

Mechanisms for Interferon- α -Induced Depression and Neural Stem Cell Dysfunction

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<http://dx.doi.org/10.1016/j.stemcr.2014.05.015>

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SUMMARY

New neurons generated by the neural stem cells (NSCs) in the adult hippocampus play an important role in emotional regulation and respond to the action of antidepressants. Depression is a common and serious side effect of interferon- α (IFN- α), which limits its use as an antiviral and antitumor drug. However, the mechanism(s) underlying IFN-induced depression are largely unknown. Using a comprehensive battery of behavioral tests, we found that mice subjected to IFN- α treatment exhibited a depression-like phenotype. IFN- α directly suppressed NSC proliferation, resulting in the reduced generation of new neurons. Brain-specific mouse knockout of the IFN- α receptor prevented IFN- α -induced depressive behavioral phenotypes and the inhibition of neurogenesis, suggesting that IFN- α suppresses hippocampal neurogenesis and induces depression via its receptor in the brain. These findings provide insight for understanding the neuropathology underlying IFN- α -induced depression and for developing new strategies for the prevention and treatment of IFN- α -induced depressive effects.

INTRODUCTION

New neurons are continuously generated in the hippocampal dentate gyrus (DG) throughout life in mammals, including rodents (Altman and Das, 1965; Kaplan and Hinds, 1977; Kuhn et al., 1996), nonhuman primates (Gould et al., 1999; Kornack and Rakic, 1999), and humans (Eriksson et al., 1998; Manganas et al., 2007). In the DG, neural stem cells (NSCs) residing in the subgranular zone (SGZ), a thin cell layer between the granule cell layer (GCL) and the dentate hilus, generate transit-amplifying intermediate progenitors that give rise to new neurons (Gage, 2002; Zhao et al., 2008). The newly generated neurons then migrate into the GCL, where they differentiate into mature granule cells to be integrated into the hippocampal circuitry (Mathews et al., 2010; Toni et al., 2007; van Praag et al., 2002). Evidence suggests that neurogenesis in this region plays a role in emotional regulation (Eisch and Petrik, 2012; Samuels and Hen, 2011).

Decreased neurogenesis in the adult DG is implicated in the pathophysiology of depression, a common psychiatric

disorder. Clinical imaging studies demonstrated reduced volume and altered metabolism in the hippocampus of depressed patients (Block et al., 2009; Campbell et al., 2004; Gilbertson et al., 2002; Huang et al., 2010). Hippocampal neurogenesis is downregulated in animal models of depression induced by exposure to chronic psychosocial stress (Jacobs et al., 2000; Kempermann and Kronenberg, 2003). Conversely, chronic treatment with antidepressants enhances hippocampal neurogenesis (Anacker et al., 2011; Malberg et al., 2000; Pechnick et al., 2011), which is required for the behavioral effects of these drugs in mice (Santarelli et al., 2003). However, the relationship between neurogenesis suppression and depressive symptoms remains elusive (Airan et al., 2007; David et al., 2009; Lucasen et al., 2010). Animal models of depression induced by a single ligand and its receptor would be useful for investigating these mechanisms in vivo using genetic approaches.

Interferon- α (IFN- α), a proinflammatory cytokine with potent antiviral, antiproliferative, and immunoregulatory effects, has been widely used to treat chronic viral hepatitis and several types of malignancy (Deutsch and

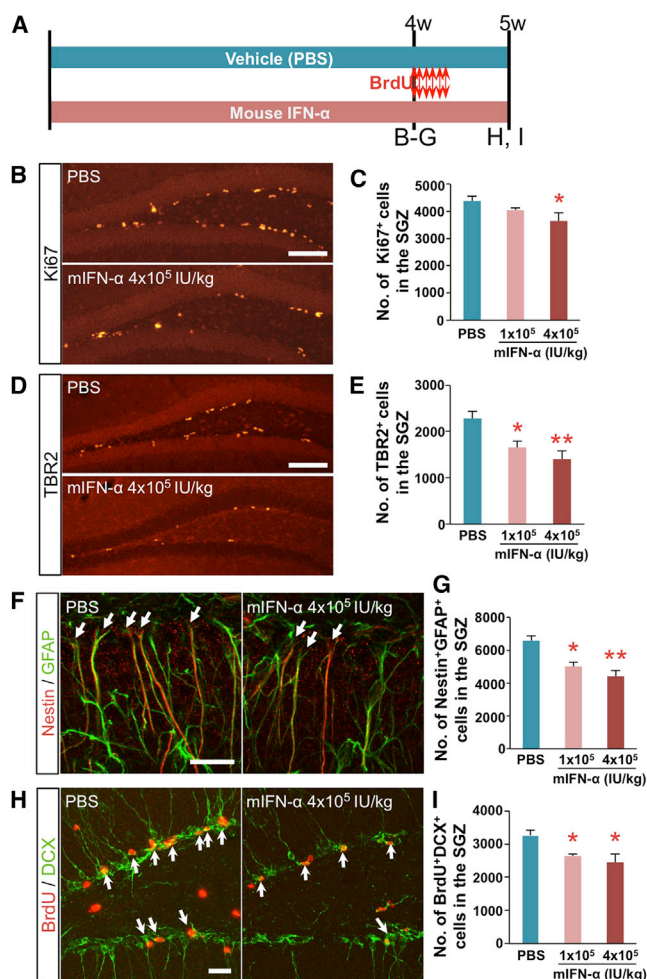


Figure 1. Chronic Treatment with mIFN- α Affects Neurogenesis in the DG of Adult Mice

(A) Experimental design.

(B–E) Effect of 4-week mIFN- α treatment on SGZ proliferative activity. The numbers of Ki67⁺ cells (B and C) and TBR2⁺ cells (D and E) in the SGZ were significantly reduced in mIFN- α -treated groups compared with the PBS-treated group. $n = 7$ mice per group.

(F and G) Effect of 4-week mIFN- α treatment on NSCs in the DG. The number of Nestin⁺ (red) and GFAP⁺ (green) putative NSCs in the DG (F, arrows) was significantly reduced by mIFN- α treatment (G) in a dose-dependent manner. $n = 5$ mice per group.

(H and I) Effect of mIFN- α treatment on neurogenesis in the DG. During mIFN- α treatment, BrdU was injected at the beginning of the fifth week, six times every 8 hr. The number of BrdU⁺ (red) and DCX⁺ (green) cells (H, arrows) was significantly reduced in mIFN- α -treated groups (I). $n = 5$ mice per group. * $p < 0.05$, ** $p < 0.01$ versus PBS-treated group; error bars: means \pm SEM; scale bars, 100 μ m: (B and D), 25 μ m: (F and H). See also Figure S1.

Hadziyannis, 2008; Papatheodoridis et al., 2008; Tagliaferri et al., 2005). However, long-term IFN- α treatment frequently triggers a variety of neuropsychiatric symptoms

(Dieperink et al., 2000). Depression is the most common and serious side effect, affecting approximately 30%–45% of patients receiving IFN- α treatment, resulting in occasional discontinuation of the therapy (Bonaccorso et al., 2001; Lieb et al., 2006). Despite its clinical importance, the mechanism underlying IFN- α -induced depression is still not well understood.

We previously reported that repeated IFN- α treatment suppresses cell proliferation in the SGZ of adult rats (Kaneko et al., 2006). However, little is known about how peripheral IFN- α affects brain function. Because a small fraction of peripheral IFN- α gains access to the brain (Greig et al., 1988; Smith et al., 1985), hippocampal neurogenesis can be directly affected by the increased IFN- α signaling in the brain (Wang et al., 2008). However, it is also possible that IFN- α affects brain function via secondary effectors such as humoral or cellular components of the peripheral immune system (Hayley et al., 2013; Orsal et al., 2008).

Here, we analyzed the effects of IFN- α treatment on neurogenesis and depressive behaviors using two types of interferon- α receptor (IFNAR) knockout (KO) mouse lines: a systemic KO (IFNAR^{-/-}; Müller et al., 1994) and a conditional KO in NSCs and their progenies (IFNAR^{fl/fl}:Nes-Cre; Detje et al., 2009). Our findings suggest that peripherally administered IFN- α directly suppresses the neurogenic function of NSCs and increases depression-like behaviors.

RESULTS

Chronic mIFN- α Treatment Reduces Cell Proliferation and Neurogenesis in the DG of Adult Mice

To investigate the effects of chronic mouse IFN- α (mIFN- α) treatment on cell proliferation in the DG, mice were intraperitoneally injected with PBS or mIFN- α daily for 2 or 4 weeks (Figures S1A–S1E available online and Figure 1A). The numbers of SGZ cells that were positive for the proliferation marker Ki67 (Figures 1B and 1C) and the neuronal progenitor marker TBR2 (Figures 1D and 1E) were significantly reduced by the 4-week, but not the 2-week, mIFN- α treatment (Figures S1A–S1C). The 4-week mIFN- α treatment also reduced the number of cells with radial glia-like morphology expressing the NSC markers Nestin and GFAP (Figures 1F and 1G). To quantify neurogenesis, the mice were injected with BrdU six times at 4 weeks of treatment and fixed at 5 weeks of mIFN- α treatment (Figure 1A). The number of BrdU⁺DCX⁺ new neurons in the DG was also significantly reduced in mIFN- α -treated mice compared with PBS-treated controls (Figures 1H and 1I).

To examine the effect of mIFN- α treatment on the survival and fate of the newly generated cells in the DG, we labeled the new neurons with bromodeoxyuridine (BrdU) just before the treatment and quantified the number of



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 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 (10–10³ 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 10³ 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

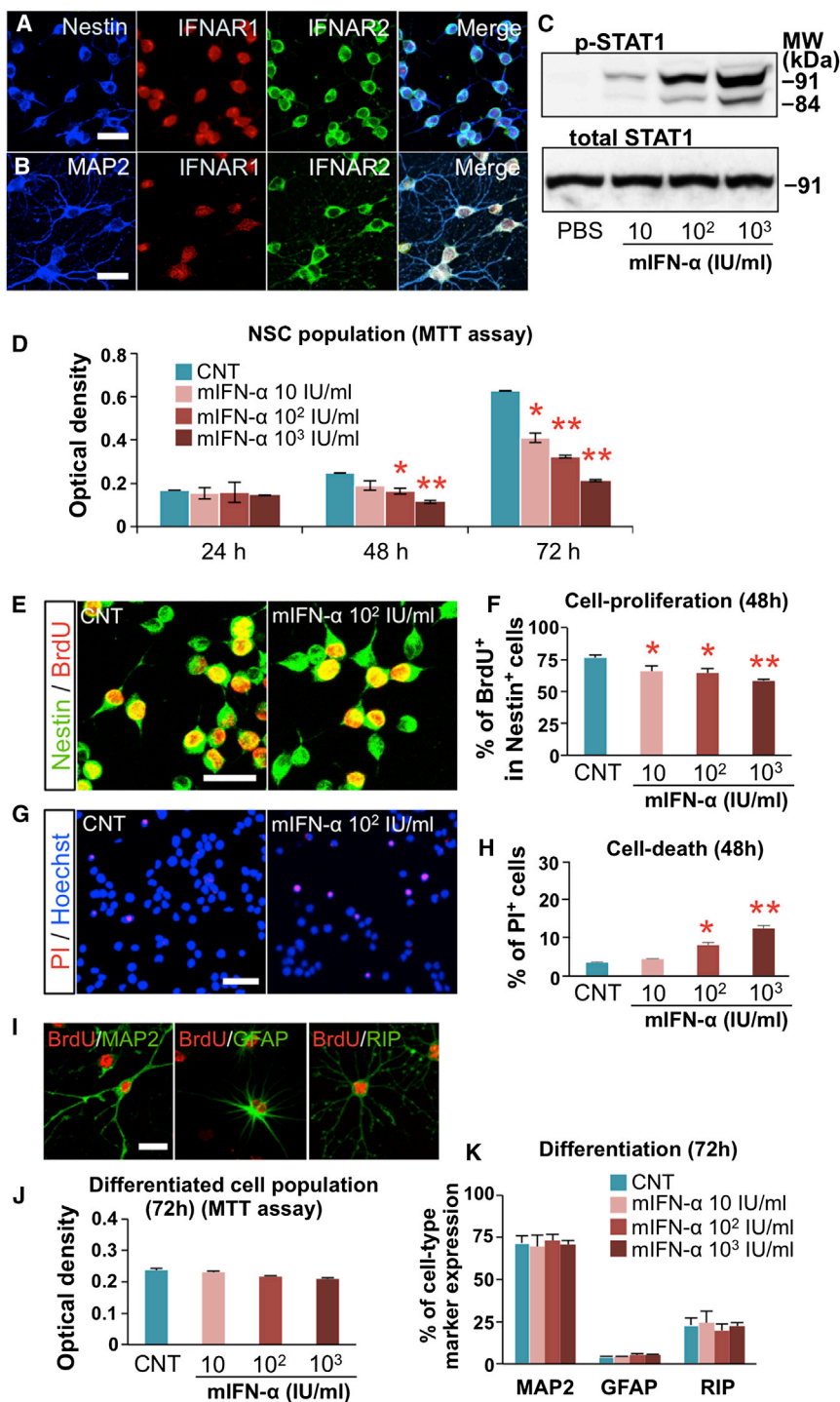


Figure 2. Treatment of Adult Hippocampal NSC Cultures with mIFN-α

(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

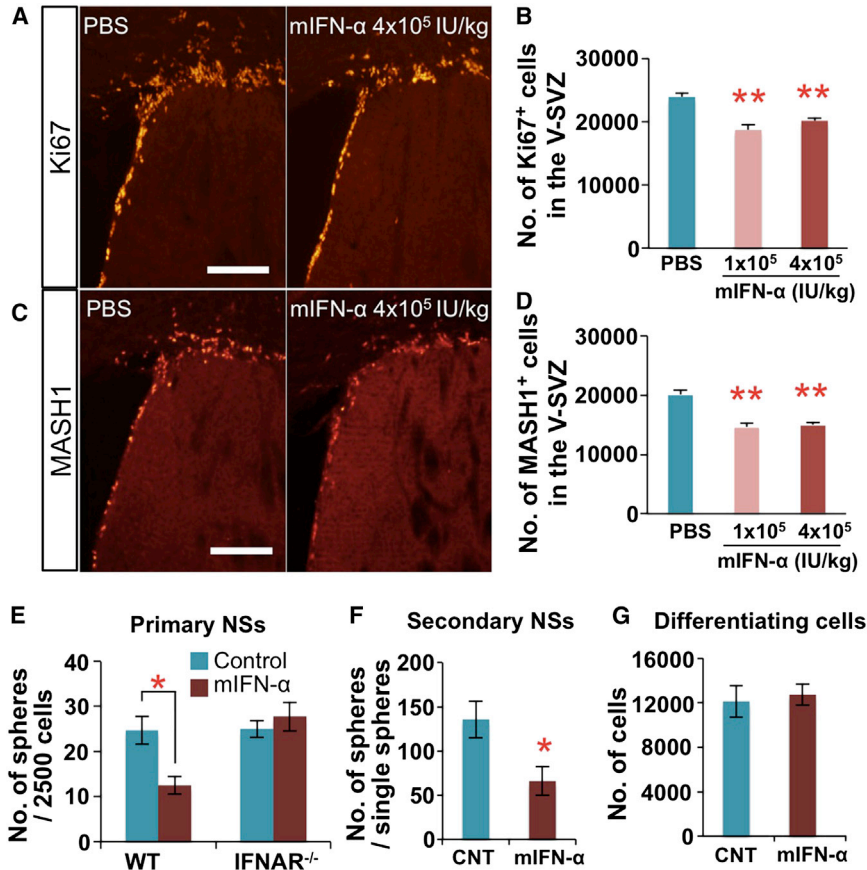


Figure 3. Effects of mIFN- α Treatment on NSCs and Neuronal Progenitor Cells in the Adult V-SVZ

(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 10³ 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 10³ 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 10³ 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 \pm SEM. The scale bars represent 100 μ m. 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

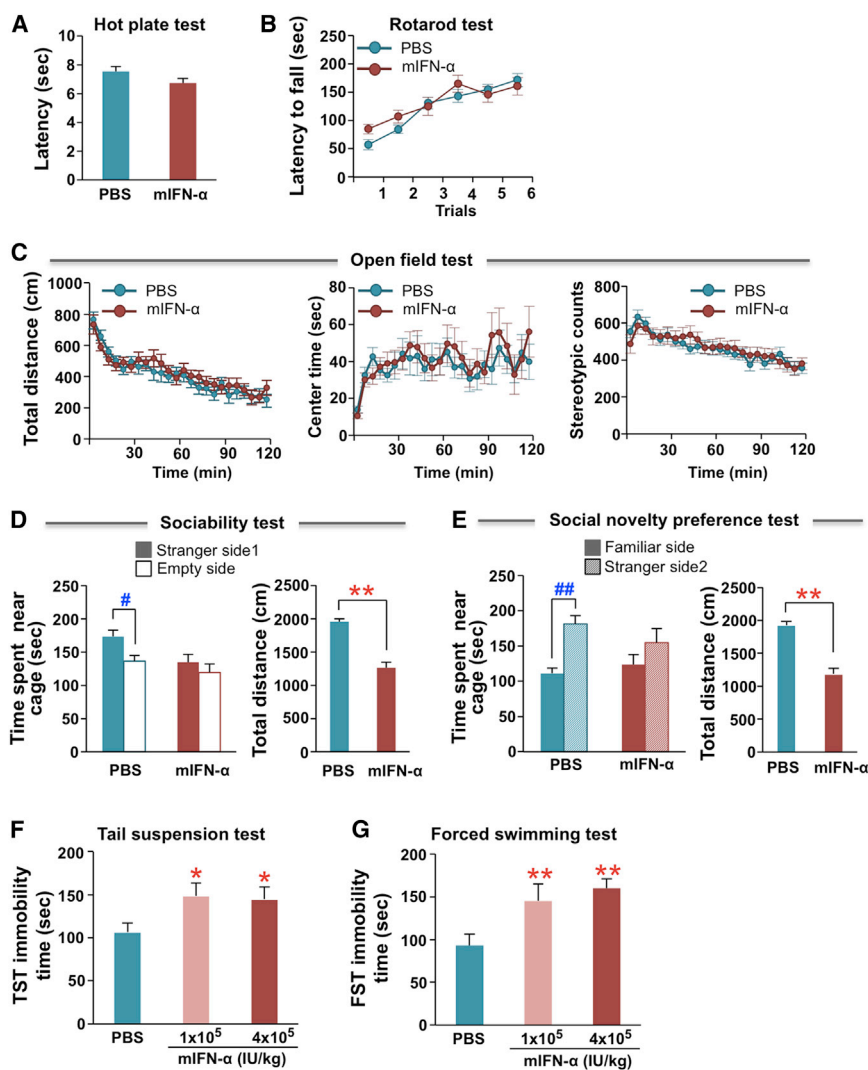


Figure 4. Effects of Chronic mIFN- α Treatment on Mouse Behavioral Profiles

(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 \pm 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

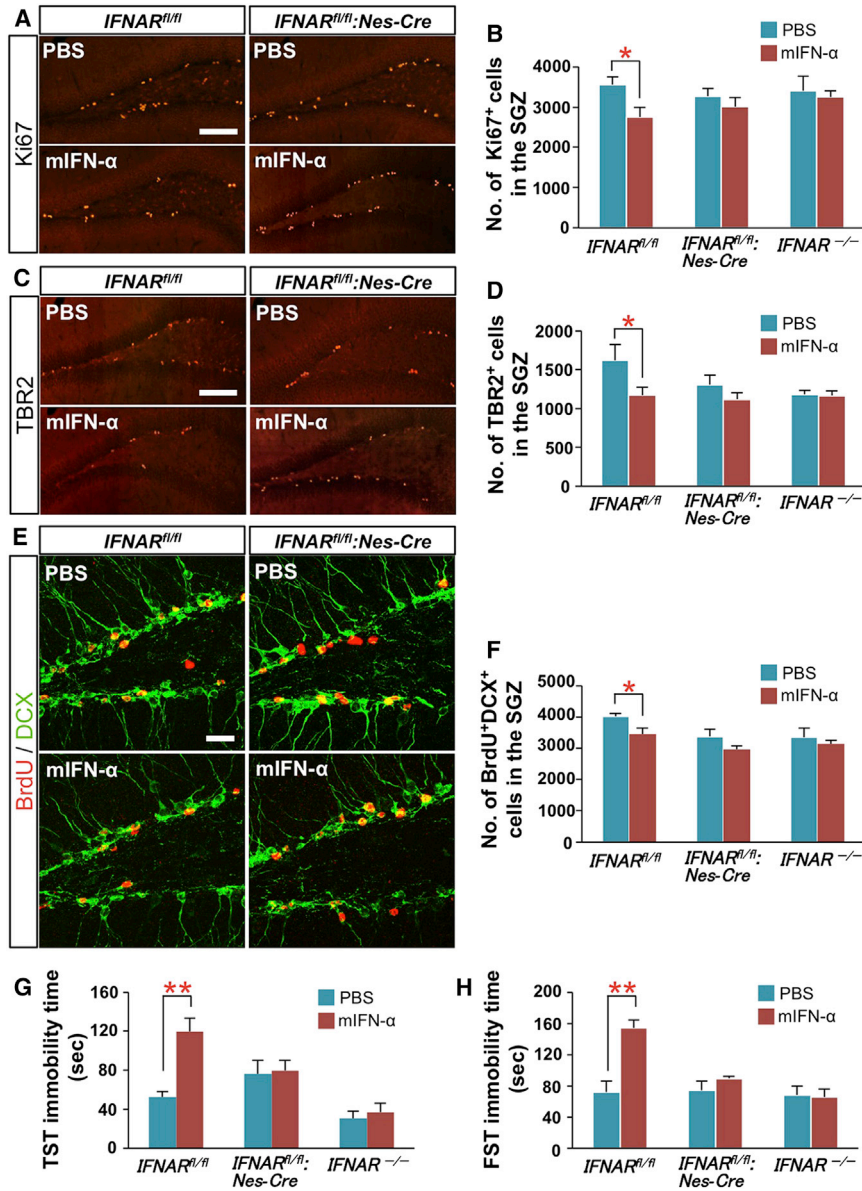


Figure 5. Effects of Chronic mIFN-α Treatment on Hippocampal Neurogenesis and Behaviors in IFNAR Knockout Mice

(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 (Gislinger 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- μ m-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 \pm 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

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