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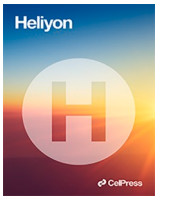

Review article

The hypoxic respiratory response of the pre-Bötzinger complex

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ABSTRACT

Since the discovery of the pre-Bötzinger Complex (preBötC) as a crucial region for generating the main respiratory rhythm, our understanding of its cellular and molecular aspects has rapidly increased within the last few decades. It is now apparent that preBötC is a highly flexible neuronal network that reconfigures state-dependently to produce the most appropriate respiratory output in response to various metabolic challenges, such as hypoxia. However, the responses of the preBötC to hypoxic conditions can be varied based on the intensity, pattern, and duration of the hypoxic challenge. This review discusses the preBötC response to hypoxic challenges at the cellular and network level. Particularly, the involvement of preBötC in the classical biphasic response of the respiratory network to acute hypoxia is illuminated. Furthermore, the article discusses the functional and structural changes of preBötC neurons following intermittent and sustained hypoxic challenges. Accumulating evidence shows that the preBötC neural circuits undergo substantial changes following hypoxia and contribute to several types of the respiratory system's hypoxic ventilatory responses.

1. Introduction

The neural network responsible for three-phasic respiration (inspiration, post-inspiration, and late-expiration) in mammals extends rostrocaudally in the ventral respiratory column (VRC) of the medulla oblongata [1–3]. This neural axis comprises the retrotrapezoid nucleus and the parafacial respiratory group (RTN/pFRG) at the most rostral end, the Bötzinger complex (BötC) and the preBötC in the middle, and the rostral and caudal ventral respiratory groups (rVRG and cVRG, respectively) at the most caudal end. Among these regions, preBötC has received particular attention because it is believed that the activity of its neurons is sufficient for generating one-phasic respiratory activity (inspiration) [2]. During regular breathing, the preBötC acts in concert with other medullary and pontine respiratory nuclei such as BötC, the parabrachial nucleus/Kölliker-Fuse complex (PBN/KF), and the post-inspiratory complex (PiCo) to generate the three-phasic respiratory rhythm [1,4,5]. The final respiratory rhythm is transmitted to respiratory pre-motoneurons, which activate the hypoglossal and phrenic motoneurons [2], as well as the motoneurons for external intercostals [6].

It is well established that individual neuronal networks reconfigure in response to hypoxic conditions to produce the most suitable output [7,8]. Neural networks responsible for brain functions that are not instantly essential for survival become deactivated, and

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critical functions such as breathing are enhanced [7]. The respiratory system of mammals responds to hypoxic conditions by altering the respiratory frequency and tidal volume. The hypoxic ventilatory response (HVR) may involve any part of the respiratory system, from peripheral chemoreceptors to respiratory motoneurons [9]. Since neurons [10,11] and glia [12–14] of the preBötC respond to local hypoxia, it is reasonable to hypothesize that this region may also contribute to the HVR. The preBötC is a complicated neural network that reconfigures state-dependently to produce various patterns of respiratory output [15–18]. *In vitro*, the preBötC generates multiple patterns of rhythmic activity based on the oxygenation level: fictive eupnea (in normoxic conditions), fictive sighing activity (augmented inspiration), and fictive gasping (in severe hypoxia) [15]. In hypoxia, the preBötC needs to reconfigure its neural elements to generate the most appropriate respiratory response to prevent the destructive consequences of hypoxia on organs, especially the brain. However, based on the pattern of hypoxic challenge, the responses of the preBötC to various types of hypoxic conditions can be varied and require special attention.

In this study, we sought to review structural and functional changes in mammalian respiratory neuronal network at the level of preBötC in response to acute, intermittent, and sustained hypoxic challenges. We primarily focus on the role of preBötC in the biphasic respiratory response to acute hypoxia. Moreover, we have explored the possible role of preBötC neurons in the long-term facilitation (LTF) response of the respiratory system to intermittent hypoxia (IH) and hypoxic ventilatory acclimatization (VAH) to sustained hypoxia (SH). To better understand how hypoxia alters the preBötC neural network, we have initially discussed some critical physiological characteristics of the preBötC.

2. Physiological characteristics of the preBötC

About three decades ago, Smith et al. [19] identified the preBötC in the ventrolateral medulla of newborn rats as the main generator of inspiratory rhythm drive. It was later shown that preBötC is also involved in generating and regulating respiratory rhythm in adult mammals [20,21]. In rats, the preBötC is a heterogeneous neural network of about 3000 interneurons on each side that is located ventral to the nucleus *ambiguus*, rostral to the ventral respiratory group (VRG), and caudal to the facial nucleus (Fig. 1) [19]. It extends around 300 μm rostrocaudally [19]. In humans, the preBötC location is slightly different, such that it is limited between the dorsal accessory of the inferior olivary nucleus and the semi-compact part of the *ambiguus* nucleus [22]. The preBötC boundaries can be identified experimentally by immunoreactivity to type 1 neurokinin receptor (NK_1R), somatostatin (SST), and transcription factor Dbx1 [23] or by the stereotypical response of its neurons (a rapid rise in burst frequency and decrease in burst amplitude) following microinjection of DL-Homocysteic acid (DLH; a glutamate analog) [24]. Without the Dbx1 gene homeobox, the preBötC does not form

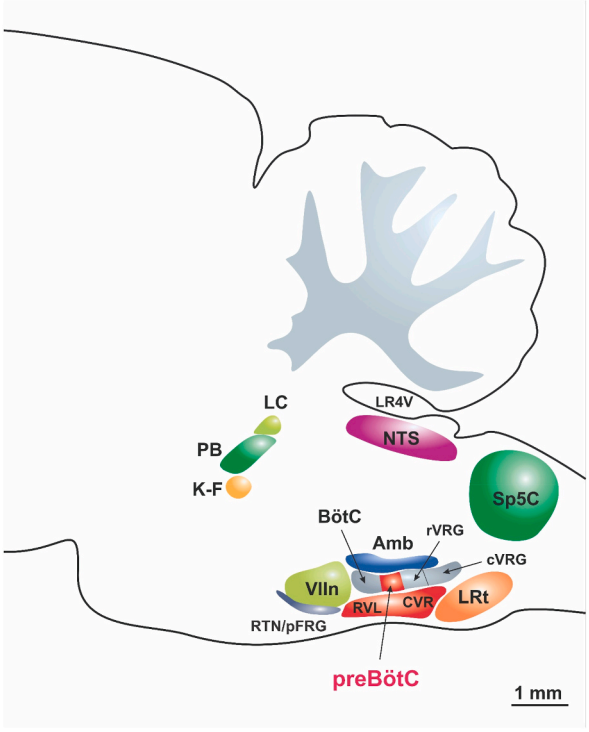

Fig. 1. The location of preBötC in rat brainstem: preBötC: the preBötzinger complex; VIIn: facial nucleus; Amb: nucleus *ambiguus*; BötC: Bötzinger complex; cVRG: caudal ventral respiratory group; rVRG: rostral ventral respiratory group; CVR: caudoventrolateral reticular nucleus; RVL: rostroventrolatelar reticular nucleus; K–F: Kölliker–Fuse nucleus; NTS: nucleus of the solitary tract; LC: locus ceruleus; PB: parabrachial nucleus; LRt: lateral reticular nucleus; LR4V: lateral recess of the fourth ventricle; RTN/pFRG: retrotrapezoid nucleus and the parafacial respiratory group [adapted from Paxinos & Watson rat brain atlas, 2006].

[25].

2.1. Types of neurons

In functional terms, respiratory neurons are categorized based on the pattern (augmenting or decrementing) and phase (inspiratory or expiratory) of their activity compared to the activity of hypoglossal or phrenic nerves (Fig. 2A) [26]: pre-inspiratory (pre-I) neurons with an augmenting activity pattern; early-inspiratory (early-I) neurons with a decrementing activity pattern; ramp-inspiratory (ramp-I) neurons with an augmenting activity pattern; post-inspiratory (post-I) neurons with a decrementing activity pattern (dec-E); and stage II expiratory (aug-E or E−2) neurons with an augmenting activity pattern [27]. The preBötC, as the main kernel of inspiratory activity, mostly comprises inspiratory-modulated neurons [28]. However, some of the preBötC neurons are active in the expiratory phase of the respiratory cycle [29,30]. Each respiratory-modulated group can be excitatory or inhibitory. There are approximately equal numbers of excitatory (glutamatergic) and inhibitory (glycinergic, GABAergic, and glycine-GABA co-expressing) neurons in the preBötC [31–34], which interact through reciprocal, fast synaptic connections [16].

It is believed that the network relies on excitatory synaptic interactions between glutamatergic neurons for inspiratory rhythmogenesis [32]. However, the role of inhibitory neurons in rhythmogenesis is a subject of discussion [35,36]. It is believed that concurrent inhibition of preBötC excitatory neurons from inhibitory neurons is crucial for network synchrony [37]. Furthermore, inhibition reduces the refractory period of excitatory neurons and, therefore, modulates the breathing frequency [38]. Excitatory glutamatergic neurons are primarily derived from the Dbx1 gene home box and have been further classified into rhythm- and pattern-generating neurons. Rhythm-generating neurons (type 1) with an augmenting pre-inspiratory activity pattern express NK_1R (NK_1R^+). These neurons fire action potentials 300–500 ms before the onset of network burst. In contrast, pattern-generating neurons (type 2) express SST (SST^+) and fire action potentials 300 ms after type-1 neurons [23,39–47]. Notably, type 1 and type 2 preBötC neurons express different types of ionic channels as such type 1 neurons express the A-type transient K^+ channel (I_A), which is believed to be essential for their pre-inspiratory activity [48], whereas type 2 neurons express the hyperpolarization-activated cationic channel (I_h), which is thought to be crucial for respiratory motor output [41].

The output of the preBötC, including commissural neurons that project to the contralateral preBötC, comprises SST^+ excitatory glutamatergic neurons, as well as inhibitory neurons [32]. The preBötC SST^+ pattern-generating neurons provide widespread reciprocal connections with multiple respiratory-related regions such as BötC, NTS, pFRG/RTN, periaqueductal gray matter, and parabrachial/Kölliker-Fuse nuclei [49–51], which place them in a critical position for processing and transmitting modulated inspiratory signal that determine the breathing pattern. It is noteworthy to mention that SST is also expressed in a subset of preBötC inhibitory

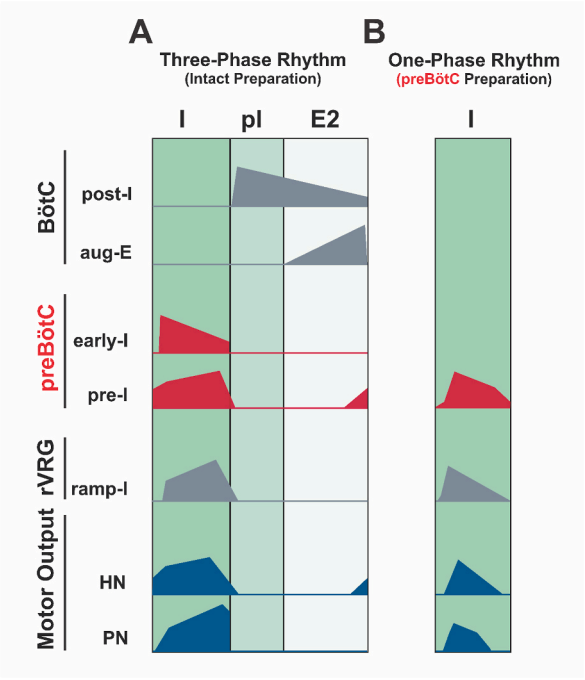

Fig. 2. Neuronal activity patterns of respiratory network in intact and reduced preparation at the level of preBötC. Activity patterns are shown within BötC, preBötC, rVRG, and from phrenic (PN) and hypoglossal (HN) nerves are from intact (**A**) and reduced preparations at the level of preBötC (**B**). Traces show the population activity pattern from distinct types of respiratory neurons. I: inspiratory; pI: post-inspiratory; E2: stage-2 expiratory; pos-I: post inspiratory; aug-E: augmented expiratory; early-I: early inspiratory; pre-I: pre-inspiratory; ramp-I: ramp inspiratory; HN: hypoglossal nerve; PN: phrenic nerve. Adapted and modified with permission from Ref. [2].

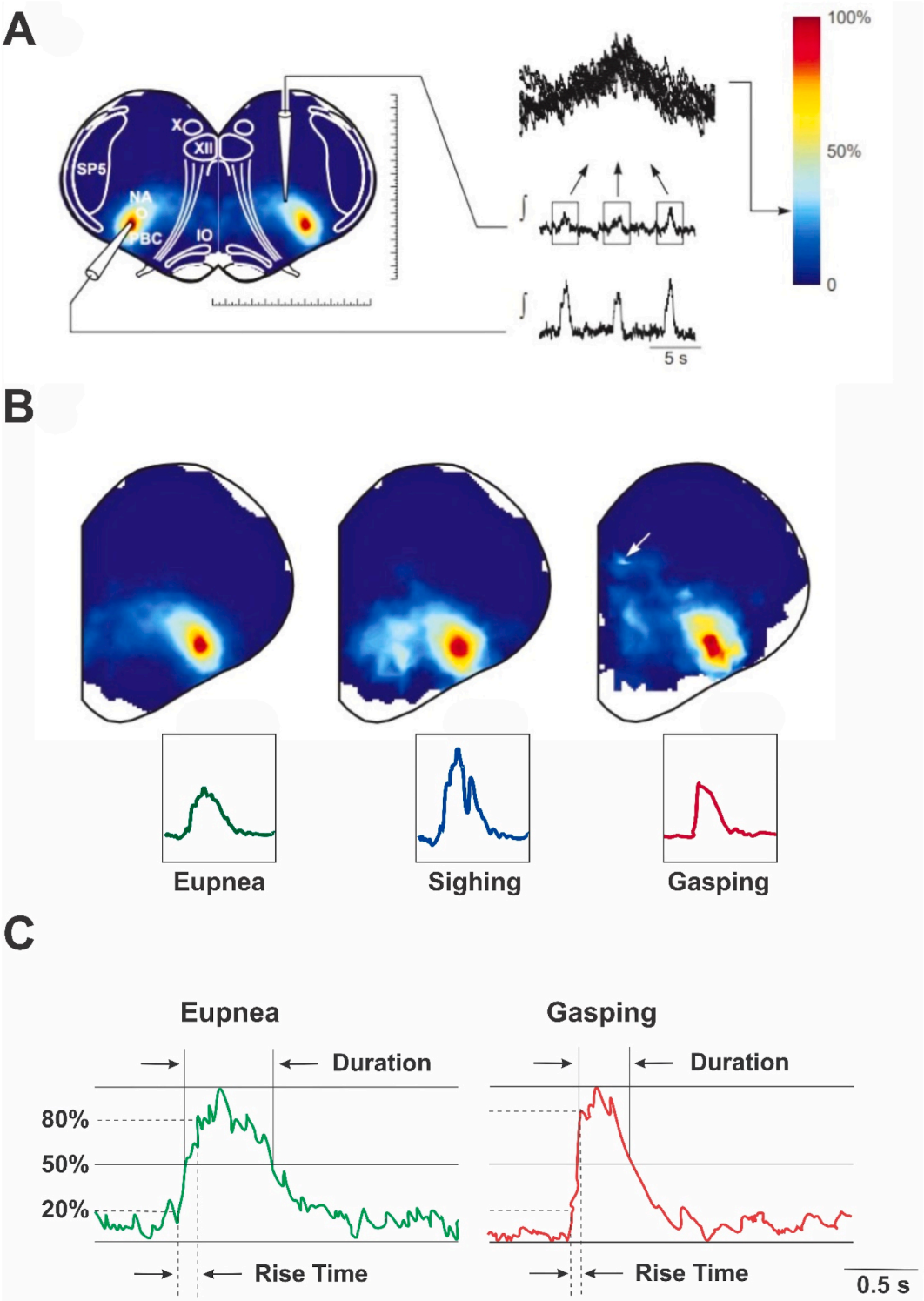

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Fig. 3. (**A**). The recorded population activity from brainstem transverse slices containing preBötC. The color scheme shows the averaged burst amplitude. Note the peak activity at the center of preBötC (lower trace). (**B**). The activation area maps were recorded from slices during eupnea, sighing, and gasping. Maps represent the distribution of averaged population activity during eupnea, sighing, and gasping. Note the greater area of activation in sighing and gasping compared to eupnea. The traces in **B** show sample eupneic (green), sighing (blue), and gasping (red) activity. (**C**) Comparison of a single eupneic (green trace) and gasping (red trace) burst recorded from preBötC. Note the significant reduction in rising time and burst duration in anoxia adapted and modified with permission from Ref. [15]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

neurons [40]. The preBötC SST^+ neurons are thought to be the major modulators of the behavioral-related lability of breathing pattern [40].

Autorhythmicity is a crucial characteristic of the preBötC neurons to generate spontaneous inspiratory output; however, most preBötC inspiratory neurons are not autonomously active (autorhythmic) because their rhythmic activity stops without synaptic inputs [52,53]. Notably, the ratio between autorhythmic and non-autorhythmic neurons depends on the network's metabolic and modulatory states [54]. Indeed, some neuromodulators, such as norepinephrine (NE), substance P (SP), or serotonin (5-HT), can induce bursting in non-autorhythmic neurons [55]. There are two types of autorhythmic neurons within the preBötC [53]: 1. Tonic spiking neurons. 2. Bursting neurons. Spiking and bursting autorhythmic neurons are not necessarily different types of neurons; on the contrary, occasionally, they may be the same neuron with "different activity states" [54]. Bursting autorhythmic neurons account for only 5 %–25 % of preBötC inspiratory neurons [52]. Two underlying ionic mechanisms have been proposed to be involved in the bursting property of preBötC neurons: 1. voltage-sensitive persistent Na^+ current (I_{NaP}) [56,57], and 2. nonspecific Ca^{2+}-activated cationic current (I_{CAN}), originally described by Peña et al., [29,58]. I_{CAN}-dependent bursters cease to fire in the presence of *cadmium* (a Ca^{2+} channel blocker) and flufenamic acid [29,52]. Due to this pharmacological approach, these neurons are known as "cadmium-sensitive" (CS) bursters [29,58]. I_{NaP}-dependent bursters are resistive to cadmium and, therefore, are known as "cadmium-insensitive" (CI) bursters [29,58,59]. However, CI bursters stop firing in the presence of *Riluzole*, a sodium channel blocker [2]. It is thought that bursting activity of CI neurons, but not CS neurons, requires endogenous release of norepinephrine acting on alpha2-noradrenergic receptors (α2-NR) [60] and 5-HT acting on 5-HT2A receptors [55].

2.2. The preBötC rhythmogenesis

In normoxia, the preBötC generates two distinct patterns of inspiratory rhythm *in vitro*: fictive eupnea and fictive sighing (Fig. 3A and B). Although most neurons are active during both rhythms, their underlying neural mechanisms are different [15,61]. Furthermore, in severe prolonged hypoxia, preBötC produces a unique type of inspiratory rhythm, termed fictive gasping [15] (Fig. 3B and C), which is thought to be the last effort of the respiratory system to save the organism's life. Besides its primary function as the main kernel of inspiratory activity, there is also evidence that preBötC acts as a master oscillator that synchronizes other oscillatory activities, such as sniffing and whisking [62,63].

Sighs are high-amplitude, usually biphasic inspiratory activity, compared to eupnea [15] which becomes more frequent in response to hypoxia [64–66]. It has been shown that fictive sighs are abolished in the presence of strychnine (a glycine receptor antagonist), cadmium (a Ca^{2+} channel blocker) [15], and MRS2279 (a purinergic $P2Y_1$ receptor antagonist) [67] *in vitro*. The sigh mechanism involves the activation of P/Q-type Ca^{2+} currents [61,68,69] and intracellular calcium (Ca^{2+}) signaling [68,70–75]. Only a subset of preBötC neurons receive glutamatergic inputs that rely on P/Q-type Ca^{2+}currents [68], suggesting that a subpopulation of preBötC neurons, probably pattern-generating glutamatergic SST^+ neurons [40,46], possess specialized synapses that are critical for the generation of sighing rhythm [72]. However, recent work by Del Negro et al. [75] doesn't support this hypothesis, suggesting that sighing and eupneic activity emerge from the same excitatory Dbx1-derived neuronal population within preBötC. In contrast to sighing, gasping, like eupnea, is insensitive to cadmium or other Na^+ channel blockers [15,76]. Gasp-like bursts can be induced by strychnine, suggesting that a decrease in synaptic inhibition is the only difference between eupnea and gasping [15]. Possible underlying mechanisms for the generation of gasping by preBötC neurons will be further discussed in its relevant section; "the response of the preBötC to acute hypoxia."

2.3. Rhythmogenic mechanisms

Despite three decades of research regarding respiratory rhythmogenesis by the preBötC, the exact underlying mechanism has not been fully understood. So far, several rhythmogenic mechanisms by the preBötC have been proposed [6,77,78]. Some of these theories are discussed below:

Pacemaker Theory: The preBötC initial identification coincided with recordings from voltage-dependent *pacemaker neurons* with autorhythmic bursting activity [19]. Later, two types of pacemaker neurons (I_{NaP}- and I_{CAN}-dependent bursters) were found to underlie the preBötC autorhythmicity [52,58,59,79,80], since the suppression of both autorhythmic bursting neurons resulted in the cessation of respiratory rhythm [58]. However, enhancing the excitability of the respiratory network with substance P (SP), for example, restores the eliminated respiratory rhythm, suggesting that other neuronal mechanisms, other than pacemaker-related ionic currents, may be involved in respiratory rhythmogenesis [52].

Hybrid Pacemaker-Network Theory: According to this model, respiratory rhythm is the result of interactions between excitatory and inhibitory neurons in the network. In this model, a pre-inspiratory/inspiratory (pre-I/I) excitatory neuron with a pacemaker property initiates the inspiration. The activity of the pre-I/I pacemaker neuron is terminated by inhibitory signals from a neural network ring

formed of early-inspiratory (early-I), post-inspiratory (post-I), and augmenting-expiratory (aug-E) neurons, which causes the cessation of inspiration [81,82]. This theory is supported by the findings that mice lacking GABA-synthesizing enzyme (GAD67) fail to generate normal respiratory rhythm [83,84]. However, there are numerous studies in which the respiratory rhythms continue even after blockade of Cl^--mediated synaptic inhibition [85–89].

Group Pacemaker (Burstlet) Theory: In this model, low-amplitude synchronized burstlets arising from a small population of rhythmogenic pre-inspiratory neurons underlie the preBötC bursting activity [90,91]. These pre-inspiratory burstlets lead to a high amplitude burst in a subset of preBötC inspiratory neurons, which is followed by a refractory period [6,42]. However, in some instances, burstlets may fail to induce a network burst required to generate motor output [92]. It is believed that activation of outward cationic currents and synaptic depression, independent of postsynaptic inhibition, underlie the transient refractory period in inspiratory neurons [6]. After the refractory period, pre-inspiratory burstlets occur again, and the next respiratory cycle starts. In hypoxia-induced gasping, preBötC rhythmogenesis is associated with the loss of pre-inspiratory spiking activity, which is inconsistent with burstlet rhythmogenic theory [58,93].

Astrocyte-Driven Theory: Astrocytes are known to have a facilitatory role in respiratory rhythmogenesis, particularly during hypoxic conditions [94,95]. The role of astrocytes in respiratory rhythmogenesis is supported by observations that the blockade of astrocyte metabolism results in the suppression of respiratory rhythm *in vitro* [96,97] and reduces respiratory frequency *in vivo* [98]. In addition, calcium imaging analysis in the preBötC slices shows that a group of astrocytes have rhythmic activity phase-locked with inspiration, and some have pre-inspiratory activity [99,100]. Furthermore, optogenetic stimulation of astrocytes triggers fictive inspiratory busting in the preBötC *in vitro* [99]. Moreover, it was shown that the blockade of vesicular release from astrocytes at the level of preBötC reduced the respiratory frequency and rhythm variability *in vivo* [101]. The role of astrocytes in preBötC rhythmogenesis is further supported by the anatomical observation that astrocytes are closely coupled with respiratory neurons [102].

The final inspiratory rhythm signal generated in the preBötC is transmitted to respiratory motor neurons, mostly indirectly: the phrenic and thoracic motor neurons are reached via rVRG and cVRG, respectively [50,103], the hypoglossal motor neurons are targeted through the para-hypoglossal reticular formation [50], and the facial nucleus via the intermediate reticular formation [50,104]. In addition, the preBötC project to other medullary and pontine respiratory nuclei such as contralateral preBötC, BötC, Kölliker-Fuse nucleus, post inspiratory complex (PiCo), and lateral parafacial nucleus [50,103,105]. It is believed that projections from preBötC neurons to their input targets are also essential for hypercapnic and hypoxic respiratory responses *in vivo* since blocking axonal transport with bilateral microinjection of colchicine (100 μg/μL, 100 nL/site) in preBötC decreases these responses [106].

2.4. The preBötC chemosensitivity

Accumulating evidence suggests that the preBötC neurons and glia sense local O_2 levels [11,12,94,107–111]. For instance, it has been shown that focal hypoxia using sodium cyanide (NaCN) at the level of the preBötC in vagotomized cat, results in augmented respiratory output *in vivo* [11]. Furthermore, a significant number of dissociated neurons cultured from preBötC were found to respond to NaCN with depolarization and increased firing frequency [10]. NaCN is known as a potent stimulator of chemoreceptors [112,113] and is usually used to induce tissue hypoxia [114,115]. Interestingly, the expression of HO-2, an enzyme that may be involved in oxygen sensing in the carotid bodies [116], in RVLM is limited to oxygen-sensitive neurons [117], suggesting that these neurons possess a functional oxygen sensor mechanism.

In addition to O_2 sensing ability, there is evidence that preBötC is also CO_2/H^+ chemosensitive [106,118–122]. For example, Solomon et al. [118] found that focal acidosis (using microinjection of the carbonic anhydrase inhibitors acetazolamide or methazolamide) at the level of the preBötC, increased peak amplitude and frequency of integrated phrenic nerve discharge, further evidence for chemosensitivity of the preBötC. It is postulated that at the preBötC level, astrocytes may act as CO_2 sensors since blocking astroglial signaling by the tetanus toxin light chain (TeLC) was associated with a 20 % and 30 % reduction of the respiratory response to CO_2 in conscious and anesthetized rats, respectively [120]. By releasing D-Serine and/or ATP, the preBötC astrocytes can modify the respiratory response to hypercapnia [123,124].

According to these findings, the preBötC can directly sense the levels of O_2 and CO_2/H^+ and can integrate the proper respiratory response to hypoxic or hypercapnic conditions. However, further detailed studies are required to illuminate the exact chemosensation mechanisms in the preBötC neurons.

3. The preBötC and the hypoxic ventilatory response

The respiratory system responds to changes in oxygenation concentration by employing several physiological mechanisms to maintain normal arterial oxygen levels. The hypoxic ventilatory response (HVR) of the respiratory system relates to the duration (acute or chronic), intensity (mild to intense), and pattern (sustained or intermittent) of hypoxic exposure. It involves various mechanisms that can facilitate or suppress tidal volume or frequency components of ventilation over seconds to years reviewed in detail by Refs. [9,125].

Within a short time domain (seconds to minutes), three distinct types of HVR can be detected in the respiratory system: acute HVR, STP (short-term potentiation), and STD (short-term depression) [125]. Acute HVR (aHVR) is the immediate ventilatory response (the first few seconds) to the onset of hypoxia, which includes increases in both respiratory frequency (f_R) and tidal volume (V_T) [125,126]. When a hypoxic stimulus lasts for more than a few seconds to minutes, STP occurs, which is known as a secondary ventilatory augmentation in addition to the initial augmentation mediated by aHVR [127]. STD usually manifests as a decreased f_R following an initial transient overshoot in f_R that can last from seconds to minutes [125]. It is proposed that the respiratory plasticity in these types

of HVR (aHVR, STP, and STD) mainly depends on transient changes in electrophysiological properties of respiratory neurons, as well as changes in synaptic transmission between first-order chemosensitive neurons in carotid bodies and second-order NTS chemosensitive neurons [9]. In prolonged time domains (hours to weeks) of sustained hypoxic exposure, an additional type of respiratory HVR, termed VAH (ventilatory acclimatization to hypoxia), can be detected. VAH is a time-dependent increase in ventilation during chronic exposure (hours to weeks) to sustained hypoxia [126]. In response to intermittent hypoxia (repeated episodes), two unique types of HVR appear: progressive augmentation (PA) and long-term facilitation (LTF) [125]. PA is an increase in successive hypoxic ventilatory responses following an episodic hypoxic stimulus [125]. LTF is a progressive increase in ventilation measured during normoxia following episodic exposure to hypoxia. Five-minute, repeated episodes of hypoxia can elicit LTF that persists for more than 60 min [128].

In intact animals, the HVR originates mainly from the activity of peripheral chemoreceptors [71,129]. However, other parts of the respiratory neural system, including central chemosensitive regions, central respiratory rhythm generators, and respiratory motoneurons, may contribute to some aspects of the respiratory system's HVR [125,130]. The preBötC, as the main source of inspiratory activity, possesses neurons and glia that directly respond to hypoxia [10,11]. Therefore, it is reasonable to expect that this region also participates in HVR. Numerous *in vitro* [11,109–111] and *in vivo* [11,131,132] studies support this hypothesis. For instance, the biphasic response to acute hypoxia can be seen in slices containing preBötC [11](Fig. 4B). Moreover, induction of focal hypoxia at the level of preBötC in dogs using hydrogen cyanide (HCN) causes biphasic changes in motor output and ventilation *in vivo* [131]. In the following sections, we will discuss the responses of the respiratory system, particularly at the level of preBötC, to acute, sustained, and intermittent hypoxia. We will mainly focus on the possible role of preBötC in the biphasic ventilatory response to acute hypoxia, the LTF response to intermittent hypoxia, and the VAH response to sustained hypoxia.

4. The response of preBötC to acute hypoxia

In response to acute hypoxia, the respiratory system of mammals shows a biphasic change in respiration, comprised of initial augmentation followed by a secondary depression [15,58,133–136] (Fig. 4). The biphasic respiratory response is a typical response of intact chemoafferent animal models, either awake or anesthetized [135,137,138]. Moreover, it is also evident in awake, but not anesthetized, peripherally chemodenervated animal models [12,131,138–141], as well as in awake humans with chronically denervated carotid bodies [130,142–147]. In anesthetized peripherally chemodenervated animals, the initial augmentation phase is absent, and the respiratory system responds to hypoxia solely with depression [135,140,141,148–150]. It is hypothesized that the respiratory augmentation mechanisms in peripherally chemodenervated animals are sensitive to anesthetic agents or state of consciousness [151, 152]. However, a study on awake, chemoafferent denervated neonate rats challenges this hypothesis, where neonate rats (P4) were found to respond to acute hypoxia with hypoxic depression without initial augmentation [153]. It is worth noting that the researchers used systemic administration of a non-selective P2 purinergic receptor antagonist (PPADS) to pharmacologically block the peripheral chemoafferents. As discussed in subsequent sections, purinergic antagonists have been shown to influence central respiratory circuits and the hypoxic respiratory response [154]. Under severe hypoxia, when the partial pressure of O_2 drops dramatically, depression terminates in apnea (cessation of breathing) [155]. When this situation persists, the eupneic activity transforms into *gasping*

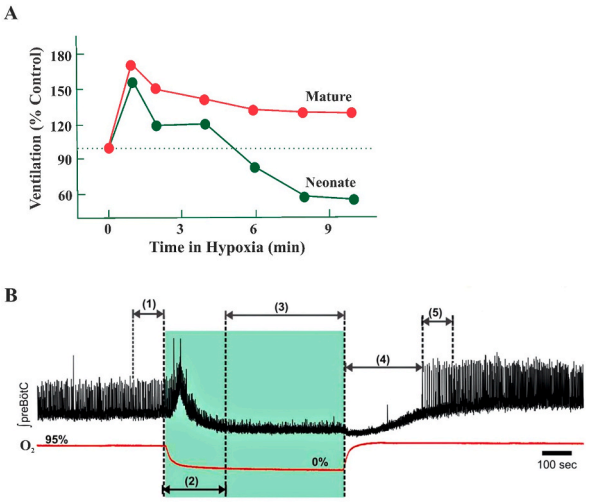

Fig. 4. Biphasic respiratory response of mammals to acute hypoxia. (A). Ventilatory responses of neonate and mature piglets to acute hypoxia. Note the milder response of mature animals to hypoxia compared to neonates (modified with permission from Ref. [392]). (B). In vitro biphasic respiratory response of the preBötC in neonatal mice (P10-13) to acute hypoxia. The neuronal network of preBötC generates a steady-state rhythmogenesis in normoxia (**1**) and responds to acute hypoxia with a biphasic change in population activity: initial augmentation (**2**) and secondary depression (**3**). After reoxygenation, a paradoxical depression in rhythmogenesis occurs (**4**). Post-hypoxic rhythmogenesis is indicated by (**5**). adapted and modified with permission from Ref. [159].

[156–158]. After reoxygenation, a paradoxical depression in rhythmogenesis occurs *in vitro*, called *post-hypoxic depression* [136,159].

The hypoxic response of mammals changes developmentally (Fig. 4A). Compared to newborns, the biphasic respiratory response to hypoxia is not prominent in adults and has slower dynamics [126,130,152,160,161]. Furthermore, it was reported that in brainstem slices of neonate mice from different age groups, hypoxia differently affects the activity of respiratory motor output [109] and its coupling with the activity of preBötC neurons [110]. Ramirez et al. [109] showed that although hypoxia increases the amplitude of XII bursts in brainstem slices from more mature neonate mice (>P8), this effect was not observed in slices from younger neonates. Moreover, they found that respiratory depression never leads to a cessation of rhythmic activity in younger neonates [109]. It seems, at least, that a part of these developmental changes in hypoxic respiratory response emerges from differences in the architecture of the central respiratory rhythm generator network [110]. For instance, in another work, Ramirez et al. [110] reported that in slices from more mature neonate mice (>P8), hypoxia increases the amplitude of rhythmic synaptic drive potentials in preBötC inspiratory neurons associated with the suppression of phasic hyperpolarizations of expiratory neurons, effects that they did not observe in slices from younger neonates [110]. Moreover, during normoxia, the coupling between the rhythmic activity of preBötC neurons and the XII burst occurs in a 1:1 manner in slices younger than P4 and in a 3:1 manner in slices older than P5. Surprisingly, although hypoxia didn't change the coupling between the activity of preBötC neurons and XII burst in slices younger than P4, it did change this coupling from a 3:1 manner to a 1:1 manner in slices older than P5 [110]. This finding may be the result of hypoxia-induced increased excitability of hypoglossal respiratory neurons during the initial augmentation phase [162].

It is documented that the hypoxic response of mammals is also gender dependent (Fig. 4B). Garcia et al. [159] showed that in rhythmically active slices of neonate mice of either sex, post-hypoxic depression occurs with a greater prevalence in males compared to females. Furthermore, following reoxygenation, time to the first inspiratory burst (TTFB) significantly delays in males' rhythms compared to females' rhythms [159]. Garcia et al. [159] proposed that these results may be because of the differences in the activity of K_{ATP} channels, since changing the activity of these channels with either diazoxide (K_{ATP} agonist) or tolbutamide (K_{ATP} antagonist) abolishes the observed differences in TTFB.

In the following subsections, we discuss the possible underlying mechanisms for respiratory augmentation, depression, and transition from eupnea into gasping at the level of preBötC.

4.1. Initial augmentation

In intact animals, hypoxic augmentation mainly results from the peripheral chemoreceptors' activity that drives the respiratory network [71,129]. First-order chemosensitive cells within carotid bodies sense decreased arterial O_2 levels and give rise to the excitation of second-order NTS chemosensitive neurons [163–165]. From NTS, excitatory fibers are relayed to various parts of the respiratory network, including the preBötC, to trigger the appropriate hypoxic response [71,166,167]. Notably, after carotid body denervation, peripheral O_2 chemoreflex initially abolishes. Surprisingly, partial or complete recovery of this response is achieved within weeks to months in different animal models [168,169]. It has been suggested that the increased sensitivity of subsidiary peripheral chemoreceptors, such as those in the proximal aorta, is the possible reason for the recovered O_2 chemoreflex [170–172]. Despite the significance of peripheral chemoreceptors, there is substantial evidence that hypoxic augmentation, at least in part, is mediated centrally [12,111,131,139,151,173,174]. For instance, respiratory augmentation can be observed in unanesthetized animals with intact, isolated, and separately perfused carotid bodies [131,139]. Further evidence to support the role of central respiratory network in hypoxic augmentation is based on studies showing respiratory augmentation in brainstem slices containing preBötC [12, 111,151,173]. In this case, hypoxic augmentation seems due to the direct and/or indirect (via the activation of astrocytes) effects of hypoxia on preBötC neurons [11,151,175].

4.1.1. Role of preBötC neurons in hypoxic augmentation

Despite increased respiratory motor output during hypoxic augmentation, most respiratory neurons exhibit no change or even a decline in their activity [134,176]. However, a small population of respiratory neurons was found in *the ventral part of the medulla* to show increased activity during hypoxic augmentation [134,177,178]. Later, Nolan et al. [179] using brainstem slice preparation of rats reported that in medullary slices, perfusing a hypoxic gas elicits a graded increase in firing frequency of most respiratory *neurons within the VLM*, which was related to the magnitude of the hypoxic stimulus. Five years later, Ramirez et al. [110] using *in vitro* recordings from RVLM showed that, during hypoxic augmentation, *preBötC inspiratory neurons* exhibited increased frequency of rhythmic depolarizing drive potentials occurring in phase with hypoglossal burst. This finding was later confirmed by Thoby-Brisson and Ramirez's [175] work, in which preBötC expiratory neurons became tonically active during anoxia, whereas inspiratory neurons depolarized and showed increased rhythmic activity frequency [175]. Another interesting finding of Thoby-Brisson and Ramirez was that after the blockade of network activity with CNQX (a competitive AMPA/kainate receptor antagonist), most inspiratory neurons became tonically active and stopped firing during anoxia. In contrast, a subset of inspiratory neurons continue to fire bursts of action potentials in the absence of network activity and later during anoxia [175]. Later, it was found that there are two types of inspiratory autorhythmic neurons (I_{NaP}- and I_{CAN}-dependent autorhythmic bursters) in the preBötC, which drive inspiratory rhythm in normoxia. However, during hypoxia, only I_{NaP}-dependent neurons remain active, while I_{CAN}-dependent neurons become tonically active and finally stop firing [58].

The question was whether the preBötC inspiratory neurons possess a functional oxygen sensor mechanism, which leads them to respond to hypoxia with increased activity, or if hypoxia affects their activity in other ways. Therefore, follow-up experiments were conducted to elucidate the putative processes underlying the enhanced activity of preBötC inspiratory neurons during hypoxia. Accumulating evidence suggests that a combination of changes in intrinsic electrical properties and intracellular molecular

mechanisms of respiratory neurons, as well as changes in synaptic transmission in the preBötC neural circuits, is responsible for increased activity of inspiratory neurons during initial augmentation.

Changes in Ionic Currents: The excitability of a neuron is determined by the balance of inward and outward ionic currents [180]. Various ion channels, such as Ca^{2+} channels [68,181–183], K^{+} channels [184,185], Na^{+} channels [186], and hyperpolarization-activated currents (I_h) [187] have been involved in the generation of the hypoxic response of respiratory neurons [181]. However, the way that hypoxia affects and alters the activity of these channels in respiratory neurons is not fully determined. Among ionic currents, Ca^{2+} current through L-type channels (Ca_L) is believed to be responsible for respiratory neuron depolarization during hypoxia [182,183,188,189]. Blocking Ca_L channels by nitrendipine or nifedipine abolishes hypoxic augmentation and leads the secondary depression to occur earlier [190], suggesting that Ca^{2+} influx through these channels contributes to hypoxic augmentation of the respiratory network [181].

Changes in Intracellular Molecular Mechanisms: Hypoxia is known to induce changes in some intracellular molecular mechanisms of respiratory neurons, such as the heme oxygenase (HO) system [117], mitochondrial K_{ATP} channels [191], and the nitric oxide (NO)-cGMP system [192]. Although HO-1 is critical for adapting the responses of the respiratory network to chronic hypoxic conditions [193–195], it is proposed that HO-2 is required for acute hypoxic response [117]. For instance, D'Agostino et al. [117] showed that HCN- or low-O_2-induced excitation of preBötC respiratory neurons critically depends on HO-2 activity. As stated before, HO-2 expression is confined to oxygen-sensing preBötC neurons, suggesting that this enzyme may be involved in oxygen sensing in the central respiratory network [64,116,117]. HO activity depends on oxygen, which converts heme into carbon monoxide (CO) and biliverdin. CO acts as an important second messenger and may also be involved in the hypoxic response of respiratory neurons [64,196,197]. In addition, it is documented that PKC-induced stimulation of mitochondrial K_{ATP} channels (mK_{ATP}) in preBötC respiratory neurons is required for initial augmentation response to acute hypoxia; blockade of these channels by 5-hydroxydecanoate (5-HD), or inactivating PKC by staurosporine prevents this response *in vitro* [191]. Furthermore, during hypoxic exposure, glutamate release from respiratory neurons activates the NO-cGMP system, which in turn increases glutamate transmission retrogradely and consequently enhances hypoxic augmentation [192].

Alterations in Synaptic Transmission: Hypoxia is known to cause changes in synaptic transmission in the respiratory network [8,181,198]. The most consistent effect of hypoxia is the suppression of synaptic inhibition in the preBötC neural network, which has been observed both *in vivo* and *in vitro* [15,175,177,199–201]. The loss of synaptic inhibition leads expiratory neurons to stop rhythmic firing [15,175] and postinspiratory neurons to fire during the inspiratory phase [15]. On the other hand, the effect of hypoxia on excitatory glutamatergic transmission is heterogeneous [8]; hypoxia suppresses a component of excitatory glutamatergic transmission [110,199,202]. However, there is another component of glutamatergic transmission, probably between preBötC rhythmogenic core neurons [203], that is resistive to hypoxia and is thought to be responsible for the synchronization of the respiratory neurons in hypoxic conditions [110,199,202]. It is proposed that suppression of synaptic inhibition leaves glutamatergic synaptic transmission unopposed, which leads to augmented respiratory activity during hypoxia [175].

4.1.2. Supportive role of astrocytes in hypoxic augmentation

In recent years, attention has shifted to the role of the astroglial system in the hypoxic respiratory response of the preBötC. This is supported by *in vivo* findings that, during hypoxia, the release of ATP from astrocytes enhances which in turn attenuates secondary depression [204,205]. Furthermore, the activation of $P2Y_1$ receptors causes a significant increase in the frequency of inspiratory bursts *in vitro* [206]. It is believed that ATP released from astrocytes is involved in the aHVR of the respiratory system in intact and peripherally chemodenervated animals, as blocking purinergic signaling either by preventing the vesicular release of ATP or targeting the astrocytes to express the light chain of tetanus toxin (TeLC) abolishes hypoxic augmentation in both preparations [12]. Moreover, it is documented that the astroglial system and ATP are responsible for post-hypoxic persistent respiratory augmentation (PHRA) [207]. Existing data from rodents strongly suggests that, in preBötC, astrocytes detect hypoxia [12,94,107,108]. Astrocytes respond to hypoxia with an increase in intracellular Ca^{2+} and vesicular release of ATP [151]. *In vivo* and *in vitro* studies show that astrocytes release ATP in proximity to preBötC neurons [208–211]. Released ATP from astrocytes binds its P2Y1 receptors on preBötC inspiratory neurons, causing the activation of Gq proteins and increasing intracellular Ca^{2+}, which results in the excitation of inspiratory neurons and ultimately increased inspiratory frequency [101,204,205].

4.2. Secondary depression

When hypoxia persists for more than a few minutes, the initial ventilatory augmentation is followed by secondary depression, in which a large number of respiratory neurons show decreased firing frequency [175,177]. As noted previously, respiratory depression is constantly observed in awake and anesthetized peripherally chemodenervated animals [137,138,149,212,213]. Furthermore, in chemoafferent intact animals, hypoxic depression can be observed even upon stimulation of peripheral chemoreceptors, suggesting that it is mediated centrally [152]. The decrease in $PaCO_2$ [214,215] resulting from increased ventilation cannot be the only reason for depression since hypoxic depression has also been found during isocapnic hypoxia [216–219]. Thus, several alternative mechanisms have been proposed: the depressant effect of higher brain regions on medullary respiratory centers [220–224], ventral medullary alkalosis due to an increase in cerebral blood flow [225–227], inadequate O_2 for aerobic metabolism [134,176], the role of nitric oxide synthase 1 (NOS_1) [228], increased activity of K_{ATP} channels [189,229], and the role of neuromodulators [230–237]. Among these potential mechanisms, the role of neuromodulators, particularly for adenosine, is strongly supported [189,229,238,239].

Within the respiratory network, adenosine is an inhibitory modulator that seems maladaptive. Adenosine-mediated respiratory depression during hypoxia is fatal in sudden infant death syndrome (SIDS), sudden unexpected death in epilepsy (SUDEP) [240,241],

and apnea of prematurity (AOP) [242,243]. Indeed, attenuation of the depressant actions of adenosine on the respiratory network using methylxanthines (e.g., theophylline, aminophylline, and caffeine) is an effective therapeutic approach in AOP [244]. Methylxanthines are nonselective antagonists of adenosine receptors [245]. By antagonizing the adenosine A1 receptors, these drugs reverse hypoxic depression of respiratory activity [237,246,247]. Adenosine inhibits preBötC neurons directly through its A1 receptors (A_1R) [248–251] or indirectly by activating GABAergic neurons through A2A receptors ($A_{2A}R$) [252–255]. Moreover, there are some reports that adenosine suppresses excitatory and inhibitory synaptic transmission in the respiratory network, which is mediated through its A_1R receptors [256,257]. By acting on A_1R receptors, adenosine decreases the conductance of voltage-dependent Ca^{2+} channels [258, 259] and increases the conductance of leak K^+ channels [260] in respiratory neurons, thus reducing their excitability.

As noted in previous sections, extracellular ATP (ATPe) release from preBötC astrocytes during hypoxia, by acting on purinergic $P2Y_1$ receptors, counteracts hypoxic depression [101,154,204,205]. However, the effect of ATPe on respiratory neurons does not end here [154]. In extracellular space, ectonucleotidases rapidly degrade ATPe into adenosine (ADOe), which, by acting on purinergic P1 receptors, exerts inhibitory actions on respiratory neurons [261,262]. Thus, the net effect of ATPe on preBötC depends on the balance between the excitatory effects of ATPe through the activation of P2 receptors and the inhibitory effects of ADOe via acting on P1 receptors [154] (Fig. 5).

In addition to adenosine, there is also evidence for the role of other neurotransmitters and neuromodulators such as glutamate, GABA, and 5-HT in the onset of hypoxic depression [189,229]. It is proposed that enhanced glutamate and GABA transmission during hypoxia increases K^+ current through K_{ATP} channels in respiratory neurons, resulting in respiratory depression [229]. The activity of K_{ATP} channels also increases when the intracellular ATP level falls in respiratory neurons during hypoxia, which leads to even greater depression [184,185,189,191]. Although it seems undesirable, it is believed that increased K^+ current through these channels has a protective action against Ca^{2+} overload during hypoxia, which can be toxic to neurons [184]. 5-HT receptors are expressed in respiratory neurons and are known as essential modulators [263,264]. It is believed that 5-HT is required for normal respiratory rhythm generation [55], and also participates in hypoxic responses of the respiratory network [265]. Although 5-HT has excitatory effects on preBötC rhythmogenesis by acting on its postsynaptic $5\text{-}HT_2$ receptors [264,266–268], it suppresses the respiratory network via $5HT_{1A}$ receptors [229].

Fortunately, hypoxia-induced respiratory depression is counteracted by several mechanisms to prevent total respiratory arrest in prolonged hypoxic exposure. As mentioned before, one of such mechanisms is ATPe released from astrocytes. A second mechanism appears to be mediated by hydrogen sulfide (H_2S). It has been shown that endogenous and exogenous H_2S can prevent hypoxia-induced respiratory depression in medullary slices of neonatal rats by down-regulating the expression of malondialdehyde (MDA) and of c-fos mRNA [269,270].

4.3. *Hypoxia-induced gasping*

In severe hypoxia, the respiratory network generates a unique type of inspiratory activity, referred to as *gasping*, which is thought to be the last effort of the organism to sustain life (Fig. 3). Failure to generate gasping leads to organism death. This condition can be observed in some infants with SIDS [271,272]. Both eupneic and gasping inspiratory activities emerge from almost the same neuronal

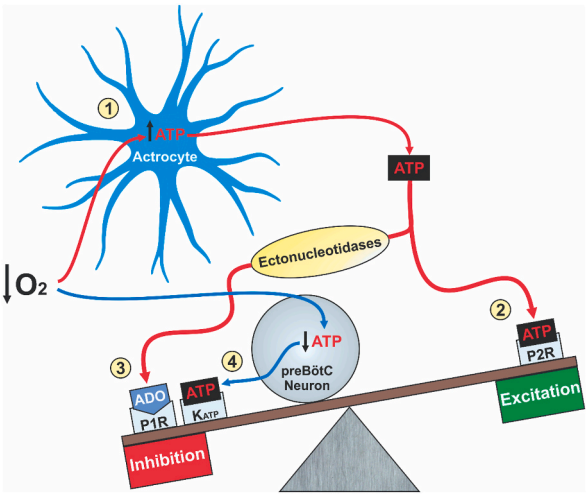

Fig. 5. The net action of extracellular ATP released from preBötC astrocytes on respiratory neurons depends on the balance between excitatory actions of ATP on P2 receptors and inhibitory actions of its main metabolite, ADO, on P2 receptors. Hypoxia causes increased production of ATP in astrocytes (**1**). ATP released from astrocytes has two main fates: 1. binding to purinergic P2 receptors (**2**) or 2. degradation by ectonucleotidases (**3**) into ADO. Extracellular ADO acting on purinergic P1 receptors exerts inhibitory actions on respiratory neurons. On the other hand, hypoxia leads to a decline in ATP production by respiratory neurons (**4**). Decreased ATP results in the activation of K_{ATP} channels, which also have an inhibitory effect on respiratory neurons adapted and modified with permission from Ref. [154].

population within preBötC [132,273,274]. Fictive gasping is generated in brainstem slices containing the preBötC and even in preBötC islands [15,60,275]. Unlike eupnea, gasping rhythmogenesis is not dependent on glutamatergic synaptic transmission and activation of excitatory amino acid (EAA) ionotropic receptors *in vivo* [275–278]. However, EAA ionotropic receptor activation in preBötC is thought to modify the expression of hypoxia-induced gasping, since blockade of these receptors by kynurenic acid, increases the onset latency to gasping, reduces the number of gasps, and prolongs the duration of gasps [275]. If ionotropic EAA receptors are not essential for gasping rhythmogenesis, then which mechanism(s) underlie the synchronization of respiratory neurons in the absence of oxygen? One possibility is that glutamate may act on its metabotropic receptors to synchronize inspiratory neurons during severe hypoxia. Another suggestion is that other excitatory neurotransmitters and/or neuromodulators in preBötC may contribute to gasping rhythmogenesis [60,198,279–282]. For instance, it is reported endogenous release of norepinephrine (NE), and activation of alpha2-noradrenergic receptors (α_2-NR) is essential for gasping rhythmogenesis in brainstem slices containing the preBötC [60]. Moreover, the blockade of hypoxia-induced gasping using α_2-NR antagonists is prevented by 5-HT_2A receptor agonists [60], indicating that the concurrent activation of α_2-NR and 5-HT_2AR acting on preBötC neurons is crucial for gasping rhythmogenesis. Notably, changes in electrical transmission via gap junctions are not involved in gasping rhythmogenesis, as blockers of gap junctions fail to disrupt the hypoxic response [283].

Compared with eupnea, gasping is identified by a fast rise in inspiratory activity, a shorter burst duration, and a lack of post-inspiratory activity both *in vivo* and *in vitro* (Fig. 3C) [15,284]. Viemari et al. [60] calculated the burst duration and rise time of preBötC population activity during normoxia and hypoxia-induced gasping. Compared to normoxia, the burst duration and rise time were significantly shorter in hypoxia-induced gasping [60,284]. It is proposed that during hypoxia, the reconfiguration of the preBötC neuronal circuit results in gasping rhythmogenesis (Fig. 8). It is believed that the loss of synaptic inhibition during severe hypoxia [15] may be the possible reason for the respiratory pattern change in gasping. Loss of synaptic inhibition during hypoxic exposure was reported by Richter et al. [177] for the first time in 1991. Later, numerous *in vitro* [15,20,175] and *in vivo* [177,200] studies supported this finding. As discussed previously, in the absence of synaptic inhibition, excitatory inputs are left unopposed, which leads to a sharper depolarization phase and decrementing pattern [15]. Loss of synaptic inhibition results in 1. discharging late-inspiratory neurons earlier during inspiration [200], 2. discharging post-inspiratory neurons in the inspiratory phase [60], and 3. Losing the activity of most expiratory neurons [175]. These changes alter the shape of the final respiratory output from a bell-shaped to a decrementing pattern, which is characteristic of gasping [60].

At the network level, it has been shown that changes in the spatiotemporal organization of the respiratory network, and the strength of connections between respiratory neurons occur during the transition from eupnea into gasping [16,203,273]. For instance, using a voltage-sensitive dye, Potts et al. [273] found that during normoxia, fluorescence activity was observed throughout the entire

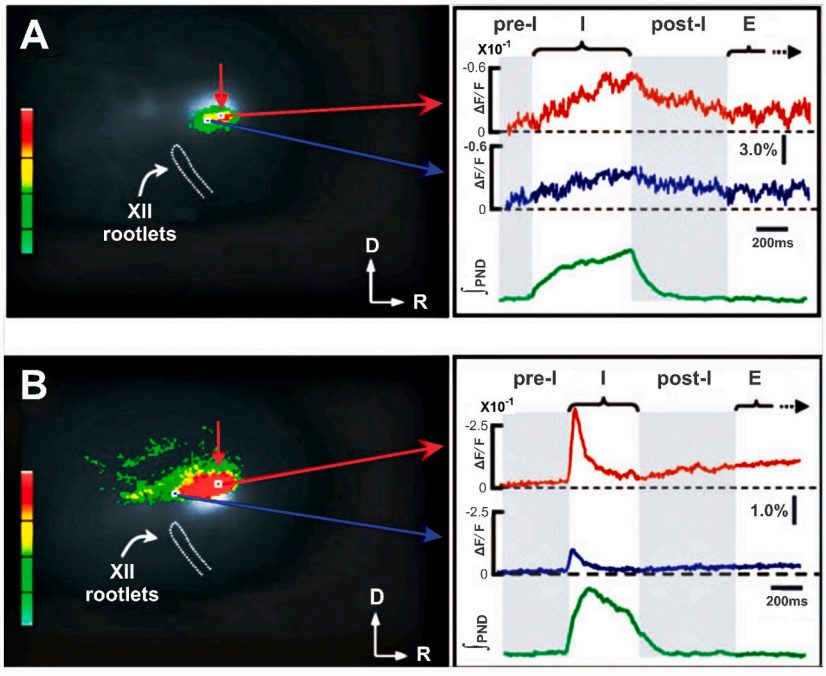

Fig. 6. The spatiotemporal pattern of fluorescence activity was obtained from the rostral ventrolateral medulla during eupnea (**A**) and gasping (**B**). The color bars on the left represent the intensity of fluorescence activity. Note the more extended area of fluorescence activity during gasping compared to eupnea (left panels), and post-inspiratory activity in eupnea, which is absent in gasping (right panels) adapted and modified with permission from Ref. [273]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

respiratory cycle (late expiratory, inspiratory, and post-inspiratory) (Fig. 6A). In contrast, in hypoxia-induced gasping (10 % O_2), there was a rapid onset of fluorescence activity, which was absent immediately after inspiration but increased monotonically during expiration (Fig. 6B). Moreover, they showed that during hypoxia-induced gasping, the active medullary areas were more extended, and the area occupied by peak fluorescence activity was far larger compared to eupnea (Fig. 6).

Another interesting finding of Potts et al. [273] was that the amplitude of peak fluorescence activity was 2.5 times greater during gasping than during eupnea. Although the authors proposed that the recruitment of post-inspiratory neurons during inspiration [285–287] underlie observed robust inspiratory activity in gasping, it appears that changes in the strength of connections between active respiratory neurons are the possible reason [203]. Using multi-electrode arrays to record the activity of dozens of respiratory neurons within preBötC, Juárez et al. showed that preBötC generates distinct types of multi-neuronal activity pattern (MAP), state-dependently [203] (Fig. 7). An interesting finding of Juárez et al. [203] was a group of respiratory neurons (constant core) in

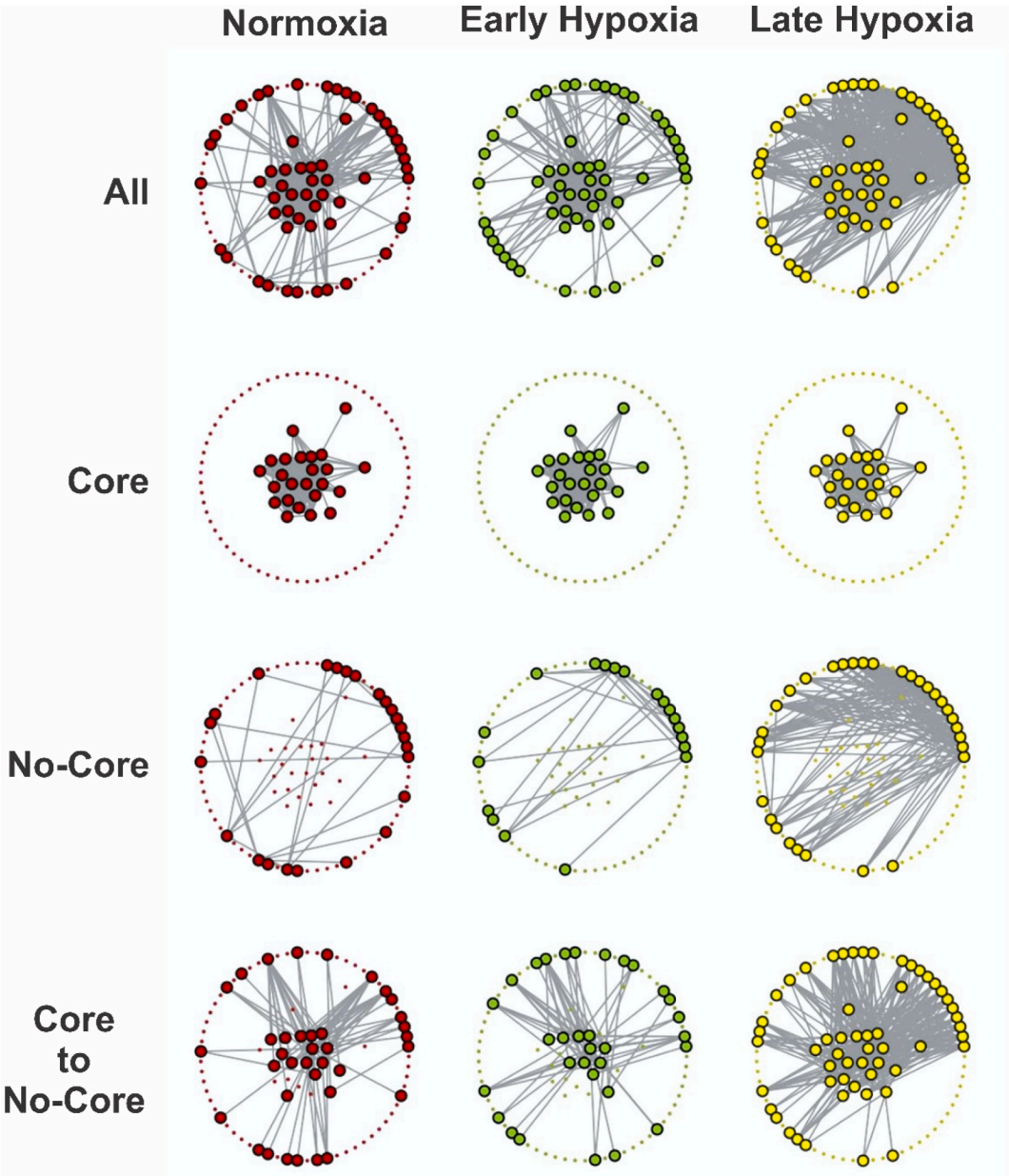

Fig. 7. Changes in the connection density between preBötC respiratory neurons during normoxia, early hypoxia, and late hypoxia. Note that in hypoxic conditions, the connection density between core neurons remains constant; however, the connection density changes only between core and non-core neurons and further between non-core neurons themselves. In late hypoxia, the reduction in connection density between respiratory neurons is even more significant. Circles represent neurons with significant coactivity, and the cells with no significant coactivity are represented by small dots adapted and modified with permission from Ref. [203].

preBötC, and their connection density (number of connections) between them remains constant in all MAPs [203]. However, in hypoxia-induced gasping, the connection density between core and non-core neurons and also between non-core neurons themselves increases (Fig. 7) [203]. It must be acknowledged that, due to their analytical approach, Juárez et al. [203] were not able to distinguish between excitatory and inhibitory neurons and their synaptic interactions.

In summary, the preBötC neuronal network reacts to acute hypoxia with a biphasic response: an initial respiratory augmentation followed by hypoxic depression. In severe, prolonged hypoxia, the eupneic activity turns into gasping. Most respiratory neurons stop firing early during hypoxia, while some autorhythmic bursting neurons, particularly CI bursters, continue to burst action potentials during the hypoxic depression phase. Synaptic inhibition decreases during hypoxia and leads to changes in the activity of most respiratory neurons, which in turn alter the pattern of respiratory output from a bell-shape to a decrementing pattern. Fig. 8 summarizes possible reasons for augmentation, depression, and gasping responses of preBötC to acute hypoxia.

5. The response of preBötC to intermittent hypoxia

Intermittent hypoxia is associated with a distinct pathological condition; obstructive sleep apnea [288–290]. Studies conducted on the effects of intermittent hypoxia on the respiratory system fall into two main categories: acute intermittent hypoxia (AIH; minutes to hours of exposure to episodic hypoxia) and chronic intermittent hypoxia (CIH; days to weeks of exposure to episodic hypoxia). Although both are episodic, their impact on the respiratory system, especially the preBötC, can be different. In the following subsections, we will review the existing literature regarding the effects of AIH and CIH on the preBötC neural network.

5.1. *Acute intermittent hypoxia*

AIH is known to induce a unique type of 5HT-dependent respiratory plasticity called long-term facilitation (LTF) [128], which is explained as an increase in minute ventilation (vLTF) after the cessation of episodic hypoxia for at least 60 min above the basal level [291]. LTF generally involves an increase in respiratory amplitude [292,293]. However, there are some reports regarding LTF of breathing frequency [128,293–296]. LTF requires episodic hypoxic exposure, as sustained hypoxia with the same exposure duration cannot elicit LTF [128,297]. It is well established that 5HT is required for the induction of vLTF [295,298] and for several models of respiratory plasticity evoked by hypercapnic exercise [299], spinal cord injury [300], cervical spinal sensory denervation [301], and chemoafferent denervation [302]. Based on studies on phrenic motoneurons, it is proposed that at least two intracellular pathways (‘*Q*’

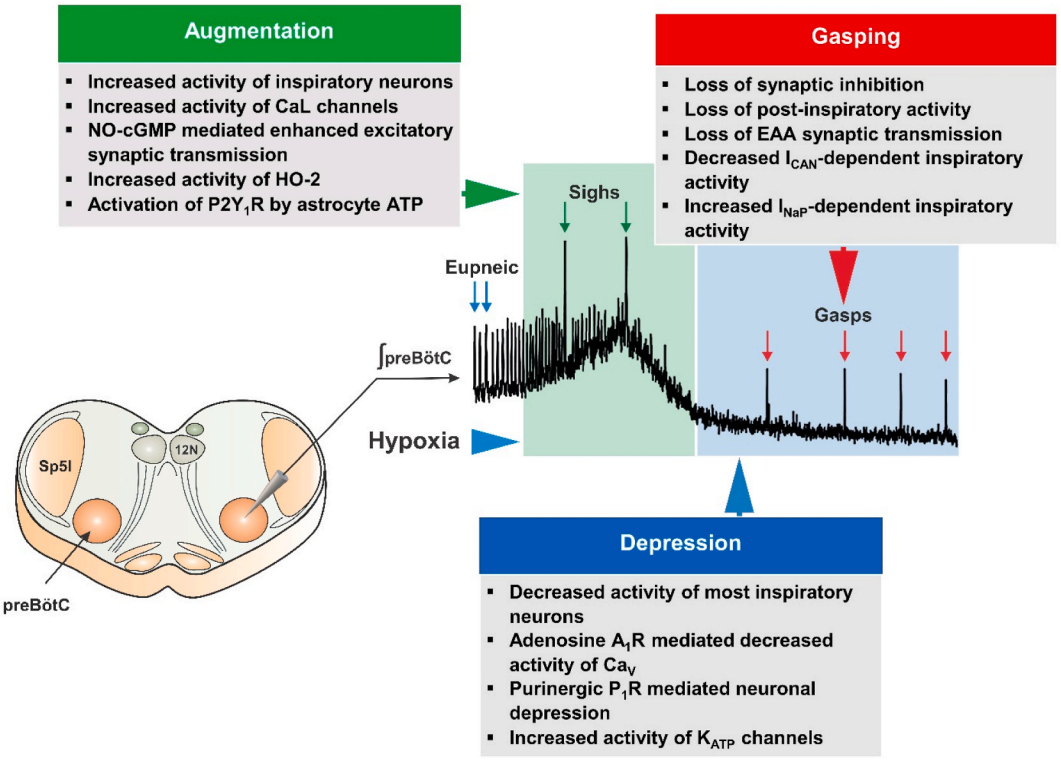

Fig. 8. The summary of changes in the preBötC neural circuit underlying different phases (augmentation, depression, and gasping) of the biphasic respiratory response to acute hypoxia. Augmentation and hypoxic depression phases are indicated by green and blue transparent rectangles, respectively The population activity recording trace is adapted with permission from Ref. [393]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and *'S'* pathways) are required for phrenic nerve LTF (pLTF) [303]. The *'Q'* and *'S'* pathways require intermittent activation of Gq and Gs proteins, respectively [304]. To induce pLTF, 5HT acts on its 5-HT2 and 5-HT7 receptors to activate Gq and Gs proteins, respectively [304]. Notably, *'S'* pathway also requires endogenous release of ADO acting on its A2A receptors [304]. Baker-Herman and Mitchell [296] suggested that besides the activation of serotonin metabotropic receptors, pLTF also requires the synthesis of brain-derived neurotrophic factor (BDNF) [305], activation of tropomyosin receptor kinase B (TrkB) [305], reactive oxygen species (ROS) [306], and glutamate NMDA receptors [307,308] at the level of phrenic motoneurons. In addition to 5HT, there is also evidence for the role of substance P (SP) in respiratory plasticity following AIH, both *in vitro* and *in vivo* [294]. Berner et al. showed that AIH induces LTF in controls, but not in $Tac1^{-/-}$ mice lacking SP [294]. It has been previously shown that SP is crucial for acute hypoxic responses of the respiratory network, including anoxia-induced sighing [309–311]. SP enhances inspiratory frequency [29], and is also crucial for the stability of respiratory rhythmogenesis both *in vivo* and *in vitro* [309,312,313].

It is postulated that stimulations from carotid bodies are required for initiation, but not for the maintenance of LTF [314], because inhibition of carotid body activity with hyperoxia in rodents and humans doesn't abolish AIH-induced LTF [128,315]. However, there are some reports that pLTF of frequency can be induced in peripherally chemodenervated rats [316–319]. Furthermore, stimulation of the carotid sinus nerve with episodic electrical currents can induce LTF [320,321]. These findings suggest that LTF is mediated centrally and does not require the enhancement of peripheral chemoreceptors. It is worth noting that CIH preconditioning is known to induce sensory LTF of carotid bodies, which likely contributes to vLTF [322]. The question is whether preBötC contributes to AIH-induced respiratory LTF. Considering that 5HT- and SP-containing neurons in the raphé nucleus directly project to the preBötC respiratory neurons [323], it is sensible to expect that respiratory plasticity following AIH also occurs at the level of the preBötC. Evidence supporting this hypothesis is based on observations that AIH induces functional [324,325] and structural [326,327] changes in preBötC neurons, which may result in the enhancement of their excitability. For instance, there are some reports regarding the increased frequency of fictive respiratory output in brainstem slices containing the preBötC following AIH [324,325,328,329]. Using whole animal and functional brainstem slices of mice containing preBötC, Zanella et al. [324] found that AIH induces a comparable increase in respiratory frequency *in vitro* compared to *in vivo* recordings. Furthermore, Zanella et al. reported that AIH also causes an increased irregularity score for amplitude and frequency of hypoglossal motor output, which can be attributable to increased spontaneous inhibitory postsynaptic potential (sIPSCs) in preBötC inspiratory neurons since these irregularities are prevented by blocking synaptic inhibition before AIH induction [324].

Although, it is a short-term hypoxic paradigm, there are some reports concerning structural changes in the preBötC neural network following AIH [326,327,330,331]. For example, it has been shown that daily AIH (dAIH) leads to increased density of NK_1R immunoreactive (NK_1R-ir) processes [327], increased activity of postsynaptic mitochondria [326,331], and increased proportion of asymmetric excitatory synapses (AS) between respiratory neurons [330]. These structural changes in the preBötC circuits following AIH may result in the generation of a more robust respiratory output, which is manifested as respiratory LTF. Thus, enhanced excitability of the preBötC neurons following AIH can be attributable to structural changes in the preBötC neural circuits.

5.2. Chronic intermittent hypoxia

Repeated peripheral chemoafferent input mediated by the NTS during chronic episodic hypoxia (CIH) induces respiratory plasticity, which is manifested as hyperventilation [126], decreased CO_2-apneic threshold [332], augmented aHVR [333], enhancement of AIH-induced LTF [333], enhanced gain of peripheral chemoafferent pathway [334,335], increased gain of the centrally generated respiratory rhythm [336,337], and an active expiratory pattern [332,338].

At the level of preBötC, CIH induces long-term changes that alter the excitability and, consequently, the basal activity of respiratory neurons in normoxia. In a series of studies on the effect of CIH on the development of sympathetic overactivity and hypertension in animal models, it was found that increased activity of RVLM presympathetic neurons was associated with changes in the excitability of expiratory neurons (Aug-E BötC neurons) in male rats [332,338–340] and of inspiratory neurons (pre-I/I and post-I neurons of preBötC) in female rats [336,341–344]. It is postulated that CIH may alter the intrinsic electrophysiological properties of preBötC neurons, leading to enhanced excitability of these neurons [341–343,345]. Enhanced excitability of pre-I/I neurons following CIH is probably mediated by downregulation of K^+ leak channels [345] and K_{ATP} channels [334].

It is believed that enhanced excitability of respiratory neurons following CIH is partly mediated by 5HT and also requires oxidative stress and ROS [297,333,346–348]. For instance, it has been shown that CIH activates the postsynaptic 5-HT/5-HT_{2A}R system associated with upregulation of phospho-protein kinase C θ (P-PKCθ) [297] and its downstream substrates (P-PKCsub) [346] in the preBötC of rats, an effect that was not observed in rats exposed to sustained hypoxia. As mentioned the 5-HT/5-HT_{2A}R system is required for AIH-induced LTF, which is expressed mainly in the phrenic nucleus [295,333], the carotid body [349], and the hypoglossal motoneurons [350].

Besides the aforementioned positive respiratory plasticity, CIH appears to have some detrimental effects on the respiratory network. CIH pretreatment has been linked to increased irregularity in preBötC rhythmogenesis [351], decreased dendritic mitochondrial activity [331], and a reduced density of excitatory synaptic interactions between respiratory neurons within the preBötC [330]. Garcia et al. [351] using electrophysiological recordings from brainstem slices containing the preBötC reported that CIH pretreatment causes irregular transmission of the preBötC respiratory signal to hypoglossal motor neurons, which is associated with an increase in the level of lipid peroxidation [351]. Garcia et al. found that CIH led respiratory neurons to generate fewer action potentials during the preBötC network burst, which culminated in intermittent transmission failure to XII motoneurons. Notably, transmission failure of the preBötC burst activity to the XII nucleus (XIIn) has been previously reported in response to AIH [324] and in conditions of reduced excitability at the level of preBötC [352]. In these conditions, the activity of preBötC neurons was insufficient to trigger

respiratory motor output. Surprisingly, Garcia et al. [351] found that in contrast to normoxic conditions, CIH preconditioning results in more stable inspiratory activity in slices during the transition to hypoxia and later during hypoxia [337], indicating that after CIH, the preBötC rhythmogenesis is less sensitive to hypoxia. They proposed that CIH increases the contribution of I_{NaP} in respiratory rhythmogenesis to counteract burst-to-burst irregularities in rhythmogenesis during hypoxia [337].

Moreover, in contrast to dAIH, it was shown that CIH treatment negatively modulates postsynaptic mitochondrial activity and synaptic interactions between preBötC respiratory neurons [330,331]. For instance, Kang et al. [331] showed that CIH results in reduced postsynaptic mitochondrial activity in the preBötC of rats [331]. They found a significant decrease in the number of moderately to darkly CO-reactive mitochondria, the length and area of dendritic mitochondria, the activity of the mitochondrial ETC enzyme, and mitochondrial membrane potential in the preBötC of CIH rats compared to the normoxic and dAIH rats. In another work, Kang J. et al. [330] reported alterations in the ultrastructure of excitatory asymmetric synapses (AS) and inhibitory symmetric synapses (SS) in the preBötC of rats exposed to either dAIH or CIH. They showed that although dAIH resulted in an increased proportion of AS, CIH increased the proportion of SS in the preBötC, suggesting increased inhibitory modulation of respiratory neurons following CIH. Moreover, Kang J. et al. [330] reported that dAIH significantly increased the expression of SST and NK1R in the preBötC, while CIH treatment led to a decrease. The CIH challenge may increase ROS production and Ca^{2+} influx in respiratory neurons, which negatively modulate the mitochondrial structure and function and synaptic interactions in the preBötC [331,351,353].

The response of preBötC to sustained hypoxia

Sustained hypoxia is associated with some diseases such as cerebrovascular accident (CVA) [354], heart failure [355], and chronic obstructive pulmonary disease (COPD) [356], as well as environmental conditions such as mountaineering [357]. Despite its importance, there are fewer studies on the effects of sustained hypoxia regarding alteration at the neuronal and network levels compared to studies of acute hypoxia. Similar to CIH conditions, prolonged exposure to hypoxia results in a decline in CO_2-apneic threshold [333,358], augmentation of aHVR in adult mammals [125,333,359,360], and an active expiratory pattern [361]. Moreover, during sustained hypoxia (hours to months), the respiratory network responds with a progressive increase in ventilation (VAH) [125,362]. Depending on the animal species, the time course for VAH can be different and may take hours to days to become completely established [9]. For instance, VAH takes four to 6 h to fully develop in goats, whereas it takes two days in rats [126,363,364], and days to weeks in humans [130].

The earliest theories explaining the time-dependent increase in ventilation during VAH were based on findings that long-term hypoxia leads to a reduction in pH in the cerebrospinal fluid (CSF) [364]. However, other experiments have not achieved the same results, and later studies reported a constant increase in CSF pH during sustained hypoxia [365]. Therefore, alternative theories emerged to explain the progressive increase in ventilation during sustained hypoxia. Since sustained hypoxia enhanced the responsiveness of the carotid body to hypoxia, it was initially proposed that VAH results primarily from carotid body plasticity [126,362]. However, there is compelling evidence supporting increased gain of central respiratory network's aHVR following prolonged sustained hypoxia [358,364,366,367]. Whether sustained hypoxia also enhances the gain of respiratory rhythmogenesis by preBötC is not fully determined. Although there is not direct electrophysiological data, there is some evidence of enhanced excitability of preBötC neurons following sustained hypoxia. For instance, using a carotid sinus nerve stimulation protocol, Dwinell and Powell [358] demonstrated that CSH leads to an *augmented frequency response* of the phrenic nerve to the stimulation of the carotid sinus nerve, with little effect on amplitude response, suggesting the enhanced excitability (gain) of respiratory rhythm generating neurons, probably those in the preBötC, to acute chemoafferent stimulation, which can be mediated by increased excitatory inputs from NTS second-order chemosensitive neurons [368,369] or the direct effect of brain hypoxia on respiratory neurons [11,370].

The molecular basis of the enhanced excitability of respiratory neurons following sustained hypoxia is not fully specified. However, based on studies on carotid bodies and NTS second-order chemosensitive neurons, the role of glutamate/NO [371,372], GABA [229], dopamine [373,374], platelet-derived growth factor (PDGF) [375], HO [376], and hypoxia-inducible factor 1 (HIF-1) is proposed [9,364,377]. At the level of preBötC, direct O_2 sensitivity based on HO can be a candidate for neuroplasticity following sustained hypoxia [10,11,64,117,370,376,378]. It has been reported that CSH for 10 consecutive days induces the expression of HO-1 mRNA, but not HO-2 mRNA in the preBötC in rats [64] and mice [376], which is associated with increased peak diaphragm electromyogram (dEMG) after 10 days of hypoxic exposure [376]. Under normoxic conditions, the brain does not express HO-1 [64,379,380]. However, chronic sustained hypoxia can stimulate the expression of HO-1 in specific hypoxia-sensitive brain regions, including RVLM [64,380]. Within the preBötC, CSH induces the expression of HO-1 within the NK_1R-expressing neurons [376]. HO-1 can change the activity of respiratory neurons through its direct effects on ion channels and its indirect effects through the actions of CO [196,381].

Further evidence supporting the role of preBötC neural circuits in VAH comes from observations that sustained hypoxia induces a morphological changes in preBötC respiratory neurons and glia [382–384]. It has been shown that short-term sustained hypoxia (10 % O_2, 1–6 h) significantly increases the number of 5HT-immunoreactive (5HT-ir) nerve cell bodies in the raphe nuclei, associated with increased *5HT-ir nerve fibers* in close contact with NK_1R-ir preBötC neurons of rats [382]. Moreover, short-term sustained hypoxia (1 h) was found to induce morphological shift of microglia to a more amoeboid state in the preBötC, suggesting increased activity of microglia following sustained hypoxia [383]. Microglia account for about 20 % of all glial cells and have been proposed to constitute the first line of defense in the brain during inflammation [385]. Furthermore, it is suggested that microglia, via crosstalk with astrocytes, are involved in the first 24 h of acclimatization to sustained hypoxia [386].

However, like CIH conditions, there are also molecular and morphological changes following sustained hypoxia, which appears deleterious for preBötC rhythmogenesis [387–390]. For instance, it has been found that short-term sustained hypoxia (8%–10 % O_2 for 1–6 h) can cause the impairment (swollen neurons and collapsed, lightly stained Nissl bodies) of some preBötC neurons [388,389],

associated with increased oxidative stress in the medulla of adult rats [388]. Moreover, CSH for ten days resulted in VLM oxidative stress, which was correlated with tauopathy (tau hyperphosphorylation) and decreased the power of local field potential (LFP) recordings at the level of the preBötC [390]. Although, at first, it seems that oxidative stress, tau hyperphosphorylation, and a decline in the power of the preBötC electrical activity are maladaptive to the respiratory rhythm, these changes may be part of a protective mechanism against CSH-induced increased glutamatergic inputs from the chemoafferent pathway and toxic Ca^{2+} overload in respiratory neurons [130,391].

Concluding remarks

Besides its primary function to generate the basal inspiratory rhythm, it is now apparent that the preBötC is a highly plastic network that reconfigures state-dependently to produce different patterns of respiratory output. To fulfill this vital role, preBötC can reconfigure its neural network by altering the activity of its neurons and the strength of connections between them. The preBötC possesses distinct types of respiratory-modulated neurons, and each group of respiratory neurons responds differently to hypoxic conditions. Most preBötC non-autorhythmic and autorhythmic spiking neurons (tonic neurons) stop firing in the early stages of hypoxia, and the only active neuronal population during severe hypoxia are autorhythmic bursting neurons, whose activity depends on persistent sodium currents. Moreover, the preBötC network responds differently to distinct types of hypoxia, from acute to sustained hypoxic conditions. In acute hypoxic conditions, only transient changes occur in ionic currents and synaptic transmission. However, prolonged exposure to hypoxia leads to structural changes at the synaptic level. The preBötC is also involved in many forms of respiratory responses to hypoxia, such as the biphasic hypoxic response to acute hypoxia, the long-term facilitation response to intermittent hypoxia, and ventilatory acclimatization to sustained hypoxia. However, further cell- and network-based experiments are required to understand the exact molecular mechanisms underlying neuroplasticity at the level of preBötC.

Data availability statement

No new data were collected as this is a review paper.

CRediT authorship contribution statement

Jamal Khalilpour: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Conceptualization. **Hamid Soltani Zangbar:** Writing – review & editing. **Mohammad Reza Alipour:** Writing – review & editing, Conceptualization. **Parviz Shahabi:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of Abbreviations

5-HT	Serotonin
Ach	Acetylcholine
ADO	Adenosine
aHVR	Acute Hypoxic Ventilatory Response
Amb	Nucleus Ambiguus
AOP	Apnea of Prematurity
ATP	Adenosine Tri Phosphate
aug-E:	Augmenting Expiratory Neuron
BötC	The Bötzinger Complex
CI	Cadmium-Insensitive
CIH	Chronic Intermittent Hypoxia
CRCs	Central Respiratory Chemosensitive Centers
CS	Cadmium-Sensitive
CSH	Chronic Sustained Hypoxia
CVR	Caudoventrolateral Reticular Nucleus
cVRG	Caudal Ventral Respiratory Group
DA	Dopamine
E−2	Stage II Expiratory Neuron
early-I:	Early-Inspiratory Neuron
GRP	Gastrin-Releasing Peptide
HD	Hypoxic Desensitization
HO	Heme Oxygenase

HVD	Hypoxic Ventilatory Depression
HVR	Hypoxic Ventilatory Response
K–F	Kölliker–Fuse Nucleus
LC	Locus Ceruleus
LRt	Lateral Reticular Nucleus
LTF	Long-Term Facilitation
MAP	Multineuronal Activity Pattern
NE	Norepinephrine
NMB	Neuromedin B
NO	Nitric Oxide
NTS	Nucleus of Solitary Tract
OSAS	Obstructive Sleep Apnea Syndrome
PA	Progressive Augmentation
PB	Parabrachial Nucleus
pFRG	Parafacial Respiratory Group
post-I:	Post-Inspiratory Neuron
pre-I:	Pre-Inspiratory Neuron
preBötC	The pre-Bötzinger Complex
ramp-I:	Ramp-Inspiratory Neuron
RVL:	Rostroventrolatelar Reticular Nucleus
rVRG:	Rostral Ventral Respiratory Group
SIDS	Sudden Infant Death Syndrome
SIUD	Sudden Infant Unexplained Death
SP	Substance P
STD	Short-Term Depression
STP	Short-Term Potentiation
TRH	Thyrotropin-Releasing Hormone
TTFB	Time to The First Breath
VIIn	Facial Nucleus
VDH	Ventilatory Desensitization from Hypoxia
VRC	Ventral Respiratory Column
$\int$PND	Integrated Phrenic Nerve Discharge

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