Supporting Information

Cole et al. 10.1073/pnas.0910280107

SI Methods

Mouse Fluid Percussion Brain Injury. Mice were divided into two groups: surgery only (sham), or surgery and fluid percussion injury (injured). The fluid percussion brain injury (FPI) protocol was carried out over 2 days. On the first day, the animal was anesthetized using a combination of ketamine (2.6 mg/kg) and xylazine (0.16 mg/kg) and placed in a mouse stereotactic frame (Stoelting). The scalp was incised and reflected. The following was conducted under 0.7–3.5× magnification. A craniectomy was performed with a trephine (3-mm outer diameter) over the right parietal area between the bregma anteriorly, the lambda posteriorly, the sagittal suture medially, and the lateral cranial ridge laterally. The dura remained intact throughout the procedure. A rigid Luer-loc needle hub (3-mm inside diameter) was secured to the skull over the opening with cyanoacrylate and dental acrylic. The skull sutures were sealed with the cyanoacrylate during this process to ensure that the fluid bolus from the injury remained within the cranial cavity. The hub was capped and the scalp sutured closed. The mouse was placed on a heating pad and returned to the home cage once ambulatory.

On the second day, the animal was placed under isoflurane anesthesia (500 mL/min) via nose cone, and respiration was visually monitored. When the animal was breathing once per 2s, the nose cone was removed, the cap over the hub removed, and dural integrity visually confirmed. The hub was filled with isotonic sterile saline and a 32-cm section of high-pressure tubing extending from the LFPI device (Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA) attached to the Luer-loc fitting of the hub. The animal was then placed on its left side and observed. After normal breathing resumed, and before sensitivity to stimulation returned, a 20-ms pulse of saline onto the dura was delivered. A pressure gauge attached to an oscilloscope was used to ensure delivered pressures between 1.4 and 2.1 atm, which generates a mild to moderate brain injury. Immediately after injury, the hub was removed from the skull and the animal was placed in a supine position. The animal was then reanesthetized with isofluorane for scalp closure. Sham animals received all of the above, with the exception of the fluid pulse. The animal was returned to a heating pad until ambulatory and then returned to the home cage.

HPLC Analysis. Specific methodology was as described earlier here and elsewhere (1, 2). Briefly, brains from injured and sham animals were harvested and regionally dissected as described above. After homogenization, samples were extracted with 1.5 M perchloric acid on a 3:1 v:w basis. After 2 min, 2 M potassium carbonate was added (1.5 v:w basis). After a 10 min incubation, the solution was centrifuged for 10 min at 2,000 *g* and 4°C. After centrifugation, 100 μL of supernatant was mixed with 900 μL of HPLC-grade water and spin filtered. An aliquot of 0.1mL was combined with 0.1 mL benzoic acid, 1.4 mL HPLC-grade water. Samples were then precolumn derivatized with σ-phthaldiahldehyde and loaded into the HPLC auto-sampler cartridge. A Supelco 3-μm reverse-phase C18 column guarded by a Supelco 40 μm reversed phase C18 column separated the amino acids. Fluorescence was detected at an excitation of 340 nm and emission of 450 nm. Amino acid standards, run separately, aided in the quantification of the amino acid quantification by the Waters model 810 baseline workstation.

Isotope Uptake. Hippocampal slices were collected from injured and sham mice as described earlier here. After allowing the slices to incubate in normal aCSF for 1 h to recover from the dissection procedure, the slices were incubated for 30 min in aCSF containing ^{15}N-leucine. After the incubation period, the slices were rinsed twice in applications of separately prepared aCSF that does not contain BCAAs, to remove any nonspecific binding. All reactions were stopped by incubating the slices for 5 min in 4% perchloric acid.

Chemicals and Antibodies. L-Leucine, L-isoleucine, L-Valine, L-glutamine, 2-amino-2-norbane-carboxylic acid (BCH), 6-diazo-5-oxo-L-norleucine (DON) α-ketoisocaproate, α-ketoisovalerate, α-ketomethylvalerate, and ^{15}N-leucine were all obtained from Sigma-Aldrich. Antibodies to BCATc and BCATm were produced by Affinity Bioreagents. Antibodies were confirmed by the labeling of single bands of the appropriate size, as predicted using Ensembl protein information. The BCKD antibody was graciously provided by Dr. Chris Lynch (Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center; Department of Cellular and Molecular Physiology). In addition to previous publications using this antibody (3), the specificity was verified by the presence of a single band of the appropriate size as predicted using Ensembl protein information. Both glutamate dehydrogenase and glutamine synthetase were obtained from Abcam. Glutamic acid decarboxylase was obtained from Sigma. Glutaminase was obtained from Novus Biologicals.

Field Recording. Recordings were conducted in the transverse slices generated from the ipsilateral hemisphere at 25°C. Perforant path (for DG) or Schaffer collateral (for area CA1) pathways were stimulated with a bipolar tungsten electrode, and fields recorded by patch electrodes filled with 3 M NaCl. For each stimulation intensity, records were averaged from three trials, and the field excitatory postsynaptic potential slope calculated by fitting a curve between two cursors placed on the linear portion of the field excitatory postsynaptic potential. For in vitro application of treatments (BCAAs, BCKAs, BCH, Gln), a baseline I/O curve in normal aCSF was generated in the subregion of interest, and then a second I/O curve generated after a 20-min superfusion of modified aCSF containing the substrate of interest. All amino acids were applied together or singly, when appropriate, at a concentration of 100 μM each. Inhibition of glutaminase with DON (50 μM) was achieved by superfusing the inhibitor for 10 min and then simultaneously applying both DON (50 μM) and either BCAAs (100 μM) or glutamine (100 μM). Fiber volley slopes were calculated by fitting a curve between two cursors placed on the linear portion of the fiber volleys.

Subregional Dissection of Hippocampus. Seven days after injury, the ipsilateral (injured) hippocampus from sham and injured animals were harvested and sectioned using a Brinkmann tissue chopper. The section was then placed flat on the dissecting surface so that the pyramidal cell layer, hippocampal fissure, supra, and infra granular blades of the dentate gyrus were clearly visible. CA1 and DG were microdissected and tissue then placed into lysis buffer containing protease inhibitor mixture tablets (Roche Diagnostics). Immediately after immersion in lysis buffer, the samples were frozen in liquid nitrogen and stored at −80°C for later analysis To obtain sufficient protein for analysis, regional dissected tissue was pooled from five animals for both sham and LFPI populations (one sample). This procedure was repeated to generate an overall four samples per treatment (5).

Western Blot Analysis. Western blots were performed by standard laboratory techniques (Bio-Rad Protean III Mini-Blot System). In brief, tissue samples were boiled in 3× Laemmli SDS and then 20–40 μg of protein were run on a 12% SDS polyacrylamide gel and subsequently transferred to PVDF membrane (Bio Rad) using 24 V overnight at 4°C. For each enzyme being assayed, the samples were loaded with an equal amount of protein. The membrane was then incubated with a rabbit polyclonal antibody against BCATc (1/10,000), BCKD (1/10,000), GDH (1/15,000), GAD (1/10,000), or BCATm (1/10000), or a mouse polyclonal antibody against glutaminase (1/10,000). Membranes were then incubated with a species appropriate secondary antibody (1:3,000) followed by enhanced chemiluminescence detection (Pierce). Membranes were subsequently stripped and reprobed using a rabbit polyclonal β-actin antibody (Santa Cruz Biotechnology) to confirm proper protein loading. Densitometry analysis of blots using UN-SCAN-IT gel-scanning software (Silk Scientific) was used to quantify and statistically compare protein expression between sample sets.

In Vivo BCAA Administration. Two days after the administration of the LFPI or sham procedure, the animals were randomly assigned to one of three water treatments. Mice were offered either untreated tap water (control), tap water containing a combination of valine, leucine, and isoleucine (100 mM each; LIV), or tap water containing phenylalanine (100 mM; Phe). Each morning the drinking water remaining in their water bottle was measured to determine the intake of both water and the BCAAs and fresh treatment given. Mice were weighed at the onset of the drinking water treatment and then immediately before euthanasia at the end of treatments, to determine whether the dietary treatment affected body weight.

Mouse Handling and Conditioned Fear Response Training and Testing. Classical conditioning experiments were performed using the fear conditioning paradigm as previously described (4). All animals received 2 days of handling before training. During conditioning, animals were trained individually in a conditioning chamber (Med Associates). Care was taken to minimize any variation in the procedure either between individual animals or between the conditioning and testing days. Therefore, the conditioning and testing were conducted at the same time of day, level of room light, and background noise. For each mouse, the chamber was cleaned before occupancy to eliminate any olfactory stimuli. The individual scoring the freezing behavior was blinded to the mouse's injury status and dietary treatment. For anterograde testing, 6 days after the administration of either the injury or the sham procedure, and therefore after 4 days of the diet, each mouse was placed in the chamber for 3 min before administration of a mild foot shock consisting of 1.5 mA for 2 s. The mouse was then left in the chamber for an additional 30 s. Freezing behavior was scored during training. The following day (day 7 after injury), the animals were returned to the conditioning chamber for 5 min to assay retention. For retrograde testing, 1 day before the surgery to prepare the mice for the injury or sham procedure, the animals were trained as described for the anterograde test. Then, 7 days after injury, and therefore after 5 days on the dietary treatment, the animals were returned to the chamber to assay retention, as described for the anterograde test. For both anterograde and retrograde tests, freezing behavior was scored at 5s intervals for the duration of the animals' time in the chamber during both the training period and the retention assay. Percent freezing was defined as number of "frozen" observations divided by total observations.

Statistical Procedures. Two-tailed, unpaired Student's t tests were performed to determine statistical significance at the $P < 0.05$ confidence level when comparing different treatment and animals groups. Where appropriate, data were analyzed using a single-factor ANOVA. All data are presented as group means ± SEM.

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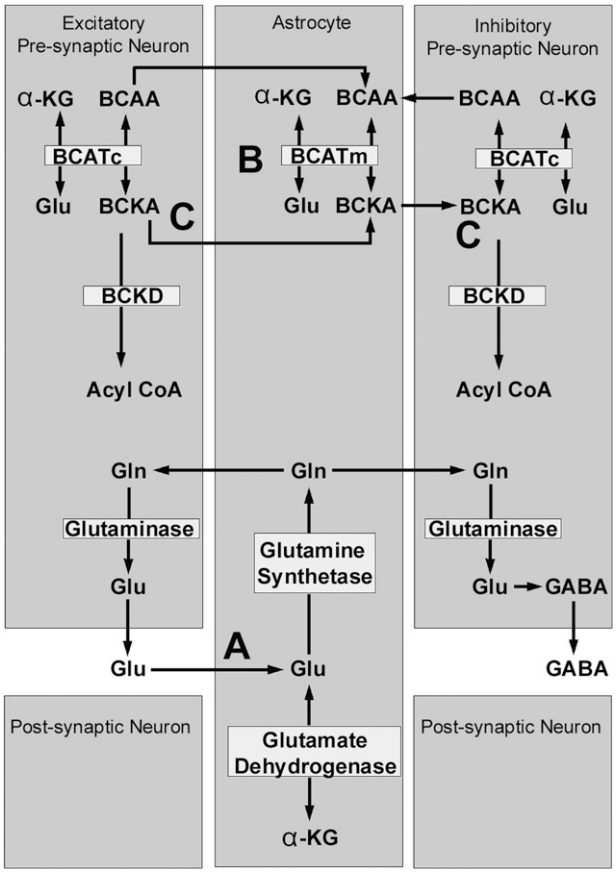

Fig. S1. Scheme depicting the coordination between the glutamate:glutamine cycle and the putative astroglial:neuronal shuttle. (*A*) Glutamate released from the presynaptic terminal is removed from the synaptic cleft by astrocytes, after they have temporarily bound to the postsynaptic receptors. Within the astrocyte, the glutamate is aminated to form glutamine for transfer back to the presynaptic neuron. However, this process is not efficient, as both glutamate and glutamine can be diverted for other processes, e.g., protein synthesis and catabolism for energy. (*B*) Therefore, astrocytes supplement the glutamate:glutamine cycle by transferring amino groups via mitochondrial branched chain aminotransferase (BCATm) from the three branched chain amino acids (BCAAs; valine, isoleucine, and leucine) to α-ketoglutarate to form glutamate. (*C*) The resultant branched chain ketoacids (BCKAs) are then transported to the neuron for either re-capitulation into BCAAs by cytosolic branched chain aminotransferase or irreversible degradation by branched chain ketoacid dehydrogenase (BCKD). GAD, glutamic acid decarboxylase; BCAA, branched chain amino acid; BCAT, branched chain aminotransferase; BCKD, branched chain keto acid dehydrogenase.

Table S1. BCAA or injury effect on fiber volleys in slices from sham and injured animals

	Sham CA1	FPI CA1	Sham DG	FPI DG
	−1.09 ± 0.08	−0.85 ± 0.15	−1.17 ± 0.15	−1.06 ± 0.11
No. (slices/animal)	9/4	8/5	12/5	10/4
LIV	−1.17 ± 0.18	−0.97 ± 0.09	−0.98 ± 0.12	−0.96 ± 0.13
No. (slices/animal)	12/5	11/4	10/5	12/6

Data are mean ± SEM. Units are in ms/mv (like fields), and the linear slope of the fiber volley was measured in the same fashion as fEPSP slope. Fiber volleys were measured from the highest (150 μA) stimulation record. None of the populations were significantly different from each other, as measured by an unpaired Student's *t* test in which significance was assessed at the 0.05 confidence level.