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Pharmacological modulation of hypoxia-induced respiratory neuroplasticity

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Abstract

Hypoxia elicits complex cell signaling mechanisms in the respiratory control system that can produce long-lasting changes in respiratory motor output. In this article, we review experimental approaches used to elucidate signaling pathways associated with hypoxia, and summarize current hypotheses regarding the intracellular signaling pathways evoked by intermittent exposure to hypoxia. We review data showing that pharmacological treatments can enhance neuroplastic responses to hypoxia. Original data are included to show that pharmacological modulation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) function can reveal a respiratory neuroplastic response to a single, brief hypoxic exposure in anesthetized mice. Coupling pharmacologic treatments with therapeutic hypoxia paradigms may have rehabilitative value following neurologic injury or during neuromuscular disease. Depending on prevailing conditions, pharmacologic treatments can enable hypoxia-induced expression of neuroplasticity and increased respiratory motor output, or potentially could synergistically interact with hypoxia to more robustly increase motor output.

Hypoxia triggers respiratory neuroplasticity

Respiratory neuroplasticity is an experience-induced and persistent change in the neural system controlling breathing (Fuller and Mitchell, 2016). Accordingly, respiratory neuroplasticity is distinct from direct, “real time” stimulation of respiratory neural circuits, such as occurs during chemoreceptor stimulation. Hypoxia can be a powerful trigger of respiratory neuroplasticity, and the pharmacological approaches that have been used to study the underlying mechanisms are the focus of this article.

Much of our current knowledge regarding cellular and molecular mechanisms of hypoxia-induced respiratory neuroplasticity comes from selective application of agonists and/or antagonists of membrane-bound neurotransmitter/neuromodulator receptors on and near respiratory-related neurons, or through pharmacologic manipulation of downstream

signaling molecules (e.g., kinases, phosphatases). These same pharmacologic approaches are useful for controlling the neuroplastic impact of hypoxia in the context of neurorehabilitation (Gonzalez-Rothi et al., 2015). This article provides an overview of pharmacological approaches that have been used to activate or inhibit hypoxia-induced respiratory neuroplasticity. Particular emphasis is placed on experimental methods including drugs and different routes of delivery used (Tables 1–3). Mechanisms of phrenic motor plasticity are highlighted since more is known about the underlying molecular pathways in comparison to other respiratory motor systems. We focus on acute exposure to single or multiple bouts of hypoxia, and conclude with a brief overview of how pharmacological strategies may enhance the development and/or optimization of neurorehabilitation protocols based on moderate hypoxia exposures. This article does not address chronic exposure to sustained hypoxia or intermittent hypoxia, and the reader is referred to several comprehensive reviews of these topics (Almendros et al., 2014; Bisgard, 1995; Fields and Mitchell, 2015; Navarrete-Opazo and Mitchell, 2014).

1.1. Hypoxia pattern and severity

Before discussing experimental methods and hypoxia-induced molecular signaling pathways, we first briefly comment regarding the importance of the hypoxia paradigm. The most salient point is that hypoxia-induced respiratory plasticity is sensitive to the pattern of hypoxia exposure. Indeed the exposure pattern rather than the total hypoxic “dose” is the primary determinant of the signaling pathways that are activated (Baker and Mitchell, 2000; Mitchell et al., 2001). For example, moderate acute *intermittent* hypoxia (AIH) evokes a sustained increase in respiratory motor output called long term facilitation (LTF). However, an acute, *sustained* bout of moderate hypoxia of similar cumulative duration used to induce LTF does not produce sustained increases in respiratory motor output (Baker and Mitchell, 2000; Devinney et al., 2016; Mitchell et al., 2001; Wilkerson et al., 2008). The relative degree of hypoxemia within bouts of intermittent hypoxia also has a profound impact on the molecular pathways leading to facilitation. It follows that there is not a single unique molecular pathway that leads to LTF; moderate AIH paradigms (i.e., PaO₂ values of ~35–45 mmHg) induce LTF via serotonergic mechanisms whereas severe AIH paradigms (i.e., PaO₂ in the range of 25–35 mmHg) activate adenosinergic mechanisms of LTF (Devinney et al., 2013). The serotonergic and adenosinergic pathways actively inhibit one another, such that only one pathway prevails in specific conditions – this interplay is termed “cross-talk inhibition” (Devinney et al., 2013). On the other hand, when serotonergic and adenosinergic mechanisms are “balanced”, the pathways offset one another, and plasticity is no longer observed (Devinney et al., 2013; Devinney et al., 2016). Lastly, when considering hypoxia exposure paradigms, it is important to keep in mind that respiratory plasticity itself adapts based on experience. This sustained change in the capacity to express plasticity after triggering experiences (e.g., chronic intermittent hypoxia) is known as metaplasticity (Fields and Mitchell, 2015). Thus, background experiences of hypoxia (e.g., sleep apnea) may facilitate or undermine plasticity elicited by AIH (reviewed in (Mateika, 2015; Mateika and Syed, 2013)).

2.0 Acute responses and short term potentiation

Mechanisms driving the rapid, acute increase in respiratory motor output (i.e., acute hypoxic ventilatory response (Powell et al., 1998)) during hypoxia are well-established and were recently reviewed (Pamenter and Powell, 2016). The acute hypoxic response is typically followed by a more gradual increase of respiratory output. Once hypoxia is terminated, respiratory activity typically drops rapidly, followed by a slow “roll off” to pre-hypoxia levels. Short term potentiation (STP) of the respiratory motor response to hypoxia includes both the gradual increase in output during hypoxia and the “roll off” in bursting after normoxic conditions are restored (Powell et al., 1998). STP may not be directly caused by hypoxia *per se*, but rather may be a response driven by non-specific increases in synaptic activity in respiratory neurons or networks. It is nevertheless a robust phenomenon having been described in a range of anesthetized animal preparations (Hayashi et al., 1993; Lee and Fuller, 2010a, b; Lee et al., 2015) and also in unanesthetized humans (Fregosi, 1991; Georgopoulos et al., 1995). Respiratory-related outcome measures showing STP include recordings of nerve activity (Hayashi et al., 1993; Lee and Fuller, 2010a, b; Lee et al., 2015), muscle EMG (Mateika and Fregosi, 1997) or direct quantification of ventilation (Fregosi, 1991; Georgopoulos et al., 1995).

Early work in anesthetized cats found that STP is unaffected by vagal nerve stimulation (Eldridge and Gill-Kumar, 1978), is affected by subthreshold respiratory drive (Eldridge, 1980), and does not require input from inspiratory neurons in the medulla (Eldridge, 1980; Eldridge and Gill-Kumar, 1980). Pharmacological methods have also been used to examine the mechanisms of respiratory STP. Early studies by Eldridge and colleagues used intravenous delivery of serotonin (5HT) antagonists to show that STP is 5HT independent (Millhorn et al., 1981). Thus, when methysergide, parachlorophenylalanine, or 5,7-dihydroxytryptamine were given, there was no change in the phrenic nerve response to carotid sinus nerve stimulation, and no effect on STP in anesthetized cats. Moreover, no effect on STP was observed following intravenous administration of antagonists for multiple other neurotransmitter receptors including the dopamine-norepinephrine antagonists alpha-methyltyrosine, and haloperidol and the endorphin antagonist, naloxone (Millhorn et al., 1981). In anesthetized rats, systemic blockade of NMethyl-D-aspartic acid receptors (NMDAR) with intravenous delivery of MK-801 causes a slower onset of phrenic STP during hypoxia, and also extends the time course of STP offset (Poon et al., 1999). Accordingly, the authors suggested that NMDA receptors function as a “molecular” switch involved with both the induction and recovery phases of STP (Poon et al., 1999). Nitric oxide may also be involved in STP since intraperitoneal delivery of the nitric oxide synthase 1 (NOS-1) inhibitor 7-nitroindazole prior to acute hypoxia attenuates or eliminates STP of ventilation (Kline et al., 2002).

Pamenter and Powell (2016) recently proposed a molecular model to explain how stimulation of carotid chemoafferent neurons leads to STP. Sustained glutamate release leads to activation of post-synaptic NMDA receptors on second-order nucleus tractus solitarius (NTS) neurons and intracellular Ca^{2+} accumulation. Increased levels of Ca^{2+} activate calmodulin-dependent protein kinase II (CaMKII). CaMKII modifies membrane-dissociated neuronal NOS (nNOS) and stimulates production of nitric oxide. Nitric oxide then defuses

back across the synaptic cleft to stimulate guanylyl cyclase-mediated production of cyclic guanosine monophosphate (cGMP). cGMP increases presynaptic glutamate release, thereby enhancing excitatory signaling that increases downstream respiratory drive and producing STP of respiratory motor output (Pamenter and Powell, 2016).

2.1 Acute sustained hypoxia – long-term responses

Sustained hypoxic exposures (e.g., >15-min) typically elicit STP onset and offset responses but do not evoke longer-lasting respiratory plasticity, such as phrenic motor facilitation (Baker and Mitchell, 2000). Devinney et al. (2016) recently proposed that lack of plasticity following acute sustained hypoxia (ASH) reflects the mutually-inhibitory interplay between competing serotonin and adenosine-dependent mechanisms (Table 1; (Devinney et al., 2016)). In their study, phrenic motor output was recorded from anesthetized rats pretreated with intrathecal drug application. With this approach, a small incision is made in the dura over the mid-cervical spinal cord, and a catheter is placed to deliver small volumes of solution (e.g., 7–15 μ l). As an internal control, the output of the hypoglossal (XII) nerve is recorded—if XII output is unchanged by the intrathecal drug delivery, it is assumed that the drugs acted locally on the spinal cord (Baker-Herman and Mitchell, 2002). To test whether hypoxia-activated serotonergic and adenosinergic signaling mechanisms (see sections 3.1–3.3) were “offsetting” following sustained hypoxia, a series of experiments were conducted using intrathecal drug delivery and either moderate or severe sustained hypoxia (Table 1). First, when adenosine 2A (A2A) receptors were blocked via intrathecal delivery of MSX-3 prior to moderate ASH ($\text{PaO}_2 \sim 45$ mmHg), a persistent increase in phrenic burst amplitude was noted (> 60 min post-hypoxia). These data suggest that A2A activation constrains plasticity following moderate ASH. Second, intrathecal administration of the broad spectrum 5HT antagonist methysergide prior to moderate ASH did not lead to any long-lasting changes in phrenic nerve output - indicating that serotonin activation following moderate ASH does not constrain the expression of plasticity. Third, dual blockade of A2A and 5HT receptors via intrathecal MSX-3 and methysergide before moderate ASH did not evoke phrenic motor facilitation (Table 1; (Devinney et al., 2016)). Taken together, these findings demonstrate that moderate ASH activates serotonin signaling pathways which are capable of evoking LTF-like plasticity, however, the response is abolished by concurrent activation of adenosinergic signaling. The Devinney paper also showed that a more severe bout of ASH ($\text{PaO}_2 \sim 30$ mmHg) did in fact evoke sustained phrenic motor facilitation that did not require drug pretreatment. Intrathecal delivery of MSX-3 prior to severe ASH abolished this facilitation, indicating adenosine-dependent signaling mechanisms. On the other hand, when the serotonin receptor antagonist methysergide was intrathecally delivered prior to severe ASH, phrenic motor facilitation was potentiated beyond that elicited by severe hypoxia alone. Thus, severe ASH activates adenosine-dependent mechanisms for long-term respiratory plasticity that are attenuated by serotonergic signaling (Table 1; (Devinney et al., 2016)).

Another proposed mechanism constraining phrenic motor plasticity following ASH is elevated protein phosphatase activity (Table 1; (Wilkerson et al., 2008)). Protein kinases and phosphatases regulate the phosphorylation state of proteins, and play critical roles in phrenic motor facilitation (Wilkerson et al., 2008; Winder and Sweatt, 2001). Intrathecal

pretreatment with the serine/threonine phosphatase inhibitor okadaic acid before a bout of ASH revealed phrenic nerve facilitation in urethane anesthetized rats (Table 1; (Wilkerson et al., 2008)). Collectively, the literature indicates that a single hypoxic exposure elicits multiple signaling mechanisms in respiratory neurons and networks, and it is the interplay between these mechanisms that determine the presence (or absence) of sustained increases in respiratory motor output after normoxic conditions are restored.

3.0. Acute intermittent hypoxia (AIH)

AIH-induced LTF has been most extensively studied in the phrenic motor system of anesthetized rats, but is also expressed in other respiratory-related motor outputs including hypoglossal (Bach and Mitchell, 1996), glossopharyngeal (Cao et al., 2010) and intercostal (Fregosi and Mitchell, 1994). Motor facilitation following AIH has also been demonstrated in somatic motor pools including lower extremities in humans with spinal cord injury (Hayes et al., 2014; Trumbower et al., 2012). The literature suggests that expression of AIH-induced LTF is fundamentally similar across different motor pools, but that distinct mechanistic features may be present within motor nuclei (Baker-Herman and Strey, 2011).

For the purposes of this review, we focused on the systemic (e.g., intravenous) and spinal (e.g., intrathecal) pharmacological approaches to block AIH-induced changes in respiratory motor output. These have been cornerstone approaches for elucidating the mechanisms of LTF in phrenic nerve activity. Tables 2 and 3 summarize key pharmacologic approaches that have informed current cellular models of AIH induced respiratory motor plasticity. Figure 1 provides an overview of current thinking regarding mechanisms of phrenic LTF following moderate or severe AIH, and was created based on the studies described in Tables 2–3. Studies with outcome measures other than phrenic nerve recordings are denoted with asterisks in the tables and detailed in the table legend.

3.1. Q pathway to phrenic LTF

Millhorn *et al.*, 1980, used intravenous delivery of the broad spectrum serotonin-receptor antagonist methysergide (and other anti-serotonin drugs including para-chlorophenylalanine and 5–7 dihydroxytryptamine) to establish that serotonin receptor activation plays a role in respiratory LTF induced by electrical activation of chemoafferent neurons (Millhorn et al., 1980). Subsequently, Bach and Mitchell demonstrated that intravenous methysergide also blocks phrenic LTF elicited by moderate episodic hypoxia (Bach and Mitchell, 1996). Intravenous delivery of the selective 5-HT_{2A/C} antagonist ketanserin following AIH does not block phrenic LTF, whereas intravenous ketanserin before AIH abolishes LTF (Fuller et al., 2001). Thus, 5-HT receptor activation is necessary to initiate (during hypoxia), but not maintain (following hypoxia) phrenic LTF once established.

A considerable number of published studies have utilized intrathecal drug delivery above the mid-cervical spinal cord to probe spinal mechanisms of phrenic LTF. The following paragraphs highlight key findings of these intrathecal drug delivery studies, and the associated pharmacological methods are summarized in Tables 2 and 3. Moderate AIH triggers **spinal** 5HT receptor (type 2) activation (Baker-Herman and Mitchell, 2002) that in

turn activates G proteins, triggering new protein synthesis necessary for phrenic LTF; since the canonical G protein in this pathway is Gq, the pathway is called the “Q-pathway” to phrenic motor facilitation (Dale-Nagle et al., 2010). The relevant Gq-protein coupled serotonin receptors initiate phrenic LTF by activating signaling cascades that include extracellular signal-regulated - mitogen-activated protein (ERK-MAP) kinase activity (Wilkerson and Mitchell, 2009), *de novo* brain-derived neurotrophic factor (BDNF) synthesis (Baker-Herman et al., 2004), tropomyosin receptor kinase B (TrkB) activation (Baker-Herman et al., 2004), and downstream PKC θ activity (Agosto-Marlin et al., 2017; Devinney et al., 2015). The 2004 study from Baker-Herman and colleagues was a landmark in terms of experimental methodology and the advancement of the cellular model of phrenic LTF. This was the first effective use of RNA interference (RNAi) *in vivo* in the adult mammalian nervous system; by applying siRNAs targeting BDNF mRNA to the cervical intrathecal space, new BDNF protein synthesis was blocked via impairment of BDNF mRNA translation. This occurred before appreciable knock down of BDNF mRNA and protein levels. This study also demonstrated that spinal tyrosine kinases are necessary for phrenic LTF since intrathecal (C4) delivery of a non-specific TrkB receptor inhibitor, K252a was sufficient to block the response. In a separate set of experiments, phrenic motor facilitation was evoked by delivering BDNF directly to the intrathecal space above the phrenic motor nucleus—thereby demonstrating that increased spinal BDNF is sufficient to elicit phrenic motor plasticity. Collectively, the results demonstrated that new spinal BDNF synthesis is necessary and sufficient for respiratory plasticity following moderate AIH (Baker-Herman et al., 2004).

Subsequent work using intrathecal drug delivery established that 5HT2A and 5HT2B receptor activation are both sufficient to evoke phrenic motor facilitation (Table 2). Pretreatment with nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase inhibitors prior to episodic spinal application of 5HT2A or 5HT2B selective agonists demonstrated that phrenic motor facilitation evoked by 5HT2B receptor activation is NADPH-oxidase dependent, whereas phrenic motor facilitation from 5HT2A receptor activation is not (MacFarlane et al., 2011). Episodic spinal NO donor application (sodium nitroprusside; SNP) is also sufficient to elicit phrenic motor facilitation (MacFarlane et al., 2014) and pretreatment with a nNos inhibitor demonstrated that spinal nNos regulates phrenic motor facilitation (MacFarlane et al., 2014). NADPH oxidase subunits are a major source of reactive oxygen species (ROS, (MacFarlane et al., 2009)). Systemic or spinal pretreatment with a superoxide anion scavenger (MnTMPyP) blocks pLTF, demonstrating that ROS near the phrenic motor nucleus are necessary for mAIH-induced pLTF (MacFarlane and Mitchell, 2008).

The sequence of events leading to phrenic motor facilitation via the Q pathway is predicted to phosphorylate glutamate receptor subunits on respiratory (phrenic) motoneurons. Consistent with this hypothesis, spinal cord blockade of NMDA receptors via intraperitoneal injection of MK-801 prevents phrenic LTF (McGuire et al., 2008).

3.2. S Pathway to phrenic LTF

Severe AIH (e.g., PaO₂ 25–35 mmHg) also evokes phrenic LTF, but via distinct cellular pathways (Nichols et al., 2012). This stimulus activates a 5HT₂-independent pathway known as the “S pathway,” since the initiating receptors are Gs protein coupled (Dale-Nagle et al., 2010). As summarized in Table 3, intrathecal drug delivery has been used to demonstrate that the S pathway to phrenic LTF can be evoked by activation of 5HT₇ receptors (Hoffman and Mitchell, 2011) or adenosine 2A receptors (A_{2A}) (Nichols et al., 2012), cyclic AMP signaling (Fields and Mitchell, 2017) via exchange protein activated by cAMP (EPAC; (Fields et al., 2015)), activation of protein kinase B/Akt (Golder et al., 2008; Hoffman and Mitchell, 2013), mammalian target of rapamycin signaling (mTorr; (Dougherty et al., 2015)), and new synthesis of an immature TrkB isoform (Hoffman and Mitchell, 2013). The cell signaling mechanisms that lead from immature TrkB synthesis to phosphorylated glutamate receptors remain to be determined (Fig. 1).

3.3. Cross-talk inhibition between Q and S pathways

The Q and S pathways to phrenic motor facilitation interact via cross-talk inhibition. In some circumstances, a single pathway predominates with the other acting as a brake to phrenic motor facilitation (Dale-Nagle et al., 2010; Devinney et al., 2013). This relationship is revealed when the constraining pathway is blocked; for example, when adenosine 2A (Hoffman et al., 2010) or serotonin 7 receptor antagonists (Hoffman and Mitchell, 2013) are delivered prior to moderate AIH, phrenic LTF is nearly doubled due to loss of S to Q pathway inhibition. In some conditions, cross-talk inhibition between the Q and S pathways can be equal and opposite, effectively canceling plasticity. Thus, whereas intrathecal delivery of a 5HT_{2B} (BW 723C86) or 5HT₇ receptor agonist (AS-19) elicits phrenic motor facilitation when applied alone (via the Q and S pathways, respectively), plasticity is no longer observed with concurrent agonist administration (Perim et al., 2017). These data demonstrate strong, mutual inhibition between the Q and S pathways to phrenic motor facilitation.

Cross-talk inhibition can enable emergent properties, such as the sensitivity of phrenic LTF to the pattern of hypoxia (Devinney et al., 2013). For example, whereas moderate acute intermittent hypoxia elicits phrenic LTF, moderate sustained hypoxia does not (Baker and Mitchell, 2000). On the other hand, if the serotonin-dependent Q or the adenosine-dependent S pathway is blocked via cervical spinal administration of a receptor antagonist, phrenic motor facilitation is revealed after moderate sustained hypoxia, demonstrating that phrenic LTF pattern-sensitivity arises from cross-talk interactions between competing pathways to phrenic motor facilitation (Devinney et al., 2016).

The S pathway inhibits Q pathway-dependent facilitation via cAMP-dependent activation of protein kinase A (PKA) (Fields and Mitchell, 2017; Hoffman and Mitchell, 2013). Although the mechanism whereby PKA inhibits the Q pathway is uncertain, these authors hypothesized that it inhibits MEK/ERK activation (Fig. 1). Further, with concurrent Q and S pathway activation via intrathecal agonist administration, PKA inhibition reveals Q-pathway plasticity (Fields and Mitchell, 2017). Less is known concerning mechanisms whereby the Q

pathway inhibits S pathway-dependent phrenic motor facilitation. Preliminary studies suggest that NADPH oxidase plays a key role. Specifically, with concurrent Q and S pathway activation, phrenic motor plasticity is revealed by intrathecal cervical application of the NADPH oxidase inhibitor apocynin (Perim et al., 2017), potentially by inhibiting adenylate cyclase or EPAC (Fig. 1).

Mechanistic studies are currently on-going, and our understanding of the functional significance of cross-talk inhibition is incomplete. However, both are interesting topics for further research. The emerging picture is that both mechanisms can be activated to variable degrees by hypoxia, depending on the severity and/or pattern of hypoxia. This activation balance will determine which pathway predominates and which will act instead as a “brake,” titrating the magnitude of plasticity expression. The balance between the Q and S pathways, and the strength of their cross-talk interactions may differ among different populations of motor neurons. Such motor neuron heterogeneity may confer differential properties to different motor pools so that their response is appropriate to their role in critical movements, such as breathing.

4.0 Pharmacological strategies to *enhance* hypoxia induced plasticity

There are multiple reasons to investigate methods to enhance hypoxia induced plasticity. One consideration is that while moderate AIH is safe (Navarrete-Opazo and Mitchell, 2014), it may still be beneficial to use pharmacological methods reduce the necessary AIH “dose” (e.g., severity, number of hypoxic events, or duration) for triggering plasticity. This could be a practical consideration for clinical AIH exposures targeting neurorehabilitation (Gonzalez-Rothi et al., 2015). Second, regardless of hypoxia dose, it may be possible to *enhance* the functional impact of hypoxia paradigms through pharmacologic treatments. As three possible examples, enhancing glutamatergic signaling (Turner et al., 2016a), blocking A2A receptor signaling (Hoffman et al., 2010; Hoffman and Mitchell, 2013; Navarrete-Opazo et al., 2017; Navarrete-Opazo et al., 2015; Navarrete-Opazo et al., 2014) or reducing inflammation (reviewed in (Hocker et al., 2017)) are strategies to enhance the relative magnitude of AIH induced respiratory neuroplasticity with potential functional benefits in the context of neurorehabilitation. However, to date the concept of “priming” the respiratory neural control system (or other motor circuits) to enhance hypoxia-induced motor output has received relatively little formal evaluation.

4.1 Ampakines enhance hypoxia-induced respiratory plasticity

Glutamatergic synaptic transmission is critical in both the generation and transmission of the neural drive to breathe (Liu et al., 1990). Pharmacologic modulation of glutamatergic currents therefore has potential to increase respiratory motor output, both in health and disease. In this regard, a class of compounds known as ampakines appears to be particularly effective. Ampakines are designed to enhance AMPA-mediated glutamatergic neurotransmission (Lynch, 2006) but are not AMPA receptor agonists. Rather, ampakines act as positive allosteric modulators of AMPA receptors and do not impact NMDA or kainate receptors directly (Arai and Kessler, 2007).

Ampakines successfully stimulate breathing after opioid overdose (Lorier et al., 2010) and in several neuromuscular disorders (ElMallah et al., 2015; Ogier et al., 2007). However, AMPA receptors are widely distributed on respiratory-related neurons (Feldman et al., 2003) making it challenging to localize the primary site at which ampakines impact the respiratory network. The Greer laboratory has demonstrated that ampakines can enhance inspiratory rhythmogenesis in the brainstem, and can act directly on respiratory motoneurons (Lorier et al., 2010; Ren et al., 2009; Ren et al., 2013). Current data indicate that ampakines have relatively little impact on normal baseline or “eupneic” respiratory patterns, but can have a strong impact on brainstem rhythm-related circuits when the prevailing level of respiratory drive is relatively low (Greer and Ren, 2009; Ren et al., 2009; Turner et al., 2016a). Under conditions of excessive inhibition such as occurs following opiate overdose, intravenous ampakines robustly increase the rate of breathing, but with little impact on the amplitude of inspiratory efforts (Ren et al., 2009). However, ampakines also stimulate depressed inspiratory burst amplitudes. We recently observed this in a murine model of a rare neuromuscular condition (Pompe disease) that is associated with respiratory motoneuron pathology (ElMallah et al., 2015). In those experiments, intraperitoneal delivery of ampakine CX717 produced a > 300% increase in inspiratory burst amplitude recorded in the phrenic and XII nerves of Pompe (*Gaa*^{-/-}) mice. In contrast, ampakines had relatively little impact on wild-type mice without neuromuscular pathology (ElMallah et al., 2015). Interestingly, genome wide mRNA screening in the Pompe mouse spinal cord indicates downregulation of AMPA receptors and attenuated glutamatergic signaling pathways (Turner et al., 2016b). One possibility is that overall downregulation of AMPA receptors resulted in enhanced sensitivity of the remaining receptors, and this adaptation explains the robust increase in discharge following delivery of ampakines.

Ampakines can also augment the capacity for long-term synaptic plasticity in the hippocampus (Lynch, 2006), and this recently lead us to explore the impact of ampakine pretreatment on AIH-induced respiratory neuroplasticity (Turner et al., 2016a). After intraperitoneal pretreatment with ampakine (CX717; 15mg/kg), the magnitude of LTF recorded in the hypoglossal (XII) nerve of anesthetized mice was dramatically enhanced. When ampakine CX717 was given 10 min prior to a moderate AIH paradigm (1 min 15% O₂ for 3 cycles), XII burst amplitude was 400 ± 75% of baseline at 60 min post AIH. Vehicle (10% hydroxypropyl-beta-cyclodextrin (HPCD)) pretreated mice also showed LTF of XII burst amplitude at 60-min post-hypoxia, but the value was much less 189 ± 30% baseline. Despite the significant increase in average LTF following ampakine, within group, variability in the magnitude of LTF was observed. Post-hoc analyses indicated that ampakine pretreatment was effective at enhancing LTF only if the initial (baseline) XII burst amplitude was relatively high as compared to maximum output (Turner et al., 2016a). Respiratory rate was not different between ampakine and vehicle treated groups suggesting little to no impact of drug treatment on respiratory rhythmogenic circuits. This observation is consistent with a motoneuron site of action of the ampakine (Lorier et al., 2010).

In recent experiments, we tested whether ampakine CX717 would increase the probability that a single bout of acute hypoxia would produce a persistent increase in XII bursting (Figure 2). In urethane anesthetized and ventilated adult mice (see (Turner et al., 2016a) for description of the basic experimental preparation), intraperitoneal delivery of ampakine

CX717 (15 mg/kg in 10% HPCD) altered the respiratory neuroplastic response to a short bout of moderate hypoxia ($\text{FIO}_2=0.15$, duration 1-min). Specifically, ampakine pretreatment shifted the distribution of XII motor responses such that facilitation of burst amplitude was statistically increased after a single bout of hypoxia as evaluated using 2-way repeated measures analysis of variance (2-way RM ANOVA, vehicle vs. ampakine treatment effect, $P=0.022$). In contrast, respiratory rate (burst per minute) was not different between the two groups (2-way RM ANOVA, treatment effect $p=0.115$). Since ampakine pretreatment reveals hypoglossal motor facilitation following a single hypoxia, these data suggest ampakines decrease the required hypoxic dose (i.e., 1 vs. 3 episodes of hypoxia) for eliciting facilitation of XII burst amplitude. This may occur because certain ampakines can rapidly increase levels of BDNF (Ogier et al., 2007). It is currently unknown whether the ampakine used in our studies (CX717) can modulate BDNF expression, but one possibility is that CX717 resulted in increased BDNF/TrkB signaling within XII motoneurons. In turn, the enhanced BDNF/TrkB signaling could render a single bout of hypoxia sufficient to trigger respiratory motor facilitation via the mechanisms outlined in Fig. 1.

4.2 Enhancing mAIH-induced plasticity using adenosine receptor antagonists.

In 2010 Hoffman et. al. tested the hypothesis that A2A receptor activation contributes to AIH-induced pLTF since extracellular adenosine increases during hypoxia. Contrary to the initial hypothesis, pLTF was *increased* in rats pretreated with a selective A2A antagonist – an effect that was confirmed with intrathecal and intravenous delivery of selective antagonists as summarized in Table 3 (Hoffman et al., 2010). To test whether the enhanced phrenic LTF was still serotonin-dependent, the broad spectrum serotonin antagonist methysergide was administered intrathecally, followed by the intrathecal A2A antagonist MSX-3 before mAIH. When both 5HT and A2A receptors were blocked, mAIH was not sufficient to evoke phrenic LTF; confirming that enhanced phrenic LTF is serotonin dependent (Hoffman et al., 2010). It is important to note that serotonin receptors are both Gq and Gs coupled, depending on the subtype of the receptor. To further explore the contribution of Gs coupled 5HT7 receptors in the expression of phrenic LTF, the 5HT7 selective antagonist SB-269970 was intrathecally applied to the mid-cervical cord prior to mAIH. Selective blockade of 5HT7 receptors resulted in enhanced phrenic LTF; demonstrating a second Gs coupled receptor that constrains Q pathway LTF (Hoffman and Mitchell, 2013). Based on the canonical signaling elicited by Gs protein coupled receptors, Hoffman and Mitchell subsequently hypothesized that blocking protein kinase A (PKA) activity would potentiate the magnitude of LTF. Intrathecal administration of the PKA inhibitor KT-5720 *prior* to mAIH enhanced phrenic LTF (Hoffman and Mitchell, 2013). These publications ((Hoffman et al., 2010; Hoffman and Mitchell, 2013)) show that blockade of Gs protein coupled receptor signaling in the spinal cord can “remove the brakes” on mAIH-induced phrenic motor plasticity, and that the S pathway constrains Q pathway plasticity via a PKA-dependent mechanism (see Section 3.3 for further details on cross-talk inhibition).

Antagonism of adenosine receptors may have implications for AIH-induced neurorehabilitation after SCI, as follows. The Mitchell laboratory has established that AIH exposure elicits respiratory plasticity via a serotonin-*independent* mechanism at 2 wks following cervical SCI in rats (Navarrete-Opazo et al., 2015) and a serotonin-*dependent* mechanism after 8 wks (Dougherty et al., 2017; Navarrete-Opazo et al., 2014). Based on the cellular pathways illustrated in Fig. 1, these data suggest that A2A antagonists may facilitate respiratory plasticity after chronic not acute SCI. To test this idea, rats with and without A2A antagonist treatment were exposed to AIH. Rats began daily AIH exposures at 8 wks post-cervical SCI (10, 5-min episodes, 10.5% O₂; 5-min intervals; 7days) followed by AIH 3× per week for 8 additional weeks (i.e., “reminder doses”) with/without intraperitoneal A2A receptor inhibition (KW6002) on each AIH exposure day. Following the AIH exposure paradigm, tidal volume and bilateral diaphragm electromyogram (EMG) activity were assessed. Blockade of A2A receptors significantly increased respiratory tidal volume and bilateral diaphragm activity beyond the effect of AIH alone (Navarrete-Opazo et al., 2017). Moreover, the functional benefits of AIH plus A2A receptor antagonism were maintained for 4 weeks (Navarrete-Opazo et al., 2017). Collectively, the data indicate that A2A antagonism may be a simple, safe, and effective strategy to enhance plasticity in the context of *chronic* spinal cord injury.

4.3 Mitigating inflammation to enhance respiratory plasticity

Neuroinflammation can have a powerful impact on the expression of respiratory neuroplasticity (Hocker et al., 2017). This is of particular importance for translating therapeutic applications of AIH (Gonzalez-Rothi et al., 2015) to humans since neuroinflammation is common in many chronic diseases and neurologic conditions that are associated with impaired breathing and/or locomotor function. For example, respiratory capacity is severely reduced in individuals suffering from cervical spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), and Pompe disease-persistent neuroinflammation is common to these and most other neurological conditions (Hausmann, 2003; Turner et al., 2016b; Volpe and Nogueira-Machado, 2015).

Low-grade systemic inflammation can be induced by a single lipopolysaccharide (LPS) injection (I.P., 100 µg/kg) which elicits spinal inflammatory gene expression (Hocker et al., 2017; Huxtable et al., 2013). Moderate AIH exposure 3 or 24 h hours following LPS injection does not elicit phrenic LTF (amplitude change 3–4% from baseline values). However, pretreatment with a high dose of the nonsteroidal anti-inflammatory drug ketoprofen (12.5 mg/kg, I.P.) restored the ability of AIH to induce phrenic LTF 24 h following LPS treatment (Huxtable et al., 2013). In a similar study, spinal cord inflammation was elicited by one night of a chronic intermittent hypoxia simulating that experienced during moderate sleep apnea (2 min cycles of hypoxia/normoxia, FiO₂=0.105, 8 h). This paradigm increased pro-inflammatory gene expression in the spinal cord, and abolished the ability of AIH to induce phrenic LTF (60 min post-AIH values within 5% of baseline) (Huxtable et al., 2015). However, ketoprofen (12.5 mg/kg; I.P.) or direct inhibition of spinal p38 MAP kinase (C4 intrathecal delivery) restored phrenic LTF in these rats (Huxtable et al., 2015). In the working model from the Mitchell laboratory, systemic inflammation leads to p38 MAP kinase activation which activates serine/threonine protein phosphatases that

dephosphorylate and inactivate kinases critical for mAIH-induced pLTF expression (Tadjalli et al., 2017). On-going studies suggest that intrathecal administration of the serine/threonine protein phosphatase inhibitor okadaic acid in LPS-treated rats restores mAIH-induced LTF (Tadjalli et al., 2017). Thus, LPS pretreatment and chronic intermittent hypoxia (2 min cycles; 8h) are important models for understanding how the “rules of plasticity” change based in a pro-inflammatory CNS milieu, and may suggest new protocols to enhance/restore AIH-induced respiratory plasticity.

5.0. Conclusion: potential significance to neurorehabilitation.

By focusing on short-duration exposures and mild reductions in O₂, AIH can promote beneficial neuroplastic responses without evoking pathology associated with more severe intermittent hypoxia (Navarrete-Opazo and Mitchell, 2014). Studies conducted at Rehabilitation Institute of Chicago (Trumbower et al., 2012), Emory University (Hayes et al., 2014), the Teleton Rehabilitation Institute (Chile) (Navarrete-Opazo et al., 2016), Wayne State (Sankari et al., 2015) and University of Florida (Tester et al., 2014) all show that AIH can be safely delivered to humans with spinal cord injury (Hayes et al., 2014; Navarrete-Opazo et al., 2016; Sankari et al., 2015; Tester et al., 2014; Trumbower et al., 2012). Pairing hypoxia-based therapies with pharmacologic approaches may provide a way to boost the functional impact of mild hypoxia exposures, and may also tailor the therapy to each individual. For example, in certain cases, anti-inflammatory treatments may be indicated, whereas in other conditions boosting glutamatergic signaling may be beneficial (e.g., Fig. 2; Table 4). Pharmacologic approaches may also reduce the number of hypoxic episodes necessary for activating intracellular pathways necessary for sustained increases in respiratory and somatic motor output (e.g., ampakines, see section 4.1). We suggest that further study of the interactions of drug treatments with moderate AIH paradigms is scientifically necessary and potentially clinically meaningful.

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Figure 1. Schematic illustrating cellular pathways which can lead to pLTF.

Phenotypically similar pLTF can be elicited by distinct signaling pathways that have been termed “Q” and “S” (named for associated G-protein coupled receptors). The solid lines represent signaling interactions that have been experimentally validated as reviewed in the text; the dotted lines represent hypothesized connections. See text for detailed descriptions. The red numbers correspond to the leftmost columns in Tables 1 and 2.

Figure 2. Ampakine pretreatment enables a single hypoxic exposure to evoke XII motor facilitation.

Ampakine CX717 was given via intraperitoneal injection (15 mg/kg) in anesthetized mice; see text for details. **A**) In vehicle-treated mice, mean XII inspiratory burst amplitude was maintained close to baseline values for 60-min following an acute hypoxic episode. When ampakine was given prior to acute hypoxia, a persistent facilitation in burst amplitude was sustained for 60-min post-hypoxia (treatment effect, $P = 0.022$). **B**) Relative to baseline values, burst frequency tended to be lower in the post-hypoxia period in the group pretreated with ampakine, but this did not reach statistical significance. In both A and B, the right panel shows data from 60-min post-hypoxia, and provides each individual data point as well as the mean. In the left panels, the statistical test was a 2-way repeated measures ANOVA.

Table 1.
Summary of drugs which reveal respiratory neuroplasticity following a single exposure to hypoxia.

Moderate acute sustained hypoxia (mASH) describes exposures with PaO₂ values of 35–45 mmHg; severe acute sustained hypoxia (sASH) describes studies with PaO₂ in the range of 25–35 mmHg. I.T. = intrathecal drug delivery, I.P. = intraperitoneal drug delivery, PMF = phrenic motor facilitation, A2A = adenosine 2A, 5-HT = serotonin, AMPA= α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, R = receptor.

Drug / Delivery	Primary action	Hypoxia paradigm	Outcome	Interpretation	Citation
Vehicle / I.T.	n/a	mASH	No Facilitation	mASH is not sufficient for PMF	(Devinney et al., 2016)
MSX-3 / I.T.	A2A-R block	mASH	PMF	Spinal A2A-R block reveals PMF	
Methysergide / I.T.	5-HT-R block	mASH	No Facilitation	Spinal 5-HT-R block does not reveal PMF	
MSX-3 and methysergide / I.T.	Spinal A2A-R + 5-HT-R block	mASH	No Facilitation	PMF after spinal A2A-R blockade is 5HT-dependent	
Vehicle / I.T.	n/a	sASH	PMF	sASH is sufficient for PMF	
MSX-3 / I.T.	A2A-R block	sASH	No Facilitation	sASH-induced PMF is dependent on spinal A2A-R	
Methysergide / I.T.	5-HT-R block	sASH	<i>Enhanced</i> PMF	Spinal 5-HT-R activation constrains, but does not abolish, A2A-dependent PMF	
Vehicle / I.T.	n/a	mASH	No Facilitation	mASH is not sufficient for PMF	(Wilkerson et al., 2008)
Okadaic Acid / I.T.	Protein phosphatase inhibition (PP1, 2, 5)	mASH	PMF	Spinal protein phosphatases constrain PMF	
Okadaic acid and methysergide / I.T.	Protein phosphatase inhibition + 5-HT-R block	mASH	No Facilitation	Spinal protein phosphatase inhibition reveals 5-HT-dependent PMF	
Ampakine CX717 / I.P.	Positive allosteric modulation of AMPA-R	15% inspired O ₂ for 1-min	Hypoglossal Motor Facilitation	Amapkines enable a single hypoxic exposure to evoke sustained increases in XII motor output	Figure 2

Table 2.
Summary of studies which have used drugs to study the “Q-pathway” to respiratory motor facilitation following AIH.

Numbers in the leftmost column correspond to the red numbers in Figure 1. I.V. = intravenous drug delivery, I.P. = intraperitoneal drug delivery, I.T. = intrathecal drug delivery, PMF = phrenic motor facilitation, pLTF = phrenic long term facilitation

Identifier in Fig. 1	Drug & Primary Action	Drug delivery method	Result	Citation
6	<ul style="list-style-type: none"> Phenylephrine: α1 receptor agonist Prazosin: α1 receptor antagonist 	I.V. and I.T.	Gq protein-coupled α 1 adrenergic receptors evoke PMF, but are not necessary for pLTF	(Huxtable et al., 2014)
1, 7	<ul style="list-style-type: none"> Methysergide: nonspecific 5-HT-R antagonist 	I.V.	5-HT receptors are necessary for pLTF	(Bach and Mitchell, 1996)
7	<ul style="list-style-type: none"> Ketanserin: 5HT2A/C-R antagonist 	I.V.	5-HT-R activation is necessary to initiate (during hypoxia) but not maintain (following hypoxia) pLTF	(Fuller et al., 2001)
1,7,9	<ul style="list-style-type: none"> Methysergide: nonspecific 5-HT-R antagonist Emetine: protein synthesis inhibitor Cycloheximide: protein synthesis inhibitor 	I.T.	pLTF requires spinal serotonin receptor activation and protein synthesis	(Baker-Herman and Mitchell, 2002)
1,3	<ul style="list-style-type: none"> DOI: 5-HT2A-R agonist BW723C86: 5-HT2B-R agonist Ketanserin: 5-HT2A-R antagonist SB206553: 5-HT2B-R antagonist Apocynin: NADPH oxidase inhibitor DPI: NADPH oxidase inhibitor 	Episodic I.T. 5-HT-R agonists I.T. NADPH	5-HT2A-R and 5-HT2B-R agonist-induced PMF were both blocked by selective 5HT antagonists, but not by antagonists to the opposite receptor subtype. Pre-treatment with NADPH oxidase inhibitors blocked 5-HT2B, but not 5-HT2A-induced PMF	(MacFarlane et al., 2011)
4	<ul style="list-style-type: none"> MnTMPyP: superoxide anion scavenger 	I.V. or I.T.	ROS near the phrenic motor nucleus are necessary for AIH-induced pLTF	(MacFarlane and Mitchell, 2008)
3,4	<ul style="list-style-type: none"> Apocynin: 1.NADPH oxidase inhibitor DPI: NADPH oxidase inhibitor 	I.T.	Blocking NADPH oxidase attenuates pLTF. NADPH oxidase subunits are a major source of ROS necessary for pLTF	(MacFarlane et al., 2009)
3,4	<ul style="list-style-type: none"> 5-HT Apocynin: NADPH oxidase inhibitor DPI: NADPH oxidase inhibitor 	Episodic I.T. 5-HT; I.T. NADPH	Episodic spinal 5-HT receptor activation is sufficient to elicit PMF by an NADPH oxidase-dependent mechanism	(MacFarlane and Mitchell, 2009)

Identifier in Fig. 1	Drug & Primary Action	Drug delivery method	Result	Citation
3,4,5	<ul style="list-style-type: none"> nNOS-inhibitor-1: neuronal NOS inhibitor sodium nitroprusside, SNP: NO donor SB206553: 5-HT_{2B}-R antagonist MnTMPyP: superoxide dismutase mimetic Apocynin: NOX inhibitor DPI: NADPH oxidase inhibitor KT-5823: protein kinase G (PKG) inhibitor 	I.T.	Spinal nNOS activity is necessary for AIH-induced pLTF. Episodic spinal NO is sufficient to elicit pMF by a mechanism that requires 5-HT _{2B} -R activation and NOX-derived ROS formation. AIH (and NO) elicits spinal respiratory plasticity by a nitrergic-serotonergic mechanism.	(MacFarlane et al., 2014)
8	<ul style="list-style-type: none"> U0126: MEK inhibitor PI-828: PI3K inhibitor 	I.T.	pLTF requires MEK/ERK (not PI3K/AKT) signaling. ERK is critical for the development but not maintenance of pLTF.	(Hoffman et al., 2012)
9	<ul style="list-style-type: none"> BDNF K252a: tyrosine kinase inhibitor BDNF siRNA 	I.T.	BDNF is necessary and sufficient for pLTF	(Baker-Herman et al., 2004)
10	<ul style="list-style-type: none"> siTrkB: siRNA targeting TrkB 	I.P.	pLTF requires TrkB protein within the phrenic motor nucleus	(Dale et al., 2017)
2, 11	<ul style="list-style-type: none"> Go 6983: broad-spectrum classical PKC active-site inhibitor BIS: broad-spectrum novel + classical PKC active-site inhibitor NPC: inhibitor of most PKC isoforms, except PKCμ and PKCθ CID755673: PKCμ inhibitor Sotrastaurin: broad-spectrum PKC inhibitor that potentially inhibits PKCθ TIP: block of PKCθ PKCθ siRNA PKCζ siRNA 	I.T. Drugs I.P. siRNA	pLTF requires spinal PKC isoform PKC θ .	(Devinney et al., 2015)
12	<ul style="list-style-type: none"> MK-801: NMDA-R antagonist 	I.P. or Intraspinal injection (C4)	Activation of NMDA-Rs are necessary for pLTF	(McGuire et al., 2005)
12	<ul style="list-style-type: none"> APV: NMDA-R antagonist CNQX: non-NMDA-R antagonist 	I.P.	Activation of NMDA-R, but not non-NMDA-R is necessary for induction and maintenance of ventilatory LTF	(McGuire et al., 2008)
13 21	<ul style="list-style-type: none"> BW 723C86 HCl: 5-HT_{2B}-R agonist AS-19: 5-HT₇-R agonist Apocynin: NADPH oxidase inhibitor 	I.T.	Concurrent 5-HT _{2B} -R and 5-HT ₇ -R activation undermines pMF. 5-HT ₇ activation	(Perim et al., 2017)

Identifier in Fig. 1	Drug & <i>Primary</i> Action	Drug delivery method	Result	Citation
	<ul style="list-style-type: none">Rp-8-Br-cAMPS: PKA inhibitor		inhibits the Q pathway via a PKA- dependent mechanism.	

Table 3.
Summary of pharmacological approaches used to study the “S Pathway” to respiratory motor facilitation.

The numbers in the leftmost column correspond to the red numbers in Figure 1. Moderate acute intermittent hypoxia (mAIH) describes exposures with PaO₂ values of 35–45 mmHg; severe acute intermittent hypoxia (sAIH) describes studies with PaO₂ in the range of 25–35 mmHg. I.P. = intraperitoneal drug delivery, I.T. = intrathecal drug delivery, PMF = phrenic motor facilitation, pLTF = phrenic long term facilitation

Identifier in Fig. 1	Plasticity Stimulus	Drugs & Primary Action	Drug delivery method	Result	Citation
14	Episodic 5-HT ₇ -R agonist (AS-19)	<ul style="list-style-type: none"> SB 269970: 5-HT₇-R antagonist k252a: tyrosine kinase inhibitor siRNAs preventing TrkB (but not BDNF) mRNA translation 	I.T.	PMF evoked by episodic, spinal 5-HT ₇ -R activation requires TrkB synthesis and activity	(Hoffman and Mitchell, 2011)
21	mAIH	<ul style="list-style-type: none"> SB-269970: 5-HT₇-R antagonist KT-5720: PKA inhibition 8-br-cAMP: PKA activation 	I.T.	mAIH-induced pLTF is constrained by coincident activation of 5-HT ₇ -R via activation of PKA	(Hoffman and Mitchell, 2013)
14, 15	sAIH and mAIH	<ul style="list-style-type: none"> MSX-3: A_{2A}-R antagonist Methysergide: 5-HT-R antagonist 	I.T.	sAIH shifts pLTF from a 5-HT- to A _{2A} -dependent mechanism; the S pathway inhibits the Q pathway following mAIH	(Nichols et al., 2012)
15	mAIH	<ul style="list-style-type: none"> MSX-3: A_{2A}-R antagonist ZM2412385: A_{2A}-R antagonist DPCPX: A₁-R antagonist 	I.T.	Spinal A _{2A} -R, not A ₁ -R, constrain the expression of 5-HT-dependent pLTF	(Hoffman et al., 2010)
14 16 17 19 21	Episodic 5-HT ₇ -R agonist (AS-19)	<ul style="list-style-type: none"> ESI-05: EPAC inhibitor KT-5720 PKA inhibitor 8-pCPT-2'-Me-cAMP: EPAC activator Rapamycin: mTORC1 inhibitor 	I.T.	Spinal 5-HT ₇ R activation elicits an EPAC and mTORC1 dependent pMF. Divergent cAMP signaling underlies the distinct functions of 5-HT ₇ R as they elicit (EPAC) and constrain (PKA) spinal serotonin-dependent plasticity	(Fields et al., 2015)
19	sAIH or mAIH	<ul style="list-style-type: none"> Rapamycin: mTOR complex 1 inhibitor 	I.T.	Spinal mTOR activity is required for adenosine-dependent (sAIH) but not 5-HT-	(Dougherty et al., 2015)

Identifier in Fig. 1	Plasticity Stimulus	Drugs & Primary Action	Drug delivery method	Result	Citation
				dependent (mAIH) pLTF	
15 18 20	Episodic A2A-R agonist (CGS 21680)	<ul style="list-style-type: none"> MSX-3: A2A antagonist K252a: tyrosine kinase inhibitor Emetine: protein synthesis blocker siRNA targeting TrkB mRNA 	I.T. (CGS 21680); I.P. (MSX-3 and K252a); I.T. (Emetine)	A2A-R activation induces pMF. A2A-R activation increased phosphorylation and new synthesis of immature TrkB protein, induced TrkB signaling through Akt, and strengthened synaptic pathways to phrenic motoneurons.	(Golder et al., 2008)
21	mAIH	<ul style="list-style-type: none"> KW6002: A2AR inhibitor 	I.P.	AIH induces LTF of diaphragm and external intercostal EMG bursting in spinal-intact and spinal cord injured rats. A2A-R blockade enhances AIH-induced LTF in spinal-intact but not spinally injured rats.	(Navarrete-Opazo et al., 2014)
16, 17, 21	Episodic 5-HT2A-R agonist (DOI) PKA activator (Rp-8-Br-cAMPS) or EPAC activator (8-pCPT-2'-Me-cAMP)	<ul style="list-style-type: none"> DOI: 5-HT2A-R agonist 6-Bnz-cAMP: PKA activator Rp-8-Br-cAMPS: PKA inhibitor 8-pCPT-2'-Me-cAMP: EPAC activator ESI-05: EPAC inhibitor 	I.T.	Spinal activation of PKA, EPAC and 5-HT2A-R alone are sufficient for pMF; concurrent 5-HT2A-R and PKA activation block PMF; concurrent 5-HT2A-R and EPAC enhances PMF	(Fields and Mitchell, 2017)

Table 4.
Summary drugs to manipulate the magnitude of respiratory neuroplasticity following intermittent hypoxia.

Moderate acute intermittent hypoxia (mAIH) describes

Preparation and Species	Hypoxia Paradigm	Drug & Primary Action	Drug delivery method	Result	Citation
Urethane anesthetized rat phrenic nerve recording	mAIH	Mild systemic inflammation (LPS).	I.P.	Acute LPS induced-systemic inflammation blocks pLTF	(Vinit et al., 2011)
Urethane anesthetized rat phrenic nerve recording	mAIH	<ul style="list-style-type: none"> Mild systemic inflammation (LPS). NSAID (ketoprofen) 	I.P.	Following LPS, pLTF is restored by ketoprofen	(Huxtable et al., 2013)
Urethane anesthetized rat phrenic nerve recording	sAIH or mAIH	<ul style="list-style-type: none"> Mild systemic inflammation (LPS) A_{2A}R agonist (CGS-21680) A_{2A} R antagonist (MSX-3) 	I.P. (LPS) Intrathecal (A _{2A} R)	LPS does not impair cervical A _{2A} -R induced pMF nor sAIH-induced pLTF. The adenosine-dependent pathway is insensitive to systemic inflammation	(Agosto-Marlin et al., 2017)
Urethane anesthetized rat phrenic nerve recording	mAIH	<ul style="list-style-type: none"> Mild systemic inflammation (LPS) Protein phosphatase inhibitor (okadaic acid) 	I.P. (LPS) Intrathecal (Okadaic acid)	Okadaic acid-sensitive protein phosphatases constrain pLTF following LPS	(Tadjalli et al., 2017)
Nine adult humans with chronic motor-incomplete SCI	Cycles of 9% O ₂ for 90 sec/21% O ₂ for 60 sec	Single dose NSAID (ibuprofen)	Oral	AIH increased lower extremity strength in individuals with incomplete SCI; ibuprofen did not further augment AIH induced strength increases	(Lynch et al., 2017)
Urethane anesthetized, gonadectomized rat phrenic and XII nerve recordings	mAIH	<ul style="list-style-type: none"> testosterone (T) T + aromatase inhibitor (ADT) 5α-dihydrotestosterone (DHT), a form of testosterone not converted to oestradiol 	S.Q.	Phrenic and XII LTF are restored by testosterone replacement in gonadectomized male rats; effect requires the conversion of testosterone to oestrogen	(Zabka et al., 2006)
Urethane anesthetized mouse XII nerve recording	3 cycles of 15% O ₂ for 1-min	Positive allosteric modulation of AMPA receptors (CX717)	I.P.	Ampakine CX717 enhances XII LTF	(Turner et al., 2016a)