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ORIGINAL ARTICLE

Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression

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Several lines of evidence implicate the pro-inflammatory cytokine interleukin-1 (IL-1) in the etiology and pathophysiology of major depression. To explore the role of IL-1 in chronic stress-induced depression and some of its underlying biological mechanisms, we used the chronic mild stress (CMS) model of depression. Mice subjected to CMS for 5 weeks exhibited depressive-like symptoms, including decreased sucrose preference, reduced social exploration and adrenocortical activation, concomitantly with increased IL-1β levels in the hippocampus. In contrast, mice with deletion of the IL-1 receptor type I (IL-1rKO) or mice with transgenic, brain-restricted overexpression of IL-1 receptor antagonist did not display CMS-induced behavioral or neuroendocrine changes. Similarly, whereas in wild-type (WT) mice CMS significantly reduced hippocampal neurogenesis, measured by incorporation of bromodeoxyuridine (BrdU) and by doublecortin immunohistochemistry, no such decrease was observed IL-1rKO mice. The blunting of the adrenocortical activation in IL-1rKO mice may play a causal role in their resistance to depression, because removal of endogenous glucocorticoids by adrenalectomy also abolished the depressive-like effects of CMS, whereas chronic administration of corticosterone for 4 weeks produced depressive symptoms and reduced neurogenesis in both WT and IL-1rKO mice. The effects of CMS on both behavioral depression and neurogenesis could be mimicked by exogenous subcutaneous administration of IL-1β via osmotic minipumps for 4 weeks. These findings indicate that elevation in brain IL-1 levels, which characterizes many medical conditions, is both necessary and sufficient for producing the high incidence of depression found in these conditions. Thus, procedures aimed at reducing brain IL-1 levels may have potent antidepressive actions.
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Keywords: chronic mild stress (CMS); major depression; hypothalamus–pituitary–adrenal (HPA) axis; hippocampus; pro-inflammatory cytokines; adrenalectomy

Introduction

Over the last decade, several lines of evidence have implicated inflammatory processes in general, and the pro-inflammatory cytokine interleukin-1 (IL-1) in particular, in the etiology and pathophysiology of depression, including: (1) a high incidence of depression in medical conditions characterized by inflammation and IL-1 production, particularly within the brain.[1–3] (2) Elevated levels of inflammatory markers, particularly IL-1, in patients with major depression or minor depression (dysthymia), which are correlated with the severity of depression, the duration of the current depressive episode and the age of disease onset.[3–9] (3) Induction of depressive symptoms in cytokine-treated cancer and hepatitis-C patients, which can be reversed by antidepressant treatment.[10,11] (4) Genetic association between genes of inflammatory factors, including polymorphisms in IL-1 family genes, and severity of depression and its responsiveness to antidepressant treatment.[12] (5) Cytokine-related induction of depressed mood in normal subjects following experimental exposure to immune challenges.[13–15] (6) Studies in experimental animals, showing that exposure to various immune challenges, as well as exogenous administration of IL-1, either peripherally or directly into the brain, produce depressive-like symptoms, which can be attenuated by chronic treatment with antidepressant drugs,[1,16–19] as well as by pretreatment with cytokine antagonists, particularly IL-1ra, and by manipulations in IL-1 family genes.[4,20–22] Taken together, these findings suggest that under conditions of immune activation due to physical illnesses, the production of IL-1 contributes importantly to the induction of depressive symptomatology.

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In addition to mediating the neurobehavioral effects of immune challenges, IL-1 was found to mediate many effects of stress. Exposure of humans and experimental animals to various stressors induces the expression and production of both peripheral and central IL-1.[23–29] Furthermore, the stress-induced increase in IL-1 levels has an important role in mediating many of the behavioral and neuroendocrine effects of stress.[30–32] Ample evidence indicates that exposure to stressors, particularly in individuals with genetic vulnerability, has a substantial causal association with depression.[33–35] The relations between stress and depression are reflected by the marked activation of the hypothalamus–pituitary–adrenal (HPA) axis exhibited by depressed patients,[36–38] which can be normalized following successful antidepressant therapy.[39–42] Together, these findings suggest that stress-induced, IL-1-mediated neurobehavioral and neuroendocrine processes may be involved in depression, even in situations that are not related to immune activation due to any medical condition.

Another process that has been implicated in depression and may be influenced by brain IL-1 is hippocampal neurogenesis.[4,43,44] Although a direct role of IL-1 in neurogenesis has not been established, it has been reported that treatment with IL-1 inducers, including lipopolysaccharide and radiation, results in marked suppression of this process.[45,46]

To examine the hypothesis that IL-1 plays a causal role in depression, we examined the development of depressive-like symptoms during exposure to chronic mild stress (CMS), an established model of depression in rodents[47,48] in two strains of mice with genetic impairments in IL-1 signaling, including mice with deletion of the IL-1 receptor type I (IL-1rKO) and mice with transgenic, central nervous system (CNS)-specific overexpression of IL-1ra (IL-1raTG), and their respective control strains. Furthermore, we assessed two potential mechanisms for downstream mediation of the influence of IL-1 on depression—adrenocortical activation and reduced hippocampal neurogenesis. Additionally, we examined whether chronic exposure to IL-1 in normal mice can mimic the effects of CMS on depressive-like behavior and hippocampal neurogenesis.

Methods

Subjects

Subjects were male IL-1rKO mice and their C57BL/6×129/Sv wild-type (WT) controls (Jackson Laboratories, Bar Harbor, ME, USA), as well as IL-1raTG mice and their C57BL/$6 \times$CBA WT controls (Stockholm University). IL-1rKO mice were shown to have no expression of IL-1 receptor type I, which appears to mediate all of the known biological functions of IL-1. These mice appear to have a normal physiological and behavioral phenotype, although they do not respond to either IL-1α or IL-1β in a variety of assays, including IL-1-induced fever and IL-6 secretion. Without a challenge, they have no major immunological abnormalities, however, they display a reduced acute phase response to turpentine, reduced delayed-type hypersensitivity responses and high susceptibility to infection by *Listeria monocytogenes*.[49] IL-1rKO mice also exhibit impaired learning and memory functioning, associated with reduced neural plasticity,[50] and diminished adrenocortical responsiveness to mild stressors.[32] IL-1raTG mice have astrocyte-directed overexpression of the human IL-1ra gene under the control of the murine glial fibrillary acidic protein promoter, and are insensitive to the administration of exogenous IL-1, therefore, they over-express human IL-1ra only within the brain and spinal cord.[51–53] The physiological and behavioral phenotype of this strain is overall normal, besides a small, but significant, elevation in body weight and reduced bone density.[52] Similar to the IL-1rKO mice, IL-1raTG mice are insensitive to the administration of exogenous IL-1[51] and to some manipulations that induce brain IL-1, for example, closed-head injury.[54] Animals were housed in an air-conditioned room ($22 + 1^{\circ}$C), with food and water *ad libitum*, except when specified otherwise. All the experiments were performed during the first three hours of the dark phase of a reversed 12 h light/dark cycle (lights off 1400 hours). The experiments were approved by the Hebrew University Committee on Animal Care and Use.

CMS

To induce CMS, according to the protocol adapted from Willner,[47,48] the following stimuli were administered each week in a random order, for five weeks: 3 h of 45° cage tilt, two times a week; soaked cage for 12 h, once a week, water deprivation for 14 h, once a week; lights on for 9 h during the dark phase, once a week; noise in the room for 3 h, three times a week, flashing light for 30 min, three times a week.

Enzyme-linked immunosorbent assay

To measure IL-1β and IL-6 levels, complete hippocampi were collected from both $C57 \times 129$ and $C57 \times$CBA wild-type (WT) mice, which were either untreated or subjected to 5 weeks of CMS. Hippocampi were homogenized in 300 μl cold RPMI 1640 (Sigma, Rehovot, Israel) containing 60 KIU Aprotinin (Sigma, Israel), and centrifuged for 10 min (300 *g*) at 4°C before supernatant was collected. IL-1β and IL-6 levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA). The intra- and interassay coefficients for the IL-1 kit are 10.2 and 19.2%, respectively, and for the IL-6 kit 7.8 and 9.6%, respectively. Total protein was determined by the Bradford method, and ELISA results were normalized accordingly.

Behavioral measurements

Sucrose preference. To measure sucrose preference, mice were placed for 3 h in individual measurement cages at the beginning of the dark phase, and

presented with two graduated drinking tubes, one containing tap water and the other 2% sucrose solution. Sucrose preference was calculated as the percent of sucrose consumption out of the total drinking volume.

Social exploration. Each mouse was placed in an observation cage and allowed 15 min of habituation, following which a male juvenile mouse was placed in the cage. Social exploration, defined as the time of contact between the nose of the mouse and the pup, was then recorded for 2 min.

Bromodeoxyuridine immunofluorescent staining
At the end of the fifth week of CMS, mice were i.p. injected four times (2 h apart) with 75 mg/kg of bromodeoxyuridine (BrdU), and killed 24 h after the last injection. Mice were intracranially perfused with cold 4% paraformaldehyde, and brains were deep frozen in liquid nitrogen; serial 8–10 μM brain axial frozen sections were performed for immunofluorescent analysis. BrdU immunofluorescent staining was performed on 8 μm frozen brain sections. Sections were fixated in acetone (for 10 min at -20°C), dried and then incubated in 0.3% H_2O_2 in methanol for 15 min. After several phosphate-buffered saline (PBS) washes, the sections were incubated in 0.1 mg/ml proteinase K for 15 min, and then treated with 4 N HCl for 10 min and washed to neutralize the pH. Sections were incubated with anti-BrdU overnight at 4°C. A goat anti-mouse immunoglobulin G (IgG) secondary antibody, conjugated to Alexa 488, was added for 50 min at room temperature. Counterstaining was carried out with 4′,6-diamidino-2-phenylindole (DAPI). Images were taken using a fluorescent microscope (Leica) or a Nikkon E-600 fluorescent microscope.

Immunohistochemistry for doublecortin
Doublecortin (DCx) staining was performed on 8–10 μm frozen brain sections. Sections were fixated in 4% formaldehyde for 10 min at RT. After three PBS washes, sections were incubated in 3% H_2O_2 in methanol for 30 min. Following two washes with double diluted water and one wash with PBS, sections were incubated in 0.1% tween in PBS for 5 min. After one more wash with PBS, sections were incubated with the primary antibody (goat anti-DCx 1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 24 h at 4°. Sections were then incubated with the secondary antibody (biotinylated horse anti-goat, 1:100; Vector Laboratories, Burlingame, CA, USA), for 1 h at RT and visualized using an avidin–biotin–peroxidase complex system (Vectastain ABC Elite Kit, Vector Laboratories).

Determination of corticosterone levels
At the end of the fifth week of CMS, 4 h before the beginning of the dark phase, WT and IL-1rKO mice were killed by decapitation, and corticosterone was determined by radioimmunoassay (RIA), as previously described.[55] The sensitivity limit of the assay was 0.5 μg/100 ml and the intra- and interassay coefficients of variation were 6.2 and 7.5%, respectively.

Adrenalectomy
Mice were anesthetized with ketamine–xylazine and the adrenal glands were bilaterally removed. Sham operations were similar in all aspects apart from the removal of the glands. Following operation, mice were provided with 0.45% NaCl as their drinking solution instead of water. The complete removal of the adrenal glands was confirmed by measuring corticosterone levels, which were found to be below the detection limit of the kit in all the ADX-operated mice.

Chronic corticosterone administration
Corticosterone (0.5 mg dissolved in 100 μl olive oil) was subcutaneously injected daily for 4 weeks. This dose was shown to elevate plasma corticosterone to a level equivalent to those seen following stress.[56] The control group was injected with 100 μl olive oil only. At the end of the fourth week, mice were injected with BrdU and brains were collected as described above.

Minipumps implantation
Adult mice were subcutaneously implanted, under ketamine–xylazine anesthesia, with osmotic minipumps (Alzet, Cupertino, CA, USA) releasing either human IL-1β (5 μg/kg/day, R&D systems, Minneapolis, MN, USA) or saline for 4 weeks. Although in the present study the levels of IL-1 were not measured at the end of the experiment, previous studies verified the long-term activity of IL-1 secreted from minipumps in several systems, for example Lippuner *et al*.[57] and Koide *et al*.[58]

Statistical analysis
The results were analyzed by *t*-test or two-way analysis of variances followed by the Tukey *post hoc* analysis, when appropriate.

Results

*CMS increases hippocampal IL-1*β *levels*
As a first step in examining the role of IL-1 in CMS-induced depression, we assessed the effects of CMS on the production of IL-1β within the hippocampus, as well as the production of the IL-1-inducible cytokine IL-6. Mice that were exposed to 5 weeks of CMS exhibited a significant increase in IL-1β protein levels ($t_{22} = -2.56$, $P < 0.01$; Figure 1a) compared to their respective non-treated controls. No significant increase in IL-6 levels was detected (Figure 1b).

Mice with impaired IL-1 signaling show no depressive behavior following CMS
If CMS-induced IL-1β production is critically involved in mediating the development of depressive symptomatology, then mice with impaired IL-1

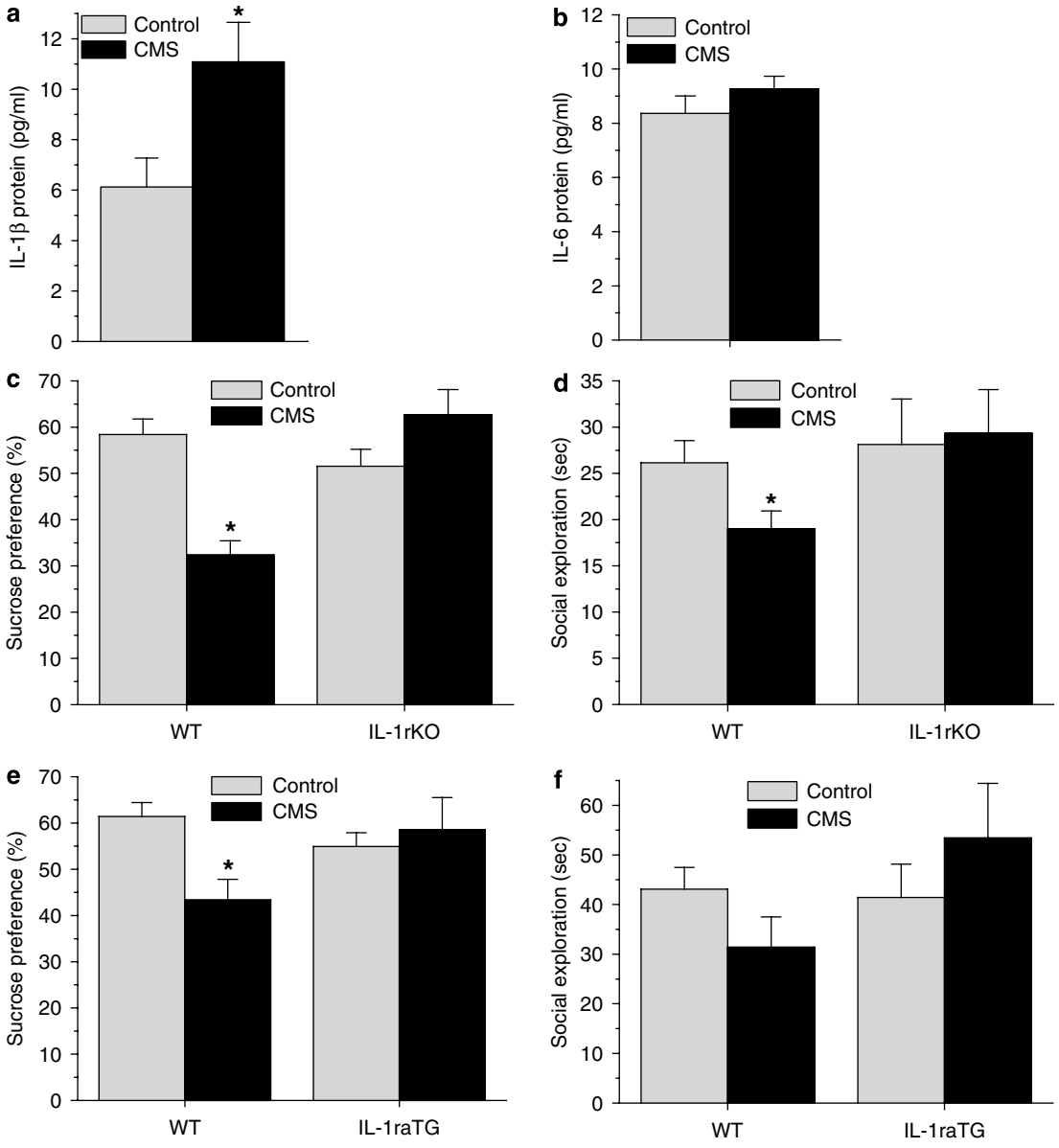

Figure 1 Chronic mild stress (CMS)-induced brain IL-1 is necessary for the manifestation of depressive symptoms. (**a**) Hippocampal IL-1 protein levels were increased in mice exposed to 5 weeks of CMS ($n=12$), compared to non-treated (Control, $n=12$) mice. $^*P<0.01$. No change in hippocampal IL-6 levels was found in these mice (**b**). (**c**) C57 × 129 wild-type (WT) mice ($n=12$), but not IL-1 receptor type-I-deficient mice (IL-1rKO, $n=12$), displayed decreased sucrose preference following 5 weeks of CMS, compared to their respective controls ($n=10$, $n=12$), $^*P<0.05$. (**d**) CMS also resulted in a reduction in social exploration in WT mice ($n=11$), whereas exploration level in IL-1rKO mice ($n=11$) remained unchanged, compared to their respective controls ($n=10$, $n=10$), $^*P<0.005$. (**e**) C57 × CBA WT mice ($n=9$), but not mice with central nervous system-specific overexpression of IL-1ra (IL-1raTG, $n=9$), displayed decreased sucrose preference following 5 weeks of CMS, compared to their respective controls ($n=12$, $n=11$), $^*P<0.05$. (**f**) CMS also resulted in a reduction in social exploration in WT mice ($n=10$), but not in IL-1raTG mice ($n=12$), compared to their respective controls ($n=9$, $n=7$), $^*P<0.05$. Data presented as mean ± s.e.m. IL, interleukin; IL-1rKO, mice with deletion of the IL-1 receptor type I; IL-1raTG, mice with transgenic, brain-specfic overexpression of IL-1 receptor antagonist; WT, wild type.

signaling should exhibit less or no effects of CMS. To examine this hypothesis, we compared the depressive-like effects of CMS in IL-1rKO and IL-1raTG mice with their respective WT controls. Following 5 weeks of CMS, the C57 × 129 WT mice exhibited a significant decrease in sucrose preference ($P<0.04$), whereas sucrose preference in IL-1rKO mice remained unchanged (Figure 1c). This finding was reflected by a significant strain by treatment interaction ($F_{3,42}=20.175$, $P<0.0001$). No difference in the total drinking volume was found between the groups. Additionally, CMS resulted in a 27% reduction in

social exploration in WT mice, whereas exploration level in IL-1rKO mice remained unchanged (Figure 1d). This finding was reflected by a significant strain by treatment interaction ($F_{3,42} = 4.411$, $P < 0.05$). Similar findings were also observed in IL-1raTG mice: Following 5 weeks of CMS, C57 × CBA WT mice exhibited a significant decrease in sucrose preference ($P < 0.04$), whereas sucrose preference in IL-1raTG mice remained unchanged (Figure 1e). This finding was reflected by a significant strain by treatment interaction ($F_{3,37} = 5.732$, $P < 0.025$). No difference in the total drinking volume was found between the groups. Additionally, CMS resulted in a 25% reduction in social exploration in WT mice, whereas exploration level in IL-1raTG mice remained unchanged (Figure 1f). This finding was reflected by a significant strain by treatment interaction ($F_{3,37} = 4.811$, $P < 0.05$).

Impaired neurogenesis is involved in mediating the role of IL-1 in CMS-induced depression

Based on previous reports that inflammatory challenges impair the neurogenesis process,[45,46] it may be suggested that CMS-induced IL-1 production is critically involved in mediating the development of depressive symptoms. To examine this hypothesis, we assessed the effects of CMS on hippocampal neurogenesis in IL-1rKO mice and their WT controls. Following 5 weeks of CMS, WT mice exhibited a significant decrease in the number of new cells (BrdU positive) in the dentate gyrus (DG) of their hippocampus ($P < 0.03$; Figure 2a–e), whereas the number of new cells in IL-1rKO mice remained unchanged (Figure 2f). This finding was reflected by a significant strain by treatment interaction ($F_{1,186} = 3.939$, $P < 0.05$). Similarly, WT mice exhibited a significant decrease in the number of new neurons (DCx positive) in the DG of their hippocampus following CMS ($P < 0.0001$; Fig 2g–i), whereas the number of new neurons in IL-1rKO mice remained unchanged (Figure 2j). This finding was reflected by a significant strain by treatment interaction ($F_{1,108} = 7.333$, $P < 0.01$).

Glucocorticoids mediate the role of IL-1 in CMS-induced depression

To examine whether glucocorticoids are involved in mediating the role of IL-1 in CMS-induced depression, we first assessed the effect of CMS on corticosterone secretion in IL-1rKO and WT mice. Following 5 weeks of CMS, WT mice exhibited a significant increase in CS levels ($P < 0.05$), whereas the levels of CS in IL-1rKO mice remained unchanged (Figure 3a). This finding was reflected by a significant strain by treatment interaction ($F_{1,42} = 6.342$, $P < 0.02$). Based on previous research, implicating glucocorticoids in depression,[59] it may be suggested that the lack of CMS-induced corticosterone secretion underlies the lack of depressive symptomatology in IL-1rKO mice. However, such a claim can be true only if glucocorticoids are causally related to CMS-induced depression. To explore this causal relationship, we examined the depressive-like effects of CMS in adrenalectomized mice. In response to 5 weeks of CMS, Sham-operated mice exhibited a significant decrease in sucrose preference ($P < 0.05$), whereas sucrose preference in ADX mice remained unchanged (Figure 3b). This finding was reflected by a significant operation by treatment interaction ($F_{1,30} = 6.311$, $P < 0.02$). No difference in the total drinking volume was found between the groups. Additionally, CMS resulted in a 28% reduction in social exploration in sham-operated mice, whereas the exploration level in ADX mice remained unchanged (Figure 3c). This finding was also reflected by a significant operation by treatment interaction ($F_{1,34} = 4.735$, $P < 0.05$).

Although these findings suggest that corticosterone mediates the involvement of IL-1 in CMS-induced depression, they do not preclude the possible involvement of other adrenal hormones, particularly adrenomedual release of catecholamines. To further establish the role of corticosterone as a downstream mediator of the involvement of IL-1 in depression, we examined whether chronic corticosterone administration is sufficient to mimic the depressive-like effects of CMS, in both WT and IL-1rKO mice. Following chronic corticosterone administration, both WT and IL-1rKO mice exhibited a significant decrease in sucrose preference (Figure 3d). This finding was reflected by a significant effect of treatment ($F_{1,29} = 8.313$, $P < 0.01$), but no effect of strain and no strain by treatment interaction. No difference in the total drinking volume was found between the groups. Additionally, chronic corticosterone administration resulted in decreased social exploration in both WT and IL-1rKO mice (Figure 3e). This finding was reflected by a significant effect of treatment ($F_{1,27} = 11.637$, $P < 0.005$), but no effect of strain and no strain by treatment interaction. Chronic corticosterone administration also induced a significant decrease in the number of new cells (BrdU positive) in the DG of both WT and IL-1rKO mice (Figure 3f). This finding was reflected by a significant effect of treatment ($F_{1,52} = 14.622$, $P < 0.0001$), but no effect of strain and no strain by treatment interaction. Furthermore, chronic corticosterone administration resulted in a significant decrease in the number of new neurons (DCx positive) in the DG of both WT and IL-1rKO mice (Figure 3g). This finding was reflected by a significant effect of treatment ($F_{1,72} = 8.395$, $P < 0.005$), but no effect of strain and no strain by treatment interaction.

Chronic IL-1β exposure induced depressive behavior and reduced neurogenesis

The findings presented above suggest that IL-1 is required for mediating the effects of CMS-induced depression. To examine whether IL-1 is sufficient for inducing depressive symptoms and reduced neurogenesis, we tested whether chronic administration of IL-1β can mimic the effects of CMS on these parameters.

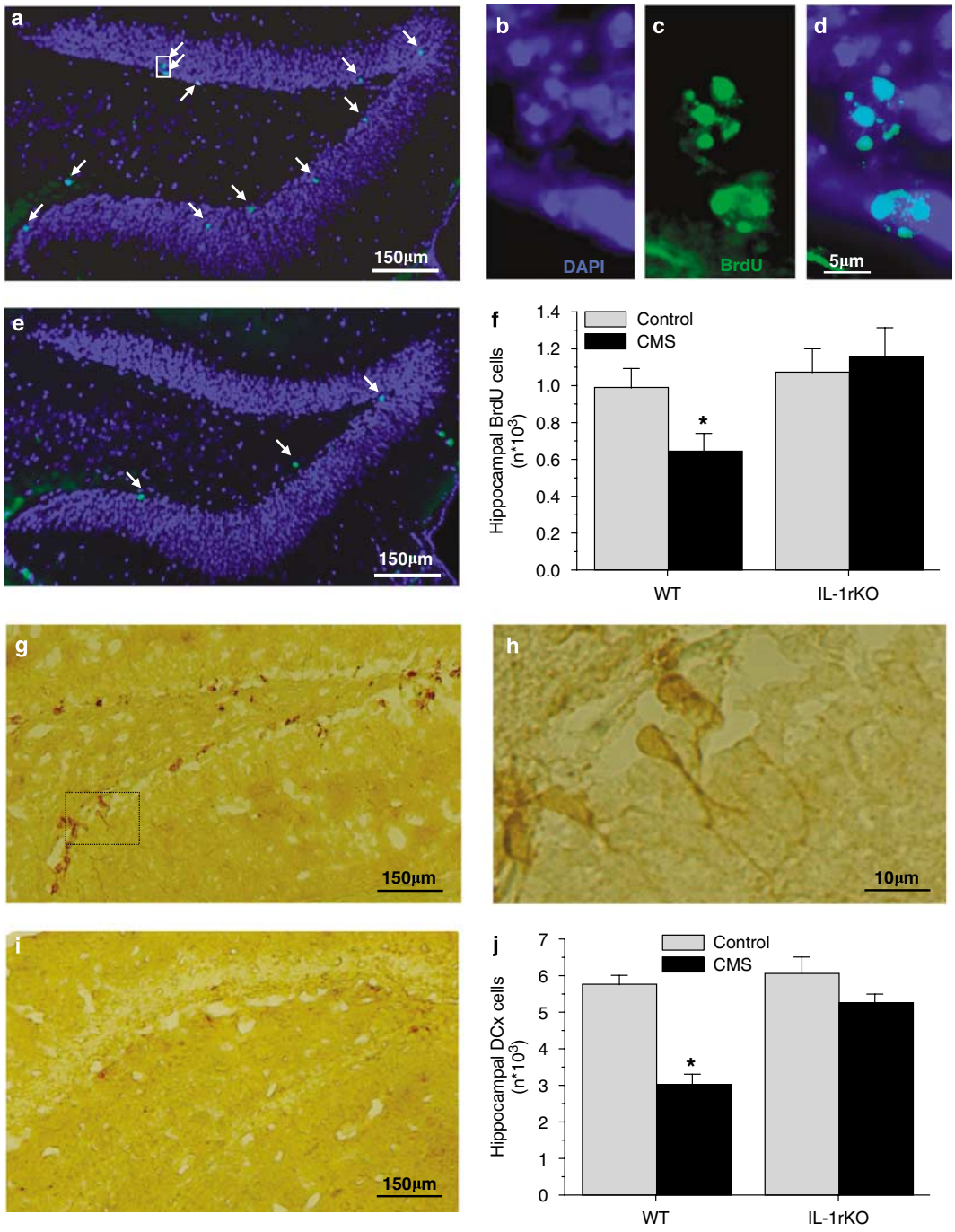

Figure 2 Impaired neurogenesis mediates the role of IL-1 in CMS-induced depressive symptoms. (**a**) Normal hippocampal cytogenesis levels were observed in the dentate-gyrus (DG) of control C57 × 129 WT mice. All nuclei are stained in blue (4′,6-diamidino-2-phenylindole), and newly generated cells that incorporated BrdU are stained in green. Double-stained cells are marked by arrowheads. A higher magnification of the double staining within the white square is shown in (**b–d**). (**e**) Smaller number of newly generated cells was seen in WT mice that were exposed to 5 weeks of CMS. (**f**) CMS exposure resulted in decreased cytogenesis in the DG of WT mice ($n=9$), whereas cytogenesis levels in IL-1rKO mice ($n=9$) remained unchanged, compared to their non-treated controls ($n=9$, $n=6$, respectively). $^*P<0.05$. (**g**) Normal hippocampal neurogenesis levels were observed in the DG of control C57 × 129 WT mice. Newly generated neurons, which express the early neuronal marker doublecortin (DCx) are stained in brown. A higher magnification of the DCx staining within the black square, in which the young neurons with their extensions can be seen, is shown in (**h**). (**i**) A smaller number of young neurons is seen in WT mice that were exposed to 5 weeks of CMS. (**j**) CMS exposure resulted in decreased neurogenesis in the DG of WT ($n=9$), but not in IL-1rKO mice ($n=4$), compared to their non-treated controls ($n=5$, $n=7$, respectively). $^*P<0.0001$. Data presented as mean ± s.e.m. IL, interleukin; IL-1rKO, mice with deletion of the IL-1 receptor type I; WT, wild type; BrdU, bromodeoxyuridine.

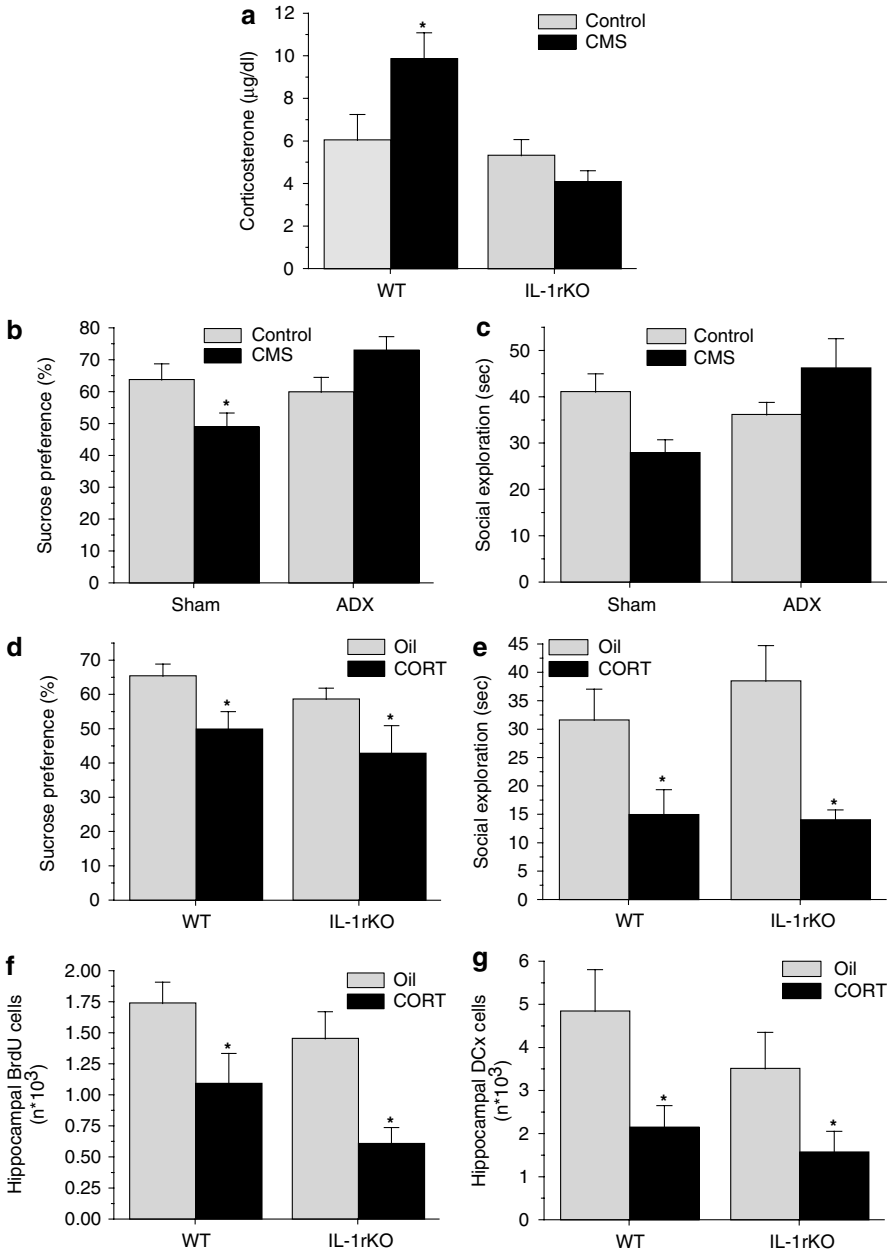

Figure 3 Corticosterone may mediate the role of IL-1 in CMS-induced depressive symptoms, and is necessary for their manifestation. (**a**) C57 × 129 wild-type (WT) mice ($n=12$), but not IL-1rKO ($n=11$), displayed increased serum corticosterone levels following 5 weeks of CMS, compared to their respective controls ($n=11$, $n=12$), $^*P<0.05$. (**b**) Sham-operated mice ($n=9$), but not adrenalectomized mice (ADX, $n=7$), displayed decreased sucrose preference following 5 weeks of CMS, compared to their respective sham and ADX non-treated controls ($n=11$, $n=7$), $^*P<0.05$. (**c**) CMS also resulted in a reduction in social exploration in sham-operated mice ($n=9$), whereas exploration level in ADX mice ($n=12$) remained unchanged, compared to their respective sham and ADX non-treated controls ($n=8$, $n=8$). (**d**) WT and IL-1rKO mice ($n=10$ and 7, respectively), displayed decreased sucrose preference following 4 weeks of corticosterone administration, compared to their respective oil-injected WT and IL-1rKO controls ($n=9$ and 7, respectively), $^*P<0.05$. (**e**) Chronic corticosterone administration also resulted in a reduction in social exploration in both WT and IL-1rKO mice ($n=9$ and 5, respectively), compared to their oil-injected WT and IL-1rKO controls ($n=7$ and 10, respectively), $^*P<0.05$. (**f**) A smaller number of newly generated cells (BrdU-positive) was found in WT and IL-1rKO mice ($n=17$ and 12, respectively) following corticosterone administration, compared to their respective oil-injected WT and IL-1rKO controls ($n=17$ and 10, respectively), $^*P<0.05$. (**g**) Chronic corticosterone administration also resulted in a reduction in number of newly generated neurons (DCx positive) in both WT and IL-1rKO mice ($n=24$ and 14, respectively), compared to their oil-injected WT and IL-1rKO controls ($n=22$ and 16, respectively), $^*P<0.05$. Data presented as mean ± s.e.m. IL-1, interleukin-1; CMS, chronic mild stress; BrdU, bromodeoxyuridine; IL-1rKO, mice with deletion of the IL-1 receptor type I; WT, wild type.

Following 4 weeks of IL-1β exposure, C57 $\times$ 129 WT mice exhibited a significant decrease in sucrose preference ($P<0.03$) compared to their saline-treated controls. No difference in sucrose preference was found between the groups following a single week of exposure to IL-1β, suggesting that similar to CMS, only long chronic exposure to IL-1 results in depressive symptoms. This finding was reflected by a significant repeated-measures time by treatment interaction ($F_{1,18}=6.205$, $P<0.025$; Figure 4a). No difference in the total drinking volume was found between the groups. Chronic IL-1β exposure for 4 weeks also resulted in a significant decrease in social exploration ($t_{18}=-2.173$, $P<0.025$; Figure 4b). Mice that were chronically exposed to IL-1β also exhibited a significant decrease in the number of both BrdU-positive new cells ($t_{65}=-3.78$, $P<0.0001$; Figure 4c) and DCx-positive new neurons ($t_{58}=-2.735$, $P<0.004$; Figure 3d) compared to their saline-treated controls.

Discussion

The present study demonstrates a critical role for IL-1 in depressive behavior, and two of its putative underlying mechanisms. Specifically, we found that IL-1rKO and IL-1raTG mice, which were previously shown not to respond to either exogenous or endogenous IL-1,[49,51] display no depressive-like symptoms following exposure to CMS. Since IL-1ra overexpression in IL-1raTG mice is under the glial fibrillary acidic protein promoter and therefore restricted to the CNS, it can be concluded that central, rather than peripheral IL-1 production and actions are mediating the depressive-like effect of CMS. Using this approach we also studied two neurobiological mechanisms for the involvement of IL-1 in depression. Based mainly on the actions of antidepressant drugs, decreased hippocampal neurogenesis and dysregulation of the HPA axis have been previously implicated in depression.[60,61] The finding of the present study, demonstrating that IL-1rKO mice did not display CMS-induced impaired neurogenesis, suggest that this processes is critically dependent on IL-1 signaling. Moreover, the findings that IL-1rKO mice did not display CMS-induced corticosterone elevation, that blockade of endogenous corticosterone release abolished CMS-induced depression and that chronic corticosterone administration mimicked the behavioral and neural effects of CMS strongly suggest that corticosterone is a downstream mediator of IL-1's effects in the CMS paradigm. Finally, using chronic exogenous administration of IL-1, we also show that this cytokine is not only necessary, but also sufficient,

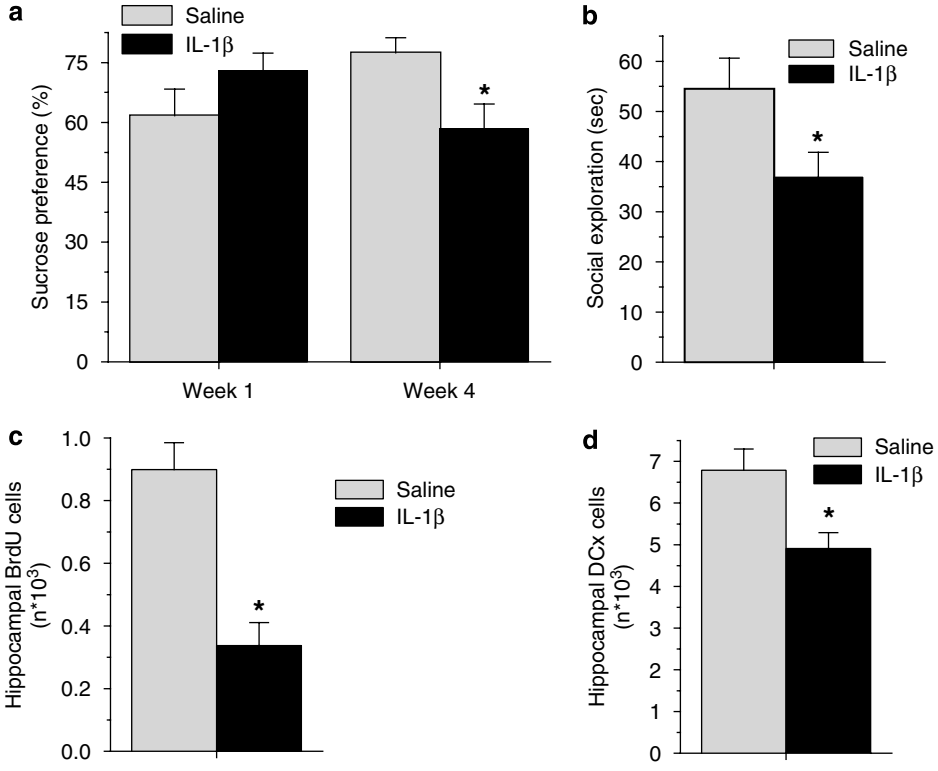

Figure 4 Chronic IL-1 exposure is sufficient for the appearance of depressive symptoms and impaired neurogenesis. (**a**) Mice that were chronically exposed to IL-1β ($n=11$) via osmotic micropumps demonstrated decreased sucrose preference after 4 weeks, but not 1 week of exposure, compared to saline-treated mice ($n=9$), $^*P<0.05$. (**b**) Four weeks of IL-1β exposure also resulted in a reduction in social exploration in these mice, $^*=P<0.05$, as well as decreased cytogenesis (**c**) and neurogenesis (**d**) in the dentate-gyrus of their hippocampus compared to their saline-treated controls. $^*P<0.005$. Data presented as mean $\pm$ s.e.m. IL-1, interleukin-1.

for the production of depressive symptoms and neurogenesis impairment.

Our finding that exposure to the CMS regime induced IL-1 protein secretion in the hippocampus is in line with the increase in peripheral IL-1 levels found in depressed patients.[5–7] These results are consistent with studies in rodents, which demonstrated an increase in hippocampal IL-1 protein levels and activity following acute severe stressors.[27–29] Studies on the effects of chronic stressors on the IL-1 system focused on the mRNA level and their results are less clear, demonstrating either increased,[62] decreased[63] or no effect[64,65] on brain IL-1 expression. The physiological significance of the increase in IL-1 protein levels following CMS is demonstrated by the findings that IL-1rKO and IL-1raTG mice did not display depressive-like symptoms, including decreased sucrose preference (which is considered to represent diminished sensitivity to rewards) and social exploration. The CMS model resembles human depression in its long duration, the depressive-like symptoms that it induces, particularly anhedonia (an inability to experience pleasure), and its responsiveness to chronic antidepressant therapy.[47,48] The finding that impaired IL-1 signaling abolished the anhedonic depressive-like effect of stress are consistent with previous reports that IL-1 is critically involved in the depressive-like cognitive effects of stress, including learned helplessness,[30] and memory impairments.[31]

Ample evidence indicates that IL-1 influences all levels of the HPA axis, inducing the secretion of corticotropin releasing hormone, adrenocorticotropic hormone and glucocorticoids and reducing the sensitivity of glucocorticoid receptors.[66] Therefore, it is possible that dysregulation the HPA axis, which is one of the most robust biological markers of depression, underlies the involvement of IL-1 in depression. We show here that whereas in WT mice corticosterone levels were increased following CMS, similar to the hypercortisolism found in depressed patients,[37,38] no such increase was observed in IL-1rKO mice, concomitant with their blunted behavioral depressive symptoms. These findings are consistent with our previous demonstration that IL-1rKO mice display reduced adrenocortical activation following exposure to acute mild stressors.[32] The necessity of corticosterone in CMS-induced depression is further demonstrated by the lack of depressive symptoms in adrenalectomized mice. This finding, together with the results of a recent study demonstrating that administration of the glucocorticoid receptor antagonist mifepristone (RU-486) abolished CMS-induced behavioral and neural alterations,[67] indicate that at least in the CMS model, glucocorticoids secretion is causally related to the development of the depressive symptoms. This conclusion is further strengthened by the finding that chronic corticosterone administration resulted in depressive symptoms similar to those displayed by mice that were exposed to CMS. Basal corticosterone may also play a permissive role in CMS-induced depression, but this issue was not addressed in this study, as adrenalectomized mice did not receive basal corticosterone replacement. Our findings are consistent with previous findings that many patients with depression exhibit hypersecretion of cortisol and its metabolites,[68,69] that treatment with mifepristone is effective in some forms of depression,[70] and with the clinical observations that patients with Cushing's syndrome, which results in hypercortisolemia, as well as patients with other diseases who are treated pharmacologically with high doses of glucocorticoids often experience severe depression.[59] The concomitant absence of CMS-induced depressive-like symptoms and corticosterone secretion in IL-1rKO, along with the findings that adrenalectomy blocks CMS-induced depression whereas chronic corticosterone mimics the effects of CMS in both WT and IL-1rKO mice demonstrate that chronic elevation in corticosterone levels are both necessary and sufficient for producing depressive symptoms, and strongly implicate corticosterone as the downstream mediator of IL-1's involvement in CMS-induced depressive symptoms.

Another possible mechanism for the involvement of IL-1 in CMS-induced depression is impaired neurogenesis. In recent years, impaired hippocampal neurogenesis has been implicated in major depression,[43,71] mainly based on studies in rodents, demonstrating that: (1) chronic administration of antidepressants increased neurogenesis in the rat DG,[72–74] (2) antidepressants prevented the reduction in neurogenesis in models of stress-induced depression,[75–78] and (3) normal levels of neurogenesis are required for the behavioral effects of antidepressants.[79] These findings may be also related to the reduction in hippocampal volume in patients with major depression, which correlates with the length of illness,[80,81] and the finding that chronic antidepressant treatment can increase hippocampal volume.[82] In support of a role for neurogenesis in depression, we show here that CMS reduces hippocampal cytogenesis and neurogenesis in WT mice, corroborating the results of two recent publications.[76,77] Furthermore, we show that in IL-1rKO mice cytogenesis and neurogenesis remained intact, concomitantly with the lack of depressive symptoms in these animals. It may be suggested that in the IL-1rKO mice the lack of CMS-induced corticosterone secretion, which was previously shown to directly impair neurogenesis,[83] is responsible for the blunted effect on neurogenesis. This conclusion is supported by the finding that chronic corticosterone administration resulted in impaired neurogenesis in both WT and IL-1rKO mice.

Finally, the present results indicate that IL-1 is not only necessary, but also sufficient for the manifestation of depressive symptoms, as mice that were chronically exposed to IL-1 via osmotic minipumps, without any exposure to stressors, demonstrated depressive symptoms similar to those expressed in mice that were exposed to CMS. As the level of IL-1 was relatively low (the dose of 5 mg/kg/24 h is half of

that of the common dose for a single injection in acute studies), no acute behavioral effects of IL-1 were observed, and no other signs of malaise were noted at this or at later time points. Only a chronic, 4 weeks long exposure, resulted in depressive symptoms. This finding also suggests that the effects of IL-1 were not caused by its possible synergy with the mild inflammation produced by the minipump implantation surgery. The finding that chronic IL-1 induces a depressive-like condition corroborates previous research demonstrating that exogenous administration of IL-1 produces anhedonia in rodents, reflected by reduced preference for sweet solutions and reduced libido.[19,84] The relationship of these findings to depression is further demonstrated by the ability of chronic antidepressant therapy to block IL-1-induced anhedonia.[19] Our results also demonstrate, for the first time, that chronic IL-1 administration impairs neurogenesis, providing additional support for the hypothesis that CMS-induced IL-1 production exerts its effect on depression via suppression of neurogenesis.

In conclusion, the findings of the present study suggest that elevated levels of brain IL-1 are causally related to various aspects of depression, including the behavioral symptomatology, adrenocortical activation and reduced neurogenesis. This conclusion is consistent with the abundant evidence that many medical conditions, including chronic infectious diseases, autoimmune diseases, stroke, neurotrauma and neurodegenerative diseases, are associated with elevation in brain IL-1 levels and with high incidence of depression. Furthermore, the elevated IL-1 levels observed in depressed patients who are not physically ill[5–7] are not merely a by-product of the depressive symptomatology, but may be caused by chronic life stressors and play a direct role in the induction of depression. Our findings suggest that antidepressant therapy may produce at least some of its beneficial effects on behavioral depression, as well as the normalization of the HPA axis functioning and neurogenesis, by modulating IL-1 signaling. Moreover, it is suggested that manipulations and interventions that directly modulate IL-1 signaling should provide novel preventive and therapeutic procedures for alleviating depression.

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