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Chronic intermittent hyperoxia alters the development of the hypoxic ventilatory response in neonatal rats

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Abstract

Chronic exposure to sustained hyperoxia alters the development of the respiratory control system, but the respiratory effects of chronic intermittent hyperoxia have rarely been investigated. We exposed newborn rats to short, repeated bouts of 30% O₂ or 60% O₂ (5 bouts h⁻¹) for 4–15 days and then assessed their hypoxic ventilatory response (HVR; 10 min at 12% O₂) by plethysmography. The HVR tended to be enhanced by intermittent hyperoxia at P4 (early phase of the HVR), but it was significantly reduced at P14–15 (primarily late phase of the HVR) compared to age-matched controls; the HVR recovered when individuals were returned to room air and re-studied as adults. To investigate the role of carotid body function in this plasticity, single-unit carotid chemoafferent activity was recorded *in vitro*. Intermittent hyperoxia tended to decrease spontaneous action potential frequency under normoxic conditions but, contrary to expectations, hypoxic responses were only reduced at P4 (not at P14) and only in rats exposed to higher O₂ levels (i.e., intermittent 60% O₂). Rats exposed to intermittent hyperoxia had smaller carotid bodies, and this morphological change may contribute to the blunted HVR. In contrast to rats exposed to intermittent hyperoxia beginning at birth, two weeks of intermittent 60% O₂ had no effect on the HVR or carotid body size of rats exposed beginning at P28; therefore, intermittent hyperoxia-induced respiratory plasticity appears to be unique to development. Although both intermittent and sustained hyperoxia alter carotid body development and the HVR of rats, the specific effects and time course of this plasticity differs.

Keywords

Developmental plasticity; Control of breathing; Carotid body; Critical window; Hyperoxia; Hypoxia

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1. Introduction

Mammals typically respond to hypoxia by increasing pulmonary ventilation. In neonatal mammals, this hypoxic ventilatory response (HVR) is biphasic (Eden and Hanson, 1987; Bissonnette, 2000; Teppema and Dahan, 2010). Initially there is a rapid increase in ventilation (early or augmentation phase) that is mediated by excitation of the carotid body, the primary arterial O₂ chemoreceptor for the control of breathing. Although carotid body excitation continues, a secondary ventilatory decline occurs (late or depressive phase) that is generally attributed to the inhibition of neurons within the central nervous system (CNS) (Bissonnette, 2000; Teppema and Dahan, 2010). As mammals mature, this biphasic ventilatory pattern shifts towards a sustained increase in ventilation during hypoxia.

In addition to this ontogenetic change, the HVR can be modified by environmental conditions experienced during an individual's lifetime (Mitchell and Johnson, 2003; Bavis and Mitchell, 2008). Rats exposed to sustained hyperoxia (30–60% O₂) for the first 1–4 postnatal weeks of life exhibit an attenuated HVR as adults (Ling et al., 1996; Fuller et al., 2002; Bavis et al., 2013). This is an example of “developmental plasticity” (i.e., a form of phenotypic plasticity unique to developing organisms) since the HVR is not altered when individuals are exposed to equivalent hyperoxic exposures as adults (Ling et al., 1996; Bavis et al., 2002). Subsequent experiments revealed that the critical window for this plasticity (i.e., the time period during which the plasticity can be elicited) is limited to the first two postnatal weeks in rats (Bavis et al., 2002; Bisgard et al., 2003).

Attenuation of the HVR by chronic sustained hyperoxia is largely due to abnormal development of the carotid body. Postnatal exposure to hyperoxia markedly reduces carotid body size by decreasing cell proliferation and increasing cell death (Dmitrieff et al., 2012); these effects are evident after as few as 4 days in 60% O₂ (Dmitrieff et al., 2012) and appear to be permanent (Fuller et al., 2002). In addition to fewer O₂-sensitive glomus cells being present in the carotid body, individual glomus cells are less responsive to hypoxic stimuli when measured immediately after the hyperoxic exposure (Donnelly et al., 2005, 2009; Kim et al., 2013). This may further diminish carotid body function during the neonatal period, but glomus cell O₂ sensitivity recovers within a few days after return to room air (Bavis et al., 2011). Sustained hyperoxia also causes degeneration of primary carotid chemoafferent neurons as evidenced by fewer unmyelinated axons in the carotid sinus nerve (CSN) and fewer dopaminergic neurons in the petrosal ganglion (PG) (Erickson et al., 1998; Chavez-Valdez et al., 2012). Collectively, these changes to the carotid body and CSN result in life-long impairment of the CSN response to hypoxia (Fuller et al., 2002; Bisgard et al., 2003).

Rats reared in sustained hyperoxia also exhibit fewer dopaminergic cells in the nucleus tractus solitarius (nTS) (Chavez-Valdez et al., 2012), suggesting that hyperoxia elicits plasticity in the central limb of the carotid chemoafferent pathway. Indeed, recent studies indicate that developmental hyperoxia alters several aspects of the central neural regulation of breathing. For example, rats reared in sustained hyperoxia exhibit a sustained increase in ventilation at 4–7 days of age rather than the biphasic HVR expected in this age range (Bavis et al., 2010, 2014a). This may be due to reduced central nervous system (CNS) inhibition during hypoxia (Hill et al., 2013) and/or a shift in the balance between inhibitory

and excitatory neuromodulation within the brainstem (Bavis et al., 2014a). Neonatal rats reared in 60% O₂ also hypoventilate when acutely returned to normoxia (Bavis et al., 2010, 2014b). While this hypoventilation may be explained partially by abnormal carotid body function (Bavis et al., 2014b), developmental hyperoxia also slows the frequency of respiratory bursts produced in isolated brainstems (Bierman et al., 2014; Bavis et al., 2014b). Taken together, these findings suggest that sustained hyperoxia impacts development of both peripheral and central components of the respiratory control system.

Studies on the effects of sustained hyperoxia have provided important insights into developmental plasticity of respiratory control, but few studies have considered the effects of chronic intermittent hyperoxia on breathing. In fact, preterm and low birth weight human infants routinely experience intermittent hyperoxia while undergoing supplemental O₂ therapy (Hagadorn et al., 2006; Sola et al., 2008; Claure and Bancalari, 2009; Bancalari and Claure, 2012; Lim et al., 2014). A previous study found that rats exposed to 60% O₂ every other hour for the first two weeks of life exhibit a modest blunting of their HVR at 6–10 weeks of age (Bavis et al., 2007), but the slow cycles of prolonged hyperoxia used in that study were not intended to approximate clinical intermittent hyperoxia.

The present study employed a model of chronic intermittent hyperoxia in which rats were exposed to five bouts of hyperoxia (30 or 60% O₂) per hour from birth through 4–15 days of age. Based on previous studies of chronic sustained hyperoxia (reviewed in Bavis et al., 2013), we hypothesized that intermittent hyperoxia would decrease normoxic ventilation, attenuate the HVR, and result in a sustained HVR (vs. biphasic) at an earlier age than in age-matched control rats. The results revealed age-dependent effects of intermittent hyperoxia on the HVR, so additional experiments investigated the potential role of the carotid body in this plasticity by examining the effects of intermittent hyperoxia on carotid chemoafferent responses to hypoxia and carotid body size. Finally, to determine whether the respiratory effects of intermittent hyperoxia are unique to developmental exposures, additional rats were exposed to intermittent hyperoxia beginning at 28 days of age (i.e., after the neonatal period).

2. Methods

2.1. Experimental animals

All experimental protocols were approved by the Bates College Institutional Animal Care and Use Committee. Timed-pregnant Sprague-Dawley rats were obtained from a commercial supplier (Charles River Laboratories) and housed in room air. For the initial experiments, the resulting litters were assigned to one of three treatment groups on the day of birth (2–8 hours after parturition): Control, intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀). To determine whether a critical window exists for intermittent hyperoxia-induced plasticity, additional rats were reared in room air until 28 days of age (i.e., P28, where P0 is the day of birth) and then assigned to Control or I₆₀ treatment groups; these rats were weaned at P21. Rat cages (pups housed with their mothers or P28 rats housed 2–3 per cage) were placed into chambers programmed to cycle between set oxygen levels at a rate of 5 cycles h⁻¹ (4.5 min at 30 or 60% O₂, 7.5 min⁻¹ at 21% O₂; 24 at 21% O₂; 24 h d⁻¹) (OxyCycler A420C controller with A-30274A chambers; BioSpherix); because of the gas

exchange dynamics of the system, rats actually spent approximately 5.4 min above 21% O₂ and 6.6 min at 21% O₂ per cycle (Fig. 1). Age-matched Control rats were housed in a large acrylic chamber (310 L) flushed with room air at flow rates sufficient to maintain approximately 21% O₂ (and less than 0.4% CO₂); the Control chamber sat adjacent to the I₃₀ and I₆₀ chambers. Chambers were opened briefly when animals were removed for study and for routine cage-cleaning as needed. Rats were exposed to a 12-h light: 12-h dark cycle and were provided food and water *ad libitum*.

In the first round of experiments (i.e., intermittent hyperoxia exposure beginning at birth), ventilation and carotid body responses to hypoxia were measured for P4, P6-7, and P14-15 rats from 6 Control, 5 I₃₀, and 6 I₆₀ litters; ventilation was measured only once for each individual. A subset of the rats studied at P14-15 was subsequently raised in room air until ventilation and carotid body volumes were determined at 4-6 months of age (i.e., as adults). Metabolic rates were measured for an additional group of rats at P14 (2 Control and 2 I₆₀ litters). In the second round of experiments (i.e., intermittent hyperoxia exposure beginning at P28), ventilation, metabolism, and carotid body volumes were measured at P41-42 (i.e., after 13–14 days of exposure) for Control and I₆₀ rats derived from a total of three litters. Sample sizes for specific treatment groups are provided in the tables and figure legends; both male and female rats were studied with approximately equal representation in each treatment group (summarized in Tables 1 and 5).

2.2. Ventilation and metabolism measurements – P4, P6-7, and P14-15

Ventilation was measured for rat pups at P4, P6-7, and P14-15 with a head-body plethysmograph; measurements began within one hour after pups were removed from the control or intermittent hyperoxia chambers. The custom-built plethysmograph consisted of two cylindrical compartments, a head compartment (4.75 cm ID; 70 ml) and a body compartment (3.1 cm ID; internal volume adjusted for the size of the animal with modeling clay). After weighing, the rat was sealed into the plethysmograph chamber with a flexible collar (layers of latex film and Parafilm (Pechiney Plastic Packaging Co.)) separating the head and body compartments; petroleum jelly was applied around the animal's neck to ensure an airtight seal. Air mixtures were passed through the head compartment using a mass flow controller (MFC-4; Sable Systems) and valves (840 series; Sierra Instruments) at a flow rate of 1.5 L min⁻¹. A pneumotach (MLT1L; ADInstruments) was attached to the body compartment and connected to a differential pressure transducer (ML141, ADInstruments) to monitor air flowing into and out from the chamber during breathing. Prior to placing the animal into the plethysmograph, 0.5 mL of air was injected into the body compartment for calibration of the pneumotach. Output from the pressure transducer was recorded at a sampling rate of 1000 Hz, passed through a high pass filter (0.1 Hz), and integrated to obtain respiratory volumes (PowerLab 8SP and LabChart 7 software with the spirometry extension; ADInstruments). Air temperature within the body compartment was monitored with a T-type thermocouple probe (IT-18; Physitemp Instruments); this temperature was maintained at 32–34°C (i.e., within the thermoneutral zone; Malik and Fewell, 2003) by placing the plethysmograph chamber in a temperature-controlled incubator.

Following a 10-min adjustment period (21% O₂, balance N₂), baseline ventilation was recorded for 2–5 minutes. Gas concentrations were then switched to 12% O₂ (balance N₂) for 10 min to assess the HVR. Baseline breathing was determined from 40–60 s of the record, excluding sighs and movement artifacts. Hypoxic ventilation was determined from 10–15 s of the record after the 1st and 10th minute of hypoxia; these time points were selected to represent the early and late phases of the HVR, respectively.

The gas flow rate through the plethysmograph head chamber was too great to measure metabolic O₂ consumption and CO₂ production with sufficient resolution. Therefore, flow-through respirometry was performed on a separate group of P14 rats to determine whether intermittent hyperoxia alters metabolic responses to hypoxia. After weighing, the rat was sealed into a clear plastic box (420 ml) that was supplied with a continuous flow of air. The respirometer chamber was placed into an incubator so that it could be maintained at 32–34°C; air temperature was monitored with a T-type thermocouple (IT-18; Physitemp Instruments). Airflow through the chamber was set at 300 ml min⁻¹ STPD using a gas mixing mass flow controller (MFC-4; Sable Systems) and valves (840 series; Sierra Instruments); the gas was humidified prior to entering the respirometry chamber by bubbling through 40 ml deionized water. Fractional concentrations of O₂ and CO₂ in the air exiting the respirometer chamber were measured (ML206 gas analyzer; ADInstruments) and recorded to computer at a sampling rate of 20 Hz (PowerLab 8SP and LabChart 7 software; ADInstruments); air was dried (Direrite, W.A. Hammond Drierite Co.) prior to passing through the gas analyzer. The concentrations of O₂ and CO₂ in the air entering the chamber were determined immediately after 20–25 min in 21% O₂ (balance N₂), and then the incurrent gas was switched to 12% O₂ (balance N₂). O₂ and CO₂ measurements were repeated after 15 min. O₂ consumption and CO₂ production rates were calculated from the average excurrent gas concentrations recorded over the final minute of each exposure (i.e., 21% or 12% O₂) with equations from Frappell et al. (1992).

2.3. Ventilation and metabolism measurements – P41-42 and adults

Ventilation was measured for P41-42 and adult (4-6 months) rats by whole-body plethysmography. Since this technique requires knowledge of the animal's body temperature, temperature transponders (E-mitter G2 or PDT 4000 G; Starr Life Sciences) were surgically implanted into adult rats under isoflurane anesthesia (2.5% isoflurane, balance O₂) 2–4 weeks prior to ventilation measurements. Transponders were inserted into the abdominal cavity through a ventral midline incision. Carprofen (Rimadyl; Pfizer) was provided as an analgesic (5 mg kg⁻¹; i.p.). Body temperature was determined by rectal thermometer for juvenile rats (see below), so no surgical preparation was necessary for this age group.

Unrestrained rats were placed on a raised platform inside a clear, acrylic plethysmograph chamber (19 cm inner diameter; 2.8 L functional volume). Gases were warmed and humidified prior to passage through the chamber at 2 l min⁻¹ STPD using a gas mixing mass flow controller (MFC-4; Sable Systems) and valves (840 series; Sierra Instruments). A differential pressure transducer (DP45 transducer and CD 15 carrier demodulator; Validyne Engineering) was used to measure breathing-related fluctuations in pressure; the system was

pre-calibrated by repeatedly injecting 0.3 ml of air into the chamber. Pressure fluctuations and chamber temperature (IT-18; Physitemp) were recorded to a computer at a sampling rate of 400 Hz (PowerLab 8SP and LabChart 7, ADInstruments), and body temperature for adult rats was continuously recorded via telemetry (VitalView 4.1; Starr Life Sciences). These measurements were used to calculate tidal volume (Drorbaugh and Fenn, 1955), respiratory frequency, and minute ventilation. O₂ and CO₂ concentrations entering and exiting the plethysmograph chamber were measured (S-3A and CD-3A analyzers, respectively; AEI Technologies) to calculate metabolic O₂ consumption and CO₂ production using the equations of Frappell et al. (1992); air was dried (Drierite; W.A. Hammond Drierite Co.) prior to passing through the gas analyzers.

On the day of the experiment, rats were weighed and placed into the plethysmograph chamber. The rat initially was exposed to 21% O₂ (balance N₂) for a 1-h adjustment period. For juveniles (P41-42), the plethysmograph chamber was opened briefly 30 min into this adjustment period to determine the baseline rectal temperature (Physitemp rectal probe coupled to a Eutech Instruments digital thermometer). After the adjustment period, and once the rat appeared calm, airflow through the chamber was interrupted and 45–60 s of baseline breathing was recorded. O₂ concentration was then lowered to 12% O₂ (balance N₂) for 15–20 min and measurements were repeated; for juveniles, rectal temperature was measured again immediately following the hypoxic exposure (for calculating hypoxic tidal volumes).

2.4. Single-unit carotid chemoafferent activity

Rats were deeply anesthetized with CO₂ and decapitated at P4 or P13-14. The carotid bifurcation and petrosal-nodose ganglion complex were removed *en bloc* and placed in an ice-cold, oxygenated (95% O₂, 5% CO₂) saline solution containing (in mM): 125 NaCl, 5 KCl, 2 CaCl₂, 1 Na₂HPO₄, 1 MgSO₄, 24 NaHCO₃, and 5 dextrose. Tissue was removed from the carotid body preparation using a solution of 0.1–0.15% collagenase (collagenase P; Roche Diagnostics) and 0.02–0.03% protease (type XIV; Sigma-Aldrich) in oxygenated saline with gentle agitation for 30 min at 37°C. The preparation was then cleaned until only the carotid body, carotid sinus nerve (CSN), glossopharyngeal nerve, and petrosal ganglion remained. The cleaned preparation was transferred to a perfusion chamber (RC-22C; 140 µL; Warner Instruments) on the stage of an inverted microscope (Eclipse TE-300; Nikon). The chamber was perfused with saline solution bubbled with 21% O₂ (5% CO₂, balance N₂) that was delivered at a rate of ~3 mL min⁻¹ through stainless steel tubing (0.030 in. I.D.; Upchurch Scientific). The solution was passed through an in-line heater so that bath temperature was approximately 37°C.

A suction electrode placed into the petrosal ganglion was used to record single-unit nerve activity. An extracellular amplifier (EX-1; Dagan Instruments) was employed to amplify pipette potential (2,000–5,000 ×), which was passband-filtered (0.1–2 kHz), digitized (10 kHz sample rate), and recorded to a computer (Powerlab 8/30 and Chart 6 software; AD Instruments).

For identification of individual chemoafferent cells under baseline conditions (21% O₂, 5% CO₂, balance N₂), the carotid body was stimulated (~200 µA × 0.05 ms pulse duration) at 0.5–1 Hz (Isostim A320; World Precision Instruments) using a glass electrode (filled with 1

M NaCl). If a stimulus elicited a single orthodromic action potential, the stimulator was turned off and baseline nerve activity was recorded for 2 min. The response to acute hypoxia (5% O₂, 5% CO₂, balance N₂) was then recorded for 2 min. One recording was made for each carotid body-petrosal ganglion complex. Single-unit chemoreceptor activity was discriminated off-line (Spike Histogram Module v.1; ADInstruments). The number of spikes was counted for the final 1 min of baseline and in 1-s bins throughout the duration of acute hypoxia. Peak firing activity in hypoxia was determined from a 3-s moving average of spike frequency.

2.5. Carotid body histology

P41-42 and adult rats were sacrificed after ventilation measurements. Rats were euthanized with CO₂ and transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS) (pH 7.4). Rats were then decapitated for dissection of the carotid bifurcations, which were fixed for 1 hour in 4% PFA in PBS. Following cyoprotection in 30% sucrose in PBS (24 h at 4°C), bifurcations were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek), flash frozen on dry ice, and stored at -80°C. Samples were sectioned at 12 µm with a cryostatic microtome and mounted onto slides (Fisherbrand Superfrost Plus). Slides were then stained with hematoxylin and eosin and imaged with a Nikon Eclipse 80i microscope attached to a 2 megapixel camera and NIS Elements Software. Carotid body area within each section was calculated using Image J software (version 1.43u; National Institutes of Health). Carotid body volume was calculated from the area of the carotid body in each section, section thickness, and total number of sections containing the carotid body.

2.6. Statistical analysis

Body mass was compared among treatment groups at each age by two-way analysis of variance (ANOVA) (factor 1: developmental treatment; factor 2: sex) followed by Student-Newman-Keuls (SNK) *post hoc* tests where appropriate. Since preliminary analysis showed that male and female rats responded similarly to intermittent hyperoxia treatments, ventilation and metabolism data were pooled across sexes for subsequent analysis. Ventilation and metabolism were compared among groups separately at each age by two-way repeated measures ANOVA (factor 1: developmental treatment; factor 2: time or inspired O₂ level) followed by SNK *post hoc* tests. If the raw data did not meet the equal variance assumption for parametric tests, data were transformed prior to running the ANOVA (i.e., logarithm transformation for juvenile and adult body mass; square root transformation for P4 respiratory frequency). Baseline chemoafferent neuron discharge rates were not normally distributed, so comparisons among treatment groups were made using the nonparametric Kruskal-Wallis test and Dunn's *post hoc* tests. Peak chemoafferent discharge rates in hypoxia and carotid body volume were compared across treatment groups by one-way ANOVA followed by SNK *post hoc* tests. All statistical tests were run using SigmaPlot 12.5 (Systat Software) with statistical significance determined at $P < 0.05$. Values are presented as mean $\pm$ SEM unless otherwise indicated.

3. Results

3.1. Intermittent hyperoxia beginning at birth

3.1.1. Body mass—Body mass varied across treatment groups at P4 and P6-7 (main effect for developmental treatment, $P<0.001$ at both ages), but there were no differences in body mass among treatment groups at P13-15 or after the rats recovered in room air until 4-6 months of age (treatment and treatment $\times$ sex, all $P>0.05$) (Table 1). Specifically, I₃₀ rats were heavier than Control rats at P4 (+12%) and at P6-7 (+10%), while I₆₀ rats weighed less than Controls at both ages (−11% and −12%, respectively). Male rats weighed significantly more than female rats beginning at P6-7 (main effect for sex, $P=0.03$, 0.05 , <0.001 for P6-7, P13-15, and adult, respectively).

3.1.2. Ventilation at P4—At P4, minute ventilation did not vary among treatment groups under baseline conditions or during hypoxia when expressed in raw units (main effect for developmental treatment, $P=0.70$; treatment $\times$ time, $P=0.10$) (Fig. 2A, left panel). When the ventilatory response was expressed as a percentage change from baseline, I₆₀ rats had a greater increase in ventilation during the first minute of hypoxia than Controls (treatment $\times$ time, $P=0.05$; *post hoc*, $P=0.02$ vs. Control), but this enhancement did not persist in the late phase of the HVR (i.e., minute 10; *post hoc*, $P=0.76$ vs. Control) (Fig. 2A, right panel). I₃₀ rats did not differ from Controls at any time point. All three treatment groups showed a biphasic HVR, as ventilation at minute 10 was lower than at minute 1 whether data were plotted as raw units (main effect for time, $P<0.001$; *post hoc*, $P<0.001$ vs. minute 1) or as percentage change from baseline (*post hoc*, all $P<0.05$ vs. minute 1) (Fig. 2A). This decrease in minute ventilation by minute 10 was caused by decreases in both respiratory frequency and tidal volume (*post hoc*, both $P<0.05$ vs. minute 1) (Table 2); although respiratory frequency remained elevated compared to baseline during minute 10 ($P<0.001$), tidal volume was no longer different from baseline.

3.1.3. Ventilation at P6-7—There was a significant difference in minute ventilation among treatment groups at P6-7 (treatment, $P=0.01$). I₆₀ rats had greater ventilation than Controls across all time points ($P=0.02$) (Fig. 2B, left panel) due to both increased tidal volume (treatment, $P=0.01$; *post hoc*, $P=0.02$) and respiratory frequency (treatment, $P=0.004$; *post hoc*, $P=0.003$) (Table 2). I₃₀ rats tended to have higher respiratory frequency than Controls as well ($P=0.01$), but this did not translate into greater minute ventilation ($P=0.82$). The overall greater ventilation in I₆₀ rats reflected increased baseline ventilation rather than an enhanced HVR: the HVR was similar among treatment groups whether expressed in raw units (treatment $\times$ time, $P=0.36$) or as a percentage increase from baseline (treatment, $P=0.81$; treatment $\times$ time, $P=0.96$) (Fig. 2B, right panel). The HVR was biphasic in all treatment groups (i.e., minute 10 vs. minute 1; all $P<0.001$) (Fig. 2B). The decrease in minute ventilation by minute 10 was caused by decreases in both respiratory frequency and tidal volume (*post hoc*, both $P<0.05$ vs. minute 1), but both of these variables remained elevated compared to baseline (*post hoc*, both $P<0.001$) (Table 2).

3.1.4. Ventilation at P14-15—There was a significant effect of intermittent hyperoxia on the HVR at P14-15 (treatment $\times$ time, $P=0.02$) (Fig. 2C, left panel). Although baseline

ventilation was similar among groups, hypoxic ventilation was reduced in I₃₀ rats (−19%) and I₆₀ rats (−12%) during the late phase of the HVR (*post hoc*, $P=0.002$ and 0.03 vs. Control, respectively); similar results were obtained when ventilation was normalized as a percentage increase from baseline (treatment $\times$ time, $P=0.02$) (Fig. 2C, right panel). In both I₃₀ and I₆₀ rats, the blunted response to hypoxia at minute 10 was explained by a smaller increase in respiratory frequency (treatment $\times$ time, $P<0.001$) (Table 2); there were no differences in the tidal volume response in the late phase of the HVR.

Although blunted ventilatory responses were apparent in I₃₀ and I₆₀ rats during the late phase of the HVR (i.e., minute 10), ventilation was more variable during the early phase (i.e., minute 1). Minute ventilation tended to be reduced during the early phase in I₆₀ rats, but this trend did not reach statistical significance ($P=0.07$) (Fig. 2C, left panel). However, the respiratory frequency response was attenuated in both I₃₀ and I₆₀ rats during the first minute of hypoxia (both $P<0.05$ vs. Control) (Table 2). In contrast, I₃₀ rats exhibited greater tidal volumes than Controls during the first minute of hypoxia ($P=0.002$) (Table 2), resulting in a similar overall minute ventilation. Rats from all three treatment groups exhibited a sustained increase in ventilation throughout the HVR at P14–15 (minute 10 vs. minute 1, all $P>0.05$) (Fig. 2C).

3.1.5. Metabolic responses to hypoxia at P14—To determine whether differences in the HVR at P14–15 could be attributed to altered metabolic responses to hypoxia, CO₂ production were measured in an additional group of P14 Control and I₆₀ rats. Rates of CO₂ production were similar between the treatment groups (main effect for developmental treatment, $P=0.43$) (Fig. 3). Although CO₂ production decreased during hypoxia (main effect for O₂ level, $P<0.001$), the magnitude of the metabolic depression was comparable between Control and I₆₀ rats (treatment $\times$ O₂ level, $P=0.50$). Similar results were obtained when metabolic rate was expressed in units of O₂ consumption (data not shown).

3.1.6. Ventilation and metabolism in adult rats—To determine whether blunting of the HVR persists after return to normoxia, subsets of Control, I₃₀, I₆₀ rats were returned to room air at P15 and reared under standard conditions until studied again as adults (4–6 months of age). Adult rats in each treatment group increased minute ventilation in response to hypoxia (main effect for inspired O₂ level, $P<0.001$), but there were no differences among treatment groups in this response (main effect for developmental treatment, $P=0.41$; treatment $\times$ O₂ level, $P=0.65$) (Fig. 4A). Likewise, frequency and tidal volume responses were not affected by developmental treatments (treatment and treatment $\times$ O₂ level, all $P>0.05$) (Table 3).

Mass-specific CO₂ production decreased upon exposure to hypoxia (O₂ level, $P=0.04$), but this response did not differ among treatment groups (treatment, $P=0.58$; treatment $\times$ O₂ level, $P=0.32$) (Fig. 4B). Accordingly, no differences were detected in the ventilation-to-metabolism ratio (treatment, $P=0.15$; treatment $\times$ O₂ level, $P=0.24$) (Fig. 4C). Similar results were obtained when metabolism was expressed as O₂ consumption (data not shown).

3.1.7. Carotid body responses to hypoxia—To determine whether changes in the HVR observed at P4 (section 3.1.2) and P14–15 (section 3.1.4) are associated with changes

in carotid body function, carotid body responses to hypoxia were measured for these age groups. At P4, single-unit chemoafferent activity under baseline conditions (saline equilibrated with 21% O₂) was reduced in the I₆₀ group relative to Controls (median discharge rate = 0.07 vs. 0.23 impulses s⁻¹; treatment, $P=0.04$; *post hoc*, $P<0.05$) (Fig. 5A). There was no significant difference between the baseline discharge rate of the I₃₀ group (0.18 impulses s⁻¹) and Controls at this age. The peak discharge rate of I₆₀ chemoafferent neurons during hypoxia (5% O₂) was reduced by 43% relative to Controls (treatment, $P=0.02$; *post hoc*, $P=0.04$), but the hypoxic response in the I₃₀ group was not affected ($P=0.89$) (Fig. 5B). Similar results were obtained when chemoafferent responses were expressed as the change from baseline (discharge rate, impulses s⁻¹: Control: 12.6±2.0, I₃₀: 12.4±1.3, and I₆₀: 7.2±0.9) (treatment, $P=0.03$; *post hoc* for I₆₀ vs. Control, $P=0.05$).

At P13-14, baseline carotid chemoafferent neuron discharge rate was significantly reduced in both the I₃₀ and I₆₀ treatment groups compared to Controls (median discharge rates = 0.27, 0.35, and 0.96 impulses s⁻¹, respectively; treatment, $P=0.01$; *post hoc*, both $P<0.05$ vs. Control) (Fig. 5C). However, there was no significant difference in the response to hypoxia across treatment groups (treatment, $P=0.81$) (Fig. 5D). Similar results were obtained when chemoafferent responses were expressed as a change from baseline (discharge rate, impulses s⁻¹: Control: 12.5±1.4, I₃₀: 13.5±2.2, and I₆₀: 16.1±1.8) (treatment, $P=0.36$).

3.1.8. Carotid body size—Carotid body volumes were determined for Control, I₃₀, and I₆₀ rats after being reared from P15 to adulthood in room air. Mass-specific carotid body volume varied among groups (main effect for developmental treatment, $P=0.001$), with carotid bodies being 24% smaller in I₃₀ rats ($P=0.037$) and 50% smaller in I₆₀ rats ($P=0.001$) than in Controls (Fig. 4D). Similar results were obtained when carotid body volumes were compared without normalizing to body mass (data not shown).

3.2. Intermittent hyperoxia beginning at P28

3.2.1. Body mass—P41-42 rats exposed to intermittent hyperoxia (I₆₀) beginning at P28 weighed 5% less than age-matched Controls (main effect for developmental treatment, $P=0.009$) (Table 4); this effect did not vary by sex (treatment × sex, $P=0.44$). Male rats weighed more than female rats (main effect for sex, $P<0.001$).

3.2.2. Ventilation and metabolism—Control and I₆₀ rats (exposed beginning at P28) increased minute ventilation in response to hypoxia (main effect for inspired O₂ level, $P<0.001$), but this response did not differ between treatment groups (main effect for developmental treatment, $P=0.63$; treatment × O₂ level, $P=0.75$) (Fig. 6A). Likewise, frequency and tidal volume responses were not affected by intermittent hyperoxia (treatment and treatment × O₂ level, all $P>0.05$) (Table 5).

Mass-specific CO₂ production decreased upon exposure to hypoxia (O₂ level, $P<0.001$), but this response did not differ among treatment groups (treatment, $P=0.92$; treatment × O₂ level, $P=0.72$) (Fig. 6B). Accordingly, no differences were detected in the ventilation-to-metabolism ratio (treatment, $P=0.95$; treatment × O₂ level, $P=0.76$) (Fig. 6C). Similar results were obtained when metabolism was expressed as O₂ consumption (data not shown).

3.2.2. Carotid body size—Control and I₆₀ rats (exposed beginning at P28) had similar carotid body sizes whether normalized to body mass ($P=0.40$; Fig. 6D) or compared in absolute units (data not shown).

4. Discussion

Chronic intermittent hyperoxia during postnatal development altered respiratory control in neonatal rats. Two weeks of exposure to intermittent hyperoxia (either I₃₀ or I₆₀) attenuated the HVR as has been previously reported for rats reared in sustained hyperoxia (Bavis et al., 2010). Interestingly, there was a general trend for the HVR to be enhanced in the youngest rats studied (i.e., I₆₀ rats at P4, see Fig. 2A) and to become progressively blunted with continued exposure to intermittent hyperoxia; a similar pattern was noted for rats reared in sustained hyperoxia (Bavis et al., 2010, 2013). Moreover, this plasticity appears to be unique to intermittent hyperoxia exposures that occur during early life. When intermittent hyperoxia was initiated in older rats (i.e., at P28), the HVR and carotid body size were unaffected. The present study did not have the resolution to identify the specific bounds of the critical window, but its timing is consistent with that previously established for sustained hyperoxia (i.e., the first two postnatal weeks; Bavis et al., 2002).

Despite these similarities, there are important differences in the respiratory plasticity elicited by intermittent and sustained hyperoxia. Whereas neonatal rats reared in sustained hyperoxia hypoventilate when returned to room air (Bavis et al., 2010, 2014b), rats reared in intermittent hyperoxia exhibited normal, or somewhat greater (I₆₀ rats at P6-7), normoxic ventilation. Furthermore, rats reared in sustained hyperoxia obtain a mature, sustained HVR earlier than rats reared in room air (Bavis et al., 2010, 2014a). However, there was no evidence for earlier maturation of the HVR during chronic intermittent hyperoxia: there was a progressive shift from a strongly biphasic HVR at P4 to a sustained HVR by P14-15 that tracked the normal postnatal maturation of age-matched control rats. Finally, the blunted HVR elicited by postnatal intermittent hyperoxia did not persist into adulthood despite morphological impairment of the carotid body. In contrast, 1–4 weeks of sustained hyperoxia (30–60% O₂) causes life-long attenuation of the HVR in rats (Ling et al., 1996; Fuller et al., 2002; Bavis et al., 2002, 2007, 2008).

4.1. Carotid body and CNS plasticity

Metabolic responses to hypoxia at P14 were unaffected by developmental exposure to intermittent hypoxia, so the blunted HVR observed in this age group after two weeks of intermittent hyperoxia appears to reflect plasticity in respiratory control rather than differences in O₂ consumption or CO₂ production. Based on the effects of sustained hyperoxia on respiratory control (Bavis et al., 2013), we anticipated that this plasticity would involve abnormal development of the peripheral arterial chemoreceptors. Carotid body volume was reduced in adult I₃₀ and I₆₀ rats, consistent with the reduced number of O₂-sensitive glomus cells and overall smaller carotid body sizes reported after perinatal exposure to sustained hyperoxia (e.g., Erickson et al., 1998; Fuller et al., 2002; Dmitrieff et al., 2012; Bavis et al., 2013). Since intermittent hyperoxia exposures ended at P15 in the present study, it is likely that the carotid bodies were already smaller at P14-15 and that

carotid body hypotrophy contributes to the blunted HVR observed at that age. Other morphological changes to the carotid chemoafferent pathway were not studied after intermittent hyperoxia, but diminished carotid body size may also decrease carotid body-derived trophic support to primary chemoafferent neurons (Hertzberg et al., 1994). This may further diminish carotid body function (at the whole-organ level) by disrupting carotid body innervation and/or altering the neurochemical phenotype of the chemoafferent pathway (Hertzberg et al., 1994; Erickson et al., 1998; Chavez-Valdez et al., 2012).

Since decreased carotid body size persisted into adulthood in the present study, recovery of the adult HVR after intermittent hyperoxia could indicate compensatory plasticity elsewhere in the respiratory control system. There is indirect evidence for such compensatory plasticity after sustained hyperoxia. Specifically, phrenic nerve responses to electrical stimulation of carotid sinus nerve stimulation are normal in rats exposed to 60% O₂ for the first postnatal month of life (Ling et al., 1997; Fuller et al., 2002) despite fewer axons being present in the nerve (Erickson et al., 1998). This suggests an increased CNS gain for afferent input from peripheral chemoreceptors, although CNS plasticity is not sufficient to fully restore the HVR in that model of plasticity (Fuller et al., 2002). Alternatively, recovery of the HVR could reflect upregulation of extracarotid peripheral and/or central chemoreceptors after return to normoxia.

There was a poor correlation between single-unit carotid chemoafferent activity and ventilation in the present study under both normoxic and hypoxic conditions. Peripheral chemoreceptors contribute to eupneic ventilatory drive (Teppema and Dahan, 2010), and abnormal carotid body development contributes to the hypoventilation observed in rats reared in sustained hyperoxia (Bavis et al., 2014b). Although intermittent hyperoxia reduced baseline chemoafferent activity in the present study (e.g., at P4 and P13-14 for I₆₀, at P13-14 for I₃₀), this did not reduce normoxic ventilation. Likewise, the relationship between chemoafferent and ventilatory responses to hypoxia were opposite to what one might predict. Chemoafferent responses to hypoxia were blunted after only four days of intermittent 60% O₂ while the HVR tended to be augmented in this group of P4 rats; a similar pattern (i.e., normal or enhanced HVR in the face of diminished carotid body size and O₂ sensitivity) has also been noted for P4 rats exposed to sustained hyperoxia beginning just prior to birth (Bavis et al., 2013). Moreover, intermittent hyperoxia-treated rats exhibited normal chemoafferent responses to hypoxia at P13-14 while their HVR was blunted. Collectively, these data suggest that hyperoxia-induced plasticity in central respiratory neurons may obscure (or partially compensate for) impaired carotid body function.

Although not directly assessed in the present study, plasticity has been demonstrated in CNS regions critical to respiratory control after chronic exposures to sustained hypoxia (reviewed in Powell et al., 2000), intermittent hypoxia (e.g., Kline et al., 2007; Mayer et al., 2015), and sustained hyperoxia (Chavez-Valdez et al., 2012; Bierman et al., 2014; Bavis et al., 2014b). In particular, the nTS, which is the location of the first central synapse of carotid chemoafferent neurons, is sensitive to variation in oxygen levels (e.g., Kline et al., 2007; Chavez-Valdez et al., 2012; Pamentier et al., 2014; Mayer et al., 2015); these changes may reflect the direct effects of hypoxia/hyperoxia on nTS neurons or they could be secondary to

changes in sensory input from the carotid body. It remains to be investigated, but plasticity in the central limb of the carotid chemoafferent pathway (such as increased excitability at the nTS) could explain how the early phase of the HVR is enhanced in P4 I₆₀ rats despite their diminished carotid body responses. In contrast, the blunted HVR at P14-15 was most apparent in the late phase of the HVR. The late phase of the HVR is greatly influenced by signaling pathways in the nTS and other CNS regions that modulate (over a slower time course) the respiratory response to excitatory input from the peripheral chemoreceptors (Bissonnette, 2000; see also Hill et al., 2012). Thus, it is tempting to speculate that two-week exposures to intermittent hyperoxia strengthen the relative influence of inhibitory neuromodulators within the CNS during the HVR. However, there was a trend for the early phase of the HVR to be blunted as well in I₆₀ rats ($P=0.07$), and both I₃₀ and I₆₀ rats had blunted frequency responses during the first minute of hypoxia, so additional mechanisms cannot be ruled out.

4.2. Potential explanations for the contrasting effects of intermittent and sustained hyperoxia

It is unclear whether the qualitative and quantitative differences in the respiratory plasticity elicited by intermittent hyperoxia and sustained hyperoxia reflect different cumulative dosages of O₂, varying opportunities for “recovery” between bouts of hyperoxia, or different cellular mechanisms altogether. In the present study, neonatal rats spent approximately 45% of their time at greater than 21% O₂, amounting to only 6–7 days of cumulative hyperoxia over the course of the 2-week exposure (and less than 2 days for the 4-day exposures). However, 4–7 days of sustained hyperoxia (60% O₂) is sufficient to depress normoxic ventilation, hasten maturation of the biphasic HVR, and cause life-long impairment of the HVR in rats (Bavis et al., 2010, 2013). Since none of these forms of plasticity were apparent after intermittent hyperoxia in the present study, it does not appear that qualitative differences between intermittent and sustained hyperoxia reflect a simple dose-response relationship.

By raising arterial O₂ partial pressures, hyperoxia is expected to substantially reduce glomus cell depolarization and the generation of action potentials by chemoafferent neurons. Neuronal activity regulates the expression of hundreds of genes (Flavell and Greenberg, 2008), thus one mechanism by which developmental hyperoxia may attenuate hypoxic responses is by altering the transcription and translation of genes involved in O₂ sensing, carotid body growth, and neuronal development (Bavis et al., 2013). Similarly, hyperoxic inhibition of glomus cell depolarization could diminish the release of key signaling molecules such as brain-derived neurotrophic factor at the carotid body or downstream neurons (Hertzberg et al., 1994; Chavez-Valdez et al., 2012; Bavis et al., 2013). If one or both of these hypotheses is correct, periods of normal carotid body activity (and thus periods of relatively normal gene expression and/or signaling) between bouts of hyperoxia could be sufficient to rescue the developing respiratory control system.

In addition to elevated O₂ partial pressures, developmental hyperoxia is expected to increase the production of reactive oxygen species (ROS). ROS have been implicated in several forms of excitatory respiratory plasticity after acute and chronic intermittent hypoxia,

including enhanced carotid body activity / O₂ sensitivity and centrally mediated phrenic long term facilitation (e.g., MacFarlane et al., 2008; Prabhakar et al., 2010; Prabhakar, 2011). Although sustained exposure to 60% O₂ alters the redox status of some tissues in neonatal rats (e.g., reduced glutathione potential in lung and liver, but not brain; Prieto-Lloret et al., 2015), antioxidant supplementation does not ameliorate the effects of sustained hyperoxia on respiratory control (Bavis et al., 2008; Prieto-Lloret et al., 2015). Thus, ROS production does not contribute to the impaired carotid body growth, diminished carotid body sensitivity, or blunting of HVR caused by sustained exposure to moderate hyperoxia (60% O₂). However, it remains possible that increased ROS production influences the respiratory phenotype observed after intermittent hyperoxia. Indeed, fluctuating O₂ levels may generate more ROS than stable O₂ levels *in vivo* (Fabian et al. 2004), potentially overwhelming endogenous antioxidant defenses and altering intracellular signaling cascades. For example, the differential production of ROS by intermittent versus sustained hypoxia contributes to the pattern-dependence of phrenic long term facilitation (which is more readily initiated by acute intermittent hypoxia than sustained hypoxia; MacFarlane et al., 2008; Wilkerson et al., 2008). Similarly, ROS production during intermittent hyperoxia may be great enough to elicit excitatory plasticity at the carotid body or within the CNS (i.e., favoring increased O₂ sensitivity and/or respiratory drive) that opposes the inhibitory effects of high O₂ partial pressures on carotid body development and central respiratory drive; the balance between these opposing stimuli could differ across cell populations (e.g., based on the expression of NADPH oxidase) or over the course of the hyperoxic exposure (e.g., initial augmentation of carotid body activity may succumb to progressive carotid body hypotrophy and chemoafferent degeneration). Additional experiments measuring ROS production and/or the effects of antioxidant supplementation during developmental intermittent hyperoxia are needed, but the transient augmentation of the early, carotid body-mediated phase of HVR at P4 and normoxic ventilation at P6-7 observed in rats exposed to intermittent 60% O₂ are consistent with this hypothesis.

4.3. Clinical relevance of intermittent hyperoxia

Recent studies have begun to investigate the impacts of intermittent (versus sustained) hyperoxic exposures in animal models of retinopathy of prematurity and bronchopulmonary dysplasia (e.g., McColm et al., 2004; Chang et al., 2013; Winners-Mendizabal et al., 2014), but comparable studies on respiratory control are largely absent. At least in the rat model, the present results suggest that intermittent cycling reduces the impact of hyperoxia on the developing respiratory control system. However, even transient respiratory dysfunction in the neonatal period may be detrimental to the health of vulnerable infants. Both augmented and blunted HVR may lead to maladaptive responses to apneic or rebreathing events.

In order to model the hyperoxic exposures experienced by preterm human infants in neonatal intensive care units, we considered published reports that suggest that preterm infants may experience 5–10 prolonged bouts of hyperoxia per hour and may be hyperoxic (relative to SpO₂ targets) 30–60% of the time while on supplemental O₂ (Hagadorn et al., 2006; Sola et al., 2008; Claure and Bancalari, 2009; Bancalari and Claure, 2012; Lim et al., 2014). Although the I₃₀ and I₆₀ exposure paradigms used in the present study do not capture that variability in magnitude and/or timing of hyperoxic events encountered clinically, the

basic model (~5-min episodes, 5 episodes h⁻¹) more closely resembles clinical hyperoxia than previous studies using sustained hyperoxia or the long-duration episodes (1-h episodes, 0.5 episodes h⁻¹) employed by Bavis et al. (2007). Even so, the potential combinations of O₂ profiles and timing are seemingly limitless and additional experiments exploring this parameter space are warranted.

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Highlights

Neonatal rats were reared in intermittent hyperoxia (30 or 60% O₂, 5 bouts h⁻¹).

The hypoxic ventilatory response was blunted at P14-15 but recovered by adulthood

Chronic intermittent hyperoxia reduced carotid body size.

This plasticity is not elicited when intermittent hyperoxia occurs later in life.

Fig. 1. Representative traces of O_2 concentration measured during (A) intermittent 30% O_2 (I₃₀) and (B) intermittent 60% O_2 (I₆₀) exposures. O_2 concentrations cycled continuously (24 h d⁻¹).

Fig. 2.

Hypoxic ventilatory responses for (A) P4, (B) P6-7, and (C) P14-15 rats exposed to 21% O₂ (Control), intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀) beginning at birth.

Ventilation is expressed in raw units (left panels) and as a percentage increase from baseline (BL) during a 10-min exposure to 12% O₂. Values are mean $\pm$ SEM; sample sizes (Control, I₃₀, I₆₀) are P4: 20, 21, 20; P6-7: 19, 18, 20; and P14-15: 16, 16, 18. Where there was a significant treatment $\times$ time interaction, † $P < 0.05$ vs. Control at the same time point and ‡ $P < 0.05$ vs. 1st minute of hypoxia within the same treatment group. Where the interaction was not significant, * $P < 0.05$ vs. Control across all time points (*post hoc* test for main effect of developmental treatment) and # $P < 0.05$ vs. the 1st minute of hypoxia across all treatment groups (*post hoc* test for main effect of time).

Fig. 3.

Metabolic responses to hypoxia for P14 rats exposed to 21% O₂ (Control; n=10) or intermittent 60% O₂ (I₆₀; n=12) beginning at birth. CO₂ production was measured in 21% O₂ (baseline, BL) and in hypoxia (12% O₂). Values are mean $\pm$ SEM. No significant differences were detected between developmental treatment groups.

Fig. 4.

Ventilatory and metabolic responses to hypoxia and carotid body size for adult rats exposed from birth through P15 to 21% O₂ (Control), intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀); all rats were reared in room air from P15 until studied as adults. (A) Minute ventilation, (B) metabolism (CO₂ production), and (C) ventilation-to-metabolism ratio were measured in 21% O₂ and in response to hypoxia (12% O₂); n = 16 per treatment group. (D) Mass-specific carotid body volume was measured for a subset of rats (n= 6 Control, 6 I₃₀, 5 I₆₀). Values are mean ± SEM. * P<0.05 vs. Control.

Fig. 5.

Carotid chemoafferent nerve activity for P4 and P13-14 rats exposed to 21% O₂ (Control), intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀) beginning at birth. Single-unit carotid chemoafferent activity was recorded *in vitro* under baseline conditions (panels A and C; horizontal line indicates median) and during acute exposure to 5% O₂ (panels B and D; mean $\pm$ SEM). Sample sizes (Control, I₃₀, I₆₀) are P4: 11, 13, 11 and P13-14: 14, 12, 13. * $P < 0.05$ vs. Control.

Fig. 6.

Ventilatory and metabolic responses to hypoxia and carotid body size for P41-42 rats exposed to 21% O₂ (Control) or intermittent 60% O₂ (I₆₀) beginning at P28. (A) Minute ventilation, (B) metabolism (CO₂ production), and (C) ventilation-to-metabolism ratio were measured in 21% O₂ and in response to hypoxia (12% O₂); n = 12 Control and 16 I₆₀ for panels A and 11 Control and 14 I₆₀ for panels B and C. (D) Mass-specific carotid body volume was measured for a subset of rats (n= 12 per group). Values are mean ± SEM. No significant differences were detected between developmental treatment groups.

Table 1

Body mass for rats reared in 21% O₂ (Control), intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀) from birth. Adult rats were studied after being raised in room air from PI5 until 4-6 months of age. Data are pooled across all experiments from the present study.

		Male			Female			Statistical comparisons ($P \leq 0.05$) ^a
		Control	I ₃₀	I ₆₀	Control	I ₃₀	I ₆₀	
P4	n	12	10	11	8	11	9	
	Mass (g)	10.5 ± 0.3	11.6 ± 0.6	9.0 ± 0.4	10.3 ± 0.5	11.7 ± 0.2	9.6 ± 0.4	I ₃₀ > C > I ₆₀
P6-7	n	10	7	9	9	11	11	
	Mass (g)	16.4 ± 1.5	17.7 ± 0.7	14.8 ± 0.6	15.3 ± 0.2	17.3 ± 0.5	13.1 ± 0.8	I ₃₀ > C > I ₆₀ ; M > F
P13-15	n	14	14	22	20	12	18	
	Mass (g)	30.5 ± 1.0	33.5 ± 1.5	31.3 ± 0.8	28.8 ± 1.2	30.9 ± 1.7	30.1 ± 0.9	M > F
Adult	n	8	8	8	8	8	8	
	Mass (g)	451 ± 13	467 ± 15	483 ± 18	284 ± 7	266 ± 6	280 ± 8	M > F

Values are means ± SEM.

^aFor statistical comparisons: M, male; F, female; C, Control.

Table 2

Respiratory frequency and tidal volume during baseline conditions (21% O₂) and during the 1st and 10th minutes of hypoxia (12% O₂) for rats reared in 21% O₂ (Control), intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀) beginning at birth.

Age	Treatment	n	Respiratory Frequency (breaths min ⁻¹)			Tidal Volume (ml 100g ⁻¹)		
			Baseline	1 min	10 min	Baseline	1 min	10 min
P4	Control	20	139±5	165±7	155±6 [#]	0.68±0.04	0.81±0.05	0.73±0.04 [#]
	I ₃₀	21	152±5	180±6	174±6 [#]	0.66±0.03	0.75±0.04	0.65±0.04 [#]
	I ₆₀	20	137±5	169±8	161±7 [#]	0.71±0.05	0.92±0.05	0.73±0.05 [#]
P6-7	Control	19	147±4	176±4	165±5 [#]	0.57±0.04	0.72±0.04	0.68±0.04 [#]
	I ₃₀	18	157±4 [*]	198±5 [*]	187±5 ^{##}	0.51±0.03	0.67±0.05	0.63±0.04 [#]
	I ₆₀	20	157±5 [*]	185±5 [*]	181±6 ^{##}	0.66±0.04 [*]	0.88±0.06 [*]	0.85±0.07 ^{##}
P14-15	Control	16	125±4	192±6	185±7	0.74±0.02	0.88±0.03	0.94±0.02
	I ₃₀	16	126±4	159±8 [†]	154±6 [†]	0.74±0.03	1.01±0.03 [†]	0.91±0.04
	I ₆₀	18	137±4	168±4 [†]	176±5	0.67±0.02	0.89±0.03	0.88±0.03

Data are expressed as mean ± SEM. Where there was a significant developmental treatment × time interaction (i.e., P14-15),

[†] P<0.05 vs. Control at the same time point. Where the interaction was not significant,

^{*} P<0.05 vs. Control (post hoc test for main effect of developmental treatment)

[#] P<0.05 vs. the 1st minute of hypoxia (post hoc test for main effect of time)

Table 3

Respiratory frequency and tidal volume during baseline conditions (21% O₂) and hypoxia (12% O₂) for adult rats reared in 21% O₂ (Control), intermittent 30% O₂ (I₃₀), or intermittent 60% O₂ (I₆₀) from birth through P15. Data are expressed as mean $\pm$ SEM.

Treatment	n	Respiratory Frequency (breaths min ⁻¹)		Tidal Volume (ml 100g ⁻¹)	
		21% O ₂	12% O ₂	21% O ₂	12% O ₂
Control	16	90 $\pm$ 5	101 $\pm$ 4	0.59 $\pm$ 0.03	0.71 $\pm$ 0.03
I ₃₀	16	92 $\pm$ 5	112 $\pm$ 5	0.58 $\pm$ 0.03	0.70 $\pm$ 0.03
I ₆₀	16	106 $\pm$ 9	105 $\pm$ 3	0.50 $\pm$ 0.03	0.68 $\pm$ 0.03

Table 4

Body mass for P41-42 rats exposed to 21% O₂ (Control) or intermittent 60% O₂ (I₆₀) beginning at P28.

	Male		Female		Statistical Comparisons (<i>P</i> 0.05) ^a
	Control	I ₆₀	Control	I ₆₀	
n	5	10	7	6	
Mass (g)	209 ± 5	201 ± 3	160 ± 3	149 ± 3	C > I ₆₀ ; M > F

Values are means ± SEM. For statistical comparisons: M, male; F, female; C, Control.

Table 5

Respiratory frequency and tidal volume during baseline conditions (21% O₂) and hypoxia (12% O₂) for P41-42 rats exposed to 21% O₂ (Control) or intermittent 60% O₂ (I₆₀) beginning at P28. Data are expressed as mean $\pm$ SEM.

Treatment	n	Respiratory Frequency (breaths min ⁻¹)		Tidal Volume (ml 100g ⁻¹)	
		21% O ₂	12% O ₂	21% O ₂	12% O ₂
Control	12	144 $\pm$ 9	137 $\pm$ 5	0.57 $\pm$ 0.02	0.81 $\pm$ 0.02
I ₆₀	16	135 $\pm$ 5	130 $\pm$ 4	0.60 $\pm$ 0.02	0.85 $\pm$ 0.02