Published in final edited form as:

Respir Physiol Neurobiol. 2009 December 31; 169(3): 333–337. doi:10.1016/j.resp.2009.09.015.

Prenatal nicotine exposure alters respiratory long term facilitation in neonatal rats

DD Fuller¹, BJ Dougherty¹, MS Sandhu¹, NJ Doperalski¹, CR Reynolds², and LF Hayward²

¹ University of Florida, College of Public Health and Health Professions, Department of Physical Therapy, PO Box 100154, 100 Newell Dr, Gainesville, FL 32610, USA

² University of Florida, College of Veterinary Medicine, Department of Physiological Sciences, PO Box 100144, 1600 SW Archer Rd, Gainesville, FL 32610, USA

Abstract

Intermittent hypoxia can evoke persistent increases in ventilation ($\dot{V}_E$) in neonates (i.e. long-term facilitation, LTF) (Julien *et al.* *Am J Physiol Regul Integr Comp Physiol* 294: R1356–R1366, 2008). Since prenatal nicotine (PN) exposure alters neonatal respiratory control (Fregosi & Pilarski. *Respir. Physiol. Neurobiol.* 164: 80–86, 2008), we hypothesized that PN would influence LTF of ventilation ($\dot{V}_E$) in neonatal rats. An osmotic minipump delivered nicotine (6 mg/kg/day) or saline to pregnant dams. $\dot{V}_E$ was assessed in unanesthetized pups via whole body plethysmography at post-natal (P) days 9–11 or 15–17 during baseline (BL, 21% O₂), hypoxia (10 × 5 min, 5% O₂) and 30 min post-hypoxia. PN pups had reduced BL $\dot{V}_E$ ($p < 0.05$) but greater increases in $\dot{V}_E$ during hypoxia ($p < 0.05$). Post-hypoxia $\dot{V}_E$ (i.e. LTF) showed an age × treatment interaction ($p < 0.01$) with similar values at P9–11 but enhanced LTF in saline (30 ± 8 %BL) vs. PN pups (6 ± 5 %BL; $p = 0.01$) at P15–17. We conclude that the post-natal developmental time course of hypoxia-induced LTF is influenced by PN.

Keywords

developmental plasticity; hypoxia; ventilation; nicotine; prenatal; neonatal

1. Introduction

Considerable evidence indicates that prenatal nicotine (PN) exposure alters the normal developmental progression of respiratory control mechanisms (reviewed in Hafström *et al.* 2005; Fregosi and Pilarski 2008; Mahliere *et al.* 2008). For example, both the eupneic pattern of breathing (Huang *et al.* 2004) and the ability to increase minute ventilation ($\dot{V}_E$) during hypoxia (Hafström *et al.* 2005; Mahliere *et al.* 2008) are altered in neonatal rats following PN. Recently, it has been shown that intermittent hypoxia exposures (i.e. intermittent hypoxia) can induce persistent increases in respiratory motor output in neonates (i.e. long term facilitation or LTF; McKay *et al.* 2004; Tadjalli *et al.* 2008; Julien *et al.* 2008). While its physiological significance is not precisely known, recent studies indicate that LTF in humans is associated with reductions in upper airway airflow resistance (Pierchala *et al.* 2008). Accordingly,

Corresponding Author: David D. Fuller, Ph.D., Phone: (352) 273 6634, Fax: (352) 273 6109, ddf@phhp.ufl.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

prolonged increases in upper airway muscle activity following intermittent hypoxia may be one important functional aspect of LTF (Harris *et al.* 2006). Studies in anesthetized adult animals indicate that activation of serotonin (5-hydroxytryptamine or 5-HT) receptors is both necessary and sufficient to induce LTF of respiratory motor output (Baker-Herman *et al.* 2004). Because PN alters the post-natal development of serotonergic neurons (Slotkin *et al.* 2007), we examined the impact of PN on LTF in neonatal rats. We hypothesized that PN exposure would attenuate LTF of $\dot{V}_E$ following intermittent hypoxia in unanesthetized neonatal rats.

2. Methods

2.1. Overview

Pregnant female Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA, USA). Alzet osmotic minipumps (2ML4, Alzet, Cupertino, CA, USA) containing nicotine or saline (see below) were implanted in pregnant dams on gestational day 5. All pups were then studied on P9-11 or 15-17 at baseline conditions (21% O₂) and many were also studied during and following intermittent hypoxia (details provided below). At baseline conditions the sample sizes were as follows: P9-11, N=27 saline, N=26 nicotine; P15-17, N=28 saline, N=26 nicotine. During and following hypoxia, the total number of pups were: P9-11, N=16 saline, N=13 nicotine; P15-17, N=14 saline, N=16 nicotine.. Experiments were approved by the Institutional Animal Care and Use Committee at the University of Florida.

2.2. Osmotic pumps and PN exposure

These procedures were based on prior reports (Huang *et al.* 2004; Luo *et al.* 2004). Pumps were filled with saline or nicotine hydrogen tartrate (6 mg/Kg/day, Sigma Chemical) and released fluid at ~2.5 μ l/hr. Dams were briefly anesthetized (2–3% isoflurane) to a surgical plane and an incision was made over the scapulae to enable subcutaneous insertion of the pump. The incision was closed with wound clips. An analgesic (buprenorphine, 0.03 mg/kg, *s.q.*) and an anti-inflammatory drug (carprofen, 5.0 mg/kg, *s.q.*) were given every 12 hrs for 2 days.

2.4. Plasma nicotine measurement

Nicotine was measured in a subset of rats (n=2) to confirm that the delivery system was working. Dams were sacrificed at one or two weeks following implantation of an osmotic minipump, and plasma was collected and analyzed by HPLC using methods previously described by Gosheh *et al.* (2000).

2.5. Plethysmography

A whole-body plethysmography system (Buxco Inc., Wilmington, NC, USA) was used to study the pattern of breathing. The system was calibrated by injecting known gas volumes into a ~400 ml Plexiglas recording chamber using a 1 ml syringe. The chamber pressure, temperature and humidity, rat rectal temperature, and atmospheric pressure were used to calculate respiratory volumes including $\dot{V}_E$ (mL/min) and tidal volume (V_T , mL/breath) (Drorbaugh and Fenn, 1955). Calibration experiments indicated that the accuracy of volume measurements was $\pm 10\%$ over the range of 0.05 to 1.0 ml. In this study, however, our primary objective was to quantify relative changes in respiratory volumes (i.e. within an experimental session) in each animal. Overall breathing frequency (fR, min⁻¹) and the duration of inspiration (T_i) and expiration (T_e) were calculated from the airflow traces. Gas mixtures flowed through the chamber at 1 L/min. Baseline recordings lasted ~ 30 min while the chamber was flushed with 21% O₂ (balance N₂). Rats were then exposed to 10 hypoxic episodes (5 min, 5% O₂, balance N₂) separated by baseline conditions. It took ~ 30 sec for complete equilibration of the

chambers after switching gases. The relatively severe hypoxic challenge (5% O₂) was chosen because both the relative magnitude and time course of LTF in neonates has been reported to correlate with the degree of hypoxia (Tadjalli *et al.* 2007). Ten hypoxic episodes were used because the probability of evoking $\dot{V}_E$ LTF increases with greater number of hypoxic episodes (McGuire and Ling 2005). Chamber temperature was maintained at 34°C using an incubator.

2.8. Data Analyses

Plethysmography data were analyzed over a stable 10-min period prior to hypoxia (*i.e.* baseline), over the last min of the first hypoxic challenge, and at 25–30 min post-intermittent hypoxia. A two-way analyses of variance (ANOVA) was used to compare outcome measures between groups: factor 1 = age (P9-11 or P15-17), factor 2 = treatment (nicotine or saline). To enable comparisons across groups, the hypoxia and LTF data were expressed relative to baseline values (Simakajornboon *et al.*, 2004; Baker-Herman *et al.* 2004). Male and female pups were grouped together as in prior reports (Huang *et al.* 2004; Simakajornboon *et al.*, 2004; Luo *et al.* 2004, 2007). Data are presented as the mean $\pm$ SEM; statistical significance was assumed when $P < 0.05$.

3. Results

3.1. Body weight and plasma nicotine

Body weight was not significantly different between groups (Table 1). However, there was a tendency for PN pups to be slightly heavier (2 way ANOVA, treatment effect $P = 0.094$), and accordingly we expressed respiratory volume data ($\dot{V}_E$, V_T) relative to body weight. It was previously reported that PN pups are heavier than saline controls at day P21 (Mahlière *et al.* 2008). To confirm that the osmotic minipumps were functional, plasma nicotine and its metabolite cotinine were measured in a small sample ($n = 2$) of dams. At one week following pump implantation plasma nicotine was 28 ng/ml and cotinine was 182 ng/ml; at two weeks nicotine was 24 ng/mL and cotinine was 170 ng/ml.

3.2. Baseline $\dot{V}_E$

Representative airflow traces are provided in Fig. 1. Assessment of baseline fR revealed a significant age $\times$ treatment interaction ($p = 0.042$; Fig. 2A). Similar age $\times$ treatment interactions were also observed for baseline T_I ($p = 0.026$) and T_E ($p = 0.003$) (Table 1). Accordingly, the normal developmental changes in the pattern of breathing are influenced by PN (Fig. 2A). Significant treatment effects were present for both baseline V_T ($p = 0.044$) and $\dot{V}_E$ ($p = 0.045$). Specifically, PN pups had reduced V_T and $\dot{V}_E$ relative to age matched saline controls (Fig. 2A).

3.3. Hypoxic ventilatory response and LTF

The hypoxic ventilatory response (HVR) (*i.e.* $\dot{V}_E$ expressed as % baseline) was greater in PN *vs.* saline pups ($p = 0.046$) which is consistent with at least two prior reports (Fregosi *et al.* 2006; Mahlière *et al.* 2008). The increase in fR during hypoxia was indistinguishable between the two treatment groups ($p = 0.920$, Fig. 2B) suggesting that the enhanced $\dot{V}_E$ was driven by greater V_T in PN pups. However, differences in hypoxic V_T did not reach statistical significance as evaluated with 2 way ANOVA ($p = 0.103$, Fig. 2B). T_I was not substantially altered by hypoxia in P9-11 pups (saline: 105 ± 3 ; nicotine: 107 ± 3 % baseline). In contrast, at P15-17 hypoxia reduced T_I to 82 ± 3 % baseline in both groups (age effect: $p < 0.001$). In P9-11 pups, hypoxic T_E was 81 ± 3 (PN) and 83 ± 3 % baseline (saline). These values were reduced to 58 ± 3 (PN) and 59 ± 3 (saline) at P15-17 (age effect: $p < 0.001$). No treatment effects were found for hypoxia T_I ($p = 0.649$) or T_E ($p = 0.607$).

$\dot{V}_E$ was assessed at 30 min post-intermittent hypoxia to evaluate LTF. A significant age $\times$ treatment interaction ($p=0.005$) was observed for $\dot{V}_E$ (Fig. 2C). Post-hoc tests indicate that LTF (i.e. $\dot{V}_E$, % baseline) was not different between saline and PN pups at P9-11 ($p=0.204$), but was greater in saline pups at P15-17 ($p=0.011$; Fig. 2C). An age $\times$ treatment interaction was also observed for V_T LTF ($p=0.020$) indicating that $\dot{V}_E$ LTF in P15-17 saline pups was driven by primarily by V_T (Fig. 2C). Indeed, V_T (% baseline) tended to be greater saline vs. PN pups at P15-17 ($p=0.051$). At 30 min post-hypoxia, fR was not different between PN and saline pups ($p=0.277$) and the age $\times$ treatment interaction did not reach significance ($p=0.078$). Consistent with this, T_I ($p=0.155$) and T_E ($p=0.709$) were not different between PN and saline pups at 30 min post-hypoxia.

Additional experiments were conducted in which pups remained in the plethysmograph chamber for a similar length of time but without exposure to intermittent hypoxia (i.e. a “time control”). In saline pups, $\dot{V}_E$ was 103 ± 5 (P9-P11, N=6) and 98 ± 6 % baseline (P15-17, N=7) at the end of the time control trial. PN nicotine pups had similar values of 102 ± 5 (P9-P11, N=6) and 92 ± 7 % baseline (P15-P17, N=7). These time control data indicate that the $\dot{V}_E$ LTF observed in P15-P17 saline pups (Fig. 2C) was not the result of the extended period in the plethysmograph recording chamber.

4. Discussion

These data are consistent with earlier work showing that PN can influence the normal developmental progression of breathing pattern in neonatal animals. We observed that PN exposure attenuates $\dot{V}_E$ during eupnea but enhances the $\dot{V}_E$ response to a 5% O_2 challenge. In addition, we report for the first time that intermittent hypoxia-induced LTF of $\dot{V}_E$ is blunted after PN in P15-17 rat pups.

4.1. Pattern of breathing during normoxia and hypoxia following PN

There is variability across prior studies regarding how PN alters the pattern of breathing during the post-natal period (Bamford et al. 1996; St. John and Leiter, 1999; Huang et al. 2004; Simakajornboon et al., 2004; Mahlière et al. 2008; Pendelbury et al. 2008). Over the age ranges used in our study, there are reports of unchanged (Simakajornboon et al., 2004), reduced (Huang et al. 2004) and finally increased (Mahlière et al. 2008) $\dot{V}_E$ during normoxic or “eupneic” conditions following PN. The present data, however, are consistent with the findings of Huang et al. (2004) who observed reduced V_T ($p<0.05$) and a tendency for reduced $\dot{V}_E$ ($p=0.067$) in PN pups at P14 and P18 during eupnea. In contrast, Mahlière et al. (2008) report that baseline $\dot{V}_E$ was *increased* in both P7 and P11 PN pups reflecting greater V_T . A commentary regarding these apparently conflicting results is provided below (section 4.2).

The observation that the HVR was enhanced in PN pups could theoretically reflect an adaptation to an impaired metabolic response to hypoxia. For example, early neonatal rats typically achieve hyperventilation via a reduction in metabolic rate (Mortola 2001). This effect is most pronounced in very young pups (e.g. P2) and as pups age they begin to hyperventilate during hypoxia by increasing ventilation with progressively lower changes in metabolism (Mortola 2001). We did not measure metabolic rate in the current experiments, and this should be an important component of future studies. However, preliminary evidence indicates that PN does not impact overall metabolic rate during hypoxia (R.F. Fregosi, personal communication).

The elevated HVR following PN occurred due to an increased V_T (Fig. 2). Previously, Mahlière et al. (2008) also reported that PN pups (P11) had an increased HVR (10% O_2 challenge, 10 min duration) associated with an increased V_T . Similarly, Fregosi and colleagues (R.F. Fregosi et al., *The FASEB Journal*. 20:A1213, 2006) found an increased HVR at P12 and P18 in PN pups (10% O_2 , 3 min), and this response also reflected an increased V_T . To the contrary, two

studies have reported no changes in the HVR in similarly aged pups following PN (Bamford et al. 1996; Simakajornboon et al. (2004). Bamford et al. (1996) assessed $\dot{V}_E$ in the 11th min of a 10% O₂ challenge and observed similar HVR in PN and control pups at P19. It should be noted, however, that in Bamford's study an increase in the breathing frequency response to hypoxia ($P < 0.001$) was observed in slightly younger (P8) PN pups. Simakajornboon and colleagues found change in the HVR at P10, P 15, and P 20 following PN. However, similar to Bamford et al. (1996), some differences were noted in younger pups as PN was associated with a decreased HVR and attenuated hypoxic ventilatory roll-off at P5.

4.2. Commentary regarding conflicting results

Differences across published reports likely do not reflect nicotine dosage as each of the studies reviewed here report a dose of 6 mg/kg/day. We speculate that differences in genetics (Fuller et al. 2001), temperature (Pendlebury et al. 2008) or possibly the nicotine delivery method could contribute to the conflicting results. For example, respiratory-related plasticity can differ between rat substrains (e.g. Sprague-Dawley rats originating from different breeding colonies) (Fuller et al. 2001). In regards to temperature, Pendlebury and colleagues (2008) found that the ambient temperature influenced breathing more profoundly in P7 rats exposed to prenatal cigarette smoke vs. control animals. Finally, studies producing apparently conflicting results have used surgically implanted pellets to release nicotine (Mahlière et al. 2008) vs. osmotic minipumps (Huang et al. 2004; current study). Thus the method of nicotine delivery may ultimately influence the development of plasticity. In any case, despite the conflicting results regarding the manner in which breathing pattern changes after PN across laboratories and experimental preparations, there is a growing consensus that PN alters the normal development of respiratory control mechanisms (Hafström et al. 2005; Fregosi & Pilarski 2008).

4.3. Respiratory LTF in neonates

Respiratory LTF has been observed in anesthetized P3-P7 rat pups (Mckay et al. 2004), *in situ* heart-brainstems from P15-P25 rats (Tadjalli et al. 2008), and unanesthetized P10 pups (Julien et al. 2008). In the latter study robust $\dot{V}_E$ LTF was induced by 10 episodes of hypoxia (10% O₂) and was driven by increased V_T with little change in fR. Since the $\dot{V}_E$ LTF observed by Julien et al. was not statistically significant until ~50 min post-hypoxia, their finding does not contradict our observation that $\dot{V}_E$ LTF was absent at 30 min post-hypoxia in P9-P11 rats. In this paper we extend these earlier observations by reporting that PN blunts LTF in P15-17 pups. In our preparation, LTF was not apparent in the P9-11 pups of either treatment group.

These studies were not intended to address the mechanism(s) responsible for the impact of PN on changes in breathing during or following hypoxia, but possibilities include changes in the ability of nicotinic acetylcholine receptors to modulate the pre-synaptic release of GABA and glutamate (Fregosi and Pilarski 2008) as well as changes in serotonergic function (Slotkin et al. 2007). A relationship between altered LTF and serotonergic function after PN is an attractive hypothesis because LTF is serotonin-dependent (at least in adults; Baker-Herman et al. 2004), and PN can lead to persistent alterations in the serotonergic nervous system (Slotkin et al. 2007). Another intriguing hypothesis is that PN may affect LTF expression via changes in the neuropeptide orexin. Recent work has implicated orexin in the expression of respiratory LTF. For example, LTF of both ventilation (Terada et al. 2008) and phrenic activity (Toyama et al. 2009) is blunted in orexin knockout mice. Nicotine can activate orexinergic neurons (Pasumarthi et al. 2006; Pasumarthi and Fadel 2008), and orexinergic neurons in turn send dense projections to brainstem serotonergic neurons (Baldo et al. 2003; Peyron et al. 1998). In addition, orexin release, can be regulated by serotonergic feedback (Muraki et al. 2004).

Acknowledgments

Support for this work was provided by a grant from the James & Esther King Biomedical Research Program. Support was also provided by NIH grant T32HD043730T32 (BJD). The authors thank Dr. Ralph F. Fregosi (University of Arizona) for help with the osmotic pumps and advice regarding the data. We also thank Ms. Heather Carr for help with data collection and analyses.

References

- Baker-Herman TL, Fuller DD, Bavis RW, Zabka AG, Golder FJ, Doperalski NJ, Johnson RA, Watters JJ, Mitchell GS. BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia. *Nat Neurosci* 2004 Jan;7(1):48–55. [PubMed: 14699417]
- Baldo B, Daniel RA, Berridge CW, Kelley AE. Overlapping distributions of orexin/hypocretin- and dopamine-Beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. *J Comp Neurol* 2003;464:220–237. [PubMed: 12898614]
- Bamford OS, Schuen JN, Carroll JL. Effect of nicotine exposure on postnatal ventilatory responses to hypoxia and hypercapnia. *Respir Physiol* 1996;106:1–11. [PubMed: 8946572]
- Drorbaugh JE, Fenn WO. A barometric method for measuring ventilation in newborn infants. *Pediatrics* 1955;16:81–87. [PubMed: 14394741]
- Fregosi RF, Pilarski JQ. Prenatal nicotine exposure and development of nicotinic and fast amino acid-mediated neurotransmission in the control of breathing. *Respir Physiol Neurobiol* 2008;164:80–86. [PubMed: 18585984]
- Fregosi RF, Brown AR, Huang YH, Costy-Bennett S. Influence of prenatal nicotine exposure on development of ventilatory responses to hypoxia and asphyxia. *The FASEB Journal* 2006;20:A1213.
- Fuller DD, Baker TL, Behan M, Mitchell GS. Expression of hypoglossal long-term facilitation differs between substrains of Sprague-Dawley rat. *Physiol Genomics* 2001;4:175–181. [PubMed: 11160996]
- Ghosheh OA, Browne D, Rogers T, deLeon J, Dwoskin LP, Crooks PA. A simple high performance liquid chromatographic method for the quantification of total cotinine, total 3'-hydroxycotinine and caffeine in the plasma of smokers. *J Pharm Biomed Anal* 2000;23:543–549. [PubMed: 10933548]
- Hafström O, Milerad J, Sandberg KL, Sundell HW. Cardiorespiratory effects of nicotine exposure during development. *Respir Physiol Neurobiol* 2005;149:325–41. [PubMed: 15970470]
- Harris DP, Balasubramaniam A, Badr MS, Mateika JH. Long-term facilitation of ventilation and genioglossus muscle activity is evident in the presence of elevated levels of carbon dioxide in awake humans. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R1111–9. [PubMed: 16627688]
- Huang YH, Brown AR, Costy-Bennett S, Luo Z, Fregosi RF. Influence of prenatal nicotine exposure on postnatal development of breathing pattern. *Respir Physiol Neurobiol* 2004;143:1–8. [PubMed: 15477168]
- Julien C, Bairam A, Joseph V. Chronic intermittent hypoxia reduces ventilatory long-term facilitation and enhances apnea frequency in newborn rats. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R1356–R1366. [PubMed: 18287216]
- Luo Z, Costy-Bennett S, Fregosi RF. Prenatal nicotine exposure increases the strength of GABA(A) receptor-mediated inhibition of respiratory rhythm in neonatal rats. *J Physiol* 2004;561:387–93. [PubMed: 15513949]
- Luo Z, McMullen NT, Costy-Bennett S, Fregosi RF. Prenatal nicotine exposure alters glycinergic and GABAergic control of respiratory frequency in the neonatal rat brainstem-spinal cord preparation. *Respir Physiol Neurobiol* 2007;157:226–34. [PubMed: 17321805]
- Mahlière S, Perrin D, Peyronnet J, Boussouar A, Annat G, Viale JP, Pequignot J, Pequignot JM, Dalmaz Y. Prenatal nicotine alters maturation of breathing and neural circuits regulating respiratory control. *Respir Physiol Neurobiol* 2008;162:32–40. [PubMed: 18455969]
- Mortola, JP. *Respiratory Physiology of Newborn Mammals—A Comparative Perspective*. The Johns Hopkins University Press; 2001.
- McGuire M, Ling L. Ventilatory long-term facilitation is greater in 1- vs. 2-mo-old awake rats. *J Appl Physiol* 2005;98:1195–201. [PubMed: 15591293]
- McKay LC, Janczewski WA, Feldman JL. Episodic hypoxia evokes long-term facilitation of genioglossus muscle activity in neonatal rats. *J Physiol* 2004;557:13–8. [PubMed: 15047768]

- Muraki Y, Yamanaka A, Tsujino N, Kilduff TS, Goto K, Sakurai T. Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. *J Neurosci* 2004;24:7159–7166. [PubMed: 15306649]
- Pasumarthi RK, Reznikov LR, Fadel J. Activation of orexin neurons by acute nicotine. *Eur J Pharmacol* 2006;535:172–6. [PubMed: 16545369]
- Pasumarthi RK, Fadel J. Activation of orexin/hypocretin projections to basal forebrain and paraventricular thalamus by acute nicotine. *Brain Res Bull* 2008;77:367–73. [PubMed: 18950690]
- Pendlebury JD, Wilson RJ, Bano S, Lumb KJ, Schneider JM, Hasan SU. Respiratory control in neonatal rats exposed to prenatal cigarette smoke. *Am J Respir Crit Care Med* 2008;177:1255–61. [PubMed: 18310476]
- Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998;18:9996–10015. [PubMed: 9822755]
- Pierchala LA, Mohammed AS, Grullon K, Mateika JH, Badr MS. Ventilatory long-term facilitation in non-snoring subjects during NREM sleep. *Respir Physiol Neurobiol* 2008;160:259–66. [PubMed: 18088567]
- Simakajornboon N, Vlastic V, Li H, Sawnani H. Effect of prenatal nicotine exposure on biphasic hypoxic ventilatory response and protein kinase C expression in caudal brain stem of developing rats. *J Appl Physiol* 2004;96:2213–9. [PubMed: 14752122]
- Slotkin TA, MacKillop EA, Rudder CL, Ryde IT, Tate CA, Seidler FJ. Permanent, sex-selective effects of prenatal or adolescent nicotine exposure, separately or sequentially, in rat brain regions: indices of cholinergic and serotonergic synaptic function, cell signaling, and neural cell number and size at 6 months of age. *Neuropsychopharmacology* 2007;32:1087–1097.
- St-John WM, Leiter JC. Maternal nicotine depresses eupneic ventilation of neonatal rats. *Neurosci Lett* 1999;267:206–8. [PubMed: 10381012]
- Tadjalli A, Duffin J, Li YM, Hong H, Peever J. Inspiratory activation is not required for episodic hypoxia-induced respiratory long-term facilitation in postnatal rats. *J Physiol* 2007;585:593–606. [PubMed: 17932158]
- Tadjalli A, Duffin J, Peever J. Intermittent hypoxia induces respiratory long-term facilitation in postnatal rats. *Adv Exp Med Biol* 2008;605:233–8. [PubMed: 18085278]
- Terada J, Nakamura A, Zhang W, Yanaqisawa M, Kuriyama T, Fukuda Y, Kuwaki T. Ventilatory long-term facilitation in mice can be observed during both sleep and wake periods and depends on orexin. *J Appl Physiol* 2008;204:499–507. [PubMed: 18032578]
- Toyama S, Sakurai T, Tatsumi K, Kuwaki T. Attenuated phrenic long-term facilitation in orexin neuron-ablated mice. *Respir Physiol Neurobiol* 2009;168:295–302. [PubMed: 19664728]

Figure 1. Representative airflow traces depicting the pattern of breathing in saline (top panel) and nicotine exposed (bottom panel) P16 rat pups

Data are shown during quiet breathing (BL), hypoxia, and 30 min following intermittent hypoxia (see methods). Data are presented with two time scales: a compressed file to show many breaths (scale bar = 1 min) and an expanded trace depicting just a few breaths (scale bar = 1 sec). The scaling of the data is the same in the top (saline) and bottom panels (nicotine).

Figure 2. The impact of prenatal nicotine on the pattern of breathing in neonatal rats at P9-11 and P15-17

Data are presented from saline (●) and nicotine exposed pups (○) at baseline, hypoxia and 30 min post-intermittent hypoxia (see methods). The statistical significance symbols denote the following: #, treatment effect, $p < 0.05$; †, age effect, $p < 0.05$; ‡, age $\times$ treatment interaction, $p < 0.05$; *, different than the corresponding nicotine data point, $p < 0.025$.

Table 1

Body weight and baseline inspiratory (T_I) and expiratory duration (T_E) in control, saline treated pups and nicotine exposed pups.

	Treatment	P9-11	P15-17
Weight (g)	Saline	23.2±0.8	40.6±0.8
	Nicotine	25.4±0.8	41.1±0.8
T_I (sec)	Saline	0.118±0.002	0.164±0.002
	Nicotine	0.115±0.002	0.172±0.002*
T_E (sec)	Saline	0.338±0.008	0.339±0.008
	Nicotine	0.312±0.008*	0.361±0.008*

*, p<0.05 vs. saline