Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Respir Physiol Neurobiol. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Respir Physiol Neurobiol. 2018 October ; 256: 15–20. doi:10.1016/j.resp.2017.10.005.

Intermittent but not sustained moderate hypoxia elicits longterm facilitation of hypoglossal motor output

Julia E.R. Wilkerson[1,3], **Michael Devinney**[1,4], **Gordon S. Mitchell**[1,2]

[1]Department of Comparative Biosciences, University of Wisconsin, Madison, WI 53706

[2]Center for Respiratory Research and Rehabilitation, Department of Physical Therapy and, McKnight Brain Institute, University of Florida, Gainesville, FL, 32610.

[3]Present address: Department of Neuroscience, University of Texas Southwestern, Dallas, TX 75390

[4]Present address: Department of Anesthesiology, Duke University, Durham, NC, 27710

Abstract

Phrenic long-term facilitation (pLTF) is a form of serotonin-dependent respiratory motor plasticity induced by moderate acute intermittent hypoxia (AIH), but not by moderate acute sustained hypoxia (ASH) of similar cumulative duration. Thus, moderate AIH-induced pLTF is sensitive to the pattern of hypoxia. On the other hand, pLTF induced by severe AIH protocols is neither pattern sensitive nor serotonin dependent (it converts to an adenosine-dependent mechanism). Although moderate AIH also induces hypoglossal LTF (hLTF), no data are available concerning its sensitivity/insensitivity to the pattern of hypoxia. Since hLTF following moderate hypoxia is serotonin-dependent, we hypothesized that hLTF is pattern-sensitive, similar to serotonin-dependent pLTF. Integrated hypoglossal nerve activity was recorded in urethane-anesthetized, vagotomized, paralyzed, and ventilated rats exposed to isocapnic AIH (3, 5 min episodes of 11% O_2) or ASH (a single 25 min episode of 11% O_2). Similar to previous studies of pLTF, hypoglossal motor output was elevated for more than 1 h following AIH (50 ± 20%, $P < 0.01$), but not ASH (−6 ± 9%, $P > 0.05$). Frequency LTF was not observed following either hypoxic exposure. Thus, in agreement with our hypothesis, hypoglossal LTF following moderate AIH is pattern-sensitive, similar to phrenic LTF.

Keywords

plasticity; hypoxia; pattern; control of breathing

[*]***Corresponding author:*****Gordon S. Mitchell,** Department of Physical Therapy, University of Florida, 330 Center Drive, Gainesville, FL, USA 32610, Phone: 352-273-6107 gsmitche@phhp.ufl.edu.

Contributions: JERW collected and analyzed all data. JERW, MJD and GSM contributed to ideas, data analysis and manuscript preparation. All authors have approved the manuscript.

Conflicts of Interest: The authors declare no competing financial interests.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Introduction

Patterned stimulation is often more effective than continuous stimulation at inducing plasticity in the central nervous system. For example, stimulus presentations spaced over time are more effective at inducing long term memory formation versus an equal duration continuous presentation (Ebbinghaus, 1913; Beck *et al.*, 2000; Sutton *et al.*, 2002; Cepeda *et al.*, 2006). This phenomenon, known as the "spacing effect," is expressed widely throughout the animal kingdom, and has been extensively studied in several animal models and humans because of its potential utility in education (Kerfoot, 2010), psychology (Goverover *et al.*, 2009b), advertising (Janiszewski *et al.*, 2003; Appleton-Knapp *et al.*, 2005) and physical rehabilitation (Goverover *et al.*, 2009a). Consistent with the spacing effect in learning and memory, patterned stimulation (intermittent vs. continuous) is also more effective at eliciting synaptic plasticity in the nervous system. For example, intermediate-term memory formation and synaptic facilitation in *Aplysia* (Mauelshagen *et al.*, 1998; Sutton *et al.*, 2002), and hippocampal long-term potentiation in rodents (Kauer, 1999; Nguyen *et al.*, 2000; Scharf *et al.*, 2002) exhibit pattern sensitivity, with intermittent more effective than continuous stimulus presentations. Despite its importance to neuroplasticity, mechanisms giving rise to pattern sensitivity are not well known.

Some forms of hypoxia-induced respiratory motor plasticity exhibit clear pattern sensitivity (Baker & Mitchell, 2000; Wilkerson *et al.*, 2008). For example, serotonin-dependent phrenic long-term facilitation (pLTF) can be elicited by moderate acute intermittent (AIH), but not moderate acute sustained hypoxia (ASH) of similar cumulative duration (Mitchell *et al.*, 2001; Baker & Mitchell, 2000). Both severe intermittent and sustained hypoxia also elicit pLTF, although this form of pLTF is mediated by a distinct, adenosine-dependent mechanism (Nichols et al., 2012; Devinney et al., 2016). While episodic spinal serotonin receptor activation is required to elicit phrenic motor facilitation in rats, a single, larger serotonin injection fails to elicit the response (MacFarlane & Mitchell, 2009a). Further, whereas intermittent but not sustained serotonin receptor activation elicits phrenic long-term facilitation in neonatal brainstem-spinal cord preparations, similar pattern sensitivity is not observed in inspiratory intercostal activity (Lovett-Barr *et al.*, 2006). Thus, all forms of respiratory motor plasticity do not exhibit similar pattern sensitivity to the inducing stimulus.

Another form of hypoxia-induced respiratory motor plasticity is hypoglossal long-term facilitation (hLTF; Bach and Mitchell, 1996). Similar to pLTF, hLTF is expressed as a progressive increase in hypoglossal motor output induced by moderate AIH (Bach & Mitchell, 1996; Baker-Herman and Strey, 2011). Hypoglossal LTF might stabilize the upper airways, maintaining upper airway patency during sleep (Fuller, 2005; Mahamed and Mitchell, 2007; Baker-Herman and Strey, 2011). Thus, greater understanding of distinctions between the mechanisms giving rise to pLTF versus hLTF could lead to new therapeutic approaches that minimize certain forms of obstructive sleep apnea (Baker-Herman and Strey, 2011). However, little is known concerning similarities and differences in the mechanisms of pLTF and hLTF, and there are no reports concerning pattern-sensitivity of hLTF with moderate hypoxia. Here, we demonstrate that hLTF exhibits pattern sensitivity, similar to moderate AIH-induced pLTF.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Materials and Methods

Experiments were performed on 3–5 month old male Sprague-Dawley rats (colony PO4, Charles River Inc., Wilmington, MA). Rats were individually housed in a controlled environment (12h light/dark cycle), with food and water *ad libitum*. The University of Wisconsin, School of Veterinary Medicine Animal Care and Use Committee approved all protocols.

Surgical Preparation and Nerve Isolation.

Rats were initially anesthetized in a closed chamber containing isoflurane followed by isoflurane administration through a nose cone (3.0 – 3.5% in 50% O_2, balance N_2). The trachea was cannulated to enable pump-ventilation (tidal volume, 2 – 2.5 mL; $FIO_2 = 0.50$; Rodent Respirator model 682, Harvard Apparatus, South Natick, MA). A bilateral vagotomy was performed at the mid-cervical level to prevent entrainment of respiratory motor output with the ventilator. Catheters were placed into the tail vein for fluid administration (1:11 by volume $NaHCO_3$:lactated Ringer's; 2.5 mL/hr) and the femoral artery for blood pressure measurement and to draw blood samples for blood gas analysis. Body temperature was maintained at 37.5 ± 1.0 °C using a rectal probe and custom-designed heated table. The left hypoglossal nerve was isolated using a dorsal approach, cut distally, desheathed and placed on a bipolar silver electrode. Rats were slowly converted to urethane anesthesia (1.6 g/kg, i.v.) and then paralyzed with pancuronium bromide to prevent spontaneous breathing movements (2.5 mg/kg, i.v., supplemented as necessary). End-tidal CO_2 was measured throughout the experiment using a flow-through capnograph (Capnogard, Model 1265, Novametrix; Wallingford, CT) with sufficient response time to measure expiratory gases in rats.

Experimental Protocols.

The CO_2 apneic threshold was determined by decreasing CO_2 levels and/or increasing the ventilator rate until nerve activity ceased. Inspired CO_2 levels were then increased; the end-tidal CO_2 at which phrenic activity resumed was taken as the recruitment threshold (Mahamed & Mitchell, 2007). End-tidal CO_2 levels were then set 1–2 mmHg above the recruitment threshold. A stable hypoglossal neurogram was established and an initial blood sample was taken to establish baseline PaO_2, $PaCO_2$, pH, and base excess values (0.3 ml in 0.5 ml heparinized glass syringe; ABL-500, Radiometer, Copenhagen, Denmark; unused blood was returned to the animal). Rats were given 3, 5-min episodes of hypoxia (i.e., AIH; $FIO_2 = 0.11 \pm 0.1$, $PaO_2 = 39 \pm 1$ mmHg), separated by 5 min of baseline conditions ($FIO_2 = 0.5$, $PaO_2 > 250$ mm Hg), or a single, cumulative 25-min hypoxic episode (i.e., ASH; $FIO_2 = 0.11 \pm 0.1$, $PaO_2 = 38 \pm 1$ mmHg). Hypoglossal activity was monitored 60 min post-hypoxia to determine LTF magnitude. Arterial blood samples were drawn and analyzed during the final minute of the first hypoxic episode, and 15, 30 and 60 min after the final hypoxic episode. Additional rats that did not receive hypoxia (time controls) were used to verify the stability of nerve output over a similar time period in this preparation. Throughout the protocol, isocapnic conditions (± 1 mmHg from baseline $PaCO_2$) were maintained by adjusting ventilator frequency and/or inspired CO_2.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Electrophysiological methods.

Hypoglossal nerve activity was amplified (x 10,000), band pass filtered (100 Hz to 10 kHz; Model 1700, A-M Systems, Inc., Carlsborg, WA), and integrated (time constant = 50 ms, Model MA-821RSP, CWE Inc., Ardmore, PA). Integrated signals were digitized and processed with commercially available software (WINDAQ software, DATAQ Instruments, Akron, OH). Peak integrated hypoglossal burst amplitude, burst frequency, and mean arterial blood pressure were calculated over a 60 second period just prior to the first hypoxic episode (baseline), at the end of the first hypoxic episode or the equivalent time point during sustained hypoxia (short-term hypoxic response), and 30 and 60 min post-hypoxia. Data were included in the analysis only if isocapnic conditions were successfully maintained. Amplitude data are expressed as the change in hypoglossal burst amplitude, expressed as a percent change from baseline values. Frequency data are reported as a change from baseline in bursts per minute (delta burst frequency). Data were compared using a one-way ANOVA or two-way ANOVA with repeated measures design as applicable (Fisher LSD *post-hoc* test; SigmaStat 2.03, SPSS Inc., Chicago, IL).

Results

Physiological variables

No significant differences were observed in the CO_2 recruitment threshold for rats treated with (IH: 41 ± 1 mmHg, n=9; SH: 40 ± 1 mmHg, n=14) or without hypoxia (42 ± 1 mmHg, n=14; $p > 0.05$). Under baseline conditions, mean arterial blood pressure was not different between treatment groups (IH: 112 ± 5 mmHg, SH: 110 ± 4 mmHg; Table 1; $p > 0.05$), or time controls that did not receive hypoxia: (112 ± 5 mmHg; Table 1, $p > 0.05$). Similar to other studies from our laboratory, mean arterial blood pressure significantly decreased during the hypoxic stimulus versus baseline (IH: 62 ± 4 mmHg, SH: 65 ± 7 mmHg; $p < 0.05$), but not in time controls (112 ± 5 mmHg; Table 1, $p > 0.05$). We did not observe significant changes in mean arterial blood pressure at 30 min post-hypoxia in any group; however, small but significant decreases were found at the 60 min time point in the AIH (105 ± 4 mmHg), ASH (100 ± 3 mmHg), and time control (105 ± 5 mmHg) (Table 1, $p < 0.05$). No significant treatment effects were found between groups at any time for mean arterial blood pressure ($p > 0.05$). $PaCO_2$ was successfully maintained within 1 mmHg of the baseline value throughout experimental protocols. Thus, changes in hypoglossal burst amplitude or frequency were not caused by changes in arterial PCO_2 from baseline values.

Hypoglossal long-term facilitation: AIH versus ASH

Representative integrated hypoglossal neurograms before, during and 60 min following AIH or ASH are presented in Figure 1. During hypoxic exposures, hypoglossal burst amplitude and frequency significantly increased in rats exposed to AIH (203 ± 31% baseline and 16 ± 3 bursts/min, respectively) and ASH hypoxia (236 ± 34% baseline and 8 ± 2 bursts/min, respectively) (Figure 2). There were no significant differences in hypoglossal burst amplitude or frequency during AIH versus ASH (Figure 2, $p > 0.05$ for both). Time controls showed no significant difference in hypoglossal burst amplitude or delta burst frequency at equivalent times (0 ± 3% baseline and −3 ± 2 bursts/min, respectively; Figure 2; $p > 0.05$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Group data for hypoglossal burst amplitude 60 minutes post-hypoxia are presented in Figure 3. Hypoglossal burst amplitude was significantly increased from baseline 60 min (50 ± 20% baseline, $p < 0.01$) post-AIH, indicative of hypoglossal LTF (Figure 3A). In contrast, hypoglossal LTF was not observed following ASH (−6 ± 9% baseline, $p > 0.05$, Figure 3A). Hypoglossal burst amplitude was stable in time control rats; there were no significant changes in hypoglossal burst amplitude versus baseline at 60 minutes in this group (13 ± 9 %baseline, $p > 0.05$).

Frequency long-term facilitation

Whereas hypoglossal burst frequency increased during hypoxia (Figure 2B; $p < 0.05$), no significant changes were observed in hypoglossal delta burst frequency 60 min post-AIH (4 ± 3 bursts/min) or post-ASH (1 ± 2 bursts/min; both $p > 0.05$; Figure 3B). Time control rats did not show significant changes in hypoglossal burst frequency at an equivalent time (2 ± 1 bursts/min; $p > 0.05$, Figure 3B). Since there were no significant differences between groups 60 minutes post-treatment ($p > 0.50$), there was no evidence for frequency LTF in these experiments.

Discussion

Surprisingly, despite numerous investigations concerning phrenic LTF and pLTF pattern-sensitivity (Baker & Mitchell, 2000; Wilkerson et al., 2008; Dale-Nagle et al., 2010; Devinney et al., 2013; Devinney et al., 2015; Devinney et al., 2016), there have been no previous reports concerning the existence of pattern sensitivity in hypoglossal LTF (Baker-Herman and Strey, 2011). Using AIH and ASH protocols similar to those previously used to demonstrate pLTF pattern-sensitivity, we report that moderate AIH elicits hLTF, yet moderate ASH does not. Thus, although serotonin-dependent facilitation of intercostal motor activity lacks pattern-sensitivity in neonatal rats (Lovett-Barr *et al.*, 2006), and hLTF differs from pLTF in certain key characteristics (Baker-Herman and Strey, 2011), the hypoglossal and phrenic motor pools exhibit similar sensitivity to the pattern of moderate hypoxia in adult rats.

We do not yet have a full understanding of mechanisms giving rise to pattern-sensitivity of hypoxia-induced respiratory motor plasticity in any motor pool. We do not even know if hLTF pattern-sensitivity occurs via the same mechanisms as pLTF pattern-sensitivity, nor if hLTF is less pattern-sensitive when severe hypoxia is applied (Devinney et al., 2016). A full understanding of mechanisms giving rise to hLTF and hLTF pattern-sensitivity will be essential if we are to fully appreciate the biological and clinical significance of this form of hypoxia-induced plasticity in motor pools innervating the upper airways.

Potential mechanisms of pattern sensitivity in hLTF

Hypoglossal and phrenic LTF exhibit many similarities and differences (Baker-Herman and Strey, 2011). Prominent differences include their responses to sex, sex hormones and ageing (Zabka et al., 2001a, 2001b, 2003, 2005, 2006; Behan et al., 2002, 2003), strain and sub-strain (Fuller et al., 2000; Mitchell et al., 2001; Fuller et al., 2001a; Baker-Herman et al., 2010), and the relative influence of alpha-adrenergic receptors in their underlying

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

mechanisms (Neverova et al., 2007; Huxtable et al., 2014). On the other hand, both hLTF and pLTF require 5-HT_2 receptor activation (Fuller et al., 2001b) and reactive oxygen species formation (MacFarlane & Mitchell, 2008a; MacFarlane et al. 2008b), suggesting fundamental similarities in their cellular/synaptic mechanisms.

Although there are no previous studies concerning mechanisms of hLTF sensitivity to the pattern of hypoxia *in vivo*, one study in medullary slices from juvenile rats showed that episodic, but not continuous, serotonin receptor activation elicits hLTF and increases AMPA-mediated inspiratory currents in hypoglossal motor neurons (Bocchiaro & Feldman, 2004). Similarly, hLTF is elicited by episodic alpha-adrenergic receptor activation in brainstem slice preparations (Neverova et al., 2007). These studies provide at least suggestive evidence that hypoxia-induced hLTF may also be pattern sensitive in adult rats. In contrast, phrenic and intercostal motor outputs differ in their pattern sensitivity to serotonin receptor activation *in vitro*, suggesting that different motor pools may vary in the extent of pattern-sensitivity to the inducing stimulus (Lovett-Barr *et al.*, 2006).

In previous studies of pLTF pattern sensitivity, we demonstrated that inhibition of protein phosphatases with okadaic acid revealed pLTF following moderate ASH (Wilkerson *et al.*, 2008). Since AIH-induced pLTF requires reactive oxygen species formation (MacFarlane et al., 2008a, 2008b, 2009a, 2009b), and intrathecal okadaic acid restores pLTF in rats with diminished reactive oxygen species formation (MacFarlane et al., 2008b), we suggested that pLTF pattern sensitivity arises through suppression of serine/threonine protein phosphatases that normally constrain pLTF expression via increased reactive species formation that is characteristic of AIH and not ASH (MacFarlane et al., 2008b; Wilkerson et al., 2007; Wilkerson et al., 2008). The relevant reactive oxygen species formation most arises from increased NADPH oxidase activity elicited by episodic serotonin spinal receptor activation (MacFarlane et al., 2009a). Since this form phrenic motor facilitation requires episodic serotonin receptor activation, and is not activated by a single serotonin injection of the same cumulative dose, we suggested that pLTF pattern sensitivity arises downstream from spinal serotonin receptor activation (MacFarlane and Mitchell, 2009a). The protein tyrosine phosphatase corkscrew regulates pattern sensitivity in *Drosophila* long-term memory induction (Pagani *et al.*, 2009), suggesting that phosphatases play a role in multiple forms of pattern-sensitive neuroplasticity. The requirement for serotonin-dependent reactive oxygen species formation, and suppression of okadaic acid sensitive protein phosphatases in hLTF has not been investigated.

Additional insights concerning mechanisms of pLTF pattern sensitivity were inspired by the realization that multiple competing cellular mechanisms are capable of giving rise to long-lasting phrenic motor facilitation (Dale-Nagle et al., 2010; Devinney et al., 2013). The “Q pathway” to phrenic motor facilitation is initiated by Gq protein coupled metabotropic receptors such as serotonin type 2 receptors, and gives rise to pLTF following moderate AIH (Dale-Nagle et al., 2010). In contrast, the “S pathway” to phrenic motor facilitation is initiated by Gs protein coupled metabotropic receptors such as adenosine 2A receptors (Dale-Nagle et al., 2010). These pathways interact via cross talk inhibition, suggesting that there is a point where they are equal and opposing, cancelling the expression of phrenic motor facilitation (Devinney et al., 2013). Whereas the serotonin-dependent Q pathway

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

predominates with moderate AIH, we proposed that serotonin and adenosine receptor activation are more balanced with ASH, effectively cancelling pLTF expression (Devinney et al., 2016). Selective inhibition of spinal serotonin or adenosine 2A receptors disrupts this balance, revealing pLTF following moderate ASH (Devinney et al., 2016). Similar studies exploring hLTF pattern-sensitivity have not been done.

Significance of pattern sensitivity in respiratory neuroplasticity

The importance of patterned stimulation in evoking plasticity is described in many different models of plasticity, yet the mechanism remains unknown. For example, intermediate-term memory formation and synaptic facilitation in the sea slug *Aplysia* (Mauelshagen *et al.*, 1998; Sutton *et al.*, 2002), habituation in the crab (Freudenthal *et al.*, 1998), olfactory memory formation in fruit flies (Isabel *et al.*, 2004), and hippocampal long-term potentiation in rodents (Kauer, 1999; Nguyen *et al.*, 2000; Scharf *et al.*, 2002) all exhibit apparent pattern sensitivity to the induction protocol. However, there are certain instances of pattern-insensitive plasticity. In the whole brainstem-spinal cord preparation, long term facilitation of thoracic intercostal motor output is elicited by a continuous infusion of 5-HT (Lovett-Barr *et al.*, 2006). Another example of pattern insensitive respiratory plasticity is ventilatory acclimatization to chronic hypoxia, such as occurs during exposure to high altitude (Dwinell & Powell, 1999). Thus, by describing pattern sensitivity in respiratory neuroplasticity, we may yield insights as to why pattern sensitivity exists in some motor pools, and mechanisms giving rise to that pattern sensitivity. Understanding pattern-sensitive aspects of respiratory neuroplasiticity will also guide its optimal utilization in strategies aiming to increase respiratory motor output through induction of plasticity.

Significance of hLTF

The physiological significance of any form of respiratory plasticity is not completely understood (Mitchell *et al.*, 2001; Mahamed and Mitchell, 2007; Fields and Mitchell, 2015). However, hLTF represents one potential mechanism to increase upper airway tone, thereby preserving upper airway patency and possibly minimizing obstructive apneas due to upper airway collapse in normal individuals or those with sleep-disordered breathing (Mitchell et al., 2001; Fuller, 2005; Mitchell, 2007; Mahamed and Mitchell, 2007; Baker-Herman and Strey, 2011). Furthermore hLTF might represent mechanisms enabling long-term adaptations of hypoglossal motor output during normal ageing (Zabka et al., 2005), or central nervous system injury or disease (Mitchell *et al.*, 2001; Mitchell, 2007). Understanding mechanisms of hypoglossal motor facilitation will aid attempts to utilize hLTF to increase upper airway tone for therapeutic advantage. Elucidating mechanisms of hLTF pattern sensitivity will increase basic understanding of pattern sensitivity in neuroplasticity, and could guide strategies that utilize plasticity to treat disease and/or injury.

In humans, upper airway collapse increases from evening to morning, independent of sleep stage (Mateika et al., 2015, Sforza et al., 1998, Charbonneau et al., 1994). Further, intermittent hypoxia in humans elicits a distinct form of respiratory plasticity known as progressive augmentation (Powell et al., 1998), which increases the number of flow limiting breathing events (Yokhana et al., 2012, Mateika and Syed, 2013; Mateika and Narwani, 2009). Consequently, LTF of upper airway muscle activity may help offset otherwise

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

detrimental forms of respiratory plasticity (El-Chami et al., 2017, Mateika and Komnenov, 2017).

Acknowledgements:

This work was supported by the National Institutes of Health (Grants HL080209 and HL111598, GSM).

Support provided by NIH Grants HL080209 and HL111598 (GSM).

References

Appleton-Knapp SL, Bjork RA, Wickens TD, 2005 Examining the Spacing Effect in Advertising: Encoding Variability, Retrieval Processes, and Their Interaction. The Journal of Consumer Research 32, 266–276.

Bach KB, Mitchell GS, 1996 Hypoxia-induced long-term facilitation of respiratory activity is serotonin dependent. Respir Physiol 104, 251–260. [PubMed: 8893371]

Baker TL, Mitchell GS, 2000 Episodic but not continuous hypoxia elicits long-term facilitation of phrenic motor output in rats. J. Physiol. (Lond.) 529 Pt 1, 215–219. [PubMed: 11080263]

Baker-Herman TL, Bavis RW, Dahlberg JM, Mitchell AZ, Wilkerson JE, Golder FJ, Macfarlane PM, Watters JJ, Behan M, Mitchell GS, 2010 Differential expression of respiratory long-term facilitation among inbred rat strains. Respir Physiol Neurobiol 170, 260–7. [PubMed: 20036763]

Baker-Herman TL, Strey KA, 2011 Similarities and differences in mechanisms of phrenic and hypoglossal motor facilitation. Respir Physiol Neurobiol 179, 48–56. doi:10.1016/j.resp.2011.06.022 [PubMed: 21745601]

Beck CD, Schroeder B, Davis RL, 2000 Learning performance of normal and mutant Drosophila after repeated conditioning trials with discrete stimuli. J. Neurosci 20, 2944–2953. [PubMed: 10751447]

Behan M, Zabka AG, Mitchell GS, 2002 Age and gender effects on serotonin-dependent plasticity in respiratory motor control. Respir Physiol Neurobiol 131, 65–77. [PubMed: 12106996]

Behan M, Zabka AG, Thomas CF, Mitchell GS, 2003 Sex steroid hormones and the neural control of breathing. Respir Physiol Neurobiol 136, 249–63. [PubMed: 12853015]

Bocchiaro CM, Feldman JL, 2004 Synaptic activity-independent persistent plasticity in endogenously active mammalian motoneurons. Proc. Natl. Acad. Sci. U.S.A 101, 4292–4295. doi:10.1073/pnas.0305712101 [PubMed: 15024116]

Cepeda NJ, Pashler H, Vul E, Wixted JT, Rohrer D, 2006 Distributed practice in verbal recall tasks: A review and quantitative synthesis. Psychol Bull 132, 354–380. doi:10.1037/0033-2909.132.3.354 [PubMed: 16719566]

Charbonneau M, Marin JM, Olha A, Kimoff RJ, Levy RD, Cosio MG. Changes in obstructive sleep apnea characteristics through the night. Chest. 1994 12;106(6):1695–701. [PubMed: 7988186]

Dale-Nagle EA, Hoffman MS, MacFarlane PM, Mitchell GS, 2010. Multiple pathways to long-lasting phrenic motor facilitation. Adv Exp Med Biol. 2010;669:225–30. doi: 10.1007/978-1-4419-5692-7_45. [PubMed: 20217354]

Devinney MJ, Nichols NL, Mitchell GS, 2016 Sustained hypoxia elicits competing spinal mechanisms of phrenic motor facilitation. J Neurosci. 36(30):7877–85. doi: 10.1523/JNEUROSCI.4122-15.2016. [PubMed: 27466333]

Devinney MJ, Fields DP, Huxtable AG, Peterson TJ, Dale EA, Mitchell GS, 2015 Phrenic long-term facilitation requires PKCθ activity within phrenic motor neurons. J Neurosci. 35(21):8107–17. doi: 10.1523/JNEUROSCI.5086-14.2015. [PubMed: 26019328]

Devinney MJ, Huxtable AG, Nichols NL, Mitchell GS, 2013 Hypoxia-induced phrenic long-term facilitation: emergent properties. Ann N Y Acad Sci. 1279:143–53. doi: 10.1111/nyas.12085.10.1152/japplphysiol.00204.2017. [PubMed: 23531012]

Dwinell MR, Powell FL, 1999 Chronic hypoxia enhances the phrenic nerve response to arterial chemoreceptor stimulation in anesthetized rats. J. Appl. Physiol 87, 817–823. [PubMed: 10444644]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ebbinghaus H, 1913 Memory: a contribution to experimental psychology. New York City, Teachers College, Columbia University.

El-Chami M, Sudan S, Lin HS, Mateika JH. Exposure to intermittent hypoxia and sustained hypercapnia reduces therapeutic CPAP in participants with obstructive sleep apnea. J Appl Physiol (1985). 2017 7 6:jap.00204.2017. doi:10.1152/japplphysiol.00204.2017.

Fields DP, Mitchell GS 2015 Spinal metaplasticity in respiratory motor control. Front Neural Circuits. 11;9:2, 143–6. doi: 10.3389/fncir.2015.00002

Freudenthal R, Locatelli F, Hermitte G, Maldonado H, Lafourcade C, Delorenzi A, Romano A, 1998 Kappa-B like DNA-binding activity is enhanced after spaced training that induces long-term memory in the crab Chasmagnathus. Neurosci. Lett. 242, 143–146. [PubMed: 9530926]

Fuller DD, 2005 Episodic hypoxia induces long-term facilitation of neural drive to tongue protrudor and retractor muscles. J. Appl. Physiol 98, 1761–1767. doi:10.1152/japplphysiol.01142.2004 [PubMed: 15640385]

Fuller DD, Bach KB, Baker TL, Kinkead R, Mitchell GS 2000 Long term facilitation of phrenic motor output. Respir Physiol 121, 135–146. [PubMed: 10963770]

Fuller DD, Baker TL, Behan M, Mitchell GS, 2001a Expression of hypoglossal long-term facilitation differs between substrains of Sprague-Dawley rat. Physiol. Genomics 4, 175–181. [PubMed: 11160996]

Fuller DD, Zabka AG, Baker TL, Mitchell GS, 2001b Phrenic long-term facilitation requires 5-HT receptor activation during but not following episodic hypoxia. J. Appl. Physiol 90, 2001–2006; discussion 2000. [PubMed: 11299296]

Goverover Y, Arango-Lasprilla JC, Hillary FG, Chiaravalloti N, DeLuca J, 2009a Application of the Spacing Effect to Improve Learning and Memory for Functional Tasks in Traumatic Brain Injury: A Pilot Study. The American Journal of Occupational Therapy 63, 543–548. doi:10.5014/ajot.63.5.543 [PubMed: 19785253]

Goverover Y, Hillary FG, Chiaravalloti N, Arango-Lasprilla JC, DeLuca J, 2009b A functional application of the spacing effect to improve learning and memory in persons with multiple sclerosis. J Clin Exp Neuropsychol 31, 513–522. doi:10.1080/13803390802287042 [PubMed: 18720184]

Huxtable AG, MacFarlane PM, Vinit S, Nichols NL, Dale EA, Mitchell GS, 2014 Adrenergic α_1 receptor activation is sufficient, but not necessary for phrenic long-term facilitation. J Appl Physiol (1985). 116(11):1345–52. doi:10.1152/japplphysiol.00904.2013. [PubMed: 24526581]

Isabel G, Pascual A, Preat T, 2004 Exclusive consolidated memory phases in Drosophila. Science 304, 1024–1027. doi:10.1126/science.1094932 [PubMed: 15143285]

Janiszewski C, Noel H, Sawyer AG, 2003 A Meta-analysis of the Spacing Effect in Verbal Learning: Implications for Research on Advertising Repetition and Consumer Memory. Journal of Consumer Research: An Interdisciplinary Quarterly, Journal of Consumer Research: An Interdisciplinary Quarterly 30, 138–49.

Kauer JA, 1999 Blockade of hippocampal long-term potentiation by sustained tetanic stimulation near the recording site. J. Neurophysiol 81, 940–944. [PubMed: 10036292]

Kerfoot BP, 2010 Adaptive spaced education improves learning efficiency: a randomized controlled trial. J. Urol. 183, 678–681. doi:10.1016/j.juro.2009.10.005 [PubMed: 20022032]

Lovett-Barr MR, Mitchell GS, Satriotomo I, Johnson SM, 2006 Serotonin-induced in vitro long-term facilitation exhibits differential pattern sensitivity in cervical and thoracic inspiratory motor output. Neuroscience 142, 885–892. doi:10.1016/j.neuroscience.2006.06.036 [PubMed: 16893610]

MacFarlane PM, Mitchell GS, 2009a Episodic spinal serotonin receptor activation elicits long-lasting phrenic motor facilitation by an NADPH oxidase-dependent mechanism. J. Physiol. (Lond.) 587, 5469–5481. doi:10.1113/jphysiol.2009.176982 [PubMed: 19805745]

MacFarlane PM, Satriotomo I, Windelborn JA, Mitchell GS, 2009b NADPH oxidase activity is necessary for acute intermittent hypoxia-induced phrenic long-term facilitation. J. Physiol. (Lond.) 587, 1931–1942. doi:10.1113/jphysiol.2008.165597 [PubMed: 19237427]

MacFarlane PM, Mitchell GS, 2008a Respiratory long-term facilitation following intermittent hypoxia requires reactive oxygen species formation. Neuroscience 152, 189–197. doi:10.1016/j.neuroscience.2007.12.003 [PubMed: 18207649]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

MacFarlane PM, Wilkerson JE, Lovett-Barr MR, Mitchell GS, 2008b Reactive oxygen species and respiratory plasticity following intermittent hypoxia. Respir Physiol Neurobiol 164, 263–71. doi: 10.1016/j.resp.2008.07.008 [PubMed: 18692605]

Mahamed S, Mitchell GS, 2007 Is there a link between intermittent hypoxia-induced respiratory plasticity and obstructive sleep apnoea? Exp. Physiol 92, 27–37. doi:10.1113/expphysiol.2006.033720 [PubMed: 17099064]

Mateika JH, El-Chami M, Shaheen D, Ivers B. Intermittent hypoxia: a low-risk research tool with therapeutic value in humans. J Appl Physiol (1985). 2015 3 1;118(5):520–32. doi: 10.1152/japplphysiol.00564.2014. [PubMed: 25549763]

Mateika JH, Komnenov D. Intermittent hypoxia initiated plasticity in humans: A multipronged therapeutic approach to treat sleep apnea and overlapping co-morbidities. Exp Neurol. 2017 1;287(Pt 2):113–129. doi: 10.1016/j.expneurol.2016.05.011. [PubMed: 27170208]

Mateika JH, Narwani G. Intermittent hypoxia and respiratory plasticity in humans and other animals: does exposure to intermittent hypoxia promote or mitigate sleep apnoea? Exp Physiol. 2009 3;94(3):279–96. doi: 10.1113/expphysiol.2008.045153. [PubMed: 19060117]

Mateika JH, Syed Z. Intermittent hypoxia, respiratory plasticity and sleep apnea in humans: present knowledge and future investigations. Respir Physiol Neurobiol. 2013 9 15;188(3):289–300. doi: 10.1016/j.resp.2013.04.010.

Mauelshagen J, Sherff CM, Carew TJ, 1998 Differential induction of long-term synaptic facilitation by spaced and massed applications of serotonin at sensory neuron synapses of Aplysia californica. Learn. Mem 5, 246–256. [PubMed: 10454368]

Mitchell GS, Baker TL, Nanda SA, Fuller DD, Zabka AG, Hodgeman BA, Bavis RW, Mack KJ, Olson EB, 2001 Invited review: Intermittent hypoxia and respiratory plasticity. J. Appl. Physiol 90, 2466–2475. [PubMed: 11356815]

Neverova NV, Saywell SA, Nashold LJ, Mitchell GS, Feldman JL 2007 Episodic stimulation of alpha1-adrenoreceptors induces protein kinase C-dependent persistent changes in motoneuronal excitability. J Neurosci. 27(16):4435–42. [PubMed: 17442828]

Nichols NL, Dale EA, Mitchell GS, 2012 Severe acute intermittent hypoxia elicits phrenic long-term facilitation by a novel adenosine-dependent mechanism. J Appl Physiol (1985). 112(10):1678–88. doi: 10.1152/japplphysiol.00060.2012. [PubMed: 22403346]

Nguyen PV, Duffy SN, Young JZ, 2000 Differential maintenance and frequency-dependent tuning of LTP at hippocampal synapses of specific strains of inbred mice. J. Neurophysiol 84, 2484–2493. [PubMed: 11067991]

Pagani MR, Oishi K, Gelb BD, Zhong Y, 2009 The phosphatase SHP2 regulates the spacing effect for long-term memory induction. Cell 139, 186–198. doi:10.1016/j.cell.2009.08.033. [PubMed: 19804763]

Powell FL, Milsom WK, Mitchell GS. Time domains of the hypoxic ventilatory response. Respir Physiol. 1998 5;112(2):123–34. [PubMed: 9716296]

Scharf MT, Woo NH, Lattal KM, Young JZ, Nguyen PV, Abel T, 2002 Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. J. Neurophysiol 87, 2770–2777. [PubMed: 12037179]

Sforza E, Krieger J, Petiau C. Nocturnal evolution of respiratory effort in obstructive sleep apnoea syndrome: influence on arousal threshold. Eur Respir J. 1998 12;12(6):1257–63. [PubMed: 9877474]

Sutton MA, Ide J, Masters SE, Carew TJ, 2002 Interaction between Amount and Pattern of Training in the Induction of Intermediate- and Long-Term Memory for Sensitization in Aplysia. Learning & Memory 9, 29–40. doi:10.1101/lm.44802 [PubMed: 11917004]

Wilkerson JER, Macfarlane PM, Hoffman MS, Mitchell GS, 2007 Respiratory plasticity following intermittent hypoxia: roles of protein phosphatases and reactive oxygen species. Biochem. Soc. Trans 35, 1269–1272. doi:10.1042/BST0351269 [PubMed: 17956327]

Wilkerson JER, Satriotomo I, Baker-Herman TL, Watters JJ, Mitchell GS, 2008 Okadaic acid-sensitive protein phosphatases constrain phrenic long-term facilitation after sustained hypoxia. J. Neurosci 28, 2949–2958. doi:10.1523/JNEUROSCI.5539-07.2008 [PubMed: 18337426]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Yokhana SS, Gerst DG 3rd, Lee DS, Badr MS, Qureshi T, Mateika JH. Impact of repeated daily exposure to intermittent hypoxia and mild sustained hypercapnia on apnea severity. J Appl Physiol (1985). 2012 2;112(3):367–77. doi: 10.1152/japplphysiol.00702.2011. [PubMed: 22052874]

Zabka AG, Mitchell GS, Behan M, 2006 Conversion from testosterone to oestradiol is required to modulate respiratory long-term facilitation in male rats. J Physiol. 576(Pt 3):903–12. [PubMed: 16931552]

Zabka AG, Mitchell GS, Behan M, 2005 Ageing and gonadectomy have similar effects on hypoglossal long-term facilitation in male Fischer rats. J Physiol. 563(Pt 2):557–68. [PubMed: 15613371]

Zabka AG, Mitchell GS, Olson EB Jr, Behan M, 2003 Selected contribution: chronic intermittent hypoxia enhances respiratory long-term facilitation in geriatric female rats. J Appl Physiol (1985). 95(6):2614–23; discussion 2604. [PubMed: 12937027]

Zabka AG, Behan M, Mitchell GS, 2001a Selected contribution: Time-dependent hypoxic respiratory responses in female rats are influenced by age and by the estrus cycle. J Appl Physiol (1985). 91(6):2831–8. [PubMed: 11717252]

Zabka AG, Behan M, Mitchell GS, 2001b Long term facilitation of respiratory motor output decreases with age in male rats. J Physiol. 531(Pt 2):509–14. [PubMed: 11230522]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Highlights:

- Long-term facilitation of hypoglossal motor output induced by moderate hypoxia is pattern-sensitive
- Intermittent, but not sustained, moderate hypoxia, elicits hypoglossal long-term facilitation
- Long-term facilitation of hypoglossal motor output results from enhanced hypoglossal burst amplitude versus frequency

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

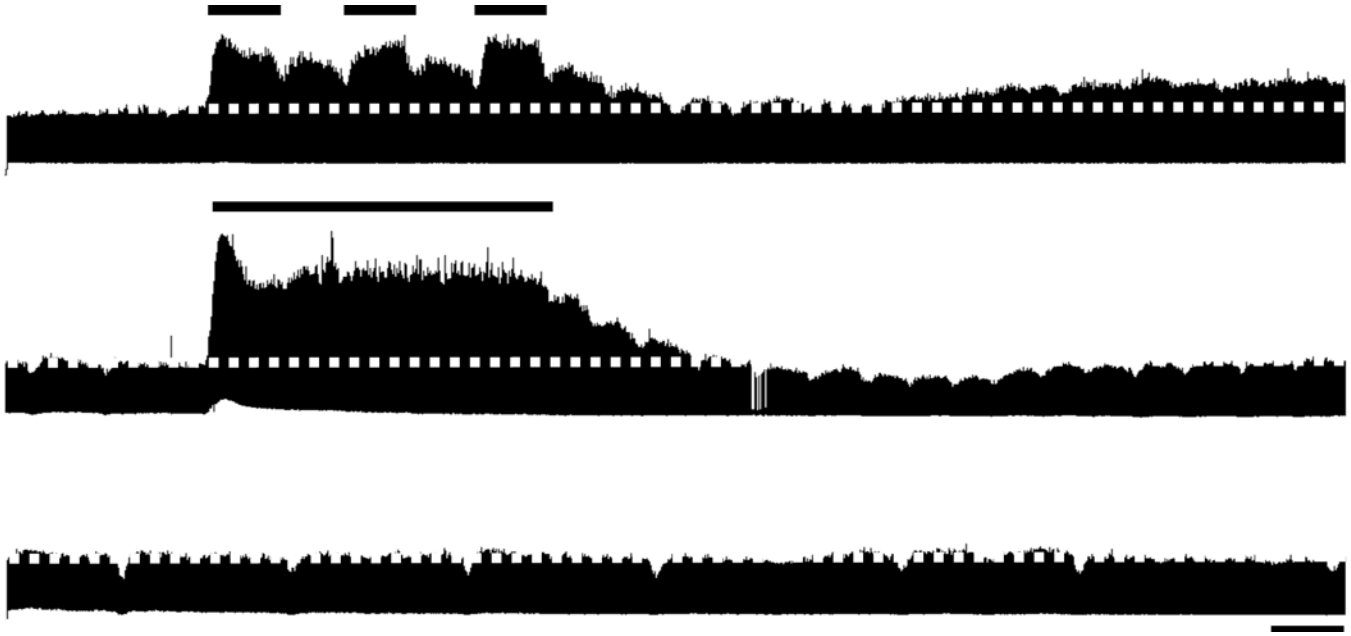

Figure 1. Intermittent, but not sustained, hypoxia, elicits long-term facilitation (LTF) of hypoglossal (XII) motor output.

Representative integrated hypoglossal neurograms before, during, and 60 min following intermittent (*top*) or sustained (*middle*) hypoxia or no hypoxia (time control, *bottom*). Relative to baseline (indicated by dotted line), hypoglossal burst amplitude was increased 60 min following intermittent, but not sustained hypoxia. When hypoxia is not presented, hypoglossal motor output remains stable over time (time control). Short bar = 5 min; long bar = 25 min.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

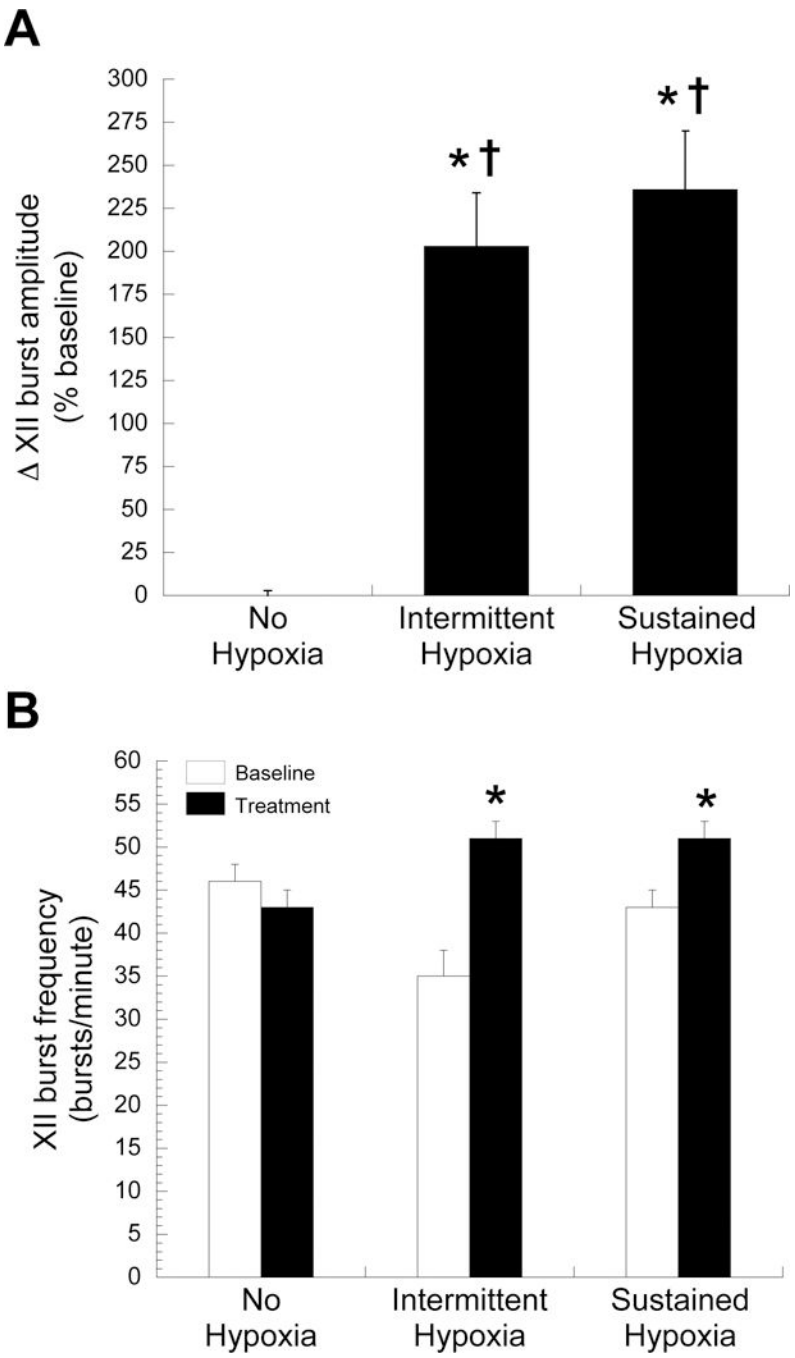

Figure 2. Hypoglossal (XII) motor output is similar during intermittent or sustained hypoxia.

Short-term response of hypoglossal burst amplitude (**A**) and frequency (**B**) during intermittent or sustained hypoxia or without hypoxia. Data are presented as mean ± SEM. * Significantly increased from baseline ($p < 0.05$)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

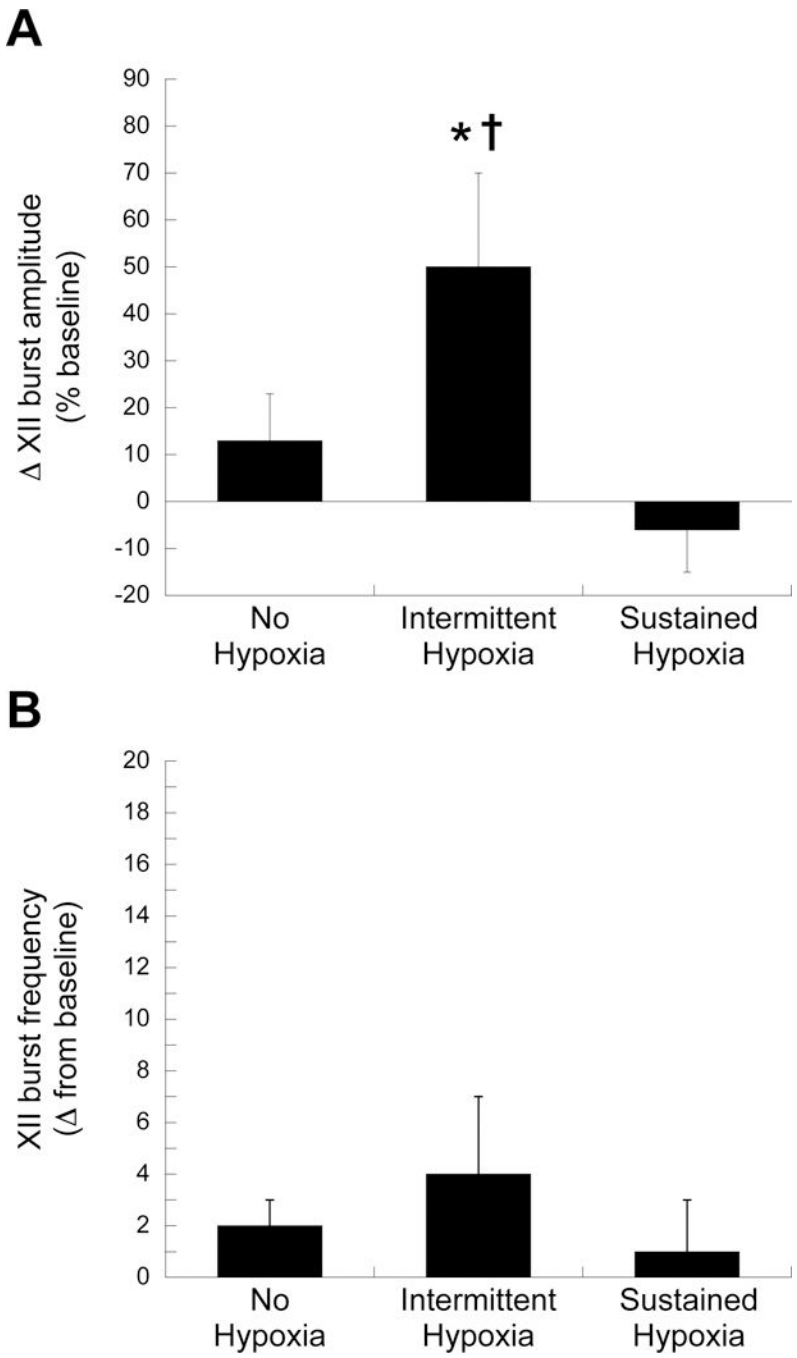

Figure 3. Long-term facilitation (LTF) of hypoglossal (XII) burst amplitude, but not burst frequency, is observed following intermittent, but not sustained, hypoxia.

XII burst amplitude (**A**) but not delta burst frequency (**B**) is enhanced 60 min following intermittent, but not sustained hypoxia. Data are presented as mean ± SEM. *Significantly increased from baseline ($p < 0.05$).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1.

Temporal changes in mean arterial blood pressure (MABP), $PaCO_2$, and PaO_2 in rats

Relative to baseline, mean arterial blood pressure (MABP), arterial partial pressure of CO_2 ($PaCO_2$), and arterial partial pressure of O_2 (PaO_2) were not significantly different over time in rats that did not receive hypoxia ($p > 0.05$). As expected PaO_2 significantly decreased during hypoxia exposure ($P < 0.05$), but returned to baseline values following exposure. Rat groups treated with intermittent or sustained hypoxia showed similar significant decreases in MABP during hypoxia ($p < 0.05$) and small but significant decreases in MABP 60 min post-hypoxia compared to baseline ($p < 0.05$). Overall, there was not a significant treatment effect on MABP ($p > 0.05$).

Treatment	N	Baseline	1st HX	60 min post
No hypoxia	14	112 ± 5 (MABP)	112 ± 5	105 ± 5
		47.7 ± 1.2 ($PaCO_2$)	47.7 ± 1.2	47.6 ± 1.2
		267.2 ± 4.0 (PaO_2)	265.9 ± 4.7	252.2 ± 8.7
Intermittent hypoxia	9	112 ± 5	68 ± 4 *	105 ± 4 *
		45.2 ± 1.2	44.0 ± 1.7	45.9 ± 1.1
		275.7 ± 6.5	38.9 ± 0.8 *	262.0 ± 8.1
Sustained hypoxia	14	110 ± 4	65 ± 7 *	100 ± 3 *
		44.4 ± 1.8	43.9 ± 0.8	44.0 ± 0.9
		257.0 ± 4.9	37.9 ± 1.2 *	245.4 ± 8.0

* Significantly different from baseline within treatment group