BrdU$^+$ cells that had differentiated into mature new neurons (BrdU$^+$NEUN$^+$ cells) after the 4-week mIFN-α treatment (Figure S1F). There were no significant differences in the numbers of BrdU$^+$ or BrdU$^+$NEUN$^+$ cells in the DG (Figures S1G–S1I) or in the percentage of NEUN$^+$ cells in the BrdU-labeled population (data not shown) between the treatment groups. We further examined the effects of IFN treatment on the morphological phenotypes of the new neurons. New neurons were labeled by injecting a retroviral vector-encoding DsRed (red fluorescent protein) into the DG 1 day before 4-week mIFN-α treatment. The dendrites of the DsRed-labeled new neurons were then analyzed. There were no significant differences in the total length or number of branching points of dendrites in the DsRed-labeled new granule cells between the groups (Figures S1J–S1L), suggesting that mIFN-α did not affect the neuronal differentiation or the survival of the newly produced cells.

We next examined whether mIFN-α treatment affected oligodendrocyte progenitor cells, which are widely distributed and proliferate continuously in the adult brain. There was no significant difference in the density of OLIG2$^+$ oligodendrocyte progenitor cells between the groups in any of the brain areas studied (Figure S1M). Taken together, these data suggest that chronic mIFN-α treatment reduces the proliferation of neural stem/progenitor cells, reducing the production of new neurons in the DG.

Treatment of Cultured Neural Stem Cells with mIFN-α

Cultured hippocampal NSCs (Gage et al., 1995) were used to study the direct effects of mIFN-α on NSCs. Immunocytochemistry revealed that both of the interferon receptor subunits, IFNAR1 and IFNAR2, were expressed in all the Nestin$^+$ NSCs (IFNAR1: 100%; IFNAR2: 100%; n = 352 cells; Figure 2A) and in most of the MAP2+ neurons (IFNAR1: 100%; IFNAR2: 99.17%; n = 121 cells; Figure 2B), GFAP+ astrocytes (IFNAR1: 99.07%; IFNAR2: 99.53%; n = 214 cells; Figure S2), and RIP+ oligodendrocytes (IFNAR1: 98.85%; IFNAR2: 100%; n = 87 cells; Figure S2). A 15 min treatment with mIFN-α dose-dependently increased the phosphorylation level of STAT1 (a downstream effector of the IFN-α/IFNAR-signaling pathway; Figure 2C), demonstrating that hippocampal NSCs were responsive to IFN-α.

To examine mIFN-α's effects on proliferating hippocampal NSCs, we incubated NSCs with different concentrations of mIFN-α (10–10^3 IU/ml). After 24 hr of treatment, there were no significant differences in cell numbers as determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. However, after further expansion of NSCs at 48 and 72 hr, there was a significant dose-dependent reduction in cell numbers of the IFN-α treatment groups (Figure 2D). To label proliferating cells, NSCs were incubated with BrdU during the last 4 hr of culture with PBS or mIFN-α (Figure 2E). mIFN-α dose-dependently reduced the number of BrdU$^+$ proliferating cells in the Nestin$^+$ NSC populations of 24 hr (data not shown) and 48 hr (Figure 2F) cultures. The percentage of PI$^+$ apoptotic cells in the culture was increased by mIFN-α treatment at higher doses compared with the control (Figures 2G and 2H), but this effect only accounted for a small part of the mIFN-α-mediated reduction in NSC expansion (Figure 2D). These data suggested that mIFN-α directly suppresses hippocampal NSC proliferation.

To determine whether mIFN-α affects NSC differentiation, as well as survival of the differentiated cells, the NSCs were labeled with BrdU and allowed to differentiate for 72 hr with or without mIFN-α (Figure 2I). mIFN-α had no effect on the total number of differentiated cells (Figure 2J) or on the differentiation of BrdU-labeled cells into MAP2$^+$ neurons, GFAP$^+$ astrocytes, or RIP$^+$ oligodendrocytes (Figure 2K). These findings suggest that mIFN-α did not alter the survival or fates of hippocampal NSCs during their differentiation. Taken together, our in vivo and in vitro results indicate that mIFN-α treatment specifically and directly modulates the proliferation of NSCs.

mIFN-α Treatment Decreases the Proliferative Activity of Neural Stem/Progenitor Cells in the V-SVZ

We further investigated the effects of mIFN-α on neural stem/progenitor cells in the ventricular-subventricular zone (V-SVZ), another region populated with NSCs in the adult brain. The numbers of cells positive for Ki67 (Figures 3A and 3B) or MASH1 (Figures 3C and 3D), a marker for transit-amplifying neuronal progenitors, were significantly reduced in the V-SVZ of mice treated with mIFN-α for 4 weeks. Because the characteristics of the NSCs in the V-SVZ vary according to region (Kelsch et al., 2007; Merkle et al., 2007, 2014), we separately quantified and compared the number of Ki67$^+$ cells in the medial, cortical, dorsal, lateral, and ventral V-SVZ areas (Merkle et al., 2007) of each group. The Ki67$^+$ cell population was significantly decreased in the dorsal and lateral areas in the mIFN-α-treated groups (Figure S3A).

To examine the effect of mIFN-α on the V-SVZ-derived NSCs, a neurosphere assay was conducted. The number of primary neurospheres generated from the V-SVZ of adult wild-type mice was significantly reduced when the V-SVZ-derived cells were cultured with 10^3 IU/ml mIFN-α (Figures 3E and S3B). Moreover, the number of secondary neurospheres formed by dissociated single primary neurospheres was significantly reduced by mIFN-α (Figure 3F). These data suggest that mIFN-α inhibited the proliferation and self-renewal of the V-SVZ NSCs. Notably, sphere formation from the V-SVZ of *IFNAR*$^{-/-}$ mice was not affected by mIFN-α treatment (Figure 3E), indicating that the suppressive effects of mIFN-α were mediated by the IFNAR expressed on NSCs. We also examined the differentiating cells

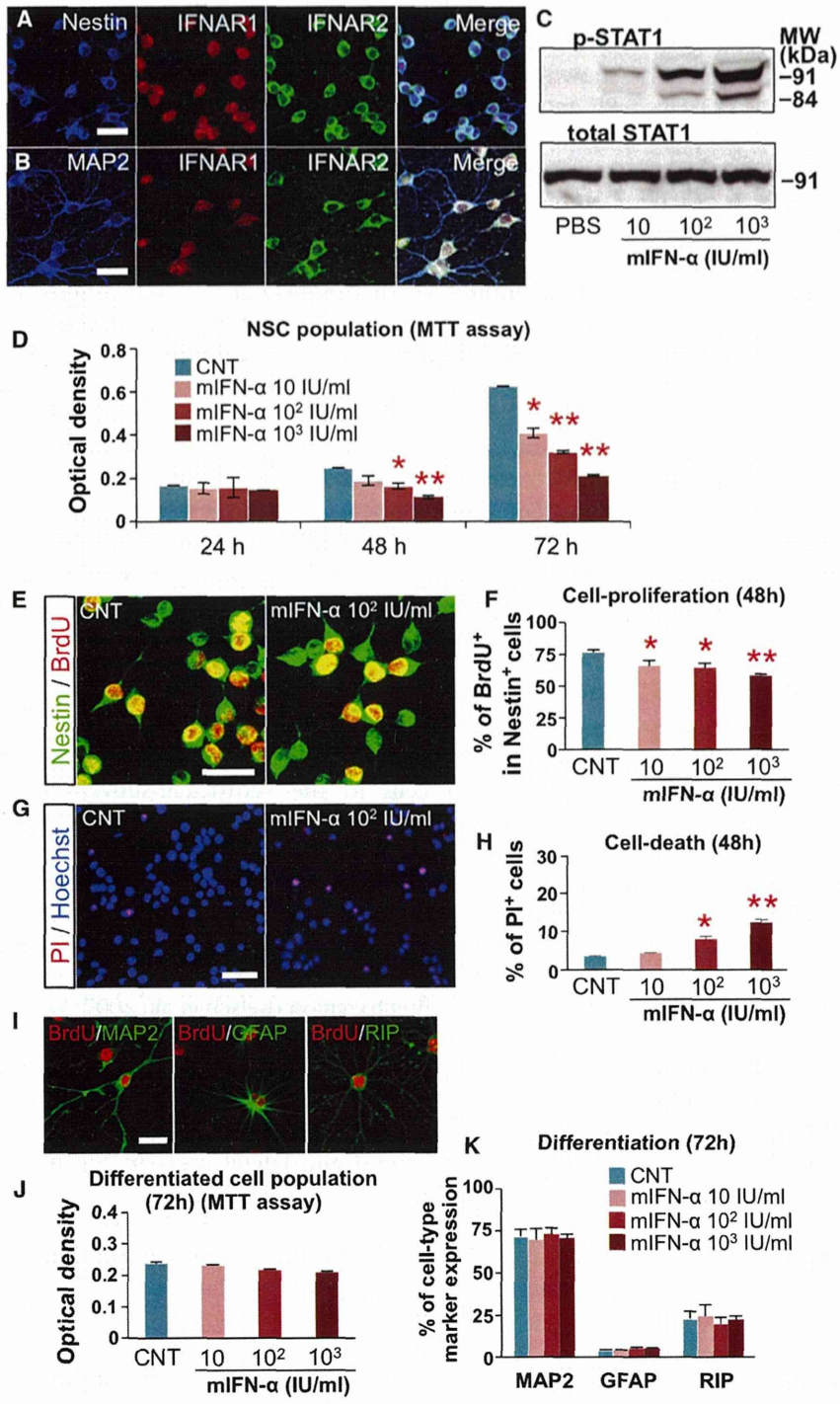

Figure 2. Treatment of Adult Hippocampal NSC Cultures with mIFN-α

(A–C) Interferon receptor expression and STAT1 phosphorylation in cultured adult hippocampal NSCs. All Nestin$^+$ (A) and MAP2$^+$ (B) cells (blue) in the culture expressed both interferon receptor subunits, IFNAR1 (red) and IFNAR2 (green). A 15 min incubation with mIFN-α resulted in dose-dependent increases in STAT1 phosphorylation (p-STAT1), as detected by western blotting, whereas total STAT1 levels were unchanged (C).

(D–H) Effects of mIFN-α on survival and proliferation of hippocampal NSCs. The numbers of live cells quantified by the MTT assay were significantly reduced in the mIFN-α-treated groups, compared with the control groups, at 48 and 72 hr, but not 24 hr (D; n = 3 or 4 wells). The proliferating NSCs were labeled with BrdU during the last 4 hr of incubation with mIFN-α (E and F). After 48 hr of incubation with mIFN-α, the percentage of Nestin$^+$ cells (E, green) that were BrdU$^+$ (E, red) was reduced in a dose-dependent manner (F; n = 3 wells). The percentage of PI$^+$ apoptotic cells (G, red; Hoechst, blue) was significantly increased after the 48 hr incubation with mIFN-α (H; n = 3 wells).

(I–K) Effects of mIFN-α on NSC differentiation. The NSCs labeled with BrdU were allowed to differentiate for 72 hr with or without mIFN-α (I). There were no significant differences in the number of differentiated cells as quantified by MTT assay (J; n = 3 wells) or the percentage of BrdU-labeled cells that differentiated into neurons (MAP2$^+$), astrocytes (GFAP$^+$), or oligodendrocytes (RIP$^+$; K; n = 6 wells) among the groups. *p < 0.05, **p < 0.01 versus PBS-treated group; error bars: means ± SEM; scale bars, 25 μm: (A, B, E, G, and I).

See also Figure S2.

dissociated from the primary neurospheres from wild-type mice. mIFN-α did not affect the survival (Figure 3G) or neuronal differentiation of these cells (data not shown). Taken together, these results suggest that mIFN-α directly suppresses proliferation/self-renewal of the NSCs in the V-SVZ as well as in the SGZ.

Chronic mIFN-α Treatment Induces Depressive Behavioral Phenotypes and Decreases Social Interactions in Mice

We next studied the effects of chronic mIFN-α treatment on mouse behavior (Figures 4A–4G and S4A–S4L). Mice were injected with PBS or mIFN-α for 4 weeks and then subjected

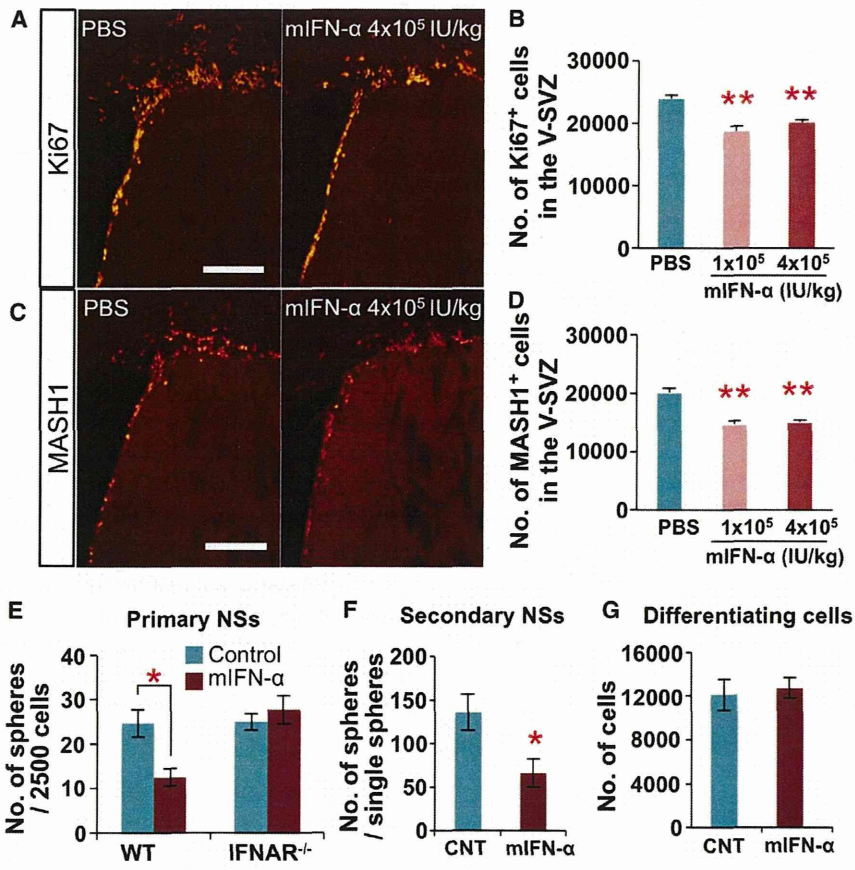

Figure 3. Effects of mIFN-α Treatment on NSCs and Neuronal Progenitor Cells in the Adult V-SVZ

(A–D) Effect of 4-week mIFN-α treatment on V-SVZ proliferative activity. Following mIFN-α treatment, the numbers of Ki67$^+$ cells (A and B) and MASH1$^+$ cells (C and D) in the V-SVZ were significantly reduced in the mIFN-α-treated group compared with the PBS-treated group. n = 7 mice per group.

(E–G) Effects of mIFN-α treatment on V-SVZ-derived NSC cultures. The number of primary neurospheres formed by the dissociated V-SVZ cells from wild-type mice cultured for 7 days was significantly reduced with 10^3 IU/ml of mIFN-α. This effect was not observed with V-SVZ-derived cells from *IFNAR*$^{-/-}$ mice (E; n = 4 mice per group). The number of secondary neurospheres formed by the cells dissociated from single wild-type primary neurospheres treated with 10^3 IU/ml of mIFN-α was significantly reduced compared with untreated control cells (F; n = 12 mice per group). When dissociated cells derived from primary neurospheres were cultured with 10^3 IU/ml mIFN-α for 4 days after induction of differentiation, there was no significant difference in the number of cells compared with the control group, as quantified by the ATP luminescence assay (G; n = 4 cultures). *p < 0.05, **p < 0.01 versus no treatment group; error bars: means ± SEM. The scale bars represent 100 μm. CNT, control; NS, neurosphere; WT, wild-type.

See also Figure S3.

to a comprehensive battery of behavioral tests. Raw data and a summary of these tests are shown in the Mouse Phenotype Database (http://www.mouse-phenotype.org/). General health, muscular strength (Figures S4A–S4D), sensorimotor function (Figures 4A, pain sensitivity, and 4B, motor coordination), and locomotor activity (Figure 4C) were not affected by mIFN-α treatment.

To evaluate anxiety in these mice, we performed the light/dark transition test (Figures S4E–S4H) and the elevated plus maze test (Figures S4I–S4L). We also measured the time spent in the center in the open field test (Figure 4C, middle). mIFN-α treatment significantly decreased the distance traveled in the elevated plus maze test (Figure S4K), but did not affect performance in the other tests, suggesting that the mIFN-α-treated mice exhibited a slightly increased sensitivity to stressful environmental changes.

Depression-like behaviors in these mice were examined using the tail suspension test (Steru et al., 1985) and the Porsolt forced swimming test (Porsolt et al., 1977). In these tests, depression levels are determined based on immobility times, which can be elongated by decreased escape-oriented behaviors. In both tests, the immobility times were not altered by a 2-week mIFN-α treatment (Figures S1D and S1E) but were significantly increased by a 5-week treatment (Figures 4F and 4G), indicating that chronic mIFN-α treatment induced depressive behavioral phenotypes, consistent with previous reports (Fahey et al., 2007; Felger et al., 2007).

To further characterize the IFN-α-induced depression, we used Crawley's three-chamber social approach test, which consists of a sociability test and a social novelty preference test. This test is useful for monitoring social withdrawal, one of the typical symptoms of depression, relatively independent of their changes in locomotor activity in mice (Moy et al., 2004). In the sociability test, whereas one cage was empty, another cage contained a mouse stranger to the experimental animal (stranger side 1). PBS-treated mice, placed in the central compartment between these two cages, spent more time near the cage with the stranger

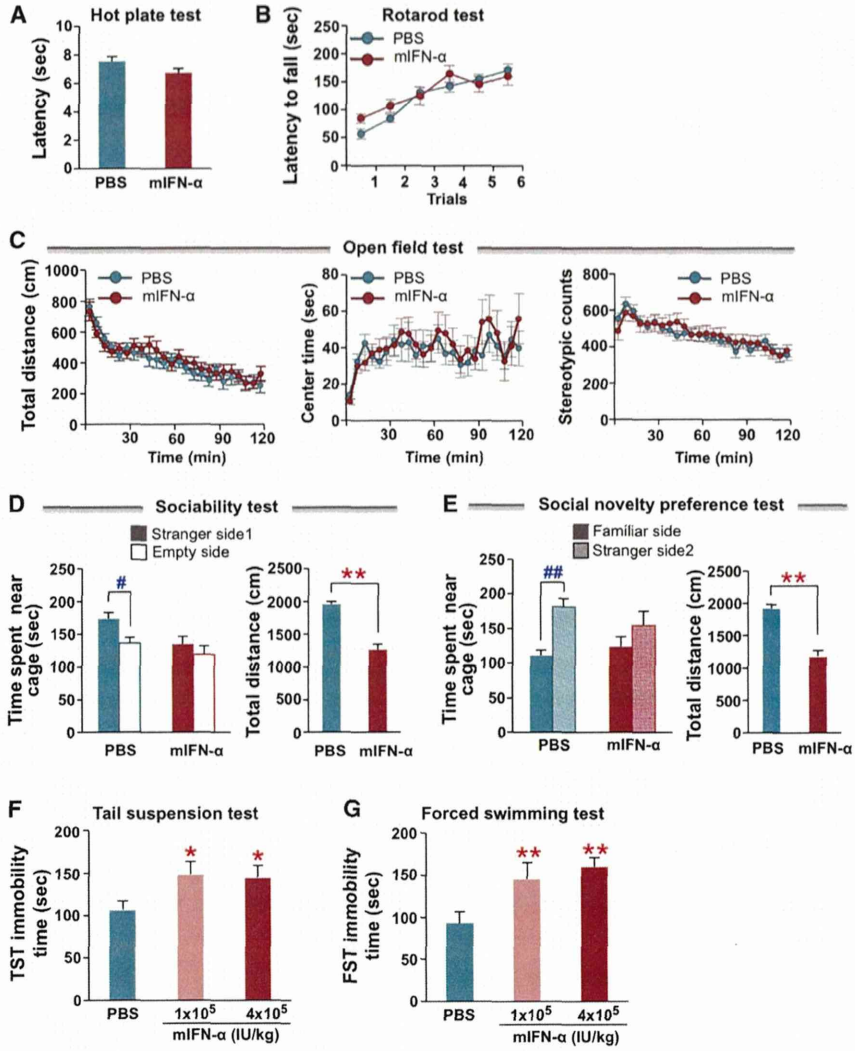

Figure 4. Effects of Chronic mIFN-α Treatment on Mouse Behavioral Profiles

(A–C) Sensorimotor function tests and the open field test. There were no significant differences in mouse responses to the hot plate test (A), the rotarod test (B), or the open field test (C) between mIFN-α- and PBS-treated groups. n = 20 to 21 mice per group.

(D and E) Crawley's social interaction test. The time spent near the cage with an unfamiliar (stranger 1) mouse in the sociability test (D, left) or near the cage with a second unfamiliar (stranger 2) mouse in the social novelty preference test (E, left) was significantly longer than the time spent near an empty cage or the cage containing a familiar mouse in the PBS-treated group, but not in the mIFN-α treated group. The total distance traveled during both tests was significantly reduced in the mIFN-α-treated groups compared with the controls (D and E, right). n = 20 to 21 mice per group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ stranger side versus empty (D) or familiar (E) side, paired-t test; $^{**}p < 0.01$ versus PBS-treated group.

(F and G) Tail suspension test (TST) and forced swimming test (FST). Immobility times were significantly increased in mIFN-α-treated groups in both the TST (F) and FST (G), compared with the PBS-treated groups. n = 10 mice per group; $^{*}p < 0.05$, $^{**}p < 0.01$ versus PBS-treated group. Error bars represent means ± SEM.

See also Figure S4.

mouse than the empty cage (Figure 4D, left). Next, in the social novelty preference test, the same animal that had been a stranger in the sociability test was used as the familiar one (familiar side), and a new mouse was introduced into the other cage as a stranger (stranger side 2). PBS-treated mice also spent a significantly longer time near the cage with the stranger than with the familiar mouse (Figure 4E, left), because normal mice show more interest and interaction with novel conspecific mice. In contrast, mIFN-α-treated mice did not show this preference in either test (Figures 4D and 4E, left), suggesting that mIFN-α treatment impaired the social affiliation/motivation and social novelty of these mice. In addition, the decrease in total distance traveled by the mIFN-α-treated mice compared with the PBS-treated mice (Figures 4D and 4E, right) may reflect increased anxiety during socially stressful conditions. We conclude that chronic mIFN-α treatment of mice induced depressive behaviors and impaired social interactions without affecting general health or sensorimotor functions.

mIFN-α Increases Depression-like Behavior and Reduces Neurogenesis via IFN Receptor Expressed in the CNS

The direct effects of IFN-α on cultured NSCs (Figures 2 and 3) suggested that receptors for IFN-α expressed in the brain are involved in suppressing hippocampal neurogenesis and inducing depressive behaviors. To test this possibility, we used two types of knockout mice targeting IFNAR1, an IFN receptor subunit essential for IFN-α signal transduction (Müller et al., 1994): conditional CNS-specific knockout mice (*IFNAR*$^{fl/fl}$:*Nes-Cre*; Detje et al., 2009) and conventional knockout mice (*IFNAR*$^{-/-}$; Müller et al., 1994). These mice were treated with mIFN-α for 5 weeks and injected with BrdU six times at 8 hr intervals after 4 weeks of treatment using the same protocol as described

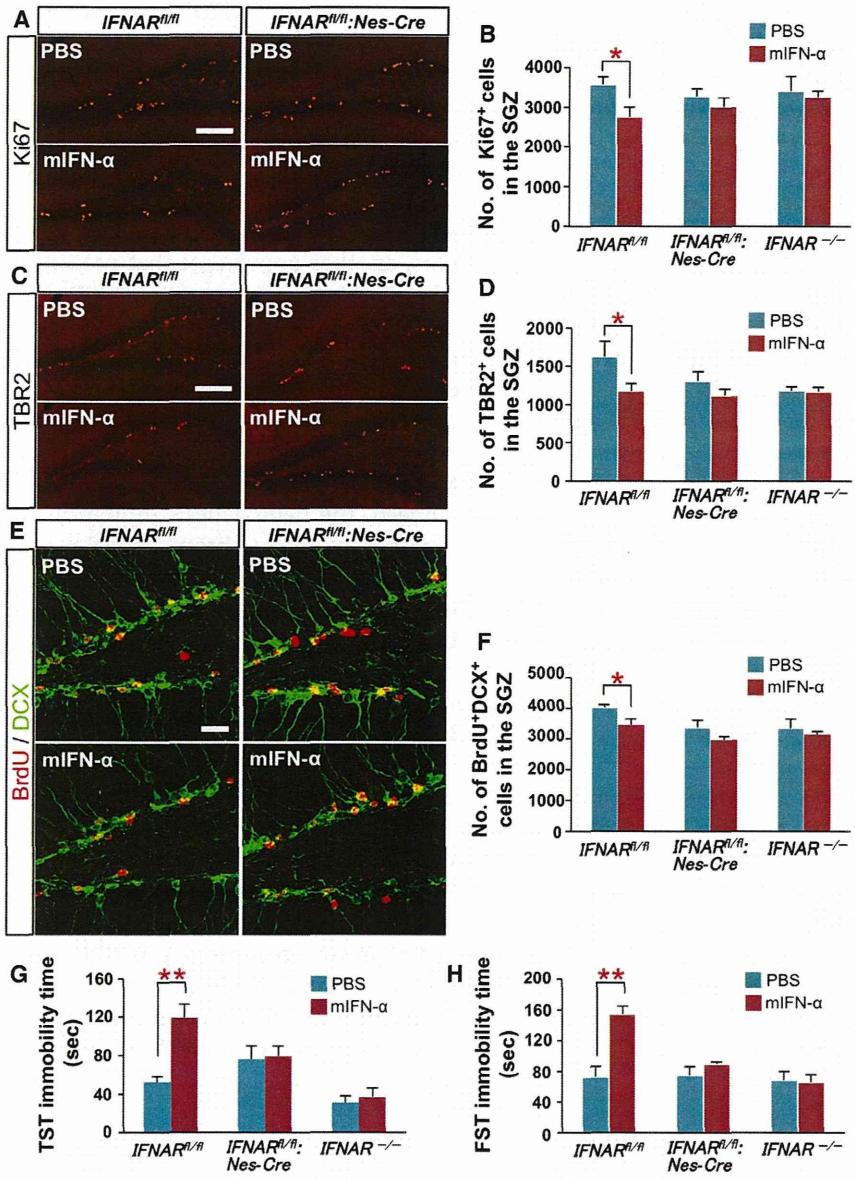

Figure 5. Effects of Chronic mIFN-α Treatment on Hippocampal Neurogenesis and Behaviors in *IFNAR* Knockout Mice

(A–F) Effects of 5-week mIFN-α treatment on neurogenic activity in the DG of *IFNAR*$^{fl/fl}$, *IFNAR*$^{fl/fl}$:*Nes-Cre*, and *IFNAR*$^{-/-}$ mice. The numbers of Ki67$^+$ proliferating cells (A and B), TBR2$^+$ neuronal progenitor cells (C and D), and BrdU$^+$DCX$^+$ newly generated neurons (E and F) were significantly reduced in the mIFN-α-treated group compared with the PBS-treated group in *IFNAR*$^{fl/fl}$ mice, but not in *IFNAR*$^{fl/fl}$:*Nes-Cre* or *IFNAR*$^{-/-}$ mice. n = 5 mice per group.

(G and H) Effects of mIFN-α treatment on the TST and FST. The immobility times in the TST (G) and FST (H) were significantly increased in the mIFN-α-treated groups compared with PBS-treated groups in *IFNAR*$^{fl/fl}$ mice, but not in *IFNAR*$^{fl/fl}$:*Nes-Cre* or *IFNAR*$^{-/-}$ mice. n = 10–13 mice per group. *p < 0.05, **p < 0.01 versus PBS-treated group. The error bars represent means ± SEM. The scale bars represent 100 μm (A and C) and 25 μm (E).

above (Figure 1A). After mIFN-α treatment, the mice were subjected to the tail suspension test and the Porsolt forced swimming test and then sacrificed, and the neural progenitor cells were analyzed (Figures 5A–5F). Although the TBR2$^+$ cell population in the PBS-treated groups tended to be smaller in *IFNAR*$^{-/-}$ and *IFNAR*$^{fl/fl}$:*Nes-Cre* mice compared with *IFNAR*$^{fl/fl}$ mice having normal IFNAR expression, these differences were not statistically significant (Figure 5D). In contrast, chronic mIFN-α treatment significantly decreased the numbers of Ki67$^+$ (Figures 5A and 5B), TBR2$^+$ (Figures 5C and 5D), and BrdU$^+$DCX$^+$ (Figures 5E and 5F) cells in the DG of *IFNAR*$^{fl/fl}$ mice, similar to the effect in wild-type mice (Figure 1). These effects were not observed in the *IFNAR*$^{-/-}$ or *IFNAR*$^{fl/fl}$:*Nes-Cre* mice (Figures 5A–5F). Similarly, mIFN-α treatment significantly increased the immobility times in the tail suspension test and the Porsolt forced swimming test in *IFNAR*$^{fl/fl}$, but not *IFNAR*$^{fl/fl}$:*Nes-Cre* or *IFNAR*$^{-/-}$ mice (Figures 5G and 5H). Therefore, IFN-α induced hippocampal neurogenesis suppression and depression-like behavioral changes via IFNAR expressed in the CNS.

DISCUSSION

Depression is one of the most common and serious side effects of IFN, which can limit its success as an antiviral or antitumor therapy. In this study, we used a simple

depression model with definitive molecular targets, IFN-α and IFNAR. Rodents treated with mIFN-α were reported to show depressive behaviors and/or increased anxiety (Fahey et al., 2007; Makino et al., 1998, 2000b; Yamano et al., 2000), although several groups failed to reproduce those behavioral alterations (De La Garza et al., 2005; Loftis et al., 2006). This discrepancy might result from variations in experimental paradigms, including the types of IFN-α used, animal species or lines employed, treatment regimens, and behavioral tests performed. Because the interaction of IFN with IFNAR is highly species specific (Wang et al., 2008), we chose mouse IFN-α for our animal experiments. Clinical studies showed that patients frequently develop depressive symptoms after several weeks of IFN-α administration, but not within the first few weeks (Hauser et al., 2002; Raison et al., 2005). Similarly, 2-week mIFN-α treatments did not induce behavioral changes in our mice (Figure S1). Therefore, we used mice treated with mIFN-α for over a month to evaluate behavioral changes.

Systemic IFN treatment could affect various brain functions other than emotional regulation, which might influence performance in tests assessing depressive-like behaviors. However, no studies have comprehensively investigated neurological and/or psychological alterations of IFN-treated animals. Using a battery of behavioral tests, we assessed a variety of brain functions. Whereas continuous mIFN-α treatment had no significant effect on general health or sensorimotor functions within the first 8 weeks, we noticed a gradual loss of body weight beginning in the ninth week (data not shown). Therefore, we included only the data obtained within the first 8 weeks in this report (Figures 4 and S4) and performed the depression-like behavioral tests (the tail-suspension test and the Porsolt forced swimming test) separately from the other tests, using mice immediately after the 5-week mIFN-α treatment. As a result, we found that mIFN-α treatment increased depression-like behaviors and impaired social interactions (Figures 4 and S4), consistent with the clinical symptoms of depression and independent of somatic conditions or sensorimotor functions. Taken together, we conclude that the IFN-α-treated mice are a reliable model for patients with IFN-induced depression, which is useful for analyzing the relationship between IFN-α's effects on neural stem/progenitor cell function and on the induction of depressive behaviors.

IFN-α is reported to induce depression via upregulation of the hypothalamic-pituitary-adrenal (HPA) axis, alteration of monoamine neurotransmission, and induction of proinflammatory cytokines (Reyes-Vázquez et al., 2012; Schaefer et al., 2002). IFN-α can directly interact with opioid receptors (Jiang et al., 2000), which are also implicated in the induction of depressive behaviors (Makino et al., 2000a). Several proinflammatory cytokines, including interleukin (IL)-1 and IL-6, have been shown to modify the neurogenic behavior of NSCs (Gonzalez-Perez et al., 2012; Kohman and Rhodes, 2013). We previously found that IFN-α treatment suppresses cell proliferation in the hippocampal neurogenic region (Kaneko et al., 2006), which might mediate depression. Here, close examination revealed that chronic IFN-α treatment reduced the number of NSCs by nearly 40%, but not that of oligodendrocyte progenitors, another population that proliferates continuously in the adult brain (Figures 1 and S1M). Additionally, in vitro experiments showed that IFN-α significantly inhibited NSC proliferation, but did not affect their survival or neuronal differentiation, despite the presence of IFNAR on differentiated neurons, astrocytes, and oligodendrocytes (Figures 2, 3, and S2). Taken together, our findings indicate that NSCs in the adult brain may be a primary target of IFN-α. Indeed, the neurogenesis inhibition and depressive-behavior induction by chronic mIFN-α treatment were completely abrogated by CNS-specific and systemic IFNAR knockouts (Figure 5), suggesting that IFNAR in the brain mediates both of these effects of IFN-α.

Although the IFN-α molecule is large, a small fraction of systemically administered IFN-α penetrates the brain in areas where the blood brain barrier is more permeable (Biddle, 2006; Pan et al., 1997). In addition, IFN-α treatment increased the expression of endogenous IFN-α in the hippocampus (data not shown). Therefore, both exogenously administered and locally produced IFN-α can be involved in activating IFNAR signaling in the brain.

Although IFN-α treatment inhibited hippocampal neurogenesis and caused depressive behaviors, it is still unclear whether decreased neurogenesis directly affects mood and emotional regulation. New neurons have electrophysiological features that are distinct from those of mature granule cells and play a critical role in the plasticity of hippocampal circuitry (Nakashiba et al., 2012; Schmidt-Hieber et al., 2004), which is considered to be important for adaptation to environmental changes and stress coping (Eisch and Petrik, 2012). However, because ablation of hippocampal neurogenesis does not always cause depressive-like symptoms (Jayatissa et al., 2010), it is controversial whether new neurons participate in mood or emotional control (Eisch and Petrik, 2012). Impaired social behavior coincides with depression-like behaviors in some mouse lines, such as heat shock factor 1 knockout (Uchida et al., 2011) and RGS2 mutant (Lifschytz et al., 2012) mice. However, little is known about the neuronal circuits responsible for depression-like and/or social behaviors. Further studies are needed to understand how IFN treatment affects sociability.

Some proinflammatory cytokines, including IL-1 and IL-6, induce the secretion of glucocorticoid (Dunn, 2000), a negative regulator of adult hippocampal neurogenesis.

IFN-α also induces glucocorticoid secretion by stimulating the release of corticotropin-releasing hormone in the hypothalamus, followed by activation of the HPA axis (Gisslinger et al., 1993). Moreover, hippocampal neurogenesis negatively regulates the HPA axis, thereby reducing stress responses (Snyder et al., 2011). Because excessive activation of the HPA axis is thought to play a role in depression (Nestler et al., 2002), it is possible that the decreased neurogenesis caused by IFN-α (Figures 1 and 5) leads to depression via HPA axis dysregulation. Thus, although the precise relationships among depression, neurogenesis, and the HPA axis remain unclear, their interactions could amplify the depression-promoting effects of IFN-α.

In conclusion, we demonstrated that chronic peripheral administration of mIFN-α inhibited neurogenesis and induced depressive behavioral phenotypes via IFNAR expressed in the brain. The NSCs were remarkably responsive to IFN stimulation, exhibiting reduced proliferation and survival. Although more comprehensive studies are needed to elucidate the mechanism, these findings improve our understanding of the neuropathology of IFN-α-induced effects and may lead to new strategies targeting NSCs and/or neurogenesis for the prevention and treatment of IFN-α-induced depression. Furthermore, our simple pharmacologically induced depression model may be useful for analyzing the molecular mechanisms of neurogenesis-dependent mood and emotional regulation.

EXPERIMENTAL PROCEDURES

Animals

Male 8-week-old C57BL/6J mice were purchased from SLC. *IFNAR*$^{-/-}$ (Müller et al., 1994), *IFNAR*$^{fl/fl}$ (Detje et al., 2009), and *IFNAR*$^{fl/fl}$:*Nes-Cre* mice (Detje et al., 2009; Tronche et al., 1999; at least 10-fold backcrossed to the C57BL/6J background) were described previously. All experiments using live animals were performed in accordance with the guidelines and regulations of Nagoya City University and National Institute for Physiological Sciences.

mIFN-α Treatment and BrdU Labeling

PBS or mouse IFN-α (mIFN-α; 1×10^5 or 4×10^5 IU/kg) diluted with PBS was intraperitoneally injected into mice once a day for 2, 4, or 5 weeks. BrdU was intraperitoneally injected (50 mg/kg) six times at 8 hr intervals during the first 2 days of the fifth week or just prior to mIFN-α treatment. The fixed brains were processed to generate 50-μm-thick floating sections as previously described (Kaneko et al., 2010).

Neural Stem Cell Culture

Adult rat hippocampal NSCs were kindly provided by Dr. Fred Gage (Salk Institute). Neurosphere cultures were prepared as previously described (Hitoshi et al., 2002). For details, see Supplemental Information.

Behavioral Testing

A comprehensive battery of behavioral tests was performed as previously described (Miyakawa et al., 2003; Takao et al., 2010) using mice that had received a 4-week mIFN-α (4×10^5 IU/kg) treatment. For details, see Supplemental Information.

Statistical Analysis

All data were expressed as the mean ± SEM. Differences between means were determined by two-tailed Student's t test, one-way ANOVA, or two-way repeated-measures ANOVA followed by Tukey-Kramer multiple comparison tests, unless specified otherwise. A p value of <0.05 was considered significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2014.05.015.

AUTHOR CONTRIBUTIONS

L.-S.Z., S.H., N.K., K.T., and H.X. performed experiments and analyzed data. N.K., Y.T., T.M., K.K., S.K., U.K., K.I., and K.S. designed the experiments. L.-S.Z., S.H., N.K., and K.S. wrote the manuscript, and the whole project was supervised by N.K. and K.S.

ACKNOWLEDGMENTS

We thank Dr. Wataru Ukai (Sapporo Medical University) for his valuable comments on the manuscript; Dr. Fred H. Gage (Salk Institute) for providing the adult hippocampal progenitor cells; Dr. Rudiger Klein (Max Planck Institute) for providing Nes-Cre mice; Dr. Kinichi Nakashima (Kyushu University), Mr. Hiroshi Ishikawa (Nara Institute of Science and Technology), Dr. Ichiro Miyoshi, and Ms. Sayuri Nakamura (Nagoya City University) for technical support; and members of the K.S. laboratory for valuable discussions. This work was supported by a research grant from the Japanese Ministry of Health, Labour and Welfare (to S.H., Y.T., N.K., and K.S.), the Funding Program for the Next Generation World-Leading Researchers grant number LS104 (to K.S.), JSPS KAKENHI grant numbers 23680041 and 25111727 (to N.K.), grants from Takeda Science Foundation (to N.K. and K.S.), Terumo Life Science Foundation (to K.S.), and Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

Received: December 28, 2013
Revised: May 21, 2014
Accepted: May 21, 2014
Published: June 26, 2014

REFERENCES

Airan, R.D., Meltzer, L.A., Roy, M., Gong, Y., Chen, H., and Deisseroth, K. (2007). High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science *317*, 819–823.

Altman, J., and Das, G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol. *124*, 319–335.

Anacker, C., Zunszain, P.A., Cattaneo, A., Carvalho, L.A., Garabedian, M.J., Thuret, S., Price, J., and Pariante, C.M. (2011). Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol. Psychiatry *16*, 738–750.

Biddle, C. (2006). The neurobiology of the human febrile response. AANA J. *74*, 145–150.

Block, W., Träber, F., von Widdern, O., Metten, M., Schild, H., Maier, W., Zobel, A., and Jessen, F. (2009). Proton MR spectroscopy of the hippocampus at 3 T in patients with unipolar major depressive disorder: correlates and predictors of treatment response. Int. J. Neuropsychopharmacol. *12*, 415–422.

Bonaccorso, S., Puzella, A., Marino, V., Pasquini, M., Biondi, M., Artini, M., Almerighi, C., Levrero, M., Egyed, B., Bosmans, E., et al. (2001). Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res. *105*, 45–55.

Campbell, S., Marriott, M., Nahmias, C., and MacQueen, G.M. (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. Am. J. Psychiatry *161*, 598–607.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron *62*, 479–493.

De La Garza, R., 2nd, Asnis, G.M., Pedrosa, E., Stearns, C., Migdal, A.L., Reinus, J.F., Paladugu, R., and Vemulapalli, S. (2005). Recombinant human interferon-alpha does not alter reward behavior, or neuroimmune and neuroendocrine activation in rats. Prog. Neuropsychopharmacol. Biol. Psychiatry *29*, 781–792.

Detje, C.N., Meyer, T., Schmidt, H., Kreuz, D., Rose, J.K., Bechmann, I., Prinz, M., and Kalinke, U. (2009). Local type I IFN receptor signaling protects against virus spread within the central nervous system. J. Immunol. *182*, 2297–2304.

Deutsch, M., and Hadziyannis, S.J. (2008). Old and emerging therapies in chronic hepatitis C: an update. J. Viral Hepat. *15*, 2–11.

Dieperink, E., Willenbring, M., and Ho, S.B. (2000). Neuropsychiatric symptoms associated with hepatitis C and interferon alpha: A review. Am. J. Psychiatry *157*, 867–876.

Dunn, A.J. (2000). Cytokine activation of the HPA axis. Ann. N Y Acad. Sci. *917*, 608–617.

Eisch, A.J., and Petrik, D. (2012). Depression and hippocampal neurogenesis: a road to remission? Science *338*, 72–75.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H. (1998). Neurogenesis in the adult human hippocampus. Nat. Med. *4*, 1313–1317.

Fahey, B., Hickey, B., Kelleher, D., O'Dwyer, A.M., and O'Mara, S.M. (2007). The widely-used anti-viral drug interferon-alpha induces depressive- and anxiogenic-like effects in healthy rats. Behav. Brain Res. *182*, 80–87.

Felger, J.C., Alagbe, O., Hu, F., Mook, D., Freeman, A.A., Sanchez, M.M., Kalin, N.H., Ratti, E., Nemeroff, C.B., and Miller, A.H. (2007). Effects of interferon-alpha on rhesus monkeys: a nonhuman primate model of cytokine-induced depression. Biol. Psychiatry *62*, 1324–1333.

Gage, F.H. (2002). Neurogenesis in the adult brain. J. Neurosci. *22*, 612–613.

Gage, F.H., Coates, P.W., Palmer, T.D., Kuhn, H.G., Fisher, L.J., Suhonen, J.O., Peterson, D.A., Suhr, S.T., and Ray, J. (1995). Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc. Natl. Acad. Sci. USA *92*, 11879–11883.

Gilbertson, M.W., Shenton, M.E., Ciszewski, A., Kasai, K., Lasko, N.B., Orr, S.P., and Pitman, R.K. (2002). Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. Nat. Neurosci. *5*, 1242–1247.

Gisslinger, H., Svoboda, T., Clodi, M., Gilly, B., Ludwig, H., Havelec, L., and Luger, A. (1993). Interferon-alpha stimulates the hypothalamic-pituitary-adrenal axis in vivo and in vitro. Neuroendocrinology *57*, 489–495.

Gonzalez-Perez, O., Gutierrez-Fernandez, F., Lopez-Virgen, V., Collas-Aguilar, J., Quinones-Hinojosa, A., and Garcia-Verdugo, J.M. (2012). Immunological regulation of neurogenic niches in the adult brain. Neuroscience *226*, 270–281.

Gould, E., Reeves, A.J., Fallah, M., Tanapat, P., Gross, C.G., and Fuchs, E. (1999). Hippocampal neurogenesis in adult Old World primates. Proc. Natl. Acad. Sci. USA *96*, 5263–5267.

Greig, N.H., Soncrant, T.T., Wozniak, K.M., and Rapoport, S.I. (1988). Plasma and tissue pharmacokinetics of human interferon-alpha in the rat after its intravenous administration. J. Pharmacol. Exp. Ther. *245*, 574–580.

Hauser, P., Khosla, J., Aurora, H., Laurin, J., Kling, M.A., Hill, J., Gulati, M., Thornton, A.J., Schultz, R.L., Valentine, A.D., et al. (2002). A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol. Psychiatry *7*, 942–947.

Hayley, S., Scharf, J., and Anisman, H. (2013). Central administration of murine interferon-α induces depressive-like behavioral, brain cytokine and neurochemical alterations in mice: a mini-review and original experiments. Brain Behav. Immun. *31*, 115–127.

Hitoshi, S., Alexson, T., Tropepe, V., Donoviel, D., Elia, A.J., Nye, J.S., Conlon, R.A., Mak, T.W., Bernstein, A., and van der Kooy, D. (2002). Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev. *16*, 846–858.

Huang, Y., Chen, W., Li, Y., Wu, X., Shi, X., and Geng, D. (2010). Effects of antidepressant treatment on N-acetyl aspartate and choline levels in the hippocampus and thalami of post-stroke depression patients: a study using (1)H magnetic resonance spectroscopy. Psychiatry Res. *182*, 48–52.

Jacobs, B.L., van Praag, H., and Gage, F.H. (2000). Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol. Psychiatry *5*, 262–269.

Jayatissa, M.N., Henningsen, K., Nikolajsen, G., West, M.J., and Wiborg, O. (2010). 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 *13*, 95–105.

Jiang, C.L., Son, L.X., Lu, C.L., You, Z.D., Wang, Y.X., Sun, L.Y., Cui, R.Y., and Liu, X.Y. (2000). Analgesic effect of interferon-alpha via mu opioid receptor in the rat. Neurochem. Int. *36*, 193–196.

Kaneko, N., Kudo, K., Mabuchi, T., Takemoto, K., Fujimaki, K., Wati, H., Iguchi, H., Tezuka, H., and Kanba, S. (2006). Suppression of cell proliferation by interferon-alpha through interleukin-1 production in adult rat dentate gyrus. Neuropsychopharmacology *31*, 2619–2626.

Kaneko, N., Marín, O., Koike, M., Hirota, Y., Uchiyama, Y., Wu, J.Y., Lu, Q., Tessier-Lavigne, M., Alvarez-Buylla, A., Okano, H., et al. (2010). New neurons clear the path of astrocytic processes for their rapid migration in the adult brain. Neuron *67*, 213–223.

Kaplan, M.S., and Hinds, J.W. (1977). Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science *197*, 1092–1094.

Kelsch, W., Mosley, C.P., Lin, C.W., and Lois, C. (2007). Distinct mammalian precursors are committed to generate neurons with defined dendritic projection patterns. PLoS Biol. *5*, e300.

Kempermann, G., and Kronenberg, G. (2003). Depressed new neurons—adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol. Psychiatry *54*, 499–503.

Kohman, R.A., and Rhodes, J.S. (2013). Neurogenesis, inflammation and behavior. Brain Behav. Immun. *27*, 22–32.

Kornack, D.R., and Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. Proc. Natl. Acad. Sci. USA *96*, 5768–5773.

Kuhn, H.G., Dickinson-Anson, H., and Gage, F.H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. *16*, 2027–2033.

Lieb, K., Engelbrecht, M.A., Gut, O., Fiebich, B.L., Bauer, J., Janssen, G., and Schaefer, M. (2006). Cognitive impairment in patients with chronic hepatitis treated with interferon alpha (IFNalpha): results from a prospective study. Eur. Psychiatry *21*, 204–210.

Lifschytz, T., Broner, E.C., Zozulinsky, P., Slonimsky, A., Eitan, R., Greenbaum, L., and Lerer, B. (2012). Relationship between Rgs2 gene expression level and anxiety and depression-like behaviour in a mutant mouse model: serotonergic involvement. Int. J. Neuropsychopharmacol. *15*, 1307–1318.

Loftis, J.M., Wall, J.M., Pagel, R.L., and Hauser, P. (2006). Administration of pegylated interferon-alpha-2a or -2b does not induce sickness behavior in Lewis rats. Psychoneuroendocrinology *31*, 1289–1294.

Lucassen, P.J., Meerlo, P., Naylor, A.S., van Dam, A.M., Dayer, A.G., Fuchs, E., Oomen, C.A., and Czéh, B. (2010). Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. Eur. Neuropsychopharmacol. *20*, 1–17.

Makino, M., Kitano, Y., Hirohashi, M., and Takasuna, K. (1998). Enhancement of immobility in mouse forced swimming test by treatment with human interferon. Eur. J. Pharmacol. *356*, 1–7.

Makino, M., Kitano, Y., Komiyama, C., Hirohashi, M., and Takasuna, K. (2000a). Involvement of central opioid systems in human interferon-alpha induced immobility in the mouse forced swimming test. Br. J. Pharmacol. *130*, 1269–1274.

Makino, M., Kitano, Y., Komiyama, C., and Takasuna, K. (2000b). Human interferon-alpha increases immobility in the forced swimming test in rats. Psychopharmacology (Berl.) *148*, 106–110.

Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J. Neurosci. *20*, 9104–9110.

Manganas, L.N., Zhang, X., Li, Y., Hazel, R.D., Smith, S.D., Wagshul, M.E., Henn, F., Benveniste, H., Djuric, P.M., Enikolopov, G., and Maletic-Savatic, M. (2007). Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. Science *318*, 980–985.

Mathews, E.A., Morgenstern, N.A., Piatti, V.C., Zhao, C., Jessberger, S., Schinder, A.F., and Gage, F.H. (2010). A distinctive layering pattern of mouse dentate granule cells is generated by developmental and adult neurogenesis. J. Comp. Neurol. *518*, 4479–4490.

Merkle, F.T., Mirzadeh, Z., and Alvarez-Buylla, A. (2007). Mosaic organization of neural stem cells in the adult brain. Science *317*, 381–384.

Merkle, F.T., Fuentealba, L.C., Sanders, T.A., Magno, L., Kessaris, N., and Alvarez-Buylla, A. (2014). Adult neural stem cells in distinct microdomains generate previously unknown interneuron types. Nat. Neurosci. *17*, 207–214.

Miyakawa, T., Leiter, L.M., Gerber, D.J., Gainetdinov, R.R., Sotnikova, T.D., Zeng, H., Caron, M.G., and Tonegawa, S. (2003). Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc. Natl. Acad. Sci. USA *100*, 8987–8992.

Moy, S.S., Nadler, J.J., Perez, A., Barbaro, R.P., Johns, J.M., Magnuson, T.R., Piven, J., and Crawley, J.N. (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes Brain Behav. *3*, 287–302.

Müller, U., Steinhoff, U., Reis, L.F., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., and Aguet, M. (1994). Functional role of type I and type II interferons in antiviral defense. Science *264*, 1918–1921.

Nakashiba, T., Cushman, J.D., Pelkey, K.A., Renaudineau, S., Buhl, D.L., McHugh, T.J., Rodriguez Barrera, V., Chittajallu, R., Iwamoto, K.S., McBain, C.J., et al. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. Cell *149*, 188–201.

Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., and Monteggia, L.M. (2002). Neurobiology of depression. Neuron *34*, 13–25.

Orsal, A.S., Blois, S.M., Bermpohl, D., Schaefer, M., and Coquery, N. (2008). Administration of interferon-alpha in mice provokes peripheral and central modulation of immune cells, accompanied by behavioral effects. Neuropsychobiology *58*, 211–222.

Pan, W., Banks, W.A., and Kastin, A.J. (1997). Permeability of the blood-brain and blood-spinal cord barriers to interferons. J. Neuroimmunol. *76*, 105–111.

Papatheodoridis, G.V., Manolakopoulos, S., Dusheiko, G., and Archimandritis, A.J. (2008). Therapeutic strategies in the

management of patients with chronic hepatitis B virus infection. Lancet Infect. Dis. *8*, 167–178.

Pechnick, R.N., Zonis, S., Wawrowsky, K., Cosgayon, R., Farrokhi, C., Lacayo, L., and Chesnokova, V. (2011). Antidepressants stimulate hippocampal neurogenesis by inhibiting p21 expression in the subgranular zone of the hipppocampus. PLoS ONE *6*, e27290.

Porsolt, R.D., Bertin, A., and Jalfre, M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. *229*, 327–336.

Raison, C.L., Borisov, A.S., Broadwell, S.D., Capuron, L., Woolwine, B.J., Jacobson, I.M., Nemeroff, C.B., and Miller, A.H. (2005). Depression during pegylated interferon-alpha plus ribavirin therapy: prevalence and prediction. J. Clin. Psychiatry *66*, 41–48.

Reyes-Vázquez, C., Prieto-Gómez, B., and Dafny, N. (2012). Interferon modulates central nervous system function. Brain Res. *1442*, 76–89.

Samuels, B.A., and Hen, R. (2011). Neurogenesis and affective disorders. Eur. J. Neurosci. *33*, 1152–1159.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science *301*, 805–809.

Schaefer, M., Engelbrecht, M.A., Gut, O., Fiebich, B.L., Bauer, J., Schmidt, F., Grunze, H., and Lieb, K. (2002). Interferon alpha (IFNalpha) and psychiatric syndromes: a review. Prog. Neuropsychopharmacol. Biol. Psychiatry *26*, 731–746.

Schmidt-Hieber, C., Jonas, P., and Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature *429*, 184–187.

Smith, R.A., Norris, F., Palmer, D., Bernhardt, L., and Wills, R.J. (1985). Distribution of alpha interferon in serum and cerebrospinal fluid after systemic administration. Clin. Pharmacol. Ther. *37*, 85–88.

Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., and Cameron, H.A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature *476*, 458–461.

Steru, L., Chermat, R., Thierry, B., and Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl.) *85*, 367–370.

Tagliaferri, P., Caraglia, M., Budillon, A., Marra, M., Vitale, G., Viscomi, C., Masciari, S., Tassone, P., Abbruzzese, A., and Venuta, S. (2005). New pharmacokinetic and pharmacodynamic tools for interferon-alpha (IFN-alpha) treatment of human cancer. Cancer Immunol. Immunother. *54*, 1–10.

Takao, K., Tanda, K., Nakamura, K., Kasahara, J., Nakao, K., Katsuki, M., Nakanishi, K., Yamasaki, N., Toyama, K., Adachi, M., et al. (2010). Comprehensive behavioral analysis of calcium/calmodulin-dependent protein kinase IV knockout mice. PLoS ONE *5*, e9460.

Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H., and Gage, F.H. (2007). Synapse formation on neurons born in the adult hippocampus. Nat. Neurosci. *10*, 727–734.

Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P.C., Bock, R., Klein, R., and Schütz, G. (1999). Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat. Genet. *23*, 99–103.

Uchida, S., Hara, K., Kobayashi, A., Fujimoto, M., Otsuki, K., Yamagata, H., Hobara, T., Abe, N., Higuchi, F., Shibata, T., et al. (2011). Impaired hippocampal spinogenesis and neurogenesis and altered affective behavior in mice lacking heat shock factor 1. Proc. Natl. Acad. Sci. USA *108*, 1681–1686.

van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H. (2002). Functional neurogenesis in the adult hippocampus. Nature *415*, 1030–1034.

Wang, J., Campbell, I.L., and Zhang, H. (2008). Systemic interferon-alpha regulates interferon-stimulated genes in the central nervous system. Mol. Psychiatry *13*, 293–301.

Yamano, M., Yuki, H., Yasuda, S., and Miyata, K. (2000). Corticotropin-releasing hormone receptors mediate consensus interferon-alpha YM643-induced depression-like behavior in mice. J. Pharmacol. Exp. Ther. *292*, 181–187.

Zhao, C., Deng, W., and Gage, F.H. (2008). Mechanisms and functional implications of adult neurogenesis. Cell *132*, 645–660.