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Qiang Liu  
*Barrow Neurological Institute*, qiang.liu@dignityhealth.org

Wei Xin

Ping He

Dharshaun Turner

Junxiang Yin

*See next page for additional authors*

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Authors
Qiang Liu, Wei Xin, Ping He, Dharshaun Turner, Junxiang Yin, Yan Gan, Fu Dong Shi, and Jie Wu
Interleukin-17 inhibits Adult Hippocampal Neurogenesis

Qiang Liu1,2*, Wei Xin1*, Ping He3, Dharshaun Turner1, Junxiang Yin1, Yan Gan1, Fu-Dong Shi1,2 & Jie Wu1,4

1Divisions of Neurology, Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, Phoenix, AZ, USA, 2Department of Neurology, Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin 300052, China, 3Department of Chemical Engineering, Arizona State University, Tempe, AZ, USA, 4Department of Physiology, Shantou University of Medical College, Shantou, Guangdong, China.

Interleukin 17(A) (IL-17) is a potent pro-inflammatory cytokine that acts as a central regulator of inflammatory response within the brain, but its physiological roles under non-inflammatory conditions remain elusive. Here we report that endogenous IL-17 ablates neurogenesis in the adult dentate gyrus (DG) of hippocampus. Genetic deletion of IL-17 increased the number of adult-born neurons in the DG. Further, we found that IL-17 deletion altered cytokine network, facilitated basal excitatory synaptic transmission, enhanced intrinsic neuronal excitability, and increased expression of proneuronal genes in neuronal progenitor cells (NPCs). Our findings suggest a profound role of IL-17 in the negative regulation of adult hippocampal neurogenesis under physiology conditions.

Neurogenesis in the subgranular zone (SGZ) of dentate gyrus is a major mechanism that occurs in the adult hippocampus to maintain its function and plasticity in response to extrinsic and intrinsic changes1–3. Abnormal neurogenesis in SGZ is likely to exacerbate major neurological disorders such as Alzheimer’s disease, depression and drug addiction1–4. An understanding of how adult-born neurons develop and integrate into preformed neural networks may suggest targets for therapeutic intervention and assist in developing stem cell therapies for repairing damaged neuronal tissue5,6.

Environmental factors such as inflammatory mediators can influence neurogenesis9,10. Among these inflammatory mediators, interleukin 17 (A) (IL-17) has been found to play a pivotal role in the pathogenesis of numerous inflammatory diseases in the central nervous system (CNS), such as multiple sclerosis and stroke11,12. IL-17 acts synergistically with tumor necrosis factor (TNF) and IL-1 to shape CNS inflammation. Besides Th17 cells, a variety of cell types can produce IL-17 including γδ T cells, natural killer (NK) cells, NKT cells and lymphoid tissue inducer cells. In addition to immune cells, glial cells in the CNS also express IL-17 under physiology conditions13–15. IL-17 functions through a distinct ligand-receptor signaling system, IL-17 receptor (IL-17R), which is widely expressed and binds IL-17 with high affinity16. IL-17R deficiency results in reduced chemokine levels17 and its signaling has been implicated in both innate and adaptive immunity18. Recently, the expression of IL-17R has been detected within the CNS and upregulated under inflammatory conditions19,20. However, little is known about the effects of IL-17 on neurogenesis under non-inflammatory conditions.

In this study, we examined the role of endogenous IL-17 in hippocampal neurogenesis under physiological condition. Our results demonstrate that IL-17 is a negative regulator of adult hippocampal neurogenesis in the DG of hippocampus. Using IL-17 KO mice, we provided evidence that the absence of IL-17 significantly improved neurogenesis in the DG, enhanced synaptic function, reduced expression of inflammatory cytokines, and increased expression of proneuronal genes in NPCs.

Methods

Animals. All mice were C57BL/6 background littermates and used at 2–3 months of age. IL-17 (A) KO mice were provided by Y. Iwakura (University of Tokyo, Tokyo, Japan). All experiments were performed in accordance with approved guidelines set by the Barrow Neurological Institute Ethical Committee.

Real-time RT-PCR. Total RNA was extracted from brain tissues or cultured progenitors with TRIzol (Invitrogen, CA). First-strand cDNA of each sample was synthesized using a reverse transcription kit (Invitrogen, CA). RT-PCR was performed as we previously described21, using an ABI Prism 7900 HT sequence system (Applied Biosystems, CA) with the QuantiTect SYBR Green PCR kit (QIAGEN, CA), in accordance with the manufacturer’s instructions. The GAPDH gene was amplified and served as an endogenous control. All primer sequences used in this study were listed in supplemental Table 1. 1 μl of first-strand cDNA product was amplified with platinum Taq polymerase (Invitrogen,
CA) and gene-specific primer pairs. Each sample was assayed in triplicate and experiments were repeated twice. The relative amounts of mRNA were calculated by plotting the Ct (cycle number). The mean relative expression was determined by the 2^-ΔΔCt comparative method and expressed by relative expression levels against housekeeping gene (i.e., GAPDH).

**Cell cultures.** The method for cultures of neurons from adult DG of mouse hippocampus is the standard procedure as we previously described. Briefly, the DG was dissected under a stereotrophic microscope. Tissue was minced with scissors in ice-cold Neurobasol medium (Invitrogen, Carlsbad, CA) and then digested with Papain (20 unit/mg, Worthington, NJ) at 30°C for 20 min in tubes shaken at 120 rpm in a water bath shaker. After enzyme digestion, the reaction was ceased by adding inactivated fetal bovine serum into the medium. Then, digested tissue was filtered and transferred into 15 ml tubes. After trituration, tissue was centrifuged at 1500 rpm for 5 min to form pellets containing dissociated cells, and the supernatant was removed and replaced with Neurobasol medium, which was used to resuspend the pellets. This process was repeated three times. After the final centrifugation, the supernatant was replaced with Neurobasol medium supplemented with 0.5% (w/v) L-glutamine and 2% B27 serum-free supplement. Cells were re-suspended and counted based on Trypan blue exclusion and plated at a density of 4.5-5.0 × 10^5 cells/cm² in 35 mm Falcon culture dishes (BD Biosciences, San Jose, California). Cells were kept within the 37°C/5% CO₂ incubator for further use.

Primary cultures of mouse astrocytes were prepared from the DG of C57BL/6 newly born mice (postnatal 1-3 days) as previously described. Briefly, the DG was dissected under sterile conditions and the tissues were dissociated in 0.25% trypsin (Invitrogen, CA) for 10 minutes at 37°C. The cell suspension was separated by centrifugation at 1500 rpm for 5 minutes, and the cells were transferred to poly-D-lysine precoated cell culture flasks in DMEM containing 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Before the experiments, the purity of astrocytes in cultures was >95% determined by an astrocyte marker, GFAP (Santa Cruz, CA). Cells were used for ELISA assay after 10-14 days in culture.

To prepare primary microglial cells as previously described, after reaching a confluent monolayer of glial cells (10–14 days), microglia were separated from astrocytes by shaking off for 5 h at 100 rpm and re-plated on 24-well plates. After plating the microglia-enriched population for 48 h, cells were used for ELISA assay. The purity was >98% as determined by a microglia marker, Iba1 (Ike, NY).]

NPC cultures. NPC cultures derived from adult mice hippocampus were performed as previously described. Briefly, mice were deeply anesthetized using halothane and decapitated, and brains were removed. Hippocampi were dissected, pooled, and dissociated in DMEM (Invitrogen, CA) containing 2.5 U/ml papain, 1 U/ml dispase II, and 250 U/ml DNase I (Invitrogen, CA). Cells were used for ELISA assay after 10–14 days in culture.

ELISA. Measurement of IL-17 levels in mouse DG homogenates and cultured cell lysates was performed with an ELISA kit specific to murine IL-17 (A). Procedures were carried out according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). Results are expressed in pg/ml protein for samples from DG homogenates or for samples from cultured cell lysates. Data were from three individual experiments performed in triplicate. A Multi-Analyte ELISArray kit (SABioscience) was used to measure inflammatory cytokines in homogenates of WT and IL-17 KO DG tissues, according to the manufacturer’s instructions, as we previously described.

Flow cytometry. Single cell suspensions (0.5 × 10^6 cells) were prepared from cultured neuron, astrocytes, and NPCs and stained with fluorochrome-conjugated antibodies (eBioscience and Santa Cruz). Antibodies were directly labeled with one of the following fluorescent tags: FITC, PE, APC, or PerCP. Flow cytometric data were collected on a FACSAria flow cytometer and analyzed with Diva software.

BrdU labeling. BrdU was injected intraperitoneally at 10 mg/ml in sterile 0.9% NaCl to yield a single dose of 50 µg/g body weight on each of 4 consecutive days. Mice were singly housed for 2 or 4 weeks after the initial injection until tissue preparation. After perfusion, all steps were performed blind to genotype.

Tissue Preparation and immunohistochemistry. Mice were anesthetized by intraperitoneal injection of 10 mg/ml ketamine and 1 mg/ml xylazine in sterile 0.9% NaCl at a volume of 1.0 ml/20 g body weight. The punch was used to detach effectiveness of anesthesia. Animals were transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). Whole brain was removed and post-fixed in 4% PFA in PBS overnight, then equilibrated in 30% sucrose. Next, the tissue was coronal slices (40 µm) were cut using a cryostat microtome and processed for BrdU immunohistochemistry.

For BrdU and neuron-specific nuclear protein (NeuN) double-labeled immunofluorescence, images were captured and quantitated by observers who were blind to genotype. Free-floating sections were denatured in 2N HCl for 30 min at 37°C, rinsed in KPBS (to neutralize pH), preincubated with 2% goat and donkey serum in 0.3% Triton X-100 for 1 h, and incubated overnight with rabbit anti-BrdU antibody (1:100; Santa Cruz, CA) and mouse anti-NeuN antibody (1:100; Millipore, MA) or rabbit anti-DCX (1:100; Sigma, St. Louis, MO) or rabbit anti-GFAP (1:100; Millipore, MA) at 4°C. Sections were then rinsed and incubated in the dark for 2 h with Alexa Fluor 685 goat anti-rat IgG antibody (1:1000; Invitrogen, CA) and Alexa Fluor 685 donkey anti mouse IgG antibody (1:1000; Invitrogen, CA). Sections were washed with PBS 3 times for 10 minutes. Sections were then mounted on glass slides, and coverslipped with glycerol-based mounting medium (for fluorescence microscopy). After immunostaining, counts were made of BrdU-positive cells in the first third of the granule cell layer in every fourth section through the entire hippocampus. Adult-born neurons were defined as the first third of the granule cell layer. Imaging was performed within 48 hours of immunostaining using a Leica TCS STED CW confocal microscope. For BrdU-1 cells counts in brain sections, images were captured mainly from germinal regions but other areas were also screened to ensure a complete analysis.

Colocalization of BrdU-positive cells with NeuN was validated using a confocal scanning light microscope (MRC1024UV, Bio-Rad, Hemel Hempstead, UK) with krypton/argon 488 and 568 excitation filter. A double-stained cell was defined as having the strongest intensity of both stainings within the same or directly neighboring 1-µm-thick optical section through the cell in a consecutive Z-series of at least four sections, with an overlap of the stainings in at least three sections. Stereological estimations of the total number of BrdU+ cells in the SGZ of the dorsal hippocampal formation were performed using the optical fractionator method.

Hippocampal slice preparation. Hippocampal slices that were prepared as described previously. After isoflurane anesthesia, brain tissue was quickly removed and placed in oxygenated ACSF (artificial cerebral spinal fluid) (ACSF): 119 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl₂, 1.25 mM MgCl₂, and 11.0 mM glucose. The ACSF was continuously bubbled with 95% O₂/5% CO₂ for 30 minutes. Tissue was cut using a vibratome (Vibroslice 725 M, WPI, Sarasota, FL) and transferred to a holding chamber and incubated at room temperature (21 ± 1°C) for at least 60 min prior to recording.

Electrophysiology. Current-clamp recordings to measure neuronal excitability were performed with a modified protocol as previously described. Electrophysiology recordings were performed by experimenters who were blind to genotype. All recordings were performed at 31 ± 1°C. Neuronal intrinsic excitability was measured as the number of spikes in response to a series of fixed 500 ms current injection steps (0, 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, and 0.21 nA). Data were acquired by Axonpatch 200 amplifier at 2 kHz with pClamp 9.2 software (Molecular Devices, Sunnyvale, CA) and Mini Analysis software (Synaptosoft, Decatur, CA). For field potential recordings from slices of hippocampal DG, a stimulating electrode was placed in the angular bundle of the perforant path and a recording electrode in the hilus of the DG. A single slice was transferred to a liquid-air interface chamber (Fine Science Tools, Inc., Foster City, CA, USA) and transferred on a nylon net at the liquid-air interface in a bath of continuously dripping oxygenated ACSF (2–2.5 ml/min).

**Drugs and Solutions.** All drugs used in this study were purchased from Sigma (St. Louis, MO). Mouse IL-17 was purchased from Cell Signaling (Cell Signaling, MA). For patch-clamp recording from hippocampal cultures, the standard extracellular solution contained 140 mM NaCl, 3.3 mM CaCl₂, 2 mM MgCl₂, 10 mM d-glucose, 10 mM HEPES, adjusted to pH 7.4 with Tris base. The internal solution for current-clamp recordings to measure intrinsic excitability contained 130 mM KMeSO₄, 10 mM KCl, 10 mM HEPES/K-HEPES, 2 mM MgSO₄, 0.5 mM EGTA and 3 mM ATP; the pH was adjusted to 7.3 with KOH. For AMPA receptor-mediated miniature EPSC (mEPSC) recordings, 0.3 µM TTX, 50 µM picrotoxin, and 50 µM APV were added into external solution. For whole-cell recordings of ligand-gated ion channels, the internal solution contains the following: 140 mM potassium glutamate, 5 mM KCl, 10 mM HEPES, 0.2 mM MgATP, 2 mM MgCl₂, 4 mM MgATP, 0.3 mM Na₂GTP and 10 mM Na₂-picosphosphatase (pH 7.3 with KOH). For field potential recordings, the internal solution was 2 M NaCl.

**Results**

**IL-17 and IL-17R are expressed in the DG of hippocampus.** To evaluate the expression of IL-17 and its receptor (IL-17R) in the DG of hippocampus, we performed ELISA assay and found that IL-17 mRNA and protein are expressed in WT DG tissues (Figure 1A–B). To identify the source of IL-17 in WT DG tissues, we cultured astrocytes, microglia, neurons and NPCs. Thereafter, we used fluorescent-activated cell sorting (FACS) with intracellular staining to compare the expression of IL-17 among these cells. We found that
astrocytes are the major source of IL-17 under physiological condition (Figure 1C–D). To assess the expression of IL-17R on NPCs, we examined the expression of IL-17R mRNA and protein via RT-PCR and FACS, respectively. We found that majority of NPCs expressed IL-17R (Figure F–G).

Genetic deletion of IL-17 promotes neurogenesis in the DG of hippocampus. To determine whether endogenous IL-17 influences adult-born neuron proliferation and survival, we birthdated adult-born neurons in wild-type (WT) and IL-17 knockout (KO) mice by injecting BrdU and following their fate. Hippocampal slices were taken for BrdU immunostaining after 2 and 4 weeks to allow quantification before and after the critical period for activity-dependent adult-born neuron proliferation and survival. A significant increase in the number of BrdU-labeled cells was seen in the dentate gyrus of IL-17 KO mice as compared to age-matched WT mice at both 2 and 4 weeks (Figure 2A–B). The results indicate that IL-17 signaling appears to negatively regulate adult-born neurogenesis.

We then explored whether the absence of IL-17 can influence the formation of immature and mature neurons. IL-17 KO and WT mice were injected with BrdU once daily for 4 days and sacrificed 2 or 4 weeks. We found that the BrdU⁺ cells were distributed mainly in the SGZ/GCL. Genetic deletion of IL-17 caused a significant increase in the number of BrdU⁺ cells double labeled for both the specific marker of immature neurons (DCX) at 2 weeks after BrdU injections and mature neurons (NeuN) in the SGZ/GCL compared to WT animals at 4 weeks after BrdU injections (Figure 2C–F). These results suggest that deletion of IL-17 increases both adult-born immature and mature neurons in the DG of hippocampus.

Endogenous IL-17 regulates cytokine network in the DG of hippocampus. IL-17 participates in the cytokine network in the DG of hippocampus. Our finding that the deletion of IL-17 promoted neurogenesis suggests that endogenous IL-17 may influence cytokine environment in the DG. To test this possibility, we compared expression profile of key pro-inflammatory cytokines in the DG of hippocampus from IL-17 KO and WT mice. Using an ELISA array kit, we found that the deletion of IL-17 reduced the expression of TNF-α, IL-1β, IL-6, and IFN-γ, as compared to WT controls (Figure 3). These data suggest that IL-17 deletion may regulate the cytokine network in the DG, where progenitors reside.

Deficiency of IL-17 alters synaptic functions in the DG of hippocampus. To investigate the neurophysiological consequences of IL-17 deletion in the DG of hippocampus, we examined baseline synaptic transmission and synaptic short- and long-term potentiation (LTP) in the DG of hippocampus in both IL-17 KO and WT mice. For field potential recordings from slices of hippocampal DG, the stimulating electrode was placed in the angular bundle of the perforant path and a recording electrode in the hilus of the DG. We conducted a systematic evaluation of changes in DG synaptic functions in IL-17 KO mice (Figure 4A). Although no changes in LTP was observed (Figure 4B), we found that the input-output (I/O) curve of field EPSP (fEPSP) slopes in response to different stimulation intensities were shifted to the left when compared with those of WT controls (Figure 4C), suggesting that basal synaptic transmission was significantly enhanced in the DG of IL-17 KO mice. We next examined synaptic short plasticity using paired-pulse stimulation protocol and found that the paired-pulse facilitation (PPF) was indistinguishable between IL-17 KO mice and WT controls at 50 ms inter-pulse intervals. However, PPF was significantly enhanced when the inter-pulse intervals were 100, 150 and 500 ms (Figure 4D). Therefore, short-lived presynaptic plasticity was increased in IL-17 KO mice.

Increased intrinsic excitability and excitatory synaptic activities on adult-born IL-17 KO neurons from the DG of hippocampus cultures. Next, we compared neuronal intrinsic excitability and glutamatergic inputs between these adult-born IL-17 KO neurons and WT controls by performing somatic whole-cell current-clamp recordings in cultured hippocampal DG neurons dissociated from mice injected with BrdU (i.p. injected with BrdU for 4 days before cell dissociation). After DIV for over 14 days, the cultured cells were recorded and biocytin was microinjected afterward. Then biocytin-injected cells were stained with streptavidin conjugated antibody and anti-BrdU antibody to confirm that the recorded cells were newly born from the DG of hippocampus following BrdU injections (Figure 5A). We examined the effects of IL-17 deletion on intrinsic membrane excitability in response to injection of a 90 pA current from cultured BrdU⁺ neurons. Results showed that IL-17 deletion increased neuronal excitability represented as an increase in spike
numbers (Figure 5B). I/O relationships derived from plots of spike numbers occurring each 500 ms as a function of injected, different depolarizing currents were used to evaluate neuronal excitability. IL-17 KO mice exhibited a significantly increased neuronal excitability represented as a left shift of I-O relationship curve (in response to injected currents ranging from 0-180 pA) compared to WT controls (Figure 5C). These results suggest that IL-17 deletion enhances intrinsic excitability in the newly born neurons from DG of hippocampus.

To test the impact of IL-17 deletion on synaptic excitatory glutamatergic activities, we examined the AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid) receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) that occur spontaneously in cultured neurons in the presence of tetrodotoxin. We found a significant increase in both mEPSC amplitude (Figure 5D–E) and frequency (Figure 5D–F) in cultured IL-17 KO neurons as compared to WT controls. Statistical analysis demonstrated that the mEPSC amplitude was increased in IL-17 KO neurons as compared to that of WT controls (Figure 5E). Meanwhile, mEPSC frequency was enhanced in IL-17 KO neurons as well (Figure 5F). These results suggest that deletion of IL-17 results in increased mEPSC amplitude and frequency, which may be caused by increased post-synaptic AMPARs levels and the probability of presynaptic vesicular glutamate release. In the absence of IL-17, adult-born neurons receive more synaptic activity, offering a possible explanation for the increased neurogenesis in the DG of hippocampus.

IL-17 regulates the expression of the proneuronal and proglial genes in adult-born DG neural stem cells. Since most of the neuronal progenitors are regulated by proneural genes, we asked whether the expressions of these proneural genes were changed in IL-17 KO progenitors. By using real-time RT-PCR techniques, we measured the messenger expression levels of neurogenin 2 (Ngn2), mammalian achaete-scute homolog 1 (Mash1), and neurogenic...
function of IL-17 is much less studied. One recent report suggests the ligand are directly mediated by IL-17R on these cells. Furthermore, IL-17R is expressed by various CNS cells. One recent study also showed that IL-17 treatment can reduce NPC proliferation and differentiation by acting on IL-17R in vitro. In the present study, we identified astrocytes as a major source of IL-17 in the DG tissues, and IL-17R is expressed by NPCs. Our results show the anti-proneurogenic effects of proinflammatory cytokines, because loss of endogenous IL-17 potentiates neurogenesis, as evidenced by the experiments with IL-17 KO mice in vivo. Nevertheless, the role of IL-17 in neurogenesis during pathological conditions and in the cross-talk between glial cells and NPCs require further investigation, which could be a relevant molecular link during the development of neuroinflammation and brain repair.

Neurogenesis is affected by their local cytokine environment. For example, several studies show that TNF-α, IL-1β and IL-6 inhibit neurogenesis by activating their receptors. The net effects of IL-17 on neurogenesis are most likely dependent on its levels and on the expression of IL-17R in the progenitor cells. IL-17 binds to IL-17R with high affinity. The altered CNS inflammatory environment may underlie this enhanced neurogenesis in the DG. On the other hand, the intracellular mechanisms mediating the actions of IL-17 through its receptor IL-17R on progenitor proliferation and survival in vivo are largely unknown. Previous findings demonstrated that activation of IL-17R induced cell apoptosis by activating caspase-3, caspase-9 and up-regulating the ratio of Bax/Bcl-2. We observed
that both IL-17 and IL-17R were expressed in DG tissues as well as in cultured progenitors (Figure 2), although at a relatively low amount, which is similar to what has been reported previously in rats and humans\(^1\)\(^3\)\(^4\)\(^5\). Thus, the ligand acting on the IL-17R could originate from the CNS-resident cells including hippocampal progenitors themselves. In addition, we found that deletion of endogenous IL-17 alters the cytokine environment in the DG tissues, including several proinflammatory cytokines that may be detrimental for neurogenesis. This altered cytokine microenvironment may be involved in the enhanced neurogenesis caused by the deletion of IL-17 in the DG of hippocampus.

Our findings on the deletion of IL-17 promoting basal neurotransmission and intrinsic excitability do not conclude that increased neurogenesis in IL-17 KO mice results from the neurophysiological consequences of IL-17 deletion. Recent evidence suggests that neurogenesis can be directly coupled with neuronal excitation\(^4\)\(^6\)\(^7\), and the survival of NPC-derived immature neurons through the critical period depends on their excitatory inputs\(^4\)\(^6\)–\(^8\). Meanwhile, several lines of studies also support that increased neurogenesis can modulate synaptic transmission and neuronal activities\(^4\)\(^9\)–\(^5\)\(^1\). These studies have highlighted the link between neurogenesis and neuronal network activities. In this study, we found that deletion of IL-17 increased basal synaptic transmission, as well as the intrinsic excitability and glutamatergic inputs of newly born neurons. These results suggest that endogenous IL-17 can regulate neuronal network activities. Further investigations are warranted to reveal whether or not the increased neurogenesis seen in IL-17 KO mice results from these neurophysiological consequences of IL-17 deletion.

Our results show altered expression of proneural and glia genes in cultured NPCs from IL-17 KO mice. These findings are consistent with previous studies that inhibited expression of glia fate genes but increased proneural genes promotes neurogenesis\(^3\)\(^0\)–\(^3\)\(^1\). We found that

**Figure 5** Adult-born neurons lacking IL-17 exhibit increased intrinsic excitability and receive more excitatory synaptic activity. (A). A representative cultured neuron from the adult DG of hippocampus (i.p. injected with BrdU for 4 days before cell dissociation) was recorded and microinjected with biocytin then immunostained with streptavidin conjugated antibody (green) and BrdU antibody (red). (B). Typical traces of action potential generation in response to 90 pA current injection (note the -60 mV holding potential and the time course and membrane potential calibration bars) show that deletion of IL-17 signaling causes increased neuronal excitability in BrdU\(^+\) primary cultured neuron from the adult DG of hippocampus. (C). Calculated input-output relationships for action potential spike activity (ordinate) as a function of currents injected (0-180 pA) in cultured BrdU\(^+\) cortical neurons prepared from the adult DG of hippocampus. Results show that deletion of IL-17 induces the increase of action potential numbers as compared to WT controls. (D–F). Deletion of IL-17 also increases the amplitude and frequency of mEPSCs recorded from cultured BrdU\(^+\) neurons from the DG of hippocampus as compared to those from WT controls. \(n = 8\). Means ± SEM; *\(p < 0.05\); **\(p < 0.01\).

**Figure 6** Endogenous IL-17 regulates the expression of proneuronal and proglial genes. Real-time RT-PCR analysis of the messenger expression levels of proneural genes (NGN2, MASH1, and NeuroD1) and proglia genes (HES1 and Id2) in cultured NPCs from IL-17 KO and WT mice. The mRNA expression of NGN2, Mash1, and NeuroD1 were increased in IL-17 KO progenitor cells compared with WT controls. Proglial gene expressions of HES1 and Id2 were decreased in IL-17 KO progenitors as compared to WT controls. Data were obtained from four individual experiments performed in triplicate. Means ± SEM; *\(p < 0.05\); **\(p < 0.01\).
deletion of IL-17 down-regulates Hes1 and Id2 and up-regulates MASH1, NGN2, and NeuroD messengers in cultured NPCs, which may be related to the increased differentiation toward neurons seen in IL-17-KO progenitors.

Our data shows that endogenous IL-17 can negate regulation of adult neurogenesis in the DG of hippocampus. However, caveats should be taken when extrapolate these findings to all adult neurogenesis, including neurogenesis in the subventricular zone. Future studies are needed to further clarify whether endogenous IL-17 can influence neurogenesis in other brain regions as well.

In summary, we have demonstrated the involvement of endogenous IL-17 in the negative regulation of adult hippocampal neurogenesis under non-inflammatory conditions. Given that circumstantial evidence indicates a link between hippocampal neurogenesis and cognitive function, our findings suggest that the ablation of endogenous IL-17 may serve as a potential strategy to promote adult hippocampal neurogenesis.

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Author contributions
Q.L. designed and performed experiments, and wrote the main manuscript text. W.X. designed and performed experiments, and data analysis. P.H. performed cell biological experiments and data analysis. D.T. performed electrophysiological experiments and data analysis. J.X.Y. performed immunohistochemical staining and contributed to the Fig. 2. Y.G. performed some immunological experiments and contributed to the part of Fig. 1, 3. F.D.S. designed experiments and revised MS. J.W. designed experiments, wrote part of MS, and revised MS. All authors reviewed the manuscript.

Additional information
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