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Review

Structural and functional architecture of respiratory networks in the mammalian brainstem

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Neural circuits controlling breathing in mammals are organized within serially arrayed and functionally interacting brainstem compartments extending from the pons to the lower medulla. The core circuit components that constitute the neural machinery for generating respiratory rhythm and shaping inspiratory and expiratory motor patterns are distributed among three adjacent structural compartments in the ventrolateral medulla: the Bötzinger complex (BötC), pre-Bötzinger complex (pre-BötC) and rostral ventral respiratory group (rVRG). The respiratory rhythm and inspiratory–expiratory patterns emerge from dynamic interactions between: (i) excitatory neuron populations in the pre-BötC and rVRG active during inspiration that form inspiratory motor output; (ii) inhibitory neuron populations in the pre-BötC that provide inspiratory inhibition within the network; and (iii) inhibitory populations in the BötC active during expiration that generate expiratory inhibition. Network interactions within these compartments along with intrinsic rhythmogenic properties of pre-BötC neurons form a hierarchy of multiple oscillatory mechanisms. The functional expression of these mechanisms is controlled by multiple drives from more rostral brainstem components, including the retrotrapezoid nucleus and pons, which regulate the dynamic behaviour of the core circuitry. The emerging view is that the brainstem respiratory network has rhythmogenic capabilities at multiple hierarchical levels, which allows flexible, state-dependent expression of different rhythmogenic mechanisms under different physiological and metabolic conditions and enables a wide repertoire of respiratory behaviours.

Keywords: breathing; brainstem; respiratory rhythm and pattern generation; pons;
pre-Bötzinger complex; ventral respiratory column

1. INTRODUCTION

Vital circuits in the mammalian brainstem control respiratory movements and maintain homeostasis of the internal physiological state of the brain and body. Breathing is a primal homeostatic neural process, regulating levels of oxygen and carbon dioxide in blood and tissues, which are crucial for life. Rhythmic respiratory movements must occur continuously throughout life and originate from neural activity generated by specially organized circuits in the brainstem. The automatic and seemingly simple nature of the act of inspiration and expiration belies the complexity of the neural machinery involved. Indeed, although neuroscientists have been conducting intensive investigations of neural circuits involved in respiratory rhythm and pattern generation for more than a century, the underlying neural mechanisms are still not fully understood, and many key issues remain unresolved. Here, we provide a synopsis of new emerging views on the spatial and functional architecture of brainstem respiratory circuits.

Innate rhythmic movements such as breathing are thought to be produced by central pattern generator (CPG) networks. These are specialized neuronal circuits in the central nervous system that are intrinsically capable of generating rhythmic activity and motor output without rhythmic inputs from other central circuits and sensorimotor feedback signals (Marder & Calabrese 1996; Grillner *et al.* 2005). The patterned rhythmic activities generated by CPGs emerge from a combination of synaptic interactions among spatially distributed populations of neurons and intrinsic cellular properties of the neurons comprising the CPG. In addition, the CPG circuits are incorporated into

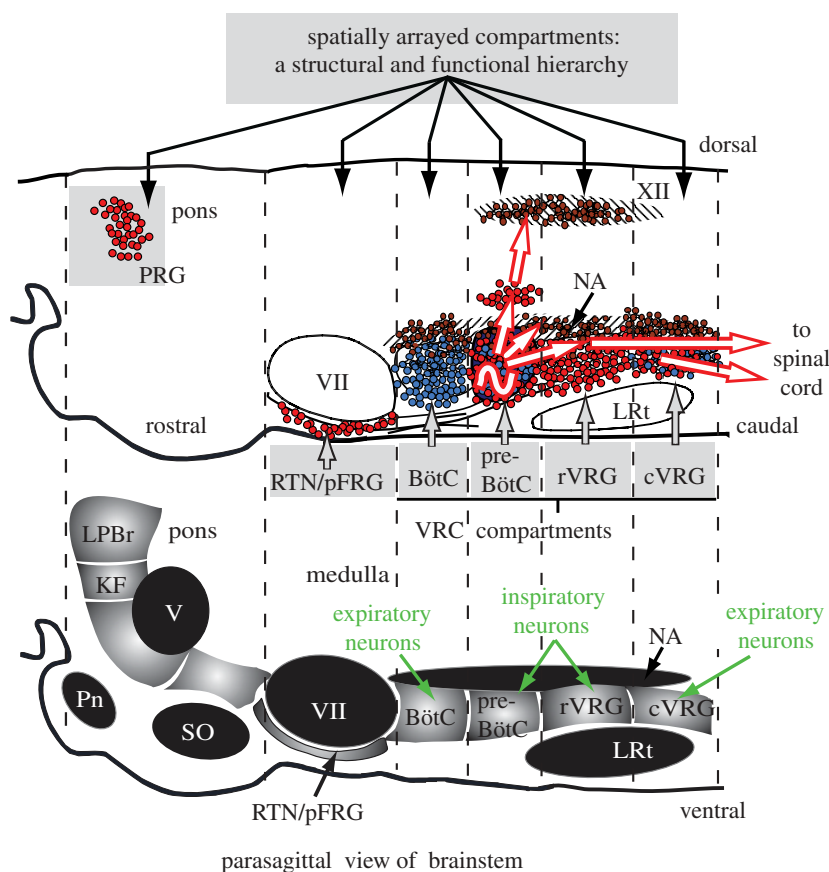*Author for correspondence (jsmith@helix.nih.gov).

Figure 1. Spatially arrayed respiratory compartments extending in the brainstem from the pons to caudal areas of the medulla are illustrated in a schematic parasagittal brainstem section at the level of nucleus ambiguus (NA), VRC, facial nucleus (VII) and the pons, including rostral dorsolateral pontine respiratory regions (PRG consisting of LPBr, lateral parabrachial region and KF, Kölliker–Fuse nucleus). The top schematic illustrates concentrations of excitatory neurons (red circles) and inhibitory neurons (blue circles) in different compartments (cVRG, rVRG, pre-BötC, BötC, RTN/pFRG and PRG). The schematic at the bottom presents a simplified structural view of the serially arranged medullary and pontine compartments, also indicating distributions of the main populations of expiratory and inspiratory neurons within the medullary VRC compartments. Excitatory pre-BötC neurons (top schematic) project (white on red arrows) to rVRG excitatory inspiratory bulbospinal neurons and via premotor circuits to cranial motoneurons (brown circles, hypoglossal XII motoneurons illustrated dorsally). Excitatory expiratory bulbospinal neurons of the cVRG project to thoracic and abdominal spinal expiratory motoneurons. See text for descriptions of functional properties of each compartment and roles of the different populations of excitatory/inhibitory neurons. V, motor nucleus of the trigeminal nerve; Pn, ventral pontine nucleus; LRt, lateral reticular nucleus; SO, superior olive.

larger neural systems and operate under the control of various central and peripheral sensory inputs that modify the CPG-generated motor pattern, adjusting it to the internal and/or external environment, current motor task and organismal needs. Such inputs regulate not only the frequency and amplitude of the output rhythmic motor activity, but can also dramatically reconfigure the CPG by transforming the operational rhythmogenic and neural pattern formation mechanisms. Thus, CPG circuits not only serve as generators of spatio-temporal patterns of neural activity, but also function as neural substrates for sensorimotor integration. Understanding the complex neural processes involved in the operation of different CPGs, and in particular the respiratory CPG, including the mechanisms underlying the circuit dynamic reconfiguration under different conditions represents a central and challenging problem in neuroscience.

Indeed, breathing is a dynamically mutable motor behaviour that not only performs a vital homeostatic function, but is also integrated with many other physiological functions controlled by the brainstem and

higher CNS circuits, such as suckling, swallowing, sniffing, chewing, coughing, vomiting and vocalization. This requires a robust yet highly flexible network organization that can permit multiple state-dependent modes of operation. At the brainstem level, this functional flexibility appears to result from a hierarchically arranged architecture of neural circuits with structurally and functionally compartmentalized components, whose interactions can be dynamically re-organized to produce multiple rhythmic respiratory motor behaviours.

2. SPATIALLY ARRAYED FUNCTIONAL RESPIRATORY COMPARTMENTS: OVERVIEW
Breathing movements are produced by a spatially distributed pontine–medullary respiratory network generating rhythmic patterns of alternating inspiratory and expiratory activities that drive and coordinate the activity of spinal and cranial motoneurons (Cohen 1979; Bianchi *et al.* 1995; Feldman & Smith 1995; Richter 1996). The motor pattern during normal breathing is considered to consist of three phases:
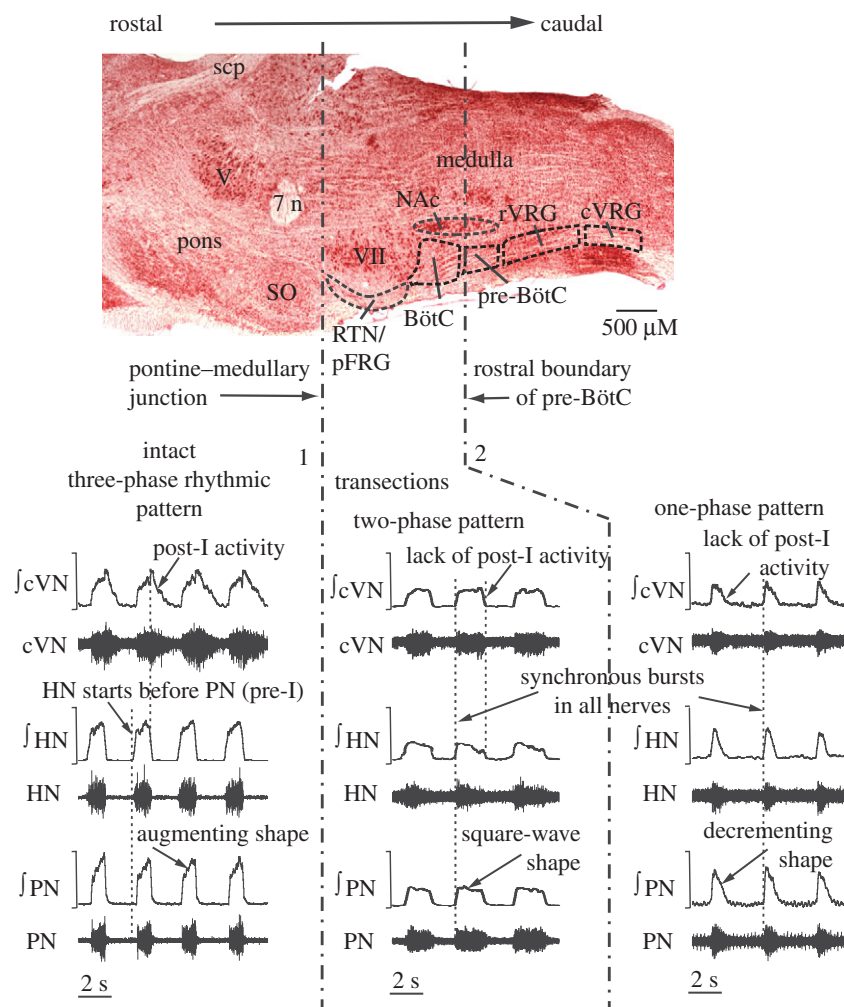
Figure 2. Transformations of respiratory rhythm and pattern following sequential brainstem transection in the *in situ* arterially perfused brainstem–spinal cord preparation from juvenile rat, revealing three rhythmic states of the respiratory network as the circuitry is progressively reduced. Top: parasagittal section (Neutral Red stain) of the mature rat brainstem (NAc, compact and semi-compact subdivisions of NA, both within outlined region; V, trigeminal motor nucleus; VII, facial nucleus; 7n, facial nerve; scp, superior cerebellar peduncle; SO, superior olive). Dimensions indicated are typical for a four- to five-week-old rat. Bottom: representative activity patterns of phrenic (PN), hypoglossal (HN) and central vagus (cVN) nerves recorded from the intact preparation (left) and from reduced preparations obtained by transections at the pontine–medullary junction performed to remove the pons (vertical dot-dashed line 1, middle panel) and at the rostral boundary of the pre-BötC made to remove all compartments rostral to pre-BötC (vertical dot-dashed line 2, right panel). Each panel shows raw (bottom traces) and integrated (upper traces) recordings of motor nerve discharge. Vertical dashed lines in the left panel indicate onsets of HN inspiratory burst and the post-I component of cVN activity characteristic of the intact three-phase rhythmic pattern, which also includes a ramping PN discharge. Dashed lines in middle and right panels indicate synchronous onset of inspiratory bursts in all nerves characteristic of the two-phase and one-phase rhythmic patterns. Motor nerve discharges have square-wave and decrementing shapes in the two-phase and one-phase patterns, respectively, which also characterize these rhythmic states (after Smith *et al.* 2007).

inspiration, post-inspiration (post-I or P-I) and the later stage 2 (E-2) of expiration (Richter 1996). This pattern originates within interconnected bilateral columns of medullary neurons, the ventral respiratory columns (VRCs), and is controlled by inputs from other medullary structures including the retrotrapezoid nucleus (RTN), raphé nuclei and more rostrally pontine circuits. The VRC includes several rostro-caudally arranged compartments (figure 1) (see also Alheid & McCrimmon 2008): the Bötzinger complex (BötC), the pre-Bötzinger complex (pre-BötC) and the rostral (rVRG) and caudal (cVRG) parts of the ventral respiratory group (VRG). Respiratory neurons in these compartments are typically classified based on their firing patterns (e.g. decrementing and

augmenting) and their phases of activity relative to the breathing cycle, such as: early-inspiratory (early-I) neurons, with a decrementing firing pattern during inspiration; ramp-inspiratory (ramp-I) neurons, with an augmenting firing pattern during inspiration; post-I neurons, with a decrementing firing pattern during expiration; augmenting expiratory (aug-E) neurons, with an augmenting firing pattern during expiration; and a heterogeneous population of pre-inspiratory (pre-I or also referred to as pre-I/I) neurons that start firing before the onset of inspiration and continue throughout the inspiratory phase (see Richter 1996, for detailed review).

The new concept we propose is that these specific populations of respiratory neurons are arrayed in the

spatially distinct compartments described earlier and constitute microcircuits that interact within and between these compartments. Furthermore, the microcircuits within each compartment are hypothesized to represent functionally distinct network building blocks. A synopsis of functional properties of these compartments and circuit components is provided in the following.

(a) Pontine circuits

The pontine respiratory regions, called the pontine respiratory group (PRG), include neuronal populations in the Kölliker–Fuse (KF) nucleus and parabrachial (PB) complex (lateral, LPB, and medial, MPB, nuclei) in the rostral dorsolateral pons (figure 1) and several areas in the ventrolateral pons. Although the functional role of the pons in the generation and control of respiratory rhythm and pattern has not been fully established, the above-mentioned pontine regions have been shown to interact with multiple medullary compartments; these interactions provide strong modulation of medullary respiratory network activity and control respiratory phase transitions (e.g. Cohen 1979; St-John 1998; Okazaki *et al.* 2002; Alheid *et al.* 2004; Cohen & Shaw 2004; Ezure 2004; Song & Poon 2004; Dutschmann & Herbert 2006; Ezure & Tanaka 2006). Pontine connections to caudal medullary circuits appear to be critical for coordinating the activity of expiratory muscles and upper airway musculature during expiration and for expression and regulation of post-I activity (Dutschmann & Herbert 2006; reviewed in Mörschel & Dutschmann 2009). There is also an emerging consensus that pontine circuits may play a fundamental role in sensorimotor integration (Song & Poon 2004; Potts *et al.* 2005).

(b) Retrotrapezoid nucleus/parafacial respiratory group compartment

Neuronal clusters that constitute the RTN (Smith *et al.* 1989) are located below and extend through the rostro-caudal levels of the facial motor nucleus. RTN contains neurons whose activity is sensitive to the brain carbon dioxide level and also receives input from peripheral, oxygen-sensitive chemoreceptors and hence is thought to function as a major chemosensory region (Mulkey *et al.* 2004; Guyenet *et al.* 2005). RTN provides chemosensitive excitatory drives to most respiratory populations of the VRC, which modulates the activity of the medullary respiratory network and adapts the activity to the metabolic state of the system (Nattie 1999; Mulkey *et al.* 2004; Guyenet *et al.* 2005). Onimaru *et al.* (1988, 2006) and Onimaru & Homma (1997), studying this area in neonatal rodent *in vitro* brainstem–spinal cord preparations, found a group of 'pre-I' oscillatory neurons located in the so-called parafacial respiratory group (pFRG) that appears to spatially overlap the RTN. They have proposed that the pFRG provides a primary rhythmic excitatory drive that entrains the pre-BötC inspiratory oscillator (below). In contrast, other investigators have proposed that pFRG activity represents an expiratory oscillator that interacts with the inspiratory oscillator in the pre-BötC to generate

coordinated patterns of inspiratory and expiratory activity (Feldman & Del Negro 2006; Janczewski & Feldman 2006, also see the following). Thus, an emerging view is that the RTN/pFRG compartment may contain functionally distinct populations of neurons involved in several interrelated basic functions, including coordinating inspiratory and expiratory activities in a metabolic state-dependent manner (see Champagnat *et al.* 2009 for additional information on structural/functional organization, including developmental origins).

(c) Bötzinger complex compartment

The BötC, with predominately expiratory neurons (post-I and aug-E), is considered to be a major source of expiratory activity in the system during normal breathing (Ezure 1990; Jiang & Lipski 1990; Tian *et al.* 1999; Ezure *et al.* 2003). This function of the BötC and its interactions with other VRC compartments have been extensively investigated (Fedorko & Merrill 1984; Long & Duffin 1986; Ezure & Manabe 1988; Ezure 1990; Jiang & Lipski 1990; Tian *et al.* 1999; Ezure *et al.* 2003; Shen *et al.* 2003), although an exclusive role of BötC neurons in the generation of expiration has been recently debated (see RTN/pFRG mentioned earlier). Recent work indicates that the BötC contains critical respiratory network elements generating two major forms of expiratory activity (Smith *et al.* 2007), which is consistent with a basic role of the BötC in expiratory pattern generation. This role arises from the presence of rhythmic expiratory inhibitory neurons making widely distributed interconnections with other compartments (see §4). The BötC neurons are critically involved in the control of the transition between inspiratory and expiratory activities in the network, which is fundamental for the rhythmic inspiratory–expiratory alternation essential for normal breathing.

(d) Pre-Bötzinger complex compartment

Adjacent and caudal to the BötC, the pre-BötC contains interneuron circuitry essential for generating inspiratory activity (Smith *et al.* 1991, 2000; Rekling & Feldman 1998; Koshiya & Smith 1999; Feldman & Del Negro 2006). The pre-BötC has been of intense interest because it is thought to function as a kernel structure that is a main source of rhythmic inspiratory excitatory drive to premotor circuits. Furthermore, pre-BötC circuits can express autorhythmic or pacemaker-like activity that generates a rudimentary pattern of inspiratory activity when the structure is experimentally isolated *in vitro* (Koshiya & Smith 1999; Johnson *et al.* 2001). This activity is proposed to be based on excitatory synaptic interactions within the pre-BötC and intrinsic cellular mechanisms involving persistent sodium current (I_{NaP}, Butera *et al.* 1999a,b; Smith *et al.* 2000; Rybak *et al.* 2003, 2004b; Purvis *et al.* 2007; Koizumi & Smith 2008). The intrinsic rhythmic activity of the network may also involve calcium-activated nonselective cationic current (I_{CAN}), which in combination with excitatory synaptic interactions can also provide cellular- and network-level rhythmic bursting (Ramirez *et al.* 2004; Del Negro *et al.* 2005; Pace *et al.* 2007). Collectively, these (and other) neuronal currents and excitatory synaptic interactions provide mechanisms for

regenerative initiation, maintenance and termination of inspiratory network activity in the isolated pre-BötC *in vitro*. Mechanisms underlying rhythmic inspiratory pattern generation in the pre-BötC under more physiological conditions (i.e. when the pre-BötC is embedded in the intact brainstem) are more complex, because there is a dynamic overlay of rhythmic inhibitory, tonic excitatory and other modulatory inputs that converge on the pre-BötC excitatory network (Smith *et al.* 2000, 2007; see §4). Furthermore, the cellular composition of the pre-BötC is heterogeneous, containing different electrophysiological phenotypes (Smith *et al.* 2000; Del Negro *et al.* 2001, 2002*a*,*b*; Stornetta *et al.* 2003; Ramirez *et al.* 2004; Koizumi & Smith 2008), including subpopulations of cranial pre-motoneurons (Koizumi *et al.* 2008) and populations of rhythmically active GABAergic (Kuwana *et al.* 2006) and glycinergic inhibitory neurons (Winter *et al.* 2009). These latter inhibitory neurons function critically in dynamically coordinating inspiratory and expiratory activity (discussed subsequently). Thus, pre-BötC circuits subserve two basic functions: (i) generation of rhythmic excitatory inspiratory drive, including the pre-I component of this drive (Schwarzacher *et al.* 1995; Smith *et al.* 2000, 2007; Feldman & Del Negro 2006) that is important for initiation of the inspiratory phase and (ii) coordination of inspiratory–expiratory pattern formation via inspiratory inhibition provided by the pre-BötC inhibitory neurons.

(e) Rostral ventral respiratory group compartment

This compartment contains the main bilateral clusters of bulbospinal inspiratory (ramp-I) excitatory neurons that project to spinal phrenic and intercostal inspiratory motoneurons and shape the inspiratory motor output pattern (Bianchi *et al.* 1995; Richter 1996). These neurons are driven by the pre-BötC excitatory neurons and are inhibited during expiration by the BötC inhibitory neurons; both of these inputs along with other modulatory drives shape and control the characteristic ramping pattern of inspiratory rVRG activity. Thus, the rVRG is also a compartment with multiple convergent input drives essential for inspiratory pattern formation but in contrast to the pre-BötC, this neuronal population as a whole does not have intrinsic rhythmogenic capability (Smith *et al.* 2007).

(f) Caudal ventral respiratory group compartment

Excitatory bulbospinal expiratory neurons that project to spinal thoracic and lumbar expiratory motoneurons are concentrated in the retroambiguus area (Ezure 1990). This compartment is presumed to be the expiratory counterpart to the inspiratory rVRG. Convergent inputs including those from BötC that are synaptically integrated in the cVRG, locally shape the patterns of excitatory and inhibitory expiratory bulbospinal drives.

3. A SPATIAL AND FUNCTIONAL HIERARCHY OF MULTIPLE RHYTHMOGENIC MECHANISMS

The spatial and functional organization of the brainstem respiratory network has been recently studied in

an *in situ* arterially perfused rat brainstem–spinal cord preparation (Paton 1996; Pickering & Paton 2006) using sequential rostral-to-caudal microtransections through the brainstem while recording cranial and spinal motor outflow, as well as compartmental neuronal population activity to observe transformations of network behaviour (Rybak *et al.* 2007; Smith *et al.* 2007). This approach is similar to that applied in neonatal rat brainstem–spinal cord preparations *in vitro* that resulted in the original discovery of the pre-BötC (Smith *et al.* 1991), but now applied in a mature rat nervous system generating neural activity patterns more similar to those *in vivo*. This approach was designed to test the concept that there exists structural and functional compartmentalization of circuit elements as outlined earlier. Informed by the anatomy, it was hypothesized that there is a rostral-to-caudal stacking of network building blocks subserving distinct circuit functions, which are fully integrated in the intact system, but can be revealed as particular compartments are removed.

The major results obtained from these studies are that sequential reduction of the network progressively re-organizes network dynamics, such that new rhythmogenic mechanisms emerge. Specifically, starting from a transection at the pontine–medullary junction (figure 2), the normal three-phase respiratory pattern is transformed to a two-phase rhythmic pattern lacking the post-I phase. With more caudal transections made close to or at the rostral boundary of pre-BötC (figure 2), the respiratory activity transforms to 'one-phase' inspiratory oscillations originating within the pre-BötC, without critical involvement of phasic expiratory inhibition (Rybak *et al.* 2007; Smith *et al.* 2007). From these results, we concluded that: (i) generation of the normal three-phase rhythmic pattern requires the presence of the pons (i.e. excitatory drive from pontine neurons to the VRC); (ii) generation of the two-phase pattern is intrinsic to reciprocal inhibitory synaptic interactions between the BötC and the pre-BötC and may also involve the RTN to provide excitatory drive to generate stable behaviour; and (iii) the one-phase inspiratory oscillations are generated within the pre-BötC and rely on intrinsic cellular mechanisms operating in the context of the pre-BötC excitatory network. The latter is inferred since systemic application of riluzole, a blocker of I_{NaP}, abolished the one-phase oscillations in contrast to the three-phase and two-phase patterns that persisted in the presence of riluzole (Smith *et al.* 2007). Remarkably, the disturbances of the pre-BötC inspiratory oscillations by blocking I_{NaP} in the reduced mature neuraxis *in situ* were very similar to those obtained with the pre-BötC compartment isolated *in vitro* within neonatal rat medullary slices (Koizumi & Smith 2008).

This approach of sequentially reducing the pontine–medullary network, coupled with the analysis of network activity patterns and systematic probing for pre-BötC autorhythmic mechanisms, has provided a new unified view that integrates several earlier models (Smith *et al.* 2000; Richter & Spyer 2001; Rybak *et al.* 2004*a*). The basic conclusion is that
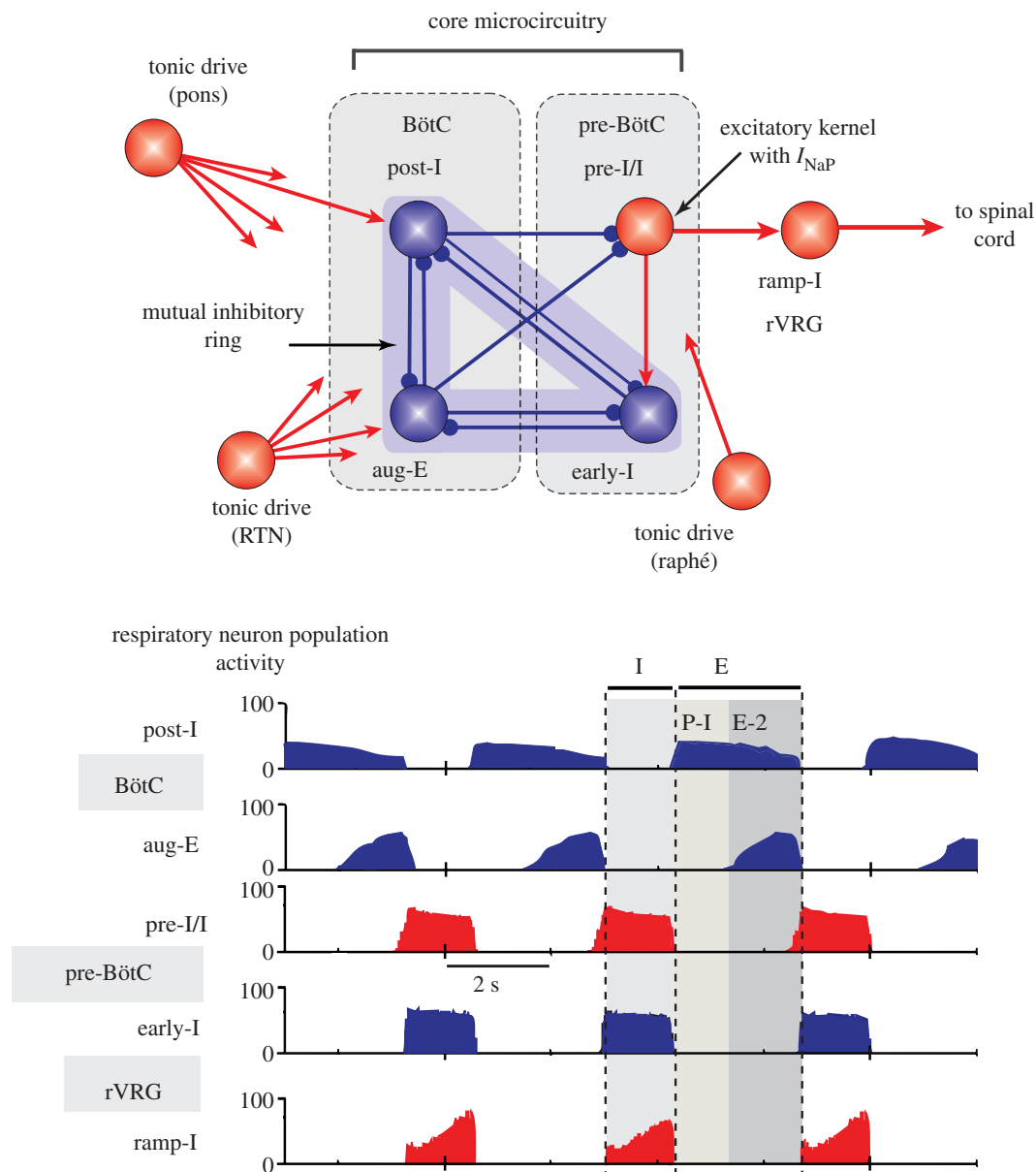
Figure 3. Schematic of the core microcircuitry within BötC and pre-BötC respiratory compartments and activity patterns of respiratory neuron populations of the core circuitry. Shown are the main interacting neuronal populations in these compartments operating under the control of external excitatory drives from the pons, RTN and raphé nuclei. Spheres represent neuronal populations (excitatory, red, including tonic drives to the different populations; inhibitory, blue). Red arrow, excitatory connection; small blue circles, inhibitory connection. Inhibitory neurons distributed in the BötC and pre-BötC compartments are interconnected in a mutual inhibitory ring-like circuit (blue background shading, see bottom for activity patterns) that dynamically interacts with the excitatory kernel network in the pre-BötC. The excitatory (glutamatergic) pre-BötC neurons with pre-I/inspiratory discharge patterns have persistent sodium current (I_{NaP}) that underlies an important cellular-based oscillatory mechanism contributing to the autorhythmic properties of the pre-BötC network when this structure is isolated and generates a one-phase rhythmic inspiratory pattern. Pre-BötC excitatory neurons rhythmically excite neurons of the rVRG that generate a ramping pattern of excitatory activity (ramp-I) that then drives inspiratory spinal motoneurons such as phrenic motoneurons in the cervical spinal cord. Respiratory neuron population activity patterns, shown below by the colour-filled activity profiles representing integrated population activity (population spike frequency histograms, units are spikes s^{-1} per neuron), are obtained from a computational model of the network that reproduces the patterns of activity recorded from neuron populations in the BötC, pre-BötC and rVRG compartments (see Smith *et al.* 2007 for details of the computational model and electrophysiologically recorded activity patterns of the different neuronal populations). The three phases of the respiratory cycle—inspiration (I), and the two phases of expiration, P-I and stage 2 of expiration (E-2)—are indicated. Neurons with peak activity during post-I define this phase (although post-I neuron population activity also typically extends into the next expiratory phase), while neurons with augmenting (aug-E) discharge patterns define E-2. See text for additional descriptions.

there exists a spatial and dynamical hierarchy of interacting pontine, BötC and pre-BötC circuits, each of which controls different aspects of respiratory rhythm generation and pattern formation, which can

be revealed as the network is progressively reduced. The expression of each rhythmogenic mechanism is state-dependent and produces specific motor patterns likely to underpin distinct motor behaviours.
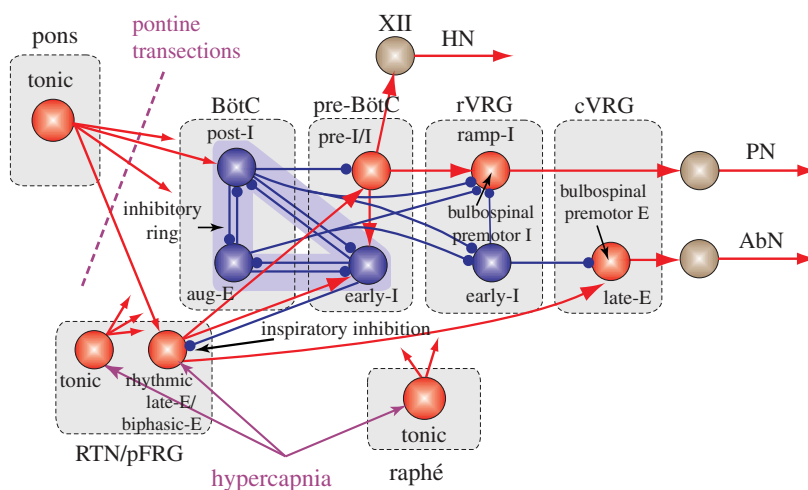
Figure 4. Schematic of an extended model of the brainstem respiratory network indicating possible state-dependent neuronal interactions between the RTN/pFRG, the core BötC and pre-BötC circuitry and more caudal compartmentalized circuit components. The schematic shows interacting neuronal populations within the major brainstem respiratory compartments (pons, BötC, pre-BötC, rVRG and cVRG) and includes proposed interactions of a late-E neuronal population in the RTN/pFRG, activated by hypercapnia, with the other respiratory neuron populations. The late-E population in the RTN/pFRG receives a necessary excitatory drive from the pons, which under quiet eupneic breathing conditions is not sufficient to evoke rhythmic activity in this population. We propose that during quiet breathing, these neurons are not rhythmically active and are inhibited by early-I inhibitory neurons located in the pre-BötC (see connection labelled inspiratory inhibition). Hypercapnia excites late-E neurons in the RTN/pFRG, so that these cells may start generating bursts in advance of bursts of the pre-BötC pre-I/I population. This expressed RTN/pFRG late-E activity excites abdominal expiratory motoneurons possibly via the excitatory bulbospinal premotor neurons located in cVRG as indicated, resulting in late-E activity in abdominal motor nerves (AbN). We also suggest, as indicated in the schematic, that the late-E population excites the pre-I/I population in the pre-BötC, contributing to an earlier onset and enhancement of the pre-I component of hypoglossal nerve (HN) discharge. Thus this schematic incorporates the concept that cells in the RTN/pFRG exhibit both CO_2-sensitive tonic and rhythmic activity profiles, the rhythmic cells are also controlled by pontine inputs and these cells have excitatory actions on both inspiratory (including early-I) and expiratory neuronal components of the circuitry. Other cell populations that provide tonic excitatory drive to the network, such as raphé serotonergic neurons, have also been proposed to provide state-dependent excitatory drive to the network, including in response to alterations in blood/brain CO_2, as depicted in the schematic. Red arrow, excitatory connection; small blue circles, inhibitory connection; red sphere, excitatory population; blue sphere, inhibitory population; brown sphere, motoneurons.

4. CORE CIRCUITRY OF THE RESPIRATORY PATTERN GENERATION NETWORK

A minimal network configuration proposed to represent core circuitry (figure 3) that incorporates the multiple rhythmic states described earlier and their transformations as observed experimentally includes: (i) a mutually inhibitory circuit with a ring-like architecture composed of post-I and aug-E neurons of the BötC compartment, and early-I neurons within the pre-BötC; and (ii) a pre-BötC kernel of excitatory pre-I/inspiratory neurons, with persistent sodium current (I_{NaP})-dependent intrinsic bursting properties. The latter participate dynamically in the expiratory–inspiratory phase transition and generation of the inspiratory phase. Detailed descriptions and computational models of the dynamic operation of this core network and the circuit elements participating in each rhythmic state can be found in a series of related publications (Rybak *et al.* 2007; Smith *et al.* 2007; Rubin *et al.* 2009). Figure 3 shows examples of simulations from these computational models illustrating activity patterns of each population within the core BötC and pre-BötC compartments and the resulting activity pattern downstream in the rVRG, which shapes and provides the essential excitatory drive to spinal inspiratory motoneurons.

The rhythmogenic mechanisms expressed by this core circuitry are postulated to depend on 'excitatory drives' that carry state-characterizing information provided by multiple sources distributed within the brainstem, particularly the pons, RTN, raphé nuclei and nucleus of the solitary tract (not shown in figure 3). These drives include those considered to be major chemoreceptor sites sensing CO_2/pH and/or receiving input from peripheral chemoreceptors sensing CO_2/pH and low O_2 (i.e. RTN and raphé nuclei, Nattie 1999; Richerson 2004; Guyenet *et al.* 2005). Although currently not completely defined, these drives appear to have a spatial organization that maps specifically on the spatial organization of the network. These drives are conditionally represented in the core circuit schematic (figure 3) by three separate sources located in the pons, RTN and raphé, the latter of which provides excitatory serotonergic modulatory inputs to the pre-BötC and other network components (Richerson 2004; Ptak *et al.* 2009). These serotonergic inputs have been implicated in state-dependent regulation of respiratory network activity including that in response to hypercapnia (figure 4) and also in pathological respiratory behaviour including sudden infant death syndrome (Paterson *et al.* 2006). The computational models incorporating the core

circuitry and these three major drives (figure 3) have been able to reproduce the three-, two- and one-phase rhythmic regimes and their transformations (Rybak *et al.* 2007; Smith *et al.* 2007; Rubin *et al.* 2009). Importantly, recent modelling has shown that the core circuitry is a robust dynamical structure in which each element of the circuitry represents a node for control of oscillatory frequency by the input drives (Rubin *et al.* 2009). For example, augmenting net excitatory drive to the pre-BötC excitatory kernel can increase inspiratory frequency by a factor of three, accompanied by a reduction in expiratory duration and a smaller decrease in inspiratory duration. In contrast, increasing drive to the BötC aug-E population can reduce frequency by a factor of three owing to the associated prolongation of expiratory phase duration (Rubin *et al.* 2009).

5. OTHER OSCILLATORY MECHANISMS IN THE NETWORK

The studies of Onimaru & Homma (1997, 2003) and Janczewski & Feldman (2006; see also Feldman & Del Negro 2006) have lead to different proposals for fundamental respiratory rhythm generation mechanisms. It has been suggested that a neural oscillator in the pFRG interacts with the pre-BötC inspiratory oscillator to generate coordinated patterns of inspiratory and expiratory activity in neonatal rodents. While there is currently no clear consensus about the nature and functional role of this mechanism, there has been intense general interest in properties of the pFRG, including developmental origins (Champagnat *et al.* 2009). This has been bolstered by data of Guyenet *et al.* (2005), who have shown that the RTN, which as noted earlier spatially overlaps the pFRG, appears to provide a fundamental chemosensory drive to the VRC. Furthermore, developmental studies show that RTN/pFRG cells embryologically have a transcription factor (Phox2b) expression phenotype and that deletion of these cells in mutant mice causes a congenital central hypoventilation-like syndrome associated with irregular breathing, lack of responsiveness to hypercapnia and fatality at birth (Dubreuil *et al.* 2009).

Recent studies investigating the nature of RTN/pFRG activity and its potential role as an oscillatory mechanism within *in situ* perfused brainstem–spinal cord preparations from mature rats (Abdala *et al.* in press) suggest that a subpopulation of RTN/pFRG cells become rhythmically active during hypercapnia. This emergent activity is associated with a transformation of activity of motor outflows generating expiratory muscle activity, particularly abdominal nerve (AbN) motor outflow. During eupnoeic-like respiratory activity, only low-amplitude post-I discharge was observed in AbN expiratory discharge, but AbN late-expiratory (late-E) activity, immediately preceding inspiratory phrenic nerve bursts, occurred during hypercapnia (2–5% elevation of CO_2). This AbN activity constitutes active/forced expiration. This activity did not occur after removing the pons by pontine–medullary transections or after chemical inactivation of the RTN region by site-specific

application of a $GABA_A$ receptor agonist (isoguvacine) to locally silence neurons.

One plausible conclusion from these results is that there are distinct subpopulations of RTN/pFRG neurons operating under control of pontine circuits and the metabolic state of the system that provide the necessary excitation to activate an additional RTN/pFRG oscillatory mechanism. This mechanism operates only under conditions of high respiratory drive to generate a central late-E activity projected to AbN. We hypothesize that this oscillatory mechanism is not a necessary component of the respiratory CPG, but constitutes an adaptive mechanism activated under critical metabolic conditions to entrain both inspiration and active expiration. This view is congruent with the ideas that the pFRG provides both tonic (Guyenet *et al.* 2005) and rhythmic (Onimaru *et al.* 2003) pre-I excitatory drives to the BötC, pre-BötC and other VRC circuit elements, and that this region is involved in the generation of active expiration *in vivo* (Feldman & Del Negro 2006). This late-E/pre-I activity that emerges under specific metabolic conditions is provided by rhythmogenic mechanisms that are distinct from, and additional to, the mechanisms operating within the core VRC circuitry (figure 3). Figure 4 shows a schematic of our extended model of the core circuitry with state-dependent rhythmic interactions between the core respiratory network and the RTN/pFRG that incorporates this additional rhythmogenic mechanism emerging during hypercapnic conditions. As depicted in this figure, it is possible that the RTN/pFRG not only subserves a central chemosensory function but also contains cells integrating a variety of state-dependent signals, including those from the pons to coordinate the activity of abdominal and upper airway musculature, particularly at high levels of respiratory drive.

6. GENERAL INSIGHTS INTO THE FUNCTIONAL ORGANIZATION OF BRAINSTEM RESPIRATORY CIRCUITS

A number of models describing the functional organization of brainstem respiratory networks have been proposed over the past two decades. These include three-phase models involving predominantly inhibitory network interactions (Richter 1996; Rybak *et al.* 1997; Richter & Spyer 2001), hybrid pacemaker-network models (Smith *et al.* 2000; Rybak *et al.* 2004*a*, 2009; Rubin *et al.* 2009) in which the pre-BötC with autorhythmic properties is dynamically controlled by inhibitory networks, a dual inspiratory pacemaker neuron model (Ramirez *et al.* 2004), an alternating inspiratory–expiratory two-oscillator model (Feldman & Del Negro 2006; Janczewski & Feldman 2006) and a coupled dual excitatory oscillator model (Onimaru & Homma 2003; Onimaru *et al.* 2006). However, the actual spatial and dynamical organization of the brainstem respiratory network has been difficult to define unequivocally. The core circuitry described earlier and its interactions with other brainstem elements suggest that the pontine–medullary respiratory network has a specific spatial and functional organization extending from the rostral pons to the caudal VRC.

Although some respiratory neuron types (e.g. post-I, aug-E and early-I) are not strictly localized within particular medullary compartments, but rather are distributed throughout the VRC (Segers *et al.* 1987; Ezure 1990; Bianchi *et al.* 1995), each compartment nevertheless contains dominant populations that may define each compartment's specific functional role. A basic principle is that each compartment operates under the control of more rostral compartments (Smith *et al.* 2007), constituting a rostro-caudal functional hierarchy of interacting circuit building blocks. Specifically, at the caudal end of the VRC, the cVRG bulbospinal expiratory neurons are controlled by the BötC and inputs from more rostral compartments (e.g. arising within the RTN). Similarly, inspiratory activity of rVRG bulbospinal premotor inspiratory neurons is formed by an excitatory inspiratory synaptic drive from the pre-BötC excitatory neurons and phasic inhibition from BötC expiratory neurons. In turn, the pre-BötC is controlled by the rostrally placed BötC that inhibits the pre-BötC during expiration, whereas the RTN and pontine nuclei provide excitatory drives to BötC, pre-BötC and rVRG. The pontine activation of expiratory BötC populations (especially post-I neurons) provides a widely distributed inhibition within the network during expiration, which appears critical for rhythm and pattern generation under normal conditions. At the level of RTN, at least some neurons (late-E) appear to operate under state-dependent control by the rostral pons.

7. FUNCTIONAL SIGNIFICANCE OF MULTIPLE RHYTHMOGENIC MECHANISMS

The functional diversity of the respiratory CPG necessitates that respiratory circuits have a flexible organization, permitting multiple state-dependent modes of operation. The experimental results reviewed earlier indicate that respiratory circuits incorporate at least four oscillatory mechanisms (others may exist) and that such rhythmic states can occur under different physiological and pathophysiological conditions. Changes in metabolic conditions (levels of carbon dioxide/pH, or oxygen) that alter the balance of excitatory/inhibitory drives and excitability of pontine, RTN, BötC and pre-BötC neuronal populations can change network interactions, producing transformations from the normal three-phase rhythmic state to the other rhythmogenic states inherent in the system. For example, severe hypoxia transforms the respiratory system to generate a one-phase, I_{NaP}-dependent inspiratory gasping rhythm (Paton *et al.* 2006) that represents a functional unembedding of I_{NaP}-dependent oscillatory mechanisms intrinsic to the pre-BötC. Furthermore, the I_{NaP}-dependent mechanism operating in reduced states such as *in vitro* may not be exclusive, and the pre-BötC may have several intrinsic mechanisms of inspiratory rhythm generation as noted earlier. Thus the pre-BötC, as one of the key compartments, when functionally embedded in the spatially distributed brainstem network, can operate in multiple modes of rhythm generation, depending on interactions with other compartments. Other studies have shown that hypocapnia can convert the

network three-phase to a two-phase rhythmic pattern (A. P. L. Abdala, J. C. Smith, I. A. Rybak & J. F. R. Paton 2007, unpublished data; Sun *et al.* 2001). Emergence of other oscillatory mechanisms such as those underlying active expiration at high levels of respiratory drive (e.g. hypercapnia) was discussed earlier.

8. SUMMARY AND PERSPECTIVES

An emerging view is that the architecture of respiratory CPG circuits incorporates multiple rhythmogenic mechanisms that are inherent in the spatial and functional organization of the brainstem respiratory network. This network has rhythmogenic capabilities at multiple levels of cellular and network organization with oscillatory mechanisms ranging from inhibitory network-based synaptic interactions, to neuronal conductance-based endogenous rhythmogenic mechanisms. The emergence of each oscillatory mechanism depends on the metabolic and functional state of the system and is controlled by multiple sources of drives located within the medulla and pons, some of which are sensitive to the levels CO_2, O_2 and pH that provide for homeostatic regulation, while others arise from multiple descending systems involved in sensorimotor integration. The architecture of the core circuitry with an embedded excitatory kernel provides multiple nodes for external control of respiratory oscillation frequency and phase durations as well as for dramatically changing the mode of respiratory CPG operation.

Further experimental investigations are required to more precisely define the neuronal connection patterns and dynamic interactions of the core circuit elements and input drives to test and refine the concept of the hierarchical organization of the respiratory CPG. It remains to be established how the brain exploits this architecture and alters network interactions and cellular properties to transform the respiratory rhythm and pattern to behaviourally adapt breathing to different physiological and metabolic states.

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