Respiratory Physiology & Neurobiology 264 (2019) 19–27

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Comparison of the CO_2 ventilatory response through development in three rodent species: Effect of fossoriality

Ryan J. Sprenger[a,*], Anne B. Kim[a], Yvonne A. Dzal[b], William K. Milsom[a]

[a] Department of Zoology, University of British Columbia, #4200-6270 University Blvd., Vancouver, B.C, V6T 1Z4, Canada
[b] Department of Biology and Centre for Forest Interdisciplinary Research, University of Winnipeg, 515 Portage Avenue, Winnipeg, MB, R3B 2E9, Canada

ARTICLE INFO

Keywords:
Hypercapnia
Semi-fossorial
Development
Carbon dioxide
Ventilation
Rodent

ABSTRACT

Burrowing rodents have a blunted ventilatory response to CO_2 in comparison to non-burrowing rodents. Non-burrowing rats display a period during development where ventilatory responses to hypercarbia become transiently blunted. This study examined the ventilatory responses to CO_2 of rats, hamsters and ground squirrels through neonatal development to determine whether the blunted adult response of burrowing species is a retention of the blunting period seen in rats or present from birth. All three species increased ventilation in response to hypercarbia on the day of birth (70–170% in response to 5% CO_2; 100–250% in response to 7% CO_2). Rats in our study exhibited the triphasic ventilatory response (when expressed as %Δ) to CO_2 previously described. In golden-Syrian hamsters, the ventilatory response slowly and progressively waned to a blunted adult response while in the 13-lined ground squirrels, the early ventilatory response to CO_2 decreased within days and remained attenuated through development. Our study shows three distinct developmental patterns in the hypercarbic ventilatory response.

1. Introduction

Elevated environmental CO_2 (hypercarbia) is a strong respiratory stimulant in mammals (Feldman, 1986; Nattie and Li, 2009; Tenney and Boggs, 1986; Walker et al., 1985). Typically, when given hypercarbic air, mammals respond with an increase in ventilation ($\dot{V}_E$) due to increases in both tidal volume (V_T) and breathing frequency (*f*) (Walker et al., 1985). In contrast, adult fossorial rodents (rodents that live in underground burrows) are known to have a high tolerance for hypercarbia compared to non-fossorial rodents like rats, presumably due to their burrowing nature (Boggs and Birchard, 1989; Boggs et al., 1984). Though burrow structures are variable, depending on species, (Tenney and Boggs, 1986), burrows can be hypercarbic and hypoxic because gas diffusion through soil is inadequate for the amount of CO_2 produced and O_2 consumed by their occupants (Wilson and Kilgore, 1978b). The level of CO_2 in burrows can rise to between 1–8% (Tenney and Boggs, 1986), and since developing neonates of burrowing rodents are born and remain in these conditions until weaning, this may contribute to their tolerance and reduced sensitivity. For example, the threshold for eliciting a ventilatory response in the fully-fossorial (lives permanently underground) naked mole rat is ˜5% CO_2, (Arieli and Ar, 1979; Boggs et al., 1984) while that for the semi-fossorial (spends time both above and below ground) echidna is ˜3% CO_2 (Parer and Hudson, 1974). Humans and dogs, on the other hand, begin to hyperventilate at ˜0.5% inspired CO_2 (Tenney and Boggs, 1986) and adult rats at about 2% inspired CO_2 (Arieli and Ar, 1979). In addition to higher thresholds, fully and semi-fossorial rodents also have an attenuated ventilatory response after their threshold is surpassed. For example, in hypercarbia (5% CO_2) golden-Syrian hamsters increase $\dot{V}_E$ by about 40% of their normocarbic ventilation compared to the 120% increase under the same conditions reported in rats (Boggs and Birchard, 1989).

Non-fossorial rodents do have a developmental window during which their hypercapnic ventilatory response (HCVR) is also blunted, however (Stunden et al., 2001; Wickström et al., 2002). In rats for example, a distinct triphasic response pattern has been consistently observed where an early postnatal (P0-2) response becomes blunted (P7-9), but subsequently rises again to adult response levels (P14-adult) (Putnam et al., 2005; Stunden et al., 2001; Wickström et al., 2002). This raises the question of whether neonates of semi-fossorial species are born with an attenuated response to hypercarbia, or develop an attenuated response, as do rats, but one that is then retained. We used Sprague-Dawley rats (*Rattus norvegicus;*), golden-Syrian hamsters (*Mesocricetus auratus,* facultative hibernators that are semi-fossorial), and 13-lined ground squirrels (*Ictidomys tridecemlineatus*; seasonal

* Corresponding author at: #4200-6270 University Blvd, Vancouver, B.C., V6T 1Z4, Canada.
E-mail addresses: sprenger@zoology.ubc.ca (R.J. Sprenger), annekim@zoology.ubc.ca (A.B. Kim), y.dzal@uwinnipeg.ca (Y.A. Dzal), milsom@zoology.ubc.ca (W.K. Milsom).

https://doi.org/10.1016/j.resp.2019.03.006
Received 15 January 2019; Received in revised form 27 February 2019; Accepted 18 March 2019
Available online 29 March 2019

hibernators that are fully-fossorial during the hibernating season) to address this question in more detail. Thus these species range in degree of fossoriality and heterothermy.

We hypothesized that the developmental pattern in the HCVR of semi-fossorial mammals would differ from the triphasic rat response, and that the attenuated adult fossorial response would be present at birth. Furthemore, we hypothesized that the two semi-fossorial mammals would have the same pattern of development of the HCVR. Thus the HCVR of these three species was examined over the developmental period from birth (postnatal day zero (P0)) to adulthood (P29-30) in animals raised under normocarbic conditions to separate out genetic factors from developmental plasticity.

2. Methods

2.1. Animals

All procedures were conducted under a protocol approved by the UBC Animal Care Committee (A17-0018) and were in compliance with the policies of the Canadian Council on Animal Care. Gravid Sprague-Dawley rats and golden-Syrian hamsters were acquired commercially from Charles River (Wilmington, Massachusetts). Gravid 13-lined ground squirrels were acquired by trapping in Carman, MB, Canada (49°30′ N, 98°01′ W) and transferred to an animal care housing facility at the University of British Columbia in Vancouver, BC, Canada. 13-lined ground squirrels were trapped with the approval of Manitoba Conservation and Water Stewardship, under wildlife scientific permit WB15027. Wild female 13-lined ground squirrels were treated with Ivermectin and Droncit for endoparasites (0.4 mg/kg, subcutaneous), and flea spray for ectoparasites. Any pup born before the mother was treated, was dosed with Ivermectin and Droncit once a minimum body mass was met (50 g). All animals were kept in a temperature-controlled chamber (20 ± 2 °C) on a photoperiod that matched the daily outdoor photoperiod in Vancouver. Sprague-Dawley rats and golden-Syrian hamsters were fed a rodent chow diet (Lab Chow) supplemented with assorted cereals and nuts *ad libitum*. 13-lined ground squirrels were fed IAMS large chunk dog chow supplemented with apples, assorted cereals, peanuts, and sunflower seeds *ad libitum*. All species were allowed access to water *ad libitum*.

2.2. Measurement of ventilation and oxygen consumption

2.2.1. Ventilatory measurements

During the ages when pups could not maintain a large enough body temperature/ambient temperature (T_b/T_a) difference for whole body plethysmography (˜P0-P20) (Ivy and Scott, 2017) ventilation was measured using pneumotachography. A small facemask was made from the barrel of a 5, 10, or 30 ml syringe tube depending on the size of the animal to reduce dead space. The mask was sealed tightly around the head (caudal to the ears) of the animal with a rubber cuff. A rubber cork was used to seal the open end of the mask. Inflow and outflow ports were inserted into the rubber cork. A pneumotachograph was attached on the outflow side of the facemask. Pressure changes across the pneumotachograph were measured with a differential pressure transducer (Validyne model DP103-18; Validyne Engineering Northridge, California) and amplified with a Gould DC amplifier (Gould; Valley View, Ohio). Inflow gases were metered with a rotameter (Matheson 4334; Whitby, Ontario) and a flow rate of 20–120 ml/min was used based on the oxygen consumption of the animal. The system was calibrated for tidal volume by injecting known volumes (0.1, 0.2 and 0.3 ml) across the pneumotachograph on top of the constant gas flow (at a frequency similar to the respiratory frequency of each respective species).

Whole body plethysmography (barometric method) was used to measure ventilation during the ages when pups were able to maintain a large enough T_b/T_a difference (> 4 °C) for accurate measurement (˜P20-29, 30) (Drorbaugh and Fenn, 1955; Jacky, 1980; Malan, 1973). For this study, the minimum T_b/T_a difference seen was 7 °C with most T_b/T_a differentials ranging from 10 °C to 15 °C. Pups were placed in one of two identical plexiglass chambers (large set: 10 cm x 10 cm x 10 cm, medium set: 9 cm x 6 cm x 7 cm, small set: 7 cm x 5 cm x 7 cm (length x width x height)) that allowed them to move freely. These experimental chambers were housed in a temperature-controlled chamber to allow for a consistent known ambient temperature. A constant flow (100–120 ml/min) was maintained through both chambers that were each connected to a pressure transducer (Validyne model DP103-18)). Ports on two sides of the chamber allowed for gas movement into and out of the chamber, and a port on the top allowed for connection to the pressure transducer. Pressure changes from the warmed and humidified expired air in the animal chamber were detected by the pressure transducer (Validyne model DP103-18)) and amplified with a Gould DC amplifier (Gould; Valley View, Ohio). The system was calibrated for tidal volume, and gas concentrations as described above but by injecting known volumes directly into the chamber. The plethysmograph remained closed for the duration of breathing pattern measurement and calibration.

2.2.2. Metabolic measurements

Flow-through respirometry was used to measure O_2 consumption and CO_2 production ($\dot{V}O_2$ and $\dot{V}CO_2$) as indicators of metabolic rate. For the younger animals (P0-P20), the facemask also served as a respirometer. Gas flow rate through the mask was 20–120 ml/min set such that the fractional composition of the O_2 in the outflow gas did not fall by more than 1%. For older animals (P20-P29, P30) the whole body plethysmograph served as a respirometer and a constant flow (100–120 ml/min) was maintained through the animal chamber again set such that the fractional composition of the O_2 in the outflow gas did not fall by more than 1%. In both cases, outflow gas was connected to a desiccant (silica beads) and then to a field metabolic system (FMS; Sable Systems International; North Las Vegas, Nevada) or to Beckman gas analyzers (OM-11 and LB-2; Beckman Coulter Indianapolis, Indiana) for analysis of O_2 and CO_2 composition. Inflow gases were analyzed for gas composition at the start of each trial at each respective flow rate. O_2 and CO_2 analyzers were calibrated to correct for drift with pre-mixed gases of known concentrations (0, 5, and 7% CO_2 with 21% O_2, balanced with N_2) daily.

2.3. Measurement of temperature

Chamber temperature was monitored with a thermal probe connected to a physitemp (Bat-12) analyzer and maintained between 21–30 °C, adjusted to ensure animals remained at normal body temperatures (˜38 °C). Animal T_b and nasal temperature (T_n) were monitored with a FLIR (forward looking infrared) thermal monitoring system (FLIR; Wilsonville, Oregon, United States) throughout the experimental trials in order to make adjustments to ambient temperature to allow for a normal T_b (˜38 °C). FLIR thermal imaging provided a non-invasive method to monitor temperature in neonatal rodents that are imperfect thermal regulators (Tattersall, 2016). Animal chambers were housed in a temperature-controlled unit to help maintain temperature.

2.4. Experimental protocol

For pneumotachography, pups were taken from the litter and placed on a heating pad. A mask was custom fit to each pup (as previously described in section 2.2.1) and the body of the animal was placed in a plastic syringe open on both sides. This body chamber was clipped to the facemask chamber, limiting the animal from excessive movement. Animals were allowed to acclimate while breathing normocarbic air (21% O_2 balanced with N_2) for 1 h prior to the administration of the hypercarbic gas mixtures.

For whole body plethysmography, pups were taken from the litter

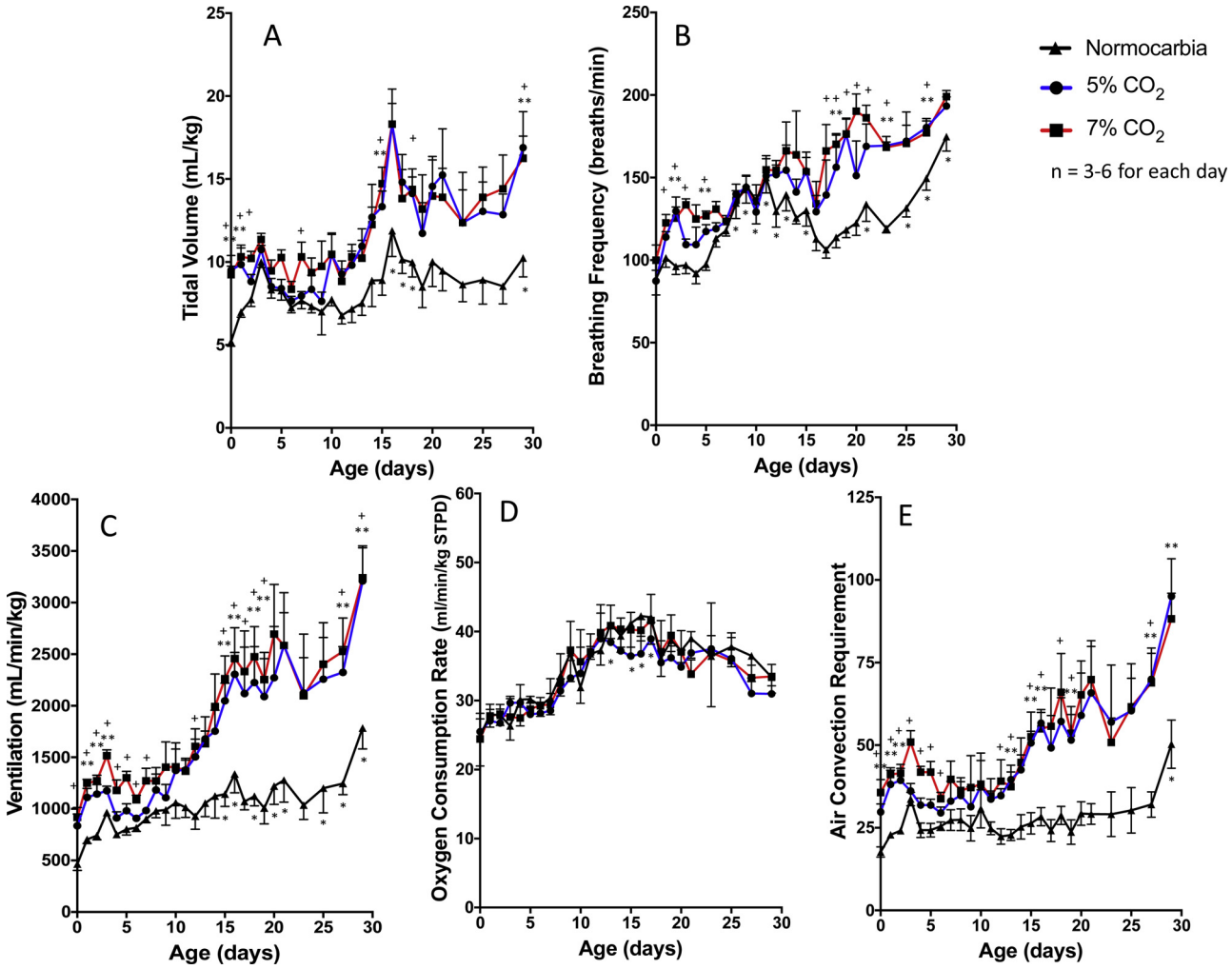

Fig. 1. Breathing frequency (breaths/minute)(A), tidal volume (mL/kg) (B), and total ventilation (mL/min/kg) (C), O_2 consumption rate (mL/min/kg) (D), and the air convection requirement (E) in Sprague-Dawley rats through development (P0-30). Rats were given normocarbic (open triangles), and hypercarbic (5% CO_2, (open circles) and 7% CO_2 (closed circles)) gas mixtures. (n = 3–6 for each day). Error bars show SEM. * denotes a significant differences ($P < 0.05$) from day 0 within the normocarbic treatment, and ** (5% CO_2) and + (7% CO_2) denote differences ($p < 0.05$) between hypercarbic and normocarbic gas on that day (ANOVA with Tukey's post hoc test).

and placed in a plexiglass chamber with bedding and allowed a 1-hour acclimation period breathing normoxic air prior to the administration of the hypercarbic gas mixtures. Bedding was changed before the start of each trial.

In all cases, pups were exposed to pre-mixed gasses (Praxiar Canada, Vancouver B.C.) containing 0%, 5%, and 7% CO_2 combined with 21% O_2 (balanced with N_2) in random order. Each gas combination was administered for 15 min with normoxic air supplied for 15 min in between each CO_2 trial. Total run time for each experiment was about 120 min. Pups from multiple litters (golden-Syrian hamsters: 3 litters, Sprague-Dawley rats: 2 litters, 13-lined ground squirrels: 5 litters) were required to obtain a sample size of 5–6 for each day of development (P0-P29 or 30) for each species. Given litter size, the minimum time between testing of the same pup was ˜4 days and this time increased with litter size. All data, except body and nasal temperature, were acquired using a PowerLab 16/32 data acquisition system (ADInstruments; Colorado Springs, Colorado).

2.5. Data analysis

Data analysis was done on Labchart v8.1.9 (ADInstruments; Colorado Springs, Colorado). Two 1–2 min widows from the middle and end of each trial (during ˜6–8th and 13–15th min) were analyzed. The respiratory variables tidal volume (V_T; ml/kg) and breathing frequency (*f*; breaths/min) were averaged for each gas trial on each animal. The oxygen and CO_2 fractional concentration values for calculating O_2 consumption and CO_2 production (ml/min/kg) were taken from the minute before the trial (inflow) and the same time intervals over which the respiratory variables were measured (outflow). Ventilation ($\dot{V}E = V_T$ x *f*; ml/min/kg) and the air convection requirement ($ACR = \dot{V}E/\ \dot{V}O_2$) were calculated from the measured variables. $\dot{V}O_2$ was calculated using the following equation;

$$\dot{V}O2 = fR\{Fio2 - \frac{(1 - Fio2 - Fico2)}{(1 - Feo2 - Feco2)}(Feo2)\}$$

where *fR* is the flow rate of the inflow gas; Fio_2 is the fraction of inspired oxygen; Feo_2 is the fraction of expired oxygen; $Fico_2$ is the fraction of inspired carbon dioxide; and $Feco_2$ is the fraction of expired carbon dioxide (Lighton, 2008). For pneumotachography, V_T was determined by integrating the differential pressure signal produced by the change in flow due to each breath. For whole body plethysmography, V_T was calculated using the following equation;

$$Tidal\ Volume = \frac{Pm\ x\ \dot{V}cal\ x\ TA(PB - PCH2O)}{PCal(TA(PB - PCH2O) - TC(PB - PAH2O))}$$

where Pm is the measured pressure deflection; Vcal the calibration

volume; TA the body temperature; PB the barometric pressure; PCH_2O the water vapor pressure at chamber temperature; PCal the measured pressure deflection of the calibration volume; PAH_2O the water vapor pressure at animal body temperature. These tidal volumes were then corrected for the difference in whole body versus nasal temperature with the following equation;

$$\frac{\dot{V}}{VCOR} = 1 - \left(\frac{TI}{TTOT}\right)\left(1 - \frac{TA}{TN}\right)$$

where VCOR is the corrected volume; TI the inspiratory time; TTOT the total time for inspiration and expiration; TA the body temperature and TN the nasal temperature (Drorbaugh and Fenn, 1955; Jacky, 1980).

2.6. Statistical analysis

A two-way ANOVA was used to determine the effect of age (within treatment group compared to the starting value (P0)) and treatment (between CO_2 treatments on the same day) on respiratory and metabolic variables. A separate two-way ANOVA was used to determine the effect of age and species on the percent increase in ventilation to 5 and 7% inspired CO_2. A Shapiro-Wilks test was used to test for normality, and a Levene test was used to test for equal variance in all two- way ANOVAs. Tukey's post-hoc tests were used for comparisons and a significance level of $p < 0.05$ was used throughout. All statistics were run with R Studio statistical software (RStudio Version 1.1.447) with the exception of the species comparison, and all comparisons made on percent change data, which were run with GraphPad Prism 7 statistical software. All values are reported as mean ± S.E.M.

3. Results

3.1. Sprague-Dawley rats

3.1.1. Normocarbia

Breathing frequency increased significantly with age in Sprague-Dawley rats as did V_T (Fig. 1). Thus $\dot{V}_E$ (ml/kg/min) also increased significantly with age (Fig. 1). Mass specific oxygen consumption ($\dot{V}_{O2}$; ml/min/kg) initially increased with age; P13-17 animals had a significantly larger $\dot{V}_{O2}$ compared to P0 animals ($p < 0.05$) (Fig. 1). Shortly after, $\dot{V}_{O2}$ fell to adult levels, which were still elevated but not significantly different from values for P0 animals (Fig. 1). The air convection requirement (ACR; $\dot{V}_E/\dot{V}_{O2}$) did not change with age with the exception of the oldest (P29-P30) animals, which had a significantly larger ACR in comparison to animals of all other ages ($p < 0.05$) (Fig. 1).

3.1.2. Hypercarbia

Hypercarbia (5 and 7% CO_2) significantly increased *f*, V_T, and $\dot{V}_E$ early in development (roughly P1-7) ($p < 0.05$) and again later in development (roughly P12-29) ($p < 0.05$) (Figs. 1 and 2) but did not have any significant effect on ventilation between ˜P8-12. Hypercarbia had no effect on oxygen consumption at any time during development (Fig. 1). Thus, the ACR also significantly increased in hypercarbia early in development (P0-6) and again later in development (P12-29) (Fig. 1). The $\dot{V}_E$ responses to 5 and 7% inspired CO_2 were not significantly different with the exception of two days (P3 and P5) where $\dot{V}_E$ increased significantly more in 7% CO_2.

3.2. Golden-Syrian hamsters

3.2.1. Normocarbia

In golden-Syrian hamsters *f* increased significantly throughout development until roughly day 20 ($p < 0.05$) (Fig. 3), while V_T remained relatively constant (Fig. 3). $\dot{V}_E$ increased significantly until age P15, largely due to the increases in *f* (Fig. 3). Mass-specific $\dot{V}_{O2}$ (ml/min/kg) increased significantly with age and reached a maximum at P21 before decreasing again to adult levels (Fig. 3). The ACR did not change significantly with age (Fig. 3).

3.2.2. Hypercarbia

In golden-Syrian hamsters, hypercarbia (5 and 7% CO_2) only increased *f* and V_T significantly early in development (Fig. 3). However, when the net effects of the changes in both were combined, $\dot{V}_E$ increased significantly under hypercarbic conditions from P0-P20 (Figs. 3 and 4). There was no effect of hypercarbia on oxygen consumption (Fig. 3). The ACR increased significantly in hypercarbia early in development but this effect slowly tapered off (Fig. 3). When expressed as % change, the increase in all ventilatory variables due to hypercarbia progressively decreased throughout development (Fig. 4). The $\dot{V}_E$ responses to 5 and 7% inspired CO_2 were not significantly different with the exception of two days (P1 and P2) where $\dot{V}_E$ increased significantly more in 7% CO_2.

3.3. 13-lined ground squirrels

3.3.1. Normocarbia

During early postnatal development (P0-P7) *f* increased significantly but then leveled off and declined to levels that were not significantly different from values recorded for P0 animals (Fig. 5). V_T, on the other hand, did not increase with age early on, but did increase significantly starting at age P16 (Fig. 5). As a result, $\dot{V}_E$ increased reaching a peak in animals between the ages of P16-P26 ($p < 0.05$) but then declined to values similar to those of P0 animals (Fig. 5). Mass-specific $\dot{V}_{O2}$ increased significantly with age (Fig. 5) leveling off after P17 but remained elevated in comparison to P0 animals (Fig. 5). The ACR did not change with age (Fig. 5).

3.3.2. Hypercarbia

Hypercarbia did not significantly alter *f* in the 13-lined ground squirrels (Figs. 5 and 6). V_T and $\dot{V}_E$, however, increased significantly during the first half of development (Figs. 5 and 6) but these responses were reduced throughout the second half. Hypercarbia did not have an effect on oxygen consumption in 13-lined ground squirrels (Fig. 5). Thus, the ACR also increased significantly during the first half of development (Fig. 5). When expressed as % change, the initial increase in breathing frequency due to hypercarbia on P0 decreased rapidly but then rose again in late development (Fig. 6). The initial increase in V_T deceased progressively. The net effect was that the initial increase in $\dot{V}_E$ due to hypercarbia was rapidly reduced and the net response remained low throughout the rest of development. The $\dot{V}_E$ responses to 5 and 7% inspired CO_2 were not significantly different with the exception of one day (P18) where $\dot{V}_E$ increased significantly more in 7% CO_2.

4. Discussion

The pattern of development of the HCVR in semi-fossorial golden-Syrian hamsters and 13-lined ground squirrels differs from that of the non-fossorial rat. Our results also show that the pattern of development of the HCVR in the two semi-fossorial species differ from each other (Fig. 7) (Putnam et al., 2005). In both cases, the attenuated HCVR seen in the adults of the semi-fossorial species was not present at birth (Fig. 7). Indeed, the newborns of the semi-fossorial species in the present study were more responsive to hypercarbia than newborns of the non-fossorial rat (Fig. 7).

4.1. Changes in the HCVR through development in non-fossorial neonates

In Sprague-Dawley rats we saw a triphasic pattern in the HCVR through development (an initial robust increase in ventilation at birth that became attenuated and reached a nadir around P15 before rising again into adulthood) as has been reported by others (Putnam et al., 2005; Stunden et al., 2001). The initial HCVR present at birth (P0-1) in

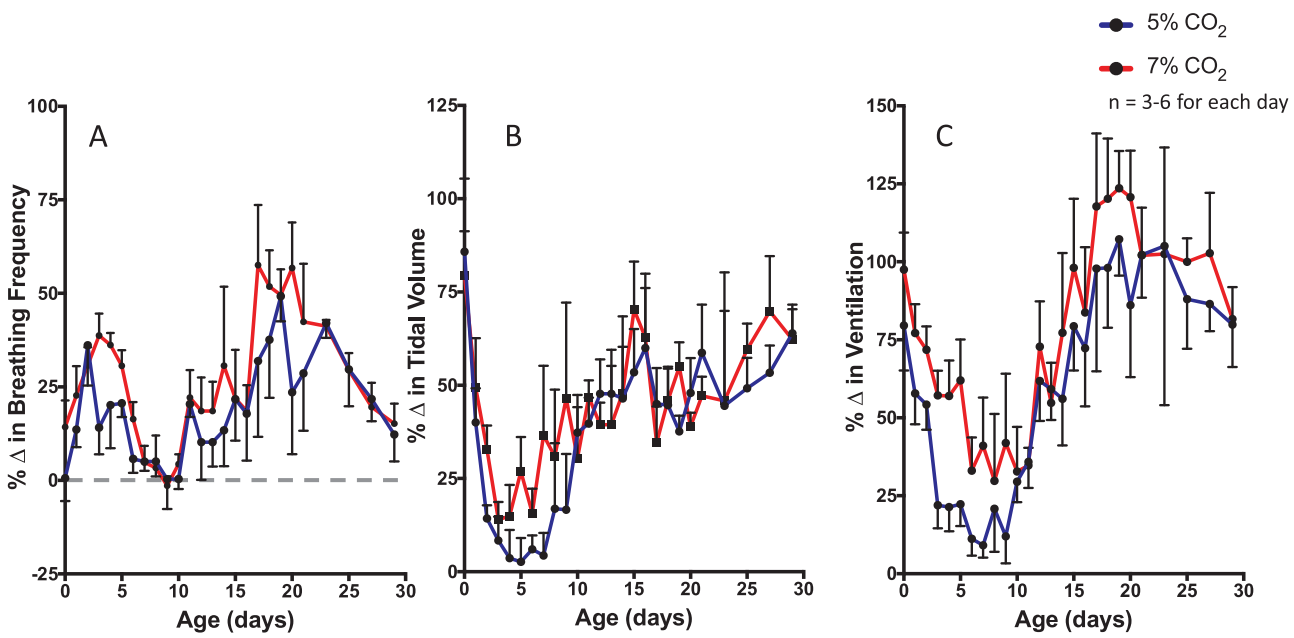

Fig. 2. Percent change from normocarbia in breathing frequency (A), tidal volume (B), and ventilation (C) in Sprague-Dawley rats through development (P0-30) when administered 5% CO_2 (open circles), and 7% CO_2 (closed circles). (n = 3–6 for each day) Error bars show SEM.

Sprague-Dawley rats appears to have been due to increases in V_T only (Fig. 2) with contributions from breathing frequency developing over the next few days. The subsequent nadir in the HCVR was due to reductions in both V_T and *f*, both of which increase again during late development (P11-29). However, the nadir was mostly due to reductions in tidal volume. Previous studies show a similar trend, where tidal volume contributes more to the changes in ventilation early in development, with breathing frequency making larger contributions later in development (Putnam et al., 2005; Stunden et al., 2001). A lower HCVR in neonates compared to adults has also been reported in other non-fossorial mammals including neonates of humans, dogs, and pigs (Nattie and Edwards, 1981; Putnam et al., 2005; Søvik and Lossius, 2004; Wolsink et al., 1992). In pigs, as in the Sprague-Dawley rats, the ventilatory response reached a nadir around postnatal day 15 before rising again (Wolsink et al., 1992) while in humans and dogs, the secondary rise to adult response levels occurred over the first few (˜ 2–3) postnatal weeks with no reported drop in sensitivity (Nattie and Edwards, 1981; Putnam et al., 2005; Søvik and Lossius, 2004). Because hypercarbia (5 and 7% CO_2) did not affect oxygen consumption, but did increase ventilation, early (˜P0-5) and late (˜P12-29) animals hyperventilated in hypercarbia and the magnitude of the hyperventilation, as reflected in the increases in the air convection requirement, increased significantly through development (ACR, Fig. 1).

4.2. Changes in the HCVR through postnatal development of semi-fossorial rodents

The initial response to hypercarbia seen in animals immediately after birth (P0-2) was largest in golden-Syrian hamsters (represented as percent change from normocarbia to 5% and 7% CO_2) compared to the other species examined in this study (Fig. 7). Shortly after, the ventilatory response fell transiently (P3), rose again (P5), and then gradually fell to the attenuated adult response (Figs. 4, 7). Like golden-Syrian hamsters, 13-lined ground squirrels are semi-fossorial. Examination of their HCVR through development reveals another pattern when expressed as percent change from normocarbia. In this species, there was an early ventilatory response to CO_2 (P0-1) like the Sprague-Dawley rats and golden-Syrian hamsters, however, this response fell almost immediately and remained attenuated through development (Fig. 7). There was a small increase in the HCVR in 13-lined squirrels at the same age at which the HCVR in Sprague-Dawley rats began to increase to adult levels (P8, Fig. 7). However, this trend was not significant and not sustained.

Because of the large HCVR at birth and the low metabolic rate, the effect of hypercarbia on the ACR was initially very large. Unlike the Sprague-Dawley rats, however, through development, the relative increase in ventilation was reduced while metabolic rate increased and the ACR, and hence the magnitude of the hyperventilation, was reduced in golden-Syrian hamsters (Fig. 3). In the 13-lined ground squirrels, ventilation tracked metabolism and hence the ACR, and increases in the ACR in hypercarbia, remained relatively constant through much of development (until roughly P20). The ACR and hence the relative hyperventilation in hypercarbia, declined as they reached adulthood (Fig. 5).

The response to CO_2 seen in golden-Syrian hamsters was produced by equal increases in both V_T, and *f* (Fig. 4) (Stunden et al., 2001; Wickström et al., 2002) throughout development (Fig. 4). Interestingly, the age at which the HCVR in golden-Syrian hamsters began to asymptote (˜P9-12) coincided with the time at which the hypercarbic sensitivity began to increase again in the Sprague-Dawley rats (Figs. 4 and 7). The initial increase in ventilation in hypercarbia in the 13-lined ground squirrels was due to equal contributions from increases in V_T and *f* when given 5% CO_2, but when given 7% CO_2, *f* did not increase beyond the 5% value while V_T did (Fig. 6). This change will result in an increase in effective ventilation, as all the increase will reach the alveoli and will not result in any increase in dead space ventilation.

4.3. Underlying basis of differences in developmental patterns of the HCVR

The specific underlying changes that produce the different developmental patterns in the HCVR remain unknown. In rats, there are changes in both peripheral and central chemosensory regions that coincide with the blunting phase (Gao et al., 2011; Liu and Wong-Riley, 2005; Nottingham et al., 2001; Vincent et al., 2004; Wang and Richerson, 1999; Wong-Riley and Liu, 2005). Centrally, there is a precipitous fall in glutamate (excitatory neurotransmitter) and its NMDA receptors in chemosensitive brainstem regions (Vincent et al., 2004; Wong-Riley and Liu, 2005). During this drop, increases in $GABA_A$, $GABA_B$, and glycine receptors (all involved in inhibitory pathways), are seen in the same areas (Wong-Riley and Liu, 2005). This transient pre-

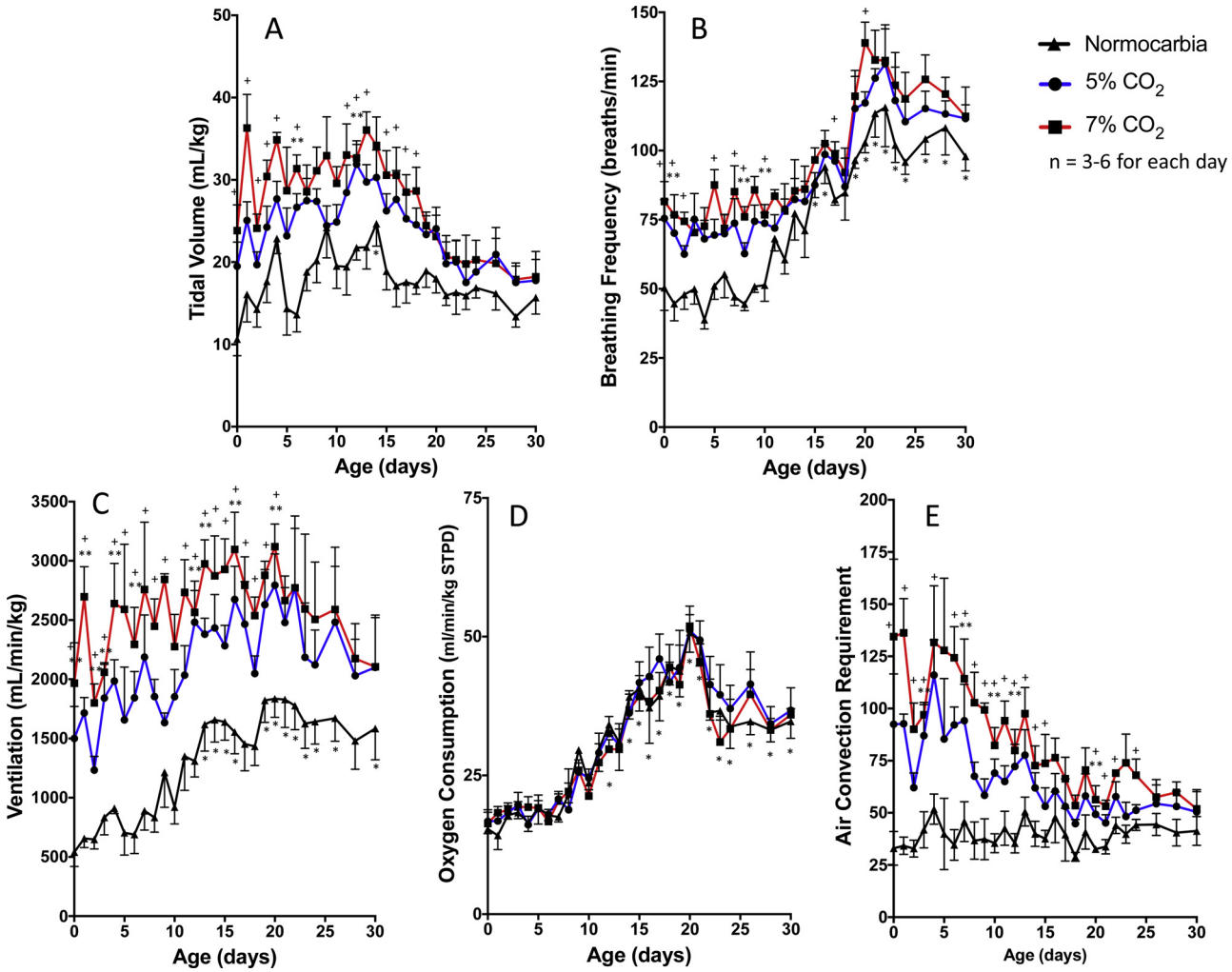

Fig. 3. Breathing frequency (breaths/minute)(A), tidal volume (mL/kg) (B), and total ventilation (mL/min/kg) (C), O_2 consumption rate (mL/min/kg) (D), and the air convection requirement (E) in golden-Syrian hamsters through development (P0-30). Hamsters were given normocarbic (open triangles),and hypercarbic (5% CO_2, (open circles) and 7% CO_2 (closed circles)) gas mixtures. (n = 3–6 for each day). Error bars show SEM. * denotes a significant differences ($P < 0.05$) from day 0 within the normocarbic treatment, and ** (5% CO_2) and + (7% CO_2) denote differences ($p < 0.05$) between hypercarbic and normocarbic gas on that day (ANOVA with Tukey's post hoc test).

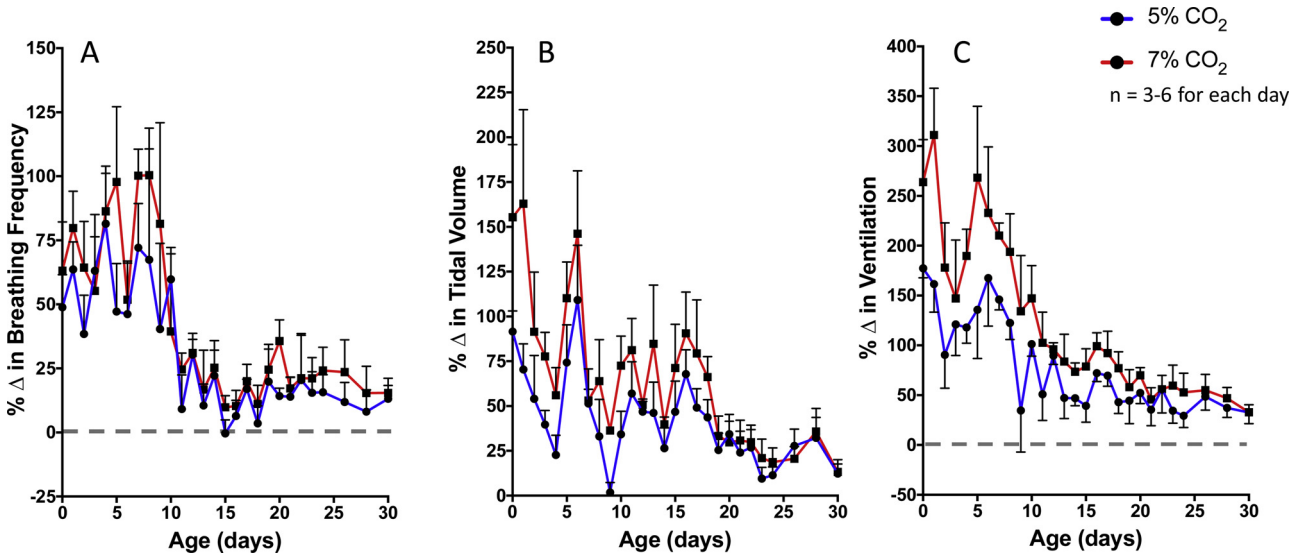

Fig. 4. Percent change from normocarbia in breathing frequency (A), tidal volume (B), and ventilation (C) in golden-Syrian hamsters through development (P0-30) when administered 5% CO_2 (open circles), and 7% CO_2 (closed circles). (n = 3–6 for each day) Error bars show SEM.

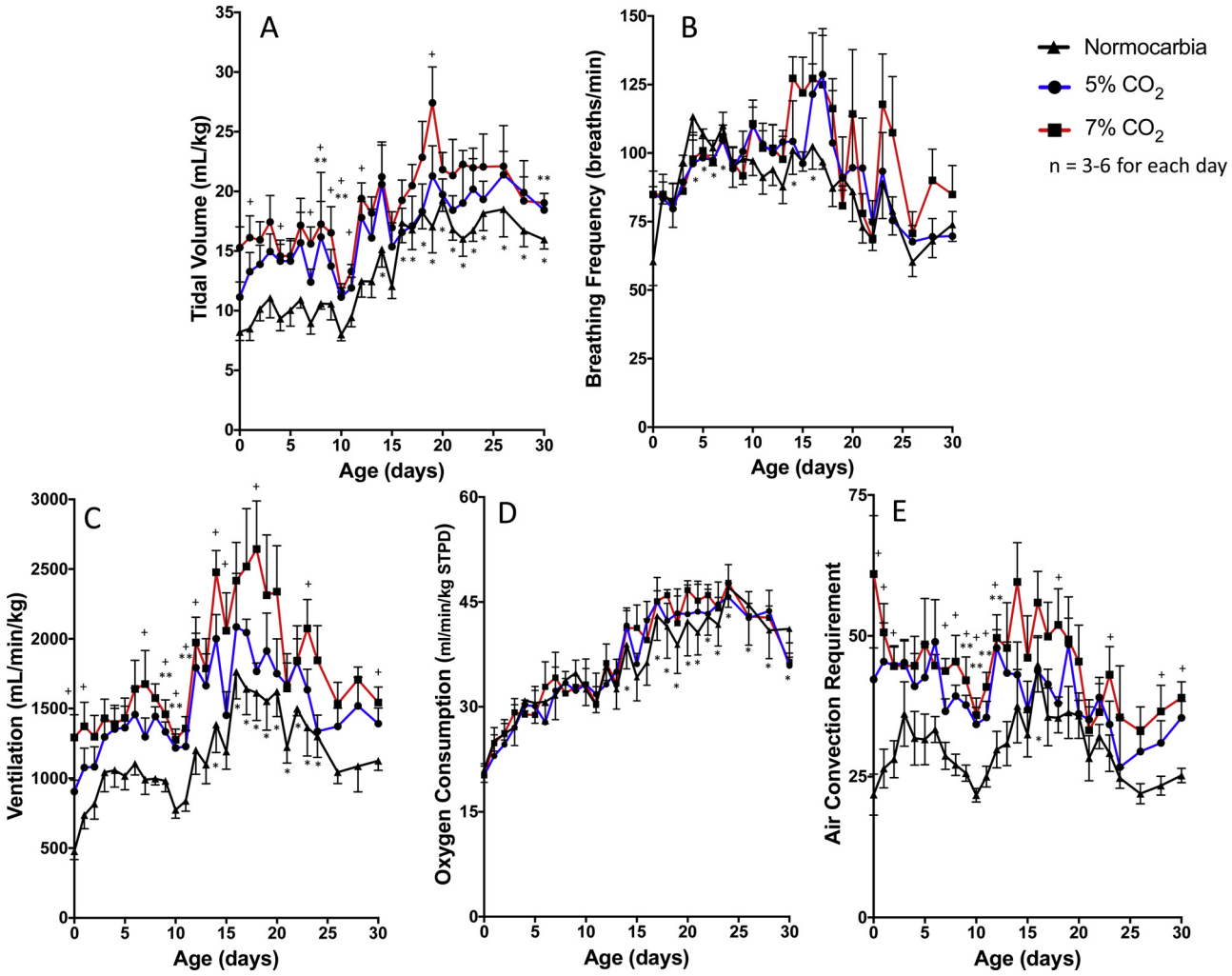

Fig. 5. Breathing frequency (breaths/minute)(A), tidal volume (mL/kg) (B), and total ventilation (mL/min/kg) (C), O_2 consumption rate (mL/min/kg) (D), and the air convection requirement (E) in 13-lined ground squirrels through development (P0-30). Squirrels were given normocarbic (open triangles), and hypercarbic (5% CO_2, (open circles) and 7% CO_2 (closed circles)) gas mixtures. (n = 3–6 for each day). Error bars show SEM. * denotes a significant differences ($P < 0.05$) from day 0 within the normocarbic treatment, and ** (5% CO_2) and + (7% CO_2) denote differences ($p < 0.05$) between hypercarbic and normocarbic gas on that day (ANOVA with Tukey's post hoc test).

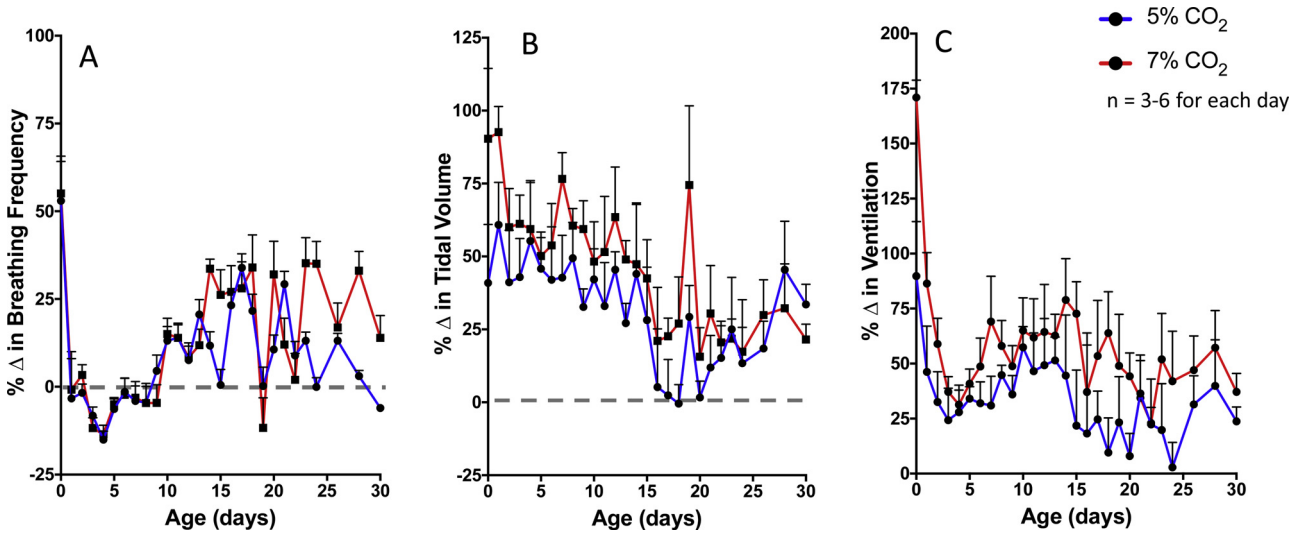

Fig. 6. Percent change from normocarbia in breathing frequency (A), tidal volume (B), and ventilation (C) in 13-lined ground squirrels through development (P0-30) when administered 5% CO_2 (open circles), and 7% CO_2 (closed circles). (n = 3–6 for each day) Error bars show SEM.

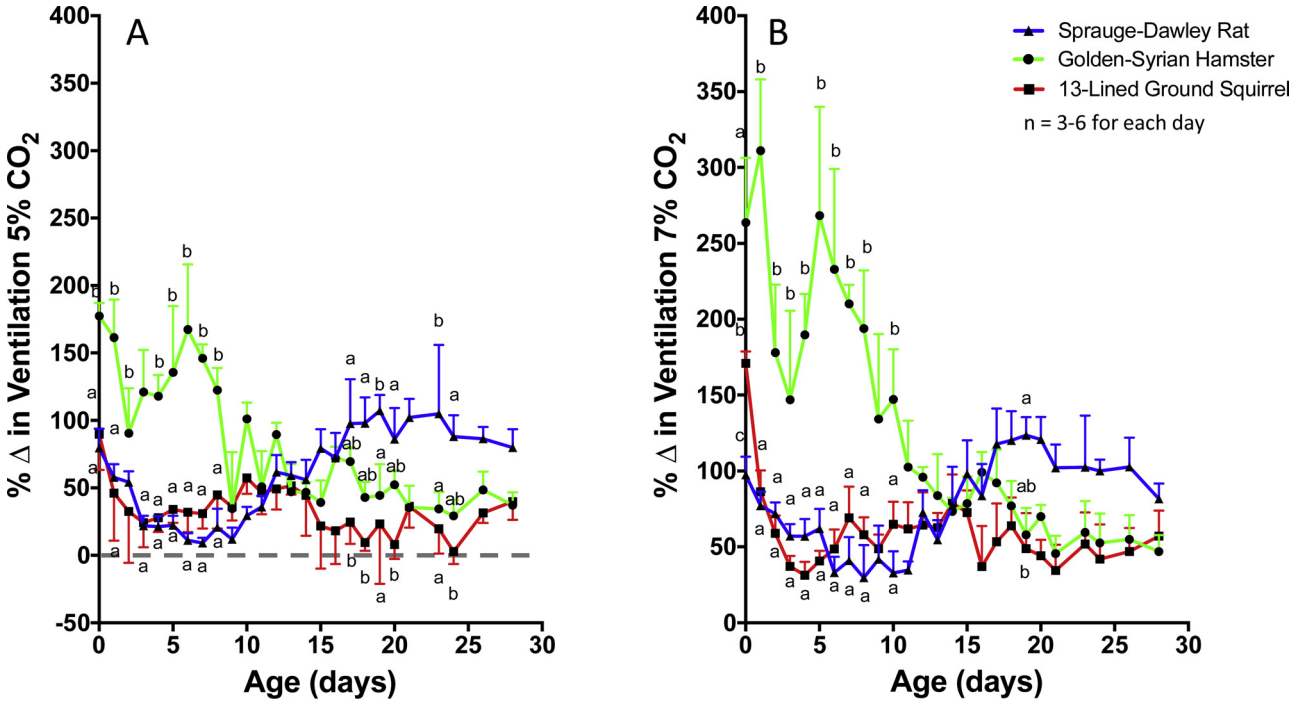

Fig. 7. Comparison of the developmental pattern of the hypercarbic ventilatory response of Sprague-Dawley rats (blue), golden-Syrian hamsters (green), and 13-lined ground squirrels (red). Figure shows the percent change in ventilation from breathing 0%–5% CO_2 (A) and from 0% to 7% CO_2 (B) (n = 3–6 for each day). Letter differences denote significant differences ($p < 0.05$) between the species on each day (ANOVA with Tukey's post hoc test).

eminence of inhibitory over excitatory neurotransmission has been reported to occur around postnatal days 3–5 in rats (Gao et al., 2011; Wong-Riley and Liu, 2005), which could contribute to the fall in responsiveness of the central chemoreceptors in this study (Putnam et al., 2005) (Parisian et al., 2004). Peripherally, the carotid body also undergoes postnatal changes (Bamford et al., 1999; Hertzberg et al., 1990; Ling et al., 1997; Pepper et al., 1995). At birth, the carotid body goes from a hypoxic environment to a hyperoxic environment, which could contribute to the immediate fall in responsiveness to respiratory stimuli after birth (Hertzberg et al., 1990). This fall coincides with both the blunting phase seen in rats, as well as the period (P3) in which the carotid body is least responsive to hypoxic and hypercarbic stimuli (Bamford et al., 1999; Pepper et al., 1995).

With age, $GABA_A$, and $GABA_B$ receptor density begins to fall, starting at postnatal day 5 and reaches a nadir at postnatal day 11 (Liu and Wong-Riley, 2005; Wong-Riley and Liu, 2005). During this period, glutamate begins to increase in the nucleus tractus solitarius (NTS) and nucleus ambiguus (Liu and Wong-Riley, 2005; Wong-Riley and Liu, 2005). The postnatal development of the carotid body could also contribute to the secondary rise in rats. The size of the carotid body has been shown to increase early in development in mammals (Wang and Bisgard, 2005). This is mostly due to type I cell hyperplasia, and an increase in type II cell proliferation and synapse formation between type I and II cells (Wang and Bisgard, 2005; Bamford et al., 1999; Pepper et al., 1995). Following this, both hypoxic and hypercarbic responses increase and reach adult responsiveness at ˜P8 (CO_2) and ˜P16 (O_2) (Bamford et al., 1999). Though carotid body input to the HCVR has been shown to be non-essential in neonates (Forster et al., 2000), it may still contribute to the patterns we report in this study.

Postnatal development of the lung and respiratory related musculature may also contribute to the differences we report in the development of the HCVR. Rodents are typically born altricial, and underdeveloped in comparison to other mammalian species. The lung is immature at birth and develops with age. In rats, growth of the lung is slow in newborns and starts to increase in volume faster relative to body weight between ˜P5 and P14 (Thurlbeck, 1975) before slowing again. Additionally, respiratory related musculature such as the diaphragm and intercostal muscles, as well as the shape of the thorax all change with age (Mortola, 2001). Differences in respiratory mechanics could affect the ability of the neonates to produce chest wall, and diaphragmatic movement in hypercarbia.

The differences seen in the developmental response patterns in the golden-Syrian hamsters and 13-lined ground squirrels could arise from differences in underlying mechanisms or simply from differences in the time course of the same underlying mechanisms.

We did not distinguish between the responses of males versus females in our study. It has been shown that P10–15 includes a critical developmental period in male but not female rats. Compared to age-matched females, P12–13 male rats had lower ventilation in normoxia and hypercapnia, which correlated with increased levels of circulating estradiol (Holley et al., 2012). This effect will be in part offset in our study by the use of pups of both genders and should not have had an effect on the pattern of change that we observed.

4.4. Conclusions

The present study shows three distinct patterns in the development of the HCVR in three different species. While the present study cannot attribute these differences to lifestyle directly, differences in lifestyle most likely play a partial role. Both golden-Syrian hamsters and 13-lined ground squirrels are semi-fossorial, and the mothers of each species raise their litters in the burrow. Litter sizes are slightly different between the two species. 13-lined ground squirrels have smaller litters (6–12 pups) (Vaughan et al., 2006), with litters as small as 4 (personal observation). Golden-Syrian hamsters have less variable, but larger litters (8–14) (Schneider and Wade, 1990). Being contained in the burrow, larger litters likely produce greater perturbations in ambient gas concentrations particularly as the animals' age. Both semi-fossorial species are solitary in the burrows as adults. Soil type may vary between the two species which would contribute to the gas diffusion rate (Burda et al., 2007; Wilson and Kilgore, 1978a). Golden-Syrian hamsters naturally reside in sand or clay-like soils and have a mean burrow

depth of 65 cm. Details on the structure of 13-lined ground squirrel burrows are not well known, but the soils this species burrow in are a moist top soil found in grassy fields (personal observation).

The triphasic developmental pattern in the HCVR seen in the rat has been reported in other non-fossorial species (Stunden et al., 2001; Wolsink et al., 1992). While the time course and pattern of the blunting phase of the HCVR differs between the two semi-fossorial species, the absence of the secondary increase in sensitivity is consistent. The differences seen between the two semi-fossorial species could lie either in the level of fossoriality, which differs between the two, or in the hibernation lifestyle seen in the 13-lined ground squirrel. The second of the two proposed possibilities seems less likely given that golden-Syrian hamsters have been shown to enter multi-day torpor bouts (Ibuka and Fukumura, 1997). However, the characteristics of torpor differ between the two (Carey et al., 2003; Ibuka and Fukumura, 1997), and this study cannot attribute the differences seen to the use of torpor directly. Whether any differences seen arise from similar or different underlying causes is not clear but either way it suggests there has been selection for sustaining a blunted response in semi-fossorial species.

It is also apparent that both fossorial species are not born with attenuated responses but rather they develop, even when the neonates are raised in normocarbic/normoxic conditions. It remains unknown how raising the neonates in a “burrow-like” hypoxic/hypercarbic environment would alter the developmental pattern. In rats, it has been shown that chronic perinatal hypercapnia transiently reduces the HCVR but it then returns to a normal adult response after removal from hypercarbia (Bavis and Kilgore, 2001; Saiki and Mortola, 1996). This is also true for mice (Birchard et al., 1984). The effect of perinatal hypercapnia in semi-fossorial species remains to be explored.

Declarations of interest

None.

Acknowledgements

The authors would like to thank Jessica Chiang and Grace Wong for their technical assistance, Dr. Phillip Matthews for helpful comments on the manuscript, Nicha Boonpattrawong for assistance with the prism software, Ellie, Larry and Helen Beckman, Jacelyn Shu for assistance with statistical analysis, and Dr. Dana Merriman for the use of her FLIR system.

This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC of Canada) (NSERC 22R87150).

References

Arieli, R., Ar, A., 1979. Ventilation of a fossorial mammal (*Spalax ehrenbergi*) in hypoxic and hypercapnic conditions. J. Appl. Physiol. 47, 1011–1017.
Bamford, O.S., Sterni, L.M., Wasicko, M.J., Montrose, M.H., Carroll, J.L., 1999. Postnatal maturation of carotid body and type I cell chemoreception in the rat. Am. J. Physiol. 276, L875–84.
Bavis, R.W., Kilgore Jr, D.L., 2001. Effects of embryonic CO2 exposure on the adult ventilatory response in quail : does gender matter? Respir. Physiol. 126, 183–199.
Birchard, G.F., Boggs, D.F., Tenney, S.M., 1984. Effect of perinatal hypercapnia on the adult ventilatory response to carbon dioxide. Respir. Physiol. 57, 341–347.
Boggs, D.F., Birchard, G.F., 1989. Cardiorespiratory responses of the woodchuck and porcupine to C02 and hypoxia. J. Comp. Physiol. B 641–648.
Boggs, D.F., Kilgore Jr, D.L., Birchard, G.F., 1984. Respiratory physiology and of burrowing mammals and birds. Comp. Biochem. Physiol. 77A, 1–7.
Burda, H., Sumbera, R., Begall, S., 2007. Microclimate in Burrows of Subterranean Rodents – Revisited. In Subterranean Rodents. pp. 21–33.
Carey, H.V., Andrews, M.T., Martin, S.L., 2003. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol. Rev. 83, 1153–1181.
Drorbaugh, J.E., Fenn, W.O., 1955. A barometric method for measuring ventilation in newborn infants. Pediatrics 81–87.
Feldman, J.L., 1986. Neurophysiology of breathing in mammals. Compr. Physiol. 463–524.
Forster, H.V., Pan, L.G., Lowry, T.F., Serra, A., Wenninger, J., Martino, P., 2000. Important role of carotid chemoreceptor afferents in control of breathing of adult and neonatal mammals. Respir. Physiol. 119, 199–208.
Gao, Xping, Liu, Qsong, Liu, Q., Wong-Riley, M.T.T., 2011. Excitatory-inhibitory imbalance in hypoglossal neurons during the critical period of postnatal development in the rat. J. Physiol. 589, 1991–2006.
Hertzberg, T., Hellstrom, S., Lagercrantz, H., Pequignot, J.M., 1990. Development of the arterial chemoreflex and turnover of carotid body catecholamines in the newborn rat. J. Physiol. 211–225.
Holley, H.S., Behan, M., Wenninger, J.M., 2012. Age and sex differences in the ventilatory response to hypoxia and hypercapnia in awake neonatal, pre-pubertal and young adult rats. Respiratory Physiology and Neurobiology 180, 79–87.
Ibuka, N., Fukumura, K., 1997. Unpredictable deprivation of water increases the probability of torpor in the syrian Hamster. Physiol. Behav. 62, 551–556.
Ivy, C.M., Scott, G.R., 2017. Ventilatory acclimatization to hypoxia in mice: methodological considerations. Respir. Physiol. Neurobiol. 235, 95–103.
Jacky, J.P., 1980. Barometric measurement of tidal volume: effects of pattern and nasal temperature. J. Appl. Physiol. 49, 319–325.
Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual for Scientists, 1st ed. Oxford University Press.
Ling, L., Olson Jr., E.B., Vidruk, E.H., Mitchell, G.S., 1997. Developmental plasticity of the hypoxic ventilatory response. Respir. Physiol. 110, 261–268.
Liu, Q., Wong-Riley, M.T.T., 2005. Postnatal developmental expressions of neurotransmitters and receptors in various brain stem nuclei of rats. J. Appl. Physiol. 98, 1442–1457.
Malan, A., 1973. Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. Respir. Physiol. 17, 32–44.
Mortola, J.P., 2001. Respiratory Physiology of Newborn Mammals Mechanical Behavior of the Respiratory Pump. Johns Hopkins University Press.
Nattie, E.E., Edwards, W.H., 1981. CSF acid-base regulation and ventilation during acute hypercapnia in the newborn dog. J. Appl. Physiol. 50, 566–574.
Nattie, E., Li, A., 2009. Central chemoreception is a complex system function that involves multiple brain stem sites. J. Appl. Physiol. 106, 1464–1466.
Nottingham, S., Leiter, J.C., Wages, P., Buhay, S., Erlichman, J.S., 2001. Developmental changes in intracellular pH regulation in medullary neurons of the rat. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 281, 1940–1951.
Parer, J.T., Hudson, W.A., 1974. Respiratory studies of monotremes. IV. Normal respiratory functions of echidnas and ventilatory response to inspired oxygen and carbon dioxide. Respir. Physiol. 21, 307–316.
Parisian, K., Wages, P., Smith, A., Jarosz, J., Hewitt, A., Leiter, J.C., Erlichman, J.S., 2004. Ventilatory effects of gap junction blockade in the NTS in awake rats. Respir. Physiol. Neurobiol. 142, 127–143.
Pepper, D.R., Landauer, R.C., Kumar, P., 1995. Postnatal development of CO2-O2 interaction in the rat carotid body in vitro. J. Physiol. 485, 531–541.
Putnam, R.W., Conrad, S.C., Gdovin, M.J., Erlichman, J.S., Leiter, J.C., 2005. Neonatal maturation of the hypercapnic ventilatory response and central neural CO2 chemosensitivity. Respir. Physiol. Neurobiol. 149, 165–179.
Saiki, C., Mortola, J.P., 1996. Effect of CO2 on the metabolic and ventilatory responses to ambient temperature in conscious adult and newborn rats. J. Physiol. 491 (Pt 1), 261–269.
Schneider, J.E., Wade, G.N., 1990. Effects of ambient temperature and body fat content on maternal litter reduction in syrian hamsters. Physiol. Behav. 49, 135–139.
Søvik, S., Lossius, K., 2004. Development of ventilatory response to transient hypercapnia and hypercapnic hypoxia in term infants. Pediatr. Res. 55, 302–309.
Stunden, C.E., Filosa, J.A., Garcia, A.J., Dean, J.B., Putnam, R.W., 2001. Development of in vivo ventilatory and single chemosensitive neuron responses to hypercapnia in rats. Respir. Physiol. 127, 135–155.
Tattersall, G.J., 2016. Infrared thermography: a non-invasive window into thermal physiology. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 202, 78–98.
Tenney, S.M., Boggs, D.F., 1986. Comparative mammalian respiratory control. Handb. Physiol. Sect. 3 Respir. Syst. Vol. II Control breathing, Part 2. pp. 833–855.
Thurlbeck, W., 1975. Postnatal growth and development of the lung. Am. Rev. Respir. Dis. 111, 803–844.
Vaughan, D.K., Gruber, A.R., Michalski, M.L., Seidling, J., 2006. Capture, care, and captive breeding of 13-lined ground squirrels, Spermophilus tridecemlineatus. Lab Anim. (NY) 35, 33–40.
Vincent, A., Kessler, J.P., Baude, A., Dipasquale, E., Tell, F., 2004. N-methyl-D-aspartate receptor activation exerts a dual control on postnatal development of nucleus tractus solitarii neurons in vivo. Neuroscience 126, 185–194.
Walker, B.R., Adams, E.M., Voelkel, N.F., 1985. Ventilatory responses of hamsters and rats to hypoxia and hypercapnia. J. Appl. Physiol. 59, 1955–1960.
Wang, Z., Bisgard, G.E., 2005. Postnatal growth of the carotid body. Respir. Physiol. Neurobiol. 149, 181–190.
Wang, W., Richerson, G.B., 1999. Development of chemosensitivity of rat medullary raphe neurons. Neuroscience 90, 1001–1011.
Wickström, R., Hökfelt, T., Lagercrantz, H., 2002. Development of CO2-response in the early newborn period in rat. Respir. Physiol. Neurobiol. 132, 145–158.
Wilson, K.J., Kilgore, D.L., 1978a. The effects of location and design on the diffusion of respiratory gases in mammal burrows. J. Theor. Biol. 71, 73–101.
Wilson, K.J., Kilgore, D.L., 1978b. The effects of location and design on the diffusion of respiratory gases in mammal burrows. J. Theor. Biol. 71, 73–101.
Wolsink, J.G., Berkenbosch, A., DeGoede, J., Olievier, C.N., 1992. The effects of hypoxia on the ventilatory response to sudden changes in CO2 in newborn piglets. J. Physiol. 39–48.
Wong-Riley, M.T.T., Liu, Q., 2005. Neurochemical development of brain stem nuclei involved in the control of respiration. Respir. Physiol. Neurobiol. 149, 83–98.