Brain metabolic sensing and metabolic signaling at the level of an astrocyte

Nephtali Marina1,2 | Egor Turovsky3 | Isabel N Christie1 | Patrick S Hosford1
Anna Hadjihambi1 | Alla Korsak1 | Richard Ang1 | Svetlana Mastitskaya1
Shahriar Sheikhbahaei1 | Shefeeq M Theparambil1 | Alexander V Gourine1

1Centre for Cardiovascular and Metabolic Neuroscience, Neuroscience, Physiology & Pharmacology, University College London, London, WC1E 6BT, United Kingdom
2Research Department of Metabolism and Experimental Therapeutics, Division of Medicine, University College London, London, WC1E 6JJ, United Kingdom
3Laboratory of Intracellular Signalling, Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia

Correspondence
Nephtali Marina, Research Department of Metabolism and Experimental Therapeutics, Division of Medicine, University College London, London WC1E 6JJ, UK.
Email: n.marina@ucl.ac.uk
Alexander V. Gourine, Neuroscience, Physiology & Pharmacology, University College London, London WC1E 6BT, UK.
Email: a.gourine@ucl.ac.uk

Abstract
Astrocytes support neuronal function by providing essential structural and nutritional support, neurotransmitter trafficking and recycling and may also contribute to brain information processing. In this article we review published results and report new data suggesting that astrocytes function as versatile metabolic sensors of central nervous system (CNS) milieu and play an important role in the maintenance of brain metabolic homeostasis. We discuss anatomical and functional features of astrocytes that allow them to detect and respond to changes in the brain parenchymal levels of metabolic substrates (oxygen and glucose), and metabolic waste products (carbon dioxide). We report data suggesting that astrocytes are also sensitive to circulating endocrine signals—hormones like ghrelin, glucagon-like peptide-1 and leptin, that have a major impact on the CNS mechanisms controlling food intake and energy balance. We discuss signaling mechanisms that mediate communication between astrocytes and neurons and consider how these mechanisms are recruited by astrocytes activated in response to various metabolic challenges. We review experimental data suggesting that astrocytes modulate the activities of the respiratory and autonomic neuronal networks that ensure adaptive changes in breathing and sympathetic drive in order to support the physiological and behavioral demands of the organism in ever-changing environmental conditions. Finally, we discuss evidence suggesting that altered astroglial function may contribute to the pathogenesis of disparate neurological, respiratory and cardiovascular disorders such as Rett syndrome and systemic arterial hypertension.

KEYWORDS
brainstem, breathing, chemoreception, food intake, gut hormone, metabolism

1 INTRODUCTION

Living cells generate a constant supply of adenosine triphosphate (ATP) to provide the energy required to carry out fundamental cellular processes, such as cytoskeleton assembly, maintenance of membrane potential and excitability, membrane transport, cell movement/migration, intracellular and intercellular signaling. A complex hierarchy of behavioral, physiological and biochemical mechanisms ensure adequate delivery of metabolic substrates and effective elimination of metabolic waste products from all tissues of the body (Fell, 1997).
changes in membrane excitability, neurotransmitter release and gene expression. This, in turn, results in adaptive physiological responses that control multiple aspects of energy balance such as oxygen delivery, carbon dioxide elimination and feeding behavior, among others. More recently it has become evident that CNS metabolic sensing is more complex than originally thought and may involve glial cells in an active role.

In this special issue of GLIA we discuss the emerging evidence supporting the hypothesis that astrocytes function as versatile metabolic sensors of CNS milieu and by doing so play an important role in the maintenance of brain metabolic homeostasis. Cellular features of astrocytes allow them to detect and respond to changes in the brain parenchymal levels of metabolic substrates and metabolic waste products. Astrocytes are also sensitive to circulating hormones that modulate the activities of the neuronal circuits controlling food intake and energy balance. Recent evidence suggests that astrocytes modulate the activities of vital respiratory and autonomic neuronal networks that control breathing and autonomic balance while compromised astroglial function may contribute to the development and progression of disparate neurological, respiratory and cardiovascular diseases.

2 | ASTROCYTES

Astrocytes support neuronal function by providing structural and nutritional support as well as by facilitating neurotransmitter trafficking and recycling. There is also significant evidence to suggest that astrocytes contribute to CNS information processing (Halassa et al., 2009; Papouin, Dunphy, Tolman, Foley, & Haydon, 2017). The morphological and functional adaptations of astrocytes ideally position them to act as physiological sensors of brain metabolic milieu: (i) Sensory input: perturbations in metabolic milieu as well as systemic hormonal signals are detected by astrocytes residing in the hypothalamus and the brainstem (Chowen et al., 1999; Cheunsuang & Morris, 2005; Angelova et al., 2015; Garcia-Caceres et al., 2016; Turovsky et al., 2016). Astroglial processes and end feet surrounding the cerebral vasculature form one of the key elements of the brain–blood barrier. As a result, astrocytes are ideally placed to sense blood-borne metabolic and endocrine signals (Kacem, Lacombe, Seylaz, & Bonvento, 1998; Sofroniew & Vinters, 2010); (ii) Transduction mechanisms: astrocytes are not electrically excitable but display so-called “Ca2+ excitability” responding to various stimuli (e.g., chemical, mechanical, etc.) and certain neuronal cues with increases in intracellular [Ca2+] (Zheng et al., 2015; Bazargani & Attwell, 2016) followed by intracellular changes and/or the release of various signaling molecules (“gliotransmitters”). (iii) Neuromodulatory output: astrocytes have a dense network of finely branching processes that enwrap neuronal synapses forming one of the components of the so-called “tripartite synapse” (Perea, Navarrete, & Araque, 2009). These processes contain membrane proteins that play important roles in ensuring effective synaptic transmission such as glutamate transporters (Chaudhry et al., 1995), potassium channels (Higashi et al., 2001; Olsen, 2012), aquaporins (Thrane et al., 2011), and lactate transporters (Puchades, Sogn, Maehlen, Bergersen, & Gundersen, 2013). Increases in intracellular [Ca2+] in astrocytes can also trigger the release of gliotransmitters that interact with pre- and post-synaptic receptors and can potentially control neuronal network activity via modulation of synaptic transmission and neuronal excitability (Perea et al., 2009). Several molecules have been suggested to function as gliotransmitters, including ATP/adenosine, polyphosphate, d-serine, glutamate, GABA, and lactate (Volterra & Meldolesi, 2005; Rollenhagen et al., 2007; Holmstrom et al., 2013; Tang et al., 2014; Marina et al., 2015; Martin, Bajo-Graneras, Moratalla, Perea, & Araque, 2015; Papouin et al., 2017).

Thus, astrocytes appear to be strategically positioned to monitor the chemical composition of the arterial blood entering the brain, integrate it with the metabolic signals arising from the brain parenchyma and communicate this information to intermingled neuronal networks, enabling the initiation of coordinated adaptive physiological and behavioral responses that ensure homeostasis in dynamic environmental conditions (Gourine, 2005; Gourine & Kasparov, 2011; Teschemacher, Gourine, & Kasparov, 2015). Astrocytes are also at the center of the neurovascular interface and are able to release vasoactive molecules that regulate cerebral blood flow in accord with prevailing neuronal activity. This facilitates the supply of oxygen and glucose and the removal of CO2 in a process known as neurovascular coupling (Attwell et al., 2010).

3 | ASTROCYTES AS CNS METABOLIC SENSORS

3.1 | Sensing oxygen

Aerobic respiration is the key cellular process which breaks down metabolic substrates to produce molecules of ATP. In air-breathing animals the supply of oxygen and the removal of carbon dioxide involve the transfer of air between the atmosphere and the lungs by the process of alveolar ventilation, the diffusion of gas between alveoli and the pulmonary blood and the transport of oxygen and carbon dioxide to and from all tissues of the body, respectively. The partial pressure of oxygen (PO2) in the arterial blood is sensed by the peripheral oxygen chemoreceptors located in the carotid bifurcation and in the aortic arch (in some species). The chemosensitive glomus cells of the carotid body are traditionally considered to be the primary (and only) respiratory oxygen sensors in mammals. When activated by hypoxia, carotid bodies initiate a chemoreflex that results in activation of the respiratory and sympathetic circuits located in the brainstem. This leads to rapid respiratory and cardiovascular responses directed towards restoring the arterial PO2 (Guyenet, 2006; Kumar & Prabhakar, 2012). However, there is significant evidence that all mammals can survive denervation of the peripheral oxygen sensors and that hypoxic ventilatory response recoverers in experimental animals whose carotid bodies have been surgically denervated or removed, suggesting that the brain may also contain functional respiratory oxygen sensors (Davenport, Brewer, Chambers, & Goldschmidt, 1947; Miller & Tenney, 1975; Olson, Vidrik, & Dempsey, 1988; also see Gourine & Funk, 2017 for a comprehensive review).

Results of recent studies suggested that astrocytes may function as physiological sensors of brain oxygenation (Figure 1: Angelova et al., 2015). This was demonstrated using two-photon imaging of cortical astrocytes in anesthetized and mechanically ventilated rats, where decreases in inspired O2 (from 21% to 15% or 10%) were found to trigger robust increases in astroglial [Ca2+], (Angelova et al., 2015; Marina et al., 2015).
Figure 1: Hypothesized cellular mechanisms underlying astroglial oxygen and CO₂/pH sensitivities. (a) The astroglial signaling cascade triggered by hypoxia involves inhibition of mitochondrial respiration, facilitated formation of reactive oxygen species (ROS), lipid peroxidation, activation of phospholipase C (PLC), IP₃ receptors, release of Ca²⁺ from the intracellular stores and enhanced vesicular release of ATP. Hypoxia may also alter opening probability of connexin (Cx) hemichannels permeable to ATP and lactate. (b) Increases in PCO₂ gate open Cx hemichannels in Ca²⁺ and pH-independent manner allowing rapid egress of ATP in response to hypercapnia. If hypercapnic stimulus is persistent, intracellular acidification will occur and will eventually close Cx hemichannels. In conditions of decreased pH, astrocytes continue to release ATP by Ca²⁺-dependent exocytotic release mechanism. Intracellular acidification activates Na⁺/HCO₃⁻ cotransport (NBC) which brings Na⁺ inside the cell. Raising [Na⁺], activates Na⁺/Ca²⁺ exchanger (NCX) to operate in a reverse mode leading to Ca²⁺ entry. Released ATP acting in autocrine and paracrine manner spreads astroglial Ca²⁺ signals within the neuropil and enhances respiratory and sympathetic activities via excitation of the respiratory rhythm generating circuits of the pre-Bötzinger complex (preBötC), retrotrapezoid nucleus (RTN) neurons and sympathoexcitatory (pre-sympathetic) neurons of the brainstem.

Figure 2. In vitro experiments revealed that oxygen sensitivity is a general feature of brain astrocytes and that the hypoxia sensor is located in the mitochondria. Simultaneous measurements of mitochondrial membrane potential (ΔΨm) and [Ca²⁺], in cultured astrocytes showed that a decrease in PO₂ causes a significant decrease in ΔΨm, and that this response precedes increases in [Ca²⁺], (Figure 2d). Inhibition of mitochondrial respiration in these conditions was accompanied by increases in mitochondrial reactive oxygen species (ROS) production. Both hypoxia-induced ROS production and [Ca²⁺] responses in astrocytes were markedly reduced or abolished by mitochondrial uncoupler (FCCP), mitochondrial antioxidant (MitoQ) or ROS scavenger (α-tocopherol). Subsequent pharmacological analysis of Ca²⁺ responses suggested a feasible hypoxia-sensitive signaling pathway: in astrocytes hypoxia leads to inhibition of mitochondrial respiration, increased production of free radicals, lipid peroxidation, activation of phospholipase C and recruitment of Ca²⁺ from IP₃-sensitive intracellular stores (Figure 1) (Angelova et al., 2015).

It was reported that the PO₂ hypoxia threshold required to trigger Ca²⁺ responses in astrocytes is ~17 mmHg (Angelova et al., 2015). Normal level of arterial PO₂ is ~100 mmHg. Normal level of brain parenchymal PO₂ is between 20 and 30 mmHg with little difference between the mammalian species (Erecinska & Silver, 2001). A study conducted in climbers of Mt Everest reported mean arterial PO₂ of ~25 mmHg in four individuals breathing ambient air at 8400 m above sea level (Grocott et al., 2009). Although we do not know what was the brain tissue PO₂ in these conditions, these fascinating data suggest that the brain can operate in a very low oxygen environment (the participants were able to perform complex tasks), with parenchymal PO₂ sufficiently low to trigger astroglial activation and downstream sympathetic, respiratory and regional cerebrovascular responses (see below).

Experiments conducted to visualize vesicular fusion events in cultured rat brainstem astrocytes using total internal reflection fluorescence microscopy demonstrated that reductions in PO₂ facilitate exocytosis of ATP-containing vesicles (Angelova et al., 2015; Figure 1). Earlier in vitro and in vivo experiments using amperometric enzymatic ATP biosensors (Gourine et al., 2007; Gourine et al., 2008) showed that brain tissue hypoxia is indeed associated with the release of ATP, specifically in the brainstem areas which harbor the respiratory and sympathetic neuronal circuits (Gourine, Llaudet, Dale, & Spyer, 2005). Activation of these networks by ATP triggers compensatory cardiorespiratory responses that facilitate central respiratory drive, alveolar ventilation and oxygen transport.

The rhythm and pattern of breathing are generated by complex neuronal interactions within the so-called brainstem ventral respiratory column that extends from the dorsolateral pons to the caudal regions of the medulla oblongata (Feldman, Mitchell, & Nattie, 2003; Feldman, Del Negro, & Gray, 2013). The brainstem respiratory network ensures that the frequency, depth and pattern of lung ventilation are always adequate to maintain blood and brain PO₂, PCO₂ and pH within physiological ranges. Neurons that constitute the brainstem respiratory-rhythm generating circuits express ATP receptors and are strongly excited by ATP (Thomas & Spyer, 2000; Gourine, Atkinson, Deuchars, & Spyer, 2003; Huxtable et al., 2009). Recent data demonstrated that ATP, released by brainstem astrocytes in response to hypoxia, contributes to the development of the hypoxic ventilatory response (Angelova et al., 2015; Rajani et al., 2017). In rats, blockade of astroglial signaling
in the ventral regions of the brainstem by overexpression of ATP-degrading enzymes or targeted astrocyte-specific expression of tetanus toxin light chain (to block vesicular release mechanisms) was found to be associated with a significant reduction of the hypoxic ventilatory response. These data strongly suggested that the central stimulatory effect of hypoxia on breathing is mediated by an astroglial purinergic signaling mechanism (Angelova et al., 2015; Rajani et al., 2017).

The hypoxic ventilatory response is also accompanied by a coordinated cardiovascular response to ensure effective delivery of the oxygenated blood to all tissues of the body. Oxygen delivery is determined by cardiac output that is controlled (among other factors) by the activities of the sympathetic and parasympathetic branches of the autonomic nervous system. Groups of sympathoexcitatory (pre-sympathetic) brainstem neurons, including bilateral populations of catecholaminergic C1 neurons, are essential for the generation of cardiovascular sympathetic tone (Ross et al., 1984; Reis, Golanov, Ruggiero, & Sun, 1994; Guyenet, 2006; Marina et al., 2011; Wenker et al., 2017). These neurons send monosynaptic projections to sympathetic preganglionic neurons in the intermediolateral spinal cord, which in turn project to sympathetic ganglia innervating peripheral targets, including the heart, kidneys and the resistance arterioles of the skeletal muscle (Guyenet, 2006; Guyenet et al., 2013). There is strong evidence that the activities of the brainstem pre-sympathetic neuronal circuits are modulated by astrocytes (Marina et al., 2013; Marina et al., 2015). It was reported that [Ca\(^{2+}\)] response to hypoxia in brainstem slices in vitro was induced by gradual displacement of oxygen in the incubation medium with argon; (c) hypoxia-induced [Ca\(^{2+}\)], responses of brainstem astrocytes (PO\(_2\) threshold of activation 15 mmHg). In this example astrocytes were identified and their responses to hypoxia were assessed in organotypic brainstem slices in vitro using genetically encoded Ca\(^{2+}\) sensor Case12 expressed under the control of GFAP promoter; (d) simultaneous imaging of hypoxia-induced changes in 

The physiological significance of astroglial control of pre-sympathetic brainstem neurons becomes apparent during central hypoxia. These sympathoexcitatory neurons are known to be highly sensitive to decreases in local tissue PO\(_2\) or cytotoxic hypoxia, responding with profound excitation leading to generalized increases in central sympathetic drive (Sun & Reis, 1994; D’Agostino, Mazza, & Neubauer, 2009; Marina et al., 2015). It appears that the sensitivity of C1 pre-sympathetic neurons to hypoxia is largely indirect, and mediated by the actions of ATP and lactate released by neighboring astrocytes (Figure 1). First, hypoxia-induced excitation of C1 neurons was found to be markedly reduced by blockade of either metabolic ATP receptors or inhibition of glycogenolysis (Marina et al., 2015). Second, excitation of C1 neurons following optogenetic stimulation of astrocytes was significantly reduced in the presence of an ATP-degrading enzyme apyrase (Marina et al., 2013). Third, exogenous ATP and lactate were shown to mediate the excitation of C1 neurons in brainstem slices in vitro.
lactate induced potent excitation of C1 neurons in vitro and triggered sympatoexcitatory effects similar to those observed following optogenetic activation of brainstem astrocytes or brain hypoxia in vivo (Sun, Wahlestedt, & Reis, 1992; Horuchi, Potts, Tagawa, & Dampney, 1999; Ralevic, 2000; Marina et al., 2015). Finally, hypoxia facilitated release of both ATP (Gourine et al., 2005) and lactate (Karagiannis et al., 2016; Hadjihambi et al., 2017) in brainstem regions containing populations of pre-sympathetic neurons. While the effect of ATP on C1 neuronal activity is mediated by P2X and P2Y receptors (Sun et al., 1992; Ralevic, 2000; Wenker, Sobrinho, Takakura, Mulkey, & Moreira, 2013), the mechanisms underlying the excitatory effects of lactate remain unknown but appear to involve activation of as yet uncharacterized lactate-sensitive G coupled receptor (Tang et al., 2014).

The experimental data reviewed and discussed above suggest that astrocytes are intrinsically sensitive to hypoxia and play an important role in the development of coordinated ventilatory and cardiovascular responses to decreases in brain (stem) PO2. There is also evidence that astroglial oxygen sensitivity may contribute to the pathogenesis of certain diseases associated with brain hypoxia. Congestive heart failure and systemic arterial hypertension are highly prevalent conditions characterized by sustained increases in sympathetic nerve activity, which is generally believed to have long-term detrimental effects and contribute to the disease progression (Naughton et al., 1995; Esler et al., 2001; Mansfield et al., 2003). Human and experimental animal studies revealed that both heart failure and systemic arterial hypertension are associated with lower brain PO2 even when arterial PO2 is within the normal physiological range (Rifai, Winters, Friedman, & Silver, 2012; Marina et al., 2015; Turlejski, Humoud, Desai, Smith, & Marina, 2016; Hosford, Millar, Ramage, & Marina, 2017). The mechanisms underlying compromised brain oxygenation in these conditions are complex and remain poorly understood (Cates, Steed, Abdala, Langton, & Paton, 2011; Cates, Dickinson, Hart, & Paton, 2012; Marina et al., 2015; Marina, Teschemacher, Kasparov, & Gourine, 2016), however, brainstem parenchymal hypoxia might contribute to sustained excitation of the pre-sympathetic circuits via the actions of ATP and lactate released by astrocytes at low tissue PO2 (Marina et al., 2013; Marina et al., 2015). To test this hypothesis, two studies in rats used viral gene targeting of pre-sympathetic regions of the brainstem to overexpress a potent ectonucleotidase—transmembrane prostatic acid phosphatase. Activity of this enzyme effectively prevents vesicular accumulation of ATP (Wells et al., 2015) and facilitates degradation of extracellular ATP (Marina et al., 2013). Blockade of ATP-mediated signaling within the pre-sympathetic brainstem regions slowed the progression of cardiac remodeling in animals with myocardial infarction-induced heart failure (Marina et al., 2013) and reduced systemic arterial blood pressure in spontaneously hypertensive rats (Marina et al., 2015). Subsequent studies provided further evidence of brainstem tissue hypoxia and astrogliosis in hypertensive rats (Turlejski et al., 2016). Together these data support the hypothesis that sustained astrogial activation in conditions of brainstem tissue hypoxia might be responsible for maintaining heightened sympathetic drive that contributes to the development and progression of cardiovascular disease (Marina et al., 2013; Marina et al., 2015; Marina et al., 2016).

Finally, there is evidence that brain tissue PO2 is the key metabolic factor that determines the direction of cerebral arteriole response (constriction at high PO2 and dilatation at physiological/low PO2) that follow astrogial [Ca2+]i elevations (Gordon, Choi, Rungta, Ellis-Davies, & MacVicar, 2008). Since Ca2+-dependent release of vasoactive substances by astrocytes can alter cerebral blood flow (Attwell et al., 2010; Mishra et al., 2016; Bazargani & Attwell, 2016), the mechanism of direct oxygen sensing by astrocytes (Angelova et al., 2015) may be important for the regulation of cerebral microcirculation in conditions of increased local oxygen demand or regional brain tissue hypoxia. While this hypothesis awaits experimental scrutiny, the available data strongly suggest that detection of hypoxia by brainstem astrocytes stimulates the networks of the respiratory and pre-sympathetic neurons and contributes to the development of the ventilatory and cardiovascular responses which ensure appropriate oxygenation and delivery of the arterial blood.

### 3.2 | Sensing glucose

Glucose is an important source of energy and a substrate for many biochemical reactions. Blood glucose level fluctuates between ~70 and 100 mg dl−1 throughout a 24-hr period in fasting conditions and may increase up to 140 mg dl−1 within the first 2 hr after ingestion of a meal. Intricate neural and hormonal control mechanisms operate to maintain blood glucose level within a physiological range and to ensure that the metabolic demands of all tissues of the body, and of the brain in particular, are satisfied.

Diabetic patients treated with insulin and sulphonylureas are at increased risk of acute hypoglycemia. Hypoglycemia can have profound deleterious effects on the neuronal function, leading to permanent brain damage and even death (Frier, 2014). Sustained elevations in plasma glucose can also have various adverse effects on vital organs, including the brain. Therefore, physiological glucose sensing is critically important for homeostasis which is ensured via recruitment of the hormonal (insulin and glucagon secretion), autonomic (liver glucose production) and behavioral (feeding initiation and termination) mechanisms.

There is evidence that brainstem astrocytes may function as CNS glucose sensors. Mice with genetic deletion of the glucose transporter type 2 (GLUT2) were not able to respond to systemic hypoglycemia with increased glucagon secretion (Marty et al., 2005). However, the capacity to release glucagon in response to hypoglycemia was restored by selective re-expression of GLUT2 in the brainstem glial cells, but not in neurons (Marty et al., 2005). An earlier study reported that hypoglycemia-induced activation of neurons in the hypothalamus and the brainstem was blocked in conditions when astroglial glutamate metabolism was compromised by application of glutamine synthetase inhibitor methionine sulfoximine (Young, Baker, & Montes, 2000). Together, these data suggest that detection of hypoglycemia by the brain may require metabolic coupling and signaling between astrocytes and neurons. Consistent with this hypothesis, astrocytes have been shown to control (via release of lactate) the activities of hypothalamic orexin neurons which promote arousal, stimulate food intake and
hepatic glucose production (Parsons & Hirasawa, 2010). However, the cellular and molecular mechanisms underlying glucose sensitivity of astrocytes remain to be determined.

Recently, Garcia-Caceres and colleagues (2016) reported data suggesting that astroglial insulin signaling modulates hypothalamic glucose sensing and systemic glucose metabolism. The authors demonstrated that ablation of insulin receptors in hypothalamic astrocytes reduced glucose-induced activation of pro-opio-melanocortin neurons and impaired physiological responses to changes in glucose availability. Following systemic glucose administration, cerebrospinal fluid accumulation of glucose and insulin were found to be reduced in mice lacking astroglial insulin receptors. The authors concluded that brain glucose sensing and, therefore, systemic glucose metabolism are controlled, at least in part, by insulin acting at hypothalamic astrocytes. Moreover, the data reported by Garcia-Caceres et al. (2016) also suggested that astroglial insulin receptors play an important role in modulating glucose transfer across the blood–brain barrier.

### 3.3 | Sensing carbon dioxide and pH

Metabolic homeostasis also requires effective elimination of waste products. Carbon dioxide is generated in proportion to the metabolic rate and the amount of metabolic substrates utilized. At rest, our body produces ~12 mmol kg$^{-1}$ h$^{-1}$ of CO$_2$; most of which is removed with expired air through the process of alveolar ventilation. The partial pressure of CO$_2$ (PCO$_2$) in the arterial blood is directly proportional to the rate of CO$_2$ production and inversely proportional to the rate of CO$_2$ elimination by the respiratory system. Increased CO$_2$ production and/or impaired CO$_2$ elimination facilitates generation of hydrogen ions (respiratory acidosis), a condition that needs to be rapidly corrected by adaptive changes in the ventilatory and cardiovascular activities in order to ensure effective CO$_2$ removal.

It is generally believed that changes in the arterial and brain PCO$_2$/pH are monitored by specialized pH-sensitive neurons residing in the brainstem (Loeschcke, 1982). Current models of central respiratory CO$_2$ chemosensitivity (the mechanism that adjusts breathing in accordance with changes in brainstem parenchymal PCO$_2$/pH) are focused on a group of pH-sensitive neurons of the retrotrapezoid nucleus (RTN) located near the ventral surface of the brainstem. Garcia-Caceres et al. (2016) also suggested that astroglial insulin receptors play an important role in modulating glucose transfer across the blood–brain barrier.

3.3 | Sensing carbon dioxide and pH

Metabolic homeostasis also requires effective elimination of waste products. Carbon dioxide is generated in proportion to the metabolic rate and the amount of metabolic substrates utilized. At rest, our body produces ~12 mmol kg$^{-1}$ h$^{-1}$ of CO$_2$; most of which is removed with expired air through the process of alveolar ventilation. The partial pressure of CO$_2$ (PCO$_2$) in the arterial blood is directly proportional to the rate of CO$_2$ production and inversely proportional to the rate of CO$_2$ elimination by the respiratory system. Increased CO$_2$ production and/or impaired CO$_2$ elimination facilitates generation of hydrogen ions (respiratory acidosis), a condition that needs to be rapidly corrected by adaptive changes in the ventilatory and cardiovascular activities in order to ensure effective CO$_2$ removal.

It is generally believed that changes in the arterial and brain PCO$_2$/pH are monitored by specialized pH-sensitive neurons residing in the brainstem (Loeschcke, 1982). Current models of central respiratory CO$_2$ chemosensitivity (the mechanism that adjusts breathing in accordance with changes in brainstem parenchymal PCO$_2$/pH) are focused on a group of pH-sensitive neurons of the retrotrapezoid nucleus (RTN) located near the ventral surface of the brainstem (Guyenet, & Mulkey, 2010). This view is supported by the results of the experimental studies which demonstrated that the permanent loss or acute silencing of RTN neurons abolishes or significantly reduces ventilatory CO$_2$ sensitivity (Dubreuil et al., 2008; Guyenet et al., 2009; Guyenet & Mulkey, 2010; Marina et al., 2010; Ramanatsoa et al., 2011). Although other notable groups of brainstem neurons, including 5-HT neurons of the raphe nuclei, are intrinsically chemosensitive (Teran, Massey, & Richerson, 2014) and contribute to the development of the ventilatory response to CO$_2$ (Ray et al., 2011), the current prevailing view is that pH-sensitive RTN neurons play the key role (Guyenet et al., 2016).

However, there is evidence that pH-sensitivity of RTN neurons is, to a large extent, indirect and mediated by the responses triggered by the chemosensory stimuli in the neighboring astrocytes (Gourine et al., 2010). Experiments conducted in anesthetized and mechanically ventilated rats demonstrated that astrocytes residing near the ventral surface of the brainstem (within the RTN region) respond to decreases in pH with robust elevations in intracellular [Ca$^{2+}$] (Gourine et al., 2010; Figure 3). This triggers Ca$^{2+}$-dependent vesicular release of ATP (Gourine et al., 2010; Kasymov et al., 2013) which propagates Ca$^{2+}$ excitation among neighboring astrocytes, activates RTN neurons (Gourine et al., 2010) as well as respiratory neurons that constitute other functional divisions of the ventral respiratory column (Gourine et al., 2003).

The mechanisms linking the detection of PCO$_2$/[H$^+$] increases with [Ca$^{2+}$]$_i$ responses in brainstem astrocytes are dependent on the activities of certain membrane transporters (Turovsky et al., 2016). Astroglial [Ca$^{2+}$]$_i$, responses triggered by CO$_2$-induced acidification (respiratory acidosis) were found to be preceded by Na$^+$ entry, reduced by inhibition of the Na$^+$/HCO$_3^-$ cotransporter (NBC) or Na$^+$/Ca$^{2+}$-exchange (NCX) and abolished in the absence of extracellular Na$^+$. Acidification-induced Ca$^{2+}$ responses were also dramatically reduced in brainstem astrocytes of mice deficient in the electrogenic Na$^+$/HCO$_3^-$ cotransporter NBCe1 (Turovsky et al., 2016). Thus, coupled NBC and NCX activities appear to underlie functional pH-sensitivity of brainstem astrocytes leading to the increases in intracellular [Ca$^{2+}$]$_i$ (Figure 1).
This CO\textsubscript{2}-induced release of ATP was dependent upon the structural integrity of the subpial astrocyte layer and occurred prior to the increases in central respiratory drive (Gourine et al., 2005; Huckstepp et al., 2010). Furthermore, CO\textsubscript{2}-induced ventilatory responses were significantly reduced following blockade of ATP receptors at the sites of release (Gourine et al., 2005). The effect of CO\textsubscript{2} on breathing was mimicked by application of ATP to the brainstem sites of release as well as by optogenetic stimulation of brainstem astrocytes (Gourine et al., 2005; Gourine et al., 2010; Figueiredo et al., 2011).

Impaired astroglial mechanisms may contribute to the development of abnormal breathing patterns observed in some prototypical neurological disorders. In humans, mutations of the transcriptional regulator methyl-CpG-binding protein 2 (MeCP2) gene lead to a neurodevelopmental disorder called Rett syndrome, which is characterized by irregular breathing pattern and blood gas instability (Southall et al., 1988; Viemari et al., 2005; Ramirez, Ward, & Neul, 2013). MeCP2 is highly expressed in astrocytes (Yasui et al., 2013) and loss of MeCP2 leads to astrogial dysfunction (Okabe et al., 2012). In a mouse model of Rett syndrome (global MeCP2 gene knockout), sensitivity of brainstem astrocytes to changes in P\textsubscript{CO2}/pH is markedly reduced (Turovsky, Karagiannis, Abdala, & Gourine, 2015) and ventilatory CO\textsubscript{2} sensitivity is severely impaired (Bissonnette, Schaevitz, Knopp, & Zhou, 2014). Moreover, it was reported that in mice, conditional astrocyte-specific deletion of MeCP2 is sufficient to dramatically impair CO\textsubscript{2}-induced ventilatory response (Garg, Lioy, Knopp, & Bissonnette, 2015). Remarkably, in global MeCP2 gene knockout mice, selective re-expression of MeCP2 in astrocytes rescues the normal respiratory pattern (Lioy et al., 2011). These data indicate that the brainstem networks of the respiratory neurons, including chemo-sensitive RTN neurons, are not able to mount an appropriate ventilatory response to CO\textsubscript{2} when astroglial function and pH-sensitivity are compromised, supporting the idea of a critical role played by astroglial pH-sensitivity in the CNS mechanisms.
which transmit changes in brain parenchymal $\text{PCO}_2$/$\text{pH}$ into a modified pattern of breathing. It remains to be determined whether astroglial dysfunction may also contribute to the expression of altered breathing patterns (e.g., central sleep apnoea) observed in some other pathological conditions.

Finally, a recent study has suggested that astrocytes may also mediate cerebrovascular responses to $\text{CO}_2$. Howarth and colleagues (2017) reported that in anesthetized mice, increases in the level of inspired $\text{CO}_2$ trigger $[\text{Ca}^{2+}]_i$ responses in cortical astrocytes, which in turn may evoke cerebral vessel dilations via stimulation of COX-1 activity followed by Pgf$_2$ release and its action on cerebrovascular smooth muscle cells (Howarth et al., 2017).

## 4 | ASTROCYTES AS CNS SENSORS OF METABOLIC ENDOCRINE SIGNALS

Metabolic homeostasis is ensured by coordinated adaptive physiological and behavioral responses to a multitude of endogenous and environmental factors and most importantly by the availability of nutrients. The control of food intake in particular is generally believed to depend on the ability of specialized neurons located in the hypothalamus and the brainstem to detect and integrate various humoral and afferent neuronal signals which provide information about the nutritional state and energy demands of the organism (Caron & Richard, 2017). Considering that astrocytes function as CNS metabolic sensors we next explored whether these glial cells are sensitive to endocrine metabolic signals released from the gut and the adipose tissue. The data reported below were obtained using standard experimental models and protocols described in detail in our previous publications (Gourine et al., 2010; Kasymov et al., 2013; Angelova et al., 2015; Turovsky et al., 2015; Turovsky et al., 2016).

### 4.1 | Ghrelin

Ghrelin (also known as growth hormone-releasing peptide) is the only circulating peptide known to stimulate appetite and increase food intake. Ghrelin is mainly produced and released by oxyntic glands of the gastric fundus and its CNS actions increase food intake, produce weight gain, and promote adiposity via increased production of orexigenic neuropeptides such as neuropeptide Y and Agouti-related peptide (AGRP) by the neurons of the arcuate nucleus of the hypothalamus, which in turn may evoke cerebral vessel dilations via stimulation of COX-1 activity followed by Pgf$_2$ release and its action on cerebrovascular smooth muscle cells (Howarth et al., 2017).

Ghrelin (also known as growth hormone-releasing peptide) is the only circulating peptide known to stimulate appetite and increase food intake. Ghrelin is mainly produced and released by oxyntic glands of the gastric fundus and its CNS actions increase food intake, produce weight gain, and promote adiposity via increased production of orexigenic neuropeptides such as neuropeptide Y and Agouti-related peptide (AGRP) by the neurons of the arcuate nucleus of the hypothalamus (Wren et al., 2001; Greenman et al., 2004). There is evidence that the effect of ghrelin on food intake is suppressed by activation of astrocytes which inhibit hypothalamic AGRP-producing neurons via the release of ATP/adenosine acting at A1 receptors (Yang, Qi, & Yang, 2015).

To determine the effect of ghrelin on astrocytes we used dissociated neuroglial cultures transduced to express a genetically encoded $\text{Ca}^{2+}$ indicator GCaMP6f. Astrocytes were transduced using an adeno-associated viral vector designed to drive the expression of GCaMP6f under the transcriptional control of the GFAP promoter (Jiang, Hausstein, Sofroniew, & Khakh, 2014). The majority of astrocytes expressing GCaMP6f responded to application of a prototypical glial signaling molecule ATP (10 $\mu$M) with elevations in $[\text{Ca}^{2+}]_i$ (Figure 4a–e). Ghrelin triggered robust $[\text{Ca}^{2+}]_i$ responses in $\sim$30% of brainstem astrocytes that were activated by ATP (Figure 4a). Strong $[\text{Ca}^{2+}]_i$ responses in astrocytes were induced following application of ghrelin in concentrations as little as 1 nM (Figure 4a). Ghrelin-induced $[\text{Ca}^{2+}]_i$ responses in astrocytes were effectively blocked by ghrelin receptor (GHSR1a) antagonist [(D-Lys3)-GMPR-6] (Figure 4d).

These data suggest that astrocytes (at least in culture) express functional ghrelin receptors and respond to physiological concentrations of ghrelin with elevations in intracellular $[\text{Ca}^{2+}]_i$. It remains to be determined whether astrocytes in situ are sensitive to ghrelin and may ultimately mediate or modulate the effects of ghrelin on neighboring neurons.

### 4.2 | Glucagon-like peptide (GLP-1)

GLP-1 is one of many hormones known to induce satiety. GLP-1 belongs to the family of incretin peptides that are produced by the intestinal epithelial L-cells (Tian & Jin, 2016). Actions of GLP-1 include stimulation of glucose-dependent insulin secretion, inhibition of glucagon secretion and stimulation of somatostatin secretion (Tian & Jin, 2016). GLP-1 also delays gastric emptying, inhibits gastrointestinal motility, and contributes to the physiological control of feeding behaviour (Holst, 2007). GLP-1 receptors (GLP-1R) are expressed in the brain regions involved in the control of energy metabolism, such as mediodorsal hypothalamus and the caudal brainstem (Cork et al., 2015) and central actions of GLP-1 reduce food intake (Tang-Christensen et al., 1996; Turton et al., 1996). There is recent evidence suggesting that astrocytes may mediate the effects of central GLP-1R activation on feeding behavior. Results of the experiments conducted in rat brainstem slices showed that astrocytes residing in the dorsal brainstem respond to GLP-1R activation with elevations in $[\text{Ca}^{2+}]_i$. (Reiner et al., 2016). Moreover, poisoning of dorsal brainstem astrocytes with fluorocitrate attenuated the effect of GLP-1R activation on food intake when GLP-1 analog Exendin-4 (Ex-4) was administered into the same brainstem site (Reiner et al., 2016). However, these data should be interpreted with some caution since fluorocitrate is not a selective “glial toxin” and depending on the concentration and the experimental conditions could potentially inhibit tricarboxylic acid cycle of all cells.

Experiments conducted using cultures of brainstem astrocytes transduced to express GCaMP6f revealed that relatively high concentrations of GLP-1R agonist Ex-4 are needed to elicit $[\text{Ca}^{2+}]_i$ responses (Figure 4b). The threshold dose of Ex-4 required to trigger robust $[\text{Ca}^{2+}]_i$ elevations in cultured astrocytes was found to be 100 nM (Figure 4b). $[\text{Ca}^{2+}]_i$ responses to GLP-1R activation were observed in $\sim$30% of astrocytes that responded to the application of ATP. Ex-4-induced $[\text{Ca}^{2+}]_i$ responses in astrocytes were abolished in the presence of the GLP-1R antagonist Exendin 9-39 (Figure 4e). Interestingly, astrocytes that displayed $[\text{Ca}^{2+}]_i$ responses to ghrelin were also sensitive to GLP-1R activation (Figure 4c). Blockade of GLP-1R had no effect on $[\text{Ca}^{2+}]_i$ responses triggered by ghrelin, while blockade of GHSR1a receptors had no effect on Ex-4-induced $[\text{Ca}^{2+}]_i$ transients (Figure 4d.e). These data suggest that individual brainstem astrocytes
are able to respond to various endocrine signals with apparently opposing central physiological actions.

There is evidence that the central effects of GLP-1 analogs are associated with excitation of pre-sympathetic C1 neurons, increases in central sympathetic drive, systemic arterial blood pressure and heart rate (Yamamoto et al., 2002). Although, these effects are likely to be attributed to direct activation of neuronal GLP-1Rs, the data reported and discussed above suggest that brainstem astrocytes may contribute to GLP-1-induced sympathoexcitation via Ca²⁺-dependent release of signaling molecules that activate pre-sympathetic circuits (e.g., ATP). This hypothesis can be tested in the future by recording the sympathoexcitatory brainstem regions in conditions when astroglial signaling pathways are blocked using molecular approaches.

4.3 | Leptin

Leptin is a hormone produced mainly by the white and brown adipose tissue that plays an important role in the control of energy metabolism. Leptin crosses the blood–brain barrier through a saturable transport system (Banks, Kastin, Huang, Jaspan, & Maness, 1996) and induces receptor (ObR)-mediated inhibition of the release of orexigenic peptides (neuropeptide Y and AGRP) produced by the neurons of the hypothalamic arcuate nucleus (Elias et al., 1999; van den Top, Lee, Whymont, Blanks, & Spanswick, 2004). Leptin also stimulates the release of anorectic peptides (e.g., pro-opio melanocortin; Elias et al., 1999; Cowley et al., 2001). These actions of leptin inhibit appetite and increase energy expenditure.

Previously the central effects of leptin were thought to be primarily mediated via its direct actions on hypothalamic neurons and endothelial cells but recent evidence suggests that some of these effects are mediated by astrocytes. Astrocytes express several ObR splice variants and leptin application has been shown to trigger strong and sustained [Ca²⁺] responses in primary astrocytes from mouse hypothalamus (Hsuchou, Pan, Barnes, & Kastin, 2009). Mice with genetic- and diet-induced obesity show an upregulation of ObR expression in hypothalamic astrocytes and a concomitant downregulation of ObR expression in neurons (Hsuchou et al., 2009; Pan et al., 2008). Another study reported enhanced leptin uptake by hypothalamic neurons in the presence of fluorocitrate, suggesting that astrocytes may play a certain role in the distribution of this hormone among different cellular compartments (Pan et al., 2011).
Several studies conducted in rats reported that direct administration of leptin to the pre-sympathetic regions of the brainstem triggers profound and sustained increases in central sympathetic drive and systemic arterial blood pressure (Haynes, Morgan, Walsh, Mark, & Sivitz, 1997; Barnes & McDougal, 2014). Moreover, leptin actions in the brainstem were found to enhance the baseline respiratory activity and ventilatory sensitivity to CO₂ in leptin deficient mice (ob/ob; Bassi et al., 2014). These effects of leptin on the respiratory and sympathetic activities mirror the effects of astroglial activation. However, it remained unclear whether the cardiorespiratory effects of leptin result from its direct actions on the respiratory and pre-sympathetic neuronal circuits or secondary to the responses elicited by this hormone in neighboring astrocytes.

We evaluated the effect of leptin on [Ca²⁺] in cultured brainstem astrocytes transduced to express the genetically encoded Ca²⁺ indicator. These effects of leptin on the respiratory and sympathetic activities mirror the effects of astroglial activation. However, it remained unclear whether the cardiorespiratory effects of leptin result from its direct actions on the respiratory and pre-sympathetic neuronal circuits or secondary to the responses elicited by this hormone in neighboring astrocytes. We evaluated the effect of leptin on [Ca²⁺] in cultured brainstem astrocytes transduced to express the genetically encoded Ca²⁺ indicator.
indicator Case12 under the control of the GFAP promoter (Gourine et al., 2010). Leptin evoked robust \([\text{Ca}^{2+}]_i\) responses in \(~40\%\) of brainstem astrocytes that responded to ATP application (Figure 5a,b). The threshold dose of leptin required to trigger \([\text{Ca}^{2+}]_i\) elevations in these astrocytes was found to be 5 nM (Figure 5b).

To test whether changes in the neuronal activity induced by leptin might be secondary to astroglial activation and mediated by the release and actions of gliotransmitters, we next determined the effect of leptin on \([\text{Ca}^{2+}]_i\) in astrocytes and neurons recorded in organotypic brainstem slices (cut at the "pre-sympathetic level") bulk-loaded with the \([\text{Ca}^{2+}]_i\) indicator Oregon Green-488 BAPTA-1 AM (OGB-488). Astrocytes were identified by labeling with sulforhodamine 101 (SR101) as described previously (Turovsky et al., 2015). Cells labeled with SR101 displayed robust \([\text{Ca}^{2+}]_i\) responses to ATP application and were insensitive to KCl (Figure 5c), indicating that these cells are astrocytes. Leptin-induced \([\text{Ca}^{2+}]_i\) responses in astrocytes were unaffected in the presence of P2Y receptor antagonist MRS2179 or ATP-hydrolyzing enzyme apyrase (Figure 5d). In contrast, leptin-induced \([\text{Ca}^{2+}]_i\) responses in brainstem neurons (identified by lack of SR101 labeling, and robust \([\text{Ca}^{2+}]_i\) responses to KCl) were abolished by either MRS2179 or apyrase (Figure 5d). Although the phenotype of the neurons recorded in these experiments was not characterized, these results suggest that the CNS behavioral and physiological effects of leptin may involve recruitment of astroglial signaling pathways, release of gliotransmitter ATP and activation of the neuronal purinergic receptors.

Together, these data suggest that astrocytes residing in brain regions involved in the control of energy metabolism (hypothalamus and the brainstem) are sensitive to key hormonal factors whose central actions provide important information about the nutritional state and energy demands of the organism. It remains to be determined whether these sensitivities are exclusive features of astrocytes residing in brain areas involved in metabolic control. It also remains to be determined whether chronic exposure to the elevated levels of these hormones may alter astroglial function and signaling mechanisms. This may have a significant impact on the control of feeding behavior and cardiorespiratory homeostasis and ultimately contribute to the pathogenesis of metabolic and/or cardiovascular disease.

| SUMMARY |
|---------------------------------------------|
| There is growing evidence to suggest that astrocytes actively monitor CNS metabolic milieu and contribute to the development of adaptive physiological respiratory, cardiovascular and behavioral responses which maintain metabolic homeostasis. Anatomical and functional features of astrocytes allow them to detect and respond to changes in the brain parenchymal levels of metabolic substrates (O\(_2\) and glucose), metabolic by products (CO\(_2\)), and hormonal metabolic factors involved in the CNS mechanisms controlling food intake and energy balance. In the brainstem, astrocytes modulate the activities of the neuronal circuits responsible for the generation of the respiratory and autonomic rhythms and the development of the adaptive changes in breathing and sympathetic nerve activity in conditions of increased metabolic demand. The key signaling molecule which mediates communication between astrocytes and the brainstem cardiorespiratory networks appears to be ATP, although other gliotransmitters (e.g., lactate) may also play a role. Furthermore, there is evidence that altered astroglial function may contribute to the pathogenesis of disparate respiratory and cardiovascular disorders such as Rett syndrome, heart failure and systemic arterial hypertension. |

| ACKNOWLEDGMENT |
|-----------------|
| Results of the authors’ experimental studies described in this review article were obtained with generous support of The Wellcome Trust (Refs: 095064 and 200893), British Heart Foundation (Ref: RG/14/4/30736), and the Medical Research Council (Ref: MR/N02589X/1). A.V.G. is a Wellcome Trust Senior Research Fellow. N.M. was also supported by the British Heart Foundation Intermediate Basic Science Research fellowship (FS/13/5/29927). |

| ORCID |
|-------|
| Neptali Marina [http://orcid.org/0000-0001-9921-660X] |
| Shahriar Sheikhbahaei [http://orcid.org/0000-0003-4119-9979] |
| Alexander V Gourine [http://orcid.org/0000-0003-3068-491X] |

| REFERENCES |
|-------------|
| Anand, B. K., Chhina, G. S., Sharma, K. N., Dua, S., & Singh, B. (1964). Activity of single neurons in the hypothalamic feeding centers: Effect of glucose. American Journal of Physiology, 207, 1146–1154. |
| Angelova, P. R., Kasymov, V., Christie, I., Sheikhbahaei, S., Turovsky, E., Marina, N., ..., Gourine, A. V. (2015). Functional Oxygen Sensitivity of Astrocytes. Journal of Neuroscience, 35, 10460–10473. |
| Attwell, D., Buchan, A. M., Charpak, S., Lauritzen, M., Macvicar, B. A., & Newman, E. A. (2010). Glial and neuronal control of brain blood flow. Nature, 468, 232–243. |
| Banks, W. A., Kastin, A. J., Huang, W., Jaspan, J. B., & Maness, L. M. (1996). Leptin enters the brain by a saturable system independent of insulin. Peptides, 17, 305–311. |
| Barnes, M. J., & McDougall, D. H. (2014). Leptin into the rostral ventral lateral medulla (RVLM) augments renal sympathetic nerve activity and blood pressure. Frontiers in Neuroscience, 8, 232. |
| Bassi, M., Furuya, W. I., Menani, J. V., Colombari, D. S., do Carmo, J. M., da Silva, A. A., … Colombari, E. (2014). Leptin into the ventrolateral medulla facilitates chemorespiratory response in leptin-deficient (ob/ob) mice. Acta Physiologica (Oxf), 211, 240–248. |
| Bazargani, N., & Attwell, D. (2016). Astrocyte calcium signaling: The third wave. Nature Neuroscience, 19, 182–189. |
| Bissonnette, J. M., Schaevitz, L. R., Knopp, S. J., & Zhou, Z. (2014). Respiratory phenotypes are distinctly affected in mice with common Rett syndrome mutations MeCP2 T158A and R168X. Neuroscience, 267, 166–176. |
| Caron, A., & Richard, D. (2017). Neuronal systems and circuits involved in the control of food intake and adaptive thermogenesis. Annals of the New York Academy of Science, 1391, 35–53. |
| Cates, M. J., Steed, P. W., Abdala, A. P., Langton, P. D., & Paton, J. F. (2011). Elevated vertebrobasilar artery resistance in neonatal spontaneously hypertensive rats. Journal of Applied Physiology, (1985), 111, 149–156. |
regulation by the retrotrapezoid nucleus. *Journal of Physiology*, 594, 1529–1551.

Hadjihambi, A., De Chiara, F., Hosford, P. S., Habtezion, A., Karagiannis, A., Davies, N., . . . Jalan, R. (2017). Ammonia mediates cortical hemichannel dysfunction in rodent models of chronic liver disease. *Hepatology*, 65, 1306–1318.

Halassa, M. M., Florian, C., Fellin, T., Munoz, J. R., Lee, S. Y., Abel, T., . . . Frank, M. G. (2009). Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron*, 61, 213–219.

Haynes, W. G., Morgan, D. A., Walsh, S. A., Mark, A. L., & Sivitz, W. I. (2013). Differential sensitivity of brainstem versus cortical astrocytes to changes in pH reveals functional regional specialization of astroglia. *Journal of Neuroscience*, 33, 435–441.

Haywood, P., Kuo, F., Bellemare, L., Perez, D., Dubreuil, T., & Mulkey, D. (2014). Conditional knockout of Kir4.1 in astrocytes blunts the hypercapnic respiratory response in awake mice. *Faseb J*, 28 (872), 7.

Higashi, K., Fujita, A., Inanobe, A., Tanemoto, M., Doi, K., Kubo., & Kuraoka, Y. (2001). An inwardly rectifying K+ channel, K+4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. *American Journal of Physiology - Cell Physiology*, 281, C922–C931.

Holmstrom, K. M., Marina, N., Baev, A. Y., Wood, N. W., Gourine, A. V., & Abramov, A. Y. (2013). Signalling properties of inorganic polyphosphate in the mammalian brain. *Nature Communications*, 4, 1362.

Holst, J. J. (2007). The physiology of glucagon-like peptide 1. *Physiological Reviews*, 87, 1409–1439.

Horiiuchi, J., Potts, P. D., Tagawa, T., & Dampney, R. A. (1999). Effects of activation and blockade of P2X receptors in the ventrolateral medulla on arterial pressure and sympathetic activity. *Journal of the Autonomic Nervous System*, 76, 118–126.

Hosford, P. S., Millar, J., Ramage, A. G., & Marina, N. (2017). Abnormal oxygen homeostasis in the nucleus tractus solitarius of the spontaneously hypertensive rat. *Experimental Physiology*, 102, 389–396.

Howarth, C., Sutherland, B., Choi, H. B., Martin, C., Lind, B. L., Khennouf, L., . . . MacVicar, B. A. (2017). A critical role for astrocytes in hypercapnic vasodilation in brain. *Journal of Neuroscience*, 37, 2403–2414.

Hsichou, H., He, Y., Kastin, A. J., Tu, H., Markadakis, E. N., Rogers, R. C., . . . Pan, W. (2009). Obesity induces functional astrocytic leptin receptors in hypothalamus. *Brain*, 132, 889–902.

Hsichou, H., Pan, W., Barnes, M. J., & Kastin, A. J. (2009). Leptin receptor mRNA in rat brain astrocytes. *Peptides*, 30, 2275–2280.

Huckstepp, R. T., Id Bihi, R., Eason, R., Spyer, K. M., Dicke, N., Willecke, K., . . . Dale, N. (2010). Connexin hemichannel-mediated CO2-dependent release of ATP in the medulla oblongata contributes to central respiratory chemosensitivities. *Journal of Physiology*, 588, 3901–3920.

Huckstepp, R. T., Llaudet, E., & Gourine, A. V. (2016). CO2-induced ATP-dependent release of acetylcholine on the ventral surface of the medulla oblongata. *PLoS One*, 11, e0167861.

Huxtable, A. G., Zwicker, J. D., Poon, B. Y., Pagliardini, S., Vrouwe, S. Q., Greer, J. J., & Funk, G. D. (2009). Tripartite purinergic modulation of central respiratory networks during perinatal development: The influence of ATP, ectonucleotidases, and ATP metabolites. *Journal of Neuroscience*, 29, 14713–14725.

Jiang, R., Haustein, M. D., Sofroniew, M. V., & Khakh, B. S. (2014). Imaging intracellular Ca2+ signals in striatal astrocytes from adult mice using genetically-encoded calcium indicators. *Journal of Visualized Experiments*, 93, e51972.

Kacem, K., Lacombe, P., Seylaz, J., & Bonvento, G. (1998). Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: A confocal microscopy study. *Glia*, 23, 1–10.

Karagiannis, A., Sylantyev, S., Hadjihambi, A., Hosford, P. S., Kasparov, S., & Gourine, A. V. (2016). Hemichannel-mediated release of lactate. *Journal of Cerebral Blood Flow & Metabolism*, 36, 1202–1211.

Kasyrov, V., Larina, O., Castaldo, C., Marina, N., Patrushev, M., Kasparov, S., & Gourine, A. V. (2013). Differential sensitivity of brainstem versus cortical astrocytes to changes in pH reveals functional regional specialization of astroglia. *Journal of Neuroscience*, 33, 435–441.

Kumar, P., & Prabhakar, N. R. (2012). Peripheral chemoreceptors: Function and plasticity of the carotid body. *Comparative Physiology*, 2, 141–219.

Lioy, D. T., Garg, S. K., Monaghan, C. E., Raber, J., Foust, K. D., Kaspar, B. K., . . ., Mandel, G. (2011). A role for glia in the progression of Rett’s syndrome. *Nature*, 475, 497–500.

Loeschcke, K. H. (1982). Central chemosensitivity and the reaction theory. *Journal of Physiology*, 332, 1–24.

Mansfield, D., Kaye, D. M., Brunner La, R. H., Solin, P., Estler, M. D., & Naughton, M. T. (2003). Raised sympathetic nerve activity in heart failure and central sleep apnea is due to heart failure severity. *Circulation*, 107, 1396–1400.

Marina, N., Abdala, A. P., Trapp, S., Li, A., Nattie, E. E., Hewinson, . . . , Gourine, A. V. (2013). Essential role of Pхо2b-expressing ventrolateral brainstem neurons in the chemosensory control of inspiration and expiration. *Journal of Neuroscience*, 30, 12466–12473.

Marina, N., Abdala, A. P., Korsak, A., Simms, A. E., Allen, A. M., Paton, J. F., & Gourine, A. V. (2011). Control of sympathetic vasomotor tone by catecholaminergic C1 neurons of the rostral ventrolateral medulla oblongata. *Cardiovascular Research*, 91, 703–710.

Marina, N., Tang, F., Figueiredo, M., Mastitiskaya, S., Kasimov, V., Mohamed-All, V., . . ., Gourine, A. V. (2013). Purinergic signalling in the rostral ventro-lateral medulla controls sympathetic drive and contributes to the progression of heart failure following myocardial infarction in rats. *Basic Research Cardiology*, 108, 317.

Marina, N., Ang, R., Machhada, A., Kasimov, V., Karagiannis, A., Hosford, P., . . ., Gourine, A. V. (2015). Brainstem hypoxia contributes to the development of hypertension in the spontaneously hypertensive rat. *Hypertension*, 65, 775–783.

Marina, N., Teschemacher, A. G., Kasparov, S., & Gourine, A. V. (2016). Glia, sympathetic activity and cardiovascular disease. *Experimental Physiology*, 101, 565–576.

Martin, R., Bajo-Graneras, R., Moratalla, R., Perea, G., & Araque, A. (2015). Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. *Science*, 349, 730–734.

Marty, N., Dallaporta, M., Foretz, M., Emery, M., Tarusso, M., Bady, I., . . ., Torens, B. (2005). Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. *Journal of Clinical Investigation*, 115, 3545–3553.

Meigh, L., Greghalgh, S. A., Rodgers, T. L., Cann, M. J., Roep, D. I., & Dale, N. (2013). CO2 directly modulates connexin26 by formation of carbonic bridges between subunits. *Elife*, 12, e01213.

Miller, M. J., & Tenney, S. M. (2005). Hypoxia-induced tachypnea in cotard-deafferented cats. *Respiratory Physiology*, 23, 31–39.

Mishra, A., Reynolds, J. P., Chen, Y., Gourine, A. V., Rusakov, D. A., & Attwell, D. (2016). Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nature Neuroscience*, 19, 1619–1627.

Naughton, M. T., Benard, D. C., Liu, P. P., Rutherford, R., Rankin, F., & Bradley, T. D. (1995). Effects of nasal CPAP on sympathetic activity in patients with heart failure and central sleep apnea. *American Journal of Respiratory and Critical Care Medicine*, 152, 473–479.

Okabe, Y., Takahashi, T., Mitsumasa, C., Kosai, K., Tanaka, E., & Matsumi, T. (2012). Alterations of gene expression and glutatione
clearance in astrocytes derived from an MeCP2-null mouse model of Rett syndrome. PLoS One, 7, e35354.

Olsen, M. (2012). Examining potassium channel function in astrocytes. Methods in Molecular Biology, 814, 265–281.

Olsen, E. B., Jr., Vidruk, E. H., & Dempsey, J. A. (1988). Carotid body excision significantly changes ventilatory control in awake rats. Journal of Applied Physiology, 64, 666–671 (1985).

Oomura, Y., Kimura, K., Maeno, T., Iki, M., & Kuniyohsi, M. (1964). Reciprocal activities of the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. Journal of Neuroscience, 4, 474–494.

Oomura, Y., Nakamura, T., Sugimori, M., & Yamada, Y. (1975). Effect of free fatty acid on the rat lateral hypothalamic neurons. Physiology & Behavior, 14, 483–486.

Pan, W., Hsouch, H., He, Y., Sakharkar, A., Cain, C., Yu, C., & Kastin, A. J. (2008). Astrocyte leptin receptor (ObR) and leptin transport in adult-onset obese mice. Endocrinology, 149, 2799–2806.

Pan, W., Hsouch, H., Xu, C., Wu, X., Bouret, S. G., & Kastin, A. J. (2011). Astrocytes modulate distribution and neuronal signalling of leptin in the hypothalamus of obese A (vy) mice. Journal of Molecular Neuroscience, 43, 478–484.

Papouni, T., Dunphy, J., Tolman, M., Foley, J. C., & Haydon, P. G. (2017). Astrocytic control of synaptic function. Philosophical Transactions of the Royal Society of London B Biological Sciences, 372, 1715.

Parsons, M. P., & Hirasawa, M. (2010). ATP-sensitive potassium channel-mediated lactate effect on orexin neurons: Implications for brain energetics during arousal. Journal of Neuroscience, 30, 8061–8070.

Perea, G., Navarrete, M., & Araque, A. (2009). Tripartite synapses: Astrocytes process and control synaptic information. Trends in Neurosciences, 32, 421–431.

Puchades, M., Uvera, C. J., Burgues, L. H., & Gundersen, V. (2013). Altered lactate and glucose transporter levels in the MPTP mouse model of Parkinson’s disease. Journal of Parkinson’s Disease, 3, 371–385.

Rajani, V., Zhang, Y., Jalubula, V., Rancic, V., Sheikhbahaei, S., Zwicker, J. D., … Funk, G. D. (2017). Release of ATP by pre-Bötzinger complex astrocytes contributes to the hypoxic ventilatory response via a Ca^{2+}-dependent P2Y1 receptor mechanism. Journal of Physiology, (in press). https://doi.org/10.1113/JP274727

Ralevic, V. (2000). P2 receptors in the central and peripheral nervous systems modulating sympathetic vasomotor tone. Journal of the Autonomic Nervous System, 81, 205–211.

Ramanantsoa, N., Hirsch, M. R., Thoby-Brisson, M., Dubreuil, V., Bouvier, J., Ruffault, P. L., … Goridis, C. (2011). Breathing without CO(2) chemosensitivity. Science, 333, 637–642.

Ray, R. S., Corcoran, A. E., Brust, R. D., Kim, J. C., Richerson, G. B., Nattie, E., & Dymekci, S. M. (2011). Impaired respiratory and body temperature control upon acute serotoninergic neuron inhibition. Science, 333, 637–642.

Reiner, D. J., Mietlicki-Baase, E. G., McGrath, L. E., Zimmer, D. J., Bence, K. K., Sousa, G. L., … Hayes, M. R. (2016). Astrocytes regulate GLP-1 receptor-mediated effects on energy balance. Journal of Neuroscience, 36, 3531–3540.

Reis, D. J., Golovin, E. V., Ruggiero, D. A., & Sun, M. K. (1994). Sympatho-excitatory neurons of the rostral ventrolateral medulla are oxygen sensors and essential elements in the tonic and reflex control of the systemic and cerebral circulations. Journal of Hypertension, Supplement, 12, S159–S180.

Rifai, L., Winters, J., Friedman, E., & Silver, M. A. (2012). Initial description of cerebral oximetry measurement in heart failure patients. Congestive Heart Failure, 18, 85–90.

Rolenhagen, A., Satzler, K., Rodriguez, E. P., Jonas, P., Frotscher, M., & Lubke, J. H. (2007). Structural determinants of transmission at large hippocampal mossy fiber synapses. Journal of Neuroscience, 27, 10434–10444.

Ross, C. A., Ruggiero, D. A., Park, D. H., Joh, T. H., Sved, A. F., Fernandez-Pardal, J., … Reis, D. J. (1984). Tonic vasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. Journal of Neuroscience, 4, 474–494.

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: Biology and pathology. Acta Neuropathologica, 119, 7–35.

Southall, D. P., Kerr, A. M., Tiros, E., Amos, P., Lang, M. H., & Stephenson, J. B. (1988). Hyperventilation in the awake state: Potentially treatable component of Rett syndrome. Archives of Disease in Childhood, 63, 1039–1048.

Sun, M. K., & Reis, D. J. (1994). Hypoxia selectively excites vasomotor neurons of rostral ventrolateral medulla in rats. American Journal of Physiology, 266, R245–R256.

Sun, M. K., Wahlestedt, C., & Reis, D. J. (1992). Action of externally applied ATP on rat reticulospinal vasomotor neurons. European Journal of Pharmacology, 224, 93–96.

Tang, F., Lane, S., Korsak, A., Paton, J. F., Gourine, A. V., Kasparov, S., & Teschemacher, A. (2014). Lactate-mediated glia-neuronal signalling in the mammalian brain. Nature Communications, 5, 3284.

Tang-Christensen, M., Larsen, P. J., Goke, R., Fink-Jensen, A., Jessop, D. S., Moller, …, & Sheikh, S. P. (1996). Central administration of GLP-1 (7-36) amide inhibits food and water intake in rats. American Journal of Physiology, 271, R848–R856.

Teran, F. A., Massey, C. A., & Richerson, G. B. (2014). Serotonin neurons and central respiratory chemoreception: Where are we now? Progress in Brain Research, 209, 207–223.

Teschemacher, A. G., Gourine, A. V., & Kasparov, S. (2015). A role for astrocytes in sensing the brain microenvironment and neuro-metabolic integration. Neurochemical Research, 40, 2386–2393.

Thomas, T., & Spyer, K. M. (2000). ATP as a mediator of mammalian central CO2 chemoreception. Journal of Physiology, 523(Pt 2), 441–447.

Thrane, A. S., Rappold, P. M., Fujita, T., Torres, A., Bekar, L. K., Takano, T., & Nagelhus, E. A. (2011). Critical role of aquaporin-4 (AQP4) in astrocytic Ca^{2+} signaling events elicited by cerebral edema. Proceedings of the National Academy of Science U.S.A, 108, 846–851.

Tian, L., & Jin, T. (2016). The incretin hormone GLP-1 and mechanisms underlying its secretion. Journal of Diabetes, 8, 753–765.

Turelski, T., Humoud, I., Desai, R., Smith, K. J., & Marina, N. (2016). Immunohistochemical evidence of tissue hypoxia and astroglisis in the rostral ventrolateral medulla of spontaneously hypertensive rats. Brain Research, 1650, 178–183.

Turovsky, E., Karagiannis, A., Abdala, A. P., & Gourine, A. V. (2015). Impaired CO2 sensitivity of astrocytes in a mouse model of Rett syndrome. Journal of Physiology, 593, 3159–3168.

Turovsky, E., Theparambil, S. M., Kasymov, V., Deitmer, J. W., Del Arroyo, A. G., Ackland, G. L., …, Gourine, A. V. (2016). Mechanisms of CO2/H+ sensitivity of astrocytes. Journal of Neuroscience, 36, 10750–10758.

Turton, M. D., O’Shea, D., Gunn, I., Beak, S. A., Edwards, C. M., Meeran, K., …, Bloom, S. R. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. Nature, 379, 69–72.
van den Top, M., Lee, K., Whyment, A. D., Blanks, A. M., & Spanswick, D. (2004). Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nature Neuroscience*, 7, 493–494.

Viemari, J. C., Roux, J. C., Tryba, A. K., Saywell, V., Burnet, H., Pena, F., ... Hilaire, G. (2005). MeCP2 deficiency disrupts norepinephrine and respiratory systems in mice. *Journal of Neuroscience*, 25, 11521–11530.

Volterra, A., & Meldolesi, J. (2005). Astrocytes, from brain glue to communication elements: The revolution continues. *Nature Reviews Neuroscience*, 6, 626–640.

Wells, J. A., Christie, I. N., Hosford, P. S., Huckstepp, R. T., Angelova, P. R., Viiko, P., ... Gourine, A. V. (2015). A critical role for purinergic signalling in the mechanisms underlying generation of BOLD fMRI responses. *Journal of Neuroscience*, 35, 5284–5292.

Wenker, I. C., Sobrinho, C. R., Takakura, A. C., Mulkey, D. K., & Moreira, T. S. (2013). P2Y1 receptors expressed by C1 neurons determine peripheral chemoreceptor modulation of breathing, sympathetic activity, and blood pressure. *Hypertension*, 62, 263–273.

Wenker, I. C., Abe, C., Viar, K. E., Stornetta, D. S., Stornetta, R. L., & Guyenet, P. G. (2017). Blood pressure regulation by the rostral ventrolateral medulla in conscious rats: Effects of hypoxia, hypercapnia, baroreceptor denervation, and anesthesia. *Journal of Neuroscience*, 37, 4565–4583.

Wren, A. M., Small, C. J., Abbott, C. R., Dhillo, W. S., Seal, L. J., Cohen, M. A., ... Bloom, S. R. (2001). Ghrelin causes hyperphagia and obesity in rats. *Diabetes*, 50, 2540–2547.

Yamamoto, H., Lee, C. E., Marcus, J. N., Williams, T. D., Overton, J. M., Lopez, M. E., ... Elmquist, J. K. (2002). Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *Journal of Clinical Investigation*, 110, 43–52.

Yang, L., Qi, Y., & Yang, Y. (2015). Astrocytes control food intake by inhibiting AGRP neuron activity via adenosine A1 receptors. *Cell Reports*, 11, 798–807.

Yasui, D. H., Xu, H., Dunaway, K. W., Lasalle, J. M., Jin, L. W., & Maezawa, I. (2013). MeCP2 modulates gene expression pathways in astrocytes. *Molecular Autism*, 4, 3.

Young, J. K., Baker, J. H., & Montes, M. I. (2000). The brain response to 2-deoxy glucose is blocked by a glial drug. *Pharmacology, Biochemistry and Behavior*, 67, 233–239.

Zheng, K., Bard, L., Reynolds, J. P., King, C., Jensen, T. P., Gourine, A. V., & Rusakov, D. A. (2015). Time-resolved imaging reveals heterogeneous landscapes of nanomolar Ca2+ in neurons and astroglia. *Neuron*, 88, 277–288.

How to cite this article: Marina N, Turovsky E, Christie IN, et al. Brain metabolic sensing and metabolic signaling at the level of an astrocyte. *Glia*. 2018;66:1185–1199. https://doi.org/10.1002/glia.23283