

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

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1 **Summary**

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

14

15 **Keywords**

16 K_{ATP} channel, pyramidal cell, interneuron, glucose, single cell RT-PCR, ATP.

17

18 **Highlights**

- 19
- Most cortical neurons subtypes express pancreatic beta-cell like K_{ATP}

20 channels.

 - Lactate enhances spiking activity via its uptake and closure of K_{ATP} channels.

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 - Cortical neurons take up and oxidize lactate.

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 - Cortical neurons produce ATP mainly by oxidative phosphorylation.

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1 **Introduction**

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

16
17 Based on the observations that various by-products released during glutamatergic
18 transmission stimulate astrocyte glucose uptake, aerobic glycolysis and lactate
19 release (Pellerin and Magistretti, 1994;Voutsinos-Porche et al., 2003;Ruminot et al.,
20 2011;Choi et al., 2012;Sotelo-Hitschfeld et al., 2015;Lerchundi et al., 2015), lactate
21 has been proposed to be shuttled from astrocytes to neurons to meet neuronal
22 energy needs. This hypothesis is supported by the existence of a lactate gradient
23 between astrocytes and neurons (Machler et al., 2016), the preferential use of lactate
24 as an energy substrate in cultured neurons (Bouzier-Sore et al., 2003;Bouzier-Sore
25 et al., 2006), and its ability to support neuronal activity during glucose shortage
26 (Schurr et al., 1988;Rouach et al., 2008;Wyss et al., 2011;Choi et al., 2012).
27 However, the use of different fluorescent glucose analogues to determine whether
28 astrocytes or neurons take up more glucose during sensory-evoked neuronal activity
29 has led to contradicting results (Chuquet et al., 2010;Lundgaard et al., 2015).
30 Furthermore brain slices and *in vivo* evidence have indicated that synaptic and
31 sensory stimulation enhanced neuronal glycolysis and potentially lactate release by
32 neurons (Ivanov et al., 2014;Diaz-Garcia et al., 2017), thereby challenging the
33 astrocyte-neuron lactate shuttle hypothesis. Hence, the relative contribution of
34 glucose and lactate to neuronal ATP synthesis remains unresolved.

1 ATP-sensitive potassium channels (K_{ATP}) act as metabolic sensors controlling
2 various cellular functions (Babenko et al., 1998). Their open probability (P_o) is
3 regulated by the energy charge of the cell (*i.e.* the ATP/ADP ratio). While ATP
4 mediates a tonic background inhibition of K_{ATP} channels, cytosolic increases of ADP
5 concentrations that occur as a sequel to enhanced energy demands, increase the P_o
6 of K_{ATP} channels. In neurons, electrical activity is accompanied by enhanced sodium
7 influx, which in turn activates the Na^+/K^+ ATPase. Activity of this pump alters the
8 submembrane ATP/ADP ratio sufficiently to activate K_{ATP} channels (Tanner et al.,
9 2011). The use of fluorescent ATP/ADP biosensors has demonstrated that K_{ATP}
10 channels are activated ($P_o > 0.1$) when ATP/ADP ratio is ≤ 5 (Tantama et al., 2013).

11
12 K_{ATP} channels are heterooctamers composed of four inwardly rectifying K^+ channel
13 subunits, Kir6.1 or Kir6.2, and four sulfonylurea receptors, SUR1 or SUR2, the later
14 existing in two splice variants (SUR2A and SUR2B) (Sakura et al., 1995; Aguilar-
15 Bryan et al., 1995; Inagaki et al., 1995b; Isomoto et al., 1996; Inagaki et al.,
16 1996; Chutkow et al., 1996; Yamada et al., 1997; Li et al., 2017; Martin et al., 2017; Lee
17 et al., 2017; Puljung, 2018). The composition in K_{ATP} channel subunits confers
18 different functional properties, pharmacological profiles as well as metabolic
19 sensitivities (Isomoto et al., 1996; Inagaki et al., 1996; Gribble et al., 1997; Yamada et
20 al., 1997; Okuyama et al., 1998; Liss et al., 1999). K_{ATP} channel subunits are
21 expressed in the neocortex (Ashford et al., 1988; Karschin et al., 1997; Dunn-Meynell
22 et al., 1998; Thomzig et al., 2005; Cahoy et al., 2008; Zeisel et al., 2015; Tasic et al.,
23 2016) and have been shown to protect cortical neurons from ischemic injury (Heron-
24 Milhavet et al., 2004; Sun et al., 2006) and to modulate their excitability (Gimenez-
25 Cassina et al., 2012) and intrinsic firing activity (Lemak et al., 2014). K_{ATP} channels
26 could thus be leveraged to decipher electrophysiologically the relative contribution of
27 glucose and lactate to neuronal ATP synthesis. Here, we apply single-cell RT-PCR
28 (scRT-PCR) to identify the mRNA subunit composition of K_{ATP} channel across
29 different neocortical neuron subtypes and demonstrate lactate as the preferred
30 energy substrate that also enhances firing activity.

31

1 **Results**

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

3 We first sought to determine whether K_{ATP} channel subunits were expressed in
4 different neuronal subtypes from the neocortex. Neurons (n=277) of the juvenile rat
5 barrel cortex from layers I to IV (Table S1) were functionally and molecularly
6 characterized in acute slices by scRT-PCR (Figure 1), whose sensitivity was
7 validated from 500 pg of total cortical RNAs (Figure S1A). Neurons were segregated
8 into 7 different subtypes according to their overall molecular and electrophysiological
9 similarity (Figure 1A) using unsupervised Ward's clustering (Ward, 1963), an
10 approach we previously successfully used to classify cortical neurons (Cauli et al.,
11 2000; Gallopin et al., 2006; Karagiannis et al., 2009). Regular spiking (RS, n=63) and
12 intrinsically bursting (IB, n=10) cells exhibited the molecular characteristics of
13 glutamatergic neurons, with very high single-cell detection rate (n=69 of 73, 95%) of
14 vesicular glutamate transporter 1 (vGluT1) and low detection rate (n=7 of 73, 10%) of
15 glutamic acid decarboxylases (GADs, Figure 1B-E and Table S2), the GABA
16 synthesizing enzymes. This group of glutamatergic neurons distinctly displayed
17 hyperpolarized resting membrane potential (-81.2 ± 0.8 mV), possessed a large
18 membrane capacitance (108.6 ± 3.6 pF), discharged with wide action potentials (1.4
19 ± 0.0 ms) followed by medium afterhyperpolarizations (mAHs). These neurons did
20 sustain only low maximal frequencies (35.4 ± 1.6 Hz) and showed complex spike
21 amplitude accommodation (Table S3-7). In contrast to RS neurons, IB neurons were
22 more prominent in deeper layers (Table S1) and their bursting activity affected their
23 adaptation amplitudes and kinetics (Figure 1C and Tables S4-5), spike broadening
24 (Figure 1C and Tables S6) and the shape of mAHs (Figure 1C and Tables S7).

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

33 Other GABAergic interneurons co-expressed somatostatin (SOM) and calbindin (CB)
34 as well as neuropeptide Y (NPY) to a lesser extent and functionally corresponded to

1 Adapting SOM neurons (n=24, Figure 1B and Table S2). They displayed depolarized
2 resting membrane potential, pronounced voltage sags, low rheobases and
3 pronounced afterdepolarizations (Figure 1C and Table S3-6). In another group of
4 GABAergic adapting interneurons located in superficial layers, mRNA for NPY was
5 detected at a high rate (n=31 of 56, 55%). In these Adapting NPY interneurons
6 mRNA for nitric oxide synthase-1 (NOS-1) was detected at a lower rate (Figure 1B
7 and Tables S1-2). In response to suprathreshold depolarizing current steps, these
8 interneurons showed very little spike frequency adaptation (Figure 1C and Table S4).
9 Finally, parvalbumin (PV) was observed in virtually all neurons of a subpopulation
10 termed Fast Spiking-PV interneurons (FS-PV, n=37 of 38, 97%, Figure 1B and Table
11 S2). In comparison to all other cortical neurons described above, they were
12 characterized by low membrane resistance ($201 \pm 13 \text{ M}\Omega$), fast time constant, high
13 rheobase, very short spikes ($0.6 \pm 0.0 \text{ ms}$) with sharp fast afterhyperpolarizations
14 (fAHs) and the ability to sustain high firing rates ($139.9 \pm 6.8 \text{ Hz}$) with little to no
15 frequency adaptation (Figure 1C and Tables S3-7). These data thus identified
16 different neuronal subtypes based on their distinctive electrophysiological and
17 molecular features (Ascoli et al., 2008) confirming our previous classification
18 schemes (Cauli et al., 2000; Gallopin et al., 2006; Karagiannis et al., 2009).

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

1 **Characterization of K_{ATP} channels in cortical neurons**

2 To assess whether K_{ATP} channels are functional in identified cortical neurons ($n=18$,
3 Figure 2A), we measured the effects of different K_{ATP} channel modulators on whole-
4 cell currents ($Q_{(3,18)}=32.665$, $p=3.8 \times 10^{-7}$, Friedman test) and membrane resistances
5 ($Q_{(3,18)}=40.933$, $p=6.8 \times 10^{-9}$). Pinacidil (100 μ M), a SUR2-preferring K_{ATP} channel
6 opener (Inagaki et al., 1996;Moreau et al., 2005), had little or no effect on current (4.1
7 ± 3.7 pA, $p=0.478$) and membrane resistance (-9.6 ± 3.7 %, $p=0.121$, Figure 2B-C).
8 By contrast, diazoxide (300 μ M), an opener acting on SUR1 and SUR2B-containing
9 K_{ATP} channels (Inagaki et al., 1996;Moreau et al., 2005), consistently induced an
10 outward current (45.0 ± 9.6 pA, $p=4.8 \times 10^{-5}$) and a decrease in membrane
11 resistance ($-34.5 \pm 4.3\%$, $p=3.6 \times 10^{-5}$) indicative of the activation of a hyperpolarizing
12 conductance (Figure 2B-C). The sulfonylurea tolbutamide (500 μ M, Figure 2B-C), a
13 K_{ATP} channel blocker (Ammala et al., 1996;Isomoto et al., 1996;Gribble et al.,
14 1997;Isomoto and Kurachi, 1997), did not change whole-cell basal current (-6.6 ± 3.0
15 pA, $p=0.156$) or membrane resistance ($20.5 \pm 7.5\%$, $p=3.89 \times 10^{-2}$). Conversely,
16 tolbutamide dramatically reversed diazoxide effects on both current ($p=4.1 \times 10^{-8}$)
17 and membrane resistance ($p=5.8 \times 10^{-10}$).

18
19 In virtually all neuronal subtypes ($H_{(6,43)}=2.274$, $p=0.810$, Kruskal–Wallis H test) or
20 groups ($t_{(42)}=0.3395$, $p=0.736$, Student's t-test), the diazoxide-tolbutamide
21 current/voltage relationship reversed very close to the theoretical potassium
22 equilibrium potential ($E_K=-106.0$ mV, Figure 2D-F) confirming the opening of a
23 selective potassium conductance. Besides its effects on plasma membrane K_{ATP}
24 channels, diazoxide is also a mitochondrial uncoupler (Drose et al., 2006) which
25 increases reactive oxygen species (ROS) production. This might stimulate Ca^{2+}
26 sparks and large-conductance Ca^{2+} -activated potassium channels (Xi et al., 2005)
27 leading to potential confounding effects. This possibility was ruled out by the
28 observation that Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP, 25 μ M), a
29 ROS scavenger (D'Agostino et al., 2007), did not reduce the diazoxide-tolbutamide
30 responses on current ($t_{(10)}=0.76559$, $p=0.462$, Figure S2A,B) and conductance
31 ($t_{(10)}=1.24758$, $p=0.241$, Figure S2A,C).

32
33 Cortical neurons exhibited K_{ATP} conductances of similar value between their subtypes
34 ($H_{(6,63)}=5.6141$, $p=0.468$) or groups ($U_{(9,54)}=233$, $p=0.855$, Mann–Whitney U test,

1 Figures S3A,B). K_{ATP} channels activated by diazoxide essentially doubled the whole
2 cell conductance in the subthreshold membrane potential compared to control or
3 tolbutamide conditions, regardless of neuronal subtypes ($H_{(6,63)}=5.4763$, $p=0.484$) or
4 groups ($t_{(61)}=1.324$, $p=0.191$, Figures 2G,H). Also, K_{ATP} current density was similar
5 ($H_{(6,63)}=4.4769$, $p=0.612$, $U_{(9,54)}=240.5$, $p=0.965$, Figures S3C,D). Together with the
6 pinacidil unresponsiveness, these data indicate that the large majority of cortical
7 neurons expressed functional SUR1-mediated K_{ATP} channels in a homogeneous
8 fashion across different subpopulations. To confirm that Kir6.2 is the pore-forming
9 subunit of K_{ATP} channels in cortical neurons, we used a genetic approach based on
10 Kir6.2 knock-out mice (Miki et al., 1998). We first verified the expression of Kir6.2 and
11 SUR1 subunits in pyramidal cells from wild type mice by scRT-PCR (Figure 3A,B).
12 We next used a dialysis approach by recording neurons with an ATP-free pipette
13 solution (Miki et al., 2001) enriched in sodium (20 mM) to stimulate submembrane
14 ATP depletion and ADP production by the Na^+/K^+ ATPase, which is known to activate
15 K_{ATP} channels (Figure 3H). We confirmed that ATP1a1 and ATP1a3 (Figure 3B) were
16 the main α -subunits of the Na^+/K^+ ATPase pump in pyramidal neurons (Zeisel et al.,
17 2015; Tasic et al., 2016). Dialysis of ATP-free/20 mM Na^+ -pipette solution indeed
18 induced a standing outward current in Kir6.2^{+/+} neurons within 5 minutes (46.7 ± 19.0
19 pA at -50 mV, $n=26$) that was reversing close to E_K (Figure 3C,E). In contrast, this
20 current was not observed in Kir6.2^{-/-} neurons (-59.9 ± 11.9 pA, $n=22$, $U_{(26,22)}=78$,
21 $p=2.4221 \times 10^{-6}$, one-tailed, Figure 3D-G). Collectively, these data show that cortical
22 neurons predominately express functional K_{ATP} channels composed of Kir6.2 and
23 SUR1 subunits.

24

25 **Modulation of neuronal excitability and activity by K_{ATP} channel**

26 Despite their large diversity, cortical neurons display a homogenous functional
27 expression of K_{ATP} channels, questioning how these channels integrate the metabolic
28 environment to adjust neuronal activity. To address this question, we first evaluated
29 in identified cortical neurons ($n=39$) the ability of K_{ATP} channels to modulate neuronal
30 excitability, notably by measuring membrane potentials ($Q_{(2,39)}=38.000$, $p=5.6 \times 10^{-9}$)
31 and membrane resistances ($Q_{(2,39)}=40.205$, $p=1.9 \times 10^{-9}$), as well as spiking activity
32 ($Q_{(2,39)}=28.593$, $p=6.2 \times 10^{-9}$). Following electrophysiological identification, the K_{ATP}
33 channel blocker tolbutamide was applied, which resulted in a slight depolarization
34 ($\Delta V_m=2.6 \pm 0.8$ mV, $p=1.74 \times 10^{-2}$, Figure 4A,D) and increase in membrane

1 resistance ($\Delta R_m = 78 \pm 31 \text{ M}\Omega$, $p = 1.52 \times 10^{-3}$, Figure 4B,E). These effects were strong
2 enough to trigger and stimulate the firing of action potentials ($\Delta F = 0.3 \pm 0.1 \text{ Hz}$, $p =$
3 9.21×10^{-3} , Figure 4A,C,F). By contrast, diazoxide hyperpolarized cortical neurons ($-$
4 $4.0 \pm 0.6 \text{ mV}$, $p = 1.87 \times 10^{-4}$, Figure 4A,D), decreased their membrane resistance (-39
5 $\pm 23 \text{ M}\Omega$, $p = 1.52 \times 10^{-3}$, Figure 4B,E) but did alter their rather silent basal spiking
6 activity ($-0.1 \pm 0.1 \text{ Hz}$, $p = 0.821$, Figure 4A,C,F).

7
8 Most cortical neurons ($n = 32$ of 39) showed modulation of neuronal excitability by
9 both K_{ATP} channel modulators and were considered to be responsive. A similar
10 proportion of responsive neurons was observed between neuronal subtypes (Figure
11 S4A, $\chi^2_{(5)} = 7.313$, $p = 0.1984$) or groups (Figure S4B, $p = 0.9999$, Fisher's exact test).
12 The apparent relative lack of responsiveness in FS-PV interneurons (Figure S4A),
13 despite a whole-cell K_{ATP} conductance similar to that of other neuronal types (Figure
14 S3A), is likely attributable to their low input resistance (Table S3) making K_{ATP}
15 channels less effective to change membrane potential. Overall, K_{ATP} channels
16 modulated membrane potential, resistance and firing rate by up to $7.6 \pm 0.9 \text{ mV}$, $76 \pm$
17 17% and $0.5 \pm 0.2 \text{ Hz}$, respectively. This modulation of neuronal excitability (Figure
18 4G-J) and activity (Figure S4C,D) was similar between neuronal subtypes or groups
19 (Figure 4H-J and S4C-E). Thus, K_{ATP} channels modulate the excitability and activity
20 of all subtypes of cortical neurons.

21

22 **Enhancement of neuronal activity by lactate via modulation of K_{ATP} channels**

23 The uniform expression of metabolically highly sensitive K_{ATP} channels by cortical
24 neurons suggests their ability to couple the local glycolysis capacity of astrocytes
25 with spiking activity. We therefore evaluated whether extracellular changes in
26 glucose and lactate could differentially shape the spiking activity of cortical neurons
27 through their energy metabolism and K_{ATP} channel modulation. Importantly, to
28 preserve intracellular metabolism, neurons were recorded in perforated patch-
29 configuration. Stable firing rates of about 4 Hz inducing ATP consumption by the
30 Na^+/K^+ ATPase (Attwell and Laughlin, 2001) were evoked by applying a depolarizing
31 current and continuously monitored throughout changes in extracellular medium
32 (Figure 5A, $Q_{(2,16)} = 22.625$, $p = 1.222 \times 10^{-5}$).

1 Decreasing extracellular glucose from 10 mM to a normoglycemic concentration of
2 2.5 mM (Silver and Erecinska, 1994;Hu and Wilson, 1997b) did not change firing rate
3 (Figure 5A,B, $p=0.2159$) of cortical neurons ($n=16$). By contrast, supplementing
4 extracellular 2.5 mM glucose with 15 mM lactate, a concentration observed in the
5 blood after an intense physical exercise (Quistorff et al., 2008) and corresponding to
6 the same number of carbon atoms as 10 mM glucose, roughly doubled the firing rate
7 compared to both 2.5 ($p=7.829 \times 10^{-4}$) and 10 mM glucose ($p=4.303 \times 10^{-6}$)
8 conditions. Firing rate enhancement by lactate was dose-dependent ($H_{(8,76)}=35.142$,
9 $p=1.052 \times 10^{-5}$) and reached statistical significance above 5 mM (Figure 5C). We
10 reasoned that this effect could be mediated by K_{ATP} channel closure. Indeed, the
11 increase in firing rate by lactate ($209 \pm 49\%$) was strongly reduced by the K_{ATP}
12 channel activator diazoxide ($71 \pm 18\%$, $p=3.346 \times 10^{-3}$, Figure 5D). Tolbutamide
13 reversed diazoxide's effect ($160 \pm 17\%$, $p=9.345 \times 10^{-3}$) but did not increase firing
14 rate further ($p=0.5076$). This occlusion of tolbutamide's effect by 15 mM lactate also
15 suggests that this concentration reaches saturating levels and is the highest
16 metabolic state that can be sensed by K_{ATP} channels. Enhancement of neuronal
17 activity by lactate was also observed in Kir6.2^{+/+} cortical neurons ($147 \pm 25\%$,
18 $p=2.840 \times 10^{-2}$) but not in Kir6.2^{-/-} mice ($112 \pm 32\%$, $p=0.8785$, Figure 5E). These
19 observations indicate that lactate enhances neuronal activity via a closure of K_{ATP}
20 channels (Figure 5F).

21

22 **Mechanism of lactate-sensing**

23 To determine whether lactate-sensing involves intracellular lactate oxidative
24 metabolism and/or extracellular activation of the lactate receptor GPR81, we next
25 probed the expression of monocarboxylate transporters (MCT), which allow lactate
26 uptake. Consistent with mouse RNAseq data (Zeisel et al., 2015;Tasic et al., 2016),
27 MCT1 and MCT2 were the main transporters detected in rat cortical neurons by
28 scRT-PCR, although with relatively low single cell detection rates (54 of 277, 19.5%
29 and 78 of 277, 28.2%, for MCT1 and 2, respectively, Figure 6A and S5).

30 The expression of monocarboxylate transporters in cortical neurons is compatible
31 with lactate uptake and metabolism leading to the closure of K_{ATP} channels and an
32 increase in firing rate. We thus evaluated whether lactate uptake was needed for
33 lactate-sensing. We used 250 μ M α -cyano-4-hydroxycinnamic acid (4-CIN), a
34 concentration blocking lactate uptake while only moderately altering mitochondrial

1 pyruvate carrier in brain slices (Schurr et al., 1999;Ogawa et al., 2005;Galeffi et al.,
2 2007). 4-CIN reversed the increased firing rate induced by lactate (Figure 6B, $T(9)=0$,
3 $p=7.686 \times 10^{-3}$) indicating that facilitated lactate transport is required for K_{ATP} channel
4 closure and in turn firing rate acceleration.

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

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

26 The requirement of monocarboxylate transport and the similar effect of lactate and
27 pyruvate on neuronal activity suggest that once taken up, lactate would be oxidized
28 into pyruvate and metabolized by mitochondria to produce ATP, leading in turn to a
29 closure of K_{ATP} channels and increased firing rate. The apparent absence of glucose
30 responsiveness in cortical neurons also suggests that glycolysis contributes modestly
31 to ATP production. To determine the relative contribution of glycolysis and oxidative
32 phosphorylation to ATP synthesis, we transduced the genetically encoded
33 fluorescence resonance energy transfer (FRET)-based ATP biosensor AT1.03^{YEMK}
34 (Imamura et al., 2009) using a recombinant Sindbis virus. AT1.03^{YEMK} fluorescence

1 was mostly observed in pyramidal shaped cells (Figure 6G), consistent with the
2 strong tropism of this viral vector towards pyramidal neurons (Piquet et al., 2018).
3 Blocking glycolysis with 200 μ M iodoacetic acid (IAA) decreased modestly the FRET
4 ratio by $2.9 \pm 0.2\%$ (Figure 6H, $p=2.55 \times 10^{-9}$). By contrast, adding potassium cyanide
5 (KCN, 1mM), a respiratory chain blocker, reduced the FRET ratio to a much larger
6 extent ($52.3 \pm 0.6\%$, Figure 6H, $p=2.55 \times 10^{-9}$). KCN also induced a strong NADH
7 fluorescence increase (Figure S6A-B, $U_{(12,42)}=0$, $p=5.83 \times 10^{-12}$), indicating a highly
8 active oxidative phosphorylation in cortical neurons.

1 **Discussion**

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

13

14 **K_{ATP} channel subunits in cortical neurons**

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

30

31 Consistent with the preferred expression of Kir6.1 in mural and endothelial cells
32 (Bondjers et al., 2006; Zeisel et al., 2015; Tasic et al., 2016; Aziz et al.,
33 2017; Vanlandewijck et al., 2018; Saunders et al., 2018), this subunit was only
34 observed in one out of 277 cortical neurons analyzed. Similarly, SUR2B, the SUR2

1 variant expressed in forebrain (Isomoto et al., 1996) and cortex (Figure S1), whose
2 presence is largely restricted to vascular cells (Zeisel et al., 2015), was not observed
3 in cortical neurons.

4

5 **Relative sensitivity of cortical neurons to glucose, lactate and pyruvate**

6 Consistent with previous observations (Yang et al., 1999), decreasing extracellular
7 glucose from standard slice concentrations down to a normoglycemic level did not
8 alter firing rates of cortical neurons. However, their activity is silenced during
9 hypoglycemic episodes through K_{ATP} channels activation (Yang et al., 1999;Zawar
10 and Neumcke, 2000;Molnar et al., 2014;Sada et al., 2015). This relative glucose
11 unresponsiveness is in contrast with pancreatic beta cells and hypothalamic glucose-
12 excited neurons whose activity is regulated over a wider range of glucose
13 concentrations by K_{ATP} channels also composed with Kir6.2 and SUR1 subunits
14 (Aguilar-Bryan et al., 1995;Inagaki et al., 1995a;Miki et al., 1998;Yang et al.,
15 1999;Miki et al., 2001;Tarasov et al., 2006;Varin et al., 2015). The inability of cortical
16 neurons to regulate their spiking activity at glucose levels beyond normoglycemia is
17 likely due to the lack of glucokinase, a hexokinase which catalyzes the first step of
18 glycolysis and acts as a glucose sensor in the millimolar range (German, 1993;Yang
19 et al., 1999). As earlier reported, hexokinase-1 (HK1) is the major isoform in cortical
20 neurons (Zeisel et al., 2015;Tasic et al., 2016;Piquet et al., 2018). Since this enzyme
21 has a micromolar affinity for glucose and is inhibited by its product, glucose-6-
22 phosphate (Wilson, 2003), HK1 is likely already saturated and/or inhibited during
23 normoglycemia thereby limiting glycolysis. Nonetheless, HK1 saturation/inhibition can
24 be mitigated when energy consumption is high (Attwell and Laughlin, 2001;Wilson,
25 2003;Tantama et al., 2013), and then glucose can probably modulate neuronal
26 activity via a high affinity mechanism, as evidenced by slow oscillations of spiking
27 activity involving synaptic transmission (Cunningham et al., 2006) or by the use of
28 glucose-free whole-cell patch-clamp solution (Kawamura, Jr. et al., 2010) that mimics
29 high glucose consumption (Piquet et al., 2018;Diaz-Garcia et al., 2019).

30

31 Similarly to glucose-excited hypothalamic neurons (Yang et al., 1999;Song and
32 Routh, 2005), but in contrast with pancreatic beta cells (Newgard and McGarry,
33 1995), cortical neurons were dose-dependently excited by lactate. This lactate
34 sensitivity is consistent with lactate transport and oxidization in hypothalamic and

1 cortical neurons (Ainscow et al., 2002;Sada et al., 2015;Diaz-Garcia et al., 2017)
2 which are low in beta cells (Sekine et al., 1994;Pullen et al., 2011). Pyruvate had a
3 similar effect to lactate in cortical neurons under normoglycemic condition whereas it
4 only maintains the activity of hypothalamic glucose-excited neurons during
5 hypoglycemia (Yang et al., 1999) and barely activates pancreatic beta cells (Dufer et
6 al., 2002). Thus, cortical neurons display a peculiar metabolic sensitivity to
7 monocarboxylates. Our data also suggest that under normoglycemic conditions a
8 portion of K_{ATP} channels are open when cortical neurons fire action potentials.

9

10 **Mechanism of lactate-sensing**

11 Our pharmacological, molecular and genetic evidence indicates that the closure of
12 K_{ATP} channels is responsible for the firing rate enhancement by lactate. Since K_{ATP}
13 channels can be modulated by G protein-coupled receptors (Kawamura, Jr. et al.,
14 2010), lactate-sensing might have been mediated by GPR81, a G_i protein-coupled
15 lactate receptor expressed in the cerebral cortex (Lauritzen et al., 2014). This
16 possibility is however unlikely since the activation of GPR81 inhibits cultured cortical
17 neurons (Bozzo et al., 2013;de Castro Abrantes H. et al., 2019) and we show here
18 that enhancing effect pyruvate on neuronal activity was similar to that of lactate,
19 although pyruvate does not activate GPR81 (Ahmed et al., 2010).

20

21 We found that lactate-sensing was critically dependent on lactate transport and we
22 confirmed the capacity of cortical neurons to take up and oxidize lactate (Bittar et al.,
23 1996;Laughton et al., 2000;Bouzier-Sore et al., 2003;Wyss et al., 2011;Choi et al.,
24 2012;Sada et al., 2015;Machler et al., 2016). LDH metabolites, including pyruvate
25 and oxaloacetate, can lead to K_{ATP} channel closure (Dhar-Chowdhury et al.,
26 2005;Sada et al., 2015) and could mediate lactate-sensing. An intermediate role of
27 oxaloacetate in lactate-sensing is compatible with enhanced Krebs cycle and
28 oxidative phosphorylation, which leads to an increased ATP/ADP ratio and the
29 closure of K_{ATP} channels (Figure 7). In contrast to oxaloacetate, intracellular ATP was
30 found to be ineffective for reverting K_{ATP} channel opening induced by LDH inhibition
31 (Sada et al., 2015). Interestingly, hippocampal interneurons were found to be
32 insensitive to glucose deprivation in whole cell configuration (Sada et al., 2015) but
33 not in perforated patch configuration (Zawar and Neumcke, 2000) whereas almost
34 the opposite was found in CA1 pyramidal cells. Whether altered intracellular

1 metabolism by whole-cell recording accounted for the apparent lack of ATP
2 sensitivity remains to be determined.

3

4 **Lactate as an energy substrate for neurons and an enhancer of spiking activity** 5 **and neuronal plasticity**

6 We confirmed that the ATP produced by cortical neurons was mostly derived from
7 oxidative phosphorylation and marginally from glycolysis (Almeida et al., 2001; Hall et
8 al., 2012). Together with the enhancement of spiking activity through K_{ATP} channels
9 by lactate, but not by glucose, our data support both the notion that lactate is a
10 preferred energy substrate over glucose for cortical neurons (Bouzier-Sore et al.,
11 2003) and the astrocyte-neuron lactate shuttle hypothesis (Pellerin and Magistretti,
12 1994). Although the local cellular origin of lactate has been recently questioned (Lee
13 et al., 2012; Diaz-Garcia et al., 2017), a growing number of evidence indicates that
14 astrocytes are major central lactate producers (Almeida et al., 2001; Choi et al.,
15 2012; Sotelo-Hitschfeld et al., 2015; Karagiannis et al., 2015; Le Douce J. et al.,
16 2020; Jimenez-Blasco et al., 2020).

17 Glutamatergic synaptic transmission stimulates blood glucose uptake, astrocyte
18 glycolysis, as well as lactate release (Pellerin and Magistretti, 1994; Voutsinos-Porche
19 et al., 2003; Ruminot et al., 2011; Choi et al., 2012; Sotelo-Hitschfeld et al.,
20 2015; Lerchundi et al., 2015) and diffusion through the astroglial gap junctional
21 network (Rouach et al., 2008). This indicates that local and fast glutamatergic
22 synaptic activity would be translated by astrocyte metabolism into a widespread and
23 long-lasting extracellular lactate increase (Prichard et al., 1991; Hu and Wilson,
24 1997a), which could in turn enhance the firing of both excitatory and inhibitory
25 neurons (Figure 7). Such a lactate surge would be spatially confined by the gap
26 junctional connectivity of the astroglial network, which in layer IV represents an
27 entire barrel (Houades et al., 2008).

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

1 formation (Stella et al., 1997), hamper lactate production by astrocytes (Jimenez-
2 Blasco et al., 2020).

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

13

14 **Lactate-sensing compensatory mechanisms**

15 Since excitatory neuronal activity increases extracellular lactate (Prichard et al.,
16 1991;Hu and Wilson, 1997a) and lactate enhances neuronal activity, such a positive
17 feedback loop (Figure 7) suggests that compensatory mechanisms might be
18 recruited to prevent an overexcitation of neuronal activity by lactate supply. A
19 metabolic negative feedback mechanism could involve the impairment of astrocyte
20 metabolism and lactate release by endocannabinoids (Jimenez-Blasco et al., 2020)
21 produced during intense neuronal activity (Stella et al., 1997).

22 Another possibility would consist in a blood flow decrease that would in turn reduce
23 the delivery of blood glucose and subsequent local lactate production and release but
24 also blood-borne lactate. Some GABAergic interneuron subtypes (Cauli et al.,
25 2004;Uhlirva et al., 2016;Krawchuk et al., 2019), but also astrocytes (Girouard et al.,
26 2010), can trigger vasoconstriction and blood flow decrease when their activity is
27 increased. This could provide a negative hemodynamic feedback restricting spatially
28 and temporally the increase of spiking activity by lactate.

29 PV-expressing and SOM-expressing interneurons exhibit higher mitochondrial
30 content and apparent oxidative phosphorylation than pyramidal cells (Gulyas et al.,
31 2006) suggesting that interneurons would more rapidly metabolize and sense lactate
32 than pyramidal cells. These inhibitory GABAergic interneurons might therefore
33 silence the cortical network, thereby providing a negative neuronal feedback loop.
34 Active decrease in blood flow is associated with a decrease in neuronal activity

1 (Shmuel et al., 2002;Shmuel et al., 2006;Devor et al., 2007). Vasoconstrictive
2 GABAergic interneurons may underlie for both processes and could contribute to
3 returning the system to a low lactate state.

4

5 **Conclusion**

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

13

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20

1 Author contributions

2 Conceptualization, T.G., J. Roeper and B.C.; Formal Analysis, J.N., B.C.;
 3 Investigation, A.K., T.G., A.L., F.P., J.P., H.G., R.H., B.C., Resources, B.L., J.
 4 Rossier, H.I., S.S., J. Roeper, B.C.; Writing –Original Draft, B.C.; Writing –Review &
 5 Editing, J.N., B.L.G., R.E., D.L., B.L., J.F.S., J. Roeper, B.C., Visualization, A.K.,
 6 B.C.; Supervision, B.C.; Project Administration, B.C., Funding Acquisition, B.C.

8 Competing interests

9 The authors declare no competing interests.

10

11 Materials and methods

12 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 <i>Bacillus aneurinolyticus</i> (<i>Bacillus brevis</i>)	Sigma-Aldrich	G5002
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		
Rat: Wistar	Janvier Labs	jHan:W1
Mouse: Wild type C57BL/6RJ	Janvier Labs	C57BL/6RJ
Mouse: Kir6.2 knockout (KO)	Miki et al., 1998	MGI:2178821
Oligonucleotides		
PCR primer rat vGluT1 external sense: 5'-GGCTCCTTTTTCTGGGGGTAC-3'	(Gallopín et al., 2006)	N/A
PCR primer rat/mouse vGluT1 external antisense: 5'-CCAGCCGACTCCGTTCTAAG-3'	(Gallopín et al., 2006)	N/A
PCR primer rat vGluT1 internal sense: 5'-TGGGGGTACATTGTCACCTAGA-3'	(Gallopín et al., 2006)	N/A
PCR primer rat vGluT1 internal antisense: 5'-ATGGCAAGCAGGGTATGTGAC-3'	(Gallopín 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'-TCCTCCAGATTTTGCAGTTG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat GAD65 internal sense: 5'-TGAGAAGCCAGCAGAGAGCG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat GAD65 internal antisense: 5'-TGGGGTAATGGAAATCAATCACTT-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat GAD67 external sense: 5'-ATGATACTTGGTGTGGCGTAGC-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat GAD67 external antisense: 5'-GTTTGCTCCTCCCGTCTTAG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat GAD67 internal sense: 5'-CAATAGCCTGGAAGAGAAGAGTCG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat GAD67 internal antisense: 5'-GTTTGCTCCTCCCGTCTTAG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat NOS-1 external sense: 5'-CCTGGGGCTCAAATGGTATG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat NOS-1 external antisense: 5'-CACAAATCCACACCAGTCGG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat NOS-1 internal sense: 5'-CCTCCCGCTGTGTCCAA-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat NOS-1 internal antisense: 5'-GAGTGGTGGTCAACGATGGTCA-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'-CCCACACATTTTGATTCCCTG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat CB internal sense: 5'-ATGGGCAGAGAGATGATGGG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat CB internal antisense: 5'-TATCATCCACGGTCTTGTGTTGC-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat NOS-1 internal antisense: 5'-GAGTGGTGGTCAACGATGGTCA-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat PV external sense: 5'-GCCTGAAGAAAAGAGTGCGG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat PV external antisense: 5'-GTCCCGTCCCTTGCTCCAG-3'	(Karagiannis et al., 2009)	N/A
PCR primer rat PV internal sense: 5'-GCGGATGATGTGAAGAAGGTG-3'	(Karagiannis et al., 2009)	N/A
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PCR primer rat CR internal antisense: 5'-ATTCTCTCGGTTGGCAGGA-3'	(Karagiannis et al., 2009)	N/A
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PCR primer rat NPY internal antisense: 5'-GTTCTGGGGGCATTTTCTGTG-3'	(Karagiannis et al., 2009)	N/A
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PCR primer rat VIP internal antisense: 5'-GAATCTCCCTCACTGCTCCTCT-3'	(Karagiannis et al., 2009)	N/A
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PCR primer rat SOM external antisense: 5'-GCCTCATCTCGTCCGTCTCA-3'	(Férezou et al., 2007)	N/A
PCR primer rat SOM internal sense: 5'-GCATCGTCCCTGGCTTTGGG-3'	(Karagiannis et al., 2009)	N/A
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PCR primer rat MCT1 external antisense: 5'-TCCGCTTCTGTTCTTTGGC-3'	This paper	N/A
PCR primer rat MCT1 internal sense: 5'-TTGTTGCGAATGGAGTGTGC-3'	This paper	N/A
PCR primer rat MCT1 internal antisense: 5'-CAGCCACAAGCCCAGTATG-3'	This paper	N/A

PCR primer rat MCT2 external sense: 5'-GCGAAGTCTAAAAGTAAGGTTGGC-3'	This paper	N/A
PCR primer rat MCT2 external antisense: 5'-ATTTACCAGCCAGGGGAGGG-3'	This paper	N/A
PCR primer rat MCT2 internal sense: 5'-CCGTATGCTAAGGACAAAGGAGT-3'	This paper	N/A
PCR primer rat MCT2 internal antisense: 5'-GGGAAGAACTGGGCAACACT-3'	This paper	N/A
PCR primer rat MCT4 external sense: 5'-CATTGGTCTCGTGCTGT-3'	This paper	N/A
PCR primer rat MCT4 external antisense: 5'-CCCCGTTTTTCTCAGGCTCT-3'	This paper	N/A
PCR primer rat MCT4 internal sense: 5'-TGTGGCTGTGCTCATCGGAC-3'	This paper	N/A
PCR primer rat MCT4 internal antisense: 5'-CCTCTTCCCTTCCCGATGC-3'	This paper	N/A
PCR primer rat LDH-A external sense: 5'-GAAGAACAGGTCCCCCAGAA-3'	This paper	N/A
PCR primer rat LDH-A external antisense: 5'-GGGTTTGAGACGATGAGCAGT-3'	This paper	N/A
PCR primer rat LDH-A internal sense: 5'-CAGTTGTTGGGGTTGGTGCT-3'	This paper	N/A
PCR primer rat LDH-A internal antisense: 5'-TCTCTCCCTTTGCTGACGG-3'	This paper	N/A
PCR primer rat LDH-B external sense: 5'-ACTGCCGTCCCGAACAA-3'	This paper	N/A
PCR primer rat LDH-B external antisense: 5'-ACTCTCCCCCTCCTGCTGG-3'	This paper	N/A
PCR primer rat LDH-B internal sense: 5'-TCTGGGGAAGTCTCTGGCTGA-3'	This paper	N/A
PCR primer rat LDH-B internal antisense: 5'-TTGGCTGTACGGAGTAATCTTT-3'	This paper	N/A
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
LUMPlan FI /IR 60x/0.90 W	Olympus	N/A
LUMPlan FI /IR 40x/0.80 W	Olympus	N/A
CoolSnap HQ2	Roper Scientific	N/A
Axopatch 200B	Molecular Devices	N/A
Digidata 1440A	Molecular Devices	N/A
S900 stimulator	Dagan corporation	N/A
pE-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
Dotd Gradient Contrast	Luigs and Neumann	200-100 200 0155
725 DCSPXR	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,
3 and will be fulfilled by, the lead contact, B. Cauli (bruno.cauli@upmc.fr).

4
5 **Experimental model and subject details**

6 Wistar rats, C57BL/6RJ or Kir6.2 KO mice were used for all experiments in
7 accordance with French regulations (Code Rural R214/87 to R214/130) and
8 conformed to the ethical guidelines of both the directive 2010/63/EU of the European
9 Parliament and of the Council and the French National Charter on the ethics of
10 animal experimentation. A maximum of 3 rats or 5 mice were housed per cage and
11 single animal housing was avoided. Male rats and mice of both genders were housed
12 on a 12-hour light/dark cycle in a temperature-controlled (21–25°C) room and were
13 given food and water *ad libitum*. Animals were used for experimentation at 13-24
14 days of age.

1 **Cortical slice preparation**

2 Rats or mice were deeply anesthetized with isoflurane. After decapitation brains were
3 quickly removed and placed into cold (~4°C) oxygenated artificial cerebrospinal fluid
4 (aCSF) containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 26
5 NaHCO₃, 10 glucose, 15 sucrose, and 1 kynurenic acid. Coronal slices (300 μm
6 thick) containing the barrel cortex were cut with a vibratome (VT1000S, Leica) and
7 allowed to recover at room temperature for at least 1h in aCSF saturated with O₂/CO₂
8 (95 %/5 %) as previously described (Karagiannis et al., 2009;Devienne et al., 2018).

9

10 **Whole-cell patch-clamp recording**

11 Patch pipettes (4-6 MΩ) pulled from borosilicate glass were filled with 8 μl of RNase
12 free internal solution containing in (mM): 144 K-gluconate, 3 MgCl₂, 0.5 EGTA, 10
13 HEPES, pH 7.2 (285/295 mOsm). Whole-cell recordings were performed at 25.3 ±
14 0.2°C using a patch-clamp amplifier (Axopatch 200B, Molecular Devices). Data were
15 filtered at 5-10 kHz and digitized at 50 kHz using an acquisition board (Digidata 1440,
16 Molecular Devices) attached to a personal computer running pCLAMP 10.2 software
17 package (Molecular Devices). For ATP washout experiments neurons were recorded
18 in voltage clamp mode using an ATP-free internal solution containing in (mM): 140
19 KCl, 20 NaCl, 2 MgCl₂, 10 EGTA, 10 HEPES, pH 7.2.

20

21 **Cytoplasm harvesting and scRT-PCR**

22 At the end of the whole-cell recording, lasting less than 15 min, the cytoplasmic
23 content was aspirated in the recording pipette. The pipette's content was expelled
24 into a test tube and reverse transcription (RT) was performed in a final volume of 10
25 μl, as described previously (Lambolez et al., 1992). The scRT-PCR protocol was
26 designed to probe simultaneously the expression of neuronal markers, K_{ATP} channels
27 subunits or some key elements of energy metabolism. Two-steps amplification was
28 performed essentially as described (Cauli et al., 1997;Devienne et al., 2018). Briefly,
29 cDNAs present in the 10 μl reverse transcription reaction were first amplified
30 simultaneously using all external primer pairs listed in the Key Ressources Table.
31 Taq polymerase and 20 pmol of each primer were added to the buffer supplied by the
32 manufacturer (final volume, 100 μl), and 20 cycles (94°C, 30 s; 60°C, 30 s; 72°C, 35
33 s) of PCR were run. Second rounds of PCR were performed using 1 μl of the first
34 PCR product as a template. In this second round, each cDNA was amplified

1 individually using its specific nested primer pair (Key Ressources Table) by
2 performing 35 PCR cycles (as described above). 10 μ l of each individual PCR
3 product were run on a 2 % agarose gel stained with ethidium bromide using Φ X174
4 digested by *Hae*III as a molecular weight marker.

5

6 **Perforated patch-clamp recording**

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

20

21 **NADH imaging**

22 Recordings were made in layer II-III of the rat somatosensory cortex. Wide-field
23 fluorescent images were obtained using a double port upright microscope BX51WI,
24 WI-DPMC, Olympus) with a 60x objective (LUMPlan FI /IR 60x/0.90 W, Olympus)
25 and a digital camera (CoolSnap HQ2, Roper Scientific) attached on the front port of
26 the microscope. NADH autofluorescence was obtained by 365 nm excitation with a
27 Light Emitting Device (LED, pE-2, CoolLED) using Imaging Workbench 6.0.25
28 software (INDEC Systems) and dichroic (FF395/495/610-Di01-25x36, Semrock) and
29 emission filters (FF01-425/527/685-25, Semrock). Infrared Dodt gradient contrast
30 images (IR-DGC, (Dodt and Zieglgansberger, 1998)) were obtained using a 780 nm
31 collimated LED (M780L3-C1, Thorlabs) as a transmitted light source and DGC optics
32 (Luigs and Neumann). Autofluorescence and IR-DGC images were collected every
33 10s by alternating the fluorescence and transmitted light sources. In parallel, infrared
34 transmitted light images of slices were also continuously monitored on the back-port

1 of the microscope using a customized beam splitter (725 DCSPXR, Semrock) and an
2 analogic CCD camera (XC ST-70 CE, Sony). The focal plane was maintained
3 constant on-line using infrared DGC images of cells as anatomical landmarks
4 (Lacroix et al., 2015).

5

6 **Subcloning and viral production**

7 The coding sequence of the ATP sensor ATeam1.03YEMK (Imamura et al., 2009)
8 was subcloned into the viral vector pSinRep5. Sindbis virus was produced as
9 previously described (Piquet et al., 2018). Recombinant pSinRep5 and helper
10 plasmid pDH26S (Invitrogen) were transcribed in vitro into capped RNA using the
11 Megascript SP6 kit (Ambion). Baby hamster kidney-21 cells (ATCC) were
12 electroporated with sensor-containing RNA and helper RNA ($2 \cdot 10^7$ cells, 950 μ F, 230
13 V) and incubated for 24 h at 37°C in 5% CO₂ in Dulbecco's modified Eagle Medium
14 supplemented with 5% fetal calf serum before collecting cell supernatant containing
15 the viruses. The virus titer (10^8 infectious particles/ml) was determined after counting
16 fluorescent baby hamster kidney cells infected using serial dilution of the stock virus.

17

18 **Brain slice viral transduction**

19 Brain slices were placed onto a millicell membrane (Millipore) with culture medium
20 (50% minimum essential medium, 50% Hank's balanced salt sodium, 6.5 g/l glucose
21 and 100 U/ml penicillin-streptomycin (Sigma-Aldrich) as previously described (Piquet
22 et al., 2018). Infection was performed by adding $\sim 5 \times 10^5$ particles per slice. Slices
23 were incubated overnight at 35°C in 5% CO₂. The next morning, brain slices were
24 equilibrated for 1h in aCSF containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2
25 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 10 glucose, 15 sucrose. Slices were then placed into
26 the recording chamber, heated at ~ 30 °C and continuously perfused at 1-2 ml/min.

27

28 **FRET imaging**

29 Recordings were made from visually identified pyramidal cells in layer II-III of the rat
30 somatosensory cortex. Wide-field fluorescent images were obtained using a 40x
31 objective and a digital camera attached on the front port of the microscope. The ATP
32 sensor ATeam1.03YEMK was excited at 400 nm with a LED using Imaging
33 Workbench 6.0.25 software and excitation (FF02-438/24-25, Semrock) and dichroic
34 filters (FF458-Di02-25x36, Semrock). Double fluorescence images were collected

1 every 15s by alternating the fluorescence emission filters for the CFP (FF01-483/32-
2 25, Semrock) and the YFP (FF01-542/27-25, Semrock) using a filter wheel (Lambda
3 10B, Sutter Instruments). The focal plane was maintained constant on-line as
4 described above.

5

6 **Pharmacological studies**

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

18

19 **Quantification and statistical analysis**

20 **Analysis of somatic features**

21 The laminar location determined by infrared videomicroscopy and recorded as 1-4
22 according to a location right within layers I, II/III or IV. For neurons located at the
23 border of layers I-II/III and II/III-IV, the laminar location was represented by 1.5 and
24 3.5, respectively. Somatic features were measured from IR DGC of the recorded
25 neurons. Briefly, the soma was manually delineated using Image-Pro Analyzer 7.0
26 software (MediaCybernetics) and length of major and minor axes, perimeter and area
27 were extracted. The soma elongation was calculated as the ratio between major and

28 minor axis. Roundness was calculated according to: $\frac{perimeter^2}{4\pi \times area}$; a value close to 1 is
29 indicative of round somata.

30

1 **Analysis of electrophysiological properties**

2 32 electrophysiological properties chosen to describe the electrophysiological
3 diversity of cortical neurons (Ascoli et al., 2008) were determined using the I-clamp
4 fast mode of the amplifier as previously described (Karagiannis et al., 2009).
5 Membrane potential values were corrected for theoretical liquid junction potential (-
6 15.6 mV). Resting membrane potential was measured just after passing in whole-cell
7 configuration, and only cells with a resting membrane potential more negative than -
8 55 mV were analyzed further. Membrane resistance (R_m) and membrane time
9 constant (τ_m) were determined on responses to hyperpolarizing current pulses
10 (duration, 800 ms) eliciting voltage shifts of 10-15 mV negative to rest (Kawaguchi,
11 1993;Kawaguchi, 1995). Time constant was determined by fitting this voltage
12 response to a single exponential. Membrane capacitance (C_m) was calculated
13 according to $C_m = \tau_m / R_m$. Sag index was quantified as a relative decrease in
14 membrane conductance according to $(G_{sag} - G_{hyp}) / G_{sag}$ (Halabisky et al., 2006) where
15 G_{hyp} and G_{sag} correspond to the whole-cell conductance when the sag was inactive
16 and active, respectively. G_{sag} was measured as the slope of the linear portion of a
17 current–voltage (I–V) plot, where V was determined at the end of 800 ms
18 hyperpolarizing current pulses (-100 to 0 pA) and G_{hyp} as the slope of the linear
19 portion of an I–V plot, where V was determined as the maximal negative potential
20 during the 800 ms hyperpolarizing pulses. Rheobase was quantified as the minimal
21 depolarizing current pulse intensity (800 ms duration pulses, 10 pA increments)
22 generating at least one action potential. First spike latency (Gupta et al., 2000;Ascoli
23 et al., 2008) was measured at rheobase as the time needed to elicit the first action
24 potential. To describe different firing behaviors near threshold, spike frequency was
25 measured near spike threshold on the first trace in which at least three spikes were
26 triggered. Instantaneous discharge frequencies were measured and fitted to a
27 straight line according to $F_{threshold} = m_{threshold} \cdot t + F_{min.}$, where $m_{threshold}$ is the slope
28 termed adaptation, t the time and $F_{min.}$, the minimal steady state frequency. Analysis
29 of the action potentials waveforms was done on the first two spikes. Their amplitude
30 (A1 and A2) was measured from threshold to the positive peak of the spike. Their
31 duration (D1 and D2) was measured at half amplitude (Kawaguchi, 1993;Cauli et al.,
32 1997). Their amplitude reduction and the duration increase were calculated
33 according to $(A1 - A2) / A1$ and $(D2 - D1) / D1$, respectively (Cauli et al., 1997;Cauli et al.,
34 2000). The amplitude and the latency of the fast and medium afterhyperpolarization

1 (fAH and mAH) were measured for the first two action potentials as the difference
2 between spike threshold and the negative peak of the AHs (Kawaguchi, 1993). The
3 amplitude and latency of afterdepolarization (AD) following single spikes (Haj-
4 Dahmane and Andrade, 1997) were measured as the difference between the
5 negative peak of the fAH and the peak of the AD and between the spike threshold
6 and the peak of the AD, respectively. When neurons did not exhibit mAH or AD,
7 amplitude and latency were arbitrarily set to 0. A complex spike amplitude
8 accommodation during a train of action potentials, consisting in a transient decrease
9 of spikes amplitude, was measured as the difference between the peak of the
10 smallest action potential and the peak of the following largest action potential (Cauli
11 et al., 2000). Maximal firing rate was defined as the last trace before prominent
12 reduction of action potentials amplitude indicative of a saturated discharge. To take
13 into account the biphasic spike frequency adaptation (early and late) occurring at
14 high firing rates (Cauli et al., 1997;Cauli et al., 2000;Gallopín et al., 2006),
15 instantaneous firing frequency was fitted to a single exponential (Halabisky et al.,
16 2006) with a sloping baseline, according to : $F_{Saturation} = A_{sat} \cdot e^{-t/\tau_{sat}} + t \cdot m_{sat} + F_{max}$, where
17 A_{sat} corresponds to the amplitude of early frequency adaptation, τ_{sat} to the time
18 constant of early adaptation, m_{sat} to the slope of late adaptation and F_{max} to the
19 maximal steady state frequency.

20

21 **Unsupervised clustering**

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

32

1 **Analysis of voltage clamp recordings**

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

16 During ATP washout experiments, whole-cell currents and I/V relationships were
17 measured every 10 s at a holding potential of -50 mV and during voltage ramps from
18 -40 to -120 mV, respectively. Washout currents were determined by subtracting the
19 whole-cell currents measured at the beginning and the end of the whole cell-
20 recording, respectively.

21 **Analysis of current clamp recordings**

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

30 **Analysis of perforated patch recordings**

31
32 Mean firing frequency was measured every 10 s. Quantification of spiking activity
33 was determined by averaging firing frequency over a period of 5 min preceding a
34

1 change in extracellular aCSF composition. Firing frequencies were normalized by the
2 averaged mean firing frequency measured under control condition.

3

4 **NADH imaging**

5 Shading correction was applied off-line on the NADH autofluorescence images using
6 the "Shading Corrector" plugin of FIJI software (Schindelin et al., 2012) and a blank
7 field reference image. To compensate for potential x-y drifts all IR-DGC images were
8 realigned off-line using the "StackReg" and "TurboReg" plugins (Thevenaz et al.,
9 1998) of FIJI software and the same registration was applied to the corrected
10 NAD(P)H autofluorescence images. To determine somatic regions of interest (ROIs)
11 the soma was manually delineated on IR-DGC images. The mean NADH
12 autofluorescence was measured at each time point using the same ROIs. Variations
13 of fluorescence intensity were expressed as the ratio $(F-F_0)/F_0$ where F corresponds
14 to the mean fluorescence intensity in the ROI at a given time point, and F₀
15 corresponds to the mean fluorescence intensity in the same ROI during the 5 min
16 control baseline prior to changes in aCSF composition. Effect of monocarboxylate
17 superfusion or oxidative phosphorylation blockade was quantified by averaging the
18 normalized ratio (R/R_0) during the last five minutes of drug application.

19

20 **FRET imaging**

21 All images were realigned off-line as described above using the YFP images as the
22 reference for registration. Fluorescence ratios were calculated by dividing the
23 registered YFP images by the registered CFP images using FIJI. The somatic ROIs
24 were manually delineated on the YFP images as described above. The mean ratio
25 was measured at each time point using the same ROIs. Variations of fluorescence
26 ratio were expressed as the ratio $(R-R_0)/R_0$ where R corresponds to the
27 fluorescence ratio in the ROI at a given time point, and R₀ corresponds to the mean
28 fluorescence ratio in the same ROI during the 10 min control baseline prior to drug
29 application. Effect of glycolysis or oxidative phosphorylation blockade was quantified
30 by averaging the normalized ratio during the last five minutes of drug application.

31

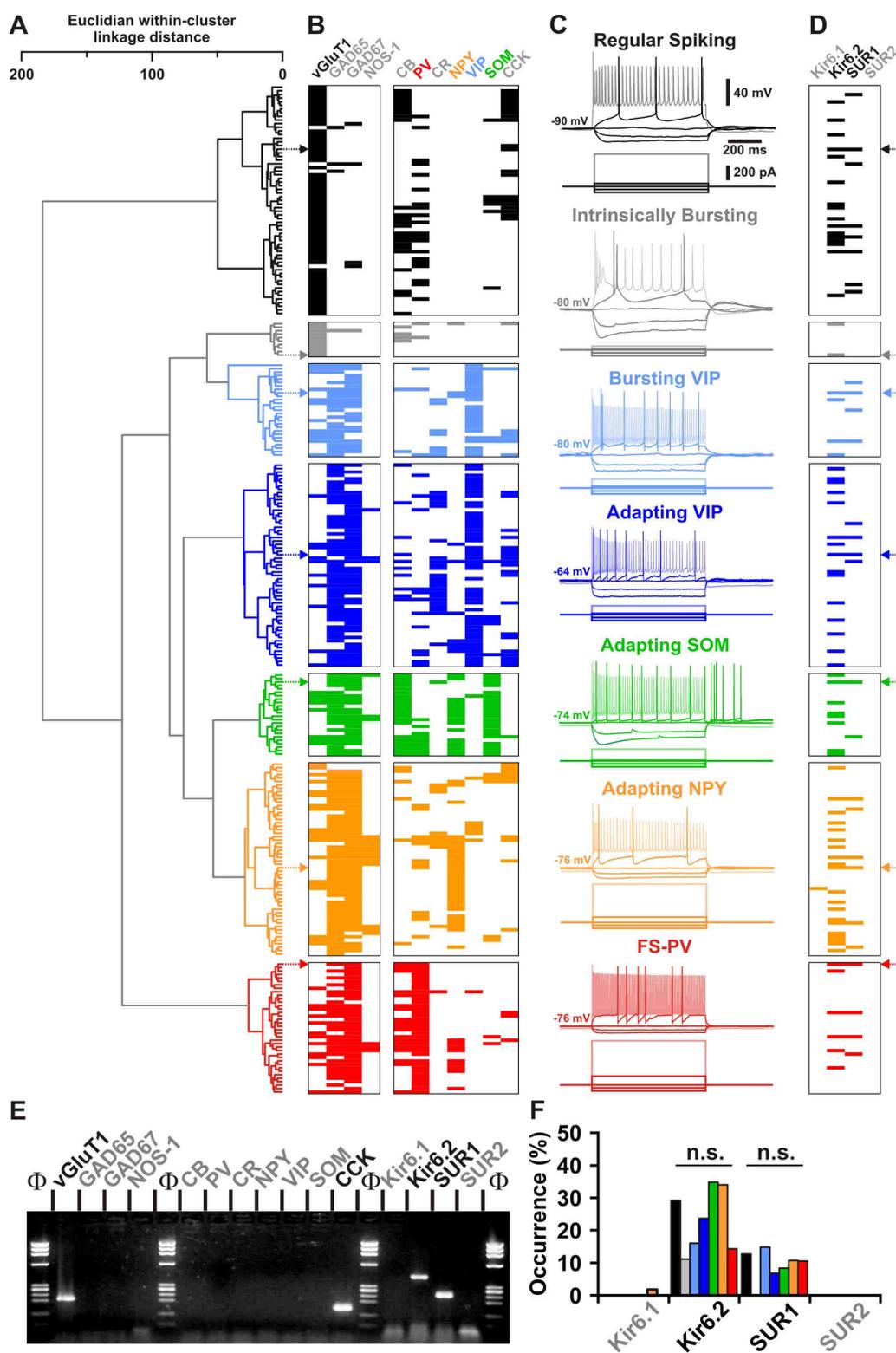
1 **Statistical analysis**

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

9 Statistical significance of morphological and electrophysiological properties of
10 neurons was determined using the Mann-Whitney U test. Comparison of the
11 occurrence of expressed genes and of responsiveness of K_{ATP} channel modulators
12 between different cell types was determined using Fisher's exact test. Statistical
13 significance of the effects of K_{ATP} channel modulators was determined using the
14 Friedman and post hoc Dunn's tests. Significance of the effect of the ROS scavenger
15 was determined using one-tailed unpaired student t-test. Comparison of K_{ATP} channel
16 properties was determined using Mann-Whitney U, Student-t, or Kruskal-Wallis H
17 tests. Comparison of responses between Kir6.2^{+/+} and Kir6.2^{-/-} neurons was
18 determined using Mann-Whitney U test. Statistical significance of the effects of
19 energy substrates and drug applications on evoked firing in perforated patch
20 recordings was determined using Friedman and Dunn's tests. Comparison of the
21 effects of monocarboxylates and cyanide on NADH fluorescence was determined
22 using Mann-Whitney U test. Statistical significance of the effects of metabolic
23 inhibitors on intracellular ATP was determined using Friedman and Dunn's tests.

1 **Figure legends**

2 **Figure 1. Cortical neuron subtypes express Kir6.2 and SUR1 K_{ATP} channel**
 3 **subunits.**

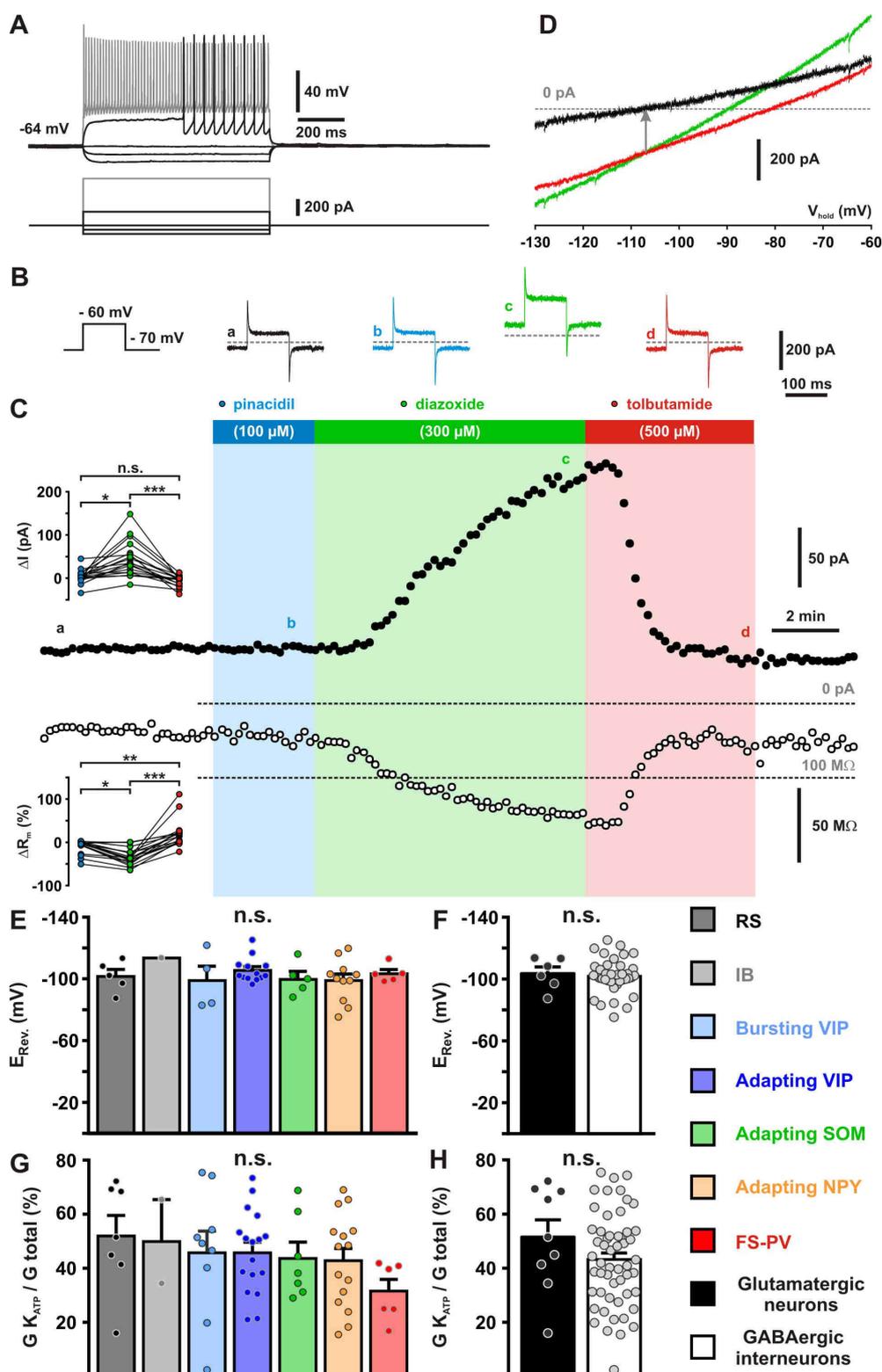


4

5

- 1 (A) Ward's clustering of 277 cortical neurons (left panel). The x axis represents the
- 2 average within-cluster linkage distance, and the y axis the individuals.
- 3 (B) Heatmap of gene expression across the different cell clusters. For each cell,
- 4 colored and white rectangles indicate presence and absence of genes, respectively.
- 5 (C) Representative voltage responses induced by injection of current pulses (bottom
- 6 traces) corresponding to -100, -50 and 0 pA, rheobase and intensity inducing a
- 7 saturating firing frequency (shaded traces) of a Regular Spiking neuron (black), an
- 8 Intrinsically Bursting neuron (gray), a Bursting VIP interneuron (light blue), an
- 9 Adapting VIP interneuron (blue), an Adapting SOM interneuron (green), an Adapting
- 10 NPY interneuron (orange), and a Fast Spiking-Parvalbumin interneuron (FS-PV, red).
- 11 The colored arrows indicate the expression profiles of neurons whose firing pattern is
- 12 illustrated in (C).
- 13 (D) Heatmap of the expression of the subunits of the K_{ATP} channels in the different
- 14 clusters.
- 15 (E) scRT-PCR analysis of the RS neuron depicted in (A-D).
- 16 (F) Histograms summarizing the expression profile of K_{ATP} channel subunits in
- 17 identified neuronal types. n.s. not statistically significant.
- 18

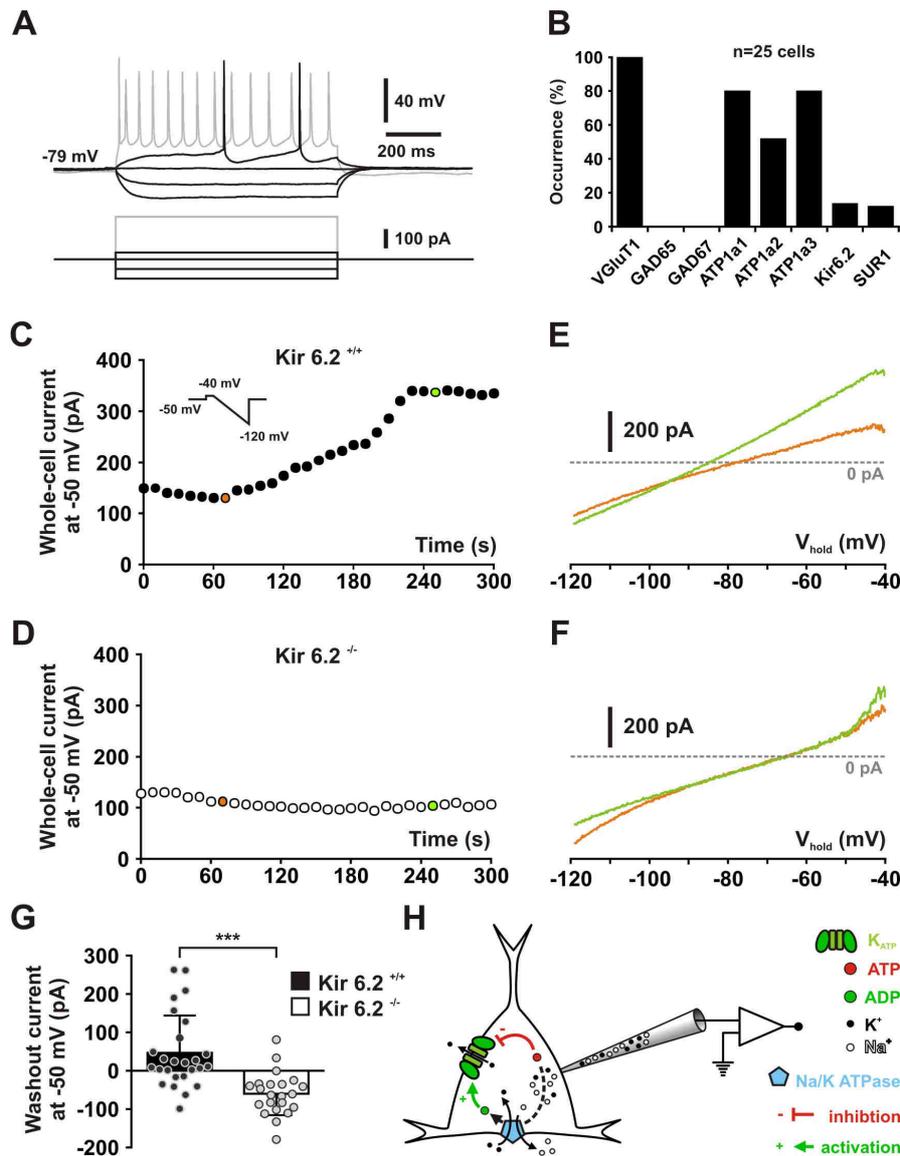
1 **Figure 2. Pharmacological and biophysical characterization of K_{ATP} channels in**
 2 **cortical neurons.**



3
 4
 5

1 (A) Representative voltage responses of a FS-PV interneuron induced by injection of
2 current pulses (bottom traces).
3 (B) Protocol of voltage pulses from -70 to -60 mV (left trace). Responses of whole-
4 cell currents in the FS-PV interneurons shown in (A) in control condition (black) and
5 in presence of pinacidil (blue), piazoxide (green) and tolbutamide (red) at the time
6 indicated by a-d in (C).
7 (C) Stationary currents recorded at -60 mV (filled circles) and membrane resistance
8 (open circles) changes induced by K_{ATP} channel modulators. The colored bars and
9 shaded zones indicate the duration of application of K_{ATP} channel modulators. Upper
10 and lower insets: changes in whole-cell currents and relative changes in membrane
11 resistance induced by K_{ATP} channel modulators, respectively.
12 (D) Whole cell current-voltage relationships measured under diazoxide (green trace)
13 and tolbutamide (red trace). K_{ATP} I/V curve (black trace) obtained by subtracting the
14 curve under diazoxide by the curve under tolbutamide. The arrow indicates the
15 reversal potential of K_{ATP} currents.
16 (E-H) Histograms summarizing the K_{ATP} current reversal potential (E,F) and relative
17 K_{ATP} conductance (G,H) in identified neuronal subtypes (E,G) or between
18 glutamatergic and GABAergic neurons (F,G). Data are expressed as mean \pm s.e.m.,
19 and the individual data points are depicted. n.s. not statistically significant.

1 **Figure 3. Kir6.2 is the pore forming subunit of K_{ATP} channels in cortical**
 2 **neurons.**



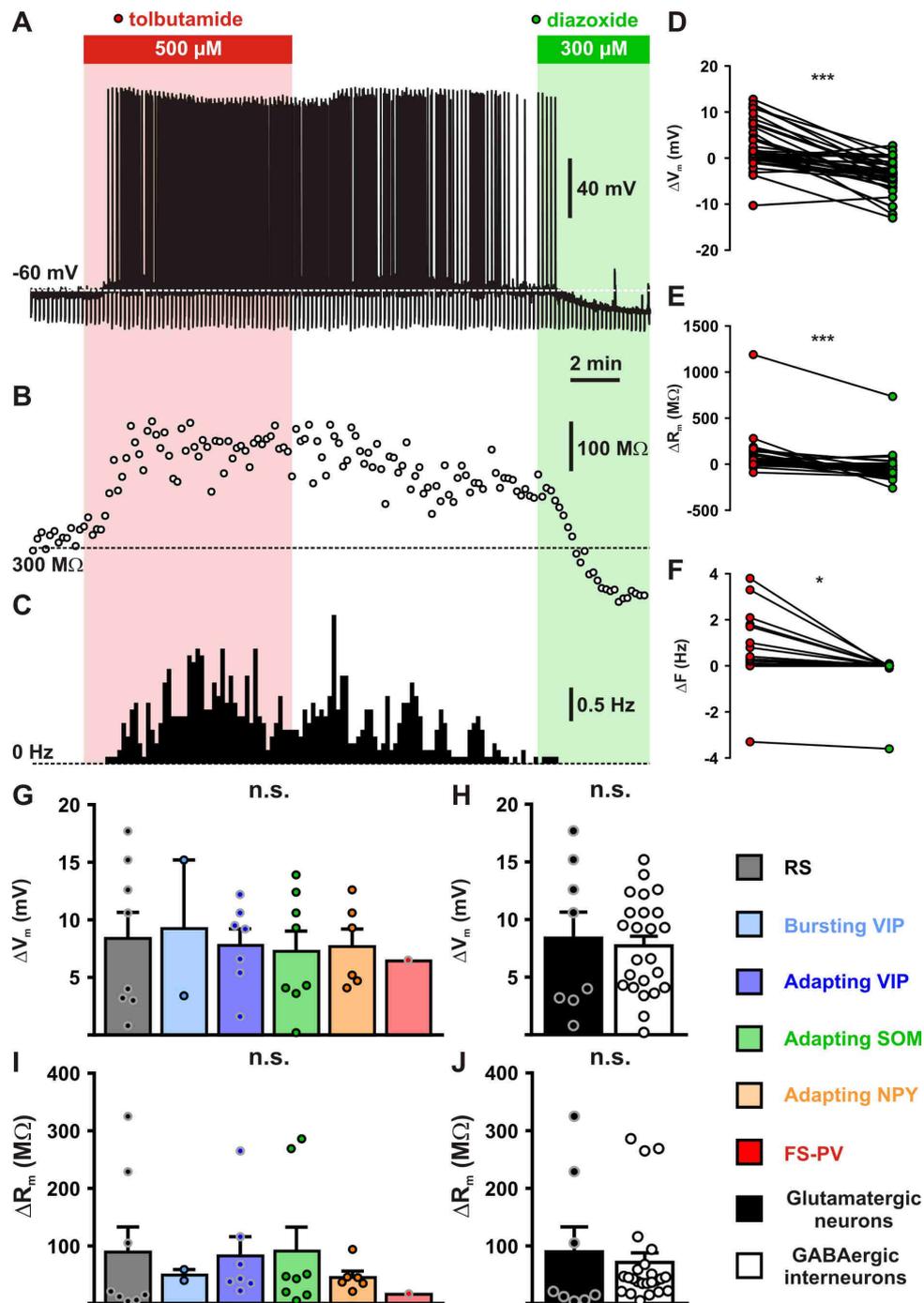
3
 4
 5 (A) Representative voltage responses of a mouse layer II/III RS pyramidal cell
 6 induced by injection of current pulses (bottom traces).

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

10 (C, D) Whole-cell stationary currents recorded at -50 mV during dialysis with ATP-
 11 free pipette solution in RS neurons of Kir6.2^{+/+} (C) and Kir6.2^{-/-} (D) mice. Inset;
 12 voltage clamp protocol.

- 1 (E, F) Current-voltage relationships obtained during ATP washout at the time
- 2 indicated by green and orange circles in (C, D) in RS neurons of Kir6.2^{+/+} (E) and
- 3 Kir6.2^{-/-} (F) mice.
- 4 (G) Histograms summarizing the whole-cell ATP washout currents in Kir6.2^{+/+} (black)
- 5 and Kir6.2^{-/-} (white) RS neurons. Data are expressed as mean \pm s.e.m., and the
- 6 individual data points are depicted.
- 7 (H) Diagram depicting the principle of the ATP washout experiment.
- 8

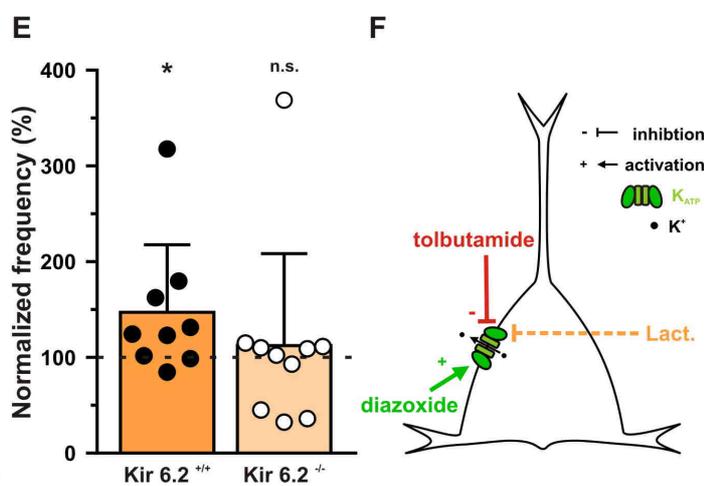
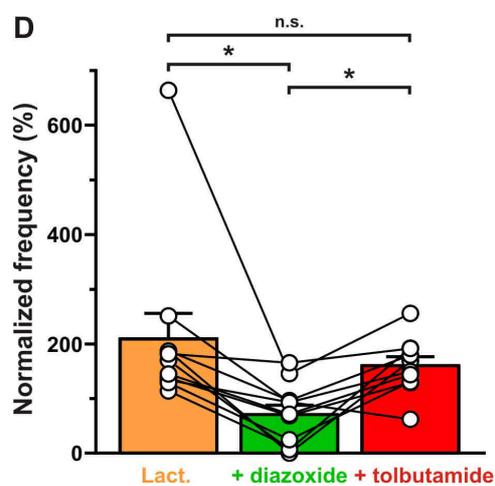
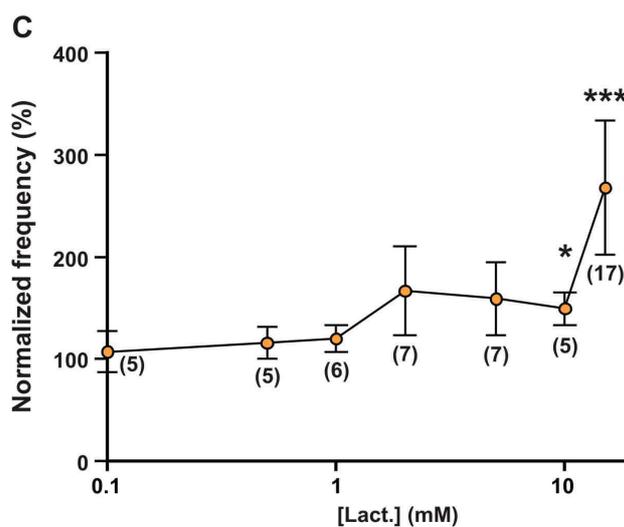
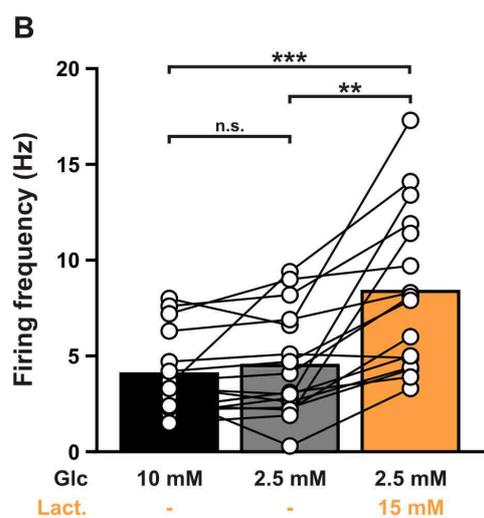
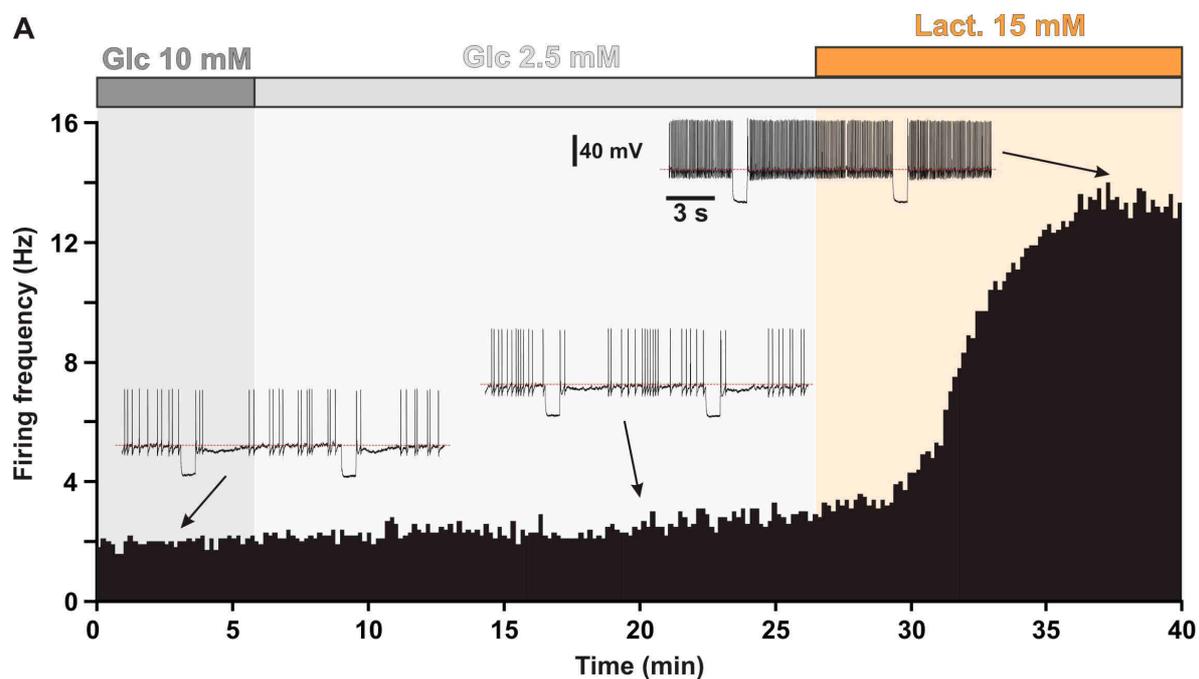
1 **Figure 4. Modulation of cortical neuronal excitability and activity by K_{ATP}**
 2 **channels.**



3
 4
 5 (A-C) Representative example showing the changes in membrane potential (A),
 6 resistance (B, open circles) and spiking activity (C) induced by application of
 7 tolbutamide (red) and diazoxide (green). The colored bars and shaded zones indicate
 8 the application duration of K_{ATP} channel modulators.

1 (D-F) Relative changes in membrane potential (D), resistance (E) and firing rate (F)
2 induced by tolbutamide and diazoxide in cortical neurons.
3 (G-J) Histograms summarizing the modulation of membrane potential (G,
4 $H_{(5,32)}=0.14854$, $p=0.999$, and H, $U_{(8,24)}=95.5$, $p=0.991$) and resistance (I,
5 $H_{(5,32)}=3.00656$, $p=0.699$, and J, $U_{(8,24)}=73$, $p=0.329$) by K_{ATP} channels in neuronal
6 subtypes (G, I) and groups (H, J). Data are expressed as mean \pm s.e.m., and the
7 individual data points are depicted. n.s. not statistically significant.
8

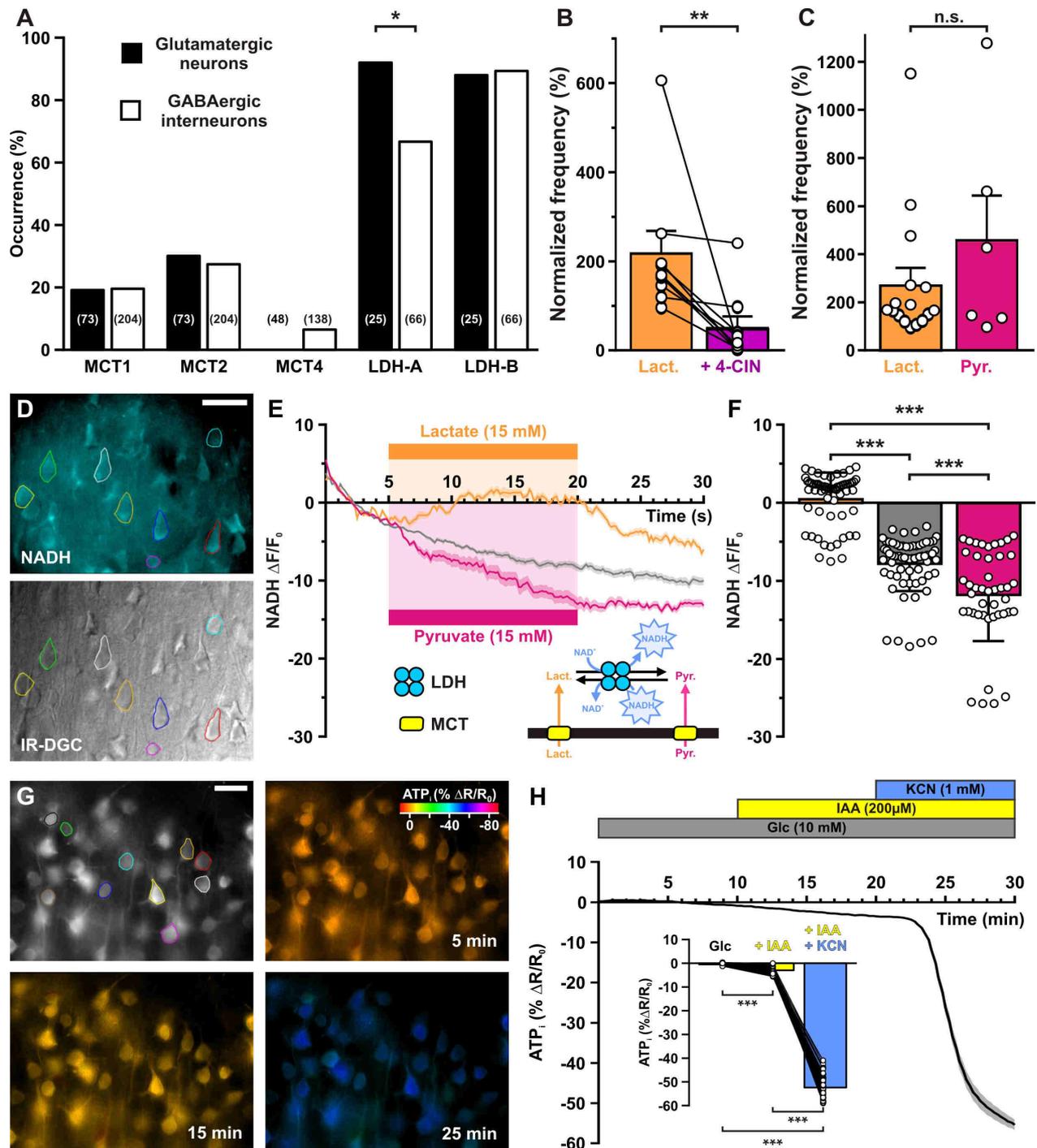
1 **Figure 5. Lactate enhances cortical neuronal activity via K_{ATP} channel**
 2 **modulation.**



3
4

- 1 (A) Representative perforated patch recording of an adapting VIP neuron showing
2 the modulation of firing frequency induced by changes in the extracellular
3 concentrations of metabolites. The colored bars and shaded zones indicate the
4 concentration in glucose (grey) and lactate (orange). Voltage responses recorded at
5 the time indicated by arrows. The red dashed lines indicate -40 mV.
- 6 (B) Histograms summarizing the mean firing frequency during changes in
7 extracellular concentration of glucose (black and grey) and lactate (orange). Data are
8 expressed as mean \pm s.e.m., and the individual data points are depicted. n.s. not
9 statistically significant.
- 10 (C) Dose-dependent enhancement of firing frequency by lactate. Data are normalized
11 by the mean firing frequency in absence of lactate and are expressed as mean \pm
12 s.e.m. Numbers in brackets indicate the number of recorded neurons at different
13 lactate concentrations.
- 14 (D) Histograms summarizing the normalized frequency under 15 mM lactate (orange)
15 and its modulation by addition of diazoxide (green) or tolbutamide (red). Data are
16 expressed as mean \pm s.e.m., and the individual data points are depicted. n.s. not
17 statistically significant.
- 18 (E) Histograms summarizing the enhancement of normalized frequency by 15 mM
19 lactate in Kir6.2^{+/+} (orange) and Kir6.2^{-/-} (pale orange) mouse cortical neurons. The
20 dash line indicates the normalized mean firing frequency in absence of lactate. Data
21 are expressed as mean \pm s.e.m., and the individual data points are depicted.
- 22 (F) Diagram depicting the enhancement of neuronal activity by lactate via modulation
23 of K_{ATP} channels.

1 **Figure 6. Lactate enhancement of cortical neuronal activity involves lactate**
 2 **uptake and metabolism.**



3
 4
 5 (A) Histograms summarizing the expression profile of the monocarboxylate
 6 transporters MCT1, 2 and 4 and LDH A and B lactate dehydrogenase subunits in
 7 glutamatergic neurons (black) and GABAergic interneurons (white). The numbers in
 8 brackets indicate the number of analyzed cells.

1 (B) Histograms summarizing the enhancement of normalized frequency by 15 mM
2 lactate (orange) and its suppression by the MCTs inhibitor 4-CIN (purple). Data are
3 expressed as mean \pm s.e.m., and the individual data points are depicted.

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

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

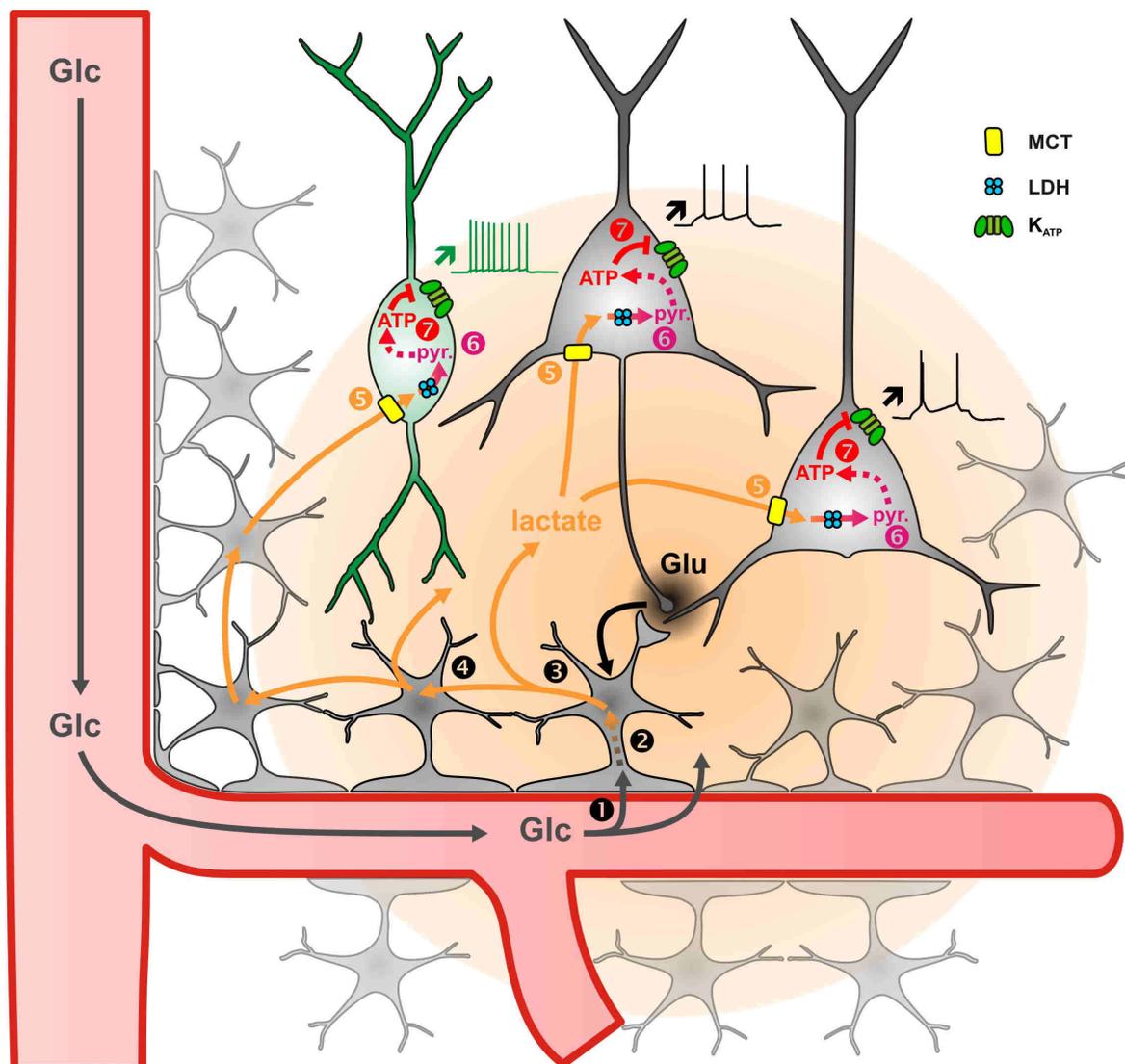
10 (E) Mean relative changes in NADH autofluorescence in control condition (grey) and
11 in response to 15 mM lactate (orange) or pyruvate (magenta). The colored bars
12 indicate the duration of applications. Data are expressed as mean \pm s.e.m. Inset:
13 diagram depicting the NADH changes induced by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

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

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

25 (H) Mean relative changes in intracellular ATP (relative YFP/CFP ratio) measured
26 under 10 mM extracellular glucose (grey) and after addition of IAA (yellow) and KCN
27 (blue). Data are expressed as mean \pm s.e.m. The colored bars indicate the time and
28 duration of metabolic inhibitor application. Inset: Histograms summarizing the mean
29 relative changes in intracellular ATP (relative YFP/CFP ratio) ratio under 10 mM
30 extracellular glucose (grey) and after addition of IAA (yellow) and KCN (blue). Data
31 are expressed as mean \pm s.e.m., and the individual data points are depicted.

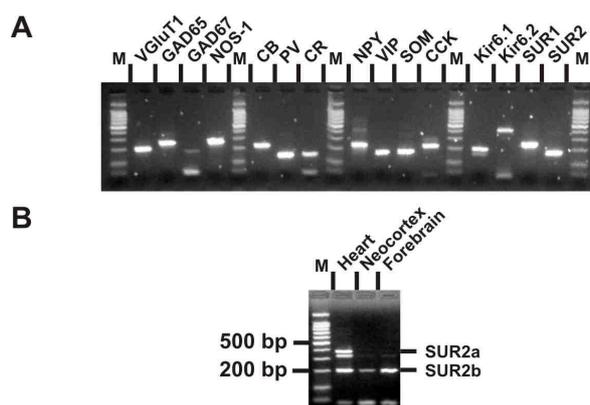
1 **Figure 7. Diagram summarizing the mechanism of lactate-sensing in the**
 2 **cortical network.**



3
 4 Glutamate (Glu) released during synaptic transmission stimulates **1** blood glucose
 5 (Glc) uptake in astrocytes, **2** aerobic glycolysis, **3** lactate release and **4** diffusion
 6 through the astrocytic network. Lactate is then **5** taken up by neurons via
 7 monobarboxylate transporters (MCT) and **6** oxidized into pyruvate by lactate
 8 dehydrogenase (LDH). The ATP produced by pyruvate oxidative metabolism **7**
 9 closes K_{ATP} channels and increases the spiking activity of both pyramidal cells (black)
 10 and inhibitory interneurons (green). The color gradient of the circles represents the
 11 extent of glutamate (black) and lactate (orange) diffusion, respectively. Dashed
 12 arrows indicate multisteps reactions.

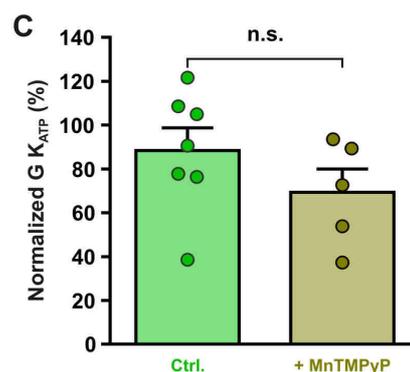
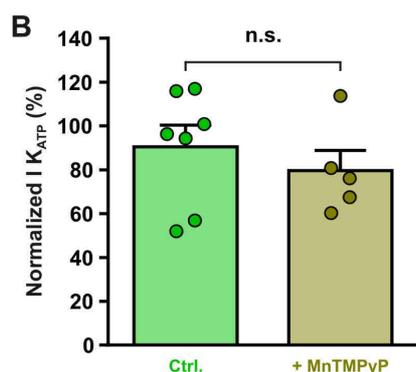
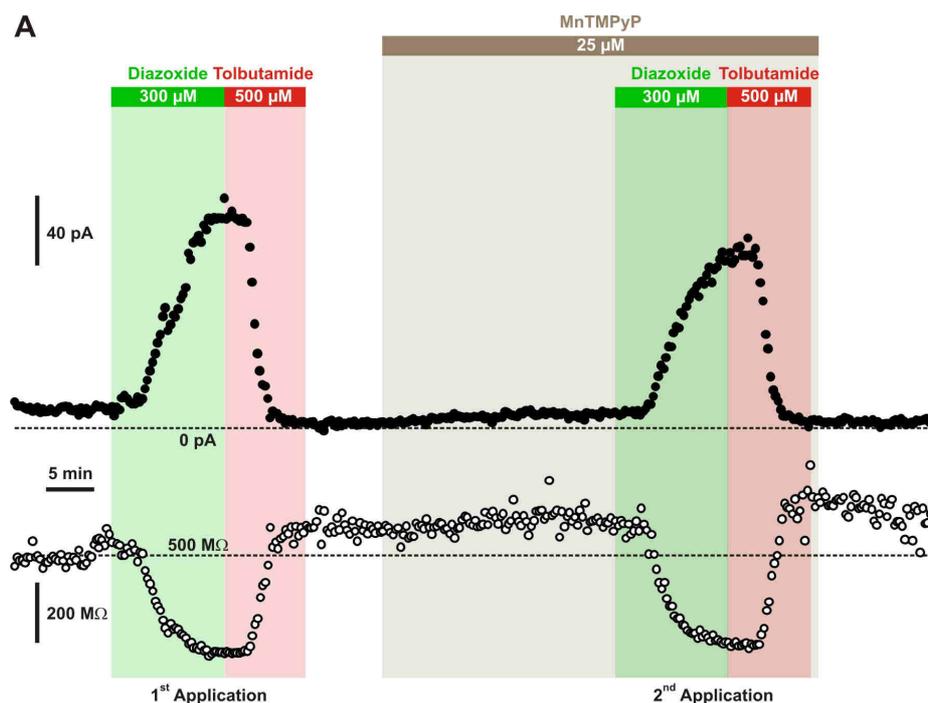
13

1 **Figure S1. Molecular expression of K_{ATP} channels.**



- 2
3
4 (A) RT-PCR products generated from 500 pg of total cortical RNAs. M: 100 bp ladder
5 molecular weight marker.
6 (B) SUR2 splice variants-specific RT-PCR analysis of 1 ng total RNAs from rat heart,
7 neocortex and forebrain.
8

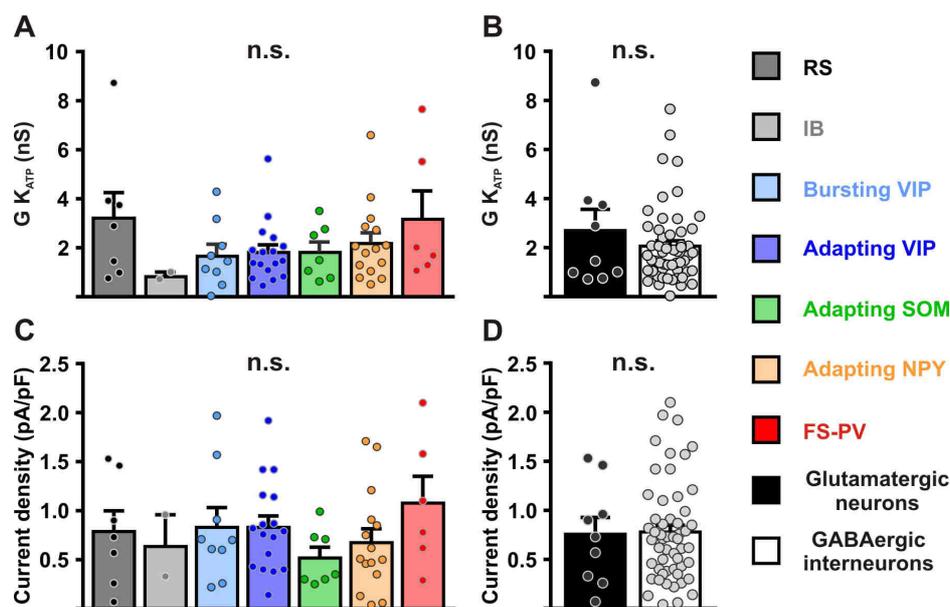
1 Figure S2. Diazoxide-induced current is independent of ROS production.



2
3 (A) Representative stationary currents at -60 mV (filled circles) and membrane
4 resistance (open circles) changes induced by diazoxide and tolbutamide under
5 control condition and in presence of the superoxide dismutase and catalase mimetic,
6 MnTMPyP. The colored bars and shaded zones indicate the duration of application.
7 (B-C) Histograms summarizing the relative K_{ATP} currents (B) and relative whole-cell
8 K_{ATP} conductance (C) evoked by two consecutive diazoxide and tolbutamide
9 applications in control condition (Ctrl.) and after the presence of MnTMPyP. Data are
10 normalized by the data measured during first application, expressed as mean \pm
11 s.e.m., and the individual data points are depicted. n.s. not statistically significant.

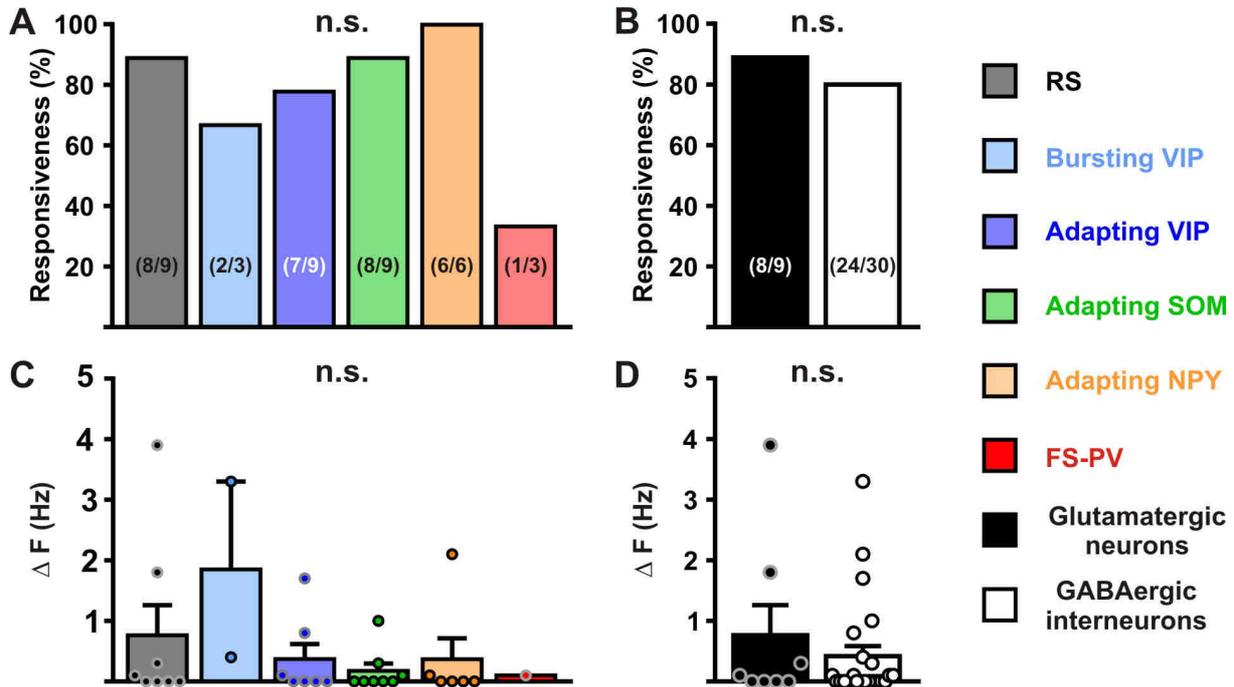
12

1 **Figure S3. Characterization of K_{ATP} channels in different cortical neurons.**



2
3 (A-D) Histograms summarizing the whole-cell K_{ATP} conductance (A, B) and K_{ATP}
4 current density (C, D) and K_{ATP} current reversal potential in identified neuronal
5 subtypes (A,C) or between glutamatergic and GABAergic neurons (B,D). Data are
6 expressed as mean \pm s.e.m., and the individual data points are depicted. n.s. not
7 statistically significant.

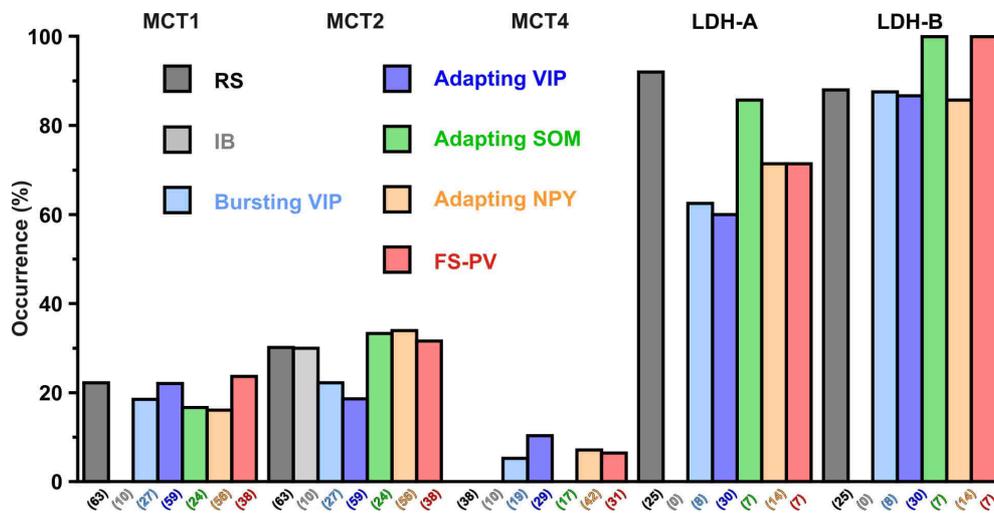
1 **Figure S4. Modulation of neuronal activity in different cortical neurons by K_{ATP}**
 2 **channels.**



3
 4
 5 (A-D) Histograms summarizing the proportion of responsive neurons (A,
 6 $K^2_{(5)}=7.3125$, $p=0.1984$, and B, $p=0.9999$) and modulation firing rate (C,
 7 $H_{(5,32)}=5.69107$, $p=0.337$, and D, $U_{(8,24)}=87.5$, $p=0.6994$) by K_{ATP} channels in
 8 neuronal subtypes (A,C) and groups (B,D). The numbers in brackets indicate the
 9 number of responsive cells and analyzed cells, respectively. Data are expressed as
 10 mean \pm s.e.m., and the individual data points are depicted. n.s. not statistically
 11 significant.

1 **Figure S5. Expression profile of monocarboxylate transporters and lactate**
 2 **dehydrogenase subunits in different cortical neuronal types.**

3



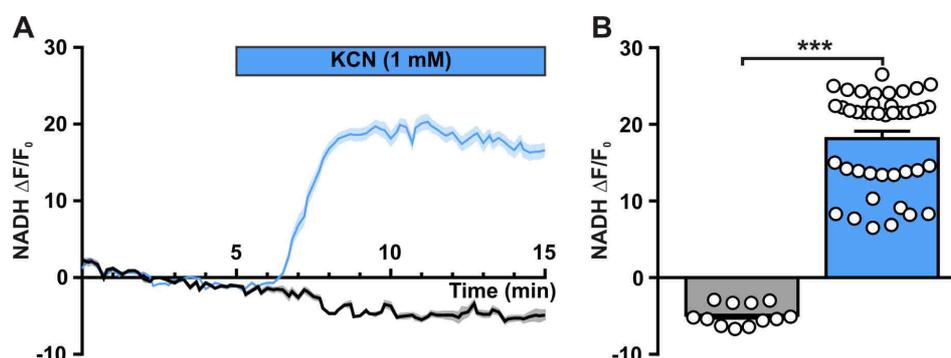
4

5

6 Histograms summarizing the expression profile of the monocarboxylate transporters
 7 MCT1, 2 and 4 and LDH A and B lactate dehydrogenase subunits in different
 8 neuronal subtypes. The numbers in brackets indicate the number of analyzed cells.

9

1 **Figure S6. Neuronal NADH autofluorescence increase by blockade of oxidative**
2 **phosphorylation.**



- 3
4
5 (A) Mean relative changes in NADH autofluorescence in control condition (grey) and
6 in response to 1 mM KCN (blue). The colored bar indicates the duration of KCN
7 applications. Data are expressed as mean \pm s.e.m.
8 (B) Histograms summarizing the mean relative changes in NADH autofluorescence
9 measured during the last 5 minutes of 1 mM KCN application (blue) and
10 corresponding time in control condition (grey). Data are expressed as mean \pm s.e.m.,
11 and the individual data points are depicted.

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