Supporting Information for:

Pharmacological Mitigation of Tissue Damage during Brain Microdialysis

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EXPERIMENTAL SECTION

Fast Scan Cyclic Voltammetry. Carbon fiber microelectrodes were constructed by pulling borosilicate capillaries (0.58 mm I.D., 1.0 mm O.D., Sutter Instruments, Novato, CA) to fine tips around a single carbon fiber (7 μm diameter, T650, Cytec Carbon Fibers LLC., Piedmont, SC) with a vertical puller (Narishing Tokyo, Japan). The tips were sealed with a low viscosity epoxy (Spurr Epoxy, Polysciences Inc., Warrington, PA) and cured overnight at 70 ºC. The exposed fibers were cut to a length of 400 μm. The capillaries were filled with mercury and a nichrome wire (Goodfellow, Oakdale, PA) was inserted to complete electrical contact. Microelectrodes were pre-treated by soaking the tip in isopropyl alcohol (Sigma Aldrich, St. Louis, MO) containing carbon decolorizing (Fisher Scientific, Pittsburgh, PA) for one hour.

Fast scan cyclic voltammetry was performed using a computer controlled potentiostat (EI-400, Ensman Instruments, Bloomington, IN) and CV Tarheels version 4.3 software (Michael Heien, University of Arizona, Tucson AZ). The potential was applied between a Ag/AgCl reference electrode and a carbon fiber working electrode. The potential was ramped at a scan rate of 400 V/s from 0 V to 1 V, then to -0.5 V, and then back to 0 V. Scans were performed at 2.5 Hz. Dopamine (DA) oxidation peaks were monitored between 0.6 V and 0.8 V on the initial potential sweep. DA voltammograms were created by background subtraction. DA current was converted to DA concentration by post-calibration of electrodes in a flow cell with at least three different concentrations of DA (Sigma Aldrich, St. Louis, MO) dissolved in nitrogen purged artificial cerebrospinal fluid (aCSF).

Animal and Surgical Procedures. All procedures involving animals were approved by the Institutional Animal Care and Use of Committee of the University of Pittsburgh. Male Sprague-Dawley rats (250-350 g) were intubated, anesthetized with isofurane (0.5 % by volume,

Baxter Healthcare, Deerfield, IL), placed in a stereotaxic frame and wrapped in a heating blanket. The scalp was shaved and the skull was exposed. Three holes were drilled through the skull and the dura was carefully cut away to expose the brain. The incisor bar was adjusted so that the dorsal ventral measurements at lamda and bregma were no more than 0.01 mm apart (flat skull).

Evoked DA release was measured with microelectrodes 70-100 μm away from probes. Electrical connection between the brain and a Ag/AgCl reference electrode was achieved by creating a salt bridge with a Kimwipe soaked in aCSF and placed in a plastic pipet tip. A microelectrode was implanted into the striatum at a 5° angle from the vertical (0.7 mm anterior from bregma and 5.0 mm below dura). A bipolar stimulating electrode (MS303-1-untwisted, Plastics One, Roanoke, VA) was lowered into the ipsilateral medial forebrain bundle (MFB, 4.3 mm posterior from bregma, 1.2 mm lateral from midline, and 7.2 mm below dura) until evoked DA release was detected in the striatum.

Evoked release was measured three times at 20 min intervals. A microdialysis probe perfused with aCSF, aCSF with dexamethasone (DEX) or aCSF with XJB-5-131(XJB) was lowered into the striatum over the course of 30 min in the same coronal plane as the microelectrode (0.7 mm anterior to bregma, 2.5 mm lateral from midline, and 7 mm below dura). Two hours after implantation, stimulation of the MFB resumed at 20 min intervals. Nomifensine (20 mg/kg) was administered by intraperitoneal (i.p.) injection after the third stimulus and evoked release was measured 25 min later.

Statistics. Statistical analysis was performed with SPSS software. The statistical tests used for each analysis are explained in the results and discussion section.

Tissue Processing and Immunohistochemistry. The tissue was fixed by transcardial perfusion with 200 mL of 0.2 g/mL phosphate buffer saline (1x PBS: 155 mM NaCl, 100 mM phosphate, pH 7.40) followed by 250 mL of 4 % paraformaldehyde (PFA) and 50 mL of 0.1 % fluorescent nanobeads (Invitrogen, Eugene, OR). After the brains were removed, the tissue was postfixed for 2 h in 4 % PFA, soaked overnight in 30 % sucrose at 4 °C for cryoprotection, and stored at -80 °C until sliced. For TH staining, brains were postfixed for 24 h in cold 4 % PFA and then sectioned.

Horizontal tissue sections (perpendicular to the probes) were cut at 30 or 40 µm using a cryostat. For NeuN and ED-1 labeling, the slides were stored at -20 °C, washed three times in 1x PBS, incubated with Triton X-100 in PBS for 15 min, washed in 0.5 % bovine serum albumin (BSA), and soaked in 2 % BSA for 45 min. Then, the sections were incubated with antibody (NeuN or ED-1) for 1 h at room temperature, rinsed repeatedly in 0.5 % BSA, and incubated with the secondary antibody in 0.5 % BSA for 1 h at room temperature. Sections were rinsed in 0.5 % BSA and 1x PBS. For TH labeling, the sections were rinsed multiple times in 1x PBS at room temperature for 1 h, incubated in a blocking solution containing 2 % BSA, 0.3 % Triton X-100 in 1x PBS for 1 h at room temperature. Free floating sections were incubation with the TH antibody in the blocking solution at room temperature for 2 h followed by incubation at 4 °C for 48 h. Sections were rinsed three times for 10 min each in 1x PBS and incubated with the secondary antibody in blocking solution for 1 h at room temperature. Sections were then covered with gelvatol mounting medium (polyvinyl alchohol, gycerol, Tris buffer pH 8.5, and sodium azide in water) and coverslipped.

Image Processing and Quantification. As explained in the main text (see Fluorescence Microscopy and Image Processing), normalized counts of the four histochemical markers were

obtained from images after the images were treated with a threshold to eliminated scattered light and after the use of a freehand graphics cursor to eliminate the probe track and its edges (in the case of tissues labeled for TH) from the region of interest. Fig S-1 illustrates these procedures by means of an example. Fig S-1a is a raw image (not yet processed) of tissue surrounding a microdialysis probe track labeled with a primary antibody for tyrosine hydroxylase and a secondary antibody tagged with CY3. Note that the entire image is at least slightly red, including the center of the probe track that contains no tissues. This pale red background is due to light scattering. The first step in image processing is to set a threshold value to eliminate the background (Fig S-1b): setting the threshold is a built-in function in Metamorph. Once the user has set the threshold intensity, the software turns off any pixel with a subthreshold intensity value. This removes the pale background (note in Fig S-1b that the center of the probe track now appears black) but does not affect other features of the image. Fig S-1c shows a line drawn by means of a freehand graphics cursor tool in the software package. This line is placed around the probe track and its brightly labeled edges. In TH-labeled images such as this, the edges of the probe track exhibited bright fluorescence but this is most likely nonspecific binding. Inspection of the edge feature at high magnification did not reveal the expect pattern of labeling of axons or terminals. The software is then instructed to define the region of interest as that portion of the image outside the freehand line. Prior to counting, the software turns each pixel with an intensity above the threshold to white and leaves pixels with sub-threshold intensity black (Fig S-1d). The software then counts and reports the number of black and white pixels in the defined region of interest. Note, this procedure reports just a count of the number of above-threshold pixels and does not attempt to account for variations in fluorescence intensity. This is a typical approach to quantifying images such as these, because the fluorescence intensity can be affected by several

factors, such has how well the antibody penetrates the tissue slice, etc., that are difficult to control from one image to the next.

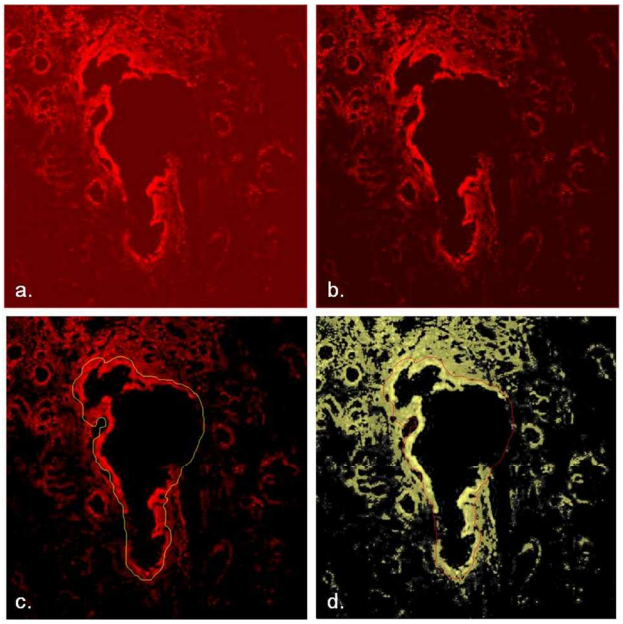

Fig S-1. Representative images of labeled brain tissue surrounding a microdialysis probe track. The tissue has been labeled with primary antibody for tyrosine hydroxylase and secondary antibody tagged with CY3. Images were processed using Metamorph software. The probe track is in the center of the each image. a) The raw image as imported into Metamorph. b) The same image after setting the threshold. c) The thin line was placed with a freehand graphics cursor. The region of interest is the portion of the image outside the freehand line. d) Prior to counting, pixels above the threshold are set to white and pixels below the threshold are left black. The software reports the number of white and black pixels in the region of interest.