

Developmental changes in GABAergic neurotransmission to presympathetic and cardiac parasympathetic neurons in the brainstem

[Olga Dergacheva](#), [Carie R. Boychuk](#), and [David Mendelowitz](#)

Department of Pharmacology and Physiology, The George Washington University, Washington, District of Columbia

✉ Corresponding author.

Address for reprint requests and other correspondence: O. Dergacheva, Dept. of Pharmacology and Physiology, The George Washington Univ., 2300 Eye St. NW, Washington, DC 20037 (e-mail: olgad@gwu.edu).

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Abstract

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Cardiovascular function is regulated by a dynamic balance composed of sympathetic and parasympathetic activity. Sympathoexcitatory presympathetic neurons (PSNs) in the rostral ventrolateral medulla project directly to cardiac and vasomotor sympathetic preganglionic neurons in the spinal cord. In proximity to the PSNs in the medulla, there are preganglionic cardiac vagal neurons (CVNs) within the nucleus ambiguus, which are critical for parasympathetic control of heart rate. Both CVNs and PSNs receive GABAergic synaptic inputs that change with challenges such as hypoxia and hypercapnia (H/H). Autonomic control of cardiovascular function undergoes significant changes during early postnatal development; however, little is known regarding postnatal maturation of GABAergic neurotransmission to these neurons. In this study, we compared changes in GABAergic inhibitory postsynaptic currents (IPSCs) in CVNs and PSNs under control conditions and during H/H in *postnatal day 2–5* (P5), *16–20* (P20), and *27–30* (P30) rats using an *in vitro* brainstem slice preparation. There was a significant enhancement in GABAergic neurotransmission to both CVNs and PSNs at age P20 compared with P5 and P30, with a more pronounced increase in PSNs. H/H did not significantly alter this enhanced GABAergic neurotransmission to PSNs in P20 animals. However, the frequency of GABAergic IPSCs in PSNs was reduced by H/H in P5 and P30 animals. In CVNs, H/H elicited an inhibition of GABAergic neurotransmission in all ages studied, with the most pronounced inhibition occurring at P20. In conclusion, there are critical development periods at which significant rearrangement occurs in the central regulation of cardiovascular function.

Keywords: presympathetic, parasympathetic, neurons, brainstem, development

SYMPATHETIC AND PARASYMPATHETIC control of cardiovascular function undergoes significant alterations after birth. Tonic sympathetic regulation of the heart begins before birth in rats and is present in 7- to

10-day-old pups; however, sympathetic influence on heart rate decreases around 20 days of age ([Adolph 1967](#); [Kasparov and Paton 1997](#)). In contrast, tonic cardiac vagal motor activity has little influence on the heart at birth but progressively reaches a mature level between the second and third weeks of postnatal age in rats ([Adolph 1967](#); [Kasparov and Paton 1997](#); [Tucker and Johnson 1984](#)). Altered autonomic functions have been implicated in some disorders prevalent at specific postnatal ages, such as sudden infant death syndrome (SIDS) ([Hunt 2006](#); [Kluge et al. 1988](#); [Leistner et al. 1980](#); [Schechtman et al. 1988](#); [Southall et al. 1988](#)). SIDS incidence peaks during a narrow developmental time window, specifically between 2 and 6 mo of infant age, which represents an unstable time for many physiological systems ([Filiano and Kinney 1994](#); [Willinger et al. 1991](#)). However, little information is known regarding changes in presympathetic and parasympathetic brainstem mechanisms underlying the maturation of autonomic cardiovascular control.

A major central mechanism responsible for the generation of sympathetic outflow includes the activity of presympathetic neurons (PSNs) in the rostral ventrolateral medulla (RVLM) ([Dampney 1994](#); [Wang et al. 2009](#)). PSNs project directly to cardioacceleratory and vasomotor sympathetic preganglionic neurons in the intermediolateral nucleus of the upper thoracic spinal cord ([Dampney et al. 2002](#); [Zagon and Smith 1993](#)). Electrical or chemical stimulation of the RVLM produces elevations of arterial pressure, tachycardia, and sympathoexcitation ([Ross et al. 1984a](#)) indicating PSNs in the RVLM play a crucial role in generating sympathetic cardiovascular activity ([Calaresu and Yardley 1988](#); [Schobel et al. 2002](#); [Wang et al. 2009](#)). Electrophysiological studies have shown PSNs under resting conditions display two distinct tonic firing rates and receive synaptic inputs, including neurotransmission from GABAergic neurons ([Brown and Guyenet 1985](#); [Schreihöfer and Guyenet 2002](#)).

Parasympathetic activity dominates the control of heart rate under resting conditions in the adult and primary originates from preganglionic cardiac vagal neurons (CVNs) within the nucleus ambiguus (NA) ([Mendelowitz 1999](#); [Wang et al. 2001a, b](#)). CVNs send their fibers through the vagi nerves to cardiac ganglia ([Mendelowitz 1999](#); [Nosaka et al. 1982](#)). Focal electrical or chemical stimulation of the NA region elicits decreases in heart rate mediated by the activation of CVNs ([Ciriello and Calaresu 1982](#); [Ciriello et al. 2003](#)). These neurons are intrinsically silent, and electrophysiological studies have identified major synaptic pathways including activation of GABAergic pathways and postsynaptic receptors in CVNs ([Dergacheva et al. 2012](#); [Mendelowitz 1996](#); [Neff et al. 2004](#); [Wang et al. 2001a](#)).

SIDS and other cardiorespiratory developmental disorders are often accompanied by apneas and episodes of hypoxia and hypercapnia (H/H) ([Cummings et al. 2011](#); [Meny et al. 1994](#); [Poets et al. 1999](#)). In addition to their activity under control conditions, the activity of both PSNs and CVNs is strongly modulated by challenges such as H/H or hypoxia alone ([Dergacheva et al. 2009](#); [Kamendi et al. 2009](#); [Sun and Reis 1994a, b](#)). In this study, we examined changes in maturation of central sympathetic and parasympathetic control of cardiovascular function, and, more specifically, we compared changes in GABAergic neurotransmission to fluorescently identified bulbospinal PSNs and parasympathetic CVNs both under control conditions and during H/H in *postnatal day 2–5* (P5), *P16–20* (P 20), and *P27–30* (P30) rats.

MATERIALS AND METHODS

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This study was performed on Sprague-Dawley rats (Hilltop, Scottsdale, PA). All animal procedures were performed in compliance with the institutional guidelines at George Washington University and are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication *Guide for the Care and Use of*

Laboratory Animals. All procedures were approved by the GWU IACUC. All efforts were made to minimize the number of animals used and any possible discomfort.

To retrogradely label parasympathetic CVNs, rat pups (*postnatal days 1–3*) were anesthetized with hypothermia and received a right thoracotomy. The heart was exposed, and 0.05 ml of 1–5% rhodamine (XRITC; Molecular Probes, Eugene, OR) was injected into the pericardial sac (see [Fig. 1](#)). Specificity of the cardiac vagal labeling has been confirmed in a previous study by the absence of any labeled neurons in the brainstem when rhodamine is injected either outside the pericardial sac or within the pericardial sac if the cardiac branch of the vagus nerve is sectioned ([Bouairi et al. 2004](#)).

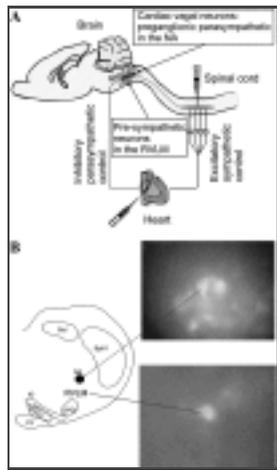


Fig. 1.

Schematic drawing of the experimental preparation. *A*: both bulbospinal presympathetic neurons (PSNs) and parasympathetic cardiac vagal neurons (CVNs) reside in the medulla oblongata. Retrograde labeling of PSNs was performed by injecting of the tracer ...

The retrograde labeling of bulbospinal PSNs was performed as described previously ([Boychuk et al. 2012](#); [Hayar and Guyenet 1999](#); [Li et al. 1995](#); [Li and Guyenet 1996](#)). Rat pups (*postnatal days 1–3*) were anesthetized with hypothermia. Upper thoracic spinal cord was exposed, and the retrograde tracer cholera toxin subunit B conjugated with Alexa Fluor 555 (1%, Invitrogen, Carisban, CA) was injected bilaterally into T2–T4 spinal segments (1–2 deposits per side, total volume, 50 nl). While the injection sites were not restricted to the intermediolateral cell column because of the small size of the spinal cord ([Hayar and Guyenet 1999](#)), RVLM neurons that were retrogradely labeled from the thoracic spinal cord have been shown to selectively innervate the intermediolateral cell column with no projections to thoracic dorsal or ventral horns ([Ross et al. 1984b](#)). Neurons retrogradely labeled from thoracic spinal cord have been found in the ventrolateral, ventromedial, and dorsomedial medulla ([Li et al. 1995](#)). However, only labeled PSNs within the triangular region bounded by the apex of the rostral portion of the NA (immediately caudal to the facial nucleus), the ventral border of the spinal trigeminal tract, and the lateral border of the pyramidal tract, which corresponds to the location of the RVLM ([Lipski et al. 1998](#); [Moon et al. 2002](#)), were used for experiments in this study (see [Fig. 1](#)).

On the day of experiment, P5 pups were anesthetized with isoflurane and killed by rapid cervical dislocation. Under a dissection microscope, the brain was removed, and a single slice of the medulla (300- μ m thickness) was obtained using a vibratome in cold (4°C) buffer (140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 5 mM glucose, and 10 mM HEPES) continually gassed with 100% O₂.

To obtain viable brain slices from older P20 and P30 animals, the methodology by Ye and colleagues ([Ye et al. 2006](#)) was adopted. Following this approach, glycerol-based artificial cerebrospinal fluid (aCSF) was used for cardiac perfusion and brainstem sectioning. Glycerol-based aCSF contained (in mM): 252 glycerol, 1.6 KCl, 1.2 NaH₂PO₄, 1.2 MgCl, 2.4 CaCl₂, 26 NaHCO₃, and 11 glucose. On the day of experiment, the older animals were anesthetized with isoflurane, and glycerol-based aCSF (4°C) was perfused transcidentally before death. The brain was removed and embedded in 2% low-melt agarose and mounted for coronal brainstem sectioning on a VF300 compressstone (Precisionary Instruments, San Jose, CA). A single slice of the medulla (300- μ m thickness) was obtained.

The obtained slices (from animals of all ages) were transferred to solution of the following composition (in mM): 110 *N*-methyl-*D*-glucamine (NMDG), 2.5 KCl, 1.2 NaH₂PO₄, 25 NaHCO₃, 25 glucose, 110 HCl, 0.5 CaCl₂, and 10 mM MgSO₄ equilibrated with 95% O₂ and 5% CO₂ (pH 7.4) at 34°C for 15 min. This brief protective recovery step using NMDG-based aCSF reduces neuronal swelling during rewarming and is critically important for viable brain slice preparation in animals ([Zhao et al. 2011](#)). The slices were then transferred from NMDG-based aCSF to a recording chamber, which allowed perfusion (5–10 ml/min) of aCSF at room temperature (25°C) containing (in mM): 125 NaCl, 3 KCl, 2 CaCl₂, 26 NaHCO₃, 5 glucose, and 5 HEPES equilibrated with 95% O₂ and 5% CO₂ (pH 7.4) for at least 1 h before experiments were conducted.

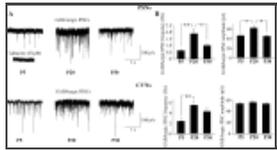
Individual CVNs in the NA, or, in another set of experiments, PSNs in the RVLM were identified by the presence of the appropriate fluorescent tracer ([Fig. 1](#)). These identified neurons were then imaged with differential interference contrast optics, infrared illumination, and infrared-sensitive video detection cameras to gain better spatial resolution. Patch pipettes (2.5–3.5 MΩ) were filled with a solution consisting (in mM) of 150 KCl, 2 MgCl₂, 2 EGTA, 10 HEPES, and 2 Mg-ATP, pH 7.4. With this pipette solution, the chloride current induced by activation of GABA receptors was recorded as an inward current. Voltage clamp whole cell recordings were made at a holding potential of –80 mV with an Axopatch 200B and pClamp 8 software (Axon Instruments, Union City, CA).

GABAergic inhibitory postsynaptic currents (IPSCs) were isolated by focal release of strychnine (1 μM), *D*-2-amino-5-phosphonovalerate (50 μM), and 6-cyano-7-nitroquinoxaline-2,3-dione (50 μM) to block glycine, *N*-methyl-*D*-aspartate, and non-*N*-methyl-*D*-aspartate glutamatergic receptors, respectively, using a pneumatic picopump pressure system (WPI, Sarasota, FL). A drug pipette was positioned within 30 μm of the patched CVN or PSN. The maximum range of drug application was determined previously to be 100–120 μm downstream from the drug pipette and was considerably less behind the drug pipette ([Wang et al. 2002](#)). After the end of experiments, the GABAergic receptor antagonist gabazine (25 μM) was bath applied to abolish GABAergic IPSCs. In all neurons ($n = 55$) successfully exposed to gabazine (25 μM) at the end of the recordings that focused on GABAergic IPSCs, gabazine reduced the event frequency to $3.4 \pm 1.2\%$ (average) of control frequency, indicating that prior focal application of glycinergic and glutamatergic blockers provided near complete blockage of non-GABAergic postsynaptic currents. All drugs used in this study were purchased from Sigma-Aldrich Chemical (St. Louis, MO).

Spontaneous GABAergic IPSCs were recorded for 10 min in control aCSF. Slices were then exposed to H/H by changing control aCSF to an identical solution bubbled with 85% N₂, 6% O₂, and 9% CO₂ (pH 7.1). Slices were exposed to H/H for 10 min, and then slices were reoxygenated for 20 min by returning the perfusate to the control aCSF.

Analysis of GABAergic IPSCs was performed using MiniAnalysis (version 5.6.12; Synaptosoft, Decatur, GA). Threshold was set at root-mean-square noise multiplied by 5. Data were analyzed during the last 2 min of the control period, the first 2 min of H/H, min 4–5, and the last 2 min of 10-min H/H period as well as during the first 2 min of post-H/H, min 4–5, and 9–10 min post-H/H. Results are presented as means \pm SE. To test for differences in GABAergic IPSC amplitude and frequency under control conditions as well as IPSC frequency percentage change from control levels during H/H among P5, P20, and P30 animals, the results were statistically compared using an ANOVA and Tukey post hoc test. To test for changes in IPSC amplitude and frequency elicited by H/H in each experimental group, the results were statistically compared using one-way repeated measures ANOVA and Dunnett's post hoc test. Significant difference was set at $P < 0.05$. Only one experiment was performed in each

Developmental changes in GABAergic neurotransmission to PSNs under control conditions. P20 animals possessed an increased GABAergic neurotransmission to PSNs under control conditions compared with younger P5 or older P30 animals. The frequency of GABAergic IPSCs in PSNs was significantly higher in P20 animals ($n = 15$) than those in P5 ($P < 0.01$, $n = 15$) and P30 ($P < 0.05$, $n = 15$) animals ([Fig. 2](#)). Similarly, the amplitude of GABAergic IPSCs was significantly higher in P20 animals ($P < 0.05$, $n = 15$) compared with both P5 ($n = 15$) and P30 ($n = 15$) animals ([Fig. 2](#)).

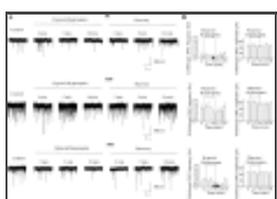


[Fig. 2.](#)

Developmental changes in GABAergic neurotransmission to PSNs and CVNs under control conditions. Examples of GABAergic inhibitory postsynaptic currents (IPSCs), isolated by focal application of strychnine (1 μ M), D-2-amino-5-phosphonovalerate (50 ...

Developmental changes in GABAergic neurotransmission to CVNs under control conditions. There were no statistically significant differences ($P > 0.05$) in the amplitude of GABAergic IPSCs in parasympathetic CVNs between P5 ($n = 16$), P20 ($n = 16$), and P30 ($n = 16$) animals under control conditions. However, the GABAergic IPSC frequency was significantly ($P < 0.01$) higher in P20 animals ($n = 16$) than those in P5 pups ($n = 16$, [Fig. 2](#)). No significant differences ($P > 0.05$) in the GABAergic IPSC frequency were detected between either P5 ($n = 16$) and P30 ($n = 16$) or between P20 ($n = 16$) and P30 ($n = 16$) animals ([Fig. 2](#)).

Developmental changes in GABAergic neurotransmission to PSNs during H/H. H/H (85% N₂, 6% O₂, and 9% CO₂, pH 7.1) elicited a reversible decrease in the IPSC frequency in PSNs in P5 animals ($P < 0.05$, at min 9–10 of H/H, $n = 15$, see [Fig. 3](#)). In contrast, in P20 animals H/H did not evoke a significant alteration in the frequency of IPSCs ($P > 0.05$, $n = 15$, [Fig. 3](#)). Similar to P5 rats, in P30 animals H/H elicited a significant decrease the IPSC frequency ($P < 0.05$, at min 9–10 of H/H, $n = 15$, see [Fig. 3](#)). The recovery to the control frequency occurred at min 4–5 post-H/H ([Fig. 3](#)). The amplitude of the GABAergic IPSCs in PSNs was not significantly altered by H/H in P5, P20, or P30 animals ([Fig. 3](#)).



[Fig. 3.](#)

Developmental changes in GABAergic neurotransmission to PSNs during H/H. No significant changes in the GABAergic IPSC amplitude in bulbospinal PSNs occur during hypoxia and hypercapnia (H/H) (85% N₂, 6% O₂, and 9% CO₂; pH 7.1) in P5 ($n = 15$), P20 ($n = ...$

Developmental changes in GABAergic neurotransmission to CVNs during H/H. In P5 animals H/H elicited a biphasic alteration in GABAergic IPSCs in parasympathetic CVNs, composed of an initial increase ($P < 0.05$, at min 1–2 of H/H, $n = 16$) followed by a decrease ($P < 0.001$, at min 9–10 of H/H, $n = 16$) in the GABAergic IPSC frequency. The recovery to the control frequency occurred at min 4–5 post-H/H ([Fig. 4](#)). In P20 animals, a biphasic response to H/H was replaced by a gradual decrease in

the GABAergic IPSC frequency. In P20 animals, a statistically significant decrease occurred at min 4–5 of H/H ($P < 0.001$, $n = 16$), which was 5 min faster than in P5 animals. In addition, the decrease in GABAergic IPSC frequency was more profound in P20 animals ($86 \pm 3\%$ reduction in GABAergic frequency in P20 animals, $n = 16$, comparatively to $52 \pm 5\%$ reduction in P5 pups, $n = 16$, at min 9–10 of H/H, $P < 0.001$). Similar to P5 animals, the recovery to the control frequency occurred at min 4–5 post-H/H (Fig. 4). In P30 animals, a biphasic response to H/H was restored to that seen in P5 pups (see Fig. 4). H/H evoked an initial increase ($P < 0.05$, $n = 16$) in the GABAergic IPSC frequency at min 1–2 of H/H. The following decrease in the GABAergic IPSC frequency first occurred at min 4–5 of H/H ($P < 0.001$, $n = 16$) and continued at min 9–10 of H/H ($P < 0.001$, $n = 16$). At this point, the reduction of control frequency was $82 \pm 5\%$ in P30 rats ($n = 16$), which was significantly greater ($P < 0.001$) than in P5 animals ($52 \pm 5\%$, $n = 16$) and not significantly different ($P > 0.05$) from P20 animals ($86 \pm 3\%$, $n = 16$). The recovery of the control frequency of IPSCs occurred at min 4–5 post-H/H (Fig. 4). H/H did not alter the amplitude of the GABAergic IPSCs in P5 or P30 animals. In contrast, the IPSC amplitude was significantly and reversibly diminished ($P < 0.05$, at min 9–10 of H/H, $n = 16$) in P20 animals (Fig. 4).

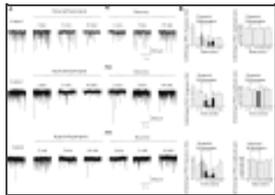


Fig. 4.

Developmental changes in GABAergic neurotransmission to CVNs during H/H. A biphasic response to H/H, comprising an initial facilitation followed by inhibition of GABAergic IPSC frequency in CVNs, was detected in P5 ($n = 16$) and P30 ($n = 16$) animals. ...

DISCUSSION

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The major findings from this study include the following. 1) There is a significant enhancement in GABAergic neurotransmission to both CVNs and PSNs at postnatal age P20 compared with animals at P5 and P30, with more pronounced enhancement in PSNs than in CVNs. 2) This enhanced GABAergic neurotransmission to PSNs is not altered by H/H in P20 animals; however, GABAergic neurotransmission to PSNs is inhibited by H/H in P5 and P30 animals. 3) Although H/H decreases GABAergic neurotransmission to CVNs at all ages studied (P5, P20, and P30), the most pronounced inhibition occurs in P20 animals. In addition, a transient increase in GABAergic IPSC frequency in CVNs occurs in P5 and P30 animals but is absent in P20 animals.

Thus, the results from this study indicate that maturation of the brainstem cardiovascular sympathetic and parasympathetic control systems includes an important transition stage, particularly at age P20 in rats, when significant and transient changes occur in the spontaneous synaptic activity as well as responses to H/H in both bulbospinal PSN and parasympathetic CVNs. Similarly, our previously published data indicate that orexinergic presynaptic inhibitory modulation of GABAergic neurotransmission to parasympathetic CVNs in the NA first occurs at age P20 in rats (Dergacheva et al. 2012). Both CVNs and PSNs play a key role in central regulation of cardiovascular function. Whereas CVNs in the NA generate the parasympathetic control of heart rate (Mendelowitz 1999; Wang et al. 2001a, b), PSNs in the RVLM are responsible for the generation of sympathetic outflow to the heart and blood vessels (Dampney 1994; Wang et al. 2009). Developmental changes in activity of these neurons would result in developmental changes in cardiovascular regulation and function. In accordance with our in vitro study results, the data from in vivo studies indicate there is an important postnatal age, between the second and third weeks in rats, for developmental changes in the

cardiovascular control system. Specifically, tonic parasympathetic control of heart rate has been shown to reach mature levels between the second and third weeks of postnatal age in rats with no additional changes from the adolescent to adult ([Adolph 1967](#); [Kasparov and Paton 1997](#); [Tucker and Johnson 1984](#)). Sympathetic influence on heart rate decreases around 20 days of postnatal age ([Adolph 1967](#); [Kasparov and Paton 1997](#)). These systemic findings are in accordance with the results from our study indicating a profound enhancement in inhibitory neurotransmission to PSNs in P20 rats. Similar to rats, early postnatal developmental changes occur in human infants including an increase in parasympathetic control of heart rate and a decrease in sympathetic modulation of blood pressure with advancing postnatal age until 6 mo of age ([Yiallourou et al. 2012](#)).

The results from previous studies suggest that the activities of both bulbospinal PSNs and CVNs are strongly modulated by H/H or hypoxia alone ([Boychuk et al. 2012](#); [Dergacheva et al. 2009](#); [Kamendi et al. 2009](#); [Sun and Reis 1994a, b](#)). A recent study conducted on P5-P8 rats has demonstrated H/H reduces the frequency of both glycinergic and GABAergic IPSCs in bulbospinal PSNs in the RVLM ([Boychuk et al. 2012](#)). Consistent with this previous report, the results from this study indicate H/H evokes an inhibition of the frequency of GABAergic IPSCs in PSNs in P5 and P30 animals. However, GABAergic neurotransmission to PSNs is enhanced under control conditions and not significantly altered by H/H in P20 animals. GABAergic neurotransmission and receptors have been shown to play an important role in determining PSN activity. Activation of GABAergic receptors of PSNs in the RVLM increases an inwardly rectifying K^+ conductance, hyperpolarizes PSNs, and reduces the intrinsic firing frequency of PSNs ([Li and Guyenet 1996](#); [Lin et al. 1998](#)). These mechanisms of inhibition of PSN activity likely contribute to the regulation of the basic sympathetic tone generation in the RVLM ([Li and Guyenet 1996](#)). As shown by results from in vivo studies, acute hypoxia increases arterial pressure and activity in sympathetic nerves ([Sun and Reis 1994a](#)). In addition, acute hypoxia increases the firing activity of bulbospinal PSNs in the RVLM with peripheral chemoreceptors denervated suggesting that PSNs can be directly stimulated by hypoxia ([Sun and Reis 1994a](#)). This hypothesis has been supported by the results from a recent in vitro study indicating that H/H facilitates the firing activity of a subpopulation of slow-firing PSNs, and this facilitation is dependent on the depression of GABAergic and glycinergic neurotransmission to PSNs ([Boychuk et al. 2012](#)). Thus, the data from previous studies and results from this study, taken together, suggest that the inhibition of GABAergic neurotransmission to PSNs by H/H likely contributes to facilitation of firing activity of PSNs and activation of sympathoexcitatory cardiovascular responses to H/H in P5 and P30 animals. However, enhanced GABAergic neurotransmission to PSNs under normal conditions and blunted changes during H/H in P20 animals may result in impaired sympathetically mediated cardiovascular responses to H/H in P20 animals. A blunted change in heart rate at least during hypoxia has been shown in similarly aged rodents ([Boychuk and Hayward 2010](#)).

Unlike in bulbospinal PSNs, H/H evokes the most pronounced inhibition of GABAergic neurotransmission to CVNs in P20 animals. Parasympathetic CVNs in the NA are intrinsically silent, and inhibitory synaptic inputs, including GABAergic and glycinergic, play a substantial role in determining CVN activity ([Mendelowitz 1996](#); [Neff et al. 2004](#); [Wang et al. 2001a](#)). Hyperpolarizing GABAergic and glycinergic synaptic inputs inhibit CVNs both directly and by removing inactivation of K^+ current ([Mendelowitz 1996](#)). Prolonged hypoxia evokes an increase in heart rate followed by a parasympathetically mediated bradycardia ([Deshpande et al. 1999](#); [Neff et al. 2004](#); [Taylor and Butler 1982](#)). This biphasic heart rate response to hypoxia has been suggested to be mediated by biphasic changes in inhibitory neurotransmission to CVNs in the NA ([Neff et al. 2004](#)). Indeed, hypoxia evokes an excitation following by inhibition of GABAergic and glycinergic IPSC frequency in CVNs in P4-P8

rats ([Neff et al. 2004](#)). Consistently, the results from this study indicate that H/H elicits an initial increase followed by a decrease in GABAergic neurotransmission to CVNs in P5 and P30 animals. However, this biphasic response to hypoxia is replaced by a gradual inhibition in GABAergic neurotransmission to CVNs in P20 rats. Interestingly, the sensitivity of GABAergic signaling to H/H increases with postnatal age of animals. Indeed, H/H evokes $52 \pm 5\%$ reduction of GABAergic IPSC frequency in P5 animals; however, H/H elicits significantly ($P < 0.001$) greater reduction of GABAergic IPSC frequency in older P20 and P30 animals ($86 \pm 3\%$ and $82 \pm 5\%$, respectively). In addition, the significant decrease in GABAergic IPSC frequency in response to H/H occurs 5 min faster in both P20 and P30 animals than in P5 pups. In P20 animals, this fast and profound decrease in GABAergic IPSC frequency is accompanied by a decrease in GABAergic IPSC amplitude. The depression of GABAergic neurotransmission to CVNs would evoke a disinhibition of CVN activity and subsequent activation of parasympathetic influences to the heart, which would result in bradycardia. Since the most robust decrease in GABAergic IPSCs occurs in P20 animals, this would predict H/H would evoke the most profound bradycardia in P20 animals (compared with P5 and P30 animals).

The age P20 in rats is roughly equivalent to human infancy when the highest incidence of SIDS occurs ([Darnall et al. 2010](#)). Interestingly, 90% of the sudden infant deaths occur before 6 mo of age, with 75% of these deaths between 2 and 4 mo of age ([Cummings et al. 2011](#); [Mathews and MacDorman 2008](#)). Although human developmental age between 2 and 6 mo has been characterized as an unstable time for many physiological systems ([Filiano and Kinney 1994](#); [Willinger et al. 1991](#)), the developmental neurophysiological mechanisms underlying this unique period of vulnerability remains unknown ([Hunt and Brouillette 1987](#)). The results from this study elucidate important developmental changes in the sympathetic and parasympathetic central mechanisms of cardiovascular regulation and provide a possible explanation for the unique period of vulnerability associated with this period and increased risk of SIDS. Infants that succumb to SIDS typically experience a progressive bradycardia and hypotension during the lethal event ([Fewell and Smith 1998](#); [Harper 2000](#); [Hunt and Brouillette 1987](#); [Ledwidge et al. 1998](#); [Meny et al. 1994](#)), suggesting that sympathoinhibition and/or exaggerated parasympathetic activity to the heart could be likely mechanisms of SIDS ([Harper 2000](#)). In addition, hypoxia has been suggested to play an important role in SIDS ([Cummings et al. 2011](#)). Indeed, postnatal risk factors for SIDS include exposure to noxious stimuli such as rebreathing of air which may result in H/H ([Farrell et al. 2002](#)). As discussed earlier, the specific postnatal age P20 in rats is characterized by a transient increase of inhibitory GABAergic neurotransmission to PSNs under both control conditions and during H/H and diminished GABAergic neurotransmission to CVNs during H/H, which would result in sympathoinhibition and parasympathetic overactivity in response to H/H, and these results would predict altered cardiovascular responses to H/H including severe bradycardia and/or life-threatening hypotension that would be exaggerated at P20 compared with P5 and P30 animals.

These results support the hypothesis that neurophysiological mechanisms responsible for SIDS include an age-dependent reorganization of sympatho-parasympathetic influences to the heart and vessels, and, as a result, altered cardiovascular responses to physiological challenges such as H/H. Supporting this hypothesis, the results from other animal studies suggest that the cardiorespiratory responses to hypoxia strongly depend on the postnatal age of animals. For example, postnatal age influences the ability of rats to autoresuscitate from apnea ([Fewell et al. 2000](#)). In particular, increases in postnatal age, up to 20 days of postnatal age, decreases the time to last gasp and the number of successful autoresuscitations following repeated hypoxic exposures ([Fewell et al. 2000](#)). In addition, results from

mice ([Jacobi and Thach 1989](#)) indicate the ability to recover from hypoxic apnea by gasping is profoundly diminished in P17-P20 mice compared with younger (P1-P16) and adult animals ([Jacobi and Thach 1989](#)). Thus, integrating the results from this and previous studies would indicate there is a critical age in rodents, particularly P20, in which GABAergic neurotransmission and function of both presympathetic and parasympathetic cardiac vagal neurons are altered that would lead to these animals being more vulnerable to challenges such as H/H.

Conclusion. In conclusion, this study elucidates important maturation of the sympathetic and parasympathetic cardiovascular control systems. There is a critical development time window in which there are significant rearrangements in central cardiovascular control mechanisms, particularly at the P20 developmental period. The results from this study not only yield insight into normal developmental processes of autonomic brainstem cardiovascular control system but also are relevant to an understanding of the pathophysiological mechanisms of disorders that likely occur in a brief period of development, and could increase the risk of adverse cardiovascular events at this critical period of development and increase the incidence of diseases such as SIDS.

GRANTS

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DISCLOSURES

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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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O.D. conception and design of research; O.D. and C.R.B. performed experiments; O.D. and C.R.B. analyzed data; O.D. interpreted results of experiments; O.D. prepared figures; O.D. drafted manuscript; O.D. and D.M. edited and revised manuscript; D.M. approved final version of manuscript.

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Present address of C. R. Boychuk: Dept. of Physiology, University of Kentucky, MS-508 Chandler Medical Center, 800 Rose St., Lexington, KY 40536-0298.

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