Neurochem Res (2015) 40:2386–2393
DOI 10.1007/s11064-015-1562-9

ORIGINAL PAPER

A Role for Astrocytes in Sensing the Brain Microenvironment and Neuro-Metabolic Integration

A. G. Teschemacher[1] · A. V. Gourine[2] · S. Kasparov[1]

Received: 12 December 2014 / Revised: 27 January 2015 / Accepted: 7 February 2015 / Published online: 3 April 2015

Abstract Astrocytes occupy a strategic position in the brain where they can act as an interface between neurones and blood vessels, and neurones and the cerebro-spinal fluid. This location is ideal for functioning as interoceptors, as they may sense changes in brain microenvironment and contribute to the adaptive homeostatic responses coordinated by neuronal networks. Here we briefly review some of the recent evidence, which implicates the involvement of astrocytes in the central nervous control of breathing, sympathetic tone and blood glucose levels. L-lactate appears a potentially crucial signaling molecule in the communication between astrocytes and neurones. Based on the available evidence, we conclude that astrocytes contribute to the homeostasis by playing a significant role in the brain's interoceptive mechanisms.

Keywords Astrocytes · Chemosensitivity · Lactate · G-protein coupled receptors · Signalling

Special Issue: In Honor of Dr. Gerald Dienel.

✉ A. G. Teschemacher
Anja.Teschemacher@Bristol.ac.uk

✉ S. Kasparov
Sergey.Kasparov@Bristol.ac.uk

[1] School of Physiology and Pharmacology, University of Bristol, Bristol BS8 1TD, UK

[2] Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, UK

Introduction

Astrocytes occupy a unique anatomical and functional niche in the brain. These numerous cells not only fill the spaces between neurones but also isolate most of the central nervous system (CNS) neurones from the rest of the body. The tightness of the blood brain barrier is to a large extent dependent on astrocytes as all small blood vessels in the brain are completely covered and are sealed by the astrocytic end feet [1]. On one hand, this implies that traffic of nutrients and metabolites between blood and neurones to a large extent goes via the perivascular end feet (in case of glucose for example this is an entirely passive, gradient-driven process). On the other, it puts the astrocyte in an ideal position to sense metabolic signals and modulate local neuronal networks to trigger adaptive changes.

An astrocyte does not fulfill the criteria for a computational element in a neuronal circuit because it does not generate action potentials and lacks the mechanisms for targeted release of a chemical signal towards a clearly defined downstream element. In fact, one of the most significant problems in the field at present time is how to even define the state of astrocyte activation. For many years, second messenger activity, such as changes in $[Ca^{2+}]_i$, was taken as a surrogate index of astrocytic activation. However, work which used IP_3R_2 knock-out mice has challenged this concept because in these animals release of Ca^{2+} from intracellular stores of astrocytes is suppressed, at least in the somatic region, but the mice show no major deficits in glutamatergic transmission or LTP [2]. Nevertheless, there is a vast body of literature, which demonstrates beyond doubt that, in response to various stimuli (including increased neuronal activity), astrocytes can affect activity of neighboring neurons by release signaling molecules such as ATP, D-serine, lactate, glutamate, etc.

which all are neuro-active. Many studies have documented activation of astrocytes that appears secondary to the activity of local neurones [3–5]. Other studies have shown that acute stressors such as tail pinch or mechanical stimulation of whiskers in mice lead to propagating Ca^{2+} signals in astrocytes, which seem to be dependent on noradrenergic input [6–8]. However, there are processes where the information flows in the opposite direction, i.e. from the activated astrocytes to the local neuronal networks. It follows that, if astrocytes can sense the brain microenvironment (i.e. function as interoceptors) and respond to changes in internal milieu by modulating neuronal network activity, they become active players rather than passive witnesses.

In this review, we briefly discuss recent data, which suggest that astrocytes could play an active role in a number of CNS homeostatic mechanisms, acting as primary detectors of the internal environment and/or signal amplifiers. Various aspects of this discussion are illustrated in Fig. 1.

Role of Astrocytes in Central Respiratory CO_2 Chemosensitivity

CO_2 is an essential driver for respiration in mammals and our respiratory networks are silent in the absence of CO_2. PCO_2 is detected at both peripheral and central levels. Peripheral chemoreceptors are located within the carotid and aortic bodies where they are ideally positioned to monitor the gas composition of the arterial blood. However, it was estimated that up to 80 % of the overall ventilatory response to CO_2 is mediated via its action on respiratory chemosensors located within the CNS [9]. Chemosensitivity was detected in a number of brain areas (mostly in the brainstem) but the cellular identity of central chemosensors remained controversial for many years.

Evidence is now mounting to support the idea of significant regional specialisation of astrocytes. While discussion of this issue is outside of the scope of this review, we would like to strass that it is more than likely that astrocytes within the lower and evolutionary old parts of

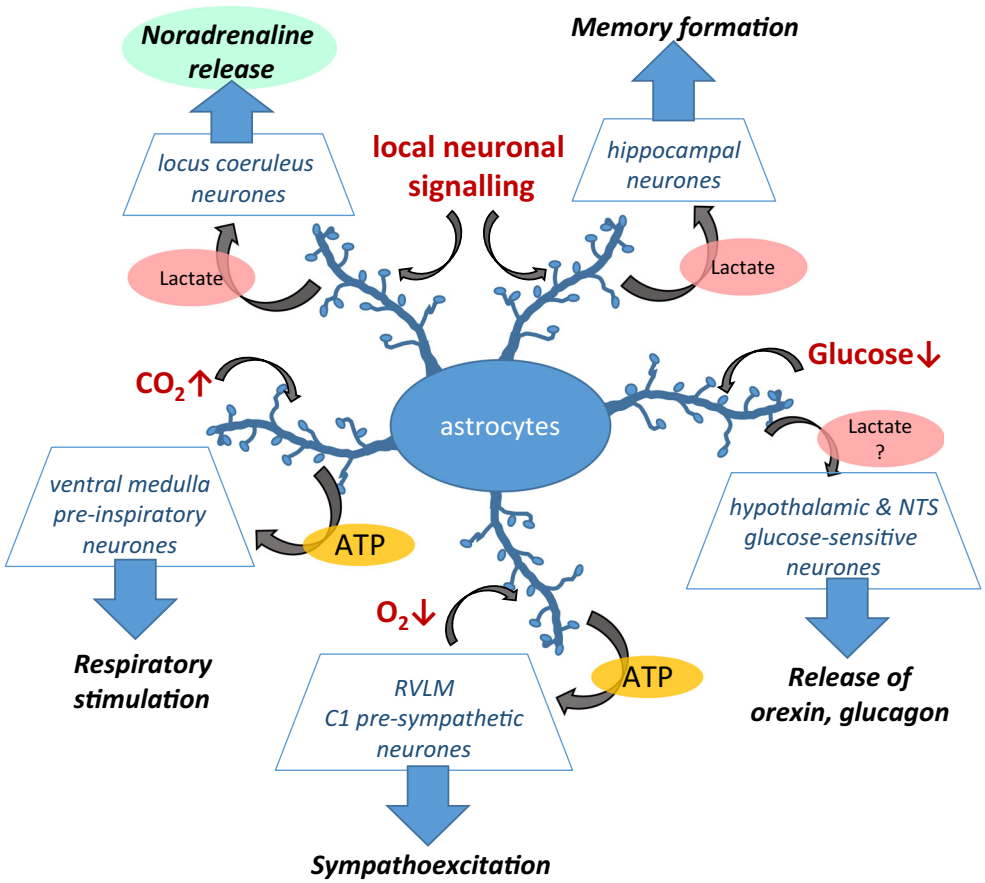

Fig. 1 Astrocytes interface between brain microenvironments, blood vessels and local neuronal networks. This position allows them to sense and integrate fluctuations in respiratory gases and metabolic/signals and influence a range of neuronal circuits that control homeostatic adaptation

the brain (hypothalamus, pons and medulla oblongata) are in many ways different to astrocytes in the cortex or hippocampus. This is perhaps not surprising because brainstem contains nuclei essential for highly specific and critical functions such as respiration, control of concentrations of blood solutes and glucose, autonomic (sympathetic and parasympathetic) control and others. In addition, medulla and pons contain large clusters of cells which synthesise and release neuromodulators such as norepinephrine which project to other parts of the brain and also the spinal cord.

Several studies suggested a role for brainstem astrocytes in central respiratory chemoreception [10, 11]. In 2005 [12] demonstrated CO_2-evoked release of ATP from the structures located at and near the ventral surface of the medulla oblongata, perhaps the best documented central chemosensitive area. In light of the existing data and given that ATP is one of the best characterised and ubiquitous gliotransmitters, we hypothesized that medullary astrocytes contribute to the detection of PCO_2 and pH changes and drive the adaptive changes in the activity of the respiratory network [13]. Using a combination of in vitro and in vivo approaches, imaging, electrophysiology and optogenetics, we found that astrocytes of this "classical" chemoreceptor area are indeed highly chemosensitive. They have an intrinsic ability to respond to acidification in the absence of the neuronal cues. We found that ventral medullary astrocytes respond to small physiological decreases in pH with vigorous elevations in intracellular Ca^{2+} and release of ATP. Based on the effects of brefeldin A and bafilomycin A (non-selective blockers of exocytotic release) on acidification-induced Ca^{2+} elevations in astrocytes, we concluded that ATP release occurs via vesicular exocytosis. Interestingly astrocytes from the front brain seem to be unresponsive to similar pH challenges.

The exocytotic nature of ATP release from astrocytes cultured from the ventral medullary surface was recently confirmed directly using total internal reflection fluorescence microscopy [14]. That study also demonstrated that fusion of the putative ATP-containing vesicles requires $[Ca^{2+}]_i$ elevations and that astrocytes including those from the ventral surface of the medulla express vesicular nucleotide transporter. Interestingly, the fusion of an individual vesicle in response to acidification could be detected in sequential images over periods of $\sim$1.5 s (Fig. 1C in [14]), possibly suggesting that the exocytotic machinery in astrocytes is much slower than in neurones.

In order to mimic pH-evoked $[Ca^{2+}]_i$ excitation [13], astrocytes were transduced with channelrhodopsin-2 using a viral vector with an enhanced GFAP promoter [15]. Optogenetic stimulation of ventral brainstem astrocytes resulted in ATP-dependent activation of the local retrotrapezoid nucleus (RTN) chemoreceptor neurones and triggered robust respiratory responses in vivo. That study was the first successful application of optogenetic technology for astrocyte research, which was later adopted by many other groups [4, 16–18]. In conclusion, our experiments have demonstrated that astrocytes located on the ventral surface of the medulla are capable of sensing changes in pH/PCO_2 levels of the arterial blood, integrating it with pH/PCO_2 level of the brain parenchyma and then imparting this information on the activity of the brainstem respiratory network. While these data did not exclude the existence of specialized chemosensitive neurones, they suggest that astrocytes act as true central respiratory chemoreceptors.

A later study confirmed the importance of purinergic signalling for CO_2 chemosensitivity in the medulla oblongata [19], but the results were more consistent with ATP release from astrocytes via connexin hemichannels [20].

Finally some studies suggested that astrocytes in other areas of the brain such as nucleus tractus solitarius might be also pH sensitive via different mechanisms (modulation of transporters) but this hypothesis is much less well studied [21].

Activation of Astrocytes Contributes to the Development of Heart Failure Secondary to Myocardial Infarction

The RTN chemosensitive area, mentioned in the previous section, is located in close proximity to another medullary region critical for central autonomic and cardio-respiratory control—the C1 group of noradrenergic neurones of the rostral ventro-lateral medulla (RVLM). The RVLM is thought to provide a major descending excitatory drive to the spinal sympathetic preganglionic neurones. These sympathoexcitatory RVLM neurones respond to changes in PO_2 and contribute to the increases in sympathetic cardiac and vasomotor activities during systemic hypoxia [22].

Following myocardial infarction, a significant proportion of patients develop congestive heart failure, which is often associated with an increased activity of the sympathetic nervous system. It is generally believed that such increase in sympathetic tone is detrimental, damages the heart and contributes to the progression of the disease [23]. Moreover, in the developing heart failure not only the circulation but also respiration is affected with up to 38 % of heart failure patients presenting with episodes of central sleep apnoea [24, 25]. Interestingly, hypoxia has been demonstrated to trigger release of ATP from the ventral medullary regions located within and in close proximity to the RVLM [12] and RVLM neurones are highly sensitive to ATP [26]. They are excited in response to application of exogenous ATP or ATP receptor agonists while activation of ATP receptors in the RVLM evokes profound increases

in the systemic arterial blood pressure, heart rate and renal sympathetic nerve activity [27, 28].

Additional evidence underpinning the idea of hypoxia-evoked, astrocyte-dependent purinergic excitation of RVLM neurones includes demonstration of $P2Y_1$ receptor-mediated excitation of RVLM neurones in response to peripheral chemoreceptor activation [29], excitation of RVLM neurones by hypoxia [30], and hypoxia-induced $[Ca^{2+}]_i$ elevations in astrocytes [31]. A caveat in these latter studies is that the level of hypoxia was not well controlled and experimental conditions may rather have resembled those in brain ischaemia (stroke) than in a chronic state of brain hypoperfusion during heart failure. Nevertheless, these observations raised a possibility that brain tissue hypoxia could contribute to the mechanisms underlying sympathetic activation in heart failure.

We hypothesised that, in developing heart failure, hypoperfusion and hypooxygenation of the brain exacerbated by recurrent episodes of sleep apnoea may result in activation of astrocytes within the ventral medulla. This could lead to an increase in extracellular concentration of ATP, enhanced activity of the RVLM sympathoexcitatory neurones and increased central sympathetic drive [17]. In order to test this hypothesis a new approach was needed to chronically interfere with ATP-mediated signalling in a specified location within the medulla oblongata in a rat model of heart failure secondary to myocardial infarction. To this end, we generated a lentiviral vector, which drives expression of a purine-metabolising enzyme—transmembrane prostatic acid phosphatase (TMPAP)—on the external surface of cell membranes. TMPAP can cleave a broad spectrum of substrates and is highly active at neutral pH. It is most potent in conversion of ADP to AMP and AMP to adenosine and because of the shift in the kinetics of the upstream reaction behaves as a very potent inhibitor of ATP-mediated signalling. This was verified using a very well established in vitro paradigm in which confluent cultured astrocytes are grown on a coverslip and one or two cells are mechanically stimulated with a patch pipette. Mechanical stimulus triggers local release of ATP which acts on the adjacent astrocytes to elicit $[Ca^{2+}]_i$ waves which propagate across the culture via vesicular release of ATP [32]. Expression of TMPAP in cultured astrocytes almost completely blocked propagation of ATP-mediated $[Ca^{2+}]_i$ waves [17]. By chronically suppressing ATP-mediated signalling in the RVLM of rats subjected to myocardial infarction, we were able to slow the remodelling process and heart failure progression as evidenced by normalisation of end diastolic left ventricular pressure and an improvement in the left ventricular contractile function. In addition, we also demonstrated that optogenetic activation of RVLM astrocytes leads to ATP-dependent activation of C1 neurones in vitro and to a profound sympathoactivation in vivo. Taken together, these observations suggest that astrocytes in the RVLM may, via release of ATP, contribute to sympathetic hyperactivity characteristic of heart failure and that activation of this pathway by low brainstem parenchymal PO_2 is one of the pathogenic mechanisms contributing to the progression of this common pathology [17].

Central Glucose Sensitivity

Glucose sensors of the hypothalamus and dorsal brainstem represent another group of functional brain interoceptors of unclear identity. They sense changes in glucose level and contribute to the autonomic control of pancreatic α-cells' secretory activity, inducing glucagon secretion in response to hypoglycaemia. Glucagon restores blood and brain levels of glucose by stimulating hepatic glucose production and release. Glucose transporter type 2 (GLUT2) deficiency in mice results in abnormal control of plasma glucagon and plasma glucagon insensitivity to systemic or central hypoglycaemia [33, 34]. Interestingly, transgenic re-expression of GLUT2 selectively in brainstem astrocytes fully restored glucagon responses to hypoglycaemia as well as systemic and central application of glucoprivic stimuli (injections of non-metabolisable 2-deoxy-D-glucose [34]). Although the Marty et al. (2005) report did not demonstrate that astrocytes were directly sensitive to or activated by low glucose, it implicated astrocytes in the central mechanism, which triggers glucagon release in response to hypoglycaemia. Recent studies also suggested that astrocytes located within the nucleus of the solitary tract, which is the key relay of the visceral primary afferents, are glucosensitive and potentially could contribute to the regulation of glucose levels in the body [35, 36]. It has been further demonstrated [37] that the effect of glucoprivation (central administration of 2-deoxyglucose) on the activity of the neurones of the nucleus of the solitary tract and the dorsal motor nucleus of the vagus nerve can be blocked by fluorocitrate. Fluorocitrate is sometimes used as "astrocytic inactivator" based on its ability to preferentially inhibit the Krebs cycle in astrocytes vs neurones [38]. While the actual link between the astrocytes and neurons within the dorsal vagal complex remains to be established, studies reviewed in the next section suggest that in the hypothalamus signalling between astrocytes and glucoresponsive neurones could be mediated by astrocyte-derived lactate.

Lactate as a Glia-Neuronal Metabolic Signal

Much of what is currently known about brain lactate metabolism stems from the work of Dienel et al. [39–42]—to mention just a few publications. Indeed, lactate is one of

the most studied metabolites in the brain and has been in the focus of intensive debate over the last 15 years, mainly in context of the so-called lactate shuttle hypothesis [43–45]. According to this hypothesis, astrocytes produce lactate and export it to neurones, which use it to support their energy needs during periods of prolonged activity. While the whole idea of the reliance of neurones on lactate (rather than glucose) as their energy source is actively debated, it is nevertheless clear that, upon brain activation, consumption of glucose increases disproportionally more than consumption of oxygen which implies an increase in the rate of glycolysis (and therefore pyruvate/lactate overflow). Curiously, there are drastic and still unexplained regional variations in this phenomenon. This was demonstrated by imaging studies in humans suggesting that the rate of lactate production and its fate may be radically different in different parts of the CNS, for example in cortex and cerebellum [46]. Overall, activated brain produces too much lactate which then even overspills into the systemic circulation [40]. At rest, extracellular concentrations of lactate in the brain have been estimated by several studies to be less than 1 mM but episodes of seizures or ischemia can lead to increases to 2 mM and higher [47–49]. Both neurones and astrocytes can theoretically produce and release lactate, but neuronal transporters are likely to be saturated even at relatively low levels of intracellular lactate, rendering neurones poor lactate exporters [40]. In contrast, astrocytes have a powerful lactate producing and handling machinery and they are the only type of brain cell that stores glycogen. Glycogen supplies in astrocytes are quickly recruited by neuronal activity (such as sensory stimulation) [50] but even after severe physical exercise brain glycogen level only drops by $\sim$50 % followed by supercompensation within the next few hours [51]. Therefore, astrocytes are a stable reserve of glucose and its metabolites. This raises the interesting possibility of lactate acting as a signalling molecule between astrocytes and neurones. This pathway could be engaged when lactate is released by astrocytes either under conditions favouring glycolysis (hypoxia) or when astrocytes mobilise their stores of glucose from glycogen in response to stimulation of certain receptors (for example receptors for noradrenaline).

Publications from several groups state that some neurones are responsive to lactate. One interesting cell type are orexin-producing neurones in the hypothalamus. These cells are thought to play a role in the homeostatic mechanisms of nutrient control. Hypothalamic orexin neurones induce arousal, stimulate food intake and hepatic production of glucose [52–54] and are sensitive to glucose, but the mechanisms of this glucosensitivity remain unclear. [55] found that glucose hyperpolarised orexin neurones in brain slices of mice. Interestingly, lactate (5 mM) caused depolarisation of these cells, resulting in a significant increase in the firing rate. It is important to note that the experiments were performed using solutions containing 1 mM glucose (a typical artificial cerebrospinal fluid [aCSF] solution for in vitro experiments contains 5–10 mM) and no glucose in the patch pipette (again, intracellular solutions used by many laboratories contain glucose), although it contained ATP. Therefore, the neurones probably had low basal levels of pyruvate and lactate. The mechanism of the hyperpolarising action of glucose was not established in that study [55] and surprisingly, also non-metabolisable 2-deoxyglucose had effects similar to D-glucose. Nevertheless, this study clearly demonstrated excitatory action of lactate on orexin neurones.

Somewhat different conclusions were reached by Parsons and Hirasawa [56], who studied orexin-producing neurones in hypothalamic slices of rats. This study also used 1 mM glucose-containing aCSF and found that *removal* of extracellular glucose hyperpolarised orexin-producing neurones and this hyperpolarisation was reversed by application of either lactate or acetate (which hints at the involvement of astrocytes which can metabolise acetate much better than neurones). Fluoroacetate was used to inhibit glial metabolism and caused hyperpolarisation of orexin neurones in the presence of glucose suggesting that a factor released from astrocytes was keeping neurones depolarised. In agreement with this idea, hyperpolarisation caused by inhibition of glial metabolism could be prevented by bath application of lactate. In zero glucose solution application of lactate was found to have an excitatory effect, the threshold for the lactate effect was $\sim$1 mM and it reached its peak at $\sim$5 mM. Pharmacological analysis suggested that in these cells lactate was acting by altering the activity of K_{ATP} channels, a mechanism reminiscent of that of pancreatic β-cells, where closure of the K_{ATP} channels leads to depolarisation and insulin secretion [56, 57].

Taken together the two previous studies agree that lactate is excitatory to orexin neurones but suggest different reasons for why that may be the case.

However, there is evidence that in the brain lactate may also act via a completely different route as a gliotransmitter with its own cognate receptor. There is a known G-protein coupled receptor for lactate which was originally known as GPR81 and later renamed to HCA-1 [58]. HCA-1 was initially described in lipid tissue where it takes part in the control of lipid metabolism [59, 60]. Although at low levels, HCA-1 is also expressed in various regions of the CNS [61]. HCA-1 is a G_i coupled receptor and in neurones activation of these receptors typically has an inhibitory effect, causing hyperpolarisation and suppression of transmitter release (exemplified by $GABA_B$ or α_2-adrenoceptors). Indeed, a recent study described an inhibitory effect of lactate in cultured mouse cortical neurones which would be consistent with an action via HCA-1 [62].

However, HCA-1 has extremely low affinity for lactate and in the study of [62] EC_{50} for lactate was estimated to be 4.2 mM, which is considerably higher than the extracellular lactate concentrations measured in the normal brain (discussed in the previous sections). Therefore, the physiological role of HCA-1 receptors in the CNS remains unclear. Possibly, signalling via this receptor becomes significant under conditions of tissue hypoxia or extremely high neuronal activity such as during seizures.

An alternative pathway of lactate-mediated signalling was recently described by us in the locus coeruleus (LC), the largest cluster of CNS neurones which produce and release norepinephrine [63]. In that study we optogenetically excited LC astrocytes in organotypic brain slices while recording from the LC neurones using patch clamp. We found that after a delay of $\sim$1 min LC neurones responded with depolarisation and increased rate of action potential firing. This signalling between astrocytes and LC neurones was found to be mediated by lactate because it could be blocked by a range of chemicals, which either prevent mobilisation of glycogen or block conversion of pyruvate into lactate, or by application of D-lactate. Interestingly, a commonly used inhibitor of monocarboxylate transporters (4-CIN) was ineffective. Application of exogenous lactate was also able to excite LC neurones with EC_{50} of $\sim$680 μM, corresponding to a physiological concentration of lactate in the brain. Exogenous lactate only depolarised LC neurones if applied extracellularly, but not when dialysed into the cell. Blockers of adenylate cyclase and protein kinase A prevented the excitatory effects of lactate on the activity of the LC neurones. Qualitatively similar effects were observed using fast cyclic voltammetry, a method which detects norepinephrine release. Pyruvate and acetate were ineffective in either assay. Interestingly, neurones from another brain area (hippocampus) were not responsive to 2 mM lactate. We, therefore, hypothesised that lactate could be acting on an unknown receptor, probably a member of GPCRs operating via stimulation of cAMP production, typical for Gs-coupled receptors [63]. The physiological paradigms, which lead to activation of this lactate-mediated signalling pathway between astrocytes and LC neurones and its molecular identity remain to be explored. It is tempting to speculate that this link could couple release of noradrenaline to the local neuronal activity in various parts of the front brain via a lactate-mediated positive feedback loop between noradrenergic varicosities and local astrocytes.

Taken together, our observations [63] and results of others [61, 62] open a possibility that various metabolic signals which converge on astroglial lactate metabolism could utilise lactate as a glio-transmitter acting on its own cognate receptor and capable of modulating the activities of adjacent neurones.

Conclusions

In summary, astrocytes have been implicated in many physiological mechanisms at cellular and whole body level. While these cells do not possess the swiftness required for circuit computations, they are ideally positioned to act as integrators and detectors of various metabolic signals and can trigger adaptive physiological responses via modulation of the activity of neuronal networks in both, physiological and pathological states. Recent data, which suggests a novel role for lactate as a glio-transmitter, may eventually change the way we think about this ubiquitous metabolite. In the future it will be important to further consolidate the evidence for the involvement of astrocytes in various aspects of interoreception (glucose, salt, pH) and get a much more accurate picture of the fate of lactate and glucose in the brain including measurements in extra and intracellular microdomains. The idea of the signalling role of lactate is also attractive and probably could help to explain some of the current controversies.

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