Leptin restores adult hippocampal neurogenesis suppressed by chronic unpredictable stress and reverses glucocorticoid-induced inhibition of GSK3β/β-catenin signaling

Jacob C. Garza, Ph.D.[1], **Ming Guo, Ph.D.**[1], **Wei Zhang, M.D., Ph.D.**[1], and **Xin-Yun Lu, M.D., Ph.D.**[1,2,*]

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229

[2]Department of Psychiatry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229

Abstract

Stress and glucocorticoid stress hormones inhibit neurogenesis, whereas antidepressants increase neurogenesis and block stress-induced decrease of neurogenesis. Our previous studies have shown leptin, an adipocyte-derived hormone with antidepressant-like properties [1], promotes baseline neurogenesis in the adult hippocampus [2]. The present study aimed to determine whether leptin is able to restore stress-induced suppression of neurogenesis in a rat chronic unpredictable stress (CUS) model of depression. Chronic treatment with leptin reversed the CUS-induced reduction of hippocampal neurogenesis and depression-like behaviors. Leptin treatment elicited delayed long-lasting antidepressant-like effects in the behavioral despair test, and this effect was blocked by ablation of neurogenesis with X-irradiation. The functional isoform of the leptin receptor, LepRb, and the glucocorticoid receptor (GR) were colocalized in hippocampal neural stem/progenitor cells *in vivo* and *in vitro*. Leptin treatment reversed the GR agonist dexamethasone (DEX)-induced reduction of proliferation of cultured neural stem/progenitor cells from adult hippocampus. Further mechanistic analysis revealed that leptin and DEX converged on GSK3β and β-catenin. DEX decreased Ser9 phosphorylation and increased Tyr216 phosphorylation of GSK3β, while leptin increased Ser9 phosphorylation and attenuated the effects of DEX at both Ser9 and Tyr216 phosphorylation sites of GSK3β. Moreover, leptin increased total level and nuclear translocation of β-catenin, a primary substrate of GSK3 β and a key regulator in controlling neural progenitor proliferation, and reversed the inhibitory effects of DEX on β-catenin. Together, our results suggest that adult neurogenesis is involved in the delayed long-lasting antidepressant-like behavioral effects of leptin, and leptin treatment counteracts chronic stress and glucocorticoid-induced suppression of hippocampal neurogenesis *via* activating the GSK3β/β-catenin signaling pathway.

*To whom correspondence should be addressed: Xin-Yun Lu, M.D., Ph.D., Department of Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229, Phone: 210-567-0803, Fax : 210-567-4303,: lux3@uthscsa.edu.

Conflict of Interest: The authors declare no conflict of interest.

Supplementary information is available at *Molecular Psychiatry's* website.

Keywords

leptin; chronic unpredictable stress; glucocorticoids; neurogenesis; depression; glycogen synthase kinase 3β; β-catenin

INTRODUCTION

New neurons are continuously generated throughout adulthood from a pool of neural stem/progenitor cells in the subgranular zone of the dentate gyrus of the hippocampus. In a tightly regulated process, these cells divide and give rise to granule cells that extend axons along the mossy fiber pathway and are capable of integrating into functional hippocampal circuitry [3–5]. These processes are modulated both positively or negatively by neurotransmitters [6–12], hormones [13–22], neurotrophic factors [6, 21, 23–29], pharmacological agents and environmental factors [30–32].

Among the regulatory factors of hippocampal neurogenesis, stress, a participating and precipitating factor of depression, potently inhibits neurogenesis in adult animals [18, 33–37]. For example, chronic stress paradigms including chronic unpredictable stress (CUS), chronic mild stress, and chronic social defeat stress that can induce depression-like behaviors, have been reported to decrease proliferation of neural progenitor cells in the dentate gyrus [38–49]. Also, a decrease in hippocampal neurogenesis has been demonstrated in other rodent models of stress such as repeated restraint stress [50, 51], predator odor [52–55], and footshock stress [56–59]. These findings suggest that reduction of hippocampal neurogenesis is a common feature of various types of stress. The mechanisms underlying stress-induced decrease of neurogenesis is most likely to be mediated by activation of the hypothalamic-pituitary-adrenal axis and subsequent elevation of glucocorticoid stress hormones during stress. Indeed, elevation of glucocorticoid stress hormones or administration of exogenous glucocorticoids decreases hippocampal neurogenesis [14, 15, 49, 60–66], and depletion of glucocorticoids by adrenalectomy attenuates stress-reduced neurogenesis [52]. Chronic treatment with a variety of antidepressants, including selective serotonin reuptake inhibitors, norepinephrine reuptake inhibitors, monoamine oxidase inhibitors, increases basal adult hippocampal neurogenesis, and reverses the inhibitory effects of stress and glucocorticoids on neurogenesis [10, 28, 35, 49, 56, 63, 64, 67–69]. Further studies indicate that some behavioral effects of antidepressants are neurogenesis-dependent under chronic stress or glucocorticoid treatment conditions [49, 64].

Our earlier studies demonstrate that leptin, an adipocyte-derive hormone with antidepressant-like efficacy [1], promotes adult hippocampal neurogenesis under basal conditions [2]. This finding provides evidence for adipostatic control of adult neurogenesis. However, it remains unknown whether leptin can reverse or oppose the reduction of neurogenesis induced by stress. Leptin is known to enter the brain by a saturable transport system [70], where it exerts its biological functions *via* interacting with leptin receptors. Among six isoforms of the leptin receptor (a–f) that have been identified [71, 72], LepRb is the only functional isoform as it possesses all the intracellular motifs required for signal transduction. We have previously shown that LepRb is expressed in neural stem/progenitor

cells of adult hippocampus [2]. The glucocorticoid receptor (GR) has also been reported to be present in neural stem/progenitor cells [73, 74], suggesting a possible interaction between leptin signaling and glucocorticoid signaling in the regulation of neurogenesis. In the present study, we examined the effects of leptin on hippocampal neurogenesis and behaviors in a chronic unpredictable stress (CUS) model of depression and the involvement of hippocampal neurogenesis in the antidepressant-like behavioral effects of leptin. Further *in vitro* mechanistic studies investigated the effects of leptin on glucocorticoid-induced inhibition of neurogenesis and the underlying molecular mechanisms involving the GSK3β/β-catenin signaling pathway.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats, weighing 250–300 g, were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed in groups of three. All animals were maintained on a 12-h light/dark cycle (lights on at 0700 h) with *ad libitum* access to food and water. Animals were habituated to housing conditions for 7–10 days prior to the beginning of experimental procedures. All procedures were carried out in accordance with the National Institutes of Health Guide.

Drug treatment

Recombinant rat leptin (R&D Systems, Minneapolis, MN) was dissolved in sterile saline at a concentration of 1 mg/ml and intraperitoneally (i.p.) injected into animals daily at a dose of 1 mg/kg body weight.

Chronic unpredictable stress paradigm

The chronic unpredictable stress (CUS) procedure was carried out in rats as describe in our earlier report [1]. This paradigm was designed to maximize unpredictability and mildness of the stress intensity. It consisted of a variety of stressors applied randomly and at varying times of the day for 21 days (see Supplemental Table 1 for detailed protocol). Rats were first divided into two groups, i.e. handled control (n = 7) and CUS (n = 13) groups. All stressors were applied to animals outside of their housing area in a separate procedure room. After the animals were stressed, they were kept in the procedure room for 1–2 h to allow the stress odor to disappear. All control and stressed animals were housed in groups of 2–3 except when they were subjected to isolation, high-density housing or behavioral testing. Control rats (n = 7) were handled daily in the housing room. Twenty-one days after the beginning of the CUS procedure, CUS rats were further divided into two groups. One group received intraperitoneal injection of leptin (1 mg/kg) (n = 5) and the other received vehicle (saline) injection (n = 8), given 30 min before the application of stress daily. This dose of leptin was chosen based upon its effectiveness in inducing antidepressant-like effects [1, 75]. During the 14 days of leptin or vehicle treatment, CUS rats were continuously exposed to stressors as shown in supplementary Table 1. Handled control rats received only vehicle treatment. All animals were subjected to three behavioral tests to confirm induction of depression-like behaviors in CUS rats as described below.

Behavioral testing

The open field test was performed to examine exploratory activity and fear response to novelty. The apparatus was made of black acrylic plastic. The light intensity in the four corners of the arena was adjusted to ensure the arena had uniform illumination. At the beginning of the test, the animal was placed in the center of the arena and allowed to freely explore for 5 minutes. Animals were video-tracked using the Noldus EthoVision 3.0 system (Noldus Information Technology, Leesburg, VA). The apparatus was cleaned after each testing session to prevent any odors deposited by the rats from influencing the following rat. Total distance travelled, movement velocity, and freezing time were measured.

For the sucrose preference test, in order to habituate the animals to the two bottle situation and to avoid neophobia during testing, two bottles, one with water and one with 1% sucrose solution, were placed in each rat cage two days before the CUS procedure and 21 days after CUS exposure began. A 1% sucrose solution was chosen to assess anhedonia based upon previous studies reported by us as well as other groups [1, 76–79]. Rats were tested for sucrose preference on days 30 and 35. On testing days, animals were individually housed and provided with a free choice between plain water and 1% sucrose right before the dark cycle. The bottle position was balanced within the group and rotated between tests to avoid side preference. The amount of water and sucrose consumed over the dark cycle was measured by weight. Sucrose preference was expressed as a ratio of sucrose intake volume over water intake volume.

The forced swim test was performed as described previously [1]. Briefly, animals were placed in a cylindrical tank (60 cm height, 30 cm diameter) filled with tap water (25°C) to a depth of 45 cm for 5 min. The behaviors in the forced swim test were recorded by two video cameras with one positioned on top of the swimming tank and another positioned on the side. The recorded behavior of the rats was scored using a time sampling technique to rate the predominant behavior over a 5 sec interval as described by Lucki [80]. Immobility was defined as floating or no active movements made other than what is necessary to keep their nose above water. Swimming was defined as active motions throughout the swim tank and crossing into another quadrant. Climbing was evaluated as upward-directed movements of the forepaws against the wall.

X-ray irradiation

X-ray irradiation was performed using a modified protocol from previously reported studies [81–83]. Rats were anesthetized and subjected to cranial irradiation using a Faxitron X-ray system (58 Kv, 3.0 mA). A lead shield was designed to protect the entire body of the rat from X-ray exposure while leaving a small oval-shaped area (1.5 cm × 2 cm) over the hippocampus open to X-ray exposure. On two consecutive days, 10 Gy (X-irradiation) each day was delivered over 14.7 minutes to anesthetized animals placed in a plastic restrainer. This paradigm and dose for irradiated rats has been well established and used in previous studies to reduce hippocampal neurogenesis without adverse side effects [81, 82, 84, 85]. Sham controls were taken to the X-ray facility and anesthetized, but not subjected to irradiation. After a 4-week recovery period, leptin (1mg/kg) or saline was administered to the rats i.p. once per day for 14 consecutive days.

In vivo adult hippocampal neurogenesis

BrdU injection—BrdU was used to label proliferating cells and track the fate of newly proliferated cells. The same rats used for behavior testing were injected with BrdU 2 h after the last drug administration or 24 h after the last stressor was applied to CUS rats. The length of S-phase was estimated to be approximately 8 h [86]. Thus, three injections of BrdU were given i.p. (50 mg/kg) to the animals, at 8-h intervals over 24 h. The rate of cell proliferation was determined by sacrificing the animals 2 h after the third BrdU injection. To track the fate of BrdU-labeled cells, animals were sacrificed 28 d after the last BrdU injection.

Tissue Preparation—Animals were anesthetized with an intramuscular (i.m.) injection of an anesthetic cocktail (43 mg/kg ketamine, 9 mg/kg xylazine and 1.4 mg/kg acepromazine in saline) and perfused through the ascending aorta using 0.1 M sodium phosphate buffer (PBS) followed by 4% paraformaldehyde in PBS. The brain was removed and fixed overnight in 4% paraformaldehyde, and then transferred to 30% sucrose in PBS. Brains were cut into 40 μm coronal sections on a cryostat and stored in cryoprotectant (30% sucrose, 30% ethylene glycol, 1% polyvinyl pyrrolidone, 0.05 M sodium phosphate buffer) until processing for immunohistochemistry.

Immunohistochemistry—Immunohistochemistry for BrdU was performed as described previously [2]. Briefly, free floating sections were washed in 0.1 M PBS followed by incubation in a solution of $1\times$ saline-sodium citrate buffer (SSC) and 50% formamide for 2 h at 60°C. Next, the sections were treated with 2 N HCL for 30 min at 37°C followed by incubation in 0.1 M boric acid buffer. One percent hydrogen peroxide in PBS was used to quench the endogenous peroxidase of the sections. The tissue was then incubated in immuno blocking buffer (3% goat serum, 1% BSA, 0.3% triton-X 100, in PBS) for 1 h followed by mouse anti-BrdU primary antibody (1:200, Roche, Indianapolis, IN) in blocking solution overnight at 4°C. After rinsing in PBS buffer, the sections were then incubated with a biotinylated goat anti-mouse antibody in blocking solution (1:400) for 1 h at room temperature. The tissue was washed in PBS followed by subsequent incubation with avidin-biotin complex (1:100, Vector Laboratories, Burlingame, CA) for 1 h. BrdU immunoreactivity was revealed using 3′-diaminobenzidine (DAB; Vector Laboratories, Burlingame, CA).

To determine if BrdU-labeled cells expressed neuronal or glial phenotypes, brain sections were processed for BrdU, neuronal nuclei (NeuN) or anti-glial fibrillary acidic protein (GFAP) fluorescent double labeling. The tissue was first treated for BrdU immunohistochemistry as described above with some modifications. To detect BrdU, the tissue was incubated with rat-anti-BrdU antibody (1:200, Accurate Chemical, Westburg, NY) and mouse anti-NeuN (1:400, Chemicon, Temecula, CA) or rabbit anti-GFAP (1:1000, Chemicon, Temecula, CA). After overnight incubation in primary antibody, the sections were washed in PBS and incubated for 4 h with fluorescent secondary antibodies: Alexa Fluor 488 goat anti-rat IgG to reveal immunoreactivity of BrdU and Alexa Fluor 546 goat anti-mouse IgG or Alexa Fluor 546 goat anti-rabbit IgG to reveal immunoreactivity of NeuN or GFAP, respectively (1:400 for all three antibodies, Molecular Probes, Eugene, OR).

Then, the sections were rinsed in PBS and mounted onto poly-lysine coated glass slides and coverslipped with fluorescence mounting medium.

Cell counting—Every sixth section throughout the entire rostral-caudal extent of the hippocampus was used to determine the number of BrdU-labeled cells within the dentate gyrus. Counting was accomplished using 400× magnification on an Olympus BX51 microscope. Cells were counted bilaterally throughout the dentate gyrus. The number of BrdU-labeled cells was multiplied by 6 to obtain the total number of cells per dentate gyrus. For cell differentiation experiments, a total of 70 BrdU-positive cells were counted for BrdU/NeuN and BrdU/GFAP and visualized on an Olympus FV-1000 confocal microscope. Colocalization with NeuN or GFAP was confirmed by examining multiple optical planes for each cell on the z-axis. The percentage of BrdU-positive cells double-labeled for NeuN or GFAP was determined.

Adult hippocampal neural stem/progenitor cell culture—Adult hippocampal stem cells isolated from adult Fisher rats (Millipore, Billerica, Ma) were grown and maintained on poly-L-ornithine and laminin coated plates as a monolayer in Neural Expansion Media (DMEM:F12 nutrient mix plus B-27 supplement and 20 ng/mL β-FGF). The cultured adult hippocampal stem cells were tested for the expression of neural stem cell marker nestin and showed no expression of markers for differentiated cells.

Triple labeling method combining *in situ* hybridization and immunohistochemistry *in vivo* and *in vitro*—For *in vivo* detection of LepRb mRNA and glucocorticoid receptor (GR) and nestin proteins in the dentate gyrus, *in situ* hybridization was used in combination with immunohistochemistry. Rats were transcardially perfused with 2% paraformaldehyde, post-fixed for 2 h, and subsequently immersed in 30% sucrose in PBS. Brains were sectioned at 20 μm and thaw mounted onto poly-lysine coated glass slides. To detect LepRb mRNA, with *in situ* hybridization, brain sections were fixed with 4% paraformaldehyde in PBS and then washed in 2× SSC buffer (pH 7.2). The tissue was then acetylated using 0.1 M triethanolamine (pH 8.0) with 0.25% acetic anhydride for 10 min and subsequently dehydratedthrough a graded series of alcohol (50–100%). An antisense cRNA probe directed against the C-terminal sequence of LepRb, specific for LepRb, was labeled with Biotin-16-UTP using the standard transcription system as described in our previous studies [87]. Hybridization was performed by incubating the tissue sections in 70 μl of hybridization buffer containing 5 μl of labeled probe at 55 °C overnight in humidified chambers. The tissue sections were then rinsed in 2× SSC and incubated in RNase A buffer (200 μg/ml) for 1 h at 37 °C followed by a series of washes of increasing stringency (2×, 1×, and 0.5× SSC for 5 min each). Finally, the tissue slides were washed with 0.1× SSC at 70 °C for 1 h. The Tyramide Signal Amplification (TSA) Plus Biotin Kit (Perkin Elmer, Waltham, MA) was used to detect the biotin-labeled LepRb probe. First, the slides were washed in 0.05 M phosphate buffer (PBS) (pH 7.4) followed by 2% H_2O_2 for 30 min and then incubated in the *in situ* blocking solution for 1h. Next, the slides were incubated in streptavidin conjugated to horseradish peroxidase (HRP) (1:1000, Roche, Indianapolis, IN) in blocking solution followed by incubation in the biotinyl tyramide in amplification diluent provided with the TSA Plus Biotin Kit. Following three rinses in 0.05

M PBS and incubation in 2% H_2O_2 for 30 min, immunohistochemical staining for GR and nestin was performed before completion of LepRb detection. The tissue slides were rinsed and then incubated in immunoblocking buffer containing 3% goat serum, 1% BSA, and 0.3% triton-X 100 in PBS, followed by incubation with antibodies for GR (1:500, Santa Cruz) and nestin (1:100, Chemicon) overnight. The next day, after the tissue sections were rinsed, Alexa Fluor 546 goat anti-rabbit IgG was used to reveal immunoreactivity of GR, and Alexa Fluor 647 goat anti-mouse IgG was used to detect nestin. Then, the tissue sections were incubated in the *in situ* blocking solution and streptavidin-conjugated to alkaline phosphatase (1:100) and subsequently, the Enzyme-Labeled Fluorescence-97 Endogenous Phosphatase Detection kit (Molecular Probes, Eugene, OR) was used to visualize LepRb signals.

For detection of triple-labeling of LepRb mRNA and the glucocorticoid receptor (GR) and nestin proteins *in vitro*, adult hippocampal stem/progenitor cells were seeded onto poly-L-ornithine and laminin coated glass coverslips and grown overnight. The cells were processed for *in situ* hybridization as described for *in vivo* studies above. To detect biotin labeled LepRb probe, the Tyramide Signal Amplification (TSA) Plus Cyanine 2 System (Perkin Elmer, Waltham, MA) was used. Coverslips were washed in 0.1 M phosphate buffer (pH 7.4) followed by incubation with 2% H_2O_2 for 20 min. Next, blocking solution was applied for 1 h at room temperature and streptavidin conjugated to horseradish peroxidase was added to detect biotin-labeled LepRb probes. LepRb signals were subsequently detected using the TSA Plus Cyanine 2 System. Then, coverslips were incubated in 2% H_2O_2 for 20 min followed by immunoblocking buffer (1% BSA, 3% Goat Serum, 0.3% Triton X-100) for 1 h and then primary antibodies diluted in blocking solution (mouse anti-nestin, 1:1000; rabbit anti-GR, 1:500). Next, coverslips were washed in PBS and subsequently incubated in secondary antibodies diluted in blocking solution (biotinylated goat anti-mouse antibody, 1:400; Alexa Fluor 546 goat anti-rabbit antibody, 1:400, Molecular Probes) for 4 h. Then streptavidin conjugated to Alexa Fluor 647 (1:1000, Molecular Probes, Eugene, OR) was added to visualize nestin immunoreactivity.

Immunocytochemistry for BrdU and β-catenin—Adult hippocampal neural stem/progenitor cells were seeded on poly-ornithine and laminin coated 8-well chamber slides. A total of four wells were treated for each condition as follows: vehicle, 1 nM leptin, 10 μM DEX, 10 μM DEX with 0.1 nM, 1 nM or 3 nM leptin, and grown for 48 h. BrdU (10 μM) was added to the media for the last 4 h after which the cells were fixed with 4% paraformaldehyde in PBS. After washing in PBS, the cells were incubated in 2 N HCl for 30 min at 37 °C followed by 0.1 M boric acid buffer. Immuno blocking solution was added to each well of the chamber slide for 1 h. For BrdU detection, the cells were incubated in mouse anti-BrdU (Roche, Indianapolis, Inc.) primary antibody diluted in blocking solution overnight at 4 °C. The slides were washed in PBS followed by incubation in secondary antibody, Alexa Fluor 488 goat anti-mouse IgG and coverslipped using fluorescence mounting media. Cell proliferation was determined by counting BrdU-labeled cells in each well. Five random fields were chosen under the 20× objective, and the total number of BrdU-labeled cells was counted.

To detect nuclear translocation of β-catenin, adult hippocampal stem/progenitor cells were seeded at a density of 2×10^4 cells per ml onto poly-ornithine and laminin coated glass coverslips in 12-well plates. The cells were treated with vehicle, 10 μM DEX, 10 μM DEX with 1 nM leptin, or 1 nM leptin and grown for 48 h, after which the cells were fixed with 4% paraformaldehyde in PBS. The cells were then rinsed in PBS followed by treatment with immuno blocking buffer (3% goat serum, 1% BSA, 0.3% triton X-100 in PBS) and subsequently incubated with rabbit anti-β-catenin antibody (1:500, Abcam, Cambridge, Ma) overnight at 4 °C. The cells were washed in PBS followed by incubation in Alexa Fluor 488 goat anti-rabbit IgG and mounted using fluorescent mounting media.

Western blot—Cells were platted on polyornithine and laminin-coated 6-well polystyrene plates and grown in neural expansion media for 24 h. The cells were treated with vehicle, 10 μM dexamethasone (DEX), 10 μM DEX with 1 nM leptin, or 1 nM leptin. The cells were harvested after 20 min, and total protein was extracted using cell lysis buffer (50 mM Hepes, pH 7.6, 1% Triton X-100, 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF) containing a mixture of phosphatase inhibitors (leupeptin, aprotinin, sodium orthovanadate, phenylmethylsulfonyl fluoride, Ser/Thr phosphatase inhibitor mixture, Tyr phosphatase inhibitor mixture). The total protein concentration was assessed using the Bradford assay and run on an SDS-PAGE gel. The proteins were then transferred to a nitrocellulose membrane and blocked in a solution of 1% BSA and 0.05% Tween 20 in $1\times$ Tris-buffered saline. The membranes were incubated in blocking solution containing primary antibody (anti-GSK3β, 1:1000, Cell Signaling; anti-phosphorylated GSK3β on Ser9, 1:1000, Cell Signaling; anti-GSK3β phosphorylated on Tyr216, 1:1000, Abcam; anti-β-catenin, 1:5000, Abcam) overnight at 4 °C. After washing the membrane was incubated in anti-rabbit secondary antibody conjugated to horseradish peroxidase (1:5,000) in blocking solution for 1 h. Next, the membrane was washed in Tris-buffered saline and the electrogenerated chemiluminescence (ECL) reaction solutions were added (solution 1: 0.1 M Tris-HCl, luminol, *p*-coumaric acid; solution 2: 0.1 M Tris-HCl, hydrogen peroxide) for 1 min. The membrane was exposed to X-ray film (Phenix Research Products, Candler, NC) for visualization.

Nuclear protein extraction—Nuclear lysates were isolated using the nuclear extraction kit (Chemicon International). Cells were grown to approximately 50% confluency on 10 cm poly-ornithine and laminin coated plates and treated for 48 h with vehicle, 10 μM DEX, 1 nM leptin, or a combination of 10 μM DEX and 1 nM leptin. Cells were harvested and nuclear extract was isolated. The cell pellet was resuspended in cytoplasmic lysis buffer containing 0.5 mM DTT and protease inhibitors and disrupted using a 27G syringe needle. Once the cytoplasmic portion of the cells was purified, nuclear extraction followed using nuclear extraction buffer containing 0.5 mM DTT and protease inhibitors. Total β-catenin present in the nuclear extract was evaluated using Western blot with an antibody specific for β-catenin (anti-β-catenin, 1:5000, Abcam) and normalized to β-actin.

Statistical analysis—Results were expressed as mean ± SEM. Statistical analyses were performed with one-way ANOVA with repeated measures on body weight and one-way ANOVA without repeated measures on behavioral tests, cell proliferation and

differentiation, and two-way ANOVA on the X-irradiation study, phosphorylation of GSK3β and β-catenin levels. Student Newman Keuls or Tukey/Kramer (for unequal n) *post hoc* comparisons followed ANOVAs. Two-tailed *t*-test was used for the rest of the experiments.

RESULTS

Suppression of cell proliferation in adult hippocampus and induction of behavioral depression by chronic unpredictable stress

First, we determined whether adult hippocampal neurogenesis can be suppressed in a rat CUS model of depression. Rats subjected to a CUS procedure daily for 21 days (detailed in Supplementary Table 1) were assessed in three behavioral tests. In the open field test, CUS rats displayed decreased exploration ($P < 0.01$) and moving velocity ($P < 0.01$) and increased freezing ($P < 0.01$). In the sucrose preference test, the CUS rats showed anhedonia-like behavior ($P < 0.05$), as indicated by reduced preference for 1% sucrose solution. In the forced swim test, CUS rats displayed despair behavior as indicated by increased time of immobility ($P < 0.01$) (Figure S1A-C). To examine whether adult hippocampal neurogenesis is impaired by chronic stress, cell proliferation in the dentate gyrus was evaluated by detecting BrdU labeled cells at 2 h after BrdU injection. CUS exposure reduced the number of BrdU-labeled cells in the dentate gyrus ($P < 0.05$) (Figure S1D). These results support the validity of the rat CUS procedure as an animal model of depression and as an effective model to examine the effect of leptin on chronic stress-induced suppression of adult hippocampal neurogenesis.

Reversal of chronic unpredictable stress-induced suppression of adult hippocampal neurogenesis and depression-like behaviors by administration of leptin

Using the CUS model established above, the effects of leptin on the stress-induced suppression of adult hippocampal neurogenesis and behavioral deficits were examined. Rats were first exposed to the CUS procedure for 21 days followed by 14 days of leptin treatment (1 mg/kg) or vehicle daily, during which CUS exposure continued (Supplementary Table 1) (Figure 1A). For behavioral tests, ANOVA indicated there were significant differences among three treatments in their effect on open field behaviors: Exploratory activity ($F_{(2,17)} = 20.11$, $P < 0.001$), moving velocity ($F_{(2,17)} = 7.89$, $P < 0.005$) and freezing time ($F_{(2,17)} = 7.88$, $P < 0.005$). *Post hoc* tests revealed vehicle-treated CUS rats exhibited a significant decrease in exploration ($P < 0.0001$) and moving velocity ($P < 0.0001$) and an increase in freezing ($P < 0.0001$) compared to the vehicle-treated control rats, but leptin treatment had no significant effect on any open field measure ($P > 0.5$) (Figure 1B). ANOVA showed a main effect of treatment on sucrose preference ($F_{(2, 13)} = 7.19$, $P < 0.01$). *Post hoc* tests indicated that vehicle-treated CUS rats had a significantly lower preference for sucrose solution than the vehicle-treated control rats ($P < 0.05$), and leptin treatment reversed the CUS-induced decrease in sucrose preference ($P = 0.01$) (Figure 1C). In the forced swim test, ANOVA revealed main effects of treatment on immobility ($F_{(2, 26)} = 8.78$, $P = 0.001$), swimming ($F_{(2, 26)} = 10.74$, $P < 0.0005$) and climbing ($F_{(2, 26)} = 4.01$, $P < 0.05$). *Post hoc* tests demonstrated leptin treatment significantly decreased immobility time ($P < 0.001$) and increased swimming activity in CUS rats ($P < 0.0001$) (Figure 1D). Reversal of CUS-induced 'behavioral despair' and 'anhedonia' by chronic leptin treatment confirmed the

antidepressant potential of leptin. In addition, the effects of CUS and leptin on body weight were monitored (Figure 1E). ANOVA with repeated measures indicated that the CUS exposure significantly decreased body weight gain prior to leptin or vehicle treatment ($F_{(2,46)}$= 8.789, $P < 0.0001$). After rats received leptin or vehicle administration, ANOVA showed a main effect of treatment on body weight gain ($F_{(2, 170)}$ = 7.90, $P < 0.005$). *Post hoc* tests revealed a significant decrease in body weight gain in vehicle-treated CUS rats compared to vehicle-treated control rats. Leptin treatment further decreased body weight gain in CUS rats compared to vehicle treatment ($P < 0.01$).

The effect of leptin on adult hippocampal neurogenesis in CUS rats was assessed by analyzing BrdU labeling (Figure 2A). Cell proliferation was assessed at 2 h after BrdU labeling. ANOVA revealed a main effect of treatment on total number of BrdU-positive cells ($F_{(2,17)}$ = 5.56, $P = 0.01$). CUS exposure significantly decreased the total number of BrdU-positive cells compared to control (Figure 2B, $P < 0.05$), and this effect of CUS was significantly reversed by chronic leptin treatment ($P < 0.01$). The survival of newly proliferated cells in the dentate gyrus was examined 28 days after BrdU administration (Figure 2C). ANOVA indicated a significant effect of treatment on total number of survived BrdU-positive cells ($F_{(2,17)}$ = 17.57, $P < 0.0001$). The vehicle-treated CUS group exhbibited a significantly lower number of survived BrdU-positive cells than in the vehicle-treated control group ($P < 0.01$), and CUS-induced decrease in the number of survived BrdU-labeled cells was partially reversed by leptin treatment ($P = 0.01$) (Figure 2C left panel). The majority of survived BrdU-positive cells exhibited a neuronal phenotype, i.e. co-localizing with NeuN, however, there was no significant effect of treatment on percentage of BrdU-labeled cells that were double-labeled for NeuN ($F_{(2,16)}$ = 1.89, $P = 0.184$) (Figure 2C right panel and D). A low percentage of survived BrdU-positive cells were co-localized with GFAP (Figure 2C right panel and D). CUS exposure decreased the percentage of BrdU-positive cells differentiated into glia ($P < 0.001$), and this was reversed by treatment with leptin. Together, this data supports that leptin is capable of reversing the CUS-induced suppression of neurogenesis in the adult hippocampus.

Involvement of hippocampal neurogenesis in mediating the long lasting antidepressant-like behavioral effects of leptin

To test the involvement of leptin-induced hippocampal neurogenesis in mediating the antidepressant-like effects of leptin, animals were first exposed to a sham procedure or X-irradiation (10 Gy/day for 2 consecutive days) to induce ablation of hippocampal neurogenesis [81, 82]. At 28 days after exposure to the sham procedure or X-irradiation, one set of animals was injected with BrdU to examine the effectiveness of X-irradiation in blocking neurogenesis. As reported in previous studies [81, 82], we found that X-irradiation at this dose greatly reduced BrdU-labeled cells in the dentate gyrus (Figure 3A), which confirmed ablation of hippocampal neurogenesis.

To determine whether new neurons stimulated by leptin contribute to its antidepressant-like efficacy, 28 days after X-irradiation animals were treated for 14 days with leptin to upregulate neruogenesis, followed by a 14-day delay to permit functional integration of new-born neurons during leptin treatment. ANOVA revealed a significant effect of

irradiation ($F_{(1,22)}$ = 5.34, P < 0.05) but not treatment on immobility ($F_{(1,22)}$ = 3.42, P = 0.08). There was a marginally significant effect of irradiation × treatment interaction on immobility ($F_{(1,22)}$ = 3.81, P = 0.06). *Post hoc* analysis indicated that leptin treatment significantly reduced immobility time (P < 0.05), whereas X-irradiation abolished the effect of leptin on immobility (P < 0.01) (Figure 3C). In addition, ANOVA demonstrated a significant effect of irradiation ($F_{(1,22)}$ = 9.04, P < 0.01) but not treatment on swimming time ($F_{(1,22)}$ = 2.70, P = 0.12). There was a significant interaction between irradiation and treatment on swimming time ($F_{(1,22)}$ = 6.64, P < 0.05). *Post hoc* analysis indicated that leptin treatment significantly increased swimming time (P < 0.05), whereas X-irradiation abolished the effect of leptin on swimming (P < 0.01) (Figure 3C). Climbing time was not affected by irradiation or treatment ($F_{(1,22)}$ = 0.06, P = 0.81 for irradiation; $F_{(1,22)}$ = 1.09, P = 0.31 for treatment; $F_{(1,22)}$ = 0.03, P = 0.82 for irradiation × treatment). To determine the immediate effects on immobility and swimming in the forced swim test following leptin treatment, some animals exposed to the sham procedure were tested at the end of 14-day of leptin treatment. We found that leptin treatment significantly decreased immobility time by 51% and increased swimming time by 55% without a significant effect on climbing time (Figure 3B). These effects of leptin are unlikely to be related to leptin-induced neurogenesis as newborn neurons take about 4 weeks to achieve mature connectivity [88]. These results, together with our previous findings [1], indicate that leptin produces both rapid and delayed antidepressant-like behavioral effects, and these effects are likely mediated through distinct neurogenesis-dependent and–independent mechanisms.

Co-expression of LepRb and GR in adult hippocampal neural stem/progenitor cells

Stress-induced glucocorticoid surge activates GR, which is believed to mediate the suppressive effects of stress on neurogenesis [89]. Both GR and LepRb have been reported to be expressed in the dentate gyrus [90, 91]. To determine whether these two receptors coexist in neural progenitor cells, we employed a triple-labeling detection method to demonstrate the colocalization of LepRb, GR and nestin, a marker of undifferentiated neural stem/progenitor cells, in the dentate gyrus. As shown in Figure 4A, LepRb and GR were distributed in the entire dentate gyrus, whereas nestin-positive cells were restricted to the subgranular zone. Colocalization of LepRb and GR was observed in nestin-positive cells in the subgranular zone of the dentate gyrus (Figure 4A). *In vitro* studies demonstrated that GR was expressed in almost all nestin-positive cells (Figure 4B). The co-existence of LepRb, GR and nestin were confirmed in cultured hippocampal neural stem/progenitor cells (Figure 4B). These observations provide the biological basis for a possible mechanism by which leptin antagonizes the effect of glucocorticoid stress hormones on hippocampal neurogenesis.

Effects of leptin and the GR agonist DEX on proliferation of adult hippocampal neural stem/progenitor cells

Cultured hippocampal neural stem/progenitor cells were treated with DEX and various doses of leptin for 48 h. ANOVA revealed a main effect of treatment ($F_{(5,23)}$ = 24.05, P < 0.0001). *Post hoc* tests indicated that DEX at a dose of 10 μM significantly reduced the number of BrdU-positive cells (P < 0.01, Figure 4C), and this effect was attenuated by co-treatment with leptin at the doses of 1 nM and 3 nM (P = 0.01, Figure 4C). These results indicate

leptin produces a dose-related reversal of DEX-induced inhibition of adult hippocampal neural stem/progenitor cell proliferation.

Effects of leptin and DEX on the GSK3β/β-catenin signaling pathway

To identify the molecular mechanisms by which leptin reversed the effect of DEX on hippocampal neural stem/progenitor cell proliferation, we examined the GSK3β/β-catenin signaling pathway. GSK3β phosphorylationat Ser9 and Tyr216 was detected 20 min after leptin (1 nM) and/or DEX (10 μM) treatment. ANOVA revealed a main effect for both leptin and DEX treatment on GSK3β Ser9 phosphorylation ($F_{(1,15)} = 22.62$, $P < 0.001$ for leptin; $F_{(1,15)} = 4.88$, $P < 0.05$ for DEX) and only a significant main effect for DEX on Tyr216 phosphorylation ($F_{(1,15)} = 0.93$, $P = 0.35$ for leptin; $F_{(1,15)} = 5.99$, $P < 0.05$ for DEX). There was no significant interaction between leptin and DEX ($F_{(1,15)} = 2.26$, $P = 0.16$ for Ser9 phosphorylation; $F_{(1,15)} = 1.04$, $P = 0.32$ for Tyr216 phosphorylation). DEX decreased GSK-3β Ser9 phosphorylationand increased Tyr216 phosphorylation compared to vehicle treatment ($P < 0.05$), while leptin increased Ser9 phosphorylation ($P < 0.001$) and had no significant effect on Tyr216 phosphorylation (Figure 5A). When leptin (1 nM) was co-administered with DEX, it reversed DEX-induced reduction of Ser9 phosphorylation ($P < 0.001$) and attenuated DEX-induced increase in Tyr216 phosphorylation($P = 0.1$) (Figure 5A). Similar results were obtained after 48 h treatment with leptin and/or DEX (Figure S2).

It is known that Ser9 phosphorylation inhibits GSK-3β activity and Tyr216 phosphorylation increases GSK-3β activity [92–94]. As active GSK3β promotes β-catenin degradation, a reduction of GSK-3β activity would increase cytosolic levels of β-catenin and allow for its translocation from the cytoplasm to the nucleus [95, 96]. Therefore, the effects of leptin and DEX on the total cellular level of β-catenin and nuclear level of β-catenin were determined. The immunohistochemical staining showed that β-catenin immunoreactivity was primarily in cytosol in neural stem/progenitor cells treated with DEX, and co-treatment with leptin increased β-catenin immunoreactivity in the nucleus as well as in the cytosol (Figure 5B). This result was confirmed by measuring β-catenin levels in the total cell and nuclear fractions using Western blot assay. ANOVA revealed a main effect of DEX and leptin on total cellular level of β-catenin ($F_{(1,11)} = 6.77$, $P < 0.05$ for DEX, $F_{(1,11)} = 31.46$, $P < 0.001$ for leptin). *Post hoc* analysis revealed that total β-catenin level was significantly decreased by DEX treatment alone ($P < 0.05$) and increased by leptin treatment alone ($P < 0.01$), compared to the vehicle treated group (Figure 5C). DEX-induced decrease in total β-catenin was significantly reversed by co-treatment with leptin ($P < 0.001$). Moreover, ANOVA revealed a significant main effect of DEX and leptin on nuclear β-catenin levels ($F_{(1,8)} = 4.913$, $P < 0.05$ for DEX, $F_{(1,8)} = 50.147$, $P < 0.001$ for leptin). *Post hoc* analysis showed that the level of β-catenin in the nucleus was reduced by DEX treatment ($P < 0.05$) and increased by leptin treatment ($P < 0.001$) when compared to the vehicle treated group. The reduction in nuclear β-catenin induced by DEX was reversed by co-treatment with leptin (Figure 5D, $P < 0.01$). It is noteworthy that there was a greater magnitude of increase in nuclear β-catenin (7-fold) than total β-catenin (1.5-fold) following leptin treatment, suggesting that leptin facilitates nuclear translocation of β-catenin. Together, these results suggest that the GSK3β/β-catenin signaling pathway may underlie the effects of leptin and glucocorticoids on hippocampal neural stem/progenitor cell proliferation.

DISCUSSION

In recent years, evidence has emerged that leptin plays a neurotrophic role in developing and adult brains [2, 97–102]. Our previous studies have demonstrated that leptin has antidepressant-like activity [1, 103] and promotes basal adult hippocampal neurogenesis in non-stressed animals [2]. This study shows that chronic leptin treatment attenuates stress-induced suppression of hippocampal neurogenesis and depression-like behaviors. Hippocampal neurogenesis contributes to the long-lasting antidepressant-like behavioral effects of leptin. Leptin and stress hormones produce opposing effects on hippocampal neurogenesis, converging on the GSK3β/β-catenin signaling pathway. The GR agonist DEX-induced inhibition of proliferation of cultured hippocampal neural stem/progenitor cells and changes in GSK3β phosphorylation and β-catenin levels in the nucleus can be reversed by leptin treatment. These findings indicate that leptin is able to combat the deleterious consequences of chronic stress and excessive glucocorticoids on neurogenesis and behaviors, possibly via acting on the GSK3β/β-catenin signaling pathway.

Chronic unpredictable/variable/mild stress or chronic elevation of stress hormones have been demonstrated to induce suppression of hippocampal neurogenesis [40, 42, 43, 104] and behavioral phenotypes mimicking those seen in major depression [38, 42, 105–107], which can be reversed by administration of classic antidepressants [28, 37, 38, 56, 69, 108]. Consistent with previous findings, we showed that exposure to unpredictable stress for 21 days in rats decreased neurogenesis in the adult dentate gyrus and induced depression-like behavioral deficits including anhedonia and 'behavioral despair' as indicated by decreased sucrose preference and increased immobility in the forced swim test, respectively. The latter behavioral phenotype has been inconsistent across studies [42, 109]. Differences in the types, intensity and durations of stress and behavioral testing conditions may account for this discrepancy. Chronic leptin treatment reversed CUS-suppressed cell proliferation in the adult hippocampus, an effect accompanied with improvement of anhedonia and 'behavioral despair'. However, CUS-induced changes in exploratory activity and freezing time, a rodent fear response, were not altered. In addition, while weight loss can be a symptom of depression, CUS-induced weight loss was slightly but significantly enhanced by leptin treatment in spite of the alleviation of depression-like behaviors. These findings suggest differential effects of leptin on chronic stress-induced cellular, behavioral and metabolic phenotypes in this CUS model.

Although clear evidence for a causal link between adult neurogenesis and the development of depression is still lacking, recent studies support that behavioral actions of antidepressants might involve neurogenesis-dependent mechanisms. Some but not all behavioral effects of antidepressants can be blocked by selective ablation of neurogenesis, depending on the types of behavioral paradigms and the methods used to ablate neurogenesis [10, 49, 64, 81, 84, 110–121]. The behavioral effects of antidepressants on anhedonia and forced swimming have been reported to require neurogenesis [38, 81, 121]. These findings raise the possibility that leptin-induced increase in hippocampal neurogenesis might contribute to its antidepressant-like behavioral effects. To address this, we used a leptin treatment regimen that promotes hippocampal neurogenesis followed by a time lag to permit new neurons to integrate into hippocampal neuronal network and become fully functional [122]. We found that leptin

treatment elicited significant effects on 'behavioral despair' in the forced swim test at 14 days after the cessation of leptin administration. This delayed behavioral effect of leptin was abolished by ablation of neurogenesis with X-irradiation. This finding suggests that neurogenesis contributes to the delayed long-lasting antidepressant-like behavioral effect of leptin. Our previous studies have shown that leptin produces rapid effects on 'anhedonia' and 'behavioral despair' [1], presumably through neurogenesis-independent mechanisms. Leptin action on mature neurons in the hippocampus and other brain regions may mediate its acute and short-term behavioral effects.

Multiple lines of evidence support that inhibitory effects of stress on hippocampal cell proliferation are mediated by stress-induced glucocorticoid release [14, 15, 33, 49, 52, 60–66, 123]. This is likely to be due, at least in part, to a direct effect of glucocorticoids on neural stem/progenitor cells [73, 74]. However, the molecular mechanisms underlying stress- and glucocorticoid-induced decrease in neurogenesis remains poorly understood. As reported previously [124, 125], we demonstrated that activation of GR by the selective agonist DEX decreased proliferation of cultured neural stem/progenitor cells. The colocalization of LepRb with GR in hippocampal neural stem/progenitor cells provided a biological basis for interaction between LepRb and GR signaling on cell proliferation. Leptin-induced reversal of DEX-induced decrease in proliferation of stem/progenitor cells *in vitro* is in agreement with the *in vivo* results in CUS rats. It should be pointed out, however, that the extrapolation of *in vitro* results to the *in vivo* condition is not straightforward. *In vitro* and *in vivo* studies differ in the experimental situations and treatment protocols. In cultures the experimental conditions are well-controlled, and stem cells are isolated from differentiated neurons or glia. Thus, the effects of leptin would reflect a direct mechanism. On the other hand, *in vivo* conditions could be modulated with time and by positive and negative regulatory factors derived from hippocampus and extra-hippocampal cells. It has been reported that differentiated neurons inhibit proliferation of neural progenitor cells [126]. This might partially explain why a longer time was required for leptin treatment *in vivo* than *in vitro* to produce a significant effect on neurogenesis. The combination of using an *in vivo* model to identify the neurogenic phenomenon with a well-controlled *in vitro* system to dissect the underlying molecular mechanisms would allow us to better understand the regulation of neurogenesis by leptin.

β-catenin signaling plays an essential role in adult neurogenesis [127–132]. β-catenin is a primary substrate of GSK3β, which phosphorylates N-terminal residues of β-catenin initiating rapid ubiquitin-mediated degradation by proteosomes [95]. GSK3β activity is, in turn, regulated by phosphorylation on Ser9 and Tyr216 [92–94]. While Ser9 phosphorylation inhibits GSK3β activity, Tyr216 phosphorylation increases its activity. We found that DEX increased Tyr216 phosphorylation and decreased Ser9 phosphorylation in neural stem/progenitor cells. The former change is consistent with a recent study by Boku *et al.* [124]. However, they found that Ser9 phosphorylation was not affected by DEX treatment. This discrepancy could be due to different doses of DEX (10 μM in this study versus 5 μM in the study by Boku *et al.*) and detection times after DEX treatment (20 min in our study versus 72 h in the study by Boku *et. al.*). One possibility is that DEX-induced decrease in GSK3β-Ser9 phosphorylation in this study is a transient phenomenon. However, this possibility is

unlikely as our further study detected a significant reduction 48 h after DEX treatment. Changes in both Ser9 and Tyr216 phosphorylation induced by DEX were reversed by co-treatment with leptin. This would lead to additive decrease in GSK3β activity and subsequent β-catenin accumulation in the cytosol and nuclear translocation, promoting cell proliferation by activating the lymphoid enhancer factor/T-cell factor (LEF/TCF) family of transcription factors [96]. In consistence with this notion, leptin was shown to increase total level and nuclear translocation of β-catenin, and reverse the inhibitory effects of DEX. These findings suggest that leptin and DEX converge on GSK3β/β-catenin signaling in the regulation of neural stem/progenitor cell proliferation.

Possible signal transduction mechanisms underlying leptin regulation of GSK3β/β-catenin activity may involve activation of the phosphatidylinositol-3-kinase (PI3K)/AKT and the signal transducer and activator of transcription pathway 3 (STAT3) signaling pathways [2] (Figure 6). Both AKT and STAT3 signaling pathways are stimulated once leptin binds to LepRb [133–137]. It is well known that Akt phosphorylates GSK3β on Ser9 and thereby inhibits GSK3β activity [92, 138]. STAT3 has also been implicated in negative regulation of GSK3β. Loss of STAT3 in peripheral tissue results in a net increase in active form of GSK-3β [139]. Inhibition of GSK3β by leptin-stimulated Akt and STAT3 signaling pathways would lead to increased β-catenin signaling and thus contribute to increased neurogenesis. Interestingly, β-catenin has been reported to induce *de novo* synthesis of brain-derived neurotrophic factor (BDNF) [140], which is an important regulator of adult hippocampal neurogenesis and behavioral effects of antidepressants [23, 141–148]. Specifically, knockdown or knockout of BDNF in the dentate gyrus reduces neurogenesis, induces depression-like behavioral deficits and blocks behavioral response to antidepressants [149, 150]. Direct ablation of the BDNF receptor, TrkB, in neural progenitor cells, leads to decreased basal proliferation and insensitivity to antidepressants [143], further supporting the critical role of BDNF in neurogenesis and behavioral effects of antidepressants. Very recently, leptin was shown to increase BDNF levels in the hippocampus [151]. Whether leptin interacts with BDNF in mediating neurogenesis under basal and stress conditions awaits future investigation.

In summary, our results suggest leptin can reverse impaired hippocampal neurogenesis under chronic stress or excessive glucocorticoid conditions, and improve depression-like behaviors. These findings strengthen the new concept of adipostatic control of adult hippocampal neurogenesis. Leptin is well known for its role in the regulation of food intake and body weight; deficiency of or resistance to leptin leads to obesity in rodents and humans [152–157]. Given the findings that high fat diet induces leptin resistance and obesity [158] and impairs hippocampal neurogenesis and depressive behaviors [151, 159, 160], further research will address whether leptin signaling is an important link between obesity, stress-related disorders and neurogenic/neuroplastic abnormalities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Lu XY, Kim CS, Frazer A, Zhang W. Leptin: a potential novel antidepressant. Proc Natl Acad Sci U S A. 2006; 103(5):1593–1598. [PubMed: 16423896]

2. Garza JC, Guo M, Zhang W, Lu XY. Leptin increases adult hippocampal neurogenesis in vivo and in vitro. J Biol Chem. 2008; 283(26):18238–18247. [PubMed: 18367451]

3. Stanfield BB, Trice JE. Evidence that granule cells generated in the dentate gyrus of adult rats extend axonalprojections. Exp Brain Res. 1988; 72(2):399–406. [PubMed: 2465172]

4. Song HJ, Stevens CF, Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. Nat Neurosci. 2002; 5(5):438–445. [PubMed: 11953752]

5. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature. 2002; 415(6875):1030–1034. [PubMed: 11875571]

6. Cameron HA, McEwen BS, Gould E. Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. J Neurosci. 1995; 15(6):4687–4692. [PubMed: 7790933]

7. Gould E. The effects of adrenal steroids and excitatory input on neuronal birth and survival. Ann N Y Acad Sci. 1994; 743:73–92. discussion 92–73. [PubMed: 7802420]

8. Bursztajn S, Falls WA, Berman SA, Friedman MJ. Cell proliferation in the brains of NMDAR NR1 transgenic mice. Brain Res. 2007; 1172:10–20. [PubMed: 17803978]

9. Banasr M, Hery M, Printemps R, Daszuta A. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology. 2004; 29 (3):450–460. [PubMed: 14872203]

10. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003; 301(5634):805–809. [PubMed: 12907793]

11. Encinas JM, Vaahtokari A, Enikolopov G. Fluoxetine targets early progenitor cells in the adult brain. Proc Natl Acad Sci U S A. 2006; 103(21):8233–8238. [PubMed: 16702546]

12. Yang P, Arnold SA, Habas A, Hetman M, Hagg T. Ciliary neurotrophic factor mediates dopamine D2 receptor-induced CNS neurogenesis in adult mice. J Neurosci. 2008; 28(9):2231–2241. [PubMed: 18305256]

13. Desouza LA, Ladiwala U, Daniel SM, Agashe S, Vaidya RA, Vaidya VA. Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. Mol Cell Neurosci. 2005; 29(3):414–426. [PubMed: 15950154]

14. Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience. 1994; 61(2):203–209. [PubMed: 7969902]

15. Cameron HA, Tanapat P, Gould E. Adrenal steroids and N-methyl-D-aspartate receptor activation regulate neurogenesis in the dentate gyrus of adult rats through a common pathway. Neuroscience. 1998; 82(2):349–354. [PubMed: 9466447]

16. Banasr M, Hery M, Brezun JM, Daszuta A. Serotonin mediates oestrogen stimulation of cell proliferation in the adult dentate gyrus. Eur J Neurosci. 2001; 14(9):1417–1424. [PubMed: 11722603]

17. Tanapat P, Hastings NB, Reeves AJ, Gould E. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci. 1999; 19(14): 5792–5801. [PubMed: 10407020]

18. Duman RS, Malberg J, Nakagawa S. Regulation of adult neurogenesis by psychotropic drugs and stress. J Pharmacol Exp Ther. 2001; 299(2):401–407. [PubMed: 11602648]

19. Gould E, Tanapat P. Stress and hippocampal neurogenesis. Biol Psychiatry. 1999; 46(11):1472–1479. [PubMed: 10599477]

20. Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, Geary C, et al. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science. 2003; 299(5603):117–120. [PubMed: 12511652]

21. Shingo T, Sorokan ST, Shimazaki T, Weiss S. Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. J Neurosci. 2001; 21 (24):9733–9743. [PubMed: 11739582]

22. Mak GK, Enwere EK, Gregg C, Pakarainen T, Poutanen M, Huhtaniemi I, et al. Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. Nat Neurosci. 2007; 10(8):1003–1011. [PubMed: 17603480]

23. Scharfman H, Goodman J, Macleod A, Phani S, Antonelli C, Croll S. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. Exp Neurol. 2005; 192(2):348–356. [PubMed: 15755552]

24. Zigova T, Pencea V, Wiegand SJ, Luskin MB. Intraventricular administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb. Mol Cell Neurosci. 1998; 11(4):234–245. [PubMed: 9675054]

25. Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci U S A. 2002; 99(18):11946–11950. [PubMed: 12181492]

26. Brezun JM, Daszuta A. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. Neuroscience. 1999; 89(4):999–1002. [PubMed: 10362289]

27. Duman RS, Nakagawa S, Malberg J. Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology. 2001; 25(6):836–844. [PubMed: 11750177]

28. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci. 2000; 20(24):9104–9110. [PubMed: 11124987]

29. Nakagawa S, Kim JE, Lee R, Malberg JE, Chen J, Steffen C, et al. Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein. J Neurosci. 2002; 22(9):3673–3682. [PubMed: 11978843]

30. Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature. 1997; 386(6624):493–495. [PubMed: 9087407]

31. Ge S, Pradhan DA, Ming GL, Song H. GABA sets the tempo for activity-dependent adult neurogenesis. Trends Neurosci. 2007; 30(1):1–8. [PubMed: 17116335]

32. Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, et al. Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. J Comp Neurol. 2003; 467(4):455–463. [PubMed: 14624480]

33. Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci U S A. 1998; 95 (6):3168–3171. [PubMed: 9501234]

34. McEwen BS. Stress and hippocampal plasticity. Annu Rev Neurosci. 1999; 22:105–122. [PubMed: 10202533]

35. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. Nat Neurosci. 2007; 10(9):1110–1115. [PubMed: 17726477]

36. Campbell S, MacQueen G. An update on regional brain volume differences associated with mood disorders. Curr Opin Psychiatry. 2006; 19(1):25–33. [PubMed: 16612175]

37. Dranovsky A, Hen R. Hippocampal neurogenesis: regulation by stress and antidepressants. Biol Psychiatry. 2006; 59(12):1136–1143. [PubMed: 16797263]

38. Jayatissa MN, Bisgaard C, Tingstrom A, Papp M, Wiborg O. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology. 2006; 31(11):2395–2404. [PubMed: 16482085]

39. Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrie P. Blockade of CRF(1) or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. Mol Psychiatry. 2004; 9(3):278–286. 224. [PubMed: 14699428]

40. Mineur YS, Belzung C, Crusio WE. Functional implications of decreases in neurogenesis following chronic mild stress in mice. Neuroscience. 2007; 150(2):251–259. [PubMed: 17981399]

41. Surget A, Tanti A, Leonardo ED, Laugeray A, Rainer Q, Touma C, et al. Antidepressants recruit new neurons to improve stress response regulation. Mol Psychiatry. 2011

42. Toth E, Gersner R, Wilf-Yarkoni A, Raizel H, Dar DE, Richter-Levin G, et al. Age-dependent effects of chronic stress on brain plasticity and depressive behavior. J Neurochem. 2008; 107(2): 522–532. [PubMed: 18752645]

43. Xu Y, Ku B, Cui L, Li X, Barish PA, Foster TC, et al. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Res. 2007; 1162:9–18. [PubMed: 17617388]

44. Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology. 2006; 31(8):1616–1626. [PubMed: 16395301]

45. Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J Neurosci. 1997; 17(7):2492–2498. [PubMed: 9065509]

46. Van Bokhoven P, Oomen CA, Hoogendijk WJ, Smit AB, Lucassen PJ, Spijker S. Reduction in hippocampal neurogenesis after social defeat is long-lasting and responsive to late antidepressant treatment. Eur J Neurosci. 2011

47. Lagace DC, Donovan MH, DeCarolis NA, Farnbauch LA, Malhotra S, Berton O, et al. Adult hippocampal neurogenesis is functionally important for stress-induced social avoidance. Proc Natl Acad Sci U S A. 2010; 107(9):4436–4441. [PubMed: 20176946]

48. Yap JJ, Takase LF, Kochman LJ, Fornal CA, Miczek KA, Jacobs BL. Repeated brief social defeat episodes in mice: effects on cell proliferation in the dentate gyrus. Behav Brain Res. 2006; 172(2): 344–350. [PubMed: 16839619]

49. David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron. 2009; 62(4):479–493. [PubMed: 19477151]

50. Pham K, Nacher J, Hof PR, McEwen BS. Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. Eur J Neurosci. 2003; 17(4):879–886. [PubMed: 12603278]

51. Rosenbrock H, Koros E, Bloching A, Podhorna J, Borsini F. Effect of chronic intermittent restraint stress on hippocampal expression of marker proteins for synaptic plasticity and progenitor cell proliferation in rats. Brain Res. 2005; 1040(1–2):55–63. [PubMed: 15804426]

52. Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. J Comp Neurol. 2001; 437(4):496–504. [PubMed: 11503148]

53. Falconer EM, Galea LA. Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. Brain Res. 2003; 975(1–2):22–36. [PubMed: 12763590]

54. Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. Nat Neurosci. 2004; 7(8):841–846. [PubMed: 15273691]

55. Hill MN, Kambo JS, Sun JC, Gorzalka BB, Galea LA. Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours. Eur J Neurosci. 2006; 24(7):1845–1849. [PubMed: 17067290]

56. Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. Neuropsychopharmacology. 2003; 28(9):1562–1571. [PubMed: 12838272]

57. Shors TJ, Mathew J, Sisti HM, Edgecomb C, Beckoff S, Dalla C. Neurogenesis and helplessness are mediated by controllability in males but not in females. Biol Psychiatry. 2007; 62 (5):487–495. [PubMed: 17306770]

58. Vollmayr B, Simonis C, Weber S, Gass P, Henn F. Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness. Biol Psychiatry. 2003; 54 (10): 1035–1040. [PubMed: 14625145]

59. Fornal CA, Stevens J, Barson JR, Blakley GG, Patterson-Buckendahl P, Jacobs BL. Delayed suppression of hippocampal cell proliferation in rats following inescapable shocks. Brain Res. 2007; 1130(1):48–53. [PubMed: 17161390]

60. Brummelte S, Galea LA. Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. Neuroscience. 2010; 168(3):680–690. [PubMed: 20406669]

61. Gould E, Cameron HA, Daniels DC, Woolley CS, McEwen BS. Adrenal hormones suppress cell division in the adult rat dentate gyrus. J Neurosci. 1992; 12(9):3642–3650. [PubMed: 1527603]

62. Murray F, Smith DW, Hutson PH. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. Eur J Pharmacol. 2008; 583(1):115–127. [PubMed: 18289522]

63. Qiu G, Helmeste DM, Samaranayake AN, Lau WM, Lee TM, Tang SW, et al. Modulation of the suppressive effect of corticosterone on adult rat hippocampal cell proliferation by paroxetine. Neurosci Bull. 2007; 23(3):131–136. [PubMed: 17612590]

64. Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, et al. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol Psychiatry. 2008; 64(4):293–301. [PubMed: 18406399]

65. Wong EY, Herbert J. The corticoid environment: a determining factor for neural progenitors' survival in the adult hippocampus. Eur J Neurosci. 2004; 20(10):2491–2498. [PubMed: 15548194]

66. Karishma KK, Herbert J. Dehydroepiandrosterone (DHEA) stimulates neurogenesis in the hippocampus of the rat, promotes survival of newly formed neurons and prevents corticosterone-induced suppression. Eur J Neurosci. 2002; 16(3):445–453. [PubMed: 12193187]

67. Jacobs BL. Adult brain neurogenesis and depression. Brain Behav Immun. 2002; 16(5):602–609. [PubMed: 12401475]

68. Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocampus. 2006; 16(3):239–249. [PubMed: 16425236]

69. Czeh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, et al. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A. 2001; 98(22):12796–12801. [PubMed: 11675510]

70. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. Peptides. 1996; 17(2):305–311. [PubMed: 8801538]

71. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, et al. Abnormal splicing of the leptin receptor in diabetic mice. Nature. 1996; 379(6566):632–635. [PubMed: 8628397]

72. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, et al. Identification and expression cloning of a leptin receptor, OB-R. Cell. 1995; 83(7):1263–1271. [PubMed: 8548812]

73. Cameron HA, Woolley CS, Gould E. Adrenal steroid receptor immunoreactivity in cells born in the adult rat dentate gyrus. Brain Res. 1993; 611(2):342–346. [PubMed: 8392896]

74. Garcia A, Steiner B, Kronenberg G, Bick-Sander A, Kempermann G. Age-dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus. Aging Cell. 2004; 3(6):363–371. [PubMed: 15569353]

75. Kim CS, Huang T, Garza JC, Ramos F, Frazer A, Liu F, et al. Leptin Induces Antidepressant-Like Behavioral Effects and Activates Specific Signal Transduction Pathways in the Hippocampus and Amygdala of Mice. Neuropsychopharmacology. 2006; (31):S237–S238.

76. Orsetti M, Canonico PL, Dellarole A, Colella L, Di Brisco F, Ghi P. Quetiapine prevents anhedonia induced by acute or chronic stress. Neuropsychopharmacology. 2007; 32(8):1783–1790. [PubMed: 17213846]

77. Muscat R, Papp M, Willner P. Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. Psychopharmacology (Berl). 1992; 109(4):433–438. [PubMed: 1365858]

78. Liu J, Garza JC, Truong HV, Henschel J, Zhang W, Lu XY. The melanocortinergic pathway is rapidly recruited by emotional stress and contributes to stress-induced anorexia and anxiety-like behavior. Endocrinology. 2007; 148(11):5531–5540. [PubMed: 17673512]

79. Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc Natl Acad Sci U S A. 2008; 105(2):751–756. [PubMed: 18178625]

80. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol. 1997; 8(6–7):523–532. [PubMed: 9832966]

81. Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science. 2007; 317(5839): 819–823. [PubMed: 17615305]

82. Noonan MA, Bulin SE, Fuller DC, Eisch AJ. Reduction of adult hippocampal neurogenesis confers vulnerability in an animal model of cocaine addiction. J Neurosci. 2010; 30(1):304–315. [PubMed: 20053911]

83. Snyder JS, Hong NS, McDonald RJ, Wojtowicz JM. A role for adult neurogenesis in spatial long-term memory. Neuroscience. 2005; 130(4):843–852. [PubMed: 15652983]

84. Mizumatsu S, Monje ML, Morhardt DR, Rola R, Palmer TD, Fike JR. Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. Cancer Res. 2003; 63(14):4021–4027. [PubMed: 12874001]

85. Monje ML, Mizumatsu S, Fike JR, Palmer TD. Irradiation induces neural precursor-cell dysfunction. Nat Med. 2002; 8(9):955–962. [PubMed: 12161748]

86. Nowakowski RS, Lewin SB, Miller MW. Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol. 1989; 18(3):311–318. [PubMed: 2746304]

87. Garza JC, Kim CS, Liu J, Zhang W, Lu XY. Adeno-associated virus-mediated knockdown of melanocortin-4 receptor in the paraventricular nucleus of the hypothalamus promotes high-fat diet-induced hyperphagia and obesity. J Endocrinol. 2008; 197(3):471–482. [PubMed: 18492813]

88. Esposito MS, Piatti VC, Laplagne DA, Morgenstern NA, Ferrari CC, Pitossi FJ, et al. Neuronal differentiation in the adult hippocampus recapitulates embryonic development. J Neurosci. 2005; 25(44):10074–10086. [PubMed: 16267214]

89. Mirescu C, Gould E. Stress and adult neurogenesis. Hippocampus. 2006; 16(3):233–238. [PubMed: 16411244]

90. Scott MM, Lachey JL, Sternson SM, Lee CE, Elias CF, Friedman JM, et al. Leptin targets in the mouse brain. J Comp Neurol. 2009; 514(5):518–532. [PubMed: 19350671]

91. Van Eekelen JA, Jiang W, De Kloet ER, Bohn MC. Distribution of the mineralocorticoid and the glucocorticoid receptor mRNAs in the rat hippocampus. J Neurosci Res. 1988; 21(1):88–94. [PubMed: 2851057]

92. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature. 1995; 378(6559):785–789. [PubMed: 8524413]

93. Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR. Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. Embo J. 1993; 12(2):803–808. [PubMed: 8382613]

94. Wang QM, Fiol CJ, DePaoli-Roach AA, Roach PJ. Glycogen synthase kinase-3 beta is a dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation. J Biol Chem. 1994; 269(20):14566–14574. [PubMed: 7514173]

95. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin-proteasome pathway. Embo J. 1997; 16(13):3797–3804. [PubMed: 9233789]

96. Shimizu T, Kagawa T, Inoue T, Nonaka A, Takada S, Aburatani H, et al. Stabilized beta-catenin functions through TCF/LEF proteins and the Notch/RBP-Jkappa complex to promote proliferation and suppress differentiation of neural precursor cells. Mol Cell Biol. 2008; 28(24):7427–7441. [PubMed: 18852283]

97. Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. Science. 2004; 304(5667):108–110. [PubMed: 15064420]

98. Guo Z, Jiang H, Xu X, Duan W, Mattson MP. Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. J Biol Chem. 2008; 283(3):1754–1763. [PubMed: 17993459]

99. Weng Z, Signore AP, Gao Y, Wang S, Zhang F, Hastings T, et al. Leptin protects against 6-hydroxydopamine-induced dopaminergic cell death via mitogen-activated protein kinase signaling. J Biol Chem. 2007; 282(47):34479–34491. [PubMed: 17895242]

100. Zhang F, Wang S, Signore AP, Chen J. Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. Stroke. 2007; 38 (8): 2329–2336. [PubMed: 17600230]

101. O'Malley D, MacDonald N, Mizielinska S, Connolly CN, Irving AJ, Harvey J. Leptin promotes rapid dynamic changes in hippocampal dendritic morphology. Mol Cell Neurosci. 2007; 35(4): 559–572. [PubMed: 17618127]

102. Matochik JA, London ED, Yildiz BO, Ozata M, Caglayan S, DePaoli AM, et al. Effect of leptin replacement on brain structure in genetically leptin-deficient adults. J Clin Endocrinol Metab. 2005; 90(5):2851–2854. [PubMed: 15713712]

103. Liu J, Garza JC, Bronner J, Kim CS, Zhang W, Lu XY. Acute administration of leptin produces anxiolytic-like effects: a comparison withfluoxetine. Psychopharmacology (Berl). 2010; 207(4): 535–545. [PubMed: 19823809]

104. Jayatissa MN, Henningsen K, Nikolajsen G, West MJ, Wiborg O. A reduced number of hippocampal granule cells does not associate with an anhedonia-like phenotype in a rat chronic mild stress model of depression. Stress. 2010; 13(2):95–105. [PubMed: 19929309]

105. Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology. 2005; 52(2):90–110. [PubMed: 16037678]

106. D'Aquila PS, Brain P, Willner P. Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiol Behav. 1994; 56(5):861–867. [PubMed: 7824585]

107. Kirshenbaum GS, Saltzman K, Rose B, Petersen J, Vilsen B, Roder JC. Decreased neuronal Na(+), K(+) -ATPase activity in Atp1a3 heterozygous mice increases susceptibility to depression-like endophenotypes by chronic variable stress. Genes Brain Behav. 2011

108. Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS. Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. Biol Psychiatry. 2007; 62 (5):496–504. [PubMed: 17585885]

109. Bielajew C, Konkle AT, Kentner AC, Baker SL, Stewart A, Hutchins AA, et al. Strain and gender specific effects in the forced swim test: effects of previous stress exposure. Stress. 2003; 6 (4): 269–280. [PubMed: 14660059]

110. Parent JM, Tada E, Fike JR, Lowenstein DH. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. J Neurosci. 1999; 19(11):4508–4519. [PubMed: 10341251]

111. Peissner W, Kocher M, Treuer H, Gillardon F. Ionizing radiation-induced apoptosis of proliferating stem cells in the dentate gyrus of the adult rat hippocampus. Brain Res Mol Brain Res. 1999; 71(1):61–68. [PubMed: 10407187]

112. Blaiss CA, Yu TS, Zhang G, Chen J, Dimchev G, Parada LF, et al. Temporally specified genetic ablation of neurogenesis impairs cognitive recovery after traumatic brain injury. J Neurosci. 2011; 31(13):4906–4916. [PubMed: 21451029]

113. Jin K, Wang X, Xie L, Mao XO, Greenberg DA. Transgenic ablation of doublecortin-expressing cells suppresses adult neurogenesis and worsens stroke outcome in mice. Proc Natl Acad Sci U S A. 2010; 107(17):7993–7998. [PubMed: 20385829]

114. Revest JM, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza PV, et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry. 2009; 14 (10):959–967. [PubMed: 19255582]

115. Schloesser RJ, Lehmann M, Martinowich K, Manji HK, Herkenham M. Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. Mol Psychiatry. 2010; 15(12):1152–1163. [PubMed: 20308988]

116. Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, et al. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. Proc Natl Acad Sci U S A. 2006; 103(46):17501–17506. [PubMed: 17088541]

117. Bush TG, Savidge TC, Freeman TC, Cox HJ, Campbell EA, Mucke L, et al. Fulminant jejuno-ileitis following ablation of enteric glia in adult transgenic mice. Cell. 1998; 93(2):189–201. [PubMed: 9568712]

118. Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, et al. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat Neurosci. 2008; 11(10):1153–1161. [PubMed: 18758458]

119. Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, et al. The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry. 2009; 14(8):764–773. 739. [PubMed: 18982002]

120. Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. Nature. 2001; 410(6826):372–376. [PubMed: 11268214]

121. Perera TD, Dwork AJ, Keegan KA, Thirumangalakudi L, Lipira CM, Joyce N, et al. Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates. PLoS One. 2011; 6(4):e17600. [PubMed: 21525974]

122. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. Trends Neurosci. 2004; 27(8):447–452. [PubMed: 15271491]

123. Wong EY, Herbert J. Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. Neuroscience. 2006; 137(1):83–92. [PubMed: 16289354]

124. Boku S, Nakagawa S, Masuda T, Nishikawa H, Kato A, Kitaichi Y, et al. Glucocorticoids and lithium reciprocally regulate the proliferation of adult dentate gyrus-derived neural precursor cells through GSK-3beta and beta-catenin/TCF pathway. Neuropsychopharmacology. 2009; 34 (3):805–815. [PubMed: 19005466]

125. Kim JB, Ju JY, Kim JH, Kim TY, Yang BH, Lee YS, et al. Dexamethasone inhibits proliferation of adult hippocampal neurogenesis in vivo and in vitro. Brain Res. 2004; 1027(1–2):1–10. [PubMed: 15494151]

126. Hastings NB, Gould E. Neurons inhibit neurogenesis. Nat Med. 2003; 9(3):264–266. [PubMed: 12612572]

127. Hirabayashi Y, Gotoh Y. Stage-dependent fate determination of neural precursor cells in mouse forebrain. Neurosci Res. 2005; 51(4):331–336. [PubMed: 15740796]

128. Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, et al. Wnt signalling regulates adult hippocampal neurogenesis. Nature. 2005; 437(7063):1370–1375. [PubMed: 16251967]

129. Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. Cell. 2009; 136(6):1017–1031. [PubMed: 19303846]

130. Qu Q, Sun G, Li W, Yang S, Ye P, Zhao C, et al. Orphan nuclear receptor TLX activates Wnt/ beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nat Cell Biol. 2010; 12(1):31–40. 31–39. [PubMed: 20010817]

131. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005; 434(7035):843–850. [PubMed: 15829953]

132. Zechner D, Fujita Y, Hulsken J, Muller T, Walther I, Taketo MM, et al. beta-Catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. Dev Biol. 2003; 258(2):406–418. [PubMed: 12798297]

133. Bates SH, Myers MG Jr. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol Metab. 2003; 14(10):447–452. [PubMed: 14643059]

134. Munzberg H, Huo L, Nillni EA, Hollenberg AN, Bjorbaek C. Role of signal transducer and activator of transcription 3 in regulation of hypothalamic proopiomelanocortin gene expression by leptin. Endocrinology. 2003; 144(5):2121–2131. [PubMed: 12697721]

135. Vaisse C, Halaas JL, Horvath CM, Darnell JE Jr, Stoffel M, Friedman JM. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat Genet. 1996; 14 (1):95–97. [PubMed: 8782827]

136. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC. Defective STAT signaling by the leptin receptor in diabetic mice. Proc Natl Acad Sci U S A. 1996; 93(13):6231–6235. [PubMed: 8692797]

137. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG Jr, Schwartz MW. Intracellular signalling. Key enzyme in leptin-induced anorexia. Nature. 2001; 413(6858):794–795. [PubMed: 11677594]

138. Cross DA, Watt PW, Shaw M, van der Kaay J, Downes CP, Holder JC, et al. Insulin activates protein kinase B, inhibits glycogen synthase kinase-3 and activates glycogen synthase by rapamycin-insensitive pathways in skeletal muscle and adipose tissue. FEBS Lett. 1997; 406(1–2):211–215. [PubMed: 9109420]

139. Moh A, Zhang W, Yu S, Wang J, Xu X, Li J, et al. STAT3 sensitizes insulin signaling by negatively regulating glycogen synthase kinase-3 beta. Diabetes. 2008; 57(5):1227–1235. [PubMed: 18268048]

140. Wada A. Lithium and neuropsychiatric therapeutics: neuroplasticity via glycogen synthase kinase-3beta, beta-catenin, and neurotrophin cascades. J Pharmacol Sci. 2009; 110(1):14–28. [PubMed: 19423950]

141. Yuan Y, Sun J, Zhao M, Hu J, Wang X, Du G, et al. Overexpression of alpha-synuclein down-regulates BDNF expression. Cell Mol Neurobiol. 2010; 30(6):939–946. [PubMed: 20405200]

142. Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, et al. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur J Neurosci. 2006; 24(7):1850–1856. [PubMed: 17040481]

143. Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, Kernie SG, et al. TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. Neuron. 2008; 59 (3):399–412. [PubMed: 18701066]

144. Schmidt HD, Duman RS. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology. 2010; 35(12):2378–2391. [PubMed: 20686454]

145. Sairanen M, Lucas G, Ernfors P, Castren M, Castren E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. J Neurosci. 2005; 25(5):1089–1094. [PubMed: 15689544]

146. Lee J, Duan W, Mattson MP. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J Neurochem. 2002; 82(6):1367–1375. [PubMed: 12354284]

147. Govindarajan A, Rao BS, Nair D, Trinh M, Mawjee N, Tonegawa S, et al. Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. Proc Natl Acad Sci U S A. 2006; 103(35):13208–13213. [PubMed: 16924103]

148. Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci. 2002; 22(8): 3251–3261. [PubMed: 11943826]

149. Adachi M, Barrot M, Autry AE, Theobald D, Monteggia LM. Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. Biol Psychiatry. 2008; 63(7):642–649. [PubMed: 17981266]

150. Taliaz D, Stall N, Dar DE, Zangen A. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. Mol Psychiatry. 2010; 15(1):80–92. [PubMed: 19621014]

151. Yamada N, Katsuura G, Ochi Y, Ebihara K, Kusakabe T, Hosoda K, et al. Impaired CNS Leptin Action Is Implicated in Depression Associated with Obesity. Endocrinology. 2011

152. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372(6505):425–432. [PubMed: 7984236]

153. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature. 1997; 387(6636): 903–908. [PubMed: 9202122]

154. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995; 269(5223):540–543. [PubMed: 7624776]

155. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia. 1978; 14(3):141–148. [PubMed: 350680]

156. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 1998; 18(3):213–215. [PubMed: 9500540]

157. Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, et al. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A. 2004; 101(13):4531–4536. [PubMed: 15070752]

158. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Invest. 2000; 105(12): 1827–1832. [PubMed: 10862798]

159. Lindqvist A, Mohapel P, Bouter B, Frielingsdorf H, Pizzo D, Brundin P, et al. High-fat diet impairs hippocampal neurogenesis in male rats. Eur J Neurol. 2006; 13(12):1385–1388. [PubMed: 17116226]

160. Park HR, Park M, Choi J, Park KY, Chung HY, Lee J. A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. Neurosci Lett. 2010; 482(3):235–239. [PubMed: 20670674]
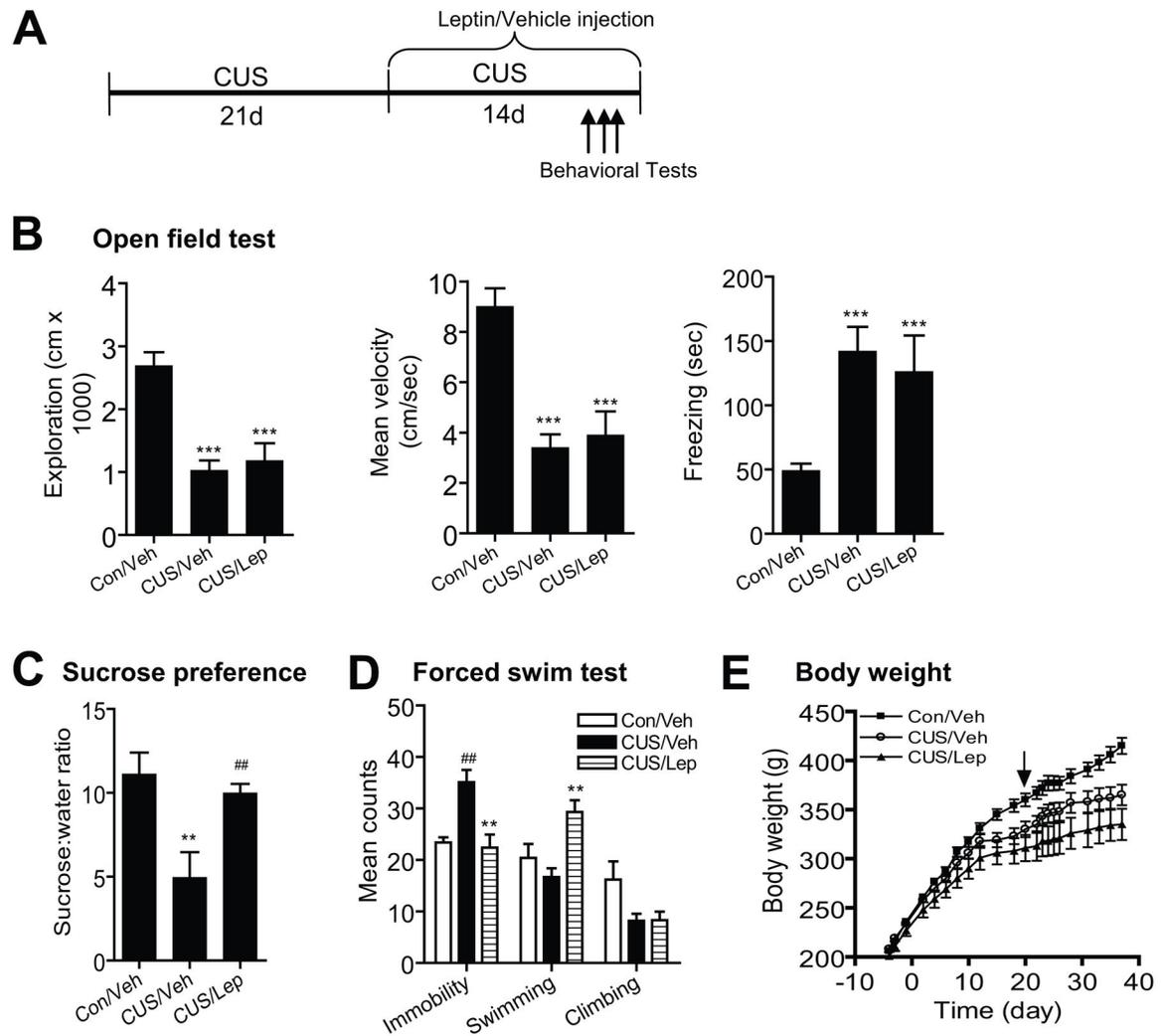
Figure 1.
Effect of chronic leptin treatment on chronic unpredictable stress (CUS)-induced depression-like behaviors. A. Schematic representation of the experimental procedure for CUS and treatments in rats. CUS rats were exposed to one stressor per day for 21 days, and then received 14 days of leptin or vehicle injections during which CUS continued. B. Open field test. Exploratory activity (total distance traveled and movement velocity) and freezing time were evaluated for a 5 min test session. C. Sucrose preference test. Sucrose preference is expressed as a ratio of sucrose solution: water intake measured within the 12 h dark cycle. D. Forced swim test. Time spent for immobility, swimming and climbing was scored for a 5 min test session. E. Body weight was measured before and after leptin treatment. The arrow indicates the beginning of leptin or vehicle administration. Results are expressed as mean ± SEM (n = 5–8 per group). $**P < 0.01$, $***P < 0.001$, compared to handling control group.
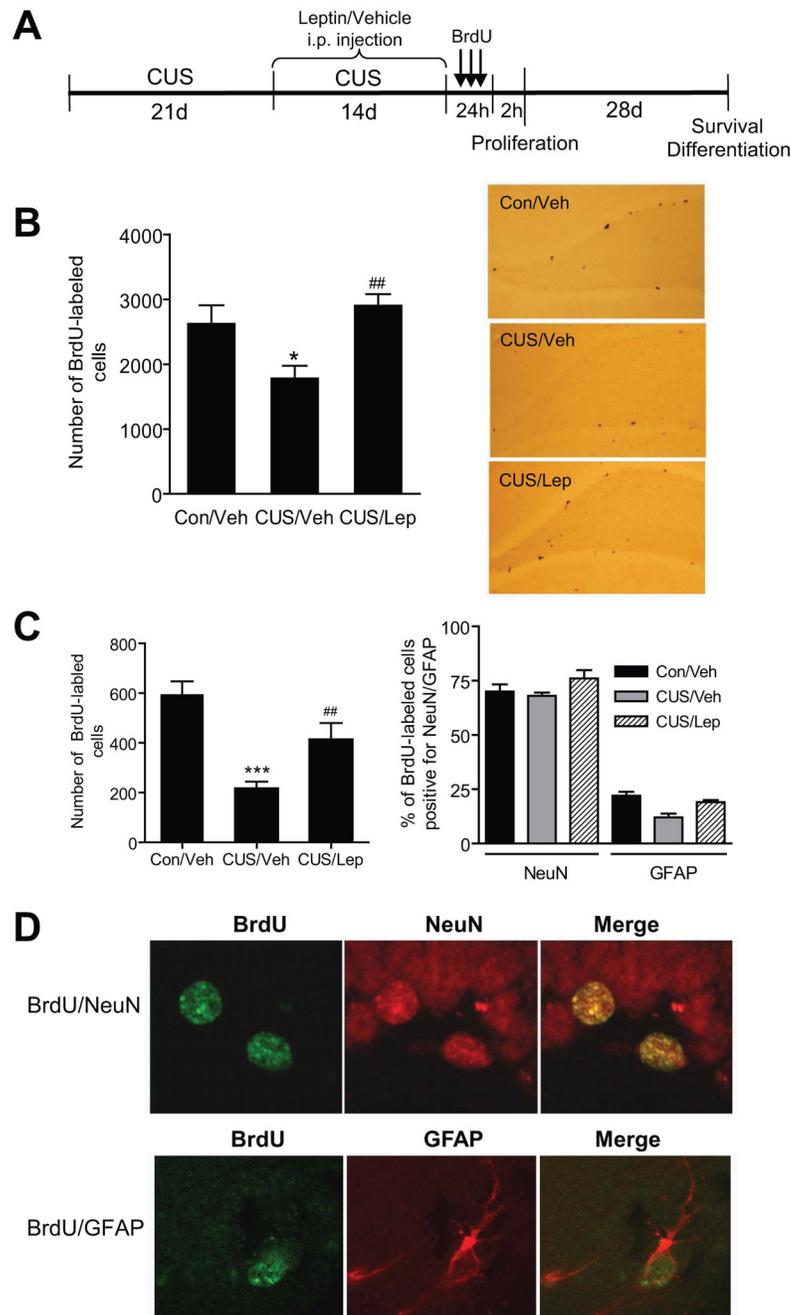
Figure 2.
Effect of chronic leptin treatment on cell proliferation and differentiation in the adult hippocampus of rats exposed to chronic unpredictable stress (CUS). A. Timeline of experimental procedures. B. Left panel, quantitative data showing the effects of CUS and leptin treatment on the number of total BrdU-positive cells in the dentate gyrus. Right panel, representative images showing BrdU-positive cells in the dentate gyrus following different treatments. C. Left panel, the number of survived BrdU-labeled cells in the dentate gyrus at 28 days after BrdU labeling. Right panel, percentage of BrdU-positive cells double-labeled

for NeuN or GFAP at 28 days after BrdU labeling. D. Confocal images showing colocalization of BrdU with NeuN or GFAP. Scale bars = 10 μm. Con/Veh, handled control rats treated with vehicle (n = 7); CUS/Veh CUS rats treated with vehicle (n = 8); CUS/Lep, CUS rats treated with leptin (n = 5). Data are expressed as mean ± SEM. *$P < 0.05$ compared to Con/Veh; ##$P < 0.01$ compared to CUS/Veh; ***$P < 0.001$ compared to Con/Veh; ##$P < 0.01$ compared to CUS/Veh; + $P = 0.07$ compared to CUS/Veh.
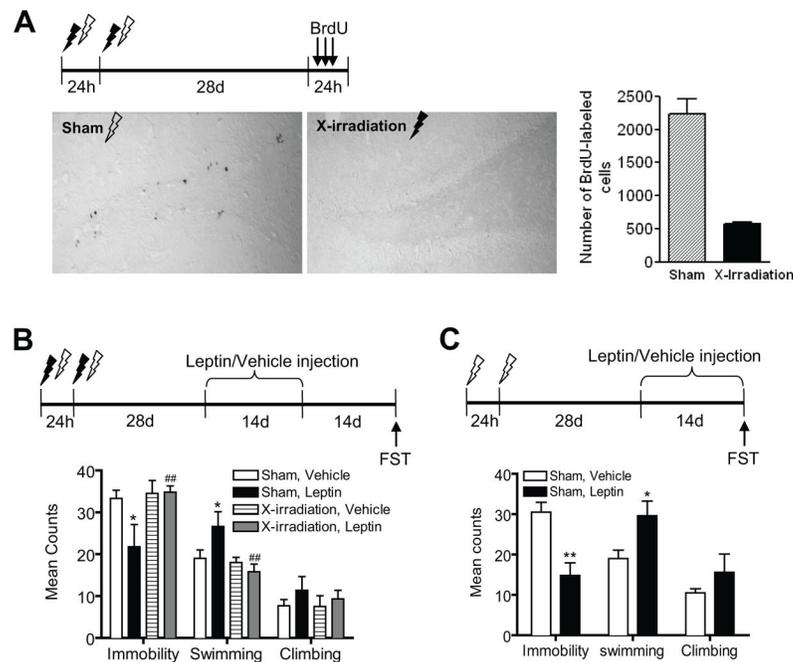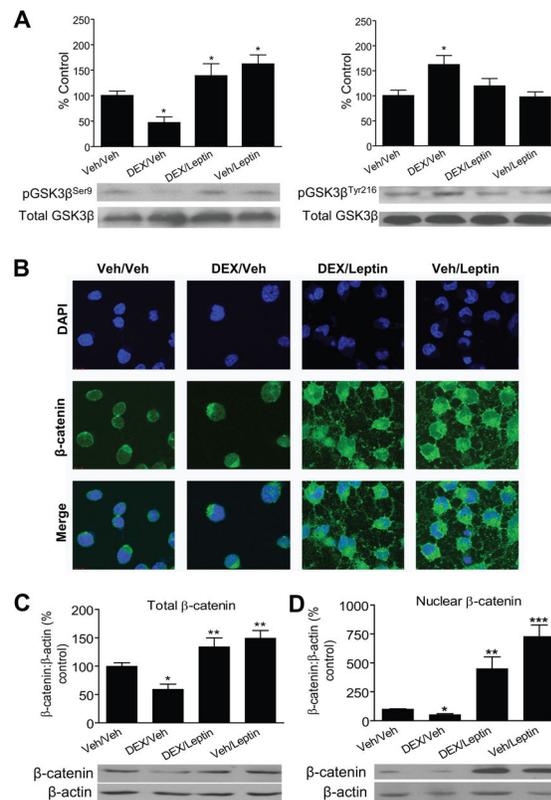
Figure 3.
Involvement of hippocampal neurogenesis in mediating the antidepressant-like efficacy of leptin. A. Ablation of hippocampal neurogenesis by X-irradiation. Top panel, timeline of experimental procedures. Bottom-left panel, representative photomicrographs showing BrdU-labeled cells 28 days after X-irradiation or sham exposure. Bottom-right panel, number of BrdU-labeled cells at 28 days after exposure to the sham procedure or X-irradiation. ***$P < 0.001$. B. Top panel, experimental design. Rats received leptin (1 mg/kg, i.p.) or vehicle injection for 14 consecutive days beginning 28 days after exposure to the sham procedure or X-irradiation. The forced swim test was performed 14 days after the cessation of leptin treatment. Bottom panel, quantitative data showing the effects of X-irradiation and leptin treatment on forced swim behaviors. Data are presented as mean ± SEM (n = 5–6/group). *$P < 0.05$ compared to the sham-vehicle group, ##$P < 0.01$ compared to the sham-leptin group. C. Antidepressant-like behavioral effects of leptin treatment. Top panel, experimental design. Rats received leptin (1 mg/kg, i.p.) or vehicle (saline) injection for 14 consecutive days beginning 28 days after exposure to the sham procedure, followed by the forced swim test. Bottom panel, effects of leptin treatment on immobility, swimming and climbing. Data are presented as mean ± SEM (n = 5–6/group). *$P < 0.05$, **$P < 0.01$ compared to vehicle-treated controls.
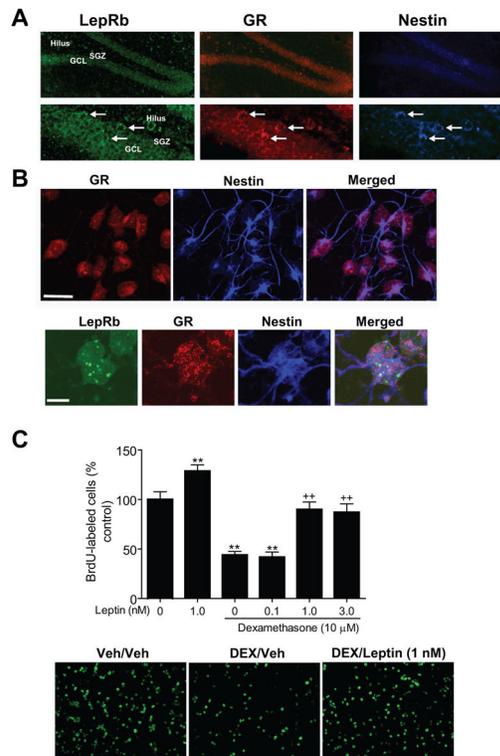
Figure 4.
Effect of leptin on dexamethasone-induced suppression of adult hippocampal neural stem/ progenitor cell proliferation. A. Colocalization of LepRb mRNA and GR protein in nestin-positive cells in the dentate gyrus of adult hippocampus. Left image, LepRb mRNA *in situ* hybridization (green); middle image, GR immunostaining (red); right image, nestin immunostaining (blue). White arrows indicate triple-labeled cells for LepRb, GR and nestin. Scale bars = 10 μm. GCL, granular layer; SGZ, subgranular zone. B. Top panel, immunohistochemical staining showing the expression of GR (red) in nestin (blue)-positive hippocampal neural stem/progenitor cells in cultures. Bottom panel, colocalization of LepRb mRNA (green) and GR (red) in nestin (blue) positive cells. C. Adult hippocampal neural stem/progenitor cells were treated with 10 μM dexamethasone (DEX), 1 nM leptin, or a combination of DEX (10 μM) and various doses of leptin (0.1, 1.0 or 3.0 nM). BrdU (10 μM) was added to label proliferating cells and was detected by immunohistochemistry. Top panel, quantitative data demonstrating the effects of leptin and DEX on the number of BrdU-labeled cells. Bottom panel, representative images showing BrdU-labeled cells under different treatment conditions. Data are presented as mean ± SEM. **P < 0.01 compared to vehicle treatment; ++*P* < 0.01 compared to the DEX treatment.
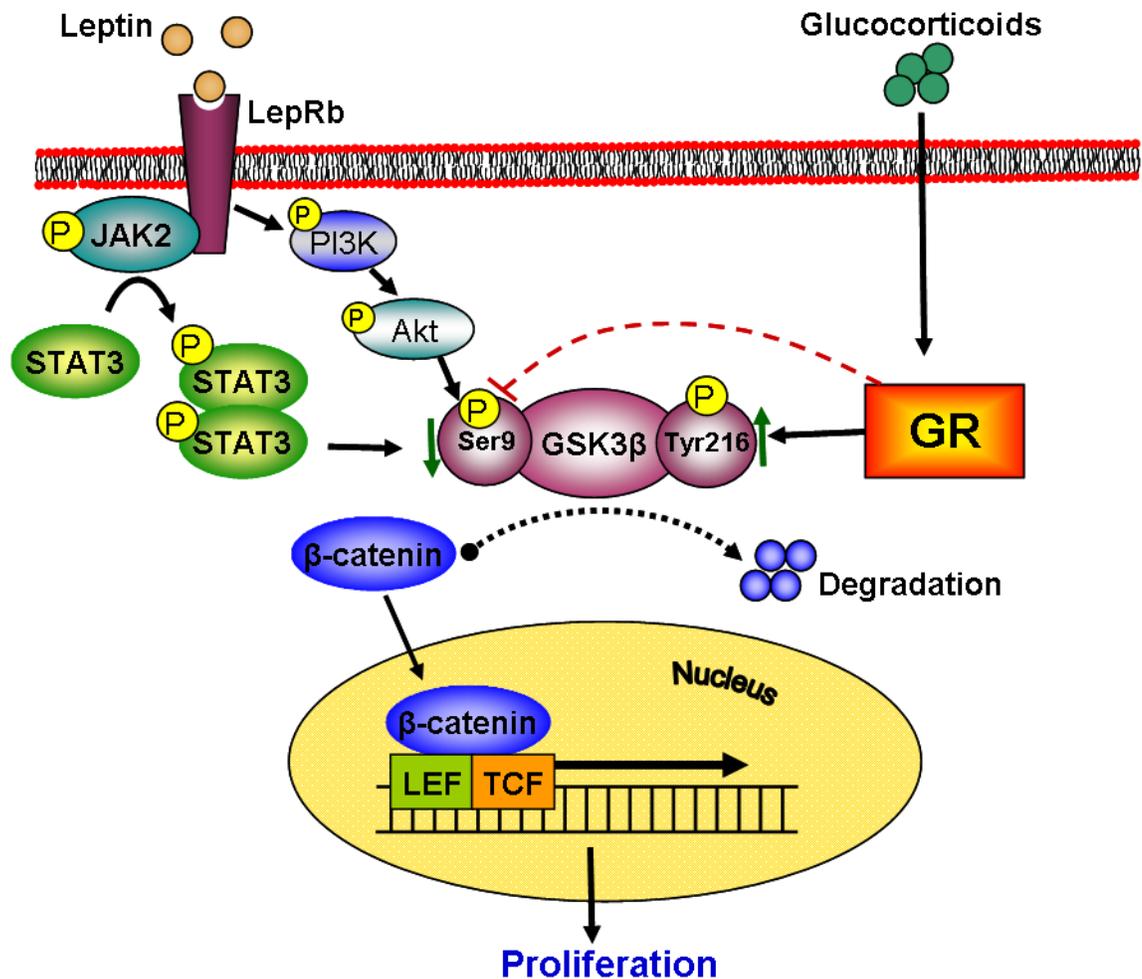
Figure 5.
Effects of leptin and dexamethasone on phosphorylation of GSK3β and nuclear translocation of β-catenin. A. Hippocampal neural stem/progenitor cells were treated with dexamethasone (DEX, 10 μM), leptin (1 nM), or a combination of leptin and DEX for 20 min. Phosphorylation of GSK3β at Ser9 (left panel) and Tyr216 (right panel) was determined using Western blot. B. Representative confocal images demonstrating immunohistochemical staining of β-catenin in cultured hippocampal neural stem/progenitor cells treated with DEX (10 μM), leptin (1 nM), or a combination of both for 48 h. Scale bar = 10 μm. C and D. Western blot showing levels of β-catenin form total cell extracts (C) and nuclear extracts (D) of hippocampal neural stem/progenitor cells treated with DEX (10 μM), leptin (1 nM), or a combination of both for 48 h. Data are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to vehicle/vehicle treatment.

Figure 6.
Proposed mechanisms of leptin and glucocorticoid stress hormones in the GSK3β/β-catenin signaling pathway. Leptin binds to the functional form of the leptin receptor, LepRb, and results in phosphorylation and activation of PI3K/AKT signaling pathway. This in turn phosphorylates GSK3β on Ser9 resulting in decreased GSK3β activity. In parallel, activated LepRb recruits JAK2 and initiates phosphorylation of STAT3. Phosphorylated STAT3 acts on GSK3β to reduce its activity. Glucocorticoids binding to GR induce phosphorylation of GSK3β on Tyr216 and decreases phosphorylation on Ser9, consequently increasing GSK3β activity. Increased GSK3β activity reduces the stability of β-catenin and leads to β-catenin degradation through ubiquitination. Decreased GSK3β activity increases the stability of β-catenin and results in accumulation of β-catenin and its translocation into the nucleus, allowing interaction with members of the lymphoid enhancer factor/T-cell factor (LEF/TCF) family of transcription factors and, as a consequence, promoting the expression of cell proliferation genes.