IL-1β is an essential mediator of the antineurogenic and anhedonic effects of stress

Ja Wook Koo*† and Ronald S. Duman†‡

*Department of Psychology, Yale University, New Haven, CT 06520-8205; and †Division of Molecular Psychiatry, Yale University School of Medicine, New Haven, CT 06508

Edited by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved November 16, 2007 (received for review August 27, 2007)

Stress decreases neurogenesis in the adult hippocampus, and blockade of this effect is required for the actions of antidepressants in behavioral models of depression. However, the mechanisms underlying these effects of stress have not been identified. Here, we demonstrate an essential role for the proinflammatory cytokine IL-1β. Administration of IL-1β or acute stress suppressed hippocampal cell proliferation. Blockade of the IL-1β receptor, IL-1RI, by using either an inhibitor or IL-1RI null mice blocks the antineurogenic effect of stress and blocks the anhedonic behavior caused by chronic stress exposure. *In vivo* and *in vitro* studies demonstrate that hippocampal neural progenitor cells express IL-1RI and that activation of this receptor decreases cell proliferation via the nuclear factor-κB signaling pathway. These findings demonstrate that IL-1β is a critical mediator of the antineurogenic and depressive-like behavior caused by acute and chronic stress.

cytokine | depression | neurogenesis

The profound consequences of stress exposure, defined as disturbances of physiological homeostasis, include a detrimental impact on certain aspects of brain function (1, 2). In particular, uncontrollable stress is a major contributing factor for neuropsychiatric disorders such as major depression and posttraumatic stress disorders (3, 4). Alterations at the cellular level in the hippocampus have been linked to the pathophysiology of stress-related mood disorders (5–7). Many studies demonstrate that stressful experiences suppress hippocampal neurogenesis, which could contribute to the hippocampal atrophy observed in depressed patients (8–10). In contrast, antidepressant treatment increases hippocampal neurogenesis, blocks the antineurogenic effects of stress (11, 12), and reduces or even reverses hippocampal atrophy (9, 11). Recent studies demonstrate that new hippocampal neurons are required for the actions of antidepressants in behavioral models of depression and anxiety (13, 14) with some exceptions (15, 16).

Despite this progress, the mechanisms underlying the antineurogenic and behavioral actions of stress remain ill defined. One possibility is that excessive proinflammatory cytokines, particularly IL-1β, contribute to the actions of stress. Animal studies demonstrate that exposure to stress increases IL-1β in several brain areas, including the hippocampus (17–20), and central administration of IL-1β produces several stress-like effects, including activation of the hypothalamic-pituitary-adrenal (HPA) axis (17, 21), inhibition of hippocampal long-term potentiation (22), down-regulation of hippocampal brain-derived neurotrophic factor (23), and impaired hippocampal-dependent contextual fear conditioning (24). In contrast, blockade of the receptor, IL-1RI that mediates the actions of IL-1β (25) inhibits these stress-like effects (23, 24) and blocks the antiproliferative effects of INF-α in the hippocampus (26).

Based on this evidence, the current study was undertaken to test the hypothesis that the antineurogenic and depressive actions of stress occur via activation of IL-1β signaling. We present *in vivo* and *in vitro* evidence in support of this hypothesis, further defining the molecular and cellular mechanisms of stress-like actions of IL-1β.

Results

IL-1β Decreases Hippocampal Cell Proliferation. Administration of exogenous IL-1β (i.c.v.) significantly decreased the number of BrdU$^+$ cells in the dentate gyrus (DG) (Fig. 1*A*). *Post hoc* analysis revealed that BrdU$^+$ cell numbers were significantly decreased at both the 20- and 100-ng-per-rat doses of IL-1β. The reduction in BrdU$^+$ cells caused by IL-1β (100 ng per rat) was completely blocked by administration of an IL-1β receptor antagonist, IL-1Ra, immediately before IL-1β infusion (Fig. 1*B*). IL-1β or IL-1Ra administration did not influence the number of BrdU$^+$ cells in the subventricular zone (SVZ) [supporting information (SI) Fig. 6], demonstrating that the effects observed in the hippocampal DG were not a result of nonspecific effects on BrdU uptake and/or labeling. TUNEL$^+$ cell number in the subgranular zone (SGZ) was not altered by IL-1β administration (Fig. 1 *C* and *F*), indicating that apoptotic mechanisms do not account for the decreased cell proliferation caused by IL-1β.

Inhibition of IL-1β Blocks the Decrease in Neurogenesis Caused by Acute Stress. Rats were administered IL-1Ra or vehicle and then exposed to either foot-shock or immobilization (Fig. 1*J*), stressors that have been shown to increase central IL-1β (19). Foot-shock significantly decreased BrdU$^+$ cell number in the DG (Fig. 1*G*), and this effect was completely blocked by preadministration of IL-1Ra. Similarly, immobilization significantly down-regulated BrdU$^+$ cell number, and this effect was also blocked by IL-1Ra (Fig. 1 *D*, *E*, and *H*). Immobilization stress and/or IL-1Ra did not influence the number of BrdU$^+$ cells in the SVZ, again demonstrating that the effect on hippocampal SGZ is region specific and not due to nonspecific effects on BrdU-labeling (SI Fig. 6). TUNEL staining demonstrates that immobilization did not influence cell death in SGZ (Fig. 1*I*).

Inhibition of IL-1β Blocks the Anhedonic and Antineurogenic Effects of Chronic Unpredictable Stress. To determine whether IL-1β underlies the effects of chronic unpredictable stress (CUS) on depressive-like behavior and hippocampal neurogenesis, IL-1Ra was administered (i.c.v., minipump) during 3 weeks of exposure to CUS. Immediately before and after CUS, rats were subjected to sucrose preference testing (Fig. 2*A*). A loss of sensitivity to reward is a model of anhedonia, a core symptom of human depression (27, 28). Exposure to CUS resulted in a significant decrease in sucrose preference in rats administered vehicle, but this effect was completely blocked by infusion of IL-1Ra (Fig. 2*B*). After CUS, IL-1Ra-infused rats (CUS + 1Ra) showed

Author contributions: J.W.K. and R.S.D. designed research; J.W.K. performed research; J.W.K. analyzed data; and J.W.K. and R.S.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

‡To whom correspondence should be addressed at: 34 Park Street, New Haven, CT 06508. E-mail: ronald.duman@yale.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0708092105/DC1.

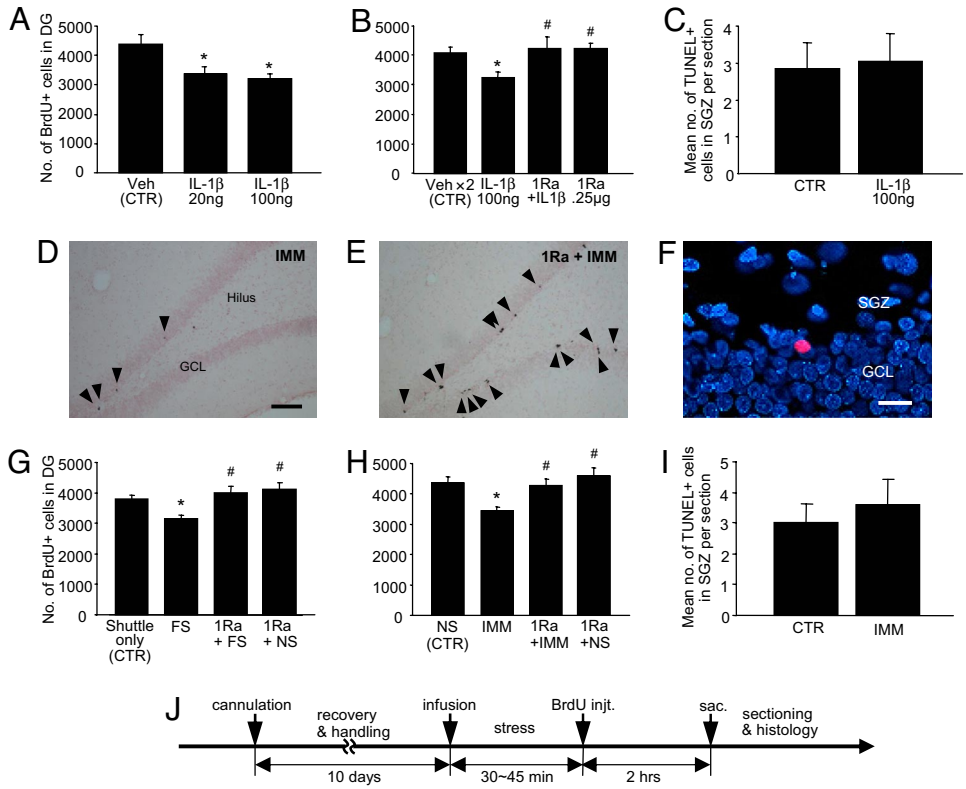

Fig. 1. Effects of IL-1β, acute stress, and/or IL-1Ra on cell proliferation and cell death in the DG of the hippocampus in rats. (*A*) IL-1β administration (20 or 100 ng per rat) decreased the number of BrdU$^+$ cells in the DG ($F_{2,26} = 4.059$, $P < 0.05$; $n = 8$–9 per group). (*B*) IL-1Ra administration (0.25 μg per rat) blocked the antiproliferative effect of IL-1β (100 ng per rat) ($F_{3,36} = 5.205$, $P < 0.01$; $n = 7$–10 per group). (*C*) IL-1β did not change the number of TUNEL$^+$ cells per DG ($t_6 = 0.674$, $P =$ n.s.; $n = 4$ per group). (*D* and *E*) Dividing BrdU$^+$ cells (black arrowheads) in the DG of immobilization (IMM)-stressed (*D*) and IL-1Ra infused IMM-stressed rats (*E*). (Scale bar, 100 μm.) (*F*) A representative TUNEL$^+$ cell (red) in the DG. Granular cell layer (GCL) is shown with DAPI+ staining (blue). SGZ, subgranular zone, (Scale bar, 20 μm.) (*G* and *H*) Inescapable foot-shock (FS) (*G*) and IMM stress (*H*) exposures also decreased the number of BrdU$^+$ cells in the DG. The antiproliferative effect of acute stress was blocked by IL-1Ra (0.25 μg per rat) (FS, $F_{3,30} = 6.298$, $P < 0.01$; IMM, $F_{3,34} = 5{,}445$, $P < 0.01$; $n = 5$–9 per group). (*I*) IMM did not affect the number of TUNEL$^+$ cells per DG ($t_6 = 1.450$, $P =$ n.s.; $n = 4$ per group). (*J*) A schematic diagram depicting the experimental procedures for acute stress. After 10-day recovery and handling, followed by cannulation, animals were infused with IL-1Ra or vehicle immediately before stress and given BrdU immediately after stress. Rats were killed (sac) 2 h after BrdU administration. *, $P < 0.05$ compared with CTR; #, $P < 0.05$ compared with 100 ng of IL-1β-infused or stressed rats in the Tukey's *post hoc* test, mean ± SEM.

significantly more preference for the sucrose solution than PBS-infused rats (CUS + PBS). The total intake of liquid (water plus sucrose solution) was unchanged between the groups (data not shown).

To assess the proliferation of cells, BrdU was administered before the last behavioral test and analyzed 14 days later. Consistent with the post-CUS sucrose preference data, there were significantly greater numbers of BrdU$^+$ cells in the CUS + 1Ra compared with the CUS + PBS rats (Fig. 2 *C*–*E*). The number of cells colabeled with doublecortin (DCX), a marker of immature neurons, and BrdU in the hippocampal DG was also significantly higher in the CUS + 1Ra rats compared with CUS + PBS rats (Fig. 2 *F* and *G*). Taken together, these results demonstrate that IL-1β is a key mediator of CUS on hippocampal neurogenesis and that IL-1β can modulate anhedonic behavior.

Effects of Acute and Chronic Stress Are Blocked in IL-1RI Null Mutant Mice. The role of IL-1β in the actions of acute and chronic stress was further tested in mice with a null mutation of the IL-1β receptor, IL-1RI. Exposure to acute stress significantly decreased BrdU$^+$ cell number in WT, but not in IL-1RI null mice (Fig. 3 *A*–*C*). There was a significant difference between WT and IL-1RI null mice exposed to stress, but not in the absence of stress. Similarly, the antiproliferative response to CUS was also blocked in IL-1RI null mice (Fig. 3*D*). CUS exposure significantly decreased BrdU$^+$ cell number in the WT mice compared with IL-1RI null mice, whereas in the absence of CUS there was no difference. A reduction in newborn neurons in response to CUS and blockade in IL-1RI null mice was confirmed by analysis of BrdU$^+$ cells colabeled with DCX (Fig. 3*E*).

The anhedonic effect of CUS was also blocked in IL-1RI null mice. CUS significantly decreased sucrose consumption in WT mice but not in IL-1RI null mice, and there was a significant difference between WT and IL-1RI null mice exposed to CUS (Fig. 3*F*). There was no difference in normal water consumption (Fig. 3*G*). Taken together, these data indicate that the CUS-induced anhedonic behavior is mediated by IL-1β and IL-1RI.

Expression of IL-1RI in the Hippocampus. Immunohistochemical analysis demonstrates that IL-1RI is densely expressed in the hippocampal formation, particularly the granule cell layer of the DG (Fig. 4), and the CA1 and CA3 pyramidal cells (SI Fig. 7 *A* and *C*). The expression pattern of IL-1RI is the same as reported (29, 30). This pattern was not observed in IL-1RI null mice and was blocked by preabsorption of the IL-1RI antibody with IL-1RI-blocking peptide in rats (SI Fig. 7 *B* and *D*). Double-labeling studies demonstrate that several different types of cells express IL-1RI. This includes a population of IL-1RI$^+$ cells located in the SGZ that express a marker of neural progenitor cells (sox2) (Fig. 4 *D*–*F*) and other IL-1RI+ cells located either in the SGZ or DG that express markers of immature (DCX) or

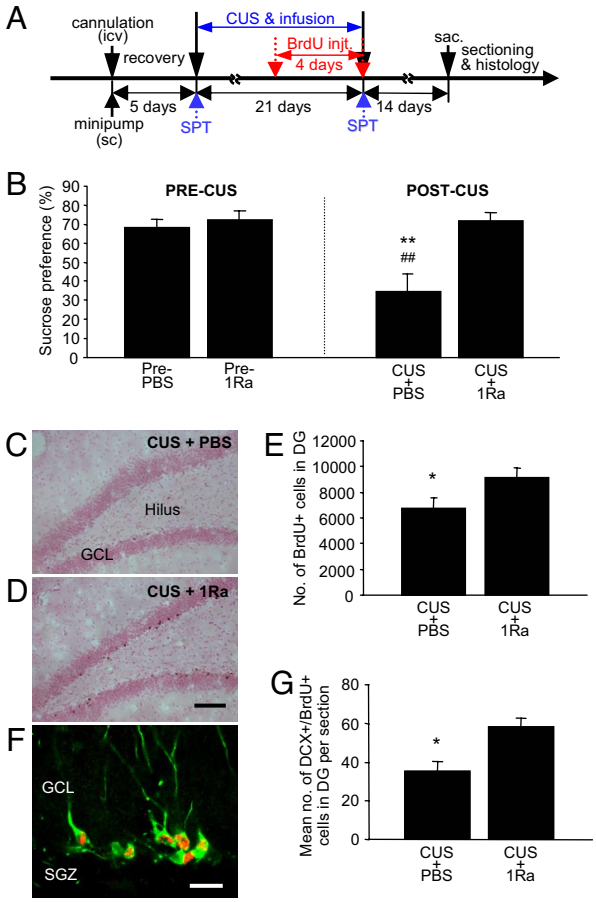

Fig. 2. Effects of IL-1Ra during chronic unpredictable stress (CUS) on sucrose preference and hippocampal neurogenesis in rats. (*A*) A schematic diagram depicting the experimental procedures for CUS in rats. After 5-day recovery, preceded by implantation of cannula (i.c.v.) and minipump (s.c.), all rats were exposed to two stressors per day for 21 days and received BrdU daily for 4 days of the last CUS period. Immediately before (PRE-CUS) and after CUS (POST-CUS), sucrose preference test (SPT) was conducted. Rats were killed 14 days after last BrdU administration. (*B*) Continuous administration of IL-1Ra during CUS restored the sucrose preference that was reduced by CUS (main effect treatment: $F_{1,27} = 11.927$, $P < 0.01$; main effect time: $F_{1,27} = 8.675$, $P < 0.01$; treatment × time: $F_{1,27} = 7.765$, $P < 0.05$; $n = 7$ per group). **, $P < 0.01$ compared with CUS + 1Ra group; ##, $P < 0.01$ compared with Pre-PBS group Tukey's *post hoc* test. (*C–E*) There was a significant difference in the number of hippocampal BrdU$^+$ cells in CUS + 1Ra rats (*D*) relative to CUS+PBS rats (*C*) ($t_{12} = 2.333$, $P < 0.05$; $n = 7$ per group). (Scale bar, 100 μm.) (*F* and *G*) IL-1Ra during CUS also increased the number of new immature neurons measured as the number of BrdU$^+$ (red) and DCX$^+$ (green) double-labeled cells per hippocampal DG ($t_6 = 3.431$, $P < 0.05$; $n = 4$ per group). *, $P < 0.05$ in the *t* test. mean ± SEM. (Scale bar, 25 μm.)

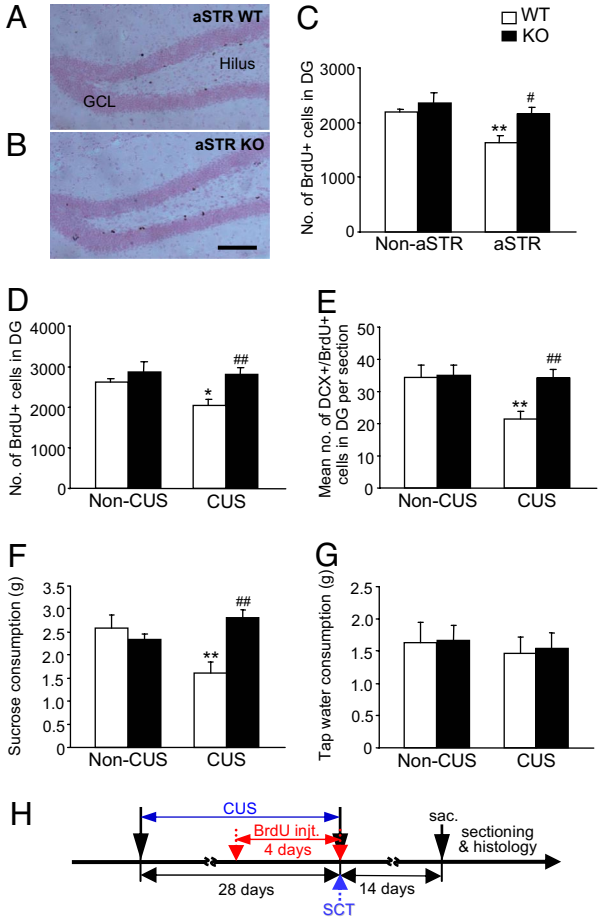

Fig. 3. Effects of acute and chronic stress on hippocampal neurogenesis (cell proliferation) and sucrose consumption in IL-1RI null mice. (*A–C*) Acute stress (aSTR, immobilization and rotation, 50 min) decreased the number of BrdU$^+$ cells in the DG of WT (*A*) but not IL-1RI null mice (KO, *B*) (main effect aSTR: $F_{1,31} = 8.297$, $P < 0.01$; main effect genotype: $F_{1,31} = 6.874$, $P < 0.05$; aSTR × genotype: $F_{1,31} = 1.559$, P = n.s.; $n = 8$ per group). Note that the number of BrdU$^+$ cells was not significantly different between nonstressed (Non-aSTR) WT and KO mice, indicating no difference in the baseline effect of IL-1RI null mutation. (*D*) Four-week CUS also significantly decreased the BrdU$^+$ cell numbers in WT mice but not in IL-1RI KO mice ($F_{1,22} = 1.933$, P = n.s.; $n = 5–6$ per group). CUS KO mice showed more BrdU$^+$ cells than CUS WT mice. There was also no difference in baseline. (*E*) CUS also decreased the number of immature neurons, determined by colabeling of BrdU with DCX, and this effect was blocked in the IL-1RI KO mice. (*F*) In the sucrose consumption test, there was no baseline difference between WT and IL-1RI KO mice. CUS significantly decreased sucrose consumption in WT but not in IL-1RI KO mice (main effect CUS: $F_{1,22} = 1.361$, P = n.s.; main effect genotype: $F_{1,22} = 5.191$, $P < 0.05$; CUS × genotype: $F_{1,22} = 11.388$, $P < 0.01$). (*G*) There was no difference in water consumption among groups (main effect CUS: $F_{1,22} = 0.305$, P = n.s.; main effect genotype: $F_{1,22} = 0.040$, P = n.s.; CUS × genotype: $F_{1,22} = 0.006$, P = n.s.). (*H*) Experimental procedures for CUS in mice. CUS mice were exposed to two or three stressors per day for 28 days and received BrdU daily for 4 days of the last CUS period. On the last CUS day, sucrose consumption test (SCT) was conducted. Mice were killed 14 days after last BrdU administration. **, $P <$ 0.01 compared with nonstressed WT mice; #, $P < 0.05$, ##, $P < 0.01$ compared with stressed WT mice in the Tukey's *post hoc* test, mean ± SEM.

mature (NeuN) neurons (data not shown). Newborn cells labeled with BrdU (2 h after injection) also expressed IL-1RI (Fig. 4 *A–C*).

Effect of IL-1β on Hippocampal Progenitor Cells *in Vitro*. To further investigate the mechanisms by which IL-1β inhibits neurogenesis, studies were conducted on cultured adult hippocampal progenitors (AHPs). Under the culture conditions used (20 ng/ml FGF2), ≈80% of DAPI$^+$ cells expressed nestin, a marker of AHP. There was no staining for astrocytes (GFAP), immature oligodendrocytes (NG2), or immature neurons (NeuN) (data not shown). When cultured in the presence of BrdU (2.5 μM, 36 h), most of the nestin$^+$ cells were also labeled with BrdU (90.3 ± 0.021%, $n = 3$), indicating that the majority of the AHPs were actively proliferating. Moreover, double-labeling studies demonstrate that all of the nestin$^+$ and BrdU$^+$ dividing AHPs expressed IL-1RI (Fig. 5 *A* and *B*). RT-PCR also confirmed that the hippocampal progenitors express IL-1RI mRNA (Fig. 5*C*).

Two-hour incubation with IL-1β significantly decreased AHP proliferation compared with vehicle control (Fig. 5 *D*, *E*, and *I*), and the effective concentration was in the range of IL-1RI

NEUROSCIENCE

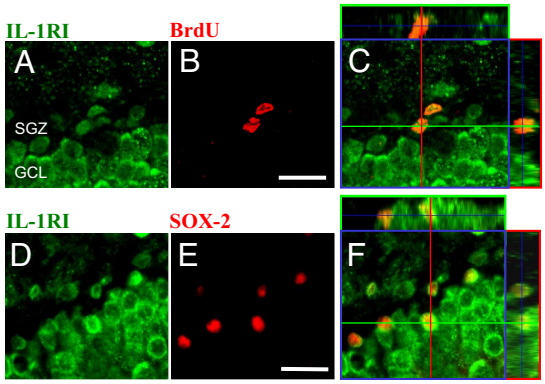

Fig. 4. Expression of IL-1RI in the hippocampus in rats. IL-1RI (green) is expressed in proliferating cells (BrdU+, red, *A–C*) and neural progenitors (*D–F*, sox^{2+}, red) in the SGZ of the hippocampus. (Scale bars, 50 μm.)

affinity (10 ng/ml or ≈0.5 nM) (25). IL-1β decreased the ratio of $BrdU^+$ to $nestin^+$ cells, but did not influence the total number of $DAPI^+$ cells or the ratio of $nestin^+$ to $DAPI^+$ cells (SI Fig. 8). IL-1Ra coincubation resulted in a dose-dependent blockade of the inhibitory effect of IL-1β (Fig. 5*J*), whereas IL-1Ra alone did not influence the number of $DAPI^+$ cells or the ratio of $nestin^+$ to $DAPI^+$ cell numbers (SI Fig. 8). IL-1β did not affect the ratio of $TUNEL^+$ to $DAPI^+$ cells (Fig. 5 *F* and *G*), indicating that the decrease observed is not a result of cell death. The ability of IL-1β to decrease AHP proliferation is also supported by studies of cyclin, a regulator of G_1 cell cycle (31). Time-course analysis demonstrated that cyclin D1 levels were dramatically reduced by incubation with IL-1β after 2–4 h (data not shown), and this effect was blocked by coincubation with IL-1Ra (Fig. 5*H*).

To examine the signaling pathways underlying the actions of IL-1β, we treated cultures with inhibitors of NF-κB, JNK, p38 MAPK, glycogen synthase kinase (GSK)-3β, or PI3K. Interestingly, the inhibitors of NF-κB/I$\kappa\kappa$ signaling (JSH-23 and SC-512, respectively) significantly blocked the antineurogenic effect of IL-1β in AHPs (Fig. 5*K*), but inhibitors of the other signaling pathways had no effect (SI Fig. 9).

Discussion

In this study, we demonstrate that acute, and chronic, stress suppress hippocampal cell proliferation, consistent with previous reports (12, 32, 33) and that the antiproliferative effect of stress is blocked by inhibition of IL-1RI. *In vitro* studies show that suppression of cell proliferation is mediated by direct actions of IL-1β on IL-1RI receptors localized on adult hippocampal progenitor cells via a reduction in cell cycling and not increased cell death. In addition, the results demonstrate that inhibition of IL-1RI blocks the reduction in behavioral reward caused by CUS exposure. Together the results demonstrate that IL-1β plays a key role in the antineurogenic and anhedonic effects of stress.

Although glucocorticoids are known to play a role in stress responses and negatively regulate neurogenesis, contradictory evidence of a dissociation among stress, glucocorticoids, and neurogenesis also exists (34), prompting the current study of

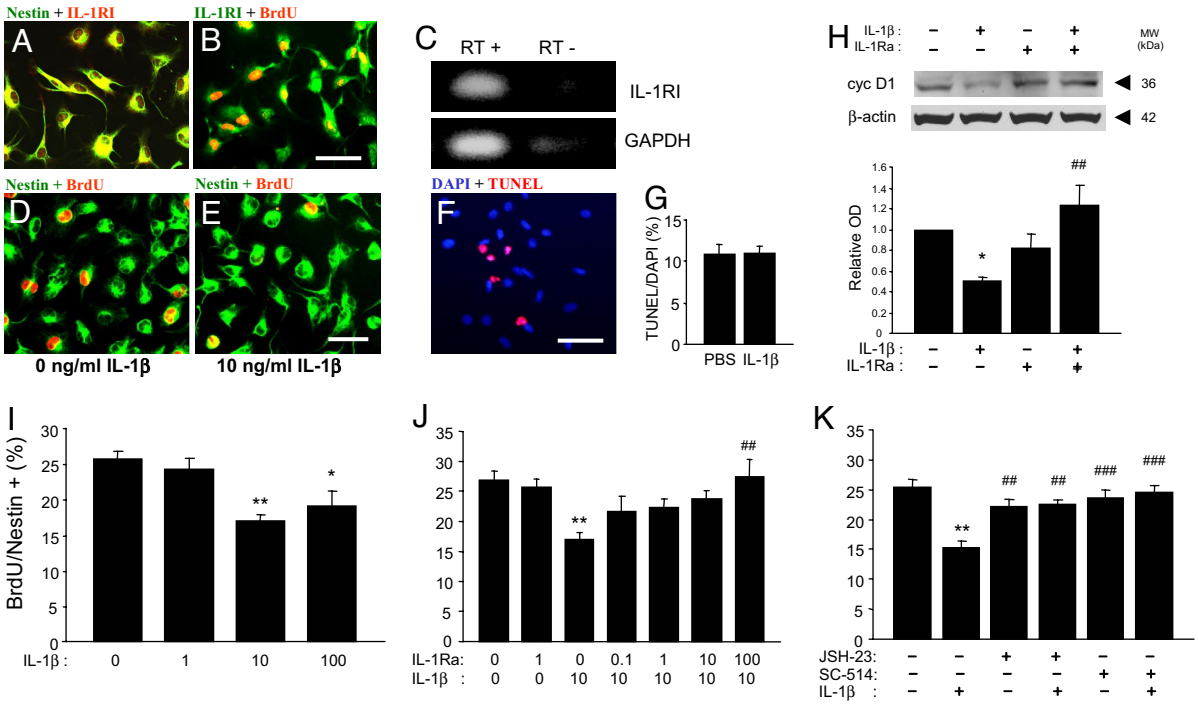

Fig. 5. Expression of IL-1RI in primary cultured AHPs and effects of IL-1β and/or IL-1Ra on AHP proliferation. (*A*) All of the AHPs ($nestin^+$, green) *in vitro* coexpressed IL-1RI (red). (*B*) Proliferating AHPs ($BrdU^+$, red) also expressed IL-1RI (green). (*C*) Expression of IL-1RI genes in cultured AHPs. RT-PCR was performed with IL-1RI and GAPDH genes prepared from cultured AHPs in the presence (left lane) or absence (right lane, as a negative control) of reverse transcriptase (RT). (*D* and *E*) Representative images of proliferating ($BrdU^+$, red) AHPs ($nestin^+$, green) in the presence of PBS (*D*) or IL-1β (*E*, 10 ng/ml). (*F* and *G*) IL-1β did not alter the percentage of $TUNEL^+$ (red) to $DAPI^+$ (blue) cells ($n = 4$) ($t_6 = 0.194$, P = n.s.; $n = 4$ per group). (*H*) IL-1Ra (100 ng/ml) blocked the IL-1β (10 ng/ml)-dependent decrease of cyclin D1 expression ($F_{3,15} = 7.329$, $P < 0.01$; $n = 3$–4 per group). β-Actin served as an internal loading control. Expression levels were measured with relative optical density (OD). (*I*) IL-1β (10–100 ng/ml) decreased the ratio of $BrdU^+$ (red) to $nestin^+$ (green) compared with CTR (0 ng/ml IL-1β) ($F_{3,33} = 7.156$, $P < 0.001$; $n = 8$–9 per group). (*J*) Coincubation of IL-1Ra (100 ng/ml) blocked the antiproliferative effect of IL-1β ($F_{6,39} = 4.198$, $P < 0.01$; $n = 5$–6 per group). (*K*) Coincubation of NF-κB (JSH-23, 25 μM) or I$\kappa\kappa$ inhibitors (SC-514, 25 μM) blocked the antiproliferative effect of IL-1β ($F_{5,35} = 8.504$, $P < 0.001$; $n = 5$–6 per group). (Scale bars, 50 μm.) *, $P < 0.05$ and **, $P < 0.01$ compared with CTR. ##, $P < 0.01$ and ###, $P < 0.001$ compared with 10 ng/ml IL-1β group in the Tukey's post hoc tests, mean ± SEM.

IL-1β. We used two different approaches, infusions of the inhibitor IL-1Ra and IL-1RI null mice. IL-1Ra is relatively selective for IL-1RI, although we cannot rule out potential actions at other receptor subtypes (35). However, the blockade observed in the IL-1RI null mice confirms that IL-1β and stress inhibit cell proliferation via this receptor subtype. In addition to blockade of acute stress, blockade of the CUS responses by either IL-1Ra or deletion of IL-1RI demonstrates that the antiproliferative actions of IL-1β are sustained over several weeks of CUS exposure. In addition, in the presence of IL-1Ra, there are significantly more newborn neurons (DCX^+) after CUS exposure relative to controls. Acute IL-1Ra did not influence neurogenesis, and the IL-1RI null mice were not different from controls, but further studies will be needed to determine whether chronic IL-1Ra, in the absence of stress, increases cell proliferation. Combined with the IL-1β infusion experiments, the results demonstrate that IL-1β is necessary and sufficient for suppression of hippocampal cell proliferation by stress.

CUS has a high degree of face, predictive, and construct validity as a behavioral model of depression (27, 28). Notably, the reduction in sucrose preference is a model for anhedonia (a core symptom of depression), is induced by realistic stress conditions, a known precipitant of depression in humans (3, 4), and is reversed by chronic, but not acute, antidepressant administration, consistent with the time course for the therapeutic actions of antidepressants (28). Blockade of the anhedonic effects of CUS by either IL-1Ra or IL-1RI deletion demonstrates that this behavioral response to CUS is mediated by IL-1β/IL-1RI. A causal relationship between the regulation of neurogenesis and behavioral responses has been suggested by studies demonstrating that the effects of chronic antidepressant administration require increased neurogenesis (13, 14), although the current findings are correlative. However, two recent studies found neurogenesis-independent effects of antidepressants (15, 16), indicating that alternate mechanisms underlie the behavioral responses (5). Previous studies have demonstrated that IL-1β produces a rapid and presumably neurogenesis-independent effect on behavioral despair (36). Future studies will be required to determine the alternate actions of IL-1β in the hippocampus, and other brain regions, such as the ventral tegmentum and nucleus accumbens (37), that could underlie the anhedonic actions of stress.

Immunohistochemical studies demonstrate that IL-1RI is expressed on hippocampal cells that express markers of neural progenitors both *in vivo* (sox2) and *in vitro* (nestin). IL-1RI-labeled cells also incorporate BrdU, indicating that these proliferating progenitors express IL-1RI, both *in vivo* and *in vitro*. The presence of IL-1RI on neural progenitor cells suggests that the actions of IL-1β occur via a direct effect and not via polysynaptic or indirect actions on cells surrounding the progenitor cells. This is confirmed by *in vitro* studies demonstrating that the proliferation of hippocampal neural progenitor cells is decreased by IL-1β, and this effect is blocked in a dose-dependent fashion by IL-1Ra. A previous study that examined the influence of IL-1β on neuronal differentiation (cell proliferation was not examined) found no effect (38). Together with the current study, the results demonstrate that IL-1β affects proliferation, but not differentiation of AHPs.

Suppression of hippocampal progenitor cell proliferation by IL-1β could result from either a loss of progenitor cells because of cell death, arrest of the cell cycle, or both. IL-1β is reported to enhance the death of human fetal brain cells and rat oligodendrocyte progenitor cells, but only when combined with TNF-α or INF-γ (39, 40). In the present study, there was no evidence from TUNEL staining that IL-1β and/or acute stress elicit hippocampal cell death either *in vitro* or *in vivo*, which is consistent with previous studies (40–42), although there is one report that severe acute stress increases apoptosis (43). In contrast, we found that incubation of AHPs with IL-1β induces a dramatic decline in levels of cyclin D1, a cell cycle regulatory protein that controls specific cyclin-dependent kinases such as cdk4 (31). Glucocorticoid-induced suppression of cyclin D1 in embryonic neural stem cells occurs via a similar mechanism (44).

The antiproliferative effects of IL-1β could occur via activation of one of several intracellular cascades such as NF-κB, JNK, or p38 MAPK pathways, which are activated by IL-1β or stress (25, 45, 46). Our present data revealed that the NF-κB pathway mediates the antineurogenic effect of IL-1β in AHPs (Fig. 5*K* and SI Figs. 9 and 10). This is consistent with previous studies in other cell types, including mouse embryo fibroblasts and human epithelial cells (47, 48), although other responses have been reported, depending on cell types and conditions (48).

In summary, this is the first report to show that the antineurogenic effects of both acute and chronic stress are mediated by IL-1β, that IL-1β directly decreases the AHP cell proliferation by arresting cell cycle, not by cell death, and that this effect occurs via activation of the NF-κB pathway in AHPs. These findings further elucidate the actions of IL-1β in stress responses, and could provide new targets for the development of novel antidepressant medications.

Methods

Animals. Adult male Sprague–Dawley (SD) rats (Charles River Laboratories) with initial weights of 230–250 g and adult male IL-1RI null mice bearing a C57BL/6 background (The Jackson Laboratory) with initial weights of 23–30 g were used for experiments. The IL-1RI null mice exhibit no overt phenotype, breed well, and have normal litter size (49). For the null mice, age- and weight-matched C57BL/6 mice (The Jackson Laboratory) were used as WT controls. Animals were housed, three per cage, under standard illumination parameters (12-h light/dark cycle) and with ad libitum access to food and water. All procedures were in accordance with Yale Animal Care and Use Committee and National Institutes of Health guidelines for animal research.

Cannula Implantation and Microinjections. A 26-gauge guide cannula (Plastics One) was implanted into either the left or right (randomly assigned) lateral ventricle of the brain of the rats for i.c.v. infusion of IL-1β (20 or 100 ng/μl; R & D Systems), IL-1Ra (0.25 μg/μl; R & D Systems) and/or 0.1% BSA/PBS (vehicle). For details, see *SI Methods*.

Acute Stress Exposure. For foot-shock stress with SD rats, animals were exposed to 30 unpredictable foot-shock (30-sec shock duration, 0.8 mA intensity, ITI = 30-sec average, 25- to 35-sec range) on the shuttle box immediately after administration of IL-1Ra or vehicle. The shock duration, intensity, and ITI were chosen based on previous work (12). For immobilization stress, rats were restrained in a Plexiglas immobilization tube (3.5-inch diameter × 7-inch length) for 45 min, which was based on previous work (32). For acute stress experiments with mice, half of IL-1RI null and WT mice were stressed for 50 min with a combination of restraint stress and rotation stress on an orbital shaker. All experiments for acute stress exposure were performed between 11:00 and 14:00.

BrdU Labeling and Quantitative Analysis. BrdU immunohistochemistry and its quantitative analysis by using a modified unbiased stereology protocol (12) was conducted as described in *SI Methods*.

Surgical Procedure and Treatments for Chronic Infusion. An i.c.v. cannula (Brain Infusion kit 2; Alzet) was inserted into the lateral ventricle of the brain of the rats for prolonged i.c.v. infusions of either vehicle or IL-1Ra (0.2 μg/μl concentration) during CUS. A miniosmotic pump (Alzet 2004; Alzet) infusing fluids at a rate of 1 μg/day was implanted s.c. and connected to the cannula. For details, see *SI Methods*.

CUS Procedures. For our CUS procedure (Figs. 2*A* and 3*H*), we used various stressors of which the sequence was intentionally designed to maximize unpredictability (50, adapted from ref. 27). CUS rats were exposed to two stressors per day for 21 days (for details, see SI Table 1). In the case of mice, two or three stressors a day were used for 28 days (for details, see SI Table 2). All animals received BrdU daily (i.p., 75 mg/kg to rats; 50 mg/kg to mice; Sigma) for 4 days of the last CUS period (CUS day 18–21 for rats; CUS day 25–28 for mice). Two weeks after BrdU injection, animals were perfused, BrdU immunohistochemistry was performed, and $BrdU^+$ cells were quantified in the hippocampus by using a modified unbiased stereology protocol (12). $BrdU^+$

cells colabeled with DCX were also quantified in DG (Three to four sections per animal) by using confocal laser microscopy (Zeiss).

Sucrose Preference Test. Rats were singly housed and habituated to a 1% (wt/vol) sucrose solution (Sigma) for 48 h (CUS days −3 and 19) to avoid neophobia during testing (27, 50). To prevent location preference, the bottle position was changed daily. On test days (CUS days 0 and 21), after 4-h water deprivation, rats were presented with two bottles containing 1% sucrose or tap water for 1 h, and intake volume was measured. Sucrose preference was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed.

Sucrose Consumption Test. Mice were singly housed on CUS day 23 and habituated first to 1% sucrose solution for 48 h (CUS days 23–24) and then to the test procedure for 3 days (CUS days 25–27, free access to 1% sucrose solution after 14- to 20-h water deprivation). On CUS day 28, mice were presented with 1% sucrose solution for 1 h after 20-h water deprivation. On the following day, the same procedure was repeated with tap water. Sucrose or tap water consumption was measured by weight.

Neural Progenitor Cell Isolation and Culture. Neural progenitor cells were isolated from the hippocampi of adult male or female SD rats (160–200 g of body weight, Charles River Laboratories) and cultured as described (38, 51). For details, see *SI Methods*.

Statistical Analysis. All data are expressed as mean ± SEM. Experiments with two groups were analyzed statistically by using unpaired *t* test. Experiments with three or more groups were subjected to a one-way ANOVA, followed by the Tukey's *post hoc* test. Sucrose preference data with rats and sucrose consumption and $BrdU^+$ cell counting data in mice were analyzed by using two-way ANOVA, followed by the Tukey's *post hoc* test. The level of statistical significance for all analysis was set at $P < 0.05$.

Additional Details. See *SI Methods* for additional routine procedures including immunofluorescence, TUNEL assay, real-time RT-PCR, and Western immunoblot analysis.

ACKNOWLEDGMENTS. We thank Drs. M. Picciotto and J. Taylor for helpful comments on manuscript preparation and S. Gourley for sucrose consumption protocol. This work was supported by US Public Health Service Grants MH45481 and 2 P01 MH25642, Veterans Administration National Center Grant for Post-Traumatic Stress Disorder, and the Connecticut Mental Health Center.

1. Kim JJ, Diamond DM (2002) *Nat Rev Neurosci* 3:453–462.
2. McEwen BS (2007) *Physiol Rev* 87:873–904.
3. Anisman H, Matheson K (2005) *Neurosci Biobehav Rev* 29:525–546.
4. Miller MM, McEwen BS (2006) *Ann N Y Acad Sci* 1071:294–312.
5. Sahay A, Hen R (2007) *Nat Neurosci* 10:1110–1115.
6. Warner-Schmidt JL, Duman RS (2006) *Hippocampus* 16:239–249.
7. Schmidt HD, Duman RS (2007) *Behav Pharmacol* 18:391–418.
8. Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999) *J Neurosci* 19:5034–5043.
9. Sheline YI, Gado MH, Kraemer HC (2003) *Am J Psychiatry* 160:1516–1518.
10. McEwen BS (1999) *Annu Rev Neurosci* 22:105–122.
11. Czeh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, Bartolomucci A, Fuchs E (2001) *Proc Natl Acad Sci USA* 98:12796–12801.
12. Malberg JE, Duman RS (2003) *Neuropsychopharmacology* 28:1562–1571.
13. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, *et al.* (2003) *Science* 301:805–809.
14. Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K (2007) *Science* 317:819–823.
15. Holick KA, Lee DC, Hen R, Dulawa SC (2008) *Neuropsychopharmacology* 33:406–417.
16. David DJ, Klemenhagen KC, Holick KA, Saxe MD, Mendez I, Santarelli L, Craig DA, Zhong H, Swanson CJ, Hegde LG, *et al.* (2007) *J Pharmacol Exp Ther* 321:237–248.
17. Shintani F, Nakaki T, Kanba S, Sato K, Yagi G, Shiozawa M, Aiso S, Kato R, Asai M (1995) *J Neurosci* 15:1961–1970.
18. Nguyen KT, Deak T, Owens SM, Kohno T, Fleshner M, Watkins LR, Maier SF (1998) *J Neurosci* 18:2239–2246.
19. Deak T, Bordner KA, McElderry NK, Barnum CJ, Blandino P, Jr, Deak MM, Tammariello SP (2005) *Brain Res Bull* 64:541–556.
20. Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, Fleshner M (2005) *Neuroscience* 135:1295–1307.
21. Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W (1987) *Science* 238:522–524.
22. Murray CA, Lynch MA (1998) *J Neurosci* 18:2974–2981.
23. Barrientos RM, Sprunger DB, Campeau S, Higgins EA, Watkins LR, Rudy JW, Maier SF (2003) *Neuroscience* 121:847–853.
24. Pugh CR, Nguyen KT, Gonyea JL, Fleshner M, Wakins LR, Maier SF, Rudy JW (1999) *Behav Brain Res* 106:109–118.
25. Dinarello CA (1996) *Blood* 87:2095–2147.
26. Kaneko N, Kudo K, Mabuchi T, Takemoto K, Fujimaki K, Wati H, Iguchi H, Tezuka H, Kanba S (2006) *Neuropsychopharmacology* 31:2619–2626.
27. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) *Psychopharmacology (Berlin)* 93:358–364.
28. Willner P (2005) *Neuropsychobiology* 52:90–110.
29. Sairanen TR, Lindsberg PJ, Brenner M, Sirén AL (1997) *J Cereb Blood Flow Metab* 17:1107–1120.
30. French RA, VanHoy RW, Chizzonite R, Zachary JF, Dantzer R, Parnet P, Bluthe RM, Kelley KW (1999) *J Neuroimmunology* 93:194–202.
31. Sherr CJ, Roberts JM (1999) *Genes Dev* 13:1501–1512.
32. Vollmayr B, Simonis C, Weber S, Gass P, Henn F (2003) *Biol Psychiatry* 54:1035–1040.
33. Shors TJ, Mathew J, Sisti HM, Edgecomb C, Beckoff S, Dalla C (2007) *Biol Psychiatry* 62:487–495.
34. Mirescu C, Gould E (2006) *Hippocampus* 16:233–238.
35. Boraschi D, Tagliabue A (2006) *Vitam Horm* 74:229–254.
36. Maier SF, Watkins LR (1995) *Brain Res* 695:279–282.
37. Berton O, Nestler EJ (2006) *Nat Rev Neurosci* 7:137–151.
38. Monje ML, Toda H, Palmer TD (2003) *Science* 302:1760–1765.
39. Chao CC, Hu S, Ehrlich L, Peterson PK (1995) *Brain Behav Immun* 9:355–365.
40. Vela JM, Molina-Holgado E, Arevalo-Martin A, Almazan G, Guaza C (2002) *Mol Cell Neurosci* 20:489–502.
41. Gould E, Woolley CS, McEwen BS (1991) *J Comp Neurol* 313:479–485.
42. Lowy MT, Wittenberg L, Yamamoto BK (1995) *J Neurochem* 65:268–274.
43. Heine VM, Maslam S, Zareno J, Joels M, Lucassen PJ (2004) *Eur J Neurosci* 19:131–144.
44. Sundberg M, Savola S, Hienola A, Korhonen L, Lindholm D (2006) *J Neurosci* 26:5402–5410.
45. Madrigal JL, Moro MA, Lizasoain I, Lorenzo P, Castrillo A, Bosca L, Leza JC (2001) *J Neurochem* 76:532–538.
46. Shen CP, Tsimberg Y, Salvadore C, Meller E (2004) *BMC Neurosci* 5:36.
47. Seitz CS, Deng H, Hinata K, Lin Q, Khavari PA (2000) *Cancer Res* 60:4085–4092.
48. Chen F, Lu Y, Castranova V, Li Z, Karin M (2006) *J Biol Chem* 281:37142–37149.
49. Glaccum MB, Stocking KL, Charrier K, Smith JL, Willis CR, Maliszewski C, Livingston DJ, Peschon JJ, Morrissey PJ (1997) *J Immunol* 159:3364–3371.
50. Banasr M, Valentine GW, Li XY, Gourley S, Taylor J, Duman RS (2006) *Biol Psychiatry* 62:496–504.
51. Palmer TD, Takahashi J, Gage FH (1997) *Mol Cell Neurosci* 8:389–404.