

Supporting Information

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SI Materials and Methods

Electrophysiological Recording in Slices. PFC slices (300 μm) were placed in a perfusion chamber attached to the fixed stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial CSF (ACSF). Bicuculline (10 μM) and CNQX (25 μM) were added in NMDAR-EPSC recordings. Bicuculline (10 μM) and D-APV (25 μM) were added in AMPAR-EPSC recordings. Cells were visualized with a 40 \times water-immersion lens and illuminated with near infrared (IR) light, and the image was detected with an IR sensitive CCD camera. A Multiclamp 700A amplifier was used for these recordings. Tight seals (2–10 G Ω) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction, and the whole-cell configuration was obtained. The access resistances ranged from 13–18 M Ω . Evoked currents were generated with a pulse from a stimulation isolation unit controlled by a S48 pulse generator (Astro Med). A bipolar stimulating electrode (FHC) was positioned approximately 100 μm from the neuron under recording. Synaptic currents evoked by the same stimulation intensity of short pulses (0.6 ms for NMDAR-EPSC; 0.06 ms for AMPAR-EPSC) were recorded in individual neurons across groups with different manipulations. Patch electrodes (5–9 M Ω) were filled with the internal solution (in mM) for eEPSC: 130 Cs-methanesulfonate, 10 CsCl, 4 NaCl, 10 Hepes, 1 MgCl₂, 5 EGTA, 2.2 QX-314, 12 phosphocreatine, 5 MgATP, 0.2 Na₃GTP, and 0.1 leupeptin, pH 7.2–7.3, 265–270 mOsm. Membrane potential was held at -70mV during AMPAR-EPSC recording. For NMDAR-EPSC measurement, the neuron (clamped at -70mV) was depolarized to $+60\text{mV}$ for 3 s before stimulation to fully relieve the voltage-dependent Mg²⁺ block. Miniature EPSCs in PFC slices were recorded with low (1 mM) MgCl₂ ACSF in the presence of TTX (1 μM),

bicuculline (10 μM), and D-APV (25 μM). The membrane potential was held at -70mV .

Data analyses were performed with Clampfit (Axon Instruments) and Kaleidagraph (Albeck Software). Synaptic currents were analyzed with Mini Analysis Program (Synaptosoft). Statistical comparisons of synaptic currents were made using the Kolmogorov-Smirnov (K-S) test. For analysis of statistical significance, ANOVA tests were performed to compare the current amplitudes between groups subjected to different treatments.

Biochemical Measurement of Surface-Expressed Receptors. PFC slices were incubated with ACSF containing 1 mg/mL sulfo-NHS-LC-Biotin (Pierce Chemical Co.) for 20 min on ice. They were then rinsed 3 times in TBS to quench the biotin reaction, followed by homogenization in 300 μL modified RIPA buffer (1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid, 50 mM NaPO₄, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, and 1 mg/mL leupeptin). The homogenates were centrifuged at 14,000 $\times g$ for 15 min at 4 $^{\circ}\text{C}$. To measure total protein, 15 μg protein were removed. For surface protein, 150 μg protein were incubated with 100 μL of 50% Neutravidin agarose (Pierce Chemical Co.) for 2 h at 4 $^{\circ}\text{C}$, and bound proteins were resuspended in SDS sample buffer and boiled.

Behavioral Tests. For tail-suspension tests, animals were suspended (60 cm) above the floor by tapping the tail to a piece of tubing. The duration of immobility within a 6-min monitoring period was recorded. For open-field tests, animals were placed in an open area (60 \times 80 cm), and the amount of time that the animal spent in the center (25 \times 25 cm) was counted. Animals were examined at 1–2 days before stress exposure and 4-h or 1-day poststress.

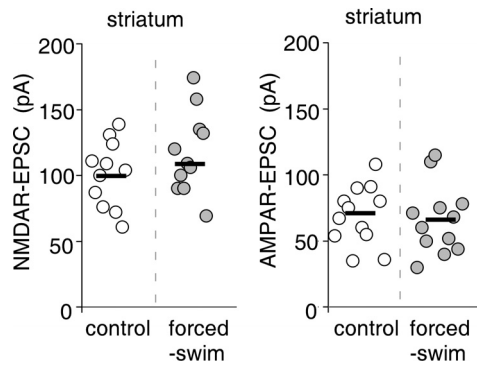


Fig. 51. Dot plots showing the amplitude of NMDAR-EPSC and AMPAR-EPSC in striatal medium spiny neurons taken from control or animals exposed to forced-swim stress.

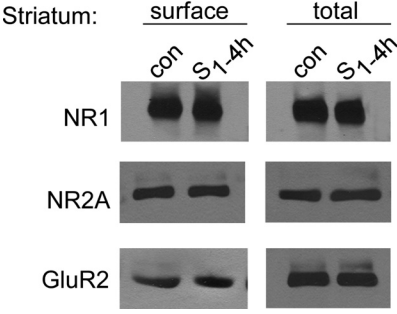


Fig. S2. Immunoblots of the surface and total NR1, NR2A, and GluR2 in lysates of striatal slices from control vs. acutely stressed animals (examined at 1–4 h poststress).