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A dual role for interleukin-1 in hippocampal-dependent memory processes

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U-curve

Summary

Ample research demonstrates that pathophysiological levels of the pro-inflammatory cytokine interleukin-1 (IL-1) produces detrimental effects on memory functioning. However, recent evidence suggests that IL-1 may be required for the normal physiological regulation of hippocampal-dependent memory. To substantiate the physiological role of IL-1 in learning and memory we examined the induction of IL-1 gene expression following a learning experience, and the effects of IL-1 signaling blockade, by either genetic or pharmacological manipulations, on memory functioning. We show that IL-1 gene expression is induced in the hippocampus 24 h following fear-conditioning in wild type mice, but not in two mouse strains with impaired IL-1 signaling. Moreover, we report that mice with transgenic over-expression of IL-1 receptor antagonist restricted to the CNS (IL-1raTG) display impaired hippocampal-dependent and intact hippocampal-independent memory in the water maze and fear-conditioning paradigms. We further demonstrate that continuous administration of IL-1ra via osmotic minipumps during prenatal development disrupt memory performance in adult mice, suggesting that IL-1 plays a critical role not only in the formation of hippocampal-dependent memory but also in normal hippocampal development. Finally, we tested the dual role of IL-1 in memory by intracerebroventricular (ICV) administration of different doses of IL-1 β and IL-1ra following learning, providing the first systematic evidence that the involvement of IL-1 in hippocampal-dependent memory follows an inverted U-shaped pattern, i.e., a slight increase in brain IL-1 levels can improve memory, whereas any deviation from the physiological range, either by excess elevation in IL-1 levels or by blockade of IL-1 signaling, results in impaired memory.

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1. Introduction

Interleukin-1 (IL-1) is a proinflammatory cytokine, produced by both glia and neurons within the brain. Signaling can be triggered by the attachment of either IL-1 α or IL-1 β to the type I receptor (IL-1R1), whereas IL-1 receptor antagonist (IL-1ra) blocks the effects of IL-1 (Dinarello, 1996). At pathophysiological levels, IL-1 can produce detrimental effects on memory. These effects are specific for the consolidation of memories that depend on the hippocampus, whereas hippocampal-independent memories are not altered (Rachal Pugh et al., 2001; Goshen and Yirmiya, 2007). Exogenous IL-1 also inhibits long-term potentiation (LTP) (Murray and Lynch, 1998; O'Connor and Coogan, 1999), a model system for the neural mechanism underlying memory, in several hippocampal pathways (Martin et al., 2000). However, recent evidence suggests that under some circumstances IL-1 may be required for the normal physiological regulation of hippocampal plasticity and memory processes: LTP in the hippocampus is accompanied by a long-lasting increase in IL-1 β gene expression, and exposure to IL-1ra impairs the maintenance of LTP (Schneider et al., 1998). Furthermore, administration of IL-1ra impairs memory in the water-maze and passive-avoidance paradigms in rats, whereas relatively low doses of IL-1 β improve avoidance memory (Yirmiya et al., 2002; Brennan et al., 2003; Song et al., 2003). The particularly high expression of IL-1, IL-1ra, and proteins belonging to the IL-1 receptor family in the hippocampus (Loddick et al., 1998) may underlie the effects of IL-1 within this structure.

Recently, we further demonstrated the importance of IL-1 signaling in hippocampal memory consolidation and neural plasticity by reporting that mice with targeted deletion of IL-1R1 (IL-1rKO) display a severely impaired hippocampal-dependent but normal hippocampal-independent memory. Furthermore, these mice demonstrate diminished short-term plasticity, and exhibit no LTP, both *in vivo* and *in vitro* (Avital et al., 2003). Since the deletion is permanent, these findings may be explained by the acute lack of IL-1 during memory consolidation and/or by abnormal brain development. The latter hypothesis may be clinically relevant since several studies in humans demonstrated that mutations in the IL-1 receptor accessory protein-like gene are involved in X-linked mental retardation (Carrie et al., 1999; Jin et al., 2000).

The data presented above indicates that on the one hand elevated IL-1 levels have detrimental effects on memory, but on the other hand low IL-1 doses can facilitate memory and blockade of IL-1 signaling is associated with impaired memory functioning. Together, the findings gathered so far suggest that the influence of IL-1 on memory follows an inverted U shaped pattern (Goshen and Yirmiya, 2007). According to this hypothesis, physiological levels of IL-1 are needed for memory formation; however, any deviation from the physiological range, either by excess elevation in IL-1 levels or by blockade of IL-1 signaling, results in impaired memory.

To further elucidate the role of IL-1 in memory processes, we investigated IL-1 gene expression following learning, and examined hippocampal-dependant memory in mice with transgenic over-expression of IL-1ra (IL-1raTG).

Additionally, we investigated the involvement of IL-1 in the development of the neural substrate mediating hippocampal-dependent memory in mice that were chronically exposed to IL-1ra during prenatal brain development. Furthermore, we systematically tested the inverted U-shape hypothesis by intracerebroventricular (ICV) administration of different doses of IL-1 β and IL-1ra following learning.

2. Materials and methods

2.1. Subjects

Subjects were 2–4 month old male IL-1raTG mice and their C57BL/6 x CBA wild type (WT) controls (Stockholm University) and IL-1rKO mice and their 129/Sv X C57BL/6 WT controls (Jackson Laboratories, Bar Harbor). IL-1raTG mice have astrocyte-directed over-expression of the human IL-1ra gene under the control of the murine glial fibrillary acidic protein (GFAP) promoter, and are insensitive to the administration of exogenous IL-1 (Lundkvist et al., 1999; Shavit et al., 2005). 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, and are reported to have a defective response to IL-1 α and IL-1 β (Labow et al., 1997). No differences in vitality and general appearance were found between these strains and their respective controls. Animals were housed in an air-conditioned room ($23 \pm 2^\circ\text{C}$), with food and water ad libitum, for several weeks before the beginning of the experiments. The behavioral experiments were conducted during the first half of the dark phase of a reversed 12h light/dark cycle. All experiments were approved by the Hebrew University Committee of Animal Care and Use.

2.2. Memory testing paradigms

2.2.1. Water maze

The water maze consisted of a round tank, 1.6m in diameter, filled with water mixed with non-toxic gouache paint to make it opaque. In the spatial memory experiment, mice were trained to find the location of a hidden platform (16cm in diameter), submerged 1cm below the water surface, using extra maze visual cues. The maximal trial duration was 120s. Training consisted of three trials per day, with a 1-h break between trials, for 3 days. In the non-spatial memory experiment, the platform was elevated 1cm above the water level and therefore was visible. Mice were tested over three trials, separated by a 1h interval. All 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 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, which monitored the latency to reach the platform and the swimming speed (VP118 tracking system, HVS Image, Hampton, UK). Mice were dried under a red light heating lamp after each trial.

2.2.2. Fear conditioning

The apparatus consists of a transparent square conditioning cage (25 × 21 × 18 cm), with a grid floor wired to a shock generator and scrambler (Campden Instruments, UK). 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. After 48 h, fear conditioning was assessed by a continuous measurement of freezing (complete immobility), the dominant behavioral fear response (Fanselow, 2000), as measured by infra-red movement detectors, connected to a computerized data collection system (Campden instruments, UK). 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. After 2 h, 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, in order to assess possible strain differences in baseline freezing in the conditioning context.

2.3. IL-1 β gene expression measurements by quantitative real-time RT-PCR

2.3.1. Contextual learning

In order to explore the influence of a hippocampal-dependent learning experience on IL-1 β gene expression we performed the following experiment: C57BL/6 × CBA WT mice were exposed to the conditioning session of the fear-conditioning paradigm, as described above, without the presentation of the tone (i.e., mice were placed in the conditioning cage for 120 s, and then a 2 s, 0.5 mA foot-shock was administered. This procedure was then repeated, and 30 s after the delivery of the second shock mice were returned to their home cage). To control for the stressful effect of shock-administration, and separate it from the learning experience, we also administered a similar pair of shocks (0.5 mA, 2 s long) to another group of mice in their home-cages. The shock was administered to the hind legs of the mouse, using a Y shaped shocker. The effect of contextual learning on IL-1 β gene expression was also assessed in two mouse strains with impaired IL-1 signaling, IL-1raTG and IL-1rKO. Mice were sacrificed 1.5, 4 or 24 h following conditioning or shock administration, the brains were rapidly removed and the hippocampi were immediately frozen in liquid nitrogen.

2.3.2. RNA extraction

RNA was extracted from total hippocampal tissue using TRI Reagent (Molecular Research Center INC, Cincinnati, OH, USA), according to the manufacturer's protocol. Briefly, tissues were homogenized with 1 ml TRI Reagent; the homogenates were incubated for 5 min at room temperature

(RT), and transferred into 1.5 ml tubes. After the addition of 100 μ l of 1-Bromo-3-chloropropane (BCP) the mixture was vigorously shaken, incubated for 10 min at RT, and centrifuged at 12,000g for 15 min at 4 °C to allow for phase separation. The RNA in the upper aqueous phase was transferred to a fresh tube, precipitated by adding 0.5 ml isopropanol, and incubated for 7 min at RT. The samples were then centrifuged at 12,000g for 8 min at 4 °C. The RNA pellet was washed with 1 ml of 75% ethanol, air dried, and solubilized with 25 μ l of DEPC treated water.

2.3.3. Real-time RT-PCR

Total hippocampal RNA (1 μ g) was reverse transcribed using ImpromII (Promega, Madison, WI, USA) in the presence of random hexamers. PCR reactions were performed in a total volume of 20 μ l using TaqMan universal PCR master mix (Applied Biosystems, Foster city, CA, USA), using 2 μ l of cDNA and gene specific Assay on demand (Applied Biosystems). PCR reactions were performed in an ABI PRISM 7000 Sequence Detector (Applied Biosystems, Serial numbers: IL-1 β Mm00434228-m1, Tbp Mm0046973-m1). Amplification was performed using the following conditions: 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Reactions were performed in duplicates, and threshold cycle (C_t) values were normalized to the house keeping gene TATA box binding protein (T_{bp}).

2.3.4. Prenatal exposure to IL-1ra

The role of IL-1 in the development of the neural substrate for hippocampal memory was investigated by exposing mice to IL-1ra in utero, and examined their memory performance in adulthood as follows: WT females were housed in groups of 3–4, with one WT male in the same cage. Pregnancy was determined at the beginning of the light phase by the presence of a vaginal plug. At day 10 of gestation, osmotic micro-pumps (Alzet[®], CA, USA) releasing either hIL-1ra (20 mg/kg/day; Amgen Inc.) or a placebo solution (Amgen, Inc.) were implanted subcutaneously under ether anesthesia, only to females that gained at least 5 g to their body weight since the detection of the vaginal plug. The time of surgery was determined based on the fact that IL-1ra blocks embryonic implantation in mice when applied on days 3–9 of gestation (Simon et al., 1994). From the 14th gestational day, females were housed singly. After birth, pups were adopted by naive females, who gave birth 48 h earlier. Pups were weaned 21 days after birth, and memory tests were performed at the age of 2 months, using no more than 2 male pups from each litter.

2.3.5. Enzyme-linked immunosorbent assay (ELISA)

To verify that hIL-1ra is present in the brains of IL-1raTG mice, and that it can cross the placenta and the blood–brain barrier (BBB) in fetuses, hIL-1ra levels were measured by ELISA as follows: adult hippocampi were collected from IL-1raTG and WT mice. Additionally, dams were implanted on day 10 of gestation with hIL-1ra-secreting micro-pumps as described above, and on day 15 of gestation fetal brains were collected. Mice from all groups were perfused with 20 ml of cold saline under ether anesthesia, before tissues were collected and weighed. Brain tissue was then homogenized in 200 μ l cold RPMI 1640 (Sigma, Israel) containing 0.5 μ l protease inhibitor cocktail (Sigma, Israel), and

centrifuged for 10 min (300g) at 4 °C before supernatant was collected. hIL-1ra levels were determined using an ELISA kit (R&D Systems, Minneapolis, MN). According to the manufacturer, the detection limit of this assay is 14pg/ml, the intra- and inter-assay coefficient of variation is 5.5% and 5.4%, respectively. In order to evaluate the normal levels of IL-1ra in the mouse strain that we used, murine IL-1ra (mIL-1ra) levels were measured in four naïve WT mice by ELISA, as we previously described (Wolf et al., 2003; Bajayo et al., 2005).

2.3.6. ICV injections of IL-1 β and IL-1ra

To test the hypothesis that the effects of IL-1 on hippocampal-dependent memory follow an inverted U-shaped pattern, we injected different doses of IL-1 β and IL-1ra into the lateral ventricles of mice following fear-conditioning and examined their memory performance as follows:

Surgery: Adult mice were anesthetized with Ketamine–Xylazine and placed in a stereotaxic apparatus. A burr hole was drilled posterior to bregma using the following formula: $[-0.4-0.66*(bl-3.8)]$ mm (bl = distance between bregma and lambda), 1.5 mm lateral to the midline, and a 26 gauge stainless-steel guide cannula (Plastics-One Inc., VA, USA) was lowered 2.2 mm below skull surface. The tip of the guide cannula was positioned 1 mm above the lateral ventricle. The guide cannula was secured to the skull with three stainless-steel screws and dental cement, and was closed by a dummy cannula (Plastics-One Inc.). Experiments were performed following 3 weeks of recovery.

Injections: Immediately following conditioning, IL-1ra (100 μ g/mouse, Amgen Inc., Thousand Oaks, USA), IL-1 β (either 1 or 10 ng/mouse, R&D Systems, Minneapolis, USA) and saline were infused in a volume of 10 μ l into the lateral ventricle through a 33 gauge stainless steel internal cannula (Plastics-One Inc.), which was 1 mm longer than the guide cannula. The internal cannula was connected to a micro-syringe pump (KD Scientific, New Hope, PA, USA) by a PE20 tube. Solutions were administered at a constant rate for 1 minute, and the injection cannula was removed 1 min following the termination of the injection to avoid spillage from the guide cannula. Mice were tested 48 h after conditioning and injections, as described above. The location of the cannula was verified by ICV injection of Trypan Blue through a cannula at the end of the experiment, and examination of the spread of the dye within the ventricles. If a cannula was found to be misplaced, the mouse was excluded from data analysis.

Statistical analysis: The results were analyzed by one- or two-way ANOVAs, with the strain or treatment as a between subjects factor and the different measures as a within-subjects, repeated measure factor. Significant ANOVAs were followed by Tukey post hoc analyses.

3. Results

3.1. IL-1 β gene expression is increased following learning

Fear conditioning induced a 2.8-fold increase in IL-1 β gene expression in the hippocampus of WT mice 24 h, but not 1.5

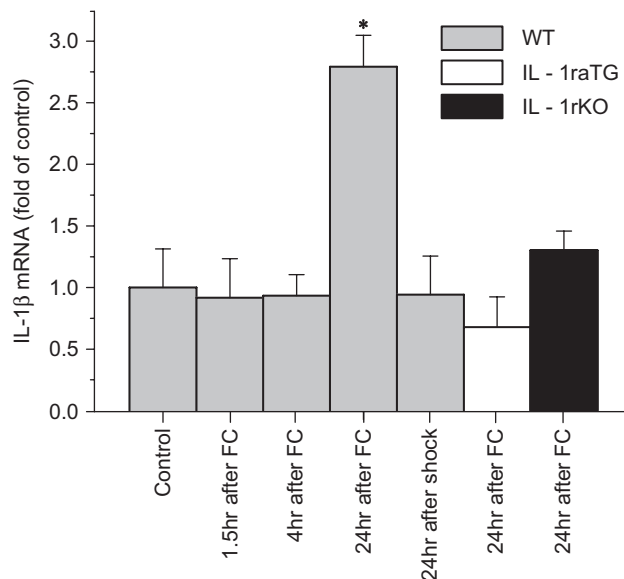


Figure 1 Fear conditioning induces IL-1 β gene expression in WT, but not in IL-1rKO or IL-1raTG mice: fear conditioning (FC) induced a 2.8-fold increase in IL-1 β expression in the hippocampus of wild type (WT) mice 24 h ($n = 3$), but not 1.5 h ($n = 4$) or 4 h ($n = 8$) after contextual learning compared to either mice that received an electrical shock in their home-cage ($n = 5$) or untreated home-cage controls ($n = 6$). In contrast, mice with transgenic over-expression of IL-1ra within the CNS (IL-1raTG; $n = 5$) and IL-1 type I receptor deficient mice (IL-1rKO, $n = 3$), did not demonstrate a similar increase in IL-1 β gene expression. * $p < 0.01$ compared to 24 h after shock and control. Data presented as the mean \pm SEM.

or 4 h after contextual learning (Figure 1) ($F(6,33) = 5.322$, $p < 0.001$). Post hoc analyses revealed a significant difference between WT mice 24 h after conditioning to WT control mice ($p < 0.0001$) and to WT mice that received only a shock at the same time ($p < 0.01$). This increase cannot be attributed to the exposure to the stress caused by the electrical shock per se, but only to the learning experience, as shock administration in the home cage did not affect IL-1 β gene expression. Interestingly, in two genetically manipulated mouse strains with deficient IL-1 signaling, IL-1rKO and IL-1raTG, the levels of IL-1 β gene expression were not increased 24 h after fear-conditioning (Figure 1).

3.2. Memory impairments in IL-1raTG mice

3.2.1. Impaired memory performance in the fear conditioning paradigm

IL-1raTG mice exhibited impaired contextual fear conditioning, i.e., they displayed a significantly shorter freezing time than their WT controls ($F(1,27) = 17.53$, $p < 0.05$) in the original conditioning context, in which they received a shock 48 h earlier (Figure 2a). This difference cannot be attributed to an inherent diminished tendency to freeze, because before shock administration there was no difference in freezing in this context between IL-1raTG and WT mice ($p > 0.5$; Figure 2a). In contrast, IL-1raTG mice displayed

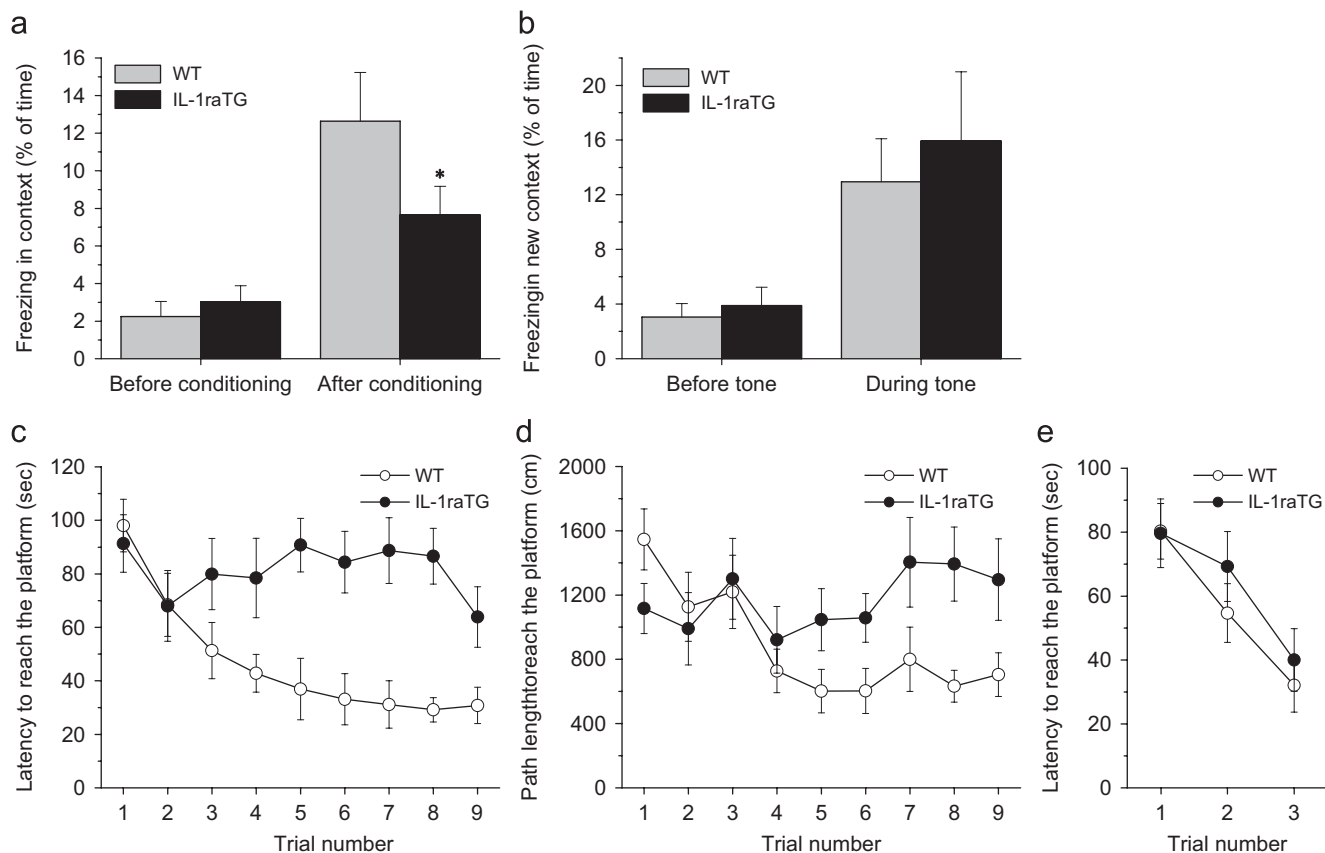


Figure 2 Impaired spatial and contextual memory in mice with central IL-1ra over-expression: in the fear-conditioning paradigm, mice with transgenic over-expression of IL-1ra within the CNS (IL-1raTG; $n = 15$) displayed contextual memory impairment (a), but no impairment in auditory-cued memory (b), compared to their wild type controls (WT; $n = 12$). In the water maze, IL-1raTG mice ($n = 12$) displayed spatial memory impairment compared to their WT controls ($n = 13$) in both the latency (c) and path length (d) to reach the hidden platform. No difference in non-spatial memory was observed (e). * $p < 0.05$ compared to WT controls. Data presented as the mean \pm SEM.

normal auditory cued fear conditioning (Figure 2b), i.e., the tone presentation had a significant effect in both groups ($F(1,27) = 19.58$, $p < 0.001$), but there were no strain differences in either the conditioned freezing to the tone that was previously paired with foot shock ($p > 0.5$), or the baseline freezing time in the different context ($p > 0.5$).

3.2.2. Impaired memory performance in the water maze

IL-1raTG mice displayed impaired spatial memory, i.e., starting from the third trial and throughout the rest of the experiment the latency to reach the platform was significantly longer in IL-1raTG mice than in their WT controls ($F(8,184) = 3.224$, $p = 0.002$) (Figure 2c). No difference in swimming speed was found between WT and IL-1raTG mice (18.78 ± 4.52 and 17.09 ± 4.56 cm/s, respectively, $p > 0.5$), and the path length to reach the hidden platform was longer in IL-1raTG mice compared to WTs. This finding was reflected by a significant strain by trials interaction ($F(8,84) = 2.547$, $p < 0.02$) (Figure 2d). In contrast with these findings, in the non-spatial memory paradigm (visible platform) IL-1raTG mice showed no difference from WT controls ($p > 0.5$, Figure 2e).

3.3. Memory impairments following prenatal exposure to IL-1ra

3.3.1. Impaired memory performance in the fear conditioning paradigm

Because there were no differences between placebo-treated and non-treated mice in any of the memory tests, these groups are presented together henceforth as controls. Mice that were prenatally treated with IL-1ra exhibited impaired contextual fear conditioning, i.e., they displayed a significantly shorter freezing time than their controls ($F(1,22) = 0.88$, $p < 0.05$) in the original conditioning context, in which they received a shock 48h earlier (Figure 3a). This difference cannot be attributed to a diminished tendency to freeze, because before shock administration there was no difference in freezing in this context between prenatal IL-1ra and control mice ($p > 0.5$; Figure 3a). In contrast, the prenatal IL-1ra-treated mice displayed normal auditory cued fear conditioning (Figure 3b), i.e., the tone presentation had a significant effect in both groups ($F(1,23) = 123.5$, $p = 0.0001$), but there was no significant effect for the prenatal treatment either in the conditioned freezing to the tone that was previously paired with foot

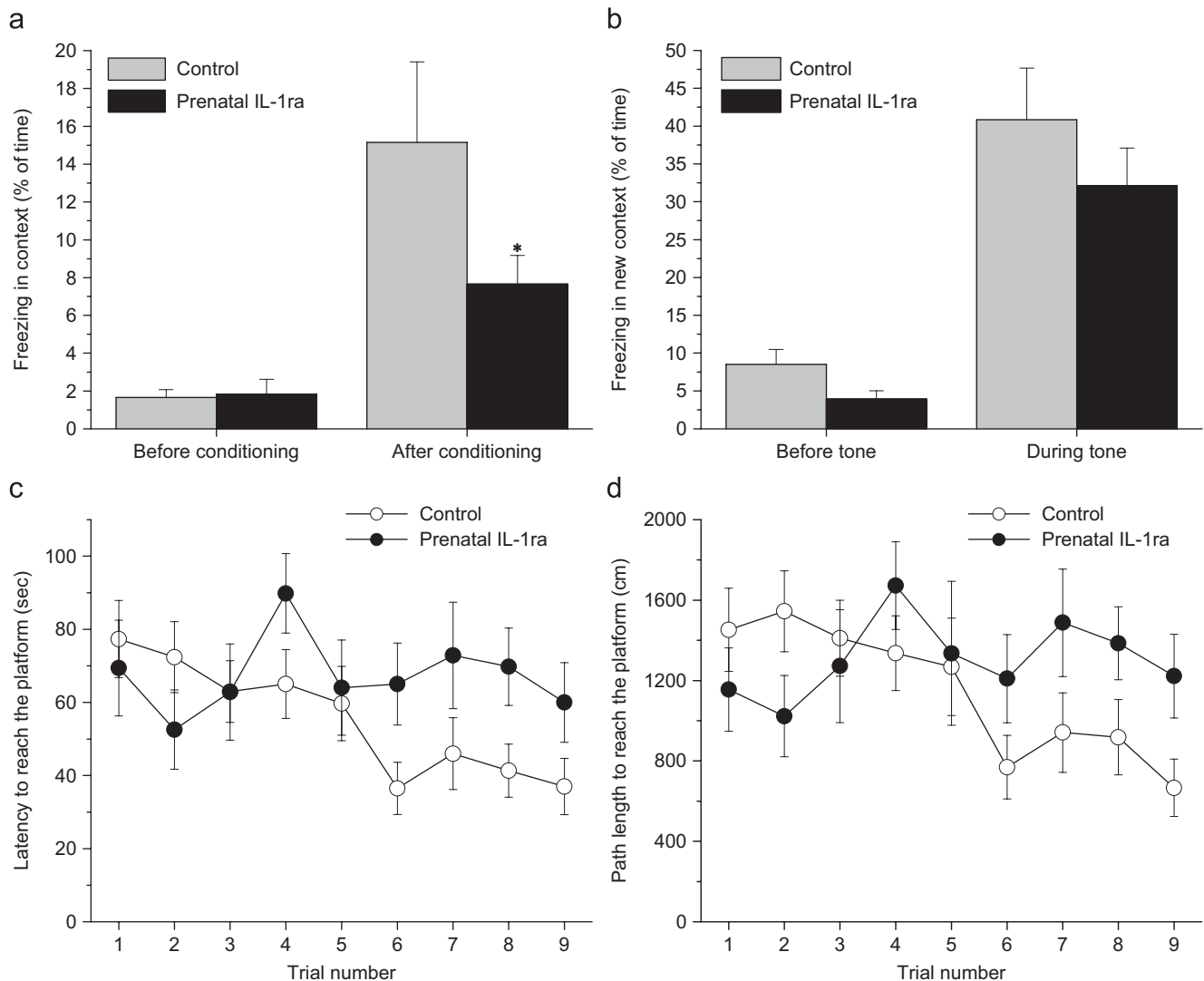


Figure 3 Impaired contextual memory after prenatal exposure to IL-1ra: In the fear-conditioning paradigm, mice that were exposed to IL-1ra in utero (Prenatal IL-1ra, $n = 9$) displayed contextual memory impairment (a), but no impairment in auditory-cued memory (b), compared to placebo-treated and non-treated mice (Control, $n = 15$). In the water maze, prenatal IL-1ra mice ($n = 13$) displayed spatial memory impairment compared to their controls ($n = 18$) in both the latency (c) and path length (d) to reach the hidden platform. * $p < 0.05$ compared to controls. Data presented as the mean \pm SEM.

shock ($p > 0.5$), or the baseline freezing time in the different context ($p > 0.5$).

3.3.2. Impaired memory performance in the water maze

Mice that were prenatally treated with IL-1ra displayed no learning in the water maze, i.e., their latency to reach the hidden platform did not shorten throughout the trials, and was longer compared to their placebo- and non-treated controls. This finding was reflected by a significant treatment by trials interaction ($F(8,232) = 2.263$, $p < 0.05$) (Figure 3c). No significant difference in swimming speed was found between the groups ($p > 0.5$), and the path length to reach the hidden platform was longer in prenatal IL-1ra mice compared to their controls. This finding was reflected by a significant treatment by trials interaction ($F(8,232) = 2.016$, $p < 0.05$) (Figure 3d).

3.3.3. Brain human IL-1ra levels

Whereas no hIL-1ra was detected in the hippocampi of WT control mice, IL-1raTG mice demonstrated a significant production of hIL-1ra protein within the hippocampus (Table 1). This level was 170 times higher than the level of murine IL-1ra found in the hippocampi of non-treated WT mice (1.13 ± 0.06 pg/mg tissue). High levels were also found in whole brains of fetuses that were exposed to hIL-1ra by implanting their mothers with hIL-1ra secreting micro-pumps. The quantitative data is presented in Table 1.

3.3.4. An inverted U-shaped influence of IL-1 on memory performance in the fear conditioning paradigm

Before conditioning there was no difference in baseline freezing in the context between the different groups ($p > 0.8$). However, 48 h after conditioning and injections a

Table 1 Brain human IL-1ra levels in the different experimental groups.

Mode of IL-1ra exposure	WT	Transgenic over-expression	Prenatal micro-pumps
Brain region	Hippocampus	Hippocampus	Whole fetal brain
hIL-1ra (pg/mg tissue)	Not detected	195 ± 10	47 ± 9
<i>n</i>	4	4	7

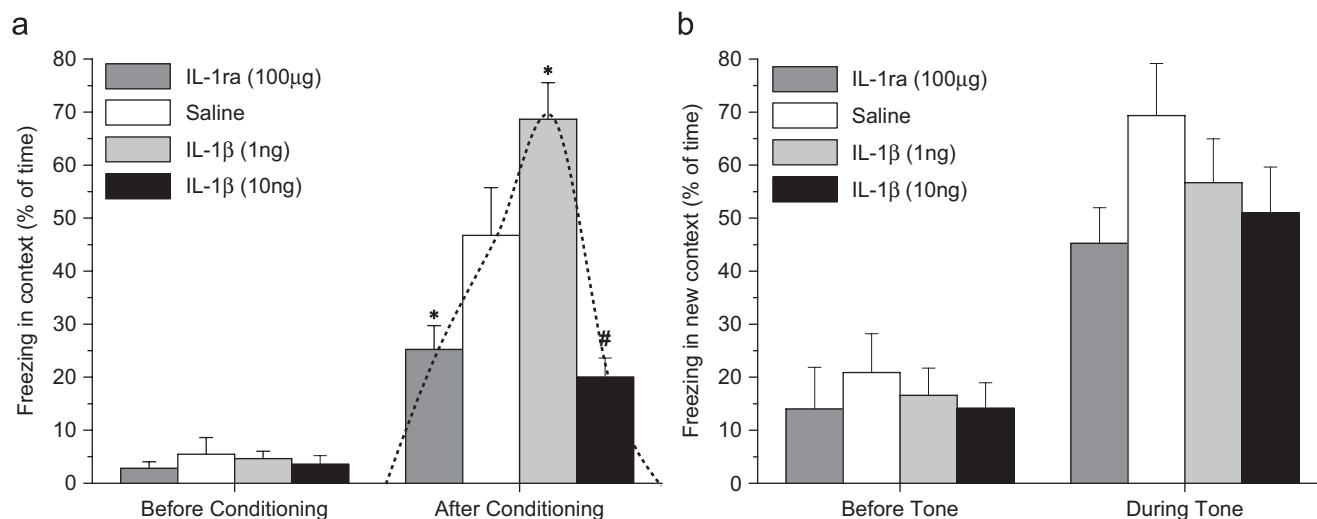


Figure 4 The effect of IL-1 on memory performance in the fear conditioning paradigm follows an inverted U-shape pattern: Mice that were ICV injected with either IL-1ra ($n = 8$) or a high dose of IL-1 β ($n = 7$) displayed contextual memory impairment, whereas a low dose of IL-1 β ($n = 8$) resulted in improved contextual memory (a) compared to saline-injected controls ($n = 11$). All groups displayed normal auditory-cued memory (b). * $p < 0.05$ and # $p < 0.015$ compared to saline injected controls. Data presented as the mean \pm SEM.

significant difference was found between the groups ($F(3,32) = 8.405$, $p < 0.0001$) (Figure 4a). Specifically, mice that were injected with either IL-1ra or the high dose of IL-1 β exhibited impaired contextual fear conditioning, i.e., they displayed a significantly shorter freezing time than saline injected mice ($p < 0.045$ and $p < 0.014$, respectively) in the original conditioning context, in which they received a shock 48 h earlier (Figure 4a). On the other hand, mice that were injected with a low dose of IL-1 β exhibited improved contextual fear conditioning, i.e., they displayed a significantly longer freezing time than saline injected mice in the conditioning context ($p < 0.034$; Figure 4a). In contrast, all groups displayed normal auditory cued fear conditioning (Figure 4b), i.e., the tone presentation had a significant effect on all groups ($F(1,29) = 123.223$, $p = 0.0001$), with no interaction between the tone presentation and the injection ($p > 0.5$), and no significant effect on the baseline freezing time in the different context ($p > 0.5$).

4. Discussion

In the present study we demonstrate in several converging strategies that IL-1 plays a critical role in hippocampal-dependent memory processes. First, we report that learning of a hippocampal-dependent task induces IL-1 gene expres-

sion in the hippocampus. Then we demonstrate that brain-specific blockade of IL-1 signaling in IL-1raTG mice specifically disrupts hippocampal-dependent memory. Furthermore we demonstrate, for the first time, the importance of IL-1 signaling during brain development, by showing impaired memory in adulthood following interruption of IL-1 signaling during prenatal developmental. Finally, we provide conclusive evidence that the involvement of IL-1 in memory processes follows an inverted U-shaped pattern i.e., physiological levels of IL-1 are needed for memory formation, and a slight increase in brain IL-1 levels can even improve memory, whereas any deviation from the physiological range, either by excess elevation in IL-1 levels or by blockade of IL-1 signaling, results in impaired memory.

The finding of increased IL-1 β gene expression 24 h after fear conditioning fits all the behavioral findings published so far (reviewed in Goshen and Yirmiya, 2007), in which the earliest time point at which a beneficial effect for IL-1 was reported is 24 h. A former study (Depino et al., 2004) examined the gene expression of IL-1 α , IL-1 β , and IL-1ra in the hippocampus at different time points (1, 4, 6, and 9 h) following a single acquisition trial of step-down passive-avoidance. In that study, IL-1 α gene expression was increased 4 h after acquisition, but no change in the expression of IL-1 β and IL-1ra were observed at any time point (Depino et al., 2004). It should be noted, however, that the control group in that study consisted of animals that

received a shock in the conditioning context but did not perform the step-down action, thus the difference is not in contextual learning per se, but in its association to the performance of a motor activity. Our findings are consistent with a previous report that IL-1 β gene expression is also substantially increased at 8 h, but not 3 h after the induction of LTP in freely moving rats (Schneider et al., 1998; Balschun et al., 2003). Interestingly, IL-1raTG and IL-1rKO mice did not display increased IL-1 β gene expression 24 h following fear-conditioning. Because IL-1 itself is one of the major triggers for the induction of further IL-1 production (Dinarello, 1996), it is possible that an initial small increase in IL-1 secretion and activation of IL-1R1 are required to induce the relatively high levels of expression at 24 h post-conditioning.

The physiological significance of the increase in IL-1 production following fear conditioning is demonstrated by our findings that both genetic and pharmacological procedures that block IL-1 signaling markedly impair hippocampal-dependent memory. Thus, IL-1raTG mice, which over-express hIL-1ra specifically within the central nervous system, and were reported to have a defective response to IL-1 α and IL-1 β (Lundkvist et al., 1999), display a severe deficit in spatial and contextual hippocampal-dependent memory, but not in hippocampal-independent tasks. This finding extends our previous demonstration of impaired memory in IL-1rKO mice (Avital et al., 2003), in which IL-1 signaling is blocked in both the brain and the periphery, by showing a similar memory disruption when the IL-1 signaling blockade is restricted to the CNS, as IL-1ra is over-expressed in IL-1raTG mice in the brain and spinal cord only (Lundkvist et al., 1999; Bajayo et al., 2005).

Because the impairment in IL-1 signaling in IL-1raTG and IL-1rKO mice is permanent, the memory disturbances in both strains may be explained by either the acute lack of IL-1 signaling during the performance of the memory task and/or by abnormal brain development. Our findings suggest that both of these explanations are feasible, as chronic IL-1ra administration in utero during the last ten days of gestation, as well as IL-1ra administration in adulthood, resulted in contextual memory impairments. Significant levels of hIL-1ra were detected in the brains of fetuses, suggesting that hIL-1ra can cross both the placenta and the fetal BBB, as was demonstrated before for the adult BBB (Gutierrez et al., 1994).

The finding that prenatal IL-1 blockade have developmental consequences that result in memory deficiency in adulthood is consistent with previous reports on the neurodevelopmental roles of IL-1. IL-1 β , IL-1ra, and IL-1 receptor genes are present during prenatal development in mouse embryos (Kruessel et al., 1997), and their proteins are detectable starting from the 2 cells stage and throughout human embryonic development (De los Santos et al., 1996), as well as in human newborns (Pillay et al., 1993). Specifically, IL-1 β was found to increase with time in human forebrain cells during the first trimester (Mousa et al., 1999). IL-1 has a direct influence on neuronal differentiation: It increases the number of progenitor cells converting into neurons and the number of extending neurons (Potter et al., 1999). IL-1 also enhances the expression and secretion of various neurotrophic factors, including NGF, CNTF, BDNF and neurotrophin-3 (Steiner et al., 1991; Kamiguchi et al.,

1995; Ishida et al., 1997), which in addition to their role in the development of the CNS, have been implicated in hippocampal-dependent memory (Chen et al., 1997; Hall et al., 2000), and thus may also contribute to the memory deficit following adult exposure to IL-1ra. The presence of IL-1 in the fetal brain, together with its ability to influence neuronal development, suggest that endogenous IL-1 is involved in the development of the neural substrate mediating hippocampal-dependent memory, whereas impaired IL-1 signaling during fetal development results in memory disturbances, as demonstrated in our present work.

Based on the data gathered so far about the role of IL-1 in memory processes (reviewed in Goshen and Yirmiya, 2007), we suggested that the influence of IL-1 on memory follows an inverted U shaped pattern. According to this hypothesis, physiological levels of IL-1 are needed for memory formation, whereas any deviation from the physiological range, either by excess elevation in IL-1 levels or by blockade of IL-1 signaling, results in impaired memory. This model was based on: (A) the beneficial role of low-dose IL-1 administration on hippocampal-memory formation (Yirmiya et al., 2002; Brennan et al., 2003; Song et al., 2003). (B) The detrimental effect of elevated IL-1 levels (induced by either exogenously administered IL-1 or by enhanced endogenous release of IL-1, stimulated by exposure to pathogens or stress) on hippocampal-dependent memory (Oitzl et al., 1993; Pugh et al., 1999; Rachal Pugh et al., 2001; Barrientos et al., 2002). (C) The fact that blockade of IL-1 signaling either pharmacologically (by IL-1ra administration) or genetically (by deletion of the IL-1 receptor or over-expression of IL-1ra) impairs hippocampal-dependent memory (Yirmiya et al., 2002; Avital et al., 2003). This collection of studies was performed by many groups, using various testing paradigms, experimental approaches (pharmacological or genetic), species, doses and administration routes. In the present study we confirm the inverted U-shape hypothesis in a systematic fashion for the first time, and demonstrate the dual role of IL-1 in memory under identical conditions. Our results show that physiological levels of IL-1 are required for memory formation and a slight increase in brain IL-1 can even improve memory performance, whereas excess elevation in IL-1 levels or blockade of IL-1 signaling, results in impaired memory. Moreover, we show that this inverted U-shaped involvement of IL-1 in memory processes is specific to hippocampal-dependent memory, whereas hippocampal-independent memory is not affected by changes in IL-1 signaling.

The results of all of the experiments presented above show dissociation between the involvement of IL-1 signaling in hippocampal-dependent memory and its lack of effect on memory tasks for which the hippocampus is not required. Specifically, we show impairments in spatial memory in the water maze, and in contextual fear conditioning, which both depend on normal hippocampal functioning (Morris et al., 1982; Maren, 2001). The impairment in contextual memory was observed following disruption of the homeostasis of IL-1 either chronically or acutely, peripherally or centrally, during brain development or in adulthood. However, visually guided memory in the water maze and auditory-cued fear conditioning, which do not require the hippocampus (Morris et al., 1982; Maren, 2001) were not affected by alterations in IL-1 signaling. Together, these findings suggest

that: (1) IL-1 is specifically involved in hippocampal memory consolidation, and (2) IL-1 exerts its influence on the development of the neural basis of memory specifically within the hippocampus.

In conclusion, the findings of the present study suggest that elevated levels of hippocampal IL-1 gene expression within the physiological range are causally related to hippocampal-dependent memory performance, whereas either excess or absence of IL-1 signaling results in memory impairments. Furthermore, the results suggest that during fetal maturation, normal IL-1 signaling has a role in the development of the neural substrate mediating hippocampal-dependent memory. These conclusions may have important implications for several medical conditions in humans, which involve alteration in the IL-1 system, accompanied by cognitive deficits. Specifically, several recent studies in humans demonstrated that mutations in the IL-1 receptor accessory protein-like gene are involved in X-linked mental retardation (Carrie et al., 1999; Jin et al., 2000). Furthermore, we have recently reported that a transient increase in plasma IL-1ra levels is associated with endotoxin-induced memory deficits in healthy volunteers (Reichenberg et al., 2001). Thus, in humans, changes in IL-1 signaling, caused by genetic, pathological or environmental factors, in utero or in adulthood, may lead to cognitive impairments.

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All of the above had no further role in study design; in the collection, analysis and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

None of the authors have any financial, personal or other conflict of interest that can influence this work.

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