Astrocytes regulate brain extracellular pH via a neuronal activity-dependent bicarbonate shuttle

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Brain cells continuously produce and release protons into the extracellular space, with the rate of acid production corresponding to the levels of neuronal activity and metabolism. Efficient buffering and removal of excess H+ is essential for brain function, not least because all the electrogenic and biochemical machinery of synaptic transmission is highly sensitive to changes in pH. Here, we describe an astroglial mechanism that contributes to the protection of the brain milieu from acidification. In vivo and in vitro experiments conducted in rodent models show that at least one third of all astrocytes release bicarbonate to buffer extracellular H+ loads associated with increases in neuronal activity. The underlying signalling mechanism involves activity-dependent release of ATP triggering bicarbonate secretion by astrocytes via activation of metabotropic P2Y1 receptors, recruitment of phospholipase C, release of Ca2+ from the internal stores, and facilitated outward HCO3− transport by the electrogenic sodium bicarbonate cotransporter 1, NBCe1. These results show that astrocytes maintain local brain extracellular pH homeostasis via a neuronal activity-dependent release of bicarbonate. The data provide evidence of another important metabolic housekeeping function of these glial cells.
Maintaining pH homeostasis is fundamentally important for uninterrupted activity of individual neurons and effective communication within neuronal circuits, which makes the brain information processing possible. Neurons constantly produce and release into the extracellular space significant amounts of acid equivalents, with the rate of acid production corresponding to the levels of neuronal activity and energy usage. Any failure to effectively counteract extracellular acid loads would compromise the function of neuronal circuits, simply because all the electrogenic and biochemical machinery of synaptic transmission is highly sensitive to pH1–7.

Buffering by CO₂/HCO₃⁻ is one of the most important mechanisms of tissue pH control. In this system, CO₂ and H₂O are in a dynamic equilibrium with H⁺ and HCO₃⁻. This equilibrium is rapidly attained by the activity of enzymes from the carbonic anhydrase family8.10. Bicarbonate buffering and carbonic anhydrase activity protect brain tissue from acidification by converting H⁺ and HCO₃⁻ to H₂O and CO₂. CO₂ is then removed by cerebral circulation and transported to the lungs to be exhaled. However, little is known about the mechanisms that maintain local CO₂/HCO₃⁻ buffer strength in the extracellular space of the brain. Indeed, the brain extracellular space occupies only ~20% of the tissue volume11,12, and the efficacy of the CO₂/HCO₃⁻ buffering system would rapidly decline if HCO₃⁻ is depleted in conditions of significant extracellular acid loads, such as during periods of increased neuronal activity. Therefore, maintaining stability of brain tissue pH necessitates an effective mechanism capable of supplying HCO₃⁻ to the extracellular space in a responsive mode, i.e., in a neuronal activity-dependent manner.

Amongst different cellular players within the synaptic neuropil, astrocytes appear to be well suited to provide active control of local brain extracellular pH (pH₄) microenvironment. A single astrocyte occupies a large volume of brain parenchyma with an extensive arborisation covering thousands of individual synapses13. This anatomical arrangement enables astrocytes to monitor local brain activity by sensing neuronal signalling molecules (such as glutamate and ATP) that escape from the synaptic cleft20,21. NBCe1 activity is modulated by intracellular signalling mechanisms involving Ca²⁺, cyclic adenosine monophosphate/protein kinase A, and phospholipase C (PLC)22.

We hypothesised that, in astrocytes, the recruitment of one (or several) of these intracellular mechanisms in response to neuronal signalling molecules can stimulate outward activity of NBCe1 and thus supply HCO₃⁻ to the extracellular space ‘on demand’, and in accord with the level of local neuronal activity. To test this hypothesis, we examined the effects of purinergic activation on HCO₃⁻ transport and intracellular pH (pH₄) regulation in astrocytes, investigated the cellular mechanisms of HCO₃⁻ release, and determined the effect of NBCe1 deletion in astrocytes on brain pH regulation. The results obtained in this study suggest that bicarbonate transport in astrocytes of the forebrain is controlled by purinergic signalling. ATP and downstream purines facilitate HCO₃⁻ release by astrocytes via PLC/Ca²⁺-mediated activation of NBCe1. This astroglial mechanism appears to play an important role in the control of local brain extracellular pH.

**Results**

**Regulation of brain extracellular pH.** In the absence of carbonic anhydrase catalytic activity, the rate of H⁺ removal could be too slow to effectively counteract extracellular acid loads associated with brain activity. Indeed, inhibition of carbonic anhydrase with acetazolamide leads to a reversible extracellular acidification (by −0.07 ± 0.02 pH units) of brain tissue (somatosensory cortex), as is evident from a significant decrease in the pH-sensitive electrochemical current recorded in the vicinity of the acetazolamide microinjection site (Fig. 1a). Profound and sustained extracellular acidification was also recorded in the brain after systemic administration of acetazolamide (Supplementary Fig. S1a). This result indicates that brain cells continuously produce and extrude H⁺ into the extracellular space. It also implies that H⁺ buffering by HCO₃⁻ and carbonic anhydrase activity that facilitates the reversible conversion of H⁺ and HCO₃⁻ to H₂O and CO₂ are essential for the maintenance of constant brain extracellular pH.

In the mouse somatosensory cortex, pH measurements with carbon fibre microelectrodes (CFM), coupled with fast scan cyclic voltammetry, showed that during periods of increased neuronal activity the extracellular pH remains unchanged or shifts in the alkaline direction during or immediately after the stimulation (regardless of the duration of the somatosensory stimulation) (Fig. 1b; Supplementary Fig. S1b). Effective buffering of the extracellular H⁺ loads would require a supply of extra HCO₃⁻, since extracellular HCO₃⁻ pools are readily depletable while H⁺ generation scales with the neuronal activity and energy use23,24. Using two-photon excitation imaging of cortical astrocytes loaded with a pH-sensitive dye BCECF (Fig. 1c), we next studied astroglial [H⁺], responses to the increases in local neuronal activity (Fig. 1d, e). On average, the population of cortical astrocytes responded to the activation of somatosensory pathways with intracellular acidification (p < 0.001). Strong intracellular acidification (change in BCECF fluorescence ≥ 2 standard deviations from the mean) was recorded in 27% of cortical astrocytes (n = 32/117 cells; Fig. 1d, e) and alkalisation in 4% of astrocytes (n = 5/117 cells; Fig. 1d). The specificity of the recorded pH₄ responses was confirmed by giving the animals 10% CO₂ to breathe. As expected, CO₂ inhalation triggered intracellular acidification in the majority (63%, n = 60/95 cells) of the recorded cortical astrocytes (Supplementary Fig. S1c, d).

In acute hippocampal slices, electrical stimulation of Schaffer collateral fibres triggered intracellular acidification in 53% of recorded CA1 astrocytes (n = 53/100 cells; Fig. 2a–d) and alkalisation in 30% of astrocytes (n = 30/100 cells; Fig. 2c,d). In baseline conditions, no significant fluctuations or drift of pH₄ values were detected over comparable recording periods (Fig. 2c). The magnitude of pH₄ changes recorded in CA1 astrocytes was dependent on the strength of the Schaffer collateral recruitment with significant pH₄ transients observed in response to a single pulse electrical stimulation (Fig. 2c, d). The proportion of astrocytes responding with changes in pH₄ to increases in the neuronal activity recorded in vitro was larger compared to that recorded in vivo, likely reflecting differences in tissue perfusion between the preparations (superfusion from the surfaces of the slice vs perfusion with the blood flow in vivo). Together, the data obtained in vivo and in vitro show that between 30–50% of all astrocytes respond to the increases in local neuronal activity with intracellular acidification. As extracellular pH is well maintained during periods of increased neuronal activity these data suggest that a significant proportion of astrocytes release HCO₃⁻ to counteract the activity-associated extracellular acid loads (Fig. 1f).

ATP modulates bicarbonate transport in astrocytes. Emerging evidence suggests that communication between neurons and astrocytes is mediated primarily by purinergic signalling. ATP is released together with glutamate at central synapses25,27, and astrocytes are well equipped with purinoceptors to sense synaptic activity by responding to changes in extracellular ATP28.
Fig. 1 Regulation of brain extracellular pH. a The role of the carbonic anhydrase: the effect of carbonic anhydrase inhibitor acetazolamide (ATZ) on extracellular pH (pHe) in the somatosensory cortex of anaesthetised rats. Microinjection of ATZ (1 mM, placed -100 μm from the tip of the recording electrode) leads to a reversible decrease in brain pHe, as evident from a reduction in pH-sensitive electrochemical current recorded by fast cyclic voltammetry. Traces illustrate averaged (means ± SEM) changes in pH-sensitive current. Box-and-whisker plot illustrates peak changes in pHe in response to ATZ: the central dot indicates the mean, the central line indicates the median, the box limits indicate the upper and lower quartiles, and the whiskers show the minimum–maximum range of the data. p value, Mann–Whitney U test. b No extracellular acidification in response to increased neuronal activity and post-stimulus alkalisation: Time course of pHe changes in the right forelimb region of the somatosensory cortex (S1FL) induced by activation of somatosensory pathways (electrical stimulation of the contralateral paw; 3 Hz, 1.5 mA, 20 s) in anaesthetised mice (n = 13). Changes in pHe, and neuronal responses were recorded simultaneously using carbon fibre microelectrodes. Traces illustrate averaged (means ± SEM) changes in pH-sensitive current and representative recordings of extracellular potential in response to somatosensory stimulation. c In vivo imaging of intracellular pH (pHi) responses in astrocytes of the S1FL cortex using two-photon laser scanning microscopy (2-PLM) in mice. (i) Cortical astrocytes loaded with a pH sensitive dye BCECF and identified by sulphorhodamine (SR101) labelling (arrows). Scale bar = 50 μm. (ii) SR101 staining was used to binarise the image, whereby (iii) astrocyte cell bodies were segmented as contiguous SR101-positive regions. All identified astrocytes were included in the analysis. (iv) A representative example of peak BCECF fluorescence changes recorded in S1FL cortical astrocytes in response to the electrical stimulation of the contralateral paw. d Representative examples of changes in BCECF fluorescence in five S1FL cortical astrocytes induced by activation of somatosensory pathways. Pie chart shows the proportion of astrocytes responding to increased neuronal activity with intracellular acidification and alkalisation recorded in vivo. e Neuronal activity-induced intracellular acidification in astrocytes suggesting that astroglia is a source of bicarbonate: Time course of pHe changes recorded in S1FL cortical astrocytes in response to electrical stimulation of the contralateral paw. Traces illustrate averaged (means ± SEM) changes in BCECF fluorescence recorded in cortical astrocytes that showed peak change in ΔF/F₀ ≥ 2 SD of baseline fluorescence (responders; 27% of the whole population) and all other astrocytes. False colour plots illustrate averaged changes in BCECF fluorescence in all SR101-labelled cells in each individual animal. f Schematic drawing of the neurovascular unit illustrating the sources of extracellular H⁺. Acid loads associated with neuronal activity are hypothesised to be buffered by HCO₃⁻ derived from astrocytes. NHE, sodium hydrogen exchanger. MCT, monocarboxylate transporter. Source data are provided as a Source Data file.

Extracellular ATP concentration increases during neuronal activation²⁹, and mediates the neurovascular coupling response at the capillary level²⁹–³¹. Therefore, we next tested the hypothesis that HCO₃⁻ transport in astrocytes is modulated by purinergic signalling.

In a bicarbonate buffered solution (26 mM [HCO₃⁻]), saturated with 5% CO₂, cultured cortical astrocytes responded to ATP with strong intracellular acidification (increase in [H⁺], by 18 ± 1 mM and 66 ± 4 mM in response to 200 μM and 1 mM ATP, respectively; p < 0.001) (Fig. 3a), which followed elevations in [Ca²⁺], (see below and Supplementary Fig. S2a). ADP (200 μM) had the same effect (increase in [H⁺], by 33 ± 2 mM, p < 0.001; Fig. 3c; Supplementary Fig. S2b). Neither ATP nor ADP had an effect on intracellular pH in conditions when CO₂/HCO₃⁻-buffer was replaced with bicarbonate-free solution (HEPES buffer saturated with O₂) (Fig. 3b, d), suggesting that the ATP/ADP-induced intracellular acidification in astrocytes develops as a result of facilitated outward HCO₃⁻ transport.

The ATP/ADP-induced decreases in intracellular pH in astrocytes were markedly reduced in the presence of pharmacological
agents that inhibit Na\(^+\)/HCO\(_3\)\(^-\) cotransporter (NBC) activity (S0859, 50 and 100 µM; 4,4\(^{-}\)Disothiocyano-2,2\(^{-}\)-stilbenedisulfonic acid, DIDS, 200 µM) (Fig. 3e, f; Supplementary Fig. S2c, d), and abolished in conditions of genetic NBCe1 deletion (Fig. 3g, h). Membrane depolarisation by raising the extracellular [K\(^+\)] (from 3 to 7 mM and then to 20 mM) reduced the magnitude of ATP-induced intracellular acidification in astrocytes (Supplementary Fig. 3a–d). This result is consistent with the properties of NBCe1 and shows that outwardly directed transport of bicarbonate by NBCe1 is thermodynamically less favourable at the membrane potentials less negative than the NBCe1 equilibrium potential (calculated at −71 mV). Collectively, these data suggest that purinergic signalling modulates NBCe1 activity in astrocytes, with ATP (and downstream purines) acting to facilitate the outward transport of HCO\(_3\)\(^-\).

This conclusion was also supported by measurements of intracellular [Na\(^+\)]. NBCe1 cotransports HCO\(_3\)\(^-\) and Na\(^+\). Therefore, in response to ATP, the direction of intracellular [Na\(^+\)] change is determined by the NBCe1-mediated outward Na\(^+\) transport, by Na\(^+\) entry via enhanced sodium–calcium exchange (secondary to Ca\(^2+\) responses), and by activation of ionotropic P2X receptors (all occurring in parallel with the background activity of Na\(^+\)/K\(^+\) ATPase)\(^{-3}\). We found that the balance of these ATP-induced actions results in a net increase in [Na\(^+\)]. (Supplementary Fig. S2e, f). Because it is predominantly the Na\(^+\)/HCO\(_3\)\(^-\) cotransport that is affected in the absence of HCO\(_3\)\(^-\), this result is consistent with the facilitation of outward NBCe1 activity in response to ATP.

**Expression of NBCe1 in the cerebral cortex.** The single-cell RNAseq data from the mouse cerebral cortex (Fig. 4a) demonstrated high expression of gene encoding NBCe1 (Slc4a4) in discrete populations (or clusters) of cells (Fig. 4b). The analysis of cell identity marker genes showed that these clusters express characteristic astrocyte-specific genes (Fig. 4c). Slc4a3 (glutamate aspartate transporter 1) and Gja1 (connexin 43) are strongly and uniformly expressed across all cell clusters that display high expression of Slc4a4 and are negative for the expression of genes associated with neurons (Syt1), pericytes (Knnj8), smooth-muscle cells (Acta2), and microglial cells (Tmem119) (Fig. 4c). We, therefore, refer to these cells as astrocyte marker-positive cells. The analysis of RNAseq data from these cells taken in isolation (6,760 cells) revealed 11 sub-clusters of astrocyte marker-positive cells with distinct RNA expression profiles (Fig. 4d). Slc4a4 and the gene encoding carbonic anhydrase 2 (Car2) showed significant differential expression within these cell clusters (Fig. 4e). A large cluster comprised of >1000 cells (Cluster 2) displayed high expression of both Slc4a4 and Car2 genes (average logFC of 0.52; adj. \(p = 1.4 \times 10^{-61}\), and 0.26; adj. \(p = 1.2 \times 10^{-20}\), respectively). The proportion of cells within the clusters that were Slc4a4-positive and/or Car2-positive, and the relative average expression of these genes (Fig. 4e), showed the heterogeneity of Slc4a4 and Car2 expression in astrocyte marker-positive cells. Clusters 1 and 2 contained the largest proportion of cells expressing high levels of both Slc4a4 and Car2, accounting for more than 30% of all astrocyte marker-positive cells (Fig. 4f).

These data indicate that in the cerebral cortex NBCe1 is expressed predominantly in astrocytes, and that in a significant proportion of these cells high NBCe1 expression is complemented by high expression of carbonic anhydrase 2.

**NBC mediates activity-dependent pH\(_i\) responses in astrocytes.** In the experiments in acute hippocampal slices we next tested the hypothesis that NBC is responsible for the neuronal activity-dependent pH\(_i\) changes in astrocytes. Pharmacological blockade of NBC activity with S0859 (50 µM) or DIDS (300 µM) markedly reduced or abolished (depending on the strength of the stimulus) pH\(_i\) responses in CA1 astrocytes triggered by the electrical
Fig. 3 Bicarbonate transport in astrocytes is modulated by purinergic signalling. a Representative example of intracellular acidification induced by ATP in cortical astrocytes in culture. The trace depicts averaged [H+]i responses of 17 astrocytes to repeated application of ATP (1 mM) in culture. b ATP-induced intracellular acidification in astrocytes is reversibly blocked by removal of extracellular HCO3−. Averaged traces of ATP-induced [H+]i responses in 9 astrocytes in the presence and absence of HCO3− in the media are shown. c Representative example of intracellular acidification induced by ADP in cortical astrocytes in culture. The trace depicts averaged [H+]i responses of 9 astrocytes to repeated application of ADP (200 µM) in culture. d ADP-induced intracellular acidification in astrocytes is reversibly blocked by removal of extracellular HCO3−. Averaged traces of ADP-induced [H+]i responses in 14 astrocytes in the presence and absence of HCO3− in the media are shown. e ATP-induced intracellular acidification in astrocytes is blocked by sodium bicarbonate co-transporter (NBC) inhibitor S0859 (100 µM). Averaged traces of ATP-induced [H+]i responses in 12 astrocytes are shown. f Summary data illustrating the effects of pharmacological inhibition of NBC (S0859, DIDS) or removal of extracellular HCO3− on peak increases in [H+]i induced by ATP or ADP in astrocytes. g Representative examples of averaged [H+]i responses to ADP or ATP in 12 cultured astrocytes of wild-type (WT) and 12 astrocytes of electrogenic sodium-bicarbonate cotransporter 1 (NBCe1) knockout (NBCe1-KO) mice. h Summary data illustrating peak changes in [H+]i induced by ATP and ADP in astrocytes of WT and NBCe1-KO mice. In the box-and-whisker plots the central dot indicates the mean, the central line indicates the median, the box limits indicate the upper and lower quartiles and the whiskers extend to 1.5 IQR from the quartiles. Numbers in parentheses indicate the numbers of individual astrocytes recorded in 3–5 separate cultures prepared from the same number of animals. *** denotes difference from the response recorded in control conditions (f) or in WT astrocytes (h) with p < 0.001 (one-way ANOVA with Tukey’s post-hoc test). Source data are provided as a Source Data file.
stimulation of Schaffer collateral fibres (Fig. 5). Activity-dependent pH$_i$ changes in individual CA1 astrocytes responding with intracellular acidification (Fig. 5a–c) and intracellular alkalisation (Fig. 5d, e) were reduced by the NBC blockade. These data are consistent with the evidence that NBCe1 in astrocytes can transport bicarbonate in both directions$^{19,33}$. These data also argue against a significant role of other potential cytosolic acid loading mechanisms, such as Cl$^−$/HCO$_3$$^−$ exchange and proton uptake via glutamate transporters in the mechanisms underlying the neuronal activity-dependent intracellular acidification in astrocytes (conclusion also supported by the results of our earlier reports$^{21,33}$).

Patch-clamp recordings in CA1 astrocytes (nine cells recorded in acute slice preparations from four animals, resting membrane potential below $−75$ mV) showed that pharmacological NBC inhibition leads to a reversible hyperpolarization. S0859 (50 µM, 10 min application) reduced the median membrane potential of CA1 astrocytes from $−80.7$ to $−85.0$ mV ($p = 0.004$; paired-sample Wilcoxon test; Supplementary Fig. 3e,f). These data provide evidence that under resting conditions, NBCe1 in astrocytes is operating in the outward mode.

Signalling mechanisms of NBCe1 activity modulation by ATP. We next investigated the signalling mechanisms responsible for the activation of the NBCe1-mediated outward transport of HCO$_3$$^−$ in response to ATP. ATP-induced intracellular acidification and [Ca$^{2+}$]$_i$ responses were markedly reduced in the presence of the broad spectrum P2 receptor antagonist PPADS (100 µM) (Fig. 6a, b). However, neither TNP-ATP (10 µM), which preferentially inhibits ionotropic P2X receptors, nor the P2X$_7$ antagonist O-ATP (100 µM) had an effect on ATP-induced pH and Ca$^{2+}$ responses in astrocytes (Supplementary Fig. S4a–d).

Extracellular ATP is rapidly broken down by ectonucleotidase activity to ADP that is predominantly active at metabotropic P2Y receptors. That the effects of ATP on intracellular pH and [Ca$^{2+}$]$_i$ in astrocytes are mimicked by ADP (Fig. 3) suggests that metabotropic P2Y receptors mediate these effects. Indeed, the
blockade of P2Y1 receptors with MRS2179 (20 µM) or MRS2500 (2 µM) reduced or abolished the ATP or ADP-induced intracellular acidification and [Ca2+]i responses (Fig. 6c, g, h; Supplementary Fig. S5c). The specific P2Y1 receptor agonist 2-Mes-ADP (20 µM) induced intracellular acidification and Ca2+ responses in astrocytes (Fig. 6d, g, h; Supplementary Fig. S5b). These responses were similar to the responses induced by ATP/ADP and were abolished by the P2Y1 receptor blocker MRS2500 (Fig. 6d, g, h).

An increase in [Na+]i following activation of a sodium–calcium exchanger is one of the potential mechanisms that can facilitate the outward NBCe1 activity34. However, the blockade of sodium–calcium exchange with SN-6 (20 µM) had no effect on ATP-induced intracellular pH and [Ca2+]i responses in astrocytes (Supplementary Fig. S4e,f). There is also evidence that PLC-mediated Ca2+ recruitment from the intracellular stores can modulate NBCe1 activity 22. Activation of P2Y1 receptors in astrocytes recruits PLC activity 28,35. ATP/ADP effects on intracellular pH and [Ca2+]i in astrocytes were found to be abolished when PLC activity was blocked with edelfosine (10 µM) or U73122 (10 µM) or when intracellular Ca2+ stores were depleted by thapsigargin (1 µM) (Fig. 6e–h, Supplementary Fig. 5a, d). These data suggest that astrocytes release bicarbonate to the extracellular space in response to ATP/ADP acting at
Fig. 6 Signalling mechanisms underlying purinergic modulation of bicarbonate transport in astrocytes. a Representative recording illustrating the effect of P2 receptor antagonist PPADS (100 μM) on [H⁺], and [Ca²⁺], responses induced by ATP (1 mM) in astrocytes. Averaged traces of [H⁺], and [Ca²⁺], changes in ten astrocytes are shown. b Summary data illustrating the effects of PPADS on peak increases in [H⁺], and [Ca²⁺], induced by ATP in astrocytes. c Intracellular acidification and Ca²⁺ responses in astrocytes induced by ADP (200 μM) are blocked by P2Y₁ receptor antagonist MRS2500 (2 μM). Averaged traces of ADP-induced [H⁺], and [Ca²⁺], responses in ten astrocytes are shown. d The effects of ATP/ADP on [H⁺], and [Ca²⁺], in astrocytes are mimicked by P2Y₁ receptor agonist 2-Mes-ADP (20 μM). Averaged traces of 2-Mes-ADP-induced [H⁺], and [Ca²⁺], responses in the absence and presence of MRS2500 in 13 astrocytes are shown; e Intracellular acidification and [Ca²⁺], responses in astrocytes induced by ADP are blocked by phospholipase C inhibitor edelfosine (10 μM). Averaged traces of ADP-induced [H⁺], and [Ca²⁺], responses in 11 astrocytes are shown. f Intracellular acidification and [Ca²⁺], responses in astrocytes induced by ADP are blocked after depletion of intracellular Ca²⁺ stores with thapsigargin (1 μM) in the absence of extracellular Ca²⁺. Averaged traces of ADP-induced [H⁺], and [Ca²⁺], responses in 16 astrocytes are shown. g, h Summary data illustrating the effects of P2Y₁ receptor blockade (MRS2179, 20 μM; MRS2500, 2 μM), phospholipase C inhibition (U73122, 10 μM; edelfosine, 10 μM), removal of extracellular Ca²⁺ (0 Ca²⁺ conditions) or removal of extracellular Ca²⁺ combined with depletion of intracellular Ca²⁺ stores with thapsigargin (1 μM) on peak increases in [H⁺], and [Ca²⁺], induced by ADP (200 μM) in astrocytes. Peak [H⁺], and [Ca²⁺], responses induced in astrocytes by 2-Mes-ADP (20 μM) in the absence and presence of MRS2500 are also shown. In the box-and-whisker plots, the central dot indicates the mean, the central line indicates the median, the box limits indicate the upper and lower quartiles and the whiskers extend to 1.5 IQR from the quartiles. Numbers in parentheses indicate the number of individual astrocytes recorded in 3–5 separate cultures prepared from the same number of animals. *** denotes difference from the response recorded in control conditions with p < 0.001 (one-way ANOVA with Tukey’s post-hoc test). Source data are provided as a Source Data file.
metabotropic P2Y1 receptors, through the downstream activation of PLC, the recruitment of Ca\textsuperscript{2+} from the intracellular stores, and the facilitation of HCO\textsubscript{3}– transport by NBCe1.

**Astrogial NBCe1 is essential for the control of brain pHe.** To understand the adaptive significance of the astrogial mechanism of NBCe1-mediated HCO\textsubscript{3}– release found in this study, we next used the NBCe1 conditional knockdown strategy to reduce the expression of this transporter specifically in astrocytes (Fig. 7a–e).

Tamoxifen treatment of NBCe1\textsuperscript{fl/oxy/}GLAST\textsuperscript{CreERT2/+} mice reduced the NBCe1 transcript level by ~25% and the density of NBCe1 immunostaining by ~40% (Fig. 7e), resulting in a mosaic pattern of NBCe1 expression in the cortex (Fig. 7d). The degree of NBCe1 knockdown with this approach was smaller than expected. Yet, even in conditions of partial NBCe1 knockdown in astrocytes, extracellular pH was not maintained, and significant extracellular acidification developed in the somatosensory cortex in response to electrical stimulation of the forepaw (Fig. 7f–h). These data suggest that astrocytes actively release HCO\textsubscript{3}– via
NBCe1 to counteract extracellular acid loads associated with increases in local neuronal activity.

**Discussion**

The results obtained in this study show that ATP and ADP (the first product of extracellular ATP breakdown) trigger bicarbonate secretion by astrocytes through the activation of metabotropic P2Y1 receptors, recruitment of PLC, release of Ca\(^{2+}\) from the internal stores, and facilitated outward HCO\(_3^-\) transport by NBCe1. Because enhanced neuronal/synaptic activity is commonly associated with increases in the local concentration of extracellular ATP\(^{27,29,30}\) and with [Ca\(^{2+}\)], elevations in neighbouring astrocytes\(^{36-38}\), this mechanism is proposed to be responsible for the neuronal activity-dependent supply of bicarbonate to the extracellular space. This process is essential for the maintenance of extracellular pH buffer strength and, therefore, the brain pH homeostasis.

The brain milieu could be prone to significant fluctuations in pH due to the activities of several cellular and membrane mechanisms that involve the generation of acid/base equivalents and their movements across the cellular membranes. These mechanisms include metabolic production of CO\(_2\)/H\(^+\), monocarboxylate transporter-mediated co-transport of H\(^+\) and lactate by neurons and glial cells, exocytosis of highly acidic neurotransmitter vesicles, the activity of plasma membrane Ca\(^{2+}\) ATPase, bicarbonate flux via anion channels, transporters and GAB\(_A_3\) receptors, and operation of H\(^+\) extruders, such as Na\(^+\)/H\(^+\) exchanger and V-type ATPase\(^8,23,39\) (Fig. 1f). The balance between these processes leads to extracellular acid loads, since protons and CO\(_2\) generated by active neurons and other brain cells must be removed if brain function is to be maintained. Yet, the brain extracellular pH remains remarkably stable, not only without variable levels in neuronal activity but also major (physiological or pathological) perturbations of systemic acid/base balance\(^40\).

The profile of activity-induced changes in extracellular pH we recorded in the mouse somatosensory cortex is broadly consistent with the pH responses to physiological stimuli recorded in vivo and reported in earlier publications, in response to increased neuronal activity extracellular pH becomes more alkaline or large population constituting of up to 50% of all astrocytes respond with intracellular acidification, consistent with the activity-dependent outward transport of HCO\(_3^-\) that prevents extracellular pH falls during periods of increased neuronal activity. A smaller population (~4% of cells recorded in vitro; 30% of cells recorded in vivo) respond with intracellular alkalinisation. Intracellular alkalinisation has been previously attributed to K\(^+\)-dependent HCO\(_3^-\) uptake via the NBCe1\(^43-48\) leading to glycolytic activation, reduction of oxygen consumption and fast lactate release by astrocytes\(^49-52\). Taken together, these results are consistent with the evidence suggesting that forebrain astrocytes are heterogeneous in terms of their resting membrane potential with a larger subpopulation having the membrane potential between ~90 to ~70 mV, and a ‘depolarised’ subpopulation with the membrane potential between ~60 to ~30 mV\(^33\). Considering that ion stoichiometry of astroglial NBCe1 is 1Na\(^+\)-2HCO\(_3^-\) and that the equilibrium potential of NBCe1 is between ~68 to ~74 mV\(^21\), this transporter would be expected to operate close to its reversal potential or in the outward mode in the majority of astrocytes. In a smaller subpopulation of astrocytes NBCe1 would operate in the inward mode.

In cortical astrocytes, a variety of extracellular acidic stimuli have been shown to trigger the rapid release of HCO\(_3^-\) via NBCe1\(^33\). In this study, recruitment of polysynaptic somatosensory pathways in the in vivo experiments revealed the heterogeneous [H\(^+\)], responses of astrocytes in the somatosensory cortex. We hypothesise that two populations of astrocytes fulfil distinct functional roles: a larger population that exports HCO\(_3^-\) and, by doing so, maintains extracellular pH homeostasis, and the second population in which inward NBCe1 activity facilitates glucose mobilisation and glycolysis, as described previously by one of our laboratories\(^49,50\). Analysis of single-cell RNAseq data of the mouse cerebral cortex from the Mouse Brain
Atlas database\textsuperscript{54} identified 11 distinct clusters of cells based on similarities in the RNA expression profile, all expressing a high level of astrocyte-specific genes (Fig. 4). Although, NBCe1 expression is almost exclusively confined to astroglial population, it remains to be determined whether the cells that constitute different astrocyte sub-clusters based on their genetic makeup are also functionally distinct.

High levels of neuronal activity leading to significant elevations of extracellular [K\textsuperscript{+}] may cause depolarisation of astrocytes and, therefore, would be expected to inhibit HCO\textsubscript{3}\textsuperscript{−} outward transport and favour NBCe1 operation in the inward mode. In pathological conditions like epilepsy, stroke, and spreading depression, when the brain extracellular [K\textsuperscript{+}] markedly increases, the astroglial mechanism of extracellular pH control described in this study is likely to be disrupted. Indeed, early reports showed that in rats direct electrical stimulation of the brain tissue or evoked spreading depression trigger large depolarisations (up to +40 mV) and intracellular alkalinisation in cortical astrocytes leading to acidification of the extracellular space\textsuperscript{40}.

Importantly, under normal physiological conditions NBCe1-mediated transport is prone to modulation by key intracellular signalling pathways\textsuperscript{22}. The activity of NBCe1 expressed in Xenopus oocytes has been shown to be facilitated in response to PIP2 degradation by PLC and intracellular Ca\textsuperscript{2+} release\textsuperscript{55}. There is also evidence that the increases in intracellular Ca\textsuperscript{2+} and NBCe1 phosphorylation can potentially alter the ion stoichiometry of the transporter and, therefore, the direction of NBCe1-mediated bicarbonate transport\textsuperscript{56,57}. The results of this study are consistent with the existing evidence that in the majority of astrocytes NBCe1 is operating in the outward mode and that the outward activity of the transporter is facilitated by P2Y\textsubscript{1} receptor activation via PLC recruitment and Ca\textsuperscript{2+} release from the internal stores. We found no evidence that this signalling pathway changes NBCe1 stoichiometry or the direction of HCO\textsubscript{3}\textsuperscript{−} transport.

Interestingly, analogous mechanisms have been shown to operate in other physiological systems, although these may utilise other membrane transporters of bicarbonate. For example, in the gut epithelial cells, ATP acting at P2Y\textsubscript{1} receptors stimulates HCO\textsubscript{3}− secretion via Cl\textsuperscript{−}/HCO\textsubscript{3}− exchanger\textsuperscript{58}. There is evidence for the existence of a local negative feedback mechanism that controls HCO\textsubscript{3}− release by these cells with the key components including extracellular alkaline phosphatase, ATP, P2Y\textsubscript{1} receptors and membrane bicarbonate transport\textsuperscript{58}. Akin to the mechanism described here, ATP stimulates HCO\textsubscript{3}− secretion leading to alkalinisation of the extracellular space. This optimises the activity of alkaline phosphatase and facilitates ATP degradation. The acidic environment inhibits alkaline phosphatase activity, prolongs ATP actions and promotes HCO\textsubscript{3}− secretion\textsuperscript{58}. We found no evidence of alkaline phosphatase involvement in the mechanism identified in this study (Supplementary Fig. 4g, h). Indeed, it seems advantageous for the extracellular concentration of ATP/ADP in the brain to be determined primarily by the level of local neuronal activity, and not by the tissue ectonucleotidase activity. There is also evidence that extracellularacidification by itself (independently of the synaptic activity) may facilitate the release of purines\textsuperscript{59,60} and, therefore, potentiate bicarbonate release by the mechanism described here.

Because of the high pH sensitivity of neurotransmitter receptors, ion channels, and biochemical cascades involved in synaptic transmission, maintaining a stable pH environment is critically important for brain function. Respiratory chemoreceptors located in the brainstem restore brain pH in response to acute changes in the blood and brain PCO\textsubscript{2}/pH by controlling the activity of the respiratory network leading to the adaptive changes in lung ventilation. There is evidence that brainstem astrocytes function as central respiratory chemoreceptors, sensitive to changes in PCO\textsubscript{2} and pH\textsuperscript{51−65}. Interestingly, the mechanisms underlying the pH sensitivity of the brainstem astrocytes also involve NBCe1 and ATP-mediated signalling\textsuperscript{63,65}. However, in contrast to cortical and hippocampal astrocytes, in the specialised chemosensory brainstem astrocytes NBCe1 operates in the inward mode\textsuperscript{63}. In these astrocytes acidification leads to NBCe1-mediated increase in intracellular [Na\textsuperscript{+}] which activates sodium/calcium exchanger to operate in a reverse mode, leading to increases in intracellular [Ca\textsuperscript{2+}] and Ca\textsuperscript{2+}-dependent release of ATP\textsuperscript{63,66}. Since brainstem astrocytes are adjacent to, and intermingled with, the neuronal respiratory control networks, they are in a position to directly modulate breathing activity (via the release of ATP\textsuperscript{61}) and, therefore, maintain systemic (arterial) pH homeostasis. The data obtained in this study demonstrate the existence of another key astroglial mechanism, essential for the maintenance of local brain pH in face of variable extracellular acid loads that depend on neuronal activity.

Clinical studies have identified homozygous mutations of Slc4a4 gene (leading to variable degree loss of NBCe1 function) causing permanent renal tubular acidosis, glaucoma and hemi plegic migraine\textsuperscript{67,68}. Heterozygous carriers of Slc4a4 mutation appear to be largely normal, but may display some of these pathological features\textsuperscript{57}. Mouse models demonstrated that NBCe1 function is critically important for homeostasis as global Slc4a4 knockout animals (with complete NBCe1 loss-of-function) do not survive beyond the third week of life\textsuperscript{69}. Lifespan in these animals is limited by severe metabolic acidosis\textsuperscript{69} where breathing deficits may contribute to this harmful phenotype, as discussed previously\textsuperscript{63}. In this study, we employed an inducible astrocyte-specific NBCe1 knockout strategy whereby the animals develop normally. Behavioural studies focusing on the assessment of basic parameters such as stress, motor skills, balance, learning and memory in conditions of astrocyte-specific NBCe1 deletion are currently in progress.

Disturbances of brain pH homeostasis have been implicated in the pathogenesis of several common neurological conditions, including epilepsy, ischaemia/stroke, as well as different psychiatric disorders such as schizophrenia, bipolar disorder and autism spectrum disorders\textsuperscript{70}. A strong correlation between full scale human intelligent quotient (IQ) and brain pH was reported in adolescents (7−13 years old), with lower pH associated with lower IQ scores\textsuperscript{71}. Astrocytes control the extracellular concentrations of key ions (potassium in particular), and metabolites (lactate, glutamate/glutamine), therefore, are critically important for the maintenance of the ionic and metabolic homeostasis of the brain milieu. This study describes another important metabolic housekeeping role of these ubiquitous glial cells. The data show that astrocytes help to maintain the stability of local brain extracellular pH via a neuronal activity-dependent shuttle of bicarbonate.

Methods

Ethical approval and animal husbandry. All animal experimentations were performed in accordance with the European Commission Directive 2010/63/EU (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes) and the UK Home Office (Scientific Procedures Act, 1986) with project approval from the Institutional Animal Care and Use Committees of the University College London and Centro de Estudios Científicos. The animals were group-housed and maintained on a 12-h light cycle (lights on 07:00) and had ad libitum access to water and food. The rats were housed in a temperature-controlled room at 22 °C with 55 ± 10% relative humidity. The mice were housed at 24 °C ambient temperature with relative humidity kept at 60 ± 5%.

Animal preparation for the in vivo studies. Young adult C57BL/6J mice of both sexes (3−4 months old) were anaesthetised with either ketamine/xylazine (100 mg kg\textsuperscript{−1}/10 mg kg\textsuperscript{−1}) or a mix of fentanyl, medetomidine and midazolam (0.03 mg kg\textsuperscript{−1}/0.3 mg kg\textsuperscript{−1}/3 mg kg\textsuperscript{−1}, respectively). Rats (Sprague-Dawley,
Fast scan cyclic voltammetry in vivo. Parenchymal pH and evoked neuronal activity in the S1FL region of the cortex were recorded using fast cyclic voltammetry. Reference 43 gives detailed description of the technique, principles of H+ detection, calibration, specificity and interference with the detection of other analytes. Cyclic voltammetry (diameter 7 μm) were advanced into the S1FL cortex until evoked extracellular potentials were detected in response to the electrical stimulation (1 Hz) of the contralateral forepaw. A series of voltage ramps (200 V s−1) from −1 V to 0 V were applied to the CFM at a frequency of 2 Hz. The resulting current was amplified, digitised and recorded for offline isolation of faradic currents corresponding to [H+]. Trains of electrical forepaw stimulation were applied three times per animal/experimenral condition with intervals of at least 3 min between the stimulations. Neuronal responses were analysed by integration of the evoked volley of extracellular potentials with the baseline responses (as described) at the end of the recordings. pH sensitivity of each CFM was determined by calibration, as described 43.

Two-photon imaging of pH, in astrocytes in vivo. During the animal preparation, the exposed area of the cortex was superfused with warm artificial cerebrospinal fluid (aCSF; containing in mM: 125 NaCl, 2 KCl, 26 NaHCO3, 1.25 NaH2PO4, 18 Glucose, 2 CaCl2, 2 MgSO4; saturated with 95% O2/5% CO2, pH 7.4). Cortical astrocytes were labelled with sulforhodamine 101 (SR101) and loaded with a pH sensitive dye BCECF. The solution containing BCECF (Invitrogen; 1 mM) was administered. The body temperature was maintained at 37 ± 1 °C. The animal was anaesthetised with α-chloralose (100 mg kg−1). Adequate anaesthesia was achieved by maintaining stable levels of the heart rate showing lack of a response to a paw pinch. If required, supplemental doses of the anaesthetics were administered. The body temperature was maintained at 37 ± 1 °C. The animal was mounted in a stereotaxic frame. For the recordings of extracellular pH using fast scan cyclic voltammetry or intracellular pH using 2P microscopy, the right forelimb of the S1FL regions were separated by dissection. After isolation, the cells were plated on poly-D-lysine-coated cover slips and maintained at 37 °C in a humidified atmosphere of 5% CO2 and 95% air for a minimum of 10 days before the experiments. Astrocyte loading with BCECF and 2P microscopy

Imaging of pH, in cultured astrocytes. Optical recordings of changes in [H+], were performed using an inverted epifluorescence (OMU-MP05, equipped with a cooled CCD camera (CLARA, ANDOR, Oxford Instruments)) 63,64. For simultaneous recordings of changes in [H+], the cells were loaded with BCECF (Invitrogen; 2 μM for 10 min incubation69) and X-Rhod1 or Rhod2 (Invitrogen; 5 μM for 10 min incubation). After incubation with the dyes, cultures were washed three times prior to the experiment. Recordings were performed in a custom-made flow-through imaging chamber at 32 °C in aCSF saturated with 95% O2/5% CO2 (pH 7.4). The rate of chamber perfusion with aCSF was 1 ml min−1. Changes in [H+], were monitored in individual cells using excitation light provided by a xenon arc lamp with the beam passing sequentially through a multi dichroic beam splitter (Chroma Technology). Emission was excited at 495/10 nm and the emission was collected at 535/30 nm (CAMERAC). Fluorescence emission was recorded at 535, 590, and 600 nm for BCECF, Rhod2 and X-Rhod1 indicators, respectively. Changes in BCECF fluorescence were converted to intracellular proton concentration ([H+]i) in nM using the Nernstian K+ calibration method 62. Changes in intracellular [Na+]i changes in astrocytes were recorded using a sodium-sensitive fluorescent dye Asante-sodium green (ANG-2-AM, Abbam; 10 μM for 45 min incubation), as described 73. ANG-2 fluorescence was excited at 495/10 nm wavelength and the emission was collected at 535/30 nm.

Conditional NBCe1 knockdown in astrocytes. To induce conditional NBCe1 knockdown in astrocytes, mice carrying a loxP-flanked NBCe1 allele (NBCe1fl/+fl+) were crossed with the mice expressing an inducible form of Cre (CreERT2) under the astrocyte-specific GLAST promoter 77. Tamoxifen (100 mg kg−1) dissolved in corn oil (750/15; 1 ml) was injected intraperitoneally 3 times per week postnatal week 7 and the expression level of NBCe1 was examined 6 weeks after the tamoxifen treatment. Breeding was organised through PCR genotyping obtained from tail DNA biopsy. First, we evaluated the cell-specificity of recombination by inducing the expression of the fluorescent reporter tdTomato in stop78 tdTomato/GLAST-CreERT2 mice of following tamoxifen treatment at postnatal week 7. Astroglial recombination specificity of GLAST-CreERT2 mice has been reported in several prior studies 75,77. Specific Cre-mediated recombination was confirmed in cortical protoplasmatic astrocytes and Bergmann glia (cerebellum) by observing the characteristic pattern of tdTomato expression in the brains of stop78 tdTomato/GLAST-CreERT2 mice postnatal week 7. Astroglial recombination of the NBCe1 locus was verified by PCR in various brain regions from NBCe1fl/+fl+/GLASTCreERT2/2 mice injected with tamoxifen and in two control groups including littermate NBCe1fl/+fl+/GLASTCreERT2/2 mice injected with vehicle (oil) and NBCe1fl/+fl+/GLASTCreERT2/2 mice injected with tamoxifen. Western blot analysis of the cortex, hippocampus, brainstem, and cerebellum, but not in the kidneys of KO mice. No NBC3 recombination was observed in tissues of control animals.
immunohistochemistry. At the end of the experiments, the animals were given an anesthetic overdose, the brains were removed, fixed in 4% paraformaldehyde and sliced (15 μm). These slices were first incubated (10 μM citric acid) for 20 min and then in the blocking solution (3% bovine serum albumin, 10% normal goat serum, 0.1% Triton X-100) for 2 h following by incubation with primary mouse monoclonal anti-NBCe1 (1:50; Santa Cruz Biotechnology, Cat.# sc-515543, Lot#122116) or mouse polyclonal anti-MAP2 antibodies (1:500; Sigma, Cat# M-1400) overnight at 4°C. The sections were subsequently incubated in secondary anti-mouse antibody Dylight 488 (1:500; ThermoFisher, Cat #35502) for 2 h at room temperature.

Images of NBCe1 immunofluorescence in the cortices of NBCe1lox/lox; GLASTCreERT2 mice treated with vehicle (oil) or tamoxifen were acquired with identical optical settings using a confocal microscope (Olympus FV1000). Images were processed using ImageJ software (version 1.52 P). For each of the images, pixel intensity frequency distribution graph was generated, and pixel intensities were quantified by making binary images using the mean intensities of the two peaks of fluorescence. By measuring the area of the high-intensity pixel population, intensity of NBCe1 immunofluorescence in the cortex was compared between NBCe1lox/lox;GLASTCreERT2+ mice treated with vehicle or tamoxifen.

Genomic recombination and genotyping analysis. Specificity of recombination was evaluated by PCR using DNA from the brain tissue of NBCe1lox/lox; GLASTCreERT2+/− mice treated with tamoxifen or vehicle using the following primers: recombination forward 5’-TGG TGG CTT AAA TTA GCAATATTGGC-3’; recombination reverse 5’-TGGGGCTTAAATTGCGAATAGC-3’; genotyping forward primer 5’-GGGAGCTTGTCAGTAAATTGGACAT-3’ and reverse primer 5’-CGTCCCCTGTTTGCTATGATG-3’, and yielded 63 distinct cell clusters. The distribution of the Slek4+ expression was then plotted across the identified clusters. Only four clusters showed average scaled Slek4+ expression of >1.5 in more than 40% of the cells and were found to be grouped together after dimensionality reduction. These clusters (9, 13 and 29) were scrutinised for the expression of characteristic cell-specific marker genes30,31. Gene expression data from these four identified clusters were then pooled and re-clustered by the same method described for the whole dataset with modifications. The KNN graph was constructed using 20 principle components with Louvain algorithm resolution set to 0.8. After visualisation by UMAP, 11 distinct astroglial clusters were identified. Seurat analysis was used to find differentially expressed genes between the identified cell clusters.

Statistical analysis. Imaging data were acquired and analysed using IQ3 imaging software (version 6.3; Andor, Oxford Instruments) or Olympus Fluoview software (version 4; Olympus). CFM recordings in vivo were acquired using Power1401 in vivo and analysed offline using Spike2 software (version 7; Cambridge Electronic Design). Electrophysiological data from the in vitro recordings were acquired and analysed using pClamp 10.2 software. Statistical analysis of the data was performed using GraphPad-Prism software (version 8). Details of the statistical tests applied are provided within the figure legends. The data are reported as individual values and means ± SEM or as box-and-whisker plots.

RT qPCR. Brain tissue from NBCe1lox/lox;GLASTCreERT2+/− mice treated with tamoxifen or vehicle were incubated in Trizol Reagent (Invitrogen) and RNA was extracted. Total RNA (2 μg) was reverse transcribed using the ImPrim-ITM Reverse Transcription System (Promega). qPCR was performed in triplicates using KAPA SYBR FASTA qPCR kit. NBCe1 expression was quantified using a comparative ΔCt method50 and presented as arbitrary units of expression, normalised to the expression of the cyclophilin gene. The primers used were the following: NBCe1 cDNA forward: 5’-GACAATGTCGAGGGTTGTTG-3’; NBCe1 cDNA reverse: 5’-CCAGCAGCCGAACTAAAGACG-3’, cyclophilin cDNA forward: 5’-GGC AGT GCT GGA GCA AAG A A-3’; cyclophilin cDNA reverse: 5’-GTAAGATCGCCCGATTCAAA-3’. Data processing and visualisation was performed using the Seurat package30 (v.3.1.4) in R (v.3.6.0, “Planting of a Tree”). Code used for the analyses of the RNAseq data is included within the Supplementary Material.

Analysis of single-cell RNAseq data. Single-cell RNA-sequencing (RNAseq) data from the mouse cerebral cortex were obtained from a publicly available database, collated and maintained by the Linnarsson group (Karolinska Institutet; http://mousebrain.org/). Cell dissociation, single-cell RNAseq and quality control methods are described in detail in the original report of the database53. Data processing and visualisation was performed using the Seurat package30 (v.3.1.4) in R (v.3.6.0, “Planting of a Tree”). Code used for the analyses is available as part of the Supplementary Material. The combined mouse cortical cell RNAseq dataset was obtained from 50,478 cells with expression data for 27,998 genes. All cells that displayed nFeatures greater than 200, but less than 4000, and a percentage of genes with expression of >1.5 in more than 40% of the cells and were found to be grouped together after dimensionality reduction. These clusters (9, 13 and 29) were scrutinised for the expression of characteristic cell-specific marker genes30,31. Gene expression data from these four identified clusters were then pooled and re-clustered by the same method described for the whole dataset with modifications. The KNN graph was constructed using 20 principle components with Louvain algorithm resolution set to 0.8. After visualisation by UMAP, 11 distinct astroglial clusters were identified. Seurat analysis was used to find differentially expressed genes between the identified cell clusters.

Data availability. The data that support the findings in this study are included within the Supplementary Material and available from the corresponding author upon request. The source data underlying Figs. 1a, b, d, e, 2d, 3f, h, 5c, e, 6b, g, h, and 7e, f, g and Supplementary Fig. 1a, d, f, g, h, and i are provided as a Source Data file. Single-cell RNA-sequencing source data underlying Fig. 4 are provided from a publicly available database (http://mousebrain.org/).

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**Acknowledgements**

This work was supported by The Wellcome Trust (A.V.G. and D.A.R.) and the Fondecyt Iniciación Grant 1190678 (I.R.). D.A.R is a Wellcome Trust Principal Research Fellow (Ref. 212251). A.V.G is a Wellcome Trust Senior Research Fellow (Ref. 200893). CECs is funded by the Chilean Government through the Centers of Excellence Base Financing Program. We thank Héctor Oyarzún, Pablo Castro and Pamela Sanhueza (CECs) for technical assistance, Gary E. Shull (Cincinnati, USA), for providing NBCe1 flox mice, Frank Kirchhoff (Hamburg, Germany) for providing GLAST-CRE ERT2 mice and Hongkui Zeng (Seattle, USA) for providing Cre-reporter tdTomato mice. We are grateful to Professor Joachim W. Deitmer for his comments on an earlier version of the manuscript.

**Author contributions**

A.V.G. conceived and directed the project; S.M.T., P.S.H., I.R., O.K., J.R.R. and P.Y.S. performed research; D.A.R. and O.K. designed the in vitro electrophysiological experiments. I.R. generated astrocyte-specific conditional NBCe1 knockout mouse. L.F.B. contributed unpublished reagents/analytic tools; S.M.T., P.S.H., I.R., O.K. and J.R.R. analysed the experimental data; P.S.H. analysed the RNAseq data. A.V.G. and S.M.T. wrote the paper. All authors revised the article critically for important intellectual content.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-18756-3.

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**Peer review information** *Nature Communications* thanks Kevin Staley and the other anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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