Drug-Dependent Requirement of Hippocampal Neurogenesis in a Model of Depression and of Antidepressant Reversal

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Background: Depression and anxiety disorders have been linked to dysfunction of the hypothalamo-pituitary-adrenal (HPA) axis and structural changes within the hippocampus. Unpredictable chronic mild stress (UCMS) can recapitulate these effects in a mouse model, and UCMS-induced changes, including downregulation of hippocampal neurogenesis, can be reversed by antidepressant (AD) treatment. We investigated causality between changes in hippocampal neurogenesis and the effects of both chronic stress and chronic ADs.

Methods: Mice were treated with either a sham procedure or focal hippocampal irradiation to disrupt cell proliferation before being confronted with 5 weeks of UCMS. From the third week onward, we administered monoaminergic ADs (imipramine, fluoxetine), the corticotropin-releasing factor 1 (CRF_1) antagonist SSR125543, or the vasopressin 1b (V_{1b}) antagonist SSR149415 daily. The effects of UCMS regimen, AD treatments, and irradiation were assessed by physical measures (coat state, weight), behavioral testing (Splash test, Novelty-Suppressed feeding test, locomotor activity), and hippocampal BrdU labeling.

Results: Our results show that elimination of hippocampal neurogenesis has no effect on animals' sensitivity to UCMS in several behavioral assays, suggesting that reduced neurogenesis is not a cause of stress-related behavioral deficits. Second, we present evidence for both neurogenesis-dependent and -independent mechanisms for the reversal of stress-induced behaviors by AD drugs. Specifically, loss of neurogenesis completely blocked the effects of monoaminergic ADs (imipramine, fluoxetine) but did not prevent most effects of the CRF_1 and the V_{1b} antagonists.

Conclusions: Hippocampal neurogenesis might thus be used by the monoaminergic ADs to counteract the effects of stress, whereas similar effects could be achieved by directly targeting the HPA axis and related neuropeptides.

Stress is a key etiological factor in anxiety and major depressive disorders (1). Most patients exhibit abnormalities of the hypothalamo-pituitary-adrenal (HPA) axis (2), which coordinates the stress response through glucocorticoid (GC) release. The hippocampus is known to negatively regulate the HPA axis and this inhibitory feedback is altered by chronic stress (3,4). The involvement of the hippocampus in depression is suggested by brain neuroimaging studies and postmortem data showing a reduction of its volume that parallels the duration of depression (5–9) as well as atrophy or neuronal loss (8,10). These alterations could involve disproportionate GC levels, which can cause structural damage in the brain including atrophy, apoptosis, and reduction of cell proliferation (11). Decreased dentate gyrus (DG) cell proliferation and neurogenesis have been observed after exposure to stressors in different species (12–18) and are related to elevated stress hormones such as GCs (19,20). Accordingly, all these data combined have led to the assumption that chronic stress could precipitate depression by altering neuroplasticity and particularly hippocampal neurogenesis.

Chronic antidepressant (AD) treatment increases cell proliferation and granule cell survival (16,21,22) and is able to reverse the stress-induced decrease of hippocampal neurogenesis (14,16,17,23). The link between hippocampal neurogenesis and AD action seems to be causal, because suppression of hippocampal neurogenesis by irradiation prevents the effects of fluoxetine and imipramine (22) as well as a putative AD with a non-monoaminergic target (24). Interestingly, the ability of AD drugs to increase hippocampal neurogenesis is a common feature of both classical ADs such as selective serotonin reuptake inhibitors (SSRI) and tricyclic drugs and atypical or potential ADs (24–27), including antagonists of corticotropin-releasing factor 1 (CRF_1) and vasopressin 1b (V_{1b}) receptors (16). For these reasons, it has been suggested that hippocampal neurogenesis might be a key factor in the action of AD drugs.

Here we have investigated the involvement of hippocampal neurogenesis in two distinct and opposing behavioral effects: the response to unpredictable chronic mild stress (UCMS) and the reversal of UCMS-induced deficits by different classes of AD drugs, focusing particularly on comparing the action of mono-aminergic drugs with known clinical efficacy and compounds acting on the HPA axis (CRF_1 and V_{1b} antagonists) that possess a potent AD-like action in preclinical models (16,28–30) and in clinical studies (31–33). A targeted irradiation procedure was used to disrupt progenitor cell proliferation only in the subgranular zone (SGZ) of the hippocampus (22). Mice were then subjected to a naturalistic model of depression, the UCMS paradigm, which is known to detect the effects of chronic but not acute treatment with ADs (34,35). The effects of ablating hippocampal neurogenesis on the response to both UCMS and
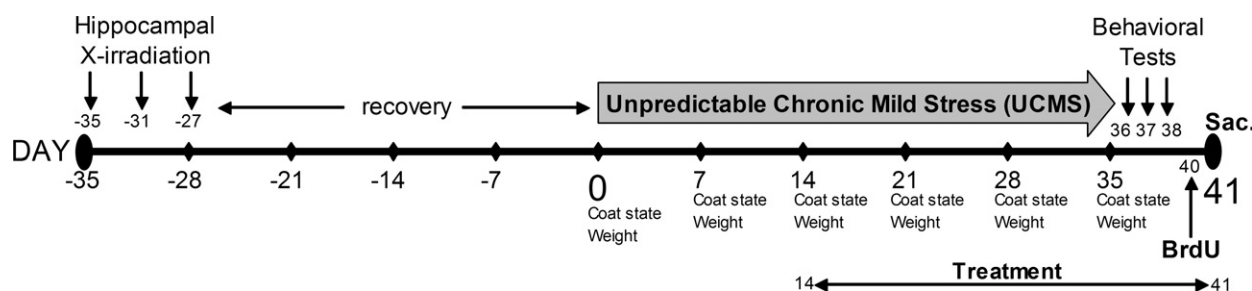
Figure 1. Schematic representation of experimental design. Five weeks before the onset of the UCMS, three focal x-irradiations were performed specifically above the hippocampus over the course of 1 week (day −35, −31, and −27). After recovery, mice were subjected to a 5-week UCMS regimen followed by behavioral tests at day 36, 37, and 38. Finally, BrdU (4 × 75 mg/kg IP, every 2 hours) was administered at day 40, mice were killed (Sac.) 24 hours after the last BrdU injection, and brains were immediately collected.

chronic AD treatment were assessed with physical measures (coat state and body weight), behavioral tests (splash test, novelty-suppressed feeding [NSF] test, and locomotor activity in an actimeter), and immunohistochemical detection of cell proliferation in the two neurogenic brain regions: the SGZ and the subventricular zone (SVZ).

Methods and Materials

Animals

Two-month-old male BALB/c mice were obtained from Taconic (Germantown, New York). All animals were housed in groups of four or five and were maintained under standard laboratory conditions (12/12-hour light/dark cycle: lights on at 8:00 PM, 22 ± 2°C, food and water ad libitum). The treatment of the animals was in accordance with the European Community Council directive 86/609/EEC and with the Guide for Care and Use of Laboratory Animals established by the National Institutes of Health of the United States of America.

Drugs

Fluoxetine (Eli Lilly, Indianapolis, Indiana), imipramine (Sigma-Aldrich, St. Louis, Missouri), SSR125543, and SSR149415 (Sanofi-Aventis, Bagneux, France) were prepared in saline (.9% sodium chloride) containing .1% Tween 80 (fluoxetine, imipramine) or 5% dimethylsulphoxide and 5% Cremophor (SSR125543, SSR149415). The solutions were administered at a volume of 10 mL/kg.

General Procedure

For each experiment, x-ray treatment was carried out on one-half of the mice. Non-irradiated mice were anesthetized and placed in the stereotaxic frame as irradiated mice but without exposure to cranial irradiation. Five weeks after the first exposure to x-irradiation, a 5-week UCMS was conducted (Figure 1). The UCMS-exposed mice were isolated in small cages (24 × 11 × 12 cm) while nonstressed mice were housed in groups of four or five in standard cages (42 × 28 × 18 cm). The first 2 weeks of UCMS were drug-free, and treatment began from the third week until the day after BrdU injection (28 days). Fluoxetine (10 mg/kg/day), imipramine (20 mg/kg/day), SSR149415 (20 mg/kg/day), SSR125543 (20 mg/kg/day) or vehicle were administered IP once/day (including for the nonstressed experiment). The body weight and the coat state were assessed weekly until the end of UCMS. The day after the last body weight and coat state measures, behavioral testing was performed: first day splash test, second day actimeter, third day NSF test. Two days after, BrdU injections were carried out. In experiments with nonstressed

mice, animals were isolated 3 hours before the splash test, and the cage of stressed mice was changed at the same time. Five to seven mice from each group were randomly chosen for immunohistochemistry.
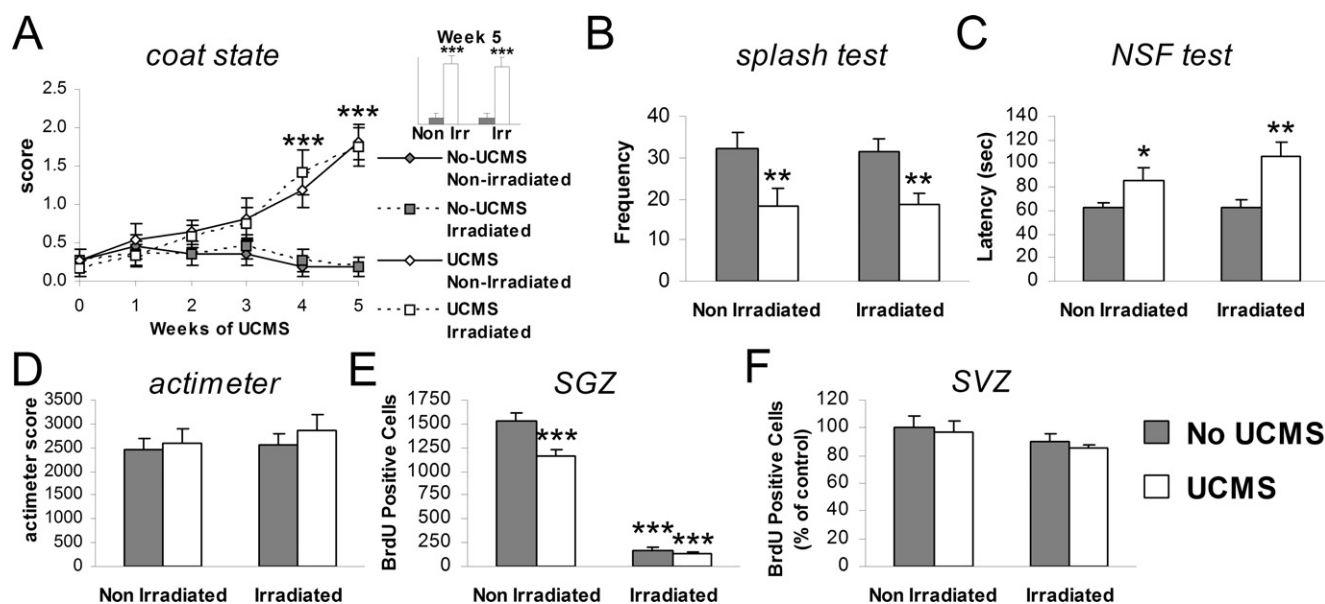
UCMS

Mice were subjected to various unpredictable stressors for 5 weeks. Alterations of the bedding (repeated sawdust changing, removal of sawdust, damp sawdust, substitution of sawdust with 21°C water), cage-tilting (45°), predator sounds (15 min), cage shift (mice were positioned in the empty cage of another male), and alterations of the light/dark cycle were used as stressors. The total score of the coat state resulted from the sum of scores from seven different body parts: head, neck, dorsal and ventral coat, tail, forepaws, and hindpaws. For each area, a score of 0 was given for well-groomed coat and 1 for an unkempt coat. This index has been pharmacologically validated in previous studies (16,22,29,30,35).

Splash Test

This test was conducted as previously described (22,36,37). It consisted of spraying a 10% sucrose solution on the mouse in its home cage. The sucrose solution dirtied the coat and induced a grooming behavior. The grooming frequency was recorded for 5 min.

NSF Test

The NSF test was a modified version of our previous study (22). The testing apparatus consisted of a 33 × 33 × 30 cm box. The floor was covered with 2 cm sawdust. Twelve hours before the test, food was removed from the cages. At the time of testing, a single pellet of food (regular chow) was placed on a white paper positioned in the box center. An animal was placed in the corner. The latency to manifestly chew the pellet was recorded within a 3-min period. This test induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the arena. This paradigm was able to reveal the effects of chronic AD treatment in nonstressed mice (22). In this study, we reduced the dimension of the apparatus by 40% and used a red light instead of white. In this way, a 4-week treatment of fluoxetine in nonstressed mice had no effect (data not shown). In addition, ADs are known to have various effects on appetite; the feeding drive of each animal was thus assessed by returning it to the home cage immediately after the test and measuring the amount of food consumed over 5 min. No difference was observed (data not shown).

Figure 2. Effects of unpredictable chronic mild stress (UCMS) and hippocampal x-irradiation on behavior and cell proliferation in the subgranular zone (SGZ) and the subventricular zone (SVZ). **(A)** The UCMS induced a deterioration of the coat state as demonstrated by increasing coat state scores, which became significant after 4 weeks of UCMS protocol. Irradiation had no effect on this measure, because the irradiated mice showed no difference with the non-irradiated mice. Detail of the results after 5-week UCMS is illustrated above legend, $n = 11–12$ mice/group. **(B)** The UCMS induced a reduction of grooming frequency during the splash test in non-irradiated and irradiated mice. Irradiation had no effect, $n = 11–12$ mice/group. **(C)** The latency to chew the pellet was increased by UCMS regimen in the novelty-suppressed feeding (NSF) test. Irradiation had no effect, $n = 11–12$ mice/group. **(D)** Locomotor activity in the actimeter was not affected by the UCMS regimen or irradiation, $n = 11–12$ mice/group. **(E)** The UCMS reduced the number of BrdU positive cells in the SGZ of the hippocampus. X-ray procedure induced a strong disruption of cell proliferation in this area, $n = 5–7$ mice/group. **(F)** No change in cell proliferation in the SVZ due to UCMS or irradiation was found, $n = 5–7$ mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: ***$p < .001$; **$p < .01$ and *$p < .05$ for both UCMS group versus their respective control no-UCMS group, excepted for **(E)**: ***$p < .001$ versus no UCMS/non-irradiated mice.

Actimeter

The actimeter assessed the activity of mice in their home cage. The cage was placed in the center of the device, which consisted of a 20×20 cm square plane with photobeam detectors crossing the plane. The movement of the animal was automatically detected when it crossed through, allowing a score to be established. The higher the score was, the more the mouse moved. Testing duration was 4 hours.

Irradiation

Irradiation was performed as previously described (22). All mice were anesthetized with ketamine/xylazine (100 mg/kg and 7 mg/kg, respectively), placed in a stereotaxic frame, and then, only for irradiated mice, exposed to cranial irradiation with a Siemens Stabilopan x-ray system (Hamburg, Germany). Animals were protected with a lead shield that covered the body but left unshielded for a 3.22×11 mm treatment field above the hippocampus (interaural 3.00 to .00). The corrected dose rate was approximately 1.8 Gy/min at a source to skin distance of 30 cm. The procedure lasted 2 min 47 sec, delivering a total of 5 Gy. Three 5 Gy doses were given over the course of 1 week (day 0, 4, and 8).

BrdU Labeling

Mice were administered BrdU (Sigma-Aldrich, 4×75 mg/kg IP, every 2 hours) and killed 24 hours after the last BrdU injection. After anesthesia with ketamine/xylazine, mice were transcardially perfused: saline for 2 min, 4% paraformaldehyde (PFA)/.1 mol/L phosphate-buffered saline (PBS; pH = 7.4) for 5 min, and brains were collected, post-fixed overnight in 4% PFA at 4°C, and then cryoprotected in 30% sucrose and stored at 4°C. Serial coronal sections through the rostro-caudal brain extent

were cut (45 μm), and every third section from each brain was collected and stored in PBS. The BrdU immunohistochemistry was performed on free-floating sections as described (16). Sections were treated with 3% hydrogen peroxide/50% ethanol for 20 min, rinsed in PBS, treated with 2 nmol/L hydrochloric acid (30 min), rinsed in borate buffer for 5 min (.1 mol/L, pH = 8.4), and then rinsed in PBS and incubated with a monoclonal rat anti-BrdU antibody (1:500, Oxford Biotechnology, Oxford, United Kingdom). Forty hours later, sections were rinsed in PBS, incubated 2.5 hours with a rabbit anti-rat biotinylated antibody (1:200, Vector Laboratories, Burlingame, California), and followed by amplification with an avidin-biotin complex (Elite ABC kit, Vector Laboratories). The staining was visualized with DAB (Sigma-Aldrich). The BrdU positive cell quantification was performed as described (16).

Statistics

Analyses of variance were performed by using environment (no UCMS/UCMS), irradiation (non-irradiated/irradiated), or treatment (vehicle/treated) as main factors, followed by a Fisher post hoc analysis when required.

Results

Disruption of Hippocampal Neurogenesis Has No Effect on Sensitivity to Stress

To determine whether loss of neurogenesis alters sensitivity to stress, we compared the responses of irradiated and non-irradiated mice to UCMS. In vehicle-treated mice, UCMS induced a gradual deterioration of coat state that reached significance by 4 weeks after beginning the stress ($p < .001$) and worsened until the end of the stress procedure ($p < .001$; Figure 2A). This
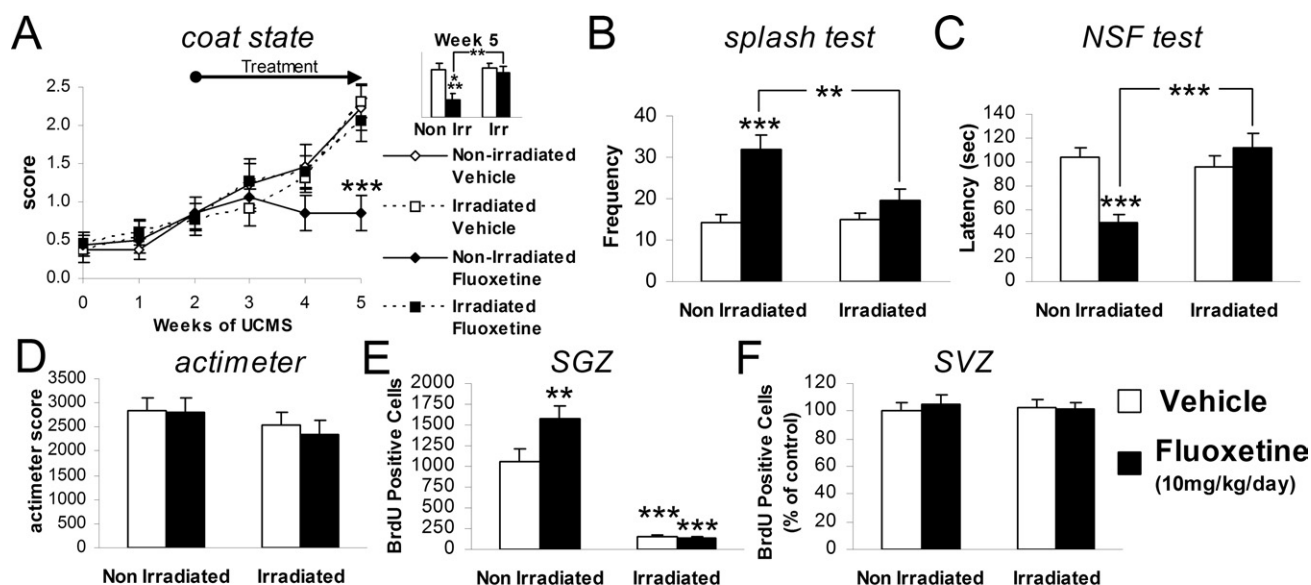
Figure 3. Hippocampal x-irradiation prevented the effects of fluoxetine (10 mg/kg/day, IP) on behavior and cell proliferation after the UCMS regimen. **(A)** Fluoxetine treatment significantly reduced the UCMS-induced deterioration of the coat state in non-irradiated mice. This fluoxetine effect was abolished in irradiated mice. Detail of the results after 5-week UCMS is illustrated above legend, $n = 13$–15 mice/group. **(B)** In the splash test, grooming behavior was increased by fluoxetine treatment only in the non-irradiated mice, $n = 13$–14 mice/group. **(C)** Fluoxetine treatment reduced the latency in the NSF test. Irradiation prevented this effect, $n = 13$–14 mice/group. **(D)** Locomotor activity in the actimeter was not affected by fluoxetine or the x-ray procedure, $n = 13$–14 mice/group. **(E)** Treatment with fluoxetine elicited a significant increase of the number of BrdU positive cells in the SGZ of the hippocampus. Irradiation potently reduced cell proliferation in the SGZ, $n = 5$–7 mice/group. **(F)** No change in cell proliferation in the SVZ due to the fluoxetine treatment or the irradiation was found, $n = 5$–7 mice/group. Data represent mean $\pm$ SEM. ANOVA following by Fisher post hoc: ***$p <$ .001 and **$p <$ .01 between line-connected groups or versus control/vehicle group; except for **(E)**: ***$p <$ .001 and **$p <$ .01 versus non-irradiated/ vehicle mice. Abbreviations as in Figure 2.

effect was accompanied by reduced grooming in the splash test ($p <$ .01; Figure 2B) and increased latency to eat in the NSF test ($p <$ .05; Figure 2C), and none of these effects were due to changes in locomotor activity (Figure 2D) or body weight (Figure 1A in Supplement 1). The UCMS decreased cell proliferation in the SGZ of non-irradiated mice ($p <$ .001), whereas irradiation almost completely abolished neurogenesis in this region ($p <$ .001; Figure 2E). Moreover, we found that neither stress nor irradiation impacted neurogenesis in the SVZ (Figure 2F). Ablation of hippocampal cell proliferation did not elicit any intrinsic effect in nonstressed mice and did not change the sensitivity to stress as measured by coat deterioration, grooming behavior, or latency to eat in the NSF test (Figure 2; Figure 1A in Supplement 1). Together these data indicate that, although chronic stress reduces hippocampal neurogenesis, this effect does not cause or contribute to the changes in behavior observed in UCMS-subjected mice. Additionally, complete loss of hippocampal neurogenesis did not accelerate or amplify the behavioral modifications induced by the UCMS procedure.

Disruption of Hippocampal Neurogenesis Prevents the Action of Monoaminergic ADs

The idea that hippocampal neurogenesis might not be essential to the pathogenesis of depression does not exclude that it is involved in the action of ADs. We have previously shown that ablation of hippocampal neurogenesis prevented the AD activity of fluoxetine (22). Here, we have sought to determine whether neurogenesis is a conserved feature in the efficacy of ADs by comparing, in irradiated or sham mice, the effects of drugs with distinct pharmacological targets: two compounds with monoam-

inergic mechanisms (the SSRI fluoxetine and the tricyclic imipramine) and two compounds targeting the HPA axis (a CRF$_1$ and a V$_{1b}$ antagonist).

We first aimed at confirming the involvement of neurogenesis in the effects of fluoxetine (22). Sham and irradiated mice were all exposed to a 5-week UCMS and, starting the third week, were administered either fluoxetine (10 mg/kg/day) or vehicle for 4 weeks. In non-irradiated mice, fluoxetine counteracted stress-induced effects on coat state ($p <$ .001; Figure 3A), grooming frequency in the splash test ($p <$ .001; Figure 3B), and latency to eat in the NSF ($p <$ .001; Figure 3C). These effects were completely abolished by hippocampal irradiation, because no effect of fluoxetine was seen in irradiated mice. These results cannot be explained by a change in body weight (Figure 1B in Supplement 1) or locomotor activity (Figure 3D). Furthermore, fluoxetine induced an increase of cell proliferation in the SGZ in non-irradiated mice ($p <$ .01), whereas irradiation caused a profound loss of cell proliferation in the SGZ of both vehicle and fluoxetine groups ($p <$ .001; Figure 3E). These effects were restricted to the hippocampus, because neither irradiation nor fluoxetine treatment altered cell proliferation in the SVZ (Figure 3F). Thus, hippocampal irradiation prevented the effects of the SSRI fluoxetine, confirming the suggestion that hippocampal neurogenesis is required for its efficacy.

To determine whether hippocampal neurogenesis is similarly required for the efficacy of other monoaminergic ADs, we repeated the aforementioned series of experiments with the tricyclic AD imipramine (20 mg/kg/day). Chronic imipramine treatment reduced the UCMS-induced deterioration of the coat state ($p <$ .01) and increased grooming frequency in non-
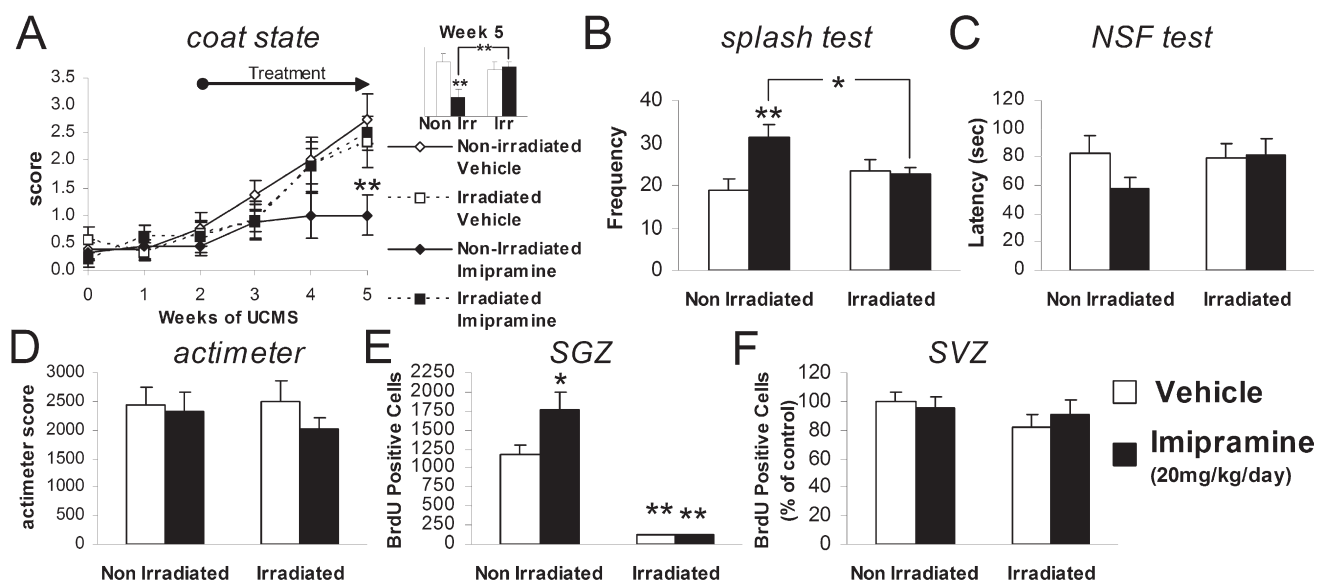
Figure 4. Hippocampal x-irradiation prevented the effects of imipramine (20 mg/kg/day, IP) on behavior and cell proliferation after the UCMS regimen. (**A**) Imipramine treatment significantly reduced in non-irradiated mice the deterioration of the coat state. This imipramine effect was abolished in irradiated mice. Detail of the results after 5-week UCMS is illustrated above legend, $n = 8$–10 mice/group. (**B**) In the splash test, imipramine treatment improved grooming behavior only in the non-irradiated mice, $n = 8$–10 mice/group. (**C**) No significant difference was found in the NSF test. $n = 8$–10 mice/group. (**D**) Locomotor activity in the actimeter was not affected by imipramine or the x-ray procedure, $n = 8$–10 mice/group. (**E**) Treatment with imipramine permitted a significant recovery in the number of BrdU positive cells in the SGZ of the hippocampus. Irradiation potently reduced cell proliferation in the SGZ, $n = 5$–6 mice/group. (**F**) No change in cell proliferation in the SVZ due to imipramine or irradiation was found, $n = 5$–6 mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: **$p < .01$ and *$p < .05$ between line-connected groups or versus control/vehicle group; except for (**E**): **$p < .01$ and *$p < .05$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

irradiated mice ($p < .05$) but not in irradiated mice (Figures 4A and 4B). There was no significant reduction of latency to eat in the NSF test (Figure 4C). Finally, imipramine increased cell proliferation in the SGZ ($p < .05$), an effect that was absent in irradiated mice (Figure 4E). Again, no effect of drug treatment was found on body weight, activity, or cell proliferation in the SVZ (Figures 4D–4F; and Figure 1C in Supplement 1). Because both monoaminergic ADs reversed the UCMS-induced effects on coat deterioration and behavioral alterations, and these effects were completely abolished by hippocampal irradiation, our results suggest that hippocampal neurogenesis is necessary for the AD action of monoaminergic compounds.

Disruption of Hippocampal Neurogenesis Does Not Prevent AD-Like Actions of CRF₁ and V₁ᵦ Antagonists

Because CRF_1 and V_{1b} antagonists also cause an increase in hippocampal neurogenesis (16), we investigated whether neurogenesis is required for the effects of the CRF_1 antagonist SSR125543 (20 mg/kg/day) and the V_{1b} antagonist SSR149415 (20 mg/kg/day). In non-irradiated mice, we found that SSR125543 (Figure 5) and SSR149415 (Figure 6) counteracted the deleterious effects of UCMS on coat state ($p < .05$ and $p < .01$, respectively), in the splash test ($p < .05$ and $p < .01$, respectively), and in the NSF test for the SSR125543 ($p < .05$), although a strong trend for a treatment effect was found with the SSR149415 [$F(1,37) = 3.86$, $p = 0.057$]. The effects of these compounds could not be explained by a change in locomotion (Figures 5D and 6D) or body weight (Figures 1D and 1E in Supplement 1). Both SSR125543 and SSR149415 increased hippocampal cell proliferation ($p < .001$; Figures 5E and 6E) without eliciting any such effect in the SVZ (Figures 5F and 6F). Although irradiation

abolished cell proliferation in the SGZ in all mice ($p < .001$; Figures 5D and 6D), the effects of both compounds on the coat state and the splash test were intact ($p < .05$; Figures 5A and 6A) and no different from their effect in non-irradiated mice, suggesting that these effects are independent of hippocampal neurogenesis. Only one aspect in the reversal of UCMS-elicited modifications was disrupted by irradiation: the SSR125543 effect in the NSF test (Figure 5C). This could suggest that the coat state and the splash test on the one hand and the NSF test on the other reflect two different features of the depressive-like state induced by UCMS. Contrary to the coat state score and the splash test, the NSF test, which was firstly developed to characterize anxiolytic properties (38), might detect UCMS-induced anxiety-like rather than depressive-like behaviors and, on the basis of the novel environment exposure, be more sensitive to hippocampal dysfunction. Nevertheless, there was a significant main effect of drug treatment [$F(1,39) = 4.8$, $p < .05$] without an interaction in the NSF test, and irradiated/SSR125543-treated mice displayed no significant difference compared with non-irradiated/SSR125543-treated mice (Figure 5C). Finally, most AD-like effects of CRF_1 and V_{1b} antagonists arose despite the suppression of hippocampal neurogenesis.

Discussion

The UCMS-induced behavioral changes were reversed by several compounds endowed with AD-like properties, such as fluoxetine and imipramine, as well as a CRF_1 antagonist (SSR125543) and a V_{1b} antagonist (SSR149415). The x-irradiation of the hippocampus had no effect per se in the UCMS procedure, suggesting that a loss of hippocampal neurogenesis does not
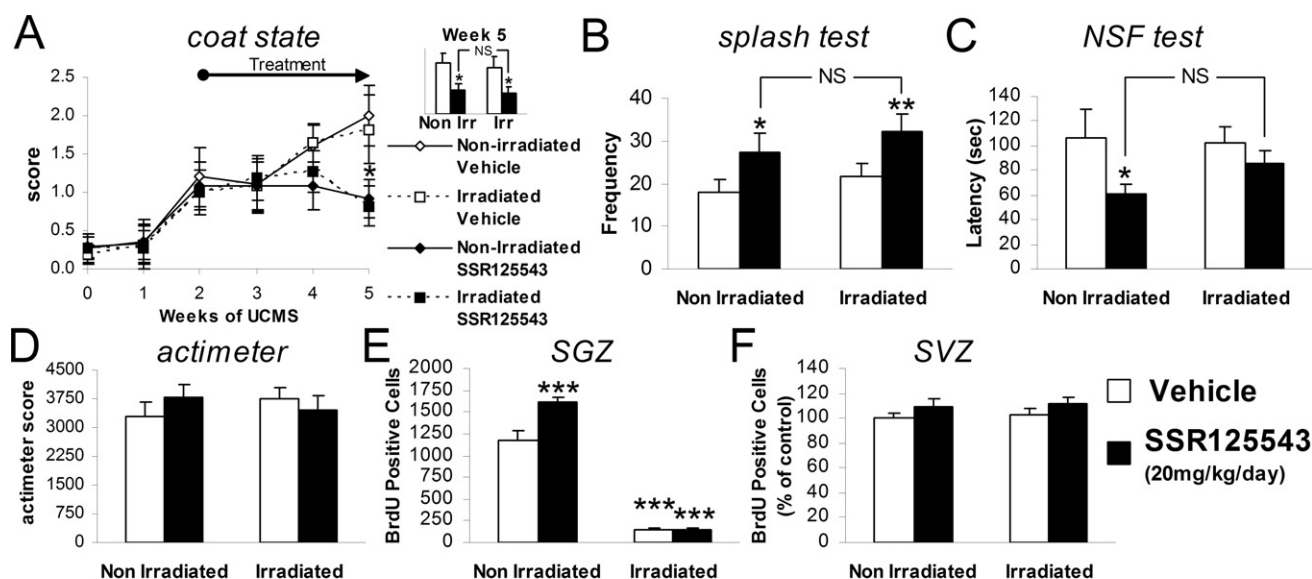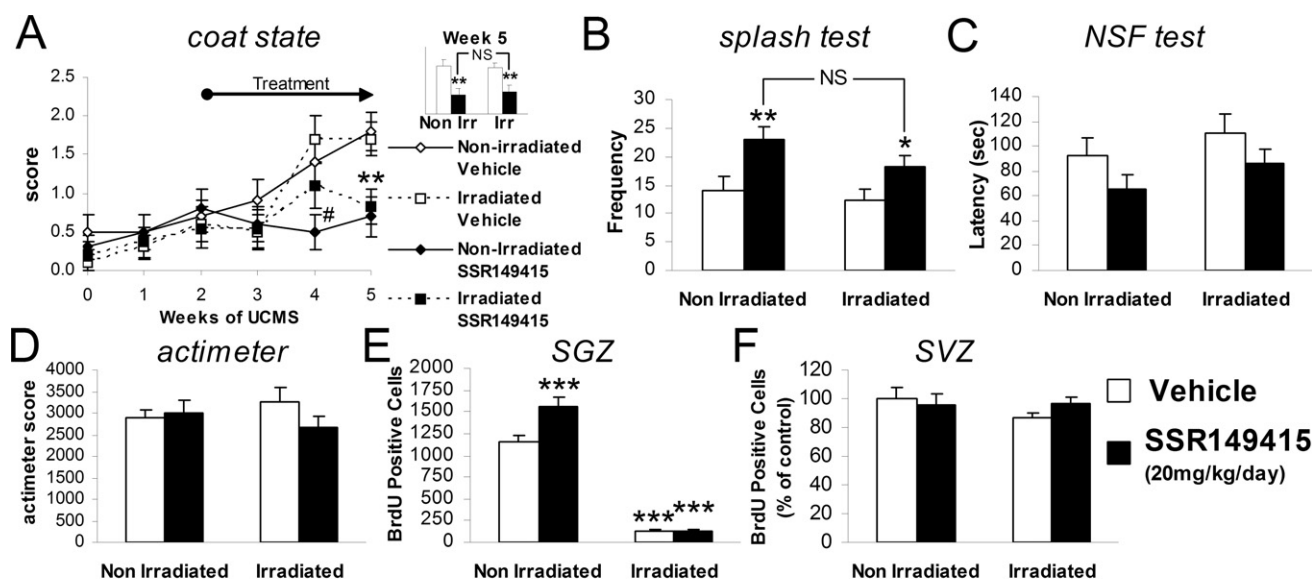
Note: all the groups have been subjected to the UCMS protocol

Figure 5. Hippocampal x-iradiation did not prevent several antidepressant-like effects of the corticotropin-releasing factor 1 (CRF_1) receptor antagonist, SSR125543 (20 mg/kg/day, IP), on behavior and cell proliferation after the UCMS regimen. (**A**) The SSR125543 treatment elicited a significant decrease in the deterioration of the state of the coat in non-irradiated and irradiated mice. The effect of SSR125543 was not blocked by the x-ray procedure. Detail of the results after 5-week UCMS is illustrated above legend, n = 10–11 mice/group. (**B**) After 5-week UCMS, the frequency of grooming was significantly increased by SSR125543 treatment in non-irradiated mice as well as in irradiated mice, n = 10–11 mice/group. (**C**) In the NSF test, the latency to chew the pellet was reduced by SSR125543 treatment in non-irradiated mice but not in irradiated mice. Nevertheless, the latency was not different when comparing non-irradiated/ SSR125543 mice versus irradiated/SSR125543 mice, n = 10–11 mice/group. (**D**) Locomotor activity in the actimeter was not affected by SSR125543 or the x-ray procedure, n = 8–9 mice/group. (**E**) The SSR125543 treatment induced a significant recovery in the number of BrdU positive cells in the SGZ of the hippocampus. Irradiation strongly reduced cell proliferation in the SGZ, n = 6 mice/group. (**F**) No change in cell proliferation in the SVZ due to SSR125543 or irradiation was found, n = 6 mice/group. Data represent mean ± SEM. ANOVA following by Fisher post hoc: **$p < .01$; *$p < .05$ and $p =$ non-significant, between line-connected groups or versus control/vehicle group; except for (**E**): ***$p < .001$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

induce a depressive-like behavior and does not worsen the deteriorations induced by the UCMS. Nevertheless, irradiation completely abolished the AD-like effects of drugs that act via a monoaminergic mechanism (fluoxetine and imipramine). In contrast, several AD-like effects of SSR125543 and SSR149415 were maintained despite irradiation. Therefore, our results support that, although hippocampal neurogenesis is required for the AD efficacy of monoaminergic drugs, it is nonessential for some AD-like effects of compounds that modulate the HPA axis. Because several AD-like effects were seen in irradiated mice, hippocampal neurogenesis might not be seen as the final neurobiological process by which ADs reverse detrimental effects of stress.

Nevertheless, some concerns need to be considered for further interpretations of the results. First, it cannot be excluded that the coat state evaluation and splash test, unlike the NSF test, are measures that are not linked to the human disease; however, this argument is questionable, because coat state evaluation is the most prevalent, reliable, and well-validated measure used in this mouse model of depression (16,22,29,30,35–37), whereas the NSF test pre-eminently examines anxiety-related behaviors (38,39). Second, because brain penetration was demonstrated for both SSR125543 and SSR149415 (40–42), these compounds could induce their AD-like effects centrally and the HPA axis could be unaffected in our model. However, we recently found that UCMS induces changes in plasmatic corticosterone that are reversed by fluoxetine and SSR125543, highlighting the implication of the HPA axis (35). Third, another confounding point might arise concerning whether irradiation induces HPA alterations, but we previously dismissed this possibility by showing

control-like HPA axis function after irradiation in both basal and stress conditions (22). Fourth, we also found that irradiation induces no modification in several other behavioral- and brain-related functions (22), although more subtle effects of irradiation could exist. Moreover, whereas irradiation induces transient inflammation, mice were allowed to recover for 5 weeks before initiating the UCMS regimen and 10 weeks before testing, a period after which the inflammatory effects of hippocampal irradiation are largely completed (43). Fifth, in contrast to our results, two studies (44,45) recently reported that fluoxetine's effects in bio-assays are independent of hippocampal neurogenesis in BALB/c mice, and that fluoxetine does not increase cell proliferation or neurogenesis in the dentate gyrus. Nevertheless, they tested fluoxetine's action in normal "non-depressed" mice, whereas we used a chronic and naturalistic model of depression in which fluoxetine's effects are examined in pathologic-like rather than baseline conditions. Considering that ADs are devoid of mood-changing effects in normal individuals, paradigms elaborated to test the action of ADs in normal "non-depressed" mice could engage different neurobiological mechanisms that are irrelevant to clinical remission. Moreover, their results are also inconsistent with several other studies using mouse models based on chronic application of stressors (16,22,46). Indeed, we have previously shown that hippocampal x-irradiation prevented fluoxetine-induced AD effects in 129SvEv as well as BALB/c strains (22), and the UCMS procedure was shown to decrease hippocampal neurogenesis in BALB/c mice (16,46). Finally, Alonso *et al.* (16) previously demonstrated that chronic fluoxetine was able to increase SGZ cell proliferation in UCMS-treated as well as control BALB/c mice.

Figure 6. Hippocampal x-irradiation did not prevent the antidepressant-like effects of the vasopressin 1b (V$_{1b}$) receptor antagonist, SSR149415 (20 mg/kg/day, IP), on behavior and cell proliferation after the UCMS regimen. (**A**) The SSR149415 treatment significantly reduced the deterioration of the state of the coat in non-irradiated and irradiated mice. The effect of SSR149415 was not blocked by the x-ray procedure. The effect of SSR125543 was not blocked by the x-ray procedure. Detail of the results after 5-week UCMS is illustrated above legend, $n = 10–11$ mice/group. (**B**) Mice treated with SSR149415 displayed more grooming behaviors than vehicle mice in the splash test. This effect was not abolished by irradiation, $n = 10–11$ mice/group. (**C**) No significant difference was found in the NSF test. $n = 10–11$ mice/group. (**D**) Locomotor activity in the actimeter was not affected by SSR149415 or the x-ray procedure, $n = 9–10$ mice/group. (**E**) The SSR149415 treatment induced a significant recovery of BrdU positive cells in the SGZ of the hippocampus. Irradiation caused a profound loss of cell proliferation in the SGZ, $n = 6–7$ mice/group. (**F**) No change in cell proliferation in the SVZ due to SSR149415 or irradiation was found, $n = 6–7$ mice/group. Data represent mean $\pm$ SEM. ANOVA following by Fisher post hoc: $^{\#}p < .05$ for the non-irradiated/SSR149415 mice versus the control non-irradiated/vehicle mice. $^{**}p < .01$; $^{*}p < .05$ and $p =$ non-significant between line-connected groups or versus control/vehicle group; excepted for (**E**): $^{***}p < .001$ versus non-irradiated/vehicle mice. Abbreviations as in Figure 2.

The fact that x-ray did not induce any "depressive-like" effects in nonstressed mice, as did a 5-week UCMS regimen, suggests that a decrease of hippocampal neurogenesis might not be causal in the pathogenesis of depression. A previous study showed that reduction of cell proliferation in the DG does not correlate with the development of learned helplessness (13); however, the limitation of this study is that the paradigm used had a short duration (approximately 10 days). Because new neurons take weeks to connect to their appropriate targets, 10 days of learned helplessness might be insufficient to elicit neurogenesis-related changes in hippocampal function, such as those thought to occur during depression. Not only did the stress procedure in our study last for 5 weeks but we also found that neurogenesis-deficient mice responded normally to the UCMS procedure. These findings are in accordance with the notion that the hippocampus is not the driver of affective disorders in humans (47) but do not exclude its contribution to AD-related effects that can restore normal brain functioning.

Initial studies showing that chronic AD treatments increase hippocampal neurogenesis have described two different patterns of action: compounds such as fluoxetine increased neurogenesis in both stressed and nonstressed mice, although conflicting results occurred with nonstressed mice (16,44,45); and CRF$_1$ and V$_{1b}$ antagonists counteracted stress-induced deficits in hippocampal neurogenesis without stimulating this process per se (16). The decrease in hippocampal neurogenesis induced by stress is mediated by the release of adrenal hormones such as GCs, because removal of the adrenal glands abolishes stress-induced decrease of cell proliferation (48). The CRF$_1$ antagonists are known to cause a reduction in CRF-mediated activation of the

HPA axis in response to stress, thus reducing the release of GCs (40,49,50). Similarly, the V$_{1b}$ antagonist SSR149415 has been shown to inhibit the elevation in plasma corticotropin observed after restraint stress (51), suggesting that this drug might also be able to block stress-induced increase of GCs. Thus, it is possible that SSR125543 and SSR149415 increase hippocampal neurogenesis under stressful conditions by preventing a GC-induced decrease rather than stimulating neurogenesis per se.

A potential explanation for our results would be that, whereas monoaminergic drugs act via a hippocampal-dependent mechanism, another mechanism might underlie the action of CRF$_1$ and V$_{1b}$ antagonists. Notably, depression is associated with abnormalities in HPA axis function, and AD effects have been suggested to occur via a normalization of these abnormalities (2,52–54). For example, monoaminergic ADs inhibit HPA axis activity by increasing GC receptor levels, thereby leading to an enhancement in GC receptor-mediated feedback inhibition (55) that might involve the hippocampus. Thus, one can propose that deficits observed in depressed patients or in stressed rodents might be related to GC-induced structural damage in the brain. Because the hippocampus exerts a negative feedback on the HPA axis that can be altered by chronic stress (4), it is conceivable that during cases of excess stress, hippocampal dysfunction might reduce this negative feedback, thus exacerbating GC release and accentuating brain damage and hippocampal atrophy. Because monoaminergic ADs do not directly or initially target the HPA axis, they might interrupt this cycle by stimulating neurogenesis; indeed, this might compensate for atrophy or neuronal loss and allow for restoration of normal hippocampal function, including inhibition of the HPA axis. By contrast, CRF$_1$
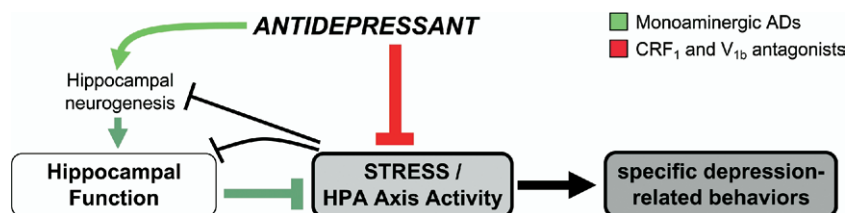
Figure 7. Antidepressant (AD) drug effects can be achieved by neurogenesis-dependent and -independent mechanisms. Stress and persistent hypothalamo-pituitary-adrenal (HPA)-axis activity can elicit changes in behavior and reduce hippocampal neurogenesis, possibly via increased glucocorticoid release. Although the hippocampus participates in the negative regulation of stress responses, the negative effects of stress on behavior and neurogenesis are not causally related. Therefore, the antidepressant effect of drugs that act directly on the HPA-axis could not require hippocampal neurogenesis. However, monoaminergic drugs might use neurogenesis to counteract the effects of stress by enhancing negative feedback provided by the hippocampus. Symbols: arrows = stimulation; bared line = inhibition. V_{1b}, vasopressin 1b; CRF_1, corticotropin-releasing factor 1.

and V_{1b} receptor antagonists might act directly on the HPA axis to restore normal GC levels, thereby counteracting such detrimental effects as hippocampal atrophy. According to this interpretation, without functional hippocampal neurogenesis, classical ADs might be either ineffective or would require a longer time to be effective, whereas the action of HPA-acting drugs would persist. This hypothesis, as summarized in Figure 7, suggests the involvement of new hippocampal neurons in the inhibitory control of the hippocampus on the HPA axis. Although this model is largely a theoretical interpretation, it was previously shown that CRF_1 and V_{1b} antagonists do not elicit significant changes in serotonin or norepinephrine levels or bind to monoaminergic receptors (unpublished results); therefore, they clearly act via different mechanisms than SSRIs and tricyclics.

In conclusion, our data suggest that hippocampal neurogenesis might not be a key factor in the pathophysiology of depression, because disruption of hippocampal neurogenesis does not induce depressive-like behaviors or alter sensitivity to chronic stress. In addition, we confirm that hippocampal neurogenesis is required for the action of monoaminergic ADs in a rodent model of depression. Finally, we show that a "therapeutic" effect can be achieved, even with ablation of hippocampal neurogenesis, by directly targeting the HPA axis and related neuropeptides.

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