Behavioral/Systems/Cognitive

Environmental Enrichment Restores Memory Functioning in Mice with Impaired IL-1 Signaling via Reinstatement of Long-Term Potentiation and Spine Size Enlargement

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Environmental enrichment (EE) was found to facilitate memory functioning and neural plasticity in normal and neurologically impaired animals. However, the ability of this manipulation to rescue memory and its biological substrate in animals with specific genetically based deficits in these functions has not been extensively studied. In the present study, we investigated the effects of EE in two mouse models of impaired memory functioning and plasticity. Previous research demonstrated that mice with a deletion of the receptor for the cytokine interleukin-1 (IL-1rKO), and mice with CNS-specific transgenic over-expression of the IL-1 receptor antagonist (IL-1raTG) display impaired hippocampal memory and long-term potentiation (LTP). We report here a corrective effect of EE on spatial and contextual memory in IL-1rKO and IL-1raTG mice and reveal two mechanisms for this beneficial effect: Concomitantly with their disturbed memory functioning, LTP in IL-1rKO mice that were raised in a regular environment is impaired, and their dendritic spine size is reduced. Both of these impairments were corrected by environmental enrichment. No deficiencies in neurogenesis or hippocampal BDNF and vascular endothelial growth factor secretion were found in IL-1rKO mice that were raised in a regular environment, and both of these variables were increased to a similar degree in enriched IL-1rKO and wild-type mice. These findings suggest that exposure to an enriched environment may be beneficial for individuals with impaired learning and memory related to genetic impairments of IL-1 signaling (and possibly other genetic causes), by reversing impairments in dentate gyrus LTP and spine size and by promoting neurogenesis and trophic factors secretion.

Introduction

Environmental enrichment (EE), comprising a variety of social and physical stimuli, is known to enhance hippocampal-dependent memory and plasticity in normal mice (Nilsson et al., 1999; Duffy et al., 2001). Furthermore, EE was shown to improve learning deficits in various disorders and injuries of the nervous system (van Praag et al., 2000; Nithianantharajah and Hannan, 2006). Human studies demonstrated a beneficial effect of different enrichment protocols on cognitive and social behavior in children that can last through adulthood (Ramey and Ramey, 1992; Campbell and Ramey, 1994; Shonkoff et al., 2000; Campbell et al., 2001).

The proinflammatory cytokine Interleukin-1 (IL-1) was shown in many studies to be involved in memory processes, specifically those related to hippocampal functioning (Goshen and Yirmiya, 2007). For example, we previously demonstrated the necessity of IL-1 for normal hippocampal memory and plasticity using two mouse models with impaired IL-1 signaling: mice with a deletion of the IL-1 receptor type I (IL-1rKO) and mice with CNS-specific transgenic overexpression of IL-1 receptor antagonist (IL-1raTG). Both IL-1rKO and IL-1raTG mice demonstrate impaired spatial memory in the water maze and impaired contextual fear conditioning, which both depend on normal hippocampal functioning, but intact performance in hippocampal-independent tasks (Avital et al., 2003; Goshen et al., 2007). Furthermore, IL-1rKO mice were found to display diminished short-term plasticity and exhibit no long-term potentiation (LTP), both *in vivo* and *in vitro* (Avital et al., 2003). These findings may be relevant to several recent studies in humans, reporting that mutations in the IL-1 receptor accessory protein-like gene are involved in X-linked mental retardation (Carrié et al., 1999; Jin et al., 2000).

In the present study, we tested the ability of environmental enrichment to improve the impaired memory performance in these IL-1 signaling deficiency mice. The effect of EE on cognitive functioning in IL-1rKO and IL-1raTG mice was assessed together with several mechanisms that may mediate the effects of EE, including alterations in LTP in the dentate gyrus (DG), dendritic spine size and density in DG granule cells, neurogenesis, and growth factors secretion.

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Materials and Methods

Subjects

Subjects were male IL-1rKO mice and their C57BL/6 × 129/Sv wild-type controls (The Jackson Laboratory), as well as IL-1raTG mice and their C57BL/6 × CBA wild-type 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 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 (Labow et al., 1997), and they exhibit diminished adrenocortical responsiveness to mild stressors (Goshen et al., 2003). 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 therefore over-expression is restricted to the brain and spinal cord (Lundkvist et al., 1999; Bajayo et al., 2005). Similar to the IL-1rKO mice, IL-1raTG mice are insensitive to the administration of exogenous IL-1 (Lundkvist et al., 1999) and were protected from neurogenesis alterations in response to acute and chronic neuroinflammation (Spulber et al., 2008). The physiological and behavioral phenotype of this strain is overall normal, besides a small elevation in body weight and reduced bone density (Bajayo et al., 2005), increased locomotion, and reduced anxiety (Oprica et al., 2005). Animals were housed in an air-conditioned room (22 + 1°C), with food and water *ad libitum*. All the experiments were performed during the first 3 h of the dark phase of a reversed 12 h light/dark cycle. The experiments were approved by the Hebrew University Committee on Animal Care and Use.

Enrichment protocol

Mice were housed in groups of 12, in large (60 × 60 × 40 cm) transparent cages. In each cage, there were running wheels, plastic-tube mazes, and ladders. The mice were exposed to the enriched environment for 6 weeks before its influence on memory and plasticity was evaluated. In the non-enriched condition, mice were housed in triads, in a small cage (25 × 20 × 15 cm), and received regular food pellets and water *ad libitum*, with no additional stimuli.

Memory assessment paradigms

Fear conditioning. The apparatus consisted of a transparent square conditioning cage (25 × 21 × 18 cm), with a grid floor wired to a shock generator and scrambler (Campden Instruments). Mice were placed in the cage for 120 s, and then a pure tone (2.9 kHz) was sound for 20 s, followed by a 2 s, 0.5 mA foot-shock. This procedure was then repeated, and 30 s after the delivery of the second shock, mice were returned to their home cage. Fear conditioning (48 h later) was assessed by a continuous measurement of freezing (complete immobility), the dominant behavioral fear response (Maren, 2001), as measured by infra-red movement detectors, connected to a computerized data collection system (Campden Instruments). To test the hippocampal dependent contextual fear conditioning (Maren, 2001), mice were placed in the original conditioning cage, and freezing was measured for 5 min. Two hours later, mice were tested for the hippocampal-independent auditory-cued fear conditioning (Maren, 2001) by placing them in a different context shaped as an opaque colorful pyramid with a smooth floor. As a control for the influence of the novel environment, freezing was measured for 2.5 min in this new environment, and then the conditioning tone was sound for 2.5 min, during which auditory-cued conditioned freezing was measured. Freezing was also measured during the first 60 s of the initial conditioning trial, before the tone and shock administration, to assess possible strain differences in baseline freezing in the conditioning context.

Water maze. The water maze consisted of a round tank, 1.6 m in diameter, filled with water mixed with nontoxic gouache paint to make it opaque. Mice were trained to find the location of a hidden platform (16 cm in diameter), submerged 1 cm below the water surface, using extra maze visual cues. Training consisted of three trials per day, with a 1 h break between trials, for 3 d. The experiments were conducted using a random protocol, in which the entrance point to the maze was varied randomly between trials, and the platform remained in a permanent position. The illumination, sound, and distal visual cues on the walls and ceiling were controlled and kept constant throughout the experiment. A video camera above the pool was connected to a computerized tracking system that monitored the latency to reach the platform, the path length, and the swimming speed in each trial (VP118 tracking system; HVS Image). Mice were dried under a red light heating lamp after each trial.

Sickness behavior measurements

To assess the ability of IL-1rKO and IL-1raTG mice to react to exogenous IL-1 administration after exposure to an enriched environment for 40 d, mice were injected intraperitoneally with either IL-1α (20 μg/kg) or saline. Spatial exploratory behavior was assessed 110 min later using the open-field test as follows: The open field apparatus is a white 80 × 80 cm plastic arena divided to 81 identical squares, with 50-cm-high walls. At the beginning of the test, the mouse was placed in the southeast corner of the arena, and the number of line crossings (with both hindpaws) was manually recorded for 180 s and served as an index for spatial explorative behavior. Social behavior was assessed 40 min later (i.e., 150 min after the injection) by the social exploration test: each mouse was placed in an observation cage, and after 15 min of habituation, 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 recorded for 3 min.

Measurements of dentate gyrus plasticity in vivo

Mice were anesthetized with urethane (21% solution: 1.2 g/kg, i.p.) and placed in a stereotaxic apparatus. A bipolar 125 μm concentric stimulating electrode was placed in the perforant path (PP) (coordinates: 0.5 mm anterior to lambda, 2.5 mm lateral to the midline; depth, 1.6–2.1 mm). A glass pipette (diameter of 2–3 μm), containing 2 M NaCl solution, was inserted into the DG of the dorsal hippocampus, using an hydraulic microdrive (coordinates: 2.0 mm posterior to bregma, 1.0 mm lateral to the midline; depth, 1.8–2.2 mm). Electrode position was optimized to record maximal population spike in response to 100 μs pulse stimulation of the PP. Evoked responses were amplified, filtered at 1 Hz–1 kHz, and stored for later off-line analysis.

After electrode insertion, recording was allowed to stabilize for 25 min. Baseline field potential responses in the DG to PP stimulation were recorded using stimulus intensity that was 50% of the intensity that evoked maximal asymptotic spike amplitude. This stimulus intensity was found to be similar in the IL-1rKO and wild-type (WT) control groups. Input–output relations were later examined using three stimulus intensities (1.5, 2.5, or 3.5 V).

To assess long-term plasticity, LTP was induced by applying high-frequency stimulation (HFS) (five trains of eight 0.4 ms, 400 Hz pulses spaced 10 s apart. Stimulus intensity was 1.5 V). Ten measurements, 10 s apart, were taken and averaged every 5 min, and LTP was computed as the change in the evoked responses measured post-HFS compared with pre-HFS responses. Data were collected and analyzed using Power Lab and Data Analysis software.

Measurements of dendritic spine size and density in the DG

After 6 weeks of enrichment, mice were killed and brains were collected and subjected to Golgi staining (FD Rapid GolgiStain Kit; FD Neurotechnologies) according to the manufacturer's instructions. Image stacks (0.3 μm apart) of third order dendrites of DG granule cells were taken using an inverted light microscope (Zeiss). Total spine size (head and neck) was measured in the picture in which it appeared at its maximal size using the ImageJ software. Reconstructions of dendrites were performed manually from three-dimensional image stacks using Neurolucida (MicroBrightField).

Western blot for synaptophysin and GluR-1 in the hippocampus

Whole hippocampi were lysed in a buffer containing 0.1% Triton X-100. Protein lysates were boiled before electrophoresis on a 7% SDS-PAGE and transferred onto a nitrocellulose membrane (40 μg protein/well). The membranes were blocked with Tris-buffered saline–Tween 20 and 3% BSA for 1 h at room temperature (RT), incubated for 1.5 h at RT with a rabbit anti-GluR1 (1:100; Alamone Labs) or mouse antisynaptophysin (1:200; Sigma), and subsequently with HRP-conjugated secondary antibodies for 45 min at RT. Immunoreactive bands were visualized using Western Blotting Luminol Reagent (EZ-ECL; Biological Industries).

BrdU and Tuj-1 immunofluorescent staining

Environmental enrichment is one of the strongest enhancers of adult neurogenesis and trophic factors secretion (van Praag et al., 2000), which were implicated in memory processes (Gross, 2000; Kempermann et al., 2004; Saxe et al., 2006). Thus, we evaluated the contribution of these factors in our experiments. After 28 d of enrichment, mice were intraperitoneally injected once a day for 12 d with 100 mg/kg of BrdU (Sigma) and killed 24 h after the last injection. Mice were intracranially perfused with cold 4% paraformaldehyde, brains were removed, kept in 4% paraformaldehyde for 72 h, washed in PBS, and stored in 75% ethanol. Brains were embedded in paraffin, and serial 8 μM axial sections were performed for immunofluorescent analysis. Sections were washed twice with Histoclear for 10 min to remove the paraffin coating and then rehydrated as follows: two washes with 100% ethanol for 1 min, two washes with 95% ethanol for 1 min, one wash with 80% ethanol for 1 min, and one wash with 50% ethanol for 1 min, followed by four washes with water. Permeability was enabled by boiling the slides in a citrate buffer (pH = 6; Zymed) for 20 min. Slides were then washed 4 times with PBS, and a blocking solution of 1% BSA in PBS was applied. The primary antibodies, mouse anti-BrdU (1:100; Amersham) and rabbit anti-Tuj-1 (1:500; Covance), were applied in a volume of 100 μl per slide, coated with a cover glass, and incubated in 4°C overnight. Slides were washed three times in PBS for 5 min. The secondary antibodies, anti-mouse coupled Fitc (1:200; Zymed) and anti-rabbit Alexa555 (1:400; Invitrogen), were applied in a volume of 100 μl per slide, coated with a cover glass, and incubated in the dark in RT for 60 min. Slides were then washed three times in PBS for 5 min, lightly dried, and mounted with 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI). Images were taken using a fluorescent microscope (Leica).

ELISA for hippocampal BDNF and vascular endothelial growth factor

Adult hippocampi were collected, and brain tissue was then homogenized in 200 μl cold RPMI 1640 (Sigma) containing 0.5 μl protease inhibitor mixture (Sigma), and centrifuged for 10 min (300 *g*) at 4°C before supernatant was collected. BDNF and vascular endothelial growth factor (VEGF) levels were determined using ELISA kits (R&D Systems).

Statistical analysis

The results were analyzed by either two-way ANOVAs with the strain and enrichment as between-subjects factors, or three-way ANOVAs with the strain and enrichment as between-subjects factors and the different measures as a within-subjects, repeated measure factor, when applicable. ANOVAs were followed by either Tukey *post hoc* or planned contrasts analyses.

Results

Environmental enrichment restores hippocampal-dependent memory in the fear conditioning and water maze paradigms

IL-1rKO mice that were raised in a regular environment displayed impaired contextual fear conditioning compared with their WT-regular controls, i.e., they displayed a significantly shorter freezing time in the original conditioning context 48 h after they received a shock there ($p < 0.05$). However, enriched IL-1rKO mice did not differ from their enriched WT controls (Fig. 1*A*). These findings were reflected by a significant strain by enrichment interaction ($F_{(1,45)} = 4.834$, $p < 0.05$). This difference cannot be attributed to an inherent diminished tendency of the IL-1rKO mice to freeze, because before shock administration, there was no difference in freezing in this context between any of the groups ($p > 0.5$; data not shown). In contrast, IL-1rKO mice that were raised in either regular or enriched environment displayed normal auditory-cued fear conditioning, compared with their respective WT controls ($p > 0.05$) (Fig. 1*B*). Specifically, the tone presentation had a significant effect on all groups ($F_{(1,43)} = 432.74$, $p < 0.0001$), with no tone by strain interaction ($p > 0.05$), and the enrichment increased auditory-cued freezing in both strains, as reflected by a significant tone by enrichment interaction ($F_{(1,43)} = 32.627$, $p < 0.0001$).

Whereas IL-1rKO mice that were raised in a regular environment displayed impaired learning in the spatial memory paradigm [i.e., the latencies to reach the platform were significantly longer in these IL-1rKO mice than in their WT controls ($p < 0.01$)], enriched IL-1rKO mice did not differ from their enriched WT controls (Fig. 1*C*). These findings were reflected by a significant triple strain by enrichment by time interaction ($F_{(1,55)} = 5.113$, $p < 0.05$).

Compared with controls, IL-1rKO mice displayed a significantly slower speed of swimming (Fig. 1*D*), as reflected by a significant strain effect ($F_{(1,55)} = 38.504$, $p < 0.0001$), suggesting that they have motor impairments. Environmental enrichment resulted in a significant increase in swimming speed ($F_{(1,55)} = 19.484$, $p < 0.0001$), but this improvement was bigger in the IL-1rKO mice, as reflected by a significant strain by enrichment interaction ($F_{(1,55)} = 4.839$, $p < 0.05$). However, the impairment in swimming speed cannot account for the slower rate of learning, because the learning deficit was also observed with respect to the path length to reach the platform, as reflected by a triple strain by enrichment by time interaction ($F_{(1,47)} = 6.175$, $p < 0.02$) (Fig. 1*E*). The path length provides a measurement of learning that does not depend on speed. Planned contrasts revealed that in the last trial, IL-1rKO mice that were raised in a regular environment displayed a significantly longer path length to reach the platform compared with their WT controls ($p < 0.02$).

IL-1raTG mice that were raised in a regular environment displayed impaired contextual fear conditioning compared with their WT controls, i.e., they displayed a significantly shorter freezing time in the original conditioning context, 48 h after receiving a shock there ($p < 0.05$). However, enriched IL-1raTG mice did not differ from their enriched WT controls, and both of these groups displayed a significant improvement in contextual fear conditioning compared with their respective controls that were raised in a regular environment ($p < 0.05$; $p < 0.005$, respectively) (Fig. 1*F*). However, there was no significant strain by enrichment interaction. The differences in contextual fear conditioning cannot be attributed to an inherent diminished tendency to freeze, because before shock administration, there was no difference in freezing in this context between any of the groups ($p > 0.5$; data not shown). In contrast, IL-1raTG mice that were raised in either regular or enriched environment displayed normal auditory-cued fear conditioning, compared with their respective WT controls ($p > 0.05$) (Fig. 1*G*). Specifically, the tone presentation had a significant effect on all groups ($F_{(1,38)} = 49.8$, $p < 0.0001$), with no tone by strain interaction ($p > 0.5$), and the enrichment increased auditory-cued freezing in both strains, as reflected by a significant tone by enrichment interaction ($F_{(1,38)} = 13.531$, $p < 0.001$).

Whereas IL-1raTG mice that were raised in a regular environment displayed impaired learning in the spatial memory paradigm ($p < 0.01$), enriched IL-1raTG mice did not differ from their enriched WT controls (Fig. 1*H*). These findings were reflected by a significant strain by enrichment interaction ($F_{(1,34)} = 4.6$, $p < 0.05$) for the last trial.

No speed differences were found between IL-1raTG and WT mice in swimming speed ($p > 0.5$) (Fig. 1*I*), and environmental enrichment resulted in a significant overall increase in swimming speed ($F_{(3,38)} = 5.92$, $p < 0.02$). Thus, the differences in the latency to reach the platform in IL-1raTG mice on the last trial cannot be attributed to a motor deficiency. However, the path length to reach the platform was also assessed and yielded a sig-

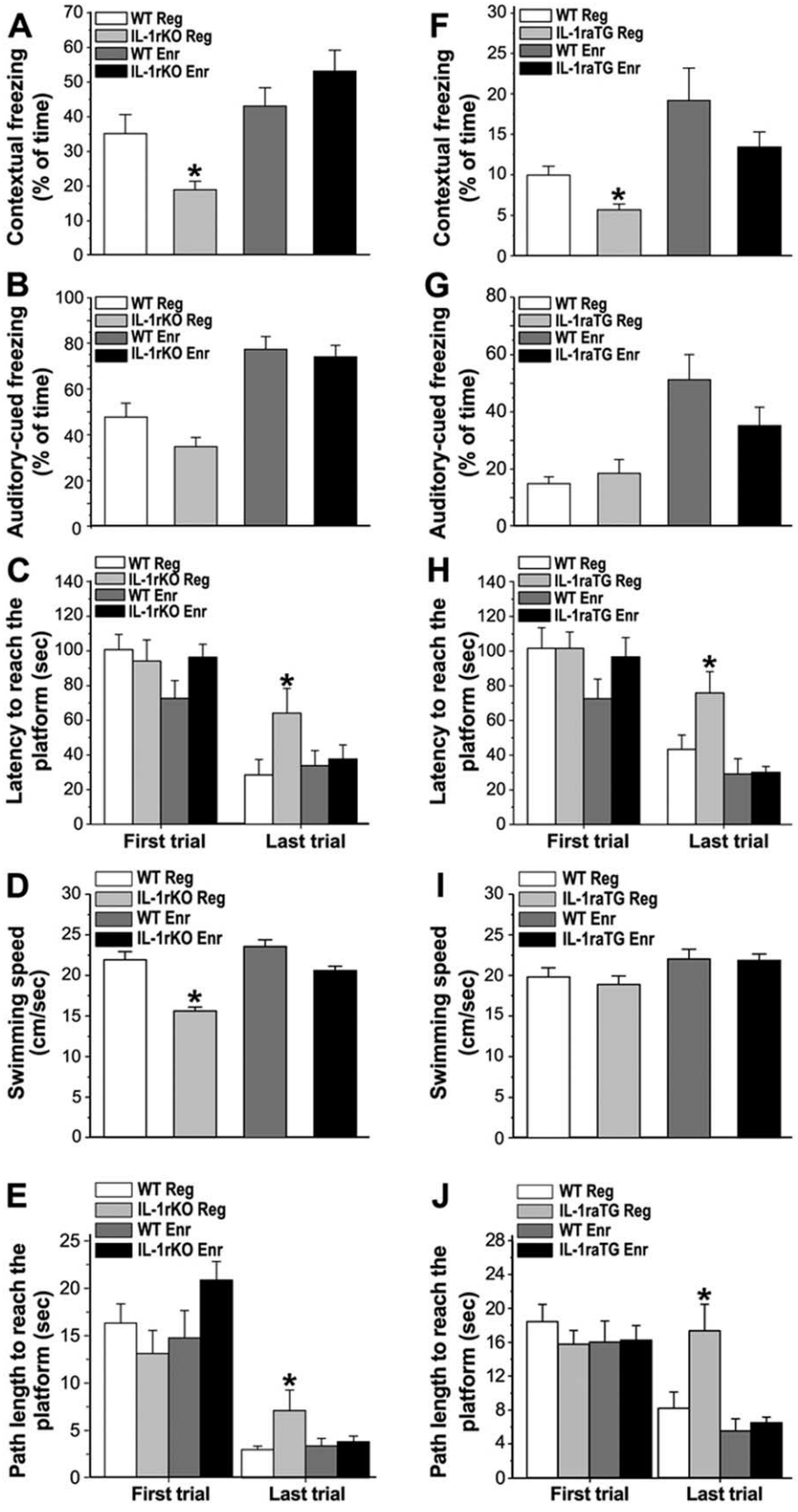

Figure 1. Environmental enrichment improves contextual and spatial memory in mice with impaired IL-1 signaling. ***A***, IL-1rKO mice that were raised in a regular environment (IL-1rKO-Reg; $n = 13$) displayed impaired contextual fear conditioning compared with their WT-regular controls (WT-Reg; $n = 9$). However, IL-1rKO mice that were raised in an enriched environment (IL-1rKO-Enr; $n = 19$) did not differ from their enriched WT controls (WT-Enr; $n = 13$). $^*p < 0.05$ compared with WT regular. ***B***, In contrast, IL-1rKO-Reg and IL-1rKO-Enr mice displayed normal auditory-cued fear conditioning, compared with their respective WT controls. Enrichment increased auditory-cued freezing in both strains. ***C***, IL-1rKO-Reg mice ($n = 13$) displayed spatial memory impairment compared with their WT controls ($n = 14$) in the latency to reach a hidden platform during the last (ninth) trial. However, after environmental enrichment, IL-1rKO mice ($n = 19$) showed normal memory compared with their enriched WT controls ($n = 13$). $^*p < 0.01$ compared with all other groups. ***D***, IL-1rKO-Reg mice demonstrated significantly lower swimming speed compared with all other groups. Environmental enrichment significantly increased the swimming speed of IL-1rKO mice, but they still differed from their WT-Enr controls. $^*p < 0.01$ compared with all other groups. ***E***, This finding cannot serve as an exclusive explanation for the longer latency of IL-1rKO-Reg mice to reach the platform, because these mice displayed spatial memory impairment in the path length to reach the hidden platform as well, a parameter independent on swimming speed. Furthermore, IL-1rKO-Enr mice, which were also slower than their WT-Enr controls, showed normal memory using this parameter. $^*p < 0.02$ compared with all other groups. ***F***, IL-1raTG-Reg mice ($n = 10$) displayed impaired contextual fear conditioning compared with WT-Reg controls ($n = 8$). However, IL-1raTG-Enr mice ($n = 12$) did not differ from their enriched WT controls ($n = 11$). $^*p < 0.05$ compared with WT-regular controls. ***G***, In contrast, IL-1raTG-Reg and IL-1raTG-Enr mice displayed normal auditory cued-fear conditioning, compared with their respective WT controls. Environmental enrichment increased auditory-cued freezing in both strains. ***H***, IL-1raTG-Reg mice ($n = 8$) displayed spatial memory impairment compared with their WT controls ($n = 6$), reflected by a longer latency to reach the hidden platform during the last trial. However, IL-1raTG-Enr mice ($n = 12$) showed normal memory compared with WT-Enr controls ($n = 12$). ***I***, IL-1raTG-Reg mice did not differ from their WT-Reg controls in swimming speed, and environmental enrichment similarly increased the swimming speed of both WT and IL-1raTG mice. ***J***, IL-1raTG-Reg mice also displayed spatial memory impairment in the path length to reach the hidden platform, whereas IL-1raTG-Enr mice showed normal memory using this parameter. $^*p < 0.01$ compared with all other groups. Data presented as the mean ± SEM.

nificant strain by enrichment interaction in the last trial ($F_{(1,31)} = 4.04$, $p < 0.05$) (Fig. 1*J*). Planned contrasts revealed that in the last trial, IL-1raTG mice that were raised in a regular environment displayed a significantly longer latency to reach the platform compared with their WT controls ($p < 0.01$).

Environmental enrichment does not restore IL-1 signaling in either IL-1rKO or IL-1raTG mice

In response to IL-1α injection, WT mice exhibited significant decreases in both social exploration and spatial exploration in the open-field compared with their saline-injected controls, whereas both social and spatial exploration in IL-1rKO mice remained unchanged (Fig. 2*A*,*B*). Enrichment had no effect on the responses to IL-1α in either WT or IL-1rKO mice. These findings were reflected by significant strain effects ($F_{(3,20)} = 17.76$, $p < 0.0001$; $F_{(3,18)} = 4.573$, $p < 0.05$, respec-

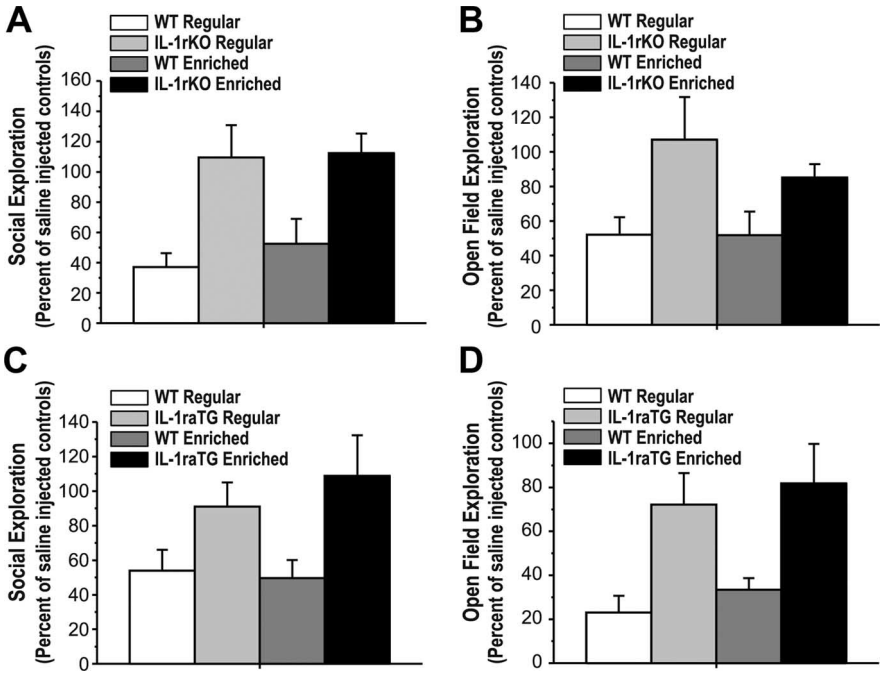

Figure 2. Environmental enrichment did not restore the ability of IL-1rKO and IL-1raTG mice to respond to IL-1. ***A***, ***B***, In response to IL-1α injection, regular and enriched WT, but not regular and enriched IL-1rKO mice ($n = 6$ in all groups), exhibited a significant decrease in social exploration (***A***) and spatial exploration in the open field (***B***) compared with their saline injected controls. ***C***, ***D***, Similarly, regular and enriched WT, but not regular and enriched IL-1raTG mice ($n = 6$ in all groups), exhibited a significant decrease in social exploration (***C***) and spatial exploration in the open field (***D***) in response to IL-1α injection compared with their saline injected controls. Data presented as the mean ± SEM.

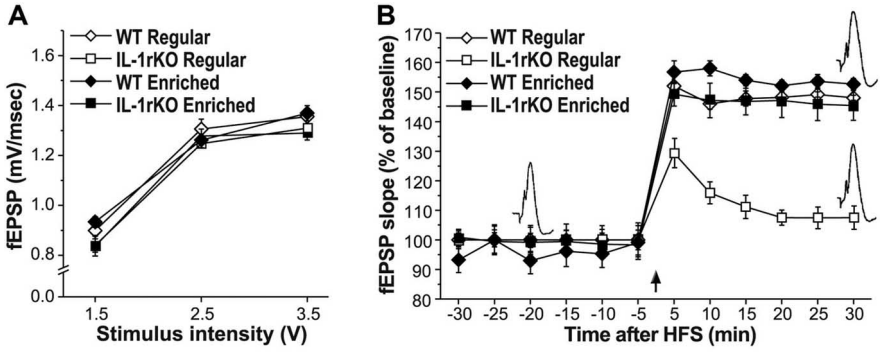

Figure 3. Environmental enrichment restores the ability to express LTP in the DG *in vivo* in IL-1rKO mice. ***A***, Baseline field potential responses in the dentate gyrus to perforant-path stimulation were similar in all groups, over a range of stimulus intensities. ***B***, After HFS, WT mice that were raised in a regular environment (WT-Reg; $n = 6$) developed LTP of fEPSP slope, whereas IL-1rKO mice (IL-1rKO-Reg; $n = 5$) displayed reduced potentiation and no LTP. However, after environmental enrichment, both IL-1rKO mice ($n = 6$) and their WT controls ($n = 6$) showed normal LTP. Environmental enrichment improved LTP in both strains, and WT mice that were raised in either regular or enriched environment demonstrated stronger LTP. Data presented as the mean ± SEM. Representative traces are presented for WT Regular (left), IL-1rKO Regular (bottom right), and IL-1rKO enriched (top right).

tively) without strain by enrichment interactions ($p > 0.5$).

Similar results were found in IL-1raTG mice: In response to IL-1α injection, WT mice exhibited significant decreases in social and spatial exploration behavior compared with their saline-injected controls, whereas social and spatial exploration in IL-1raTG mice remained unchanged (Fig. 2*C*,*D*). Enrichment had no effect on the response to IL-1α injection in either WT or IL-1raTG mice. These findings were reflected by significant strain effects ($F_{(3,17)} = 7.407$, $p < 0.02$; $F_{(3,20)} = 15.56$, $p < 0.001$, respectively) without strain by enrichment interactions ($p > 0.5$). No differences in either social or spatial exploration were found between the groups in response to saline injection (data not shown).

Environmental enrichment restores LTP in the DG of IL-1rKO mice

To assess possible differences between the groups in the dynamic range of perforant path to dentate gyrus synapses, we tested input/output relations. At all stimulus intensities, the field EPSP (fEPSP) slope was similar in all groups (Fig. 3*A*). After high-frequency stimulation, IL-1rKO mice that were raised in a regular environment displayed reduced potentiation that rapidly decreased compared with their WT controls. However, enriched IL-1rKO mice demonstrated normal potentiated fEPSP slope, as well as LTP after HFS (Fig. 3*B*). These findings were reflected by a significant time by strain by enrichment triple interaction ($F_{(11,209)} = 6.324$, $p < 0.0001$). Further repeated measures analyses revealed significant enrichment by time interactions between regular and enriched IL-1rKO mice ($F_{(11,99)} = 22.343$, $p < 0.0001$). Interestingly, enrichment also improved LTP compared with regular housing in WT mice ($F_{(11,110)} = 1.964$, $p < 0.05$). Additionally, there were significant strain by time interactions between WT and IL-1rKO mice in both regular ($F_{(11,99)} = 17.747$, $p < 0.0001$) and enriched ($F_{(11,110)} = 2.564$, $p < 0.01$) environment, i.e., in both rearing conditions, LTP was stronger in WT mice, although the difference was smaller after the enrichment.

Environmental enrichment restores normal spine size in the DG of IL-1rKO mice

IL-1rKO mice that were raised in a regular environment displayed smaller dendritic spine size compared with their WT-regular controls ($p < 0.0001$). However, IL-1rKO mice that were raised in an enriched environment did not differ from their WT controls (Fig. 4*A–E*). These findings were reflected by a significant strain by enrichment interaction ($F_{(1,2824)} = 14.426$, $p < 0.0001$). No differences in dendritic spine density were observed ($p > 0.5$) (Fig. 4*F*). The average spine density we found is higher than previous reports in the literature. This may result from the fact that we used a finer spacing (0.3 μm) for our *Z* stacks. No differences in hippocampal GluR-1 ($p > 0.5$) (Fig. 4*G*) or synaptophysin ($p > 0.5$) (Fig. 4*H*) levels were observed between any of the groups.

Environmental enrichment increases neurogenesis in the DG, as well as hippocampal BDNF and VEGF levels in both IL-1rKO and WT mice

IL-1rKO mice that were raised in a regular environment displayed similar cytogenesis levels compared with their WT-regular controls, i.e., the number of cells double-stained for BrdU (which stains newly

divided cells) (Fig. 5*F*) and DAPI (which stains all nuclei) (Fig. 5*E*) in their DG was similar (Fig. 5*A*,*B*). Enriched IL-1rKO mice also did not differ in their level of cytogenesis from their enriched WT controls. However, environmental enrichment increased cytogenesis in both strains ($p < 0.0001$; $p < 0.005$, respectively) (Fig. 5*C*,*D*). These findings were reflected by a significant effect of enrichment on cytogenesis level ($F_{(3,60)} = 38.017$, $p < 0.0001$) (Fig. 4*I*), with no strain effect or strain by enrichment interaction ($p > 0.05$).

Similar results were found when neurogenesis levels were analyzed by counting the number of DG cells triple stained (Fig. 5*H*) for BrdU, Tuj-1 (an early neuronal marker) (Fig. 5*G*), and DAPI. IL-1rKO mice that were raised in a regular environment displayed similar neurogenesis levels compared with their WT-regular controls. Enriched IL-1rKO mice also did not differ in their level of neurogenesis from their enriched WT controls. However, environmental enrichment increased neurogenesis in both strains ($p < 0.0001$ for both) (Fig. 5*J*). These findings were reflected by a significant effect of enrichment on neurogenesis level ($F_{(3,60)} = 44.396$, $p < 0.0001$), with no strain effect or strain by enrichment interaction ($p > 0.05$). A similar pattern was also observed when the percentage of Tuj1+ (neurons) among the newly generated cells was calculated (Fig. 5*K*). Again, enrichment had a significant effect ($F_{(3,60)} = 14.643$, $p < 0.0001$), with no strain effect or strain by enrichment interaction ($p > 0.5$). *Post hoc* analyses revealed a significant difference between enriched WT and IL-1rKO mice and their respective controls that were raised in a regular environment ($p < 0.05$; $p < 0.005$, respectively).

IL-1rKO mice that were raised in a regular environment displayed similar hippocampal BDNF and VEGF levels compared with their WT-regular controls, and enriched IL-1rKO mice also did not differ in their level of these trophic factors from their enriched WT controls. However, environmental enrichment increased BDNF (Fig. 5*L*) and VEGF (Fig. 5*M*) levels in both strains. These findings were reflected by a significant effect of enrichment on BDNF ($F_{(3,16)} = 7.485$, $p < 0.02$) and VEGF ($F_{(3,20)} = 9.395$, $p < 0.01$) levels, with no strain effect or strain by enrichment interactions ($p > 0.5$). *Post hoc* analyses for BDNF levels revealed a significant difference between enriched IL-1rKO mice and both WT and IL-1rKO mice that were raised in a regular environment ($p < 0.05$ for both). *Post hoc* analyses for VEGF levels revealed a significant difference between the two enrichment groups (WT and IL-1rKO) to the two groups that were raised in a regular environment ($p < 0.05$ for all).

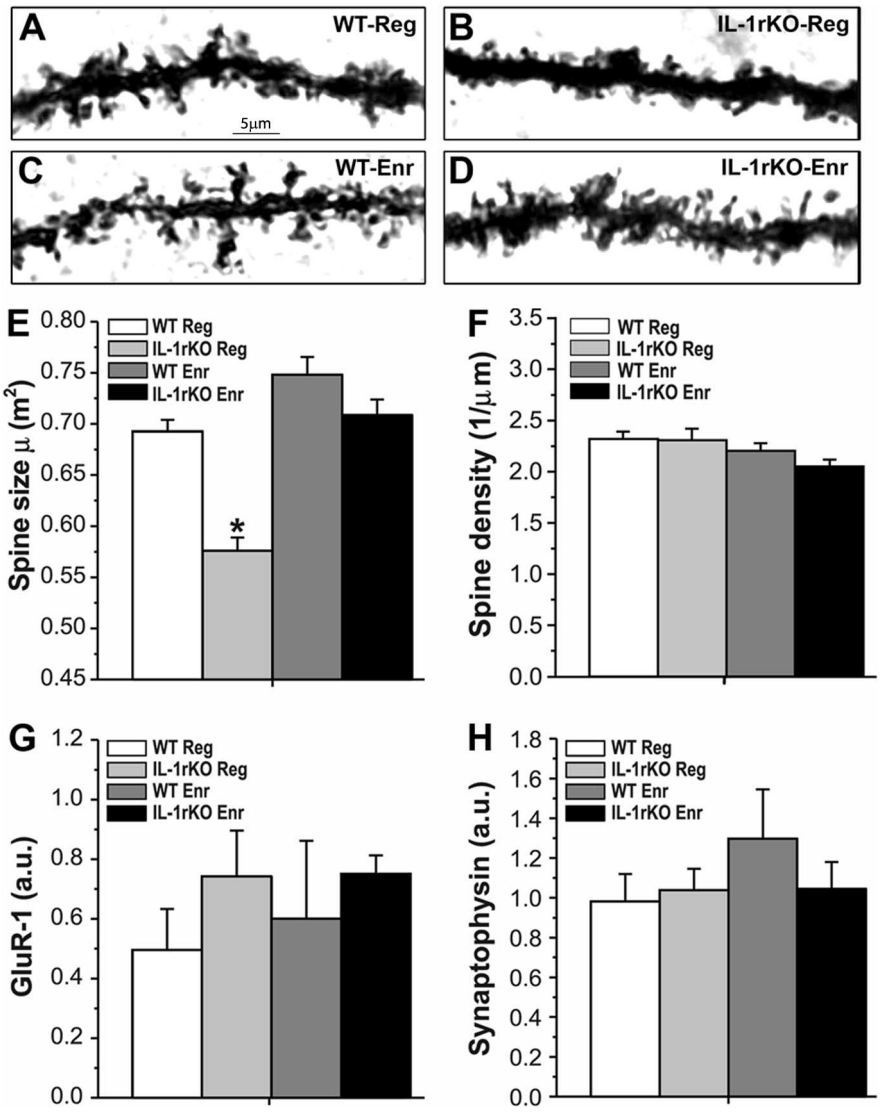

Figure 4. Environmental enrichment increases spine size in the DG *in vivo* in IL-1rKO mice. IL-1rKO-Reg mice (***B***, $n = 4$ mice, 19 dendrites, 568 spines) displayed lower dendritic spine size compared with their WT-Reg controls (***A***, $n = 4$ mice, 30 dendrites, 670 spines). However, IL-1rKO-Enr mice (***D***, $n = 4$ mice, 51 dendrites, 771 spines) did not differ from their WT-Enr controls (***C***, $n = 4$ mice, 34 dendrites, 819 spines). These data are represented quantitatively in ***E***. No differences in dendritic spine density in the DG (***F***), the levels of hippocampal AMPA receptor subunit GluR-1 (***G***), or the hippocampal presynaptic protein synaptophysin (***H***) were observed between any of the groups. Data presented as the mean ± SEM. a.u., Arbitrary units.

Discussion

The present study demonstrates the corrective effect of environmental enrichment on the impaired spatial and contextual memory in IL-1 signaling deficient mice. Furthermore, it suggests two possible mechanisms for the beneficial effect of enrichment on memory functioning in IL-1rKO mice and precludes two other mechanisms: Concomitantly with their disturbed memory functioning, LTP in IL-1rKO mice that were raised in a regular environment is impaired, and their dendritic spine size is reduced. Both of these impairments were corrected by environmental enrichment. Because both LTP and spine size were implicated in memory processes, the restoration of the ability to express LTP and to exhibit normal spine size in IL-1rKO mice may serve as possible mechanisms for the memory improvement observed in these mice after environmental enrichment. In contrast, no deficiencies in neurogenesis or hippocampal BDNF and VEGF secretion were found in IL-1rKO mice that were raised in a regular environment, and both of these variables were increased to a similar degree in environmentally enriched IL-1rKO and WT mice. Thus, they cannot explain the basic deficit in memory of IL-1rKO mice but may be involved in its improvement by enrichment.

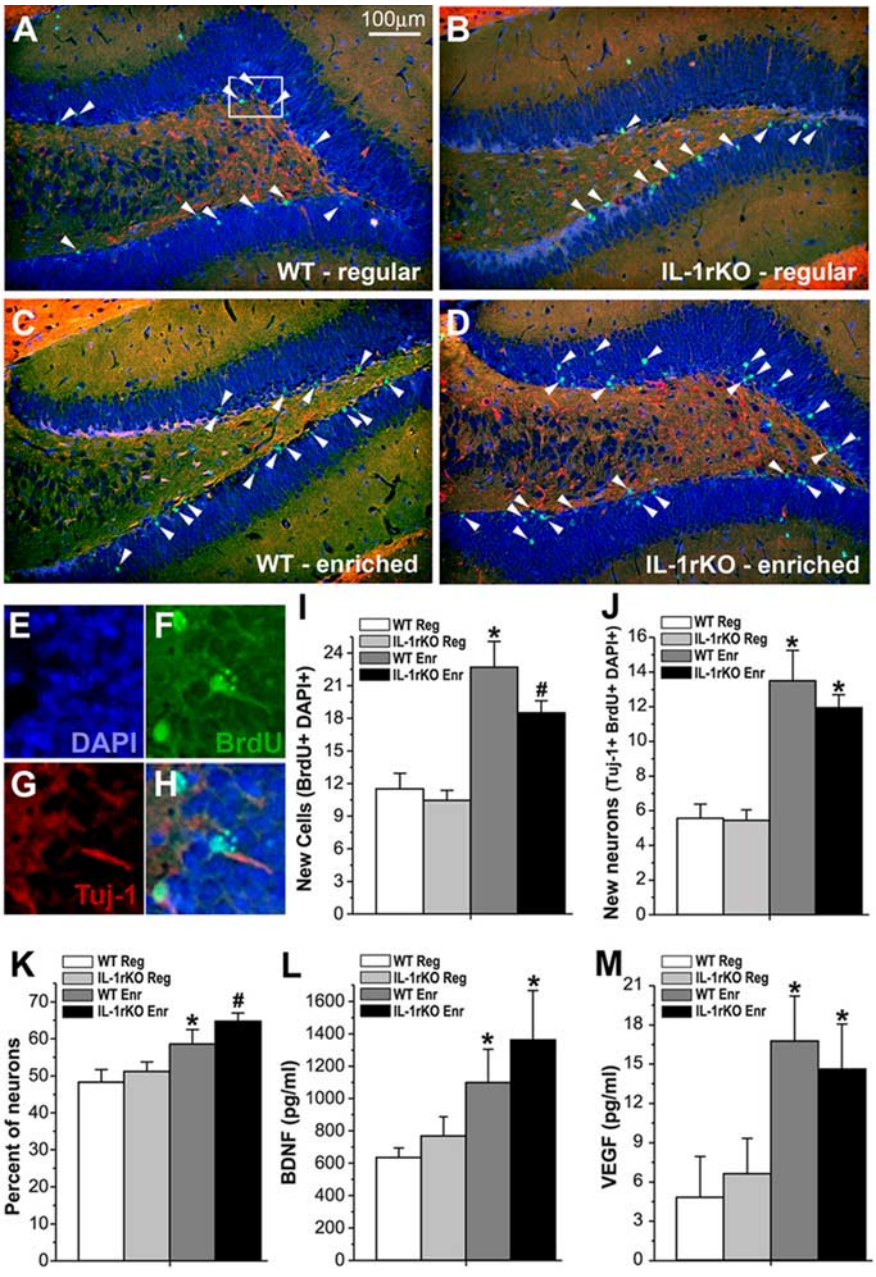

Figure 5. Environmental enrichment increases hippocampal neurogenesis, as well as BDNF and VEGF levels in both IL-1rKO and WT mice. Similar levels of cytogenesis and neurogenesis were observed in WT-Reg and IL-1rKO-Reg mice (representative figures ***A***, ***B***). Environmental enrichment increased both cytogenesis and neurogenesis levels in both WT and IL-1rKO mice (representative figures ***C***, ***D***). Slices were stained for DAPI (blue; ***E***), BrdU (green; ***F***), and Tuj-1 (red; ***G***). Cytogenesis was assessed by counting the number of cells double-stained for DAPI and BrdU (marked by arrows), and neurogenesis was assessed by counting the number of cells triple-stained for DAPI, BrdU, and Tuj-1 (***H***) (***E–H*** are a magnification of the cells marked by a white square in ***A***). A quantitative summary of the data ($n = 16$ in all groups) is presented for cytogenesis (***I***) ($^{*}p < 0.0001$ compared with WT-Reg; $^{\#}p < 0.005$ compared with IL-1rKO-Reg.), neurogenesis (***J***) ($^{*}p < 0.0001$ compared with WT-Reg and IL-1rKO-Reg), and the percentage of neurons among newly generated cells (***K***) ($^{*}p < 0.05$ compared with WT-Reg; $^{\#}p < 0.005$ compared with IL-1rKO-Reg). ***L***, Environmental enrichment also increased BDNF levels in both WT and IL-1rKO mice ($n = 5$ in all groups). $^{*}p < 0.05$ compared with WT-Reg and IL-1rKO-Reg. ***M***, Environmental enrichment increased VEGF levels in both WT and IL-1rKO mice ($n = 6$ in all groups). $^{*}p < 0.05$ compared with WT-Reg and IL-1rKO-Reg mice. Data presented as the mean ± SEM.

IL-1rKO and IL-1raTG mice that were raised in a regular environment displayed severe deficits in hippocampal-dependent memory (both spatial and contextual) measured in the water maze and fear conditioning paradigms, thus corroborating our previous reports (Avital et al., 2003; Goshen et al., 2007). The findings of rescued memory in these mice after exposure to an enriched environment are consistent with previous reports of beneficial effects of environmental enrichment on spatial and contextual memory in normal animals (Nilsson et al., 1999; Duffy et al., 2001) and its ability to restore the memory impairments that accompany disorders and injuries of the nervous system (van Praag et al., 2000; Nithianantharajah and Hannan, 2006; Fischer et al., 2007).

Environmental enrichment did not restore IL-1 signaling in IL-1rKO or IL-1raTG mice, as shown by their lack of response to IL-1α injections even after they were raised in an enriched environment. Therefore, other indirect mechanisms that can bypass the lack of IL-1 signaling are responsible for the effects of environmental enrichment on memory performance in these mice, such as LTP restoration and spine size enlargement.

The findings that environmental enrichment restored LTP in IL-1rKO mice, and strengthened LTP in their WT controls, are consistent with previous reports on the beneficial effects of environmental enrichment on hippocampal LTP in normal animals (Duffy et al., 2001; Huang et al., 2006; Li et al., 2006), even 3–5 weeks after the cessation of enrichment and transfer back to normal conditions (Artola et al., 2006), and its ability to restore impaired LTP (Yang et al., 2007). The results of the present study provide the first demonstration of the ability of environmental enrichment to facilitate LTP in normal animals and to restore impaired LTP in the dentate gyrus *in vivo*. All the former reports of such effects in the literature are based on studying LTP within the CA1 region, *in vitro*, in hippocampal slices. Only one study (Irvine et al., 2006) examined the effect of enrichment on LTP *in vivo* in freely moving rats, and reported no effect on LTP in the first 30 min, but faster decay in the following days. However, the rats in that study were exposed to enrichment for 19 d only, making it difficult to compare the results of that study to ours. Because synaptic plasticity was implicated in memory storage (Bliss and Collingridge, 1993; Eichenbaum, 1996), the restoration of the ability to express LTP in IL-1rKO mice may serve as a possible mechanism for the memory improvement observed in these mice after environmental enrichment.

The accumulation of many studies from the last decade suggests that morphological changes in spines may be related to memory consolidation and storage (Segal, 2005). LTP was shown repeatedly to induce the formation of new spines (Engert and Bonhoeffer, 1999), and spatial learning increases the number of spines per μm^3 in the DG (O'Malley et al., 2000), as well as the number of mushroom-shaped spines in the CA1 region (Hongpai-

san and Alkon, 2007). A recent study showed that in aged mice spatial memory deficiencies are accompanied by shorter spine length (but not spine density) in DG neurons (von Bohlen und Halbach et al., 2006). Furthermore, TG2576 mice (which constitute a model for Alzheimer's disease) demonstrate a concomitant decrease in spine density, LTP, and fear conditioning before Aβ deposition (Jacobsen et al., 2006). However, over-expression of the TRPC6 channel concomitantly increases water maze performance and spine density (Zhou et al., 2008). In the present study, we found that of several memory and plasticity-related molecular and morphological parameters that were investigated in the brains of IL-1rKO and WT mice, reduction in spine size was the only parameter that differed between the two strains. This finding suggests that normally IL-1 is involved in regulation of spine size and therefore reduced spine size in IL-1rKO mice may contribute to their deficits in memory and LTP. The effects of IL-1 on spine size may be exerted either during prenatal or perinatal development, as we have shown that exposure to IL-1ra *in utero* can produce memory deficits similarly to those observed in IL-1rKO mice (Goshen et al., 2007). Alternatively, IL-1 signaling may be needed acutely, to promote learning and LTP-induced increases in spine size. The latter hypothesis is consistent with the impairments in learning and LTP produced by acute administration of IL-1ra (Schneider et al., 1998; Ross et al., 2003; Goshen et al., 2007). Obviously, mechanisms other than IL-1 signaling are also responsible for spine size regulation, as evidenced by the marked increase in this parameter in enriched IL-1rKO mice, despite the persistent lack of IL-1 signaling in these mice. It is thus possible that EE activates mechanisms that bypass the need for IL-1 in controlling spine size. These mechanisms probably do not include alterations in the level of AMPA receptors, as no differences in hippocampal GluR-1 were observed. Because in our experiments extractions of whole hippocampi were assessed, our measurements may have missed specific synaptic changes, like the increase in GluR1 that was observed specifically in mushroom type spines after fear conditioning (Matsuo et al., 2008). Changes in the levels of the presynaptic marker synaptophysin were not detected as well. It should be noted, that because the causal relations between spines, memory and plasticity remain to be established, the reduced spine size in IL-1rKO mice does not necessarily underlie their memory and LTP deficiency. Alternatively, the inability of these mice to remember or execute efficient LTP may prevent their spines from developing.

No differences in neurogenesis levels in the DG were found between IL-1rKO that were raised in either a regular or an enriched environment and their WT controls. Although neurogenesis was implicated in memory formation (Gross, 2000; Kempermann et al., 2004; Saxe et al., 2006), the impairment in hippocampal-dependent memory in IL-1rKO mice is accompanied by normal neurogenesis. Environmental enrichment increased neurogenesis in both strains, thus it may explain its corrective effect on memory formation in IL-1rKO mice. However, environmental enrichment was recently shown to improve memory independently of neurogenesis (Meshi et al., 2006). Both BDNF and VEGF were also implicated in memory formation (Hall et al., 2000; Cao et al., 2004). However, no differences were found between IL-1rKO mice that were raised in a regular environment and their WT controls in either BDNF or VEGF hippocampal levels. Environmental enrichment increased BDNF and VEGF levels in the hippocampus of both strains. Thus, although these trophic factors are not involved in the initial differences in memory functioning between IL-1rKO and WT mice, their elevation after exposure to enriched environment may be involved in the memory corrective effect of this procedure in the IL-1rKO mice.

The motivation for the experiments presented above was to explore possible ways to improve the cognitive functioning of mice with deficient IL-1 signaling, which manifest marked deficits in learning, memory, and neural plasticity. It should be noted that several recent studies in humans demonstrated that mutations in the IL-1 receptor accessory protein-like (IL-1rAcP-L) gene are involved in X-linked mental retardation (Carrié et al., 1999; Jin et al., 2000), although the functional relationship between IL-1 and this receptor is not clear. Human studies demonstrated a beneficial effect of different enrichment protocols on cognitive and social behavior in children (Shonkoff et al., 2000). Intensive, high-quality preschool programs in children from high-risk families resulted in improved IQ and higher scores on both cognitive and academic tests. These effects were still apparent at early adulthood (Ramey and Ramey, 1992; Campbell and Ramey, 1994; Campbell et al., 2001). Retarded children exposed to an enriched home environment and targeted instruction demonstrate more adaptive behavior (Horner, 1980; Hessl et al., 2001). The findings presented above suggest that exposure to an enriched environment may be beneficial for children who suffer from mental retardation related to disturbances in the IL-1 system.

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