considerable relevance as we consider distinctions between ventilatory plasticity versus plasticity in respiratory-related motor output to the upper airways, which may stabilize or de-stabilize breathing depending on complex interactions between upper airway patency versus chemoreflex gain and apneic threshold. Further studies are needed to answer these questions.

An understanding of complex interactions between mechanisms of respiratory plasticity has considerable importance as we develop strategies to harness intermittent hypoxia induced motor plasticity to treat clinical disorders that impair breathing and other movements (*eg*. ref 31). While much research has been focused on characterizing new forms of plasticity, we are just beginning to understand the significance of interactions between them. These interactions, if understood and controlled, may enable us to optimize therapeutic protocols of rAIH in the treatment of spinal injury, neurodegenerative diseases and even sleep disordered breathing.[27]

Acknowledgments

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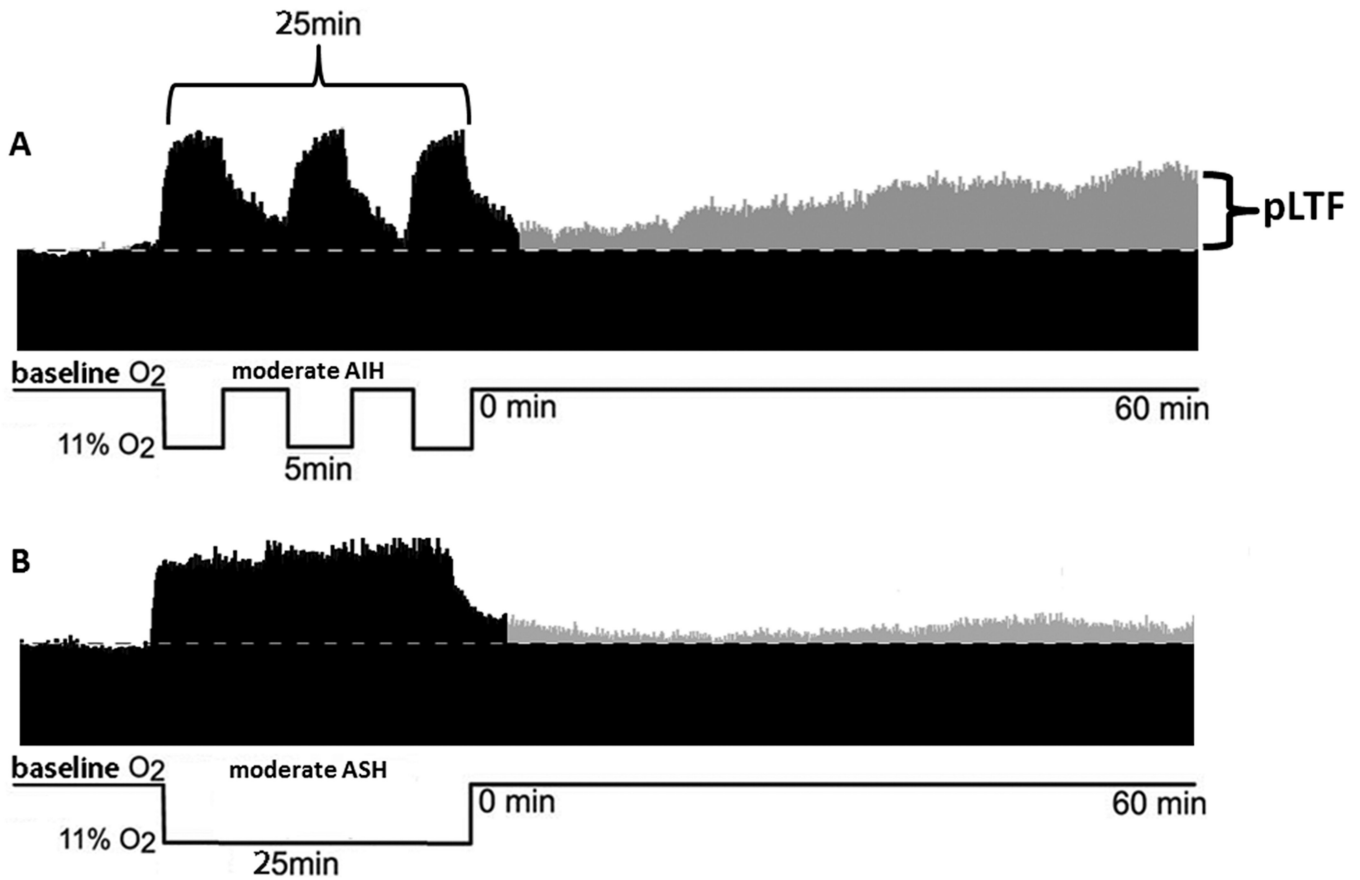
Figure 1.
Pattern sensitivity of phrenic long-term facilitation (pLTF). pLTF is elicited by moderate, acute intermittent hypoxia (AIH), but not by moderate, acute sustained hypoxia (ASH). **A:** Representative tracing of phrenic nerve activity from an anesthestized, vagotomized, paralyzed and pump ventilated rat exposed to moderate AIH. Following AIH, there is a progressive increase in phrenic nerve burst amplitude, indicating pLTF. **B:** Representative tracing from a rat exposed to moderate ASH. Following ASH, there is little increase in phrenic nerve burst amplitude from baseline.
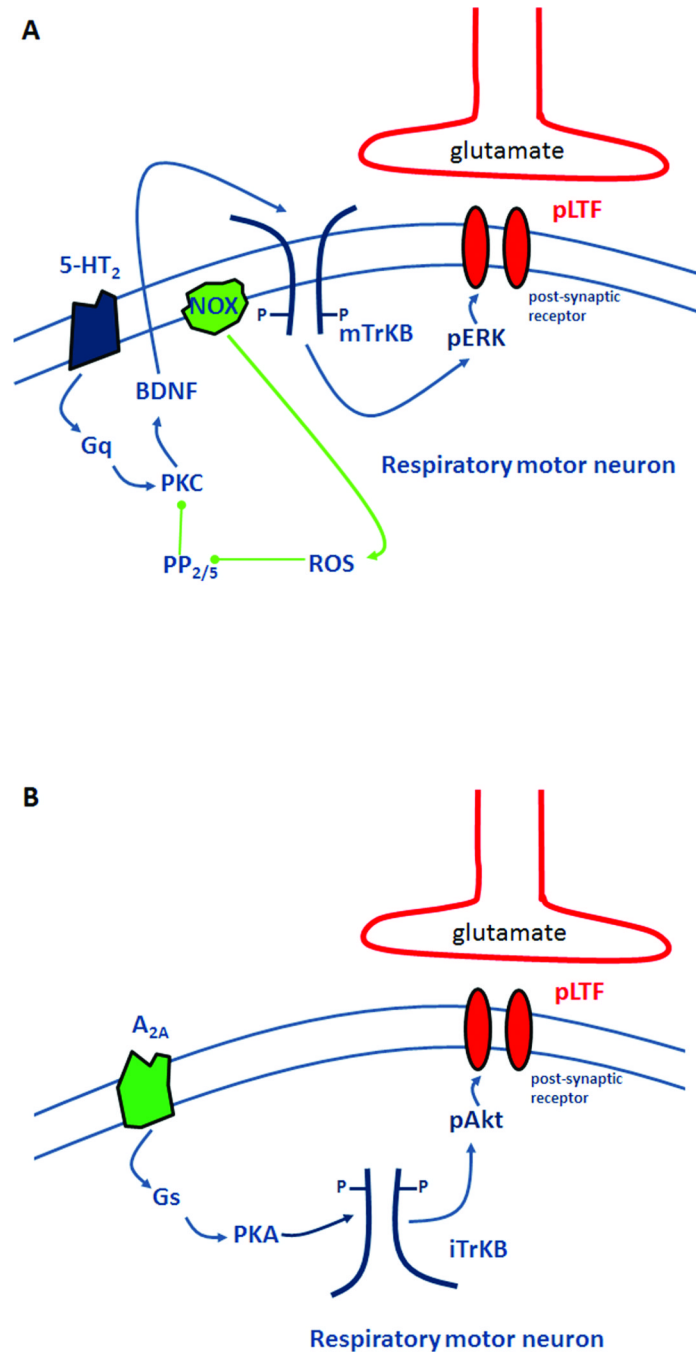
Figure 2.
Working models of cellular pathways giving rise to phrenic motor facilitation following AIH. **A:** The "Q pathway" is initiated by activation of the G_q-protein coupled 5-HT_2 receptor, leading to protein kinase C (PKC) activation and new synthesis of brain-derived neurotrophic factor (BDNF). BDNF then activates its high affinity receptor, TrkB. TrkB activation phosphorylates extracellular signal-related kinase (ERK) MAP kinase, which facilitates inputs to phrenic motor neurons by an unknown mechanism (possibly glutamate receptor trafficking). The Q pathway is regulated by protein phosphatases 2A and/or 5, which can inhibit PKC activation. NADPH oxidase-induced ROS formation inhibits these

phosphatases, enabling pLTF expression. Thus, NADPH oxidase and $PP_{2A/5}$ constitute a "regulatory cassette" for pLTF. **B:** The "S pathway" is induced by the G_s-protein coupled adenosine 2A (A_{2A}) receptor, subsequently activating protein kinase A (PKA). PKA stimulates new synthesis of an immature TrkB isoform, which auto-activates and phosphorylates and activates Akt. Subsequent to Akt activation, synaptic inputs to phrenic motor neurons are facilitated by an unknown mechanism.
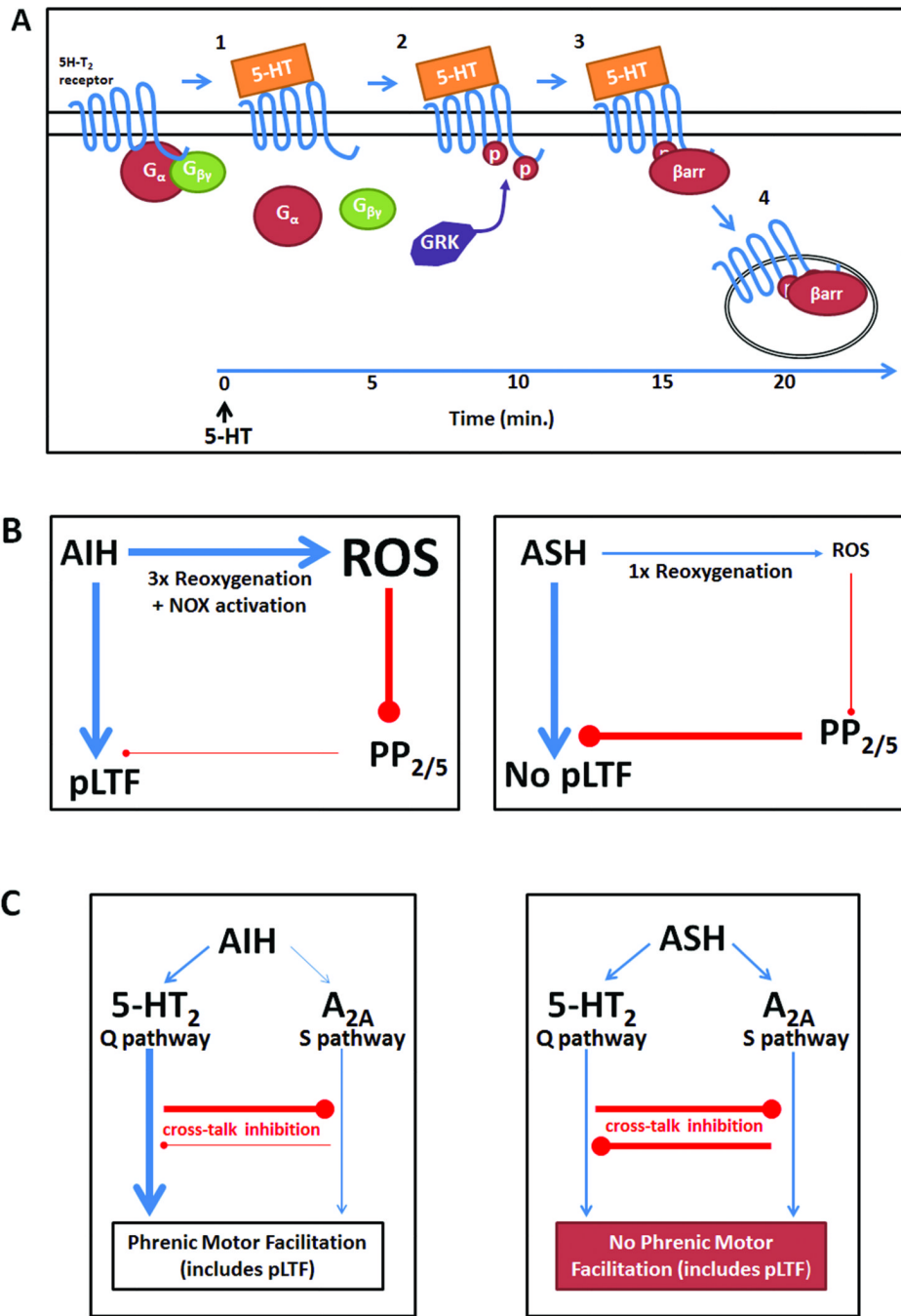
Figure 3.
Possible mechanisms giving rise to pLTF pattern sensitivity. **A:** 5-HT$_2$ receptor desensitization might occur with prolonged 5-HT release, such as during ASH: 1) 5-HT binds to its receptor, releasing Gα and G$\beta\gamma$ proteins, which activate downstream second messengers; 2) after prolonged agonist exposure (~10 min), 5-HT$_2$ receptors are phosphorylated by G-protein coupled receptor kinase (GRK), preventing subsequent activation of G-proteins (ie. functional uncoupling) and promoting β-arrestin (βarr) binding to the 5-HT$_2$ receptor; 3) β-arrestin binding prevents further 5-HT$_2$ receptor activation while promoting receptor internalization; 4) receptors are internalized via a clathrin-dependent

mechanism, decreasing the number of receptors available to elicit pLTF. **B:** Differential reactive oxygen species (ROS) formation during AIH *vs*. ASH may underlie pattern sensitivity. During AIH, ROS production during repeated reoxygenation events and NADPH oxidase (NOX) activation. Increased ROS would inhibit protein phosphatases 2 and 5, disinhibiting PKC (or other kinases) and enabling forward progression of pLTF. During ASH, insufficient ROS formation due to a single reoxygenation event may not inhibit the protein phosphatases sufficient to relieve their constraint to pLTF. **C:**Inhibitory interactions between the Q and S pathways to pMF may underlie pattern sensitivity of pLTF. 1) During AIH, the Q pathway is dominant because of 5-HT$_2$ receptor activation with relatively little adenosine accumulation. 2) During ASH, greater adenosine release/accumulation may cause sufficient S pathway activation to offset Q pathway activation. Balanced activation of both pathways may prevent any pLTF expression due to balanced cross-talk inhibition.
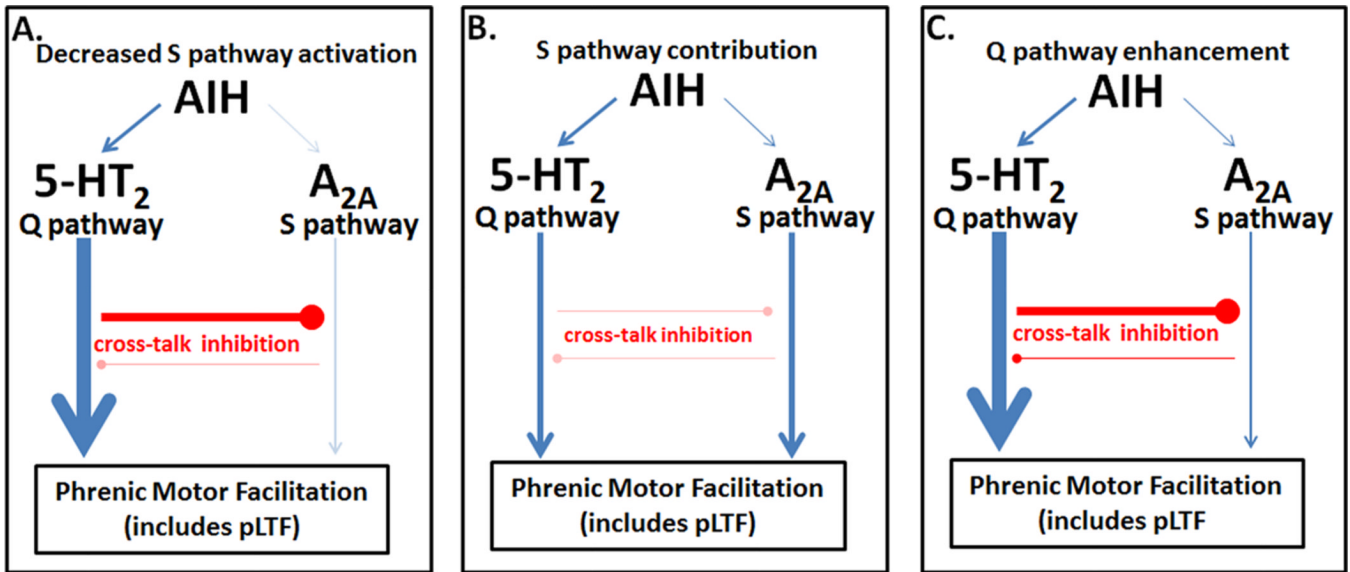
Figure 4.
Possible mechanisms of metaplasticity, enhancing pLTF following repettive intermittent hypoxia. **A:** Decreased S pathway activation, thereby eliminating S to Q inhibition, could explain metaplasticity in pLTF following rAIH. **B:** Reduced cross-talk inhibition, enabling a positive S pathway contribution could enhance pLTF. **C:** Increased Q pathway signaling ("hypertrophy"), possibly involving increased $5-HT_2$ receptors, ROS production, or BDNF synthesis could explain greater pLTF following rAIH.