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Developmental hyperoxia attenuates the hypoxic ventilatory response in Japanese quail (*Coturnix japonica*)

Ryan W. Bavis^{*} and Julia C. Simons

Department of Biology, Bates College, Lewiston, ME 04240 USA

Abstract

Early life experiences can influence development of the respiratory control system. We hypothesized that chronic hyperoxia (60% O₂) during development would attenuate the hypoxic ventilatory response (HVR) of Japanese quail (*Coturnix japonica*), similar to the effects of developmental hyperoxia in mammals. Quail were exposed to hyperoxia during prenatal development, during postnatal development, or during both prenatal and postnatal development (for approximately 2 or 4 weeks). HVR (11% O₂) was subsequently assessed in adults (>6 weeks old) via barometric plethysmography and compared to quail raised in normoxia (i.e., control). The HVR of quail exposed to hyperoxia both prenatally and postnatally was reduced 50–60% compared to control quail whereas postnatally exposed quail exhibited normal HVR. The effects of prenatal hyperoxia on HVR were equivocal and depended on how HVR was expressed. We conclude that developmental exposure to 60% O₂ attenuates the HVR in quail and that the critical period for this plasticity encompasses the late prenatal and early postnatal periods.

Keywords

Birds; Control of breathing; Critical period; Developmental window

1. Introduction

Environmental conditions during early life can have profound effects on the development of the respiratory control system (Carroll, 2003; Bavis and Mitchell, 2008). This developmental plasticity has been studied primarily in rats and other mammals. In these species, factors such as hypoxia, hyperoxia, hypercapnia, maternal separation stress, and exposure to various drugs during perinatal development have been found to durably alter eupneic ventilation and/or protective ventilatory chemoreflexes (reviewed in Bavis and Mitchell, 2008).

Postnatal exposure to chronic hyperoxia, for example, greatly reduces the hypoxic ventilatory response (HVR) in adult rats (Ling et al., 1996; Bavis et al., 2003, 2007, 2008). These effects, which may be permanent, have been linked to abnormal development of the carotid body, the primary peripheral chemoreceptor responsible for sensing arterial O₂ levels (Ling et al., 1997a; Fuller et al., 2002). Specifically, hyperoxia-treated rats have smaller carotid bodies with fewer O₂-sensitive (type I) cells and fewer neurons in the carotid sinus nerve to carry afferent

^{*}Corresponding author: Ryan W. Bavis, PhD, Department of Biology, Bates College, 44 Campus Ave., Carnegie Science Hall, Lewiston, ME 04240 USA, Phone: 207-786-8269, Fax: 207-786-8334, Email: rbavis@bates.edu.

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information to the brainstem (Erickson et al., 1998; Bisgard et al., 2003; Prieto-Lloret et al., 2004; Wang and Bisgard, 2005). Hypoxic chemotransduction may also be impaired in surviving chemoreceptor cells (Prieto-Lloret et al., 2004; Donnelly et al., 2005). The persistent effects of hyperoxia on the carotid body and HVR are specific to development, with the critical period (or developmental window) during which rats are susceptible to hyperoxia apparently closing after the second postnatal week (Erickson et al., 1998; Bavis et al., 2002); any long-lasting respiratory effects of prenatal exposure to hyperoxia have not been studied in rats.

When studying developmental plasticity, one must consider whether experimental treatments act directly on the developing animal or act indirectly through its parents (e.g., stress responses of the mother, changes in parental care, etc.). These “maternal effects” are particularly problematic when studying species that develop internally (e.g., placental mammals) and altricial species that require extensive postnatal care. In addition, cardiorespiratory responses of the mother may buffer changes in environmental gas concentrations making it difficult to manipulate the conditions experienced by the embryo *in utero*. Thus, it may be preferable to study species such as precocial birds (e.g., Japanese quail, chicken) that develop in external eggs and do not require parental care. Importantly, as in mammals, the avian respiratory control system is sensitive to environmental conditions during development. Adult zebra finches (Williams and Kilgore, 1992) and Japanese quail (Bavis and Kilgore, 2001; Foster et al., 2008) exhibit attenuated hypercapnic ventilatory responses following perinatal exposure to moderate hypercapnia. Similarly, chickens exposed to hypercapnia (Szdzyu and Mortola, 2008) or hypoxia (Szdzyu and Mortola, 2007) as embryos have reduced ventilatory responses to both hypercapnia and hypoxia for at least one day after hatching.

The effects of developmental hyperoxia on respiratory control have not been studied in birds. However, given the homology of the mammalian and avian carotid body (Milsom and Bursell, 2007), we hypothesized that hyperoxia would reduce the HVR in birds as previously observed in mammals. Therefore, the purpose of the present study was to determine whether hyperoxia elicits long-lasting changes in the HVR of Japanese quail (*Coturnix japonica*) and to identify the critical period for this plasticity.

2. Methods

All experimental procedures were approved by the Animal Care and Use Committee at Bates College.

2.1 Experimental animals

Fertile Japanese quail eggs were obtained from a commercial supplier (Boyd's Birds, Pullman, WA USA). Eggs were placed into circulated-air incubators equipped with automatic egg turners (Hova-Bator; G.Q.F. Manufacturing Co., Savannah, GA USA); temperature and humidity were regulated in accordance with the manufacturer's operating instructions. A low flow of air or of a mixture of air and O₂ was introduced to the incubator to maintain the desired gas concentration (21% O₂ or 60% O₂; see below); incubators were opened briefly (1–2 minutes) every 2–3 days to add water for humidity regulation and/or to remove the egg turner 1–2 d prior to hatch. Upon hatching, chicks were moved into poultry box brooders (G.Q.F. Manufacturing Co.) that were completely enclosed in clear plastic sheeting. Air or a mixture of air and O₂ were forced through the brooder at sufficient rates to maintain the desired gas concentration (21% O₂ or 60% O₂; see below) and to keep CO₂ levels below 0.4%; brooders were opened briefly (<5 min) each day for cleaning and to refresh food and water. When chicks were two weeks of age, the plastic sheeting was removed and all chicks were subsequently raised in room air (21% O₂) until studied as adults (>6 weeks of age).

This study consisted of five treatment groups: a control group and four groups of quail that were exposed to hyperoxia during prenatal and/or postnatal development (Fig. 1). Control quail ($n=52$) were exposed to 21% O_2 throughout prenatal and postnatal development (normoxia/normoxia, NN). A second group of quail ($n=31$) was exposed to 60% O_2 throughout the 16–17 days of incubation and for the first two weeks after hatching; thus, these quail were exposed to hyperoxia during both the prenatal and postnatal periods for a little more than 4 weeks total (hyperoxia/hyperoxia, HH-4wk). The remaining groups of quail were exposed to shorter durations of hyperoxia (approximately 2 weeks) at different developmental stages to determine whether quail are susceptible to hyperoxic exposures during specific time periods. Specifically, the third group of quail ($n=15$) was exposed to 60% O_2 from the ninth day of incubation (i.e., 8 days after the onset of incubation) through the first postnatal week; thus, these quail were exposed to hyperoxia during both the prenatal and postnatal periods, but for only a little more than 2 weeks total (hyperoxia/hyperoxia, HH-2wk). The fourth group of quail ($n=17$) was exposed to 60% O_2 only during the prenatal period (hyperoxia/normoxia, HN), from the onset of incubation until the day before chicks were expected to hatch. The fifth group of quail ($n=17$) was exposed to 60% O_2 primarily during the postnatal period (normoxia/hyperoxia, NH), from the day before chicks were expected to hatch (i.e., the approximate time of internal pipping and initiation of lung ventilation; Vince and Cheng, 1970) through the second postnatal week.

2.2 Surgical preparation

At least one week prior to ventilation measurements, temperature transponders (E-mitter G2; Respironics, Bend, OR USA) were surgically implanted into the abdominal cavity of all quail. Anesthesia was induced with isoflurane in a closed box and subsequently maintained via nose cone (2–2.5% isoflurane, balance O_2); adequacy of anesthesia was assessed by lack of a withdrawal response to toe pinch. Transponders were placed into the abdominal cavity through a ventral, midline or lateral incision. When using a ventral approach, care was taken when opening the abdomen to avoid rupturing the air sacs while inserting the transponder. Carprofen (30 mg kg^{-1} , i.m.; Pfizer, New York, NY USA) was provided immediately after surgery as an analgesic (Hocking et al., 2005).

2.3 Ventilation and metabolism measurements

Ventilation was measured on unrestrained adult quail resting in an opaque, whole-body barometric plethysmograph chamber (15 cm inner diameter, 2.1 l functional volume). Air was forced through the chamber at 2 l min^{-1} (STPD) using a gas mixing mass flow controller (MFC-4; Sable Systems, Las Vegas, NV USA) and valves (840 series; Sierra Instruments, Monterey, CA USA). To ensure that the chamber air was saturated with water vapor, air was humidified before entering the chamber and the chamber floor was covered with water beneath the animal. When sealed, respiratory-related pressure fluctuations within the chamber were measured with a differential pressure transducer (DP45 transducer and CD 15 carrier demodulator; Validyne Engineering, Northridge, CA USA). The system was pre-calibrated by repeated 0.2 ml air injections; rubber stoppers approximating the volume of the experimental animal were placed inside the chamber during the calibration procedure. Pressure fluctuations and chamber temperature (T-type thermocouple) were recorded to computer (PowerLab 8SP and Chart 5.2 software, ADInstruments, Colorado Springs, CO USA). Body temperature (T_b) was continuously recorded by telemetry (VitalView 4.1, Respironics). These measurements were used to calculate tidal volume (V_T) (Drorbaugh and Fenn, 1955), respiratory frequency (f_R), and ventilation ($\dot{V}_E$). Fractional concentrations of O_2 and CO_2 in the air entering and exiting the plethysmograph chamber were measured (PowerLab Gas Analyzer ML206, ADInstruments, Colorado Springs, CO USA) and recorded to computer to calculate metabolic O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$); air was dried (Direrite, W.A. Hammond Drierite Co., Xenia, OH USA) prior to passing through the gas analyzer.

On the day of the experiment, quail were weighed and placed into the plethysmograph chamber during the light portion of their light:dark cycle. Quail were initially exposed to 21% O₂ (balance N₂) for 1–2 hours until they appeared calm. Once the quail were resting quietly, O₂ and CO₂ exiting the plethysmograph were recorded for approximately 30 s. The chamber was then briefly sealed (~2 min) to record respiratory-related pressure fluctuations. Our objective was to obtain approximately 60 s of continuous breathing that was free from movement artifacts and sighs, and measurements were repeated after an additional 5–10 min (to reestablish steady state conditions) if necessary. Quail were then exposed to 11% O₂ (balance N₂) for 15 minutes before repeating metabolism and ventilation measurements. Barometric pressure was recorded during each ventilation measurement and averaged 752±5 mmHg (mean±SD).

2.4 Data analysis

Ventilatory variables (V_T , f_R and $\dot{V}_E$) were calculated from analysis of 30–60 s of breathing data. Metabolic O₂ consumption ($\dot{V}_{O_2}$) and CO₂ production ($\dot{V}_{CO_2}$) were calculated from 20–30 s recordings of O₂ and CO₂ concentrations of gas entering and exiting the plethysmograph chamber and used to derive the ventilation-to-metabolism ratio, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$. HVR was calculated as the increase in ventilation during hypoxia, both in raw units and as a percentage of the baseline value.

The quail from the NN and HH-4wk treatment groups were obtained from two (HH-4wk) or three (NN) independent batches of eggs. Preliminary analyses revealed no differences in any of the respiratory or metabolic variables measured among batches within these treatment groups, so data within each treatment group were pooled for all statistical analyses.

Statistical comparisons were made among groups by two-way ANOVA (developmental treatment, sex). Where ANOVA revealed a significant treatment effect, all groups were compared to the control (NN) group using Bonferroni *post hoc* tests. Where necessary (e.g., $\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$), a logarithmic transformation was applied to the data to meet the assumptions for parametric tests; for clarity, non-transformed data are reported in the text and tables. All statistical tests were run using SigmaStat v.3.0 (Systat Software, Inc., Point Richmond, CA USA). Differences were considered significant at $P \leq 0.05$. Values in the text are means ± SEM unless otherwise stated.

3. Results

3.1 Baseline ventilation

Baseline ventilation and metabolic rate data are presented in Table 1 for quail breathing 21% O₂. Quail exposed to hyperoxia only during the prenatal period (i.e., HN quail) exhibited a greater baseline V_T than control (NN) quail ($P < 0.001$). The greater V_T in these quail was partially offset by a trend toward lower f_R , such that $\dot{V}_E$ was not significantly different from the NN group. However, accounting for variation in metabolic rate, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ were significantly greater in HN quail than in NN quail (both $P \leq 0.01$). No other developmental treatment groups (HH-4wk, HH-2wk or NH) differed from the NN group for any of the baseline variables measured (all $P > 0.05$).

Male quail exhibited a deeper and slower breathing pattern than female quail. No differences were detected between the sexes for mass-corrected $\dot{V}_E$ or for $\dot{V}_E/\dot{V}_{CO_2}$ (both $P > 0.05$), although $\dot{V}_E/\dot{V}_{O_2}$ was marginally lower in males ($P = 0.05$). These sex differences were independent of the developmental treatment group (sex × treatment, all $P > 0.05$).

3.2 Hypoxic ventilatory response (HVR)

The HVR of adult quail exposed to hyperoxia during development are presented in Figures 2 and 3. When expressed as a percentage increase from baseline, significant differences were detected among developmental treatment groups for the $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ responses to hypoxia (i.e., main effect for treatment, all $P < 0.001$; Fig. 2C–E); HVR did not differ between males and females (sex and sex $\times$ treatment, all $P > 0.05$). Quail exposed to hyperoxia during both the prenatal and postnatal periods (i.e., HH-4wk and HH-2wk) exhibited, on average, a ~60% reduction in their $\dot{V}_E$ responses when compared to NN quail ($P < 0.001$ and $P < 0.01$, respectively; Fig. 2C). This reflected significantly smaller increases in f_R during hypoxia in these groups (both $P \leq 0.01$ vs. NN; Fig. 2A), with no detectable change in the $\dot{V}_T$ response ($P > 0.05$; Fig. 2B). Similar results were obtained for the $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ responses to acute hypoxia, with HH-4wk and HH-2wk quail increasing $\dot{V}_E/\dot{V}_{O_2}$ (Fig. 2D) and $\dot{V}_E/\dot{V}_{CO_2}$ (Fig. 2E) only about half as much as NN quail (all $P \leq 0.001$).

The $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ responses of quail exposed to hyperoxia primarily during the postnatal period (i.e., NH) did not differ from the NN group (all $P > 0.05$; Fig. 2C–E). Similarly, the $\dot{V}_E$ response of quail exposed to hyperoxia only during the prenatal period (HN) was unaltered ($P > 0.05$ vs. NN; Fig. 2C). The $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ responses of HN quail were somewhat reduced, however, when expressed as a percentage of baseline (both $P < 0.01$ vs. NN; Fig. 2D,E). Since HN quail exhibited relatively high $\dot{V}_E/\dot{V}_{CO_2}$ while breathing 21% O_2 , which could influence HVR when calculated as a percentage of baseline (Packard and Boardman, 1999; Reeves and Gozal, 2005), HVR was also analyzed in raw units (i.e., the difference between the hypoxic and baseline values, not normalized as a percentage of baseline) (Fig. 3). Again, the increases in $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ were significantly reduced in HH-4wk quail (all $P < 0.001$) and HH-2wk quail ($P = 0.02$, $P = 0.001$ and $P < 0.001$, respectively) when compared to NN quail and the responses of NH quail were normal (all $P > 0.05$ vs. NN). For HN quail, however, the $\dot{V}_E$ ($P = 0.59$), $\dot{V}_E/\dot{V}_{O_2}$ ($P = 0.27$), and $\dot{V}_E/\dot{V}_{CO_2}$ ($P = 0.07$) responses no longer differed significantly from those of the NN quail when expressed this way. Similarly, the average values for $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ were quite similar between NN and HN quail while breathing 11% O_2 ($\dot{V}_E/\dot{V}_{O_2}$: 27.0 ± 1.1 vs. 26.9 ± 1.7 and $\dot{V}_E/\dot{V}_{CO_2}$: 38.3 ± 1.1 vs. 38.6 ± 1.9 , respectively, pooled across sexes).

4. Discussion

Japanese quail exposed to moderate hyperoxia (60% O_2) during both prenatal and postnatal development exhibited attenuated HVR as adults. Specifically, quail in the HH-4wk and HH-2wk groups increased ventilation only about half as much as age-matched control quail. These results are qualitatively similar to those previously reported in kittens (Hanson et al., 1989) and adult rats (Ling et al., 1996; Bavis et al., 2003, 2007, 2008) exposed to 30–60% O_2 in the early postnatal period. As in rats, the reduced HVR in quail is primarily explained by a smaller increase in respiratory frequency when exposed to acute hypoxia. This plasticity did not differ between male and female quail.

The effects of hyperoxia on the HVR were age-dependent and were no longer evident in quail exposed primarily during the postnatal period (see section 4.2, below), indicating that this plasticity is specific to early development. Maternal effects cannot explain these long-lasting effects of hyperoxia on respiratory control development since quail were exposed as embryos and chicks in the absence of adults.

4.1 Methodological considerations

In rats, attenuation of the HVR after developmental hyperoxia may be less apparent during acute exposures to severe hypoxia (Ling et al., 1997b; Prieto-Lloret et al., 2004; S.E. Piro and

R.W. Bavis, unpublished observations). Therefore, to increase the likelihood of detecting differences between developmental treatment groups, we elected to study the HVR of adult Japanese quail at a moderate level of hypoxia (11% O₂). Preliminary studies indicate that the hypoxic ventilatory threshold for Japanese quail occurs at an inspired PO₂ of approximately 100 mmHg (R.W. Bavis and D.L. Kilgore, Jr., unpublished data), which is equivalent to 13% O₂ at Bates College. Since 11% O₂ is fairly close to the hypoxic ventilatory threshold for this species, it is not surprising that the observed increases in ventilation were modest and that the magnitude of the HVR was variable within treatment groups (presumably reflecting individual variation in the ventilatory threshold).

4.2 Critical period for hyperoxia-induced plasticity

It is well established in rats that only hyperoxic exposures that occur during development have long-lasting consequences on the HVR. Rats exposed to one month of 60% O₂ *as adults* exhibit normal ventilatory (Ling et al., 1996) and phrenic nerve (Ling et al., 1997b) responses to acute hypoxia. Indeed, the critical period during which rats are susceptible to long-term changes in respiratory control appears to close after the second postnatal week. Although one-week exposures to 60% O₂ during the first or second postnatal week reduce the phrenic nerve response to hypoxia into adulthood in rats, similar exposures during the third or fourth postnatal week have no lasting impact (Bavis et al., 2002). Likewise, rats exposed to 60% O₂ beginning at three weeks of age show no loss of tyrosine hydroxylase-positive neurons in the petrosal ganglion (i.e., carotid chemoafferent neurons) compared to a 32% loss of neurons when the hyperoxic exposure begins at birth (Erickson et al., 1998).

The present study suggests that the long-lasting effects of hyperoxia on quail respiratory control are specific to development as well. Indeed, the HVR of quail exposed to hyperoxia primarily during the postnatal period (i.e., NH group; 60% O₂ from the approximate time of internal pipping through the second postnatal week) did not differ from age-matched controls. This contrasts with quail exposed to a similar duration of hyperoxia but shifted to earlier developmental ages (i.e., HH-2wk group; 60% O₂ from one week prior to internal pipping through the first postnatal week). Thus, as in rats, there appears to be a specific critical period during development in which relatively short exposures to hyperoxia durably alter the HVR. However, these data do not preclude the possibility that longer (>2 weeks) or more severe (>60% O₂) exposures to hyperoxia in the postnatal period or in adulthood might also impair the HVR in quail.

To better define the critical period for this developmental plasticity, we also considered the effects of hyperoxic exposures confined to the prenatal period (i.e., HN group; 60% O₂ from onset of incubation through the approximate time of internal pipping). When expressed as the percentage change from baseline, there was a statistically significant reduction in the $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ responses (but not the $\dot{V}_E$ response) to 11% O₂. However, this analysis is complicated by higher normoxic $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ in this group. Since no other hyperoxia-treated group exhibited changes in normoxic breathing, including the HH-4wk group that encompassed the same age range, it is unlikely that the higher normoxic $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ in this group are an effect of prenatal hyperoxia *per se* and instead may have occurred by chance (i.e., type I statistical error). Importantly, the HVR did not appear blunted when expressed as a simple increase in $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$ or $\dot{V}_E/\dot{V}_{CO_2}$ in hypoxia (Fig. 3) nor did this group exhibit the characteristic blunting of the respiratory frequency response to hypoxia (Fig. 2A).

Together, these observations suggest that the critical period for this plasticity spans both the prenatal and early postnatal periods in quail and that hyperoxia may have to occur during both periods to elicit long-lasting changes in HVR. Specifically, since the HVR was nearly identical in quail from the HH-4wk and HH-2wk groups, we propose that the critical period for this

plasticity lies within a developmental window roughly corresponding to our HH-2wk exposure (Fig. 1). The actual critical period may occupy only a portion of this age range, but our experimental design cannot address that possibility. Moreover, since the time of internal pipping was not determined for individuals, the trend toward reduced HVR in HN quail may reflect variability in hatching times among quail – some quail may have internally pipped earlier than expected and, therefore, have been exposed to hyperoxia for several hours after initiating lung ventilation. Individual quail were not marked at the time of hatching, so it is not possible to correlate hatching times with HVR in the present study.

4.3 Potential mechanisms for hyperoxia-induced plasticity

The mechanisms underlying hyperoxia-induced respiratory plasticity have not been studied in birds, but presumably the reduced HVR reflects impairment of carotid body function. As in mammals, the carotid body is the primary arterial chemoreceptor in birds and is chiefly responsible for the HVR (Powell, 2000). Developmental hyperoxia is known to alter the morphology and function of the carotid body and its afferent neurons in rats (Ling et al., 1997a; Erickson et al., 1998; Fuller et al., 2002; Bisgard et al., 2003; Prieto-Lloret et al., 2004; Donnelly et al., 2005; Wang and Bisgard, 2005), and carotid body O₂ sensitivity was also reduced in kittens (12–23 days of age) maintained in hyperoxia from birth (Hanson et al., 1989); the cellular and molecular pathways by which hyperoxia impairs carotid body development in mammals are poorly understood (reviewed in Bavis and Mitchell, 2008). It will be important for future studies to directly test the effects of developmental hyperoxia on the function of the avian carotid body. However, given the homology of the avian and mammalian carotid bodies (Milsom and Burleson, 2007), it seems likely that hyperoxia will influence carotid body development through similar pathways.

Interestingly, the critical period for this plasticity is also consistent with carotid body involvement. In Japanese quail, the carotid body begins to form about the sixth day of incubation, approximately one-third of the way through embryonic development (Fontaine, 1973). This fits well with the critical period for this plasticity since no additional effect on the HVR was observed if the hyperoxic exposure began prior to the ninth day of incubation (i.e., similar HVR in HH-4wk and HH-2wk groups). Although care must be taken when comparing development across taxonomic groups, it is also notable that the critical period for hyperoxia-induced plasticity occurs somewhat earlier in Japanese quail than in rats – hyperoxic exposures solely in the postnatal period can blunt the adult HVR in rats (Bavis et al., 2002) but apparently not in quail. In newborn rats and other mammals, the carotid body exhibits little or no tonic activity or O₂ sensitivity and undergoes considerable postnatal maturation (Carroll and Kim, 2005; Donnelly, 2005). In contrast, carotid body function appears well developed in birds prior to hatching based on ventilatory responses to acute hypoxia and hyperoxia in externally pipped chicken embryos (Menna and Mortola, 2003; Mortola, 2004). Thus, we propose that the early functional development of the carotid body in precocial birds may contribute to the relatively early critical period for this plasticity in quail.

4.4 Implications

It was recently reported that hyperoxia can also have long-lasting effects on respiratory control in the zebrafish (*Danio rerio*) (Vulesevic and Perry, 2006). Specifically, zebrafish that were exposed to PO₂ = 350–400 mmHg for the first seven days post-fertilization, the period when gill O₂ chemoreceptors are innervated, exhibited increased respiratory frequency during normoxia and attenuated respiratory responses to acute hypoxia, cyanide and hypercapnia. Respiratory control was unchanged when adult fish were exposed to a comparable 7-day exposure (Vulesevic and Perry, 2006). Importantly, the gill O₂ chemoreceptors appear to be homologous with the mammalian and avian carotid bodies (Milsom and Burleson, 2007), and reduced respiratory responses to cyanide suggest impaired development of these

chemoreceptors. Thus, when these data are considered alongside the results of the present study in quail and previous studies in mammals, it appears that the effects of developmental hyperoxia on peripheral chemoreceptor development and the HVR may be widespread among vertebrate classes.

These data emphasize the important role that the environment can play in the normal and pathological development of the respiratory control system. Clinical disorders such as sudden infant death syndrome (SIDS) and central hypoventilation disorder may involve abnormal development of the respiratory control system (Cohen and Katz-Salamon, 2005; Weese-Mayer et al., 2005; Gauda et al., 2007), and peripheral and central chemoreceptors participate in homeostatic processes beyond breathing *per se* (Kara et al., 2003). Indeed, human infants that receive O₂ therapy in neonatal intensive care units exhibit abnormal chemoreceptor function (Calder et al., 1994; Katz-Salamon and Langercrantz, 1994; Katz-Salamon et al., 1996), although a causal link between O₂ therapy and impaired chemoreflexes has not been established experimentally in humans. It is therefore critical to understand how environmental conditions in early life influence development of protective chemoreflexes. Japanese quail, along with other precocial birds, may be a useful comparative model for studying long-lasting impacts of the gaseous environment on respiratory control.

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Fig. 1.

Rearing protocols used in this experiment. Hatching normally occurs after 16–17 days of incubation in Japanese quail (dotted line). In this diagram, open bars represent time periods during which quail were exposed to normoxia (21% O₂) whereas shaded bars represent time periods during which quail were exposed to hyperoxia (60% O₂). Thus, quail in the NN group were exposed to normoxia throughout development and quail in the HH-4wk group were exposed to hyperoxia for a little more than 4 weeks (31 days) spanning both prenatal and postnatal development. The remaining groups were exposed to hyperoxia for approximately two weeks (15–16 days) during the prenatal and postnatal periods (HH-2wk), during the prenatal period (HN), or primarily during the postnatal period (NH). The arrow indicates the approximate time of internal pipping (when lung ventilation begins), which usually occurs 1–1.5 days before hatching in this species (Vince and Cheng, 1970). All quail were raised in room air from approximately two weeks of age (31 days after onset of incubation) until studied as adults.

Fig. 2.

Ventilatory response to hypoxia (11% O₂) for control quail (NN) and for quail exposed to 60% O₂ during both prenatal and postnatal development (HH-4wk or HH-2wk), during prenatal development only (HN), or during postnatal development (NH); see Fig. 1 for details on the treatment groups. Panels A–E present the increases in respiratory frequency (f_R), tidal volume (V_T), minute ventilation ($\dot{V}_E$) and ventilation-to-metabolism ratio ($\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$) normalized as a percentage increase from baseline. Values are means $\pm$ S.E.M., pooled across sexes; *n* = 52 NN, 31 HH-4wk, 15 HH-2wk, 17 HN and 17 NH. * *P* < 0.05 versus the NN group.

Fig. 3. Ventilatory response to hypoxia (11% O_2) for control quail (NN) and for quail exposed to 60% O_2 during development; see Fig. 1 for details on the treatment groups. In contrast to Figure 2, $\dot{V}_E$ (panel A), $\dot{V}_E/\dot{V}_{O_2}$ (panel B) and $\dot{V}_E/\dot{V}_{CO_2}$ (panel C) responses are presented as the increase

Table 1
Baseline respiratory characteristics of control quail (NN) and of quail exposed to 60% O₂ during both prenatal and postnatal development (HH-4wk or HH-2wk), during prenatal development only (HN), or during postnatal development (NH); see Fig. 1 for details on the treatment groups.

Group	n	Age (d)	Mass (g)	T _b (°C)	V _T (ml 100g ⁻¹)	f _R (breaths min ⁻¹)	V _E (ml min ⁻¹ 100g ⁻¹)	V _{O₂} (ml O ₂ min ⁻¹ 100g ⁻¹)	V _{CO₂} (ml CO ₂ min ⁻¹ 100g ⁻¹)	V _E /V _{O₂}	V _E /V _{CO₂}
Control (NN)											
Male	27	59 ± 2	106 ± 2	40.9 ± 0.1	2.2 ± 0.1	26.9 ± 1.2	57.4 ± 2.7	4.0 ± 0.2	2.8 ± 0.1	13.1 ± 0.8	21.1 ± 1.1
Female	25	54 ± 2	126 ± 3	41.0 ± 0.1	2.0 ± 0.1	32.1 ± 1.5	60.0 ± 3.0	4.0 ± 0.2	2.9 ± 0.1	14.3 ± 1.0	21.6 ± 1.2
All	52	57 ± 1	115 ± 2	41.0 ± 0.1	2.1 ± 0.1	29.4 ± 1.0	58.7 ± 2.0	4.0 ± 0.1	2.8 ± 0.1	13.7 ± 0.6	21.3 ± 0.8
HH-4wk											
Male	14	54 ± 2	104 ± 2	41.1 ± 0.1	2.4 ± 0.2	28.5 ± 1.8	66.5 ± 5.0	4.7 ± 0.3	3.2 ± 0.3	12.5 ± 1.0	21.6 ± 1.6
Female	17	58 ± 3	134 ± 4	40.8 ± 0.2	1.7 ± 0.1	36.9 ± 1.9	59.5 ± 2.8	4.1 ± 0.4	2.9 ± 0.1	14.5 ± 1.2	21.0 ± 0.7
All	31	56 ± 2	120 ± 3	41.0 ± 0.1	2.0 ± 0.1	33.1 ± 1.5	62.6 ± 2.8	4.4 ± 0.2	3.0 ± 0.1	13.5 ± 0.8	21.3 ± 0.8
HH-2wk											
Male	8	52 ± 2	104 ± 3	41.5 ± 0.1	2.2 ± 0.2	32.9 ± 4.2	69.5 ± 8.8	4.7 ± 0.4	3.1 ± 0.2	14.7 ± 1.3	22.7 ± 2.2
Female	7	51 ± 2	129 ± 6	41.1 ± 0.1	1.9 ± 0.2	33.1 ± 3.6	60.7 ± 5.6	4.1 ± 0.2	2.7 ± 0.2	14.7 ± 1.2	22.6 ± 1.9
All	15	51 ± 1	115 ± 4	41.3 ± 0.1	2.0 ± 0.1	33.0 ± 2.7	65.4 ± 5.3	4.5 ± 0.2	2.9 ± 0.1	14.7 ± 0.8	22.6 ± 1.4
HN											
Male	9	55 ± 1	108 ± 3	40.6 ± 0.1	3.0 ± 0.2	20.0 ± 1.1	59.6 ± 4.5	3.6 ± 0.3	2.6 ± 0.2	16.3 ± 1.0	23.2 ± 1.5
Female	8	57 ± 2	125 ± 4	40.9 ± 0.1	2.4 ± 0.2	31.3 ± 3.3	72.7 ± 7.3	3.7 ± 0.2	2.5 ± 0.1	19.3 ± 2.5	29.4 ± 3.4
All	17	56 ± 1	116 ± 3	40.7 ± 0.1	2.7 ± 0.1	25.3 ± 2.1	65.8 ± 4.4	3.6 ± 0.2	2.6 ± 0.1	17.7 ± 1.3	26.1 ± 1.9
NH											
Male	8	56 ± 2	111 ± 3	40.6 ± 0.2	2.3 ± 0.1	24.1 ± 1.8	55.4 ± 5.2	3.7 ± 0.3	2.7 ± 0.2	15.0 ± 1.3	20.6 ± 1.8
Female	9	55 ± 2	125 ± 5	40.9 ± 0.1	2.1 ± 0.3	31.0 ± 3.1	60.9 ± 7.5	3.3 ± 0.1	2.4 ± 0.1	17.5 ± 1.8	25.7 ± 3.0
All	17	56 ± 1	119 ± 3	40.8 ± 0.1	2.2 ± 0.2	27.8 ± 2.0	58.3 ± 4.6	3.5 ± 0.2	2.5 ± 0.1	16.3 ± 1.1	23.3 ± 1.9
Male vs. Female ^a		ns	M < F	ns	M > F	M < F	ns	ns	ns	M < F	ns

* P < 0.05 vs. NN group

^a main effect for sex (P < 0.05); M, male; F, female; ns, non-significant