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Title: CrossTalk proposal: A central hypoxia sensor contributes to the excitatory hypoxic ventilatory response

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Running Title: A respiratory chemosensor for hypoxia in the CNS

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A central hypoxia sensor contributes to the excitatory hypoxic ventilatory response

Gregory D Funk and Alexander V Gourine

The high-energy demands of the mammalian brain are met primarily through oxidative metabolism. With minimal capacity for energy storage, the brain depends on a constant supply of oxygen and metabolic substrates to meet these demands. A host of adaptive responses have evolved to protect brain O_2 delivery. Prominent among these is the biphasic hypoxic ventilatory response (HVR). The classical view of the HVR is that specialized peripheral chemosensors in the carotid bodies (CBs) (and aortic bodies in some species) detect decreases in the arterial PO_2 and activate brainstem respiratory centers, causing adaptive increases in ventilation. Ventilation peaks in the first minute and is followed by a secondary hypoxic respiratory depression that is most pronounced in premature mammals and attributed to the central depression of the brainstem respiratory network, decreases in CB output or a reduction in metabolic rate (Bissonnette, 2000; Teppema & Dahan, 2010)(Fig 1A). This traditional view posits that the excitatory component of the HVR originates from the CB and that monitoring peripheral arterial PO_2 is sufficient to ensure brain O_2 homeostasis; i.e., the only contribution of the CNS to the HVR is the depression of ventilation. We disagree and here review evidence that the brainstem neuroglial network controlling breathing is specialized through cellular or emergent network properties to orchestrate a homeostatic response to acute hypoxia that includes network excitation and an increase in ventilation that counteracts the hypoxic depression of breathing. Cardiovascular and sympathetic nervous system responses mediated by hypoxia-sensitive presympathetic neurons (Sun & Reis, 1994) mechanisms of neuronal hypoxia sensing that are not related to the HVR (Haddad & Jiang, 1997), responses to intermittent hypoxia and mechanisms of gasping are beyond the scope of this article.

Maintaining brain metabolic homeostasis a significant challenge (Marina *et al.*, 2017). O_2 profiles vary significantly throughout the brain parenchyma, reflecting complex spatiotemporal differences in local neuronal activities and metabolic demands. These regional differences in brain PO_2 cannot be detected by the CB chemoreceptors. Neurovascular coupling mechanisms, which do not involve O_2 sensing per se, cause changes in regional cerebral blood flow in accordance with changes in neuronal activity that are communicated to the vasculature via astrocytes releasing vasoactive substances (Attwell *et al.*, 2010). Astrocytes are ideally positioned to monitor neuronal activity to adjust blood flow in accordance with local energy demands, but they are equally well-positioned to modulate neuronal activity in accordance with parenchymal metabolic signals, including brain tissue PO_2 (Teschemacher *et al.*, 2015). When brain PO_2 falls acutely, the predominant neurochemical response is an increase in extracellular adenosine (ADO), synaptic depression and decreased neuronal activity (Lipton, 1999; Ramirez *et al.*, 2007; Mukandala *et al.*, 2016). This response is adaptive at a local level, as it reduces O_2 demands, enhancing the capacity of brain tissue to survive periods of limited O_2 supply, but hypoxic depression of respiratory network activity is maladaptive; i.e., ventilation and sympathetic activity must increase to ensure recovery. There is significant evidence that brainstem respiratory network can mount an adaptive excitatory response to hypoxia, independent of CB activity.

The literature describing the expression of the HVR after CB denervation (or silencing) is confusing. The majority of mammalian studies show that hypoxia-induced increases in ventilation are abolished following acute CB denervation (Bissonnette, 2000; Teppema &

Dahan, 2010). However, we argue that data interpretation is confounded by the inhibitory effects of anaesthesia on central hypoxia sensing mechanisms, and incomplete understanding of how central and peripheral chemosensory inputs are integrated (Smith *et al.*, 2010; Gourine & Funk, 2017). CB denervation could also have an impact on other mechanisms that minimize brain hypoxemia (e.g., increases in cerebral blood flow and arterial blood pressure, hypometabolism), such that a specific hypoxic stimulus could produce a lower parenchymal PO_2 in CB-denervated compared to intact animals. Direct assessment of brain PO_2 is needed to resolve the significance of this issue. Never the less, when allowed to recover from CB denervation and studied without anesthetic, mice (Soliz *et al.*, 2005), rats (Martin-Body *et al.*, 1986; Roux *et al.*, 2000; Angelova *et al.*, 2015), cats (Miller & Tenney, 1975; Gautier & Bonora, 1980), dogs (Davenport *et al.*, 1947), goats (Daristotle *et al.*, 1991) and ponies (Bisgard *et al.*, 1976) show partial or almost complete (rats) recovery of the HVR. This might reflect compensatory plasticity of non-CB peripheral chemoreceptors (Hodges & Forster, 2012), or recovery from the disruption of brainstem chemosensory/respiratory network excitability following CB denervation. The latter is supported by experiments in unanesthetized dogs (Curran *et al.*, 2000) and goats (Daristotle *et al.*, 1991) with intact, isolated and separately perfused CBs. Animals respond to central hypoxia with an increase in ventilation, but denervation of the normoxic/normocapnic CBs abolished/attenuated the ventilatory response to central hypoxia, suggesting that brain O_2 sensing mechanisms require permissive/facilitatory inputs from the periphery.

In humans, CB denervation consistently abolishes the HVR (Timmers *et al.*, 2003b; Teppema & Dahan, 2010), but again data interpretation is challenging. Denervation studies commonly involve subjects with chronic lung disease who may have altered chemoreflex function. Thus, it may be significant that some CB-denervated subjects (2/8) without a history of lung disease showed a HVR under hypercapnic conditions (Timmers *et al.*, 2003a). CB-resected asthma subjects showed a similar dependence of the HVR on hypercapnia (Swanson *et al.*, 1978), possibly suggesting an interaction between central hypoxia-sensitive mechanism and other chemosensory inputs (Gourine & Funk, 2017). Finally, failure to record an increase in ventilation in response to hypoxia does not exclude the existence of a centrally-mediated excitatory HVR. Maintenance of ventilation during hypoxia (i.e., no depression) following chronic CB denervation (Swanson *et al.*, 1978) may be evidence of an excitatory HVR.

Brainstem astrocytes (especially those in the preBotzinger complex [preBötC]) are emerging as important in coordinating the central component of the HVR. A key observation in anesthetized rats was the slow-onset release of the gliotransmitter ATP from the ventral surface of the medulla oblongata during hypoxia (Gourine *et al.*, 2005). A reduction of the steady-state component of the HVR following microinjections of P2 receptor antagonists into the preBötC (Gourine *et al.*, 2005; Rajani *et al.*, 2017) led to the hypothesis that hypoxia evokes ATP release from astrocytes, which stimulates breathing and attenuates the hypoxic respiratory depression (Gourine *et al.*, 2005). Consistent with this, astrocytes cultured from the brainstem respond to physiologically-relevant levels of hypoxia with an increase in $[Ca^{2+}]_i$ and vesicular release of ATP (Angelova *et al.*, 2015). Astrocytes are known to change their properties in culture, but accumulating evidence supports the physiological relevance of these observations. First, the HVR is reduced in anesthetized paralyzed rats following unilateral pharmacological inhibition of $P2Y_1$ receptors in the preBötC (Gourine *et al.*, 2005; Rajani *et al.*, 2017), and in CB-denervated awake rats in conditions of virally-induced expression of transmembrane prostatic acid phosphatase (TMPAP) in the preBötC to degrade extracellular ATP (Angelova *et al.*, 2015; Sheikhbahaei *et al.*, 2018). Second, viral approaches that expressed the light chain of tetanus

toxin (TeLC) or dnSNARE to disrupt vesicular release mechanisms selectively in preBötC astrocytes reduced the HVR in: i) anesthetized paralyzed rats; ii) awake CB-denervated rats and; iii) most importantly, awake rats with intact peripheral chemoreceptors as this addressed the potentially confounding effects of anesthesia and CB denervation (Fig 1B)(Angelova *et al.*, 2015; Rajani *et al.*, 2017; Sheikhbahaei *et al.*, 2018). It will be important to demonstrate that the reduced HVR following TeLC or dnSNARE expression in preBötC astrocytes is not due to nonspecific disruption of glial function and impaired ability of the preBötC to increase ventilation. However, this possibility is unlikely because TeLC and dnSNARE expression specifically target astrocytic vesicular release mechanisms. They do not severely disrupt baseline breathing as might be expected if the general housekeeping functions of astrocytes were impaired. In addition, the HVR is also reduced via manipulations of P2 receptor signaling that have minimal effect on glial function (Angelova *et al.*, 2015; Rajani *et al.*, 2017).

Whether the hypoxia-induced, ATP release by astrocytes and local network excitation is unique to the preBötC is not known. Astrocytes in other areas of the brain respond to hypoxia with increases in $[Ca^{2+}]_i$ (Angelova *et al.*, 2015), but purinergic signaling in the preBötC (which is determined by local P2 and P1 receptors, ectonucleotidases and a host of transporters and intracellular enzymes that interact to determine the extracellular profile of P2/P1 receptor ligands) may be uniquely organized to favor network excitation.

While many mechanistic details remain unresolved, the (partial) recovery of the HVR in unanesthetized CB-denervated mammals, the hypoxia-evoked release of ATP by brainstem astrocytes and the reduction in the HVR following disruption of astrocytic signaling in the preBötC of awake, CB-intact rodents provide strong evidence that the conventional view of the CB as the only hypoxic respiratory chemosensor should be revisited.

Figure Caption

Figure 1. A. Ventilatory responses of young and older (anesthetized) piglets to hypoxia (6%). (modified with permission from Moss, RA. Respir Physiol 2000, 121, 185-97). B. Summary data illustrating hypoxia-induced changes in minute lung ventilation in carotid body intact and peripherally chemodenervated (10 weeks) conscious rats expressing CatCh (calcium translocating channel rhodopsin variant that was fused with EGFP and used as a control here) or TeLC bilaterally within astrocytes of the preBötC network. Ventilation measurements were obtained during the last 5 min of the 10 min hypoxia period. TeLC caused similar reductions in the HVR of intact and CB-denervated rats. (modified with permission from Angelova et al., 2015).

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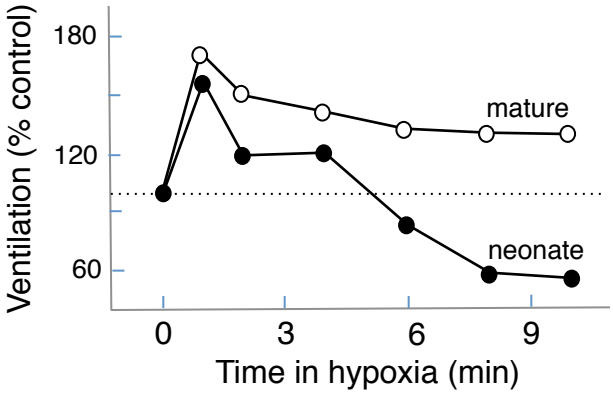
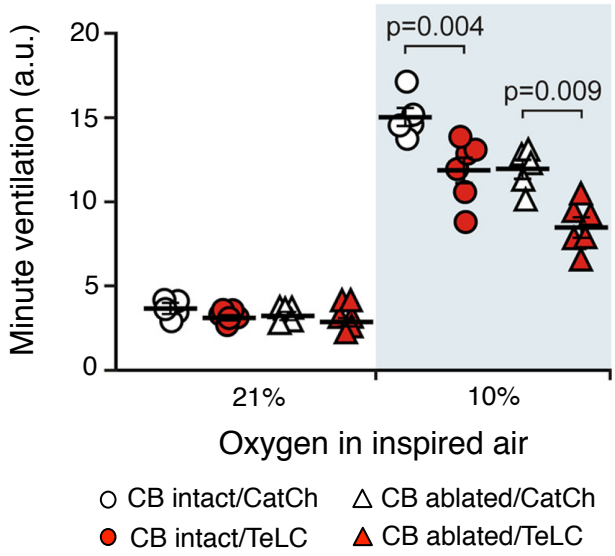
A. Pigs



B. Rats



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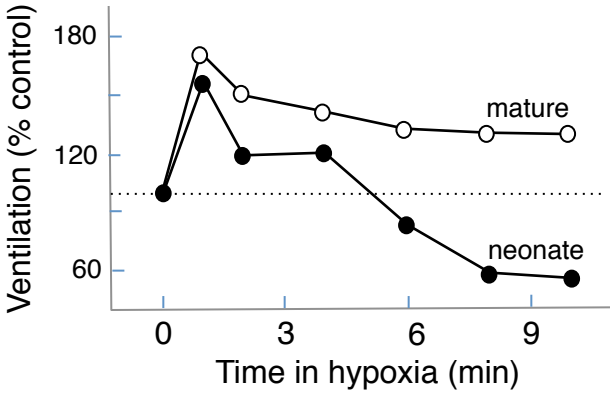

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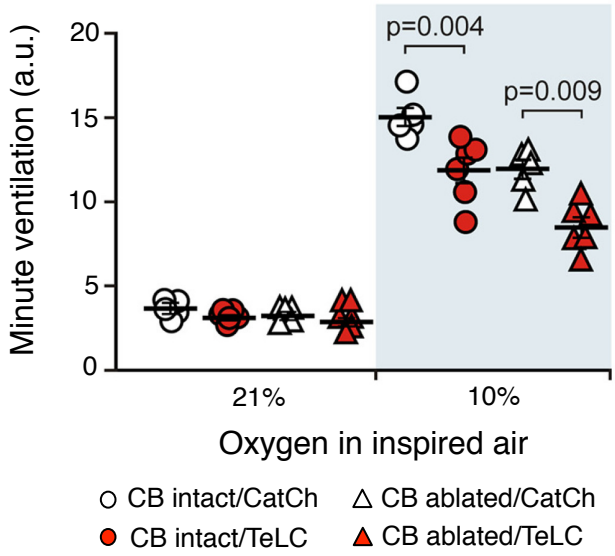

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