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Full-length Article

Microglial production of TNF-alpha is a key element of sustained fear memory

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ABSTRACT

The proinflammatory cytokine productions in the brain are altered in a process of fear memory formation, indicating a possibility that altered microglial function may contribute to fear memory formation. We aimed to investigate whether and how microglial function contributes to fear memory formation. Expression levels of M1- and M2-type microglial marker molecules in microglia isolated from each conditioned mice group were assessed by real-time PCR and immunohistochemistry. Levels of tumor necrosis factor (TNF)-α, but not of other proinflammatory cytokines produced by M1-type microglia, increased in microglia from mice representing retention of fear memory, and returned to basal levels in microglia from mice representing extinction of fear memory. Administration of inhibitors of TNF-α production facilitated extinction of fear memory. On the other hand, expression levels of M2-type microglia-specific cell adhesion molecules, CD206 and CD209, were decreased in microglia from mice representing retention of fear memory, and returned to basal levels in microglia from mice representing extinction of fear memory. Our findings indicate that microglial TNF-α is a key element of sustained fear memory and suggest that TNF-α inhibitors can be candidate molecules for mitigating posttraumatic reactions caused by persistent fear memory.

1. Introduction

Microglia represent the major population of immune cells of the central nervous system, which produce inflammatory cytokines in response to various environmental stimuli. Microglia are involved in the inflammatory response to harmful substances, such as pathogens, damaged cells, or irritants, and in acute stress response to stimuli such as pain by regulating the production of proinflammatory cytokines. For example, repetitive inescapable tail shocks elevate the levels of the proinflammatory cytokine interleukin (IL)-1β in the hypothalamus of rats (O'Connor et al., 2003), and this response is reversed by the microglial inhibitor minocycline (Blandino et al., 2006, 2009). Moreover, hippocampal microglia isolated from rats exposed to tail-shock stress produce significantly higher levels of IL-1β and the proinflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α) in response to lipopolysaccharide (LPS) (Frank et al., 2012).

These proinflammatory cytokines are involved in the stress response to electric shock and regulation of contextual fear memory formation caused by electric shock. Contextual fear memory is an associative memory in the context of conditioned fear and arises from stimuli such as an electrical footshock. To generate a long-term contextual fear memory, the unpleasant memory is stabilized through memory consolidation. In contrast, memory extinction induced by continuous or repeated retrieval of fear memory inhibits fear responses. For example, mice with experimental autoimmune encephalomyelitis (EAE) with elevated levels of IL-1β in the hypothalamus exhibit suppressed fear memory consolidation (Acharjee et al., 2013). Furthermore, social isolation induces elevated levels of IL-1β in the hippocampus and cerebral cortex that are associated with suppressed contextual fear conditioning (Pugh et al., 1999).

Administration of IL-6 to the amygdala impairs the acquisition and extinction of conditioned fear in a dose-dependent manner (Hao et al., 2014). LPS-induced elevation of hippocampal levels of

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IL-6 and its downstream transcription factor, signal transducer and activator of transcription 3 (STAT3), which serves as a marker of IL-6 signaling activity, suppresses contextual fear memory consolidation. This suppression is reversed by the inhibition of IL-6 signaling by administering sgp130 (endogenous soluble IL-6 receptor inhibitor) (Burton and Johnson, 2012; Jostock et al., 2001). Intra-amygdala infusion of the proinflammatory cytokine TNF-α delays significantly the acquisition and extinction of auditory fear conditioning (Jing et al., 2014). Collectively, these findings indirectly suggest that the elevation of proinflammatory cytokine levels in the cerebral parenchyma suppress fear memory acquisition, consolidation, and extinction, depending on the affected regions of the brain.

The two subtypes of microglia, M1 and M2, are distinguished by their cell surface markers. M1-type microglial markers include CD16 (Fc gamma III Receptor) and CD32 (Fc gamma II Receptor), and M2-type microglial markers include CD206 (MMR: macrophage mannose receptor) (Kobayashi et al., 2013) and CD209 (DC-SIGN: **d**endritic **c**ell-**s**pecific **i**ntercellular adhesion molecule-3-**g**rabbing **n**on-integrin) (Durafourt et al., 2012). Activated microglia are widely known to exert proinflammatory M1 and anti-inflammatory M2 functions (Boche et al., 2013). M1 cells release proinflammatory cytokines such as IL-1β, IL-6, and TNF-α. In contrast, M2 cells release anti-inflammatory cytokines such as IL-4, IL-5, IL-10, and neurotrophic factors such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), transforming growth factor beta (TFG-β), glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (Fumagalli et al., 2011).

All cytokines involved in the acute response to footshock and the suppression of fear memory formation are produced by M1 microglia. Although limited data are available regarding the association between M2 microglial products, such as anti-inflammatory cytokines and growth factors, and the acute response or fear memory, mice lacking microglial BDNF exhibit a significantly decreased freezing fear response to conditioned auditory cues (Parkhurst et al., 2013), indicating that M2 microglial products, including BDNF, may facilitate fear memory formation. These findings suggest that microglial polarization into the M1 and M2 types may be at least partially involved in fear memory formation in an opposite manner; however, this possibility requires investigation.

Here, we aimed to determine whether and how characteristic alterations of microglia are involved in the formation and extinction of fear memory. We show that of the M1-derived proinflammatory cytokines, TNF-α levels increased during retention of fear memory and returned to basal levels during fear memory extinction. Further, the inhibition of TNF-α facilitated the extinction of fear memory. Our findings indicate that microglial TNF-α is a key contributor to sustaining fear memory and that the inhibitors of TNF-α such as minocycline may facilitate extinction of fear memory. Considering that sustained fear memory is a key component of the pathophysiology of posttraumatic stress disorder (PTSD) (Goswami et al., 2013; Yehuda, 2002), inhibition of microglial TNF-α may be a druggable target in developing treatments to alleviate symptoms of PTSD.

2. Materials and methods

2.1. Animals

The animal ethics committee of Tohoku University Graduate School of Medicine approved the experimental protocols. C57BL/6 male mice (SLC Inc., Japan) weighing 20–30 g were individually housed and maintained on a 12:12 light/dark schedule with ad libitum access to food and water throughout the course of the entire experiment.

2.2. Behavioral procedures

Mice were placed into the training chamber (17.5 × 17.5 × 15 cm) with a stainless steel rod floor used to deliver footshocks (Ohara & Co. Ltd.). Each mouse was transferred from its home cage to the training chamber and allowed to explore it for 148 s before receiving a single footshock (2 s, 0.4 mA) following a standardized protocol (Suzuki et al., 2004). After the footshock, mice were left in the training chamber for 30 s and then transferred to their home cage. Twenty-four hours after conditioning, mice were re-exposed to the training chamber for varying durations as follows: 0 min (FS0; footshocked mice with no reexposure), 3 min (FS3; footshocked mice with 3 min of reexposure) or 30 min (FS30; footshocked mice with 30 min of reexposure) without receiving a footshock, and then transferred to their home cage. No (0 min) or short-duration (3 min) reexposure to the context are supposed to result in retention of fear memory, while long-duration (30 min) reexposure is supposed to facilitate extinction of fear memory.

To validate the effect of no, short- or long-duration reexposure on retention or extinction of fear memory, the percentage of time for which the mice exhibited freezing behavior was measured every 5 min during the 30 min exposure session (Mamiya et al., 2009; Suzuki et al., 2004). Control animals were similarly trained without receiving an electric footshock and were re-exposed to the context for 0 min (Con0), 3 min (Con3) or 30 min (Con30). To evaluate their behavior, mice were again transferred to the training chamber 24 h after the reexposure, and the percentage of time the mice engaged in freezing behavior during a 5 min observation (freezing time) was measured as an indicator of the behavioral outcome of fear memory. For analyses of microglial cells, mice were trained, re-exposed to the context in the same manner as the mice in the behavioral evaluation studies, and decapitated 24 h after the reexposure. This procedure was performed instead of behavioral monitoring to avoid the effect of tertiary exposure to the training chamber on the molecular phenotype of microglial cells.

2.3. Isolation of microglia

After the mice were decapitated, the brain was prepared as a single-cell suspension using a neural tissue dissociation kit and a gentleMACS Dissociator (Miltenyi Biotec Bergisch Gladbach, Germany). CD11b-positive microglia were isolated using CD11b-labeled MicroBeads and a magnetic cell separator (Miltenyi Biotec) (Yu et al., 2015). To verify the purity of the isolated microglia samples, cells separated with CD11b-labeled MicroBeads were stained by incubating with rat anti-mouse CD11b FITC-conjugated monoclonal antibodies (mAb) (Miltenyi Biotec) for 5 min at 4 °C. After the mAb-stained subjects were washed, cells were subjected to flow cytometry with an ACCURI Flow Cytometer (Accuri Cytometers, Inc., Ann Arbor, MI, USA). Purities of CD11b-positive cells were above 97% for all subjects (Suppl. Fig. 1).

2.4. Quantitative real-time PCR

Total RNA was extracted from CD11b-positive microglia and used as the template for cDNA synthesis conducted using random primers and the SuperScript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA). The relative copy number of each transcript in each cDNA sample was measured using specific primers and the iQ SYBR Green Supermix (Bio-Rad Inc., Hercules, CA, USA). Representative cell surface markers, cytokines, and growth factors specifically produced by M1 or M2 phenotypes of microglia were

selected for the assay (Durafourt et al., 2012; Fumagalli et al., 2011; Kobayashi et al., 2013; Miron et al., 2013). The genes encoding mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18 S rRNA (data not shown) were used as internal controls for normalizing the data. A standard curve was constructed for each assay to adjust for differences in the amplification efficiencies between primer sets. The complete list of primers is given in Suppl. Table 1.

2.5. Immunohistochemical analysis of mouse microglia

Immunohistochemistry was performed using standard methods (Yu et al., 2015). To determine the expression of markers of mouse microglia phenotypes in vivo, mice were divided into control and footshock groups subjected to different reexposure training regimens according those described in Methods section. The footshock groups were treated in the same manner employed in the behavioral and the real-time PCR studies. Because the significant differences were not detected among the non-footshocked groups (Con0, Con3 and Con30) using real-time PCR analysis, the control group in the immunohistochemical study was not divided into three groups according to different reexposure times. Control mice were moved into the training cage without exposure to a footshock and decapitated 48 h after being transferred to their home cage. Hippocampal slices (30-μm thick) dissected from frozen brains were reacted with the antibodies as follows: rabbit anti-mouse Iba-1 (1:500; Wako Pure Chemical Co., Osaka, Japan), goat anti-mouse TNF-α (1:400; R&D Systems, Minneapolis, MN, USA), goat anti-mouse macrophage mannose receptor (MMR) (CD206, 1:50; R&D Systems), or rat anti-mouse CD209a (1:50; BD Bioscience, Mountain View, CA, USA). Secondary antibodies were as follows: Alexa Fluor 488-conjugated anti-rat IgG, Alexa Fluor 488-conjugated anti-rabbit IgG and Alex Fluor 594-conjugated anti-goat IgG (1:300; Invitrogen). Nuclei present in the slices were stained using 4,6-diamidino-2-phenylindole (DAPI) (Invitrogen). Images of cells were acquired using a Leica DAS Mikroskop microscope (Leica Microsystems, Wetzlar, Germany). Levels of TNF, MMR or CD209a signal throughout the whole Iba-1-positive cell were obtained using ImageJ 1.42 software (NIH Image, Bethesda, MD, USA).

2.6. Minocycline treatment

Minocycline is a broad-spectrum tetracycline antibiotic. It is widely known to inhibit activation of microglia, particularly M1-type microglia (Kobayashi et al., 2013). Minocycline (Wako, Japan), 45 mg kg^{-1} body weight, was injected intraperitoneally 30 min after a contextual footshock or without footshock. After the first injection, the mice received three injections of minocycline every 12 h until the mice were sacrificed (Fig. 4A). Control mice received an equal volume of saline using the same time course as minocycline injections. The dose and timing of minocycline treatment were set according to the condition utilized in the traumatic brain injury model, in which postinjury administration of minocycline significantly inhibited M1-type microglial activation and reduced M1-type microglial markers TNF-α and IL-1β production (Raghavendra et al., 2003; Sanchez Mejia et al., 2001). Freezing times and hippocampal TNF-α levels were evaluated in minocycline-treated and control mice 47.5 h and 5 weeks after the first infection.

2.7. ELISA measurements of mouse serum TNF-α

Since the serum levels of TNF-α are significantly higher in patients with PTSD compared with healthy controls (Guo et al., 2012), serum TNF-α from trained mice that were re-exposed to the context in the same manner as the mice in the behavioral evaluation studies and were decapitated 24 h after the reexposure was measured using the Mouse TNF-α ELISA MAX™ Standard kit (BioLegend, Inc., San Diego, CA, USA). Optical density at 450 nm was measured with a SpectraMAX 190 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

2.8. Statistical analysis

A two-tailed unpaired Student's *t*-test was used to evaluate the differences in mean values between two groups. One- or two-way analysis of variance (ANOVA) followed by Tukey's or Sidak *post-hoc* test was used for the other studies. Statistical analysis was performed using GraphPad Prism version 6 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Effects of reexposure duration on fear memory formation

We evaluated the validity of behavioral experimental modeling of fear memory retention and extinction by determining the effects of the duration of reexposure to the training context on freezing responses. Mice were trained with or without a single footshock and re-exposed 24 h later to the training context for 3 different durations: 0 min (FS0), 3 min (FS3), or 30 min (FS30). Two-way ANOVA revealed significant effects of footshock treatment ($F_{1,\ 60} = 346.8$; $P < 0.001$) and reexposure time ($F_{2,\ 60} = 9.777$; $P < 0.001$) on freezing time, with a significant interaction ($F_{2,\ 60} = 10.16$; $P < 0.0002$). In subsequent Tukey's *post-hoc* analyses, the footshocked groups with 3 different reexposure periods (FS0, FS3, and FS30) showed significantly longer freezing times compared with non-footshocked groups of the three respective reexposure periods (Con0, Con3 and Con30), suggesting that fear memory was successfully consolidated in the footshock-stressed groups (Fig. 1A). No significant difference in freezing times was found among mice not receiving a footshock for the three reexposure durations (Con0, Con3 and Con30) (Fig. 1A). Footshocked mice with long-duration reexposure (FS30) showed a significantly shorter freezing time than those of footshocked mice with no reexposure (FS0) ($P < 0.001$) and footshocked mice with short-duration reexposure (FS3) ($P < 0.001$), while there was no difference in freezing time between FS0 and FS3 (Fig. 1A). The shortening of freezing time due to 30 min of reexposure represents extinction of the fear memory, while sustained freezing time following 0 min or 3 min of reexposure represents retention of fear memory. During the 30-min reexposure session 24 h after the footshock stress, the extent of freezing decreased with time. When the percentage of freezing time was measured every 5 min during the 30 min exposure session, the fourth (15–20 min) and fifth (20–25 min) segments showed significantly shorter freezing times compared with the first segment (0–5 min; $P < 0.01$, see Fig. 1B). These observations were consistent with those of previous studies (Mamiya et al., 2009; Suzuki et al., 2004), which showed that 30 min of reexposure facilitated fear memory extinction.

3.2. Expression of M1-type and M2-type microglia-specific molecules in fear memory retention and extinction

Among the M1 microglia markers examined, only the levels of *Tnf* mRNA significantly increased (42.1%, $P < 0.01$) in microglia of the footshocked mice with non-reexposure group (FS0) compared with the non-footshocked mice with non-reexposure group (Con0) (Fig. 2A). On the other hand, in comparisons among the footshock-treated groups, the level of *Tnf* mRNA of the long-duration reexposure group (FS30), i.e. fear memory extinction

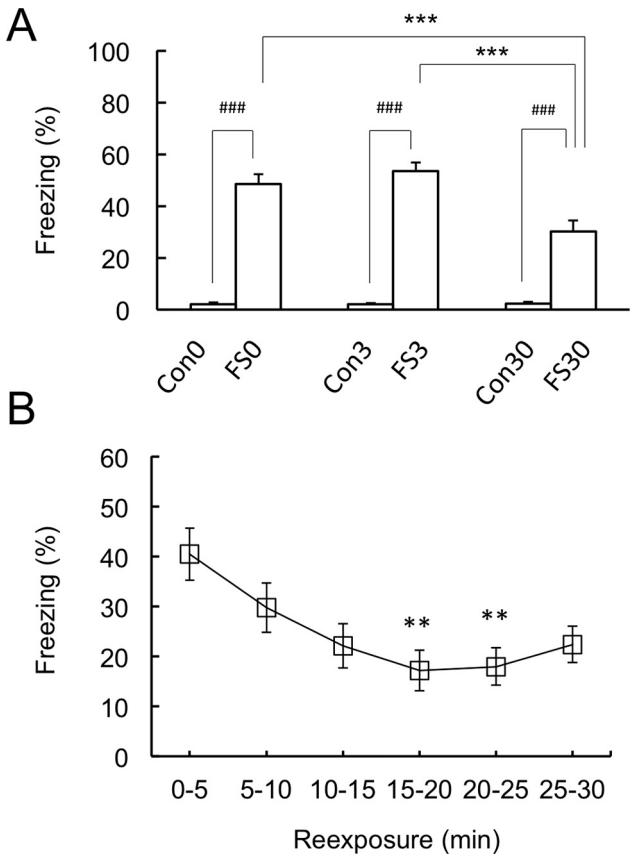

Fig. 1. Effects of reexposure duration on freezing. (A) Freezing times on the third day after footshock treatment or non-treatment of groups with 0, 3, or 30 min reexposure (Time: $F_{2,60} = 9.777$; $P < 0.001$. Stress: $F_{1,60} = 346.8$; $P < 0.001$. Interaction: $F_{2,60} = 10.160$; $P < 0.001$). Con0, no footshock 0-min reexposure; Con3, no footshock 3-min reexposure; Con30, no footshock 30-min reexposure; FS0, footshock 0-min reexposure; FS3, footshock 3-min reexposure; FS30, footshock 30-min reexposure. Time, reexposure time. Stress, footshock treatment. Interaction, time vs stress. $^{###}p < 0.001$, compared with Con0, Con3 or Con30 groups. $^{***}p < 0.001$, compared with FS30 group. Two-way ANOVA followed by Tukey's *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM ($n = 11$ per group). (B) Freezing scores in 5 min blocks are presented during 30-min reexposure ($F_{5,\ 60} = 4.042$; $P < 0.01$). $^{**}P < 0.01$, compared with 0–5 min. One-way ANOVA followed by Tukey's *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM ($n = 11$ per group).

group, was significantly decreased by 17.8% ($P < 0.01$) and 20.2% ($P < 0.05$) compared with those of the non- and short-duration reexposure groups (FS0 and FS3), respectively, i.e. both of the fear memory retention groups. There were no significant changes in the levels of mRNAs encoding the other M1-specific cell surface markers *Cd16*/*32* and cytokines *Il1b* and *Il6*, regardless of being footshocked, or duration of reexposure (Suppl. Fig. 2).

Contrary to the expression profiling of TNF-α, the transcript levels of M2 microglia cell surface markers *Cd206* and *Ym1* were significantly decreased by 37.7% ($P < 0.01$, Fig. 2B) and 64.2% ($P < 0.05$, Fig. 2D) in the footshocked mice with non-reexposure group (FS0), compared with the non-footshocked mice with non-reexposure group (Con0), and the transcript levels of *Cd206*, *Ym1* as well as *Cd209a* were significantly decreased by 52% ($P < 0.01$), 64.5% ($P < 0.05$), and 36.3% ($P < 0.05$), in the footshocked mice with short-duration reexposure group (FS3), compared with the non-footshocked mice with short-duration reexposure group (Con3), respectively (Fig. 2B-D). On the other hand, in comparisons among the footshock-treated groups, transcript levels of *Cd206*, *Cd209a*, and *Ym1* of the long-duration reexposure group (FS30), i.e. fear memory extinction group, were significantly increased by 46.3% ($P < 0.05$), 75.8% ($P < 0.01$), and 152.5% ($P < 0.05$) compared with those of the non-reexposure group (FS0), respectively, and by 76.9% ($P < 0.05$), 97.5% ($P < 0.001$), and 159.5% ($P < 0.05$) compared with those of the short-duration reexposure group (FS3), respectively, i.e. both of the fear memory retention groups.

Regarding transcript levels of neurotrophic factors released from M2 microglia, *Igf1* of the footshocked mice with long-duration reexposure group (FS30), i.e. fear memory extinction group, were significantly increased by 38.0% ($P < 0.05$) compared with those of non-footshocked mice with long-duration reexposure (Con30), as well as 50.8% ($P < 0.01$), and 34.9% ($P < 0.05$) compared with the footshocked mice with non- and short-duration reexposure groups (FS0, and FS3), i.e. both of fear memory retention groups, respectively (Fig. 2E). The levels of microglial *Tgfb1* mRNA of the footshocked mice with long-duration exposure group (FS30), i.e. fear memory extinction group, was significantly increased by 15.8% ($P < 0.05$) compared only with footshocked mice with short-duration reexposure group (FS3) of the fear memory retention groups (Fig. 2F).

The levels of mRNAs encoding other M2-type microglia-derived cytokines such as *IL4*, *Il5*, and *Il10* and those of *Igf1*, *Vegf*, and *Arg1* were not changed among the groups, regardless of being footshocked, or duration of reexposure, representing each fear memory process (Suppl. Fig. 3). The levels of the mRNAs of genes encoding other M2-type microglial-derived neurotrophic factors *Bdnf*, *Gdnf*, and *Hgf* were below the limit of detection of the real-time PCR assays (results not shown).

3.3. Verification of M1-type and M2-type microglia-specific molecule expression in the hippocampus in fear memory retention and extinction

Because memory consolidation and extinction are associated with hippocampal function (Alberini, 2005), protein expression of the M1-type microglia-specific cytokine TNF-α and M2-type microglial cell surface markers CD206 and CD209a were evaluated in the hippocampus of mice representing fear memory retention and extinction. TNF-α expression increased significantly by 46% in hippocampal microglia in brain slices from the footshocked mice with non-reexposure group (FS0) compared with that of the non-footshocked controls (Con0) ($P < 0.05$, Fig. 3A and Suppl. Fig. 4). In contrast, TNF-α levels of hippocampal microglia from the footshocked mice with long-duration reexposure group (FS30) and the non-footshocked control group (Con30) were equivalent. TNF-α level of hippocampal microglia of the footshocked mice with long-duration reexposure group (FS30), i. e. fear memory extinction group, was significantly decreased by 55.1% compared with the footshocked mice with no reexposure group (FS0), i.e. fear memory retention group ($P < 0.05$, Fig. 3A and Suppl. Fig. 4).

The levels of microglial CD206 and CD209a of hippocampal slices from the footshocked mice with no reexposure group (FS0) were significantly decreased by 43.9% ($P < 0.05$) and 63.2% ($P < 0.01$), and those of the footshocked mice with short-duration reexposure group (FS3) were significantly decreased by 83.4% ($P < 0.01$) and 64.1% ($P < 0.01$) compared with that of the non-footshocked controls (Con0 and Con3), respectively (Fig. 3B and C, and Suppl. Figs. 5 and 6). In contrast, the levels of CD206 and CD209a of the footshocked mice with long-duration reexposure group (FS30) and non-footshocked control group (Con30) were equivalent. The levels of CD206 and CD209a of the footshocked mice with long-duration reexposure group (FS30), i.e. fear memory extinction group, were significantly increased by 99.3% ($P < 0.05$) and 137.1% ($P < 0.01$) compared with the footshocked mice with no reexposure (FS0), respectively, and by 464.5% ($P < 0.01$) and 143.4% ($P < 0.01$) compared with the footshocked mice with short-duration reexposure group (FS3), respec-

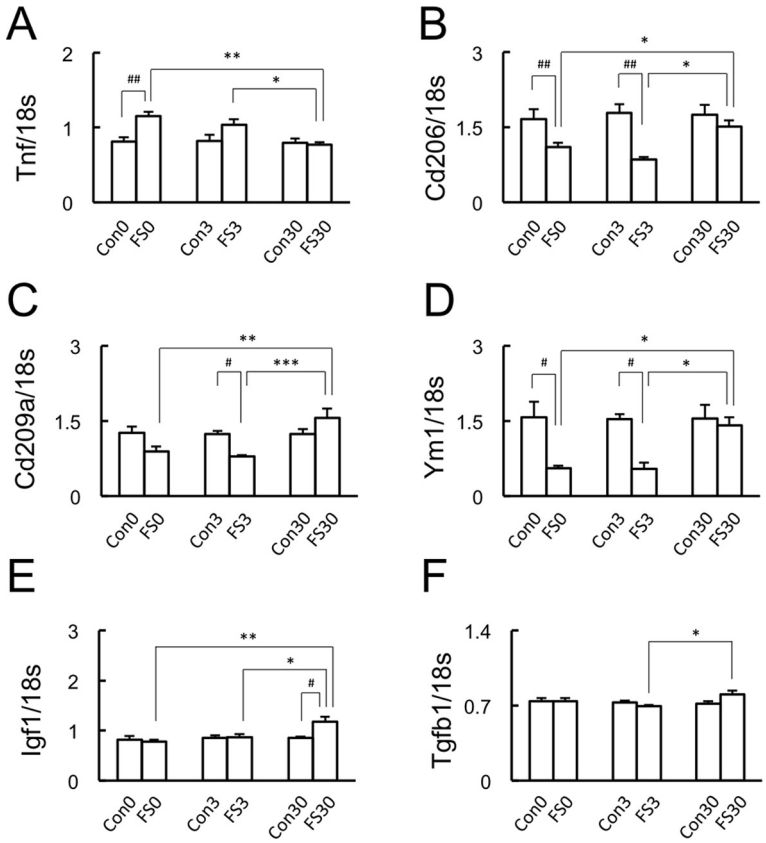

Fig. 2. The effects of reexposure durations on the transcriptional levels of microglial phenotypic markers were measured using qRT-PCR. The mRNA levels of the markers are normalized to the level of ribosomal RNA S18. (A) M1-type microglial marker Tnf (Time: $F_{2,30} = 5.372$; $P < 0.05$. Stress: $F_{1,30} = 11.86$; $P < 0.01$. Interaction: $F_{2,\ 30} = 4.566$, $P < 0.05$). (B) M2-type microglial marker Cd206 (Time: $F_{2,30} = 4.322$; $P < 0.05$. Stress: $F_{1,30} = 32.13$; $P < 0.001$. Interaction: $F_{2,\ 30} = 3.370$, $P < 0.05$). (C) M2-type microglial Cd209a (Time: $F_{2,30} = 7.160$; $P < 0.01$. Stress: $F_{1,30} = 3.546$; $P > 0.05$. Interaction: $F_{2,\ 30} = 7.465$, $P < 0.01$). (D) M2-type microglial Ym1 (Time: $F_{2,30} = 3.201$; $P > 0.05$. Stress: $F_{1,30} = 19.47$; $P < 0.001$. Interaction: $F_{2,\ 30} = 3.209$, $P > 0.05$). (E) *Igf1* (Time: $F_{2,30} = 5.734$; $P < 0.001$. Stress: $F_{1,30} = 3.471$; $P > 0.01$. Interaction: $F_{2,\ 30} = 4.682$, $P < 0.05$). (F) *Tgfb1* (Time: $F_{2,30} = 1.852$; $P > 0.05$. Stress: $F_{1,30} = 2.064$; $P > 0.05$. Interaction: $F_{2,\ 30} = 2.010$, $P > 0.05$). Con0, no footshock 0-min reexposure; Con3, no footshock 3-min reexposure; Con30, no footshock 30-min reexposure; FS0, footshock 0-min reexposure; FS3, footshock 3-min reexposure; FS30, footshock 30-min reexposure. Time, reexposure time. Stress, footshock treatment. Interaction, time vs stress. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with Con0, Con3 or Con30 group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with FS30 group. Two-way ANOVA followed by Tukey's *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM (n = 6 mice per group).

tively, i.e. both of the fear memory retention groups (Fig. 3B and C, and Suppl. Figs. 5 and 6). In addition, differences were not observed in Iba-1 expression among the non-footshocked control, FS0, FS3 and FS30 groups (data not shown).

3.4. Effects of minocycline on fear memory

Minocycline selectively inhibits M1-type microglial activity (Kobayashi et al., 2013) and reduces the levels of TNF-α produced by microglia (Lee et al., 2004; Wang et al., 2005). To investigate the effects of inhibiting M1 microglial function on fear memory, we compared the effects of reexposure on freezing times between mice administered minocycline or saline after footshock. Two-way ANOVA revealed significant effects of footshock treatment ($F_{1,\ 55} = 116.508$; $P < 0.001$), minocycline treatment ($F_{1,\ 55} = 13.263$; $P < 0.01$) and reexposure time ($F_{2,\ 55} = 14.041$; $P < 0.001$) on freezing time (Test 1, Fig. 4A and B). The freezing times of mice treated with 4 times of minocycline after footshock and subjected to either no reexposure (M + FS0, Fig. 4A) or short-duration reexposure (M + FS3) were shorter by 34.1% and 27.3%, respectively, compared with saline-treated controls (S + FS0 and S + FS3), although the difference did not reach statistical significance in Tukey's *post-hoc* analyses following the ANOVA (Fig. 4B). The freezing times of the minocycline-treated footshocked mice with long-duration reexposure group (M + FS30) were significantly shorter by 92.9% compared with those of the saline-treated group (S + FS30, Fig. 4B). To evaluate the effect of minocycline on behavioral changes during the reexposure process, the freezing time percentages of minocycline- and saline-treated footshocked mice were measured during the 3 min and 30 min reexposure groups (Test 0, Fig. 4A). Freezing time of the minocycline-treated group decreased by 40.2% compared with that of the saline-treated control group; however, the difference did not reach statistical significance ($P = 0.093$) (Fig. 4C). When the 30 min reexposure session was divided into six 5-min segments, ANOVA of repeated measures indicated a statistically significant decrease in freezing time of the minocycline-treated group compared with that of the saline-treated group ($F_{5,\ 50} = 7.786$, $P < 0.001$). Tukey's *post-hoc* analysis indicated a significant decrease in freezing time during the third (10–15 min), fourth (15–20 min), and fifth (20–25 min) segments compared with the first segment (0–5 min) ($P < 0.05$). There was no significant interaction between time and drug effects (Fig. 4D).

The long-term effect of minocycline administration on fear memory was evaluated 5 weeks after its administration (Test 2, Fig. 4A) to evaluate the effect of minocycline on the occurrence of the phenomenon termed "spontaneous recovery," which is the re-emergence of a previously extinguished conditioned fear memory within an interval after fear memory extinction was once established (Rescorla, 2004). The freezing time of the saline-treated post-footshock with long-duration reexposure

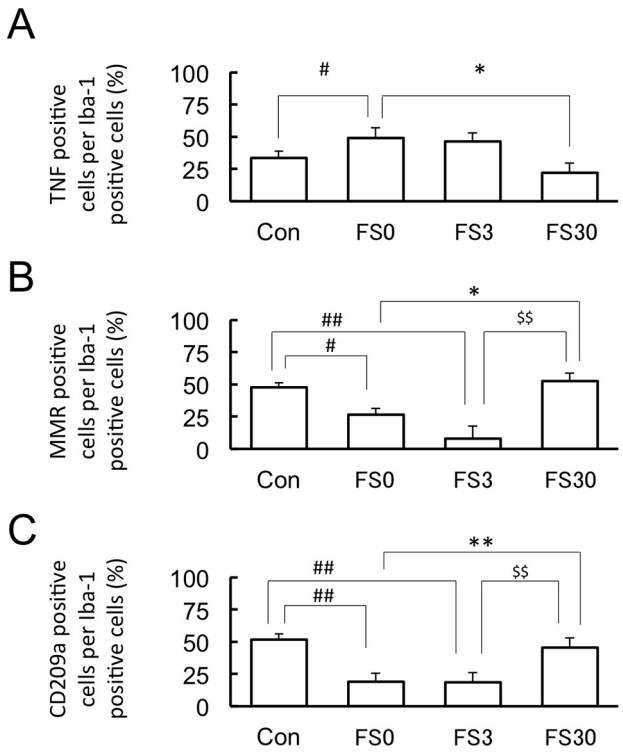

Fig. 3. Immunohistochemical analysis of the expression of microglial phenotypic markers as a function of reexposure times. (A) Percentage of TNF-α-positive M1 cells among Iba-1-positive microglia per field ($F_{3,\ 12} = 7.694$, $P < 0.01$). (B) Percentage of MMR-positive M2 cells among Iba-1-positive microglia per field ($F_{3,\ 12} = 13.66$, $P < 0.001$). (C) Percentage of CD209a-positive M2 cells among Iba-1-positive microglia per field ($F_{3,\ 12} = 13.26$, $P < 0.001$). Con3, No footshock; FS0, footshock 0-min reexposure; FS3, footshock 3-min reexposure; FS30, footshock 30-min reexposure. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with Con. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with FS0. $^{\$\$}P < 0.01$, compared with FS3. One-way ANOVA followed by Tukey's *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM (n = 4 mice per group).

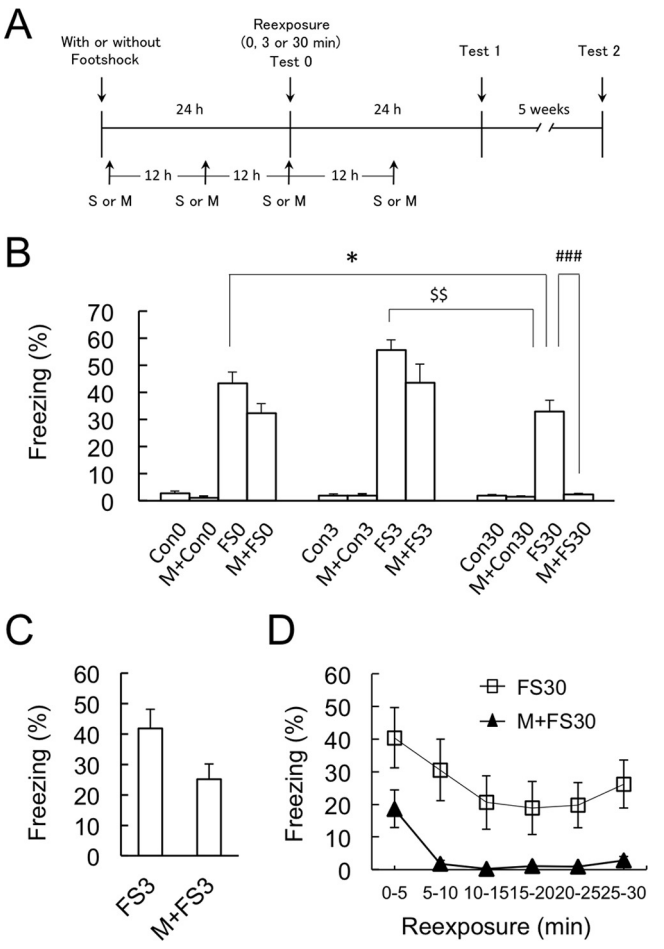

Fig. 4. Effects of minocycline administration on fear memory formation. (A) Mice were exposed to footshock and injected with saline or minocycline 4 times (30 min, 12 h, 24 h, and 36 h after the footshock). On the second day, mice were re-exposed to contextual conditions for 0, 3, or 30 min. Freezing times were assessed during the reexposures (Test 0), 48 h after the footshock (Test 1), and 5 weeks later (Test 2). S, Saline; M, minocycline. (B) Freezing times were measured 48 h after the footshock (Time: $F_{2,\ 55} = 14.041$; $P < 0.001$. Stress: $F_{1,\ 55} = 116.508$. $P < 0.001$. M: $F_{1,\ 55} = 13.263$; $P < 0.01$) on freezing time. Con0, no footshock 0-min reexposure; Con3, no footshock 3-min reexposure; Con30, no footshock 30-min reexposure; FS0, footshock 0-min reexposure; FS3, footshock 3-min reexposure; FS30, footshock 30-min reexposure. Time, reexposure time. Stress, footshock treatment. M, minocycline. $^{*}P < 0.05$, compared with FS0. $^{\$\$}P < 0.01$, compared with FS3. $^{\#\#\#}P < 0.001$, compared with FS30. One-way ANOVA followed by Tukey's *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM (n = 6 mice per group). (C) Percentage of reexposure time spent in freezing was measured for footshocked mice with 3 min of reexposure with or without minocycline (Test 0). FS3, footshock 3 min reexposure; M, minocycline. A two-tailed unpaired Student *t* test was applied to this comparison. Data are presented as the mean ± SEM (n = 6 mice per group). (D) A 30 min reexposure session was divided into six 5 min intervals and the percentage of freezing time in each was plotted for the groups treated with or without minocycline, and the effects of time during the reexposure session and the drug (minocycline vs saline) were evaluated using repeated-measure ANOVA ($F_{5,\ 50} = 0.539$, $P > 0.05$). FS30, footshock 30-min reexposure, M, minocycline. Open square, FS30; closed triangle, M + FS30. Repeated-measures ANOVA followed by the Sidak *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM (n = 6 mice per group).

group measured at 5 weeks after fear conditioning was significantly longer by 82.5% compared with freezing time measured 3 days after the fear conditioning (Suppl. Fig. 7A and B), indicating the occurrence of spontaneous recovery. The freezing time of the M + FS30 group decreased significantly by 27.4% compared with that of the S + FS30 group 5 weeks after administration of minocycline ($P < 0.05$, Suppl. Fig. 7B), which suggests long-term efficacy of minocycline on maintaining fear memory extinction.

3.5. Effects of minocycline on microglial production of TNF-α

The effects of minocycline treatment on hippocampal TNF-α in mice with variable fear memory conditions were evaluated in the same manner as described in Section 3.3. The effect of minocycline on decreasing freezing time reached statistical significance only when administered to footshocked mice with long-duration reexposure (M + FS30) but not those with non- or short-duration reexposure (M + FS0 or M + FS3). The two-way ANOVA revealed significant effects of minocycline treatment ($F_{1,\ 30} = 66.44$; $P < 0.001$) and reexposure time ($F_{2,\ 18} = 4.860$; $P < 0.05$) on TNF-α expression. Following Tukey's *post-hoc* analyses, the TNF-α level of hippocampal microglia in the footshocked mice with long-duration-reexposure group (FS30), i.e., the fear memory extinction group, was significantly decreased (by 51.3%) compared with the footshocked mice with no reexposure group (FS0), i.e. the fear memory retention group ($P < 0.05$, Fig. 5). Minocycline significantly decreased the levels of microglial TNF-α in hippocampal slices in all three footshocked groups, by 82.1% ($P < 0.01$), 80.8% ($P < 0.01$) and 81.9% ($P < 0.01$), compared with each saline-treated control (FS0, FS3, and FS30) (Fig. 5), respectively. Minocycline also exerts long-term effects (5 weeks after being footshocked) on repressing hippocampal TNF-α by 52.6% ($P < 0.05$), in parallel to the suppression of spontaneous recovery (Suppl. Fig. 7C and D). Minocycline had no effect on the levels of the M2 microglial markers MMR (Suppl. Fig. 8) and CD209a (Suppl. Fig. 9).

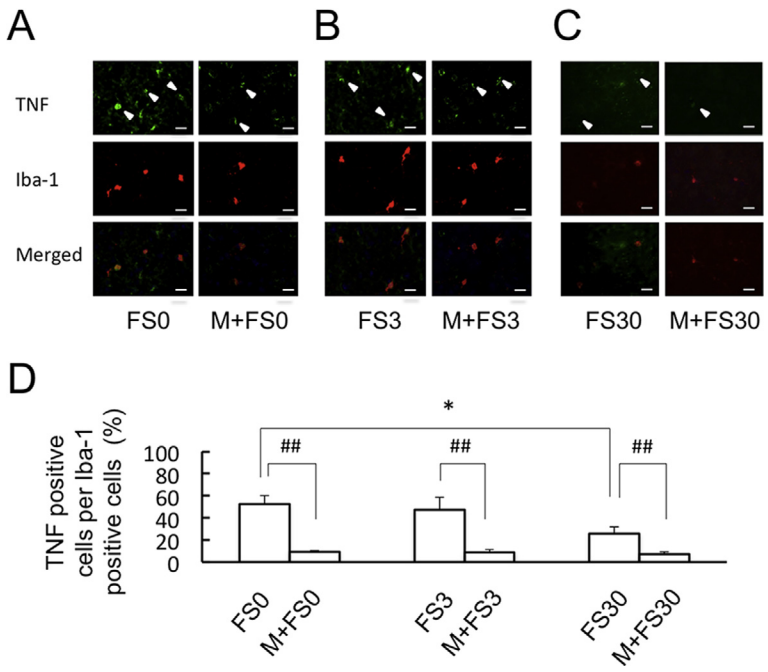

Fig. 5. Effect of minocycline administration on TNF-α expression. With or without minocycline treatment after the footshock, the mice were re-exposed for 0 min (A), 3 min (B) or 30 min (C) to the context memory on the second day. The images show microglial TNF-α (M1 microglia production) levels in the hippocampus on day 3 after the footshock (Time: $F_{2,30} = 4.860$; $P < 0.05$. M: $F_{1,30} = 66.44$; $P < 0.001$. Interaction: $F_{2,30} = 2.308$; $P = 0.117$). FS0, footshock 0-min reexposure, FS3, footshock 3-min reexposure, FS30, footshock 30-min reexposure, Time, reexposure time. Interaction, time vs M. M, minocycline. (A-C) Effect of minocycline on the microglial TNF-α levels in the mouse hippocampus (M1 marker, upper, green) and IBA-1 (microglia marker, middle, red). TNF-α levels without or with minocycline on day 3 after the footshock. The merged image shows the distribution of TNF-α/IBA1 (lower). Nuclei were stained with 4,6-diamidino-2-phenylindole (blue). Scale bar = 20 μm. Arrows indicate single TNF-α -positive cells. (D) Percentage of TNF-α-positive cells per IBA-1-positive microglia. M, minocycline. $P < 0.05$, in comparison between FS30 and FS0. $^{\#\#}P < 0.01$, in comparison between minocycline-treated and control groups. Two-way ANOVA followed by Tukey's *post-hoc* test was applied to all comparisons. Data are presented as the mean ± SEM (n = 6 mice per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.6. Expression of serum TNF-α in processes of fear memory retention and extinction

There were no significant differences in the levels of serum TNF-α among the groups regardless of being footshocked or duration of reexposure, representing retention or extinction of fear memory (Suppl. Fig. 10).

4. Discussion

In the present study, we observed characteristic changes of microglia in fear memory. For example, M1-microglial production of TNF-α was significantly increased in contextual fear memory retention and returned to baseline in fear memory extinction. Further, the inhibition of TNF-α significantly promoted fear memory extinction (Suppl. Table 2). The levels of M1-specific cell markers CD16/CD32 and M1-related proinflammatory cytokines, other than TNF-α, were not altered during retention or extinction of fear memory. These findings suggest the possibilities as follows: (1) polarization into M1-microglia is unrelated to fear memory retention; (2) microglial TNF-α production is specifically activated by acute fearful stimuli, which may in turn facilitate fear memory retention; (3) microglial TNF-α production decreases in fear memory extinction; and (4) sustained microglial TNF-α production disturbs fear memory extinction, whereas inhibition of microglial TNF-α production facilitates fear memory extinction.

In contrast, the levels of the M2-microglial cell surface markers CD206 (MMR), CD209 (DC-SIGN), and YM1 were significantly decreased in fear memory retention, and the levels of these M2-microglial cell surface markers returned to the baseline along with the increase in the mRNA levels of the M2-type microglia-related neurotrophic factors *Igf1* and *Tgfb1* in fear memory extinction (Suppl. Table 2). The levels of the mRNAs encoding the M2 microglia-marker *Arg1*; the M2 microglia-related cytokines *Il4*, *Il5*, and *Il10*; and the neurotrophic factors *Igf1*, *Vegfa*, *Vefgb*, and *Vefgc* were not altered in fear memory retention or extinction. Moreover, minocycline treatment did not detectably affect the levels of M2-related molecules.

The C-type lectin receptors MMR and DC-SIGN mediate internalization of self-antigens, cell–cell adhesion, immune responses to pathogens, and apoptosis (Lech et al., 2012; Vazquez-Mendoza et al., 2013), and the number of MMR or DC-SIGN molecules per macrophage decrease when macrophages are activated by LPS or human herpesvirus 8 (Rappocciolo et al., 2006; Shepherd et al., 1990). Therefore, our present findings indicate that changes in the levels of MMR and DC-SIGN did not alter the levels of the several M2-related molecules in the retention and extinction of fear memory. Moreover, these changes may not reflect the number of M2-type cells, but implicate changes in the functions of each M2-microglial cell, such as internalization of self-antigens, cell–cell adhesion, or apoptosis.

Our findings suggest the following: (1) MMR- and DC-SIGN-related M2-type microglial functions such as internalization of self-antigens, cell–cell adhesion, or apoptosis may be suppressed in fear memory retention. (2) M2-type cell surface molecule-related microglial functions may be reactivated in fear memory extinction. (3) M2-specific anti-inflammatory cytokines or neurotrophic factors may not contribute to fear memory retention. (4) M2-type microglia may produce the neurotrophic factors IGF-1 and TGF-β1 during fear memory extinction.

As described previously, the present study was motivated by indirect evidence indicating that increased levels of the proinflammatory cytokines, IL-1β, IL-6, and TNF-α in the cerebral

parenchyma may suppress fear memory retention and extinction. However, we present direct evidence that microglial production of IL-1β and IL-6 is not involved in retention or extinction of fear memory. Increases in the levels of IL-1β in the hypothalamus of mice with EAE and in the hippocampus and cerebral cortex of socially isolated mice suppress fear memory consolidation (Acharjee et al., 2013; Pugh et al., 1999). Similarly, an increase in the level of IL-6 in the hippocampus of LPS-administered mice suppresses fear memory consolidation (Burton and Johnson, 2012). These studies evaluated the interaction between the levels of proinflammatory cytokines and fear memory consolidation in the mouse under conditions of increased inflammation or stress. EAE, LPS, or social isolation should exert significant effects on cytokine levels, and the design of these studies may not reveal the physiological associations between cytokine levels and fear memory formation.

Moreover, administration of IL-6 and TNF-α to the amygdala impairs fear memory extinction (Hao et al., 2014; Jing et al., 2014). However, these studies do not provide information regarding the role of endogenous cytokines on fear memory retention. The current study provides direct evidence that among proinflammatory cytokines, only microglial TNF-α production was a key component of the mechanism of sustaining fear memory.

In fear memory retention, increased production of TNF-α by M1-type microglial cells may enhance the activation of the entire population of microglia (Mantovani et al., 2004; Sica and Mantovani, 2012) and may negatively influence neuronal networking. For example, high and low concentrations of TNF-α are neurotoxic or impair synaptic strength, respectively (Beattie et al., 2002). Because TNF-α resides primarily in the microglial cell membrane and microglial cell-surface TNF-α binds to TNF-α receptors expressed on the synapses of neurons (Grau et al., 2014; McCoy and Tansey, 2008), our data suggest that inter-microglia-neuron interactions modulated by increased TNF-α signaling may represent a key element that contributes to sustained fear memory.

M2 microglia potentially provide trophic support to neurons in distress (Fumagalli et al., 2011; Hu et al., 2015). Furthermore, neurotrophic factors are involved in memory. For example, IGF-2-receptor blockade reduces fear memory extinction (Agis-Balboa et al., 2011), and TGF-β1 treatment disturbs spatial learning (Nakazato et al., 2002). Further, IGF-1 and TGF-β abrogate microglial oxidative stress, regulate activation of microglia, and inhibit TNF-α production (Grinberg et al., 2013; Lodge and Sriram, 1996). Our present results show that M2 microglia expressed high levels of mRNAs encoding IGF-1 and TGF-β1, indicating decreased TNF-α production in fear memory extinction.

Minocycline exerted drastic suppression of microglial TNF-α production in the hippocampus of all of the footshocked mice followed by 0, 3 or 30 min of reexposure to the context memory, by 82.1% ($P < 0.01$), 80.8% ($P < 0.01$) and 81.9% ($P < 0.01$), respectively. On the other hand, minocycline also drastically facilitated a decrease in freezing time in the footshocked mice followed by long-duration (30 min) reexposure to context memory by 92.9% ($P < 0.001$), whereas the effect of minocycline was minimal in the footshocked mice followed by non- or short-duration reexposure to context memory. Considering microglial expression profiles of TNF-α in concordance with retention and extinction of fear memory in general, TNF-α should have an important role in facilitating fear memory extinction; however, there may also be unknown factors other than TNF-α that make a difference in freezing time between the non- or short-duration reexposure groups and the long-duration reexposure group. Long-duration exposure may reduce some unknown factors along with TNF-α to hinder extinction processes. Different biochemical mechanisms have been reported to contribute to consolidation and extinction (Suzuki et al., 2004). For example, blockade of microglial cannabinoid receptor 1 (CB1) signaling does not affect fear memory consolidation but impairs fear memory extinction (Marsicano et al., 2002). Interestingly, administration of cannabinoids to microglial cells that express CB1 inhibits the production of LPS-induced proinflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α (Puffenbarger et al., 2000). Thus, microglial CB1-signaling can be a candidate alternative mechanism to modulate fear memory extinction in cooperation with TNF-α. Application of -omics technologies to grasp comprehensive molecular profiling of microglia may shed a light on the remaining unelucidated mechanisms linking microglial function and fear memory formation.

Inadvertent activation of microglia due to isolation procedures may be a critical issue and probably impossible to avoid in the study design, indicating a limitation of this study. All we can discuss in this paper is the difference in expression levels of the M1 and M2 microglial markers in the microglia among mice with or without a footshock stress with variable reexposure time, all of which were isolated under the exact same condition. Although there might be inadvertent activations of microglia due to isolation procedures, they may be offset in comparison among the groups.

Factors that aggravate PTSD may be relevant to the factors that disturb fear memory extinction (Quirk and Mueller, 2008). Our present data provide new indications of microglial TNF-α production as a possible aggravating factor of PTSD. Although no studies have evaluated TNF-α levels in the CNS of patients with PTSD, it was shown that serum levels of TNF-α were significantly higher in patients with PTSD compared with controls (Guo et al., 2012) and LPS-induced TNF-α production by whole blood cells was significantly higher in female patients with PTSD compared with healthy controls (Gill et al., 2008). Furthermore, the severity of PTSD symptoms was associated with the levels of plasma TNF-α (von Kanel et al., 2007). Contrary to these previous clinical findings, our results showed no significant differences in the levels of serum TNF-α among mice with or without retained fear memories, which suggests that peripheral TNF-α may not reflect merely retained fear condition but also other components of the pathophysiology of PTSD.

Finally, our discovery that the TNF-α inhibitor minocycline enhances fear memory extinction, indicates that drugs that inhibit TNF-α production may be beneficial for the treating patients with PTSD.

Conflict of interest statement

All authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2016.08.011.

References

Acharjee, S., Nayani, N., Tsutsui, M., Hill, M.N., Ousman, S.S., Pittman, Q.J., 2013. Altered cognitive-emotional behavior in early experimental autoimmune encephalitis–cytokine and hormonal correlates. Brain Behav. Immun. 33, 164–172.

Agis-Balboa, R.C., Arcos-Diaz, D., Wittnam, J., Govindarajan, N., Blom, K., Burkhardt, S., Haladyniak, U., Agbemenyah, H.Y., Zovoilis, A., Salinas-Riester, G., Opitz, L., Sananbenesi, F., Fischer, A., 2011. A hippocampal insulin-growth factor 2 pathway regulates the extinction of fear memories. EMBO J. 30, 4071–4083.
Alberini, C.M., 2005. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? Trends Neurosci. 28, 51–56.
Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S., Malenka, R.C., 2002. Control of synaptic strength by glial TNFalpha. Science 295, 2282–2285.
Blandino Jr., P., Barnum, C.J., Deak, T., 2006. The involvement of norepinephrine and microglia in hypothalamic and splenic IL-1beta responses to stress. J. Neuroimmunol. 173, 87–95.
Blandino Jr., P., Barnum, C.J., Solomon, L.G., Larish, Y., Lankow, B.S., Deak, T., 2009. Gene expression changes in the hypothalamus provide evidence for regionally-selective changes in IL-1 and microglial markers after acute stress. Brain Behav. Immun. 23, 958–968.
Boche, D., Perry, V.H., Nicoll, J.A., 2013. Review: activation patterns of microglia and their identification in the human brain. Neuropathol. Appl. Neurobiol. 39, 3–18.
Burton, M.D., Johnson, R.W., 2012. Interleukin-6 trans-signaling in the senescent mouse brain is involved in infection-related deficits in contextual fear conditioning. Brain Behav. Immun. 26, 732–738.
Durafourt, B.A., Moore, C.S., Zammit, D.A., Johnson, T.A., Zaguia, F., Guiot, M.C., Bar-Or, A., Antel, J.P., 2012. Comparison of polarization properties of human adult microglia and blood-derived macrophages. Glia 60, 717–727.
Frank, M.G., Thompson, B.M., Watkins, L.R., Maier, S.F., 2012. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. Brain Behav. Immun. 26, 337–345.
Fumagalli, M., Lecca, D., Abbracchio, M.P., 2011. Role of purinergic signalling in neuro-immune cells and adult neural progenitors. Front. Biosci. (Landmark Ed.) 16, 2326–2341.
Gill, J., Vythilingam, M., Page, G.G., 2008. Low cortisol, high DHEA, and high levels of stimulated TNF-alpha, and IL-6 in women with PTSD. J. Trauma. Stress 21, 530–539.
Goswami, S., Rodriguez-Sierra, O., Cascardi, M., Pare, D., 2013. Animal models of post-traumatic stress disorder: face validity. Front. Neurosci. 7, 89.
Grau, J.W., Huie, J.R., Lee, K.H., Hoy, K.C., Huang, Y.J., Turtle, J.D., Strain, M.M., Baumbauer, K.M., Miranda, R.M., Hook, M.A., Ferguson, A.R., Garraway, S.M., 2014. Metaplasticity and behavior: how training and inflammation affect plastic potential within the spinal cord and recovery after injury. Front. Neural Circuits 8, 100.
Grinberg, Y.Y., Dibbern, M.E., Levasseur, V.A., Kraig, R.P., 2013. Insulin-like growth factor-1 abrogates microglial oxidative stress and TNF-alpha responses to spreading depression. J. Neurochem. 126, 662–672.
Guo, M., Liu, T., Guo, J.C., Jiang, X.L., Chen, F., Gao, Y.S., 2012. Study on serum cytokine levels in posttraumatic stress disorder patients. Asian Pac. J. Trop. Med. 5, 323–325.
Hao, Y., Jing, H., Bi, Q., Zhang, J., Qin, L., Yang, P., 2014. Intra-amygdala microinfusion of IL-6 impairs the auditory fear conditioning of rats via JAK/STAT activation. Behav. Brain Res. 275, 88–95.
Hu, X., Leak, R.K., Shi, Y., Suenaga, J., Gao, Y., Zheng, P., Chen, J., 2015. Microglial and macrophage polarization-new prospects for brain repair. Nat. Rev. Neurol. 11, 56–64.
Jing, H., Hao, Y., Bi, Q., Zhang, J., Yang, P., 2014. Intra-amygdala microinjection of TNF-alpha impairs the auditory fear conditioning of rats via glutamate toxicity. Neurosci. Res.
Jostock, T., Mullberg, J., Ozbek, S., Atreya, R., Blinn, G., Voltz, N., Fischer, M., Neurath, M.F., Rose-John, S., 2001. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. Eur. J. Biochem. 268, 160–167.
Kobayashi, K., Imagama, S., Ohgomori, T., Hirano, K., Uchimura, K., Sakamoto, K., Hirakawa, A., Takeuchi, H., Suzumura, A., Ishiguro, N., Kadomatsu, K., 2013. Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis. 4, e525.
Lech, M., Susanti, H.E., Rommele, C., Grobmayr, R., Gunthner, R., Anders, H.J., 2012. Quantitative expression of C-type lectin receptors in humans and mice. Int. J. Mol. Sci. 13, 10113–10131.
Lee, S.M., Yune, T.Y., Kim, S.J., Kim, Y.C., Oh, Y.J., Markelonis, G.J., Oh, T.H., 2004. Minocycline inhibits apoptotic cell death via attenuation of TNF-alpha expression following iNOS/NO induction by lipopolysaccharide in neuron/glia co-cultures. J. Neurochem. 91, 568–578.
Lodge, P.A., Sriram, S., 1996. Regulation of microglial activation by TGF-beta, IL-10, and CSF-1. J. Leukoc. Biol. 60, 502–508.
Mamiya, N., Fukushima, H., Suzuki, A., Matsuyama, Z., Homma, S., Frankland, P.W., Kida, S., 2009. Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory. J. Neurosci. 29, 402–413.
Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M., 2004. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 25, 677–686.
Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.
McCoy, M.K., Tansey, M.G., 2008. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J. Neuroinflamm. 5, 45.
Miron, V.E., Boyd, A., Zhao, J.W., Yuen, T.J., Ruckh, J.M., Shadrach, J.L., van Wijngaarden, P., Wagers, A.J., Williams, A., Franklin, R.J., French-Constant, C., 2013. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat. Neurosci. 16, 1211–1218.
Nakazato, F., Tada, T., Sekiguchi, Y., Murakami, K., Yanagisawa, S., Tanaka, Y., Hongo, K., 2002. Disturbed spatial learning of rats after intraventricular administration of transforming growth factor-beta 1. Neurol. Med. Chir. (Tokyo) 42, 151–156, discussion 157.
O'Connor, K.A., Johnson, J.D., Hansen, M.K., Wieseler Frank, J.L., Maksimova, E., Watkins, L.R., Maier, S.F., 2003. Peripheral and central proinflammatory cytokine response to a severe acute stressor. Brain Res. 991, 123–132.
Parkhurst, C.N., Yang, G., Ninan, I., Savas, J.N., Yates 3rd, J.R., Lafaille, J.J., Hempstead, B.L., Littman, D.R., Gan, W.B., 2013. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155, 1596–1609.
Puffenbarger, R.A., Boothe, A.C., Cabral, G.A., 2000. Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. Glia 29, 58–69.
Pugh, C.R., Nguyen, K.T., Gonyea, J.L., Fleshner, M., Wakins, L.R., Maier, S.F., Rudy, J. W., 1999. Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. Behav. Brain Res. 106, 109–118.
Quirk, G.J., Mueller, D., 2008. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33, 56–72.
Raghavendra, V., Tanga, F., DeLeo, J.A., 2003. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J. Pharmacol. Exp. Ther. 306, 624–630.
Rappocciolo, G., Jenkins, F.J., Hensler, H.R., Piazza, P., Jais, M., Borowski, L., Watkins, S.C., Rinaldo Jr., C.R., 2006. DC-SIGN is a receptor for human herpesvirus 8 on dendritic cells and macrophages. J. Immunol. 176, 1741–1749.
Rescorla, R.A., 2004. Spontaneous recovery. Learn Mem. 11, 501–509.
Sanchez Mejia, R.O., Ona, V.O., Li, M., Friedlander, R.M., 2001. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. Neurosurgery 48, 1393–1399, discussion 1399–1401.
Shepherd, V.L., Abdolrasulnia, R., Garrett, M., Cowan, H.B., 1990. Down-regulation of mannose receptor activity in macrophages after treatment with lipopolysaccharide and phorbol esters. J. Immunol. 145, 1530–1536.
Sica, A., Mantovani, A., 2012. Macrophage plasticity and polarization: in vivo veritas. J. Clin. Invest. 122, 787–795.
Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silva, A.J., Kida, S., 2004. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J. Neurosci. 24, 4787–4795.
Vazquez-Mendoza, A., Carrero, J.C., Rodriguez-Sosa, M., 2013. Parasitic infections: a role for C-type lectins receptors. Biomed. Res. Int. 2013, 456352.
von Kanel, R., Hepp, U., Kraemer, B., Traber, R., Keel, M., Mica, L., Schnyder, U., 2007. Evidence for low-grade systemic proinflammatory activity in patients with posttraumatic stress disorder. J. Psychiatr. Res. 41, 744–752.
Wang, A.L., Yu, A.C., Lau, L.T., Lee, C., Wu le, M., Zhu, X., Tso, M.O., 2005. Minocycline inhibits LPS-induced retinal microglia activation. Neurochem. Int. 47, 152–158.
Yehuda, R., 2002. Post-traumatic stress disorder. N. Engl. J. Med. 346, 108–114.
Yu, Z., Ono, C., Aiba, S., Kikuchi, Y., Sora, I., Matsuoka, H., Tomita, H., 2015. Therapeutic concentration of lithium stimulates complement C3 production in dendritic cells and microglia via GSK-3 inhibition. Glia 63, 257–270.