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Neurochemical and physiological correlates of a critical period of respiratory development in the rat

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Abstract

Despite its vital importance to life, respiration is not mature at birth in mammals, but rather, it undergoes a great deal of growth, refinement, and adjustments postnatally. Many adjustments do not follow smooth paths, but assume abrupt changes during certain postnatal periods that may render the animal less capable of responding to respiratory stressors. The present review focuses on neurochemical and physiological correlates of a critical period of respiratory development in the rat. In addition to an imbalanced expression of reduced excitatory and enhanced inhibitory neurotransmitters, a switch in the expressions of GABA_A receptor subunits from $\alpha 3$ to $\alpha 1$ occurs around postnatal day (P)12 in the Pre-Bötzing nucleus and the ventrolateral subnucleus of the solitary tract nucleus. Possible subunit switches in a number of other neurotransmitter receptors are discussed. These neurochemical changes are paralleled by ventilatory adjustments at the end of the second postnatal week. At P13 and under normoxia, respiratory frequency reaches its peak before assuming a gradual fall, and both tidal volume and minute ventilation exhibit a significant rise prior to a plateau or a gradual decline until P21. The response to acute hypoxia is markedly reduced between P12 and P16, being lowest at P13. Thus, the end of the second postnatal week can be considered as a critical period of respiratory development, during which multiple neurochemical and physiological adjustments and switches are orchestrated at the same time, rendering the system extremely dynamic but, at the same time, vulnerable to externally imposed perturbations and insults. The critical period embodies a time of multi-system, multifaceted growth and adjustments. It is a plastic, transitional period that is also a part of the normal development of the respiratory system.

Keywords

Brain stem; development; neurochemical; receptor subunit switch; respiration; ventilation

1. Introduction

Respiration is a key function vital for the survival of all living cells and for the organism. In mammals, automatic inspiratory and expiratory functions are mediated by a network of nuclei residing in the brain stem and the spinal cord, and these, in turn, regulate the activity of pump and airway respiratory muscles via the phrenic nerves and specific cranial and intercostal nerves. The orchestration of coordinated activities among all of these components is indeed

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complex and yet almost completely autonomous, without significant conscious control by the individual. Such orchestration appears to occur virtually instantaneously at birth, when the infant mammal expands its lungs and adapts to atmospheric breathing. However, not all components are fully mature at birth. A period of refinement has been shown to take place during early postnatal development.

A hint of instability during postnatal development comes from observations of human infants who died from Sudden Infant Death Syndrome (SIDS). Most of these infants did not exhibit any overt, life-threatening respiratory pathology or abnormality before the catastrophic event, and the highest incidence is not at birth, but rather, between the second and the fourth months after birth (Moon et al., 2007). The culmination of available evidence led Filiano and Kinney to propose the Triple Risk Model in 1994 (Filiano and Kinney, 1994). This model states that SIDS occurs, and only occurs, when (a) a vulnerable infant encounters (b) an external stressor or stressors during (c) a critical period of postnatal development.

Is there a critical period of postnatal development during which a vulnerable infant may succumb to external stressor/s? Most studies on rodents indicate that the first two postnatal weeks are critical for the postnatal development of respiratory control. The exact timing reported, however, varies among studies because typically only a few selected time points were examined. The general pattern of neurochemical development in various brain stem respiratory nuclei has been reviewed previously (Wong-Riley and Liu, 2005). The present review concentrates on neurotransmitter receptor subunit switches during development and on postnatal changes in ventilation during normoxia and acute hypoxia. The overall objective is to gain a better understanding of neurochemical and physiological bases of a sensitive or critical period in the development of the respiratory system in rats.

2. Neurochemical development of brain stem respiratory nuclei

Neurotransmitters and receptors are expected to undergo postnatal changes in their expressions as synapses get established, pruned, and mature. What is surprising, however, is that the developmental trends of some of the major neurochemicals do not follow smooth paths. Rather, they take brief and abrupt turns within a narrow time window. In this remarkable window (around postnatal day P12 in the rat), multiple neurochemicals exhibit changes at the same time. These include a sudden decrease in the immunohistochemical expressions of the excitatory neurotransmitter glutamate and its receptors (*N*-methyl-D-aspartate or NMDA), a marked increase in the immunohistochemical expressions of the inhibitory neurotransmitter GABA and the inhibitory transmitter receptors (GABA_B and glycine), and an abrupt reduction in the activity of an energy-generating enzyme, cytochrome oxidase, all in individual neurons of several brain stem respiratory nuclei (Liu and Wong-Riley, 2002; Liu and Wong-Riley, 2003; Liu and Wong-Riley, 2005)(reviewed in (Wong-Riley and Liu, 2005)). Notably, the AMPA (α amino-3-hydroxy-5-methylisoxazole-4-propionic acid) glutamate receptor subunit 2 (GluR2), which decreases the permeability of receptors to Ca²⁺ and decreases neuronal excitation (Geiger et al., 1995), also shows an abrupt rise in its expression at P12 (Liu and Wong-Riley, 2002). This is in line with a general shift toward decreased neuronal excitability at this time. If the critical period of sensitivity is “a time in which processes undergoing rapid maturational changes are particularly vulnerable” (Slotkin et al., 1986), then the sudden, dichotomous, and imbalanced expressions of inhibitory and excitatory neurochemicals would signify such a critical period.

3. Receptor subunit switches in postnatal development

The action of any neurotransmitter is not governed solely by its inherent properties, but by the type of receptors that mediate the postsynaptic response. This fact was made clear more than 70 years ago, when Otto Loewi (in 1921) discovered that acetylcholine slowed the heart (Loewi,

1956), and Henry Dale (in 1936) found that the same chemical mediated skeletal muscular contraction (Dale et al., 1936). The receptor types were found to be muscarinic and nicotinic, respectively (Purves, 1976). Since then, the number of receptor subtypes for various neurotransmitters has mushroomed. Glutamate receptors hold a commanding lead, with NMDA and non-NMDA receptors as the two major subtypes, and AMPA, kainate, and metabotropic receptors under the non-NMDA umbrella (Keinanen et al., 1990; Nakanishi, 1992). GABA receptors encompass A, B, and C categories, each with its own kinetic and pharmacological properties (Johnston, 1996). In recent years, however, attention has been paid to the subunit composition of each receptor type and subtype. Subunit composition not only determines the physiological and pharmacological properties of the receptor, but it also undergoes developmental changes by switching from one major subunit type to another (see below). These changes often occur without any external cue, are likely to be genetically determined, and coincide temporally with other neurochemical, physiological, and maturational adjustments during a sensitive or critical period of postnatal development.

In the respiratory system, GABA_A receptor α subunit switch is the one that is best studied (see below). AMPA receptor subunit 2 (GluR2) also undergoes distinct postnatal changes. This review will examine such findings as well as switches in other neurotransmitter receptors elsewhere in the brain as an incentive for future investigations in the respiratory system.

3.1 GABA_A receptor subunit switch in development

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. It acts mainly on GABA_A receptors to mediate fast transmission and on GABA_B receptors for slow transmission. The native GABA_A receptor is a heteropentameric protein, most often consisting of two α (α 1–6), two β (β 1–3) and a fifth subunit, which can be either a third β , a γ (γ 1–3), δ , σ (σ 1–2), or an ϵ subunit (Hevers and Luddens, 1998; Farrar et al., 1999; Klausberger et al., 2001; Baumann et al., 2002). The structure and assembly of these different subunits determine the functional characteristic of GABA_A receptors (Hevers and Luddens, 1998), which undergo significant postnatal changes in kinetics and functional properties, such as from slow to fast kinetics and from depolarizing to hyperpolarizing transmission (Cherubini et al., 1991; Hollrigel and Soltesz, 1997; Kapur and Macdonald, 1999; Hutcheon et al., 2000; Taketo and Yoshioka, 2000; Banks et al., 2002; Bosman et al., 2002). These changes are postulated to be the outcome of, at least in part, changes in GABA_A receptor subunit composition (Hornung and Fritschy, 1996; Banks et al., 2002), especially (Bosman et al., 2002) the α as well as a change in intracellular chloride concentration due to a shift from a predominance of Na⁺-K⁺-2Cl⁻ cotransporter to that of K⁺-coupled Cl⁻ transporter (Plotkin et al., 1997; Lee et al., 2005). The majority of GABA_A receptor subunit changes reported thus far involve a developmental decrease in α 2 or α 3 expression and an (Laurie et al., increase 1992; Fritschy et al., 1994; Hornung and Fritschy, 1996; Okada et al., 2000; Bosman et al., 2002; Heinen et al., 2004). The proposal is that α 2 (or α 3) is correlated with depolarizing GABA transmission in early life (Hornung and Fritschy, 1996), whereas the onset of α 1 subunit expression in the maturing brain signifies a new receptor subtype involved in synaptic inhibition (Fritschy et al., 1994). Although direct information is not yet available about the relationship between subunit composition and postsynaptic potentials mediated by GABA_A receptors, the fact that α 2 and/or α 3 subunit is expressed predominantly in neurons with excitatory GABA_A receptors supports that relationship (Cherubini et al., 1990; Mercuri et al., 1991; Michelson and Wong, 1991; Reichling et al., 1994). Furthermore, the GABA-induced increase in intracellular calcium observed in immature rat cortical neurons disappears when GABA_A receptor α 2 subunit is replaced by α 1 subunit (Lin et al., 1994).

The time course of transition from depolarizing to hyperpolarizing GABA transmission and the timing of the GABA_A receptor subunit switch most likely vary among different species,

different brain regions, and different neuronal types. In the rat hippocampus, approximately 85–93% of CA3 neurons exhibit spontaneous giant depolarizing potentials mediated by GABA between P0 and P8. By P11–12, only 25% of neurons show that characteristic, and after P12 they are no longer present (Ben-Ari et al., 1989), indicating that GABA transmission has switched to exclusive hyperpolarization. The reversal potential of synaptic and GABA_A receptor-mediated responses undergoes a developmental shift from –55 mV at P0–2 to –74 mV at P13–15, as the effect of GABA transitions from excitation to inhibition some time around the second postnatal week in the rat hippocampus (Isaev et al., 2007; Tyzio et al., 2007). GABA_A receptor-mediated currents shift from slower rise and decay kinetics to faster, more mature ones around the end of the second postnatal week in dentate granule cells (Hollrigel and Soltesz, 1997). In the murine respiratory system, the reversal potential of GABA_A receptors reportedly mediates the current switch from depolarizing to hyperpolarizing within the first postnatal week (Ritter and Zhang, 2000). However, depolarizing GABA transmission is reportedly not found in respiratory neurons of P0–P2 rats in vitro (Shao and Feldman, 1997; Brockhaus and Ballanyi, 1998). Future in vivo studies may shed more light on this issue. A switch from $\alpha 2$ - to $\alpha 1$ -subunit occurs during the first postnatal week in the mesencephalon and hypothalamus, and during the second and third weeks in the thalamus, pallidum, and medulla (Fritschy et al., 1994). A switch from $\alpha 3$ - to $\alpha 1$ -subunit starts around P11 in the visual cortex of rats, i.e., before eye opening, and it is not prevented by dark rearing, indicating that it is not triggered by visual input (Heinen et al., 2004), but rather, is genetically pre-programmed.

Possible switches in GABA_A receptor subunits in the brain stem respiratory nuclei were not examined until recently, when the expressions of $\alpha 1$, $\alpha 2$, and $\alpha 3$ were followed at a closely timed sequence from P0 to P21 in the rat (Liu and Wong-Riley, 2004; Liu and Wong-Riley, 2006). In the pre-Bötzinger nucleus (PBC), a postulated center of respiratory rhythmogenesis (Smith et al., 2000), $\alpha 3$ is expressed at relatively high levels at P0 and declines with age, whereas $\alpha 1$ is expressed at relatively low levels at birth but increases with age. Significantly, the two developmental trends intersect close to P12 (Fig. 1A). The expression of $\alpha 2$ subunit remains relatively constant during development (Liu and Wong-Riley, 2004). Similar trends are observed in the ventrolateral subnucleus of the solitary tract nucleus (NTS_{VL}), which receives input from the carotid body (Finley and Katz, 1992) and relays slowly-adapting pulmonary stretch receptor information via its pump cells (which are mainly GABAergic) to other regions of the NTS (such as the commissural nucleus) and to the ventrolateral medulla and pons (Miyazaki et al., 1999; Otake et al., 2001; Kubin et al., 2006). Whether these trends exist in the commissural and other subnuclei of NTS is unknown at this time. Subunit $\alpha 1$ expression gradually increases from birth, peaks at P12, and remains high thereafter. Subunit $\alpha 3$ expression is prominent during the first postnatal week, but is significantly reduced from P11 to P12 and remains low until P21 (Fig. 1B) (Liu and Wong-Riley, 2006). Thus, for both PBC and NTS_{VL}, the switch from $\alpha 3$ to $\alpha 1$ subunit dominance occurs around P12. Such switches may contribute to alterations in the kinetics of postsynaptic potentials mediated by GABA_A receptors, rather than to the reversal potential of GABA_A receptor mediated current, in affecting the function of GABA transmission. In this regard, $\alpha 3$ subunit is thought to be responsible for slow decay time and slow kinetics at early stages of visual cortical development, whereas $\alpha 1$ subunit is for fast decay time and fast kinetics in the adult (Bosman et al., 2002). The same analogy is applied to the murine superior colliculus (a switch from $\alpha 3$ to $\alpha 1$) (Juttner et al., 2001) and the rat thalamus (a switch from $\alpha 2$ to $\alpha 1$) (Okada et al., 2000). Thus, GABA_A α subunit switching may prove to be a common theme in neuronal development, but the precise timing of the switch may vary among regions. In the non-respiratory cuneate nucleus, for example, the apparent switch occurs approximately two days earlier than those in the PBC and NTS_{VL} (Fig. 1C) (Liu and Wong-Riley, 2006).

A delicate balance between excitation and inhibition maintains the normal functioning of the central nervous system. Small changes in GABA-mediated inhibition can profoundly alter

neuronal excitability (Mody et al., 1994). Switching of $\alpha 3$ and $\alpha 1$ subunits in GABA_A receptors around P12 in the PBC and NTS_{VL} may result in a transient imbalance between excitation and inhibition, i.e., increased inhibitory drive and decreased excitatory drive. The temporal correlation between GABA_A α subunit switching and an imbalance in the expressions of inhibitory and excitatory neurochemicals in various brain stem respiratory nuclei around P12 (Liu and Wong-Riley, 2002) underscores a state of dynamic synaptic adjustments during this critical period. The second postnatal week is also a time when GABAergic terminals reportedly undergo reorganization in the NTS, thereby contributing to possible local adjustment in the cardiorespiratory network (Yoshioka et al., 2006). The PBC likewise undergoes other developmental changes, such as the disappearance of most bulbospinal projections when transitioning from the neonate to the adult, although the exact timing of such a change has not been defined (Ellenberger, 1999).

3.2 Possible subunit switches in other neurotransmitter receptor types during development

3.2.1 Glycine receptors—Besides GABA_A α subunits, receptor subunits of undergo developmental switches as well. Similar to GABA, glycine receptors also mediate depolarization during development, are heteropentameric ($\alpha 1-4$, 1β) -gated anionligand channels, and complete their subunit switches by P20 in the rat CNS (Lynch, 2004; Kirsch, 2006). In the rat hypoglossal motoneurons, glycinergic postsynaptic potentials transition from depolarizing and prolonged in the neonate to hyperpolarizing and fast during the first two postnatal weeks (Singer et al., 1998). This is accompanied by a switch from $\alpha 2$ - to $\alpha 1$ -subunit mRNA expression, an increase in the miniature inhibitory postsynaptic currents, and a faster kinetic of evoked inhibitory postsynaptic response (Singer et al., 1998; Singer and Berger, 1999). A detailed analysis of the time course of glycine receptors subunit switch in brain stem respiratory nuclei awaits future investigation.

3.2.1 NMDA receptors—Experience-dependent synaptic plasticity has been postulated in the visual cortex and elsewhere in the brain to be associated with a change in the complement of postsynaptic glutamate receptors, specifically the NMDA receptors, thereby altering the functional properties of these receptors (Quinlan et al., 1999). During the second postnatal week of cerebellar development, there is evidence for a switch in the dominance of subunit composition of the NMDA receptors from NR2B to NR2A and NR2C; at the same time, the decay time of NMDA receptor-mediated excitatory postsynaptic current is significantly reduced (Sasner and Buonanno, 1996; Rumbaugh and Vicini, 1999; Cathala et al., 2000). The switch from NR2B to NR2A is also found in murine somatosensory cortex and thalamus during the second postnatal week of development (Liu et al., 2004). Similar switches are also reported in the forebrain and midbrain during development (van Zundert et al., 2004; Kubota and Kitajima, 2008), with the implication that it potentiates rapid and stable growth of immature synapses and regulates synaptic plasticity. Virtually nothing is known about possible switches in NMDA receptor subunits in the brain stem respiratory network.

3.2.2 AMPA receptors—In the respiratory system, the excitatory neurotransmitter glutamate acts mainly at non-NMDA receptors, such as AMPA and kainate, although metabotropic receptors may also be involved (Liu et al., 1990; Pierrefiche et al., 1994; Dogas et al., 1995). In the brain stem and elsewhere, evidence is emerging that subunit composition undergoes developmental changes.

In the cerebral cortex, pyramidal neurons reportedly undergo a switch in Ca²⁺ permeability of their glutamate AMPA receptors through an alteration of subunit composition (Kumar et al., 2002). AMPA receptors are known to have 4 major subunits, GluR1–4, all except GluR2 are permeable to Ca²⁺ (Brorson et al., 1999). AMPA receptors with GluR2 are found to increase with age in the rat neocortex, indicating a developmental switch in AMPA receptor subunit

composition from ones dominated by Ca^{2+} -permeable subunits to those that incorporate more of the Ca^{2+} -impermeable GluR2 subunits (Pellegrini-Giampietro et al., 1992; Kumar et al., 2002). This radically changes their current-voltage relationship, their response to external or internal polyamines, and their susceptibility to cell death. It also has strong implications for plasticity. Remarkably, GluR2 expression during postnatal development in six brain stem nuclei involved in respiratory control (pre-Bötzing complex, ventrolateral subnucleus of nucleus tractus solitarius, nucleus ambiguus, hypoglossal nucleus, dorsal motor nucleus of the vagus, and medial accessory olivary nucleus) exhibits a significant increase at P12, in stark contrast to a concurrent but notable decrease in the expressions of glutamate and NMDA receptors (Liu and Wong-Riley, 2002; Liu and Wong-Riley, 2005). Such a rise in GluR2 at P12 in brain stem respiratory nuclei is consistent with the increase described above in the neocortex. However, it remains to be determined if the other AMPA receptor subunits also undergo developmental adjustments, and if AMPA receptors in brain stem respiratory nuclei alter their physiological properties during postnatal development.

3.3 Serotonin receptors

The actions of serotonin (5-hydroxytryptamine or 5-HT) differ among systems, nuclear groups, age, and the type of receptors, which exist in multiple forms (Barnes and Sharp, 1999; Taylor et al., 2005). Transient expression of 5-HT_{1A} receptors has been reported in the hypoglossal and other somatic motoneurons during development (Talley and Bayliss, 2000). Likewise, age-dependent changes in the expressions of 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} have been noted in postnatal dissociated hypoglossal, facial, and cervical spinal motoneurons (Volgin et al., 2003). For example, 5-HT_{1B} mRNA expression is transiently reduced at P14 in rat hypoglossal motoneurons, and dendritic labeling of 5-HT_{2A} in hypoglossal motoneurons does not appear until after P12 (Volgin et al., 2003).

In the hippocampus of rats, serotonin receptors linked to phosphoinositide hydrolysis undergo a subunit switch from 5-HT_{2A} to 5-HT_{2C} between first and third weeks of life (Ike et al., 1995). Comparable switches have not been reported for brain stem respiratory nuclei. However, the fact that the effect of 5-HT evolves from inhibitory to excitatory in the hypoglossal motoneurons through development strongly implies that changes in receptor subunit expression may be an underlying mechanism (Volgin et al., 2003).

In short, neurotransmitter receptor subunit or subtype switches may play a significant role in transitioning from a neonatal to a more mature form of synaptic transmission during the critical period of respiratory network development. Such changes are likely to be genetically programmed, but can, nonetheless, be influenced by external perturbations. Subunit switches in receptors of different neurotransmitters may be developmentally staggered or temporally coincidental. Future studies will determine if switching converges around the end of the second postnatal week in the respiratory network of rats.

4. Physiological correlates of a critical period of respiratory development

Are there physiological correlates to the significant neurochemical changes occurring toward the end of the second postnatal week in rats? What is the normal developmental trend in ventilatory response during the first three postnatal weeks? What are the features that stand out during development and when do they take place? Is there any indication that the ventilatory response to hypoxia is different around the time of the presumed critical period? These questions are discussed next in this review.

4.1 Developmental changes in respiratory response to normoxia

The normal development of respiration in normoxia has been examined mostly in the context of hypoxia or hypercapnia, and typically only a few selected time points were chosen (Mortola et al., 1986; Eden and Hanson, 1987; Cameron et al., 2000; Stunden et al., 2001). When a detailed, daily study was carried out for the first three postnatal weeks in rats (Liu et al., 2006), striking features were revealed (Fig. 2). Respiratory frequencies undergo a steady increase with age, peaking at postnatal day (P) 13 and decline thereafter until P21 (Fig. 2A). Absolute tidal volume (V_T) before adjustment to body weight assumes a relative plateau during the first postnatal week, followed by a steady rise until P21, with statistically significant peaks at P8 and P13 (Fig. 2B). When V_T is normalized to body weight, it exhibits a steady decrease with age, with a small but significant fall at P1 and P12, a significant increase at P13, followed by a relative plateau until P21 (Fig. 2C). The \dot{V}_E response without adjustment to body weight shares a trend similar to that of V_T (Fig. 2D). When it is normalized to body weight, \dot{V}_E assumes a fluctuating pattern from P0 through P12, with a slight dip at P7 and P12 but a significant increase at P13, followed by a gradual decline until P21 (Fig. 2E).

The newborn rat has a greater surface area-to-body mass ratio that requires a higher metabolic rate per unit body weight than older animals to compensate for greater heat loss (Mortola, 1984). Thus, during normoxia, neonatal rat pups have relatively high V_T and \dot{V}_E values when adjusted to body weight. During the first postnatal week, the absolute tidal volume in response to normoxia changes very little, while the V_T adjusted to body weight exhibits a steady decline with age. During the second week, both frequency and absolute V_T sharply escalate, resulting in an increase in \dot{V}_E that peaks at P13. This corresponds to the development of the lungs, in which the rapid outgrowth of secondary septa is largely completed by the end of the 2nd postnatal week and the surface area for gas exchange is increasing (Burri, 1974; Burri et al., 1974; Blanco, 1995). When adjusted to body weight, V_T still shows a small but significant increase at P13, while \dot{V}_E reaches its highest value on that day, most likely reflecting the highest peak attained for respiratory frequency at P13. From P14 onward and during the 3rd postnatal week, frequency values steadily decline, while those of absolute V_T increase, denoting the attainment of more mature, deeper, and slower breaths. At the same time, there is an enormous thinning of the alveolar septa in the lung (Burri, 1974), indicating a tremendous increase in the efficiency of gas exchange. Thus, the alveolar ventilation is actually increasing even though the minute ventilation stays relatively constant. The developmental pattern of frequency, with a rise in the first 2 weeks and a fall in the 3rd postnatal week is also evident in Huang et al.'s data (Huang et al., 2004), which include only 6 time points.

The reason for increased baseline ventilation at and around P13 is not entirely clear at this time. Many factors are expected to be involved, such as maturational process of neurotransmission, imbalance of excitatory versus inhibitory synapses, conversion of neonatal to mature forms of receptor subunit composition, hypothalamic and other supra-brain stem influences, maturational process of the gas exchange mechanism in the lung, adjustment of body temperature, and maturational process of locomotion and sleep. The end of the second postnatal week is a period of multiple developmental changes that may impinge upon respiratory behavior of the animal. However, even though the baseline ventilation is increased, the animals are less capable of responding to hypoxia (see below).

4.2 The hypoxic ventilatory response undergoes postnatal developmental changes

The response to hypoxia, or hypoxic ventilatory response (HVR), undergoes developmental regulation via a variety of mechanisms (Berquin et al., 2000; Waters and Gozal, 2003; Simakajornboon and Kuptanon, 2005). Mammalian HVR to acute hypoxia is known to be biphasic, consisting of an initial increase in ventilation followed by a later ventilatory suppression that is termed hypoxic ventilatory depression (HVD) or hypoxic ventilatory roll-

off (Mortola, 1984; Easton et al., 1986; Eden and Hanson, 1987; Vizek et al., 1987; Gershan et al., 1994; Powell et al., 1998; Mortola, 1999; Moss, 2002). HVR is a complex process in which several excitatory, inhibitory, and modulatory components are involved. The early HVR may be mediated by glutamate and its N-methyl-D-aspartate (NMDA) receptors (Kazemi and Hoop, 1991; Ohtake et al., 1998; Richter et al., 1999) via carotid body-nucleus tractus solitarius (NTS) pathway and perhaps other central pathways (Mizusawa et al., 1994; Ohtake et al., 2000). It also involves the activation of protein kinase C, neuronal nitric oxide synthase, and tyrosine kinase, and such activation increases with age and NMDA receptor expression (reviewed in (Simakajornboon and Kuptanon, 2005)). HVD is postulated to be attributable to the central depressant effect of hypoxia (Vizek et al., 1987), but peripheral (arterial chemoreceptor) mechanism may not be excluded (reviewed in (Bissonnette, 2000)). One possibility is that HVD may act by depressing synaptic pathways activated by arterial chemoreceptors (Powell et al., 1998). Several neurotransmitters and neuromodulators, such as GABA (Kneussl et al., 1986; Kazemi and Hoop, 1991; Richter et al., 1999), serotonin (Di Pasquale et al., 1992; Richter et al., 1999), adenosine (Neylon and Marshall, 1991; Elnazir et al., 1996; Richter et al., 1999), and platelet-derived growth factor (PDGF- β) (Gozal et al., 2000; Simakajornboon and Kuptanon, 2005)) may play important roles in HVD. In addition, hypoxic hypometabolism (Mortola, 1999) may contribute to HVD, and nitric oxide has been implicated in both excitatory and inhibitory components of the HVR (Gozal et al., 1997). Gasping, which is activated in severe hypoxia or apnea, is reportedly mediated by 5-HT_{2A} receptors (Tryba et al., 2006).

HVD differs between the neonate and the adult. In neonatal mammals exposed to sustained hypoxia, the minute ventilation (\dot{V}_E) gradually decreases to levels near or below those observed in normoxia (baseline) (Eden and Hanson, 1987; Mortola and Rezzonico, 1988; Fung et al., 1996; Cohen et al., 1997; Martin et al., 1998), whereas in adult mammals, HVD displays a milder ventilatory decline to levels that remain higher than the baseline (Easton et al., 1986; Vizek et al., 1987; Gershan et al., 1994; Maxova and Vizek, 2001). Is there a critical period during postnatal development when the animal is less responsive to hypoxia? The answer to this question requires a more closely-spaced temporal study.

A comprehensive, daily monitoring of ventilation in normoxia and hypoxia during the first three postnatal weeks of rats was conducted recently (Liu et al., 2006). The response to acute hypoxia (10% O₂ + 90% N₂ for 5 minutes every fifth day for each of the animals tested) is indeed biphasic, especially from P3 onward, with ventilation in the first 30 seconds to 1 minute being higher than the rest of the 5-min period. Significantly, this biphasic response undergoes developmental changes during the first 3 postnatal weeks. From P0 to P11, the ratio of \dot{V}_E in hypoxia to that in normoxia throughout the 5-min period is > 1 (Fig. 3C), indicating that the system is able to respond adequately to hypoxia. However, between P12 to P16, much of the late phase of HVR is < 1, suggesting that the depressive effect of hypoxia is much more prominent at this time, despite the fact that ventilation during normoxia is quite high (Liu et al., 2006). This reduction is due mainly to decreased frequency response, but on P13, when HVR is at its lowest, the decrease is caused by a distinct suppression of both frequency and V_T (Fig. 3A, B, and C). P13 is the only time during development when the $V_T(\text{Hypoxia}):V_T(\text{Normoxia})$ ratio falls below 1. The marked reduction in HVR at the end of the second postnatal week is consistent with a predominance of inhibitory neurotransmitter expression, a switch in GABA_A receptor subunits from a neonatal to an adult form, as well as heightened expression of GluR2 receptors occurring at the same time in multiple brain stem respiratory nuclei (discussed above and reviewed in (Wong-Riley and Liu, 2005)), all of which point to a stronger inhibitory suppression that may dampen the hypoxic ventilatory response. This can be tested in the future by recordings of respiratory nuclei, including the phrenic motor nerve nuclei, in intact animals exposed to hypoxia at the end of the second postnatal week versus those at other ages. Between P17 and P21, HVR returns to a level above the baseline,

indicating that, once again, the system regains a better capability to cope with hypoxia. This renewed ability is due primarily to increasing tidal volume (Fig. 3B), despite decreasing frequency of respiration, with age (Fig. 3A). Thus, a deep and slow breathing pattern in response to hypoxia is characteristic of a more mature response, as it improves the efficiency of \dot{V}_E by maximizing alveolar ventilation (Frappell and Mortola, 1994).

The period around P13 (P12–P15) stands out as developmentally distinct in the rat (Liu et al., 2006). Both frequency and \dot{V}_E have reached their highest peaks at P13 under normoxia, and HVR is lowest at P13, with values at P12–P15 being lower than the rest of the 3 postnatal weeks. The validity of these findings is strengthened by the relatively large number of animals (between 12 to 14 animals from 7 litters per day) examined each day of the first 3 postnatal weeks. Thus, the end of the 2nd postnatal week is distinctly marked as a time of substantially reduced ability of the rat pups to respond to hypoxia.

Another developmental perturbation occurs at P3, the only time during the 1st postnatal week when the breathing frequency in response to hypoxia falls below baseline (Liu et al., 2006). However, HVR (\dot{V}_E) may be marginally adequate at this time because tidal volume is above the baseline level. Remarkably, significant alterations in neurochemical expressions (decrease in glutamate and NMDA receptors, and increase in GABA, GABA_B, glycine and GluR2) and a reduction in cytochrome oxidase activity take place at P3–4 in eight brain stem nuclei involved in respiratory control (Liu and Wong-Riley, 2001; Liu and Wong-Riley, 2002). These changes are comparable, though less drastic, than those around P12.

4.3 The body temperature undergoes postnatal developmental changes

The increase in body temperature (taken rectally) with age does not follow a steady path, but rather, it has a peak at P4 and another striking one at P13 (Liu et al., 2006) (Fig. 4). This may be correlated with a significant increase in respiratory frequency at P3 and an overall peak frequency at P13 (Fig. 2A). Increased core temperature especially at P13 may reflect a change in hypothalamic regulation, which may increase thyroid hormone levels and raise the basal metabolic rate, or it may reflect an uncoupling of electron transport and oxidative phosphorylation, generating heat (Dussault and Labrie, 1975; Lanks et al., 1986). If the heat generation is at the expense of ATP production, it could result in decreased energy supply for neuronal activity. Exposure to hypoxia lowers the body temperature by 1–2°C during the first 2 postnatal weeks (Liu et al., 2006). This may be due to hypoxia-induced decreases in metabolic rate, especially the component of oxygen consumption ($\dot{V}O_2$) related to thermogenesis (Mortola and Frappell, 2000). By the third postnatal week, with the acquisition of a thick coat of fur, increased body mass, and reduced frequency of respiration, the animals are then able to maintain their body temperature fairly well during 5 minutes of hypoxia (Liu et al., 2006).

4.4 Possible switches in physiological response during development

Postnatal changes in the expressions of neurotransmitters, receptors, and metabolic enzymes appear to be correlated temporally with changes in physiological response, both in normoxia and hypoxia, as discussed above. By the same token, postnatal switches in the expression of neurochemicals, such as neurotransmitter receptor subunit switches, may be paralleled by comparable “switches” in physiological responses during specific period or periods of postnatal development. A few possible switches to consider include: GABA_A receptor and chloride cotransporter-mediated switch from depolarizing to hyperpolarizing transmission (discussed above), a change in phrenic nerve output from irregular to regular (Zhou et al., 1996), a switch from a triphasic to a monophasic gasping response (Gozal and Torres, 2001), a switch in the hypoxic response from mainly a central to both central and peripheral chemoresponsive mechanisms (Saetta and Mortola, 1987) in conjunction with significant maturational changes in carotid body chemoreceptors postnatally (Donnelly, 2005), and a rearrangement of the

sympathetic and vagal control of the heart rate (Mills, 1978). The precise timing of these switches needs to be investigated further, as most studies focused on a few selected postnatal days, and profound changes can occur in a relatively short time span, sometimes even in a single day (see above). In the case of hypoxic ventilatory response in the rat, the switch from being influenced by both frequency and V_T to that mainly by V_T occurs around P13 (Liu et al., 2006) (Fig. 3).

5. Significance of the end of the second postnatal week in rats: a critical period of development

The overriding insight gained from recent studies is that the end of the 2nd postnatal week is a highly plastic and narrow window of respiratory development. This time window may be regarded as the “critical period” described previously as a period “devoted to structural and/or functional shaping of the neural system subserving respiratory control” (Carroll, 2003).

Neurochemically, there is an imbalance between excitatory and inhibitory systems and a switch in GABA_A receptor subunit expression (reviewed in (Wong-Riley and Liu, 2005) and above), and physiologically, the response to hypoxia is at its weakest but is stabilizing thereafter (Liu et al., 2006). The response to hypercapnia does not become mature until after the second postnatal week (Davis et al., 2006). The hypothalamo-pituitary-adrenal axis function is close to maturing and alveolarization of the lung is reaching completion (Giulian et al., 1974; Walker et al., 1986; Schmiedl et al., 2007). It is a time of dynamic adjustments by the respiratory network to transition from the neonatal to the adult form of ventilatory control, and it involves an intricate orchestration of multiple players at the molecular, cellular, organellar, and whole body levels. This window coincides with other bodily changes, such as the opening of eyelids, the opening of the external auditory canal, the onset of non-REM sleep, the onset of power-law distribution of wake bout distribution, the thickening of fur, a switch from polyneuronal to mononeuronal innervation of muscle fibers, the pruning of synapses onto Purkinje cells of the cerebellum, and a change from crawling to walking (Jouvet-Mounier et al., 1970; Crepel et al., 1976; Hoath, 1986; Petrosini et al., 1990; Blumberg et al., 2005). Whether one or more of these changes influence respiratory behavior is not fully understood. Other neurochemical and hormonal changes may also contribute to dynamic homeostatic regulation at this time. It is a period of growth, pruning, adjustment, adaptation, switches in various mechanisms, and an interplay between genetic and environmental factors. The end of the second postnatal week in rats is the most dynamic period, but it can also be a most vulnerable period, when the individual is uniquely susceptible to external perturbations. If such a critical period exists in humans (weeks or months in humans instead of days in rats), and if respiratory insults are introduced at this time to a vulnerable infant, especially during sleep when respiratory control system is further suppressed (Olson and Simon, 1996; Moss, 2002), it is possible that catastrophic events, such as Sudden Infant Death Syndrome, may result.

In conclusion, it cannot be overemphasized that sudden and major developmental adjustments are normal events that, by themselves, are not threatening to life. The fact that they have survived evolution indicates that a combination of external stressors and other vulnerable attributes (such as nonlethal organic defects or severe prenatal exposure to nicotine) during the critical period is still a relatively rare event, and the respiratory system, by and large, is able to survive the period of turmoil relatively unscathed to continue on to a path of normal maturity.

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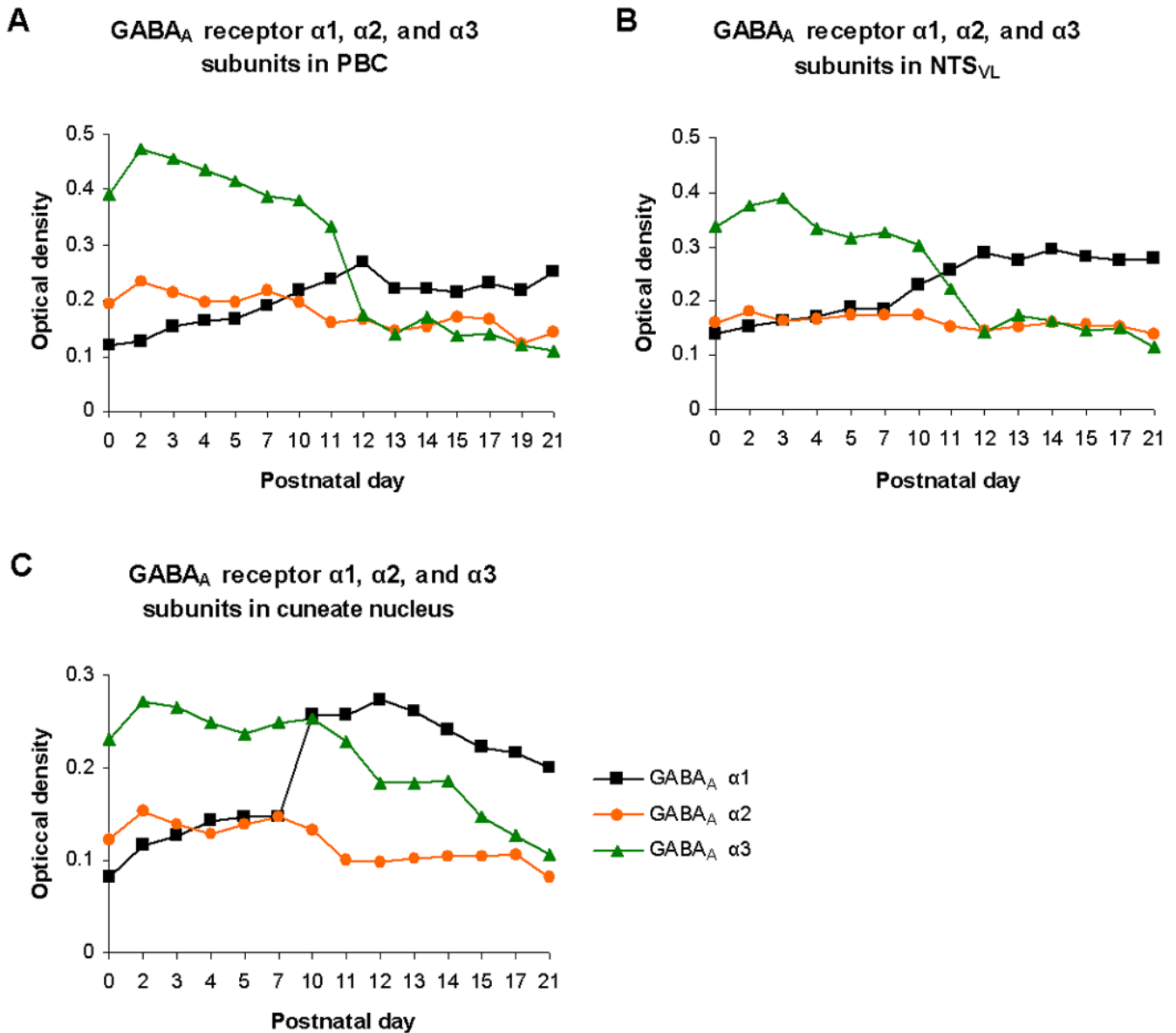
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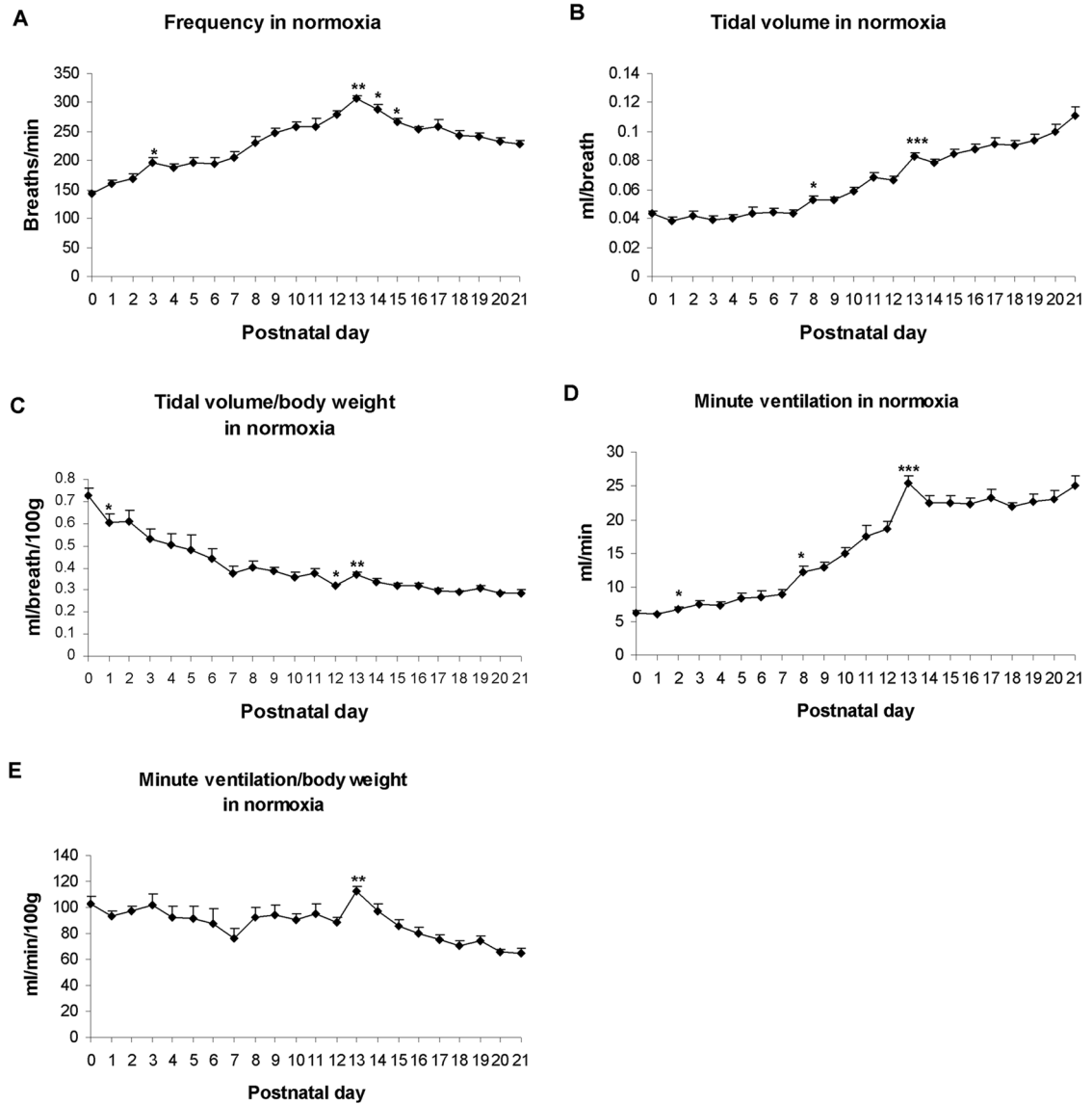
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**Fig.1.**

Composite graphs based on data of previous reports (Liu and Wong-Riley, 2004; Liu and Wong-Riley, 2006) for the developmental expressions of GABA_A receptor subunits α 1, α 2, and α 3 in the Pre-Bötzinger complex PBC (A), the ventrolateral subnucleus of the nucleus tractus solitarius NTS_{VL} (B), and the non-respiratory cuneate nucleus (C). Note that in all three nuclear groups, the expression of subunit α 1 increases with age, whereas that of subunit α 3 decreases with age. The expression of subunit α 2 remains relatively low and constant during development. Subunit α 1 reaches its peak expression at P12 in both PBC and NTS_{VL}, but earlier at P10 in the cuneate nucleus. The developmental trends of α 1 and α 3 intersect close to P12 in the PBC and NTS_{VL}, but at P10 in the cuneate nucleus.

**Fig. 2.**

Developmental trends of frequency (f) (A), tidal volume (V_T) (B), and minute ventilation (\dot{V}_E) (D) during the first 3 postnatal weeks. In normoxia, all three trends increase significantly during the 2nd postnatal week, peaking at P13 ($P < 0.001$); after which, f values fall steadily until P21, whereas V_T and \dot{V}_E values initially fall at P14 but rise again until P21. When V_T is normalized to body weight (C), its trend decreases with age, with a significant reduction from P0 to P1, and from P11 to P12 ($P < 0.05$ for both). There is a significant increase at P13 ($P < 0.01$) followed by a relative plateau until a small rise at P21. The developmental trend of \dot{V}_E normalized to body weight (E) exhibits minor fluctuations from P0 to P12. There is a significant rise in \dot{V}_E at P13 ($P < 0.01$), followed by a gradual decrease thereafter. ANOVA within each parameter reveals significant differences among ages ($P < 0.01$). Statistical comparisons (Tukey's Studentized range test) between successive age groups: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (significance between one age group and its adjacent younger age group). (Modified from Liu and Wong-Riley, 2006).

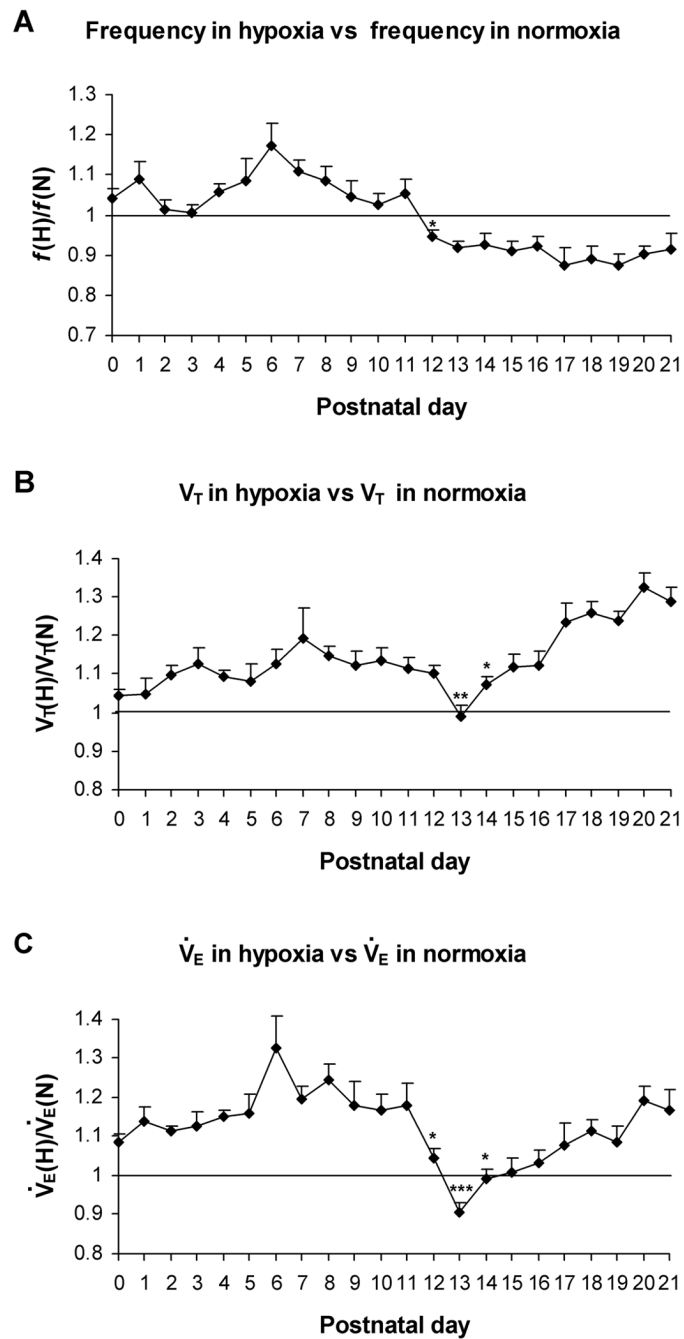


Fig. 3. Developmental changes in the mean values of frequency (f) (A), tidal volume (V_T) (B), and minute ventilation (\dot{V}_E) (C) averaged over 5-min of hypoxic ventilatory response (in 10% O_2 + 90% N_2) versus those in normoxia. These values are all above 1 from P0 to P12, with a peak at P6 (for f and \dot{V}_E) or P7 (for V_T). At P12, the f and \dot{V}_E ratios drop precipitously ($P < 0.05$ for both), and at P13, the ratios for V_T and \dot{V}_E are significantly lower than those at P12 ($P < 0.05 - 0.001$), with the V_T and \dot{V}_E ratios reaching their lowest on that day, and \dot{V}_E ratio is significantly below 1. After P13, V_T and \dot{V}_E ratios both increase with age (especially at P14, $P < 0.05$) while f ratios remain below 1 through P21. ANOVA of each parameter indicates

significant differences among ages ($P < 0.01$). Statistical comparisons (Tukey's Studentized range test) between successive age groups: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (Modified from Liu and Wong-Riley, 2006).

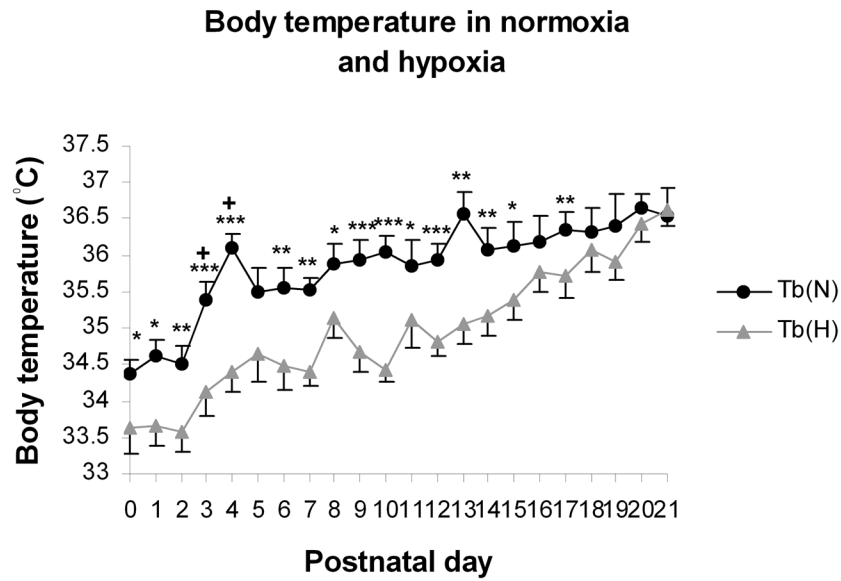


Fig.4. Changes in body temperature (T_b) and body weight with age. (A) Under normoxic conditions, T_b is lowest at P0 – P2, increases significantly between P3 and P4, then gradually increases until another peak at P13, followed by a small decline at P14 and a gradual increase with age until P21. After 5-min of exposure to hypoxia, T_b falls 1–2°C from P0–15, but close to the baseline (T_b in normoxia) thereafter, and the two trends intersect at P21. Statistical comparisons (Student's t test) between T_b in normoxia and hypoxia: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (Modified from Liu and Wong-Riley, 2006).