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Tumor necrosis factor (TNF) modulates synaptic plasticity in a concentration-dependent manner through intracellular calcium stores

Nicola Maggio [1,2,3,4] · Andreas Vlachos [5]

Abstract

The role of inflammatory signaling pathways in synaptic plasticity has long been identified. Yet, it remains unclear how inflammatory cytokines assert their pleiotropic effects on neural plasticity. Moreover, the neuronal targets through which inflammatory cytokines assert their effects on plasticity remain not well-understood. In an attempt to learn more about the plasticity-modulating effects of the pro-inflammatory cytokine tumor necrosis factor (TNF), we used two-pathway long-term potentiation (LTP) experiments at Schaffer collateral-CA1 synapses to test for concentration-dependent effects of TNF on synaptic plasticity. We report that high concentrations of TNF (1 μg/mL) impair the ability of mouse CA1 pyramidal neurons to express synaptic plasticity without affecting baseline synaptic transmission and/or previously established LTP. Interestingly, 100 ng/mL of TNF has no apparent effect on LTP, while low concentrations (1 ng/mL) promote the ability of neurons to express LTP. These dose-dependent metaplastic effects of TNF are modulated by intracellular calcium stores: Pharmacological activation of intracellular calcium stores with ryanodine (10 μM) reverses the negative effects of $TNF_{[high]}$, and the plasticity-promoting effects of $TNF_{[low]}$ are blocked when intracellular calcium stores are depleted with thapsigargin (1 μM). Consistent with this result, TNF does not promote plasticity in synaptopodin-deficient preparations, which show deficits in neuronal calcium store-mediated synaptic plasticity. Thus, we propose that TNF mediates its pleiotropic effects on synaptic plasticity in a concentration-dependent manner through signaling pathways that are modulated by intracellular calcium stores and require the presence of synaptopodin. These results demonstrate that TNF can act as mediator of metaplasticity, which is of considerable relevance in the context of brain diseases associated with increased TNF levels and alterations in synaptic plasticity.

Key messages
- TNF modulates the ability of neurons to express synaptic plasticity.
- High concentrations of TNF impair synaptic plasticity.
- Low concentrations of TNF improve synaptic plasticity.
- TNF does not affect previously established long-term potentiation.
- Plasticity effects of TNF are modulated by intracellular calcium stores.

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Introduction

Despite a wealth of information on the cellular and molecular mechanisms of neural plasticity, the relevance of synaptic plasticity under pathological conditions remains a matter of debate [8, 10, 21, 36, 39, 42, 50]. A better understanding of molecules and mechanisms that modulate the ability of neurons to express synaptic plasticity in pathological brain states is urgently needed. This knowledge could be used to restore physiological brain function and/or promote recovery after brain injury in clinically relevant settings [23, 39]. Considering that neurological and psychiatric diseases are often accompanied by inflammatory responses [52, 61], we here sought to test for the role of the pro-inflammatory cytokine tumor necrosis factor (TNF) in modulating the ability of neurons to express synaptic plasticity.

TNF has been previously linked to synaptic transmission and plasticity (for details see, [3, 5, 12, 20, 47, 49, 53, 63, 65]). However, conflicting results have been reported, and as a result, it remains unclear whether TNF promotes and/or occludes synaptic plasticity. Also, a role for TNF in both long-term potentiation (LTP) and homeostatic synaptic plasticity has been suggested [5, 26, 30, 35, 59, 60, 71], while the neuronal targets through which TNF mediates its effects on plasticity remain not well-understood. We have recently shown that systemic inflammation is accompanied by a strong increase in brain TNF expression (~ 40-fold increase in *TNF* mRNA; [62]) and alterations in LTP of Schaffer collateral-CA1 synapses [40, 62]. Conversely, in a model of traumatic brain injury, i.e., in vitro entorhinal cortex lesion [69], a moderate increase in TNF was linked to increased synaptic strength in denervated neurons [6, 7]. Interestingly, in both pathological settings, changes in the expression of the neuronal protein synaptopodin were observed, which reflected TNF-mediated changes in plasticity [62, 70]. Synaptopodin is an actin-modulating protein [14, 43] and essential component of stacked smooth endoplasmic reticulum (sER) structures, such as the spine apparatus organelle [15, 16, 19, 57], and cisternal organelles [4, 29, 34, 45]. Similar to TNF, synaptopodin has been linked to the ability of neurons to express LTP [15, 25, 67] and homeostatic synaptic plasticity [70]. In this context, evidence has been provided that synaptopodin acts through the regulation of neuronal sER calcium stores [24, 27, 28, 66, 68].

Based on these observations, it appeared well-warranted to test (1) whether TNF asserts differential concentration-dependent effects on synaptic plasticity, (2) whether intracellular calcium stores are involved in mediating the effects of TNF on synaptic plasticity, and (3) if the presence of synaptopodin is required for TNF-mediated modulation of synaptic plasticity to occur.

Materials and methods

Ethics statement

All experiments were approved by the Institutional Animal Care and Use Committee of The Chaim Sheba Medical Center (Tel HaShomer, Israel), which adheres to the Israeli law on the use of laboratory animals and NIH rules. Experimental procedures were performed also according to the German animal welfare legislation as approved by the animal welfare officer at Albert-Ludwigs-University Freiburg, Faculty of Medicine. Human material was not employed in this study.

Animals

Four- to five-month-old male C57BL/6 mice and synaptopodin-deficient mice (Synpo$^{-/-}$; available at Jackson Laboratory; [15]) and their age- and time-matched wild-type littermates were used. Mice were maintained in a 12-h light/dark cycle with food and water available ad libitum. Every effort was made to minimize distress and pain of animals.

Pharmacology

The following drugs were used at the indicated final concentrations: thapsigargin (Alomone Labs, Jerusalem, Israel), ryanodine (Tocris, UK), recombinant mouse TNF-alpha (Genescript, USA).

Electrophysiology in acute brain slices

Extracellular recordings in acute slices prepared from dorsal hippocampus were performed as previously described (e.g., [38]). Following anesthesia with ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively), animals were rapidly decapitated, the brains removed, and 400-μm slices prepared using a vibroslicer. Slices were incubated for 1.5 h in a humidified, carbogenated gas atmosphere (5% CO_2 and 95% O_2) at 33 ± 1 °C and perfused with artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 2 KCl, 26 $NaHCO_3$, 1.24 KH_2PO_4, 2.5 $CaCl_2$, 2 $MgSO_4$, and 10 glucose (pH 7.4) in a standard interface chamber. Recordings were made with a glass pipette containing 0.75 M NaCl (4 MΩ) placed in stratum radiatum of CA1. Stimulation of Schaffer collaterals was evoked using a pulse stimulator and delivered through bipolar nichrome electrodes. Input-output curves were run on each slice prior to beginning of each experiment. Before applying the tetanic stimulation, baseline values were recorded at a frequency of 0.033 Hz. LTP was induced by high-frequency stimulation (HFS) consisting of 100 pulses, delivered at a frequency of 100 Hz (1 s). Short HFS (sHFS) consisted of 25 pulses, delivered at 100 Hz.

Quantification and statistics

Data were analyzed off-line using Spike 2 software (Cambridge Electronic Design). Excitatory postsynaptic potential (EPSP) slope changes after tetanic stimulation were calculated with respect to baseline. Statistical comparisons between the two groups were performed using a t test (unpaired, two-tailed). p values smaller 0.05 were considered a significant difference between means.

Results

Concentration-dependent effects of TNF on synaptic plasticity

The effects of TNF were studied in a two-pathway experimental setting, in which two stimulating electrodes are used to independently probe and potentiate Schaffer collateral-CA1 synapses, while recording field EPSPs in CA1 stratum radiatum (see schematic in Fig. 1(A)). After acquiring a baseline of evoked EPSPs at both pathways, LTP is induced by delivering an electric stimulus consisting of 100 pulses at twice the test intensity (at a frequency of 100 Hz) to one of the two pathways (Fig. 1(B)). This leads to a robust increase in EPSP slope, leaving synaptic transmission probed at the other pathway unaffected. Eventually, the other pathway is probed/potentiated using the exact same stimulus (see arrows in Fig. 1(B)), leading to comparable levels of LTP at the second pathway without affecting pre-established LTP (Fig. 1(B)). Using this experimental setting, TNF was washed in at distinct concentrations (1 μg/mL, 100 ng/mL, 1 ng/mL, 0.1 ng/mL) after the potentiation of the first pathway. Hence, we were able to systematically test for the effects of TNF on the ability of

neurons to express LTP, and for its impact on LTP that is established prior to the exposure to TNF (Fig. 2(A–D)).

Treating hippocampal slices with a high concentration of TNF resulted in reduced LTP (Fig. 2(A)). Specifically, a tetanic stimulation applied after exposure to 1 μg/mL of TNF (referred to as TNF$_{[high]}$) resulted in a level of potentiation of 1.32 ± 0.07 compared to the 1.76 ± 0.06 at the first pathway ($p < 0.01$; $n = 12$ slices from 4 animals). Notably, TNF$_{[high]}$ did not affect LTP that was established at the first pathway prior to the treatment in the same set of slices (Fig. 2(A)). Hence, toxic effects of TNF$_{[high]}$ do not trivially explain our results. Rather, 1 μg/mL TNF affects the ability of neurons to express new LTP, while leaving pre-established LTP unaltered.

In an independent set of experiments, we tested for the effects of 100 ng/mL TNF and found no significant effect on the ability of neurons to express LTP and/or previously established LTP (1.83 ± 0.07 vs. 1.72 ± 0.07, $p = 0.08$, $n = 12$ slices from 4 animals; Fig. 2(B); c.f. Fig. 1(B)). These results suggest that a high concentration of TNF is required to induce alterations in the ability of neurons to express LTP.

Strikingly, the opposite result, i.e., an enhancement of LTP, was observed when TNF was washed in at a concentration of 1 ng/mL (Fig. 2(C)). In this setting, a tetanic stimulation to the second pathway following the application of 1 ng/mL TNF (referred to as TNF$_{[low]}$) resulted in a potentiation of 2.26 ± 0.08 vs 1.72 ± 0.07 of the control pathway (Fig. 1(C); $p < 0.001$; $n = 12$ slices from 4 animals). Similar to what we observed with TNF$_{[high]}$, no effect on established LTP at the other pathway was observed with TNF$_{[low]}$. The plasticity-enhancing effect of TNF was not statistically different between the two pathways at a concentration of 0.1 ng/mL (1.82 ± 0.07 vs 2.06 ± 0.08, $p = 0.06$, $n = 12$ slices from 4 animals; Fig. 2(D)). Taken together, we conclude that TNF asserts differential effects on the ability of neurons to express
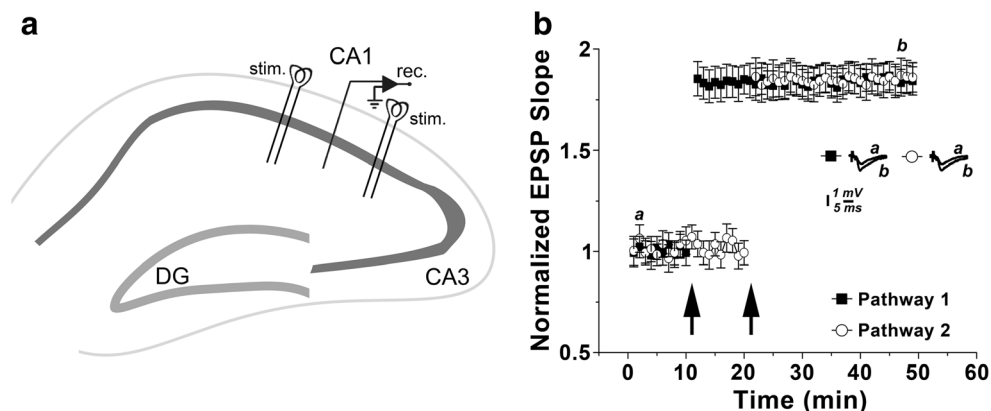

Fig. 1 Two-pathway long-term potentiation (LTP) experiments at Schaffer collateral-CA1 synapses. (**a**) Schematic illustration of the experimental setting. Two stimulating electrodes (stim.) are used to independently probe and potentiate Schaffer collateral-CA1 synapses of acute mouse hippocampal slices, while recording (rec.) field excitatory postsynaptic potentials (EPSP) in CA1 stratum radiatum (dentate gyrus, DG). (**b**) LTP is induced by delivering an electric stimulus consisting of 100 pulses at twice the test intensity at a frequency of 100 Hz (see arrows). Averaged EPSPs are plotted vs. time. Note, representative traces at indicated times (*a*, *b*). Details provided in the main text ($n = 12$ slices; from 4 animals each)

Fig. 2 Tumor necrosis factor (TNF) affects hippocampal long-term potentiation (LTP) in a concentration-dependent manner. (**a**) TNF applied at a concentration of 1 µg/mL reduces the ability of neurons to express LTP, while having no apparent effect on LTP established earlier at the other pathway. (**b**) At a concentration of 100 ng/mL, TNF does not affect LTP. (**c**) TNF at 1 ng/mL enhances LTP, while (**d**) LTP is not affected when slices are exposed to TNF concentrations of 0.1 ng/mL. Averaged EPSPs are plotted vs. time. Representative traces at indicated times (*a, b*) are shown on top of each section (*n* = 12 slices; from 4 animals each). Arrows indicate the time of tetanus delivery (100 pulses at twice the test intensity at 100 Hz). (**e**) Application of a short HFS (sHFS; 25 pulses at twice the test intensity at 100 Hz) in the presence of $TNF_{[high]}$ (1 µg/mL) neither enhances nor depresses the effects of sHFS on LTP. (**f**) The presence of 1 ng/mL $TNF_{[low]}$ enhances the ability of sHFS to induce LTP. Averaged EPSPs are plotted vs. time. Representative traces at indicated times (*a, b*) are shown on top of each section (*n* = 12 slices; from 4 animals each). Arrows indicate the time of (s)HFS delivery
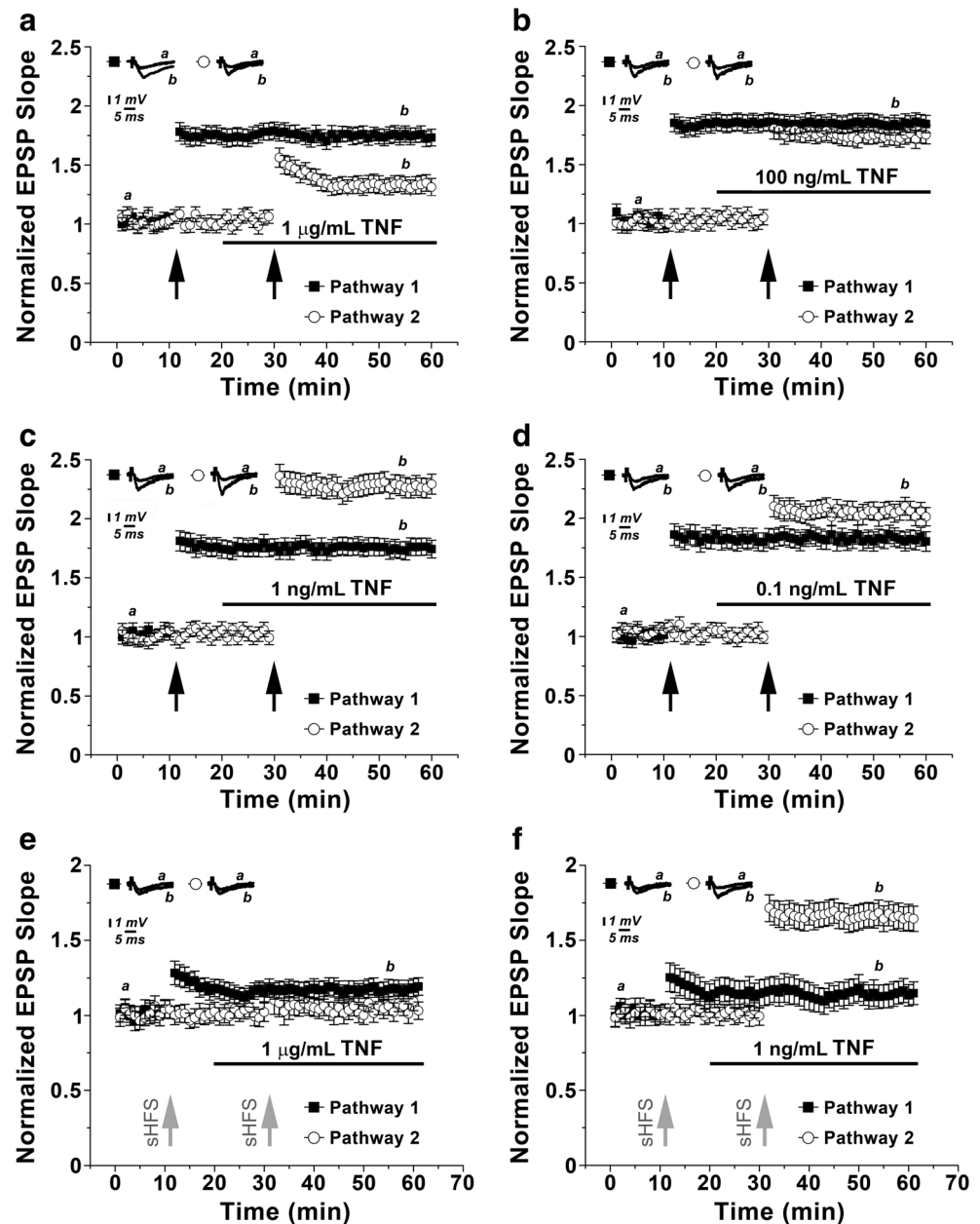
LTP, without affecting baseline synaptic transmission and/or previously established LTP (at all concentrations tested here): $TNF_{[low]}$ promotes new LTP, while $TNF_{[high]}$ impedes LTP induction.

Low-concentration TNF reduces the threshold for plasticity induction

In another set of experiments, short high-frequency stimulation was delivered (sHFS; 25 pulses at twice the test intensity at a frequency of 100 Hz), which does not induce a strong and robust LTP response per se (Fig. 2(E, F)). In this setting, application of sHFS in presence of $TNF_{[high]}$ neither enhanced nor depressed the effects of sHFS (Fig. 2(E)). In contrast, the

presence of $TNF_{[low]}$ enhanced the ability of sHFS to induce LTP, and sHFS resulted in a full-blown LTP response reaching a level of 1.64 ± 0.08 vs. 1.12 ± 0.07 (Fig. 1(F); $p < 0.001$; $n = 12$ from 4 animals). Hence, low concentrations of TNF affect the ability of neurons to express plasticity by reducing the threshold for plasticity induction.

Intracellular Ca^{2+} stores mediate the plasticity-enhancing effects of low-concentration TNF

Previous work has indicated that TNF may act on neural tissue through intracellular calcium stores [11, 18, 48, 62]. In fact, it is well-accepted that intracellular calcium stores modulate the

ability of neurons to express plasticity (reviewed in [17, 39, 55]). Specifically, metaplastic changes in the threshold of plasticity induction have been reported to depend on intracellular calcium homeostasis [22, 32, 33, 41, 51].

Indeed, enhancing calcium-release from intracellular stores with 10 μM ryanodine improved the ability of neurons to express synaptic plasticity in our experimental setting (Fig. 3(A)), similar to what we observed with TNF$_{[low]}$ (c.f. Fig. 2(C)). In turn, depletion of intracellular calcium stores with 1 μM thapsigargin—unlike TNF$_{[high]}$ (c.f. Fig. 2(A))—resulted in a slow rundown of pre-established LTP, and no major effect on the ability to induce new LTP was observed (Fig. 3(B)). Thus, activation of calcium stores mimics the effects of TNF$_{[low]}$, while pharmacological calcium store depletion does not reflect TNF$_{[high]}$ effects on synaptic plasticity. We therefore hypothesized that intracellular calcium stores mediate the plasticity-enhancing effects of TNF$_{[low]}$, while other mechanisms may account for the effects of TNF$_{[high]}$.

In view of these results, we reasoned that the depletion of intracellular calcium stores with 1 μM thapsigargin should

occlude the plasticity-enhancing effects of TNF$_{[low]}$. Indeed, applying 1 ng/mL TNF in the presence of 1 μM thapsigargin attenuated the plasticity-enhancing effects of TNF$_{[low]}$, resulting in an LTP level of 1.74 ± 0.08 (Fig. 3(C)). Moreover, when 1 μM thapsigargin was washed in after the induction of TNF$_{[low]}$-enhanced LTP (Fig. 3(D)), a substantial rundown of LTP was observed, and the ability to induce TNF$_{[low]}$-enhanced LTP at the second pathway was blocked (Fig. 3(D)). These experiments also showed that priming LTP induction at the first pathway is not required for TNF$_{[low]}$-enhanced synaptic plasticity to occur (c.f. Fig. 2(F)). We conclude that functional intracellular calcium stores are involved in mediating the plasticity-enhancing effects of TNF$_{[low]}$.

Low-concentration TNF does not enhance plasticity in synaptopodin-deficient neurons

Pharmacology acts on intracellular calcium stores in all neural compartments (i.e., neurons, astrocytes, oligodendrocytes, and microglia). In order to test for the neuronal targets of
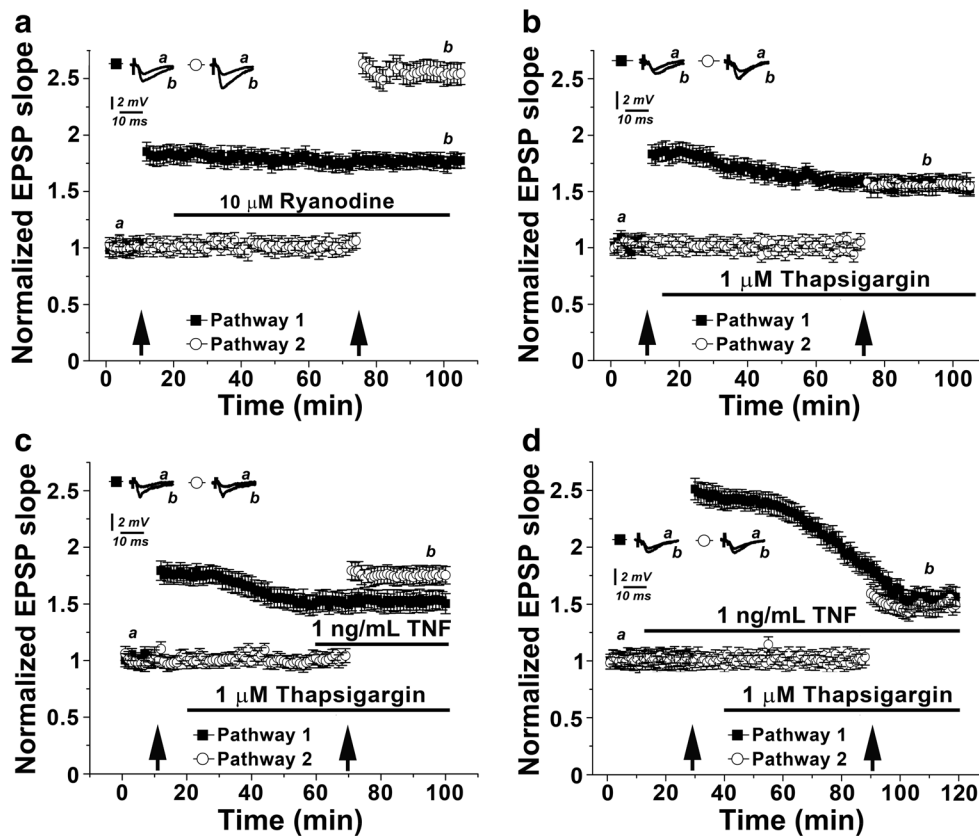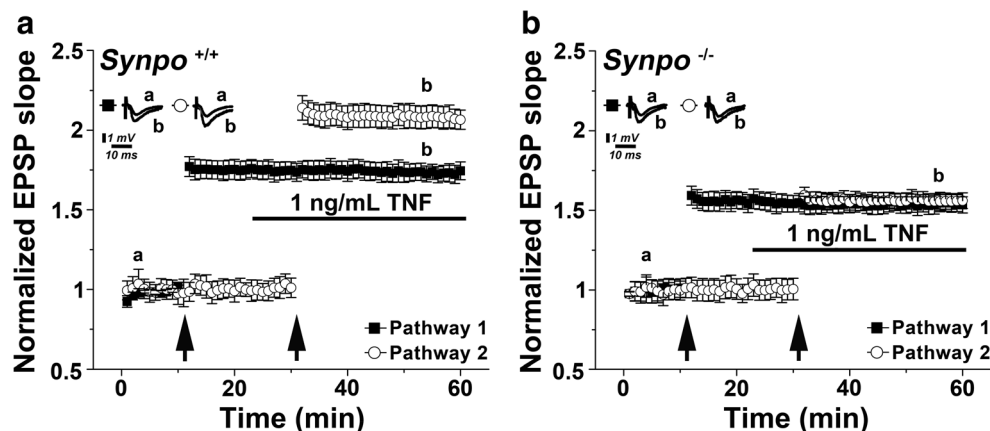

Fig. 3 Intracellular calcium stores mediate the plasticity-enhancing effects of low-centration Tumor necrosis factor (TNF). (a) Activation of calcium stores with 10 μM ryanodine enhances LTP, similar to what is observed in response to 1 ng/mL TNF, while (b) pharmacological depletions of intracellular calcium stores with 1 μM thapsigargin trigger a slowly decaying LTP. Unlike 1 μg/mL TNF, in the presence of 1 μM thapsigargin, the ability to induce LTP at the second pathway is not affected. (c) Application of 1 μM thapsigargin attenuates the plasticity-enhancing effects of 1 ng/mL TNF. (d) When TNF-enhanced LTP is induced at the first pathway, a considerable rundown of LTP is observed in the presence of 1 μM thapsigargin and the ability to express enhanced LTP is completely blocked at the second pathway in the presence of 1 μM thapsigargin and 1 ng/mL TNF. Averaged EPSPs are plotted vs. time. Representative traces at indicated times (a, b) are shown on top of each section ($n = 12$ slices; from 4 animals each). Arrows indicate the time of high frequency stimulation

Fig. 4 Low-concentration tumor necrosis factor (TNF) does not induce metaplasticity in synaptopodin-deficient neurons. (**a**) TNF (1 ng/mL) enhances LTP in age- and time-matched wild-type (Synpo$^{+/+}$), but not (**b**) in synaptopodin-deficient mice (Synpo$^{-/-}$). Averaged EPSPs are plotted vs. time. Representative traces at indicated times (a, b) are shown on top of each section (n = 6 slices; from 3 animals each). Arrows indicate the time of stimulation

TNF and based on our earlier work on the role of synaptopodin-associated neuronal intracellular calcium stores in synaptic plasticity (e.g., [31, 62]), we next assessed the plasticity-enhancing effects of TNF$_{[low]}$ on hippocampal slices prepared from synaptopodin-deficient mice and their age/time-matched wild-type littermates.

Once more, the plasticity-enhancing effect of 1 ng/mL TNF$_{[low]}$ was observed in Synpo$^{+/+}$ preparations (Fig. 4(A); c.f. Fig. 2(C)). The ability of Synpo$^{-/-}$ neurons to express LTP was reduced, consistent with earlier findings [15, 25], and TNF$_{[low]}$ was not able to further enhance LTP (Fig. 4(B)). Specifically, LTP levels upon treatment with 1 ng/mL TNF were 1.56 ± 0.05 in Synpo$^{-/-}$ vs. 2.07 ± 0.07 in Synpo$^{+/+}$ ($p < 0.001$; $n = 6$ slices from 3 animals each). Hence, TNF$_{[low]}$ requires the presence of synaptopodin to assert its plasticity-promoting effects.

Pharmacological activation of intracellular calcium stores counters the effects of TNF$_{[high]}$

Finally, we used wild-type hippocampal slices to test for the effects of thapsigargin (Fig. 5(A)) and ryanodine (Fig. 5(B))

on TNF$_{[high]}$-mediated alterations in synaptic plasticity. While the negative effects of TNF$_{[high]}$ are detectable in the presence of thapsigargin (Fig. 5(A); 1.33 ± 0.07 vs. 1.56 ± 0.07, $p < 0.001$; $n = 9$ slices from 3 animals), enhancing calcium release from intracellular stores counters the negative effects of TNF$_{[high]}$: Application of 10 μM ryanodine prior to 1 μg/mL TNF$_{[high]}$ resulted in enhanced LTP (Fig. 5; 2.25 ± 0.07 vs. 1.79 ± 0.07, $p < 0.001$; $n = 12$ from 4 animals), comparable to what we observed in our ryanodine-only experiments (c.f. Fig. 3(A)).

Discussion

The results of the present study reveal that the pro-inflammatory cytokine TNF can have opposite effects on synaptic plasticity, depending on the concentration. The plasticity-enhancing effect of 1 ng/mL TNF$_{[low]}$ is not observed when calcium stores are pharmacologically depleted. Likewise, in synaptopodin-deficient neurons, which do not form stacked sER morphologies (recently reviewed in [24]), TNF$_{[low]}$ does not promote plasticity. While the
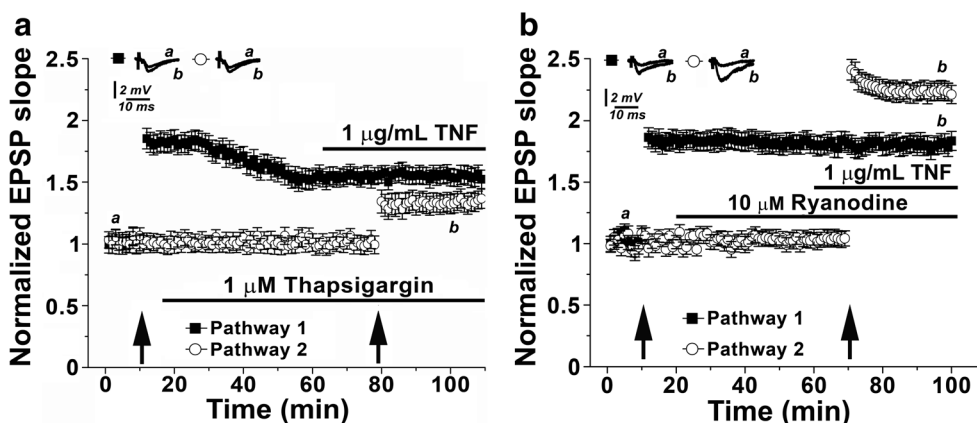

Fig. 5 Pharmacological activation of calcium stores counters the effects of high-concentration tumor necrosis factor (TNF). (**a**) In the presence of 1 μM thapsigargin and 1 μg/mL TNF a reduction in LTP is observed ($n = 9$ slices, from 3 animals each; c.f. Fig. 3(C)). (**b**) In the presence of 10 μM

ryanodine and 1 μg/mL TNF, an enhancement of LTP is observed ($n = 12$ slices, from 4 animals each). Averaged EPSPs are plotted vs. time. Note, representative traces at indicated times (a, b). Arrows indicate the time of tetanus delivery

mechanisms through which 1 μg/mL TNF[high] affects plasticity warrant further investigation, our experiments indicate that pharmacological activation of calcium stores counters the negative effects of TNF[high]. These results provide new important insight on the role of TNF in synaptic plasticity by identifying TNF as a mediator of *metaplasticity* [1, 23], which acts, at least in part, through intracellular calcium stores and synaptopodin.

A remarkable finding of our study is the observation that TNF treatment does not affect synaptic transmission and/or pre-established LTP. Rather, TNF modulates the ability of neurons to express plasticity in a concentration-dependent manner. This finding is consistent with previous work in the field of homeostatic synaptic plasticity, which has indicated a *permissive* role of TNF in synaptic plasticity: TNF modulates the ability of neurons to express homeostatic synaptic plasticity, rather than being instructive, i.e., inducing changes in synaptic strength per se [6, 58]. Yet, robust evidence exists that TNF can change synaptic strength (e.g., [5, 59]). For example, Beattie et al. [5] showed that low concentrations of TNF increase the surface expression of AMPA receptors at excitatory postsynapses in primary hippocampal neurons. It is interesting to speculate though that TNF signaling may have changed the plasticity threshold in these preparations, rather than triggering synaptic accumulation of glutamate receptors per se. Hence, network activity that is not sufficient to induce LTP under control conditions may have triggered the strengthening of glutamatergic neurotransmission in the presence of TNF. Indeed, this suggestion is supported by the results of our experiments, in which we used a mild stimulus to demonstrate that TNF[low] reduces the threshold for plasticity induction (c.f. Fig. 2(E, F)). Although more work is required to clarify the precise role of TNF in synaptic plasticity, the results of the present study provide the first experimental evidence that TNF is a component of a signaling pathway which affects the ability of neurons to express plasticity, e.g., by reducing the threshold of plasticity induction, without modifying baseline synaptic transmission and/or pre-established LTP. Hence, we are confident to conclude that besides its suggested roles in Hebbian and homeostatic plasticity, TNF also acts as a "metaplasticity mediator."

Apparently, the precise cellular and molecular mechanisms through which TNF asserts its metaplastic effects warrant further investigation. Two canonical receptors mediate the effects of TNF. TNFR1 predominantly binds soluble TNF and is constitutively expressed on most cells of the body (including neurons and astrocytes), while TNFR2 binds with high affinity to membrane-bound TNF and is mainly expressed on cells of the immune system [2, 37, 53]. The rather rapid response to TNF, i.e., within ~ 10 min, in our experimental setting, and the fact that we used soluble recombinant TNF argue for neuronal TNFR1. However, it is difficult to conceive how TNFR1- and/or TNFR2-mediated signaling pathways could mediate the

concentration-dependent effects of soluble TNF. Also, at 100 ng/mL, no apparent effect of TNF on synaptic plasticity was detected. Whether TNFR1 and TNFR2 have different affinities to soluble TNF and/or yet unknown non-canonical pathways contribute to the plasticity-modulating effects of TNF remains unknown at this point.

Regardless of these considerations, our experiments suggest a role of intracellular calcium stores in TNF-induced metaplasticity (c.f. [11, 18, 48]). Pharmacological release of calcium from intracellular stores prevented the negative effects of TNF[high] on synaptic plasticity, while the plasticity-promoting effects of TNF[low] were abolished when calcium stores were depleted. However, the results of the present study do not support a simple model in which TNF[low] activates intracellular calcium stores while TNF[high] acts by depleting intracellular stores. Even though the evidence which indicates that calcium stores mediate the plasticity-enhancing effects of TNF[low] seems robust, 1 μM thapsigargin does not mimic the effects of TNF[high]. Also, negative effects of TNF[high] on synaptic plasticity are detectable in the presence of thapsigargin. It is interesting to speculate in this context that TNF may act on a more refined level of sER morphology. Considering that spine ER is a highly dynamic structure which can enter and leave individual spines within minutes ([44, 64]; see also [68]), it is possible that TNF[high] may cause a retraction of spine ER, while dendritic ER tubules may enter spine compartments and changes their confirmation in response to TNF[low]. Such rapid regulation of sER structure could modify spine calcium signaling and homeostasis, which could explain the fast metaplastic adjustment induced by TNF.

Indeed, the work on synaptopodin supports the suggestion that TNF may act through specific changes in sER morphologies. Previous work disclosed that changes in synaptopodin cluster sizes reflect changes in the number of stacked spine ER cisternae ([70]). Moreover, a reduction in synaptopodin cluster sizes was observed in a lipopolysaccharide model of system inflammation, which is accompanied by a strong increase in brain TNF mRNA levels and alterations in the ability of neurons to express LTP [62]. Conversely, increased synaptopodin cluster sizes were reported in a model of in vitro denervation [70], which depends on local TNF release [6, 7]. Consistent with this suggestion, the results of the present study show that TNF[low] requires the presence of synaptopodin to induce its plasticity-enhancing effects. These observations motivate a rigorous study on the cellular and molecular mechanisms through which distinct concentrations of TNF modulate synaptopodin-associated calcium stores and dendritic spine plasticity.

It is interesting to note in this context that recent work has implicated intracellular calcium homeostasis and alterations in sER calcium stores to play a major role in pathological brain states [13, 39, 46, 54]. Indeed, a recent study reported that metaplastic activation of ryanodine receptors restores

plasticity in an animal model of Alzheimer's disease [33]. Considering the proposed role of TNF as a therapeutic target in Alzheimer's disease (for recent reviews, see [9, 56]), we are confident that a better understanding on the effects of TNF and other inflammatory cytokines on glial and neuronal sER calcium stores may provide an important biological basis for the development of new therapeutic strategies based on metaplasticity induction and neuromodulation.

Compliance with ethical standards

All experiments were approved by the Institutional Animal Care and Use Committee of The Chaim Sheba Medical Center (Tel HaShomer, Israel), which adheres to the Israeli law on the use of laboratory animals and NIH rules. Experimental procedures were performed also according to the German animal welfare legislation as approved by the animal welfare officer at Albert-Ludwigs-University Freiburg, Faculty of Medicine. Human material was not employed in this study.

Conflict of interest   The authors declare that they have no conflict of interest.

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