Respiratory Physiology & Neurobiology 265 (2019) 153–160

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

The parafacial respiratory group and the control of active expiration

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ARTICLE INFO

Keywords:
Parafacial respiratory group (pFRG)
Active expiration
Expiratory abdominal muscles
Sleep
Brainstem development

ABSTRACT

Breathing at rest is typically characterized by three phases: active inspiration, post-inspiration (or stage 1 expiration), and passive expiration (or stage 2 expiration). Breathing during periods of increased respiratory demand, on the other hand, engages active expiration through recruitment of abdominal muscles in order to increase ventilation. It is currently hypothesized that different phases of the respiratory rhythm are driven by three coupled oscillators: the preBötzinger Complex, driving inspiration, the parafacial respiratory group (pFRG), driving active expiration and the post-inspiratory Complex, driving post-inspiration. In this paper we review advances in the understanding of the pFRG and its role in the generation of active expiration across different developmental stages and vigilance states. Recent experiments suggest that the abdominal recruitment varies across development depending on the vigilance state, possibly following the maturation of the network responsible for the generation of active expiration and neuromodulatory systems that influence its activity. The activity of the pFRG is tonically inhibited by GABAergic inputs and strongly recruited by cholinergic systems. However, the sources of these modulatory inputs and the physiological conditions under which these mechanisms are used to recruit active expiration and increase ventilation need further investigation. Some evidence suggests that active expiration during hypercapnia is evoked through disinhibition, while during hypoxia it is elicited through activation of catecholaminergic C1 neurons. Finally, a discussion of experiments indicating that the pFRG is anatomically and functionally distinct from the adjacent and partially overlapping chemosensitive neurons of the retrotrapezoid nucleus is also presented.

1. Introduction

Rhythmic breathing behaviour is hypothesized to be generated by anatomically discrete, yet coupled, oscillators in the medulla. The current view is that each oscillator is responsible for the generation of a different phase of the respiratory cycle: the preBötzinger Complex (preBötC) is responsible for generating inspiratory rhythm (Gray et al., 2001; Smith et al., 1991; Tan et al., 2008) through recruitment of inspiratory muscles, and for coordinating the other phases of the respiratory cycle; the parafacial respiratory group (pFRG) is responsible for driving active expiration during periods of increased metabolic demand through the recruitment of expiratory abdominal (ABD) muscles (de Britto and Moraes, 2017; Huckstepp et al., 2015; Janczewski and Feldman, 2006; Janczewski et al., 2002; Pagliardini et al., 2011), and the post-inspiratory Complex (PiCo) is proposed to generate the post-inspiratory phase characterized by an eccentric contraction of the diaphragm and adduction of airway muscles to slow expiration (Anderson et al., 2016).

In resting conditions, inspiration requires the active contraction of inspiratory muscles. The preBötC is essential for the maintenance of ventilation with various preBötC neuronal subpopulations responsible for more specialized orofacial behaviours associated with breathing (Deschenes et al., 2016a, b; Gray et al., 2001; Li et al., 2016; Tan et al., 2008; Yackle et al., 2017). Conversely, expiration is passive, and, depending on species, absent or limited activity of expiratory muscles can be observed during restful breathing (Iscoe, 1998). Mounting experimental evidence suggests that the pFRG is silent at rest and becomes rhythmically active during periods of increased respiratory demand (e.g. exercise, hypercapnia, hypoxia) (de Britto and Moraes, 2017; Huckstepp et al., 2015; Pagliardini et al., 2011). The activation of pFRG promotes increase in ventilation by means of two potential and concurrent mechanisms: i) by facilitating air outflow via recruitment of expiratory pump muscles and reduction of upper airway resistance (i.e., mechanical effects), and ii) by influencing and reconfiguring the activity of the respiratory network and exciting the inspiratory oscillator, the preBötC (i.e., central effects).

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https://doi.org/10.1016/j.resp.2018.06.010
Received 23 March 2018; Received in revised form 11 June 2018; Accepted 18 June 2018
Available online 19 June 2018
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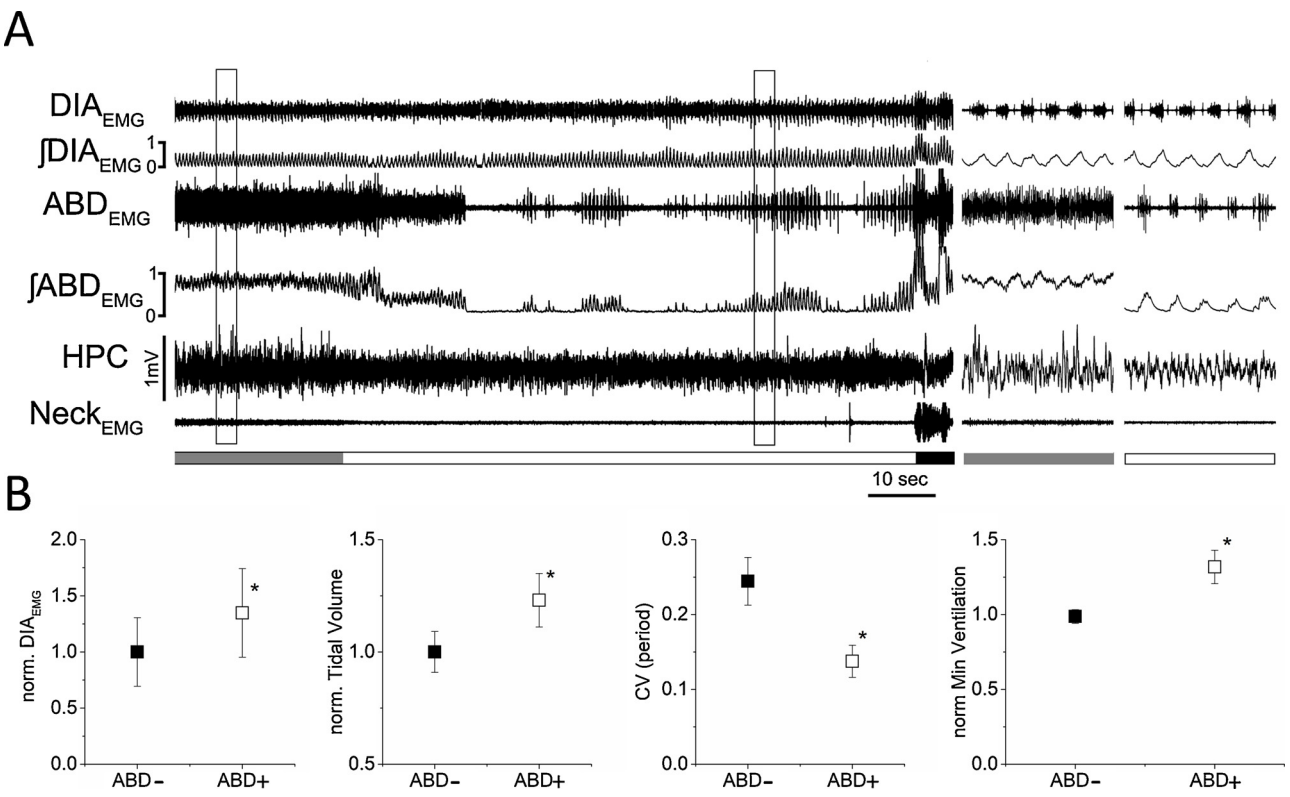

Fig. 1. Abdominal recruitment observed during REM sleep in adult normocapnic rats is associated with stabilization of breathing. (A) Abdominal muscle recruitment during transition from NREM to REM sleep. Long traces recording of diaphragm (DIA_{EMG}) and abdominal (ABD_{EMG}) muscles, their integrated EMG activity, hippocampal activity (HPC), and neck EMG activity. Details of EMG and EEG traces during NREM and REM epochs are displayed in panels on the right. Schematic blocks at the bottom of the traces indicate time spent in NREM (grey), REM (white), and wakefulness (black). (B) ABD recruitment within REM epochs significantly improves breathing. Plots display a comparison of the peak DIA_{EMG}, normalized tidal volume, coefficient of variation of the respiratory period (CV) and normalized minute ventilation before (ABD-) and during (ABD +) the occurrence of ABD recruitment within REM epochs. Figure modified from Andrews, C. G., and S. Pagliardini. 2015. 'Expiratory activation of abdominal muscle is associated with improved respiratory stability and an increase in minute ventilation in REM epochs of adult rats', *J Appl Physiol* (1985), 119: 968-74.

The anatomical origin, mechanism of generation and modulation of the inspiratory rhythm has been, and continues to be, intensely investigated (Del Negro et al., 2018). Similarly, several mathematical models have been proposed in the past decades to help unravel the mechanisms of respiratory rhythmogenesis; however, only recently the conditional activity of pFRG neurons have been included in mathematical models to explain respiratory pattern in resting conditions and under high metabolic demand (Molkov et al., 2014; Rubin et al., 2011).

Moreover, in *in vivo* experimental settings, attention has expanded to focus on the contribution of active expiration (and the expiratory oscillator) to ventilation in different physiological and vigilance states. Here, we discuss the most recent research on the generation and modulation of active expiration in rodents. We will also shed some light on the emergence of expiratory ABD activity during sleep and its contributions to ventilation in different brain states and developmental stages. Finally, we will consider whether evidence supports classification of the parafacial region into two anatomically and functionally distinct ventral and lateral subdivisions.

2. Relationship between inspiratory and expiratory oscillators across development

The relationship between inspiratory and expiratory oscillators has been studied across different developmental stages in rodents (for a concise summary, see Figure 9 in Huckstepp et al., 2016a). In the early stages of fetal development (embryonic day E14.5 in mice), rhythmic activity of the embryonic pFRG (described as embryonic paraFacial oscillator, epF in Thoby-Brisson et al., 2009) commences in mice just before the inception of preBötC activity and later on it couples with, and entrains the activity of the independently formed preBötC (Thoby-Brisson et al., 2009). With progression of fetal rodent development and within the first post-natal week, the preBötC assumes the primary role in driving rhythmic respiratory activity but remains functionally coupled with the pFRG (Mellen et al., 2003; Oku et al., 2007; Pagliardini et al., 2003; Smith et al., 1991; Thoby-Brisson et al., 2005).

The two oscillators are differentially affected by opioids: while activity of preBötC neurons and its inspiratory output are depressed, the activity in the late expiratory/pre-inspiratory neurons of pFRG persists in the first postnatal week, pacing inspiratory rhythm and providing an essential input to drive expiratory motor output and respiration (Janczewski and Feldman, 2006; Janczewski et al., 2002; Mellen et al., 2003).

In juvenile and adult rats, the pFRG is silent in resting conditions and acts as a conditional oscillator, recruited during periods of increased respiratory drive to increase tidal volume and ventilation (de Britto and Moraes, 2017; Huckstepp et al., 2015; Pagliardini et al., 2011). Interestingly, selective inhibition and stimulation of the preBötC and/or pFRG in adult rats, demonstrate that activation of pFRG induces expiratory motor output, but the pFRG is unable to sustain its activity when preBötC rhythmicity is persistently silenced (Huckstepp et al., 2016a). These results suggest that in adult rats the pFRG is unable to drive expiratory activity or a breathing rhythm in absence of input from the preBötC. However, since those experiments were performed under urethane anesthesia where autoresuscitation processes are severely disrupted (Krause et al., 2016), it will be interesting to test the ability of pFRG to support ventilation in physiological and pathological

conditions where simultaneous recordings of pFRG and preBötC neuronal activity, inspiratory and expiratory motoneuron activity and motor output can be performed without the confounding effects of anesthesia.

3. In vivo Studies on active expiration

3.1. Sleep/Wake

Studies investigating the interactions between respiratory oscillators and their corresponding motor outputs have been performed mostly in *in vitro* and *in situ* reduced preparations or in *in vivo* anesthetized rodents (Abdala et al., 2009; Iizuka, 2009; Iizuka and Fregosi, 2007; Janczewski and Feldman, 2006; Janczewski et al., 2002). Recent observations in unanesthetized and freely behaving rodents are beginning to provide insight into the contribution of active expiration to ventilation across different developmental stages and behavioural states (Andrews and Pagliardini, 2015; Leirao et al., 2017; Saini and Pagliardini, 2017). The pattern of expiratory ABD activity across sleep/wake cycles in normocapnic conditions was initially described by Sherrey et al. in 1988 and further investigated in detail by our group (Andrews and Pagliardini, 2015). Expiratory activity occurs during both NREM and REM sleep (Andrews and Pagliardini, 2015; Sherrey et al., 1988), but it is more likely to occur after the irregular breathing (i.e. apneas and high respiratory variability) observed during REM sleep (Andrews and Pagliardini, 2015). Moreover, frequent expiratory ABD recruitment in REM sleep is associated with increased respiratory stability and improved ventilation (Fig. 1; Andrews and Pagliardini, 2015). Abdominal recruitment events during NREM sleep are less frequent than in REM sleep and not associated with a significant change in respiratory frequency or respiratory variability (Andrews and Pagliardini, 2015). On the contrary, other studies have reported the absence of expiratory ADB recruitment during NREM sleep in normocapnia (Leirao et al., 2017). While factors like animal posture during sleep could differentially influence the strength of the signal recorded from oblique ABD muscles (Sherrey et al., 1988), differences in the reported occurrence of expiratory ABD activity during NREM sleep (Andrews and Pagliardini, 2015; Sherrey et al., 1988) vs (Leirao et al., 2017) could also be due to differences in the experimental procedures, such as the placement of EMG electrodes, the duration of the habituation period and recording sessions, or to differences with respect to analyses and identification of active expiration. Although the direct pFRG contribution to the recruitment of ABD activity during sleep in normocapnic and hypercapnic conditions is yet to be determined, the simultaneous recording of pFRG neurons, ABD motoneurons and respiratory motor output during natural sleep will provide further insights into the state dependence of pFRG neuronal activity in adult rats.

3.2. Development

Because the functional relationship between inspiratory and expiratory oscillators changes so much during development, a natural question is whether the ABD activity observed in adult rats during sleep is also present during postnatal development. A recent study in behaving neonate and juvenile rats under normocapnic conditions (from post-natal days 0 to 15: P0 to P15) indicates that expiratory ABD activity is recruited during both NREM and REM sleep throughout development (Saini and Pagliardini, 2017). Similar to adult rats, ABD recruitment during REM sleep in post-natal rats was associated with an increase in tidal volume and minute ventilation, as well as a reduction in respiratory variability (Fig. 2; Saini and Pagliardini, 2017). Moreover, while the rate of ABD recruitment during REM sleep remains stable across postnatal development and adulthood (Andrews and Pagliardini, 2015; Saini and Pagliardini, 2017), ABD recruitment during NREM sleep varies during postnatal development: it is highest in neonates and gradually decreases to adult levels by P15 (Andrews and Pagliardini, 2015; Saini and Pagliardini, 2017). Whether these differences reflect developmental changes in networks controlling breathing, sleep or their interactions remains to be investigated.

Experiments in medullary slices and in *en bloc* preparations of neonatal rodents indicate that both preBötC and pFRG oscillators are active and coupled during the first days after birth (Mellen et al., 2003; Oku et al., 2007; Pagliardini et al., 2003; Smith et al., 1991; Thoby-Brisson et al., 2005). This suggests that the motor output during inspiration and expiration would have an expected coupling ratio of one-to-one (inspiration always followed by expiration) during the first hours and days after birth (P0-P1). Interestingly, expiratory ABD activity during sleep in behaving neonates is not constantly present and coupled with the inspiratory intercostal (INT) muscle activity (Saini and Pagliardini, 2017) as would be expected if pFRG was continuously active and driving expiratory ABD activity. Possible explanations for this intriguing results, are: i) experimental conditions in *in vitro* preparations necessary to keep reduced preparations active (high extracellular potassium concentration, low temperature, artificial oxygenation) may promote sustained rhythmic activity of pFRG neurons and their coupling with preBötC neurons; ii) key modulatory networks are absent in the *in vitro* preparation compared to the behaving animals; this would suggest that although pFRG is rhythmic in slice and *en bloc* preparations of neonates, it may be silent in the behaving animals, becoming sporadically active during sleep; iii) the pFRG rhythmic activity observed in *in vitro* preparations of neonates is maintained in behaving animals, but its drive remains subthreshold and does not generate an expiratory motor output by itself. This would mean that either pFRG itself, or the expiratory premotor and motor neurons would require some type of additional excitatory drive to generate expiratory muscle activity.

3.3. Increased respiratory drive

Hypercapnia potentiates active expiration in every state of arousal and sleep in adult rats; however, it is not continuously recruited throughout the entire CO_2 exposure period (Leirao et al., 2017; Sherrey et al., 1988). Moreover, during hypercapnia ABD recruitment is more frequent during NREM sleep than during wakefulness (REM stage was not analyzed), and it is always associated with improvements in ventilation (Leirao et al., 2017). These results suggest that recruitment of ABD muscles by chemosensory or vigilance drives could be mediated through different neuromodulatory systems (Leirao et al., 2017; O'Halloran, 2017).

Active expiration may also be generated in hypoxic conditions (Abdala et al., 2009; Malheiros-Lima et al., 2017). Malheiros-Lima et al. (2017) propose the existence of a catecholaminergic mechanism for the generation of active expiration since selective ablation of catecholaminergic C1 neurons in the rostral ventrolateral medulla (RVLM) attenuated the late-expiratory flow observed during hypoxia (Malheiros-Lima et al., 2017).

4. Recruitment of active expiration

4.1. Disinhibition

In adult anesthetized rats, pFRG activity is tonically supressed via active synaptic inhibition (Pagliardini et al., 2011). Pharmacological disinhibition of the pFRG allows silent pFRG neurons to become rhythmically active during late expiration, driving the recruitment of expiratory ABD activity (Fig. 3; Pagliardini et al. 2011). A similar mechanism may be responsible for the activation of non-chemosensitive late expiratory pFRG neurons in hypercapnia-evoked active expiration (de Britto and Moraes, 2017). While blocking glutamatergic excitation has no effect on hypercapnia-induced active expiration, activation of GABAergic and glycinergic receptors in the pFRG eliminates hypercapnia-induced active expiration and silences non-chemosensitive late-expiratory neurons in this area (de Britto and Moraes, 2017). These

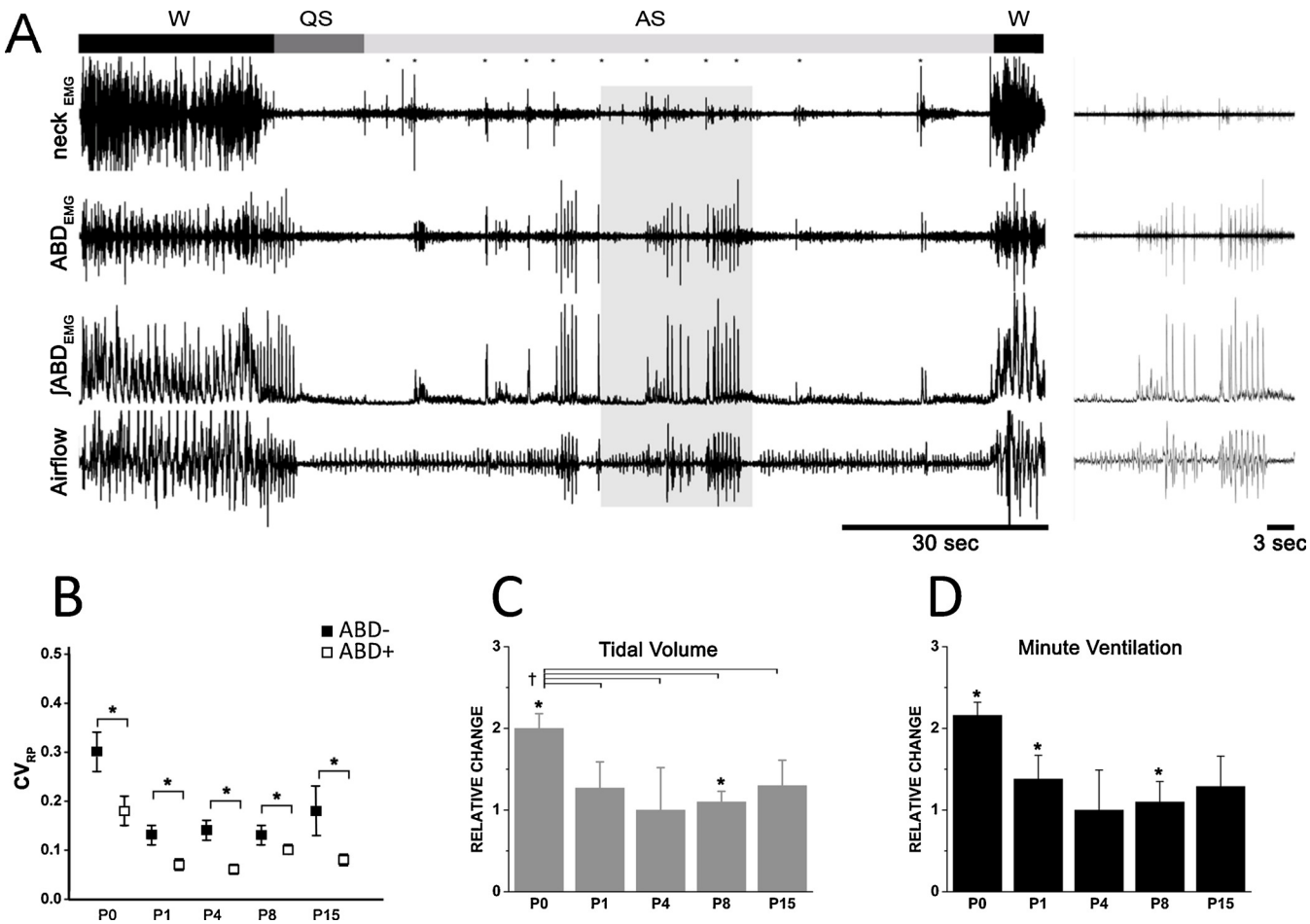

Fig. 2. The effects of abdominal recruitment during active sleep (AS) in normocapnic neonatal rats varies throughout development. (A) Abdominal muscle recruitment during transition from wakefulness (W) to quiet sleep (QS, the precursor of NREM sleep in infants) to active sleep (AS, the precursor of REM sleep in infants) in a P0 rat. Long traces recording of abdominal (ABD_{EMG}) muscles, its integrated EMG activity, neck EMG activity and whole-body plethysmograph traces (airflow) during a AS (light gray bar) epoch in which ABD muscle activity is recruited. Abdominal activity is recruited during AS, most frequently following a twitch event (indicated by *). Details of traces during AS in the grey box are displayed in the right panel. Schematic blocks at the top of the traces indicate time spent in wakefulness (W, black), QS (dark grey) and AS (light gray). (B–D) ABD recruitment within AS epochs significantly improves breathing across different age groups. (B) Comparison of the coefficient of variation (CV) of the respiratory period before (ABD-) and during (ABD +) ABD recruitment within AS sleep epochs across different ages (P0-P15) shows a significant decrease for all age groups. Relative measurements of tidal volume (C) and minute ventilation (D) indicate that there is an overall trend for tidal volume to increase with the onset of recruitment (ABD +) but this change is significant only in P0 and P8 rats, whereas minute ventilation increased with ABD recruitment at all age groups except in P4 and P15 rats. Figure modified from Saini, J. K., and S. Pagliardini. 2017. 'Breathing During Sleep in the Postnatal Period of Rats: The Contribution of Active Expiration', *Sleep*, 40.

data suggest that loss of inhibition of pFRG, and not glutamatergic excitation, may be important in the initiation of hypercapnia-induced active expiration (de Britto and Moraes, 2017). The source of the inhibition to pFRG neurons under resting conditions is currently unknown. It could come directly or indirectly from the ventral respiratory column or from chemosensitive areas of the brain like RTN, nucleus of the solitary tract, locus coeruleus and medullary raphe (de Britto and Moraes, 2017; Nattie and Li, 2012; Pagliardini et al., 2011). The Kölliker-Fuse is strongly implicated in the generation of hypercapnia-elicited active expiration through disinhibition of pFRG (Barnett et al., 2017). However, it is possible that the modulatory effects of Kölliker-Fuse on pFRG are mediated through inhibitory cells located in the Bötzinger, preBötzinger or pFRG areas (Barnett et al., 2017; Onimaru et al., 2014).

4.2. Cholinergic activation

We have recently demonstrated that local inhibition of acetylcholinesterases or the application of the cholinomimetic carbachol in the pFRG induces active expiration (Fig. 4) (Boutin et al., 2017) suggesting that pFRG is also under cholinergic modulation. The source of cholinergic input to the pFRG and its contribution to the recruitment of active expiration in different physiological scenarios is still unknown. Recent evidence suggest that acetylcholine is released by cholinergic terminals on the ventral surface of the medulla following hypercapnia-induced ATP release and increase of respiration (Huckstepp et al., 2016b). This endogenous release of acetylcholine could potentially affect the activity of both the chemosensitive neurons in the retrotrapezoid nucleus and the adjacent pFRG neurons during hypercapnia. Cholinergic mechanisms could also play an important role for the generation of active expiration during sleep (Andrews and Pagliardini, 2015; Saini and Pagliardini, 2017; Sherrey et al., 1988). If so, the pedunculopontine tegmental (PPT) and the laterodorsal tegmental (LDT) nuclei are likely candidates since they send cholinergic projections to the reticular formation (and possibly the pFRG) and are important for the generation and maintenance of REM sleep (Jones, 1990; Van Dort et al., 2015; Woolf and Butcher, 1989; Koch, Biancardi and Pagliardini, unpublished).

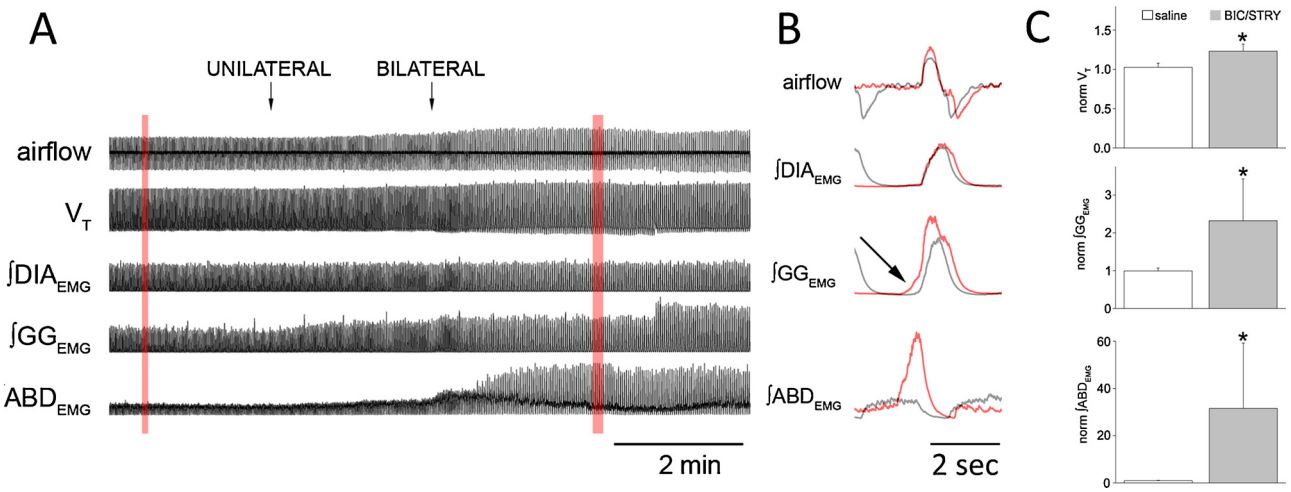

Fig. 3. Disinhibition of pFRG induces active expiration with the recruitment of ABD muscles. (A) Response to unilateral and bilateral application of (bicuculine and strychnine) BIC/STRY (50μM; 200 nl) in pFRG. The airflow, integral of abdominal (ABD_{EMG}), diaphragm (DIA_{EMG}) and genioglossus (GG_{EMG}) traces in red boxes are expanded and superimposed in (B) before (black) and after (red) BIC/STRY application. The arrow in B indicates effect of BIC/STRY on GG_{EMG} onset. (C) Plots display comparisons of the tidal volume (V_T), peak GG_{EMG}, and DIA_{EMG} amplitude in control (white bars) and after the injection of BIC/STRY (gray bars). Figure modified from Pagliardini, S., W. A. Janczewski, W. Tan, C. T. Dickson, K. Deisseroth, and J. L. Feldman. 2011.'Active expiration induced by excitation of ventral medulla in adult anesthetized rats', *J Neurosci*, 31: 2895-905.

5. The retrotrapezoid nucleus and the pFRG: two independent nuclei?

Since the discovery of the rhythmogenic properties in the region surrounding the facial nucleus, there has been an ongoing debate on whether the chemosensitive neurons in the retrotrapezoid nucleus (RTN) and the late expiratory neurons in the pFRG are the same neuronal population of identical genetic origin, or partially overlapping distinct neuronal populations.

Embryonic studies in mice identified the precursor of pFRG neurons (epF) as a unique population derived from the early growth response protein 2 transcription factor (Egr2) progenitors and expressing the transcription factor Phox2b and the neurokinin1 receptor (Thoby-Brisson et al., 2009). Rhythmogenic activity of these neurons is present in early stages of development (Onimaru and Homma, 2003; Thoby-Brisson et al., 2009), progressively declines in the postnatal period (Oku et al., 2007) and disappears in adulthood, making the identification and characterization of these adult neurons at rest challenging (de Britto and Moraes, 2017; Pagliardini et al., 2011).

Late expiratory/pre-Inspiratory pFRG neurons are sensitive to pH in P0-P2 in vitro preparations (Kawai et al., 2006; Onimaru et al., 2008). However, the pH responses were heterogeneous and allowed for the identification of distinct subpopulation of parafacial neurons: $Phox2b^+$ CO2 sensitive pre-Inspiratory neurons, $Phox2b^-$ CO2 insensitive pre-Inspiratory neurons intermingled with tonically active $Phox2b^+$ CO2 sensitive RTN neurons (Kawai et al., 2006; Onimaru et al., 2008).

In juvenile and adult rats, chemosensitive cells of the RTN have been characterized in detail: these cells are tonically activated by low pH, they express the transcription factor Phox2b in addition to glutamatergic markers (vesicular glutamate transporter 2) and NK1R (Abbott et al., 2009; Mulkey et al., 2004; Stornetta et al., 2006). Functional and anatomical studies have localized these cells mostly around the ventral and medial edges of the facial nucleus (Fig. 5A; also termed as pF_V by Huckstepp et al., 2015), they have extensive

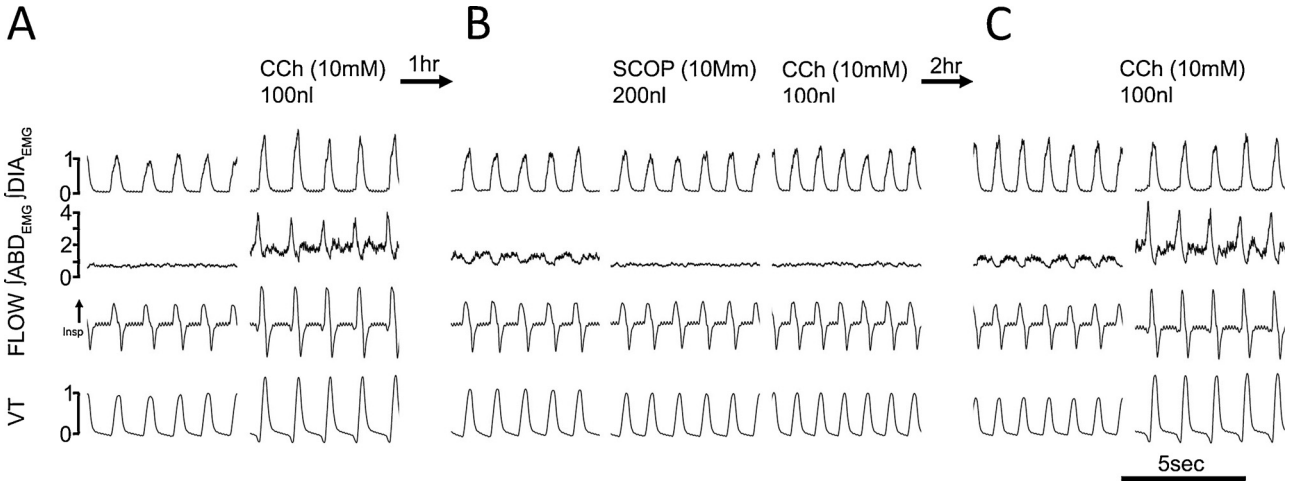

Fig. 4. Active expiration is induced by cholinergic modulation of pFRG. Traces show details of the airflow, tidal volume (VT), and the integrals of diaphragm (DIA_{EMG}) and abdominal (ABD_{EMG}) muscle activity. (A) Injection of the cholinomimetic carbachol (CCh) in pFRG induces active expiration and increases peak DIA_{EMG} amplitude and VT. (B) The injection of the muscarinic antagonist scopolamine (SCOP) in pFRG blocks the effects of CCh injection in pFRG. (C) After washout from SCOP application, the effects of CCh injection in pFRG are recovered. Figure modified from Boutin, R. C., Z. Alsahafi, and S. Pagliardini. 2017. 'Cholinergic modulation of the parafacial respiratory group', *J Physiol,* 595: 1377-92.

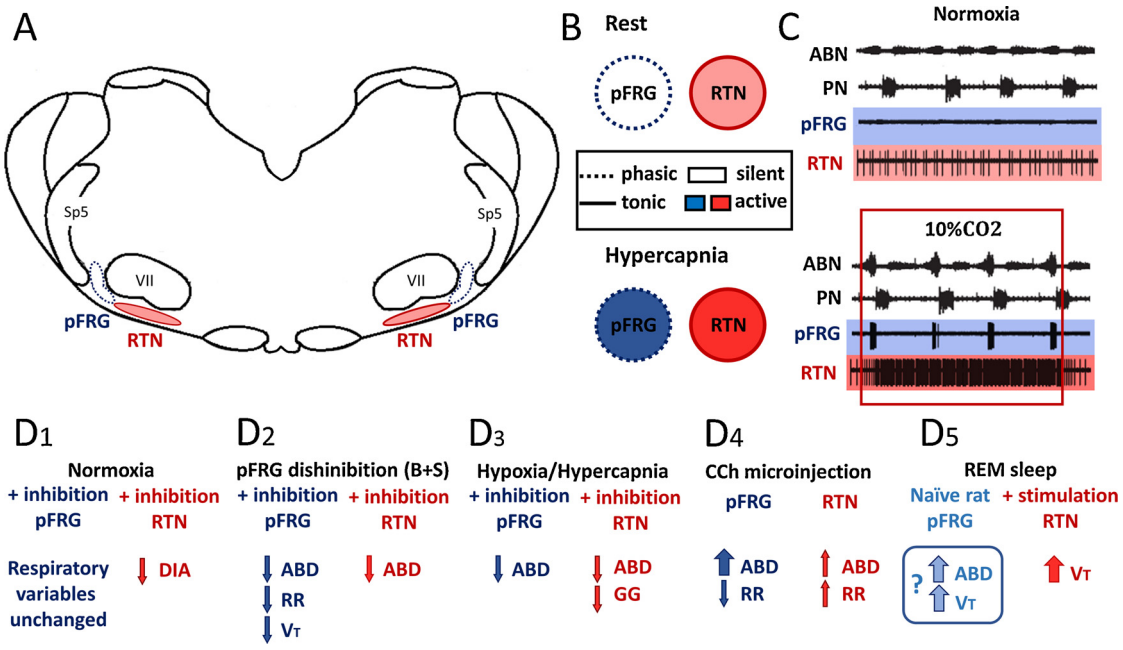

Fig. 5. Experimental evidences supporting the hypothesis that pFRG and RTN are two independent and functionally distinct nuclei. **A**: pFRG phox2B-negative neurons (area circumscribed by blue dotted line) are located ventrolateral to the facial nucleus (VII) and medial to the spinal trigeminal tract (Sp5), whereas RTN chemosensitive phox2B-positive neurons (area circumscribed by red solid line) are located ventromedial to the VII, medial to the pyramidal tract. **B:** Schematic representation of neuronal activity of pFRG and RTN in resting conditions and during hypercapnia. In resting conditions pFRG neurons are silent (white), whereas phox2B-positive RTN neurons are tonically active (light red) (de Britto and Moraes, 2017; Mulkey et al., 2004; Pagliardini et al., 2011; Stornetta et al., 2009). During hypercapnia pFRG neurons are recruited and fire rhythmically during the late expiration (blue). Conversely, RTN neurons fire tonically at a higher rate (red). **C**: Schematic view of the abdominal (ABN) and phrenic nerve (PN) activities as well as pFRG neuron firing during normoxia and hypercapnia (traces modified with permission from de Britto and Moraes, *J Physiol* 595.6 (2017) pp 2043–2064), a representative trace of RTN neuron firing has also been included based on de Britto and Moraes (2017) results. **D:** Changes in respiratory variables (DIA: diaphragm; GG: genioglossus; ABD: abdominal; RR: respiratory rate; V_T: tidal volume) after modulation of either pFRG (blue) or RTN (red) activity under similar experimental conditions. Results reported in a light blue box with a question mark are hypothesized to occur due to modulation of pFRG activity (Pisanski and Pagliardini, unpublished). Direction of the arrows indicates increase (up) or decrease (down) of the activity and the magnitude of change is proportional to the thickness of the arrows. **D1-D3**: Inhibition of pFRG (via expression of DREADD-Gi) and RTN (via expression of Allatostatin-R) during normoxia (**D1**), and during active expiration induced by either application of bicucullin and strychnine (B + S) in pFRG (pFRG dishinibition;**D2**) or hypoxia/hypercapnia (**D3**) produced different effects in various respiratory variables (Huckstepp et al., 2015). **D4**: CCh microinjections into both pFRG and RTN produced the recruitment of ABD muscles. However, the recruitment was more likely to occur when the injection was done into the pFRG and the respiratory rate was affected differently (Boutin et al., 2017). **D5**: RTN photostimulation during REM sleep increases tidal volume but does not recruit active expiration (Burke et al., 2015), however ABD recruitment is present during REM sleep in neonates and adult rats under normoxia (Andrews and Pagliardini, 2015; Saini and Pagliardini, 2017).

projections to the ventral respiratory column and their activation potentiates both inspiratory and expiratory activity (Abbott et al., 2011; Guyenet et al., 2008; Silva et al., 2016). In both juvenile and adult rats RTN/pF_V neurons fire tonically in resting conditions, increase their firing rate with lowered blood pH (Fig. 5 B,C; Mulkey et al., 2004; Stornetta et al., 2009) and acute inactivation of RTN/pF_V neurons silences chemosensitive cells, blunts responses to low pH and reduces both CO2-driven and exercise-driven inspiratory and expiratory output (Abbott et al., 2011, 2009; Huckstepp et al., 2015; Korsak et al., 2018; Marina et al., 2010). On the contrary, late expiratory neurons of pFRG have been identified on the ventral and lateral edge of the facial nucleus (also termed as pF_L by Huckstepp et al., 2015): these neurons are silent in resting conditions and when activated by hypercapnia or chemical disinhibition, they become rhythmically active during late expiration, in phase with abdominal muscle activity (Fig. 5A–C;Abdala et al., 2009; de Britto and Moraes, 2017; Huckstepp et al., 2015, 2016a; Pagliardini et al., 2011). In adult rats, late expiratory neurons do not appear to express the transcription factor Phox2b, but display NK1R immunoreactivity and are likely glutamatergic (de Britto and Moraes, 2017; Huckstepp et al., 2015; Pagliardini et al., 2011).

A clear genetic and physiological distinction between late expiratory pFRG/ pF_L neurons and the ventral chemosensitive RTN/ pF_V neurons regions remains to be established, but additional evidence supporting distinct functionalities between these two regions include: 1) selective hyperpolarization of either pF_V or pF_L induces distinct effects on various respiratory variables in normoxia, and under hypoxic and hypercapnic challenges while producing a similar attenuation of the ABD recruitment elicited by hypercapnia and hypoxia (Fig. $5D_1$–D_3; Huckstepp et al., 2015). 2) Injection of cholinomimetics in pF_L induces a stronger ABD recruitment of expiratory activity and a stronger reduction in respiratory frequency compared to similar injections in the pF_V (Fig. $5D_4$; Boutin et al., 2017). 3) Although tested in slightly different experimental conditions, optostimulation of Phox2b cells in pF_V (Abbott et al., 2011) or neurons located in pFRG/pF_L (Pagliardini et al., 2011) induced distinct respiratory responses when stimulation occurred in the inspiratory phase. When Phox2b RTN cells were stimulated, there were few effects on either respiratory rhythm, tidal volume, or phrenic nerve activity. In contrast, when pFRG/pF_L neurons were stimulated, there was complete inhibition of inspiratory effort and immediate recruitment of ABD activity immediately followed by an inspiratory event. Differences in the respiratory responses and reset plots can be directly observed in Figures 6E and 7 A in Abbott et al., 2011 and compared to Figures 7A and S1 in Pagliardini et al., 2011); 4) Photostimulation of Phox2b-positive RTN neurons in sleeping rats is only capable of producing active expiration during wakefulness (Burke et al., 2015). However, strong ABD recruitment during REM sleep has been reported in both neonates and adult rats (Figs. 1, 2; $5D_5$; Andrews and Pagliardini, 2015; Saini and Pagliardini, 2017) These results suggest

that RTN is likely not responsible for ABD recruitment in REM sleep but other mechanisms could be responsible, possibly including activation of pFRG; 5) While pFRG neurons in adult rats appear to be silent at rest and active during late expiration upon CO2 stimulation or disinhibition, activity of Phox2b-positive RTN chemosensitive neurons has consistently been reported to be tonic through the respiratory phase and not late expiratory modulated (Abdala et al., 2009; de Britto and Moraes, 2017; Marina et al., 2010; Pagliardini et al., 2011).

Based on these evidences, it is conceivable to propose that two distinct populations, located in close proximity to the facial nucleus have two important roles in respiratory control. In adult rodents, Phox2b positive, CO2 sensitive RTN neurons in pF_V control respiratory responses to changes in blood pH and affect both inspiratory and expiratory activity, while pFRG neurons in pF_L act as a conditional oscillator activated directly or indirectly by chemosensitive areas during hypercapnia and are critical for the generation of active expiration. While epF and pFRG neurons express Phox2b in the embryonic and early postnatal period, current evidence suggest that Phox2b expression may be downregulated in adulthood, similar to other cell populations in the sympathetic system (Kang et al., 2007), and therefore they may have a similar genetic origin to RTN neurons but differentiate into a unique neuronal population responsible for generation of active expiration in presence of high metabolic demands (Fig. 5).

6. Conclusions

The interaction between the inspiratory and expiratory oscillators during the early postnatal period has received much attention. However, in adult rodents this relationship and the contributions of the respiratory oscillators to ventilation require further investigation. Specific attention should be directed to whether the pFRG is able to potentiate and sustain respiratory activity when preBötC activity is impaired in unanaesthetized rodents and in sleep disordered breathing models. Simultaneous recordings of unit activity and motor output from each oscillator will be required to fully address this issue.

Recent evidence suggests that pFRG is silent in adult rats due to tonic inhibition. This inhibition may be released to generate active expiration during periods of increased respiratory and metabolic demands, such as hypercapnia. However, the sources of the inhibitory inputs to pFRG remain unknown. Chemosensitive areas could send direct or indirect projections to pFRG through the Kolliker-Fuse nucleus but this connectivity needs to be further studied. It is also unclear whether disinhibition is the only mechanism used to generate active expiration in either physiological or pathological conditions or if there are other mechanisms for the generation of active expiration; for example, through excitatory catecholaminergic or cholinergic projections. Catecholaminergic C1 neurons from the RVLM could be part of the mechanism that recruits ABD muscle activity in response to hypoxia, whereas cholinergic projections from pontine areas could be involved in REM sleep-related ABD recruitment.

Abdominal recruitment during sleep varies depending on the developmental stage, sleep state, and chemosensory status. Whether these variations imply differences in the mechanisms or nuclei involved in the recruitment of active expiration remains to be determined. Data strongly suggest that the parafacial region is responsible for the generation of active expiration, but debate concerning whether the pFRG and RTN are anatomically and functionally distinct is ongoing. However, evidence is accumulating in support of two distinct populations in the parafacial area, Phox2b-expressing tonically-active chemosensitive RTN neurons that affect both inspiratory and expiratory activity, and pFRG Phox2b-negative late expiratory neurons that act as a conditional expiratory oscillator activated by chemosensitive areas during either hypercapnia or hypoxia.

Conflict of interest

None.

Acknowledgements

Research funded by NSERC (SP), WCHRI innovation grant (SP) and CIHR Catalyst Grant on preterm birth (SP). We thank Drs. Funk and Dickson for invaluable comments on the manuscript.

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