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Review

Mechanisms contributing to the genesis of hypoglossal preinspiratory discharge

Michael G. Z. Ghali[1,2,*]

[1] *Department of Neurological Surgery, Baylor College of Medicine, 7200 Cambridge Street, Houston, TX 77030, USA*

[2] *Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 W. Queen Lane, Philadelphia, PA 19129, USA*

*Correspondence: mgzghali@gmail.com (Michael G. Z. Ghali)

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Preinspiratory discharge manifests in the neuronal recordings of the pre-Bötzinger complex, parafacial respiratory group, retrotrapezoid nucleus, and Kölliker-Fuse nucleus, as well as the efferent neural discharge of respiratory-related nerves innervating upper airway musculature. This neural component of triphasic eupnea contemporaneously contributes to the genesis of native and originate respiratory rhythmic activity, as well as the preinspiratory component of efferent neural respiratory discharges. In the course of our investigations evaluating hypoglossal discharge in response to asphyxia, we noted a curious pattern of neural respiratory recovery following postasphyxia resuscitation in hypoglossal, vagal, and phrenic neurograms in unanesthetized decerebrate rats. Specifically, we observed a gradual return of a pseudobiphasic eupnea characterized by initial transition bursts followed by robust eupneic bursts with dynamics inclusive of a gradually and progressively increasing duration of the hypoglossal eupneic bursts and duration and amplitude of the preinspiratory component of these bursts, as well as progressively lengthening expiratory interval between these bursts in the phrenic nerve discharge. This was followed by conversion to regular triphasic eupnea. We discuss our extrapolations based on these findings regarding eupneic respiratory central pattern generation and mechanisms contributing to the genesis of preinspiratory activity in hypoglossal discharge.

Keywords

Genesis; eupnea; hypoglossal; breathing; respiratory; central respiratory; rhythm generation mechanisms; pattern formation

1. Introduction

Triphasic eupnea consists of inspiratory, postinspiratory, and late expiratory neural respiratory activities (Marchenko et al., 2016; Richter, 1982; Richter et al., 1986; Smith et al., 2007), generated by the interaction of rhythm generating circuitry located within the Bötzinger and pre-Bötzinger complexes with elements in the dorsolateral metencephalon, including Kölliker-Fuse and medial parabrachial nuclei (Dutschmann and Herbert, 2006; Molkov et al., 2017; Mörschel and Dutschmann, 2009), thence relayed to the pattern generating elements of the rostral and caudal divisions of the ventral respiratory group (Figs. 1 and 2) (Anderson and Ramirez, 2017; Bonis et al., 2010a,b; Cui et al., 2016; Ramirez and Baertsch, 2018; Richter and Smith, 2014; Smith et al., 1991, 2007). Preinspiratory discharge occurs during the late expiratory phase and immediately precedes and drives inspiratory onset (Fortuna et al., 2008; Fregosi and Fuller, 1997; Ghali, 2015; Ghali and Marchenko, 2016; Lee et al., 2003, 2006, 2007a,b, 2008, 2012; Lee and Fuller, 2010a,b; Lee and Pisarri, 2001; Leiter and St-John, 2004; Molkov et al., 2017), evident in the neuronal recordings of the pre-Bötzinger complex (Malheiros-Lima et al., 2018; Marchenko et al., 2016) and parafacial respiratory group intrinsic bursting cells (Onimaru et al., 2008), Kölliker-Fuse nucleus (Kobayashi et al., 2005), as well as the efferent neural discharge of hypoglossal, vagal, and upper airway related nerve activity (Ghali, 2015; Ghali and Marchenko, 2016; Lee and Fuller, 2010a).

Hypoglossal nerve and motoneurons, as well as innervated glossal muscles, thus evidence discharge consisting of preinspiratory and inspiratory components (Fig. 3-5) (Fortuna et al., 2008; Fregosi and Fuller, 1997; Ghali, 2015; Ghali and Marchenko, 2016; Lee et al., 2003, 2006, 2007a,b, 2008, 2012; Lee and Fuller, 2010a,b; Lee and Pisarri, 2001; Leiter and St-John, 2004; Molkov et al., 2017), whereas the discharge of phrenic nerve, innervating diaphragm, consists of inspiratory and occasionally postinspiratory components (Berger, 1979; Christakos et al., 1991; Dittler and Garten, 1912; Ghali, 2015, 2018; Ghali and Marchenko, 2013, 2016; Marchenko and Rogers, 2006a,b, 2007, 2009; Marchenko et al., 2012, 2015; Rijlant, 1937; Richardson and Mitchell, 1982). The genesis of the hypoglossal discharge may alternately originate from separate volleys of incoming premotoneuronal preinspiratory and inspiratory inputs to different hypoglossal motoneurons or the contemporaneous inputs of preinspiratory inspiratory phase spanning and inspiratory premotoneuronal discharge to hypoglossal motoneurons with differential excitability (Ghali, 2015; Lee and Fuller, 2010a; Marchenko et al., 2016).

We present evidence demonstrating the gradual recovery of neural respiratory discharge in the hypoglossal, vagal, and phrenic motor outputs reflect gradual recovery of inhibitory network elements in ventral respiratory column nuclei in response to as-

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phyxia in the unanesthetized decerebrate preparation of the adult rat (Fig. 6). These findings thus evidence a curious, yet significantly informative set of hypoglossal, vagal, and phrenic neurogram responses to asphyxia and subsequent resuscitation, involving the gradual return of pseudobiphasic eupnea, clearly segmented in the phrenic motor output, though less apparently so in the hypoglossal discharge. We will thus present a discussion on the traditional understanding of the genesis of preinspiratory and inspiratory components of the hypoglossal discharge (Ghali, 2015; Ghali and Marchenko, 2016; Molkov et al., 2017), in order to contextualize our extrapolations based on our findings. Our results thus naturally give rise and lead to powerful conjectures and hypotheses illuminating mechanisms contributing to respiratory rhythm generation and pattern formation requiring empirical validation evaluating the dynamics of central neuronal and efferent neural respiratory-related discharge, as well as inhibitory network circuitry, in response to asphyxia and hypoxia, as well as its recovery from the same. We discuss several alternative hypotheses that could exist to explain these observations and present our conjectures.

2. Respiratory rhythm generation and pattern formation

Respiratory rhythm generation and pattern formation derive from the coordinately and highly synchronized activities of propriobulbar interneurons with feedforward excitatory and feedback inhibitory synaptic interactions between the Bötzinger and pre-Bötzinger complexes, supported by tonic and phasic excitatory inputs from Kölliker-Fuse, medial parabrachial, and retrotrapezoid nuclei, and thence contemporaneously relayed to pattern generating elements of the rostral and caudal divisions of the ventral respiratory group as well as the dorsal respiratory group (Figs. 1 and 2) (Anderson and Ramirez, 2017; Cui et al., 2016; Marchenko et al., 2016; Ramirez and Baertsch, 2018; Richter and Smith, 2014; Smith et al., 1991, 2007). These interactions ultimately generate triphasic eupnea, with segmentation of the neural respiratory output into inspiratory, postinspiratory and late expiratory discharges, as originally described by Richter and colleagues (Richter, 1982; Richter et al., 1986) and later demonstrated and precisely delineated by the serial transection studies of Smith et al. (2007). Such network mechanisms generating the eupneic rhythm and pattern critically require the functionality of inhibitory network elements as demonstrated by Marchenko and colleagues (Marchenko et al., 2016).

3. Pre-Bötzinger complex and parafacial respiratory group preinspiratory discharge

Ventral respiratory column nuclei and efferent neural respiratory discharge evidence preinspiratory component of neural activity (Fig. 2) (Ghali and Marchenko, 2016; Marchenko et al., 2016; Malheiros-Lima et al., 2018; Onimaru et al., 2008). Preinspiratory intrinsic bursting units in pre-Bötzinger complex (Malheiros-Lima et al., 2018; Thoby Brisson and Ramirez, 2000), described as the kernel for inspiratory rhythm generation, drive and generate the principal respiratory rhythm, as initially demonstrated by serial transection studies in the in vitro preparation of the neonatal rat by Smith and colleagues (Smith et al., 1991). Onimaru and colleagues (Onimaru et al., 1988, 1993, 2006; Onimaru and Homma, 2003) subsequently demonstrated preinsipratory bursting and biphasic discharging units in the parafacial respiratory group and retrotrapezoid nucleus zone. They posited this contributes to and drives the genesis of the pre-Bötzinger complex preinspiratory activity, while others proposed this activity also mediates genesis of hypercapnia induced abdominal expiratory neural activity (Janczewski and Feldman, 2006), as demonstrated empirically by Zoccal, Molkov and colleagues (Molkov et al., 2010, 2011; Moraes et al., 2012; Zoccal et al., 2007, 2008, 2009a,b, 2018). Hypercapnia induced late expiratory activity in abdominal nerve discharge is synchronized with sympathetic activity and parafacial respiratory group neuronal discharge, thus contributing importantly to genesis of sympathorespiratory coupling in response to hypercapnia, acute hypoxia, and chronic intermittent hypoxia (Molkov et al., 2010, 2011; Moraes et al., 2012; Zoccal et al., 2007, 2008, 2009a,b, 2018). Preinspiratory discharge is also evidenced in the central neuronal recordings of units from the Kölliker-Fuse nucleus (Kobayashi et al., 2005), which project to and provide tonic and phasic excitatory and inhibitory projections to various nuclei throughout the respiratory neuraxis (Dutschmann et al., 2007; Song et al., 2012; Yokota et al., 2007).

4. Hypoglossal discharge

Efferent neural respiratory discharge variably demonstrates a preinspiratory component of activity, most notable in hypoglossal, vagus, glossopharyngeal, facial, and trigeminal neurograms, though absent in phrenic and intercostal discharges (Fig. 3) (Ghali, 2015; Ghali and Marchenko, 2016; Lee et al., 2003, 2007a,b, 2008; Lee and Fuller, 2010a; Leiter and St-John, 2004). In the hypoglossal discharge, under vagus intact conditions, the preinspiratory discharge is absent or of significantly brief duration (Ghali, 2015; Ghali and Marchenko, 2016), though in the vagotomized condition, as shown in the unanesthetized preparation of the decerebrate rat, the preinspiratory component of hypoglossal discharge is disinhibited, becoming quite robust (Fig. 3) (Ghali and Marchenko, 2016), with marked augmentation in response to hypercapnic challenge via administration of carbon dioxide enriched hyperoxia. This evidences peripheral modulation, specifically, tonic inhibition by vagal afferents (most likely via tonic C fibers, see Ghali and Marchenko, 2016 for discussion), of a naturally centrally generated preinspiratory component of the hypoglossal discharge. The origins for the genesis of this preinspiratory component, as well as mechanisms contributing to vagal modulation of the same, remain the subject of debate (Ghali, 2015; Ghali and Marchenko, 2016), and are potentially informed by the presently reported findings.

5. Generative mechanisms of the differential activities of hypoglossal motoneurons

Hypoglossal discharge is comprised of preinspiratory and inspiratory components (Fig. 3) (Ghali, 2015; Ghali and Marchenko, 2016; Lee et al., 2003, 2007a,b, 2008; Lee and Fuller, 2010a; Leiter and St-John, 2004). A significant body of evidence and interpretations deriving therefrom support the conjecture hypoglossal preinspiratory and inspiratory components of discharge may be differentially generated activities (Ghali and Marchenko, 2016). Hypoglossal motoneurons generally exhibit two distinct patterns of

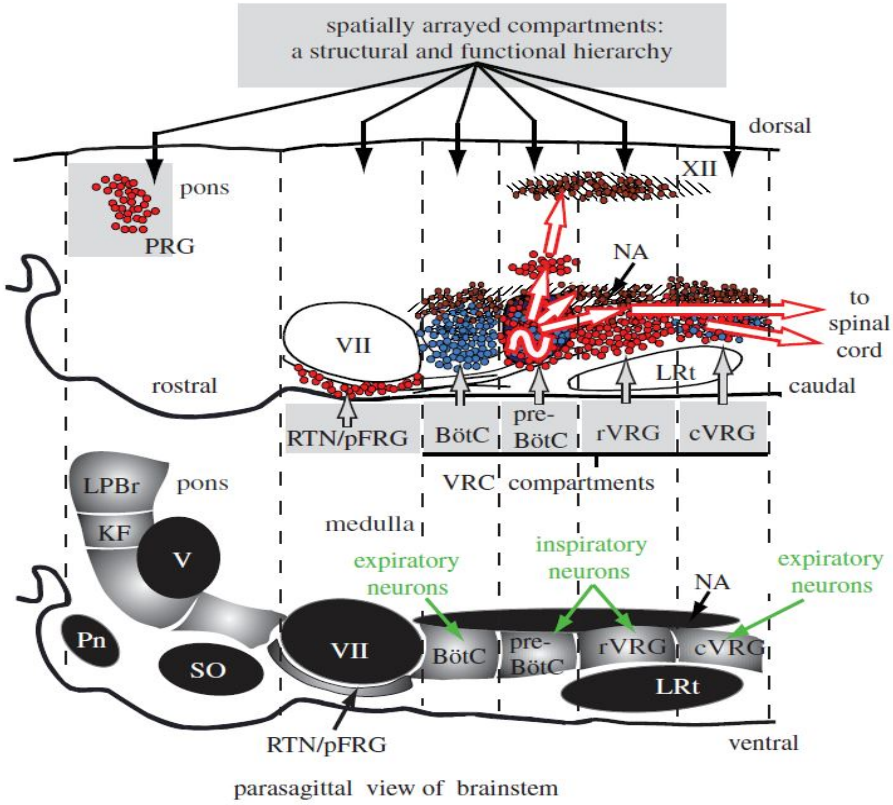

Figure 1. Schematic representation of the mammalian brainstem respiratory network architecture. Interaction between the Bötzinger and pre-Bötzinger complex generates the respiratory rhythm relayed to the caudally related pattern generator in the ventral respiratory group, divided into rostral and caudal divisions, supplying premotoneuronal inputs to inspiratory and expiratory motoneurons, respectively. The rostrally related retrotrapezoid nucleus provides tonic excitatory inputs to all neuronal types within the ventral respiratory column nuclei, whereas an overlapping group of biphasic bursting cells with preinspiratory discharge may serve as an oscillator driving the discharge of pre-Bötzinger complex preinspiratory bursting units. The Kölliker-Fuse and medial parabrachial nuclei provide tonic excitatory inputs to the Bötzinger complex postinspiratory glycinergic neurons, which themselves critically shape triphasic eupnea by providing inspiratory off switching. Sources of premotoneuronal input to the hypoglossal motor nucleus could putatively include the pre-Bötzinger complex, Kölliker-Fuse, and medial parabrachial nuclei, as well as the retrotrapezoid nucleus/parafacial respiratory group complexes. BötC, Bötzinger complex; pre-Bötzinger complex; rVRG, rostral ventral respiratory group; cVRG, caudal ventral respiratory group; LRt, lateral reticular nucleus; LPBr, lateral parabrachial nucleus; KF, Kölliker-Fuse nucleus; PRG, pontine respiratory group; V, trigeminal nucleus; VII, facial nucleus, XII, hypoglossal motor nucleus; Pn, ventral pontine nucleus; SO, superior olive; LRt, lateral reticular nucleus; NA, nucleus ambiguus. Modified with permission from Smith et al. (2007).

discharge, those with preinspiratory inspiratory phase spanning activity and those with inspiratory discharge (Fig. 4) (Lee and Fuller, 2010a), with differential spectral composition of the preinspiratory and inspiratory components further evidencing differential genesis (Leiter and St-John, 2004). We have noted in our experiments performed in an unanesthetized decerebrate rat preparation a significantly higher frequency of the high-frequency spectral band of the fast synchronous oscillatory dynamics of the preinspiratory compared to the inspiratory component of hypoglossal discharge. We discuss the possible theoretical mechanisms for genesis of these heterogeneous patterns of hypoglossal motoneuronal activity.

The genesis of preinspiratory and inspiratory components of the hypoglossal discharge could originate from incoming volleys alternately relaying preinspiratory and inspiratory premotoneuronal inputs (Fig. 5) (Ghali, 2015; Ghali and Marchenko, 2016; Lee and Fuller, 2010a). In such a model, hypoglossal motoneurons with inspiratory discharge thus reflect the summed activity of incoming premotoneuronal inspiratory volleys, whereas neurons with preinspiratory and inspiratory phase spanning discharge receive convergent inputs from separate premotoneuronal preinspiratory and inspiratory generators (Ghali, 2015; Ghali and Marchenko, 2016; Lee and Fuller, 2010a). Genesis of heterogeneous discharge patterns in the population of hypoglossal motoneurons demonstrating preinspiratory inspiratory phase spanning, as well as inspiratory discharge, could result from common premotoneuronal inputs with differential excitability of motoneurons, differential premotoneuronal inputs, or a combination thereof (Ghali, 2015; Ghali and Marchenko, 2016; Lee and Fuller, 2010a). The population of hypoglossal motoneurons could differentially receive input from either preinspiratory inspiratory phase spanning or inspiratory cells to generate this heterogeneity. Al-

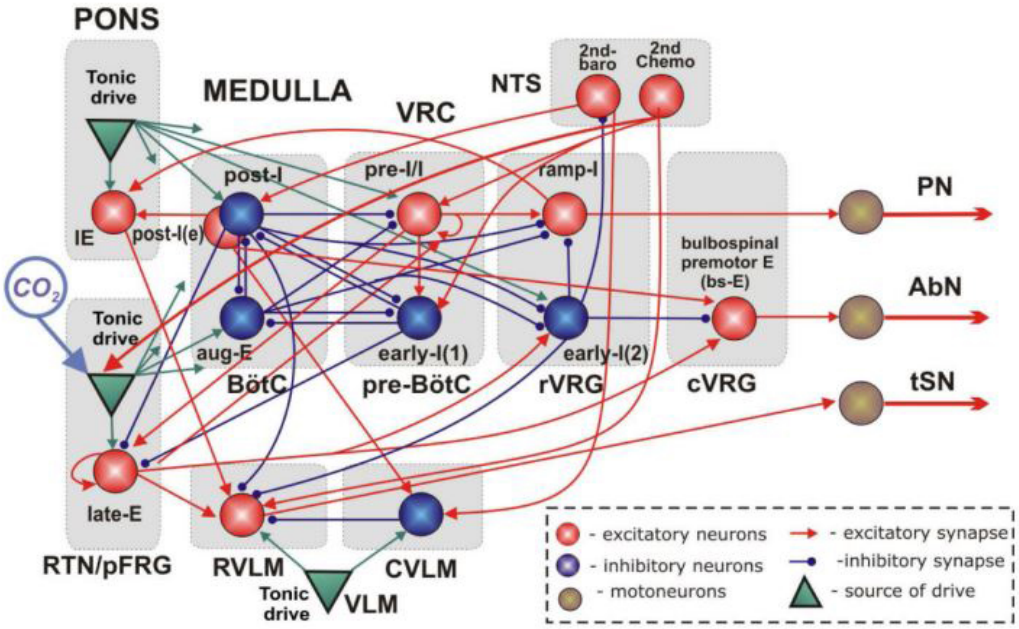

Figure 2. Brainstem respiratory circuit. The retrotrapezoid nucleus contains tonic excitatory units exquisitely sensitive to carbon dioxide and extracellular hydrogen ion concentrations, with variable respiratory modulation providing tonic excitatory drive to all respiratory nuclei, as well as late expiratory units, which receive tonic excitation from the former and supply excitation to rostral ventrolateral medulla, early inspiratory units in rostral division of ventral respiratory group and expiratory premotoneurons in caudal division of the ventral respiratory group. The parafacial respiratory group contains biphasically discharging units that may provide the excitatory drive to pre-Bötzinger complex preinspiratory neurons and serve as a respiratory oscillator. The Bötzinger complex contains decrementing glycinergic postinspiratory neurons and augmenting GABAergic late expiratory interneurons which modulate the discharge patterns of preinspiratory inspiratory phase spanning, and inhibitory decrementing early inspiratory pre-Bötzinger neurons. These provide inhibition to preinspiratory inspiratory phase spanning and early inspiratory units in the pre-Bötzinger complex as well as reciprocal inhibition. A population of Bötzinger complex excitatory postinspiratory units contributes prominently to driving a caudal ventral respiratory group of expiratory premotoneurons and the discharge of caudal ventrolateral medullary neurons. Preinspiratory inspiratory phase spanning units in the pre- Bötzinger complex project to, and drive, rostral ventral respiratory group augmenting inspiratory neurons. Decrementing early inspiratory neurons contribute prominently to shape the augmenting pattern of rostral ventral respiratory group inspiratory discharge by inhibiting units during the early component of the inspiratory epoch. Pre-Bötzinger complex preinspiratory inspiratory phase spanning units provide recurrent tonic excitation and excite inhibitory decrementing early inspiratory units, as well as late expiratory units in retrotrapezoid nucleus and parafacial respiratory group. The rostral ventral respiratory group contains augmenting inspiratory premotoneurons driving the discharge of bulbar and spinal inspiratory motoneurons, as well as early inspiratory neurons. This provides inhibition of augmenting inspiratory premotoneuronal and caudal ventral respiratory group expiratory premotoneurons, as well as inhibition of rostral ventrolateral medullary presympathetic units. Nucleus tractus solitarius baroreceptor units project to, and drive, the discharge of Bötzinger complex inhibitory decrementing postinspiratory neurons and caudal ventrolateral medullary propriobulbar inhibitory interneurons negatively modulating the discharge of rostral ventrolateral medullary neurons. Nucleus tractus solitarius chemoreceptor units drive tonic excitatory units in the retrotrapezoid nucleus and parafacial respiratory group, pre-Bötzinger complex preinspiratory inspiratory phase spanning and decrementing early inspiratory neurons, and rostral ventrolateral medullary presymapthetic neurons. The respiratory rhythm generated by the Bötzinger and pre-Bötzinger complex and modulated and shaped by rostrally related structures is thence relayed caudally to rostral and caudal divisions of the ventral respiratory group. The former supply descending bulbospinal premotoneuronal inputs to inspiratory, and the latter supplying premotoneuronal inputs to expiratory, bulbar and spinal motoneuronal nuclei. Rostral ventrolateral medulla supplies premotoneurons to preganglionic sympathetic neurons in the intermediolateral cell column and receives GABAergic inhibition from propriobulbar interneurons in the caudal ventrolateral medulla. Rostral and caudal ventrolateral medulla receive tonic excitation from propriobulbar interneurons diffusely distributed throughout ventrolateral medulla, as well as the retrotrapezoid nucleus, mediating the sympathetic chemoreflex. RTN/pFRG, retrotrapezoid nucleus/ parafacial respiratory group; BotC, Bötzinger complex; pre-BotC, preBötzinger complex; rVRG, rostral ventral respiratory group; cVRG, caudal ventral respiratory group; VRC, ventral respiratory column; NTS, nucleus tractus solitarius; RVLM, rostral ventrolateral medulla; CVLM, caudal ventrolateral medulla; IE, inspiratory expiratory; late-E, late expiratory neurons; post-I, postinspiratory neurons; aug-E, augmenting expiratory neurons; early-I (1), pre-Bötzinger complex early inspiratory neurons; pre-I/I, preinspiratory inspiratory phase spanning neurons; early-I (2), rostral ventral respiratory group early inspiratory neurons; ramp-I, ramp or augmenting inspiratory neurons; bs-E, bulbospinal expiratory neurons; PN, phrenic nerve; AbN, abdominal nerve; tSN, thoracic sympathetic nerve. Modified with permission from Molkov et al. (2017).

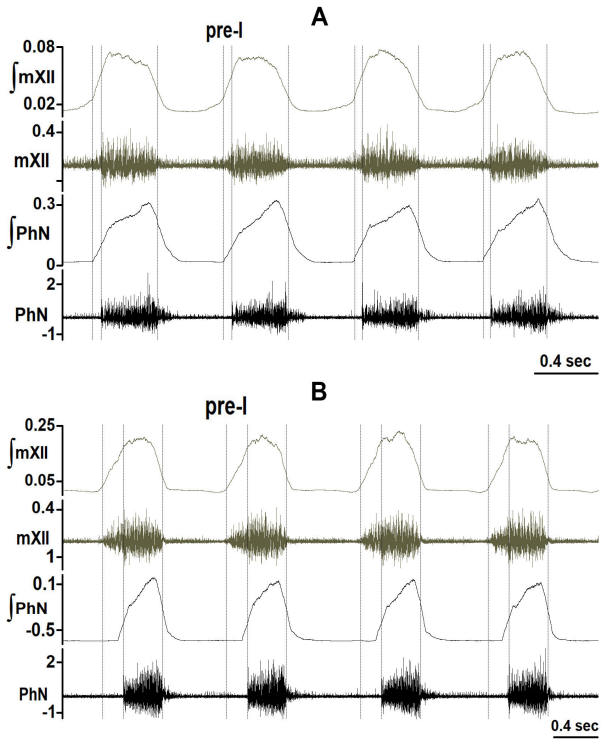

Figure 3. Responses of XII pre-I activity to hypercapnia in vagotomized animals. A: control (100% O_2). B: 5% hypercapnia (5% CO_2, 95% O_2). Integrated (τ = 0.05 s) and raw XII and PhN traces. Vertical dotted lines indicate XII onset, PhN onset, and PhN/XII inspiratory offset. The epoch between XII and PhN onset represents pre-I activity, and that between PhN onset and PhN/XII I offset represents inspiratory bursting. mXII, medial branch of XII; PhN, phrenic nerve. Timescale bar is shown in the lower right-hand corner in each panel. Neurogram amplitude shown in volts.

ternatively, hypoglossal motoneurons may all receive inspiratory premotoneuronal inputs with a group of cells also receiving preinspiratory discharge. As a possibility, all hypoglossal motoneurons could receive preinspiratory inspiratory phase spanning units with active inhibition of the preinspiratory component in some of the motoneurons. GABAergic bulbophrenic projections from the Kölliker-Fuse nucleus and Bötzinger complex inhibit phrenic motoneurons (Song et al., 2000) and similar projections to hypoglossal motoneurons could inhibit the preinspiratory component in some of these cells. Hypoglossal motoneurons do not receive inhibition from the Bötzinger complex, though they could receive Kölliker-Fuse nucleus projections inhibiting the preinspiratory component (Peever et al., 2001).

Alternatively, a common preinspiratory inspiratory premotoneuronal source may project diffusely to hypoglossal motoneurons, with the ultimate pattern manifest in the motoneuronal discharge resulting from differential excitability of motoneurons (Fig. 5) (Ghali, 2015; Ghali and Marchenko, 2016; Lee and Fuller, 2010a). Units with higher threshold and lower membrane resistance will exhibit the inspiratory motoneuronal component, whereas those with a lower threshold and higher membrane resistance will evidence the preinspiratory and inspiratory components (Lee and Fuller, 2010a). Hypoglossal motoneurons may also contemporaneously exhibit differential excitability, as well as receive differential preinspiratory, preinspiratory inspiratory phase spanning, and inspiratory inputs. Notably, the discharge patterns of the units generating the hypoglossal motor output include either preinspiratory inspiratory phase spanning or inspiratory activity, though units with intermediate discharge onsets are not described; hypoglossal motoneuronal onset occurs either commensurate with onset of the preinspiratory component of the population activity, concurrent with the onset of phrenic inspiration, or within the inspiratory epoch; units with onsets during mid-preinspiratory discharge are not observed (Lee and Fuller, 2010a). This somewhat argues against differential motoneuronal excitability as the exclusive mechanism for genesis of differential patterns of discharge in hypoglossal preinspiratory inspiratory phase spanning units, though may contribute more significantly to genesis of inspiratory motoneuronal activity with variable onsets of discharge within the inspiratory epoch and supports the presence of differential inputs from premotoneuronal and motoneuronal sources as principally contributory (Ghali, 2015; Ghali and Marchenko, 2016; Lee and Fuller, 2010a).

6. Genesis nuclei for preinspiratory discharge in hypoglossal motoneurons

Several respiratory oscillators could drive the principal inspiratory and expiratory rhythmic activities, as well as contribute to preinspiratory genesis in hypoglossal motor outputs (Anderson et al., 2016; Anderson and Ramirez, 2017; Janczewski and Feldman, 2006; Ramirez and Baertsch, 2018; Smith et al., 1991). Putative sources driving the preinspriatory discharge in hypoglossal motor output include the pre-Bötzinger complex (Sun et al., 1998), retrotrapezoid nucleus and parafacial respiratory group zone (Onimaru et al., 2006), postinspiratory complex (Anderson et al., 2016; Anderson and Ramirez, 2017), and Kölliker-Fuse nucleus (Dutschmann et al., 2007; Kobayashi et al., 2005). Since hypoglossal preinspiratory activity is eliminated by pontomedullary transection (Fig. 7) (Abdala et al., 2009) and amplified by orexin B microinjections in dorsolateral metencephalic tegmentum (Dutschmann et al., 2007), Kölliker-Fuse nucleus or parafacial respiratory group are logical premotoneuronal sources potentially generating this component of the hypoglossal discharge (Ghali, 2015; Ghali and Marchenko, 2016).

Evidence strongly suggests the retrotrapezoid nucleus parafacial respiratory group zone as contributory to the genesis of preinspiratory activity of hypoglossal motoneurons (Connelly et al., 1990; Janczewski et al., 2002; Onimaru et al., 2006; Onimaru and Homma, 2003), though remains somewhat conjectural and requires further studies in order to to more thoroughly elucidate the mechanistic basis for the same. Opioids were shown to inhibit C4 rootlet discharge, while sparing preinspiratory discharge in the parafacial respiratory group, facial nucleus, and facial nerve in the brainstem spinal cord preparation of the newborn rat, suggesting genesis of differential respiratory rhythms and preinspiratory discharge by multiply distributed oscillators (Onimaru et al., 2006). C fiber stimulation utilizing phenylbiguanidine in urethane anesthetized mice effected complete inhibition of inspiratory neuronal discharge, including hypoglossal activity via activation

of postinspiratory neurons (Paton, 1997). However, rhythmic discharge of preinspiratory and postinspiratory units continued their normal discharge, evidencing an alternate source of preinspiratory premotoneuronal inputs to the hypoglossal nucleus.

Pontomedullary transection-mediated loss of the preinspiratory component of hypoglossal nerve discharge could alternatively indicate either a premotoneuronal source of preinspiratory discharge located rostral to the pontomedullary border or the necessity of intact network connectivity and inhibitory elements for triphasic eupneic segmentation and provision of tonic excitatory inputs to a preinspiratory premotoneuroanl source located within the medulla (Dutschmann and Herbert, 2006; Molkov et al., 2017; Mörschel and Dutschmann, 2009; Smith et al., 2007). Tonic excitatory inputs to Bötzinger complex glycinergic postinspiratory neurons supplied by the Kölliker-Fuse and medial parabrachial nuclei and Bötzinger complex GABAergic augmenting late expiratory neurons from retrotrapezoid nucleus may prove necessary for consequent expiratory segmentation and genesis of preinspiratory discharge (Dutschmann and Herbert, 2006; Mörschel and Dutschmann, 2009; Smith et al., 2007).

The Kölliker-Fuse nucleus is thus aptly positioned to contribute to genesis and modulation of discharge of hypoglossal motor output and generation of preinspiratory activity (Dutschmann et al., 2007; Ezure and Tanaka, 2006; Gestreau et al., 2005; Ghali and Marchenko, 2016). In this regard, preinspiratory inspiratory phase spanning units in Kölliker-Fuse nucleus project to the hypoglossal nucleus in pentobarbital anesthetized rats (Ezure and Tanaka, 2006). Glutamate chemical stimulation of the Kölliker-Fuse nucleus induces discharge in hypoglossal nerve in pentobarbital anesthetized rats (Ezure and Tanaka, 2006) and Orexin B microinjections in Kölliker-Fuse nucleus specifically effect increases in preinspiratory, though not inspiratory, activity in hypoglossal neurograms, evidencing differential modulation and neurogenesis of these components of the rhythmic hypoglossal discharge in the in situ brainstem spinal cord preparation of the arterially-perfused juvenile unanesthetized decerebrate rat (Mörschel and Dutschmann, 2009). Hypercapnia and hypoxia effect inhibition of hypothalamic orexinergic inputs to Kölliker-Fuse nucleus, which drive the preinspiratory activity in hypoglossal motoneurons (Dutschmann et al., 2007), suggesting complex effects of hypercapnia and hypoxia in mediating respiratory rhythm and pattern, involving effects on peripheral and central chemoreceptors, activation of biphasic ventilatory response, recruiting mesencephalic mechanisms, as well as exertion of direct effects on tissue lactate and anoxic depolarization of neurons.

7. Pseudobiphasic postresuscitative neural respiratory discharge

In our investigations evaluating responses observed in respiratory-related neurograms to variable extents of hypoxia, we noted an initial hyperpneic response in hypoglossal, vagal, and phrenic nerve discharges, followed by apnea, transition bursts, and gasping in the unanesthetized decerebrate preparation of the adult rat (Fig. 6). Further, large increases in hypoglossal and vagal preinspiratory activity following asphyxia were noted, as also demonstrated during hypoxia by Lee and Fuller (2010a) and during hypercapnia by Ghali and Marchenko (2016). Following oxygen resuscitation, on inspection of the postresuscitative

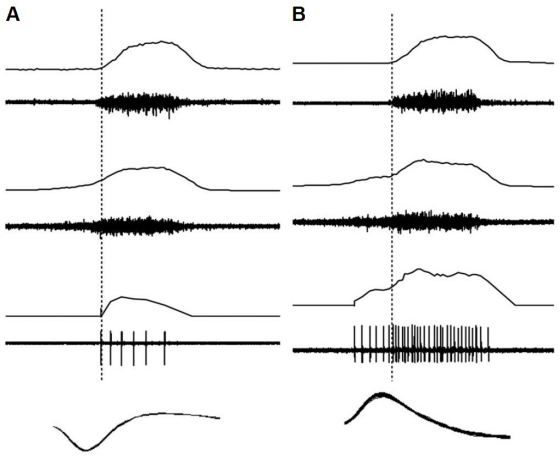

Figure 4. Hypoglossal motoneurons. A: Inspiratory hypoglossal motoneurons. B: Preinspiratory inspiratory hypoglossal motoneurons. The top pair of panels illustrate integrated, and raw phrenic nerve discharge, subsequent couple of panels illustrate integrated, and raw hypoglossal discharge, next couple of panels illustrate raw hypoglossal motoneuronal action potential discharge and integrated frequency of unit activity – [Modified with permission from Lee and Fuller (2010a)].

hypoglossal and phrenic neurograms, a most interesting pattern is observed of brief duration transition bursts followed by normal duration eupneic bursts (Fig. 6). During the earliest phases of recovery, the initial brief duration burst was more obvious in the phrenic neurogram, compared to the hypoglossal and vagal nerve discharges. The hypoglossal discharge eupneic burst was preinspiratory inspiratory phase spanning, without an expiratory period between this and the preceding transition burst. In the hypoglossal discharge, the transition burst was thus immediately followed by a eupneic burst comprised of preinspiratory and inspiratory components without an intervening expiratory neural component. Alternatively, for the phrenic discharge, a brief duration eupneic burst temporally synchronized to the same as the hypoglossal discharge was followed by an expiratory pause, with the genesis of an inspiratory eupneic burst contemporaneous with the inspiratory component of the greater duration hypoglossal eupneic burst.

Gradually, with recovery, there was a progressive increase in the duration of the transition and eupneic bursts, expiratory neural activity interposed between the initial brief transition bursts and greater duration eupneic bursts, and hypoglossal preinspiratory component of the greater duration burst, consistent with the gradual recovery of the eupneic pattern generator. The pseudobiphasic eupneic discharge was initially regular, transitioning into an increasing duration of expiratory duration interposed between the bursting discharges, with the inspiratory discharge becoming synchronized across hypoglossal, vagal, and phrenic respiratory neural outputs, with the preinspiratory component of hypoglossal discharge disappearing.

Return of a pseudobiphasic pattern of respiration could alternatively support a model of either genesis of disparate activities or the genesis of a unitary activity interrupted by some mechanism of expiratory inhibition. In general, we favor the former hypoth-

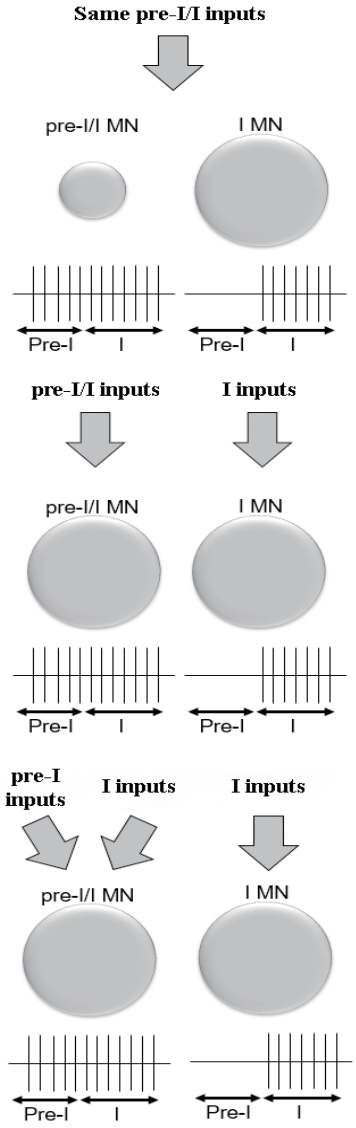

Figure 5. The putative mechanism underlying central control of pre-I and I XII motoneuronal bursting. Upper panel: XII MNs receive the same input drives, and the different bursting type (pre-I/I vs. I) reflects differential intrinsic properties (e.g., cell size, membrane resistance) of the output motoneurons. Middle panel: pre-I and I XII MN receive differential synaptic inputs. Lower panel: pre-I/I XII MN receive distinct simultaneous pre-I and I synaptic drives, while I XII MNs receive only I synaptic input.

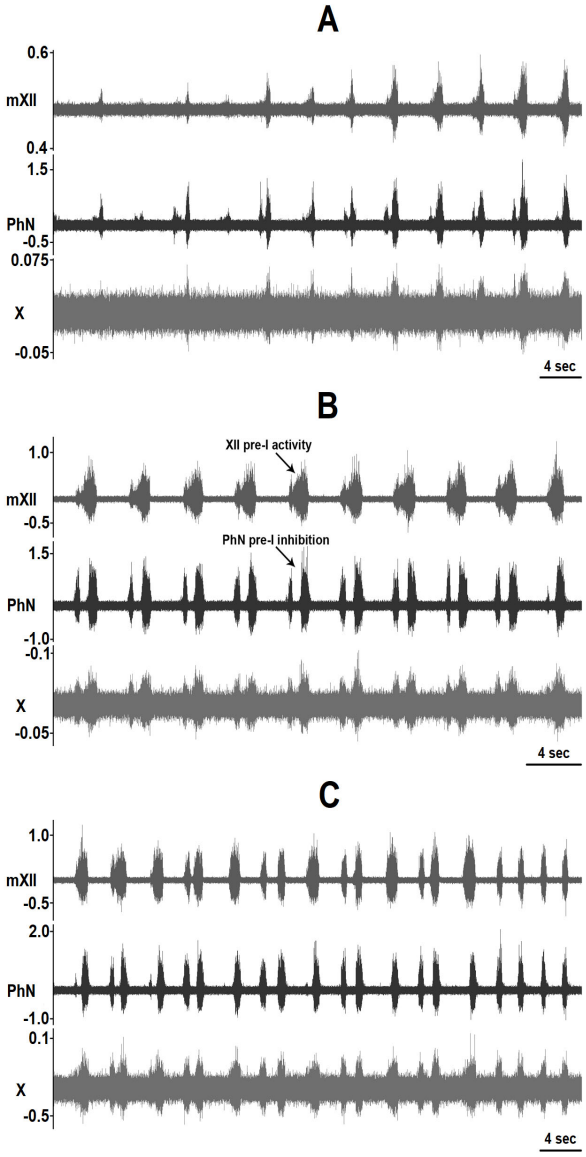

Figure 6. Post-asphyxia resuscitation. A-C: Sequential phrenic and XII activities during resuscitation following asphyxia. In panel B note biphasic inspiratory bursting in XII and PhN with interposed pre-I bursting in XII and inhibition in PhN discharge. mXII, medial branch of hypoglossal nerve; PhN, phrenic nerve; X, vagus nerve.

esis. If one closely examines panel B, a brief duration transition burst evident in and manifest between phrenic and hypoglossal discharges is followed by a normal duration preinspiratory inspiratory phase spanning hypoglossal eupneic burst and a phrenic expiratory pause and inspiratory eupneic burst synchronized with the inspiratory component of the greater duration hypoglossal eupneic burst. This pattern could reflect the gradual reconfiguration of the eupneic pattern generator (Lieske et al., 2000; Rybak et al., 2008) with the incorporation of inhibitory network elements contemporaneous with oxygen restoration. Conversely, asphyxia causes a gradual reduction of inhibitory network elements affecting reconfiguration of the respiratory, central pattern generator with transition from triphasic eupnea, to loss of expiratory segmentation, and development of monophasic bursting with inspiratory discharge synchronized across central neuronal and efferent neural discharge, as schematically depicted by Ramirez and Lieske (2003), though the complexity of excitatory and inhibitory connections amongst respiratory neuronal types is much greater during normal breathing, as characterized by Molkov et al. (2017) (Fig. 2).

The classic gasping sequence following oxygen deprivation involves hyperpnea, followed by apnea, the appearance of transition bursts, and then finally monophasic decrementing brief duration gasps (Ramirez and Lieske, 2003; Paton and St-John, 2005; Richter, 2003; St-John and Knuth, 1981; St-John et al., 1990; St-

John, 1996; St-John and Paton, 2003; St-John and Leiter, 2009), with loss of the high frequency component of highly correlated synchronous activity in the spectra of respiratory related neural discharges (Ghali and Marchenko, 2016). With the restoration of oxygen, the gasping sequence inverts and the pattern occurs in reverse en route to recovery of normal eupnea. Apnea resulting from oxymetabolic exhaustion of the gasp forming mechanisms is followed by restoration of transition bursts alternating with normal eupneic bursts. In this model, transition bursts are generated by the eupneic generator having recovered the functionality of some proportion of the inhibitory network elements, alternating with robust eupnea, generated by the network incorporating the full complement of inhibitory network elements.

Thus, we assert, the pseudobiphasic discharge evidences the eupneic generator operating in alternate modes according to the fidelity and proportion of inhibitory network elements actively involved and recruited to generate the respiratory rhythm and pattern. Studying these patterns and dissecting how these activities compete or interfere with each other as well as the study of alternative modes of the eupnea generator according to oxygen availability, inform mechanisms of both central pattern generation, and the differential genesis of preinspiratory and inspiratory components of respiratory-related neural discharge. The pattern of postresuscitative recovery supports a model whereby oxygen is critically required for support of metabolism and fast neurotransmission of inhibitory elements needed to generate robust eupnea. In conditions of severe hypoxia or anoxia, decrementing monophasic bursts or brief duration transition bursts without expiratory neural discharge (and thus no preinspiratory activity) become evident, while with moderate levels of hypoxia, brief duration bursts, neither decrementing nor augmenting, are generated in neural respiratory discharges.

With gradual reversal of hypoxia and restoration of normal oxygen levels, inhibitory elements of the eupneic pattern generator gradually appear, thus generating both transition and normal duration eupneic bursts and hypoglossal discharge with a preinspiratory component. Gradually increasing duration of the expiratory period interposed between the brief transition and greater duration eupneic burst, as well as increased duration and amplitude of the hypoglossal preinspiratory component of the greater duration eupneic burst, could result from increasing oxygen supply of inhibitory postinspiratory and late expiratory neuronal elements in the Bötzinger complex. In the present study, this was evident in hypoglossal discharge as a return of eupneic bursting composed of preinspiratory and inspiratory phases following brief duration hypoglossal and phrenic synchronized transition bursts, evidencing the eupneic generator operating in alternate modes contemporaneously.

The postresuscitation pattern following the restoration of hyperoxia eventually transitioned from pseudobiphasic eupnea to regular rhythmic eupnea with synchronized hypoglossal, vagus, and phrenic inspiratory neural discharge. We note this as a most peculiar finding, as there is a preinspiratory component of hypoglossal discharge present during the initial recovery of eupnea following resuscitation, although it eventually disappears with the restoration of pansynchronized neural inspiratory discharge. These results could evidence oxygen dependence of vagal inhibitory network elements mechanistically contributing to tonic inhibition of hypoglossal preinspiratory component (Ghali, 2015; Ghali and Marchenko, 2016). Such vagal inhibitory elements may be highly oxygen requiring and compromised by asphyxia. Thus, restoration of eupneic generator activity first involves recovery of

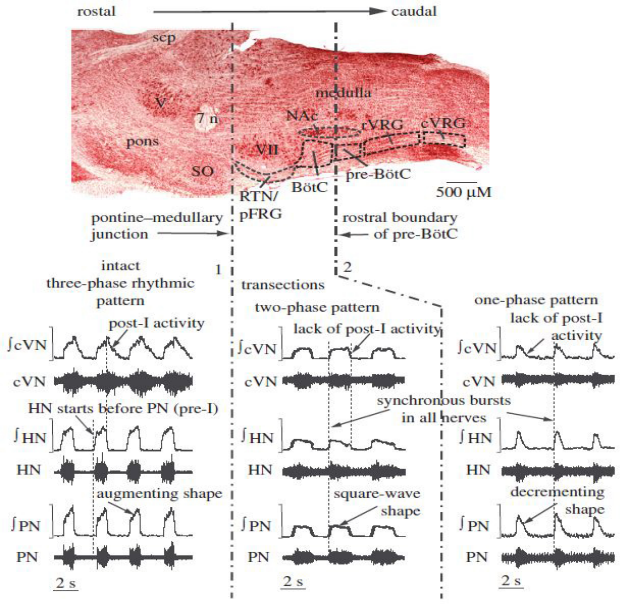

Figure 7. Mammalian brainstem respiratory circuitry. Top panel: Sagittal slice through the ventrolateral pontomedullary respiratory column nuclei. The generation of the respiratory rhythm requires mutual and reciprocal inhibitory interactions between units in the Bötzinger and pre-Bötzinger complexes. Normal triphasic eupnea consists of neural inspiration, postinspiration, and late expiration evident in the discharge patterns of central neurons as well as peripheral neurogram activity. Phrenic nerve discharge contains inspiratory and occasionally postinspiratory discharge. A hypoglossal nerve contains inspiratory discharge and, in unanesthetized conditions, exhibits preinspiratory discharge which becomes significantly more prominent with vagotomy and hypercapnia. Cervical vagus nerve exhibits both inspiratory and postinspiratory activity. Pontomedullary transection removing the tonic excitatory inputs of the Kölliker-Fuse and medial parabrachial nuclei propriobulbar interneurons to Bötzinger complex glycinergic postinspiratory neurons, as well as tonic excitatory inputs of the retrotrapezoid nucleus to Bötzinger complex GABAergic augmenting late expiratory neurons, generates the transition from normal triphasic eupnea to a two-phase pattern consisting of inspiration and nonsegmented expiration. Transection between the Bötzinger and pre-Bötzinger complexes generates a monophasic inspiratory pattern of respiratory discharge, without neural expiration, similar to gasping. Thus, the progressive reduction of the respiratory circuitry by progressively removing inhibitory elements gradually shifts the complexity of the classic triphasic eupneic rhythm. SO, superior olive; V, trigeminal nucleus; 7n, facial nucleus; RTN, retrotrapezoid nucleus; pFRG, parafacial respiratory group; scp, superior cerebellar peduncle; BotC, Bötzinger complex; pre-BotC, pre-Bötzinger complex; rVRG, rostral ventral respiratory group; cVRG, caudal ventral respiratory group. Modified with permission from Smith et al. (2007).

the inhibitory network elements necessary for generation of neural expiration and disinhibited hypoglossal preinspiratory activity, followed by the reinstitution of the inhibitory elements conveying vagal tonic inhibition of the hypoglossal preinspiratory discharge.

The argument could justifiably be made, and it may be conceivably conjectured that, as opposed to representing a biphasic activity, the initial brief duration burst could represent the concurrent onset of eupneic inspiratory activity in both PhN and XII discharge, interrupted in PhN discharge by XII pre-I activity, thus appearing in a XII neurogram as preinspiratory motoneuronal output immediately following the phrenic hypoglossal synchronized brief duration eupneic burst, and evident in PhN neurogram as a transient inspiratory inhibition of what would otherwise be a solitary inspiratory activity. For this hypothesis to hold, one must unrestrain the definition of preinspiratory activity from the neural respiratory activity occurring immediately before inspiration and the existence of an independent generator with state-dependent loss of phase regulation must be considered. Thus, asphyxia and the subsequent oxygen restoration effect a reorganization of the respiratory network in such a manner that preinspiratory activity intrudes into inspiratory activity.

If the aforementioned hypothesis holds, a new definition of pre-I naturally emerges: the central neural respiratory activity which has the capacity to drive inspiratory neuronal discharge, though may be generated independently of, and intrude upon, the same when the eupneic generator is operating in alternate modes, during transition conditions in response to lung inflation and deflation (Lee et al., 2007a), hypoxia (Lee and Fuller, 2010a,b) and hypercapnia (Ghali and Marchenko, 2016), activation of tonically discharging C fiber vagal afferents (Lee et al., 2003, 2007a,b, 2008, 2012), as well as recovery from the same. Furthermore, we may conjecture, though tenuously, the possibility of the genesis of a preinspiratory inspiratory phase spanning activity by monosynaptic projections from pre-Bötzinger complex units, in the intact normoxic condition, contemporaneously relayed to all inspiratory neural discharges, with active inhibition of preinspiratory discharge in some, though not other, neural respiratory outputs (Song et al., 2000). In such a scenario, discharge of the hypoglossal nerve, as well as other nerves innervating the upper airway musculature, thus manifests preinspiratory and inspiratory phase spanning activity, as a consequence of lack of active inhibition of the former component, with concurrent active inhibition of the preinspiratory component in phrenic motoneurons. Studies have demonstrated preinspiratory units in the Kölliker-Fuse nucleus and the Bötzinger complex projecting to phrenic motoneurons and the nature of their interaction may be inhibitory (Song et al., 2000). GABAergic projections from the Bötzinger complex to phrenic motoneurons were previously described and support a hypothesis of descending phasic inhibition of spinal respiratory motoneuronal outputs (Song et al., 2000). During resuscitation, then, there is a return of both preinspiratory and inspiratory pattern generator activity. However, this precedes recovery of the mechanism coordinating their phase relationship, which itself manifests as biphasic bursting. Decoupled bursting, or rather, discharge in upper airway related neurograms out of phase with the inspiratory discharge in phrenic, as well as dissociation of the preinspiratory and inspiratory components of upper airway related, neurograms demonstrated in previous studies occurring in response to perturbations such as increasing positive end-expiratory pressure, evidences capacity for differential genesis of these activities (Lee et al., 2007a). Alternatively, this activity may represent a biphasic XII pattern of "pre-I-less I" followed immediately by a coupled pre-I-I burst with biphasic PhN inspiratory bursting.

The following alternate mechanisms could account for the genesis of this post-resuscitative pattern observed in phrenic nerve discharge. In one mode, the activity represents dissociation of preinspiratory and inspiratory components of eupneic discharge, with conveyance of the preinspiratory component of discharge to the phrenic motor output, contemporaneously with the pseudodissociated hypoglossal preinspiratory activity. Separation of the preinspiratory and inspiratory components of the hypoglossal discharge, as well as various respiratory outputs innervating upper airway musculature, and genesis of bursts in neural respiratory discharge innervating upper airway musculature out of phase with the inspiratory discharge in phrenic motor output, are elicited by various experimental interventions, including positive end-expiratory pressure, an effect which varies in dose-dependent fashion (Lee et al., 2007a). It could alternatively be argued that the initial brief duration bursts contemporaneously appearing in the discharge of phrenic nerve indicates this pattern appears to represent neither decoupled XII bursting (Ezure et al., 2003; Saito et al., 2002) nor dissociation of the preinspiratory and inspiratory components of hypoglossal bursting described in other studies. If so, such an interpretation renders unlikely these findings represent the dissociation of preinspiratory and inspiratory discharge, which would support the model of differential generation. Possibly, both transition and eupneic bursts represent interrupted eupneic inspiratory activity.

We suggest genesis of the initial brief duration burst is more consistent with a transition burst generated by a eupneic generator operating without the full complement of inhibitory network elements (Marchenko et al., 2016). In another conceptualization, there is a normal expiratory neural period with postinspiratory and late augmenting expiratory neurons discharging sequentially, activating preinspiratory or preinspiratory inspiratory phase spanning units, generating the brief eupneic discharge, though early decrementing inspiratory neurons, perhaps of higher threshold, are less activated, or a lower fraction of this population is activated, leading to insufficient inhibition of the postinspiratory and augmenting expiratory neurons in the Bötzinger complex (Ezure and Manabe, 1988; Ezure et al., 2003; Ezure and Tanaka, 2006). This results in a premature reactivation of the Bötzinger complex postinspiratory neurons, though Bötzinger augmenting late expiratory neurons remain refractory and a wholly postinspiratory expiration without late expiratory discharge is observed, followed by rapid reactivation of both pre-Bötzinger complex preinspiratory and preinspiratory inspiratory phase spanning units, as well as a more considerable fraction of the early decrementing inspiratory neuronal population, resulting in genesis of a more prominent duration eupneic burst, followed by an expiration composed of normal postinspiratory and late expiratory epochs (Molkov et al., 2017). Inspection of the hypoglossal neurogram demonstrates a somewhat dimorphic burst with early and late components, most consistent with a pattern of transition bursting followed by preinspiratory and inspira-

tory phase spanning eupneic burst generation. The expiratory period between the brief and prominent phrenic eupneic bursts may thus represent a common coupling mechanism contemporaneously driving both postinspiratory mediated inspiratory off switching and preinspiratory activity, thus allowing the resumption of the formation of the normal eupneic ramp.

The genesis of the preinspiratory component of hypoglossal discharge that follows resuscitation from asphyxia could potentially originate from, and be driven by, expiratory neurons as a consequence of reconfiguration of the eupneic pattern generator consequent upon oxygen depletion compromising inhibitory elements and increasing the relative balance of excitatory glutamatergic compared to inhibitory $GABA_A$ergic and glycinergic expiratory neuronal elements. Rather than being driven by classic sources of preinspiratory activity, this component of the hypoglossal discharge following resuscitation may thus result from reconfigured eupneic pattern generator network elements recruiting excitatory expiratory neuronal elements, to drive hypoglossal preinspiratory discharge and generate the pause between the biphasic eupneic bursts of phrenic discharge.

8. Clinical Relevance

These hypotheses further emphasize and strengthen the critical importance of inhibitory network elements for generation and maintenance of the normal triphasic eupneic pattern of respiration (Bongianni, 2010; Marchenko et al., 2016), as well as recovery from anoxia induced monophasic inspiratory bursting and apnea to normal neural breathing. The mechanistic underpinnings of neural respiratory recovery following asphyxia may thus figure quite prominently in understanding the pathophysiology and neural network sequelae of hypoxia and asphyxia occurring with central and obstructive forms of sleep apnea (Dempsey et al., 2010). Though the precise mechanistic details may be beyond the scope of therapeutic relevance, if asphyxia causes progressive reduction of the inhibitory network circuitry and resuscitation involves the recovery of these elements, various drugs could enhance or strengthen inhibitory neurotransmission and recovery of normal triphasic eupnea following apneic episodes (Adén, 2011). Low dose respiratory stimulants, such as phosphodiesterase inhibitors, may putatively be useful in the treatment of sleep apnea (Qureshi and Lee-Chiong, 2005), sensitizing central chemoreceptors, increasing the duration and amplitude of the preinspiratory component of upper airway respiratory-related nerve discharge, enhancing excitatory drive to excitatory glutamatergic neurons and amplifying the amplitude of inspiratory nerve discharge, as well as GABAergic and glycinergic neurons, thus decreasing the risk of upper airway collapse and enhancing recovery from apneic episodes. The effects of chronic intermittent hypoxia may be extrapolated and generalized to further elucidate the pathophysiology of sleep apnea and the effects of hypoxia on neural respiratory circuitry. Chronic intermittent hypoxia causes short term potentiation, as well as long term facilitation of phrenic nerve responses to hypoxia, augmenting phrenic nerve amplitude during normoxia (Fuller et al., 2003; Ling et al., 2001), enhancing excitatory signaling and thus neural respiratory power by serotonergic mechanisms, potentially amplifying coherence and power of fast synchronous oscillations and strengthening inhibitory network elements necessary for genesis of the normal triphasic eupneic pattern and prevents posthypoxic ventilatory decline of phrenic nerve frequency (Fuller et al., 2003; Ling et al., 2001).

9. Conclusions

The dynamics of neural respiratory recovery following resuscitation from asphyxia powerfully elucidate and inform the mechanisms of central respiratory rhythm generation and pattern formation. Thus, following asphyxia, hyperpnea occurs as a result of peripheral chemoreceptor stimulation (carotid bodies), central chemoreceptor stimulation (fastigial nucleus), and anoxic depolarization. Apnea results from a compromise of eupneic generator inhibitory elements requiring oxygen. Transition bursts are generated by eupneic generator elements accumulating adenosine triphosphate during periods of apnea, in trying to generate normal eupneic bursts, followed by gasping reflecting anoxic depolarization mediated monophasic decrementing inspiratory discharge. Resuscitation is followed by a curious pattern of recovery, involving postgasping apnea and reflecting a quiescent eupneic generator, from the depletion of oxygen and adenosine triphosphate. The first evidence of recovery involves the generation of biphasic eupnea with initial brief duration bursts synchronized between hypoglossal and phrenic discharges, followed by a brief expiratory pause in phrenic then greater duration eupneic burst and followed immediately by a greater duration eupneic burst with preinspiratory activity replacing the phrenic expiratory pause inhypoglossal discharge. The duration of the interposed phrenic expiratory pause between the brief and greater duration eupneic bursts and the preinspiratory component of hypoglossal discharge gradually increases, presumably paralleling increased oxygen levels supplying the Bötzinger complex decrementing postinspiratory and augmenting late expiratory inhibitory network elements. This pattern reflects the eupneic generator operating in two functional modes, generating transition bursts and normal eupneic bursts.

Ethical approval

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors declare no conflict of interest.

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