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Cardio-respiratory coupling depends on the pons

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ABSTRACT

Cardio-respiratory coupling is reciprocal; it is expressed as respiratory-modulated sympathetic nerve activity and pulse-modulated respiratory motor activity. In the brainstem, the neuraxis controlling cardio-respiratory functions forms a ventrolateral cell column which extends to the dorsolateral (dl) pons. Our general working hypothesis is that these control systems converge at points with the common purpose of gas exchange and that neural activity along this axis coordinates both arterial pulse pressure and breathing. Here, we review the data showing that pontine nuclei modulate heart rate, blood pressure and breathing, and present new results demonstrating a vagal influence on pontine activity modulated with both arterial pulse pressure and phrenic nerve activity in the decerebrate cat. Generally with the vagi intact, dl pontine activity was weakly modulated by both arterial pulse pressure and respiratory pattern. After bilateral vagotomy, the strength and consistency of respiratory modulation increased significantly, although the strength and consistency of arterial pulse pressure modulation did not change significantly for the group; a decrease in some (62%) was offset by an increase in others (36%) neurons. Thus, the vagus shapes the envelope of the cycle-triggered averages of neural activity for both the respiratory and cardiac cycles. These data provide insight into the neural substrate for the prominent vagal effect on the cardio-respiratory coupling pattern, in particular respiratory sinus arrhythmia. While these results support convergence of inputs to neural populations controlling breathing and cardiovascular functions, the physiologic role of balancing ventilation, vascular resistance, heart rate and blood flow for the benefit of tissue oxygenation, remains hypothetical.

1. Introduction

Sympatho-respiratory coupling expressed as respiratory-modulated sympathetic activity was first described by Adrian and Bronk (1932). They reported increased cervical sympathetic nerve activity at the end of inspiration in the anesthetized rabbit (Adrian and Bronk, 1932). This and similar observations have been reported for other sympathetic nerves from head, or at least neck, to tail in various mammalian species (Habler and Janig, 1995; Pilowsky, 1995). Conversely, the expression of arterial pulse pressure modulation in respiratory motor activity was described recently for activity of single motoneurons that innervate the upper airway musculature (Dick et al., 2005). Thus, cardio-respiratory coupling is reciprocal, supporting the concept that control of homeostasis involves integrated functions such as gas exchange rather than being effector-specific; *e.g.* independent control of just vascular resistance or just respiratory muscle activity.

1.1. Coupling in neural networks

Even prior to the article by Adrian and Bronk, a respiratory sinus arrhythmia in heart rate was observed by Traube (1865). Potential reflexogenic as well as central mechanisms for the increase in heart rate during inspiration have been presented for well over 100 years and are still debated presently (Brodie, 1900; Anrep et al., 1936a,b; Eckberg, 2009; Julien et al., 2009; Karemaker, 2009a, b). Brainstem neurons that control heart rate and sympathetic tone express respiratory-modulated activity patterns (Gilbey et al., 1984; Mendelowitz, 1999; Mandel and Schreihofer, 2006). For instance, cardiac vagal pre-ganglionic neurons, "pressor" (pre-) pre-ganglionic neurons in the rostrolateral medulla (RVLM) as well as "depressor" neurons in the caudal ventrolateral medulla (CVLM) all can have respiratory-modulated patterns of activity (Guyenet and Koshiya, 1995; Guyenet, 2000). On the other hand, cardio-sympathetic neurons respond to activation of vagal pulmonary afferents (Brodie, 1900; Daly, 1986). Thus, coupling between respiratory and cardiovascular control networks is mediated by both afferent input but also by interaction at the level of the pattern generators.

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Intracellular recordings from vagal, cardio-inhibitory neurons in the nucleus ambiguous showed that these neurons were inhibited during inspiration (Gilbey et al., 1984; Mendelowitz, 1999). While intracellular recordings from RVLM neurons revealed respiratory-modulated changes in membrane potential at increased levels of respiratory drive (Lipski et al., 1996; Granata and Cohen, 2004), extracellular recordings from bulbospinal and barosensitive RVLM neurons have revealed distinct patterns of respiratory-modulated activity (McAllen, 1987). In the RVLM, neurons had either peak or nadir activity during inspiration and in a subset, the peak inspiratory activity was followed by a depression during postinspiration (McAllen, 1987). In the baroreflex pathway, CVLM neurons are excited by baroreceptor activation and inhibit RVLM neurons. Types of respiratory modulation similar to that of RVLM have been recorded from CVLM neurons, *i.e.*, a peak or nadir during inspiration (Mandel and Schreihofer, 2006). Further, in a subset of CVLM neurons the inspiratory peak was followed by a decrease in activity during postinspiration and a fourth group had their peak activity during postinspiration (Mandel and Schreihofer, 2006). These studies substantiate that brainstem neurons directly controlling BP, both heart rate and vascular resistance, express respiratory activity.

The converse, arterial pulse modulation of activity expressed by medullary respiratory-modulated, and presumably, respiratory-related neurons may be the expression of a reciprocally controlled and complementary system (Dick and Morris, 2004; Dick et al., 2005). Highly modulated medullary respiratory neurons have activity that is modulated by baroreceptor inputs. In decerebrate, paralyzed, and vagotomized cats, approximately half of the respiratory-modulated units expressed pulse-modulated activity. This activity was preferentially expressed in expiratory activity, which is consistent with the increase in the duration of expiration evoked by increases in blood pressure (Bishop, 1974; Sapru et al., 1981; Lindsey et al., 1998; McMullan et al., 2009).

1.2. Mechanisms

Even though this review focuses on coupling between central neurons, the role of sensory input cannot be minimized. Vagal sensory inputs arising from numerous types of receptors influence not only cardio-respiratory coupling but also the respiratory pattern and vascular tone. For instance, the activation of pulmonary stretch receptors shortens the duration of inspiration, lengthens the duration of expiration, decreases vascular resistance and slows heart rate (Daly, 1986; Dick et al., 2008). In contrast arterial pressure modulated activity presumably depends on baroreceptor inputs of the carotid sinus nerves (Dick and Morris, 2004; Dick et al., 2005).

The mechanisms of cardio-respiratory reciprocal coupling have been defined only in general terms (Koepchen et al., 1981). Basically, the networks can be coupled through peripheral feedback and/or central interconnections. For sympathetic activity to be coupled with respiratory rhythm, the neural components of peripheral (*e.g.* pulmonary stretch receptor afferents) or central (*e.g.* respiratory control network) can impose a respiratory rhythm on sympathetic activity by physically exciting and/or inhibiting neurons of the sympathetic control pathways (*e.g.* CVLM, RVLM neurons). The arterial pulse rhythm expressed in respiratory control networks and effectors could also arise from direct input of arterial baroreceptors, indirectly from the sympathetic or parasympathetic control networks or from a central oscillator coupled to respiratory control as well as baroreceptors (Morrison et al., 1984; Barman and Gebber, 2000; Barman et al., 2000; Granata and Cohen, 2004).

1.3. Measurements

To examine cardio-respiratory coupling more critically, we developed statistical measures, termed η^2 and δ^2, to quantify the "respiratoriness" and "cardiovascularness" of neural activity in the brainstem (Orem and Dick, 1983; Dick and Morris, 2004). These statistics indicate the magnitude and consistency of the modulation in the activity pattern from breath-to-breath (η^2 value) or arterial pulse-to-pulse (δ^2 value). These statistics are based on the analysis of variance and compare the variance across trials to the total variance. So if the activity is highly modulated such that much of the variance in the activity pattern is accounted for by the breath pattern or cardiac cycle with little variance from breath-to-breath or heart beat to heart beat, then the η^2 value or δ^2 value respectively approaches 1 and the neuron is referred to as having a high η^2 or δ^2 value. In practise, we have recorded activity with much higher η^2 values in the vl medulla than dl pons as well as much higher η^2 than δ^2 values. Activity with a high η^2 values was not as responsive to afferent input as activity with low η^2 values (Orem, 1987). Thus, neurons with high η^2 values may be locked into or even elements of the pattern generator whereas those with low η^2 values may receive many inputs and be the interface between the pattern generator and sensory inputs (Orem, 1987).

Vagal input in the anesthetized animal influences brainstem neural activity, especially in the pons. Following vagotomy, there is a significant increase in η^2 values and greater respiratory modulation of neural activity (Shaw et al., 1989; Cohen and Shaw, 2004; Dick et al., 2008). As a first approximation, pontine neuronal activity reflects the balance of inputs it receives. Thus, we postulate that vagal activity shifts the balance of respiratory and arterial pulse modulated inputs, such that η^2 values increase and δ^2 values decrease.

2. Methods

2.1. General procedures

The unpublished data in this review were acquired during two recently published studies. In the first study (Baekey et al., 2008), recordings were made in *in situ* perfused preparations from rats; the second (Dick et al., 2008), in *in vivo* decerebrate, neuromuscular blocked (pancuronium bromide (0.4 mg/kg/h)), and ventilated cats. For the first study, surgical, experimental and euthanasia procedures conformed to the United Kingdom Animals (Scientific Procedures) Act 1986 and were approved by the University of Bristol ethical review committee; for the second, procedures conformed to the guidelines of American Association for Accreditation of Laboratory Animal Care and of National Institutes of Health and National Research Council, and approved by the University of South Florida's Animal Care and Use Committee.

Briefly for the first study, experiments were performed on juvenile male

Wistar rats ($n = 8$, postnatal day 21–28, 60–100 g) using the arterially perfused *in situ* rat preparation (Paton, 1996). Rats were perfused with oxygenated artificial cerebrospinal fluid as described (Paton, 1996; Baekey et al., 2008). In the second study, adult cats ($n = 9$, 2.5–4.1 kg, either gender) were initially anaesthetized with isoflurane (2–5%), a femoral artery and vein and the trachea were catheterized for monitoring blood pressure and gases, for administering fluids and for ventilating the animal. To decerebrate the animals, external carotid arteries were ligated, a craniotomy was formed in the parietal plates and the brainstem was transected midcollicularly. Neural tissue rostral to the transection was aspirated.

2.2. Recordings

In both studies, phrenic nerve activity (PNA) was recorded. The nerve signal was amplified and filtered (band pass 0.1–5 kHz, Grass P511 amplifiers). In the first study we also recorded thoracic

sympathetic (thSNA), perfusion pressure and electrocardiogram (ECG) for heart rate. In the second study, we recorded arterial blood pressure and ensemble neuronal activity extracellularly.

For the ensemble recordings, the right side of the pons was searched with an array of tungsten microelectrodes ($n = 24$, $Z = 10–12\ M\Omega$). Signals were amplified, filtered (band pass 0.1–5 kHz, Grass P511 amplifiers) monitored and recorded for later analysis. The stereotaxic coordinates of the electrodes were referenced to the obex. Activity was recorded from areas in the dorsolateral (dl) pons at the following stereotaxic coordinates: 0.3 rostral to 2 mm caudal to the caudal edge of the inferior colliculus, 3.2–5.5 mm lateral to midline, 0.9–5.5 mm below the dorsal pontine surface. The brainstem surface was covered with a pool of warm mineral oil.

2.3. Experimental protocols

In the first study, the experimental protocol consisted of recording PNA, thSNA and heart rate before and after a brainstem transection at the ponto medullary border (Figs. 1 and 2). Before brainstem transection, nerve activity was recorded at baseline for at least 15 min then through a series of stimuli, which included baroreceptor activation produced by increasing perfusion pressure, peripheral chemo-receptor activation by a bolus cyanide injection (CN^-, 0.03% dissolved in distilled H_2O, 0.02–0.05 ml depending on the response). Then the brainstem was transected, a new baseline recording was established, and the series of stimuli repeated.

In the second study, the experimental protocol consisted of recording nerve activity and the spike trains of single pontine neurons before and after vagotomy (Figs. 3–7). The vagi were isolated from the carotid artery and the aortic depressor and cervical sympathetic nerves in the neck through the ventral midline incision made for the tracheotomy. A ligature was tied loosely around the cervical vagi. The ends of the ligature were labelled and externalized. Thus, we could evulse the cervical vagi with minimal mechanical disruption of the recording. Before vagotomy, activity was recorded for at least 15 min with the animals ventilated by a cycle-triggered pump. Inflation was triggered by the onset of the PNA; the duration of the inflation was approximately the duration of "neural" inspiration defined by the PNA.

2.4. Data entry

In both studies, all data analysis was performed off line. In the first study, cycle-triggered averages (50 respiratory cycles, triggered from the IE transition) of ∫PNA, ∫thSNA, perfusion pressure, and heart rate were made from stable periods of baseline activity before and after transection. We used these averages to characterize the respiratory modulation of the recorded signals by comparing activity among the phases of respiration in response to stimuli before

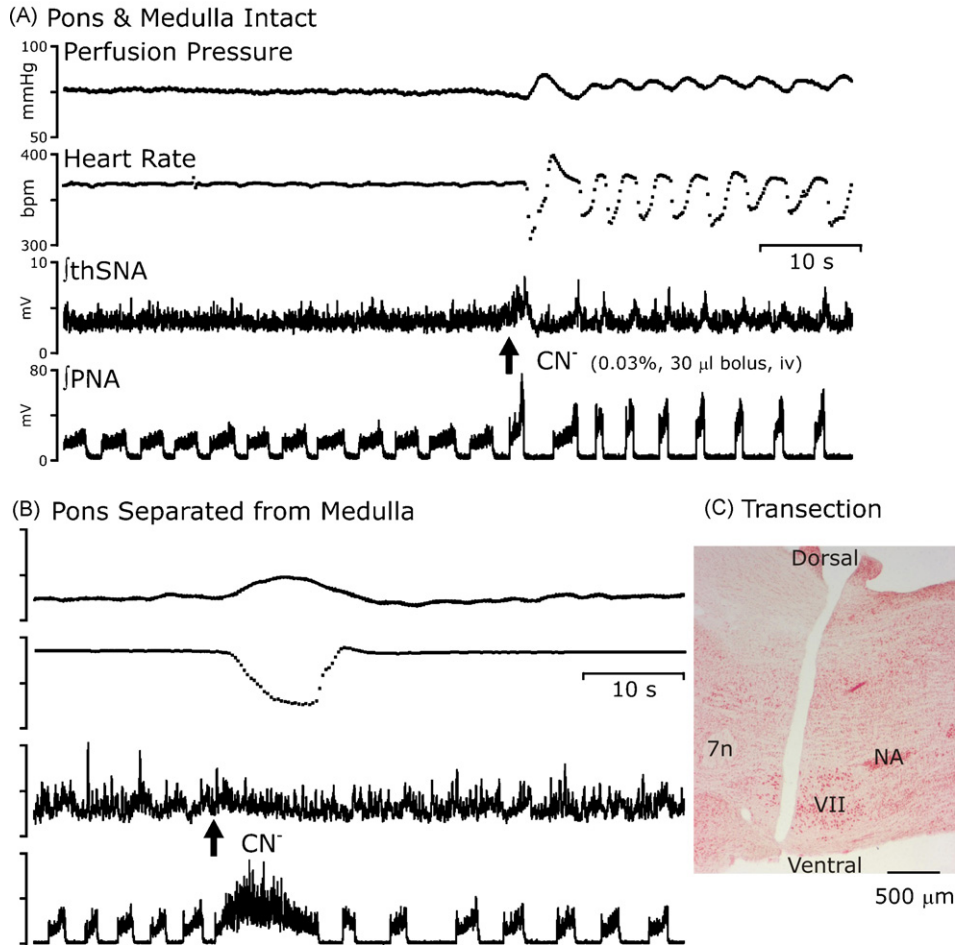

Fig. 1. Pons influences coordinated sympathetic-respiratory responses in the arterially perfused *in situ* rat preparation. (A) Response to bolus injection of CN^- with pons intact. (B) Ponto-medullary transection attenuated the sympathetic nerve response and altered the phrenic nerve pattern. (C) Sagittal section shows the location of the transection, which bisected the facial motor nucleus (VII) ventrally. The nucleus ambiguus (NA) was caudal to the transection and the seventh cranial nerve (7n) was located rostrally. The transection separated numerous pontine structures, that modulate to cardio-respiratory activity (Parabrachial Nuclei, Kölliker Fuse nuclei, Intertrigeminal regions and A5 areas) from the medullary cardio-respiratory rhythm generating nuclei.

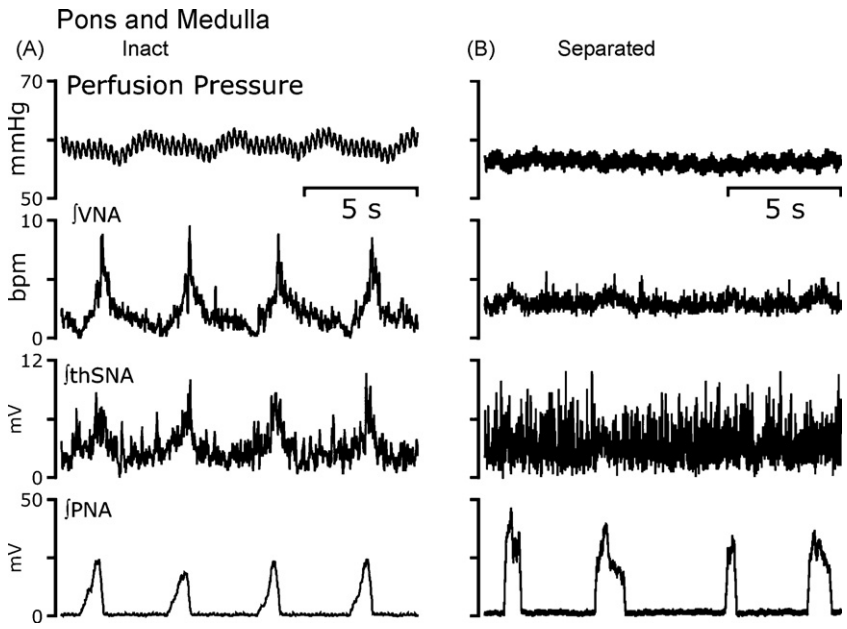

Fig. 2. Ponto-medullary transection eliminated respiratory modulation in cardio sympathetic effectors and produced apneustic breaths. (A) The ponto-medullary brainstem was intact and perfusion pressure, efferent vagal nerve activity (VNA), thSNA were entrained with the respiratory rhythm evident in the PNA. In addition this pattern of activities appeared stable from respiratory cycle to respiratory cycle. (B) The ponto-medullary brainstem was transected causing variable and, in cases, apneustic breaths in the same animal as (A) Associated with the change in breathing pattern, the transection eliminated Hering–Traube Waves in the perfusion pressure, post-inspiratory activity in the VNA, and late-inspiratory activity in thSNA. However, although attenuated, inspiratory modulated activity persisted in the VNA.

and after ponto-medullary transection. The respiratory cycle was divided into early (e–I) and late (l–I) inspiration, postinspiration (PI), and expiration (E). During each respective phase, average activity for thSNA, HR, and perfusion pressure was compared before and after transection using a two-way repeated measure analysis of variance with alpha of 0.05. These tests were followed by a post-hoc analysis for multiple comparisons (Student–Newman–Keuls test).

In the second study, action potentials were identified on the basis of eight characteristics of the waveform and converted to times of occurrence with spike-sorting software (Datawave Tech. Corp.) The action potentials and other digital and analogue signals were displayed graphically using XSCOPE (Lindsey et al., 1992), a program that allowed event codes to be added and data segments to be selected for later analysis. The following analyses were performed on brainstem neuron spike trains recorded during a 15-min period: (1) Auto-Correlation Histograms (ACHs), 2) Respiratory Cycle-Triggered Histograms (rCTHs) using the inspiratory-to-expiratory transition as the triggering event, and (3) Cardiac Cycle-Triggered Histograms (cCTH) using the nadir of arterial pulse pressure as the triggering event. The consistency and strength of the modulated activity were evaluated using statistical tests; for rCTH, we calculated the η^2 value (Orem and Dick, 1983) and for cCTH we used δ^2 value (Dick and Morris, 2004). We plotted the distribution of δ^2 values and η^2 values and correlation δ^2 values to η^2 values before and after bilateral vagotomy.

3. Results

3.1. Cardio-respiratory coupling decreases after ponto-medullary transection

In the *in situ* perfused brainstem preparation, perfusion pressure, heart rate and thoracic sympathetic chain activity can be respiratory-modulated (Fig. 1A, right-hand side of the Figure). In particular, similar to our recordings for the splanchnic sympathetic nerve activity in the *in vivo* preparation; the thoracic sympathetic activity increased following inspiration. Perfusion pressure increased later in expiration and after the increase in sympathetic activity. Heart rate was greatest in inspiration, which is also consistent with respiratory sinus arrhythmia *in vivo*.

The first indication that the pons was important for the cardio-respiratory patterning was observed during the recovery period immediately following the surgical procedures (performed in an ice bath) to create the *in situ* preparation (Fig. 1A, left-hand side of the Figure). During the recovery period, PNA had an apneustic pattern, one with a prolonged inspiratory effort and a TI-to-TE ratio greater than one. The thSNA was tonic even though the pons was intact. After a bolus injection of cyanide (NaCN, 0.03%, 30 μl bolus *iv*) the PNA pattern had an augmenting ramp-like pattern and perfusion pressure, heart rate and thoracic sympathetic chain activity became respiratory-modulated (Figs. 1 and 2).

We associate the modulation of cardiovascular and respiratory effectors with the re-establishment of a 'eupneic-like' respiratory pattern. A eupneic-like breathing pattern, including the motor expression of post-inspiratory efferent vagal activity, depends on a functioning dl pons (Fig. 2). With the pons intact and an established eupneic-like augmenting ramp pattern in the PNA, the envelopes of VNA and thSNA, as well as perfusion pressure, showed a profound modulation with the respiratory rhythm (Fig. 2A). After pontine transection (Fig. 2B, as in Fig. 1B), the modulation of vagal efferent activity as well as the other cardio-respiratory effectors was attenuated. Therefore, we hypothesized that coupling of cardio-respiratory effectors depends on the pons.

To test this hypothesis we examined coupling of cardio-respiratory effectors (Fig. 1B and C). After pontomedullary transection (Fig. 1C), the phrenic pattern is apneustic as well (Fig. 1B). In contrast to the evoked effect in establishing a functional *in situ* preparation, a bolus injection of cyanide evoked a sustained increase in PNA and did not enhance cardiovascular coupling (Fig. 1B). Thus, even though the baseline patterns were similar, the response to bolus injections of cyanide depended on the pons.

In a recent study (Baekey et al., 2008), we described the dependence of respiratory modulation of SNA on the pons. Here in Fig. 1A,

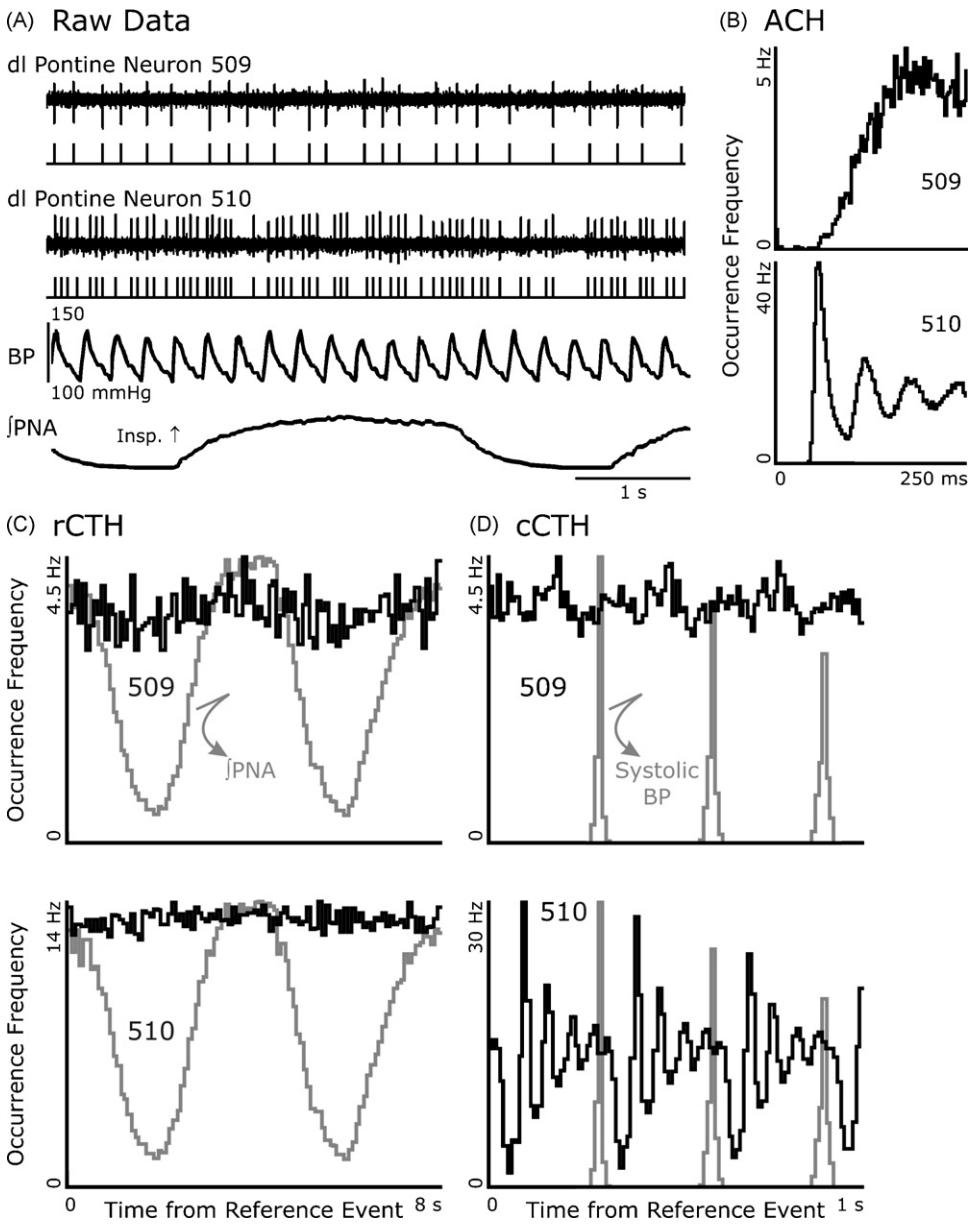

Fig. 3. Pontine activity modulated with both respiratory and cardiac rhythms. (A) Activities of two simultaneously recorded dl pontine neurons were discriminated (acceptance pulses displayed below each recording). Adult cat was decerebrated and vagotomized. Arterial blood pressure and integrated phrenic nerve activity were recorded also. Modulation with either respiration or blood pressure was not apparent in the raw tracings during the displayed respiratory cycle. (B) Autocorrelation histograms (ACH) indicated a refractory period between discriminated spikes, consistent with single-unit recordings. (C) Respiratory cycle-triggered histograms (rCTH) indicated a weak relationship between the respiratory cycle as indexed by integrated phrenic nerve activity (∫PNA) and pontine activity. (D) Cardiac cycle-triggered histograms (cCTH) indicated a modulation (robust for 510) between the cardiac cycle as indexed by arterial blood pressure (BP) and pontine activity.

we show an example of the response to a bolus (30 μl) of cyanide (0.03%) that was administered directly into the abdominal perfusate to activate chemo-afferent fibres. This was done in the first few minutes of establishing a viable preparation that expressed a stable 3-phase respiratory rhythm (see integrated efferent vagal activity in Fig. 2A) with an augmenting ramp in PNA. Cyanide injections evoked abrupt increases in perfusion pressure, SNA, and PNA. After the immediate effects of the injection, perfusion pressure, heart rate and SNA became and remained respiratory-modulated (Fig. 1A). In contrast after transection (Fig. 1B), cyanide injections still evoked an acute response but did not evoke a sustained increase in respiratory modulation of cardiovascular variables. Consequently, with the pons intact once the system had been activated, cardio-respiratory coupling was maintained while after pontomedullary transection this did not occur (Fig. 1A).

The general sympatho-respiratory coupling pattern in the *in situ* preparation was that the magnitude of thSNA was the greatest during late inspiration and least during late expiration (Fig. 2A). This particular activity pattern was reflected in the pulse pressure after an electro-mechanical delay (Fig. 2A). After this animal was transacted the efferent vagal nerve had only inspiratory activity and no apparent respiratory modulation in the perfusion pressure or thSNA (Fig. 2B). For the group of animals, there was no significant difference in thSNA, heart rate or perfusion pressure associated with the respiratory phases (Baekey et al., 2008).

In addition to the effector patterning, the baroreflex was modulated with the respiratory rhythm. The greatest effectiveness in the reflexly evoked decrease in heart rate and thSNA was during postinspiration and expiration respectively. Respiratory modulation of the baro-reflex was also abolished by ponto-medullary transection.

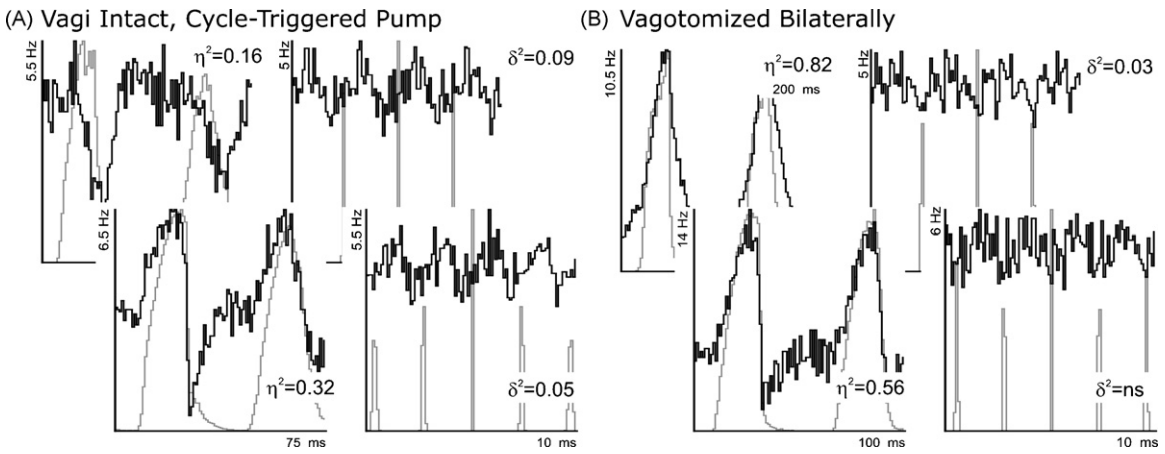

Fig. 4. Differential effects of vagotomy on respiratory and cardiac modulated pontine neuron activity in the adult decerebrated cat. (A) rCTH (left) and cCTH (right) indicated that dl pontine activities were modulated with both respiratory and cardiac rhythms when the vagi were intact. (B) After bilateral vagotomy, the respiratory modulation of both neurons transformed to pronounced augmenting inspiratory (I-Aug) discharge patterns. For both neurons, the η^2 values increased whereas pulse modulation (δ^2 values) decreased.

In summary with an intact pons, cyanide injection converts apneusis to a eupneic pattern including cardio-respiratory coupling. These results suggest that apneustic breathing, such as that pursuant to pontine transection, does not necessarily preclude cardio-respiratory coupling and the response to cyanide. In the absence of the pons the coupling and post-inspiratory vagal efferent activity response to cyanide is prevented.

3.2. Respiratory- and pulse-modulated activity recorded in single pontine neurons

We recorded dl pontine activity, in which visual inspection of their spike times did not reveal respiratory- or pulse-modulation (Fig. 3A). However, CTHs showed weak modulation with PNA but surprisingly strong modulation with arterial pulse, especially in the

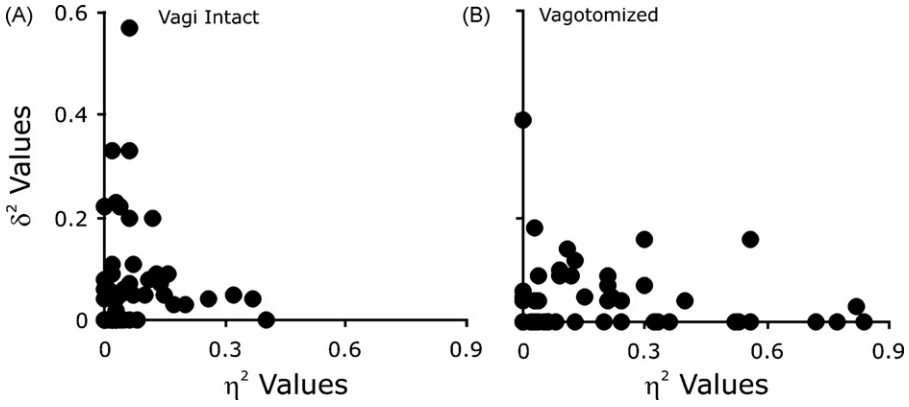

Fig. 5. Examples of pontine neurons with respiratory and cardiac rhythms before and after vagotomy in the adult decerebrated cat. (A) rCTH (left) and cCTH (right) indicated that dl pontine activities were weakly modulated with PNA and well modulated with pulse even when the vagi were intact. (B) After bilateral vagotomy, even though activity developed a recognizable I-Aug pattern, it remained weakly modulated with respect to PNA. In contrast pulse modulation decreased but remained significant.

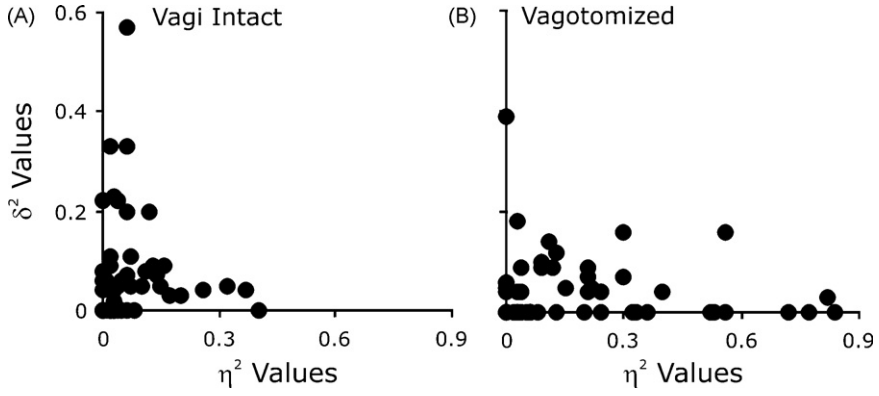

Fig. 6. Values of respiratory- and pulse-modulated pontine activity are vagal dependent. Plotted points are the dl pontine neurons ($n = 47$) that had pulse-modulated activity with significant δ^2 values before (A) and/or after (B) vagotomy (A), represent dl pontine activities that were modulated with respiratory and cardiac rhythms when the vagi were intact. However, there was a paucity of higher η^2 values. (B) After vagotomy, the number of respiratory-modulated neurons with η^2 values >0.3 increased whereas the number of pulse-modulated neurons with δ^2 values >0.2 decreased. Nevertheless, there were activity patterns with moderate η^2 and δ^2 values.

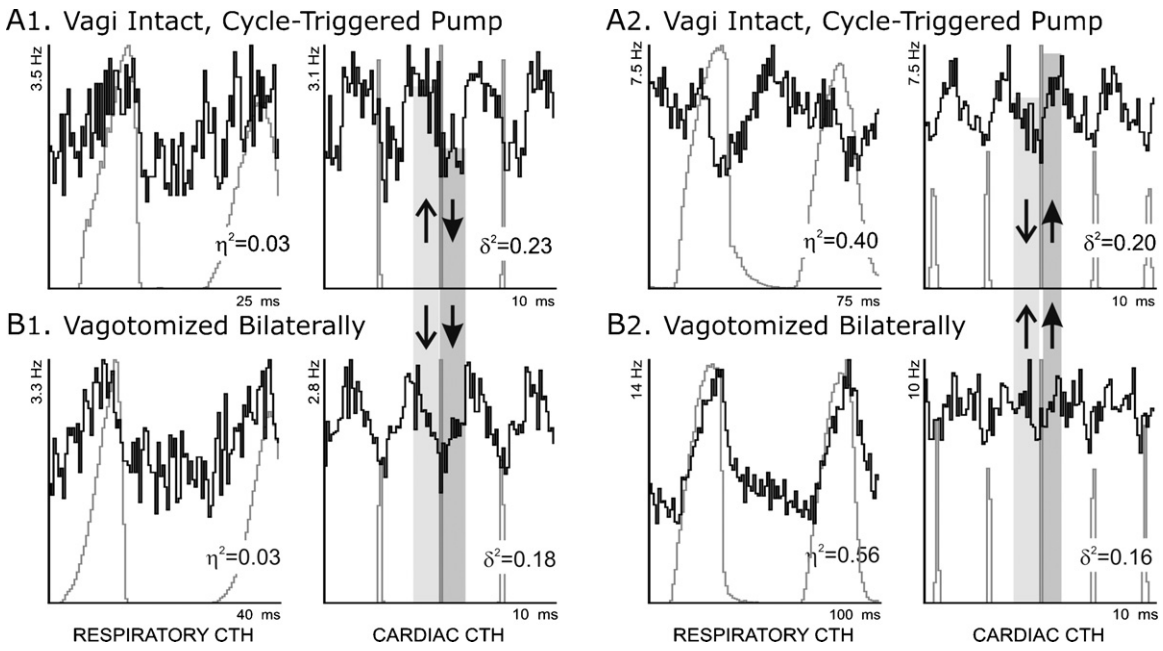

Fig. 7. Pattern of pulse-modulated pontine activity modulated by vagal input. (A1) Vagi were intact and the rCTH (left) indicated I-Aug pattern. The cCTH (right) indicated that dl pontine activities were more active during the inter-beat interval, activity was lowest just after the pulse (solid arrow, 20% grey shading) and greatest just before the next pulse (stick arrow, 10% grey shading). (B1) Vagi were transected and the I-Aug pattern remained. Pump-modulated activity remained greatest between pulses but now decreased prior to the next beat (stick arrow, 10% grey shading). The δ^2 values remained similar before (0.23) and after (0.18) vagotomy. (A2) With vagi intact, the predominant feature in the rCTH (left) was a decrease in activity in Late I. The cCTH (right) indicated an increase inactivity during the inter-beat interval, activity increased just after the pulse (solid arrow, 20% grey shading) and decreased prior to the next pulse (stick arrow, 10% grey shading). (B2) After bilateral vagotomy, the neuron's activity developed an I-Aug pattern and became more modulated with PNA (η^2 value increased from 0.40 to 0.56). In contrast, δ^2 values remained similar before (0.20) and after vagotomy (0.16) but the pattern changed; while activity still increased immediately after the pulse (solid arrow, 20% grey shading), it increased rather than decreased before the next pulse (stick arrow, 10% grey shading).

case of neuron 510 (Fig. 3C). Further analysis of this activity indicated that the signals were recorded from well-discriminated single neurons; the ACHs revealed an absence of short interspike intervals (Fig. 3B). While these initial data showed that pulse-modulated activity was expressed by dl pontine neurons, we were limited in understanding how this activity functioned in the context of cardiorespiratory control.

Given these preliminary results, we developed tools and an experimental approach that would discriminate a functional context for this activity. In particular, we built on our experience with the η^2 statistic that measures the consistency and magnitude of the respiratory modulation of activity. The η^2 statistic is a value between zero and one and approaches one if the activity is the same from breath to breath and if the activity varies from one part of the cycle to another (Orem and Dick, 1983). We developed the δ^2 statistic, which measures the consistency and magnitude of modulation of activity as it relates to the arterial pulse (Dick and Morris, 2004). The experimental protocol involved quantifying η^2 and δ^2 values before and after vagotomy. The activity of respiratory neurons depends on vagal input and increases following vagotomy. We recently published the results regarding the change in η^2 values (Dick et al., 2008). Here we focus on the changes in δ^2 values and the relationship between η^2 and δ^2 values.

With the vagi intact, respiratory-modulated activity in the dl pons is generally weak (Takagi and Nakayama, 1958; Cohen and Wang, 1959; Feldman et al., 1976; St John, 1987; Shaw et al., 1989). After vagotomy, dl pontine activity becomes more modulated with respiration (Feldman et al., 1976); generally, expressing an augmenting inspiratory (I-Aug) pattern and an increasing η^2 values (Shaw et al., 1989; Dick et al., 2008). While this general pattern is consistent with vagal-dependent presynaptic inhibition precluding respiratory-modulated afference from being received by pontine neurons, we proposed another model emphasizing the balance of convergent inhibitory and excitatory inputs in determining activity patterns (Dick et al., 2008). The advantage of the 'balance' model is that it explains the varied activity patterns observed in the pons especially abrupt and transient changes in activity at the phase transitions

The statistics η^2 and δ^2 values may reflect the summation of afference received by the neurons, especially in the absence of membrane properties that would reshape the input. Vagotomy increases η^2 values or the expression of 'respiratoriness' of activity; reflecting an increase in respiratory-modulated afference (perhaps inspiratory efference copy from the medulla) to the dl pons, whereas the relative balance of pulse-modulated or baro-receptor input should diminish. Thus, we hypothesized that neurons related to respiratory control would increase their η^2 value after vagotomy because their role in determining respiratory phase duration after vagotomy. In conjunction, with an increase in η^2 value, δ^2 values should decrease because a greater amount of spike-time variability would be determined by respiratory- rather than baro-receptor-modulated input.

Less than a quarter of the neurons (36/144) expressed arterial-pulse-modulated activity with the vagi intact. Although individual pontine neurons showed changes in cardiac modulation, as a whole the sample group did not change in δ^2 values significantly (Student's paired t-test $p > 0.05$); decreases in δ^2 values of some neurons (29 of 47) were offset by increases in others (17 of 47, including 11 recordings that were not pulse-modulated with the vagi intact became pulse-modulated after vagotomy). However, an increase in η^2 values in the recorded dl pontine activity was associated with a decrease in δ^2 associated with vagotomy (Figs. 4–6). This happened in neurons that were significantly modulated with respiration and pulse before vagotomy. In the examples (Fig. 4), both neurons expressed an I-Aug discharge pattern with high η^2 values post-vagotomy; their pulse modulation diminished (Fig. 4).

Similarly, neurons that were weakly coupled with PNA with the vagi intact and that became I-Aug with low but significant η^2 values after vagotomy had a complementary, reciprocal change in δ^2 (Fig. 5). With the vagi intact, these neurons had two of the highest δ^2 values observed.

3.3. Correlation between δ^2 and η^2 Values

We plotted δ^2 values against η^2 values before and after vagotomy for the group (Fig. 6). As expected with the vagi intact, the distribution of values was skewed toward lower η^2 values and higher δ^2 values than those after the vagotomy (Fig. 6). Nevertheless the distributions reflected a continuum rather than a dichotomy, indicating a convergence of respiratory- and pulse-modulated afference on dl pontine neurons before and after the vagi were cut. First, after vagotomy two neurons had δ^2 values of approximately 0.2 and η^2 values of 0.3 and 0.56. In both these cases, η^2 values increased but δ^2 values remained essentially the same after vagotomy. We would have predicted a decrease in δ^2 values. Second, the recording with the highest δ^2 value after vagotomy was not modulated with respiration (similar to neuron 510 in Fig. 3). Activity of this neuron had a δ^2 value of 0.11 and a weak but significant η^2 value before vagotomy and a δ^2 value of 0.39 and a non-significant η^2 value after vagotomy. In other words, vagotomy increased the pulse and decreased respiratory modulation. While this is consistent with the concept of balanced inputs shifting the activity patterns toward respiratory or cardiovascular control, it is the opposite of the predicted shift in activity patterns evoked by vagotomy.

3.4. Changes in coupling patterning for pulse-modulated activity associated with vagotomy

In 15 of the 47 neurons with pulse-modulated activity after vagotomy, δ^2 values increased more than 0.03 (including the activity described in the previous paragraph) and 9 remained similar after vagotomy (increase or decrease less than 0.03). These changes were associated with altered discharge patterns time-locked to the cardiac pulse; examples revealed by cCTHs are shown in Fig. 7. Features of the cCTH were altered even though heart rate remained the same (note, same number of bins between systolic peaks in pulse pressure before and after vagotomy in Fig. 7 comparing A1 to B1 and A2 to B2). In these examples, activity profiles immediately before systole (stick arrows on 10% grey shading) changed, while that after pulse remained the same (solid arrows on 20% grey shading). Such alterations could occur in the absence (Fig. 7A1–B1) or presence of (Fig. 7A2–B2) of change in the rCTH profile.

4. Discussion

The research summarized here has focused on cardioventilatory control; in particular, coupling between the cardio-sympathetic systems controlling blood flow and respiratory system controlling blood gases. Our results support several conclusions. First, the pons plays a role in mediating cardio-respiratory coupling and in the response to baroreceptor stimulation as well as chemo-receptor activation. Second, differential control of the cardio-respiratory system forms a continuum not a dichotomy. We have demonstrated that vagotomy not only alters the consistency and magnitude of respiratory modulation but also alters pulse modulation. Constitutive elements of the networks express activity related to both rhythms. Third, we have shown that this cardioventilatory control is reciprocal; not only are pulse and respiratory rhythms expressed in constitutive elements of both control systems but both rhythms are also evident in both motor outputs. Cardio-sympathetic effectors have a well-known respiratory rhythm in the heart beat and sympathetic nerve activity. Similarly respiratory motor output express pulse-modulated activity. These findings are consistent with coupled systems controlling blood pressure, blood flow, and blood gases for the common goal of gas exchange.

4.1. Cardio-respiratory coupling decreases after ponto-medullary transection

Sympathorespiratory coupling depends on a functional pons. In our previous study, ponto-medullary transection diminished sympathorespiratory coupling expressed in the thoracic sympathetic chain; whereas in another study disfacilitation of activity the dl pons attenuated respiratory modulation of splanchnic sympathetic nerve activity (Morrison et al., 1994; Morrison, 1996). However in these studies, the reduction in sympathorespiratory coupling was associated with changes in the respiratory pattern; prolonged inspiration and reduced phrenic nerve amplitude, an apneustic pattern. In other words, the decrease in coupling could be due to the apneustic breathing pattern or the loss of pontine activity. Unfortunately, an apneustic breathing pattern has not been dissociated from loss of pontine function. When an apneustic pattern is observed experimentally and or clinically, then pontine function is compromised (Plum and Posner, 1980; Dutschmann and Herbert, 2006).

Apneustic breathing can be observed initially in the *in situ* preparation as it recovers from being chilled and not being perfused. We assume that it is because the pons is not functioning as a part of the pattern generator. Under these conditions, often a bolus injection of cyanide normalizes the pattern; perhaps through pontine activation and reengaging the pons in the network. With normalization of the pattern, which includes the induction of post-inspiratory activity (Dutschmann and Herbert, 2006), sympatho-respiratory coupling is established. These observations cannot distinguish the neural substrate underlying the pattern formation and coupling, but rather emphasize the interdependence and co-existence of the two phenomena.

In this and our previous study, we demonstrated attenuation of: (i) respiratory coupling in neural activity, (ii) the response to hypoxia (as mimicked by intra-arterial CN^- injection), and (iii) the increase in respiratory modulation of SNA or coupling that normally occurs following hypoxia (Dick et al., 2004) (Fig. 1A). The dependence of the hypoxic response on an intact pons has been reported by other investigators. Bilateral lesions in the dl pons decrease the response to hypoxia (St John, 1979; Mizusawa et al., 1995; Song and Poon, 2009a, b) as well as the loss of post-hypoxic changes in patterning (Coles and Dick, 1996; Dick and Coles, 2000). Our results emphasize the role of the pons in coupling the relationship between breathing and sympathetic nerve activity.

4.2. Pulse modulation of respiratory neural activity in the pons

We have reported pulse modulation of respiratory activity in the medulla as well as in respiratory motoneurons, specifically in neurons associated with the control and expression of breathing (Dick and Morris, 2004; Dick et al., 2005). But ironically, our first recordings that we analyzed for co-expression of respiratory- and pulse-modulated activity were performed in the pons (Fig. 3). The limitation of not knowing the context in which they functioned became apparent in identifying neurons with weak respiratory modulation and weak or high pulse modulation (see Fig. 3). Because pontine activity associated with respiratory control becomes more modulated after vagotomy, we examined respiratory and pulse modulation before and after vagotomy. Further, we hypothesized that the increase in respiratory modulation would result in a decrease in pulse modulation.

The data that we analyzed for this manuscript were selected from a set of activity recordings of dl pontine neurons (for histology,

see (Dick et al., 2008)). Specifically, we selected the recordings for pulse-modulated analysis on the basis of visual inspection of their pulse-triggered histograms indicating the possibility that the activity of these neurons was correlated to pulse or cardiac rhythm. In this subpopulation, 68% of the neurons were modulated with respiration before vagotomy; and 70%, after vagotomy. While this does not appear to be a significant difference, before vagotomy over half (55%) had very weak η^2 values (<0.06) and only 10%, had high values (>0.2); whereas after vagotomy, only 20% had η^2 values less than 0.06 but nearly half (45%), greater than 0.2. This result is the expected effect of vagotomy (Shaw et al., 1989; Cohen and Shaw, 2004). In contrast vagotomy both increased and decreased expression of pulse-modulated activity in the activity of pontine neurons. The neurons with the 5 highest δ^2 values before vagotomy lost their pulse-modulation after vagotomy whereas the neurons with the 5 highest δ^2 values after vagotomy had significant pulse modulation in their activity pattern prior to vagotomy.

The loss of pulse-modulated activity is consistent with our hypothesis and could be related to a gain in respiratory-modulated inputs or a loss of baro-receptor input or even other cardiac afference with pulse-modulated activity that travels with the vagus nerve. The aortic depressor nerve carries most of the baro-information from the aortic arch and this nerve was transected along with the vagal nerve. In the dog, section of intrathoracic vagal afferent branches increases arterial pressure even if the aortic depressor nerve had been sectioned prior to the vagal sectioning (Ito and Scher, 1979).

Gain of function may be related to pontine gating of baroreceptor input (Felder and Mifflin, 1988; Mifflin and Felder, 1990; Hayward et al., 1993; Hayward and Felder, 1998). The dorsolateral pons conditions baroreceptor input from the carotid sinus. Activation of the dl pons weakens the efficacy of carotid sinus nerve activation in the NTS and decreases the sympathetic response to barostimulation (Felder and Mifflin, 1988; Mifflin and Felder, 1990; Hayward et al., 1993; Hayward and Felder, 1998). Thus, increased respiratory-modulated activity in the dl pons may impose respiratory modulation on baroreceptor afferent input.

In summary, the mix of increases and decreases in pulse modulation, together with the shift in patterning of activity, is consistent with our model suggesting that activity patterns, and changes in them reflect a balance of inhibitory and excitatory inputs and alterations in that balance (Dick et al., 2008).

4.3. Cardio-respiratory coupling depends on the dl-pons

Sympatho-respiratory Coupling (SRC) is a dynamic property of homeostasis. Hypoxia as well as other reflexes can evoke or enhance SRC. For example, SRC is enhanced after single and repetitive exposures to hypoxia (Dick et al., 2004, 2007) and during and after the diving reflex (Dutschmann and Herbert, 1998). Further, in hypertensive animals the respiratory-related discharge of sympathetic nerve activity increases in expiration (Czyzyk-Krzeska and Trzebski, 1990; Zoccal et al., 2008; Simms et al., 2009). This increase in sympathetic activity may be related to the development of hypertension (Simms et al., 2009). We speculate that the dynamic property of SRC is related to dl pons. We demonstrated baseline SRC depends on activity in the dl pons.

Cardio-respiratory coupling, specifically the sinus arrhythmia, depends on the pons. Sinus arrhythmia defined as an increased heart rate during inspiration is mediated by a decrease in activity of on cardiac preganglionic vagal neurons whose cell bodies are in the principal column of the nucleus ambiguus (McAllen and Spyer, 1975, 1976; Daly, 1985; Bouairi et al., 2004). These neurons can express respiratory-modulated activity (Rentero et al., 2002; O'Leary and Jones, 2003; Evans et al., 2005). These early studies were performed in urethane-anesthetized animals where the sinus arrhythmia increased heart rate in expiration rather than in inspiration (Bouairi et al., 2004) and showed an increased activity in cardiac preganglionic neurons in either inspiratory or expiratory phases (Rentero et al., 2002; O'Leary and Jones, 2003). In our study, the sinus arrhythmia was such that heart rate was the greatest in inspiration and the lowest during the post-inspiratory period (the animal were decerebrated and unanesthetized). Consequently, cardiac preganglionic vagal neurons should have their least activity during inspiration and their greatest activity during post-inspiration. The medullary substrate for inspiratory inhibition or post-inspiratory excitation has been investigated by Mendelowitz and co-workers (Mendelowitz, 1999; Neff et al., 2003; Evans et al., 2005; Frank et al., 2009). In contrast our study has shown a pontine input modulates the respiratory sinus arrhythmia. In this regard, the expression of post-inspiratory period to depends on the pons, specifically the Kölliker-Fuse neurons (Dutschmann and Herbert, 2006; Rybak et al., 2007; Smith et al., 2007). Therefore, we speculate that in addition to the medullary processes, that pontine input excites cardiac vagal neurons decreasing heart rate in that phase. The transection eliminates this input and blocks the modulation. More research is required to determine the ponto-medullary interaction in determining respiratory sinus arrhythmia.

4.4. Respiratory modulation of the baroreflex depends on the pons

In our previously published study (Baekey et al., 2008), we demonstrated respiratory modulation of the baroreflex responses in the perfused *in situ* preparation. Increases in arterial pressure evoke a decrease in HR and blood pressure accompanied by a decrease in sympathetic activity as well as an increase in cardiac vagal efferent discharge. However, the magnitude of the decrease in HR depends on when in the respiratory cycle the increase in blood pressure occurs suggesting a gating of transmission within the central reflex arc (Potter, 1981). Consistent with this, Gilbey and co-workers have demonstrated that the baroreflex bradycardia appears most sensitive when the afferents are activated in early expiration—a time when cardiac vagal motor neurons are most excitable electrically (Gilbey et al., 1984). We have demonstrated a parallel effect on the magnitude of the baroreflex evoked decrease in SNA.

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