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SYMPOSIUM REVIEW

Advances in cellular and integrative control of oxygen homeostasis within the central nervous system

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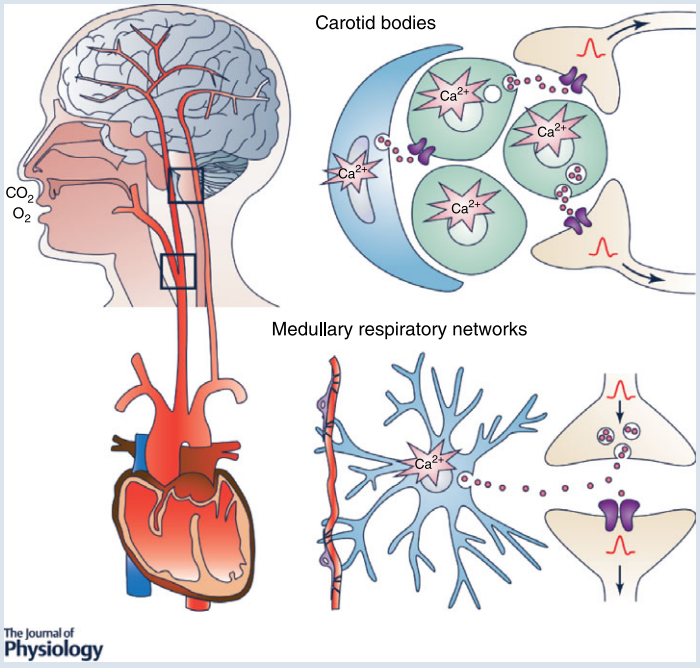

Abstract Mammals must continuously regulate the levels of O_2 and CO_2, which is particularly important for the brain. Failure to maintain adequate O_2/CO_2 homeostasis has been associated

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with numerous disorders including sleep apnoea, Rett syndrome and sudden infant death syndrome. But, O_2/CO_2 homeostasis poses major regulatory challenges, even in the healthy brain. Neuronal activities change in a differentiated, spatially and temporally complex manner, which is reflected in equally complex changes in O_2 demand. This raises important questions: is oxygen sensing an emergent property, locally generated within all active neuronal networks, and/or the property of specialized O_2-sensitive CNS regions? Increasing evidence suggests that the regulation of the brain's redox state involves properties that are intrinsic to many networks, but that specialized regions in the brainstem orchestrate the integrated control of respiratory and cardiovascular functions. Although the levels of O_2 in arterial blood and the CNS are very different, neuro-glial interactions and purinergic signalling are critical for both peripheral and CNS chemosensation. Indeed, the specificity of neuroglial interactions seems to determine the differential responses to O_2, CO_2 and the changes in pH.

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Abstract figure legend Chemoreceptors that detect changes in arterial oxygen supply are located in the glomeruli of the carotid bodies at the bifurcation of the carotid artery and in the ventral respiratory column located in the medulla. Cells in these areas utilize similar mechanisms to detect alterations in arterial oxygen content, and release modulators that lead to an increase in intracellular calcium. Modulators also bind to various receptors on nerve terminals that alter activity to ensure adequate oxygenation of the brain.

Introduction

Endothermy gave mammals and birds distinct evolutionary advantages. It allowed them to move quickly and over long distances irrespective of their surrounding environmental temperatures. This enabled them to conquer novel ecological niches. However, endothermy came with a substantially higher metabolic demand (Clarke & Pörtner, 2010). This demand is best met by aerobic metabolism, since oxygen releases substantial energy per electron transfer (Ramirez *et al.* 2007). Aerobic metabolism is particularly important to maintain the brain's state of persistent activity (Raichle *et al.* 2001; Raichle, 2015; Mitra & Raichle, 2016).

The dependency on aerobic metabolism is challenging for two important reasons. Firstly, O_2 cannot effectively be stored; consequently, mammals and birds cannot survive a prolonged cessation of breathing and heartbeat. Secondly, the dependency on the molecule with the largest energy release per electron transfer poses major regulatory challenges because too little oxygen is as detrimental as too much oxygen, a topic of great clinical significance (Semenza & Prabhakar, 1985; Haddad & Jiang, 1997; D'Agostino *et al.* 2007; Huang *et al.* 2012; Popa-Wagner *et al.* 2013; Igbal & Eftekharpour, 2017). Indeed, the P_{O_2} within the CNS is maintained within a narrow range of approximately 1–4% O_2 (Mulkey *et al.* 2001). This suggests that neuronal microcircuits in the brain must maintain persistent activity in an oxidative microenvironment that is only slightly higher than the threshold for aerobic metabolism, which lies around 1% O_2 (Clemens *et al.* 2001; Hill *et al.* 2011). Thus, maintaining a stable redox state requires precise and dynamic O_2 sensing and response mechanisms, which is achieved through neurovascular coupling involving neurons, smooth muscle cells, astrocytes (Filosa & Blanco, 2007; Ndunuizu & LaManna, 2007; Kim *et al.* 2016; Iadecola, 2017; Kisler *et al.* 2017) and possibly oligodendrocytes (Roth & Núñez, 2016).

The neuronal responses to hypoxia are differentiated, and involve various mechanisms (Haddad & Jiang, 1994; Bickler & Donohoe, 2002; Björklund *et al.* 2008). In general, acute exposure to hypoxia leads to a rapid decrease in neuronal activity and synaptic depression in many regions of the brain (Garcia *et al.* 2010*a*,*b*; Mukandala *et al.* 2016). While this may be protective, it also leads to the loss of synaptic plasticity (Lyubkin *et al.* 1997) and learning deficits (Row *et al.* 2003). During anoxia this homeostatic response breaks down as neurons depolarize within minutes until they lose their ionic gradients across the membranes (Haddad & Jiang, 1993; Fung & Haddad, 1997; Folklow *et al.* 2008). Interestingly, diving mammals have developed specialized neuroglial adaptations to withstand prolonged periods of anoxia (Folklow *et al.* 2008; Mitz *et al.* 2009; Ramirez *et al.* 2011; Czech-Damal *et al.* 2014).

The neuronal responses to hyperoxia are as differentiated (Garcia *et al.* 2010*a*,*b*). The reactive oxygen species (ROS) superoxide anion and H_2O_2 serve

neuromodulatory functions. In midbrain dopaminergic neurons, H_2O_2 activates K_{ATP} channels to reduce neuronal excitability (Avshalumov *et al.* 2005). At the neuromuscular junction, H_2O_2 differentially modulates presynaptic Ca^{2+} entry (Giniatullin & Giniatullin, 2003). The superoxide anion facilitates phrenic and hypoglossal motor outputs (MacFarlane & Mitchell, 2008; MacFarlane *et al.* 2008), and can induce plasticity (Kamsler & Segal, 2003; MacFarlane & Mitchell, 2008). Similar modulatory effects have been described for the carotid body (CB) (Peng et al. 2003, 2009).

The response to changes in oxygen is of critical importance in areas that are responsible for controlling O_2 supply. The preBötzinger complex (preBötC) is a microcircuit critical for different forms of inspiration that range from normal breathing to sighing and gasping (Smith *et al.* 1991; Lieske *et al.* 2000; Hayes *et al.* 2012; Wang *et al.* 2014). This network is located within the medulla (Smith *et al.* 1991; Schwarzacher *et al.* 2011) and is essential for breathing (Ramirez, 1998; Gray *et al.* 2001; Tan *et al.* 2008) (Fig. 1). Neuronal and glial functions within this network are responsive to hypoxia even when the network is isolated in a slice preparation (Peña & Ramirez, 2004; Peña *et al.* 2004; Tryba *et al.* 2006; Gourine *et al.* 2010; Huckstepp *et al.* 2010*a*; Hill *et al.* 2011; Nieto-Posadas *et al.* 2014; Rivera-Angula & Peña-Ortega, 2014; Angelova *et al.* 2015; Lorea-Hernandez *et al.* 2016; Peña-Ortega, 2017). Hypoxia evokes a biphasic response: a rapid augmentation with the generation of sighs is followed by a respiratory depression (Fig. 2; Wilken *et al.* 1998; Ramirez *et al.* 1998*bb*; Telgkamp & Ramirez, 1999; Lieske *et al.* 2000; Thoby-Brisson & Ramirez, 2000; Telgkamp *et al.* 2002; Peña & Ramirez, 2005). This hypoxic sensitivity of the preBötC neurons was also demonstrated *in vivo* (Solomon *et al.* 2000). Hypoxia also evokes increased activity in hypoglossal (XII) neurons (Donnelly et al. 1992, 2009; Jiang *et al.* 1992; Jiang & Haddad, 1994; Telgkamp & Ramirez, 1999) and in pre-sympathetic neurons of the rostral ventrolateral medulla (RVLM) (Sun *et al.* 1992; Sun & Reis, 1994), while hypoxia hyperpolarizes the dorsal vagal motor nucleus (Trapp & Ballanyi, 1995; Kulik *et al.* 2002; Ballanyi, 2004; Balfour & Trapp, 2007). The central responses to hypoxia within the preBötC, XII, presympathetic and parasympathetic neurons will likely contribute to an increased respiratory and sympathetic drive and a decreased parasympathetic drive (Dyavanapalli *et al.* 2014). These examples of sensitivity to hypoxia within brainstem respiratory circuits illustrate that central oxygen-sensitive mechanisms exist and locally regulate the activity of microcircuits in an adaptive manner.

This raises an important question: are these neuronal responses controlled by discrete central oxygen sensors, such as the specialized cellular interactions within the CB (Prabhakhar, 2013; Nurse, 2014; Nanduri *et al.* 2015*a*; Prabhakhar & Peng, 2017; Rakoczy & Wyatt, 2018), or do these responses emerge from multiple oxygen sensitivities intrinsic to the networks themselves? Here we propose that the hypoxic response involves both emergent network properties and specialized chemosensitive neuroglial interactions. From a functional perspective the responses to changes in O_2, CO_2 and pH must be different. Indeed, there is increasing evidence that different networks seem to specialize in sensing primarily hypoxia or hypercapnia. Yet, the strikingly different network responses seem to rely on neuroglial interactions in which astrocytes are instrumental in differentiating chemosensory responses into specific O_2 as well as CO_2 sensitivities. Thus, although this review focuses on oxygen homeostasis and the hypoxic response of the CNS, we will consider the differential O_2 and CO_2 sensitivities when discussing the neuroglial interactions. Ultimately, the organism needs to respond to changes in both blood gases in a synergistic and adaptive manner.

Unravelling the network mechanisms underlying peripheral and central O_2 sensing

To mount an effective response to changes in blood gases, O_2 sensing mechanisms within the CNS must be tightly coordinated with inputs derived from peripheral chemosensory mechanisms (Basting *et al.* 2016; Wilson & Teppema, 2016; Guyenet *et al.* 2018). Exactly how these peripheral mechanisms are integrated within the central neuronal networks in the brainstem is not fully understood, and is a source of controversy (as reviewed in Smith *et al.* 2010). At the organismal level, the hypoxic ventilatory response (HVR) is biphasic (Bissonnette, 2000): an initial augmentation is followed by a ventilatory depression (Moss, 2000). The augmentation has been associated with an excitatory drive from the CB, the depression with central regulatory activity (Teppema & Dahan, 2010; Rajani *et al.* 2018). However, it is not quite this simple. Some experiments suggest that CB denervation eliminates the augmentation phase (Bureau *et al.* 1985; Wang *et al.* 1996; Izumizaki *et al.* 2004; Hill *et al.* 2011), while others suggest that the initial augmentation during hypoxia is preserved in peripherally chemoreceptor denervated animals (Moyer & Beecher, 1942; Miller & Tenney, 1975; Richter *et al.* 1991). Indeed elegant studies have convincingly demonstrated that specific CNS hypoxia stimulates ventilation during wakefulness and sleep (Engwall *et al.* 1985; Smith *et al.* 1993; Curran *et al.* 2000). This is consistent with surgical CB denervation, which does not lead to obvious catastrophic physiological consequences. Thus, CB denervation became a procedure performed on patients in instances of carotid sinus syndrome, asthma or pulmonary disease. (For

more details on benefits and risks see Holton & Wood, 1965; Marschke *et al.* 1965; Wood *et al.* 1965; Toorop *et al.* 2010; Fitzgerald, 2014; Gourine & Funk, 2017; Iturriaga, 2018).

Limitations and caveats associated with studying hypoxic response

The discussion in the previous section is represents the challenges faced when exploring oxygen homeostasis and the neuronal response: O_2 supply and delivery depend on experimental conditions that vary widely and any experimental manipulation can have complex ramifications that are difficult to control and often difficult to interpret. This is not only the case for *in vivo* studies, but also for studies that are performed in reduced preparations in which oxygen-depth profiles differ, e.g. in the working heart–brainstem preparation (Wilson *et al.* 2001), the isolated brainstem spinal cord preparation (Brockhaus *et al.* 1993; Okada *et al.* 1993), as well as brain slices (Bingmann & Kolde, 1982; Mulkey *et al.* 2001; Garcia *et al.* 2010*a*; Hill *et al.* 2011). Oxygen profiles even differ within a given preparation, because oxygen levels depend on neuronal activity that varies between different regions of a slice (Bingmann & Kolde, 1982). Brain slices are typically studied at cooler temperatures. By decreasing metabolic consumption tissue oxygenation increases within the core, but the superficial layers are rendered hyperoxic. Thus, the neuronal networks will be exposed concurrently to hyperoxic and hypoxic conditions that will affect neuronal activity. Oxygenation is also influenced by the rate and method of superfusion, the exact composition of the artificial cerebrospinal fluid, as well as the ambient barometric pressure (Jiang *et al.* 1991; Mulkey *et al.* 2001; Fong *et al.* 2008). Experimental conditions also depend on the research questions. Studying the postnatal development of a network will be complicated by the fact that mature and neonatal slices vary in their oxygenation profile (Jiang *et al.* 1991; Mulkey *et al.* 2001; Hill *et al.* 2011). Characterizing network interactions between different regions also require slices to be cut in different thicknesses (D'Agostino *et al.* 2007; Ballanyi & Ruangkittisakul, 2009; Hill *et al.* 2011; Gourevitch & Mellen, 2014; Anderson & Ramirez, 2017).

Yet, to achieve a complete understanding of the central hypoxic response, different preparations and approaches have to be combined. The introduction of modern transgenic, optogenetic and molecular biological methods significantly increased the experimental repertoire and allows for more specific manipulations and characterizations of identified neuron classes in preparations that range from brain slices to alert and freely behaving animals (Angelova *et al.* 2015; Burke *et al.* 2015;

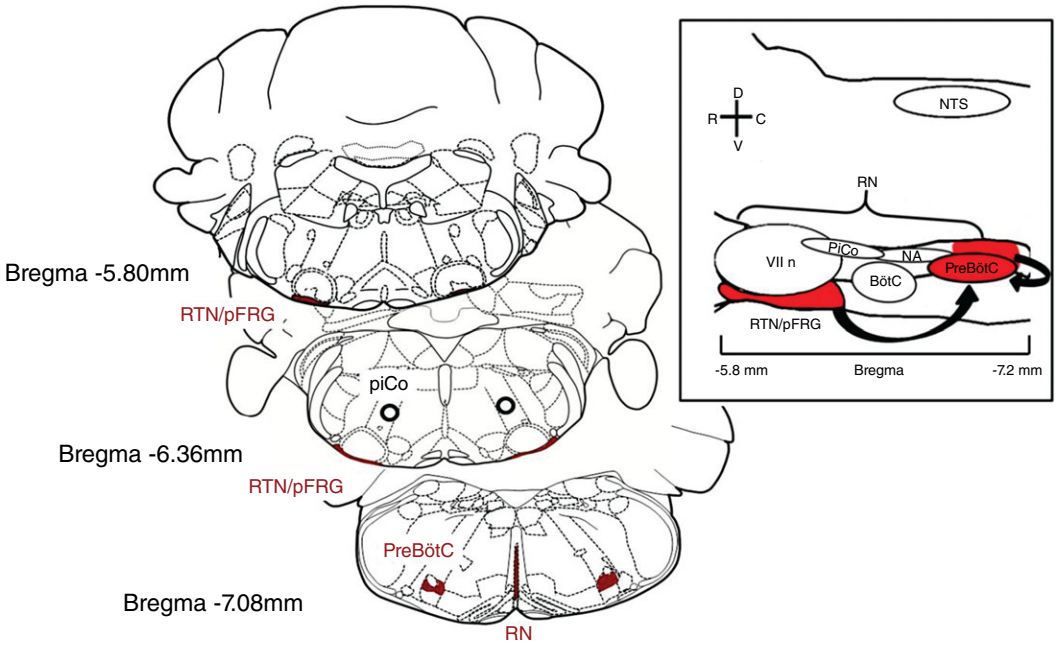

Figure 1. Anatomical schematic representation of medullary network involved in chemosensitivity
Sagittal view of ventral medullary respiratory group and the raphe nucleus (RN). The respiratory group consists of the retrotrapezoid nucleus (RTN)–parafacial respiratory group (pFRG) complex (RTN/pFRG), the Bötzinger complex (BötC), the postinspiratory complex (PiCo) and the pre-Bötzinger complex (preBötC). Structures in red have been extensively studied and mentioned in this review contributing to chemosensitivity in the CNS. Black arrows suggest communication between medullary network, RTN/pFRG and RN signal the preBötC ultimately leading to changes in breathing.

Guyenet & Bayliss, 2015; Guyenet *et al.* 2016; Rajani *et al.* 2018).

Reconfiguration of the respiratory network during hypoxia

Much has been learned about the hypoxic response of the preBötC. The respiratory rhythm in this microcircuit depends on glutamatergic neurons that are primarily derived from progenitor cells characterized by the transcription factor Dbx1 (Bouvier *et al.* 2010; Gray *et al.* 2010; Cui *et al.* 2016), and inhibitory neurons that can be subdivided into glycinergic and GABAergic neurons (Ramirez *et al.* 1997; Janczewski *et al.* 2013; Sherman *et al.* 2015). These neurons possess a variety of intrinsic membrane properties. Upon synaptic isolation respiratory neurons are silent, tonically active or possess intrinsic bursting properties (Viemari & Ramirez, 2006; Carroll & Agarwal, 2010; Morgado-Valle *et al.* 2010). These bursting properties are mediated by two principal inward currents: the persistent sodium current (I_{Nap}) and the calcium-dependent non-specific cation current (I_{CAN}) (Thoby-Brisson & Ramirez, 2001; Peña & Ramirez, 2004; Crowder *et al.* 2007; Rubin *et al.* 2009; Del Negro *et al.* 2011; Dunmyre *et al.* 2011). Early during hypoxia, I_{CAN}-dependent bursting ceases but bursting persists in neurons that depend on I_{Nap} (Fig. 2). This differential sensitivity impacts the network's dependency on these two properties. Rhythmogenesis persists when I_{Nap} is blocked with riluzole in control, but it ceases when I_{Nap} is blocked during hypoxia (Peña *et al.* 2004). At the concentration used, riluzole specifically blocked bursting, but not action potential generation (Peña *et al.* 2004), suggesting that the 'bursting property' is critical for rhythmogenesis in hypoxia. However, these pharmacological experiments cannot exclude that riluzole also altered other properties, such as synaptic transmission. Yet, modulators unrelated to riluzole had similar effects: blocking 5-HT_{2A} or α_2-adrenergic receptors blocked I_{Nap} and respiratory activity during hypoxia, but not in controls (Tryba *et al.* 2006; Viemari *et al.* 2011). These data imply that the respiratory network changes from a 'normoxic' state that depends on multiple, heterogeneous membrane properties to a 'hypoxic' (i.e. gasping) state that is particularly sensitive to the blockade of I_{Nap} (Peña & Ramirez, 2004; Paton *et al.* 2006). This hypoxic network state is characterized not only by an

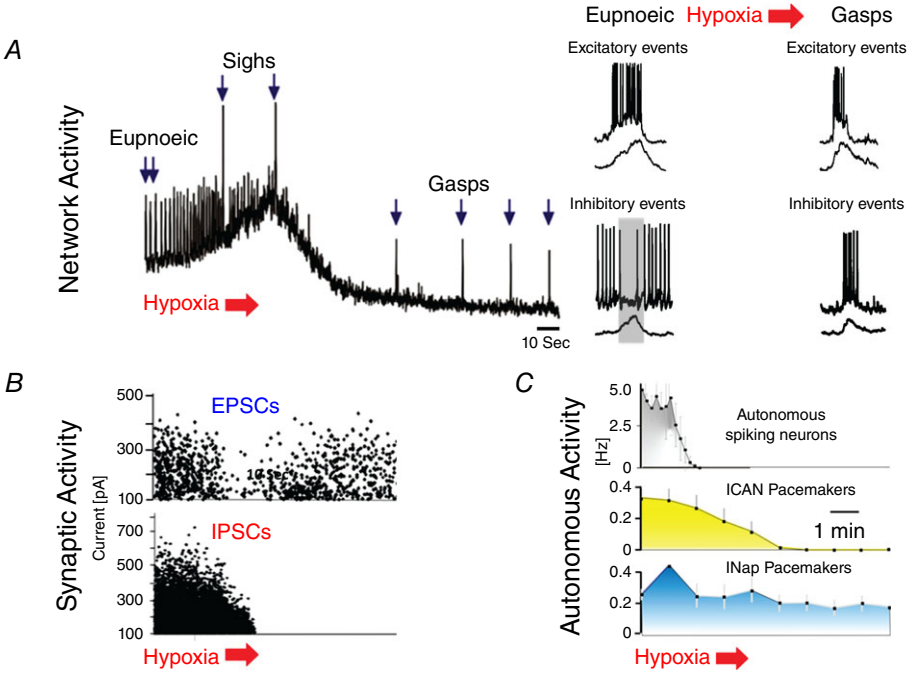

Figure 2. The effect of hypoxia on the preBötC in medullary slice recording (population and single cell recordings)
A, the respiratory network shows a biphasic response: an initial augmentation during which the respiratory frequency is enhanced and sighs are generated is followed by a depression during which the network reconfigures into gasping. *B*, synaptic changes occur during the hypoxia-induced reconfiguration as exemplified by transient changes in synaptic excitation and a suppression of synaptic inhibition. *C*, hypoxia alters bursting properties: it inhibits bursting properties that depend on the Ca^{2+}-activated non-specific cation current (I_{CAN}), while bursting mechanisms that depend on the persistent sodium current (I_{Nap}) remain relatively unaffected.

increased dependency on I_{Nap}, but also by weakened connectivity between respiratory neurons (Nieto-Posadas *et al.* 2014).

The selective dependency of the hypoxic state on the activation of the 5-HT_{2A} receptor subtype is interesting in the context of sudden infant death syndrome (SIDS). Children that die from SIDS breathe normally under normoxic conditions, but fail to gasp during hypoxia (Poets *et al.* 1999; Garcia *et al.* 2013). Various studies also demonstrated dysregulation of 5-HT in SIDS (Broadbelt *et al.* 2012; Massey *et al.* 2013; Rognum *et al.* 2014; Haynes et al. 2016, 2017; Bright *et al.* 2017). Thus, infants with disturbed 5-HT mechanisms might be protected under normal oxygenated conditions, but become vulnerable to genetic mutations that affect serotonergic neurons when the network transitions into a hypoxic state (Tryba *et al.* 2006; Garcia *et al.* 2013).

Importantly, the reconfiguration of the preBötC can only describe a small aspect of a wider network reconfiguration that will include additional microcircuits located rostral to the preBötC. This includes the retrotrapezoid nucleus (RTN)/parafacial respiratory group (pFRG), which is critical for the generation of active expiration (Janczewski & Feldman, 2006; Pagliardini *et al.* 2011; Huckstepp *et al.* 2016), and the postinspiratory complex (PiCo), which is critical for generating post-inspiration (Anderson *et al.* 2016); since postinspiratory neurons lose their inhibitory input during the inspiratory phase, we suggest these neurons might synchronize with inspiratory activity (Schmidt *et al.* 1995; Ramirez *et al.* 1998*a*; Richter & Smith, 2014). This synchronization of the network can be an acute endogenous survival response to extreme environmental changes, such as hypoxia (Michiels, 2004; Peña-Ortega, 2017).

The effect of intermittent hypoxia on the cardiorespiratory network and implications for obstructive sleep apnoea

Hypoxic conditions are often experienced in the form of intermittent hypoxia. It is a characteristic condition in patients suffering from obstructive sleep apnoea, familial dysautonomia (Weese-Mayer *et al.* 2008*a*,*b*; Carroll *et al.* 2012), Rett syndrome (Weese-Mayer *et al.* 2006; Schüle *et al.* 2008; Janc *et al.* 2016), mitochondrial disease (Brown & Squier, 1996; Quintana *et al.* 2012; Herst *et al.* 2017), epilepsy (Cohen-Gadol *et al.* 2004; Farrell *et al.* 2016) and many other disorders characterized as 'dysautonomia'. These disorders are often associated with breathing disturbances, increased heart rate, decreased heart rate variability and other forms of disturbed cardio-respiratory coupling.

Chronic exposure to intermittent hypoxia (CIH) results in increased levels of hypoxia-inducible factor (HIF) 1α and decreased HIF2, which cause an imbalance between the hypoxic and antioxidant system and a build-up of reactive oxygen species (Semenza & Prabhakar, 2007; Nanduri *et al.* 2008; Nanduri *et al.* 2015*b*). CIH seems to act directly on the CB, which then affects CNS networks through the release of neurogenic ROS. This conclusion is based on the observation that CB lesioning abolishes many of the detrimental consequences associated with obstructive sleep apnoea (Semenza & Prabhakar, 1985; Prabhakhar & Semenza, 2016). CIH leads to an upregulation of haem oxygenase 1 (Sunderram *et al.* 2016) and to an increased desynchronization of the inspiratory neurons within the preBötC (Garcia *et al.* 2016; Garcia *et al.* 2017). Incompletely synchronized preBötC bursts fail to evoke a population burst within the XII motor nucleus (Garcia *et al.* 2016), which could contribute to a pharyngeal collapse (Ramirez *et al.* 2013). The transmission failures from the preBötC to the XII can be prevented with ROS scavengers, suggesting that the CIH-induced changes involve a build-up of ROS and oxidative stress within the brainstem (Fig. 3; Garcia *et al.* 2016). The CIH-induced amplitude fluctuations in the preBötC are reminiscent of fluctuations also seen in an animal model of Rett syndrome (Fig. 4; Viemari *et al.* 2005). These mice and also human patients are characterized by increased oxidative stress (De Felice et al. 2012, 2014; Janc & Muller, 2014; Ciccoli *et al.* 2015; Filosa *et al.* 2015; Janc *et al.* 2016; Pecorelli *et al.* 2016). It is therefore conceivable that the oxidative stress seen after CIH also contributes to the breathing disturbances in Rett syndrome including the characteristic fluctuations in tidal volume (Fig. 4; Weese-Mayer *et al.* 2006). However, CIH and ROS production affects not only the preBötC but also other CNS sites, including the nucleus tractus solitarii (NTS; Kline, 2010), where CIH alters neurotransmission, neuromodulation (de Paula *et al.* 2007; Kline *et al.* 2007; Zhang *et al.* 2008; Kline *et al.* 2009; Costa-Silva *et al.* 2012; Shell *et al.* 2016), neuroprotection and plasticity by altering proteins such as TrkB and brain-derived neurotrophic factor (Almado *et al.* 2012; Moreau & Ciriello, 2015). CIH also enhances sympathetic drive and alters the baroreflex by acting differentially on central respiratory neurons (Moraes et al. 2016, 20136; Souza et al. 2016, 2017; Machado *et al.* 2017). Taken together, these studies show the close interaction between the central respiratory and cardiovascular response; however, it is also important to take careful consideration concerning CIH studies, as paradigms can vary widely among experimenters. It seems that the changes in sympathetic discharge and the levels of arterial pressure are due to the changes in the central respiratory network (Machado *et al.* 2017). This interaction occurs via connections from the respiratory microcircuits to the brainstem neurons that control sympathetic, but also parasympathetic activity. How whether the recently discovered excitatory post-

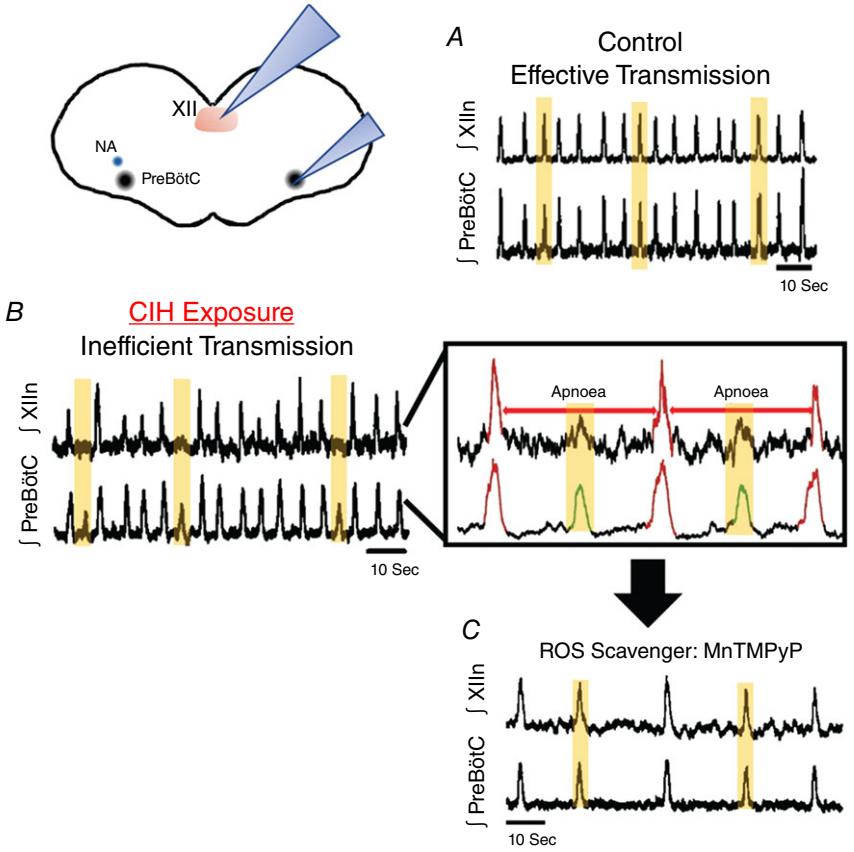

Figure 3. The effect of chronic intermittent hypoxia (CIH) on respiratory centres
A and *B*, integrated population recordings from the hypoglossal (XII, upper traces) and preBötC (lower traces) indicate that CIH exposure results in transmission failures reflected in the XII output. *C*, These transmission failures translate in 'XII apnoeas' that are prevented by treatment with cell-permeant SOD mimetic manganese(III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP).

inspiratory complex (Anderson *et al.* 2016) contributes to the integration of the sympathetic nervous system is still an open question, but a recent study indicates that there are anatomical interactions between PiCo, preBötC and the RVLM (Dempsey *et al.* 2017). For the preBötC it has been shown that it contributes to the activity of vagal neurons and parasympathetic control (Dergacheva *et al.* 2010) involving GABAergic neurons (Frank & Mendelowitz, 2012).

It is important to emphasize that the degree and specific pattern of hypoxia determine whether the consequences are detrimental or beneficial (Navarrete-Opazo & Mitchell, 2014; Wilkerson *et al.* 2018), as intermittent hypoxia can decrease (Edge & O'Halloran, 2015) or increase long-term facilitation (Fuller & Mitchell, 2017; Dougherty *et al.* 2018). Thus, under the right conditions, intermittent hypoxia has been successfully used to induce plasticity that is very beneficial during the rehabilitation following spinal cord injury (Trumbower *et al.* 2012; Dale *et al.* 2014; Fields & Mitchell, 2015; Gonzalez-Rothi *et al.* 2015).

These studies also revealed a close link to inflammation, which can suppress some aspects of the plasticity evoked by intermittent hypoxia (Vinit *et al.* 2011; Huxtable *et al.* 2013; Huxtable *et al.* 2015), while other pathways that lead to facilitation are resistant to inflammation (Agosto-Marlin *et al.* 2017). How hypoxia and inflammation interact within the CNS is an interesting and emerging area of research, with important implications for the respiratory system and the clinic (Gresham *et al.* 2011; Jafri *et al.* 2013; Lorea-Hernandez *et al.* 2016; Ribeiro *et al.* 2017). A commonly used approach to study inflammation is the use of lipopolysaccharide (LPS; Gresham *et al.* 2011; Balan *et al.* 2012; Master *et al.* 2016; Ribeiro *et al.* 2017), which via the vagal nerve causes neuroinflammation (Balan *et al.* 2011; de La Serre *et al.* 2015; Le Maitre *et al.* 2017). The relationship between inflammation and CIH is particularly relevant for premature infants, which are susceptible to lung injuries and have unstable periodic breathing (Di Fiore *et al.* 2013). Both CIH and LPS-induced inflammation modulate CB development with long-lasting consequences (Abbott *et al.* 2011) for the control of breathing, including attenuated hypoxic and hyperoxic responses (Gauda *et al.* 2013; Master *et al.* 2016). Unravelling these interactions will be critical to understanding the relationship between respiratory infections and the resulting changes in breathing that are characteristic of small infants (Gresham *et al.* 2011; Balan *et al.* 2012).

There is increasing evidence that astrocytes play a central role in the response to inflammation and hypoxia. These cells are intrinsically sensitive to hypoxic insults,

and they have been implicated in the inflammatory response (Bellaver *et al.* 2015; Forster & Reiser, 2016), the control of the cardiorespiratory responses and the modulation of sympathetic drive (Kasymov *et al.* 2013; Angelova *et al.* 2015; Marina et al. 2015, 2016*a*). The hypoxic environment increases ATP and lactate release by astrocytes, which is thought to lead to overexcitation of sympathetic circuits affecting cardiorespiratory control (Marina *et al.* 2016*a*,*b*). A recent review by Marina and colleagues has detailed how researchers have tackled the astrocyte hypothesis by blocking ATP-mediated signalling, which leads to slow progression of cardiac remodelling in rats and reduced systemic blood pressure in hypertensive rats (Marina et al. 2017). The role of glia and purinergic signalling will be discussed in more detail in the next section.

The role of purinergic signalling and neuroglial interaction in sensing O_2 and CO_2

There is an increasing consensus that neuroglial interactions play critical roles in sensing changes not only in P_{O_2} but also in P_{CO_2}/H^+. Indeed, it seems that specialized glial cells determine whether a given region, organ or network is sensitive to P_{O_2} or P_{CO_2}/H^+. These glial cells then communicate with neurons and other glia through transmitter release (Pascual *et al.* 2005; Gourine *et al.* 2010); in particular, ATP (Guthrie *et al.* 1999), D-serine (Schnell *et al.* 1995; Beltrán-Castillo *et al.* 2017) and glutamate (Parpura *et al.* 1994). The concept of specialized cell-to-cell interactions among neurons is emerging for astrocytes within the medulla, but also the cortex (Kasymov *et al.* 2013), and they may confer differential sensitivity to P_{O_2} and P_{CO_2}/H^+ depending on location within the ventral respiratory column (VRC) (Grass *et al.* 2004; Oku *et al.* 2016; Beltrán-Castillo *et al.* 2017; Forsberg *et al.* 2017). Interestingly, the neuroglial interactions that seem to underlie $P_{O_2}/P_{CO_2}/H^+$ sensitivity in the central nervous system are strikingly similar to those that occur peripherally in the carotid bodies (Figs 5 and 6) (Kumar & Prabhakar, 2012).

Therefore, the CB could provide critical insights into our understanding of how the CNS responds to hypoxia and CO_2. The CB consists of two primary cell types: type I (glomus) and type II (sustentacular) cells. These cells are bundled tightly in groups, and located in close contact with capillary beds. Afferent sensory nerves leading to the carotid sinus nerve receive autonomic innervation from the petrosal ganglion, and connect to the NTS to control breathing (Housley *et al.* 1987; López-Barneo *et al.* 2008; Kumar & Prabhakar, 2012; Prabhakhar *et al.* 2015). Type I cells are of neural origin (Duchen *et al.* 1988; López-Barneo *et al.* 2008; Pakkarato *et al.* 2015) and there are proposed to be several subtypes (McDonald

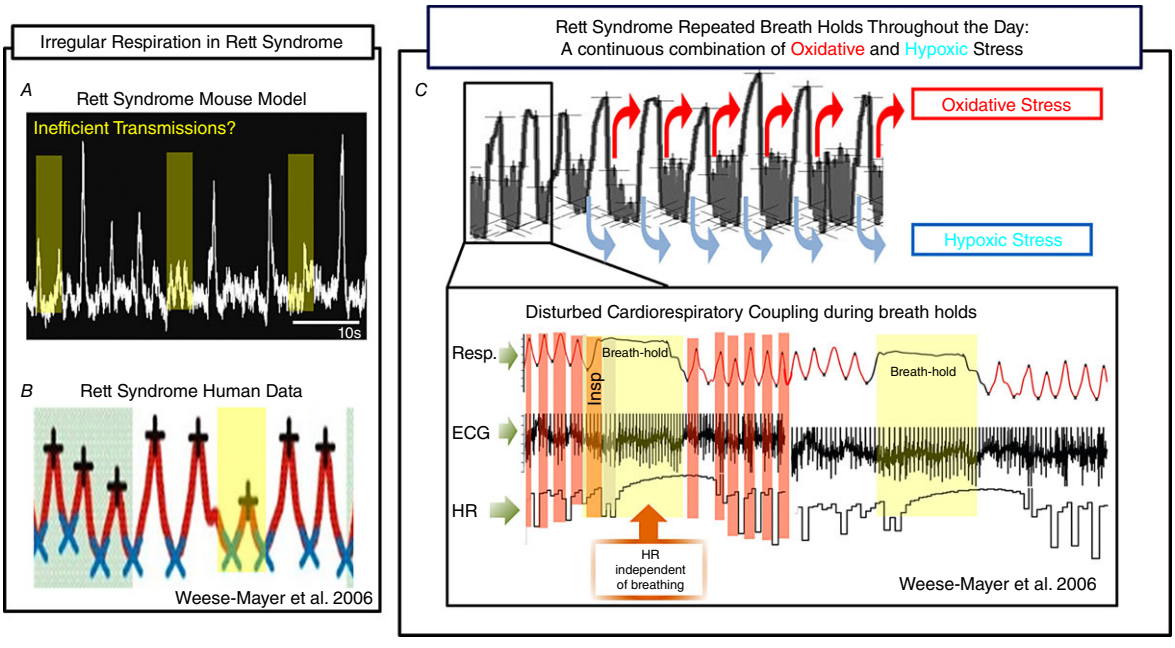

Figure 4. Respiratory irregularities in Rett syndrome
A, slices obtained from a MeCP2 KO male mouse, a model for Rett syndrome, show amplitude fluctuations in the integrated population recordings from the PreBötC that resemble those seen after CIH exposure. *B*, human data from Rett syndrome patients show large fluctuations in tidal volume. *C*, breath holds during Rett syndrome elicit oxidative and hypoxic stress. During these breath hold episodes cardiorespiratory coupling is compromised as the heart rate (HR) becomes independent from the breathing rhythm. Adapted with permission from Weese-Mayer *et al.* 2006.

& Mitchell, 1975; Chen & Yates, 1984; Prabhakhar *et al.* 2012). The responsiveness of type I cells to changes in $P_{O_2}/P_{CO_2}/H^+$ may therefore be representative of the heterogeneous hypoxic responses of central respiratory neurons (St John & Wang, 1977; Richter *et al.* 1991; Ballanyi *et al.* 1994; Peña *et al.* 2004; Hill *et al.* 2011; Beltrán-Castillo *et al.* 2017). In contrast, type II cells are glial-like and are located in close proximity to groups of type I cells, where they unsheathe type I cells with thin proccesses and are arranged into glomeruli (Fig. 5; Kumar & Prabhakar, 2012).

Both CB cell types possess distinct electrophysiological properties (Clarke & de Burgh Daly, 1981; Duchen *et al.* 1988), and there is significant crosstalk between them. ATP released from type I cells during hypoxia or hypercapnia leads to a rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$), followed by a delayed, secondary $[Ca^{2+}]_i$ increase in proximal type II cells (Murali *et al.* 2014; Murali & Nurse, 2016). A depolarization of type I cells results in sensory output to the petrosal ganglion and on to the carotid sinus nerve, mediating the cardiorespiratory response in the NTS (Housley *et al.* 1987; Iturriaga & Alcayaga, 2004). The primary transmitter, ATP, activates $P2X_{2/3}$ receptors on afferent nerve terminals (Wood *et al.* 1965; Prasad *et al.* 2001; Murali & Nurse, 2016), possibly through pannexin-1 channels, which release ATP after activation of $P2Y_2$ receptors (Prabhakhar, 2013; Murali *et al.* 2014; Prabhakhar & Joyner, 2015; Prabhakhar & Semenza, 2015).

Mechanisms proposed to underlie chemoreception in the RTN/pFRG, raphe nucleus (RN), NTS, preBötC and other areas of the respiratory network similarly rely on purinergic signalling involving astrocytes (Guthrie *et al.* 1999; Gourine *et al.* 2010; Huxtable *et al.* 2010; Huda *et al.* 2012; Turovsky *et al.* 2016; Gourine & Funk, 2017). Much has been learned about the RTN/pFRG as an important site for P_{O_2} and P_{CO_2}/H^+ sensing, but there are additional areas with varying sensitivity to hypoxia and hypercapnia in the VRC (Gourine *et al.* 2005). Purinergic signalling also plays a critical role in the central control of the cardiovascular system (Burnstock, 2006; Hawkins *et al.* 2017; Nishimura *et al.* 2017).

In principle, the astrocytic response to local changes in $P_{O_2}/P_{CO_2}/H^+$ results in elevated levels of $[Ca^{2+}]_i$, which then leads to release of ATP, which further propagates the astrocytic Ca^{2+} signal in a feedforward manner. This

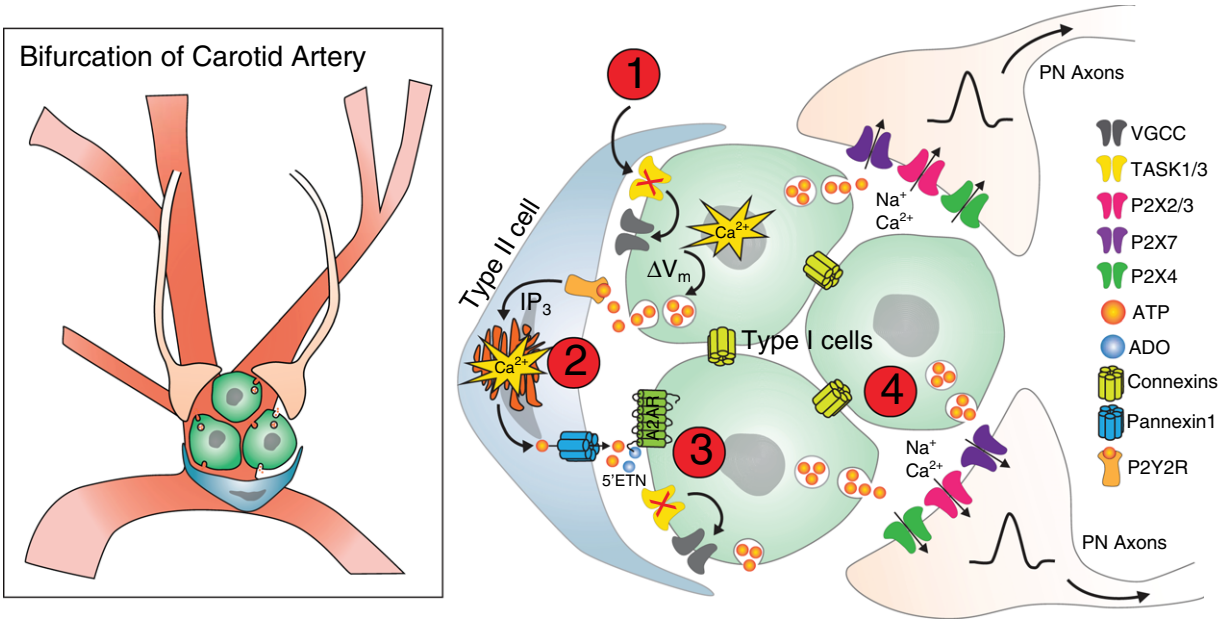

Figure 5. Illustration of the proposed mechanisms underlying chemosensitivity in the carotid bodies, located at the bifurcation of the aortic artery
Glomeruli are made up of type I cells ensheathed by type II cells, which relay changes in blood gas levels to the heart and brain through the petrosal ganglion and carotid sinus nerve. (1) Hypercapnia/H^+ or hypoxia triggers a rise in $[Ca^{2+}]_i$ in type I cells by inhibition of TASK1/3 K^+ channels, which is followed by a secondary increase in $[Ca^{2+}]_i$ in type II cells. (2) type I cells depolarize, again creating an increase in $[Ca^{2+}]_i$ resulting in neuro-/gliotransmitter release, primarily ATP, binding to $P2Y_2R$ receptors in type II cells and allowing ATP release through pannexin-1 channels. ATP is broken down through 5′-endonucleotidase activity and converted to ADO, which (3) binds to A2ARs on type 1 cells. (4) This cascade creates a positive feedback loop, followed by Na^+/Ca^{2+} release from type I cells that activates afferent axons in the petrosal axons through a variety of P2X channels (Nurse & Piskuric, 2013). It has been hypothesized that the connexin family of gap junction channels may also play a role in facilitating electrical coupling (Murali *et al.* 2014; Nurse, 2014; Murali & Nurse, 2016). VGCC, voltage-gated calcium channel.

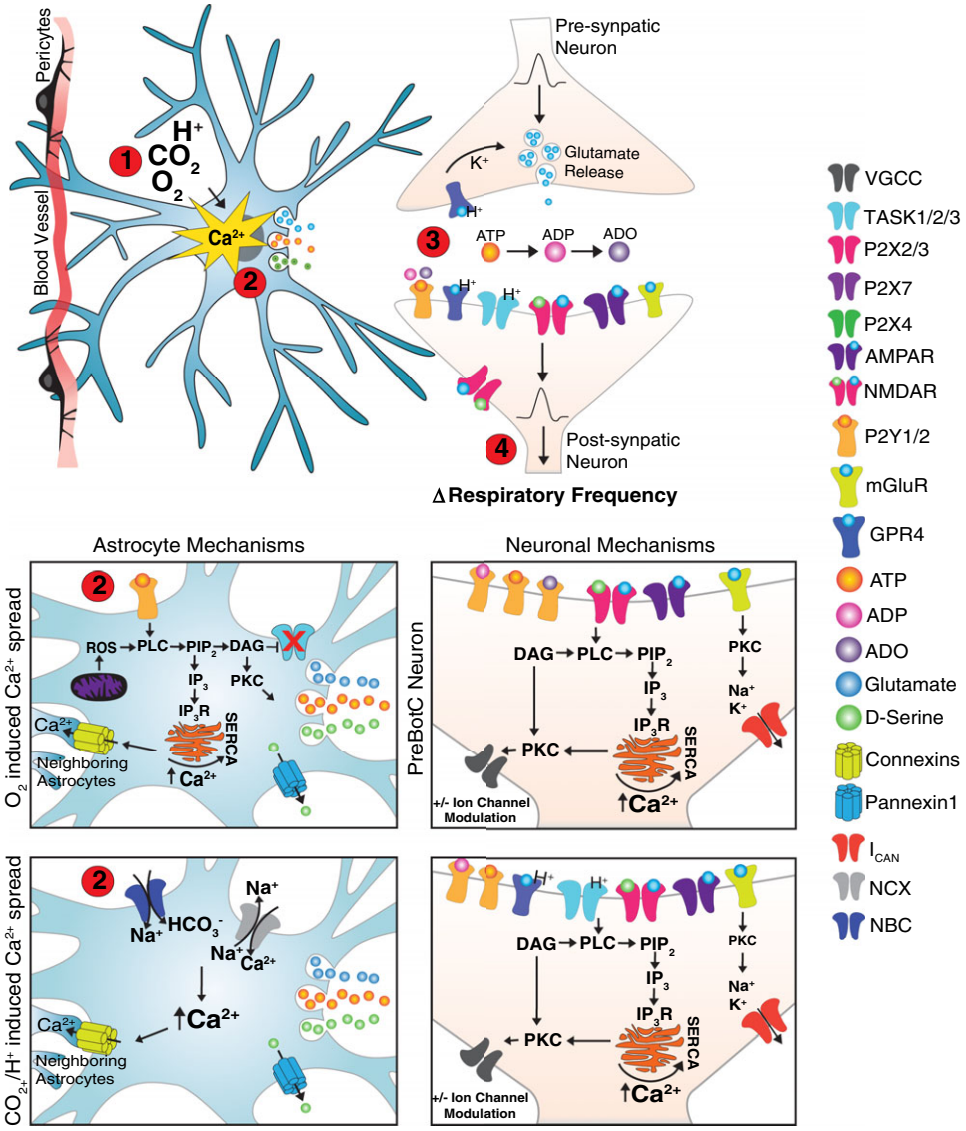

Figure 6. Illustration of the proposed mechanisms underlying sensitivity of the VRC to changes in blood gases

(1) Hypercapnia/H^+ or hypoxia triggers a cascade of events through which mitochondrial release of ROS or NBC/NCX transporters leads to a rise in $[Ca^{2+}]_i$ in astrocytes close to the ventral medullary surface near blood vessels (Gourine *et al.* 2010; Angelova *et al.* 2015; Turovsky *et al.* 2016; Rajani *et al.* 2018). (2) Increase in astrocytic $[Ca^{2+}]_i$ results in vesicular release of gliotransmitters, such as ATP (Guthrie *et al.* 1999; Angelova *et al.* 2015; Holloway *et al.* 2015), glutamate (Huxtable *et al.* 2010; Holloway *et al.* 2015) and D-serine (Beltrán-Castillo *et al.* 2017). ATP release has been proposed to be facilitated by connexin hemichannels (Huckstepp *et al.* 2010*aa*,*b*,,). (3) ATP and its derivatives (ADP and ADO; Burnstock, 2006; Robson *et al.* 2006; Funk, 2013), glutamate and D-serine are released and bind to respective receptors on neurons (or ATP to P2YRs on other astrocytes, facilitating $[Ca^{2+}]_i$ spread) (Kumar *et al.* 2015; Beltrán-Castillo *et al.* 2017; Rajani *et al.* 2018). Different mechanisms have been proposed for responses of the preBötC and RTN/pFRG, where the RTN appears to respond to changes in astrocytic Ca^{2+} through $TASK_2$/P1YR activation (Mulkey et al. 2004, 2006, 2007*bb*; Gestreau *et al.* 2009; Wang *et al.* 2013*b*) and preBötC neurons may respond by releasing glutamate that binds to NMDAR/AMPAR/mGluRs on the postsynaptic neuron or onto P2YRs on astrocytes. (4) Neuronal $[Ca^{2+}]_i$ release is then mediated by diacylglycerol (DAG)/inositol 1,4,5,-trisphosphate (IP_3), further activating protein kinase C (PKC) to modulate ion channels, thereby altering respiratory frequency (Mulkey *et al.* 2006; Lorier *et al.* 2008). GPR4, G-protein-coupled receptor 4; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; VGCC, voltage-gated calcium channel.

in turn increases the activation of chemosensitive neuron populations that are directly activated by the release of ATP (Fig. 6; Hartel *et al.* 2009; Gourine *et al.* 2010; Okada *et al.* 2012; Wang *et al.* 2013*bb*). Depending on the microcircuits in which these neuroglial interactions occur, there will be different responses at the organismic level. For example, the RTN or preBötC will mount different aspects of the systems-level responses to changes in CO_2 and O_2. The direct effect on astrocytes seems relatively clear, as blocking neuronal responses or injection of current does not alter the astrocytic Ca^{2+} response (Gourine *et al.* 2010). Additionally, ATP antagonists diminished pH-induced $[Ca^{2+}]_i$ signals (Gourine *et al.* 2010). The Ca^{2+} spread is partially due to gap junctions (Gourine *et al.* 2010), and is mediated by release of gliotransmitters (for review of Ca^{2+} spread in astrocytes, see Chemes & Ciaume, 2006). Interestingly, acidosis of the cortex and dorsal areas of the brainstem caused no change in the astrocytic $[Ca^{2+}]_i$ response, supporting the hypothesis that central chemoreception is localized to specific area(s) within the respiratory network (Gourine *et al.* 2010; Turovsky *et al.* 2016).

Precise mechanisms behind astrocytic chemosensitivity and the role of various P2X and P1/2Y receptors in modulating respiratory frequency have been partially revealed for the preBötC (Lorier et al. 2007, 2008; Huxtable *et al.* 2009; Zwicker *et al.* 2011; Rajani *et al.* 2018). These mechanisms are possibly similar for the RTN/pFRG. However, this is still debated as the sensitivity of the RTN/pFRG to ATP does not appear to be dependent on P2-related mechanisms (Fig. 6; Mulkey *et al.* 2006; Wenker et al. 2012, 2013).

In slices, $P2Y_1$ receptor ($P2Y_1R$) activation in the preBötC by ATP creates a 2- to 4-fold increase in the frequency of fictive inspiratory burst activity (Lorier *et al.* 2007). ATP released by astrocytes in the preBötC during the HVR also mediates an increase in inspiratory frequency and reduces the secondary depression phase through activation of $P2Y_1Rs$ (Rajani *et al.* 2018). Astrocytes in the preBötC sense changes in P_{O_2} and release $[Ca^{2+}]_i$, translating into release of ATP (and possibly other gliotransmitters), and activation of $P2Y_1Rs$ on neurons (Rajani *et al.* 2018). It is hypothesized that astrocytes detect changes in blood gas levels through mitochondria, relaying this information through a ROS and phosphatidylinositol 4,5-bisphosphate (PIP_2)-mediated cascade that leads to the commonly detected increase in $[Ca^{2+}]_i$ (Angelova *et al.* 2015). As mentioned above, the effect of $P2Y_1R$ activation in the RTN/pFRG is somewhat unclear. Several experiments have shown that although $P2Y_1Rs$ are expressed in RTN/pFRG neurons, they may only play a partial role in modulating CO_2- (Mulkey *et al.* 2004) or pH- (Mulkey *et al.* 2006) driven excitation, as these responses show experimental cell to cell variablity *in vitro* versus *in vivo*. However, with local application of $P2Y_1R$ antagonists during hypercapnia, there is a resultant increase in amplitude and frequency of phrenic nerve output (Burnstock, 2006; Wenker *et al.* 2012). Further downstram effects of receptor activation occur to create changes in respiratory output. Breakdown of ATP results in neuroactive metabolites, such as ADP and adenosine (ADO), which are agonists of P2YRs and P1YRs, respectively (Robson *et al.* 2006; Funk, 2013). Both of these byproducts have additional effects on respiratory frequency, as ADP is excitatory (Lorier *et al.* 2007) while ADO may have an inhibitory effect in neonates (Fig. 6; Herlenius, 2011; for review on $P2Y_1Rs$ in respiration, see Rajani *et al.* 2016).

An emergent concept is that astrocytes form different functional subpopulations. This is best illustrated by astrocytes that exist in the RTN/pFRG and preBötC performing different functions in modulating respiratory network activity (Grass *et al.* 2004; Schnell *et al.* 2011; Oku *et al.* 2016; Forsberg *et al.* 2017). Forsberg *et al.* recently produced novel evidence of two astrocyte subtypes within the RTN/pFRG and preBötC, a portion of which exhibited rhythmic calcium oscillations, and another group that maintained a state of inactivity. They also found sensitivity differences in regions of the VRC. Selective activation of astrocytes in the RTN/pFRG and preBötC increased oscillatory activity; but RTN/pFRG astrocytes released prostaglandin E_2, resulting in neural activation, whereas neurons in the preBötC had no response to an increase in calcium oscillations (Forsberg *et al.* 2017). However, as already discussed, the interpretation of findings like this needs to carefully consider experimental caveats. In normoxia, high levels of glutamate seem to be required to create astrocyte–neuron coupling. As hypothesized, these non-physiological levels of glutamate could potentially only occur during hypoxia or when blocking neuronal glutamate uptake (Schnell *et al.* 2011). Regardless of these uncertainties, it appears that each respiratory microcircuit is sensitive to changes in $P_{O_2}/P_{CO_2}/H^+$ and that multiple astrocytic subtypes may have different support functions, which is reminiscent of the situation in the CBs (Kasymov *et al.* 2013). This concept has been raised for networks throughout the CNS, not only the respiratory network (Ben Haim & Rowitch, 2017).

Further support for the concept of astrocyte subtypes and differential sensitivity in the respiratory networks comes from evidence that astrocytes also respond to changes in CO_2 by releasing D-serine (Beltrán-Castillo *et al.* 2017). It is thought that D-serine in the RN and VRC, but not NTS, increases respiratory frequency under control conditions and hypercapnia through NMDAR-dependent mechanisms (Papouin & Oliet, 2014; Beltrán-Castillo *et al.* 2017). Astrocytes appear to interact not only with neurons, but also with pericytes.

Pericytes are responsive to lactate, which results in contraction and relaxation under normal and hypoxic conditions, respectively. Lactate has been shown to be released by astrocytes located in the respiratory groups (Erlichman et al. 2008, 2010; Lazarenko *et al.* 2009; Erlichman & Leiter, 2010; Funk *et al.* 2015; Marina *et al.* 2015).

Astrocytes *versus* neurons as the primary target in oxygen sensing

As discussed above, there is support for the notion that astrocytes are directly targeted by changes in $P_{O_2}/P_{CO_2}/H^+$, while neurons are indirectly controlled by the astrocytes. Specifically, it is hypothesized that changes in blood gases are detected by astrocytes (Angelova *et al.* 2015), which in turn elicit an increase in $[Ca^{2+}]_i$, a subsequent release of gliotransmitters and neuronal activation (Hartel *et al.* 2009). However, most likely, this hypothesized mode of chemosensory transmission is much more complex, and there is still much to be learned when it comes to specific neuroglial interactions. Moreover, as discussed before, the responsiveness and mechanisms may vary for different regions.

However, can neurons be intrinsically sensitive to changes in O_2 or CO_2? In the preBötC, synaptically isolated pacemaker neurons respond to hypoxia with transient increases in rhythmicity, which is followed by cessation of the endogenous rhythm during extended exposure to hypoxia, indicating that pacemakers play a direct role in the hypoxic response (Thoby-Brisson & Ramirez, 2000). While these hypoxic responses persist after synaptic isolation, it is important to emphasize that this study does not exclude a possible involvement of gliotransmitter involving purinergic signalling. Thus, it will be necessary to demonstrate that the hypoxic responses are maintained when physically isolated, as has been shown for Raphe neurons and RVLM neurons for the CO_2 response (Wang *et al.* 1998; Wang & Richerson, 2000; D'Agostino *et al.* 2009; Sunderram *et al.* 2016). For the preBötC and C1 region, it has been shown that cells express haem oxygenase (HO-1), but these cells were anatomically identified, and it is not clear whether they play a role in the hypoxic response (Mazza *et al.* 2001; D'Agostino *et al.* 2009). Some neurons in the RTN/pFRG have also been reported to respond directly to changes in P_{CO_2}/H^+ (Guyenet & Bayliss, 2015). These neurons are purported to detect P_{CO_2} via TASK2 receptors and G-protein-coupled receptor 4 (Fig. 6; Gestreau *et al.* 2009; Guyenet & Bayliss, 2015; Kumar *et al.* 2015; Ruffault *et al.* 2015). Yet, these neurons seem to obtain this information also from surrounding astrocytes and peripheral chemoreceptors (Gourine *et al.* 2010). In this study, blocking activity of RTN/pFRG neurons had no effect on the astrocytic Ca^{2+} increase (Gourine *et al.* 2010). It has also been reported that inward currents in preBötC astrocytes occurring in phase with rhythmic neuronal oscillations under normoxia are due to neuronal release of K^+ and glutamate (Schnell *et al.* 2011).

Thus, while neurons may have intrinsic sensitivity to O_2 and CO_2, there seems to be more evidence to support the notion that astrocytes are the primary sensors for pH and hypoxic conditions. Moreover, astrocytes within the medulla are found to be in close proximity to blood vessels (Gourine *et al.* 2010) and exhibit radial processes that are in contact with vessels (Wenker *et al.* 2010). They have reversal potentials near K^+ equilibrium potential (E_K), and are blocked with barium and desipramine, a blocker of Kir4.1 channels; this has led to later experiments that have helped to uncover specific channels that astrocytes use in sensing changes in oxygen or pH (Wenker *et al.* 2010).

Ion channels and the mechanisms of O_2 and CO_2 sensing

The studies from the previous section cohesively define the role of astrocytes within the ventrolateral medulla as chemosensitive units, but specific mechanisms underlying $P_{O_2}/P_{CO_2}/H^+$ sensing and what role each specific area of the respiratory network plays in chemosensitivity have yet to be completely uncovered. Our limited understanding is partly rooted in the aforementioned experimental challenges in isolating independent mechanisms in slices or *in vivo*. This is exemplified by the amount and variety of ion channels tied to the oxygen sensing abilities of astrocytes and neurons. Clearly, multiple ion channels are involved in chemoreception. However, which channels and what particular role they play are dependent on cell type, anatomical location and experimental conditions (Lazarenko *et al.* 2010). Gap junction channels facilitate Ca^{2+} spread (Gourine *et al.* 2010), and several types of K^+ channels contribute to chemotransduction pathways within both astrocytes and neurons in the respiratory groups, especially within the RTN/pFRG (Bayliss *et al.* 2001; Mulkey *et al.* 2007*b*; Gestreau *et al.* 2009; Lazarenko *et al.* 2009; Wang *et al.* 2013*a*; Rajani *et al.* 2016; Sobrinho *et al.* 2017). K^+ channels such as the Kir4.1 inward rectifying channel have been implicated in regulation of the astrocyte resting membrane potential throughout the brain in several studies (Nwaobi *et al.* 2016), and could explain astrocytic activation through voltage-dependent mechanisms (Olsen *et al.* 2015). Interestingly, the application of fluorocitrate had no effect on astrocytes when applied to the NTS or raphe neurons (Sobrinho *et al.* 2017). The possibility of TASK channels playing a role in chemoreception was proposed in 2001, as these channels are prominent in brainstem motor nuclei and exhibit high sensitivity to pH (Bayliss et al. 2001, 2014). However, the situation may be more differentiated,

since TASK1/3 knock-out mice seem to exhibit no signs of health issues (Mulkey *et al.* 2007*aa*), while the loss of TASK2 channels in the RTN/pFRG blunted the response to pH changes. Indeed, these three subunits are in different subgroups of the same family of K2P channels, and thus the intrinsic sensitivities of TASK1/3 channels and TASK2 channels are different. Subunits 1 and 3 both exhibit a very tight range of pH sensitivity, while TASK2 has a much broader sensitivity to changes in alkalinity (Lesage & Barhanin, 2011; Bayliss *et al.* 2014). These subtle differences could underlie different mechanisms of chemosensitivity within networks of the VRC, and also explain why knockout and mutation studies were not severely detrimental to respiratory activity. TASK1 channels have similarly been suggested to have a role in controlling the background current in the carotid bodies, as mRNA and protein expression data show that they are expressed within the atrium and ventricles of heart tissue (Jones *et al.* 2002; O'Connell *et al.* 2002; Buckler, 2010).

It has been well established in recent years that ATP plays a major role in astrocytic detection of changing $P_{O_2}/P_{CO_2}/H^+$ levels. However, it would be an over-simplification to imply that purinergic signalling is the only mechanism involved. Indeed, some studies indicate that sensing O_2 and CO_2/H^+ could involve entirely different pathways within astrocytes. Turovsky *et al.* demonstrated that the Na^+–HCO_3^- cotransport (NBC) and the Na^+/Ca^{2+} exchanger (NCX) are required for increases in calcium fluctuations in astrocytes in response to changes in pH (Fig. 6; Turovsky *et al.* 2016). It will be interesting to learn whether mechanisms of mitochondrial activation and activation by NBC/NCX can occur in one astrocyte population, or if specialized subtypes exist that are specific to detecting O_2 and CO_2/H^+ changes.

As already mentioned, the mechanisms for sensing changes in $P_{O_2}/P_{CO_2}/H^+$ levels in both the carotid bodies and the CNS are surprisingly similar. For the astrocytes in the RN and VRC it has been proposed that CO_2-evoked D-serine release is due to gap junction hemi-channels, specifically pannexin1 (Beltrán-Castillo *et al.* 2017), similar to type II cells in the CB (Murali *et al.* 2014). Moreover, NMDARs are expressed in CB glomus cells and CNS astrocytes. Thus, the role of D-serine in the VRC (Liu *et al.* 2009) may bear semblance to its role in the CB. Connexins, specifically connexin 26, respond to increases in CO_2 and mediate ATP release (Huckstepp *et al.* 2010*a*,*b*), while connexins 36 and 43 are expressed in the carotid bodies and myenteric plexus in mice and are known to play a role in CO_2 detection (Chen *et al.* 2001; Frinchi *et al.* 2013).

Taken together, there is accumulating evidence for a central chemosensory component to the HVR that acts through mechanisms not dissimilar from those proposed in the carotid bodies. With the understanding that the ventrolateral medulla plays a role in the HVR, we postulate that localized responsiveness to O_2 or CO_2/H^+ in the preBötC and RTN/pFRG, respectively, emerge through specialized neuroglial interactions. Each of these regions exhibits a high sensitivity to either O_2 or CO_2/H^+, which involves regionalized astrocytes with specialized sensitivity to either O_2 or CO_2/H^+. Thus, it seems that the differences between the RTN/pFRG and preBötC are a function of the proportion of each astrocyte subtype that exists in each location (Fig. 6).

Summary

This review discussed the necessity and complexity involved in chemosensation in the CNS. We highlighted the evolutionary importance of aerobic metabolism and how our bodies have developed impeccable mechanisms to maintain homeostasis between O_2 and CO_2, which is particularly critical for normal brain function. Tightly regulated networks in the medulla that include the NTS, RN, RTN/pFRG and preBötC are primarily responsible for the homeostatic response and the regulation of blood gases. The preBötC, involved in inspiration, responds to changes in O_2 in a biphasic manner by rapidly increasing neuronal activity followed by respiratory depression that leads to gasping. This biphasic response to O_2 is attributed to a dynamic reconfiguration involving changes in ionic channel dependencies, excitatory and inhibitory conductances and the synchronization of respiratory neurons. Abnormalities in this and other medullary networks lead to disorders that affect cardiorespiratory coupling and elicit inflammatory responses exacerbating these conditions. At the core of these central chemosensory network responses are highly differentiated neuroglial interactions involving purinergic signalling. Although the chemosensitive processes in RTN/pFRG and preBötC involve neuroglial interactions including several receptors and neuromodulators that are strikingly similar to those described for the CB, it is also important to emphasize their differences, as astrocytic subtypes imbue different regions with different response properties. Unravelling the differential roles of astrocytes as the primary target in O_2 and CO_2 sensing is a riveting process, and is a departure from the ideas that (1) astrocytes are all similar and (2) neurons are the primary foci of study in the CNS. While this review emphasizes the importance of chemosensitivity, we also wanted to highlight the need for more research that will be required to unravel how the body controls its most necessary function, respiration.

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Additional information

Competing interests

The authors have no competing interests to declare.

Author contributions

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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