Supplemental Methods

Permanent middle cerebral artery occlusion (pMCAO)

Experiments were performed on adult male Sprague-Dawley rats (250-300 g: Harlan Laboratories, Barcelona, Spain). All the experimental procedures were performed in accordance with the guidelines of the Animal Welfare Committee of the Universidad Complutense (DC 86/609/EU). Rats were anaesthetized with isofluorane (1.5-2% in a mixture of 80% synthetic air/20% oxygen) and their body temperature was maintained at 37 ± 0.5°C during surgery using a servo-controlled rectal probe heating pad. The pMCAO was achieved by ligature as described previously (36), while in sham-operated control rats (sham) the MCA was exposed but not occluded. All rats were returned to their cages following surgery and sacrificed 24 hours later, except for those used in fMRI experiments.

Blood samples *(*100 μL) were collected from the femoral artery after cannulation and treated with 3.15% citrate buffer before it was centrifuged at 1,164 x g for 5 minutes to obtain plasma. Samples were frozen at -80ºC until the glutamate content was assayed. Further details of other methods can be found in Supplemental Methods.

Determination of infarct area

The brain of each rat was removed 24 h after pMCAO and a series of coronal brain slices (2 mm: Brain Matrix, WPI, UK) were stained with 1% TTC (2,3,5-triphenyl-tetrazolium chloride: Merck) in 0.2 M phosphate buffer. Infarct size was determined as described previously (1); digital images (Nikon Coolpix 990, Nikon Corporation, Tokyo, Japan) of stained sections were used to delineate the infarct borders by an observer blind to the experimental conditions using an operator-controlled cursor. The infarct area that was not stained was determined by counting the pixels within the outlined regions of interest, expressed in square millimeters (ImageJ 1.33u software; National Institutes of Health, Bethesda, MD, USA). The results were expressed as a percentage **o**f infarct volume.

Rat peritoneal dialysis

A port pre-attached 4 French silicone catheter (ID 0.25mm, OD 0.47mm) with dacron cuff and cross holes (Access Technologies, Skokie, Illinois, USA) was used for rat peritoneal dialysis. One hour before starting peritoneal dialysis, the catheter was implanted surgically under general anesthesia with the help of a needle. A midline incision was made through the abdominal skin and a hole was pierced close to linea alba using a 2mm diameter needle (16Gx2'', OD 1.70mm, long 51 mm, Terumo Europe, Leuven, Belgium). The catheter (1.5 cm) was inserted in the incision and secured by suture of the superficial abdominal muscle fascia. Peritoneal dialysis was initiated by the infusion by gravity (flow rate 1.5-2 ml/min) of 10 ml dialysate containing: glucose, 8.3 mM, NaCl 140 mM, $CaCl_2$ 1.75 mM, $MgCl_2$ 0.5 mM, lactic acid 3.5 mM, NaH_2PO_4 10 mM (pH 6.5). The dialysate was maintained in the peritoneal cavity for 20 min, and then removed for glutamate analysis. This was followed by a second infusion with fresh dialysate.

Human peritoneal dialysis

Peritoneal dialysis was performed by means of a permanent Tenckhoff silicone catheter with two cuff (Covidien, Madrid, Spain) located within the peritoneal cavity using the Seldinger blind technique and according to standard medical practice. The procedure takes 15 minutes. Peritoneal dialysate samples were collected at different times (0, 0.5, 1, 2, 3 and 4 hours) after infusion of 2000 cc of Physioneal (3.86%: Baxter, Madrid, Spain) and the samples were obtained by partial draining of the effluent at each time point. Glutamate levels were also quantified in dialysate samples obtained after 10 hours of dialysis from a previous nocturnal session.

Two venous blood samples were collected before and after the diurnal dialysis session and after 30 min at room temperature, the serum was centrifuged at 1,500 x g for 10 min and the supernatant was collected. Peritoneal effluent and serum samples were immediately frozen at -80ºC.

Fluorimetric determination of glutamate

Glutamate content in rat plasma, human serum and human peritoneal dialysate samples was assayed by on-line fluorometry, in which the increased glutamate concentration is reflected by an increase in fluorescence due to NADPH formation in the presence of glutamate dehydrogenase.

Electrode implantation and microstimulation for fMRI experiments

Animals were anesthetized with urethane (1.2-1.5 g/kg, i.p.) and secured in a stereotaxic device. Stimulating electrodes were implanted using standard surgical and stereotaxic procedures, as described previously (2,3). A twisted platinum-iridium Teflon-coated bipolar electrode (200 μm diameter, 10-15 kΩ: A-M Systems, WA, US) was positioned in the corpus callosum for widespread activation of the cerebral cortex (0 mm anteroposterior and 1 mm lateral relative to bregma; 2.3 mm ventral to the dural surface (4). The stimulating electrode was secured to the skull with dental cement and its position confirmed by means of anatomical scans, connecting the stimulating electrode to a constant current source and a pulse generator once in the scanner (STG2004, Multichannel Systems, Reutlingen, Germany). The stimulation protocol was a block design consisting of 10 periods of 4 s stimulation epochs, each followed by a resting epoch of 26 s (10 min in total), which was repeated 5 times per animal. Stimulation pulses were of 0.1 ms in duration, 0.5 mA in intensity and at a frequency of 10 Hz.

MR imaging

For MRI experiments, urethane-anesthetized animals were placed in a custom-made animal holder with adjustable bite and ear bars, and positioned on the magnet chair. Their temperature, heart rate, SpO_2 and breathing rate were monitored throughout the session, and the experiments were carried out in a horizontal 7 Tesla scanner with a 30

cm diameter bore (Biospec 70/30v, Bruker Medical, Ettlingen, Germany). Functional MRI was performed in 15 coronal slices using a GE-EPI sequence applying the following parameters: FOV, 25 x 25mm; slice thickness, 1 mm; matrix, 96 x 96; segments, 1; FA, 60°; TE, 15ms; TR, 2000 ms. T2 weighted anatomical images were collected using a rapid acquisition relaxation enhanced sequence (RARE), applying the following parameters: FOV, 25 x 25 mm; 15 slices; slice thickness, 1 mm; matrix, 192 x 192; TEeff, 56 ms; TR, 2 s; RARE factor, 8. A 1H rat brain receive-only phase array coil with integrated combiner and preamplifier, and no tune/no match, was employed in combination with the actively detuned transmit-only resonator (Bruker BioSpin MRI GmbH, Germany).

Functional MRI data were analyzed offline using our own software developed in MATLAB, which included statistical parametric mapping packages (SPM2, www.fil.ion.ucl.ac.uk/spm). After linear detrending, temporal (0.015-0.2 Hz) and spatial filtering (3 x 3 gaussian kernel of 1.5 sigma) of voxel time series, a general linear model or cross-correlation analysis, was applied with a simple boxcar model shifted forward in time, typically by 2 sec, or a boxcar convoluted with a gamma probability density function (MatLab), in order to account for the hemodynamic delay in the BOLD signal. Functional maps were generated from voxels that had a significant component for the model ($P<0.0001$, uncorrected) and they were clustered together in space. Similar results were obtained with the different analytical methods.

Limb-use asymmetry test

Forelimb use during explorative activity was analyzed by videotaping rats in a transparent cylinder (20 cm diameter and 30 cm height) for 3-10 min depending on the degree of movement maintained during the trial. This test is appropriated to evaluate sensorimotor function in unilateral models of central nervous system injury (5). We scored simultaneous use of both forelimbs for contacting the wall of the cylinder during a full rear.

Statistical analyses

Data are expressed as the means ± SEM, and an analysis of variance (ANOVA Tukey´s multiple comparison test) was used to compare the blood glutamate values before and at different times after pMCAO. The analysis of variance was also used to compare the infarct volume values in the different conditions (pMCAO, + dialysis (2.5h), + dialysis (5h), + dialysis (2.5h) 400μM GLU) with the corresponding value in the pMCAO condition. ANOVA followed by Bonferroni´s test was used to compare the functionality index in the different conditions (control, pMCAO, + dialysis 2.5h). Two tailed Student´s t-test was used to compare the following mean values: the volume of T2-hyperintensity in pMCAO and +dialysis (2.5h); the forelimb placing score in the different conditions (pMCAO, + dialysis 2.5h) with control; serum glutamate before and after a 4 hours of peritoneal dialysis session in patients. The relationship between plasma glutamate and infarct size was analyzed using the Pearson´s correlation coefficient. In all tests, a $p<0.05$ was considered significant.

References for Materials and Methods

1.- Hurtado O, et al. Delayed post-ischemic administration of CDP-choline increases EAAT2 association to lipid rafts and affords neuroprotection in experimental stroke. *Neurobiol. Dis.* 2008; 29(1): 123–131.

2.- Canals S, Beyerlein M, Murayama Y, Logothetis NK. Electric stimulation fMRI of the perforant pathway to the rat hippocampus. *Magn. Reson. Imaging* 2008; 26(7): 978-986.

3.- Canals S, Beyerlein M, Merkle H, Logothetis NK. Functional MRI evidence for LTP-induced neural network reorganization. *Curr Biol*. 2009; 19(5): 398-403.

4.- Paxinos G, Watson C. Eds. *Elsevier.* The Rat Brain in Stereotaxic Coordinates, Academic Press; 2007.

5.- Schallert T, Fleming SM, Laesure JM, Tillerson JL, Bland ST. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. *Neuropharmacology* 2000; 39(5): 777-787.