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Morphological differences of the carotid body among C57/BL6 (B6), A/J, and CSS B6A1 mouse strains

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Abstract

The C57/BL6 (B6) mouse strain exhibits post-hypoxic frequency decline and periodic breathing, as well as greater amount of irregular breathing during rest in comparison to the A/J and to the B6a1, a chromosomal substitution strain whereby the A/J chromosome 1 is bred onto the B6 background (Han et al., 2002; Yamauchi et al., 2008a,b). The hypothesis was that morphological differences in the carotid body would associate with such trait variations. After confirming strain differences in post-hypoxic ventilatory behavior, histological examination ($n = 8$ in each group) using hematoxylin and eosin (H&E) staining revealed equivalent, well-defined tissue structure at the bifurcation of the carotid arteries, an active secretory parenchyma (type I cells) from the supportive stromal tissue, and clustering of type I cells in all three strains. Tyrosine hydroxylase (TH) immunohistochemical staining revealed a typical organization of type I cells and neurovascular components into glomeruli in all three strains. Image analysis from 5 μ m sections from each strain generated a series of cytological metrics. The percent carotid body composition of TH+ type I cells in the A/J, B6 and B6a1 was $20 \pm 4\%$, $39 \pm 3\%$, and $44 \pm 3\%$, respectively ($p = 0.00004$). However, cellular organization in terms of density and ultrastructure in the B6a1 is more similar to the B6 than to the A/J. These findings indicate that genetic mechanisms that produce strain differences in ventilatory function do not associate with carotid body structure or tyrosine hydroxylase morphology, and that A/J chromosome 1 does not contribute much to B6 carotid body morphology.

Keywords

Hypoxia; Carotid body; Murine breathing; Type I cell; Chromosomal substitution strain

1. Introduction

In mammals, the carotid body is a small neural-crest derived organ located at or about the bifurcation of the common carotid artery into the internal and external carotid arteries

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(Heath, 1983; Lopez-Barneo et al., 2008). It is a small cluster of encapsulated cells that reside at this vascular junction, and provides afferent physiological input to the brainstem concerning blood oxygen and pH levels. At this strategic and high-flow location, the carotid body is the first point for detecting and responding to transient, acute hypoxic events (Garcia-Fernandez et al., 2007; Peers and Buckler, 1995). In rats the size of the carotid body is larger in spontaneously hypertensive rats compared to normotensive strains (Habeck et al., 1987), and in mice there are reported differences in the A/J and DBA/2J strains in terms of structure and function, with the A/J having blunted hypoxic response and a lower TH cell quantity and irregular morphology (Yamaguchi et al., 2003).

Previous work has documented that the B6 is an interesting strain, showing respiratory arrhythmogenesis (Stettner et al., 2008b), and displaying periodic breathing (PB) in the post-hypoxic period defined as clustered breathing with either waxing and waning of ventilation or consistently recurrent end-expiratory pauses (apnea) of 2 average breath durations (Han et al., 2002). The post-hypoxic pauses increase after administration of a neural nitric oxide synthetase inhibitor (Price et al., 2003). The B6a1 recombinant inbred strain, whereby an A/J chromosome 1 is bred onto the B6 background, more closely resembles the A/J post-hypoxic ventilatory behavior, namely regular breathing; furthermore, at rest the substitution with an A/J chromosome 1 (or buspirone, a partial 5-HT_{1A} agonist) is associated with a reduction in the appearance of spontaneous pauses and post-sigh apneas (Yamauchi et al., 2008b).

Aside from abundant neurovascular and stromal (collagenous tissue) elements, the carotid body is morphologically comprised of two main cell types: type I and type II. The type II cell type has been described as a sustentacular, glial-type cell that supports the functional role of the type I cell. Dissociated type I cells respond to acute hypoxia with membrane depolarization and subsequent calcium-modulated neurotransmitter release (Duchen and Biscoe, 1992). This neuroendocrine-like cell produces and responds to catecholamines (dopamine and norepinephrine) (Wang and Bisgard, 2002). The marker for this pathway is tyrosine hydroxylase (TH), a lower amount of which has been linked to lower hypoxic response (Yamaguchi et al., 2003).

Therefore, we used strain differences as a surrogate for genetic manipulation to examine the hypothesis that glomus cell TH morphology of the carotid body correlates with variations in ventilatory behavior known to differ among C57BL/6 (B6), A/J, and B6a1 mouse strains. The general morphology was generally assessed as well as specific markers for vascular architecture (von Willebrand factor, vWF) (Vischer, 2006), nitric oxide transduction (neural nitric oxide synthase, nNOS, given the response to 7-NI) (Kline et al., 1998; Price et al., 2003), and mitochondrial location and density (pyruvate dehydrogenase, PDH) (Papandreou et al., 2006).

2. Materials and methods

2.1. Experimental animals

We used mice from each strain for physiological ($n = 6$) and morphological ($n = 8$) analysis. The animals were purchased from Jackson Laboratories (Bar Harbor, Maine USA) and

animals were all 6-week-old males. The animals were housed in the same room under 12:12:L:D cycles and fed the same mouse chow and water ad libitum at least 2 weeks prior to experimentation (approved by the Louis Stokes CDVAMC IACUC). The animals were euthanized via 2 min of exposure to CO₂ in a designated necropsy chamber and then underwent cervical dislocation as mandated by the IACUC protocol.

2.2. Measurement of ventilatory behavior

Ventilatory behavior was measured by placing animals in a 600 ml Lucite cylindrical plethysmography chamber, and this apparatus has been described previously (Han et al., 2002; Price et al., 2003; Yamauchi et al., 2008b). Briefly, we measured breathing data via pressure change across a pressure transducer (Validyne DP45, Validyne Engineering, Northridge, CA 91324, USA), which was also attached to a reference chamber of 600 ml. The variations of pressure in the chamber were expressed as voltage swings by the transducer output, and were recorded using Lab View 7.1 (National Instruments, Austin, TX 78710, USA). The output from Lab View, was then analyzed and scored using a program custom written for our lab (Breath Detect).

2.3. Breathing data analysis

Baseline ventilation was obtained by scoring normoxic breathing from all of the animals, excluding sniffs and sighs. This resting breathing data were averaged for each strain, and strain specific averages for *f*, time per breath, and TV (estimated from voltage changes) were obtained. Post-hypoxic breathing was measured for the first minute of reoxygenation as well as the fifth minute of reoxygenation. Sniffs were purged from the data, and spontaneous apneas were defined as a pause for 2 respiratory cycles; sighs identified and noted in the event there were post-sigh apneas (Yamauchi et al., 2008b). If a significant ($p < 0.05$) increase or decrease in the *f* occurred from baseline to the post-hypoxic period, the strain was found to have short term potentiation or post-hypoxic frequency decline, respectively (Yamauchi et al., 2008a). Baseline and post-hypoxic respiratory parameters were compared among the three strains and significance was determined using ANOVA. Pairwise significant differences were reported if any two strains differed significantly ($p < 0.05$) according to unpaired *t*-test analysis. Values are expressed as mean $\pm$ standard deviation.

2.4. Carotid body extraction

The carotid body was located using standard anatomical landmarks as described by Donnelly and Rigual (2000). Upon extraction, the carotid bifurcation was immediately immersed in neutrally buffered 10% formalin. The collected samples were kept at 4 °C overnight (~12 h).

2.5. Morphological analysis

One carotid body per animal ($n = 8$ per strain) was used for morphological analysis. Extracted carotid bodies were fixed in formalin for 24 h and were thereafter stored in 0.01 M PBS at 4 °C. Samples were embedded in paraffin and serial 5 μ m sections were obtained. Hematoxylin and eosin (H&E; Sigma–Aldrich, St. Louis, MO, USA) staining was

performed at 5 sequential levels (50 μ m separation between levels) of the carotid body to compare general cellularity and morphology. All sections were imaged using a brightfield microscope and high resolution digital camera (Olympus BX51, Olympus America Inc.). Images were cropped in ImageJ (ImageJ 1.43t) prior to analysis such that only the carotid body was obtained (that is peripheral vasculature, adipose tissue, connective tissue and other stromal elements were excluded). ImageJ is a well-regarded Java-based freeware developed at the National Institutes of Health and routinely used for image processing by investigators in the life sciences (Rasband, 1997–2010). Identical thresholds for circularity, color, and cell size were used for all sections imaged in each of the three strains. As the use of these thresholds only allowed for a rough approximation of glomus cell density, immunostaining against TH was employed, as outlined below.

2.6. Immunohistochemistry

H&E stained slides were used to determine corresponding sections (eleven slides per strain) for tyrosine hydroxylase (TH) immunohistochemical staining. The same process was repeated for neural nitric oxide synthase (nNOS), von Willebrand factor (vWF) and pyruvate dehydrogenase (PDH). For all four immunohistochemical stains, the general protocol was the same. Images stained with TH were analyzed for specific cellularity using ImageJ as described above. This software was used to calculate area and pixel value statistics of immunohistochemically defined selections with intensity threshold to count objects. Due to the selective staining of glomus cells using this method, a confident estimate of cell density was obtained. Strains were compared using an unpaired *t*-test, equal variances assumed.

3. Results

3.1. General anatomy and histology of the carotid body

The *en bloc* extraction (see Supplementary Methods—Anatomy) was performed using well-known anatomical landmarks and tissue macrostructures (e.g. trachea, sternohyoideus, major salivary glands, anterior belly of the digastric, and vagus nerve). Variations in the orientation of the carotid body to the common carotid artery as well as to collateral vasculature in the site of interest occur in humans (Gulsen et al., 2009; Itirezote et al., 2009; Prendes et al., 1980), and was present; but did not appear to be correlated to strain. A conserved characteristic across the three strains was the presence of the carotid sinus branch of the glossopharyngeal nerve (cranial nerve IX) in H&E section (Figs. 1 and 2). Histologically, it resembled a light-purple wavy bundle of tissue entering the organ and further disseminating its branches throughout the carotid body parenchyma. Another commonly observed element was the passage of the vagus nerve just lateral to the common carotid artery (Figs. 1 and 2). The proximity of the carotid body to the common carotid bifurcation explains the appearance of this nerve in section as all three aforementioned structures course in or are situated along the carotid sheath (which contains the common carotid, vagus nerve, internal jugular vein, and presumably a portion or whole of the carotid body).

As shown by hematoxylin and eosin (H&E) stain in Figs. 1 and 2, in each strain the carotid body presented itself as a globular, highly basophilic clustering of parenchymal cells nestled

in a web of stromal architecture typical of the carotid fascia which includes collagenous connective tissue and varying degrees of adipose tissue. The type I cells are identified in H&E by their ovoid appearance and they are relatively equal in diameter across strains (~10 μ m). The type II sustentacular, glial-type cells are also present with their thin and spindly nuclei. These cells are typically woven throughout the neurovascular elements of the organ. The carotid body has prominent micro-capillary vasculature (provided mostly by small branches of the external carotid artery and other collateral sources in the mouse) and innervation. Visual examination of the TH stain in carotid body sections across the three strains of mice did not disclose saliently discernable differences in size or shape of type I cells (Fig. 3).

3.2. Cytological analysis

A comparison across strains using cytological metrics in H&E section revealed no differences in cellular composition or gross morphological makeup (Table 1). There appeared to be no significant differences in total area per cut of non-sustentacular basophilic cells (putative type I cells) across the three strains. In the metric of % carotid body composition of type I cells as determined by size in H&E sections, all three strains displayed similar values (Table 1). Although there appears to be a trend in cell density (B6a1 > B6 > A/J), this metric does not reach statistical significance.

We performed a similar analysis using TH immunochemical stained sections to definitively identify secretory type I cells (Table 2). There exists a discrepancy between the % carotid body composition of type I cells between the H&E and TH stained sections. This should be considered an artifact of counting cells as each stain is designed to perform separate tasks. The H&E provides a survey of the cellular landscape in the carotid body and thus includes more cells that may or may not be actual type I cells. This presents the need for the TH stain which only selectively stains type I cells. The metric that reaches statistical significance is percent carotid body composition of type I cells (Table 2, Row 3, p value = 0.00004) with the percentage of the carotid body composed of type I cells (type I Cell total area/Carotid Body total area) lowest in the A/J. The B6 and B6a1 have similar values, despite strain-specific differences in ventilatory patterning at rest and after hypoxia (Yamauchi et al., 2008b).

3.3. Additional immunohistochemistry stains

Three additional immunohistochemical markers were employed to investigate possible morphological idiosyncrasies across strains with regard to vascular histoarchitecture, mitochondrial enzymatic activity, and nitric oxide transduction (Fig. 4). No prominent differences were observed among the strains for these immunohistochemical markers. It appears that all major detectable capillary, mitochondrial and nitric oxide histoelements were present with little interstrain inhomogeneity.

3.4. Respiratory data

Under normoxic conditions, no significant differences were found between the B6a1, B6, or A/J in f , V_T , or V_E (Supplementary Material Table 1). During the first minute of hypoxia, the B6a1 and A/J animals demonstrated a greater increase in f , V_T , and V_E over baseline

than the B6 (Supplementary Material Table 2 and Fig. 5). By the fifth minute, the A/J continued to demonstrate a larger increase in respiratory frequency than the B6, but the B6a1 are at this point indistinguishable from the B6. The AJ animals possessed a larger V_E than both the B6 and B6a1 during the fifth minute of hypoxia (Supplementary Material Table 3).

Following hypoxia – during the first minute of reoxygenation with 100% O_2 – both A/J and B6a1 displayed STP, whereas no significant change was noted in the B6 animals (Fig. 5). In addition, A/J and B6a1 animals demonstrated a significantly larger post-hypoxic f to baseline f ratio than the B6 animals (Supplementary Material Table 3). Representative tracings of breathing data in the three strains can be found in Han et al. (2001). Also during this 1st minute of post-hypoxic period, all 6 of the B6 animals displayed one or more spontaneous pauses, whereas none of the B6a1 or A/J animals experienced spontaneous pauses (Supplementary Material Table 3). By the 5th minute of reoxygenation, neither the A/J nor the B6a1 still exhibited STP, but the B6 did display PHFD (Fig. 5). At this time, only one of the B6 animals displayed any apneic activity, and the AJ still exhibited a larger post-hypoxic/baseline frequency than the B6 (Supplementary Material Table 3).

4. Discussion

4.1. Respiratory phenotype

Data from the present study confirm previous reports of intrastrain differences in respiratory stability. Our findings support previous reports that A/J mice breathe at a higher frequency than B6 mice under acute hypoxic exposure, and that the A/J, but not the B6, displays post-hypoxic STP (Han et al., 2001).

Respiratory phenotypes have previously been associated with chromosome 1 in the mouse. Prior investigations suggest that substituting A/J chromosome 1 onto a B6 genetic background rescues the B6 phenotype of the post-sigh respiratory apneas (Yamauchi et al., 2008a). Also, a recent intercross study linked variation in minute ventilation in response to hypercapnic hypoxia in the mouse to a region of chromosome 1 (Tankersley and Broman, 2004). Moreover, a diverse and growing body of documented QTLs implicates loci on chromosome 1 to clinically relevant pulmonary traits ranging from lung cancer susceptibility to hyperoxic acute lung injury survival (Cho and Kleeberger, 2007; Festing et al., 1998; Prows et al., 2009; Tripodis et al., 2001; Wang et al., 2004).

4.2. Carotid body morphology

The carotid body of the A/J mouse has the lowest proportion of TH+ type I cells per unit area compared to B6 and chromosomal substitution B6a1 strains of mice (Table 2). However, this findings as well as the similarity in discernible histoarchitectural features in H&E stains do not correlate with ventilatory phenotypes with hypoxia or upon reoxygenation. Additional immunohistochemical strain-wise comparisons showed similar morphological elements for vascular organization, mitochondrial distribution, and neural nitric oxide synthase.

Prior reports on A/J carotid body morphology described the organ as having ambiguous lines of demarcation in relation to the stromal connective tissue and surrounding neurovascular elements. Furthermore, the carotid body was described as being volumetrically smaller than in the comparison strain (DBA/2J). Additionally, the A/J carotid body was described as having poorly developed type I cells (Yamaguchi et al., 2003). We confirmed a more sporadic and rarefied distribution of type I cells in terms of TH positive immunohistochemical staining (lowest TH+ proportion) in the A/J compared to the other two strains, but did not find cytoarchitectural differences. We suspect that variations in the orientation of the carotid body to the common carotid artery as well as to the collateral vasculature could contribute to variations during surgical extraction and therefore affect cellular estimates or structural inhomogeneity. Such anatomic variations in location of the carotid body around the bifurcation of the common carotid are found in humans (Gulsen et al., 2009; Iterezote et al., 2009; Prendes et al., 1980), so presumably genetic or epigenetic factors operate to architecturally position this organ.

4.3. Additional immunohistochemical staining

Three other stains were used to characterize the three strains and likewise did not associate with the functional traits. Von Willebrand's factor (vWF) is a well regarded endothelial glycoprotein marker used to identify vascular organization (Vischer, 2006). Pyruvate dehydrogenase (PDH) is an enzyme that is found exclusively in the mitochondria and has been shown recently to be involved in a hypoxia-triggered metabolic shunt that maintains ATP production and lowers toxic ROS production (Kim et al., 2006). Nitric oxide (NO) is a messenger molecule involved in the regulation of respiration and specifically with neuronal NOS having been shown, in part, to influence the respiratory response to hypoxia and reoxygenation in the mouse (Kline et al., 1998; Price et al., 2003). Its presence in type I cells is not unprecedented (Dvorakova and Kummer, 2005; Li et al., 2010; Leite et al., 2010). Type I cells are derived from neural crest (Pearse et al., 1973). All three strains showed similar patterns, so either the stain appropriately identified the neural-like properties or a non-specific staining occurred equally in all strains. Furthermore, NOS blockade by non-specific NOS inhibitor has been shown to elicit strain differences in a rat model in regard to metabolism and post-hypoxic ventilatory response (Subramanian et al., 2002). Morphological observation did not reveal any prominent differences in these immunohistochemical comparisons among strain. We can say with certainty that although the respiratory phenotype of the B6 and B6a1 are different, their carotid bodies look similar.

4.4. Future directions

Like Yamaguchi et al. (2003) we would have concluded from comparison of only two strains that differences in ventilatory phenotype might be correlated with carotid body type I TH+ glomus cells. However, the 'tiebreaker' is the B6a1 strain which has a ventilatory phenotype more closely resembling the A/J, but has a carotid body morphological configuration more close to that of the B6. We know by study of chromosomal substitution strains that substitution of other A/J chromosomes onto the B6 background alters the B6 phenomena of post-hypoxic ventilatory decline to that of potentiation, as well as alter the effects of 7-NI, even while post-hypoxic period breathing is preserved (Yamauchi et al., 2010). In this study we did not do functional studies of the isolated carotid body. We cannot

conclude that the carotid body does not contribute to trait differences, but such a correlation is not at a morphological level. These studies can be performed when there is identification of the genetic polymorphism(s) that contribute to hypoxic sensitivity.

On the other hand, the lack of correlation of carotid body TH morphologic differences to ventilation might indicate a role for central controller effects on the trait. This idea is supported by the work showing that spontaneous post-inspiratory apneas is present in the B6 in *in situ* working heartbeat-brainstem preparations in the complete absence of higher brain centers and afferent stimulation; this finding and the present study suggest that the origin of the respiratory arrhythmia is in the ponto-medullary central pattern generator (Stettner et al., 2008b). Furthermore, this might include an effect of serotonergic tone in the raphe nuclei projecting to regions essential for central respiratory regulation (Hilaire and Duron, 1999). Additionally, it has been proposed by Richter et al. that 5-HT_{1A} receptor activation stabilizes respiratory arrhythmias by attenuating the excitability of respiratory rhythmogenic neurons (Richter et al., 2003). 5-HT_{1A} receptor agonists have been shown to decrease the occurrence of spontaneous apneas in awake, unrestrained B6 mice (Yamauchi et al., buspirone paper) and abolish them altogether in an *in situ* working heartbeat-brainstem preparation (Stettner et al., 2008a). Additionally, the retrotrapezoid nucleus (RTN) receives inputs from the carotid bodies and has been proposed to exert a tonic excitatory drive to the central pattern generator of respiratory centers in the brainstem (Guyenet et al., 2009), and may be an important integration site for peripheral chemoreceptive and somatic inputs. The role of 5HT_{1A} receptors in the medullary rhythmogenic centers of the brainstem and their modulation by genetic polymorphisms is suggested by our study and becomes a tractable question using chromosomal substitution models.

4.5. Conclusion

In summary, we have shown that the morphology of the carotid body in terms of its size and shape did not differ significantly among the A/J, B6, and B6a1 strains of mice. In finding that the A/J strain had the lowest proportion of type I cells among strains we confirmed previous work that described the A/J carotid body as having “abnormal” characteristics. However, aside from this observation, all other morphological characteristics typically found in the carotid body appeared to be present and identifiable across a wide spectrum of staining methodologies in the A/J. Although substitution of A/J chromosome 1 rescues the instability of breathing phenotype, the carotid body morphology of the B6 is controlled by determinants on other chromosomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.resp.2011.04.021.

Fig. 1.

[1a–3b] Representative 5 μ m sections (paraffin embedded, hematoxylin & eosin stain) of the carotid body (CB) from the A/J, B6, and B6a1 mouse strains at 10 $\times$ followed by a 20 $\times$ close-up of the same section. The common carotid artery and carotid sinus nerves have been labeled. The carotid body and local stromal elements are present (including but not limited to loose connective tissue, adipose tissue, smooth muscle and vascular endothelial cells, erythrocytes). See text for further discussion. Scale bar represents 2310.05 pixels to 1.0 mm at 10 $\times$ and 4644.19 pixels to 1.0 mm at 20 $\times$.

Fig. 2.

[Panel A] Representative 5 μ m sections of the carotid body (CB) from the A/J, B6, and B6a1 at 40 $\times$ magnification. [Panel B] Representative 5 μ m sections of cranial nerve X (vagus nerve) as it takes a parallel course to the common carotid artery (CCA) in the AJ, B6 and B6a1 at 10 $\times$ magnification. This neuroelement was consistently used as a navigational landmark during the extraction of the CB, and images are from sections inferior to the appearance of the CB at the bifurcation of the CCA into the internal carotid artery (ICA) and the external carotid artery (ECA). [Panel C] Representative 5 μ m sections (paraffin embedded, hematoxylin & eosin stain) of the carotid sinus branch of the cranial nerve IX (glossopharyngeal nerve) to the CB from the A/J, B6, and B6a1 at 20 $\times$ magnification. Arrows points to unique appearances of the carotid sinus branch as it emerges from the stromal connective tissue to innervate the carotid body. The nerve is displayed in longitudinal section and has a wavy purple appearance (axons and myelin sheaths) with Schwann cells strewn throughout. See text for further discussion. Scale bar represents 4644.19 pixels to 1.0 mm at 20 $\times$ and 9300.19 pixels to 1.0 mm at 40 $\times$.

A. A/J Carotid Body (Tyrosine Hydroxylase, 20x)

B. B6 Carotid Body (Tyrosine Hydroxylase, 20x)

C. B6a1 Carotid Body (Tyrosine, Hydroxylase, 20x)

Fig. 3.

Representative 5 μ m sections of the carotid body (CB) from the A/J, B6, and B6a1 mouse strains at 20 $\times$ magnification evaluating for tyrosine hydroxylase. The glomeruli are present and identifiable throughout the sections. See text for further discussion. Scale bar represents 4644.19 pixels to 1.0 mm at 20 $\times$.

Fig. 4.

[Panels A–D] Representative 5 μ m sections of the carotid body (CB) from the A/J, B6, and B6a1 mouse strains at 40 $\times$ magnification. Strainwise comparison of CB immunoreactivity was evaluated for tyrosine hydroxylase, von Willebrand factor, pyruvate dehydrogenase, and neural nitric oxide synthase. See text for further discussion. Scale bar represents 9300.19 pixels to 1.0 mm at 40 $\times$.

Fig. 5. Comparison of baseline to post-hypoxic breathing at 1 min and 5 min of reoxygenation. Bars represent the mean and the SD of values for respiratory frequency for the three strains. See text for discussion. *Relationship significant at $p < 0.05$ according to paired t -test.

Table 1

Quantitative description of carotid body morphology in H&E stained sections. Cytometric values in H&E stained sections.

	A/J	B6	B6a1	Strain effect <i>p</i>-value
Total area of non-sustentacular basophilic cell (μm^2)	6.74 ± 0.70	6.05 ± 0.51	5.01 ± 0.53	0.1433
% carotid body composition of type I glomus cells	$37 \pm 2\%$	$38 \pm 1\%$	$35 \pm 3\%$	0.5990
Cell density (cells/ μm^2)	5.85 ± 0.39	6.84 ± 0.50	7.31 ± 0.55	0.1292

p-Values obtained from strain-wise ANOVA with post hoc strain ID.

Table 2

Quantitative description of carotid body morphology in TH stained sections. Cytometric values in tyrosine hydroxylase (TH) stained sections.

	A/J	B6	B6a1	Strain effect <i>p</i>-value
Total area of non-sustentacular basophilic cell (μm^2)	3.51 ± 0.68	5.18 ± 1.1	8.14 ± 1.90	0.0621
% carotid body composition of type I glomus cells	$20 \pm 4\%$	$39 \pm 3\%$	$44 \pm 3\%$	0.00004
Cell density (cells/ μm^2)	5.94 ± 0.84	9.079 ± 0.85	7.73 ± 1.11	0.0838

p-Values obtained from strain-wise ANOVA with post hoc strain ID.