Supporting Information

O'Leary et al. 10.1073/pnas.1404090111

SI Materials and Methods

Animals. $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice were generated on a BALB/c genetic background as previously described by converting the initiation codon of each isoform into a stop codon using a knockin approach (1). Previous studies have demonstrated the complete absence of $GABA_{B(1a)}$ and $GABA_{B(1b)}$ protein in $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice, respectively, confirming that mutation of the initiation codons prevents translation of the individual subunits (see figure 1c in ref. 1). Male and female wild-type (WT), $GABA_{B(1a)}^{-/-}$, and $GABA_{B(1b)}^{-/-}$ mice used in this study were bred from homozygote parents at our breeding facility at University College Cork. These homozygote pairs were obtained by breeding heterozygous mice as previously described (2) and according to the recommendations proposed by The Jackson Laboratory to obviate genetic drift and the formation of substrains (http://jaxmice.jax.org/genetichealth/GQCprogram.html). WT mice were generated by breeding WT siblings generated from $GABA_{B(1a)}^{+/-}$ and $GABA_{B(1b)}^{+/-}$ heterozygous breeding. Animals were housed under standard conditions under a 12-h light/dark cycle (lights on at 7:00 AM). All experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) and approved by the Animal Experimentation Ethics Committee of University College Cork.

Helpless H/Rouen mice and their nonhelpless counterparts were bred in Centre de Recherche en Neurosciences de Lyon INSERM U1028-CNRS 5292, Lyon, France, as previously described (3), and tissue was shipped to University College Cork. Briefly, mice from an original stock of Swiss albino CD1 mice (Charles River) were selectively bred for high or low spontaneous "helplessness" in the tail suspension test (TST) (3). The chosen selection criteria, which were the same for each generation, were a high immobility score (>115 s) for "helpless" (H/Rouen) and a low immobility score (<35 s) for "nonhelpless" (NH/Rouen) in the TST. This produced a mouse line that consistently shows depression-like behavior in a variety of preclinical tests including reduced sucrose preference, and responds to antidepressant treatment and exhibits alterations in a number of neurobiological markers associated with depression or antidepressant action (3–6). Test mice were group-housed five per cage under controlled conditions (temperature 20–21 °C, 55–60% humidity) under a 12-h light/dark cycle (lights on 7:00 AM) and provided with chow and water ad libitum. All experiments were performed with male mice from generation S35 aged 9–18 wk.

Social Defeat Stress Experimental Design. A 10-d social defeat (SD) stress paradigm was used as a chronic stressor in adulthood. We have previously shown that this paradigm effectively induces social avoidance behavior in BALB/c mice, the background strain used in the present study (7). The experimental design for the SD stress experiment is illustrated in Fig. 1*A*. WT, $GABA_{B(1a)}^{-/-}$, and $GABA_{B(1b)}^{-/-}$ mice were exposed to a 10-d SD stress. Effects on anhedonia were examined by measuring preference for a 0.1% saccharin solution over water from 8:00 PM to 8:00 AM the night before initiation of the first SD and the night before the social interaction test (which was used to measure social avoidance behavior).

Social Defeat Stress. The 10-d SD stress was conducted as previously described (7) and consisted of daily placing a test mouse in the home cage of a new aggressive resident CD1 mouse until the occurrence of a first aggressive attack from the aggressor that resulted in defeat from the intruder mouse. A 3-min cutoff for latency of attack was used to maintain a short interaction between the mice. If a test mouse was not attacked by an aggressor within 3 min, the aggressor was encouraged to move and attack. Thereafter, the test mouse and the aggressor were physically separated by a perforated transparent Plexiglas wall and remained in sensory contact for 24 h until the next defeat by a different aggressor. Control mice were left undisturbed and housed in pairs in their own home cage with another control mouse of the same strain and under the same sensory conditions as stressed mice (i.e., separated by a transparent perforated wall) but without any physical interaction allowed.

Social Interaction Test. Social defeat stress induces social avoidance behavior whereby defeated animals spend less time exploring the area around an aggressive mouse (social target) compared with the same area when the mouse is absent (nonsocial target) (7, 8). To determine the effects of genotype on SD stress-induced social avoidance, we thus used the social interaction test on the morning following the last defeat (7, 8). Briefly, mice were placed in a white-painted open arena (40 × 30 × 25 cm^3, length × width × height), comprising an empty wire-mesh cage of 10 × 6 cm^2, on one side of the box for two 2.5-min sessions as illustrated in ref. 7. During the first trial ("no target"), mice were placed in an open arena facing the wall at the opposite side of an empty wire-mesh cage. Mice were allowed to explore the empty arena for 2.5 min. Mice were then placed back in their home cage for 1 min. Meanwhile, an aggressive mouse that had been used during the SD stress was placed inside the wire-mesh cage of the open arena. The aggressor (CD1 mouse) was different for every test mouse. Test mice were then placed back into the arena for a second trial of a 2.5-min social exploration ("target") with the aggressor. At the end of the second session, mice were returned to their home cage. Social interaction boxes were cleaned between each mouse with 70% (vol/vol) ethanol to avoid odor cues. Experiments occurred under red-light conditions between 10:00 AM and 2:00 PM and were videotaped with an infrared camera. Behavior was measured and analyzed posttest with the EthoVision tracking system (Noldus). Social avoidance behavior was assessed by measuring the time spent in the zone of interaction, an area surrounding the wire-mesh cage, as previously described by Savignac et al. (7). Locomotor activity was also assessed by measuring the distance moved.

Saccharin Preference Test for SD Stress Experiment. Anhedonia, the loss of interest in previously pleasurable activities, is a core feature of depression and can be assessed in rodents by measuring their preference for a sweet solution over water. Mice susceptible to SD stress exhibit reduced preference to drink a sweet solution of 1% sucrose (9). In the present work, we used a sweet solution of 0.1% saccharin to ensure preference was not associated with its calorific value. The concentration was chosen based on the findings of others who have shown that antidepressant treatment in mice can prevent the effects of chronic mild stress on preference for a 0.1% saccharin solution (10). Pilot studies in our laboratory indicated that 12-h exposure to this concentration of saccharin was sufficient to induce preference in nonstressed mice. Therefore, we measured preference for a 0.1% saccharin solution over water from 8:00 PM to 8:00 AM the night before initiation of the first SD and the night before the last SD stress.

Unpredictable Maternal Separation Combined with Unpredictable Maternal Stress. Accumulating evidence suggests that traumatic events particularly during early life (e.g., parental loss or neglect)

coupled with genetic factors are important risk factors for the development of depression and anxiety disorders (11–13). Moreover, the brain is particularly vulnerable to the effects of stress during this period (14). Maternal separation (MS) in rodents is a useful model of early-life stress that results in enduring physiological and behavioral changes that persist into adulthood, including increased hypothalamic–pituitary–adrenal (HPA)–axis sensitivity, increased anxiety, and visceral hypersensitivity, particularly in rats (15). Such effects of MS tend to be more subtle in mice (16, 17), and thus MS may be a useful tool to unmask genetic risk factors associated with susceptibility to the negative effects of early-life stress. Because models of MS are notoriously difficult for producing reliable effects on depression-related behavior in mice as opposed to rats (17), we chose to use the model characterized by Mansuy and colleagues. In this paradigm, pups are separated at an unpredictable time during the light or dark cycle and the mother is also exposed to a brief unpredictable stressor at an unpredictable time point during the period of separation (18). Mansuy and colleagues have reported that this paradigm of unpredictable maternal separation combined with unpredictable maternal stress (MSUS) can increase depression-like behavior in mice (18). We have previously shown that this paradigm can induce visceral hypersensitivity in mice (16), a characteristic of irritable bowel syndrome, a disorder often comorbid with stress-related psychiatric disorders.

MSUS was conducted essentially as previously described (18). One week following mating, the male breeder was removed from the cage to avoid any contact with the offspring. Only dams that gave birth within 1 wk of each other were used. Litters with fewer than two pups were not included in the study. Maternally separated pups underwent unpredictable maternal separation combined with MSUS from postnatal day (PND) 1 through 14; the day of birth was considered as day 0. Control pups (non-maternally separated; NMS) were left undisturbed except for cage changing on PND2, PND8, PND15, and PND21. The MSUS procedure involved separating the pups from the dams for 3 h daily at an unpredictable time either during the light cycle or early dark cycle. During separation, dams and pups were housed in the same cage but were separated by a transparent Plexiglas barrier, close enough to have olfactory and visual contacts. The cages used during the separation procedure were clean with both food and bedding provided. At some point during the 3 h of maternal separation, dams underwent an unpredictable stress every day from day 1 to day 14 of the MSUS protocol. The stress was applied randomly and consisted of 6 min of forced swim stress in cold water (18 °C) or 20 min of restraint in a plastic tube with air holes. Animals were weaned on PND21 and, once weaned, were grouped in three or four mice per cage. Mice of the same genotype were grouped in the same cages but were mixed with pups from different dams to avoid a litter effect.

Behavioral Testing Battery for the MSUS Experiment. The battery of behavioral tests used in the MSUS experiment is illustrated in Fig. 1*B*. Ultrasonic vocalization measurements from MS pups were done on PND1 and PND7 between 9:00 AM and 11:00 AM. All other tests were performed on both NMS [males: WT $n = 14$; $GABA_{B(1a)}^{-/-}$ $n = 12$; $GABA_{B(1b)}^{-/-}$ $n = 14$; females: WT $n = 11$; $GABA_{B(1a)}^{-/-}$ $n = 6$; $GABA_{B(1b)}^{-/-}$ $n = 14$] and MS (males: WT $n = 14$; $GABA_{B(1a)}^{-/-}$ $n = 12$; $GABA_{B(1b)}^{-/-}$ $n = 14$; females: WT $n = 10$; $GABA_{B(1a)}^{-/-}$ $n = 10$; $GABA_{B(1b)}^{-/-}$ $n = 13$) mice in adulthood beginning at 8 wk of age. Behavioral tests were conducted in the following order: stress-induced hyperthermia (SIH), open field (OF), tail suspension test (TST), elevated plus maze (EPM), saccharin preference test (SPT)/female urine sniffing test (FUST), and forced swim test (FST). Anhedonia was assessed in female mice using the SPT and in male mice using the FUST. Animals were allowed to recover for 1 wk before testing in the subsequent behavioral test. Each test was performed during the light cycle starting at 11:00 AM. The OF, EPM, and FUST were performed under red-light conditions because BALB/c mice are an innately anxious mouse strain. All tests, except for the SIH, were conducted in a separate room from the holding room and animals were allowed to habituate to the new room for 3 h before behavioral testing.

Assessment of Maternal Care Behaviors. Maternal care behavior was observed and recorded at PND7 to determine whether $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ dams exhibit altered maternal care behaviors compared with WT mice, because alterations in stress-related behaviors in offspring later in adulthood could be a reflection of differences in the level of maternal care received during postnatal development (19, 20). The behaviors evaluated were arch-back nursing, licking/grooming, arch-back nursing associated with licking/grooming, nesting, arch-back nursing associated with nesting, blanket nursing, carrying, self-grooming on-nest and off-nest, and time off-nest. Self-grooming off-nest and time off-nest were considered low care behaviors, whereas the remaining behaviors were collectively considered high care behaviors. Maternal care behaviors were analyzed for 1 min at 6-min intervals during a 30-min period 2–3 h following separation. The data are presented as the percentage of time engaged in high care or low care behaviors.

Ultrasonic Vocalization Measurements. Rodents produce alarm calls (typically 20–30 kHz) in response to anxiogenic stimuli such as maternal separation (21, 22). The number of ultrasonic vocalizations (USVs) produced has been proposed to be a measure of anxiety-like behavior (23) and is reduced by treatment with anxiolytics (24). Pups were individually placed in a Plexiglas isolator box and vocalizations were recorded using a Mini-3 bat detector (Ultravox, Noldus Information Technology) for 3 min. USV recordings were done using an automated system (UltraVox; Noldus Information Technology) consisting of an audio filter, an analog digital analog-digital converter, and a computer with analysis software (UltraVox 2.0; Noldus Information Technology). Settings were determined based on the literature (25, 26) and experimentally adjusted to avoid unwanted sound detection. Specifically, inputs were recorded between 40 and 60 kHz, and only inputs that lasted longer than 10 ms and separated by the previous input by at least 20 ms were recorded.

Stress-Induced Hyperthermia. The stress-induced hyperthermia paradigm is a well-characterized test used as a physiological index of anxiety (27). The SIH was adapted from that reported by ref. 28 and was conducted as previously described (29). Briefly, animals were singly housed 1 d before the test. Rectal temperature was measured twice with a 15-min interval using a lubricated temperature-sensitive probe. Due to the stress experienced during the first temperature measurement, the temperature of the second measurement (T2) is higher than that of the first (T1). This difference in temperature ($\Delta T = T2 - T1$) is defined as the SIH response. The SIH response is reduced by different classes of anxiolytics (30).

Open Field. The open field was used to assess locomotor activity. The OF apparatus consisted of an empty gray box ($40 \times 30 \times 25$ cm^3, length × width × height) as previously described (17). Animals were allowed to explore the box for 10 min and behavior was recorded using a ceiling-mounted camera. Data were analyzed with EthoVision software. Fecal pellet output was also measured as an index of the physiological response to an anxiety-provoking stimulus (31).

Tail Suspension Test. The tail suspension test is a well-characterized test used to assess antidepressant-like behavior (32, 33). Mice are suspended to an elevated bar (60 cm) by a piece of adhesive tape attached 1 cm before the tip of their tail for a period of 6 min.

The behavioral parameter scored is time spent immobile. Treatment with antidepressant drugs decreases the time spent immobile. The test was video-recorded by a tripod camera and the time of immobility was scored manually by an investigator blind to the experimental conditions.

Elevated Plus Maze. The elevated plus maze is one of the most widely used behavioral tests to screen anxiety-related behaviors (34, 35). The EPM was conducted as previously described (2). The Plexiglas maze consists of a plus-shaped apparatus with two open and two enclosed arms ($50 \times 5 \times 15$ cm^3 walls) elevated from the floor by 1 m. The animal was placed in the center of the EPM apparatus facing an open arm and was allowed to explore it for a total period of 6 min. The apparatus was cleaned with 70% (vol/vol) ethanol after each subject to prevent olfactory cues from the previous mouse. Time spent in the open/closed arms, time spent in the center, and the number of transitions were analyzed manually. All four paws of the mouse must have entered any given arm to be considered an entry. Mice often exhibit increased defecation when placed in an anxiety-provoking environment (31), and prior administration with anxiolytic drugs decreases this fecal output in the EPM (36). Therefore, fecal output was also measured in this test.

Saccharin Preference Test. Water and 0.1% saccharin were presented in plastic 15-mL Falcon tubes with a drinking hole in the bottom. Mice were singly housed and trained to drink water from the plastic tubes over a period of 3 d. On the fourth day, mice were given access to both a water tube and a saccharin tube for a total period of 48 h. Saccharin and water intake was measured every 12 h at 8:00 AM and 8:00 PM each day. Every 12 h, the position of the plastic tubes was reversed to avoid the development of preference for drinking from one side of the cage.

Female Urine Sniffing Test. The female urine sniffing test is also a test of anhedonia and takes advantage of the fact that rodent males are commonly attracted by pheromonal odors from the opposite sex. Rodents that exhibit learned helplessness spend less time sniffing urine than water; this effect is attenuated by chronic treatment with the antidepressant citalopram, and thus reductions in the time spent sniffing the urine versus water is taken as an index of anhedonic-like behavior (37). The FUST was conducted essentially as described previously (37, 38). One hour before the test, mice were habituated to a sterile cotton-tipped applicator inserted into their home cage. At the beginning of the test, mice were exposed to a cotton tip dipped in sterile water and time spent sniffing the cotton-tipped applicator was measured over a 3-min period. Forty-five minutes later, mice were exposed to a cotton tip infused with fresh urine collected from females of the same strain that were in the estrus stage of the estrus cycle, and time spent sniffing the urine was recorded over a 3-min period.

Forced Swim Test. The forced swim test is the most widely used experimental paradigm to assess antidepressant activity (39, 40). The FST was conducted as previously described (41). In this test, mice are forced to swim for 6 min in a glass cylinder (24×21 cm) filled with 23–25 °C tap water to a depth of 17 cm. The FST was videotaped from a tripod-mounted camera positioned above the swim tank. The behavioral parameter scored is immobility during the last 4 min of the 6-min test. Antidepressant drugs decrease the time spent immobile in this test (39).

Measurement of Stress-Induced Plasma Corticosterone Concentrations. In the maternal separation experiment, the FST was used as a stressor to activate HPA-axis activity. Thirty minutes after the FST, trunk blood was collected and plasma was obtained by centrifugation ($2000 \times g$) and stored at −80 °C until analysis. All samples were collected between 11:00 AM and 3:00 PM during the light cycle. Plasma corticosterone concentrations were measured using an immunoassay kit (R&D Systems). The sensitivity of this assay is <27.0 pg/mL.

c-Fos Immunohistochemistry Experiment. Female WT, $GABA_{B(1a)}^{-/-}$, and $GABA_{B(1b)}^{-/-}$ mice underwent unpredictable maternal separation combined with unpredictable maternal stress from PND1 to PND14. Mice were weaned on PND21. At 12 wk of age, mice were acutely stressed for 2 h by restraint and killed 2 h later. Restraint is a well-established model for inducing stress in rodents (42, 43). In this study, restraint stress was induced by placing mice in 50-mL plastic Falcon tubes with air holes placed at both ends to allow ventilation for a period of 2 h. Restraint stress was performed in the light cycle from 10:00 AM to 2:00 PM. The procedure was conducted in a different room from the holding room, and the animals were allowed to habituate to the new room for 2 h before initiation of the stress procedure. Following stress, animals were immediately returned to the holding room. The peak of c-Fos expression is detected 2–3 h following an acute stimulus (44), and thus animals were killed 2 h after the end of the restraint procedure to measure the number of c-Fos–positive cells (43).

Two hours following restraint stress, deeply anesthetized animals were perfused transcardially with phosphate buffer solution followed by cold paraformaldehyde [4% (wt/vol) in PBS]. Brains were postfixed overnight at 4 °C and placed in 20% (wt/vol) sucrose before freezing with isopentane and storage at −80 °C. Samples were cut using a cryostat (Leica Microsystems) in 35 μm-thick coronal sections. Sections were serially collected in a cryoprotectant solution composed of 25% 0.2 M PBS, 30% ethylene glycol, 25% glycerol, 20% H_2O (all vol/vol) and stored at −80 °C until use.

c-Fos immunohistochemistry was conducted as previously described (43). Specifically, sections were washed three times for 5 min each in 0.01 M PBS (pH 7.4) and incubated in freshly prepared 0.75% H_2O_2 for 20 min to inhibit endogenous peroxidase. Sections were incubated at room temperature for 20 min in 10% (vol/vol) normal goat serum (NGS), 0.1% Triton X-100 in PBS to prevent nonspecific binding. Sections were then incubated overnight at room temperature with rabbit anti–c-Fos polyclonal antibody (1:5,000; Santa Cruz Biotechnology) in 0.01 M PBS with 1% (vol/vol) NGS. Sections were washed and incubated for 90 min at room temperature with a secondary biotinylated anti-rabbit antibody (1:100; Vectastain Elite ABC Kit; Vector Laboratories) followed by incubation in ABC reagent (Vectastain Elite ABC Kit; Vector Laboratories). c-Fos–positive cells were detected by incubation with 3,3-diaminobenzidine tetrahydrochloride [DAB; 0.02% (wt/vol); Sigma] with 0.0075% H_2O_2 in PBS.

Sections were visualized using an Olympus BX51 microscope; images were captured by an Olympus DP71 digital camera with $Cell^F$ software (Olympus) and c-Fos–positive cells were counted. Coordinates of coronal plates and limits of the structures analyzed were defined according to a mouse brain atlas (45). For each region, three consecutive sections were analyzed and c-Fos–positive cells were counted bilaterally by an investigator blind to the experimental conditions ($n = 7$ per group).

In Situ Hybridization. In situ hybridization was conducted as previously described (46) using oligodeoxynucleotide (cDNA) probes complementary to $GABA_{B(1a)}$ mRNA [595–636 bp; National Center for Biotechnology Information (NCBI) Nucleotide Database reference no. NM_019439.3] and $GABA_{B(1b)}$ mRNA (39–82 bp; NCBI Nucleotide Database reference no. AF120255), labeled with a digoxigenin (DIG) oligonucleotide 3′ OH Tailing Kit (Roche Molecular Biochemicals).

Frozen coronal brain sections (10 μm-thick) were mounted on glass slides and postfixed for 30 min in 4% (wt/vol) paraformaldehyde. After treatment with 0.001% proteinase K

(Sigma), 0.25% acetic anhydride in 0.1 M triethanolamine, the tissues were dehydrated through a graded series of ethanol [70%, 95%, and 100% (all vol/vol)]. Then, the samples were delipidated in chloroform for 5 min. Later the tissues were rinsed with ethanol (95% vol/vol) and air-dried before hybridization. The tissues were then incubated overnight at 37 °C with hybridization solution [50% (vol/vol) formamide, 4× saline and sodium citrate buffer (SSC), 1× Denhart solution (from a 50× stock: 1% Ficoll 400, 1% polyvinylpyrrolidone, and 1% BSA), 6.25 mg/mL sheared salmon DNA, 125 μg/mL tRNA, and cDNA probe at a fixed concentration of 100 pmol/mL]. Then, the samples were rinsed with 4× SSC and blocked with blocking reagent (Roche Molecular Biochemicals). Detection was carried out with an anti-DIG antibody (1:500 dilution prepared in Roche's blocking reagent with 1% FBS, 0.1% Triton X-100), which is conjugated with alkaline phosphatase (Roche Molecular Biochemicals). Finally, substrate solution was added (nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate; Sigma), and the reaction was stopped when a precipitate was present on the tissues. The slides were then left to air-dry, coverslipped, and photographed. Negative controls were generated by using a 100-fold excess of the respective unlabeled oligodeoxynucleotide. For semiquantitative analysis, densitometric measurements of the hippocampus microphotographs were performed using Science Lab Multi Gauge version 2.2 software (Fuji Photo Film). Pictures were analyzed in gray scale, and values correspond to the intensity of pixels (with the darkest staining corresponding to the highest intensity) in a given area (density of pixels). Values for each animal represent the average from four or five brain sections (analyzed on both brain hemispheres). Analysis of the pictures was carried out in a random fashion, and treatments of each animal were blinded to the experimenter to prevent a bias in the analysis.

Ki67 and BrdU Immunohistochemistry Experiment. WT and $GABA_{B(1b)}^{-/-}$ male mice underwent unpredictable maternal separation combined with unpredictable maternal stress from PND1 to PND14 and were weaned on PND21. The proliferation and survival of newly born cells in the adult hippocampus were assessed using the same samples (Fig. 4). Cell proliferation was measured using Ki67 immunohistochemistry, an established endogenous marker for proliferating cells, whereas the survival of newly born cells was analyzed using bromodeoxyuridine (BrdU) immunohistochemistry, a marker of the cells in the S phase of the cell cycle. To assess the survival of newly born cells in the hippocampus, mice (9-wk-old) received four injections of BrdU (4 × 75 mg/kg; 10 mL/kg; i.p.) 2 h apart as previously described (41, 47) and were perfused 4 wk following the last BrdU injection.

Animals were deeply anesthetized and transcardially perfused with PBS (pH 7.4), followed by 4% (wt/vol) paraformaldehyde in 100 mM phosphate buffer (pH 7.4). Brains were postfixed overnight at 4 °C and placed in 20% (wt/vol) sucrose before freezing with isopentane and storage at −80 °C. Samples were cut using a cryostat (Leica Microsystems) in 35 μm-thick coronal sections. Sections were serially collected in a cryoprotectant solution composed of 25% 0.2 M PBS, 30% ethylene glycol, 25% glycerol, 20% H_2O (all vol/vol) and stored at −80 °C until use.

Ki67 immunohistochemistry in free-floating sections ($n = 8$ per group) was used to assess cell proliferation, whereas BrdU immunohistochemistry was used to assess the survival of newly born cells ($n = 10$ per group) and was conducted as previously described (41, 48). Briefly, sections were washed three times for 5 min each in 0.01 M PBS (pH 7.4). Sections for BrdU immunohistochemistry then underwent the additional steps of being incubated for 20 min in 0.01 M sodium citrate buffer at 85 °C for antigen unmasking, followed by denaturation in 2 N HCl and then washes in tetraborate buffer (pH 8.5; 2 × 5 min). The remainder of the protocol did not differ for Ki67 and BrdU immunohistochemistry. Sections were then incubated in freshly prepared 0.75% H_2O_2 for 20 min to inhibit endogenous peroxidase. Sections were incubated at room temperature for 1 h in 10% (vol/vol) NGS, 0.1% Triton X-100 in PBS to prevent nonspecific binding. Sections were then incubated overnight with a rabbit monoclonal anti-Ki67 antibody (1:100; Thermo Scientific Neomarkers) or anti-rat BrdU antibody (1:100; Abcam) diluted in 1% NGS, 0.1% Triton X-100 in PBS. Sections were washed and incubated for 90 min at room temperature with a secondary biotinylated anti-rabbit antibody (1:100; Vectastain Elite ABC Kit; Vector Laboratories) or a biotinylated anti-rat antibody (1:100; Vectastain Elite ABC Kit; Vector Laboratories) followed by incubation in ABC reagent (Vectastain Elite ABC Kit; Vector Laboratories). Ki67-positive and BrdU-positive cells were detected by incubation with DAB [0.02% (wt/vol); Sigma] with 0.0075% H_2O_2 in PBS.

Sections stained for Ki67 and BrdU were visualized using an Olympus BX51 microscope; images were captured by an Olympus DP71 digital camera with Cell^F software (Olympus), and Ki67/BrdU-positive cells were counted by an investigator blind to the treatment conditions. Every sixth section was analyzed. Hippocampal sections were chosen according to the Paxinos and Franklin atlas of the mouse brain (45). The dorsal hippocampus was defined as anterior-posterior (AP) −0.94 to −2.30 and the ventral hippocampus as AP −2.46 to −3.80 (41, 47, 49).

Doublecortin Immunohistochemistry. Sections from the c-Fos experiment conducted in female mice were used to assess neurogenesis in all genotypes using doublecortin (DCX) as a marker of immature neurons. Sections were washed three times for 10 min each in 0.01 M PBS (pH 7.4) with 0.1% Triton X-100 (PBS-T) and then incubated at room temperature for 1 h in 1% BSA, 0.1% Triton X-100 diluted in PBS to prevent nonspecific binding. Sections were then incubated overnight at 4 °C with goat anti-DCX polyclonal antibody (1:500; Sc-8066; Santa Cruz Biotechnology) in PBS-T. Sections were washed four times for 10 min each in PBS-T and incubated for 2 h at room temperature with an Alexa 488 donkey anti-goat antibody (1:500; A11055; Life Technologies) diluted in 1% BSA in PBS-T. Sections were washed four times for 10 min each in PBS-T and mounted with Dako fluorescence mounting medium.

Sections were visualized using an Olympus BX53 microscope, and images were captured by an Olympus DP72 digital camera with cellSens software (Olympus). DCX-positive cells were counted in every sixth section, and cell counts were multiplied by 6 to get an estimate of DCX-positive cells throughout the whole dentate gyrus. The dorsal hippocampus was defined as AP −0.94 to −2.30 and the ventral hippocampus as AP −2.46 to −3.80 according to the Paxinos and Franklin atlas of the mouse brain (41, 45, 47, 49).

Statistical Analysis. Results are presented as mean ± SEM. Statistical analyses were performed using SPSS 20. Behavioral data from the SD stress and MSUS experiments were analyzed using two-way ANOVA followed by Fisher's least significant difference (LSD) post hoc test, with the exception of the USV and FUST data. USV data were analyzed using one-way ANOVA analysis followed by Fisher's LSD post hoc test, whereas FUST data were analyzed using an unpaired Student t test between stressed and nonstressed mice of the same genotype. The effects of genotype and MSUS on plasma corticosterone, stress-induced c-Fos activation, proliferation and survival of newly born cells, and neurogenesis were analyzed using two-way ANOVA followed by Fisher's LSD post hoc test. In situ hybridization data were analyzed using an unpaired Student t test. For all comparisons, $P < 0.05$ was the criterion used for statistical significance.

SI Results

Social Defeat Stress Experiment. ***Time spent in the target zone in the absence of a social target is unaffected by social defeat stress and genotype.*** Neither stress nor genotype alters time spent in the target zone in the absence of a social target, as illustrated in Fig. S1*A* (genotype [$F(2,51) = 2.168$, $P = 0.125$]; stress [$F(2,51) = 0.478$, $P = 0.493$]; stress x genotype [$F(2,51) = 0.045$, $P = 0.956$]).
Behavior in the social interaction test is not due to alterations in locomotor activity. Neither stress nor genotype affects locomotor activity in the absence of a social target, as illustrated in Fig. S1*B*. In the absence of a social target, the distance moved in the arena was not affected by stress [$F(1,51) = 0.775$, $P = 0.383$], genotype [$F(2,51) = 0.198$, $P = 0.821$], or stress x genotype [$F(2,51) = 1.324$, $P = 0.275$]. Similarly, the distance moved in the presence of a social target was unaffected by genotype [$F(2,51) = 0.168$, $P = 0.845$] or stress x genotype [$F(2,51) = 0.59$, $P = 0.557$]. Although there appeared to be an effect of stress [$F(1,51) = 5.045$, $P = 0.029$], post hoc analysis with Fisher's LSD did not reveal any statistically significant differences between individual experimental groups.
Saccharin preference before exposure to social defeat stress. Before initiation of social defeat stress, the preference to drink a sweet solution of 0.1% saccharin over water was measured in WT, $GABA_{B(1a)}^{-/-}$, and $GABA_{B(1b)}^{-/-}$ mice (Fig. S1*C*). This was done to ensure that stress-induced changes in saccharin preference were not a result of baseline differences in preference. Baseline saccharin preference was not different between any of the experimental groups (genotype [$F(2,51) = 1.236$, $P = 0.299$]; stress [$F(2,51) = 0.003$, $P = 0.958$]; stress x genotype [$F(2,51) = 0.455$, $P = 0.637$]).

Maternal Separation Experiment. ***Saccharin preference over 36- and 48-h periods.*** The effects of maternal separation and genotype on saccharin preference over a 36-h period are shown in Fig. S2*A*. Two-way ANOVA revealed a genotype effect [$F(2,50) = 10.235$, $P < 0.001$] but not a stress effect ($P = 0.743$) or a stress x genotype interaction ($P = 0.258$). Specifically, Fisher's post hoc test revealed that NMS $GABA_{B(1a)}^{-/-}$ and NMS $GABA_{B(1b)}^{-/-}$ mice exhibited no difference in saccharin consumption compared with WT mice over a 36-h period, indicating that under baseline conditions both $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice show preference for the sweet solution. However, MS $GABA_{B(1a)}^{-/-}$ mice displayed decreased saccharin consumption ($P < 0.01$) when measured over 36 h. The same pattern of results was also obtained when saccharin preference was measure over 48 h (Fig. S2*B*; genotype [$F(2,50) = 5.857$, $P = 0.005$]; stress [$F(2,50) = 0.004$, $P = 0.949$]; genotype x stress [$F(2,50) = 1.256$, $P = 0.294$]; MS $GABA_{B(1a)}$ vs. MS WT, $P < 0.01$). These findings are similar to those we reported over a 24-h period (Fig. 1*D*).
Forced swim test in male mice. The effects of MSUS and genotype on male mice in the FST are shown in Fig. S2*C*. In males, two-way ANOVA revealed a significant genotype effect [$F(2,69) = 7.665$, $P = 0.001$] but not a stress effect [$F(2,69) = 0.617$, $P = 0.435$] or a stress × genotype interaction [$F(2,69) = 0.121$, $P = 0.886$]. Both NMS $GABA_{B(1a)}^{-/-}$ ($P < 0.05$) and NMS $GABA_{B(1b)}^{-/-}$ mice ($P < 0.05$) displayed decreased immobility compared with NMS WT mice. MS $GABA_{B(1a)}^{-/-}$ mice ($P < 0.05$) showed decreased immobility compared with MS WT mice, whereas MS $GABA_{B(1b)}^{-/-}$ mice exhibited a trend ($P = 0.08$).
Tail suspension test. The effects of MSUS and genotype on the TST are shown in Fig. S2 *E* and *F*. In males, two-way ANOVA revealed a significant genotype effect [$F(2,72) = 26.88$, $P < 0.001$] but no stress effect ($P = 0.291$), nor a stress x genotype interaction ($P = 0.856$; Fig. S3*C*). Specifically, NMS $GABA_{B(1a)}^{-/-}$ mice exhibited increased immobility compared with NMS WT ($P < 0.01$) and NMS $GABA_{B(1b)}^{-/-}$ ($P < 0.001$) mice. Moreover, NMS $GABA_{B(1b)}^{-/-}$ mice displayed decreased immobility compared with NMS WT ($P < 0.05$) and NMS $GABA_{B(1a)}^{-/-}$ ($P < 0.001$) mice. These genotype effects occurred independent of maternal separation. Indeed, MS $GABA_{B(1a)}^{-/-}$ mice still exhibited increased immobility compared with both MS $GABA_{B(1b)}^{-/-}$ ($P < 0.001$) and MS WT ($P < 0.05$) mice. Similarly, MS $GABA_{B(1b)}^{-/-}$ mice still displayed decreased immobility compared with MS $GABA_{B(1a)}^{-/-}$ ($P < 0.001$) and MS WT mice ($P < 0.01$).

In females, two-way ANOVA revealed a significant genotype effect [$F(2,56) = 33.577$, $P < 0.001$; Fig. S2*F*] but not a stress effect ($P = 0.255$) or a stress x genotype interaction ($P = 0.592$) in the TST. Like the findings in male mice, post hoc analysis revealed that female NMS and MS $GABA_{B(1a)}^{-/-}$ mice displayed increased immobility compared with $GABA_{B(1b)}^{-/-}$ mice ($P < 0.001$) and WT ($P < 0.001$). However, in contrast to male mice, female NMS and MS $GABA_{B(1b)}^{-/-}$ mice did not exhibit decreased immobility compared with WT mice, although a trend for decreased immobility was observed in MS $GABA_{B(1b)}^{-/-}$ mice ($P = 0.069$) compared with WT mice. Similar to male mice, female NMS and MS $GABA_{B(1b)}^{-/-}$ mice displayed decreased immobility compared with NMS and MS $GABA_{B(1a)}^{-/-}$ mice ($P < 0.001$).
Locomotor activity of male mice in the open field. The effects of MSUS and genotype on locomotor activity of male mice in the open field are shown in Fig. S2*D*. In male mice, two-way ANOVA revealed a significant genotype effect [$F(2,74) = 19.77$, $P < 0.001$] but not a stress effect [$F(1,74) = 1.610$, $P = 0.208$] on locomotor activity in the OF. Moreover, two-way ANOVA revealed a stress–genotype interaction [$F(2,74) = 3.481$, $P < 0.05$]. Specifically, NMS $GABA_{B(1b)}^{-/-}$ male mice displayed increased locomotor activity compared with NMS WT ($P < 0.001$) and NMS $GABA_{B(1a)}^{-/-}$ mice ($P < 0.001$). Maternal separation attenuated this effect ($P < 0.01$), although MS $GABA_{B(1b)}^{-/-}$ male mice still exhibited hyperactivity compared with MS WT ($P < 0.01$) and MS $GABA_{B(1a)}^{-/-}$ mice ($P < 0.05$).
Maternal care behaviors. The effects of genotype and MSUS on high maternal care behaviors and time off-nest are illustrated in Fig. S3 *A* and *B*, respectively. For both measures, two-way ANOVA revealed significant effects of genotype [high maternal care: $F(2,42) = 10.348$, $P < 0.001$; time off-nest: $F(2,42) = 10.348$, $P < 0.001$] and stress [high maternal care: $F(2,42) = 4.534$, $P < 0.05$; time off-nest: $F(2,42) = 4.534$, $P < 0.05$] but no stress x genotype interaction. Post hoc analysis revealed that maternal care behavior was not different between WT and $GABA_{B(1b)}^{-/-}$ mice and that MS did not subsequently affect the maternal care behaviors of WT or $GABA_{B(1b)}^{-/-}$ dams. This suggests that the resilient phenotype of $GABA_{B(1b)}^{-/-}$ mice is not due to increased maternal care. On the contrary, $GABA_{B(1a)}^{-/-}$ dams spent less time off-nest and provided higher maternal care compared with $GABA_{B(1b)}^{-/-}$ [time-off nest: NMS $GABA_{B(1a)}^{-/-}$ vs. NMS $GABA_{B(1b)}^{-/-}$, $P < 0.05$; high care: NMS $GABA_{B(1a)}^{-/-}$ vs. NMS $GABA_{B(1b)}^{-/-}$, $P < 0.05$] and WT [time off-nest: NMS WT vs. NMS $GABA_{B(1a)}^{-/-}$, $P = 0.001$; high care: NMS WT vs. NMS $GABA_{B(1a)}^{-/-}$, $P = 0.001$] dams, irrespective of whether their pups had undergone MS [time off-nest: MS $GABA_{B(1a)}^{-/-}$ vs. MS $GABA_{B(1b)}^{-/-}$, $P < 0.05$; MS WT vs. MS $GABA_{B(1a)}^{-/-}$, $P < 0.05$; high care: MS $GABA_{B(1a)}^{-/-}$ vs. MS $GABA_{B(1b)}^{-/-}$, $P < 0.05$; MS WT vs. MS $GABA_{B(1a)}^{-/-}$, $P < 0.05$].
FST-induced plasma corticosterone levels. The effect of stress and genotype on FST-induced plasma corticosterone levels is presented in Fig. S3. In males (Fig. S3*C*), two-way ANOVA revealed a significant genotype effect [$F(2,64) = 25.42$, $P < 0.001$] but no stress effect [$F(2,64) = 3.152$, $P = 0.081$] or stress–genotype interaction [$F(2,64) = 25.42$, $P = 0.805$]. Specifically, both NMS ($P < 0.01$) and MS ($P < 0.001$) $GABA_{B(1a)}^{-/-}$ mice displayed a suppression of corticosterone release compared with WT mice. On the other hand, both NMS and MS $GABA_{B(1b)}^{-/-}$ mice exhibited enhanced corticosterone release following exposure to the FST ($P < 0.05$). In contrast to males, females (Fig. S3*D*) did not show a significant effect of genotype [$F(2,44) = 2.089$, $P = 0.136$].

Similarly, there was no stress–genotype interaction [$F(2,44) = 0.879$, $P = 0.422$]. Although there was a trend toward a significant effect of stress [$F(2,44) = 4.044$, $P = 0.05$], post hoc analysis did not reveal any differences between experimental groups.

Elevated plus maze. The effects of MSUS and genotype on the elevated plus maze are shown in Fig. S4. In male mice, neither genotype [$F(2,74) = 1.938$, $P = 0.151$], stress [$F(2,74) = 1.086$, $P = 0.300$], nor stress–genotype interaction [$F(2,74) = 0.961$, $P = 0.387$] altered the time spent in the open arms of the EPM (Fig. S4*A*). Similarly, in female mice, neither genotype [$F(2,53) = 0.432$, $P = 0.651$], stress [$F(2,53) = 1.224$, $P = 0.273$], nor stress x genotype [$F(2,53) = 1.075$, $P = 0.349$] affected time spent in the open arms of the EPM (Fig. S4*E*). In male mice, the percentage of entries into the open arms was unaffected by genotype [$F(2,74) = 2.196$, $P = 0.118$], stress [$F(2,74) = 0.236$, $P = 0.629$], or stress x genotype [$F(2,74) = 0.458$, $P = 0.634$] (Fig. S4*B*). Similarly, in female mice, the percentage of open-arm entries was unaffected by genotype [$F(2,53) = 0.919$, $P = 0.405$], stress [$F(2,53) = 0.599$, $P = 0.442$], or stress x genotype [$F(2,53) = 1.839$, $P = 0.169$] (Fig. S4*F*).

Two-way ANOVA revealed a significant genotype effect in the total number of arm entries in both male [$F(2,74) = 4.684$, $P < 0.05$; Fig. S4*C*] and female [$F(2,53) = 6.784$, $P < 0.01$; Fig. S5*G*] mice. In male mice, neither a stress–genotype interaction [$F(2,74) = 0.058$, $P = 0.944$] nor a stress effect [$F(2,53) = 2.167$, $P = 0.145$] was observed (Fig. S4*C*). In females, two-way ANOVA revealed a stress–genotype interaction [$F(2,53) = 3.647$, $P < 0.05$] but no stress effect [$F(2.53) = 0.101$, $P = 0.742$]. Post hoc analysis revealed that both male and female NMS $GABA_{B(1b)}^{-/-}$ mice displayed an increased number of total arm entries compared with their corresponding WT group ($P < 0.05$). Similarly, both male and female MS $GABA_{B(1b)}^{-/-}$ mice displayed a trend toward an increased number of total arm entries ($P < 0.06$). In addition, MS $GABA_{B(1a)}^{-/-}$ female mice exhibited an increased number of total arm entries compared with NMS $GABA_{B(1a)}^{-/-}$ mice.

The effects of MSUS and genotype on fecal output during the EPM are shown in Fig. S4 *D* and *H*. In male mice, two-way ANOVA revealed a genotype effect [$F(2,44) = 4.710$, $P < 0.05$] but no effect of stress [$F(2,44) = 0.049$, $P = 0.826$] or stress x genotype interaction [$F(2,44) = 0.049$, $P = 0.952$] (Fig. S4*D*). Specifically, male NMS and MS $GABA_{B(1b)}^{-/-}$ mice displayed decreased fecal output compared with male WT mice ($P < 0.05$). However, such differences were not observed in female mice (Fig. S4*H*; genotype [$F(2,53) = 0.429$, $P = 0.653$]; stress [$F(2,53) = 0.137$, $P = 0.713$]; stress x genotype [$F(2,53) = 0.166$ $P = 0.847$]).

Fecal output in the open field. The effects of MSUS and genotype on the number of fecal pellets emitted during the open field test are shown in Fig. S5 *A* and *B*. Neither genotype, stress, nor genotype x stress affected defecation in the open field (males: genotype [$F(2,44) = 3.208$, $P = 0.05$]; stress [$F(2.44) = 0.254$, $P = 0.616$]; stress x genotype [$F(2,44) = 1.170$, $P = 0.319$]; females: genotype [$F(2,51) = 1.157$, $P = 0.322$]; stress [$F(2,51) = 0.289$, $P = 0.593$]; stress x genotype [$F(2,51) = 1.375$, $P = 0.262$]).

Stress-induced hyperthermia. The effects of MSUS and genotype on stress-induced hyperthermia are shown in Fig. S5 *C* and *D*. In male mice, two-way ANOVA revealed a significant stress effect [$F(2,66) = 5.224$, $P < 0.05$] but no genotype [$F(2,66) = 1.125$, $P = 0.331$] or stress x genotype [$F(2,66) = 0.313$, $P = 0733$] effects (Fig. S5*C*). Specifically, post hoc analysis revealed that MSUS decreased the SIH response in WT ($P = 0.05$) but not in $GABA_{B(1a)}^{-/-}$ and $GABA_{B(1b)}^{-/-}$ mice. However, this effect of MSUS was not observed in female mice (Fig. S5*D*; stress [$F(2,59) = 2.104$, $P = 0.152$]). On the other hand, there was a significant genotype effect [$F(2,59) = 3.482$, $P < 0.05$] in female mice. Specifically, MS $GABA_{B(1a)}^{-/-}$ mice displayed a decreased SIH response compared with MS $GABA_{B(1b)}^{-/-}$ mice ($P < 0.05$) (Fig. S5*D*); however, it is also important to note that there was no stress x genotype interaction [$F(2,59) = 0.232$, $P = 0.7933$].

$GABA_{B(1b)}^{-/-}$ but not $GABA_{B(1a)}^{-/-}$ mice exhibit increased adult hippocampal neurogenesis. The effects of genotype and maternal separation on the number of doublecortin-positive cells are shown in Fig. S8. Although a similar pattern of effects was observed whereby $GABA_{B(1b)}^{-/-}$ mice exhibited an increased number of doublecortin-positive cells, in the whole dentate gryus, the dorsal hippocampus (dHi) and the ventral hippocampus (vHi), statistically significant effects were observed only in the dHi.

In the total dentate gyrus, the effects of genotype, stress, and genotype x stress interaction were not statistically significant [$F(2,35) = 2.491$, $P = 0.1$; $F(1,35) = 0.671$, $P = 0.419$; $F(2,35) = 0.097$, $P = 0.908$; respectively]. In the dHi, two-way ANOVA revealed a significant effect of genotype [$F(2,35) = 4.559$, $P = 0.019$] but no stress [$F(1,35) = 0.496$, $P = 0.487$] or stress x genotype interaction [$F(2,35) = 0.248$, $P = 0.782$]. In the vHi, neither the effects of genotype, stress, nor genotype x stress interaction reached statistical significance [$F(2,35) = 1.002$, $P = 0.379$; $F(1,35) = 0.541$, $P = 0.468$; $F(2,35) = 0.104$, $P = 0.902$; respectively].

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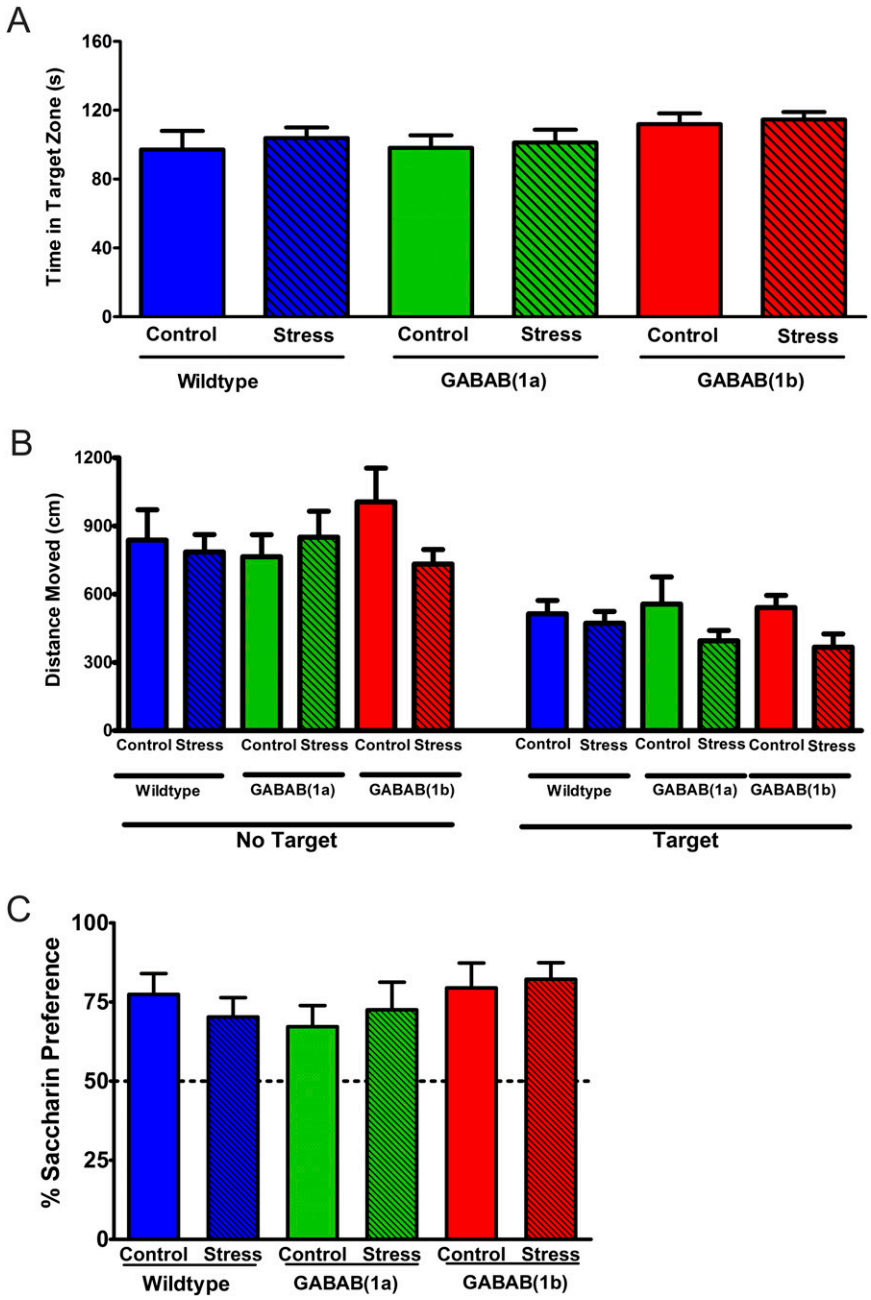

Fig. S1. (*A* and *B*) Effect of social defeat stress on time spent in the interaction zone in the absence of a social target (*A*) and distance traveled (locomotor activity) in the absence of a social target (*B*). (*C*) No differences in saccharin preference were observed across any of the treatment groups before initiation of social defeat stress. n = 9–10 males.

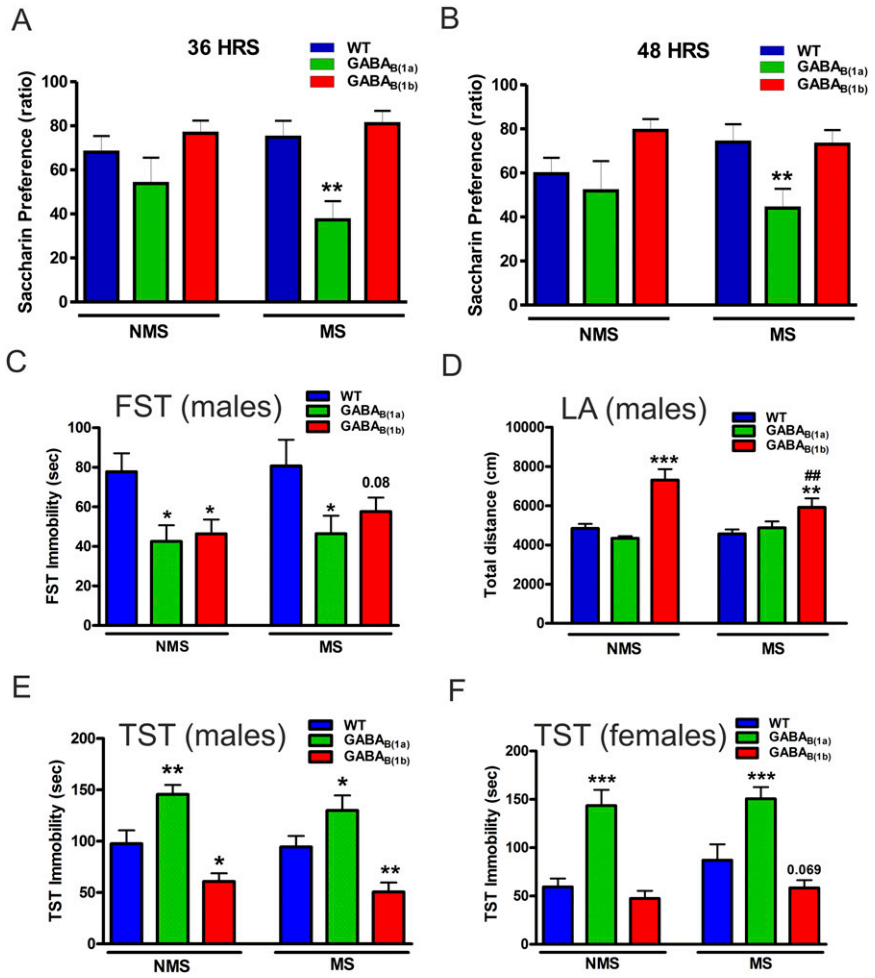

Fig. S2. Effects of maternal separation and genotype on saccharin preference, forced swim test, tail suspension test, and locomotor activity (LA) in the open field. (*A* and *B*) Maternal separation reduced saccharin preference over a 36-h (*A*) and 48-h (*B*) period in GABA$_{B(1a)}^{-/-}$ but not WT or GABA$_{B(1b)}^{-/-}$ mice. (*C*) Male GABA$_{B(1a)}^{-/-}$ and GABA$_{B(1b)}^{-/-}$ mice exhibit decreased immobility in the forced swim test. (*D*) Male GABA$_{B(1b)}^{-/-}$ mice are hyperactive in the open field. (*E*) Male GABA$_{B(1b)}^{-/-}$ mice exhibit decreased immobility, whereas GABA$_{B(1a)}^{-/-}$ mice exhibit increased immobility in the tail suspension test. (*F*) Female GABA$_{B(1a)}^{-/-}$ mice exhibit increased immobility in the tail suspension test. *Compared with WT of the same stress condition; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. $^{\#}$Compared with the respective NMS group; $^{\#\#}P < 0.01$. $n = 10$–14 males; $n = 6$–14 females.

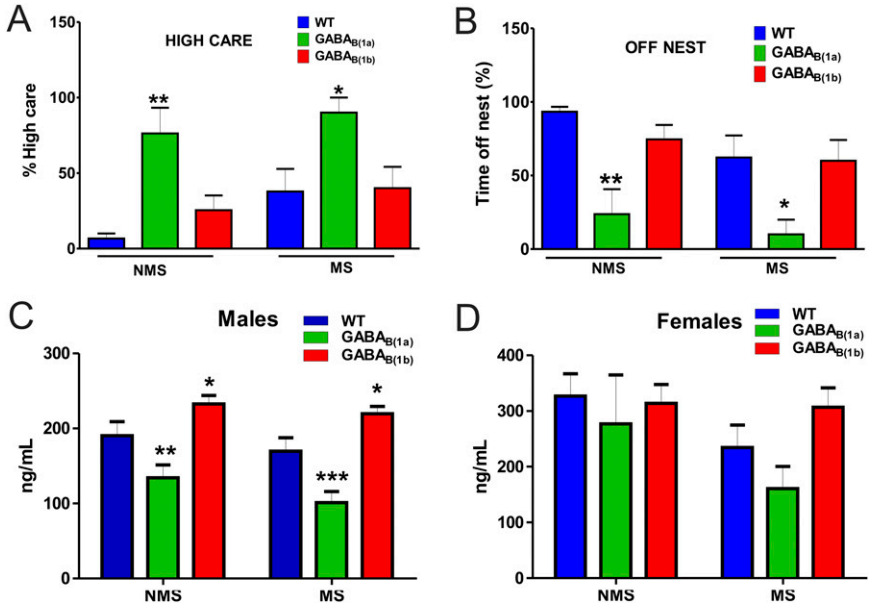

Fig. S3. Effects of genotype and maternal separation on maternal care behaviors and stress-induced plasma corticosterone concentrations. GABA$_{B(1a)}^{-/-}$ dams provide more maternal care (*A*) and spend less time off-nest than GABA$_{B(1b)}$ and WT dams (*B*) (n = 4–9 dams). Following the FST, plasma corticosterone concentrations are attenuated in male (*C*) but not female (*D*) GABA$_{B(1a)}^{-/-}$ mice and are enhanced in male but not female GABA$_{B(1b)}^{-/-}$ mice, and these effects occur irrespective of whether mice underwent maternal separation or not (n = 9–12). *Significantly different from the corresponding WT group according to Fisher's LSD post hoc test; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

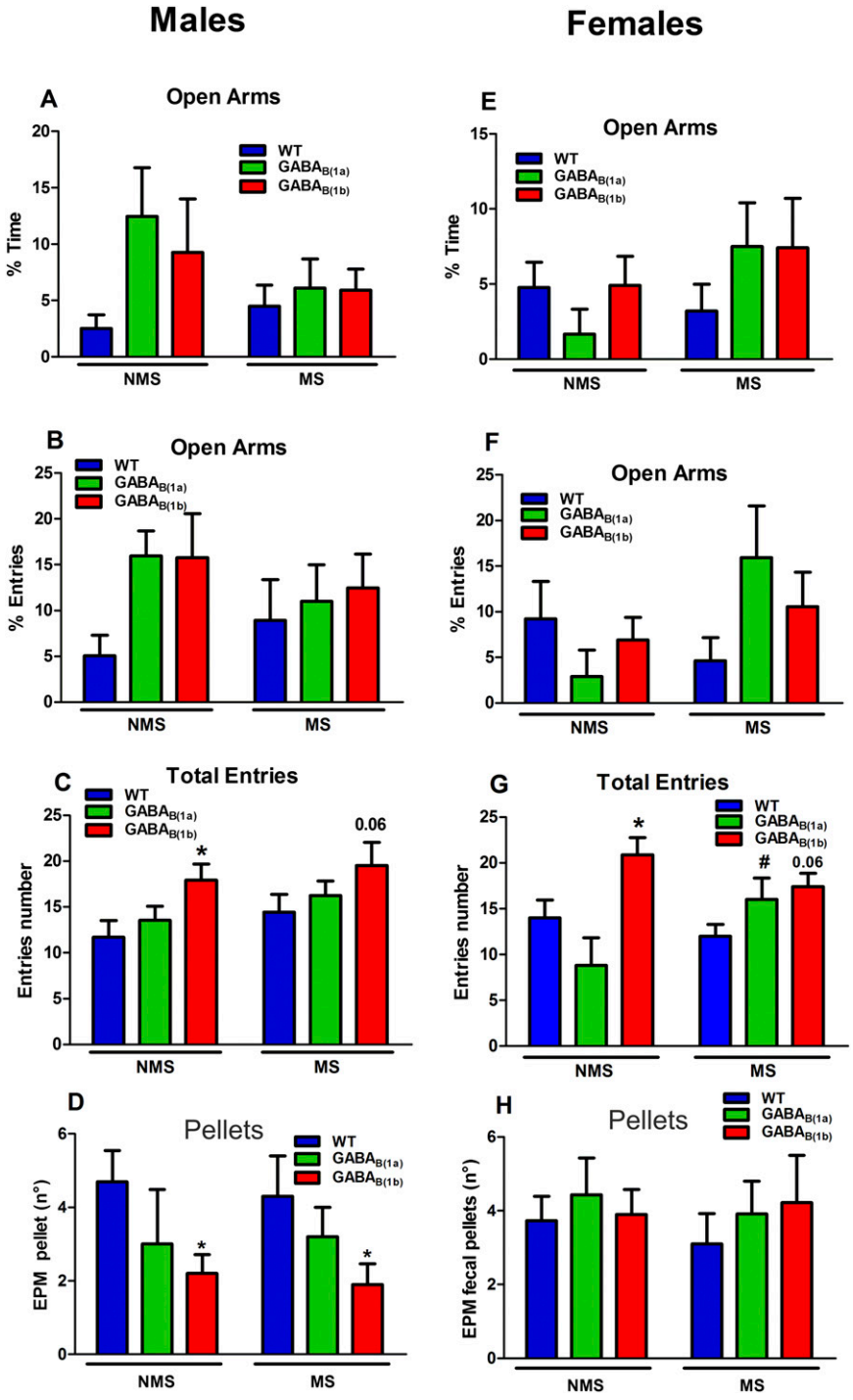

Fig. S4. Effects of maternal separation and genotype in the elevated plus maze in male (*A–D*) and female mice (*E–H*). (*A* and *E*) Time spent in open arms. (*B* and *F*) Number of entries into open arms (*B* and *F*). (*C* and *G*) Total number of arm entries. (*D* and *H*) Number of fecal pellets emitted during EPM test. *Compared with WT of the same stress condition; *$P < 0.05$. #Compared with the respective NMS group; #$P < 0.05$. n = 12–14 males; n = 6–14 females.

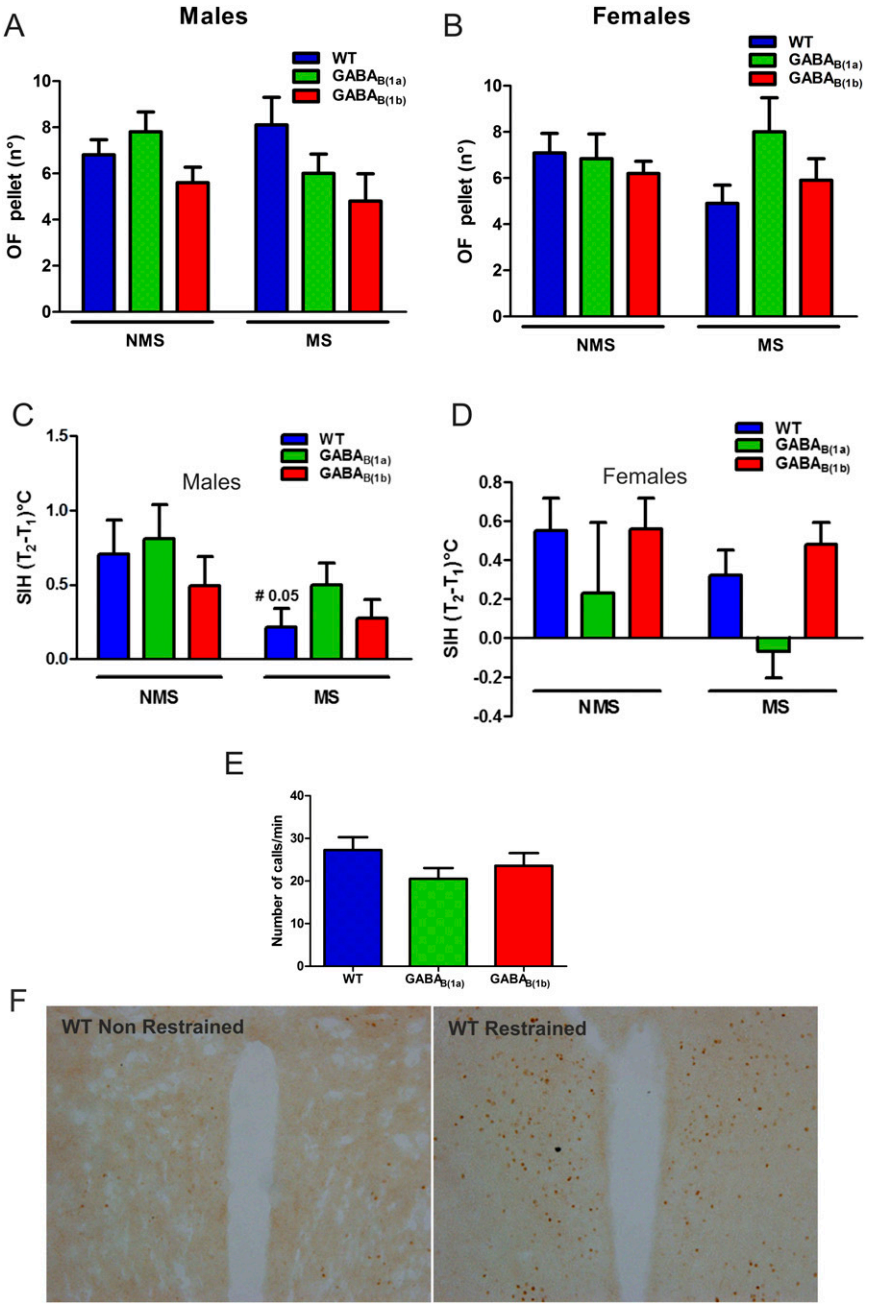

Fig. S5. Effects of maternal separation and genotype on the number of fecal pellets emitted during the open field test, stress-induced hyperthermia, and ultrasonic vocalizations during maternal separation on PND1. Acute restraint stress induction of c-Fos activation in the paraventricular nucleus of the hypothalamus of WT mice is also shown. Neither maternal separation nor genotype affects the number of fecal pellets emitted during the OF test in males (*A*; n = 12–14) or females (*B*; n = 4–14). The effects of maternal separation and genotype on SIH in males (*C*; n = 12–14) and females (*D*; n = 6–14). The effects of maternal separation and genotype on USVs during maternal separation on PND1 (*E*; n = 57–69 males and females). $^{\#}$Compared with the respective NMS group; $^{\#}P < 0.05$. Restraint stress increases the density of c-Fos–positive cells in the paraventricular nucleus of the hypothalamus of WT mice (*F*).

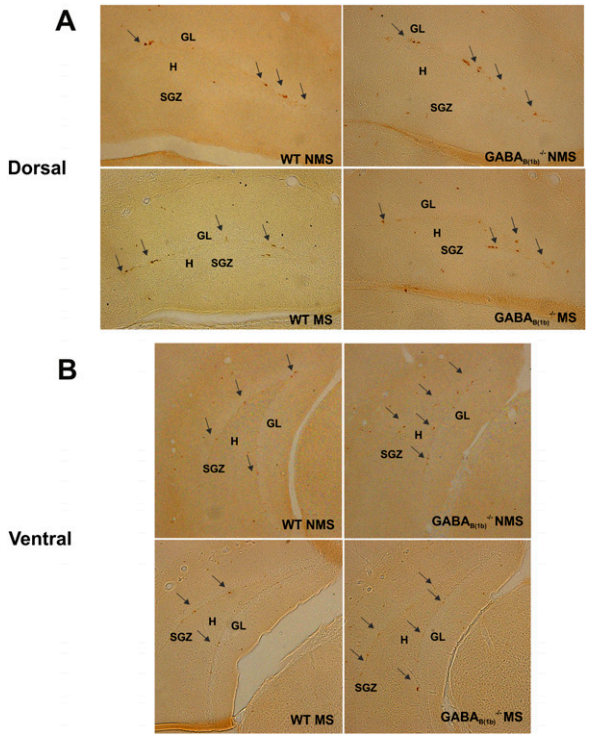

Fig. S6. Representative photographs of Ki67 immunohistochemistry investigating the effects of $GABA_{B(1b)}$ receptor subunit isoform ablation and early-life stress on cell proliferation in the dorsal (*A*) and ventral (*B*) hippocampus. GL, granular layer; H, hilus; SGZ, subgranular zone. Images were captured at 10×. Arrows point to some examples of Ki67-positive cells.

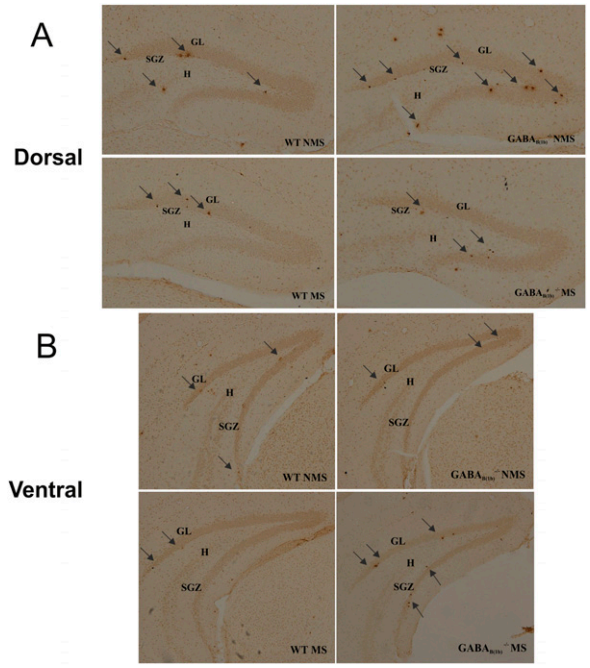

Fig. S7. Representative photographs of BrdU immunohistochemistry investigating the effects of $GABA_{B(1b)}$ receptor subunit isoform ablation and early-life stress on the survival of newly born cells in the dorsal (*A*) and ventral hippocampus (*B*). Images were captured at 10×. Arrows point to some examples of BrdU-positive cells.

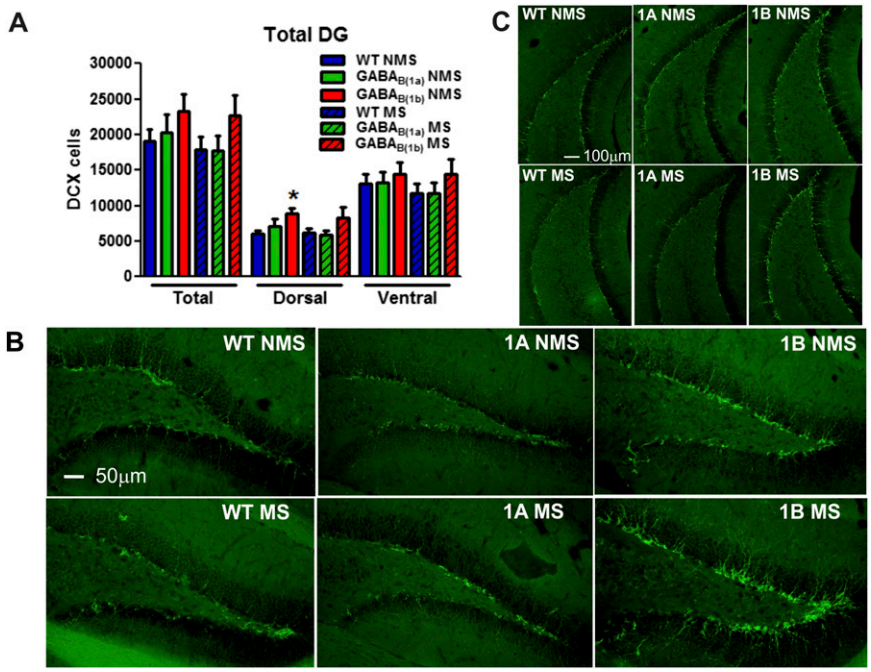

Fig. S8. Number of doublecortin-positive cells is higher in GABA$_{B(1b)}^{-/-}$ mice but not GABA$_{B(1a)}^{-/-}$ mice compared with WT mice (*A*). Representative photographs of DCX immunohistochemistry in the dorsal hippocampus captured at 20× (*B*) and ventral hippocampus captured at 10× (*C*). DG, dentate gyrus. *Significantly different from nonseparated (NMS) WT mice, $P < 0.05$.

Table S1. Summary of the effects of $GABA_B$ receptor disruption and early-life stress on stress-induced neuronal activation

							Two-way ANOVA					
	NMS			MS			Genotype		Stress		Genotype x stress	
Brain Region	WT	$GABA_{B(1a)}^{-/-}$	$GABA_{B(1b)}^{-/-}$	WT	$GABA_{B(1a)}^{-/-}$	$GABA_{B(1b)}^{-/-}$	*F*	*P*	*F*	*P*	*F*	*P*
Hippocampal formation												
Dorsal DG	39.92 ± 4.01	31.24 ± 5.38	62.78 ± 4.10**,++	25.98 ± 2.82#	27.95 ± 2.29	49.53 ± 5.67**,++,#	21.57	**<0.001**	8.396	**<0.01**	0.997	0.379
Dorsal CA1	60.90 ± 13.23	75.59 ± 21.03	106.45 ± 26	41.86 ± 6.43	54.64 ± 15.14	71.90 ± 11.47	2.590	0.089	3.276	0.079	0.126	0.882
Dorsal CA2	14.26 ± 3.39	10.93 ± 2.31	19.48 ± 3.35 +	8.90 ± 1.41	8.64 ± 1.48	15.28 ± 2.43(*),(+)	4.948	**<0.05**	3.665	0.064	0.189	0.829
Dorsal CA3	43.67 ± 5.79	35.64 ± 6.47	52.31 ± 7.21(+)	28.47 ± 3.81(#)	31.86 ± 3.81	46.5 ± 7*,(+)	4.166	**<0.05**	2.989	0.092	0.540	0.587
Ventral DG	30.07 ± 3.40	29.78 ± 6.42	47.64 ± 3.39**,++	22.14 ± 2.35	24.65 ± 2.56	39.64 ± 4.47**,+	10.79	**<0.001**	4.354	**<0.05**	0.080	0.922
Ventral CA3	44.78 ± 3.84	10.18 ± 3.85*	63.00 ± 11.06*,+++	31.69 ± 3.54(#)	27.61 ± 3.40	53.26 ± 5.03*,++	14.56	**<0.001**	2.42	0.126	0.765	0.472
PVN	151.07 ± 14.15	114.28 ± 17.26	184.86 ± 16.88 +	140.26 ± 18.17	124.05 ± 25.05	177.76 ± 16.56+	5.79	**<0.01**	0.033	0.857	0.179	0.836
NAcc	93.52 ± 10.49	88.33 ± 19.32	118.86 ± 22.48	90.00 ± 10.22	89.65 ± 14.29	128.07 ± 14.85**,++	7.729	**<0.01**	0.042	0.839	0.483	0.621
VTA	20.43 ± 5.61	8.95 ± 1.89*,~	24.57 ± 2.98	12.52 ± 4.78	10.91 ± 4.67	14.36 ± 2.15	3.401	**<0.05**	2.969	0.096	1.506	0.239
Cortical areas												
Infralimbic cortex	51.48 ± 7.17	40.68 ± 4.97	68.56 ± 11.62	54.92 ± 7.18	55.52 ± 13.42	80.36 ± 10.54	2.910	0.068	3.144	0.085	0.0509	0.605
Prelimbic cortex	58.97 ± 4.89	71.13 ± 13.39	60.54 ± 13.92	67.99 ± 25.01	70.23 ± 17.37	73.44 ± 7.83	0.059	0.942	1.647	0.208	0.018	0.981
Cingulate cortex 1	43.85 ± 4.06	41.55 ± 1.80	47.28 ± 5.52	40.21 ± 4.34	43.95 ± 10.95	51.11 ± 7.17	2.624	0.087	0.412	0.525	0.691	0.507
Amygdala												
Lateral	21.7 ± 3.43	19.30 ± 2.72	20.70 ± 2.32	19.26 ± 2.48	16.35 ± 2.80	20.86 ± 2.90	1.076	0.352	1.199	0.201	0.014	0.986
Basolateral	65.59 ± 9.50	55.68 ± 5.79	70.75 ± 8.22	59.28 ± 4.39	47.58 ± 7.96	60.81 ± 6.58	1.944	0.158	1.799	0.188	0.031	0.969
Central nucleus of amy	40.08 ± 4.93	36.23 ± 3.92	41.67 ± 6.53	34.10 ± 3.28	35.15 ± 6.69	37.36 ± 7.11	0.227	0.798	0.657	0.423	0.093	0.911
Dorsal raphe nucleus	61.89 ± 6.29	49.63 ± 13.56	69.33 ± 5.39+	51.83 ± 9.96	62 ± 16	82.07 ± 11.62*	4.025	**0.034**	0.14	0.907	0.552	0.584

Statistically significant main effects are highlighted in bold. *Significantly different from the respective WT group according to Fisher's LSD post hoc test; *$P < 0.05$; **$P < 0.01$; (*)approached significance, $P = 0.08$. +Significantly different from the respective $GABA_{B(1a)}^{-/-}$ group according to Fisher's LSD post hoc test; +$P < 0.05$; ++$P < 0.01$; +++$P < 0.001$; (+)approached significance, $P = 0.052$–0.085. #Significantly different from the respective NMS group according to Fisher's LSD post hoc test; #$P < 0.05$; (#)approached significance, $P = 0.07$–0.075. ~NMS $GABA_{B(1a)}^{-/-}$ significantly different from NMS $GABA_{B(1b)}^{-/-}$; ~$P < 0.01$. Amy, amygdala; CA1, cornu ammonis area 1; CA2, cornu ammonis area 2; CA3, cornu ammonis area 3; NAcc, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; VTA, ventral tegmental area.