Abstract
We analyzed the metabolic response to cortical spreading depression that drastically increases local energy demand to restore ion homeostasis. During single and multiple cortical spreading depressions in the rat cortex, we simultaneously monitored extracellular levels of glucose and lactate using rapid sampling microdialysis and glucose influx using 18F-fluorodeoxyglucose positron emission tomography while tracking cortical spreading depression using laser speckle imaging. Combining the acquired data with steady-state requirements we developed a mass-conserving compartment model including neurons and glia that was consistent with the observed data. In summary, our findings are: (1) Early breakdown of glial glycogen provides a major source of energy during increased energy demand and leaves 80% of blood-borne glucose to neurons. (2) Lactate is used solely by neurons and only if extracellular lactate levels are >80% above normal. (3) Although the ratio of oxygen and glucose consumption transiently reaches levels <3, the major part (>90%) of the overall energy supply is from oxidative metabolism. (4) During cortical spreading depression, brain release of lactate exceeds its consumption suggesting that lactate is only a circumstantial energy substrate. Our findings provide a general scenario for the metabolic response to increased cerebral energy demand.

Keywords
Spreading depression, glucose, lactate, glycogen, rapid sampling microdialysis, positron emission tomography

Introduction
Understanding how the brain works and how its activity is supported metabolically has been a major challenge. In particular, the regulation of energy substrate fluxes during periods of increased energy demand, i.e. of functional activation, is still puzzling. There is notably controversial discussion about the main substrate(s) used for ATP production: whether blood-borne glucose is the sole energy substrate in most situations as long claimed,1 whether cellular trafficking of lactate provides a significant amount of ATP,2 or whether an under-estimated energy storage readily available in the form of brain glycogen plays an important role.3 If more than one pathway is presumably used, it is unclear under which circumstances, one or the other will be preferentially activated, what the time sequence of the different pathways to ATP production is, and whether or not there are cellular differences. To address these issues, there is a need for in vivo data that encompass several aspects of brain metabolism simultaneously, and in the same experimental model.

In this study, we analyzed changes in energy substrate fluxes induced by cortical spreading depressions (CSDs), which cause a massive increase in energy demand both under physiological conditions and after brain injury.4,5 A CSD is a transient simultaneous depolarization of all local cells—neurons and glia.6,7 Subsequent restoration of ion homeostasis is achieved by an increased activity of transmembrane ATPase ion pumps. This active ion transport is highly dependent on the delivery of energy substrates...
for production of ATP, and any reduction in blood supply compromises CSD recovery and prolongs the depolarization. Here, we combined rapid sampling microdialysis (rsMD) and laser speckle imaging (LSI) with positron emission tomography (PET) to simultaneously monitor extracellular glucose and lactate concentrations, local cerebral blood flow (CBF), and [18F]-2-fluoro-2-deoxy-D-glucose (FDG) uptake during single and multiple CSDs in the rat cortex. rsMD measures extracellular glucose and lactate concentration changes with a time resolution of 1 min. Total influx of glucose from blood to tissue can be derived from the time-dependent uptake of FDG quantitatively measured by dynamic PET. Local blood flow changes tightly coupled to CSDs can be measured using LSI at a sub-second time resolution. Combination of the methods provided information about temporal changes in total glucose influx (FDG-PET) and two compartments of the metabolic pathway (extracellular glucose and lactate, rsMD) while the timing of CSD occurrence was derived from LSI. Based on the combined and simultaneously acquired data and on steady-state conditions, we developed a mass-conserving compartment model with linear transfer constants that is able to explain the observed dynamic fluxes of energy substrates. The patterns of substrate fluxes during CSD provide a general realistic scenario for the response of cerebral tissue to any physiological increase in energy demand, i.e. to any type of cerebral functional activation that is ATP-demanding.

Materials and methods

Surgical procedures

All animal procedures were performed in accordance with the German regulations for animal protection (TierSchG, 2006) and were approved by the local animal care committee and local governmental authorities (LANUV NRW) and written up following the ARRIVE guidelines. Fifteen male Wistar rats (Janvier, France; weight: 300 to 420 g; age 9 to 12 weeks; housed in a temperature (22°C) and humidity (55±5%) controlled room pairwise in type-4 cages filled with Lignocel; experiments performed between 9 am and 4 pm) were anesthetized with isoflurane (induced with 5% and maintained with 1.5% to 2%) in 70%/30% N2O/CO2 gases, and LSI was started. The probe laid obliquely with full membrane length (2 mm) in cortex and was perfused at 1.6 μL/min with artificial cerebro-spinal fluid. Dialysate was analyzed for glucose and lactate by rsMD every minute. During pilot and sham experiments, we verified that the presence of the MD probe did neither disturb the propagation of CSD waves nor imaging with LSI or PET.

At the end of surgery, the animals were positioned on our multimodal imaging holder in the PET gantry. The thinned skull surface was covered with warm paraffin oil, and LSI was started. The MD probe was then slowly implanted (0.5 μm/min) using a piezo-electric motor (M-663, Physics Instruments, Germany) to minimize trauma. The probe laid obliquely with full membrane length (2 mm) in cortex and was perfused at 1.6 μL/min with artificial cerebro-spinal fluid. Dialysate was analyzed for glucose and lactate by rsMD every minute. During pilot and sham experiments, we verified that the presence of the MD probe did neither disturb the propagation of CSD waves nor imaging with LSI or PET.

After LSI baseline imaging and rsMD baseline measurements, propofol infusion was started at 38 mg/kg/h via tail vein. Isoflurane was weaned off over 15 min, and propofol concentration (33 to 53 mg/kg/h) was adjusted to keep breathing rate within 60 to 80 cycles/min. Thirty minutes after isoflurane was completely off, systemic variables, CBF and rsMD glucose and lactate concentrations had stabilized.

Two mCi FDG (0.5 mL) were then injected into tail vein. PET scans were started at the time of bolus tracer injection, and emission data were acquired for 90 min. At time t = 20 min after tracer injection, either a single CSD wave was induced by a needle prick (n = 8) or multiple CSD waves were induced by epidural application of 3 M KCl through a cotton ball for 70 min (n = 7) in the left hemisphere. LSI and rsMD measurements continued uninterrupted during the whole PET scan. Animals that showed non-physiological blood pressure or arterial blood gases, unstable CBF in the right hemisphere, or abnormal baseline rsMD concentrations of glucose and lactate were excluded from this study.

Data analysis

Simultaneous LSI and PET imaging were implemented as described elsewhere. Images were co-registered
using our in-house fiduciary marker and the procedure described elsewhere.\textsuperscript{12} The position of the MD probe was readily defined on the LSI images, and co-registered regions of interest for LSI (ROIs: 1.9 × 1.9 mm\textsuperscript{2}) and volumes of interest for PET (VOIs: 1.9 mm × 1.9 mm × 2.4 mm) were analyzed around the tissue sampled by the MD probe. ROIs and VOIs in the corresponding positions in the contralateral hemisphere were also analyzed (Figure 1(a)).

Speckle contrast and inverse correlation time (ICT) were calculated on a 7 × 7 pixel sliding window using previously published algorithms.\textsuperscript{11} Percent changes in CBF were calculated from a baseline ICT value averaged over 5 min prior FDG injection. With LSI, the propagation of the CSD waves on the cortex surface was tracked, as validated in many earlier studies with electrophysiological recordings.\textsuperscript{11,13} The typical CBF changes induced by one single CSD wave and by multiple CSD waves are plotted in Figure 1(d) and (b), respectively.

PET images were corrected for attenuation using transmission data from a \textsuperscript{57}Co source. After histogramming in time frames of 5 min and Fourier rebinning, emission PET data were reconstructed using two-dimensional filtered back-projection. FDG uptake as a function of time (time-activity curve, TAC) for each VOI was calculated by spatial averaging (Figure 1(a)). To investigate the changes in FDG uptake due to CSD propagation in the left hemisphere, we calculated the FDG uptake percent change in the ipsilateral VOI compared to its counter-part contralateral VOI in each animal. Each curve was then normalized by the contralateral VOI to exclude artifacts due to the variability in FDG injection. The resulting FDG uptake curves were then averaged over \( n = 8 \) animals for the single CSD group and \( n = 7 \) animals for the multiple CSDs group (Figure 1(c) and (d)).

rsMD data were processed using our noise-removal algorithms described in Feuerstein et al.\textsuperscript{14} Dispersion and time delay from the MD probe to the rsMD analyser were accounted for after a calibration in vitro. MD recovery was estimated in vivo by the no-net flux method at steady state (see details in Supplementary material S2). Percent concentration changes were then calculated given baseline concentrations during 5 min prior to CSD induction in each animal. The time-aligned concentration changes were then averaged across all animals within each respective experimental group.

Two-cell type model

We developed a numerical model to calculate substrate fluxes after CSD assuming that substrate kinetics can effectively be described by two cell types. Details, model equations, and further assumptions are given in the Supplementary material S1.

Statistics

All data are reported here as mean values ± standard error of the mean. For statistical inference only, a 5-min moving average was performed on the rsMD data. FDG normalized TACs, and rsMD glucose and lactate were then treated identically. To verify if all data points before CSD induction were not different from control value (zero), a one-sample \( t \)-test was performed at each negative time point. Since there was no significant difference in all cases, a one-way repeated measure analysis of variance (ANOVA) was performed on the time courses of FDG and rsMD for positive time points. Tests for normality (Shapiro–Wilk test) and equal variance (Levene median test) passed. ANOVA was followed by a multiple comparison versus control group (Holm–Sidak method). Statistical significance was inferred from a \( p \) value < 0.05.

Results

Metabolic response to multiple CSD waves—experimental data

CSD waves were visualized by LSI as hyperemic waves that propagated in the left hemisphere (Figure 1(a), bottom, solid line ROI). In total, 18 to 24 CSDs propagated during 70 min of KCl application, repeating within 1.7 to 5.2 min (Figure 1(b), bottom). The CSD time occurrence was taken as the average of the hyperemia onset times over all seven animals, ignoring the last 10 min where CSD waves did not propagate in some animals (Figure 1(c), bottom).

During KCl application, FDG uptake was strongly increased in the hemisphere ipsilateral to CSD propagation compared to the contralateral hemisphere (Figure 1(a), left hemisphere), rsMD glucose dropped significantly (Figure 1(b), red) and rsMD lactate increased for a prolonged period of time (Figure 1(b), green). In the contralateral hemisphere, CBF remained stable around baseline levels (Figure 1(b), bottom, dashed line). Under the assumption that glucose consumption also remains stable, FDG uptake in the contralateral hemisphere could be regarded as a “control” normal uptake (Figure 1(b), top, dashed line). Before CSD induction, the FDG TACs in ipsi- and contralateral hemispheres were similar (Figure 1(b), top), and differences in FDG uptake between both hemispheres were not significant (Figure 1(b), top, one-sample \( t \)-test: \( p > 0.05 \) for all data points before time \( t = 0 \)). As soon as the first CSD wave reached the ipsilateral VOIs (as seen per LSI, Figure 1(b), bottom,
Figure 1. Data acquired during CSD in the rat cortex and comparison to the model output. ((a), top) Horizontal PET image of FDG accumulation over 90 min. Multiple CSDs were induced 20 min after FDG injection. ((a), bottom) LSI image of the same rat while a CSD occurrence. (b) FDG activity (μCi/cc) over time. (c) Changes in lactate and glucose concentrations (%) over time. (d) Changes in CBF, rCBV, and rMMD concentrations (%) over time. (Continued)
Metabolic response to single CSD waves—experimental data

One CSD wave caused a transient hyperemia up to 183.9 ± 12.4% above control CBF (Figure 1(d), bottom). The blood flow levels remained slightly below baseline for the next hour (80% to 90% of baseline). The hyperemic wave corresponded to a transient elevation in FDG uptake in the ipsilateral hemisphere compared to the contralateral hemisphere (Figure 1(d), top). This elevation was small (+6.5 ± 2.1%) but significant (F(8,13) = 1.826, p = 0.047). The transient change was followed by a prolonged period of decreased FDG uptake in the ipsilateral hemisphere so that differences between ipsi- and contralateral hemispheres disappeared 32 min after the CSD wave. Similarly, rsMD glucose transiently dropped to a minimum of −16.4 ± 2.1% of baseline within the first 10 min of CSD induction (Figure 1(d), red). This was followed by a prolonged period of reduced glucose concentrations stabilizing around −10% of baseline during the next 45 min (significant effect of CSD on rsMD glucose, F(4,13) = 2.7, p = 0.022). Lactate showed a monophasic transient increase, reaching a maximum of 63.4 ± 14.5% above baseline at 6 min post-CSD induction before recovering to baseline concentrations within less than 20 min after CSD induction. Although we cannot account for changes in MD recovery during CSD, the concentration changes that we here observe are consistent with other reports. Autoradiography or bioluminescence measured a peak increase in lactate, within 2 min after SD elicitation, to 180% of lactate baseline levels. Here, our MD changes, after steady state in vivo recovery corrections, yielded a peak increase of 163% of baseline lactate level. This is, therefore, very similar to 180% measured by Selman et al. However, others have reported a fourfold increase of cortical lactate, suggesting that we might underestimate the lactate change during CSD, possibly because of a drop in microdialysis recovery during CSD. Furthermore, Mies and Paschen, as well as Selman et al., both measured cortical glucose concentrations drop of—40% to −50% below baseline values within the first 2 min after the CSD. We here measured −20% decrease, which is in the same order of magnitude but not identical. Would the microdialysis recovery of glucose dramatically drop during the CSD, the glucose concentrations we here report would underestimate the true extracellular glucose concentrations, and therefore amplify the concentration drop after the CSD. However, in comparison to reported values in literature, we seem to slightly overestimate glucose concentrations, thereby suggesting that the likely MD recovery decrease due to the CSD plays a minor role. Hence, the microdialysis concentrations measured here, and corrected by the in vivo recovery at steady state and in vitro dispersion, seem to mirror extracellular concentrations of glucose and lactate, even during CSD. However, we cannot exclude that there is some uncertainty and limitation to reliably quantify the relationship between MD concentrations and tissue concentrations.

Low extracellular glucose levels and subnormal uptake of FDG are consistent with decreased glucose consumption. Thus, it seems that some energy demanding cellular process is further affected by the CSD. The metabolic response was not a sufficient condition for the CSD propagation as reported in tissue slices and in vivo. However, glucose and lactate concentrations might be sensitive indicators of the CSD propagation and may help to understand the metabolic events during CSD.
processes ($P_x$ in our model) are impaired for $\sim$1 h after a single CSD. Supportive evidence for this hypothesis is discussed in details in Supplementary material S3.

**Concentrations of energy substrates during CSD—theoretical model**

Experimental data from multiple and single CSD could be explained by the two-cell type compartment model outlined in Figure 2(a). A detailed description of the theoretical model is available in the Supplementary materials S1 and S2. The output of the model is superimposed on the experimental data in Figure 1(c) and (d) and shows good agreement with all measurements. Resulting kinetic parameters and concentrations are listed in Supplementary Tables S1, S2, and S3. Consequently, concentrations of energy substrates varied with time during single and multiple CSD according to the functions plotted in Figure 2(b) and (c). Shown outputs were derived from our mass-conserving model that complies with observed steady-state concentrations and only reacts to changes in ATP concentrations (see details in Supplementary materials S1, S2). It entails the scenario of substrate fluxes during increased energy demand described hereafter.

**ATP consumption and production**

Supplying the brain with energy means preserving cellular ATP levels (Figure 2(b) and (c)). At steady state, ATP consumption of 5.6 mM/min (value in agreement with other studies under propofol anesthesia$^{19}$), 3.6 mM/min in neurons and 2.0 mM/min in astrocytes, is matched by ATP production from glycolysis and oxidative phosphorylation (6% vs. 94%)$^{20}$ (Figure 3, prior time $t = 0$).

After depolarization of cells by a single CSD, ATP utilization rapidly shoots up (to 10 mM/min ATP in total) to provide energy for active transmembrane ion pumps (described by $P_x$, see Supplementary material S1). Glycogen breakdown is the first process that provides an essential amount of ATP: within the first 25 s after a CSD, glycogen breakdown contributes to up to 30% of astrocytic ATP formation (Figure 3(b)). Neuronal ATP production peaks only at 45 s post CSD, via increased glycolysis (to a maximum of 18% of neuronal ATP production), followed by increased glucose oxidation.

After recovery from a single CSD, the energy demand for restoration of ion homeostasis returns to normal within 4 min, which is in agreement with observed normalization of intracellular calcium levels.$^{21}$ However, the total ATP use remains reduced for up to $\sim$1 h, due to a persistent shut down of energy demanding processes (described by $P_x$ in Supplementary material S1). The tissue reacts to this state of lower energy demand by reducing the net inflow of glucose to the brain ($K_1$) and reducing ATP production via hexokinase activity ($k_3$ in the model) and via the tricarboxylic acid (TCA) cycle ($k_{OXY}$ in the model, see Supplementary material S1).

![Figure 2. Two-cell type model of brain metabolism.](image-url)

**(a)** Sketch of simplified glucose and lactate pathways in cerebral tissue. Contributions of reactions to the ATP level are indicated in red. (b) Time courses of concentrations during multiple and (c) single CSD predicted by the model. From top to bottom these are: extracellular lactate ($C_L$) and glucose ($C_E$), glycogen ($C_G$), glucose-6-phosphate ($C_{6P}$), and ATP ($\text{ATP}$). Intracellular concentrations are plotted in dashed lines for neurons, in dotted lines for astrocytes while solid are for total. CSD: cortical spreading depression.
such that the net ATP production matches the consumption (Figures 3(b) and 2(c)). A new steady state of decreased energy demand is reached at ~40 min when glycogen storages have been refilled (Figure 2(c)).

In the case of multiple CSDs, the second CSD hits the tissue before the state of low energy demand is reached and again causes an increase in energy demand, which again stimulates increased ATP production via increased glycogen breakdown followed by glycolysis and oxidative phosphorylation (Figure 3(a)). Each CSD reduces the available amount of glycogen, leading to a continuous depletion of glycogen stores (Figure 2(b)). As a consequence, the contribution of glycolysis to peak astrocytic ATP production rises from 8% after the first CSD to 16% after the 16th CSD (compared to 6% at rest), and the peak astrocytic ATP production rate decreases from 4.3 to 3 mM/min when the contribution of glycogen is negligible. In contrast, the peak neuronal ATP production rate is more stable, peaking between 5.7 and 6.5 mM/min in the acute CSD phase, with glycolysis accounting for 18% for the first CSD and 15% for the 10th CSD. In both neurons and astrocytes, ATP is predominantly produced by oxidative phosphorylation (Figure 3).

**Oxidative vs. non-oxidative metabolism**

Using our model, we calculated the time courses of the cerebral metabolic rate of oxygen (CMRO$_2$) (as the amount of oxidatively metabolized glucose multiplied by six) and CMR$_{glc}$ (rate of phosphorylation of blood-borne glucose to glucose-6-phosphate) during CSD. During the very first minute of increased ATP demand, the CMRO$_2$/CMR$_{glc}$ ratio transiently decreases (<3 in our case, Figure 6). This would be exacerbated in conditions where oxygen supply is limited, as observed by Takano et al.23 in the mouse cortex during a CSD. However, when glucose-6-phosphate ($C_{nM}$ and $C_{aM}$, Supplementary material S1) is oxidized, the CMRO$_2$/CMR$_{glc}$ ratio returns to normal and even exceeds the value of ~6 (Figure 6(b)).

Furthermore, we could assess the relative contribution of non-oxidative glycolysis vs. oxidative phosphorylation to the production of ATP. In baseline conditions, 92% of total $C_M = C_{nM} + C_{aM}$ are used for oxidative ATP production in the TCA cycle. Overall, integrated over a 10-min period after a single CSD, only 67% of $C_M$ enters the TCA cycle while at the same time a noticeable amount of lactate is released, indicating a shift from oxidative to non-oxidative ATP production.

**Figure 3.** ATP consumption and production rates in neurons and astrocytes during multiple (a) and single CSD (b). Positive values indicate production, negative values consumption of ATP. Processes are added up, so that the colored area represents the amount of ATP produced/consumed by each process and the envelope shows the total ATP production/consumption. ATP is consumed for restoration of ion homeostasis via transmembrane ion pumps ($P_{eg}$), processes that are shut down for some time after CSD ($P_x$), and processes that are not influenced by CSD ($P_{bg}$). ATP is produced by oxidative metabolism, glycolysis, and glycogen breakdown (in astrocytes only). Only the first 10 min after a single CSD are shown in (b), since the new steady state lasts for the subsequent 50 min. All following figures will also focus on these first 10 min after a single CSD. CSD: cortical spreading depression.
production (Figure 4). However, detailed analysis of our data indicates that due to the different efficiencies of glycolysis and oxidative metabolism (2 vs. 29 mol ATP/mol glucose), the changes of pathways have only minor consequences for the sources of ATP production: during CSD 92.4% of ATP is produced via oxidative metabolism vs. 93.5% in baseline conditions. Thus, even when energy demand is high, as caused by CSD, the predominant fraction of ATP is produced by oxidative metabolism.

**Sources and sinks for oxidative ATP production**

Glucose-6-phosphate ($C_M$ in our model, Figure 2 and Supplementary material S1) plays a pivotal role as branch point in the glucose pathway with several alternative metabolic fates: it can be substrate for generation of ATP by oxidative phosphorylation, substrate for release and/or use of lactate, and substrate for synthesis of glycogen. Additionally, glucose-6-phosphate can be used to generate NADPH or precursors for nucleic acid biosynthesis in the pentose phosphate pathway. However, the major fraction of glucose-6-phosphate entering this pathway is recycled to be used for energy production. In our model, we assume that the portion of glucose lost in the pentose phosphate pathway remains constant during CSD (included in the background energy, $P_{bg}$, Supplementary material S1) (see Figure 3). Apart from blood-borne glucose, lactate and glycogen are potential sources for the precursor pool for oxidative ATP production in the TCA cycle. In order to quantify their relative contributions, we analyzed the sources of $C_M$ (Figure 4). During baseline conditions, blood-borne glucose is the sole source for $C_{aM}$ in astrocytes and the major source for $C_{nM}$ (89%) in neurons. In both cell types, >90% of $C_M$ are used for ATP production by oxidative phosphorylation and <10% of $C_M$ are transformed to lactate (presumably from cellular parts with limited access to mitochondrial ATP).20,24,25

In response to a single CSD, there is an increase in neuronal $C_{nM}$ production to nearly 400% of baseline. In astrocytes, $C_{aM}$ production even increases to 800% of baseline but here the main source is glycogen (71% at the peak of $C_{aM}$ production). Note that each glycogen molecule contributes to ATP production in two ways, first by glycogen breakdown (Figure 3) and second by oxidative phosphorylation (Figure 4). A reduction of brain glycogen content during CSD is consistent with other reports.26,27 Breakdown of glycogen in astrocytes leaves more blood-borne glucose to neurons: at the peak, 80% of total blood-borne glucose influx contribute to neuronal $C_{nM}$ (Figure 4(b)). The rapid increase in ATP consumption in regions with limited access to mitochondrial ATP causes a rise in glycolysis and subsequent lactate production.

Figure 4. Sources and sinks of glucose-6-phosphate ($C_M$), the branch point between oxidative energy production (ATP) and other fates of glucose during multiple (a) and single CSD (b). Sources for $C_M$ (positive values) are: blood-borne glucose, lactate (only in neurons), and glycogen (in astrocytes only). Sinks for $C_M$ (negative values) are: ATP, lactate, and glycogen. CSD: cortical spreading depression.
Once cellular ATP levels have been restored, glycogen breakdown stops, lactate production gradually returns to normal, and glycogen stores are totally refilled within 30 min (Figure 2(c)).

In the case of multiple CSDs, the contribution of glycogen to astrocytic CaM production gradually decreases as the stored amount of glycogen decreases with each CSD (Figure 2(b)). As a result, blood-borne glucose becomes gradually the main source of CaM, from 29% to 94% of the total CaM production between the first and last CSD. In response to the 18th CSD, 60% of the total blood-borne glucose influx is used by the neurons, approximately matching the neuron/astrocyte volume ratio in rats (Figure 4(a)). To compensate for the lesser extent of blood-borne glucose available, neurons resort to another source for their ATP production: lactate. However, this usage of lactate, solely by neurons, takes place under particular conditions detailed below.

**Lactate production, removal, and utilization**

During baseline conditions, a small amount of lactate (0.032 mM/min) is transported from blood to brain, taken up by neurons, and fully oxidized to CO₂. At the same time, 9% of the blood-borne glucose is transformed to lactate and is cleared from brain tissue into the blood stream (at a rate of 0.036 mM/min, Figure 5) causing a small net release of lactate (0.004 mM/min). After CSD, energy need is elevated (in regions with limited access to mitochondrial ATP as well) and lactate release from both neurons and astrocytes increases: more than 50% of CaM are transformed to lactate (Figure 4). In the case of a single CSD, the increased extracellular lactate is cleared into the blood stream at a fast rate of ~0.3 mM/min (Figure 5(b)) and lactate levels return to normal after ~15 min (Figure 1(c) and 2(c)).

In the case of multiple CSDs, another surge for ATP (due to a CSD again spreading into this region of the brain) occurs at a time when extracellular lactate concentration is still at ~180% of baseline. In that case, neurons take up and use lactate as an additional energy substrate (Figure 4(a)). Lactate can then yield up to 70% of total neuronal glucose-6-phosphate (CaM). Simultaneously, neurons also release lactate and, on average, they produce more lactate than they consume, even during times of increased energy demand (Figure 5(a), bottom).

The time course of lactate fate is shown in Figure 5, where sources and sinks of extracellular lactate,
together with neuronal release and consumption of lactate are plotted.

Discussion

Measured data could be explained only if the following three requirements are met: (1) glycogen is rapidly metabolized to ATP in astrocytes, thus saving more blood-borne glucose for neurons, (2) astrocytes do not consume lactate, (3) neurons do consume lactate but only if extracellular lactate levels are more than 80% above normal. This theoretical framework also provides insight into how anaerobic glycolysis and oxidative phosphorylation are coupled during both increased and reduced energy demand.

The role of lactate in brain metabolism is subject of an ongoing discussion. Lactate is produced from glucose via anaerobic glycolysis. Lactate can then either be oxidized to pyruvate and as such used for oxidative ATP production or washed out of the brain via the blood stream (presumably with the support of astrocytes). In order to explain our measurements of substrate concentrations during CSD (Figure 1), we had to assume that neurons resort to lactate consumption only at highly elevated extracellular lactate levels. This hypothesis is supported by many other in vivo data.

There is indeed strong evidence that the brain switches from a net producer to a net consumer of lactate provided that plasma lactate levels are high. Quistorff et al.30 reviewed studies that measured arterio-venous differences of lactate concentrations during physical exercise and accordingly, during periods of elevated arterial lactate concentrations. In all studies cited in their review, net uptake of lactate by the brain occurred only when arterial plasma levels were high (>3 mM compared to 1 mM under normal conditions). This is a strong indication that, under conditions of increased blood lactate levels, lactate could be used as an alternative oxidative fuel, as stated by Boumezeur et al.31 “plasma lactate may be a significant net fuel for brain metabolism under conditions in which plasma lactate is elevated.” This would result in sparing glucose utilization, which is corroborated by reductions of cerebral metabolic rate of glucose (CMRglc) observed by Wyss et al.32 when plasma lactate levels were elevated by more than 300%. In the present study, we did not measure the arterial plasma lactate level. The amount of cerebral lactate release during CSD is not sufficient to modify arterial plasma lactate levels.33 But we measured significant increases of the cerebral extracellular lactate level during CSD. Given that there is a positive correlation of plasma and brain lactate levels, as observed by magnetic resonance spectroscopy,31 and that at high plasma lactate levels there is an instantaneous equilibration between plasma and brain lactate,34 the assumption that

Figure 6. Oxygen consumption rate (CMRO2), glucose consumption rate (CMRglc), and ratio of oxygen to glucose consumption rates as a function of time during multiple (a) and single CSD (b). Different timings in glucose and oxygen consumption cause a transient decrease in the ratio and a later increase above the theoretical value of 6. CSD: cortical spreading depression; CMR: cerebral metabolic rate.
utilization of lactate is driven by the extracellular level of lactate is consistent with the conclusions based on increased plasma lactate levels. In particular, our model predicts that neurons consume lactate only when extracellular lactate levels in brain tissue are elevated to ~180% of the baseline. Even then, however, neurons produced more lactate than they used during the period of increased energy demand by CSD. This could be the reason for eluding direct in vivo evidence of neuronal use of lactate up to now.

Furthermore, although in our model at the beginning we did not assume that astrocytes could not use lactate, the measured data were not consistent with astrocytic lactate consumption, which agrees with in vivo observations of Bouzier et al. This does not imply that there is no uptake of lactate by astrocytes, but only that astrocytes do not use lactate as energy substrate. Astrocytes do probably contribute to lactate clearance from the brain. In our model, this process is part of the removal of lactate from brain to blood ($k_L$, see Figure 2(a)). In agreement with our results, Cruz et al. also reported a net efflux of lactate from brain during CSD and Ackermann and Lear during activation. Thus, under normal conditions, i.e. at normal plasma and/or extracellular lactate levels, the brain is a net producer of lactate.

Another intriguing aspect of brain metabolism has long been the changes in the CMRO$_2$/CMR$_{glc}$ ratio during activation. The theoretical value of 6 has been either not met or exceeded, thus leading to opposite conclusions that either anaerobic glycolysis or oxidative phosphorylation is the main process for ATP production. Full oxidative phosphorylation includes two steps, one taking place after the other: first, glycolysis (and glycogenolysis in astrocytes) to produce glucose-6-phosphate, and second, oxidative phosphorylation of glucose-6-phosphate in the TCA cycle. During the first step, CMR$_{glc}$ is increased while CMRO$_2$ remains unchanged; during the second step, CMRO$_2$ is increased. Since the first step occurs temporally before the second, the CMRO$_2$/CMR$_{glc}$ ratio is transiently decreased (in accordance with the observations of Fox et al.). Note that oxygen supply was not limited in the model and therefore the observed CMRO$_2$/CMR$_{glc}$ ratio decrease was not due to limited oxygen availability but due to limited glucose (i.e. pyruvate) availability for oxidative phosphorylation by the mitochondria. Thereafter, the CMRO$_2$/CMR$_{glc}$ ratio transiently increased (as observed by Madsen et al.). This time course is well described by our model and we conclude that determination of the CMRO$_2$/CMR$_{glc}$ ratio after activation critically depends on the time of the measurement, which can explain the apparent contradictive results found in the literature.

Despite this temporal aspect, we clearly find that, overall, i.e., when considering the whole period of increased energy demand, oxidative phosphorylation is the main process for ATP production, in agreement with other reports. The relative amount of anaerobic glycolysis compared to oxidative phosphorylation is in fact, during periods of increased energy demand, very close to that during resting conditions. This indicates that energy supply, both during large energy demand and at rest, is primarily covered by oxidative metabolism even if the balance is transiently shifted to anaerobic glycolysis.

Finally, regulation of cerebral energy supply not only involves responses to short periods of increased demand but also includes the adjustment of substrate fluxes to match supply and demand during longer periods of altered cellular activity. After a single CSD we observe a state of reduced energy demand that lasts for about 60 min (thorough discussion of this result can be found in Supplementary material S3). Since glycogen stores are refilled at 40 min, this time is long enough for a new steady state of substrate fluxes to evolve. The comparison of the rate constants before and 45 min after CSD indicates that a reduction of energy demand (here by 23%, $P_o$) is primarily accompanied by reduction of glucose transport from blood to tissue ($K_1$ is reduced by 25%) and only secondarily by reduction of hexokinase activity ($k_3$ is reduced by 7.6%) and oxidative ATP production ($k_{OXY}$ is reduced by 1.5%). A direct consequence of this scenario is the decrease of extracellular glucose levels, which was directly measured using microdialysis (Figure 1d). The reduction of glucose transport from blood to tissue is further supported by a reduction of blood flow (Figure 1d). This leads to the conclusion that substrate fluxes, during times of reduced energy demand, are predominantly regulated by glucose transporters, while hexokinase activity controls the increase in energy supply during transient periods of elevated demand.

**Modeling of brain metabolism**

Brain metabolism critically depends on species, anesthesia, body temperature, blood pressure, plasma glucose and lactate concentrations, etc. and moreover shows high spatio-temporal variations. Furthermore, it is difficult to translate in vitro findings to the in vivo situation. The experimental data that constitute the basis of our model were recorded simultaneously in vivo, thereby reducing any uncertainties in space, time, and animal conditions. In our approach, we aimed to explain the experimental data with a model as simple as possible and as complex as necessary, i.e. with the lowest number of parameters. We started with single-cell-type model (with and without glycogen) and failed to explain the data. A two-cell type model without glycogen also failed. The successful version includes
two cell types, one with glycogen stores. Our findings, in terms of glycogen, mostly agree with the conclusions by DiNuzzo et al.28,41 astrocytic consumption of glycogen during activation alters glucose kinetics such that more blood-borne glucose is preserved for neurons.

Other models of brain metabolism have been published, supporting and disapproving lactate shuttling from astrocytes to neurons, considering or neglecting glycogen. Compared to our version these models have a much higher complexity with a much higher number of model parameters and most importantly experimental input to these models was derived from various in vivo and in vitro data performed in different conditions (see literature42–44). Our model derives from one single complete set of data. It conserves mass fluxes, complies with steady-state conditions, and reacts self-consistently to changes in ATP levels. Thus, it depicts a relatively simple but realistic scenario of substrate fluxes during increased energy demand.

**Conclusion**

We combined in vivo experimental data with a mass-conserving theoretical model to describe the metabolic response to CSD quantitatively. This led to significant conclusions about brain energy metabolism in general. The roles of oxidative phosphorylation relative to non-oxidative metabolism of glucose critically depend on the time point of the response to increased ATP demand. Rapid production of ATP in response to instant increased need is primarily controlled by hexokinase and mitochondrial breakdown of glucose, whereas down-regulation of ATP production in conditions of lower needs is mostly controlled by reduced blood-to-brain transport. Glycogen supports astrocytic energy supply to spare blood-borne glucose for neurons. Lactate is used to produce ATP only by neurons, and only provided extracellular lactate levels are highly increased.

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**Authors’ contributions**

D Feuerstein performed experiments, analyzed data, developed theoretical model, wrote manuscript. H Backes developed theoretical model, performed model calculations, analyzed data, wrote manuscript. M Gramer performed experiments, developed setup for simultaneous speckle/PET data acquisition, analyzed data. M Takagaki performed surgery and experiments. P Gabel performed experiments. T Kumagai performed surgery and experiments. R Graf designed experiments.

**Supplementary material**

Supplementary material for this paper can be found at http://jcbfm.sagepub.com/content/by/supplemental-data

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