Review

Noeud vital for breathing in the brainstem: gasping—yes, eupnoea—doubtful

Walter M. St John*

Department of Physiology, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, USA

For the past 200 years, various regions of the brainstem have been proposed as a ‘noeud vital’ for breathing—a critical region which, when destroyed, results in an irreversible cessation of breathing and death. Complicating this search for a noeud vital is the extensive network of neurons that comprises the brainstem respiratory control system of pons and medulla. Does a cessation of breathing following ablation of any region reflect the removal of a critical set of neurons whose activity generates the respiratory rhythm or does it reflect the interruption of one component of the neuronal circuit, such that this circuit cannot function, at least temporarily? An additional complication is that in contemporary neuroscience, a number of *in vitro* preparations have been introduced for the study of the generation of the respiratory rhythms. However, how are the rhythms that these preparations generate related to normal breathing? Are these rhythms similar to those of gasping, which is recruited when normal, eupnoeic breathing fails, or are these rhythms unique to the *in vitro* preparation and not related to any breathing pattern *in vivo*?

Keywords: eupnoea; gasping; *in vitro* preparations; *in situ* preparations; *in vivo* preparations

1. INTRODUCTION AND OVERVIEW

Eupnoea is normal breathing. If eupnoea fails, as in severe hypoxia or asphyxia, a second pattern, gasping, is recruited. Gasping can serve as a powerful mechanism for autoresuscitation and recommencement of eupnoea (Feldman 1986; Bianchi *et al.* 1995; St John 1998; St John & Paton 2004).

Eupnoea and gasping differ in multiple aspects including, I believe, their mechanism of generation in the brainstem respiratory control system. For more than 80 years, it has been accepted that gasping can be generated by mechanisms inherent to the medulla (Lumsden 1923a,b, 1924). Evidence is abundant that the generation and shaping of neural activities that allow for normal eupnoeic breathing require mechanisms involving both pons and medulla (St John 1998; St John & Paton 2000, 2004). This evidence is presented below.

In the context of contemporary literature, a ‘noeud vital’ for breathing has been proposed to reside in a medullary ‘pre-Bötzinger’ complex (Smith *et al.* 1991). This proposal was originally based and continues to depend heavily upon evaluations of rhythm generation in several *in vitro* preparations (Ballanyi *et al.* 1999; Ramirez & Viemari 2005; Feldman & Del Negro 2006). Yet, it remains unclear whether the rhythms generated by these *in vitro* preparations are related to normal eupnoeic breathing or are more

akin to gasping or, indeed, represent rhythms which are unique to reduced *in vitro* preparations.

Despite the ambiguity concerning *in vitro* rhythms, the pre-Bötzinger complex has been strongly advanced as a noeud vital for eupnoea (Rekling & Feldman 1998; Ballanyi *et al.* 1999; Ramirez & Viemari 2005; Feldman & Del Negro 2006; Ramirez & Garcia 2007). Such a medullary noeud vital is incompatible with the normal breathing and eupnoeic patterns of nerve activities that are observed following a complete destruction of this region (St Jacques & St John 1999; Mutolo *et al.* 2002; Krause *et al.* 2009). Following these lesions, gasping can no longer be generated (St Jacques & St John 1999). Thus, this pre-Bötzinger complex and its anatomical continuum, the ‘gasping centre’, comprise a critical and unique region for generating gasping.

In addition to its unique role in generating gasping, it is also possible, though without definitive support, that the pre-Bötzinger complex is the primary, if not exclusive, region for generating normal breathing, with mechanisms in other regions able to compensate for its removal. Yet, while acute perturbations of the pre-Bötzinger complex can markedly alter normal breathing, including inducing its complete cessation, similar changes in breathing follow acute perturbations of many other regions of the pons and medulla. These findings are also considered in detail below.

In summary, I submit that neuronal activities within the pre-Bötzinger complex represent one component, and an important integrative component, of the ponto-medullary neuronal circuit that generates normal breathing. Under conditions of severe hypoxia or

*m.stjohn@dartmouth.edu

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ischaemia, or following the removal of pontile and rostral medullary influences, a latent pacemaker mechanism, resident in some neurons in this 'pre-Bötzinger complex-gasping centre' and proximal regions, is released to generate the gasp.

2. A BRIEF CHARACTERIZATION OF NEURAL ACTIVITIES DURING EUPNOEA AND GASPING

(a) *Eupnoea*

Movement of air from the environment, through the nasal and oral passages to the lungs, requires the co-ordinated activity of multiple muscles of thorax, abdomen and upper airways. The primary muscle responsible for expansion of the lungs is the diaphragm, innervated by the phrenic nerve. Phrenic activity during eupnoeic breathing is incremental and allows the lungs to expand gradually. This period of phrenic activity defines neural inspiration; the period between phrenic bursts is neural expiration.

For air to reach the lungs, a patent upper airway is essential. Contractions of muscles in the region of the alae nasi, jaw, pharynx, tongue and larynx serve to dilate and stiffen the upper airway to prevent airway collapse by the negative pressures associated with lung inflation. These contractions are dependent upon activities of multiple cranial nerves, including the trigeminal, facial, vagus and hypoglossal. Thus, normal breathing requires balanced and coordinated activities between cranial and spinal nerves. Failure of such balanced and coordinated activities can lead to obstruction of the upper airway, as in obstructive sleep apnoea (Bartlett 1986; Feldman 1986; von Euler 1986; Bianchi *et al.* 1995; Remmers 1998; St John 1998; St John & Paton 2004).

While activities of cranial and spinal nerves are coordinated during normal breathing, these activities are not identical in either timing or pattern. Activities of the facial, hypoglossal and vagus nerves typically commence before the onset of the phrenic discharge. The hypoglossal nerve especially has an onset in the mid to late portions of neural expiration in some species (Hwang *et al.* 1983; St John & Bledsoe 1985a; Roda *et al.* 2002; Leiter & St John 2004). Such early onsets of these cranial nerves make physiological sense as these discharges can assure a patent airway before the start of phrenic discharge, lung inflation and inspiratory airflow. In terms of discharge pattern, those of the hypoglossal and facial nerve can be either incrementing or decrementing, depending upon species and experimental conditions. Activity of the recurrent laryngeal branch of the vagus, innervating the intrinsic abductor muscles of the larynx, is typically incrementing in neural inspiration (Hwang *et al.* 1983; St John & Bledsoe 1985a; Bartlett 1986; Roda *et al.* 2002; Leiter & St John 2004; St John *et al.* 2004; Berger & Sebe 2007; St John & Leiter in press).

In addition to activities during neural inspiration, the recurrent laryngeal nerve has a burst of activity at the start of neural expiration. This burst of activity leads to laryngeal adduction. This burst mirrors a powerful inhibition of inspiratory activity and defines Phase 1 of expiration. Active expiration, which can

occur with increased ventilatory drive, involves contractions of intercostals and abdominal muscles. The onset of activities of muscles of 'active expiration' occurs after Phase 1 is complete and defines Phase 2 of expiration (Richter 1982; Richter *et al.* 1986). A final, expiratory-related discharge is that produced by the mylohyoid branch of the trigeminal nerve and has activities in Phase 1 and/or Phase 2 (St John & Bledsoe 1985a,b).

The above presentation, albeit superficial, demonstrates that, in eupnoea, cranial and spinal nerves commence activity at varying times throughout the inspiratory and/or expiratory phases. Phrenic discharge is augmenting but, for other spinal and especially cranial nerves, the patterns of discharge may be incrementing, decrementing or both, depending upon the respiratory phase or experimental condition. In summary, during normal breathing, the patterns of discharge of cranial nerves, having cell bodies in pons and medulla, and spinal nerves, having cell bodies in the spinal cord, are complex yet highly coordinated. Supporting such coordination is the finding of multiple reciprocal interconnections between the neurons of pons and those of medulla (Bianchi & St John 1981, 1982; Segers *et al.* 1985, 2008). Yet this coordination is not absolute as the cranial nerves can exhibit discharges that are not related to the cyclical periods of neural inspiration and expiration. Such non-respiratory discharges, which are especially prevalent in the hypoglossal, trigeminal and facial nerves, reflect their involvement in rhythmic activities such as mastication and deglutition (Widdicombe 1980; Baekey *et al.* 2001; Roda *et al.* 2002; Morris *et al.* 2003; Gestreau *et al.* 2005).

(b) *Gasping*

In severe hypoxia or ischaemia, the frequency and tidal volume of respiration initially increase and then decline and cease. After a variable period of apnoea, breathing returns, but with a very different pattern from that of eupnoea. Each breath appears to be 'all or none'—a series of gasps (Lumsden 1923a,b, 1924; St John 1990).

Activities of cranial and spinal nerves are markedly different in gasping when compared with eupnoea. All nerves that had an inspiratory discharge in eupnoea acquire a stereotypical decrementing discharge in synchrony with phrenic discharge in gasping. The earlier onset of facial, hypoglossal and laryngeal nerves, compared with that of the phrenic nerve in eupnoea, is greatly reduced or eliminated. All these nerves now commence activity at approximately the same time, rise rapidly to a peak value soon after onset, then decline and cease. Again, as opposed to recordings during eupnoea, activities of cranial and spinal nerves during neural expiration are markedly altered in that these activities are substantially reduced or eliminated (see St John 1990, 1996, 1998 for reviews).

By what mechanisms does hypoxia or ischaemia eliminate eupnoea and recruit gasping? The mechanisms are twofold: a suppression of respiratory-modulated neuronal activities of pons and a recruitment of medullary

mechanisms for gasping (Paton *et al.* 2006; Paton & St John 2007a; St John *et al.* in press). Implicit in this statement are the concepts that gasping is generated by medullary mechanisms and that eupnoea requires pontile mechanisms for its full expression. Initial results in support of these concepts have been derived from studies involving brainstem transactions.

3. RESPIRATORY RHYTHMS FOLLOWING BRAINSTEM TRANSACTIONS: NEW FINDINGS PROVIDE ELUCIDATION OF OLD OBSERVATIONS

Lumsden (1923a,b, 1924) provided the basic characterization of the brainstem respiratory control system. In brief, Lumsden found that eupnoeic breathing was recorded in preparations having an intact pontile and medullary brainstem. Following transaction caudal to a rostral pontile 'pneumotaxic centre', respiration was altered to 'apneusis', with a sustained hold of the breath at peak inspiration. Apneusis was replaced by 'gasping' following the removal of the rest of pons by a transaction at the pontomedullary junction (Lumsden 1923a,b, 1924).

These results of Lumsden have been repeated and confirmed by many investigators, prominently including Wang *et al.* (1957) and Wang & Ngai (1964). However, Wang *et al.* produced an addendum in that, in a small minority of preparations, gasping was not obtained after the total removal of pons. Rather, in these preparations, respiration appeared to be close to normal. Other investigators had also recorded these 'non-gasping' patterns following a total removal of pons (e.g. Hukuhara 1976; St John & Leiter 2007).

The recording of non-gasping patterns following the removal of pons has provided support for the concept that eupnoea can be generated by a medullary *noeud vital*. (e.g. Rekling & Feldman 1998; Feldman & Del Negro 2006). Yet implicit to this support is the assumption that all non-gasping rhythms recorded following the removal of pons must be eupnoea or close to eupnoea. A related question concerns why seemingly identical preparations would exhibit gasping and non-gasping patterns following the removal of pons.

Removal of pons of *in vivo* preparations is invariably accompanied by a marked fall in arterial blood pressure (Wang *et al.* 1957). Of course, damage to nervous tissue following a brainstem transection extends beyond the level of the physical transection. It is thus probable that physical transections at the pontomedullary junction also caused destruction of variable portions of the rostral medulla. This probability is supported by the well-documented finding that, if a transection at the pontomedullary junction did not result in a classical gasping pattern, this gasping pattern was recorded following a further caudal transection (Wang *et al.* 1957).

This question concerning levels of brainstem transections required to produce gasping was resolved by the recent studies of Smith *et al.* (2007). These investigators produced precise brainstem transections in an *in situ* perfused preparation of the rat. In this preparation, an extracorporeal system provides perfusion of tissues and, hence, perfusion of the brainstem is

not confounded by alterations in arterial pressure. These investigators found that transections at the pontomedullary junction did not result in gasping but in a pattern of breathing that differed markedly from eupnoea. Changes from eupnoea included a change in the pattern of activities of the cranial and spinal nerves, an elimination in the differing times of onset of activities of these cranial and spinal nerves and a loss of Phase 1 expiratory activity. Respiratory activity was thus changed from the three phases of eupnoea to two phases following this transection. A further change occurred following the removal of the rostral medulla in that a one-phase pattern of gasping was produced. This lesion, which resulted in gasping, left intact the rostral medullary pre-Bötzinger complex, which is a hypothesized medullary *noeud vital* for eupnoea.

In summary, the removal of pons exclusively can result in non-gasping patterns of respiratory activity. However, these patterns differ markedly from those of eupnoea (see also St John & Leiter 2007). The differences from eupnoea and, complementarily, the importance of the pontile mechanism for eupnoea have also been shown by observations that the differences in times of onset of cranial and spinal nerves of eupnoea are reduced or eliminated by perturbations of the rostral pontile pneumotaxic centre (St John 1987; Fung & St John 1994; Smith *et al.* 2007). These results thus make tenuous the assumption that all non-gasping patterns of breathing must be eupnoea or variants thereof. No evidence excludes these patterns being 'incomplete gasps'—their rate of rise of inspiratory activity approximates that of gasps (see Smith *et al.* 2007; St John & Leiter 2007).

A reversal of the rostral-to-caudal brainstem interactions is demonstrated by the few studies in which respiratory-modulated activities have been recorded from pons following the removal of medulla. The pontile respiratory rhythms have been those recorded from the mylohyoid branch of the trigeminal nerve, which has cell bodies in the trigeminal motor nucleus (St John & Bledsoe 1985b; Huang & St John 1988). The mylohyoid nerve discharges during all or part of neural expiration in eupnoea. While altered compared with the pattern of discharge recorded when attached to the medulla, rhythmic mylohyoid activities continued following a transection of the brainstem at the pontomedullary junction. The continuance of such rhythmic activity thus supports the possibility that pons can generate rhythmic respiratory-modulated activities independent of the medulla. The possibility for rhythm generation in pons was proposed by Hugelin *et al.* (1976) and Vibert *et al.* (1976). Based upon the discharge of respiratory-modulated neuronal activities in the region of the pontile pneumotaxic centre, Hugelin proposed that an internal circuit in this region could generate a rhythmic output.

4. RHYTHMS OF *IN VITRO* PREPARATIONS: EUPNOEA, GASPING OR NEITHER?

(a) *En bloc* preparations of the neonatal rat

Suzue (1984) introduced an *en bloc in vitro* preparation of the neonatal rat for studies of the control

of breathing. This preparation generated rhythmic activities of cranial and spinal nerves that continued for extended periods. Such a preparation held great promise for the performance of studies, such as whole-cell recordings of neuronal activities, which are extremely difficult *in vivo*.

In introducing this preparation, Suzue carefully documented its limitations as well as its potential benefits. Among the most prominent limitations was the one concerning the respiratory rhythm that this preparation was expressing. Suzue raised the possibility that this pattern might be akin to gasping.

The possibility of gasping was supported by multiple observations. (i) As gasping, the discharges of cranial and spinal nerves were synchronized and decrementing (Smith *et al.* 1990; St John 1996). (ii) As gasping, these nerves displayed little or no expiratory activity in the period between phrenic bursts (Smith *et al.* 1990; St John 1996). (iii) The synchronized, decrementing pattern was not altered following the complete removal of pons, implying that this preparation was only capable of exhibiting a single respiratory pattern, akin to gasping (Monteau *et al.* 1989; Smith *et al.* 1990). This unitary 'gasp-like' rhythm and seeming lack of a role for pons in altering this rhythm may reflect the anoxia of this preparation. Oxygenation of this preparation, which is dependent upon diffusion, is only adequate for structures close to the surface; the core of the preparation is anoxic (Brockhaus *et al.* 1993; Okada *et al.* 1993). (iv) As opposed to eupnoea in which the frequency and peak height of integrated nerve activities increase with increases in respiratory drive in hypercapnia, peak heights of the en bloc preparation changed little or not at all in hypercapnia. A similar lack of change in peak height is found for gasping *in vivo* (St John 1996).

Rather than gasping, some investigators who adopted use of the en bloc preparation maintained that its rhythmic activity was that of the eupnoea of a neonatal rat (Smith *et al.* 1990, 1991; Rekling & Feldman 1998; Ballanyi *et al.* 1999; Feldman & Del Negro 2006). Three sets of observations were invoked in support of this eupnoeic activity. (i) The patterns of inspiratory airflow and integrated electromyographic activity of the diaphragm were converted from incrementing to decrementing following vagotomy in spontaneously breathing neonatal rats (Fedorko *et al.* 1988; Smith *et al.* 1991). En bloc preparations were thus argued to have similar patterns of neural discharges to *in vivo* neonates following vagotomy. (ii) As en bloc preparations, transections through caudal pons of *in vivo* rodents were reported to cause little or no alteration in the respiratory rhythm (Monteau *et al.* 1989). (iii) In an en bloc preparation with attached lungs and vagi, phrenic discharge was altered by lung inflation (Mellen & Feldman 1997, 2000, 2001). Several reviews had stated that Lumsden found that only eupnoea, and not gasping, could be altered by lung inflation (Remmers 1998; Ballanyi *et al.* 1999). Overlooked was the original article in which Lumsden had, in fact, reported that both eupnoea and gasping were altered by lung inflation (Lumsden 1923b).

Concerning the alterations in inspiratory activity to a decrementing pattern following vagotomy, the

spontaneously breathing neonatal rat preparations may have exhibited hypoventilation and, in some cases, apnoea before the decrementing discharge pattern was recorded. In paralysed and ventilated neonatal rats, integrated phrenic discharge maintained an incrementing pattern following vagotomy; a brief period of anoxia could convert this pattern to decrementing and gasping (Wang *et al.* 1996). Hence, as opposed to the en bloc preparation, phrenic activity of eupnoea of neonatal rats is incrementing following vagotomy.

In addition to the difference in phrenic discharge patterns, pontile mechanisms were found to influence ventilatory activity in rats from the day of birth. As in all other species, apneusis was recorded following bilateral ablation of the rostral pontile pneumotaxic centre and vagotomy in neonatal and adult rats (Wang *et al.* 1993; Fung & St John 1995). The minimal changes in ventilatory activity reported following caudal pontile transections in rats (Monteau *et al.* 1989) overlooked Lumsden's observation that the 'depth and duration' of apneusis declines with caudal transections of the pons (Lumsden 1923a,b).

As noted above, several reviews had misquoted Lumsden's findings concerning the influence of lung inflation upon eupnoea and gasping. In an *in situ* preparation of the rat, these results of Lumsden were confirmed in that lung inflation changed phrenic discharge in both eupnoea and gasping (Harris & St John 2003, 2005). Thus, an influence of lung inflation upon the rhythmic activity of the en bloc preparation does not preclude that this rhythmic activity might be gasping.

In summary, evidence is substantial that the rhythmic activity recorded from the en bloc preparation of the neonatal rat is fundamentally different from eupnoea, but similar to gasping. Ablation of the noeud vital for rhythm generation in this preparation, the rostral medullary pre-Bötzinger complex, irreversibly eliminates gasping *in vivo* (Fung *et al.* 1994; Huang *et al.* 1997; St Jacques & St John 1999; Gray *et al.* 2001). Such ablations only transiently alter or eliminate eupnoea and then only if large ablations of this region are produced with a short interval (St Jacques & St John 1999; Mutolo *et al.* 2002; Krause *et al.* 2009). As discussed in previous reviews, mechanisms for rhythm generation in this preparation, including its independence from inhibitory synaptic transmission, fit precisely with mechanisms that have been described *in situ* and *in vivo* for the neurogenesis of gasping, and not eupnoea (St John 1996, 1998; St John & Paton 2004; Paton & St John 2007a). A similar fit with the mechanisms for generating gasping has been derived from studies using medullary slices of the neonatal rat and mouse.

(b) *Slices of medulla of neonatal rat and mouse*

In addition to the en bloc preparation, a powerful experimental tool for the study of rhythm generation was the tangential slice of medulla, which included the regions of the pre-Bötzinger-gasping centre, hypoglossal motor nucleus and rootlets of the hypoglossal nerve. This preparation generates rhythmic 'respiratory-modulated' activities but, as with the en

bloc preparation, whether this rhythm is related to eupnoea, gasping or neither is undefined.

Basically, two different types of slices have been introduced. The first type was a thin slice from the neonatal rat, which included as little as possible beyond the limits of the pre-Bötzinger complex and hypoglossal system (e.g. Smith *et al.* 1991; Johnson *et al.* 1994). This slice displayed an invariant decrementing pattern of discharge that was the same as that of the en bloc preparation *in vitro* and gasping *in vivo* or *in situ*.

The second type of slice is a thick medullary slice of the neonatal mouse, which in fact includes most of the medulla (Lieske *et al.* 2000; Ramirez & Viemari 2005; Ramirez & Garcia 2007). The hypoglossal discharge of this preparation is reported to reflect two rhythms: (i) a 'bell-shaped' hypoglossal discharge, which is considered to represent 'eupnoea', and (ii) a decrementing hypoglossal discharge, which is considered to be gasping. As noted above, this decrementing discharge has been considered as a variant of eupnoea by others.

A significant confounding factor regarding the types of respiratory rhythms that are generated by medullary slices is the use of hypoglossal discharge to characterize these rhythms. Such characterizations are based on the assumption that the discharge of the hypoglossal nerve has the same patterns as the phrenic nerve in eupnoea and gasping. From the day of birth, phrenic discharge is fundamentally different in eupnoea and gasping, being incrementing during the great majority of cycles in the former and decrementing in the latter (Wang *et al.* 1996). Such a fundamental difference has not been found for hypoglossal discharge. For neonatal and adult rats, hypoglossal discharge can be incrementing or decrementing in different preparations or even in different cycles from the same preparation (Leiter & St John 2004; Paton *et al.* 2006; Toppin *et al.* 2007). In adult mice, a decrementing hypoglossal discharge is the predominant pattern recorded during eupnoea. Thus, in most mice, the hypoglossal nerve has the same decrementing discharge in eupnoea and gasping, and the type of respiratory rhythm could not be judged based on hypoglossal discharge alone (St John & Leiter *in press*). This use of hypoglossal discharge is further confounded by the finding that the hypoglossal nerve of *in vivo* mice of ages 0–9 days has no rhythmic discharge except during hypoxia-induced gasping. Whether anaesthesia of these neonatal mice is responsible for this elimination of hypoglossal discharge is unknown (Berger & Sebe 2007). However, a complete evaluation of hypoglossal discharge in neonatal mice *in vivo* or *in situ* would seem essential as rhythms, claimed to be eupnoea, are recorded from *in vitro* slices of mice of the same age as those having no rhythmic discharges *in vivo*, except in gasping.

Mechanisms underlying rhythm generation in the medullary slices have been strongly linked, albeit by association, to the discharge of pacemaker, burster neurons. The decrementing discharges of both slices of rat and mouse medulla are proposed to be owing to the discharge of burster neurons in which conductances through persistent sodium channels are critical for the bursting. The use of blockers of these persistent

sodium conductances eliminates the pacemaker discharge and the rhythmic hypoglossal discharge (see Paton & St John (2007a) and Ramirez & Garcia (2007) for reviews). Administration of these same blockers to *in situ* preparations also eliminates gasping (Paton *et al.* 2006). Eupnoea continues in *in situ* and *in vivo* preparations even following administrations of these blockers at many fold higher concentrations than those that eliminated gasping (St John *et al.* 2007).

In the thick medullary slice of mouse, a second group of bursters has been identified. These bursters are dependent upon calcium conductances. Blockers of these calcium conductances, administered along with those that block persistent sodium conductances, eliminate the 'eupnoeic' pattern in the slice (Ramirez & Garcia 2007). However, such simultaneous administrations do not eliminate eupnoea of *in situ* preparations (St John 2008). Moreover, as these bursters that are dependent upon calcium conductances have not yet been found in other *in vitro*, *in situ* or *in vivo* preparations, their role in respiratory rhythm generation in less reduced preparations is unclear.

A final confusing observation from the use of medullary slices is that a rhythmic output can be re-established by increasing excitation after blockers of both persistent sodium and calcium conductances are administered. This finding has led one group to abandon the concept of bursters as generating the eupnoeic rhythm. A local neuronal circuit is now proposed to generate this rhythm (Del Negro *et al.* 2002; Feldman & Del Negro 2006). Again, the correspondence between this new *in vitro* rhythm and rhythms of more intact preparations remains undefined.

In a review in the *Journal of Applied Physiology* in 2007, we noted:

... the introduction of *in vitro* preparations has stimulated much excellent work into examining mechanisms of rhythm generation for respiration. However, understanding of these mechanisms will remain confused and obscure until additional work establishes which findings from *in vitro* preparations are applicable to the generation of unequivocally defined normal breathing. The challenge is now on to put the slice back into the whole system.

(Paton & St John 2007b, p. 727)

5. ABLATION OF PONTILE AND MEDULLARY REGIONS: IDENTIFICATION OF A NOEUD VITAL FOR GASPING BUT NOT FOR EUPNOEA

A noeud vital for normal eupnoeic breathing has classically been taken to mean a unique region which, when ablated, results in irreversible apnoea and death. No such region has been identified and, based upon an accumulation of evidence, it is improbable that such a noeud vital exists. Rather, results following ablations of various components of the brainstem ventilatory control system are consistent in establishing that marked alterations in breathing occur following the removal of many regions. Yet, with time, all of these dramatic, acute changes ameliorate or are completely reversed. Such results are consistent with the concept that eupnoea is generated and supported in all its complex aspects by a

pontomedullary neuronal circuit that contains much redundancy and plasticity. As opposed to eupnoea, evidence is consistent that a region of the rostral medulla that contains components of the same neurons in the pre-Bötzinger complex and gasping centre is unique for generating gasping (St John *et al.* 1984).

An early demonstration of plasticity and redundancy within the brainstem respiratory control system was that following ablation of the rostral pontile pneumotaxic centre. Acute ablation of this region, combined with vagotomy, results in apnoea with a sustained pause in inspiration. However, if removal of the pneumotaxic centre is separated by weeks or months from vagotomy, no apnoea is observed when preparations recover from anaesthesia (St John *et al.* 1971, 1972; Gautier & Bertrand 1975). Thus, the marked alteration in breathing that follows an acute ablation is ameliorated with time.

A similar amelioration after removal of an important component of the brainstem respiratory control system was observed following acute ablation of the dorsal medullary respiratory nucleus in the region of the nucleus of tractus solitarius. This region had been proposed as a medullary *noeud vital* for eupnoea (Wyman 1976; Euler 1986). Acute ablation of this region in anaesthetized preparations resulted in prolonged apnoea. However, if ventilation was supported, a normal pattern of breathing re-appeared (Berger & Cooney 1982).

Amelioration of changes in respiratory activity has also been consistently shown to follow acute ablations of neurons of the medullary pre-Bötzinger complex (St Jacques & St John 1999; Mutolo *et al.* 2002). Based upon results from *in vitro* preparations, the region was proposed as another *noeud vital* for eupnoea (Smith *et al.* 1991). Moreover, because this pre-Bötzinger complex did not overlap completely anatomically with the gasping centre, it was strongly maintained that the pre-Bötzinger complex was not a portion of a critical region for generating gasping (Rekling & Feldman 1998).

Acute ablation of neurons of the pre-Bötzinger complex of *in vivo* decerebrate preparations by injections of neurotoxins resulted in marked changes in phrenic discharge and ultimately apnoea. However, if artificial ventilation was continued, a eupnoeic pattern of phrenic discharge returned. Following this return of eupnoea, injections of neurotoxins in high volume and high concentrations failed to cause another alteration of eupnoea. Thus, the return of eupnoea was not due to actions of remnants, if any, of the pre-Bötzinger complex but rather to a reorganization of the pontomedullary neuronal circuit for eupnoea. Importantly, gasping was eliminated irreversibly following these perturbations of the pre-Bötzinger complex (St Jacques & St John 1999).

A recent study also supports the concept that acute trauma to a neuronal circuit, rather than elimination of a *noeud vital*, is responsible for marked alterations in eupnoea following ablations of the pre-Bötzinger complex. In a previous study, it was reported that spontaneously breathing *in vivo* preparations developed irreversible apnoea after relatively large injections of neurotoxin that inactivated and destroyed much of

the pre-Bötzinger complex, and surrounding region, acutely (Wenninger *et al.* 2004a,b). However, in the recent study, multiple small injections with increasing concentrations of neurotoxins produced little or no change in breathing even though most or all of the pre-Bötzinger complex was ultimately destroyed (Krause *et al.* 2009).

Other types of experiments also add to the conclusion that substantial changes in breathing are only observed following acute perturbations of the pre-Bötzinger complex *in vivo* or *in situ*. A possible exception would be the alteration from eupnoea to ataxic breathing some days after injections of a substance that destroys neurons having NK-1 receptors; such receptors are in high concentrations in neurons of the pre-Bötzinger complex (Gray *et al.* 2001). First of all, apnoea and not ataxia would be expected if this region is the *noeud vital* for breathing. Second, as opposed to the concept of separate pre-Bötzinger complexes and gasping centres, no gasping was observed when these animals, having the ataxic breathing pattern, were exposed to severe hypoxia. Finally, these animals were apparently terminated soon after ataxic breathing was noted. Thus, we have no indication if, with time, normal breathing would have been re-established as had occurred following other acute lesions of the brainstem respiratory control system. A recent report in which viral vectors were used to silence neuronal activities within the pre-Bötzinger complex also fits with this concept of acute versus chronic alterations in breathing (Tan *et al.* 2008). The authors themselves note that sudden depression of neuronal activities of the pre-Bötzinger complex can lead to respiratory failure. I agree with this finding but believe that a similar statement could be made concerning multiple regions of the pontomedullary respiratory network.

6. SUMMARY

Evidence of a medullary *noeud vital* appears to be conclusive only for the genesis of gasping. The claim of a medullary *noeud vital* for eupnoea was premature and confounded by depending heavily upon rhythms recorded from *in vitro* preparations. The relationship of these *in vitro* rhythms with eupnoea continues to be nebulous. This nebulousness has been further increased by very different *in vitro* rhythms being considered as the fundamental unit of respiration by different laboratories. Further confounding the claim of a medullary *noeud vital* for eupnoea has been a constant reliance upon 'age and state-dependent' transformations to account for differences between findings *in vitro* and those of *in vivo* or *in situ* preparations. First of all, as noted in previous reviews (St John 1996; Paton & St John 2007a), no such transformations are required when most results from *in vitro* preparations are compared with gasping of *in situ* or *in vivo* preparations as the correspondence between *in vitro* findings and gasping is almost absolute. Second, as noted above, many hypothesized 'age-dependent' transformations have not been found when studies are actually performed using neonatal *in situ* or *in vivo* preparations.

In summary, as we have recently concluded (Paton & St John 2007b, p. 727), 'a critical problem is to determine what the rhythms generated *in vitro* actually are and how they relate to adequately defined motor behaviors (e.g. eupnoea, gasping) *in vivo* and *in situ*. Differences between *in vitro* findings, compared with those *in vivo* or *in situ*, may not reflect 'plasticity' or 'transformations' but rather fundamentally different mechanisms of rhythm generation'.

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