Supplement 3

Materials and Methods

Mice

Briefly, the neuron specific enolase-tetracycline transcriptional activator (NSE-tTA) line is on a BL6/SJL x ICR background, the TetOp-Cre line is on an ICR background, and the floxed BDNF mice are on a BL6/sv129 background. The NSE-tTA mice and the TetOP-Cre mice were maintained as homozygotes then crossed to generate the bigenic mice. The floxed BDNF mice were then crossed with the bigenic NSE-tTA/TetOp-Cre mice to generate the inducible KO mice as previous reported (1). All experiments were performed on littermates derived from this mating paradigm to ensure analysis of matched controls. Mice were bred off doxycycline to produce a late embryonic *bdnf* deletion in the forebrain (2). Nonstressed mice were handled by husbandry staff or for weekly weighing only, while stressed mice were housed in a separate room and handled as described in the following section. The female mice were studied at random points of the estrus cycle. Mice were housed two to four per cage and had *ad libitum* access to food and water except during restricted periods described in the stress methods. Mice were habituated to testing facilities one hour prior to behavioral assessment between the hours of 0800 and 1300. The female mice were housed separately from males and were examined behaviorally on the subsequent day of testing after the males. All animals were housed in a normal light cycle.

Fur state assessment

Fur score scale was designated as follows:

1. Fur is smooth and shiny with no wound or bald patches.

2. Fur is mostly normal and smooth, some patchiness or oiliness with fur spiking.
3. Fur is oily or spiking over most of the body, or has large or numerous bald patches.
4. Fur is patchy, spiky, or stained over most of the body, with wounding.

Sucrose consumption test

Briefly, group housed mice were habituated to a 1% sucrose/tap water solution for 48 hours. The mice were then habituated to water deprivation periods of 4, 14, and 19 hours followed by a 1 hour exposure to the sucrose solution for three days with intervening access to regular drinking water. To assess individual sucrose intake, the group housed mice were water deprived overnight and then housed temporarily in a new cage. Each test mouse was placed in its home cage for one hour with access to the 1% sucrose solution. The bottle of sucrose solution was weighed before and after the test to determine sucrose intake.

Novelty suppressed feeding

Briefly, group housed animals were food deprived for 24 hours and then placed in a temporary home cage for 30 minutes. For the test, individual mice were placed in a 72 x 72cm open field arena at 40 lux. A single pellet of the mouse's regular food chow was placed in the center of the open field arena. Each animal was placed in a corner of the arena and allowed to explore for up to 5 minutes. The trial ended when the mouse chewed a part of the chow. Latency to eat in the novel environment was recorded in seconds.

Tail suspension test

TST was performed on an automated tail suspension device (MedAssociates Inc., St. Albans, Vermont)(3). Animals were suspended from a strain gauge for 6 minutes. The settings for the equipment were time constant= 0.25, gain=4, threshold 1=3, and resolution=200 ms. Time spent immobile was recorded in seconds.

Forced swim test

Mice were placed in a 5000 mL glass beaker (approximately 16 cm in diameter and 25 cm high) containing 3000 mL of water at 24±1° C for 6 minutes. Water was changed between subjects. Test sessions were recorded by a camera and videotapes were analyzed and scored by an observer blind to group and genotype. Immobility duration and latency were measured during the last four minutes of testing. Mice were sacrificed 10-15 minutes after FST by decapitation, trunk blood was collected, and hippocampi were dissected and rapidly frozen.

References

1. Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, *et al.* (2007): Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry*. 61:187-197.

2. Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, *et al.* (2004): Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci U S A.* 101:10827-10832.

3. Liu X, Gershenfeld HK (2001): Genetic differences in the tail-suspension test and its relationship to imipramine response among 11 inbred strains of mice. *Biol Psychiatry*. 49:575-581.