Avian neurons consume three times less glucose than mammalian neurons

**Highlights**

- Brain tissue of awake pigeons consumes $27.29 \pm 1.57 \, \mu\text{mol glucose per 100 g per min}$
- This is equal to $1.86 \times 10^{-9} \pm 0.2 \times 10^{-9} \, \mu\text{mol glucose per neuron per minute}$
- The neuronal energy budget of pigeons is thus about $3 \times$ lower compared to mammals
- This possibly indicates more efficient neuronal processing in the avian clade

**Authors**

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**In brief**

In comparison to mammals, neuron densities in the avian brain are high, opening up the question of how birds can metabolically support their large neuron numbers. Von Eugen et al. show here that the neuronal energy budget of pigeons is about $3 \times$ lower compared to mammals, possibly indicating a more efficient neuronal processing in the avian clade.
Brains are among the most energetically costly tissues in the mammalian body. This is predominantly caused by expensive neurons with high glucose demands. Across mammals, the neuronal energy budget appears to be fixed, possibly posing an evolutionary constraint on brain growth. Compared to similarly sized mammals, birds have higher numbers of neurons, and this advantage conceivably contributes to their cognitive prowess. We set out to determine the neuronal energy budget of birds to elucidate how they can metabolically support such high numbers of neurons. We estimated glucose metabolism using positron emission tomography (PET) and 2-[18F]fluoro-2-deoxyglucose ([18F]FDG) as the radiotracer in awake and anesthetized pigeons. Combined with kinetic modeling, this is the gold standard to quantify cerebral metabolic rate of glucose consumption (CMRglc). We found that neural tissue in the pigeon consumes 27.29 ± 1.57 μmol glucose per 100 g per min in an awake state, which translates into a surprisingly low neuronal energy budget of 1.86 × 10^-9 ± 0.2 × 10^-9 μmol glucose per neuron per minute. This is approximately 3 times lower than the rate in the average mammalian neuron. The remarkably low neuronal energy budget explains how pigeons, and possibly other avian species, can support such high numbers of neurons without associated metabolic costs or compromising neuronal signaling. The advantage in neuronal processing of information at a higher efficiency possibly emerged during the distinct evolution of the avian brain.
plasma glucose levels (see STAR methods for details of the mathematical modeling).

**[18F]FDG distribution and uptake in the pigeon brain**

Quantitative CMR<sub>glc</sub> assessment was executed in 4 awake and 6 isoflurane-anesthetized pigeons. The injected dose of [18F]FDG did not differ between the awake and anesthetized group (t(8) = 0.255, p = 0.805) and ranged from 53.9 to 70.4 MBq, with an average of 65.39 ± 4.70 MBq. Post-scan venous glucose levels (12.40 ± 1.68 mmol/L) were within the normoglycemic range for birds. Raw parametric images showed that [18F]FDG was taken up and distributed throughout the entire brain in both the awake (Figure 2A) and anesthetized state (Figure S1).

To determine the AIF, continuous arterial blood was automatically sampled during the first 2 min and manually sampled at specific time intervals for the rest of the scan (Figure 2B). The modeling approach showed a good fit between the TAC and fitted TAC (Figure 2C). The residual differences (Figure 2C, inset) at almost all points were below ± 5% (see Figure S2 for the arterial input function, TAC, and fitted TAC of all individuals in both the awake and anesthetized state).

**Assessment of a pigeon-specific lumped constant**

The lumped constant (LC) describes differences in the kinetics of glucose and [18F]FDG. It is a crucial variable in the model, and some variation is present in the mammalian literature. This variation has been ascribed to methodological differences, specificities of species, or disease state. Following Backes et al., here we employed a model-dependent method in which the local LC is calculated from the rate constants of [18F]FDG. It elaborates on the idea that disentangling transport and phosphorylation, which are lumped in the LC, is more accurate than the application of a whole-brain LC. The LC can be decomposed into the underlying processes it describes, i.e., transport and phosphorylation, and the difference in efficiencies between [18F]FDG and glucose of these processes can be broken up into the following: L<sub>1</sub> = K<sub>1,FDG</sub>/K<sub>1,glc</sub>, L<sub>2</sub> = k<sub>2,FDG</sub>/k<sub>2,glc</sub>, and L<sub>3</sub> = k<sub>3,FDG</sub>/k<sub>3,glc</sub>. In humans and rats, the values of the separate ratios were found to be conserved across species and have been estimated at L<sub>1</sub> = 1.48, L<sub>2</sub> = 1.48, and L<sub>3</sub> = 0.37. The local LC in each individual pigeon can then be calculated as a function of the pigeon-specific local kinetic rate constants (see Figure 1B for the LC formula). We found that the LC values did not differ between the awake and anesthetized group (t(7) = 1.471, p = 0.185) and averaged 0.69 ± 0.037. This value is at the high end of the range employed in mammalian studies (0.45–0.71).

**Possible caveats of this approach**

This approach does assume that the kinetic rate differences of glucose and [18F]FDG for transport (L<sub>1</sub> and L<sub>2</sub>) and phosphorylation (L<sub>3</sub>) are comparable between pigeons and rats/humans. An empirical assessment of these variables would in theory be...
Figure 2. Example of [18F]FDG distribution in the awake brain, arterial input function, and model fit
(A) Cerebral [18F]FDG activity fused with a structural magnetic resonance (MR)-image in the awake state with schematic overview of the brain. Dotted lines indicate the exact plane in frontal (left), transversal (middle), and sagittal (right) sections. The heat map represents standardized uptake value, corrected for body weight (SUVbw = image activity [Bq/ml] x bw [g] / injected dose [Bq]), with red indicating high and blue low levels of uptake. The distribution in the awake state indicates relatively higher levels in the pecten oculi (not visible in these sections) and retina, as well as entopallium, which is one of the two primary visual pallial areas in birds.
See Figure S1 for the anesthetized state and Figure S3 for average CMRglc values for forebrain, cerebellum, and the rest of brain in both awake and anesthetized state.
(B) Representative arterial input function (AIF) in the awake state. Following an intravenous (i.v.) injection, the AIF shows an initial sharp peak captured by the automatic sampling device (AIF auto). The peak quickly drops and slopes off to a plateau, captured by manual sampling at later time points (AIF manual). The inset depicts the initial 5 min.
(C) Example of a model fit in the awake state. The two-tissue compartment model fit (dotted line) corresponds well to the time-activity curve of the whole brain (black triangles). The inset depicts residual differences in percentage, where, except for the first time point (~27%), all fits differ less than ± 5%.
See Figure S2 for the AIF, TAC, model fit and residuals for all pigeons. A, arcopallium; AC, anterior commissure; BS, brainstem; CN II, second cranial nerve (optic nerve); Cb, cerebellum; E, entopallium; GP, globus pallidus; H, hyperpallium; HB, hindbrain; Hp, hippocampus; LS, lateral striatum; M, mesopallium; MB, midbrain; MS, medial striatum; N, nidopallium; OT, optic tectum; Re, retina; Ro, nucleus rotundus; RH, right hemisphere; Th, rest of thalamus.
The local glucose consumption rate, expressed as $CMR_{glc}$, was calculated from the metabolic rate constant $K_i$ divided by the local LC and multiplied by the post-scan venous plasma glucose level. There was no significant difference between the awake and anesthetized state for whole brain $CMR_{glc}$. t(8) = 1.648, p = 0.069, with $27.29 \pm 1.57 \mu mol/100 \text{ g/min}$ in the awake state compared to $23.15 \pm 4.77 \mu mol/100 \text{ g/min}$ in the anesthetized state. This was surprising since in mammals, anesthesia has been found to have a diminishing effect on cerebral glucose consumption.\(^4\) One explanation could be that in these mammalian studies, only parts of the brain were analyzed as opposed to the whole brain. Indeed, when we analyzed the $CMR_{glc}$ of separate brain regions, significant effects of anesthesia were found (Figure S3). The mass-specific $CMR_{glc}$ is comparable to what has been found in humans ($26.08 \pm 1.78 \mu mol/100 \text{ g/min}$,\(19,24,25\)) and almost three times as low compared to the similarly body- and brain-sized rat ($68.00 \pm 4.00 \mu mol/100 \text{ g/min}$,\(26–28\)).

### The neuronal energy budget in the awake pigeon

To relate the calculated $CMR_{glc}$ of the pigeon to the mammalian neuronal energy budget, glucose consumption of the whole brain in the awake state was divided by the known number of neurons in the pigeon brain.\(^7\) Of course, the brain does not only consist of neurons but is a mosaic of neuronal and non-neuronal cell types that require energy in the form of glucose for both active processes and housekeeping costs.\(^4,29,30\)

In our analysis, we only included neurons, since most of the cerebral energy budget (70%–80%) has been estimated to support neuronal signaling costs.\(^4,29,30\) The determined budget can thus be understood as the glucose needed to support one neuron either directly or indirectly.\(^7\) This large contribution became evident when we estimated the glucose budget over both neurons and glial cells, revealing a highly similar pattern (Figure S4).

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**Table 1. K-parameters, LC, and $CMR_{glc}$ for whole brain per subject**

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<th>Pigeon ID</th>
<th>$K_1$ (1/min)</th>
<th>$k_2$ (1/min)</th>
<th>$k_3$ (1/min)</th>
<th>$k_4$ (1/min)</th>
<th>$K_i$ (mL/g/min)</th>
<th>LC</th>
<th>$CMR_{glc}$ (µmol/100 g/min)</th>
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<tr>
<td>Mean ± SD</td>
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<td>0.15 ± 0.0046</td>
<td>0.065 ± 0.0050</td>
<td>0.012 ± 0.0026</td>
<td>0.018 ± 0.00078</td>
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<td>877</td>
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<tr>
<td>Mean ± SD</td>
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<td>0.14 ± 0.019</td>
<td>0.059 ± 0.013</td>
<td>0.017 ± 0.0070</td>
<td>0.012 ± 0.0017</td>
<td>0.68 ± 0.040</td>
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The 2-compartment model generates four kinetic rate constants ($K_1$, $k_2$, $k_3$, and $k_4$) that describe the metabolic process of [18F]FDG. $K_i$ describes the net influx of [18F]FDG. The lumped constant (LC) describes the difference in kinetics of [18F]FDG and glucose metabolism. The outlier 645(*) of the anesthetized group was excluded from further analysis of the LC. The final cerebral metabolic rate of glucose ($CMR_{glc}$) can be calculated from the kinetic rate constants. See Table S1 for an overview of K-parameters and $CMR_{glc}$ calculated with varying blood volume percentage of the brain.
Cell size is a key factor in the metabolism of any neuron and correlates positively with energy consumption. Though no systematic survey has ever been conducted in birds, there is support for the idea that the average avian neuron size is smaller than in mammals. As mentioned above, neuron densities in the avian brain are much higher compared to similarly sized mammals, and this likely translates into small neuronal sizes and short interneuronal distances. Indeed, a mathematical approach demonstrated that neuron and non-neuronal cell density could be used as an indicator of cell size, where higher density corresponds to smaller neuron sizes. Moreover, from comparison between the macaque and mouse, it is known that some specific neuron types indeed scale positively with increasing brain size. A smaller neuron is more energy efficient in a variety of ways. For example, in line with the reduced membrane surface area and cytoplasmic volume, smaller neurons accommodate fewer receptors, ion channels, and mitochondria. They are also characterized by a lower membrane capacitance. Lastly, the overall lower number of components translates into reduced maintenance and housekeeping costs. Thus, smaller neuron sizes could explain, at least in part, the lower metabolic costs we observe in the pigeon brain.

Next to neuron size, another stark difference between birds and mammals is the higher body, and thus brain, temperature in birds. In pigeons, the core brain temperature measures 40°C–42°C compared with 36°C–37°C in the rat brain. The effect of temperature on behavior and neural activity is a widespread phenomenon, and it influences multiple cellular components and dynamics, including the resting membrane potential, generation of action potentials, synaptic transmission, and axon conduction velocity. For example, increases in temperature of 1.5 degrees have been recorded in rat hippocampus during active exploration, in concordance with changes in the waveform of action potentials. These interactions have also been found in birds. In zebra finches, the cooling of specific song-related brain nuclei had a causal decreasing effect on song tempo. This is not just an effect of experimental manipulation, because zebra finches show natural temperature fluctuations related to a diurnal cycle and social context; in correlation to

Potential factors explaining the reduced neuronal energy budget

There is ample evidence that any increase in size of parts of or the whole nervous system evolved under a selection pressure to reduce energy consumption. This can be achieved by either reducing the costs of signaling itself, with alterations in the biophysical properties of cells and circuits, or with alternative coding strategies. Understanding how energy efficiency is obtained within the brain starts with collecting detailed neuroanatomical and neurophysiological data such as neuron size, firing rates, ion channel kinetics, membrane capacitance, etc. Based on these data, computational models can test theoretical predictions of optimal energy efficiency under specific conditions. Currently, most of the crucial data are lacking for birds. This highly complicates identifying any underlying mechanism for the low neuronal energy costs. Based on what is known, we can identify two potential contributing biophysical properties that differ in birds compared with mammals: namely, neuron size and brain temperature.

Cell size is an astonishing 3.11 times lower than the average mammalian neuron. For example, neuron densities per mg are plotted on the x axis and μmol glucose consumption per 100 g per minute on the y axis, thus the graph represents CMRglc per neuron across different mammalian species and the pigeon. All mammalian species fall on the same regression line that can be described by a linear function $5.76 \times 10^{-6} X + 5.62 \times 10^{-9}$ (R² = 0.9499, p = 0.005). This corresponds to an average CMRglc per neuron of $5.79 \times 10^{-9} \pm 0.76 \times 10^{-9}$. In contrast, in the pigeon, a neuron consumes on average $1.86 \times 10^{-9} \pm 0.2 \times 10^{-9}$, which is 3.11 times lower than the average mammalian neuron. See Figure S4 for CMRglc per cell (neuronal and glial cells combined) in the whole brain across species. See Table S2 for neuronal densities, CMRglc, and the neuronal energy budget per species. CMRglc data were available for mouse, rat, baboon, and human, and combined with estimated neuron numbers. Silhouettes were obtained from http://phylopic.org under public license.

findings to mammalian data that were either collected with the same $^{18}$F-FDG-PET method as we employed or by autoradiography using the radioactively labeled glucose analog 2-deoxy-D-14C-glucose ($^{14}$C)DG). Since the CMRglc in both approaches was modeled using the same two-tissue compartment model, and several studies found no or only minor differences between the two methods, we assume that both datasets are directly comparable to our findings.

Since we found that pigeon brain tissue requires $27.29 \pm 1.57 \mu$mol glucose per 100 g per min, this translates as $1.86 \times 10^{-9} \pm 0.2 \times 10^{-9} \mu$mol glucose per neuron per minute. This is an astonishing 3.11 times lower than the mammalian whole-brain neuronal energy budget of $5.79 \times 10^{-6} \pm 0.76 \times 10^{-9} \mu$mol glucose per neuron per minute (Figure 3). This finding explains how pigeons, and possibly other avian species, are able to sustain almost twice as many neurons as a similar sized mammal, without the associated metabolic costs. What could be the underlying mechanistic explanation for this reduction in the energy budget?

Figure 3. Scaling of mean-specific CMRglc and neuron density in the whole brain across species

Neuronal densities per mg are plotted on the x axis and μmol glucose consumption per 100 g per minute on the y axis, thus the graph represents CMRglc per neuron across different mammalian species and the pigeon. All mammalian species fall on the same regression line that can be described by a linear function $5.76 \times 10^{-6} X + 5.62 \times 10^{-9}$ (R² = 0.9499, p = 0.005). This corresponds to an average CMRglc per neuron of $5.79 \times 10^{-9} \pm 0.76 \times 10^{-9}$. In contrast, in the pigeon, a neuron consumes on average $1.86 \times 10^{-9} \pm 0.2 \times 10^{-9}$, which is 3.11 times lower than the average mammalian neuron. See Figure S4 for CMRglc per cell (neuronal and glial cells combined) in the whole brain across species. See Table S2 for neuronal densities, CMRglc, and the neuronal energy budget per species. CMRglc data were available for mouse, rat, baboon, and human, and combined with estimated neuron numbers. Silhouettes were obtained from http://phylopic.org under public license.
either a decrease or increase in brain temperature, the song tempo decreased or increased, respectively. At the basis of many of these processes is the direct effect of temperature on ion channel kinetics, maximum conductance, and gating kinetics. The most prominent example of this effect is its influence on the time of overlap between Na⁺ and K⁺ currents that flow across the membrane during an action potential. Time of overlap and costs are positively correlated, since higher numbers of ions need to be pumped across the membrane by the Na⁺/K⁺-ATPase after the action potential. Higher temperatures were found to exponentially and strongly decrease the elements on neuronal dynamics generated a possible advantage in neuronal processing of information at a higher efficiency: cheap neurons with advanced processing capacity.

Evolutionary perspective

It is important to keep in mind that what is “economical” compared to a mammal can still be costly for a bird. Indeed, across different bird species, we observe many examples indicating that neurons are not a free-for-all commodity. This is clearly apparent in songbirds, which demonstrate some of the most impressive types of neuroplasticity among adult vertebrates. The most extreme example comes from the spotted towhee (Pipilo maculatus), whose HVC (a song nucleus important for learning of song) in males can increase 300% in size during a breeding season to facilitate complex song repertoires to attract mates and defend territory. Of course, it is unlikely that any organism wastes neuron numbers or other costly tissue. These extreme examples of neuroplasticity do show that avian species are under particularly high pressure to reduce (cerebral) energy consumption. In addition, the higher body temperature and costly capacity of flight might also be crucial contributing factors.

As mentioned, despite this extreme pressure, birds attain higher neuron numbers compared to similarly sized mammalian species; like mammals, these numbers positively correlate with cognitive performance. Our findings from pigeons suggest that the ceiling on neuron numbers might be raised by at least a 3-fold lower neuronal energy budget. Future studies will have to verify whether this is a class-wide phenomenon, but the high neuron densities observed across almost all avian species studied so far can be taken as a first indicator that this is the case. Importantly, the lower budget does not seem to compromise neuronal computations, since birds are considered perceptually and cognitively on par with mammals. The last common ancestor of birds and mammals existed approximately 312 mya, and in the long parallel evolution of both lineages, birds ended up with tiny brains comprising high numbers of small neurons organized in a distinct cerebral layout and situated in a warmer physiology. The combined effect of these distinct elements on neuronal dynamics generated a possible advantage in neuronal processing of information at a higher efficiency: cheap neurons with advanced processing capacity.


STAR METHODS

KEY RESOURCES TABLE

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Felix Ströckens (felix.stroeckens@hhu.de).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The research proposal was reviewed and approved by the local animal ethics committee of Nordrhein-Westfalen Germany and in accordance with the German Animal Welfare law following the recommendations of the EU directive 86/609/EEC (permission number: 84-02-04-2017-A 160). For this study, 10 adult homing pigeons (Columba livia) of unknown sex were obtained from a local breeder. All pigeons were of normal weight (464.80 ± 45.97 g). Pigeons were housed individually on a 12/12 h light/dark cycle. Food and water was provided ad libitum, except for an overnight fasting before surgeries to prevent aspirating food under anaesthesia and before scanning to facilitate similar blood glucose levels between individuals and states. To enable awake scanning, four individuals were implanted with a plastic head pedestal.63 In short, under deep anaesthesia (ketamine/xylazine 0.12 ml/100 g body weight) an incision was made in the skin to lay bare the surface of the skull. Next, a small plastic head block was secured directly on the skull with dental cement and the skin was sutured. The head block was compatible with a custom-made holding tube to prevent any head motion in the scanner (Figure 1A). To reduce stress to a minimum, pigeons underwent a habituation procedure. One week prior to scanning, pigeons were habituated to the apparatus and procedure in a mock-PET environment for increasing time.
lengths of 5, 10, 15, 30, and 45 minutes over five consecutive days. As demonstrated by Behroozi et al., this procedure reduces movement to a negligible minimum and eliminates all visible stress indicators. Namely, after a short increase, heart rate returned to normal levels within minutes and measurements of blood cortisol levels showed no increase after the habituation procedure. Therefore, we believe our results are minimally influenced by stress.

**METHOD DETAILS**

**PET data acquisition**
All PET scans were performed in a microPET Focus 220 PET scanner for small animals (CTI-Siemens, USA), see Figure 1A for experimental setup. Subjects (n = 10) were scanned in either an awake (n = 4) or anesthetized (n = 6) state in a custom-made holding tube. For the anesthetized scan, sedation was induced with 3% isoflurane in 3:7 O₂/air and maintained at 30 breaths per minute with 1 – 2% isoflurane. Body temperature was kept at 39°C via a feedback-controlled warm waterbed (medres, Germany). For scanning during the awake state, subjects were head fixated in the holding tube. At the start of each scan, subjects received an intravenous (i.v.) injection of [¹⁸F]FDG (~70 MBq in 500 µl) via a brachial vein catheter (Vasofix Safety G24) fixed with tape. Emission data were acquired for 60 min. Venous plasma glucose levels were measured after the scan with a handheld glucometer (On-Call GK Dual, ACON Laboratories Inc.), which we validated with a laboratory assay, see below. At the end of each scanning day, we performed a 10 min transmission scan using a Co point source for attenuation correction. For scanning, pigeons were sacrificed and the heads were scanned for a structural MRI in a 7-T horizontal-bore small-animal scanner (Bruker BioSpec, 70/30 USR, Germany), with a single-loop 20 mm surface coil for signal detection and an 80 mm transmit quadrature birdcage resonator for radio-frequency transmission. To acquire MR data, Bruker Paravision 5.1 software was used. T1-weighed anatomical images were acquired using multi-slice rapid acquisition (RARE) with the parameters: effective TE = 6.83 ms, TR = 100 ms, RARE factor = 2, number of average = 2, field of view = 21 x 28 x 31.5, slice thickness = 1 mm, spatial resolution = 0.07 x 0.07 x 0.07 mm, and dimensions = 400, 300, 450.

**Validation of handheld glucometer**
To validate the readings of the handheld glucometer used in this study, we took blood samples from the brachial vein of 8 pigeons (*Columba livia*). Samples were collected in ethylenediaminetetraacetic acid coated tubes and blood glucose levels were directly measured with the handheld device three times in each sample. Afterward, samples were centrifuged for 10 min at 1900 RCF at 4°C and plasma supernatant was stored at −80°C until further usage. Plasma glucose levels were acquired using the Glucose Colorimetric Assay Kit II (BioVision), strictly following the manual of the assay. Absorbance of the samples at 450 nm was measured with a Multiskan FC photometer (Thermo Fisher Scientific). Based on the absorbance readings and a standard curve, plasma glucose concentrations were then calculated for each sample with the SkanIt software (Thermo Fisher Scientific).

Mean blood glucose concentrations measured with the handheld device were at 11.23 ± 0.82 mmol/L and glucose concentrations assessed with assay kit were at 12.58 ± 0.80 mmol/L. Both measured concentrations were within normoglycemic range for birds. Statistical comparison (Wilcoxon signed-rank test, performed in SPSS Statistics, IBM) between the two measuring techniques revealed a significant difference (z = -2.38, p = 0.017), with slightly higher glucose values (12.56% on average) measured with the assay kit. This difference is still in the allowed variation range defined by the ISO norm 15197 for handheld glucometers used in humans.

**Input function**
The input function was established from plasma values. Arterial blood was sampled from the brachial artery via a catheter (Vasofix Safety G24, B. Braun, Germany) fixed just before starting the scan. Blood was sampled continuously via the catheter elongated with a heparinised tube during the first 2 minutes and radioactivity was directly measured with a custom-made blood pump with an online detector set at a pulling rate of 1 ml/min. The delay in measurement between the PET scanner and online blood detector was corrected for by dividing tube length by pulling velocity. The tube was then cut and after discarding 200 µl of catheter dead volume, discrete blood samples of 100 µl at 2, 5, 10, 20, 30, 45, and 60 min were taken. The manual blood samples were immediately put on ice. This reduces the inner blood transport of [¹⁸F]FDG from plasma to red blood cells to negligible rates. Plasma was separated by centrifuging full blood for 12 min at 1500 g in a microcentrifuge (Centrifuge 5415 R, Eppendorf) and 40 µl plasma was used for analysis. Radioactivity was measured in both the full blood and plasma sample in a Compugamma CS γ-counter (LKB/Wallac, Australia) and corrected for deadtime and radioactive decay. The plasma to full blood ratio of the radioactivity rates (counts/min) at each sampling time was calculated. The PET scanner, blood sampler and γ-counter were cross-calibrated.

**Image processing**
The emission scans were histogrammed into time frames (6 x 30, 3 x 60, 3 x 120 and 12 x 240 s) and fully 3D rebinned (span 3, ring difference 47) followed by OSEM3D/MAP reconstruction. The resulting voxel size measured 0.38 x 0.38 x 0.80 mm. Next to the attenuation correction, scans were corrected for deadtime with a global estimate based on running deadtime average and radioactive decay. Images were analysed with the VINCI Software Tool. The images were co-registered to a template structural MRI pigeon brain.
Kinetic modeling

The [18F]FDG kinetics were determined with the two-tissue compartment model\(^1\) (Figure 1B) that estimates four rate constants (K\(_1\), transport from blood to brain; k\(_2\), transport from the brain to blood; k\(_3\), phosphorylation; k\(_4\), dephosphorylation). Following Backes et al.,\(^1\)\(^2\) parametric images of the rate constants were determined using a Powell algorithm for voxel-wise fitting of

\[
C_T(t) = v_B C_p(t) + (1 - v_B) \left( A_1 \int_0^t dt C_p(t') e^{-r \cdot (t - t')} - A_2 \int_0^t dt C_p(t') e^{-r \cdot (t - t')} \right)
\]

(Equation 1)

with \(C_T(t)\) representing tissue (in this case brain) radioactivity concentration, and

\[
A_z = \frac{k_z}{r_z - r_z}
\]

(Equation 2)

\[
A_z = \frac{K_z}{r_z - r_z}(k_3 + k_4 - r_z)
\]

(Equation 3)

where \(t\) is time in minutes after injection and \(v_B\) is the fractional blood volume in brain tissue. Since the exact blood volume for these pigeons was unknown, we ran the analysis with four different fractional blood volumes (3%, 4%, 5% and 6%) to investigate the effect of this variable on the final \(k\)-parameters and CMR\(_{glc}\). We found that the different fractional blood volumes produced a negligible variation in the \(k\)-parameters and CMR\(_{glc}\), see Table S1. The value of 5% is commonly used in rats and since the blood volume per unit brain weight was found to be the same in pigeon and rat,\(^1\)\(^6\) it was also employed here for the main analysis. \(C_B(t)\) expresses whole-blood radioactivity concentration, which was measured by continuous automatic sampling during the first two minutes after tracer injection, and manual sampling thereafter. The plasma radioactivity concentration \(C_p\) was directly measured in the manual samples and calculated for the period of continuous sampling as

\[
C_p(t) = \frac{1}{a_y + (1 - a_y)(1 - e^{-vB t})} C_B(t)
\]

(Equation 4)

The parameters \(a_y\) and \(k\) were determined for each pigeon by fitting Eq. 4 to \(C_B(t)\) and \(C_p(t)\) measured in the manual samples. The net influx constant (\(K\)) for [18F]FDG results from

\[
K_i = \frac{K_1 \times k_3}{(k_2 + k_3)}
\]

(Equation 5)

which translates into CMR\(_{glc}\) (\(\mu\)mol/100 g min) via

\[
CMR_{glc} = K_i \times \frac{1}{LC} \times C_{p, glc}
\]

(Equation 6)

where \(C_{p, glc}\) is the post-scan venous plasma glucose level and LC is the local lumped constant that describes the difference of glucose versus [18F]FDG metabolism kinetics. Most commonly, a fixed value is taken for LC. It can be determined by model-independent and model-dependent methods and some variation is present in the mammalian literature.\(^1\)\(^6\) This variation has been ascribed to methodological differences, or specificities of species or disease state.\(^1\)\(^7\)\(^8\) Following Backes et al.,\(^1\)\(^2\) here we employed a model-dependent method where the local LC is determined from the local kinetic rate constants of [18F]FDG taking into account the different efficiencies in transport and phosphorylation of glucose and [18F]FDG. It elaborates on the idea that disentangling the processes of transport and phosphorylation that are lumped together in the LC is more accurate. The difference between [18F]FDG and glucose in transport and phosphorylation can be broken up into: \(L_1 = K_1,FDG/K_1,glc\), \(L_2 = k_2,FDG/k_2,glc\), and \(L_3 = k_3,FDG/k_3,glc\). Based on Michaelis-Menten formalism, which describes enzyme kinetics, the ratio of difference between two substances that use the same system is a constant, because it describes ratios between constants.\(^1\)\(^8\) This implies that there are fixed ratios of difference between glucose and [18F]FDG in transport and phosphorylation, and including these in the model will allow for calculating region-dependent LCs for each individual pigeon.\(^1\)\(^3\)\(^4\)\(^5\) The values for the separate ratios are: \(L_1 = 1.48\), \(L_2 = 1.48\) & \(L_3 = 0.37\), and were found to be similar in both human and rat.\(^1\)\(^5\)\(^1\)\(^6\) The local LC can then be calculated from the local kinetic rate constants of [18F]FDG by:

\[
LC = L_1 \left( \frac{L_3}{L_2} + \left( 1 - \frac{L_3}{L_2} \right) \frac{K_i}{K_1} \right)
\]

(Equation 7)

STATISTICAL ANALYSIS

Statistical analysis was executed with IBM Statistics SPSS 21 (IBM Corp, Released 2012, Armonk, NY). For all tests, a value was considered an outlier when it lay outside of the following range: \(^3\)\(^{rd}\) or \(^1\)\(^st\) quartile + \(0.05\) * \(^1\)\(^st\) skew interquartile range. Values are reported as mean ± standard deviation and results were significant if \(p < 0.05\). Effect sizes were described with the partial eta squared (\(\eta^2\)). The injected [18F]FDG dose and LC were compared between the awake and anaesthetized group with an independent t test. Both groups
met the assumption of normality and homogeneity of variance as tested with a Shapiro-Wilk and Levene’s test, respectively, for both factors. For the LC values, one outlier was detected (case 645 of the i.v. anaesthetized group), which was excluded from further analysis of the LC. Differences in CMR_{glc} depending on brain region (forebrain, cerebellum or rest of brain) or group (i.v. awake/i.v. anaesthetized) were assessed with a two-way mixed ANOVA. The within-subject variable was a repeated measure of “Brain Region”, and the variable “State” was assessed as between-subject variables. The main effects were more closely analysed with a post hoc test with Bonferroni correction. All group values were normally distributed, as assessed with a Shapiro-Wilk test, and met the criterium of homogeneity of variance (Levene’s test), and homogeneity of covariance matrices (Box’s M test). The CMR_{glc} per neuron was calculated by dividing the average CMR_{glc}/mg of the awake state over neuron numbers/mg. For this analysis we only used awake data since we are interested in the true neuronal energy budget without possible effects of anaesthesia. The neuron numbers of the pigeon brain (n = 3) were retrieved from Olkowicz et al. The neuronal energy budget of the pigeon was compared to mammalian data where both CMR_{glc} and neuron numbers were available. Whole brain CMR_{glc} values in an awake state were available for mouse, rat, macaque, baboon, and human.