[18F]FDG PET signal is driven by astroglial glutamate transport

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Contributions of glial cells to neuroenergetics have been the focus of extensive debate. Here we provide positron emission tomography evidence that activation of astrocytic glutamate transport via the excitatory amino acid transporter GLT-1 triggers widespread but graded glucose uptake in the rodent brain. Our results highlight the need for a reevaluation of the interpretation of [18F]FDG positron emission tomography data, whereby astrocytes would be recognized as contributing to the [18F]FDG signal.

Glucose constitutes the major source of energy in the brain, with its utilization rate correlating with neuronal activity. This key concept provides the basis for interpreting activity-dependent accumulation of the radiofluorinated glucose analog 2-deoxy-2-[18F]fluoro-d-glucose ([18F]FDG) (ref. 2), visualized using positron emission tomography (PET). For over 30 years, brain [18F]FDG PET uptake has been viewed as a proxy of neuronal activity. Despite widespread use in both clinical settings and basic research, the identity of the cell type(s) contributing to the [18F]FDG PET signal, as well as the mechanisms regulating its variations, remain highly controversial.

Over the past decades, astrocytes have been implicated in several dynamic mechanisms involving synaptic transmission and plasticity, including a crucial role in terms of glucose consumption. Indeed, strong evidence supports the hypothesis that glutamate recycling in astrocytes activates aerobic glycolysis, with neurons being partially fueled by lactate derived from astrocytes. More specifically, astroglial glutamate transport—via GLT-1 or GLAST—has been shown to act as a trigger, signaling for glucose uptake by astrocytes.

In fact, it has been shown that GLT-1- or GLAST-knockout mice have reduced [14C]-2-deoxyglucose uptake in the somatosensory cortex after whisker stimulation. Based on these observations, it seems likely that [18F]FDG PET signal may largely reflect glucose consumption in astrocytes rather than in neurons, although no PET evidence exists so far to support this claim. To further test this hypothesis, we conducted a micro-PET study using [18F]FDG to assess whether ceftriaxone (CEF), a known stimulator of astrocytic glutamate transport via GLT-1 (refs. 13,14), was capable of modulating cerebral [18F]FDG consumption in awake adult rats (Fig. 1a).

Using cultured adult cortical astrocytes, we demonstrated that acute CEF exposure enhances glutamate and glucose uptake, which is in accordance with the previously described mechanism of glutamate-induced aerobic glycolysis, without altering GLT-1 expression. Additionally, we demonstrated that the blockade of GLT-1 transport by dihydrolakalic acid (DHK) abolished CEF’s effect on glucose uptake (Supplementary Fig. 1). However, the precise molecular mechanism by which CEF acutely exerted its effect on glutamate transport via GLT-1 in astrocytes remains to be determined.

Our in vivo data demonstrate that CEF induces a global increase in [18F]FDG standard uptake values (\( t_0 = 3.309, P = 0.0091 \); Fig. 1b) without affecting spontaneous locomotion in the open field test (Supplementary Fig. 2). A voxelwise \( t \)-statistical analysis showed a marked increase in several brain regions encompassing cortical and subcortical areas, with a peak effect in the prefrontal cortex (peak \( t_0 = 5.24, P = 0.0005 \); Fig. 1c). Analysis of regional mRNA expression data from the Allen Brain Atlas suggested that regions exhibiting high GLT-1 expression were more susceptible to the CEF challenge, showing more pronounced increases in [18F]FDG uptake in our study (Supplementary Figs. 3 and 4). To support this claim, we delineated six large volumes of interest (VOIs; Fig. 1d) to estimate regional [18F]FDG uptake. Regional VOI analysis showed increased [18F]FDG standard uptake.

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values in the frontal cortex \(t_p = 3.349, P = 0.0085\), temporoparietal cortex \(t_p = 2.874, P = 0.0184\), hippocampus \(t_p = 3.918, P = 0.0035\), striatum \(t_p = 3.420, P = 0.0076\) and thalamus \(t_p = 2.975, P = 0.0156\), regions known for high GLT-1 expression. In contrast, CEF did not significantly alter \([\text{18F}]\text{FDG}\) uptake in the cerebellum \(t_p = 2.083, P = 0.070\), a region of low GLT-1 expression\(^\text{15}\) (Fig. 1c–j). For percentages of these changes, see Figure 1k.

Currently, it is well established that cerebral \([\text{18F}]\text{FDG}\) uptake is proportional to brain energy consumption and, consequently, to synaptic activity\(^\text{16,17}\). This coupling between brain activity and metabolism allows derivation of metabolic networks through intersubject \([\text{18F}]\text{FDG}\) analysis and consequent identification of patterns of brain metabolism\(^\text{18}\). In fact, through metabolic interregional analysis across the previously delineated VOIs, we found that acute activation of astroglial glutamate transport via GLT-1 altered a wide range of connections within the metabolic network. Our findings may indicate that astrocytic activation per se was capable of reshaping brain metabolic architecture \((P < 0.05\), Bonferroni corrected; Fig. 2a–d). However, we cannot rule out a regionally dependent CEF effect due to the heterogeneous expression of cerebral GLT-1. For false discovery rate (FDR)-corrected results, see Supplementary Figure 5.

Based on the coupling between neuronal activity and cerebral blood flow (CBF)\(^\text{19}\), we then decided to investigate the coupling between \([\text{18F}]\text{FDG}\) uptake and CBF response in the barrel and prefrontal cortices (Fig. 3a). The CEF challenge did not alter resting CBF in the barrel \(F_{1,888,5.656} = 1.648, P = 0.2714\); Fig. 3b) and prefrontal cortices \(F_{1,645,6.579} = 0.3977, P = 0.6495\), Fig. 3b). The evoked CBF after whisker stimulation did not differ between groups (interaction, \(F_{2,37,1.264} = 0.4291, P > 0.999\)); whiskers stimulation effect: \(F_{79, 1.264} = 60.10, P < 0.001\); group effect: \(F_{3,16} = 0.612, P = 0.6170\); Fig. 3c). Our findings revealed an uncoupling between cerebral glucose uptake and blood-flow responses following astrogial glutamate transport activation via GLT-1, which supports the hypothesis that the increased glucose utilization under CEF challenge is driven by astroglial glutamate transport.

Our results provide the first in vivo PET evidence for astrocytic glutamate transport via GLT-1 triggering glucose uptake. In fact, we show that \([\text{18F}]\text{FDG}\) PET signal was substantially affected by the activation of astrogial glutamate transport. Furthermore, combining our in vivo data with several previous studies (for review see ref. \(^\text{8}\)) argues for the view that glucose utilization by astrocytes determines part of the \([\text{18F}]\text{FDG}\) PET signal. The notion that \([\text{18F}]\text{FDG}\) PET signal also reflects astrocyte activity can change how we decode

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**Figure 2** Astrocytic glutamate transport activation via GLT-1 disrupts region-to-region metabolic synchronicity. Cross-correlation matrices: intersubject cross-correlation maps displaying region-to-region associations in (a) baseline and (b) CEF challenge conditions. Metabolic networks: 3D brain surfaces displaying large-scale metabolic cross-correlation maps in (c) baseline and in (d) CEF challenge conditions. \(n = 10\) rats per group. Data are presented as correlation values with Bonferroni-corrected thresholds; \(P < 0.05\).
brain metabolic images made with [18F]FDG PET. For example, in the context of Alzheimer’s disease, [18F]FDG hypometabolism is used as a biomarker of synaptic dysfunction of neuronal nature. Our data indicate that [18F]FDG PET signal can be driven by astrocytes, urgently calling for a reevaluation in the way we interpret imaging of brain disorders using [18F]FDG PET.

METHODS
Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Figure 3 Astrocytic glutamate transport activation via GLT-1 uncouples [18F]FDG uptake and CBF response. (a) 3D brain surface displaying highest effects of CEF challenge and probe locations. (b) Temporal resting CBF analysis during baseline and after injection of saline or CEF in the barrel (repeated-measures one-way ANOVA, $P = 0.2714$) and prefrontal cortex (repeated-measures one-way ANOVA, $P = 0.6495$). (c) Evoked CBF analysis in the barrel cortex (repeated-measures two-way ANOVA; interaction: $P > 0.999$; whisker stimulation effect: $P < 0.001$; group effect: $P = 0.6170$). $n = 5$ rats per group for evoked CBF; $n = 5$ per group for resting CBF in the prefrontal cortex; and $n = 4$ per group for resting CBF in the barrel cortex. Data are presented as mean values ± s.e.m. (shadows).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
All authors participated in the conceptualization, design and interpretation of the experiments. E.R.Z., M.J.P., A.L., H.-I.K. and P.R.-N. were responsible for conducting imaging acquisitions and analysis. E.R.Z., D.G.S. and L.P. were responsible for conducting in vitro studies in cell cultures. C.I. and E.H. were responsible for conducting laser Doppler acquisitions and analysis. All authors critically revised and approved the final version of the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Animals were kept in a room with controlled temperature (21 °C) under a 12-h light/dark cycle (lights on 7 a.m.) and had access to food and water ad libitum. All procedures were carried out according to the Guide to the Care and Use of Experimental Animals (Edz) of the Canadian Council on Animal Care. Animal experiments were performed according to the institutional guidelines of the Guangju Institute of Science and Technology and the Brazilian guidelines for animal experimentation number 11794/08, which are compliant with McGill University guidelines for experimental research (Montreal, QC, Canada).

Imaging procedures. Ten male two-month-old Sprague Dawley rats (body weight: 245–302 g) were scanned for baseline and one day after challenge. The scanning was conducted between 10:30 a.m. and 1:30 p.m. Animals received an intravenous bolus injection (0.2 mL) of [18F]FDG (mean ± s.d.: 8.14 ± 0.555 MBq) into the tail vein. Rats were maintained awake for a 40 min uptake period, followed by a 10 min static acquisition under isoflurane anesthesia. A CT transmission scan was acquired to correct for attenuation. PET measurements were performed on an Inveon micro-PET/CT scanner (Siemens Medical Solution, Knoxville, TN). The rat lay prone with the head immobilized by both a body holder and the nose cone of the anesthesia system (2% isoflurane at 0.5 l/min oxygen flow). The brain was positioned in the center of the field of view. Body temperature was maintained at 36.5 ± 1 °C, and vital signs, including respiration, heart rate and body temperature, were monitored throughout scanning procedures (BioVet; m2m Imaging Corp, Newark, NJ, USA). Images were reconstructed by a fully 3D ordered-subset expectation maximization (3D-OSEM) algorithm, normalized and corrected for scatter, dead time and decay.

Challenge experimental design. Rats were scanned twice under different pretreatment conditions. First, animals were anesthetized (2% isoflurane at 0.5 l/min oxygen flow) and positioned in the scanner. For the baseline scan, they received a tail vein injection of saline 30 min before the [18F]FDG injection. For the challenge scan, they received a tail vein injection of 200 mg/kg CEF (Rocephin, Roche, Basel, Switzerland) 30 min before the [18F]FDG injection (Fig. 1a).

Image analysis. Imaging analysis was conducted using mico-tools (http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage). Micro-PET images were manually co-registered to a standard rat histological template. Standard uptake values (SUVs) were calculated using the following equation: SUV = (radioactivity/dose injected/body weight). Regional SUV was calculated based on manually delimited VOIs, defined on the rat template for the following brain regions: frontal cortex (162.66 mm3), parietal cortex (380.36 mm3), hippocampus (63.25 mm3), striatum (88.72 mm3), thalamus (62.40 mm3) and cerebellum (155.64 mm3). For metabolic network analyses, [18F]FDG data were normalized to dose injected and body mass, to account for differences in total tracer tissue concentration. Matrices were constructed using cross-correlation coefficient symmetric analyses (VOIs as stated above, left and right hemispheres, 12 × 12 matrices). For further details about micro-PET imaging analyses, see ref. 21. Data normalization to a reference region was not recommended in our analyses due to the lack of a reference region, since GLT-1 is analyzed in total tracer tissue concentration. Matrices were constructed using cross-correlation coefficient symmetric analyses (VOIs as stated above, left and right hemispheres, 12 × 12 matrices). For further details about micro-PET imaging analyses, see ref. 21. Data normalization to a reference region was not recommended in our analyses due to the lack of a reference region, since GLT-1 is

In vivo cerebral blood flow (CBF) measurements. Two-month-old Sprague Dawley male rats (body weight: 280–320 g, n = 4 or 5 per group) were anesthetized with urethane (1 g/kg, i.p.) on a heating blanket to keep body temperature at 37 °C. The right femoral vein was cannulated for drug injection, while a heparin catheter was placed in the artery for blood pressure (MAP), heart rate and blood gas measurements (pH, pO2 and pCO2). Rats were then placed in a stereotaxic frame and bones over the left prefrontal (AP: +4.7 mm; lateral: +1 mm from bregma) and barrel (AP 2.5–3 mm, ML 6–7 mm from bregma) cortices were thinned to translucency for positioning of two laser Doppler probes (Transonic Systems). Resting CBF was continuously recorded for 10 min before CEF injection (baseline) and for 40 min following CEF injection. The CBF values from laser Doppler flowmetry were averaged every second and expressed as percent change from the mean of 10 min.

Whisker stimulation in vivo. Right whiskers were stimulated for 20 s (repeated every minute) using an electrical brush (for 20 s, –10 Hz) at baseline and 40 min after drug injection. To measure the evoked CBF response, 1 s averages were computed for 1 min 20 s (30 s before, 20 s stimulation and 30 s after stimulation).

Open-field test. Thirty (30) two-month-old Sprague Dawley rats were submitted to the open-field test under two different pretreatment conditions. Animals were briefly anesthetized (2% isoflurane at 0.5 l/min oxygen flow) and received a single tail vein injection of saline or 200 mg/kg CEF 70 min before the open-field test. The apparatus for this test was a gray acrylic box (50 cm × 50 cm × 50 cm). Experiments were conducted in a sound-attenuated room under low-intensity light (12 lx) during the light cycle. Rats were placed in the center of the box and spontaneous locomotion parameters were recorded with a video camera for 10 min. All analyses were performed using Any-maze, a computer-operated tracking system (Any-maze, Stoelting, Wood Dale, IL).

Adult astrocyte cell culture preparation and maintenance. Three-month-old male Wistar rats were killed by decapitation, and their cerebral cortices were aseptically dissected and meninges were removed. The tissue was digested using trypsin at 37 °C and papain, as previously described22. After mechanical dissociation and centrifugation, the cells were plated in 6- or 24-well plates with DMEM/F12 medium (11330-032, Life Technologies, Carlsbad, CA) supplemented with 10% FBS (12657-029, FBS, Life Technologies, Carlsbad, CA) changed regularly; during the first week, the medium was replaced once every 2 d, and from the second week on it was changed once every 4 d. From the second week on, astrocytes received medium supplemented with 20% FBS until they reached confluence (at approximately the third week). No dibutyryl cAMP was added to the culture medium, to observe the naive response of the cells. Expression of specific neuronal (anti-NeuN, MAB377, EDM Millipore, Billerica, MA) and microglial (anti-Cd11b/c, MR6200, Invitrogen, Carlsbad, CA) proteins were evaluated in order to determine the purity of the astrocyte cultures, which was found to be around 95%. Experiments involving adult culture of astrocytes were replicated at least three times.

Immunocytochemistry. Immunocytochemistry was performed as previously described22. Briefly, cell cultures were fixed with 4% paraformaldehyde for 20 min and permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature (22 ± 1 °C). After blocking overnight with 4% albumin, the cells were incubated overnight with anti-GFAP (1:400; Z0334, Dako, Carpinteria, CA), anti-GLT-1 (1:1,000; GLT11-A, Alpha Diagnostic, San Antonio, TX), anti-vimentin (1:1,000; V6630, Sigma Aldrich, St. Louis, MO) or anti-NeuN (1:50; MAB377, EDM Millipore, Billerica, MA) at 4 °C, followed by PBS washes and incubation with a specific secondary antibody (Jackson ImmunoResearch, West Grove, PA) conjugated with Alexa Fluor-488 (green staining, 111-545-003) or Alexa Fluor-594 (red staining, 315-545-003) for 1 h at room temperature. For all immunostaining-negative controls, reactions were performed omitting the primary antibody. No reactivity was observed when the primary antibody was excluded. Cell nuclei were stained with 0.2 mg/mL of 4′,6-diamidino-2-phenylindole (DAPI). Western blot analysis. Cells were solubilized in lysis solution containing 4% SDS, 2 mM EDTA, 50 mM Tris–HCl (pH 6.8). Protein content was measured, and the samples were standardized in sample buffer (62.5 mM Tris–HCl (pH 6.8), 4% (v/v) glycerol, 0.002% (w/v) bromophenol blue) and boiled at 95 °C for 5 min. Samples were separated by SDS/PAGE (10 mg protein per sample) and transferred to nitrocellulose membranes, as previously described23. Adequate loading of each sample was confirmed using Ponceau S staining. Membranes were incubated overnight (4 °C) with anti-GLT-1 (1:1,000; GLT11-A, Alpha Diagnostic, San Antonio, TX) and anti-GAPDH (1:1,000; G9545, EMD Millipore, Billerica, MA). The membranes were then washed and incubated with a peroxidase-conjugated anti-rabbit immunoglobulin (NLS934V, GE Healthcare, Little Chalfont, UK) at a dilution of 1:4,000 for 2 h. Chemiluminescence signals were detected with an Image Quant LAS4000 system (GE Healthcare) using an ECL kit. Full-length gels and membranes with molecular weight standards are shown in Supplementary Figure 6.
[\textsuperscript{3}H]-\textit{Aspartate uptake.} After the cells reached confluence, \textit{d}-aspartate uptake was performed as previously described\textsuperscript{14}, with some modifications. Briefly, cells were rinsed once with HBSS and then the medium was replaced with fresh DMEM/F12 1% FBS in the presence or absence of 0.1 \textmu M CEF (C5793, Sigma-Aldrich, St. Louis, MO) for 70 min at 37 °C. The medium was then replaced by DMEM/F12 1% FBS containing 1 mCi/mL \textit{d}-[\textsuperscript{3}H]-aspartate (NET581001MC, PerkinElmer, Boston, MA) in the presence or absence of 0.1 \textmu M CEF. Incubation was stopped after 5 min by removal of the medium and rinsing the cells three times with HBSS. The cells were then lysed in a solution containing 0.5 M NaOH. Incorporated radioactivity was measured in a scintillation counter. Unspecific uptake was carried out at 4 °C and subtracted from total uptake.

2-Deoxy-\textit{d}-[\textsuperscript{2,6}H]glucose ([\textsuperscript{3}H]2DG) uptake. Basal and CEF-stimulated deoxyglucose uptake were assessed as previously described with some modifications\textsuperscript{6}. Briefly, cells in 6- or 24- well plates were rinsed once with HBSS, and then the medium was replaced with fresh DMEM/F12 1% FBS in the presence or absence of CEF 0.1 \textmu M for 70 min at 37 °C. Adult astrocytes were then incubated with DMEM/F12 1% FBS containing 1 mCi/mL [\textsuperscript{3}H]2DG (TRK672, GE Healthcare, Little Chalfont, UK) for 20 min at 37 °C. After incubation, the cells were rinsed three times with HBSS and lysed overnight with NaOH 0.5 M. The incorporated radioactivity was measured in a scintillation counter. Cytchalasin B 10 mM (C2743, Sigma-Aldrich, Saint Louis, MO) was used as a specific glucose transporter inhibitor. Deoxyglucose uptake was determined by subtracting the uptakes of cytochalasin B from the total uptake. For GLT-1 pharmacological blockade, DHK (ab120066, Abcam, Cambridge, UK) 100 \textmu M was added to the medium 6 min before [\textsuperscript{3}H]2DG uptake.

High-performance liquid chromatography procedure. Free amino acids concentration in the culture medium was determined by high-performance liquid chromatography (HPLC) as previously described\textsuperscript{25}. Medium cell-free supernatant aliquots were used to quantify glutamate. The analysis was performed using a reverse phase column (Supelcosil LC-18, 250 mm × 4.6 mm × 5 \mu m, Supelco) in a Shimadzu Instruments liquid chromatograph (50 \mu L loop valve injection, 40 \mu L injection volume) and fluorescent detection after precolumn derivatization with 100.5 \mu L OPA (5.4 mg OPA in 1 mL of 0.2 M sodium borate, pH 9.5) plus 25.5 \mu L 4% mercaptoethanol. The mobile phase flowed at a rate of 1.4 mL/min and the column temperature was 24 °C. Buffer composition was (i) Buffer A, 0.04 mol/L sodium dihydrogen phosphate monohydrate buffer, pH 5.5, containing 80% methanol or (ii) Buffer B, 0.01 mol/L sodium dihydrogen phosphate monohydrate buffer, pH 5.5, containing 20% methanol. The gradient profile was modified according to the content of Buffer B in the mobile phase: 100% at 0.10 min, 90% at 15 min, 48% at 10 min and 100% at 60 min. Absorbance was read at 360 nm excitation and 455 nm emission in a Shimadzu fluorescence detector. Samples of 20 \mu L were used and concentration was expressed in \mu M (as mean ± s.e.m.). Glutamate was identified by its retention time and was quantitatively determined using the chromatographic peak area. A standard amino acid mixture was used for HPLC quality control and calibration.

Statistical analysis. Data distribution was first tested for normality using the Kolmogorov-Smirnov test. Sample sizes were evaluated using G’Power 3.1 for Mac (effect size dz = 1.77, noncentrality parameter \delta = 4.68, critical t = 2.44 and actual power = 0.97) based on a previous publication\textsuperscript{14}. Regional brain binding differences between baseline and challenge were estimated across the six VOIs using two-tailed paired Student’s t-tests. Comparisons between baseline and challenge were conducted at the voxel level using t-statistical analysis (Statistical Parametric Mapping, Rminc, https://github.com/Mouse-Imaging-Centre/RMINC). All analyses were conducted using minictools, which is available online at https://github.com/BIC-MNI/minc-tools. t-statistic maps were corrected for multiple comparisons using random field theory\textsuperscript{26}. Resting CBF was analyzed by repeated-measures two-way ANOVA, and evoked CBF was analyzed with repeated-measures one-way ANOVA. Data acquisitions were not performed blind to the conditions of the experiments. However, data analyses (imaging, biochemistry and behavior) were conducted in blind conditions. Animals were randomly assigned for all experiments; however, no formal randomization protocol was used in this study. Open field task and in vitro studies were analyzed by two-tailed unpaired Student’s t-test. [\textsuperscript{3}H]2DG uptake data was analyzed by one-way ANOVA. SPSS 18.0 and Matlab were used for all analysis. Differences were considered statistically significant at P < 0.05. A Supplementary Methods Checklist is available.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.