Lactate is a major energy substrate for cortical neurons and enhances their firing activity.

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Summary

Glucose is the mandatory fuel for the brain, yet the relative contribution of glucose and lactate for neuronal energy metabolism is unclear. We found that increased lactate, but not glucose concentration, enhances the spiking activity of neurons of the cerebral cortex. Enhanced spiking was dependent on ATP-sensitive potassium (K$_{ATP}$) channels formed with Kir6.2 and SUR1 subunits, which we show are functionally expressed in most neocortical neuronal types. We also demonstrate the ability of cortical neurons to take-up and metabolize lactate. We further reveal that ATP is produced by cortical neurons largely via oxidative phosphorylation and only modestly by glycolysis. Our data demonstrate that in active neurons, lactate is preferred to glucose as an energy substrate, and that lactate metabolism shapes neuronal activity in the neocortex through K$_{ATP}$ channels. Our results highlight the importance of metabolic crosstalk between neurons and astrocytes for brain function.

Keywords

K$_{ATP}$ channel, pyramidal cell, interneuron, glucose, single cell RT-PCR, ATP.

Highlights

- Most cortical neurons subtypes express pancreatic beta-cell like K$_{ATP}$ channels.
- Lactate enhances spiking activity via its uptake and closure of K$_{ATP}$ channels.
- Cortical neurons take up and oxidize lactate.
- Cortical neurons produce ATP mainly by oxidative phosphorylation.
Introduction

The human brain represents 2% of the body mass, yet it consumes about 20% of blood oxygen and glucose which are mandatory energy substrates (Clarke and Sokoloff, 1999). The majority (~50-80%) of the cerebral energy metabolism is believed to be consumed by the Na\(^+\)/K\(^+\) ATPase pump to maintain cellular ionic gradients dissipated during synaptic transmission and action potentials (Attwell and Laughlin, 2001; Lennie, 2003). Synaptic and spiking activities are also coupled with local cerebral blood flow and glucose uptake (Devor et al., 2008; Logothetis, 2008). This process, referred to as neurovascular and neurometabolic coupling, is the physiological basis of brain imaging techniques (Raichle and Mintun, 2006) and maintains extracellular glucose within a physiological range of 2-3 mM (Silver and Erecinska, 1994; Hu and Wilson, 1997b). Also, following increased neuronal activity extracellular lactate increases (Prichard et al., 1991; Hu and Wilson, 1997a) for several minutes up to twice of its 2-5 mM basal concentration despite oxygen availability (Magistretti and Allaman, 2018).

Based on the observations that various by-products released during glutamatergic transmission stimulate astrocyte glucose uptake, aerobic glycolysis and lactate release (Pellerin and Magistretti, 1994; Voutsinos-Porche et al., 2003; Ruminot et al., 2011; Choi et al., 2012; Sotelo-Hitschfeld et al., 2015; Lerchundi et al., 2015), lactate has been proposed to be shuttled from astrocytes to neurons to meet neuronal energy needs. This hypothesis is supported by the existence of a lactate gradient between astrocytes and neurons (Machler et al., 2016), the preferential use of lactate as an energy substrate in cultured neurons (Bouzier-Sore et al., 2003; Bouzier-Sore et al., 2006), and its ability to support neuronal activity during glucose shortage (Schurr et al., 1988; Rouach et al., 2008; Wyss et al., 2011; Choi et al., 2012).

However, the use of different fluorescent glucose analogues to determine whether astrocytes or neurons take up more glucose during sensory-evoked neuronal activity has led to contradicting results (Chuquet et al., 2010; Lundgaard et al., 2015). Furthermore brain slices and in vivo evidence have indicated that synaptic and sensory stimulation enhanced neuronal glycolysis and potentially lactate release by neurons (Ivanov et al., 2014; Diaz-Garcia et al., 2017), thereby challenging the astrocyte-neuron lactate shuttle hypothesis. Hence, the relative contribution of glucose and lactate to neuronal ATP synthesis remains unresolved.
ATP-sensitive potassium channels (K\textsubscript{ATP}) act as metabolic sensors controlling various cellular functions (Babenko et al., 1998). Their open probability (P\textsubscript{o}) is regulated by the energy charge of the cell (i.e. the ATP/ADP ratio). While ATP mediates a tonic background inhibition of K\textsubscript{ATP} channels, cytosolic increases of ADP concentrations that occur as a sequel to enhanced energy demands, increase the P\textsubscript{o} of K\textsubscript{ATP} channels. In neurons, electrical activity is accompanied by enhanced sodium influx, which in turn activates the Na\textsuperscript{+}/K\textsuperscript{+} ATPase. Activity of this pump alters the submembrane ATP/ADP ratio sufficiently to activate K\textsubscript{ATP} channels (Tanner et al., 2011). The use of fluorescent ATP/ADP biosensors has demonstrated that K\textsubscript{ATP} channels are activated (P\textsubscript{o}>0.1) when ATP/ADP ratio is \leq 5 (Tantama et al., 2013).

K\textsubscript{ATP} channels are heterooctamers composed of four inwardly rectifying K\textsuperscript{+} channel subunits, Kir6.1 or Kir6.2, and four sulfonylurea receptors, SUR1 or SUR2, the later existing in two splice variants (SUR2A and SUR2B) (Sakura et al., 1995; Aguilar-Bryan et al., 1995; Inagaki et al., 1996; Isomoto et al., 1996; Inagaki et al., 1996; Chutkow et al., 1996; Yamada et al., 1997; Li et al., 2017; Martin et al., 2017; Lee et al., 2017; Puljung, 2018). The composition in K\textsubscript{ATP} channel subunits confers different functional properties, pharmacological profiles as well as metabolic sensitivities (Isomoto et al., 1996; Inagaki et al., 1996; Gribble et al., 1997; Yamada et al., 1997; Okuyama et al., 1998; Liss et al., 1999). K\textsubscript{ATP} channel subunits are expressed in the neocortex (Ashford et al., 1988; Karschin et al., 1997; Dunn-Meynell et al., 1998; Thomzig et al., 2005; Cahoy et al., 2008; Zeisel et al., 2015; Tasic et al., 2016) and have been shown to protect cortical neurons from ischemic injury (Heron-Milhavet et al., 2004; Sun et al., 2006) and to modulate their excitability (Gimenez-Cassina et al., 2012) and intrinsic firing activity (Lemak et al., 2014). K\textsubscript{ATP} channels could thus be leveraged to decipher electrophysiologically the relative contribution of glucose and lactate to neuronal ATP synthesis. Here, we apply single-cell RT-PCR (scRT-PCR) to identify the mRNA subunit composition of K\textsubscript{ATP} channel across different neocortical neuron subtypes and demonstrate lactate as the preferred energy substrate that also enhances firing activity.
Results

Expression of $K_{ATP}$ channel subunits in identified cortical neurons

We first sought to determine whether $K_{ATP}$ channel subunits were expressed in different neuronal subtypes from the neocortex. Neurons (n=277) of the juvenile rat barrel cortex from layers I to IV (Table S1) were functionally and molecularly characterized in acute slices by scRT-PCR (Figure 1), whose sensitivity was validated from 500 pg of total cortical RNAs (Figure S1A). Neurons were segregated into 7 different subtypes according to their overall molecular and electrophysiological similarity (Figure 1A) using unsupervised Ward’s clustering (Ward, 1963), an approach we previously successfully used to classify cortical neurons (Caudi et al., 2000; Gallopin et al., 2006; Karagiannis et al., 2009). Regular spiking (RS, n=63) and intrinsically bursting (IB, n=10) cells exhibited the molecular characteristics of glutamatergic neurons, with very high single-cell detection rate (n=69 of 73, 95%) of vesicular glutamate transporter 1 (vGluT1) and low detection rate (n=7 of 73, 10%) of glutamic acid decarboxylases (GADs, Figure 1B-E and Table S2), the GABA synthesizing enzymes. This group of glutamatergic neurons distinctly displayed hyperpolarized resting membrane potential (-81.2 ± 0.8 mV), possessed a large membrane capacitance (108.6 ± 3.6 pF), discharged with wide action potentials (1.4 ± 0.0 ms) followed by medium afterhyperpolarizations (mAHs). These neurons did sustain only low maximal frequencies (35.4 ± 1.6 Hz) and showed complex spike amplitude accommodation (Table S3-7). In contrast to RS neurons, IB neurons were more prominent in deeper layers (Table S1) and their bursting activity affected their adaptation amplitudes and kinetics (Figure 1C and Tables S4-5), spike broadening (Figure 1C and Tables S6) and the shape of mAHs (Figure 1C and Tables S7).

All other neuronal subtypes were characterized by a high single-cell detection rate of GAD65 and/or GAD67 mRNA (n=202 of 204, 99%, Figure 1B and Table S2) and therefore likely corresponded to GABAergic interneurons. Among GAD-positive population, neurons frequently expressed mRNA for vasoactive intestinal polypeptide (VIP), and in accordance to their electrophysiological phenotypes, were segregated into Bursting VIP (n=27) and Adapting VIP (n=59) neurons. These VIP interneurons were further characterized by high membrane resistance (581 ± 27 MΩ) and small membrane capacitance (52.7 ± 2.3 pF, Figure 1B-C and Tables S2-3).

Other GABAergic interneurons co-expressed somatostatin (SOM) and calbindin (CB) as well as neuropeptide Y (NPY) to a lesser extent and functionally corresponded to...
Adapting SOM neurons (n=24, Figure 1B and Table S2). They displayed depolarized resting membrane potential, pronounced voltage sags, low rheobases and pronounced afterdepolarizations (Figure 1C and Table S3-6). In another group of GABAergic adapting interneurons located in superficial layers, mRNA for NPY was detected at a high rate (n=31 of 56, 55%). In these Adapting NPY interneurons mRNA for nitric oxide synthase-1 (NOS-1) was detected at a lower rate (Figure 1B and Tables S1-2). In response to suprathreshold depolarizing current steps, these interneurons showed very little spike frequency adaptation (Figure 1C and Table S4).

Finally, parvalbumin (PV) was observed in virtually all neurons of a subpopulation termed Fast Spiking-PV interneurons (FS-PV, n=37 of 38, 97%, Figure 1B and Table S2). In comparison to all other cortical neurons described above, they were characterized by low membrane resistance (201 ± 13 MΩ), fast time constant, high rheobase, very short spikes (0.6 ± 0.0 ms) with sharp fast afterhyperpolarizations (fAHs) and the ability to sustain high firing rates (139.9 ± 6.8 Hz) with little to no frequency adaptation (Figure 1C and Tables S3-7). These data thus identified different neuronal subtypes based on their distinctive electrophysiological and molecular features (Ascoli et al., 2008) confirming our previous classification schemes (Cauli et al., 2000; Gallopin et al., 2006; Karagiannis et al., 2009).

The functional and molecular classification of cortical neurons allowed us to probe for the single-cell expression of mRNA for K ATP channel subunits in well defined subpopulations. We observed consistent expression of Kir6.2 and SUR1 between the different cortical neuronal subtypes (Figure 1D-F). Although the scRT-PCR procedure was shown to be reliable for co-detecting all K ATP channel subunits from low amounts of total cortical RNAs (Figure S1A), the overall single-cell detection rates were low for Kir6.2 (n=63 of 248, 25%) and SUR1 (n=28 of 277, 10%). In accordance to previous single-cell studies (Liss et al., 1999; Zeisel et al., 2015; Tasic et al., 2016), our observation suggests an expression at a low copy number at the single-cell level. Apart from a single Adapting NPY neuron (Figure 1D), where Kir6.1 mRNA was detected, our data set provide evidence that for all neocortical neuron types the pattern of detected K ATP channels mRNA expression suggested the co-expression Kir6.2 and SUR1 subunits. This implies that most cortical neurons might be endowed with a pancreatic beta-cell like K ATP channel, which might operate as a metabolic sensor. We next tested this prediction with functional recordings of K ATP channel-mediated whole-cell currents in neocortical neurons.
Characterization of K\textsubscript{ATP} channels in cortical neurons

To assess whether K\textsubscript{ATP} channels are functional in identified cortical neurons (n=18, Figure 2A), we measured the effects of different K\textsubscript{ATP} channel modulators on whole-cell currents (Q\textsubscript{(3,18)}=32.665, p=3.8 x 10^{-7}, Friedman test) and membrane resistances (Q\textsubscript{(3,18)}=40.933, p=6.8 x 10^{-9}). Pinacidil (100 µM), a SUR2-preferring K\textsubscript{ATP} channel opener (Inagaki et al., 1996;Moreau et al., 2005), had little or no effect on current (4.1 ± 3.7 pA, p=0.478) and membrane resistance (-9.6 ± 3.7%, p=0.121, Figure 2B-C).

By contrast, diazoxide (300 µM), an opener acting on SUR1 and SUR2B-containing K\textsubscript{ATP} channels (Inagaki et al., 1996;Moreau et al., 2005), consistently induced an outward current (45.0 ± 9.6 pA, p=4.8 x 10^{-5}) and a decrease in membrane resistance (-34.5 ± 4.3%, p=3.6 x 10^{-5}) indicative of the activation of a hyperpolarizing conductance (Figure 2B-C). The sulfonylurea tolbutamide (500 µM, Figure 2B-C), a K\textsubscript{ATP} channel blocker (Ammala et al., 1996;Isomoto et al., 1996;Gribble et al., 1997;Isomoto and Kurachi, 1997), did not change whole-cell basal current (-6.6 ± 3.0 pA, p=0.156) or membrane resistance (20.5 ± 7.5%, p=3.89 x 10^{-2}). Conversely, tolbutamide dramatically reversed diazoxide effects on both current (p=4.1 x 10^{-8}) and membrane resistance (p=5.8 x 10^{-10}).

In virtually all neuronal subtypes (H\textsubscript{(6,43)}=2.274, p=0.810, Kruskal–Wallis H test) or groups (t\textsubscript{(42)}=0.3395, p=0.736, Student's t-test), the diazoxide-tolbutamide current/voltage relationship reversed very close to the theoretical potassium equilibrium potential (E\textsubscript{K}=-106.0 mV, Figure 2D-F) confirming the opening of a selective potassium conductance. Besides its effects on plasma membrane K\textsubscript{ATP} channels, diazoxide is also a mitochondrial uncoupler (Drose et al., 2006) which increases reactive oxygen species (ROS) production. This might stimulate Ca\textsuperscript{2+} sparks and large-conductance Ca\textsuperscript{2+}-activated potassium channels (Xi et al., 2005) leading to potential confounding effects. This possibility was ruled out by the observation that Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP, 25 µM), a ROS scavenger (D'Agostino et al., 2007), did not reduce the diazoxide-tolbutamide responses on current (t\textsubscript{(10)}=0.76559, p=0.462, Figure S2A,B) and conductance (t\textsubscript{(10)}=1.24758, p=0.241, Figure S2A,C).

Cortical neurons exhibited K\textsubscript{ATP} conductances of similar value between their subtypes (H\textsubscript{(6,63)}=5.6141, p=0.468) or groups (U\textsubscript{(9,54)}=233, p=0.855, Mann–Whitney U test,
Figures S3A,B). $K_{\text{ATP}}$ channels activated by diazoxide essentially doubled the whole cell conductance in the subthreshold membrane potential compared to control or tolbutamide conditions, regardless of neuronal subtypes ($H_{(6,63)}=5.4763, p=0.484$) or groups ($t_{(61)}=1.324, p=0.191$, Figures 2G,H). Also, $K_{\text{ATP}}$ current density was similar ($H_{(6,63)}=4.4769, p=0.612, U_{(9,54)}=240.5, p=0.965$, Figures S3C,D). Together with the pinacidil unresponsiveness, these data indicate that the large majority of cortical neurons expressed functional SUR1-mediated $K_{\text{ATP}}$ channels in a homogeneous fashion across different subpopulations. To confirm that Kir6.2 is the pore-forming subunit of $K_{\text{ATP}}$ channels in cortical neurons, we used a genetic approach based on Kir6.2 knock-out mice (Miki et al., 1998). We first verified the expression of Kir6.2 and SUR1 subunits in pyramidal cells from wild type mice by scRT-PCR (Figure 3A,B). We next used a dialysis approach by recording neurons with an ATP-free pipette solution (Miki et al., 2001) enriched in sodium (20 mM) to stimulate submembrane ATP depletion and ADP production by the Na$^+$/K$^+$ ATPase, which is known to activate $K_{\text{ATP}}$ channels (Figure 3H). We confirmed that ATP1a1 and ATP1a3 (Figure 3B) were the main $\alpha$-subunits of the Na$^+$/K$^+$ ATPase pump in pyramidal neurons (Zeisel et al., 2015;Tasic et al., 2016). Dialysis of ATP-free/20 mM Na$^+$-pipette solution indeed induced a standing outward current in Kir6.2$^{+/+}$ neurons within 5 minutes (46.7 ± 19.0 pA at -50 mV, n=26) that was reversing close to $E_K$ (Figure 3C,E). In contrast, this current was not observed in Kir6.2$^{-/-}$ neurons (-59.9 ± 11.9 pA, n=22, $U_{(26,22)}=78, p=2.422 \times 10^{-6}$, one-tailed, Figure 3D-G). Collectively, these data show that cortical neurons predominately express functional $K_{\text{ATP}}$ channels composed of Kir6.2 and SUR1 subunits.

**Modulation of neuronal excitability and activity by $K_{\text{ATP}}$ channel**

Despite their large diversity, cortical neurons display a homogenous functional expression of $K_{\text{ATP}}$ channels, questioning how these channels integrate the metabolic environment to adjust neuronal activity. To address this question, we first evaluated in identified cortical neurons (n=39) the ability of $K_{\text{ATP}}$ channels to modulate neuronal excitability, notably by measuring membrane potentials ($Q_{(2,39)}=38.000, p=5.6 \times 10^{-6}$) and membrane resistances ($Q_{(2,39)}=40.205, p=1.9 \times 10^{-6}$), as well as spiking activity ($Q_{(2,39)}=28.593, p=6.2 \times 10^{-6}$). Following electrophysiological identification, the $K_{\text{ATP}}$ channel blocker tolbutamide was applied, which resulted in a slight depolarization ($\Delta V_m=2.6 \pm 0.8$ mV, $p=1.74 \times 10^{-2}$, Figure 4A,D) and increase in membrane...
resistance ($\Delta R_m=78 \pm 31 \text{ M}\Omega$, $p=1.52 \times 10^{-3}$, Figure 4B,E). These effects were strong enough to trigger and stimulate the firing of action potentials ($\Delta F=0.3 \pm 0.1$ Hz, $p=9.21 \times 10^{-3}$, Figure 4A,C,F). By contrast, diazoxide hyperpolarized cortical neurons (-4.0 $\pm$ 0.6 mV, $p=1.87 \times 10^{-4}$, Figure 4A,D), decreased their membrane resistance (-39 $\pm$ 23 M$\Omega$, $p=1.52 \times 10^{-3}$, Figure 4B,E) but did alter their rather silent basal spiking activity (-0.1 $\pm$ 0.1 Hz, $p=0.821$, Figure 4A,C,F).

Most cortical neurons ($n=32$ of 39) showed modulation of neuronal excitability by both $K_{\text{ATP}}$ channel modulators and were considered to be responsive. A similar proportion of responsive neurons was observed between neuronal subtypes (Figure S4A, $X^2(5)=7.313$, $p=0.1984$) or groups (Figure S4B, $p=0.9999$, Fisher’s exact test). The apparent relative lack of responsiveness in FS-PV interneurons (Figure S4A), despite a whole-cell $K_{\text{ATP}}$ conductance similar to that of other neuronal types (Figure S3A), is likely attributable to their low input resistance (Table S3) making $K_{\text{ATP}}$ channels less effective to change membrane potential. Overall, $K_{\text{ATP}}$ channels modulated membrane potential, resistance and firing rate by up to 7.6 $\pm$ 0.9 mV, 76 $\pm$ 17% and 0.5 $\pm$ 0.2 Hz, respectively. This modulation of neuronal excitability (Figure 4G-J) and activity (Figure S4C,D) was similar between neuronal subtypes or groups (Figure 4H-J and S4C-E). Thus, $K_{\text{ATP}}$ channels modulate the excitability and activity of all subtypes of cortical neurons.

Enhancement of neuronal activity by lactate via modulation of $K_{\text{ATP}}$ channels

The uniform expression of metabolically highly sensitive $K_{\text{ATP}}$ channels by cortical neurons suggests their ability to couple the local glycolysis capacity of astrocytes with spiking activity. We therefore evaluated whether extracellular changes in glucose and lactate could differentially shape the spiking activity of cortical neurons through their energy metabolism and $K_{\text{ATP}}$ channel modulation. Importantly, to preserve intracellular metabolism, neurons were recorded in perforated patch-configuration. Stable firing rates of about 4 Hz inducing ATP consumption by the $\text{Na}^+/\text{K}^+$ ATPase (Attwell and Laughlin, 2001) were evoked by applying a depolarizing current and continuously monitored throughout changes in extracellular medium (Figure 5A, $Q_{(2,16)}=22.625$, $p=1.222 \times 10^{-5}$).
Decreasing extracellular glucose from 10 mM to a normoglycemic concentration of 2.5 mM (Silver and Erecinska, 1994; Hu and Wilson, 1997b) did not change firing rate (Figure 5A,B, p=0.2159) of cortical neurons (n=16). By contrast, supplementing extracellular 2.5 mM glucose with 15 mM lactate, a concentration observed in the blood after an intense physical exercise (Quistorff et al., 2008) and corresponding to the same number of carbon atoms as 10 mM glucose, roughly doubled the firing rate compared to both 2.5 (p=7.829 x 10^{-4}) and 10 mM glucose (p=4.303 x 10^{-6}) conditions. Firing rate enhancement by lactate was dose-dependent (H_{8,76} = 35.142, p= 1.052 x 10^{-5}) and reached statistical significance above 5 mM (Figure 5C). We reasoned that this effect could be mediated by K_{ATP} channel closure. Indeed, the increase in firing rate by lactate (209 ± 49 %) was strongly reduced by the K_{ATP} channel activator diazoxide (71 ± 18 %, p=3.346 x 10^{-3}, Figure 5D). Tolbutamide reversed diazoxide’s effect (160 ± 17 %, p=9.345 x 10^{-3}) but did not increase firing rate further (p=0.5076). This occlusion of tolbutamide’s effect by 15 mM lactate also suggests that this concentration reaches saturating levels and is the highest metabolic state that can be sensed by K_{ATP} channels. Enhancement of neuronal activity by lactate was also observed in Kir6.2^+/+ cortical neurons (147 ± 25 %, p=2.840 x 10^{-2}) but not in Kir6.2^-/- mice (112 ± 32 %, p=0.8785, Figure 5E). These observations indicate that lactate enhances neuronal activity via a closure of K_{ATP} channels (Figure 5F).

**Mechanism of lactate-sensing**

To determine whether lactate-sensing involves intracellular lactate oxidative metabolism and/or extracellular activation of the lactate receptor GPR81, we next probed the expression of monocarboxylate transporters (MCT), which allow lactate uptake. Consistent with mouse RNAseq data (Zeisel et al., 2015; Tasic et al., 2016), MCT1 and MCT2 were the main transporters detected in rat cortical neurons by scRT-PCR, although with relatively low single cell detection rates (54 of 277, 19.5% and 78 of 277, 28.2%, for MCT1 and 2, respectively, Figure 6A and S5). The expression of monocarboxylate transporters in cortical neurons is compatible with lactate uptake and metabolism leading to the closure of K_{ATP} channels and an increase in firing rate. We thus evaluated whether lactate uptake was needed for lactate-sensing. We used 250 µM α-cyano-4-hydroxycinnamic acid (4-CIN), a concentration blocking lactate uptake while only moderately altering mitochondrial
pyruvate carrier in brain slices (Schurr et al., 1999; Ogawa et al., 2005; Galeffi et al., 2007). 4-CIN reversed the increased firing rate induced by lactate (Figure 6B, \( T(9)=0, p=7.686 \times 10^{-3} \)) indicating that facilitated lactate transport is required for \( K_{\text{ATP}} \) channel closure and in turn firing rate acceleration.

A mechanism of lactate-sensing involving an intracellular lactate oxidative metabolism would also require the expression of lactate dehydrogenase (LDH), that reversibly converts lactate and nicotinamide adenine dinucleotide (NAD\(^+\)) to pyruvate and NADH (Figure 6E, inset). We thus also probed for the expression of LDH subunits. LDH-A and LDH-B were observed in a large majority of cortical neurons with LDH-A being more frequent in glutamatergic neurons than in GABAergic interneurons (\( p=1.61 \times 10^{-2} \), Figure 6A and S5). Nonetheless, neuron subtypes analysis did not allow to disclose which populations express less frequently LDH-A (Figure S5). To confirm the ability of cortical neurons to take up and oxidize lactate we also visualized NADH fluorescence dynamics (Chance et al., 1962) induced by bath application of lactate. Widefield somatic NADH fluorescence appeared as a diffuse labeling surrounding presumptive nuclei (Figure 6D). Consistent with lactate transport by MCTs and oxidization by LDH, NADH was increased under lactate application (\( U \)\(_{(61,67)}=196, p=1.2 \times 10^{-18} \), Figure 6E-F).

Since the lactate receptor GPR81 has been observed in the cerebral cortex (Lauritzen et al., 2014), lactate-sensing might also involve this receptor. This possibility was ruled out by the observation that pyruvate (15 mM), which is transported by MCTs (Broer et al., 1998; Broer et al., 1999) but does not activate GPR81 (Ahmed et al., 2010), enhanced firing rate to an extent similar to that of lactate (Figure 6C, \( U \)\(_{(16,6)}=43, p=0.7468 \)). In line with its uptake and reduction, pyruvate also decreased NADH (Figure 6E-F, \( U \)\(_{(44,67)}=868, p=2.08 \times 10^{-4} \)).

The requirement of monocarboxylate transport and the similar effect of lactate and pyruvate on neuronal activity suggest that once taken up, lactate would be oxidized into pyruvate and metabolized by mitochondria to produce ATP, leading in turn to a closure of \( K_{\text{ATP}} \) channels and increased firing rate. The apparent absence of glucose responsiveness in cortical neurons also suggests that glycolysis contributes modestly to ATP production. To determine the relative contribution of glycolysis and oxidative phosphorylation to ATP synthesis, we transduced the genetically encoded fluorescence resonance energy transfer (FRET)-based ATP biosensor AT1.03\(^{\text{YEMK}}\) (Imamura et al., 2009) using a recombinant Sindbis virus. AT1.03\(^{\text{YEMK}}\) fluorescence
was mostly observed in pyramidal shaped cells (Figure 6G), consistent with the strong tropism of this viral vector towards pyramidal neurons (Piquet et al., 2018). Blocking glycolysis with 200 µM iodoactic acid (IAA) decreased modestly the FRET ratio by 2.9 ± 0.2% (Figure 6H, p=2.55 x 10^{-9}). By contrast, adding potassium cyanide (KCN, 1mM), a respiratory chain blocker, reduced the FRET ratio to a much larger extent (52.3 ± 0.6%, Figure 6H, p=2.55 x 10^{-9}). KCN also induced a strong NADH fluorescence increase (Figure S6A-B, U_{12,42}=0, p=5.83 x 10^{-12}), indicating a highly active oxidative phosphorylation in cortical neurons.
Discussion

We report that extracellular lactate and pyruvate, but not glucose, enhance the activity of cortical neurons through a mechanism involving facilitated transport and the subsequent closure of $K_{\text{ATP}}$ channels composed of Kir6.2 and SUR1 subunits. ATP synthesis derives mostly from oxidative phosphorylation and weakly from glycolysis in cortical neurons. Together with their ability to oxidize lactate by LDH, these observations suggest that lactate is a preferred energy substrate over glucose in cortical neurons. Besides its metabolic importance, lactate also appears as a signaling molecule enhancing firing activity (Figure 7). This suggests that an efficient neurovascular and neurometabolic coupling could define a time window of an up state of lactate during which neuronal activity and plasticity would be locally enhanced (Suzuki et al., 2011; Jimenez-Blasco et al., 2020).

$K_{\text{ATP}}$ channel subunits in cortical neurons

Similarly to neurons of the hippocampal formation (Zawar et al., 1999; Cunningham et al., 2006; Sada et al., 2015) we found that, regardless of the neuronal type, most neocortical neurons express diazoxide-sensitive, but pinacidil-insensitive $K_{\text{ATP}}$ channels (Cao et al., 2009). In agreement with this pharmacological profile (Inagaki et al., 1996) and the absence of functional $K_{\text{ATP}}$ channels in Kir6.2$^{-/-}$ neurons, we observed Kir6.2 and SUR1 subunits as the main components of $K_{\text{ATP}}$ channels. Their low detection rate is presumably due to the low copy number of their mRNAs, as described in single cortical neurons from mice (Zeisel et al., 2015; Tasic et al., 2016), and making their observation by scRT-PCR challenging (Tsuzuki et al., 2001). Since Kir6.2 is an intronless gene, collection of the nucleus was avoided to prevent potential false positives. Thus, neurons positive for both Kir6.2 and somatostatin intron, taken as an indicator of genomic DNA (Hill et al., 2007; Devienne et al., 2018), were discarded from Kir6.2 expression analysis. Unavoidably, this procedure does reduce the amount of cytoplasm collected, thereby decreasing the detection rate of both Kir6.2 and SUR1.

Consistent with the preferred expression of Kir6.1 in mural and endothelial cells (Bondjers et al., 2006; Zeisel et al., 2015; Tasic et al., 2016; Aziz et al., 2017; Vanlandewijck et al., 2018; Saunders et al., 2018), this subunit was only observed in one out of 277 cortical neurons analyzed. Similarly, SUR2B, the SUR2
variant expressed in forebrain (Isomoto et al., 1996) and cortex (Figure S1), whose presence is largely restricted to vascular cells (Zeisel et al., 2015), was not observed in cortical neurons.

Relative sensitivity of cortical neurons to glucose, lactate and pyruvate
Consistent with previous observations (Yang et al., 1999), decreasing extracellular glucose from standard slice concentrations down to a normoglycemic level did not alter firing rates of cortical neurons. However, their activity is silenced during hypoglycemic episodes through $K_{\text{ATP}}$ channels activation (Yang et al., 1999; Zawar and Neumcke, 2000; Molnar et al., 2014; Sada et al., 2015). This relative glucose unresponsiveness is in contrast with pancreatic beta cells and hypothalamic glucose-excited neurons whose activity is regulated over a wider range of glucose concentrations by $K_{\text{ATP}}$ channels also composed with Kir6.2 and SUR1 subunits (Aguilar-Bryan et al., 1995; Inagaki et al., 1995a; Miki et al., 1998; Yang et al., 1999; Miki et al., 2001; Tarasov et al., 2006; Varin et al., 2015). The inability of cortical neurons to regulate their spiking activity at glucose levels beyond normoglycemia is likely due to the lack of glucokinase, a hexokinase which catalyzes the first step of glycolysis and acts as a glucose sensor in the millimolar range (German, 1993; Yang et al., 1999). As earlier reported, hexokinase-1 (HK1) is the major isoform in cortical neurons (Zeisel et al., 2015; Tasic et al., 2016; Piquet et al., 2018). Since this enzyme has a micromolar affinity for glucose and is inhibited by its product, glucose-6-phosphate (Wilson, 2003), HK1 is likely already saturated and/or inhibited during normoglycemia thereby limiting glycolysis. Nonetheless, HK1 saturation/inhibition can be mitigated when energy consumption is high (Attwell and Laughlin, 2001; Wilson, 2003; Tantama et al., 2013), and then glucose can probably modulate neuronal activity via a high affinity mechanism, as evidenced by slow oscillations of spiking activity involving synaptic transmission (Cunningham et al., 2006) or by the use of glucose-free whole-cell patch-clamp solution (Kawamura, Jr. et al., 2010) that mimics high glucose consumption (Piquet et al., 2018; Diaz-Garcia et al., 2019).

Similarly to glucose-excited hypothalamic neurons (Yang et al., 1999; Song and Routh, 2005), but in contrast with pancreatic beta cells (Newgard and McGarry, 1995), cortical neurons were dose-dependently excited by lactate. This lactate sensitivity is consistent with lactate transport and oxidization in hypothalamic and
cortical neurons (Ainscow et al., 2002; Sada et al., 2015; Diaz-Garcia et al., 2017) which are low in beta cells (Sekine et al., 1994; Pullen et al., 2011). Pyruvate had a similar effect to lactate in cortical neurons under normoglycemic condition whereas it only maintains the activity of hypothalamic glucose-excited neurons during hypoglycemia (Yang et al., 1999) and barely activates pancreatic beta cells (Dufer et al., 2002). Thus, cortical neurons display a peculiar metabolic sensitivity to monocarboxylates. Our data also suggest that under normoglycemic conditions a portion of K\textsubscript{ATP} channels are open when cortical neurons fire action potentials.

**Mechanism of lactate-sensing**

Our pharmacological, molecular and genetic evidence indicates that the closure of K\textsubscript{ATP} channels is responsible for the firing rate enhancement by lactate. Since K\textsubscript{ATP} channels can be modulated by G protein-coupled receptors (Kawamura, Jr. et al., 2010), lactate-sensing might have been mediated by GPR81, a G\textsubscript{i} protein-coupled lactate receptor expressed in the cerebral cortex (Lauritzen et al., 2014). This possibility is however unlikely since the activation of GPR81 inhibits cultured cortical neurons (Bozzo et al., 2013; de Castro Abrantes H. et al., 2019) and we show here that enhancing effect pyruvate on neuronal activity was similar to that of lactate, although pyruvate does not activate GPR81 (Ahmed et al., 2010).

We found that lactate-sensing was critically dependent on lactate transport and we confirmed the capacity of cortical neurons to take up and oxidize lactate (Bittar et al., 1996; Laughton et al., 2000; Bouzier-Sore et al., 2003; Wyss et al., 2011; Choi et al., 2012; Sada et al., 2015; Machler et al., 2016). LDH metabolites, including pyruvate and oxaloacetate, can lead to K\textsubscript{ATP} channel closure (Dhar-Chowdhury et al., 2005; Sada et al., 2015) and could mediate lactate-sensing. An intermediate role of oxaloacetate in lactate-sensing is compatible with enhanced Krebs cycle and oxidative phosphorylation, which leads to an increased ATP/ADP ratio and the closure of K\textsubscript{ATP} channels (Figure 7). In contrast to oxaloacetate, intracellular ATP was found to be ineffective for reverting K\textsubscript{ATP} channel opening induced by LDH inhibition (Sada et al., 2015). Interestingly, hippocampal interneurons were found to be insensitive to glucose deprivation in whole cell configuration (Sada et al., 2015) but not in perforated patch configuration (Zawar and Neumcke, 2000) whereas almost the opposite was found in CA1 pyramidal cells. Whether altered intracellular...
metabolism by whole-cell recording accounted for the apparent lack of ATP sensitivity remains to be determined.

**Lactate as an energy substrate for neurons and an enhancer of spiking activity and neuronal plasticity**

We confirmed that the ATP produced by cortical neurons was mostly derived from oxidative phosphorylation and marginally from glycolysis (Almeida et al., 2001; Hall et al., 2012). Together with the enhancement of spiking activity through $K_{ATP}$ channels by lactate, but not by glucose, our data support both the notion that lactate is a preferred energy substrate over glucose for cortical neurons (Bouzier-Sore et al., 2003) and the astrocyte-neuron lactate shuttle hypothesis (Pellerin and Magistretti, 1994). Although the local cellular origin of lactate has been recently questioned (Lee et al., 2012; Diaz-Garcia et al., 2017), a growing number of evidence indicates that astrocytes are major central lactate producers (Almeida et al., 2001; Choi et al., 2012; Sotelo-Hitschfeld et al., 2015; Karagiannis et al., 2015; Le Douce J. et al., 2020; Jimenez-Blasco et al., 2020).

Glutamatergic synaptic transmission stimulates blood glucose uptake, astrocyte glycolysis, as well as lactate release (Pellerin and Magistretti, 1994; Voutsinos-Porche et al., 2003; Ruminot et al., 2011; Choi et al., 2012; Sotelo-Hitschfeld et al., 2015; Lerchundi et al., 2015) and diffusion through the astroglial gap junctional network (Rouach et al., 2008). This indicates that local and fast glutamatergic synaptic activity would be translated by astrocyte metabolism into a widespread and long-lasting extracellular lactate increase (Prichard et al., 1991; Hu and Wilson, 1997a), which could in turn enhance the firing of both excitatory and inhibitory neurons (Figure 7). Such a lactate surge would be spatially confined by the gap junctional connectivity of the astroglial network, which in layer IV represents an entire barrel (Houades et al., 2008).

This suggests that increased astrocytic lactate induced by whisker stimulation could enhance the activity of the cortical network and fine-tune upcoming sensory processing for several minutes, thereby favoring neuronal plasticity. Along this line, lactate derived from astrocyte glycogen supports both neuronal activity and long-term memory formation (Suzuki et al., 2011; Choi et al., 2012; Vezzoli et al., 2020).

Similarly, cannabinoids, which notably alter neuronal processing and memory
formation (Stella et al., 1997), hamper lactate production by astrocytes (Jimenez-Blasco et al., 2020).

Peripheral lactate produced by skeletal muscles during intense physical exercise and consumed by the active brain (Quistorff et al., 2008) could also have a similar but more global effect on neuronal processing and plasticity. Blood-borne lactate has been shown to promote learning and memory formation via brain-derived neurotrophic factor (El Hayek L. et al., 2019). It is worth noting that the production of this neurotrophin is altered in Kir6.2⁻/⁻ mice and impaired by a K<sub>ATP</sub> channel opener (Fan et al., 2016), both conditions compromising the effect of lactate on spiking activity. Hence, the increase in astrocyte or systemic lactate could fine-tune neuronal processing and plasticity in a context-dependent manner and their coincidence could be potentially synergistic.

**Lactate-sensing compensatory mechanisms**

Since excitatory neuronal activity increases extracellular lactate (Prichard et al., 1991; Hu and Wilson, 1997a) and lactate enhances neuronal activity, such a positive feedback loop (Figure 7) suggests that compensatory mechanisms might be recruited to prevent an overexcitation of neuronal activity by lactate supply. A metabolic negative feedback mechanism could involve the impairment of astrocyte metabolism and lactate release by endocannabinoids (Jimenez-Blasco et al., 2020) produced during intense neuronal activity (Stella et al., 1997). Another possibility would consist in a blood flow decrease that would in turn reduce the delivery of blood glucose and subsequent local lactate production and release but also blood-borne lactate. Some GABAergic interneuron subtypes (Cauli et al., 2004; Uhlirova et al., 2016; Krawchuk et al., 2019), but also astrocytes (Girouard et al., 2010), can trigger vasoconstriction and blood flow decrease when their activity is increased. This could provide a negative hemodynamic feedback restricting spatially and temporally the increase of spiking activity by lactate. PV-expressing and SOM-expressing interneurons exhibit higher mitochondrial content and apparent oxidative phosphorylation than pyramidal cells (Gulyas et al., 2006) suggesting that interneurons would more rapidly metabolize and sense lactate than pyramidal cells. These inhibitory GABAergic interneurons might therefore silence the cortical network, thereby providing a negative neuronal feedback loop. Active decrease in blood flow is associated with a decrease in neuronal activity.
(Shmuel et al., 2002; Shmuel et al., 2006; Devor et al., 2007). Vasoconstrictive GABAergic interneurons may underlie for both processes and could contribute to returning the system to a low lactate state.

**Conclusion**

Our data indicate that lactate is both an energy substrate for cortical neurons and a signaling molecule enhancing their spiking activity. This suggests that a coordinated neurovascular and neurometabolic coupling would define a time window of an up state of lactate that, besides providing energy and maintenance to the cortical network, would fine-tune neuronal processing and favor, for example, memory formation (Suzuki et al., 2011; Kann et al., 2014; Galow et al., 2014; Jimenez-Blasco et al., 2020).

**Acknowledgments**

This work was supported by grants from the Human Frontier Science Program (HFSP, RGY0070/2007, BC), the Agence Nationale pour la Recherche (ANR 2011 MALZ 003 01, BC). AK was supported by a Fondation pour la Recherche Médicale fellowship (FDT20100920106). We thank the animal facility of the IBPS (Paris, France).
Author contributions
Conceptualization, T.G., J. Roeper and B.C.; Formal Analysis, J.N., B.C.; Investigation, A.K., T.G., A.L., F.P., J.P., H.G., R.H., B.C., Resources, B.L., J. Rossier, H.I., S.S., J. Roeper, B.C.; Writing –Original Draft, B.C.; Writing –Review & Editing, J.N., B.L.G., R.E., D.L., B.L., J.F.S., J. Roeper, B.C., Visualization, A.K., B.C.; Supervision, B.C.; Project Administration, B.C., Funding Acquisition, B.C.

Competing interests
The authors declare no competing interests.

Materials and methods

Key resources table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Kynurenic acid | Sigma-Aldrich | K3375 |
| Potassium D-gluconate | Sigma-Aldrich | G4500 |
| Magnesium chloride solution | Sigma-Aldrich | M1028 |
| EGTA | Sigma-Aldrich | E3889 |
| HEPES | Sigma-Aldrich | H4034 |
| Pinacidil monohydrate | Sigma-Aldrich | P154 |
| Diazoxide | Sigma-Aldrich | D9035 |
| Tolbutamide | Sigma-Aldrich | T0891 |
| Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin | Millipore | 475872 |
| Gramicidin from *Bacillus aneurinolyticus* (*Bacillus brevis*) | Sigma-Aldrich | G50002 |
| Sodium L-lactate | Sigma-Aldrich | L7022 |
| α-Cyano-4-hydroxycinnamic Acid | Sigma-Aldrich | C2020 |
| Sodium pyruvate | Sigma-Aldrich | P2256 |
| Sodium iodoacetate | Sigma-Aldrich | I2512 |
| Potassium cyanide | Sigma-Aldrich | 60178 |
| Tetrodotoxin citrate | Latoxan Laboratory | L8502 |
| Dithiothreitol | VWR | 443852A |
| Primer “random” | Roche | 11034731001 |
| dNTPs | GE Healthcare Life Sciences | 28-4065-52 |
| Mineral Oil | Sigma-Aldrich | M5904 |
| RNasin Ribonuclease Inhibitors | Promega | N2511 |
| SuperScript II Reverse Transcriptase | Invitrogen | 18064014 |
| Taq DNA Polymerase | Qiagen | 201205 |
| Penicillin-Streptomycin | Sigma-Aldrich | P4333-100ML |
| MEGAscript™ SP6 Transcription Kit | Ambion | AM1330 |

Experimental Models: Cell Lines

| Baby hamster kidney-21 | ATCC | CCL-10 |
**Experimental Models: Organisms/Strains**

| Organism/Strain | Supplier | Strain/Genotype |
|-----------------|----------|-----------------|
| Rat: Wistar     | Janvier Labs |           |
| Mouse: Wild type C57BL/6J | Janvier Labs | C57BL/6J |
| Mouse: Kir6.2 knockout (KO) | Miki et al., 1998 | MGI:2178821 |

**Oligonucleotides**

| Primer Name | Sequence (5' -> 3') | Reference | N/A |
|-------------|---------------------|-----------|-----|
| PCR primer rat vGluT1 external sense: | 5'-GGCTCCTTTTTCTGGGGGTAC-3' | (Gallopin et al., 2006) | N/A |
| PCR primer rat/mouse vGluT1 external antisense: | 5'-CCAGGGCGACCTCCTTCTAAG-3' | (Gallopin et al., 2006) | N/A |
| PCR primer rat vGluT1 internal sense: | 5'-TGGGGGTACATTGTCACTCAGA-3' | (Gallopin et al., 2006) | N/A |
| PCR primer rat vGluT1 internal antisense: | 5'-ATGGCAAGCAGGGTATGTGAC-3' | (Gallopin et al., 2006) | N/A |
| PCR primer rat/mouse GAD65 external sense: | 5'-CCAAAAGTTCACGGGCGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat/mouse GAD65 external antisense: | 5'-TCCTCCAGATTITTTGGCGGTG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat GAD65 internal sense: | 5'-TGAGAAGCCAGCAGAGGCGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat GAD65 internal antisense: | 5'-ATGGGCAGAGAGATGATGGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat GAD65 external sense: | 5'-ATGATACCTTGTGTCGCGTAGC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat GAD65 external antisense: | 5'-GTTTGCTCCTCCCAGTTTAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat GAD67 internal sense: | 5'-CAATAGCCTGGAGAGAGATGC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat GAD67 internal antisense: | 5'-GTGATCTCCTCCGTCTCAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NOS-1 external sense: | 5'-CAAATCCACACCCAGTGGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NOS-1 external antisense: | 5'-GAGTGGGTGACACGTGTC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NOS-1 internal sense: | 5'-GAGTGGTGTCAACGATGTC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NOS-1 internal antisense: | 5'-GAGTGGTGTCAACGATGTC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CB external sense: | 5'-GAAAGAAGGCTGGATTGGAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CB external antisense: | 5'-CCCACACATTTTGTCCCTGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CB internal sense: | 5'-ATGGGCGAGAAGATGTTGGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CB internal antisense: | 5'-TATCATCCACGTTCGTTG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat PV internal sense: | 5'-CCTTGCTCCTCCGTCTCAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat PV internal antisense: | 5'-GAGTGGTGATCCAGGTGTC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CR external sense: | 5'-CCCTGAAGAAGAAAGTGCGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CR external antisense: | 5'-GCTGGACGCTCTCCTTGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CR internal sense: | 5'-GCTGGACGCTCTCCTTGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CR internal antisense: | 5'-GCTGGACGCTCTCCTTGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NPY external sense: | 5'-GAGTGGTGATCCAGGTGTC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NPY external antisense: | 5'-GAGTGGTGATCCAGGTGTC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NPY internal sense: | 5'-GCTGGACGCTCTCCTTGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NPY internal antisense: | 5'-GCTGGACGCTCTCCTTGG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat NPY internal antisense: 5'-GTTCTGGGGGCAATTTTCTGTG-3' | (Karagiannis et al., 2009) | N/A |
|-----------------------------|-----------------------------|-----|
| PCR primer rat VIP external sense: 5'-TTATGATGTGTCCAGAATGCGAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat VIP external antisense: 5'-TTTTATTTGGTTTTTGCTATGGGAGAAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat VIP internal sense: 5'-TGGCAAAACGAATCAGCAGTAGC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat VIP internal antisense: 5'-GAATCTCCCTCAGCTCTCCTT-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat SOM external sense: 5'-ATGCTGCTCTCGCTGCTCCCA-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat SOM external antisense: 5'-GGCCTACTCTCCTGCTGCTCA-3' | (Férézou et al., 2007) | N/A |
| PCR primer rat SOM internal sense: 5'-GCATCGCTCCTGCTCTGTG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat SOM internal antisense: 5'-AGGCTCTTCTGGCTCTGTG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CCK external sense: 5'-TGTCTGTGCGTGGTGATGGC-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CCK external antisense: 5'-GCATAGCAACATTAGGTCTGGGAG-3' | (Karagiannis et al., 2009) | N/A |
| PCR primer rat CCK internal sense: 5'-ATACATCCGACAGCTCCGCA-3' | (Varin et al., 2015) | N/A |
| PCR primer rat Kir6.1 external sense: 5'-GTGGCGCTCCTCTCTCATC-3' | This paper | N/A |
| PCR primer rat Kir6.1 internal antisense: 5'-TTTCTCTTCCCAAACCCAATG-3' | This paper | N/A |
| PCR primer rat Kir6.2 external sense: 5'-CCCCACACGGCTCTCATTTT-3' | This paper | N/A |
| PCR primer rat Kir6.2 external antisense: 5'-AGGAGCCAGGTCGTAGAGCG-3' | This paper | N/A |
| PCR primer rat Kir6.2 internal sense: 5'-GCCGTCACAGGAACAATCCG-3' | This paper | N/A |
| PCR primer rat Kir6.2 internal antisense: 5'-CCACCCACACGCTTCTCCAT-3' | This paper | N/A |
| PCR primer rat SUR1 external sense: 5'-GGTGAAAGAAGCTCCAGATGA-3' | This paper | N/A |
| PCR primer rat SUR1 internal sense: 5'-GGTGAAAGAAGCCTCCAGATGA-3' | This paper | N/A |
| PCR primer rat SUR1 internal antisense: 5'-GGTTCGGTCCACTGTCAGAAG-3' | This paper | N/A |
| PCR primer rat SUR2 external sense: 5'-GCCCTCTGCCTCTGCTGCTG-3' | This paper | N/A |
| PCR primer rat SUR2 internal sense: 5'-GGGCAAACTACGAGGCAAGC-3' | This paper | N/A |
| PCR primer rat SUR2 internal antisense: 5'-GGGCAAACTACGAGGCAAGC-3' | This paper | N/A |
| PCR primer rat SUR2 internal antisense: 5'-TGAGAACTACGAGGCAAGC-3' | This paper | N/A |
| PCR primer rat SUR2 internal antisense: 5'-CTCGACCACTGCAAGC-3' | This paper | N/A |
| PCR primer rat SUR2 internal antisense: 5'-CAACAACACTGCAAGC-3' | This paper | N/A |
| PCR primer rat SUR2 internal antisense: 5'-AAACCATTAGGATTACGACGCTG-3' | (Hill et al., 2007) | N/A |
| PCR primer rat SOM intron external sense: 5'-GGAAATGCGTGGGACCTGCTG-3' | (Hill et al., 2007) | N/A |
| PCR primer rat SOM intron internal sense: 5'-GGTTCGGTCCACTGTCAGAAG-3' | This paper | N/A |
| PCR primer rat SOM intron internal antisense: 5'-TTCTGATGCTCCATTTTCC-3' | This paper | N/A |
| PCR primer rat SOM intron antisense: 5'-CTCTCAGGGCTGGCAGAGAAGAAG-3' | This paper | N/A |
| PCR primer rat SUR2A/B antisense: 5'-GGTCAGAGAATGCGTGGTG-3' | This paper | N/A |
| PCR primer mouse vGluT1 external sense: 5'-GGCTCTCTTTTCTGGGAGTAC-3' | (Cabezas et al., 2013) | N/A |
PCR primer mouse vGluT1 internal sense: 5'-ATTCGCAGCCAACAGGGTCT-3'  (Cabezas et al., 2013) N/A
PCR primer mouse vGluT1 internal antisense: 5'-TGGCAAGCAGGGTATGTGAC-3'  (Cabezas et al., 2013) N/A
PCR primer mouse GAD65 internal sense: 5'-CACCTGGCAGCCAAAACCT-3'  (Perrenoud et al., 2012) N/A
PCR primer mouse GAD65 internal antisense: 5'-GATTITGCGGTTGGTCTGCGC-3'  (Perrenoud et al., 2012) N/A
PCR primer mouse GAD67 external sense: 5'-TACGGGTTGTCGACAGGTGC-3'  (Férezou et al., 2002) N/A
PCR primer mouse GAD67 internal sense: 5'-CCCAGAAGTGAAGACAAAAGGC-3'  (Cabezas et al., 2013) N/A
PCR primer mouse GAD67 internal antisense: 5'-AATGCTCCGTAAACAGTCGTGC-3'  (Cabezas et al., 2013) N/A
PCR primer mouse ATP1α1 external sense: 5'-CAGGGCAGTGTTTCAGGCTAA-3'  (Devienne et al., 2018) N/A
PCR primer mouse ATP1α1 external antisense: 5'-CCGTGGAGAAGGATGGAGC-3'  (Devienne et al., 2018) N/A
PCR primer mouse ATP1α1 internal sense: 5'-TAAGCGGGCAGTAGCGGG-3'  (Devienne et al., 2018) N/A
PCR primer mouse ATP1α1 internal antisense: 5'-AGGTGTTTGGGCTCAGATGC-3'  (Devienne et al., 2018) N/A
PCR primer mouse ATP1α2 external sense: 5'-AGTGAGGAAGATGAGGGACAGG-3'  (Devienne et al., 2018) N/A
PCR primer mouse ATP1α2 internal sense: 5'-AAATCCCCTTCAACTCCACCA-3'  (Devienne et al., 2018) N/A
PCR primer mouse ATP1α2 internal antisense: 5'-GTTCCCCAAGTCCTCCCAGC-3'  (Devienne et al., 2018) N/A
PCR primer mouse Kir6.2 external sense: 5'-CGGAGAGGGCACCAATGT-3'  (Devienne et al., 2018) N/A
PCR primer mouse Kir6.2 external antisense: 5'-CACCCACGCCATTCTCCA-3'  (Devienne et al., 2018) N/A
PCR primer mouse Kir6.2 internal sense: 5'-CATCCACTCCTTTTCATCTGCC-3'  (Devienne et al., 2018) N/A
PCR primer mouse Kir6.2 internal antisense: 5'-TCGGGGCTGGTGGTCTTG-3'  (Devienne et al., 2018) N/A
PCR primer mouse SUR1 external sense: 5'-CAGTGTTGCCCCCGGAGG-3'  (Devienne et al., 2018) N/A
PCR primer mouse SUR1 external antisense: 5'-GGTCTTCTCCCTGCTGCGT-3'  (Devienne et al., 2018) N/A
PCR primer mouse SUR1 internal sense: 5'-ATCATCGGAGGCTTCTACC-3'  (Devienne et al., 2018) N/A
PCR primer mouse SUR1 internal antisense: 5'-GGTCTTCTCCCTGCTGCGT-3'  (Devienne et al., 2018) N/A
PCR primer mouse SO1 intron external sense: 5'-CTGTCCTCCCCTTACGAACTCC-3'  (Thoby-Brisson et al., 2003) N/A
PCR primer mouse SO1 intron external antisense: 5'-CCAGCACCAGGAGTCGACGCC-3'  (Thoby-Brisson et al., 2003) N/A
PCR primer mouse SO1 intron internal sense: 5'-CCAGCACCAGGAGTCGACGCC-3'  (Cea-del Rio et al., 2010) N/A
PCR primer mouse SO1 intron internal antisense: 5'-TGGAAGGGGAGGAACTCC-3'  (Cea-del Rio et al., 2010) N/A
PCR primer rat MCT1 external sense: 5'-GTGCAGCTTCTCCCTTCCA-3'  This paper N/A
PCR primer rat MCT1 external antisense: 5'-TCGGGTCTGCTTCTTTGCG-3'  This paper N/A
PCR primer rat MCT1 internal sense: 5'-TTGTGCGAATGAGTGTGCG-3'  This paper N/A
PCR primer rat MCT1 internal antisense: 5'-CACGCCACAAAAGCCAGAAT-3'  This paper N/A
| PCR primer rat MCT2 external sense: | This paper | N/A |
|-------------------------------------|------------|-----|
| 5'-GCGAAGTCTAAAAGTAAGGTTGGC-3'      |            |     |
| PCR primer rat MCT2 external antisense: | This paper | N/A |
| 5'-ATTACCCAGCCAGGGGAGG-3'            |            |     |
| PCR primer rat MCT2 internal sense:  | This paper | N/A |
| 5'-CCGTATGCCTAGGAGTACACT-3'          |            |     |
| PCR primer rat MCT2 internal antisense: | This paper | N/A |
| 5'-GGGAAGAACTGGGGAACACT-3'           |            |     |
| PCR primer rat MCT4 external sense:  | This paper | N/A |
| 5'-CATTTTCCTCTGTCCTGCTG-3'           |            |     |
| PCR primer rat MCT4 external antisense: | This paper | N/A |
| 5'-CCCCGTTTTTTCCTCAGGCTCT-3'         |            |     |
| PCR primer rat MCT4 internal sense:  | This paper | N/A |
| 5'-TGGTGCTGTCCTGCTGAC-3'             |            |     |
| PCR primer rat MCT4 internal antisense: | This paper | N/A |
| 5'-CCTCTTTCCTTCCCGATGC-3'            |            |     |
| PCR primer rat LDH-A external sense: | This paper | N/A |
| 5'-GAAGAAGAGGTTCCCAGAGA-A-3'         |            |     |
| PCR primer rat LDH-A internal sense: | This paper | N/A |
| 5'-CAGTTCTTTGTTGCTGCT-3'             |            |     |
| PCR primer rat LDH-A internal antisense: | This paper | N/A |
| 5'-TCTCTTCCCTCTGCTGACG-3'            |            |     |
| PCR primer rat LDH-B external sense: | This paper | N/A |
| 5'-ACTGCCGTCCCGAACAACA-3'            |            |     |
| PCR primer rat LDH-B internal sense: | This paper | N/A |
| 5'-TCTGGGGAAGTCTCTGGCTA-3'           |            |     |
| PCR primer rat LDH-B internal antisense: | This paper | N/A |
| 5'-TTGGCTGTCACGGAGTAATCTTT-3'        |            |     |

Recombinant DNA

- pcDNA-ATEam1.03YEMK (Imamura et al., 2009) N/A
- pSinRep5 plasmid Invitrogen K750-01
- pDH(26S) helper plasmid Invitrogen K750-01

Software and Algorithms

- Pclamp 10.2 Molecular Devices https://www.moleculardevices.com
- Matlab 2018b MathWorks https://fr.mathworks.com/
- Statistica 6.1 Statsoft http://www.statsoft.com/Products/STATISTICA-Features
- GraphPad Prism 7 GraphPad https://www.graphpad.com/
- ImagingWorkbench 6.0.25 INDEC Systems http://www.indecbiosystems.com
- FIJI (Schindelin et al., 2012) https://fiji.sc/Fiji
- Image-Pro Analyzer 7.0 MediaCybernetics http://www.mediacy.com/

Other

| VT1000S | Leica | N/A |
| Bx51WI | Olympus | N/A |
| Wi-DPMC | Olympus | N/A |
| LUMPian Fl/IR 60x/0.90 W | Olympus | N/A |
| LUMPian Fl/IR 40x/0.80 W | Olympus | N/A |
| CoolSnap H02 | Roper Scientific | N/A |
| Axopatch 200B | Molecular Devices | N/A |
| Digidata 1440A | Molecular Devices | N/A |
| S900 Stimulator | Dagan corporation | N/A |
| p-e-2 | CoolLED | N/A |
| FF395/495/610-DI01-25x36 | Semrock | N/A |
| FF01-425/527/685-25 | Semrock | N/A |
| 780 nm Collimated LED | Thorlabs | M780L3-C1 |
| Dodt Gradient Contrast | Luigs and Neumann | 200-100 200 0155 |
| 725 DCSPX | Semrock | N/A |
| XC ST-70 CE | Sony | N/A |
| Millicell | Millipore | PICM0RG50 |
|----------|-----------|-----------|
| FF02-438/24-25 | Semrock | N/A |
| FF458-DI02-25x36 | Semrock | N/A |
| FF01-483/32-25 | Semrock | N/A |
| FF01-542/27-25 | Semrock | N/A |
| Lambda 10B | Sutter Instruments | N/A |

1. **Lead contact and materials availability**
2. Further information and requests for resources and reagents should be directed to, and will be fulfilled by, the lead contact, B. Cauli (bruno.cauli@upmc.fr).

3. **Experimental model and subject details**
4. Wistar rats, C57BL/6RJ or Kir6.2 KO mice were used for all experiments in accordance with French regulations (Code Rural R214/87 to R214/130) and conformed to the ethical guidelines of both the directive 2010/63/EU of the European Parliament and of the Council and the French National Charter on the ethics of animal experimentation. A maximum of 3 rats or 5 mice were housed per cage and single animal housing was avoided. Male rats and mice of both genders were housed on a 12-hour light/dark cycle in a temperature-controlled (21–25°C) room and were given food and water *ad libitum*. Animals were used for experimentation at 13-24 days of age.
Cortical slice preparation
Rats or mice were deeply anesthetized with isoflurane. After decapitation brains were quickly removed and placed into cold (~4°C) oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 10 glucose, 15 sucrose, and 1 kynurenic acid. Coronal slices (300 µm thick) containing the barrel cortex were cut with a vibratome (VT1000S, Leica) and allowed to recover at room temperature for at least 1h in aCSF saturated with O₂/CO₂ (95%/5%) as previously described (Karagiannis et al., 2009; Devienne et al., 2018).

Whole-cell patch-clamp recording
Patch pipettes (4-6 MΩ) pulled from borosilicate glass were filled with 8 µl of RNAse free internal solution containing in (mM): 144 K-glucuronate, 3 MgCl₂, 0.5 EGTA, 10 HEPES, pH 7.2 (285/295 mOsm). Whole-cell recordings were performed at 25.3 ± 0.2°C using a patch-clamp amplifier (Axopatch 200B, Molecular Devices). Data were filtered at 5-10 kHz and digitized at 50 kHz using an acquisition board (Digidata 1440, Molecular Devices) attached to a personal computer running pCLAMP 10.2 software package (Molecular Devices). For ATP washout experiments neurons were recorded in voltage clamp mode using an ATP-free internal solution containing in (mM): 140 KCl, 20 NaCl, 2 MgCl₂, 10 EGTA, 10 HEPES, pH 7.2.

Cytoplasm harvesting and scRT-PCR
At the end of the whole-cell recording, lasting less than 15 min, the cytoplasmic content was aspirated in the recording pipette. The pipette’s content was expelled into a test tube and reverse transcription (RT) was performed in a final volume of 10 µl, as described previously (Lambolez et al., 1992). The scRT-PCR protocol was designed to probe simultaneously the expression of neuronal markers, K_ATP channels subunits or some key elements of energy metabolism. Two-steps amplification was performed essentially as described (Cauli et al., 1997; Devienne et al., 2018). Briefly, cDNAs present in the 10 µl reverse transcription reaction were first amplified simultaneously using all external primer pairs listed in the Key Resources Table. Taq polymerase and 20 pmol of each primer were added to the buffer supplied by the manufacturer (final volume, 100 µl), and 20 cycles (94°C, 30 s; 60°C, 30 s; 72°C, 35 s) of PCR were run. Second rounds of PCR were performed using 1 µl of the first PCR product as a template. In this second round, each cDNA was amplified
individually using its specific nested primer pair (Key Resources Table) by performing 35 PCR cycles (as described above). 10 µl of each individual PCR product were run on a 2 % agarose gel stained with ethidium bromide using ϕX174 digested by HaeIII as a molecular weight marker.

**Perforated patch-clamp recording**

Gramicidin stock solution (2 mg/ml, Sigma-Aldrich) was prepared in DMSO and diluted to 10-20 µg/ml (Zawar and Neumcke, 2000) in the RNAse free internal solution described above. The pipette tip was filled with gramicidin-free solution. Progress in perforation was evaluated by monitoring the capacitive transient currents elicited by -10 mV voltage pulses from a holding potential of -60 mV. In perforated patch configuration, a continuous current (52 ± 7 pA) was injected to induce the spiking of action potentials at stable firing rates of 4.1 ± 0.4 Hz obtained after an equilibration period of 3.6 ± 0.5 min. Membrane and access resistance were continuously monitored by applying -50 pA hyperpolarizing current pulses lasting 1 s every 10 s using an external stimulator (S900, Dagan) connected to the amplifier. Recordings were stopped when going into whole-cell configuration occurred, as evidenced by sudden increase of spike amplitude and decrease of access resistance.

**NADH imaging**

Recordings were made in layer II-III of the rat somatosensory cortex. Wide-field fluorescent images were obtained using a double port upright microscope BX51WI, WI-DPMC, Olympus) with a 60x objective (LUMPlan Fl/IR 60x/0.90 W, Olympus) and a digital camera (CoolSnap HQ2, Roper Scientific) attached on the front port of the microscope. NADH autofluorescence was obtained by 365 nm excitation with a Light Emitting Device (LED, pE-2, CoolLED) using Imaging Workbench 6.0.25 software (INDEC Systems) and dichroic (FF395/495/610-Di01-25x36, Semrock) and emission filters (FF01-425/527/685-25, Semrock). Infrared Dodt gradient contrast images (IR-DGC, (Dodt and Ziegglansberger, 1998)) were obtained using a 780 nm collimated LED (M780L3-C1,Thorlabs) as a transmitted light source and DGC optics (Luigs and Neumann). Autofluorescence and IR-DGC images were collected every 10s by alternating the fluorescence and transmitted light sources. In parallel, infrared transmitted light images of slices were also continuously monitored on the back-port
of the microscope using a customized beam splitter (725 DCSPXR, Semrock) and an analogic CCD camera (XC ST-70 CE, Sony). The focal plane was maintained constant on-line using infrared DGC images of cells as anatomical landmarks (Lacroix et al., 2015).

Subcloning and viral production
The coding sequence of the ATP sensor ATeam1.03YEMK (Imamura et al., 2009) was subcloned into the viral vector pSinRep5. Sindbis virus was produced as previously described (Piquet et al., 2018). Recombinant pSinRep5 and helper plasmid pDH26S (Invitrogen) were transcribed in vitro into capped RNA using the Megascript SP6 kit (Ambion). Baby hamster kidney-21 cells (ATCC) were electroporated with sensor-containing RNA and helper RNA (2.10^7 cells, 950 µF, 230 V) and incubated for 24 h at 37°C in 5% CO2 in Dulbecco's modified Eagle Medium supplemented with 5% fetal calf serum before collecting cell supernatant containing the viruses. The virus titer (10^8 infectious particles/ml) was determined after counting fluorescent baby hamster kidney cells infected using serial dilution of the stock virus.

Brain slice viral transduction
Brain slices were placed onto a millicell membrane (Millipore) with culture medium (50% minimum essential medium, 50% Hank's balanced salt sodium, 6.5 g/l glucose and 100 U/ml penicillin-streptomycin (Sigma-Aldrich) as previously described (Piquet et al., 2018). Infection was performed by adding ~5 x 10^5 particles per slice. Slices were incubated overnight at 35°C in 5% CO2. The next morning, brain slices were equilibrated for 1h in aCSF containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 10 glucose, 15 sucrose. Slices were then placed into the recording chamber, heated at ~30 °C and continuously perfused at 1-2 ml/min.

FRET imaging
Recordings were made from visually identified pyramidal cells in layer II-III of the rat somatosensory cortex. Wide-field fluorescent images were obtained using a 40x objective and a digital camera attached on the front port of the microscope. The ATP sensor ATeam1.03YEMK was excited at 400 nm with a LED using Imaging Workbench 6.0.25 software and excitation (FF02-438/24-25, Semrock) and dichroic filters (FF458-Di02-25x36, Semrock). Double fluorescence images were collected
every 15s by alternating the fluorescence emission filters for the CFP (FF01-483/32-25, Semrock) and the YFP (FF01-542/27-25, Semrock) using a filter wheel (Lambda 10B, Sutter Instruments). The focal plane was maintained constant on-line as described above.

**Pharmacological studies**
Pinacidil (100 µM, Sigma-Aldrich); Diazoxide (300 µM, Sigma-Aldrich) and Tolbutamide (500 µM, Sigma-Aldrich), Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP, 25 µM, Millipore), α-cyano-4-hydroxycinnamate (4-CIN, 250 µM, Sigma-Aldrich); iodoacetic acid (IAA, 200 µM, Sigma-Aldrich) or KCN (1 mM, Sigma-Aldrich) was dissolved in aCSF from stock solutions of pinacidil (100 mM; NaOH 1M), diazoxide (300 mM; NaOH 1M), tolbutamide (500 mM; NaOH 1M), 4-CIN (250 mM; DMSO), IAA (200 mM, water) and KCN (1 M, water). Changes in extracellular glucose, lactate or pyruvate concentration were compensated by changes in sucrose concentration to maintain the osmolarity of the aCSF constant as previously described (Miki et al., 2001; Varin et al., 2015; Piquet et al., 2018) and pH was adjusted to 7.4.

**Quantification and statistical analysis**

**Analysis of somatic features**
The laminar location determined by infrared videomicroscopy and recorded as 1-4 according to a location right within layers I, II/III or IV. For neurons located at the border of layers I-II/III and II/III-IV, the laminar location was represented by 1.5 and 3.5, respectively. Somatic features were measured from IR DGC of the recorded neurons. Briefly, the soma was manually delineated using Image-Pro Analyzer 7.0 software (MediaCybernetics) and length of major and minor axes, perimeter and area were extracted. The soma elongation was calculated as the ratio between major and minor axis. Roundness was calculated according to: \( \frac{\text{perimeter}^2}{4\pi \times \text{area}} \); a value close to 1 is indicative of round somata.
Analysis of electrophysiological properties

32 electrophysiological properties chosen to describe the electrophysiological diversity of cortical neurons (Ascoli et al., 2008) were determined using the I-clamp fast mode of the amplifier as previously described (Karagiannis et al., 2009). Membrane potential values were corrected for theoretical liquid junction potential (-15.6 mV). Resting membrane potential was measured just after passing in whole-cell configuration, and only cells with a resting membrane potential more negative than -55 mV were analyzed further. Membrane resistance ($R_m$) and membrane time constant ($\tau_m$) were determined on responses to hyperpolarizing current pulses (duration, 800 ms) eliciting voltage shifts of 10-15 mV negative to rest (Kawaguchi, 1993; Kawaguchi, 1995). Time constant was determined by fitting this voltage response to a single exponential. Membrane capacitance ($C_m$) was calculated according to $C_m = \tau_m/R_m$. Sag index was quantified as a relative decrease in membrane conductance according to $(G_{sag} - G_{hyp})/G_{sag}$ (Halabisky et al., 2006) where $G_{hyp}$ and $G_{sag}$ correspond to the whole-cell conductance when the sag was inactive and active, respectively. $G_{sag}$ was measured as the slope of the linear portion of a current–voltage (I–V) plot, where $V$ was determined at the end of 800 ms hyperpolarizing current pulses (-100 to 0 pA) and $G_{hyp}$ as the slope of the linear portion of an I–V plot, where $V$ was determined as the maximal negative potential during the 800 ms hyperpolarizing pulses. Rheobase was quantified as the minimal depolarizing current pulse intensity (800 ms duration pulses, 10 pA increments) generating at least one action potential. First spike latency (Gupta et al., 2000; Ascoli et al., 2008) was measured at rheobase as the time needed to elicit the first action potential. To describe different firing behaviors near threshold, spike frequency was measured near spike threshold on the first trace in which at least three spikes were triggered. Instantaneous discharge frequencies were measured and fitted to a straight line according to $F_{\text{threshold}} = m_{\text{threshold}} \cdot t + F_{\text{min}}$, where $m_{\text{threshold}}$ is the slope termed adaptation, $t$ the time and $F_{\text{min}}$ the minimal steady state frequency. Analysis of the action potentials waveforms was done on the first two spikes. Their amplitude (A1 and A2) was measured from threshold to the positive peak of the spike. Their duration (D1 and D2) was measured at half amplitude (Kawaguchi, 1993; Cauli et al., 1997). Their amplitude reduction and the duration increase were calculated according to $(A1-A2)/A1$ and $(D2-D1)/D1$, respectively (Cauli et al., 1997; Cauli et al., 2000). The amplitude and the latency of the fast and medium afterhyperpolarization
(fAH and mAH) were measured for the first two action potentials as the difference between spike threshold and the negative peak of the AHs (Kawaguchi, 1993). The amplitude and latency of afterdepolarization (AD) following single spikes (Haj-Dahmane and Andrade, 1997) were measured as the difference between the negative peak of the fAH and the peak of the AD and between the spike threshold and the peak of the AD, respectively. When neurons did not exhibit mAH or AD, amplitude and latency were arbitrarily set to 0. A complex spike amplitude accommodation during a train of action potentials, consisting in a transient decrease of spikes amplitude, was measured as the difference between the peak of the smallest action potential and the peak of the following largest action potential (Cauli et al., 2000). Maximal firing rate was defined as the last trace before prominent reduction of action potentials amplitude indicative of a saturated discharge. To take into account the biphasic spike frequency adaptation (early and late) occurring at high firing rates (Cauli et al., 1997; Cauli et al., 2000; Gallopin et al., 2006), instantaneous firing frequency was fitted to a single exponential (Halabisky et al., 2006) with a sloping baseline, according to: 

$$F_{\text{Saturation}} = A_{\text{sat}} e^{-\frac{t}{\tau_{\text{sat}}}} + t \cdot m_{\text{sat}} + F_{\text{max}},$$

where $A_{\text{sat}}$ corresponds to the amplitude of early frequency adaptation, $\tau_{\text{sat}}$ to the time constant of early adaptation, $m_{\text{sat}}$ to the slope of late adaptation and $F_{\text{max}}$ to the maximal steady state frequency.

### Unsupervised clustering

To classify neurons unsupervised clustering was performed using the laminar location of the soma, 10 molecular parameters (vGluT1, GAD65 and/or GAD67, NOS-1, CB, PV, CR, NPY, VIP, SOM and CCK) and the 32 electrophysiological parameters described above. Neurons positive for GAD65 and/or GAD67 were denoted as GAD positive and these mRNAs were considered as a single molecular variable as previously described (Gallopin et al., 2006). Parameters were standardized by centering and reducing all of the values. Cluster analysis was run on Statistica 6.1 software (Statsoft) using Ward's method (Ward, 1963). The final number of clusters was established by hierarchically subdividing the clustering tree into higher order clusters as previously described (Karagiannis et al., 2009).
Analysis of voltage clamp recordings

Whole-cell currents were measured from a holding potential of -70 mV and membrane resistances were determined by applying a voltage step to -60 mV of 100 ms every 5 s. The effects of K\textsubscript{ATP} channel modulators were measured at the end of drug application by averaging, over a period of 1 minute, whole cell currents and changes in membrane resistance relative to control baseline prior to the application of drugs. Whole-cell K\textsubscript{ATP} current and conductance were determined by subtracting current and conductance measured under K\textsubscript{ATP} channel activator by their value measured under K\textsubscript{ATP} channel blocker. The relative whole-cell K\textsubscript{ATP} conductance was determined by dividing the whole-cell K\textsubscript{ATP} conductance by the whole cell conductance measured under K\textsubscript{ATP} channel activator. Whole-cell K\textsubscript{ATP} current density was determined by dividing the whole-cell K\textsubscript{ATP} current by the membrane capacitance. K\textsubscript{ATP} current reversal potential was measured by subtracting I/V relationships obtained during voltage ramps from -60 to -130 mV determined under K\textsubscript{ATP} channel activator and blocker, respectively.

During ATP washout experiments, whole-cell currents and I/V relationships were measured every 10 s at a holding potential of -50 mV and during voltage ramps from -40 to -120 mV, respectively. Washout currents were determined by subtracting the whole-cell currents measured at the beginning and the end of the whole cell-recording, respectively.

Analysis of current clamp recordings

Every 10 s, membrane potential and mean firing rate were measured and membrane resistances were determined from voltage responses induced by -50 pA currents pulses lasting 1 s. K\textsubscript{ATP} voltage response and changes in membrane resistance and firing rate were determined by subtracting their value measured under K\textsubscript{ATP} channel activator by their value measured under K\textsubscript{ATP} channel blocker. Neurons were considered as responsive to K\textsubscript{ATP} channel modulators if the K\textsubscript{ATP} channel activator induced both a hyperpolarization and a decrease in membrane resistance reversed by the K\textsubscript{ATP} channel blocker.

Analysis of perforated patch recordings

Mean firing frequency was measured every 10 s. Quantification of spiking activity was determined by averaging firing frequency over a period of 5 min preceding a
change in extracellular aCSF composition. Firing frequencies were normalized by the
averaged mean firing frequency measured under control condition.

NADH imaging
Shading correction was applied off-line on the NADH autofluorescence images using
the "Shading Corrector" plugin of FIJI software (Schindelin et al., 2012) and a blank
field reference image. To compensate for potential x-y drifts all IR-DGC images were
realigned off-line using the "StackReg" and "TurboReg" plugins (Thevenaz et al.,
1998) of FIJI software and the same registration was applied to the corrected
NAD(P)H autofluorescence images. To determine somatic regions of interest (ROIs)
the soma was manually delineated on IR-DGC images. The mean NADH
autofluorescence was measured at each time point using the same ROIs. Variations
of fluorescence intensity were expressed as the ratio (F-F0)/F0 where F corresponds
to the mean fluorescence intensity in the ROI at a given time point, and F0
corresponds to the mean fluorescence intensity in the same ROI during the 5 min
control baseline prior to changes in aCSF composition. Effect of monocarboxylate
superfusion or oxidative phosphorylation blockade was quantified by averaging the
normalized ratio (R/R0) during the last five minutes of drug application.

FRET imaging
All images were realigned off-line as described above using the YFP images as the
reference for registration. Fluorescence ratios were calculated by dividing the
registered YFP images by the registered CFP images using FIJI. The somatic ROIs
were manually delineated on the YFP images as described above. The mean ratio
was measured at each time point using the same ROIs. Variations of fluorescence
ratio were expressed as the ratio (R-R0)/R0 where R corresponds to the
fluorescence ratio in the ROI at a given time point, and R0 corresponds to the mean
fluorescence ratio in the same ROI during the 10 min control baseline prior to drug
application. Effect of glycolysis or oxidative phosphorylation blockade was quantified
by averaging the normalized ratio during the last five minutes of drug application.
Statistical analysis

Statistical analyses were performed with Statistica 6.1 and GraphPad Prism 7. All values are expressed as means ± s.e.m. Normality of distributions and equality of variances were assessed using the Shapiro–Wilk test and the Fisher F-test, respectively. Parametric tests were only used if these criteria were met. Holm-Bonferroni correction was used for multiple comparisons and p-values are given as uncorrected. Statistical significance on all figures uses the following convention of corrected p-values: * p < 0.05, ** p < 0.01, *** p < 0.001.

Statistical significance of morphological and electrophysiological properties of neurons was determined using the Mann-Whitney U test. Comparison of the occurrence of expressed genes and of responsiveness of \( K_{\text{ATP}} \) channel modulators between different cell types was determined using Fisher's exact test. Statistical significance of the effects of \( K_{\text{ATP}} \) channel modulators was determined using the Friedman and post hoc Dunn's tests. Significance of the effect of the ROS scavenger was determined using one-tailed unpaired student t-test. Comparison of \( K_{\text{ATP}} \) channel properties was determined using Mann-Whitney U, Student-t, or Kruskal-Wallis H tests. Comparison of responses between Kir6.2\(^{+/+}\) and Kir6.2\(^{-/-}\) neurons was determined using Mann-Whitney U test. Statistical significance of the effects of energy substrates and drug applications on evoked firing in perforated patch recordings was determined using Friedman and Dunn’s tests. Comparison of the effects of monocarboxylates and cyanide on NADH fluorescence was determined using Mann-Whitney U test. Statistical significance of the effects of metabolic inhibitors on intracellular ATP was determined using Friedman and Dunn’s tests.
Figure legends

Figure 1. Cortical neuron subtypes express Kir6.2 and SUR1 K\_ATP channel subunits.
(A) Ward’s clustering of 277 cortical neurons (left panel). The x axis represents the average within-cluster linkage distance, and the y axis the individuals.

(B) Heatmap of gene expression across the different cell clusters. For each cell, colored and white rectangles indicate presence and absence of genes, respectively.

(C) Representative voltage responses induced by injection of current pulses (bottom traces) corresponding to -100, -50 and 0 pA, rheobase and intensity inducing a saturating firing frequency (shaded traces) of a Regular Spiking neuron (black), an Intrinsically Bursting neuron (gray), a Bursting VIP interneuron (light blue), an Adapting VIP interneuron (blue), an Adapting SOM interneuron (green), an Adapting NPY interneuron (orange), and a Fast Spiking-Parvalbumin interneuron (FS-PV, red). The colored arrows indicate the expression profiles of neurons whose firing pattern is illustrated in (C).

(D) Heatmap of the expression of the subunits of the $K_{ATP}$ channels in the different clusters.

(E) scRT-PCR analysis of the RS neuron depicted in (A-D).

(F) Histograms summarizing the expression profile of $K_{ATP}$ channel subunits in identified neuronal types. n.s. not statistically significant.
Figure 2. Pharmacological and biophysical characterization of $K_{\text{ATP}}$ channels in cortical neurons.
(A) Representative voltage responses of a FS-PV interneuron induced by injection of current pulses (bottom traces).

(B) Protocol of voltage pulses from −70 to −60 mV (left trace). Responses of whole-cell currents in the FS-PV interneurons shown in (A) in control condition (black) and in presence of pinacidil (blue), piazoxide (green) and tolbutamide (red) at the time indicated by a-d in (C).

(C) Stationary currents recorded at -60 mV (filled circles) and membrane resistance (open circles) changes induced by $K_{\text{ATP}}$ channel modulators. The colored bars and shaded zones indicate the duration of application of $K_{\text{ATP}}$ channel modulators. Upper and lower insets: changes in whole-cell currents and relative changes in membrane resistance induced by $K_{\text{ATP}}$ channel modulators, respectively.

(D) Whole cell current-voltage relationships measured under diazoxide (green trace) and tolbutamide (red trace). $K_{\text{ATP}}$ I/V curve (black trace) obtained by subtracting the curve under diazoxide by the curve under tolbutamide. The arrow indicates the reversal potential of $K_{\text{ATP}}$ currents.

(E-H) Histograms summarizing the $K_{\text{ATP}}$ current reversal potential (E,F) and relative $K_{\text{ATP}}$ conductance (G,H) in identified neuronal subtypes (E,G) or between glutamatergic and GABAergic neurons (F,G). Data are expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.
Figure 3. Kir6.2 is the pore forming subunit of $K_{ATP}$ channels in cortical neurons.

(A) Representative voltage responses of a mouse layer II/III RS pyramidal cell induced by injection of current pulses (bottom traces).

(B) Histograms summarizing the expression profile of vGluT1, GAD65 and 67, the ATP1a1-3 subunits of the Na/K ATPase and the Kir6.2 and SUR1 $K_{ATP}$ channel subunits in layer II/III RS pyramidal cells from Kir6.2$^{+/+}$ mice.

(C, D) Whole-cell stationary currents recorded at -50 mV during dialysis with ATP-free pipette solution in RS neurons of Kir6.2$^{+/+}$ (C) and Kir6.2$^{-/-}$ (D) mice. Inset; voltage clamp protocol.
(E, F) Current-voltage relationships obtained during ATP washout at the time indicated by green and orange circles in (C, D) in RS neurons of Kir6.2^{+/+} (E) and Kir6.2^{-/-} (F) mice.

(G) Histograms summarizing the whole-cell ATP washout currents in Kir6.2^{+/+} (black) and Kir6.2^{-/-} (white) RS neurons. Data are expressed as mean ± s.e.m., and the individual data points are depicted.

(H) Diagram depicting the principle of the ATP washout experiment.
Figure 4. Modulation of cortical neuronal excitability and activity by $K_{\text{ATP}}$ channels.

(A-C) Representative example showing the changes in membrane potential (A), resistance (B, open circles) and spiking activity (C) induced by application of toltubamide (red) and diazoxide (green). The colored bars and shaded zones indicate the application duration of $K_{\text{ATP}}$ channel modulators.
(D-F) Relative changes in membrane potential (D), resistance (E) and firing rate (F)
induced by tolbutamide and diazoxide in cortical neurons.

(G-J) Histograms summarizing the modulation of membrane potential (G, 
$H_{(5,32)}=0.14854$, $p=0.999$, and H, $U_{(8,24)}=95.5$, $p=0.991$) and resistance (I, 
$H_{(5,32)}=3.00656$, $p=0.699$, and J, $U_{(8,24)}=73$, $p=0.329$) by $K_{ATP}$ channels in neuronal 
subtypes (G, I) and groups (H, J). Data are expressed as mean ± s.e.m., and the 
individual data points are depicted. n.s. not statistically significant.
Figure 5. Lactate enhances cortical neuronal activity via $K_{\text{ATP}}$ channel modulation.
(A) Representative perforated patch recording of an adapting VIP neuron showing the modulation of firing frequency induced by changes in the extracellular concentrations of metabolites. The colored bars and shaded zones indicate the concentration in glucose (grey) and lactate (orange). Voltage responses recorded at the time indicated by arrows. The red dashed lines indicate -40 mV.

(B) Histograms summarizing the mean firing frequency during changes in extracellular concentration of glucose (black and grey) and lactate (orange). Data are expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.

(C) Dose-dependent enhancement of firing frequency by lactate. Data are normalized by the mean firing frequency in absence of lactate and are expressed as mean ± s.e.m. Numbers in brackets indicate the number of recorded neurons at different lactate concentrations.

(D) Histograms summarizing the normalized frequency under 15 mM lactate (orange) and its modulation by addition of diazoxide (green) or tolbutamide (red). Data are expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.

(E) Histograms summarizing the enhancement of normalized frequency by 15 mM lactate in Kir6.2 +/+ (orange) and Kir6.2 −/− (pale orange) mouse cortical neurons. The dash line indicates the normalized mean firing frequency in absence of lactate. Data are expressed as mean ± s.e.m., and the individual data points are depicted.

(F) Diagram depicting the enhancement of neuronal activity by lactate via modulation of K_{ATP} channels.
Figure 6. Lactate enhancement of cortical neuronal activity involves lactate uptake and metabolism.

(A) Histograms summarizing the expression profile of the monocarboxylate transporters MCT1, 2 and 4 and LDH A and B lactate dehydrogenase subunits in glutamatergic neurons (black) and GABAergic interneurons (white). The numbers in brackets indicate the number of analyzed cells.
(B) Histograms summarizing the enhancement of normalized frequency by 15 mM lactate (orange) and its suppression by the MCTs inhibitor 4-CIN (purple). Data are expressed as mean ± s.e.m., and the individual data points are depicted.

(C) Histograms summarizing the enhancement of normalized frequency by 15 mM lactate (orange) and pyruvate (magenta). Data are expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.

(D) Widefield NADH autofluorescence (upper panel, scale bar: 20 µm) and corresponding field of view observed under IR-DGC (lower panel). The somatic regions of interest are delineated.

(E) Mean relative changes in NADH autofluorescence in control condition (grey) and in response to 15 mM lactate (orange) or pyruvate (magenta). The colored bars indicate the duration of applications. Data are expressed as mean ± s.e.m. Inset: diagram depicting the NADH changes induced by lactate and pyruvate uptake by MCT and their interconversion by LDH.

(F) Histograms summarizing the mean relative changes in NAD(P)H autofluorescence measured during the last 5 minutes of 15 mM lactate (orange) or pyruvate (magenta) application and corresponding time in control condition (grey). Data are expressed as mean ± s.e.m., and the individual data points are depicted.

(G) Widefield YFP fluorescence of the ATP biosensor AT1.03YEMK (upper left panel, scale bar: 30 µm) and pseudocolor images showing the intracellular ATP (YFP/CFP ratio value coded by pixel hue, see scale bar in upper right panel) and the fluorescence intensity (coded by pixel intensity) at different times under 10 mM extracellular glucose (upper right panel) and after addition of IAA (lower left panel) and KCN (lower right panel).

(H) Mean relative changes in intracellular ATP (relative YFP/CFP ratio) measured under 10 mM extracellular glucose (grey) and after addition of IAA (yellow) and KCN (blue). Data are expressed as mean ± s.e.m. The colored bars indicate the time and duration of metabolic inhibitor application. Inset: Histograms summarizing the mean relative changes in intracellular ATP (relative YFP/CFP ratio) ratio under 10 mM extracellular glucose (grey) and after addition of IAA (yellow) and KCN (blue). Data are expressed as mean ± s.e.m., and the individual data points are depicted.
Figure 7. Diagram summarizing the mechanism of lactate-sensing in the cortical network.

Glutamate (Glu) released during synaptic transmission stimulates blood glucose (Glc) uptake in astrocytes, aerobic glycolysis, lactate release and diffusion through the astrocytic network. Lactate is then taken up by neurons via monobarboxylate transporters (MCT) and oxidized into pyruvate by lactate dehydrogenase (LDH). The ATP produced by pyruvate oxidative metabolism closes $K_{\text{ATP}}$ channels and increases the spiking activity of both pyramidal cells (black) and inhibitory interneurons (green). The color gradient of the circles represents the extent of glutamate (black) and lactate (orange) diffusion, respectively. Dashed arrows indicate multisteps reactions.
**Figure S1. Molecular expression of $K_{\text{ATP}}$ channels.**

(A) RT-PCR products generated from 500 pg of total cortical RNAs. M: 100 bp ladder molecular weight marker.

(B) SUR2 splice variants-specific RT-PCR analysis of 1 ng total RNAs from rat heart, neocortex and forebrain.
Figure S2. Diazoxide-induced current is independent of ROS production.

(A) Representative stationary currents at -60 mV (filled circles) and membrane resistance (open circles) changes induced by diazoxide and tolbutamide under control condition and in presence of the superoxide dismutase and catalase mimetic, MnTMPyP. The colored bars and shaded zones indicate the duration of application.

(B-C) Histograms summarizing the relative $K_{ATP}$ currents (B) and relative whole-cell $K_{ATP}$ conductance (C) evoked by two consecutive diazoxide and tolbutamide applications in control condition (Ctrl.) and after the presence of MnTMPyP. Data are normalized by the data measured during first application, expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.
Figure S3. Characterization of $K_{\text{ATP}}$ channels in different cortical neurons.

(A-D) Histograms summarizing the whole-cell $K_{\text{ATP}}$ conductance (A, B) and $K_{\text{ATP}}$ current density (C, D) and $K_{\text{ATP}}$ current reversal potential in identified neuronal subtypes (A, C) or between glutamatergic and GABAergic neurons (B, D). Data are expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.
Figure S4. Modulation of neuronal activity in different cortical neurons by $K_{\text{ATP}}$ channels.

(A-D) Histograms summarizing the proportion of responsive neurons (A, $K_2^2(5)=7.3125$, $p=0.1984$, and B, $p=0.9999$) and modulation firing rate (C, $H(5,32)=5.69107$, $p=0.337$, and D, $U(8,24)=87.5$, $p=0.6994$) by $K_{\text{ATP}}$ channels in neuronal subtypes (A,C) and groups (B,D). The numbers in brackets indicate the number of responsive cells and analyzed cells, respectively. Data are expressed as mean ± s.e.m., and the individual data points are depicted. n.s. not statistically significant.
Figure S5. Expression profile of monocarboxylate transporters and lactate dehydrogenase subunits in different cortical neuronal types.

Histograms summarizing the expression profile of the monocarboxylate transporters MCT1, 2 and 4 and LDH A and B lactate dehydrogenase subunits in different neuronal subtypes. The numbers in brackets indicate the number of analyzed cells.
Figure S6. Neuronal NADH autofluorescence increase by blockade of oxidative phosphorylation.

(A) Mean relative changes in NADH autofluorescence in control condition (grey) and in response to 1 mM KCN (blue). The colored bar indicates the duration of KCN applications. Data are expressed as mean ± s.e.m.

(B) Histograms summarizing the mean relative changes in NADH autofluorescence measured during the last 5 minutes of 1 mM KCN application (blue) and corresponding time in control condition (grey). Data are expressed as mean ± s.e.m., and the individual data points are depicted.
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