Published in final edited form as:

Respir Physiol Neurobiol. 2013 January 1; 185(1): 20–29. doi:10.1016/j.resp.2012.05.017.

Carotid chemoreceptor development in mice

Machiko Shirahata, Eric W Kostuk, and Luis E Pichard

Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

Abstract

Mice are the most suitable species for understanding genetic aspects of postnatal developments of the carotid body due to the availability of many inbred strains and knockout mice. Our study has shown that the carotid body grows differentially in different mouse strains, indicating the involvement of genes. However, the small size hampers investigating functional development of the carotid body. Hypoxic and/or hyperoxic ventilatory responses have been investigated in newborn mice, but these responses are indirect assessment of the carotid body function. Therefore, we need to develop techniques of measuring carotid chemoreceptor neural activity from young mice. Many studies have taken advantage of the knockout mice to understand chemoreceptor function of the carotid body, but they are not always suitable for addressing postnatal development of the carotid body due to lethality during perinatal periods. Various inbred strains with well-designed experiments will provide useful information regarding genetic mechanisms of the postnatal carotid chemoreceptor development. Also, targeted gene deletion is a critical approach.

Keywords

breathing; carotid sinus nerve; gene; hypoxia; inbred; outbred

1. Why mice?

The critical role of the carotid body in the regulation of breathing has been recently emphasized in both animal and human studies. In contrast to the traditional view, the input from the carotid body is essential for proper function of the central chemoreceptors in adult humans and animals (Timmers et al., 2003; Dahan et al., 2007; Blain et al., 2009; Blain et al., 2010). However, the function and morphology of the carotid body are not the same throughout life. Embryonic development of the mammalian carotid body has been investigated in many species including humans (Boyd, 1937; Hervonen and Korkala, 1972; Smith et al., 1993), calves (Smith, 1924), sheep (Blanco et al., 1984), cats (Clarke and Daly, 1985), rabbits (Kariya et al., 1990), rats (Smith, 1924; ROGERS, 1965; Kondo, 1975) and mice (Kameda et al., 2002). Further, studies of postnatal development of the carotid body include humans (Dinsdale et al., 1977), sheep (Blanco et al., 1984; Blanco et al., 1988), pigs (Mulligan, 1991), cats (Carroll et al., 1993; Carroll and Fitzgerald, 1993), rabbits (Bolle et al., 2000; Rigual et al., 2000), rats (Kholwadwala and Donnelly, 1992; Pepper et al., 1995; Bamford et al., 1999; Wasicko et al., 1999; Wang and Bisgard, 2005) and mice (Kostuk et

© 2012 Elsevier B.V. All rights reserved.

Correspondence: Dr. Machiko Shirahata, Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA, mshiraha@jhsph.edu.

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

al., 2012). We have listed here a limited number of references to point out that a variety of animals have been examined. These studies have shown that major changes in the function of the carotid body occur at postnatal periods, and the morphology of the carotid body continually develops after birth.

The fetal carotid body responds to severe hypoxia, but carotid body function is reset after birth (for reviews, (Donnelly, 2000; Carroll, 2003; Gauda et al., 2009). Function of the fetal carotid body was studied in sheep which were close to full term (Blanco et al., 1984). Functional changes of the carotid body during transition from fetus to newborn were also studied in the sheep (Blanco et al., 1984; Blanco et al., 1988). However, the developmental stages of fetuses and newborns are extremely variable among species (Evans and Sack, 1973; Sterba, 1995). The sheep is born in a matured form; i.e., a newborn sheep is covered with a developed hair coat, and is able to see and walk. On the other hand, a rodent pup is born underdeveloped with no hair, closed eyes and limited motor skills. Thus, special care must be taken when dealing with developmental stages for extrapolating data from one species to other species.

Although many animal species have been used as “model species” in neurodevelopmental research, a recent trend shows the dominant use of rats and mice (Clancy et al., 2007). Clearly, mice are the most suitable species for genetic studies because of the availability of many inbred strains and thousands of knockout mice (Collins et al., 2007). A major disadvantage of the use of mice in developmental studies of the carotid body is its size. An increasing number of studies have taken advantage of these knockout mice to understand chemoreceptor function of the carotid body, but their use has been mostly limited to adult mice (see below). Postnatal development of the mouse carotid body has been mainly estimated by measuring hypoxic or hyperoxic ventilatory response, which is an indirect assessment of the carotid body function. Why then are we bothering to use developing mice? Generally speaking, organ development of the mouse and the rat is similar (Schneider and Norton, 1979). Can we just use the data obtained from the rat, particularly in carotid sinus nerve activity? The authors’ current position is against this idea based on the data of various inbred strains of mice and the comparative studies between mice and rats. Basal respiration as well as hypoxic and hypercapnic ventilatory responses are age and strain dependent (Balbir et al., 2008; Balbir, 2008; Arata et al., 2010). Further, V_E (minute ventilation) adjusted to body size increases in mice from newborn to adult, but decreases in rats (Mortola and Noworaj, 1985). In a comparative study Mortola showed that breathing frequency and tidal volume in newborn mice (3 days old) increased in response to hypoxia (10% O_2 for 10 minutes), but in rats (2 days old) breathing frequency increased and tidal volume decreased (Mortola et al., 1989). Carotid chemoreceptor neural responses to hypoxia in adult mice differ among several strains (Figure 1). These data indicate that mice are not little rats, and that we need to investigate each species and each strain separately. Once our investigation progresses, we shall obtain a more comprehensive view as to the similarities and differences between the species and strains. Then, we can evaluate each animal model when and how the model simulates human conditions. Here, we are summarizing the past findings related to postnatal development of the mouse carotid body, and further suggest future directions of the research.

2. Carotid chemoreceptor neural output

Carotid chemoreceptor neural activity in mice has been recorded mainly using an in vitro preparation from adults (Donnelly and Rigual, 2000; Kline et al., 2002; He et al., 2002; Rong et al., 2003; Prieto-Lloret et al., 2007; Trapp et al., 2008; Peng et al., 2010). Most of these studies have compared the neural responses to hypoxia between wild type mice and knockout mice. We have observed that carotid chemoreceptor neural responses in vivo differ

in three inbred strains of mice (Figure 1). In these studies, mice were ventilated with 100% oxygen maintaining PaCO₂ ~35 mmHg. When inspired gas was changed to 10% oxygen, neural activity increased in DBA/2J mice, but barely changed in A/J mice. In FVB mice, the speed of the increase in neural activity was very fast, and activity was not sustained throughout the 10% O₂ challenge. With milder hypoxia (15% oxygen) the neural output continually increased throughout the 90 seconds of the challenge in DBA/2J and FVB mice. Again, FVB mice showed a sharp rise in neural output, and A/J mice did not show a significant increase. Carotid body responses to anoxic stimuli were seen in all mice, but the response in A/J mice was delayed and much smaller. Because these mice are raised in the same room in our facility where environments are well controlled, the differences in neural responses to hypoxia most likely reflect genetic differences. Comparative studies in these strains will provide important information as to the generation of chemoreceptor neural activity and possibly hypoxic sensing mechanisms. Currently, developmental studies in carotid chemoreceptor neural activity are lacking in mice. In rats, the carotid body chemoreceptor neural response to hypoxia increases with postnatal age. The response appeared to be negligible until ~1 week old (Kholwadwala and Donnelly, 1992), and reached a plateau between one and two weeks after birth (Kholwadwala and Donnelly, 1992; Bamford et al., 1999). It requires future investigation to confirm that this is also the case in mice. Due to its small size, recording of carotid sinus nerve activity in newborn mice would be a great challenge, but in vitro techniques could be developed for older mice (e.g. >P7).

3. Measurement of breathing parameters

Several strains of mice have been used to examine their characteristics of breathing. We have summarized breathing parameters obtained from four inbred strains (C57BL/6, BALB/c, DBA/2J, A/J), two hybrids (C57BL/6-129SvEv, C57BL/10-129SvEv), offspring (F1) of two inbred strains (C57BL/6 × BALB/c), and some outbred stocks (Swiss-IOPS, ICR, Swiss CD1, Swiss) (Table 1). The data were obtained from several publications. The timing of measurements varies among the studies. Renolleau et al., have shown detailed time dependent changes in baseline breathing as well as ventilatory responses to short-term (90 seconds) hypoxia and hypercapnia between 1 and 48 hours after birth (Renolleau et al., 2001a). Their data indicate that basal breathing patterns significantly change during the first 24 hours after birth. Respiratory frequency and tidal volume increased with time during the initial 12 hours after birth, and then decreased with time for the next 12 hours (24 hours after birth). At ages less than P1 many investigators reported frequent apneas. Changes in respiratory frequencies from P1 to P28 are summarized in Figure 2. Generally speaking, basal breathing frequencies are slow at P1, increase up to P10 and become relatively stable afterwards within a strain. We do not show tidal volume or minute ventilation here, because these values in small animals are semiquantitative in nature (Enhorning et al., 1998; Gaultier and Gallego, 2008) and extremely varied among the reports.

Before we further discuss the ventilatory responses to hypoxia, we will address possible reasons of the variability among the studies seen in Table 1 and Figure 2. These include postnatal time, strains of mice, sleep-wake states, sex, and measuring techniques such as restraint, temperature control, durations and degrees of stimuli. Surprisingly, not many studies have been performed in inbred strains of mice, in particular in very early phase after birth. Gallego and Gaultier's group, who has performed extensive investigation in ventilatory control of young mice, has used Swiss outbred stocks as well as the C57BL-120SvEv hybrid. An inbred strain is produced by sister-brother mating or parent-offspring mating for at least 20 consecutive generations. Therefore, except for the sex difference, mice of an inbred strain are as genetically alike as possible and are homozygous at virtually all of their loci. On the other hand, an inbred strain is genetically distinct from

other inbred strains (<http://research.jax.org/grs/type/inbred/index.html>). An outbred stock is a closed population of genetically variable mice that is bred to maintain maximum heterozygosity, but their genetic background is poorly characterized (Chia et al., 2005; Festing, 2010). Because of its homogenous and controlled genotype, an inbred strain can characterize the environmental effects better than an outbred stock (Festing, 2010). Arata et al. specifically addressed the strain differences in the control of breathing in their preliminary study, indicating a large variation of breathing parameters among different strains (Arata et al., 2010). Unfortunately, their data do not provide detailed recording conditions. Our data at P1 also show the difference between the DBA/2J and A/J inbred strains (Figure 2) (Balbir, 2008). More detailed studies are necessary for the early phase of respiratory changes in other inbred strains of mice such as C57BL/6 inbred mice.

Ventilatory responses to hypoxia depend on not only carotid body function but also the central respiratory control system. Sleep-wake states influence the central respiratory control system: the ventilatory response to hypoxia is attenuated during sleep compared to awake (reviewed by (Shea, 1996; Krimsky and Leiter, 2005)). Therefore, the ventilatory responses to hypoxia must be measured under a known sleep-wake state. Sleep-wake states can be classified by assessing the electroencephalogram (EEG), electromyogram (EMG) and eye movement (electrooculogram; EOG) (Brain Facts, Society for Neuroscience; <http://www.sfn.org/skins/main/pdf/brainfacts/2008/sleep.pdf>). In the adult mouse, EOG has been omitted due to the apparent technical difficulties, and EEG and EMG have been used (Schaub et al., 1998; Tagaito et al., 2001). However, EEG and EMG instrumentation in neonatal mice is challenging, and it could induce stresses which alter their behavior and ventilation. Karlsson et al. established a two-state model (sleep and wake) for neonatal rats using only EMG and behavioral criteria (Karlsson and Blumberg, 2002; Karlsson et al., 2004). Durand et al. also used EMG and behavioral indices to assess wake-sleep states in 5-day old mice (Durand et al., 2005). We examined whether we can determine sleep-wake states using exclusively behavioral indices in 7-day old DBA/2J and A/J inbred mice (Balbir et al., 2008). We found that coordinated movements (CM; defined as the prolonged movement of multiple limbs and the head) were useful indices. They were observed only during periods of high EMG activity. The duration of CMs was not significantly different from the duration of high EMG in both strains of mice. Further, the data indicate that the instrumentation of the EMG appears to cause stress in neonatal mice. Accordingly, a non-invasive method for sleep-wake assessment by monitoring the behavioral indices would be a more suitable approach to measure ventilatory responses to gas challenges in 7-day old mice. This approach has also been used in younger mice (up to 48 hours after birth) while measuring ventilation (Dauger et al., 2001): Sleep was defined as immobility in the recumbent position, and arousal was characterized by sudden neck and forepaw extension. In adult C57BL/6J mice, more than 40 seconds of continuous inactivity has been confirmed as sleep (Pack et al., 2007). Currently, we need to fill the gap to establish methods for adequately assessing sleep-wake states in mice at the ages between 7-day old and adult.

Regarding sex, many reports shown in Table 1 and Figure 2 pooled the data from both of females and males or even did not mentioned sex. Although sex differences were reported in hypoxic and hypercapnic ventilatory responses in rats (Holley et al., 2012), Renolleau et al. analyzed the sex effects and did not find any effects on breathing parameters in the C57BL/6-129SvEv hybrid strain (Renolleau et al., 2001b).

In terms of measuring techniques, Gautier and Gallego addressed the problems and difficulties of measuring ventilation from neonatal mice and recommended the use of unrestrained whole body plethysmography (Gautier et al., 2006; Gautier and Gallego, 2008). Both head out and unrestrained plethysmography were used in the studies presented in Table 1 and Figure 2. We have observed that DBA/2J and A/J mice at P7 responded to the

instrumentation of the EMG electrodes differently, the former aggressively and the latter submissively (Balbir et al., 2008). In this study the instrumentation of EMG electrode lightly restrained the movement of animals and possibly caused discomfort. Thus, the restraint due to head out plethysmography likely evokes different responses in different strains. A comparison between several mouse strains must be performed carefully in studies using this technique. Most investigators are aware of the importance of temperature control, and therefore, the recording chamber was warmed to thermoneutral ranges. Gaultier et al. assumed the thermoneutral zone for mouse pups to be 32–33 °C that is equivalent to the temperature inside the litter (Gaultier et al., 2006). One early study with head out plethysmography used room temperature (26 °C) and obtained an extremely slow breathing frequency (37 ± 15 bpm) at P0 (Burton et al., 1997). This observation is not included in the Table 1, but gives interesting insights regarding the effects of temperature on breathing.

4. Development of the hypoxic ventilatory response

As described above, breathing measurement in newborn mice requires the careful control of many factors. Therefore, to discuss the hypoxic ventilatory response we have selected data in which measurement conditions were clearly described and they were maintained in optimal conditions. Figure 3 shows the hypoxic ventilatory response during the early phase of life (<24 hours). Most data were obtained from Gallego and Gautier's laboratory (Dauger et al., 2001; Renolleau et al., 2001a; Renolleau et al., 2001b; Aizenfisz et al., 2002). In two experiments (Swiss-IOPS and C57BL/6-129SvEv) the hypoxic challenge was given using a gas mixture of 10% O₂/3% CO₂/87% N₂ for 90 seconds. We can observe a clear difference in T_{TOT} (total breathing cycle time) at 1 hour of age between the two experiments. The reason for this difference is not apparent, but genetic differences (strain differences) may be a possible explanation. A striking observation here is the presence of the hypoxic ventilatory response at very early age (1 hour) due to mostly increased V_T (tidal volume). 3% CO₂ added to the inspiratory gas may contribute to the increase in ventilation, but at 3 hours of age, severe hypoxia (5% O₂) without CO₂ clearly increased V_T and decreased T_{TOT} resulting increased V_E. These data suggest that mice show the hypoxic ventilatory response at a very early age. Within Swiss IOPS (Renolleau et al., 2001a), the hypoxic ventilatory response is weaker at 1 and 6 hours of age compared to 12 hours of age. The response did not significantly change among 12, 24 and 48 hours of age. Two studies examined the later phase of postnatal development of the hypoxic ventilatory response (Robinson et al., 2000; Bissonnette and Knopp, 2001). Both used head out plethysmography. When we focus on the early time periods in hypoxia, relative changes in ventilation decrease from P1 and the response plateaued at ~P20 in both Swiss CD1 outbred and C57BL/6 inbred mice. In summary, available data indicate that the ventilatory response to hypoxia is present even 1 hour after birth. The responses of frequency and tidal volume to hypoxia develop differently with age, but they appear to reach steady state level around 3 weeks of age.

An important consideration is whether we can use breathing parameters from newborn mice as an indication of carotid body function. Since we do not have any information about carotid body neural output of newborn mice, here we examined the data from rats, hoping that new insights may be obtained in the relationship between carotid body function and hypoxic ventilatory response during postnatal development. Liu et al. have found dynamic changes in hypoxic ventilatory responses in rats from P0 to P21 (Liu et al., 2006). Increases in breathing parameters in response to early time periods (~30 seconds) of 10% O₂ challenge was minimal at P0, but started to be evident from P1 and reached at higher steady state level between P6 and P8. During this time, both breathing frequency and tidal volume increased. There was a big dip in the hypoxic response at P13, but subsequently the response recovered and gradually reached a steady-state towards P21 with a predominant increase in tidal volume. Usually, the hypoxic ventilatory response during early time periods of the

hypoxic challenge is considered to be mediated by the carotid body. However, carotid body chemoreceptor neural activity minimally responded to anoxic stimuli until ~P7 and after P10 the response became significantly larger than the younger ages (Kholwadwala and Donnelly, 1992). A similar response pattern was seen in the calcium response of chemoreceptor glomus cells (Bamford et al., 1999). These in vitro studies used very severe hypoxia (1% O₂) or anoxia, and it is difficult to interpolate the data to intact animals. Nonetheless, it appears that the carotid body in rats did not show a vigorous response to hypoxia until ~P10, while V_E response to hypoxia between P6 and P8 was almost comparable to that of P19 and P21. In mice, the hypoxic ventilatory response is seen earlier than in rats. The dichotomy between the breathing response and the carotid body response to hypoxia at early postnatal ages in the rat suggests that the breathing responses are not appropriate to be used as a surrogate of carotid body function in neonatal rodents. It is possible that the hypoxic ventilatory response in these animals is mediated by chemosensitive organs other than the carotid body such as the pulmonary neuroepithelial body or adrenal chromaffin cells (Nurse et al., 2006). To confirm these points, more comprehensive and comparative studies are required in measuring carotid body neural output and breathing in response to similar stimuli at the same developmental stages.

5. Morphological development of the carotid body

Morphology of the carotid body also matures postnatally. It has been shown that the innervation of glomus cells is not fully matured at birth in the rabbit (Kariya et al., 1990; Bolle et al., 2000) and the rat (Kondo, 1975). The volume of the carotid body continues to rise from birth to 10 years old in humans (Dinsdale et al., 1977; Heath and Smith, 1992). Our study in DBA/2J and A/J inbred strains has shown that the growth of the carotid body is age- and strain dependent (Kostuk et al., 2012). Carotid bodies and glomus cells at 1-day and 1-week old mice demonstrated very similar gross morphology in both strains. At 2 weeks of age, the carotid body of DBA/2J strain became clearly larger compared with the carotid bodies of younger ages. In A/J mice, the size of the carotid body did not apparently change through 1 day to 4 weeks old, but the shape of the carotid body became irregular at 2 weeks old. At 4 weeks old, the carotid body of A/J strain began to exhibit a phenotype similar to that reported in the adult A/J's carotid body (Yamaguchi et al., 2003). Volumetric measurements show (Figure 4) that the carotid bodies of both strains were similar at 1 day old. There was a trend of small increases in the carotid body size from 1 day to 1 week of age in both strains. In DBA/2J mice, the carotid bodies grew rapidly between 1 and 2 weeks of age and maintained the volume between 2 and 4 weeks of age. The volume of the carotid body at 4 weeks in DBA/2J mice is significantly larger than that of the 1 day and 1 week and was comparable to the volume of the adult DBA/2J mice. The estimated total glomus cell volume at 1 day was equivalent in both strains. In the DBA/2J mice, it increased up to 2 weeks of age and was maintained till 4 weeks, but in the A/J mice, it reached maximum at 1 week, and then, decreased continually (Kostuk et al., 2012). We have recently measured the carotid body volume of C57BL/6 mice at 2 weeks of age. The mean value ($4.3 \pm 0.3 \times 10^6 \mu\text{m}^3$, n=5) was a midpoint between the carotid bodies of DBA/2J and A/J mice. Further studies at different ages will confirm whether the carotid body of the C57BL/6 strain develops slowly compared to that of the DBA/2J strain, or if the carotid body of the C57BL/6 does not reach the volume of that of the DBA/2J strain. Taken together, these data show the differential postnatal growth of the carotid body in different inbred strains and indicate that genetic factors are regulating the development of the carotid body. This explains the large variability of the carotid body volume in humans (Dinsdale et al., 1977; Heath and Smith, 1992) and cats (Clarke and Daly, 1985), because the genetic background in humans and cats is heterogeneous.

Although the size of the carotid body may not be completely coupled with the function of the carotid body, some data indicate association between the anatomy and function of the carotid body. For example, the exposure to hyperoxia during early developmental periods induces atrophy of the carotid body and decreases the number of glomus cells in rats. In these animals, the carotid sinus nerve response to hypoxia is extremely attenuated (Bisgard et al., 2003; Prieto-Lloret et al., 2004). Further, it has been shown that the synaptic nature continues to develop up to 5 weeks in the rat (Kondo, 1976) and the rabbits (Bolle et al., 2000). These studies indicate that afferent synapses continue to increase during this period. Certainly, these ultrastructural changes would contribute to functional development of the carotid body. Detailed ultrastructural changes in the carotid body of mice are not currently available.

6. Genetic factors and the carotid body development

The use of a knockout mouse provides us with a very useful tool to understand the role of a certain gene and its product in carotid body function (Table 2). Regarding the developmental aspects of the carotid body, Kameda et al have shown that *Hoxa3* (Kameda et al., 2002) and *Mash1* (*Ascl1*) (Kameda, 2005) are required for the formation of the carotid body. *Gdnf* and *Bdnf* are also required for normal development of petrosal chemosensory neurons (Erickson et al., 2001). Other studies listed in Table 2 clearly provided critical information of genetic factors in chemosensory function. For example, homozygous or heterozygous gene knockout of *Hif1a*, *Kcnk3*, *Kcnk9*, *Drd2*, *Cth*, or *P2x2* decreased carotid chemoreceptor neural response to hypoxia. However, many knockout mice die during pre- and perinatal periods (Turgeon and Meloche 2009), and therefore, except for agenesis of the carotid body, knockout mice do not always provide useful information for understanding the postnatal development of carotid body function and morphology. In this regard, inbred strains of mice may consist of better models to reveal the genetic aspects of carotid body development.

Previous studies identified two strains of inbred mice, DBA/2J and A/J, as high and low responders to hypoxia, respectively (Tankersley et al., 1994; Rubin et al., 2003; Campen et al., 2004; Campen et al., 2005). Further, we have found differences in the carotid body structure (Yamaguchi et al., 2003), as well as hypoxic sensitivity of the CB (Otsubo et al., 2011), between these two strains. We can assume that these differences are a direct result of genetics. Our study has demonstrated differences in steady-state gene expression in the carotid body between the 4-week old DBA/2J and A/J mice (Balbir et al., 2007). We have hypothesized that 1) genes related to carotid body function are expressed less in the A/J mice compared with the DBA/2J mice, and 2) gene expression levels of morphogenic and trophic factors of the carotid body are significantly lower in the A/J mice than the DBA/2J mice. Our microarray analysis, together with real-time RT-PCR, identified many genes that fit these categories and were differentially expressed between the carotid bodies of the two strains. The results indicate that many genes are involved in the development of the carotid body and associate with hypoxic chemotransduction. All of these genes could function in concert, or independently, during the early stages of life to establish functional carotid body chemotransduction processes.

In a subsequent study (Kostuk et al., 2012), we focused on glial cell line-derived neurotrophic factor (*Gdnf*), distal-less homeobox-2 (*Dlx2*), homeobox msh-like-2 (*Msx2*), and paired-like homeobox-2b (*Phox2b*) which are more expressed in the carotid body of the 4-week old DBA/2J mice. Initially, we assumed that *Gdnf*, its receptor *Gfr- α 1*, and the *Ret* pathway were responsible for the differential growth between these two strains (see Figure 4). However, in contrast to the carotid bodies of 4 weeks old mice, *Gdnf* and its receptors were not different between the two strains at 7, 10 and 14 days of age when divergence of the morphological strain differences became apparent. The only gene that showed consistent

differences with various comparison methods was *Msx2*. *Msx2* gene was significantly less expressed in the carotid body of P7 A/J mice compared with that of DBA/2J mice. *Msx2* plays a role in the survival, differentiation, and proliferation of cranial neural crest cells (Ishii et al., 2003; Ishii et al., 2005), cardiac neural crest cells (Chen et al., 2007), as well as teeth, hair follicles, and bones of the skull (Satokata et al., 2000). Thus it is tempting to suggest that *Msx2* may play an important role in the proliferation and survival of glomus cells. Future studies using knockout mice may provide some insights as to whether and how *Msx2* contributes to the morphological development of the carotid body. An alternative approach would be an addition of other strains with the use of a multifactorial design, which can take a full advantage of using inbred strains (Festing 2010). As shown in Figure 5, the carotid body of C57BL/6 appears to show different growth pattern from the carotid bodies of DBA/2J and A/J strains. Checking a panel of genes related to neural tissue growth at the time points when these carotid bodies show different growth may add some new information in the postnatal growth of the carotid body.

7. Neonatal programming

Geographical and epidemiological studies indicate that intrauterine development closely correlates with cardiovascular diseases in later life (Barker, 2007). Recent studies have further revealed the association of perinatal developmental conditions to diabetes, obesity, hypertension and even cancer (Oken and Gillman, 2003; Tamashiro and Moran, 2010; Calkins and Devaskar, 2011). It appears that this is not limited to metabolic diseases. Inflammation during the neonatal period has been shown to have long-term effects on immune function (Spencer et al., 2010) and neuroendocrine function (Kentner and Pittman, 2010). The carotid body does not escape the long-term effects of early-life events. For example, hyperoxia during perinatal periods impairs the normal development of the carotid body (Erickson et al., 1998; Prieto-Lloret et al., 2004; Dmitrieff et al., 2012) and results in inhibited respiratory responses to hypoxia and asphyxia in adulthood (Bisgard et al., 2003; Bavis and Mitchell, 2008). In particular, prolonged hyperoxia irreversibly affects carotid body function: when rats are reared in 60% hyperoxia, the hypoxic response of carotid chemoreceptor neural activity in adulthood is severely attenuated (Bisgard et al., 2003; Prieto-Lloret et al., 2004). The effect was clearly seen after 1 week of hyperoxic exposure (Bisgard et al., 2003) and reached a plateau after 2 weeks of exposure. This is intriguing, because in the rat the carotid chemoreceptor neural response to hypoxia reaches the adult level approximately at 2 weeks of age (Kholwadwala and Donnelly, 1992; Donnelly and Doyle, 1994). Although chemoafferent degeneration was also observed in hyperoxic treated rats, chemoafferent degeneration was likely caused by the impairment of the carotid body development and subsequent decreases in neurotrophic factors (Erickson et al., 1998; Dmitrieff et al., 2011). Thus, it appears that the adverse effects on the carotid body persist into adulthood, if insults are given during this developmental window (within 2 weeks from birth in the rat).

Rodents have been used as good experimental tools for human disorders during neonatal periods due to their large litter size, a short duration to mature and availability of inbred strains and knockout mice. Several knockout mice have proved to be useful for investigating disorders of respiratory control (Gaultier et al., 2006). However, many knockout mice die perinatally, and therefore, it is difficult to examine carotid body function. In some neonatal disorders, alternative approaches would be to apply the insults described above to various inbred strains, which may cause dysfunction of the carotid body. Differential outcomes among inbred strains would be valuable means to dissect the genetic background of susceptibility in the affected population.

8. Summary and Future directions

Studies examining chemoreceptor development in mice are not plentiful. We have shown that morphological development is age and strain specific. We assume that functional development of the carotid body is also age and strain dependent, because carotid body chemoreceptor function is strain specific. However, a major challenge is the size of the carotid body, and information of carotid body neural output in neonatal mice is not currently available. The hypoxic ventilatory response has been studied in neonatal mice, but it is uncertain if the response truly reflects the carotid body function. A careful comparison of hypoxic responses of chemoreceptor neural output and ventilation in rats suggests that the hypoxic ventilatory response cannot be used to assess carotid body function in neonatal rats. Whether or not this is also the case in the mouse needs further investigation. Can we get there? The development of technology is amazingly fast. For example, measuring the fetal left ventricular pressure is now possible (Le et al., 2012), which we could not imagine 10 years ago. Thus, hopefully, we will soon establish techniques for measuring carotid chemoreceptor neural activity from young mice. Then, we can investigate developmental aspects of not only hypoxia but also other natural and pharmacological stimuli. Further, we would like to emphasize that fastidious care and careful considerations are required for obtaining tissues. Because of the small size of the carotid body and the proximity to other tissues, in particular the superior cervical ganglion (see Figure 5), the contamination or misrepresentation of the tissues by personnel who are not well-trained can easily occur. We may need to develop some standards to ensure proper handling of the tissues. These may include special markers which are expressed in the carotid body but not in the superior cervical ganglion.

Because many knockout mice die during pre- and perinatal periods, various inbred strains should be used to understand genetic mechanisms for the postnatal development of the carotid body. We still have not taken a full advantage of the controlled genetics of inbred strains. Also, development of targeted gene deletion in the carotid body is a critical alternate approach.

Acknowledgments

This work was supported by HL81345 and AHA 09GRAN2080158. Luis E Pichard was supported by F31HL096450.

References

- Aizenfisz S, Dager S, Durand E, Vardon G, Levacher B, Simonneau M, Pachnis V, Gaultier C, Gallego J. Ventilatory responses to hypercapnia and hypoxia in heterozygous c-ret newborn mice. *Respir. Physiol. Neurobiol.* 2002; 131:213–222. [PubMed: 12126922]
- Arata S, Amano K, Yamakawa K, Arata A. Central respiratory failure in a mouse model depends on the genetic background of the host. *Adv. Exp. Med. Biol.* 2010; 669:21–24. [PubMed: 20217313]
- Balbir, A. PhD dissertation. Baltimore: The Johns Hopkins University; 2008. The development of respiratory control and hypoxic sensitivity in two inbred strains of mice.
- Balbir A, Lande B, Fitzgerald RS, Polotsky V, Mitzner W, Shirahata M. Behavioral and respiratory characteristics during sleep in neonatal DBA/2J and A/J mice. *Brain Res.* 2008; 1241:84–91. [PubMed: 18817755]
- Balbir A, Lee H, Okumura M, Biswal S, Fitzgerald RS, Shirahata M. A search for genes that may confer divergent morphology and function in the carotid body between two strains of mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2007; 292:L704–L715. [PubMed: 17098806]
- Bamford OS, Sterni LM, Wasicko MJ, Montrose MH, Carroll JL. Postnatal maturation of carotid body and type I cell chemoreception in the rat. *Am. J. Physiol.* 1999; 276:L875–L884. [PubMed: 10330044]

- Barker DJ. The origins of the developmental origins theory. *J. Intern. Med.* 2007; 261:412–417. [PubMed: 17444880]
- Bavis RW, Mitchell GS. Long-term effects of the perinatal environment on respiratory control. *J. Appl. Physiol.* 2008; 104:1220–1229. [PubMed: 18187608]
- Bisgard GE, Olson EB Jr, Wang ZY, Bavis RW, Fuller DD, Mitchell GS. Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia. *J. Appl. Physiol.* 2003; 95:946–952. [PubMed: 12909596]
- Bissonnette JM, Knopp SJ. Developmental changes in the hypoxic ventilatory response in C57BL/6 mice. *Respir. Physiol.* 2001; 128:179–186. [PubMed: 11812382]
- Blain GM, Smith CA, Henderson KS, Dempsey JA. Contribution of the carotid body chemoreceptors to eupneic ventilation in the intact, unanesthetized dog. *J. Appl. Physiol.* 2009; 106:1564–1573. [PubMed: 19246650]
- Blain GM, Smith CA, Henderson KS, Dempsey JA. Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO₂. *J. Physiol.* 2010; 588:2455–2471. [PubMed: 20421288]
- Blanco CE, Dawes GS, Hanson MA, McCooke HB. The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs. *J. Physiol. (Lond).* 1984; 351:25–37. [PubMed: 6747866]
- Blanco CE, Hanson MA, McCooke HB. Effects on carotid chemoreceptor resetting of pulmonary ventilation in the fetal lamb in utero. *J. Dev. Physiol.* 1988; 10:167–174. [PubMed: 3135305]
- Bolle T, Lauweryns JM, Lommel AV. Postnatal maturation of neuroepithelial bodies and carotid body innervation: A quantitative investigation in the rabbit. *J. Neurocytol.* 2000; 29:241–248. [PubMed: 11276176]
- Boyd, JD. Contributions to Embryology. Carnegie Institution of Washington; Baltimore, MD: 1937. The Development of the Human Carotid Body; p. 1-31.
- Burton MD, Kawashima A, Brayer JA, Kazemi H, Shannon DC, Schuchardt A, Costantini F, Pachnis V, Kinane TB. RET proto-oncogene is important for the development of respiratory CO₂ sensitivity. *J. Auton. Nerv. Syst.* 1997; 63:137–143. [PubMed: 9138245]
- Calkins K, Devaskar SU. Fetal origins of adult disease. *Curr. Probl. Pediatr. Adolesc. Health Care.* 2011; 41:158–176. [PubMed: 21684471]
- Campen MJ, Tagaito Y, Jenkins TP, Balbir A, O'Donnell CP. Heart rate variability responses to hypoxic and hypercapnic exposures in different mouse strains. *J. Appl. Physiol.* 2005; 99:807–813. [PubMed: 15890760]
- Campen MJ, Tagaito Y, Li J, Balbir A, Tankersley CG, Smith P, Schwartz A, O'Donnell CP. Phenotypic variation in cardiovascular responses to acute hypoxic and hypercapnic exposure in mice. *Physiol. Genomics.* 2004; 20:15–20. [PubMed: 15494473]
- Carroll JL. Plasticity in respiratory motor control - Invited review: Developmental plasticity in respiratory control. *J. Appl. Physiol.* 2003; 94:375–389. [PubMed: 12486025]
- Carroll JL, Bamford OS, Fitzgerald RS. Postnatal maturation of carotid chemoreceptor responses to O₂ and CO₂ in the cat. *J. Appl. Physiol.* 1993; 75:2383–2391. [PubMed: 8125854]
- Carroll JL, Fitzgerald RS. Carotid chemoreceptor responses to hypoxia and hypercapnia in developing kittens. *Adv. Exp. Med. Biol.* 1993; 337:387–391. [PubMed: 8109427]
- Chen YH, Ishii M, Sun J, Sucov HM, Maxson RE Jr. Msx1 and Msx2 regulate survival of secondary heart field precursors and post-migratory proliferation of cardiac neural crest in the outflow tract. *Dev. Biol.* 2007; 308:421–437. [PubMed: 17601530]
- Chia R, Achilli F, Festing MF, Fisher EM. The origins and uses of mouse outbred stocks. *Nat. Genet.* 2005; 37:1181–1186. [PubMed: 16254564]
- Clancy B, Finlay BL, Darlington RB, Anand KJ. Extrapolating brain development from experimental species to humans. *Neurotoxicology.* 2007; 28:931–937. [PubMed: 17368774]
- Clarke JA, Daly MD. The volume of the carotid body and periadventitial type I and type II cells in the carotid bifurcation region of the fetal cat and kitten. *Anat. Embryol. (Berl).* 1985; 173:117–127. [PubMed: 4073529]
- Collins FS, Rossant J, Wurst W. A mouse for all reasons. *Cell.* 2007; 128:9–13. [PubMed: 17218247]

- Dahan A, Nieuwenhuijs D, Teppema L. Plasticity of central chemoreceptors: effect of bilateral carotid body resection on central CO₂ sensitivity. *PLoS. Med.* 2007; 4:e239. [PubMed: 17676946]
- Dauger S, Aizenfisz S, Renolleau S, Durand E, Vardon G, Gaultier C, Gallego J. Arousal response to hypoxia in newborn mice. *Respir. Physiol.* 2001; 128:235–240. [PubMed: 11812388]
- Dauger S, Durand E, Cohen G, Lagercrantz H, Changeux JP, Gaultier C, Gallego J. Control of breathing in newborn mice lacking the beta-2 nAChR subunit. *Acta. Physiol. Scand.* 2004; 182:205–212. [PubMed: 15450117]
- Dauger S, Pattyn A, Lofaso F, Gaultier C, Goridis C, Gallego J, Brunet JF. Phox2b controls the development of peripheral chemoreceptors and afferent visceral pathways. *Development.* 2003; 130:6635–6642. [PubMed: 14627719]
- Dinsdale F, Emery JL, Gadsdon DR. The carotid body--a quantitative assessment in children. *Histopathology.* 1977; 1:179–187. [PubMed: 615836]
- Dmitrieff EF, Piro SE, Broge TA Jr, Dunmire KB, Bavis RW. Carotid body growth during chronic postnatal hyperoxia. *Respir. Physiol. Neurobiol.* 2012; 180:193–203. [PubMed: 22138179]
- Dmitrieff EF, Wilson JT, Dunmire KB, Bavis RW. Chronic hyperoxia alters the expression of neurotrophic factors in the carotid body of neonatal rats. *Respir. Physiol. Neurobiol.* 2011b; 175:220–227. [PubMed: 21094282]
- Donnelly DF. Developmental aspects of oxygen sensing by the carotid body. *J. Appl. Physiol.* 2000; 88:2296–2301. [PubMed: 10846048]
- Donnelly DF, Doyle TP. Developmental changes in hypoxia-induced catecholamine release from rat carotid body, in vitro. *J. Physiol. (Lond).* 1994; 475:267–275. [PubMed: 8021833]
- Donnelly DF, Rigual R. Single-unit recordings of arterial chemoreceptors from mouse petrosal ganglia in vitro. *J. Appl. Physiol.* 2000; 88:1489–1495. [PubMed: 10749846]
- Durand E, Dauger S, Pattyn A, Gaultier C, Goridis C, Gallego J. Sleep-disordered breathing in newborn mice heterozygous for the transcription factor Phox2b. *Am. J. Respir. Crit. Care Med.* 2005; 172:238–243. [PubMed: 15860752]
- Enhörning G, van Schaik S, Lundgren C, Vargas I. Whole-body plethysmography, does it measure tidal volume of small animals? *Can. J. Physiol. Pharmacol.* 1998; 76:945–951. [PubMed: 10100875]
- Erickson JT, Brosenitsch TA, Katz DM. Brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor are required simultaneously for survival of dopaminergic primary sensory neurons in vivo. *J. Neurosci.* 2001; 21:581–589. [PubMed: 11160437]
- Erickson JT, Mayer C, Jawa A, Ling L, Olson EB Jr, Vidruk EH, Mitchell GS, Katz DM. Chemoafferent degeneration and carotid body hypoplasia following chronic hyperoxia in newborn rats. *J. Physiol. (Lond).* 1998; 509(Pt 2):519–526. [PubMed: 9575300]
- Evans HE, Sack WO. Prenatal development of domestic and laboratory mammals: growth curves, external features and selected references. *Zentralbl. Veterinarmed. C.* 1973; 2:11–45. [PubMed: 4745140]
- Festing MF. Inbred strains should replace outbred stocks in toxicology, safety testing, and drug development. *Toxicol. Pathol.* 2010; 38:681–690. [PubMed: 20562325]
- Gauda EB, Carroll JL, Donnelly DF. Developmental maturation of chemosensitivity to hypoxia of peripheral arterial chemoreceptors--invited article. *Adv. Exp. Med. Biol.* 2009; 648:243–255. [PubMed: 19536487]
- Gaultier C, Gallego J. Neural control of breathing: insights from genetic mouse models. *J. Appl. Physiol.* 2008; 104:1522–1530. [PubMed: 18218910]
- Gaultier C, Matrot B, Gallego J. Transgenic models to study disorders of respiratory control in newborn mice. *ILAR J.* 2006; 47:15–21. [PubMed: 16391427]
- He L, Chen J, Dinger B, Sanders K, Sundar K, Hoidal J, Fidone S. Characteristics of carotid body chemosensitivity in NADPH oxidase-deficient mice. *Am. J. Physiol. Cell. Physiol.* 2002; 282:C27–C33. [PubMed: 11742795]
- Heath, D.; Smith, P. Diseases of the human carotid body. Springer-Verlag; London: 1992.
- Hervonen A, Korkala O. Fine structure of the carotid body of the midterm human fetus. *Z. Anat. Entwicklungsgesch.* 1972; 138:135–144. [PubMed: 4344460]

- Holley HS, Behan M, Wenninger JM. Age and sex differences in the ventilatory response to hypoxia and hypercapnia in awake neonatal, pre-pubertal and young adult rats. *Respir. Physiol. Neurobiol.* 2012; 180:79–87. [PubMed: 22067556]
- Ishii M, Han J, Yen HY, Sucov HM, Chai Y, Maxson RE Jr. Combined deficiencies of *Msx1* and *Msx2* cause impaired patterning and survival of the cranial neural crest. *Development.* 2005; 132:4937–4950. [PubMed: 16221730]
- Ishii M, Merrill AE, Chan YS, Gitelman I, Rice DP, Sucov HM, Maxson RE Jr. *Msx2* and *Twist* cooperatively control the development of the neural crest-derived skeletogenic mesenchyme of the murine skull vault. *Development.* 2003; 130:6131–6142. [PubMed: 14597577]
- Kameda Y. *Mash1* is required for glomus cell formation in the mouse carotid body. *Dev. Biol.* 2005; 283:128–139. [PubMed: 15878769]
- Kameda Y, Ito M, Nishimaki T, Gotoh N. *FRS2* alpha 2F/2F mice lack carotid body and exhibit abnormalities of the superior cervical sympathetic ganglion and carotid sinus nerve. *Dev. Biol.* 2008; 314:236–247. [PubMed: 18177855]
- Kameda Y, Nishimaki T, Takeichi M, Chisaka O. Homeobox gene *hoxa3* is essential for the formation of the carotid body in the mouse embryos. *Dev. Biol.* 2002; 247:197–209. [PubMed: 12074562]
- Kariya I, Nakjima T, Ozawa H. Ultrastructural study on cell differentiation of the rabbit carotid body. *Arch. Histol. Cytol.* 1990; 53:245–258. [PubMed: 2390325]
- Karlsson KA, Blumberg MS. The union of the state: myoclonic twitching is coupled with nuchal muscle atonia in infant rats. *Behav. Neurosci.* 2002; 116:912–917. [PubMed: 12369810]
- Karlsson KA, Kreider JC, Blumberg MS. Hypothalamic contribution to sleep-wake cycle development. *Neuroscience.* 2004; 123:575–582. [PubMed: 14698764]
- Kazemian P, Stephenson R, Yeger H, Cutz E. Respiratory control in neonatal mice with NADPH oxidase deficiency. *Respir. Physiol.* 2001; 126:89–101. [PubMed: 11348637]
- Kentner AC, Pittman QJ. Minireview: early-life programming by inflammation of the neuroendocrine system. *Endocrinology.* 2010; 151:4602–4606. [PubMed: 20668021]
- Kholwadwala D, Donnelly DF. Maturation of carotid chemoreceptor sensitivity to hypoxia: in vitro studies in the newborn rat. *J. Physiol. (Lond).* 1992; 453:461–473. [PubMed: 1464840]
- Kline DD, Buniel MC, Glazebrook P, Peng YJ, Ramirez-Navarro A, Prabhakar NR, Kunze DL. *Kv1.1* deletion augments the afferent hypoxic chemosensory pathway and respiration. *J. Neurosci.* 2005; 25:3389–3399. [PubMed: 15800194]
- Kline DD, Peng YJ, Manalo DJ, Semenza GL, Prabhakar NR. Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99:821–826. [PubMed: 11792862]
- Kline DD, Yang T, Premkumar DR, Thomas AJ, Prabhakar NR. Blunted respiratory responses to hypoxia in mutant mice deficient in nitric oxide synthase-3. *J. Appl. Physiol.* 2000; 88:1496–1508. [PubMed: 10749847]
- Kondo H. A light and electron microscopic study on the embryonic development of the rat carotid body. *Am. J. Anat.* 1975; 144:275–293. [PubMed: 1211368]
- Kondo H. An electron microscopic study on the development of synapses in the rat carotid body. *Neurosci. Lett.* 1976; 3:197–200. [PubMed: 19604886]
- Kostuk EW, Balbir A, Fujii K, Fujioka A, Pichard LE, Shirahata M. Divergent postnatal development of the carotid body in DBA/2J and A/J strains of mice. *J. Appl. Physiol.* 2012; 112:490–500. [PubMed: 22074716]
- Krimsky WR, Leiter JC. Physiology of breathing and respiratory control during sleep. *Semin. Respir. Crit. Care Med.* 2005; 26:5–12. [PubMed: 16052413]
- Le VP, Kovacs A, Wagenseil JE. Measuring left ventricular pressure in late embryonic and neonatal mice. *J. Vis. Exp.* 2012
- Liu Q, Lowry TF, Wong-Riley MT. Postnatal changes in ventilation during normoxia and acute hypoxia in the rat: implication for a sensitive period. *J. Physiol.* 2006; 577:957–970. [PubMed: 17038423]
- Mortola JP, Noworaj A. Breathing pattern and growth: comparative aspects. *J. Comp. Physiol. B.* 1985; 155:171–176.

- Mortola JP, Rezzonico R, Lanthier C. Ventilation and oxygen consumption during acute hypoxia in newborn mammals: a comparative analysis. *Respir. Physiol.* 1989; 78:31–43. [PubMed: 2813986]
- Mulligan, E. Discharge properties of carotid bodies: Developmental aspects. In: Haddad, GG.; Farber, JP., editors. *Developmental Neurobiology of Breathing*. Marcel Dekker, Inc.; New York: 1991. p. 321–340.
- Nsegbe E, Wallen-Mackenzie A, Dauger S, Roux JC, Shvarev Y, Lagercrantz H, Perlmann T, Herlenius E. Congenital hypoventilation and impaired hypoxic response in Nurr1 mutant mice. *J. Physiol.* 2004; 556:43–59. [PubMed: 14742729]
- Nurse CA, Buttigieg J, Thompson R, Zhang M, Cutz E. Oxygen sensing in neuroepithelial and adrenal chromaffin cells. *Novartis. Found. Symp.* 2006; 272:106–114. [PubMed: 16686432]
- Oken E, Gillman MW. Fetal origins of obesity. *Obes. Res.* 2003; 11:496–506. [PubMed: 12690076]
- Ortega-Saenz P, Levitsky KL, Marcos-Almaraz MT, Bonilla-Henao V, Pascual A, Lopez-Barneo J. Carotid body chemosensory responses in mice deficient of TASK channels. *J. Gen. Physiol.* 2010; 135:379–392. [PubMed: 20351062]
- Ortega-Saenz P, Pascual A, Gomez-Diaz R, Lopez-Barneo J. Acute oxygen sensing in heme oxygenase-2 null mice. *J. Gen. Physiol.* 2006; 128:405–411. [PubMed: 16966473]
- Otsubo T, Kostuk EW, Balbir A, Fujii K, Shirahata M. Differential Expression of Large-Conductance Ca-Activated K Channels in the Carotid Body between DBA/2J and A/J Strains of Mice. *Front. Cell Neurosci.* 2011; 5:19. [PubMed: 22013411]
- Pack AI, Galante RJ, Maislin G, Cater J, Metaxas D, Lu S, Zhang L, Von Smith R, Kay T, Lian J, Svenson K, Peters LL. Novel method for high-throughput phenotyping of sleep in mice. *Physiol. Genomics.* 2007; 28:232–238. [PubMed: 16985007]
- Peng YJ, Nanduri J, Khan SA, Yuan G, Wang N, Kinsman B, Vaddi DR, Kumar GK, Garcia JA, Semenza GL, Prabhakar NR. Hypoxia-inducible factor 2alpha (HIF-2alpha) heterozygous-null mice exhibit exaggerated carotid body sensitivity to hypoxia, breathing instability, and hypertension. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108:3065–3070. [PubMed: 21288809]
- Peng YJ, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. H₂S mediates O₂ sensing in the carotid body. *Proc. Natl. Acad. Sci. U. S. A.* 2010; 107:10719–10724. [PubMed: 20556885]
- Peng YJ, Nanduri J, Yuan G, Wang N, Deneris E, Pendyala S, Natarajan V, Kumar GK, Prabhakar NR. NADPH oxidase is required for the sensory plasticity of the carotid body by chronic intermittent hypoxia. *J. Neurosci.* 2009; 29:4903–4910. [PubMed: 19369559]
- Pepper DR, Landauer RC, Kumar P. Postnatal development of CO₂-O₂ interaction in the rat carotid body in vitro. *J. Physiol. (Lond).* 1995; 485:531–541. [PubMed: 7666372]
- Piruat JI, Pintado CO, Ortega-Saenz P, Roche M, Lopez-Barneo J. The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. *Mol. Cell. Biol.* 2004; 24:10933–10940. [PubMed: 15572694]
- Prieto-Lloret J, Caceres AI, Obeso A, Rocher A, Rigual R, Agapito MT, Bustamante R, Castaneda J, Perez-Garcia MT, Lopez-Lopez JR, Gonzalez C. Ventilatory responses and carotid body function in adult rats perinatally exposed to hyperoxia. *J. Physiol. (Lond).* 2004; 554:126–144. [PubMed: 14678497]
- Prieto-Lloret J, Donnelly DF, Rico AJ, Moratalla R, Gonzalez C, Rigual RJ. Hypoxia transduction by carotid body chemoreceptors in mice lacking dopamine D(2) receptors. *J. Appl. Physiol.* 2007; 103:1269–1275. [PubMed: 17673562]
- Renolleau S, Dauger S, Autret F, Vardon G, Gaultier C, Gallego J. Maturation of baseline breathing and of hypercapnic and hypoxic ventilatory responses in newborn mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2001a; 281:R1746–R1753. [PubMed: 11641148]
- Renolleau S, Dauger S, Vardon G, Levacher B, Simonneau M, Yanagisawa M, Gaultier C, Gallego J. Impaired ventilatory responses to hypoxia in mice deficient in endothelin-converting-enzyme-1. *Pediatr. Res.* 2001b; 49:705–712. [PubMed: 11328956]
- Rigual R, Almaraz L, Gonzalez C, Donnelly DF. Developmental changes in chemoreceptor nerve activity and catecholamine secretion in rabbit carotid body: possible role of Na⁺ and Ca²⁺ currents. *Pflugers Arch.* 2000; 439:463–470. [PubMed: 10678743]

- Rigual R, Rico AJ, Prieto-Lloret J, de Felipe C, Gonzalez C, Donnelly DF. Chemoreceptor activity is normal in mice lacking the NK1 receptor. *Eur. J. Neurosci.* 2002; 16:2078–2084. [PubMed: 12473075]
- Robinson DM, Kwok H, Adams BM, Peebles KC, Funk GD. Development of the ventilatory response to hypoxia in Swiss CD-1 mice. *J. Appl. Physiol.* 2000; 88:1907–1914. [PubMed: 10797156]
- Rogers DC. The development of the rat carotid body. *J. Anat.* 1965; 99:89–101. [PubMed: 14245349]
- Rong W, Gourine AV, Cockayne DA, Xiang Z, Ford AP, Spyer KM, Burnstock G. Pivotal role of nucleotide P2X2 receptor subunit of the ATP-gated ion channel mediating ventilatory responses to hypoxia. *J. Neurosci.* 2003; 23:11315–11321. [PubMed: 14672995]
- Roux JC, Dura E, Villard L. Tyrosine hydroxylase deficit in the chemoafferent and the sympathoadrenergic pathways of the Mecp2 deficient mouse. *Neurosci. Lett.* 2008; 447:82–86. [PubMed: 18834926]
- Roy A, Rozanov C, Mokashi A, Daudu P, Al mehdi AB, Shams H, Lahiri S. Mice lacking in gp91 phox subunit of NAD(P)H oxidase showed glomus cell $[Ca^{2+}]_i$ and respiratory responses to hypoxia. *Brain Res.* 2000; 872:188–193. [PubMed: 10924691]
- Rubin AE, Polotsky VY, Balbir A, Krishnan JA, Schwartz AR, Smith PL, Fitzgerald RS, Tankersley CG, Shirahata M, O'Donnell CP. Differences in sleep-induced hypoxia between A/J and DBA/2J mouse strains. *Am. J. Respir. Crit Care Med.* 2003; 168:1520–1527. [PubMed: 14512266]
- Sanders KA, Sundar KM, He L, Dinger B, Fidone S, Hoidal JR. Role of components of the phagocytic NADPH oxidase in oxygen sensing. *J. Appl. Physiol.* 2002; 93:1357–1364. [PubMed: 12235036]
- Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, Maeda T, Takano Y, Uchiyama M, Heaney S, Peters H, Tang Z, Maxson R, Maas R. Mx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. *Nat. Genet.* 2000; 24:391–395. [PubMed: 10742104]
- Schaub CD, Tankersley C, Schwartz AR, Smith PL, Robotham JL, O'Donnell CP. Effect of sleep/wake state on arterial blood pressure in genetically identical mice. *J. Appl. Physiol.* 1998; 85:366–371. [PubMed: 9655797]
- Schneider BF, Norton S. Equivalent ages in rat, mouse and chick embryos. *Teratology.* 1979; 19:273–278. [PubMed: 473080]
- Shea SA. Behavioural and arousal-related influences on breathing in humans. *Exp. Physiol.* 1996; 81:1–26. [PubMed: 8869137]
- Smith C. The origin and development of the carotid body. *Am J Anat.* 1924; 34:87–131.
- Smith P, Scraggs M, Heath D. The development of the nerve network in the fetal human carotid body and its subsequent function in cardiac disease. *Cardioscience.* 1993; 4:143–149. [PubMed: 8400021]
- Spencer SJ, Field E, Pittman QJ. Neonatal programming by neuroimmune challenge: effects on responses and tolerance to septic doses of lipopolysaccharide in adult male and female rats. *J. Neuroendocrinol.* 2010; 22:272–281. [PubMed: 20136690]
- Sterba O. Staging and ageing of mammalian embryos and fetuses. *Acta Veterinaria Brno.* 1995; 64:83–89.
- Tagaito Y, Polotsky VY, Campen MJ, Wilson JA, Balbir A, Smith PL, Schwartz AR, O'Donnell CP. A model of sleep-disordered breathing in the C57BL/6J mouse. *J. Appl. Physiol.* 2001; 91:2758–2766. [PubMed: 11717244]
- Tamashiro KL, Moran TH. Perinatal environment and its influences on metabolic programming of offspring. *Physiol. Behav.* 2010; 100:560–566. [PubMed: 20394764]
- Tankersley CG, Fitzgerald RS, Kleeberger SR. Differential control of ventilation among inbred strains of mice. *AmJPhysiol.* 1994; 267:R1371–R1377.
- Timmers HJ, Karemaker JM, Wieling W, Marres HA, Folgering HT, Lenders JW. Baroreflex and chemoreflex function after bilateral carotid body tumor resection. *J. Hypertens.* 2003; 21:591–599. [PubMed: 12640254]
- Trapp S, Aller MI, Wisden W, Gourine AV. A role for TASK-1 (KCNK3) channels in the chemosensory control of breathing. *J. Neurosci.* 2008; 28:8844–8850. [PubMed: 18753386]
- Trapp S, Tucker SJ, Gourine AV. Respiratory responses to hypercapnia and hypoxia in mice with genetic ablation of Kir5.1 (Kcnj16). *Exp. Physiol.* 2011; 96:451–459. [PubMed: 21239463]

- Turgeon B, Meloche S. Interpreting neonatal lethal phenotypes in mouse mutants: insights into gene function and human diseases. *Physiol Rev.* 2009; 89:1–26. [PubMed: 19126753]
- Wang ZY, Bisgard GE. Postnatal growth of the carotid body. *Respir. Physiol. Neurobiol.* 2005; 149:181–190. [PubMed: 15914098]
- Wasicko MJ, Sterni LM, Bamford OS, Montrose MH, Carroll JL. Resetting and postnatal maturation of oxygen chemosensitivity in rat carotid chemoreceptor cells. *J. Physiol. (Lond).* 1999; 514(Pt 2):493–503. [PubMed: 9852330]
- Yamaguchi S, Balbir A, Schofield B, Coram J, Tankersley CG, Fitzgerald RS, O'Donnell CP, Shirahata M. Structural and functional differences of the carotid body between DBA/2J and A/J strains of mice. *J. Appl. Physiol.* 2003; 94:1536–1542. [PubMed: 12626474]

Figure 1.

Carotid body chemoreceptor neural activity from three inbred strains of mice (DBA/2J, A/J and FVB strains). Top: Raw activities of carotid sinus nerve in response to hypoxic challenges (grey bars, $F_{I}O_2$ 0.15 and 0.1 for 90 seconds). Bottom: Mean chemoreceptor activity over time. The signal was processed via Fast Fourier Transformation and the Hann weighing function. It was normalized against the sum of the total power of all experiments. N_2 challenge (30 seconds) was used to confirm the viability of the nerve. The response of carotid body chemoreceptor activity to hypoxia varied among the three inbred strains. The carotid body of A/J mice responded only to severe stimulus (N_2 challenge). PaO_2 reached ~40 mmHg when mice were ventilated with 10% O_2 for 90 seconds.

Figure 2.

Changes in breathing frequency with postnatal maturation (from P1). Substantial changes in breathing frequency occur initial several days after birth. A large variation is also observed among different strains. Measuring conditions may have influenced the results as well.

Symbols and references as follows:

■

Swiss-IOPS, unrestrained whole body plethysmography (Renolleau et al., 2001a);

□

Swiss CD1, head out plethysmography (Robinson et al., 2000);

▲

Swiss, unrestrained whole body plethysmography, (Dauger et al., 2001);

▲

ICR, unrestrained whole body plethysmography, (Arata et al., 2010);

▲

BALB/c, unrestrained whole body plethysmography, (Arata et al., 2010);

F1 of BALB/c & C57BL/6, unrestrained whole body plethysmography (Arata et al., 2010);

C57BL/6, unrestrained whole body plethysmography (Arata et al., 2010)

C57BL/6, head out plethysmography (Bissonnette and Knopp, 2001);

C57BL/6, unrestrained whole body plethysmography (Dauger et al., 2004);

C57BL/6, head out plethysmography (Kazemian et al., 2001);

DBA/2J, unrestrained whole body plethysmography (Balbir, 2008)

A/J, unrestrained whole body plethysmography (Balbir, 2008).

Figure 3.

Hypoxic ventilatory response during 24 hours of age. Values within initial 1 min of hypoxic challenge were taken from figures and tables of several references (listed below). All experiments were performed using unrestrained whole body plethysmography except for the last bar (P0) which was used head out plethysmography. Gas contents during challenge varied: some are pure hypoxia (5 % or 7.4 %, indicated in the top panel) and others are 10% O_2 /3% CO_2 /87% N_2 (*). Data are mean $\pm$ SE. Swiss derived outbred stocks were mainly used for the study: Swiss-IOPS (Renolleau et al., 2001a), Swiss (Dauger et al., 2001), Swiss CD1 (Robinson et al., 2000), C57BL/6-129SvEv (Renolleau et al., 2001b), 129SvEv-C57BL/10 (Aizenfisz et al., 2002).

Figure 4.

Age-dependent changes in the volume of the carotid body in the DBA/2J and A/J strains. Carotid bodies were obtained at 1 day (1D), 1 week (1W), 2 weeks (2W), and 4 weeks (4W) of age. Scattergram shows the distribution of the volume of the carotid body in the DBA/2J (●) and A/J (○) mouse strains. Mean of the values is represented by the black line through the plotted values. No significant difference is found between the strains in the growth of the carotid body at 1D and 1W. DBA/2J strain has a significant increase in the volume of the carotid body from 1W to 2W, and has a significantly greater carotid body volume than the A/J strain at 2W and 4W. A/J strain shows no significant growth from 1D to 4W. Statistical analysis was performed using one-way ANOVA (between 4 age groups within a strain) and a Student's *t*-test (between the strains at a corresponding age group). **P* < 0.05, values significantly different between strains. §*P* < 0.05, values significantly different from 1D. #*P* < 0.05, values significantly different from 1W. (Reproduced from (Kostuk et al., 2012))

Figure 5.

Microphotographs showing the carotid body and a part of the superior cervical ganglion (SCG) from three inbred mice at 14 days old (A: DBA/2J strain; B: A/J strain; C: C57BL/6 strain). The carotid body is encircled by a solid line. The largest sections of the carotid body in each mouse are presented. A general cellular structure of the carotid body of the mouse is very similar to those of large animals, but the carotid body is much more closely located to the SCG compared to the carotid body of larger animals. Some of the neurons in the SCG are indicated by arrows.

Table 1

Breathing characteristic during first 24 hours of life.

Strain	Age (hours)	Methods	Chamber Temperature	Frequency (bpm)	T ₁₀₀ (msec)	Tidal Volume (μL/g)	References
Swiss-IOPS [‡]	1	WBP	32 °C	115 *	524 *	3.0 *	(Renolleau et al., 2001a)
	6			132 *	454 *	4.1 *	
	12			160 *	376 *	5.5 *	
	24			119 *	503 *	4.9 *	
Swiss CDI	P0 [#]	HO	35–36 °C	55 ± 7	1091 *	12.7 ± 1.3	(Robinson et al., 2000)
Swiss	3	WBP	30.5 °C	159 *	377 *	2.1 *	(Dauger et al., 2001)
	12			144 *	417 *	4.6 *	
ICR BALB/c F1 of BALB/c & C57BL/6	P0 [#]	WBP	NM	88.6 ± 21.0	677 *	39.2 ± 9.4 [‡]	(Arata et al., 2010) [§]
C57BL/6	P0 [#]			69.5 ± 13.9	863 *	26.5 ± 8.0 [‡]	
	P0 [#]			142.8 ± 45.3	420 *	15.1 ± 4.6 [‡]	
	P0 [#]			101.5 ± 26.6	591 *	20.5 ± 6.3 [‡]	
C57BL/6	6–12	HO	32–34 °C	120.1 ± 9.5	500 *	NM	(Erickson et al., 2001)
C57BL/6-129SvEv	1	WBP	28–30 °C	95 *	630 ± 54	3.1 ± 0.4	(Renolleau et al., 2001b) [§]
C57BL/10-129SvEv	10–12	WBP	32 °C	127 *	474 *	5.3 *	(Aizenfisz et al., 2002)

Data show mean values and in some cases ± SE.

[#] exact age (hour) was not provided.

[‡]The original include two sets of delivery groups (vaginal and cesarean section), but only data from vaginal delivery group are presented here. WBP: non-restrained whole body plethysmography; HO: head out plethysmography.

* these values are estimated from the figures or numbers they presented;

[‡] these values were not normalized by weight; NM: not mentioned;

[§]The pups were delivered by cesarean section.

Table 2

Gene knockout and chemoreceptor function.

Gene	Effects of Gene Deletion				Reference	
	Carotid Chemoreceptor Neural Activity		Breathing		Other Effects	
Gene Name	Alternative Name	Basal	Hypoxia	Basal	Hypoxia	
Putative Chemosensors	Cybb	gp91-phox	NT	↔	↔	[Ca ²⁺] _i ↔ (Roy et al., 2000)
	Nox2	↔	↔	NT	NT	K current inhibition ↔, [Ca ²⁺] _i ↔ (He et al., 2002)
						sLTF ↓ (Peng et al., 2009)
	Ncf1	P47-phox	↑	↑		↑ (Sanders et al., 2002)
Hmox2	Hemoxygenase 2	NT	NT	NT	NT	CA release ↓ mRNA (Cyc ↑, TH ↑, Slo1 ↓) Volume ↑ BK channel activity↔ (Ortega-Saenz et al., 2006)
Hif1a	Hif1-alpha	↔	↓ (+/-)	↔ (+/-)	↔ (+/-) ↓ Hyperoxia (+/-)	Morphology ↔ (+/-) (Kline et al., 2002)
Epas1	Hif2a	↔	↑ (+/-)	Irregular (+/-) Apnea ↑	↑ (+/-)	Morphology ↑ (+/-) CO ₂ response ↔ (+/-) (CCNA) (Peng et al., 2011)
Sdh	Sdh4	NT	NT	NT	NT	CA release ↑ (+/-) TH+ cells in the CB ↑ (+/-) K current ↔ (+/-) (Piruat et al., 2004)
K Channels	Kcna1	Kv1.1	↔	↑	↔	↑ (Kline et al., 2005)
	Kcnk3	Task1	↔	↓	↔	↓ CO ₂ response ↓ (CCNA) (Trapp et al., 2008)
		NT	NT	NT	NT	Volume ↔ (Ortega-Saenz et al., 2010)

Gene	Effects of Gene Deletion					Reference
	Carotid Chemoreceptor Neural Activity			Breathing		
Gene Name	Alternative Name	Basal	Hypoxia	Basal	Hypoxia	
Kcnk9	Task3	↑	↓	↔	↔	↔ Membrane potential ↔ K current, Ca current ↔ CA release ↔
		NT	NT	NT	NT	↔ CO ₂ response ↓ (CCNA) (Trapp et al., 2008)
Kcnj16	Kir5.1	↔	↔	↔	↓	↔ CO ₂ response ↓ (Trapp et al., 2011)
		NT	NT	Anesthetized ↓ Awake ↔	Anesthetized ↓ Awake ↓	↔ GC ↑ CO ₂ response ↔ (breathing) (Kline et al., 2000)
Nos3	Enos	NT	NT	NT	NT	↔ CA content ↔ CA secretion ↔ (Rigual et al., 2002)
		NT				
		↔	↔	↔	↔	↔ CA release ↑ (Prieto-Lloret et al., 2007)
Drd2	D2r	↔	↓	↔	↔	↔ CO ₂ response ↔ (Peng et al., 2010)
		↔	↔	↔	↔	↔ CA release ↑ (Rong et al., 2003)
P2rx2	P2x2	↔	↓	↔	↓	↔ CO ₂ response ↔ (breathing) (Rong et al., 2003)
		↔	↔	↔	↔	
		↔ (dbl-/-)	↓↓ (dbl-/-)	↔	↓ (dbl-/-)	
P2rx3	P2x3	↔	↔	↔	↔	
		↔	↔	↔	↔	
Hoxa3	Hox1e	NT	NT	NT	NT	↔ CO ₂ response ↔ (breathing) (Kameda et al., 2002)
		NT	NT	NT	NT	↔ CA release ↑ (Rong et al., 2003)
Ascl1	Mash1	NT	NT	NT	NT	↔ CA release ↑ (Rong et al., 2003)
		NT	NT	NT	NT	↔ CA release ↑ (Rong et al., 2003)

Gene	Effects of Gene Deletion				Reference	
	Carotid Chemoreceptor Neural Activity		Breathing			
Gene Name	Alternative Name	Basal	Hypoxia	Basal	Hypoxia	Other Effects
Meep2		NT	NT	↔	↑	TH staining (CB, PG) ↓ (Roux et al., 2008)
Phox2b	Pmx2b	NT	NT	↔ (+/-)	↔ (+/-) Posthypoxic (+/-)	Volume ↓ (fetal), then degenerate No TH staining CO ₂ response ↓ (breathing @ P2) PG ↓ (Dauger et al., 2003)
Nr4a2	Nurr1	NT	NT	↓, Apnea ↑	↓	Maximum diameter, TH cells ↔ (Nsegbe et al., 2004)
others	Bdnf	NT	NT	NT	NT	PG ↓ (Erickson et al., 2001)
	Gdnf	NT	NT	↓, Apnea ↑	NT	PG ↓ PG ↓↓ (dbl-/-)
	Frs2	Frs2a	NT	NT	NT	NT

Due to the availability of a large number of knockout mice, the list may not include all published work. Studies which measured only breathing parameters are not included.

- ↔ no change;
- ↑ increase;
- ↓ decrease;

NT, not tested; sLTF, long term facilitation of sensory nerve discharge; CA, catecholamine; Cyc, cyclophilin; TH, tyrosine hydroxylase; CB, carotid body; CCNA, carotid chemoreceptor nerve activity; PG, petrosal ganglion.