ONLINE METHODS

Animals

Male bitransgenic mice derived from *NSE-tTA* (line A) x *TetOp-ΔfosB* (line 11) and *NSE-tTA* (line A) x *TetOp-FLAG-ΔcJun* (line E) mice[23,50] were conceived and raised on 100 μg/ml doxycycline to suppress ΔFosB or ΔcJun expression during development. Line 11A is fully backcrossed on a C57BL/6J background. Line EA is a roughly 50:50 mixture of FVB and 129 backgrounds. This background difference explains some of the differences in baseline behavior observed between the controls of the two bitransgenic lines. However, for every experiment, littermate controls were used to limit any effects of genetic background on these studies. Thus, littermates were divided at weaning: half remained on doxycycline and half were switched to water, and the animals were used 8 to 11 weeks later when transcriptional effects of ΔFosB and ΔcJun are maximal[22,24]. For chronic social isolation stress, 8 week old C57Bl/6J mice were housed individually for a period of 8 weeks before testing as described[25]. Chronic fluoxetine was administered via subcutaneous pellets (Innovative Research, Novi, Michigan) implanted in the dorsal interscapular region under brief isoflurane anesthesia (Henry Schein, Melville, New York). Pellets were designed to administer 20 mg/kg/day of fluoxetine (or placebo) over a 20-day interval and result in clinically-relevant blood levels[18]. Mouse procedures were performed in accordance with the Institutional Animal Care and Use guidelines of University of Texas Southwestern and Mount Sinai School of Medicine.

Social defeat stress and social interaction experiment

In most experiments, 8 week old C57Bl/6J mice were submitted to social defeat stress for 4 or 10 consecutive days as described previously[10,11]. Social interaction tests were performed 1 day after the last day of defeat unless specified otherwise. Day 1 always refers to the

beginning of the social defeat procedure, such that, for example, Day 11 would refer to a social avoidance test performed 24 hr after the last day of 10 days of social defeat.

Bitransgenic mice overexpressing ΔFosB or ΔcJun, or their control littermates, were tested at 16 weeks of age and subjected to 4 or 10 days of social defeat. To examine the vulnerability of isolated mice to social defeat, the isolated mice were subjected to three consecutives defeats on the same day, and then tested for social interaction the following day. This acute defeat paradigm has been validated previously to reveal pro-susceptibility factors[10]. The segregation of defeated mice into susceptible and resilient subpopulations was performed as described previously[10]. Because most of the control mice spent more time interacting with a social target than an empty target enclosure, an interaction ratio of 100 (equal time spent in the interaction zone in the presence vs. absence of a social target) was set as a cut-off: mice with scores <100 were labeled ‘‘susceptible’’ and those with scores >100 were labeled ‘‘resilient’’. Extensive behavioral, biochemical, and electrophysiological analyses support the validity of these distinct susceptible and resilient subpopulations[10,17].

Viral-mediated gene transfer

Herpes simplex virus (HSV) vectors encoding green fluorescent protein (GFP), ΔFosB, ΔJunD, GluR2, or GluR2Q (unedited version) have been previously used and validated[10,22,52]. SC1, contained within a pSPORT vector, was inserted into the HSV amplicon HSV PrpUC and packaged into virus with helper 5dl1.2, as described previously[22]. Adeno-associated virus (AAV) overexpressing ΔJunD or eGFP have been used according to previous studies[11,22] . Expression of the HSV transgene is maximal between 1–4 days after infusion and dissipates completely by day 7[10,25]. In contrast, the AAV transgene is relatively long-lived and persists for at least several months. Importantly, we have shown in numerous published studies that both HSV and AAV vectors express their encoded transgenes within neurons only[22]. Within NAc, expression predominates in medium spiny neurons with relatively sparse expression in GABAergic and cholinergic interneurons, reflecting the neuronal makeup of this brain region. Viruses were injected into the NAc using established

stereotaxic coordinates[10,11,22,25]. Viral injection sites were confirmed for all animals with standard histologic methods (for examples, see **Supplementary Fig. 8**).

Immunohistochemistry and western blotting

Brain sections from mice subjected to social defeat were processed for immunohistochemistry as described previously[20]. Brains were perfused 18–24 hours after the last exposure to the last defeat or the last injection of fluoxetine, resulting in the degradation of any residual full-length FosB protein, such that all remaining immunoreactivity reflects ΔFosB, as confirmed by western blotting. This was confirmed by showing no significant staining with an antibody directed against the C-terminus of full length FosB which does not recognize ΔFosB (data not shown). The number of ΔFosB immunopositive cells was quantified in multiple sections through the NAc of each animal, with mean values then calculated for each animal. Each animal was considered an individual observation for statistical analysis.

Quantification of GluR2 and GluR1 immunoreactivity after social defeat, and of ΔFosB after social isolation, was performed on NAc sections and revealed using a Licor system as described previously[18]. Integrated intensities of the protein of interest, e.g., GluR2, GluR1, ΔFosB, and H1, were determined using Odyssey software. Results are presented as integrated intensity values per mm^2 and are presented as mean ± s.e.m. (n = 8 to 12 per group). Values for H1 were used as reference. Ratios of GluR2, GluR1, and ΔFosB over total H1 were analyzed and Student's t-tests were used to compare means for each brain region. Differences were considered significant when P values were <0.05. Statistical analyses were performed with GraphPad Prism.

Microdissected NAc punches from mice subjected to social defeat were processed for immunoblotting as described previously[10,18]. Western blots were probed with an antibody to ΔFosB (Cell Signaling) and GAPDH (Abcam) and then scanned and quantified using Odyssey imaging system (Licor).

Human postmortem brain tissue

Human postmortem brain tissue was obtained from the Dallas Brain Collection (DBC), where tissue is collected from the Dallas Medical Examiner's Office and the UT Southwestern Tissue Transplant Program after consent from the next-of-kin (NOK). Tissue was analyzed only from males, with 8 depressed and 8 normal cases matched for age, postmortem interval, RIN (RNA integrity number), and pH; the case demographics are given in **Supplementary Table 1**. Outstanding tissue quality was confirmed by high RIN values. Cases are subjected to a standard dissection before snap freezing in –40°C isopentane and storage at –80°C; later dissection of NAc was performed on the frozen tissue. The UT Southwestern IRB reviewed and approved the collection of this tissue for research use. A direct informant interview was carried out for each depression case at a later date, where information regarding the case's illness was documented; a consensus diagnosis of Major Depressive Disorder (MDD) was made using DSM-IV criteria by two research psychiatrists. None of the cases included in the study had blood toxicology screens positive for drugs of abuse, alcohol, or prescription drugs other than antidepressants. A minority of the cases were positive for antidepressants as indicated in **Supplementary Table 1**. Tissue samples were dispensed in a blinded fashion for analysis and processed for western blotting as described previously[10,18]. Western blots were probed with an antibody to ΔFosB (rabbit anti-FosB polyclonal antibody generated against amino acids 1–16 of FosB/ΔFosB), GluR2 (Millipore), GluR1 (Millipore), or SC1 (R&D) and β-actin (Cell Signaling), then scanned and quantified using Odyssey imaging system (Licor).

Other behavioral tests

Bitransgenic mice (line 11A and EA) were tested in open-field, elevated plus maze, light-dark, sucrose preference, and forced swim tests based on published protocols[10,11,18,25]. We used a one day forced swim test in mice. We also routinely confirmed the ability of anxiolytic benzodiazepine drugs to reduce anxiety-like measures in the open-field, elevated

plus maze, and light-dark tests. The antidepressant effects of HSV-mediated overexpression of ΔFosB or SC1 were tested in rats using a two-day forced swim test. Rats were used for this experiment because a larger range of behaviors can be measured in the forced swim test in rats compared to mice[26]. The two day forced swim test in rats was designed to test antidepressant efficacy: immobility time is increased on day two of the test and reduced by antidepressant administration. More recently, time of immobility on day one has also been proposed to reflect a stress-sensitive measure[18]. We therefore analysed immobility time on the first day of testing as well.

RNA isolation and qPCR

Two or ten days after the last defeat episode, mice were rapidly decapitated, and brains were removed and placed on ice. Dissections of NAc were taken with a 14-gauge needle punch and quickly frozen on dry ice until RNA was extracted. RNA isolation, qPCR, and data analysis were performed as previously described[18]. Data were analyzed by comparing C(t) values of the treatment condition (control vs. susceptible or resilient) with the ΔΔC(t) method.

Chromatin immunoprecipitation

ChIP was performed on pooled bilateral NAc punches from four mice 1 hr after their last defeat. A total of 20 mice per group were used. Tissues were crosslinked, washed, and kept at –80ºC. Sheared chromatin was incubated overnight with anti-FosB antibody (SC048, Santa Cruz, CA) previously bound to magnetic beads (Dynabeads M-280, Invitrogen). Non-immune IgG was used as a control. After reverse cross-linking and DNA purification, the binding of ΔFosB to the GluR2 promoter was determined by qPCR using primers spanning the portion –500 to –320 nt from the start codon, a region containing AP-1 sites. qPCR performed on a region with no AP1 sites, with primers amplifying a product from –240 to –100 from the start codon, showed no induction in defeated tissue compared to control tissue.

Electrophysiology

All electrophysiological experiments were performed on control mice, or those subjected to chronic (10 days) social defeat, 2–28 days after the last defeat episode. Coronal NAc slices (250 μm thick) were cut in ice-cold sucrose ACSF: 254 mM sucrose, 3 mM KCl, 1.25 mM NaH_2PO4, 10 mM D-glucose, 24 mM $NaHCO_3$, 2 mM $CaCl_2$, and 2 mM $MgSO_4$, oxygenated with 95% O_2 and 5% CO_2 (pH 7.35, 295–305 mOsm). After a 1 hr recovery at 37°C in ACSF (254 mM sucrose replaced by 128 mM NaCl), electrophysiological recordings were performed at 30–32°C in ACSF containing 50 μM 2-amino-phosphonovaleric acid (APV) and 50 μM picrotoxin. Patch pipettes (3–5 MΩ resistance) for whole-cell recordings were filled with an internal solution containing: 115 mM potassium gluconate, 20 mM KCl, 1.5 mM $MgCl_2$, 10 mM HEPES, 10 mM phosphocreatine, 2 mM ATP-Mg, 0.5 mM GTP, 100 μM spermine, and 1 mM QX-314 (a Na^+ channel blocker) (pH 7.2, 280–290 mOsm). Medium spiny neurons from the shell of NAc were identified under visual guidance using infrared-differential interference contrast (IR-DIC) video microscopy with a 40x objective (Olympus BX51-WI). Whole-cell patch-clamp recordings were performed with a computer-controlled amplifier (MultiClamp 700B), digitized (Digidata 1440), and acquired with Axoscope 10.1 (all from Axon Instruments, Union City, CA) at a sampling rate of 10 KHz. Electrode potentials were adjusted to zero before obtaining the whole-cell configuration, and only cells with resting membrane potential of –72 to –82 mV were used. Synaptic responses were elicited by local electrical stimulation of 0.01 to 0.25 mA square pulses 100 μsec in duration delivered every 10 sec using a tungsten bipolar stereotrode (1.0 MΩ) and DS-8000 digital stimulator (World Precision Instruments, Saratoga, FL). Evoked AMPA excitatory postsynaptic currents (EPSCs) were recorded in voltage-clamp configuration and amplitudes were measured at 0.033 msec after stimulus. An average of at least 6 EPSCs at –80 mV and +40 mV were used to determine the ratio in each cell. For 1-naphtylacetylsperimine (NASPM) experiments, AMPAR EPSCs were recorded at –80mV before (baseline) and 10

minutes after bath application of 100μM NASPM. An average of at least 6 EPSCs in each condition for each cell were used to determine the ratio reported.

Statistical analyses

Data displayed are expressed as means ± standard error of the mean (represented as error bars). One-way ANOVA was used to compare means between control, susceptible and resilient in all immunohistochemical, biochemical, and behavioral analysis. Two-way ANOVA was used to compare the effect of the overexpression of the transgene in the bitransgenic lines or with the virus, the effects of fluoxetine, or the effect of NBQX on social interaction test. Student's *t*-test was used to compare means in the social isolation experiment and between groups in the human post-mortem tissue study. Differences between experimental conditions were considered statistically significant when $P < 0.05$.