Supplemental Information

Methods and Materials: Detailed Experimental Procedures

Chronic social defeat stress (CSDS). CSDS was carried out using a method reported recently (1). Test mice were exposed to a different CD1 aggressor mouse each day prior to lights out (1600) for 10 min over a total of 10 days. During the brief exposure, all defeated mice showed signs of stress and subordination, including vocalization, active escape behavior, and submissive posturing. After the 10 min of physical contact, test mice were separated from the aggressor and placed across a plastic separator with holes, where they remained in sensory contact with the CD1 aggressor for the remainder of the 24 hours. Controls were handled daily in the palm of the hand for 30 seconds and housed in equivalent cages with members of the same strain. After the last defeat, all mice were housed individually. On Day 11, we employed a social interaction test to measure the behavioral consequences of the chronic defeat stress. This was accomplished by placing mice in a new arena with a small animal cage at one end, with their movement tracked for 2.5 min in the absence of another mouse, followed by 2.5 min in the presence of a caged, unfamiliar target CD1 mouse. The duration in the interaction zone and other measures were obtained using Ethovision 3.0 software (Noldus, Leesburg, VA). Social interaction was quantified by comparing the amount of time the test mouse spent in the interaction zone near the small animal cage in the presence vs. in the absence of the target CD1 mouse ("% target absent"). Food intake and body weights of mice were monitored daily at 1500 during the CSDS. Both 'susceptible' and 'resilient' animals as defined previously (2) were included in testing. The two social defeat experiments described in the text had similar levels of resilient mice in each group (Saline 2/10 mice and 2/10 mice respectively, and Leptin 3/10 and 1/10 respectively). This rate of resilience is similar to that reported previously.

Melanocortin 4 receptor (MC4R) null mice were back-crossed onto c57BL6/J seven generations. Heterozygote X heterozygote breeding pairs were established and all experiments were performed using homozygous null mice and wild-type litters. In order

to prevent differences in body weight due to hyperphagia, mice were individually housed from ages 6-8 weeks and MC4R null mice were pair fed compared to wild-type littermates (24.87 g vs. 24.01 g, p=0.42).

Elevated plus maze. Mice were placed in the center of an elevated plus maze (arms are 33 cm × 5 cm, with 25 cm tall walls on the closed arms) under dim lighting and their behavior was videotaped for 5 min. The time spent on the closed and open arms was determined by video tracking software, Ethovision 3.0 (Noldus). Time spent on the open arm is negatively correlated with anxiety-like behavior.

Forced swim test. The forced swim test was performed according to published protocols (1). Mice were videotaped while in a 4 L Pyrex glass beaker containing 3 L of water at 24° ± 1°C for 6 min. Two trained and blinded observers scored the videotape manually. Following a two min lead-time, latency to immobility (Latency) was determined as the first cessation of all movement for 3 seconds. Total immobility was measured as the time spent without any motion except for single limb paddling to maintain flotation.

Sucrose preference. Sucrose preference testing was performed as described previously (2). Briefly, during the last two days of social defeat stress or control handling, mice were habituated to drinking water from two fifty-ml conical tubes. Mice were then individually housed and tested for social interaction as described above. The mice received either leptin (1 mg/kg) or saline i.p. twice daily for four days. During the first two days mice received 1% sucrose in both bottles. On the final two days of injections, the mice had access to either 1% sucrose (bottle A) or water (bottle B). The total volume consumed of each bottle was measured daily and the position of the bottles was rotated to assess for position bias. Sucrose preference is presented as (volume A/(volume A +B))*100 for the final two days.

Injections. The β_3-adrenergic antagonist SR59230A (Sigma Aldrich, St Louis, MO) was made as a 10X stock (50 mg/kg) in 25% ethanol, 25% DMSO, and 50% phosphate

buffered saline (PBS). The stock was diluted to a final concentration of 5 mg/kg in PBS just prior to daily injections (0700, 1900). Vehicle injections contained 2.5% ethanol, 2.5% DMSO, and 95% PBS. The β_3-adrenergic agonist CL316243 (Tocris, Ellisville, MO) was made as a 100X stock in PBS and diluted to a final concentration (1 mg/kg) just prior to injection at 1500 hr. The specified amount of melanotan II (Bachem, Torrance, CA) was dissolved in PBS 0.2 ml and was injected i.p. four times daily (0100, 0700, 1300, 1900) for 2 days. Recombinant mouse leptin (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) was dissolved in PBS pH 7.8 to 0.1 mg/ml and injected i.p. twice daily (0700, 1900) at a final dosage of 1 mg/kg during sucrose preference and social interaction testing.

Quantitative PCR. Mice were killed by cervical dislocation, the brains were rapidly removed, the hypothalamus was grossly dissected, and epididymal fat was collected. Samples were immediately placed on dry ice and stored at -80° until processing. High quality mRNA was isolated by Trizol (Invitrogen, Carlsbad, CA) and chloroform (Sigma Aldrich) extraction and column purification following the directions of the kit (#74004, Qiagen, Valencia, CA). Complementary DNA (cDNA) was synthesized from 500 ng of mRNA by random hexamer priming (Invitrogen, #12371-019). Twenty ng of cDNA were amplified in duplicate using 8 pg of the stated primer with quantification of the DNA product by SYBR Green binding (#4367659, Applied Biosystems, Foster City, CA).

Table S1. Primer sequences

PPIB	Cyclophilin	F: GCCTGTGGAATGTGAGGGGTG R: CATCTATGGTGAGCGCTTCCCA
LEP	Leptin	F: GACCATTGTCACCAGGATCCA R: GGTGAAGCCCAGGAATGAAG
NPY	Neuropeptide Y	F: TATCCCTGCTCGTGTGTTTG R: GTTCTGGGGGCATTTTCTG
POMC	Pro-opiomelanocortin	F: ACCTCACCACGGAAAGCAA R: CGGGGATTTTCAGTCAAGG
AgRP	Agouti-related peptide	F: AGCAGACCGAGCAGAAGATG R: GACTCGTGCAGCCTTACACA

Western Blotting. In a separate experiment, 8 week old C57BL/6 mice were subjected to chronic social defeat stress as above. On Day 11 mice were tested for social interaction and on the following day received a single injection of leptin (1 mg/kg i.p.) at 1300. One hour later, the hypothalamus was collected by gross dissection and immediately frozen on dry ice and stored at -80° C until processing. Samples were resuspended in RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 5 µg/ml Aprotinin, 5 µg/ml Leupeptin, 1% Triton x-100, 1% Sodium deoxycholate, 0.1% SDS) supplemented with phosphatase inhibitors (Sigma Aldrich) and then sonicated to disrupt the tissue. Debris was cleared by centrifugation at 14,000x g for 15 minutes at 4° C. Protein concentration was determined by DC protein assay (Bio-Rad, Hercules, CA). 20 µg of protein were loaded per well and run by standard SDS-PAGE procedures. Blots were probed for STAT3 (#06-596, Upstate Biochem, Lake Placid, NY) and phospho-STAT3 (#9131S, Upstate Biochem) and visualized using SuperSignal West Dura Substrate (#34076, Pierce, Rockford, IL). Images were quantified using ImageJ software (NIH, Bethesda, MD). Data presented as fold difference of phospho-STAT3/STAT between control and social defeat mice treated with leptin. No phospho-STAT3 signal was visualized in saline treated mice.

1. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, *et al.* (2008): The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci*. 11:752-753.

2. Krishnan V, Han MH, 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.