Cortical energy demands of signaling and nonsignaling components in brain are conserved across mammalian species and activity levels

Fahmeed Hydera,b,c,1, Douglas L. Rothmana,b,c, and Maxwell R. Bennettd

*Magnetic Resonance Research Center (MRRC) and Departments of aDiagnostic Radiology and bBiomedical Engineering, Yale University, New Haven, CT 06520; and cBrain and Mind Research Institute, University of Sydney, Sydney, NSW 2050, Australia

Edited* by Robert G. Shulman, Yale University School of Medicine, New Haven, CT, and approved November 29, 2012 (received for review August 28, 2012)

The continuous need for ion gradient restoration across the cell membrane, a prerequisite for synaptic transmission and conduction, is believed to be a major factor for brain’s high oxidative demand. However, do energy requirements of signaling and nonsignaling components of cortical neurons and astrocytes vary with activity levels and across species? We derived oxidative ATP demand associated with signaling (Ps) and nonsignaling (Pns) components in the cerebral cortex using species-specific physiologic and anatomic data. In rat, we calculated glucose oxidation rates from layer-specific neuronal activity measured across different states, spanning from isoelectricity to awake and sensory stimulation. We then compared these calculated glucose oxidation rates with measured glucose metabolic data for the same states as reported by 2-deoxy-glucose autoradiography. Fixed values for Ps and Pns were able to predict the entire range of states in the rat. We then calculated glucose oxidation rates from human EEG data acquired under various conditions using fixed Ps and Pns values derived for the rat. These calculated metabolic data in human cerebral cortex compared well with glucose metabolism measured by PET. Independent of species, linear relationship was established between neuronal activity and neuronal oxidative demand beyond isoelectricity. Cortical signaling requirements dominated energy demand in the awake state, whereas nonsignaling requirements were ~20% of awake value. These predictions are supported by 13C magnetic resonance spectroscopy results. We conclude that mitochondrial energy support for signaling and nonsignaling components in cerebral cortex are conserved across activity levels in mammalian species.

Calculations

Energetics of Signaling and Nonsignaling Components. We did not sum bottom-up contributions to total oxidative ATP demand as previously done in other budgets (7, 8) but rather, tested whether unchanging values of Ps and Pns can account for measured in vivo results over a range of cortical activity levels (SI Text, section 1 and Tables S1 and S2). Although the Es term in Eq. 1 encompasses function of both neurons and astrocytes, we base our budget to neuronal activity data due to limited ability to quantify astrocytic signaling. Given that neuronal firing is statistically representative of neuropil activity (12), we used spike rate as a quantitative measure of cortical function as has been done before (7, 8). In Eq. 1, <f> is given in units of spikes/neuron/s per centimeter² and ηN and ηA are given in units of cells/cm². Tables S1 and S2 list values of neuronal activity and glucose consumption in rat and human, respectively (SI Text, section 1). Recordings of cortical signaling were reflected by layer-specific microelectrodes in rats and EEG in humans, whereas metabolic spike rate | field potentials | glutamate | functional MRI | bispectral index

The brain is one of the most energy demanding tissues in the body (1). 13C magnetic resonance spectroscopy (MRS) in the rat has shown that, in the resting awake state, ~80% of cortical energy consumption is used to support signaling as reflected by the rate of glutamate neurotransmitter release and astroglial uptake (2, 3). Cerebral energy demand is also positively correlated with the rate of pyramidal neuron firing in rat cortex (4, 5). 13C MRS findings in the human cortex have been generally consistent with the rat results (6). However, there remain questions as to how well the energy costs of specific subcellular processes needed to support synaptic transmission and conduction are conserved over different activity levels and/or across species.

Recent bottom-up energy budgets for gray matter in the mammalian brain have attempted to understand the energetic costs of neuronal and glial electrical and neurotransmission events occurring in the neuropil (7, 8) by calculating the ATP used per neuron for signaling (Ps) and nonsignaling (Pns) events. In the awake cortex, the total ATP used per cortical volume per unit time (Ef; in units of ATP/s per centimeter³) was determined by multiplying the Ps (in units of ATP/neuron per spike) and Pns (in units of ATP/neuron per second) parameters with cellular densities (n) and average cortical firing rates (<f>) to give signaling (Es) and nonsignaling (Ens) components,

\[
E_{\text{tot}} = E_s + E_{\text{ns}} = \sum P_s(f) + \sum P_{\text{ns}} n f,
\]

where the summation spans for neurons and astrocytes. These studies agreed with in vivo measurements in that the majority of brain energy consumption was used to support signaling and also concluded that both signaling and nonsignaling events in human were about two to three times more costly on a per neuron basis than in the rat. However, these budgets have limitations (7, 8); most notably, the histological and biophysical parameters used disagree with recent values, heterogeneity across cortical lamina was disregarded (9–11), and the comparison was made with only one the resting awake state. Here, we use a top-down as opposed to a bottom-up approach to assess the fundamental links between electrical and chemical events at the neuropil. We tested the hypotheses that the energy requirements per cell for signaling (Ps) and nonsignaling (Pns) are independent of the state of neuronal activity (e.g., sensory activation, awake, asleep, or anesthetized) and are conserved across species (i.e., rat and human). We used layer-specific morphologic, neurophysiologic, and metabolic data in rat brain to calculate Ps and Pns, which were then tested on human data. In contrast to views highlighting differences between rat and human brain neurometabolic couplings (7, 8), our results suggest that, on a per cellular basis, the mitochondrial energy support for mammalian cortical functions during signaling and nonsignaling are conserved.

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1To whom correspondence should be addressed. E-mail: fahmeed.hyder@yale.edu.

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measurements were made by 2-deoxyglucose (2DG) autoradiography in rats and fluoro-deoxyglucose PET in humans.

Energetics of Signaling from Layer-Specific Neuronal Recordings in the Rat Somatosensory Cortex. Cortical signaling involves events like dendritic depolarization, axonal propagation, vesicular endocytosis and exocytosis, neurotransmitter cycling, ionotropic and metabotropic receptor activity, etc. To determine $E_{\text{tot}}$ in Eq. 1, we first multiplied the spike rate of the $i$th cortical layer ($f_i$ in units of spike/s) by the cortical density in the same layer ($\eta_{i,j}$ in units of neurons/cm$^3$) to obtain the number of firing events per unit volume ($f_i \eta_{i,j}$ in units of spike.neuron/s per centimeter$^3$). Next, we estimated the fraction of the $i$th cortical layer in relation to the entire cortical thickness ($\delta_i/\sum \delta_j$). On multiplying, these two terms and summing across all layers gives $\langle f \rangle$ in Eq. 1 (in units of spike/neuron/s per centimeter$^3$):

$$\langle f \rangle = \sum (\delta_i f_i \eta_{i,j}) / \sum \delta_j.$$  \[2\]

Using values of $\delta$, $\eta$, and $f$ from recent studies, we then determined if Eq. 1 was able to fit results from experimentally measured regional metabolism across different activity levels.

Energetics of Nonsignaling Events from Isoelectric Condition in the Rat Somatosensory Cortex. We assigned the nonsignaling energy primarily to ion movement associated with maintaining neuronal and glial resting potentials described by Eq. 1. To separately calculate $P_{\text{n.s.},A}$ and $P_{\text{n.s.},N}$, we needed input resistances of neurons ($R_{i,n,N}$) and astrocytes ($R_{i,n,A}$), which describe the energy demand of leaky cell membranes at rest (SI Text, section 2, and Fig. S1) and the average neuronal ($\eta_{i,n}$) and astrocytic ($\eta_{i,a}$) densities in the cerebral cortex (Table 1). Because recent $R_{i,n,x}$ and $\eta_{i,n}$ measurements in the rat show that values for neurons and astrocytes are quite similar (13–16), the calculations were slightly simplified by assuming that $R_{i,n,N} \approx R_{i,n,A}$ and $\eta_{i,n} \approx \eta_{i,a}$ (Table 1 and SI Text, section 2). To determine $P_{\text{n.s.}}$, we needed the metabolic demand for nonsignaling, which was possible for the rat, because state of deep pentobarbital anesthesia in Table S1 induces an isoelectric condition and thus, just contains the $E_{\text{n.s.}}$ term. Because neuronal recordings do not show any significant cortical activity in the pentobarbital state, the nonsignaling energy described was uniform across all layers. By multiplying $P_{\text{n.s.}}$ (in units of ATP/cell per second) with $\eta_{i}$ (in units of cells/cm$^3$), we get $E_{\text{n.s.}}$ in Eq. 1 (in units of ATP/s per centimeter$^3$). This empirically derived $E_{\text{n.s.}}$ was held constant for all other states.

Table 1. Energy budget parameters in rat and human brains

<table>
<thead>
<tr>
<th>Source</th>
<th>$\eta_N$</th>
<th>$\eta_A$</th>
<th>$R_{i,n,N}$</th>
<th>$R_{i,n,A}$</th>
<th>$P_{\text{n.s.},N}$</th>
<th>$P_{\text{n.s.},A}$</th>
<th>$P_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study (rat)</td>
<td>4.75*</td>
<td>4.75*</td>
<td>74</td>
<td>74</td>
<td>9.20*</td>
<td>6.85*</td>
<td>4.81$^b$</td>
</tr>
<tr>
<td>This study (human)</td>
<td>1.83*</td>
<td>1.83*</td>
<td>74</td>
<td>74</td>
<td>9.20*</td>
<td>6.85*</td>
<td>4.81$^b$</td>
</tr>
<tr>
<td>Ref. 7 (rat)</td>
<td>9.2</td>
<td>9.2</td>
<td>200</td>
<td>500</td>
<td>3.42</td>
<td>1.02</td>
<td>0.71</td>
</tr>
<tr>
<td>Ref. 8 (human)</td>
<td>4.0</td>
<td>3.8</td>
<td>79</td>
<td>163</td>
<td>8.6</td>
<td>3.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Average values of cortical density of neurons ($\eta_{i,n}$) and astrocytes ($\eta_{i,a}$) in $10^7$ neurons/cm$^3$ and resistances ($R_{i,n,N}$ and $R_{i,n,A}$), $10^6$ AST/astrocyte, input resistances of neurons ($R_{i,n,N}$ and $R_{i,n,A}$), $10^6$ ATP/neuron per second), and astrocytes ($P_{\text{n.s.},A}$, $10^6$ ATP/astrocyte per second), and ATP use per signaling event per neuron ($P_s$) in 10 ATP/spike neuron.

$^a$Estimated from Fig. S1A.

$^b$Calculated from Eq. S1 and Eq. S5 (details in Fig. S1B).

Calculating Total ATP Production Rate to Cerebral Glucose Oxidation Rate. We used the following formula to convert $E_{\text{tot}}$ in Eq. 1 to rate of glucose oxidation: $\text{calcCMR}_{\text{glc(ox)}} = k E_{\text{tot}}$.

$$\text{calcCMR}_{\text{glc(ox)}} = k E_{\text{tot}},$$  \[3\]

where $k$ depends on the oxygen-to-glucose index (OGI), which is given by the ratio of cerebral metabolic rates of oxygen (CMRO$_2$) and glucose (CMR$_{\text{glc}}$) consumption, and $k$ itself is given by $10^{17}/(A_{\text{avogadro}} \times \text{OGI}$, where $p$ is the tissue density (1.05 g/mL) and $A_{\text{avogadro}}$ is the Avogadro constant (6.023 times 10$^{23}$/mol). Similarly, the measured glucose oxidation $\text{measCMR}_{\text{glc(ox)}}$ was given by

$$\text{measCMR}_{\text{glc(ox)}} = \eta \times \text{OGI} \times \text{measCMR}_{\text{glc}}.$$  \[4\]

where $\text{measCMR}_{\text{glc}}$ was obtained from 2DG autoradiography in rats and fluoro-deoxyglucose PET in humans (Tables S1 and S2); it is given by sum of oxidative [CMR$_{\text{glc(ox)}}$] and nonoxidative [CMR$_{\text{glc(no-ox)}}$] terms, whereas CMR$_{\text{glc(ox)}}$ itself has neuronal [CMR$_{\text{glc(ox)}}$] and astrocytic [CMR$_{\text{glc(ox)}}$] components. Thus, it is possible to obtain calculated forms of CMR$_{\text{glc(no-ox)}}$, CMR$_{\text{glc(ox),N}}$, and CMR$_{\text{glc(ox),A}}$ across all activity states. Finally, we compared $\text{calcCMR}_{\text{glc(ox)}}$ with $\text{measCMR}_{\text{glc(ox)}}$ by least-square fitting to determine $P_s$ and $P_{\text{n.s.}}$:

$$\sigma^2 = \sum [\text{calcCMR}_{\text{glc(ox)}} - \text{measCMR}_{\text{glc(ox)}}]^2,$$  \[5\]

where summation was over all states (Tables S1 and S2).

Calculating Signaling and Nonsignaling Energetics in the Human Visual Cortex. To test whether $P_{\text{n.s.},N}$, $P_{\text{n.s.},A}$, and $P_s$ derived from the rat (Table 1) were representative of those values in human cerebral cortex, we calculated $E_{\text{n.s.}}$ in the human by simply multiplying the $P_{\text{n.s.},N}$ and $P_{\text{n.s.},A}$ terms with $\eta_{h,n}$ and $\eta_{h,a}$, respectively [i.e., cellular density is about 2.6 times lower in human vs. rat (17)]. Neuronal activity for each state in the human was represented by EEG-measured bispectral index (BIS) values ranging from 0 to 100 (Table S2) as used intraoperatively (18). We calculated the $E_{\text{n.s.}}$ term in the human similarly as in the rat, but because the neuronal activity data in the human originated from EEG recordings (SI Text, section 3), additional steps were needed to represent Eq. 2 to convert the BIS into units similar to the rat data (i.e., in units of spike/neuron/s per centimeter$^3$):

$$\langle f \rangle_{\text{human}} = \langle f_{\text{BIS}} /\langle f_{\text{BIS,AR}} \rangle \rangle_{\text{BIS,human}} /\langle f_{\text{BIS,AR}} \rangle_{\text{BIS,human}} \times \left( \sum (\delta_i f_i \eta_{i,j})_{\text{rat,AR}} /\langle \sum \delta_j \rangle_{\text{human}} \right),$$  \[6\]

$f_{\text{BIS}}$ and $f_{\text{BIS,AR}}$ are BIS values for a given state and the awake condition (Table S2), $\eta_{\text{BIS,human}}$ and $\eta_{\text{BIS,rat}}$ are the average neuronal densities in the human and rat cortices (Table 1), $\sum (\delta_i f_i \eta_{i,j})_{\text{rat,AR}}$ is the numerator of Eq. 2 for the awake condition in the rat (Table 2), and $\langle \sum \delta_j \rangle_{\text{human}}$ is the cortical thickness in the human (Table 3). This conversion, from $f_{\text{BIS}}$ scale in humans to $\langle f \rangle$ scale in rats, accounts for differences in average neuronal density and cortical thickness between rat somatosensory and human visual cortices (15–17, 19).

Results

$P_s$ in the Rat Somatosensory Cortex. The averaged representation of normalized activity of neurons across all states showed dominant activity in the bottom two-thirds of cortical layers (Fig. S2 and Table S1), which is in good agreement with prior studies that investigated layer-specific representation of neuronal activity, 2DG autoradiography, and functional MRI (fMRI) data (9–11). The laminar activity was then used with Eqs. 1–6 to derive $\text{calcCMR}_{\text{glc(ox)}}$ and compared with $\text{measCMR}_{\text{glc(ox)}}$ by least-
square fitting to determine $P_s$ and $P_m$. An average $P_s$ value of 4.81 × 10^7 ATP/spike per neuron was calculated by fitting Eq. 5 for all activity states in the rat, which is shown in Table 1. Because the neuronal activity data for some states in Table S1 were not available for all layers, $P_s$ was estimated for those specific layers only [i.e., (0.45/1.90) × 4.81 × 10^7 ATP/spike per neuron] (Table 2). Although the current $P_s$ value is derived for the entire cortex, layer-specific $P_s$ values are unlikely to vary by more than 50% of the average value. Fig. 1A shows the goodness of fit for Eq. 5 to the rat data (red circles) for an OGI of 5.6 with an $R^2$ value of 0.96 (gray line with $\sigma^2 = 0.0182$ for 11 states), indicating that the assumption of a constant value of $P_s$ over the full activity range is well-supported.

**P$_m$ in the Rat Somatosensory Cortex.** The measured CMR$_{glc(ox)}$ for the pentobarbital state in the rat (Table S1) was fitted to the $E_m$ term (Table 2). The constant $E_m$ term for all activity states was 0.76 × 10^17 ATP/s per centimeter$^3$, leading to average $P_{m,N}$ and $P_{m,A}$ values of 9.20 × 10^15 ATP/neuron per second and 6.85 × 10^15 ATP/astrocytes per second, respectively, for an OGI range of 5.1–6.0 (20). These values of $P_m$ corresponded to an average $P_m$ value of 74 µmol/g per minute (SI Text, section 2), which is well within the in vivo range measured for neurons and astrocytes (13, 14). As shown in Table 1, despite identical cortical densities of neurons and astrocytes, $P_{m,N}$ and $P_{m,A}$ were dissimilar, because slightly different Nernst potentials for Na$^+$ and K$^+$ and resting membrane potentials were used for neurons and astrocytes (SI Text, section 2), which has been done before (7, 8). However, similarities in contributions of astrocytes and neurons to nonsignaling energy consumption is in good agreement with measurements by 13C MRS made under isoelectric pentobarbital anesthesia, supporting the accuracy of the derived values (2, 21).

### Table 2. Calculated CMR$_{glc(ox)}$ derived from neuronal activity in rat somatosensory cortex for OGI of 5.6

<table>
<thead>
<tr>
<th>Behavioral state*</th>
<th>PR†</th>
<th>US1†</th>
<th>AR†</th>
<th>AS†</th>
<th>US2†</th>
<th>CR†</th>
<th>CS†</th>
<th>HR†</th>
<th>HS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Sigma$($\sigma_i$/n$_{m,j}$) (x10$^7$ spike.neuron.cm$^{-3}$/s per centimeter$^3$)</td>
<td>0.00</td>
<td>7.051</td>
<td>9.393</td>
<td>8.858</td>
<td>9.090</td>
<td>6.024</td>
<td>8.629</td>
<td>4.233</td>
<td>6.167</td>
</tr>
<tr>
<td>$\Sigma$($\sigma_i$/n$<em>{m,j}$/n$</em>{a,j}$) (x10$^7$ spike.neuron.cm$^{-3}$/s per centimeter$^3$)</td>
<td>0.00</td>
<td>3.711</td>
<td>4.955</td>
<td>4.677</td>
<td>4.797</td>
<td>13.366</td>
<td>19.136</td>
<td>9.397</td>
<td>14.800</td>
</tr>
<tr>
<td>$E_m = \Sigma P_{m,N} \times n_{m,j}$ (x10$^{17}$ ATP/s per centimeter$^3$)</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>$E_m = P_s \times n_{m,j}$ (x10$^{17}$ ATP/s per centimeter$^3$)</td>
<td>1.79</td>
<td>2.38</td>
<td>2.24</td>
<td>2.30</td>
<td>1.53</td>
<td>1.53</td>
<td>1.53</td>
<td>1.53</td>
<td>1.53</td>
</tr>
<tr>
<td>$E_{tot} = E_m + E_s$ (x10$^{17}$ ATP/s per centimeter$^3$)</td>
<td>0.76</td>
<td>2.55</td>
<td>3.14</td>
<td>3.01</td>
<td>3.01</td>
<td>2.29</td>
<td>2.95</td>
<td>1.84</td>
<td>2.45</td>
</tr>
<tr>
<td>calcCMR$_{glc(ox)}$ (µmol/g per minute)</td>
<td>0.22</td>
<td>0.72</td>
<td>0.89</td>
<td>0.85</td>
<td>0.87</td>
<td>0.65</td>
<td>0.82</td>
<td>0.52</td>
<td>0.69</td>
</tr>
<tr>
<td>measCMR$_{glc(ox)}$ (µmol/g per minute)</td>
<td>0.20</td>
<td>0.63</td>
<td>0.90</td>
<td>0.82</td>
<td>0.91</td>
<td>0.63</td>
<td>0.90</td>
<td>0.49</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Details in Calculations. The calculated CMR$_{glc(ox)}$ [calcCMR$_{glc(ox)}$] was derived from Eq. 3. The measured CMR$_{glc(ox)}$ [measCMR$_{glc(ox)}$] was determined from Eq. 4 assuming OGI of 5.6. AR, awake rest; AS, awake stimulation; CR, α-chloralose rest; CS, α-chloralose stimulation; HR, halothane rest; HS, halothane stimulation; PR, pentobarbital; US1, urethane rest; US2, urethane stimulation.

### Table 3. Calculated CMR$_{glc(ox)}$ derived from neuronal activity in human visual cortex for OGI of 5.6

<table>
<thead>
<tr>
<th>Behavioral state*</th>
<th>VGP</th>
<th>VGA</th>
<th>PRO</th>
<th>SEV</th>
<th>HAL</th>
<th>SLP</th>
<th>AWK</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Sigma$($\sigma_i$/n$_{m,j}$) (x10$^7$ spike.neuron.cm$^{-3}$/s per centimeter$^3$)</td>
<td>282</td>
<td>1,192</td>
<td>1,317</td>
<td>1,306</td>
<td>1,959</td>
<td>2,674</td>
<td>3,407</td>
</tr>
<tr>
<td>$\Sigma$($\sigma_i$/n$<em>{m,j}$/n$</em>{a,j}$) (x10$^7$ spike.neuron.cm$^{-3}$/s per centimeter$^3$)</td>
<td>0.12</td>
<td>0.51</td>
<td>0.56</td>
<td>0.56</td>
<td>0.83</td>
<td>1.14</td>
<td>1.45</td>
</tr>
<tr>
<td>$E_m = \Sigma P_{m,N} \times n_{m,j}$ (x10$^{17}$ ATP/s per centimeter$^3$)</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>$E_m = P_s \times n_{m,j}$ (x10$^{17}$ ATP/s per centimeter$^3$)</td>
<td>0.06</td>
<td>0.24</td>
<td>0.27</td>
<td>0.27</td>
<td>0.40</td>
<td>0.55</td>
<td>0.70</td>
</tr>
<tr>
<td>$E_{tot} = E_m + E_s$ (x10$^{17}$ ATP/s per centimeter$^3$)</td>
<td>0.35</td>
<td>0.54</td>
<td>0.56</td>
<td>0.56</td>
<td>0.69</td>
<td>0.84</td>
<td>0.99</td>
</tr>
<tr>
<td>calcCMR$_{glc(ox)}$ (µmol/g per minute)</td>
<td>0.10</td>
<td>0.15</td>
<td>0.16</td>
<td>0.16</td>
<td>0.20</td>
<td>0.24</td>
<td>0.28</td>
</tr>
<tr>
<td>measCMR$_{glc(ox)}$ (µmol/g per minute)</td>
<td>0.08</td>
<td>0.14</td>
<td>0.15</td>
<td>0.14</td>
<td>0.18</td>
<td>0.24</td>
<td>0.31</td>
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</table>

Details in Calculations. To convert the EEG data in Table S2 into units of $\Sigma$($\sigma_i$/n$_{m,j}$/n$_{a,j}$), simple conversions were needed with Eq. 6. The calculated CMR$_{glc(ox)}$ [calcCMR$_{glc(ox)}$] was determined from Eq. 3. The measured CMR$_{glc(ox)}$ [measCMR$_{glc(ox)}$] was determined from Eq. 4 assuming OGI of 5.6. AWK, awake; HAL, halothane; PRO, propofol; SEV, sevoflurane; SLP, non-REM sleep; VGA, acute vegetative; VGP, persistent vegetative.

*Details in SI Text, section 1 and see Fig. 1A for comparison between calcCMRGlc(ox) and measCMRGlc(ox).
Fig. 1. Relationship between glucose oxidation [CMRglc(ox)] and neuronal activity as a function of OGI. (A) Comparison between calculated CMRglc(ox,N) [calcCMRglc(ox,N)] and measured CMRglc(ox,N) [measCMRglc(ox,N)] values. Values of measCMRglc(ox,N) were derived from 2DG autoradiography in rat brain and PET in human brain, whereas values of calcCMRglc(ox,N) were derived for an OGI of 5.6. The goodness of fit between measCMRglc(ox,N) and calcCMRglc(ox,N) for the rat data (red circles) is indicated by the gray line with an R2 value of 0.96 (σ2 = 0.0182 for 11 states). The human data (blue triangles) also showed a strong correlation (R2 = 0.91 and σ2 = 0.0023 for seven states). (B) Comparison between measured NA (measNA) and calculated glucose oxidation in neurons (calcCMRglc(ox,N)) in rat (red circles) and human (blue triangles) brains shows good correlation (R2 = 0.98, where calcCMRglc(ox,N) = 0.90(measNA + 0.12). Because the data were normalized to the awake resting state values, the intercept on the vertical axis is about ~20% of calcCMRglc(ox,N) in the awake state for both species. (C–E) Comparison of calculated total glucose oxidation [calcCMRglc], calculated glucose oxidation in neurons [calcCMRglc(ox,N)], calculated glucose oxidation in astrocytes [calcCMRglc(ox,A)], and calculated nonoxidative glucose consumption [calcCMRglc(nonox),N] and measured total glucose consumption [measCMRglc], where calcCMRglc = calcCMRglc(ox,N) + calcCMRglc(ox,A) and calcCMRglc(nonox),N = measCMRglc – calcCMRglc(ox,N). Ratios in rat brain (red), human brain (blue), and overall (purple) for (C) the value of the calcCMRglc(ox,N) in B for the nonanesthetized awake resting state [calcCMRglc(ox,N)] minus the value of the intercept [i.e., (1-intercept)calcCMRglc(ox),N,AR] and the values of (D) calcCMRglc(ox,A)/calcCMRglc(ox) and (E) calcCMRglc(nonox,normalized)/measCMRglc(ox normalized) measured across all activity levels. All error bars indicate SDs from the mean. All calculations were obtained with Pns,N = 4.81 × 10−8 ATP/spike per neuron, Pns,A = 9.20 × 10−8 ATP/neuron per second, and Pns,N = 6.85 × 10−8 ATP/astrocyte per second). Details are in Tables 1, 2, and 3.

Relative Contributions of ENS and ENS A in the Resting Awake State. To represent the magnitude of ENS vs. ENS a in the resting awake state, we plotted the calcCMRglc(ox,N) vs. the measured neuronal activity in a normalized scale (Fig. 1B). Because the intercepts on the calcCMRglc(ox,N) vertical axis—which indicates the isoelectric condition—were ~0.1 and ~0.05 μmol/g per minute for the rat and human, respectively (Fig. S3), or 15–20% of the calcCMRglc(ox,N) in the awake state (Fig. 1B), the results suggest that a significant fraction of neuronal glucose oxidation in the awake state is dedicated for maintaining resting membrane potentials (Fig. 1C). Similarly, glial glucose oxidation is about ~20% of total glucose oxidation in the awake state (Fig. 1D). Based on reported OGI values (20), nonoxidative glucose consumption is about ~6% of total glucose consumption (Fig. 1E).

Comparison with 13C MRS Studies. A similar linear relationship between neuronal activity and energy metabolism has been shown by results from 13C MRS—a method that simultaneously measures rates of total neurotransmitter cycling [Vcyc(tot)] and neuronal [CMRglc(ox,N)] as well as astrocytic [CMRglc(ox,A)] oxidative demand (2, 3). Fig. 2A shows the most up-to-date results from in vivo 13C MRS studies in rats and humans (Tables S3 and S4), which illustrate nearly a 1:1 relationship between ΔVcyc(tot) and ΔCMRglc(ox,N) just beyond the isoelectric point when Vcyc(tot) approaches zero [i.e., gray line indicates an R2 value of 0.92, CMRglc(ox,N) = 0.87 Vcyc(tot) + 0.10]. Moreover, the in vivo 13C MRS results of Vcyc(tot)/CMRglc(ox,N) and CMRglc(ox,A)/CMRglc(ox) ratios in the awake state for both species, shown in Fig. 2B, show that neurons and astrocytes in the awake state demand about ~20% of oxidative ATP for nonsignaling factors. These results are similar to our budget calculations in Fig. 1.

Discussion

Comparison of Derived PNS and PNS A with Prior Calculations. There are large differences between PNS,N and PNS,A estimates for the rat by Attwell and Laughlin (7) and our empirically derived values (Table 1). Values of PNS,N and PNS,A are susceptible to starting assumptions (SI Text, section 2 and Fig. S1). This difference principally occurs, because Attwell and Laughlin (7) assumed RNS,N and RNS,A to be around 250 and 500 Ω, respectively, based on in vitro recordings (30, 31). However, our estimates of RNS,N and RNS,A values of 74 MΩ are in close agreement with in vivo measurements (13, 14). Because PNS depends on the reciprocal of RNS (SI Text, section 2), the higher RNS,N and RNS,A values by Attwell and Laughlin (7) are the main basis for their lower PNS,N and PNS,A estimates. Additionally, however, in the case of PNS,N, there could also be differences in average neuronal density, which in the study by Attwell and Laughlin (7), were based on the mouse cortex (32), whereas our values were obtained from the rat cortex (15). The PNS,N and PNS,A values estimated by Lennie (8) for the human brain are approximately similar to our values, despite large differences in cell densities across species (Table 1). There are similar differences between P values for the rat by Attwell and Laughlin (7) and the human by Lennie (8) as discussed in detail below. However, in a recent 31P MRS study of the human brain, Zhu et al. (33) estimated a P value of 4.7 × 10−8 ATP/neuron per second from measured cerebral metabolic rates of high-energy phosphate reactions catalyzed by ATPase. Assuming that, within 1 s, there is one spike in the awake brain, this value agrees well with P estimated in the current budget (Table 1).

Gial Energy Demand and Excitatory Vs. Inhibitory Neuronal Energy Requirements. The calculated astrocytic energy demand (Fig. 1D) is consistent with 13C MRS results (Fig. 2B) but significantly higher than estimates from prior budgets (7, 8). The higher PNS,A values are because of using more recent values of astrocyte input.
resistances (14). The in vivo results were fit well with the assumption of the major energetic changes with activity being in the neurons. Relatively constant astrocytic energetics (compared with neurons) as a function of overall cortical activity may reflect the energetics associated with maintaining the high astrocyte K⁺ conductance dominating over additional functional demands (e.g., transporting glutamate and/or dealing with Ca²⁺ waves) as suggested by recent studies (34–37). ¹³C MRS results, in support of these observations, show that energy demand of astrocytes changes considerably less than neuronal energy demand over wide activity levels (Table S4). However, the glial data are relatively limited compared with neuronal data, and future studies are needed to better understand glial functional energy requirements.

As in previous bottom-up calculations (7, 8), to derive Pₚ and Pₙs, we could not separately include energy demand of inhibitory neurons, because the electrophysiological studies only included measurements of pyramidal neurons. Because CMRglc(ox),N contains the oxidative energy requirements of both excitatory and inhibitory neurons (15), the value of Pₚ likely reflects glutamatergic neurons working in conjunction with an ensemble of GABAergic neurons. Electrophysiological and ¹³C MRS studies have found that, over the range of activity that we examined, both function and energy demand of GABAergic neurons is proportional to activity/demand of glutamatergic neurons (38, 39), consistent with the fractional contribution of excitatory and inhibitory neurons to Pₚ, being constant throughout the activity range and similar to glutamatergic and GABAergic neuronal fractions measured morphologically (15).

Constitency of Pₚ and Pₙs Across Activity Levels and Species. Previously, Karbowski (40) suggested that basal metabolic cortical differences across species could be described on allometric rates. However, Herculano-Houzel (17) pointed out that absolute metabolic difference across species could be explained by neuronal number variations. Both Karbowski (40) and Herculano-Houzel (17) dealt with only the awake state values and the total neuronal number variations. Both Karbowski (40) and Herculano-Houzel (17) pointed out that astrocytes (38, 41) and if so, could lead to an overestimate of Pₙs, we fitted the data in Fig. 1B with and without the isolectric pentobarbital data and found negligible differences in the slope, intercept, and goodness of fit [rat data: CMRglc(ox) = 0.92 neuronal activity (NA) + 0.13, R² = 0.97 with pentobarbital and CMRglc(ox) = 0.94 NA + 0.11, R² = 0.93 without pentobarbital]. A fixed Pₚ does not necessitate a constant Pₙs. As shown in Fig. 1A and B, a constant value of Pₚ gave an excellent fit to the data. A nonconstant Pₚ, most likely a decrease at higher firing rates, would have shown itself as a deviation from the best linear fit. It should be recalled that bottom-up energy budgets predict that Pₚ and Pₙs differ significantly across species (Table 1), and moreover, constancy of Pₚ and Pₙs beyond the awake state had not been tested (7, 8).

The finding of a constant Pₚ across activity levels suggests that energy-consuming subcellular processes of the neuropil (e.g., ion fluxes associated with action potentials, pre- and postsynaptic potentials, vesicular exocytosis/endocytosis, neurotransmitter release/uptake, etc.) are all tightly coupled, ensuring that the fidelity of impulse trafficking across a synapse is maintained independent of signaling frequency. Although the mechanisms upholding this neurometabolic linearity are not understood, the rapid rate at which surrounding astrocytic end feet clear synaptically glutamate is probably a critical component (3). The similarity of energetic efficiency with regard to electrical (Fig. 1) and chemical (Fig. 2) events across different activity levels suggests that there are physiological factors that limit the energy associated with cortical signaling (42) and that, within the normal physiological range of neuronal signaling, the electrical and chemical events are complimentary (3). Assuming a constant energetic cost of Na⁺ and K⁺ pumping, there is a direct relationship between synaptic strength (i.e., average current induced by a signaling event at a synapse, which can be increased through either induced conductivity or higher probability of presynaptic glutamate release) and energy consumption at the synapse.

The stability of Pₚ and Pₙs across species suggests that, early in evolution, the mammalian brain reached an optimum tradeoff between energy consumption and computational power at a cellular level (43). This constancy differs from most other tissues, where there is a decrease in cellular metabolic rate by a power of relative animal size (1). These findings imply that relative energy costs of fundamental features of cortical communication (e.g., action potentials, pre- and postsynaptic potentials, glial neurotransmitter and Na⁺ transporters, etc.) as well as physiological mechanisms for supplying mitochondrial energy for neurotransmitter operations (e.g., hemoglobin, voltage-gated ion channels, glutamate and GABA receptors, and glucose and oxygen transport) are well-preserved through evolution (17, 28, 29, 44–49). A constant interspecies ATP demand of cortical activity emphasizes the relevance of animal testing in experimental neuroscience studies.
However, the approximately twofold difference in total cortical energy consumption between rats and humans may reflect limitations in blood flow in the human to supply oxygen and glucose and remove waste products and heat in relation to the number of cells that comprise each brain (50, 51).

Implications for fMRI and Resting Awake State Neuronal Activity. fMRI has become a major tool for mapping neuronal activity in humans and animals. An important question in fMRI studies is whether the change in signal during tasks, which is produced by changes in blood flow, blood volume, and oxygen consumption, directly reflects changes in neuronal activity (52). Our finding that a constant P, fits experimental data both within and across species, supports the measurement of oxygen consumption component of fMRI can provide a quantitative measure of changes in neuronal activity. This conclusion is consistent with experimental studies using calibrated fMRI and multiunit recordings that have found a linear relationship between the average pyramidal cell signaling and regional oxygen consumption (4, 5, 36, 37). Our studies using calibrated fMRI and multiunit recordings that have found a linear relationship between the average pyramidal cell signaling and regional oxygen consumption (4, 5, 36, 37). Our findings also show that the high level of neuronal activity in the resting awake state by 11C MRS is consistent with metabolic measurements of 2DG and PET as well as electrical activity measurements (spanning from EEG to extracellular recordings) for the rat and human, supporting proposals that resting state activity be incorporated into fMRI models of brain function (53). Because these results are relating macroscopic energy measurements to microscopic electrical events, we expect that the empirically derived values of P, and P* can better guide bottom-up budgets of subcellular processes representing mammalian cortical function.

Materials and Methods

Tables S1 and S2 list values of neuronal activity and glucose consumption in rat and human for 11 and 7 different states, respectively, over a range of excitability (SI Text, section 1). In summary, the measured neuronal activity data were used to calculate CMRglc(ox) and then compared with measured CMRglc(ox) to determine values of P, and P* (Tables 1, 2, and 3). The budget is described in Calculations (SI Text, section 2).

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Supporting Information

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S1 Text

Description of Neuronal Activity and Metabolic Data. Tables S1 and S2 list values of neuronal activity and cerebral metabolic rate of glucose consumption (CMRglc) in rat and human brains, respectively, for a few behavioral states. There were a total of 11 and 7 data points for rat and human, respectively, where neuronal activity was measured with microelectrodes and EEG and CMRglc was measured by 2-deoxyglucose autoradiography (2DG) and PET methods.

Table S1 shows the neuronal activity and metabolic data from the rat cerebral cortex for a few behavioral states: pentobarbital anesthesia with 120-mg/kg dose (PR), urethane anesthesia with 1.6- to 1.7-g dose (UR1), urethane anesthesia with 1.6- to 1.7-g dose and sensory stimulation (US1), awake at rest (AR), awake and sensory stimulation (AS), urethane anesthesia with 1.5-g dose (UR2), urethane anesthesia with 1.5-g dose and sensory stimulation (US2), α-chloralose anesthesia with 60-mg/kg dose (CR), α-chloralose anesthesia with 60-mg/kg dose and sensory stimulation (CS), halothane anesthesia with 1% dose (HR), and halothane anesthesia with 1% dose and sensory stimulation (HS). The neuronal activity was represented by spike rate (spike/s) as measured by microelectrodes in a layer-specific manner, where details on thickness of each cortical layer and density of excitatory neurons in each layer are in Table S1. The somatosensory cortex CMRglc (µmol/g per minute) was measured by 2DG. For PR, UR1, US1, AR, and AS, the CMRglc was averaged over all cortical layers, whereas for others, the CMRglc was averaged over cortical layers d and e. The first and perhaps, the most important fitted data point was the isoelectric condition (i.e., PR state), which allowed estimation of input resistances of neurons and astrocytes (Rin,N and Rin,A) from which it was possible to calculate the rates of ATP use per astrocyte at rest for neurons and astrocytes (Pn and Pa) as shown in Table 1. These values derived from the rat brain were also used with the human data.

In the rat, there was excellent correspondence of the anesthetic doses used in the electrophysiology and metabolic studies for each behavioral state. The PR state was achieved with deep pentobarbital anesthesia (1, 2). There were four data points—UR1, US1, AR, and AS—in which neuronal activity data were collected from each cortical layer and CMRglc was the average across the entire cortex. The pairs of UR1 and US1 (3, 4) and AR and AS (5–7) were rest and stimulation data points, respectively, for urethane-anesthetized and awake rats. In six other data points—UR2, US2, CR, CS, HR, and HS—neuronal activity data were collected from the middle cortical layers, and CMRglc was the average across specific cortical layers; each respective pair was rest and stimulation data with urethane, α-chloralose, and halothane anesthesia, respectively (4, 8–10). The PR state had the lowest neuronal activity and CMRglc values, which are in good agreement with prior 13C magnetic resonance spectroscopy (MRS) results (11). The same data without stimulation (i.e., UR1, AR, UR2, CR, and HR) had CMRglc values that were 2.5–4.2 times higher than the PR state. Sensory stimulation increased CMRglc by as little as 5% (i.e., HR to HS) or as much as 40% (i.e., UR2 to US2). The neuronal activity data were in general agreement with these trends, where the middle cortical layers showed the greatest magnitude of change.

Table S2 shows the neuronal activity and metabolic data from the human cerebral cortex for a few behavioral states: persistent vegetative state (VGP), acute vegetative state (VGA), propofol anesthesia with 0.4-mg/kg dose (PRO), sevoflurane anesthesia with 1.5% endtidal concentration (SEV), halothane anesthesia with 0.1% incremental doses up to less than 1% (HAL), nonrapid eye movement (non-REM) sleep as assessed by EEG patterns (SLP), and awake and alert (AWK). The neuronal activity was represented by bispectral index (BIS) as measured by EEG wires placed in different parts of the scalp, and CMRglc (µmol/g per minute) was measured by PET.

The SLP state represented BIS values ranging between 70 and 87 (12, 13). The HAL state was obtained from reported BIS values in the range of 50–65 (14, 15). The SEV (15–17), PRO (16, 18, 19), and VGA (20) states were quite close in BIS values, ranging between 35 and 39. The VGP state was represented by BIS values as low as 3 and as high as 36 for 84% and 16% of reported cases, respectively (21).

CMRglc values were obtained for the same behavioral states. The AWK state was taken from the average of visual cortex values, ranging from 0.21 to 0.45 µmol/g per minute (22–24). The SLP state value was obtained from several studies for non-REM sleep, where 12–33% drop in CMRglc had been reported from the AWK state (23, 24). Likewise, HAL, SEV, and PRO states represented drops of 44% (25), 44–68% (16, 17), and 50% (16, 22), respectively, in CMRglc from the AWK state. The VGA state was obtained from 50% to 60% reported drop in CMRglc from the AWK state (26, 27), whereas the VGP state was obtained from studies reporting more than 70% drop in CMRglc from the AWK state (27).

Dependency of Input Resistance on Nonsignaling Energy Demand. Each term of Ens in Eq. 1 depends on input resistances of cells (Rin in units of Ω). If the cell membrane has pumps that export 3 Na+ and import 2 K+ per ATP consumed on a time scale much slower than the membrane time constant (28, 29), then it can be shown that conductance of Na+ (gNa) and K+ (gk)—the sum of which is inversely equal to the input resistance for a given cell type [i.e., Rin = 1/(gNa + gK)]—is related to the ATP consumption rate per cell at rest, Pns (ATP/cell per second):

$$P_{ns} = \frac{A_{ns}}{\nu_{ns}} \left( \nu_{Na} - \nu_{K} \right) \left( \nu_{TP} - \nu_{K} \right) \left( \nu_{TP} + 2 \nu_{Na} - 3 \nu_{K} \right)$$

where \(\nu_{Na}\), \(\nu_{K}\), and \(\nu_{TP}\) are the Nernst potentials for Na+ and K+ and the resting membrane potential (V), respectively, and \(A_{ns}\) and \(\nu_{ns}\) are the Avogadro (mol) and Faraday (A/mol) constants, respectively.

In rat brain, recent measurements of input resistances of neurons (Rin,N) and astrocytes (Rin,A) as well as average neuronal (Rin,N) and astrocytic (Rin,A) densities in the cerebral cortex show that values for neurons and astrocytes are quite similar, thereby indicating that Rin,N ≈ Rin,A and Rn ≈ Rin,N (30–34). In vivo measured Rin,N value range is between 50 and 100 Ω (30–32), whereas observable oxygen-to-glucose index (OGI) range is between 5 and 6 (35). Thus, from Eqs. 1 and S1, an average Rin,N value of 74 Ω was determined by empirical fitting calculated CMRglc(ox) for the isoelectric condition [calcCMRglc(ox),isoelectric] to the measured CMRglc(ox) for the PR state in Table S1 [measCMRglc(ox),PR state] with Eq. 5. For Eq. S1, we assumed \(\nu_{O}_{2} = 50 \text{ mV}, \nu_{S} = -100 \text{ mV}, \nu_{TP} = -70 \text{ mV} \) for neurons and \(\nu_{O}_{2} = 50 \text{ mV}, \nu_{S} = -100 \text{ mV}, \nu_{TP} = -80 \text{ mV} \) for astrocytes. Because the value of Rn varied by a factor of three when \(\eta_{s} \approx \eta_{r} \) (Fig. S1A), we averaged Rin,N values for OGI range of 4–6 to derive values of Pns,N and Pns,A using Eqs. 5 and S1 (Fig. S1B). However, these values of Pns,N and Pns,A are vulnerable to assumed \(\nu_{TP}\) values for neurons and astrocytes (Fig. S1 C and D).
Reliability of EEG as measure of underlying neural activity and its implications for budget calculations. All of the EEG data used in the budget were represented as bispectral index (BIS) values, ranging from 0 to 100. This range of BIS values respectively reflect complete EEG silence (i.e., isoelectricity) up to awake resting condition (36). BIS is based on the weighted sum of several EEG parameters for both time and frequency domains. It is intended for monitoring anesthesia depth during surgeries where expert interpretation of the raw EEG data – either in terms of the different frequency bands or dynamic temporal patterns, both of which are known to change with overt behavioral variations – is not directly available (27, 36). While EEG is not as quantitative as microelectrode recordings, it has the advantage of non-invasively measuring small voltage fluctuations in vivo resulting from current flows within pyramidal neurons spanning cortical gray matter and thus can reflect relative variations of dynamic neural activity patterns (38, 39).

As shown in Figure S3D, there is a close to linear dependency of BIS measured by EEG with glucose oxidation (CMRglc(ox)) which was measured by PET. The BIS and CMRglc(ox) values were obtained from different studies, but for the same experimental conditions (Table S2). A best-fit of the data suggests that at isoelectricity (i.e., intercept of the plot in Fig. S3D) CMRglc(ox) is reduced to about 20% of the resting awake value. In other words, at least 80% of total energy production in the human cerebral cortex is dedicated to excitatory signaling demands. Another estimate of energy expenditure for minimal neuronal signaling can be obtained from comparing the total glucose consumption (CMRglc) values in the persistent vegetative state (0.09 μmol/g/min; see Table S2) with the resting awake state (0.34 μmol/g/min; see Table S2). The comparison suggests that about 25% of the awake CMRglc value could be assigned to isoelectricity, provided that it is assumed that the persistent vegetative state is a state with minimal cortical activity. Thus the finite intercept of the human PET and EEG data suggests non-negligible energy consumption during isoelectric situations (Figure S3D), which is in congruence with 13C MRS values from rat brain (Fig. 2).

However a limitation of this energy budget in the human is that it depends on the quantitative accuracy with which BIS reflects various behavioral states (37, 40). Studies have shown that BIS values correlate well with other clinical sedation scores (36, 37). However more studies are certainly needed to assess the BIS trends across different sedation levels for different anesthetics. Clearly the weakest data points in Fig. S3D are the vegetative states – both acute and persistent – due to tissue damage. But regardless of the fact if these data points are included or removed, the best-fits do not significantly differ (i.e., with or without the vegetative state data points, the slope is unchanged while the intercept changes from 0.06 to 0.04 μmol/g/min). The similar values for these regressions argue that some minimal metabolism is present even in vegetative states, presumably for reduced signaling but other unknown factors may also be active. More importantly, however, these tests provide rationale for depending on BIS measures as a secondary reporter of neural activity in the human brain.

Fig. S1. Dependence of input resistance ($R_{in}$) on OGI as a function of astrocytic population and rate of ATP use at rest per astrocyte ($P_{ns,A}$) and neuron ($P_{ns,N}$). 

(A) Relationships were determined by fitting the measured total glucose oxidation [CMR$_{glc(ox)}$] value for isoelectric condition in the rat (behavioral state PR in Table S1). Lower OGI values were consistent with higher $R_{in}$ values, whereas higher OGI values were consistent with lower $R_{in}$ values. The $R_{in}$ values increased with astrocytic population. In vivo measured $R_{in}$ values of neurons and astrocytes corresponded to calculated $R_{in}$ values for ~50% of astrocytic population and OGI values of 5 and 6. 

(B) Averaged values for $P_{ns,A}$ and $P_{ns,N}$ with astrocytic population ranging between 30% and 70% and OGI values ranging between 4 and 6, where averaged $R_{in}$ value was 74 MΩ for both astrocytes and neurons. Details in Table 1. 

Dependence of rate of ATP use at rest per cell ($P_{ns,x}$) as a function of resting membrane potential ($V_{rp}$) when Nernst potentials for Na$^+$ ($V_{Na}$) and K$^+$ ($V_{K}$) are varied by ±10 mV as shown in C and D, respectively. Although $P_{ns,x}$ increases as a function of $V_{rp}$, $P_{ns,x}$ is more sensitive to the assumed value for $V_{K}$ than $V_{Na}$.
Fig. S2. Averaged representation of normalized neuronal activity ($f_N$) per cortical layer in rat brain measured for numerous activity levels (Table S1), where layers a–f are located in superficial to deep cortical layers, respectively. Majority (~80%) of cortical neuronal activity is represented in the middle cortical layers (c–e). The parameter $f_N$ was calculated by the $(\delta_i f_i \eta_{NI})$ term for each $i$th cortical layer divided by the $\Sigma (\delta_i f_i \eta_{NI})$ term spanning over all cortical layers, given that $\delta_i$, $\eta_{NI}$, and $f_i$ represent the layer thickness (cm), density of neurons per layer (neurons/cm³), and spike rate per layer (spike/s).
Fig. S3.  Relationship between glucose oxidation [CMR$_{\text{glc(ox)}}$] and neuronal activity as a function of OGI. (A and B) Comparison between calculated and measured CMR$_{\text{glc(ox)}}$ values. The measured CMR$_{\text{glc(ox)}}$ values in (A) rat and (B) human brains were derived from 2DG and PET, respectively. The calculated CMR$_{\text{glc(ox)}}$ values were derived for OGI of 5.6, whereas the dashed lines represent OGI values ranging between 5.1 and 6.0. Fig. 1A shows these data on the same plot, where details of the red and orange circles are in Table 2. (C and D) Comparison between measured neuronal activity and measured glucose oxidation in neurons (CMR$_{\text{glc(ox)},N}$) in rat and human brains, respectively. The intercepts of both linear trends are about 0.1 and 0.05 μmol/g per minute, respectively. The dashed lines represent OGI values ranging between 5.1 and 6.0. Fig. 1B shows normalized plots of these results. Calculated CMR$_{\text{glc(ox)}}$ values were derived with parameters described in Table 1 (i.e., $P_s = 4.81 \times 10^8$ ATP/spike per neuron; $P_{\text{n,N}} = 9.20 \times 10^8$ ATP/neuron per second; $P_{\text{n,A}} = 6.85 \times 10^8$ ATP/astrocytes per second). Tables S1 and S2 are measured CMR$_{\text{glc(ox)}}$ and measured neuronal activity in rat and human brains, respectively. Tables 2 and 3 are calculated CMR$_{\text{glc(ox)}}$ values in rat and human brains, respectively.
Table S1. Neuronal activity (f_i) and glucose consumption (CMR_{glc}) measured across behavioral states in rat somatosensory cortex

<table>
<thead>
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<th>Layer</th>
<th>δ_i (×10^{-3} cm)</th>
<th>η_i (×10^{-3} neurons/cm^3)</th>
<th>Neuronal activity per cortical layer* (f_i; spike/s) for each behavioral state^†</th>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>168</td>
<td>0.53</td>
<td>0.00 0.16 0.24 0.10 0.05 — — — — — — 1</td>
</tr>
<tr>
<td>b</td>
<td>438</td>
<td>5.26</td>
<td>0.00 0.32 0.47 0.20 0.10 — — — — — — 8</td>
</tr>
<tr>
<td>c</td>
<td>240</td>
<td>8.57</td>
<td>0.00 0.58 0.98 1.40 1.40 — — — — — — 8</td>
</tr>
<tr>
<td>d</td>
<td>226</td>
<td>3.61</td>
<td>0.00 1.10 1.20 2.10 3.00 3.70 y 5.30 y 2.60 y 4.10 ** 3.20 **†† 3.40 **†† 25</td>
</tr>
<tr>
<td>e</td>
<td>226</td>
<td>3.61</td>
<td>0.00 3.70 4.25 3.10 3.40 3.70 y 5.30 y 2.60 y 4.10 ** 3.20 **†† 3.40 **†† 35</td>
</tr>
<tr>
<td>f</td>
<td>601</td>
<td>4.25</td>
<td>0.00 0.47 0.72 0.50 0.30 — — — — — — 13</td>
</tr>
<tr>
<td></td>
<td>CMR_{glc} (μmol/g per minute) →</td>
<td>0.21 0.68 0.96 0.88 0.97 0.68 0.96 0.52 0.73 0.65 0.68</td>
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</tbody>
</table>

Neuronal activity, represented by spike rate, was measured with microelectrodes. CMR_{glc} was measured by 2DG autoradiography. Also shown are thickness (δ_i), neuronal density (η_i), and normalized neuronal activity (f_i) in each cortical layer. AR, awake rest; AS, awake stimulation; CR, α-chloralose rest; CS, α-chloralose stimulation; HR, halothane rest; HS, halothane stimulation; PR, pentobarbital; UR1, urethane rest; UR2, urethane stimulation; US1, urethane stimulation; US2, urethane stimulation.

*Layers a–f are located in superficial to deep cortical layers with thickness, Σδ_i, of 1.90 mm for the entire cortex and where the density (η_i) is for all neurons in a given layer (Tables 1 and 2 in ref. 1 respectively show layer-specific thickness and neuronal density).

†For each behavioral state, the parameter f_i was calculated by the (δ_i; f_i; η_i) for each cortical layer divided by the Σ(δ_i; f_i; η_i) over all cortical layers—given that δ_i, η_i, and f_i represent the layer thickness (cm), density of neurons per layer (neurons/cm^3), and spike rate per layer (δ_i)—and then averaged across all behavioral states (details in Fig. S2).

‡Average of urethane-anesthetized states with spike rates of 4.6 and 2.8 Hz as measured by matrix electrodes.

§On sensory stimulation under urethane anesthesia, spike rate increased by 1.6 Hz as measured by matrix electrodes.

**Based on spike rate histograms with 84–87% at 1.0 Hz and 13–16% at 11 Hz and fractions of population three times greater than HR.

††Based on spike rate histograms with 34–35% at 6.9 Hz and 65–66% at 11 Hz and fractions of population three times greater than CR.

†‡Based on spike rate histograms with 23–27% at 8.7 Hz and 65–66% at 11 Hz and fractions of population three times greater than L. More information in SI Text, section 1.

Table S2. Neuronal activity (f_{BIS}) and glucose consumption (CMR_{glc}) measured across behavioral states in human visual cortex

<table>
<thead>
<tr>
<th>Behavioral state</th>
<th>Neuronal activity (f_{BIS}) from EEG recordings using measures of BIS (from 0 to 100)</th>
<th>CMR_{glc} (μmol/g per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AWK</td>
<td>100</td>
<td>0.33</td>
</tr>
<tr>
<td>SLP</td>
<td>79</td>
<td>0.26</td>
</tr>
<tr>
<td>HAL</td>
<td>58</td>
<td>0.19</td>
</tr>
<tr>
<td>SEV</td>
<td>38</td>
<td>0.15</td>
</tr>
<tr>
<td>PRO</td>
<td>39</td>
<td>0.16</td>
</tr>
<tr>
<td>VQA</td>
<td>35</td>
<td>0.15</td>
</tr>
<tr>
<td>VGP</td>
<td>8</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Neuronal activity represented by BIS as measured by EEG. CMR_{glc} (μmol/g per minute) was measured by PET. AWK, awake; HAL, halothane; PRO, propofol; SEV, sevoflurane; SLP, non-REM sleep; VQA, acute vegetative; VGP, persistent vegetative.

*More information in SI Text, section 1.


Table S3. Experimental results of total glutamate neurotransmitter cycling \( [V_{\text{cyt(tot)}}] \) and neuronal glucose oxidation \( [\text{CMR}_{\text{glc(ox),N}}] \) measured by \( ^{13}\text{C} \) MRS in rat and human brain

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Condition</th>
<th>( V_{\text{cyt(tot)}} )</th>
<th>( \text{CMR}_{\text{glc(ox),N}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat, pentobarbital (120 mg/kg, +30 mg/kg per hour)</td>
<td>0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>1</td>
<td>Rat, ( \alpha )-chloralose (80 mg/kg, +30 mg/kg per hour)</td>
<td>0.13</td>
<td>0.27</td>
</tr>
<tr>
<td>1</td>
<td>Rat, morphine sulfate (50 mg/kg, +25 mg/kg per hour)</td>
<td>0.40</td>
<td>0.51</td>
</tr>
<tr>
<td>2</td>
<td>Rat, pentobarbital (80 mg/kg per hour)</td>
<td>0.04</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>Rat, awake (in vivo)</td>
<td>0.51</td>
<td>0.58</td>
</tr>
<tr>
<td>4</td>
<td>Rat, halothane (1.5%), gray matter</td>
<td>0.31</td>
<td>0.40</td>
</tr>
<tr>
<td>4</td>
<td>Rat, halothane (1.5%), white matter</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>Rat, halothane (1.5%), subcortical</td>
<td>0.18</td>
<td>0.21</td>
</tr>
<tr>
<td>5</td>
<td>Rat, halothane (2–3%)</td>
<td>0.22</td>
<td>0.26</td>
</tr>
<tr>
<td>5</td>
<td>Rat, halothane (2–3%), seizure</td>
<td>0.52</td>
<td>0.57</td>
</tr>
<tr>
<td>6</td>
<td>Rat, pentobarbital (120 mg/kg, +30 mg/kg per hour)</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td>Rat, halothane (1%)</td>
<td>0.58</td>
<td>0.61</td>
</tr>
<tr>
<td>7</td>
<td>Rat, halothane (2–3%)</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>8</td>
<td>Rat, awake (ex vivo)</td>
<td>0.49</td>
<td>0.54</td>
</tr>
<tr>
<td>9</td>
<td>Rat, ( \alpha )-chloralose (80 mg/kg, +27 mg/kg per hour)</td>
<td>0.16</td>
<td>0.25</td>
</tr>
<tr>
<td>10</td>
<td>Rat, urethane (1.5 g/kg)</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>11</td>
<td>Rat, pentobarbital (60 mg/kg), in vitro</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>11</td>
<td>Rat, morphine sulfate (15 mg/kg), in vitro</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>12</td>
<td>Rat, halothane (1%)</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>13</td>
<td>Rat, ( \alpha )-chloralose (80 mg/kg, +28 mg/kg per hour)</td>
<td>0.11</td>
<td>0.23</td>
</tr>
<tr>
<td>14</td>
<td>Human, awake</td>
<td>0.26</td>
<td>0.37</td>
</tr>
<tr>
<td>15</td>
<td>Human, awake</td>
<td>0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>16</td>
<td>Human, awake</td>
<td>0.32</td>
<td>0.36</td>
</tr>
<tr>
<td>17</td>
<td>Human, awake</td>
<td>0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>18</td>
<td>Human, awake</td>
<td>0.26</td>
<td>0.42</td>
</tr>
<tr>
<td>19</td>
<td>Human, awake</td>
<td>0.26</td>
<td>0.35</td>
</tr>
<tr>
<td>20</td>
<td>Human, awake</td>
<td>0.28</td>
<td>0.38</td>
</tr>
<tr>
<td>21</td>
<td>Human, awake</td>
<td>0.29</td>
<td>0.38</td>
</tr>
<tr>
<td>22</td>
<td>Human, awake</td>
<td>0.26</td>
<td>0.36</td>
</tr>
<tr>
<td>23</td>
<td>Human, awake</td>
<td>0.17</td>
<td>0.29</td>
</tr>
<tr>
<td>24</td>
<td>Human, awake</td>
<td>0.26</td>
<td>0.40</td>
</tr>
<tr>
<td>25</td>
<td>Human, awake</td>
<td>0.16</td>
<td>0.27</td>
</tr>
</tbody>
</table>

All human and rat data were localized to the visual and somatosensory cortices, respectively. Underlined values indicate mean values. All units are in micromoles per gram per minute (Fig. 2A).


Table S4. Experimental results of glucose oxidation in astrocytes 
[CMR$_{gly(ox),A}$] compared with total glucose oxidation [CMR$_{gly(ox)}$] measured by $^{13}$C MRS in rat and human brain

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Condition</th>
<th>CMR$_{gly(ox),A}$</th>
<th>CMR$_{gly(ox)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Rat, pentobarbital (80 mg/kg per hour)</td>
<td>0.04</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>Rat, awake (in vivo)</td>
<td>0.18</td>
<td>0.76</td>
</tr>
<tr>
<td>4</td>
<td>Rat, halothane (2–3%)</td>
<td>0.06</td>
<td>0.33</td>
</tr>
<tr>
<td>4</td>
<td>Rat, halothane (2–3%), seizure</td>
<td>0.07</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>Human, awake</td>
<td>0.05</td>
<td>0.41</td>
</tr>
<tr>
<td>6</td>
<td>Human, awake</td>
<td>0.07</td>
<td>0.42</td>
</tr>
<tr>
<td>7</td>
<td>Human, awake</td>
<td>0.07</td>
<td>0.43</td>
</tr>
<tr>
<td>8</td>
<td>Human, awake</td>
<td>0.08</td>
<td>0.37</td>
</tr>
</tbody>
</table>

All human and rat data were localized to the visual and somatosensory cortices, respectively. All units are in micromoles per gram per minute (Fig. 2B).

*For all rat studies, the full pyruvate carboxylase flux was used as a maximum estimate of CMR$_{gly(ox),A}$ (details in ref. 1).

†For all rat and human studies, CMR$_{gly(ox)}$ was estimated by adding neuronal and astrocytic glucose oxidation terms.