Stress and Cocaine Trigger Divergent and Cell Type-Specific Regulation of Synaptic Transmission at Single Spines in Nucleus Accumbens

Supplemental Information

Supplementary Methods & Materials

Details of Chronic Social Defeat Stress Procedure

CD-1 mice were screened for aggressive characteristics before the start of CSDS experiments on the basis of previously described criteria (1). All mice were housed within the social defeat cages (26.7 cm wide x 48.3 cm deep x 15.2 cm high, Allentown Inc.) for at least 4 hours before the start of defeat on one side of a clear perforated Plexiglass divider (0.6 cm x 45.7 cm x 15.2 cm, Nationwide Plastics). Experimental C57Bl/6J mice were then subjected to a new CD-1 aggressor mouse for 10 min daily over 10 consecutive days. After the 10 min interaction, experimental mice were moved to the opposite side of the social defeat cage behind a Plexiglass divider, which allowed sensory contact during the next 24-hour period. Control mice were housed two per cage on opposite sides of the perforated divider and rotated daily in a manner similar to the defeat group, but never exposed to aggressive CD-1 mice. Twenty-four hours after the final social defeat stress, the experimental C57Bl/6J mice were singly housed.

Social interaction testing, performed as previously described (1), was carried out under red-light conditions. Mice were placed in a novel interaction open-field arena custom made from opaque Plexiglass (42 cm x 42 cm x 42 cm, Nationwide Plastics) with a small animal cage placed at one end. Mice were monitored and recorded using Ethovision 3.0 tracking software (Noldus Information Technology) for 2.5 min in the absence (target

absent phase) of a new CD-1 mouse. This phase was used to determine baseline exploratory behavior. We then immediately measured 2.5 min of exploratory behavior in the presence of a caged CD-1 mouse (target present phase), recording total distance traveled and duration of time spent in the interaction and corner zones. Social interaction behavior was calculated as a ratio of the time spent in the interaction zone with target present to the time spent in the interaction zone with target absent. Ratio above 1.0 was classified as resilience to CSDS and ratio below 1.0 was classified as susceptibility to CSDS based on extensive validation (1,2).

Details of Electrophysiological Recording Techniques

Artificial cerebrospinal fluid contained in mM: 128 NaCl, 3 KCl, 1.25 NaH_2PO_4, 10 D-glucose, 24 $NaHCO_3$, 2 $CaCl_2$ and 2 $MgSO_4$ (oxygenated with 95% O_2 and 5% CO_2, pH 7.35, 295-305 mOsm). Cells were visualized through a 60× water-immersion objective with either infrared differential interference contrast optics or epifluorescence to identify $EGFP^+$ (D2) MSNs. Whole-cell voltage-clamp recordings were made from D1- and D2-MSNs in the NAc shell region. D1-MSNs and D2-MSNs were identified on the basis of the respective absence and presence of EGFP fluorescence, presence of spines, and their membrane properties. Cells were held at -70 mV. Patch pipettes (3–4.5 MΩ) pulled from borosilicate glass (BF150-110-10, Sutter Instrument) were filled with an internal solution containing (in mM): 115 potassium gluconate, 20 KCl, 1.5 $MgCl_2$, 10 phosphocreatine, 10 HEPES, 2 magnesium ATP, 0.5 GTP and 50 μM Alexa 594 (pH 7.2, 285 mOsm).

Supplemental References

1. Golden SA, Covington HE, Berton O, Russo SJ (2011): A standardized protocol for repeated social defeat stress in mice. *Nat Protoc* 6: 1183–91.
2. Krishnan V, Han M-H, Graham DL, Berton O, Renthal W, Russo SJ, *et al.* (2007): Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 131: 391–404.