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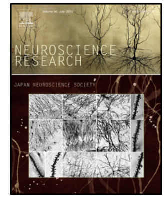

TNFα is required for the production of T-type Ca^{2+} channel-dependent long-term potentiation in visual cortex

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ABSTRACT

Monocular deprivation produces depression and potentiation of visual responses evoked in visual cortical neurons by stimulation of deprived and nondeprived eyes, respectively, during the critical period of ocular dominance plasticity. Our previous studies suggested that T-type Ca^{2+} channel-dependent long-term potentiation (LTP), induced by 2 Hz stimulation, mediates the potentiation of visual responses. However, it was proposed that the experience-dependent response potentiation is mediated by tumor necrosis factor-α (TNFα)-dependent homeostatic synaptic scaling but not by Hebbian synaptic plasticity, because the potentiation was absent in TNFα knockout (TNFα-KO) mice. In this study, we investigated whether TNFα is required for LTP induced by 2 Hz stimulation using visual cortical slices prepared from critical period mice and rats. The production of LTP was prevented by pharmacological blockade of TNFα in rats and mice. LTP production was also prevented by an inhibitor of TNFα-converting enzyme that converts membrane-bound TNFα to soluble TNFα. In TNFα-KO mice, LTP did not occur and was rescued by exogenous soluble TNFα. Soluble TNFα was required for LTP production only during a restricted time window soon after 2 Hz stimulation. These results strengthen the view that T-type Ca^{2+} channel-dependent LTP contributes to the potentiation of nondeprived eye responses following monocular deprivation.

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1. Introduction

Visual cortical neurons respond selectively to the features of visual stimulation (Hubel, 1982). This response selectivity is refined by visual experience during postnatal development (Wiesel, 1982). It has been hypothesized that activity-dependent long-term modification of synaptic transmission contributes to this experience-dependent development of cortical functions (Bear et al., 1987; Katz and Shatz, 1996; Singer, 1995; Zhang and Poo, 2001). Ocular dominance plasticity has been used to study the mechanisms of this developmental process. Visual cortical neurons undergo depression and potentiation of their visual responses to stimulation of the deprived and non-deprived eyes, respectively, after monocular deprivation during the critical period (Frenkel and Bear, 2004). Recent studies have suggested that some forms of long-term potentiation (LTP) and depression (LTD) are involved in ocular dominance plasticity. One line of evidence suggests that N-methyl-D-aspartate (NMDA) receptor-dependent LTD mediates the depression of deprived eye responses following monocular deprivation (Heynen et al., 2003; Liu et al., 2008; Yoon et al., 2009). On the other hand, we suggested the possibility that T-type Ca^{2+} channel-dependent LTP mediates the potentiation of nondeprived eye responses following monocular deprivation, because an infusion of T-type Ca^{2+} channel blocker into rat visual cortex during monocular deprivation prevented the potentiation of visual responses (Yoshimura et al., 2008). This form of LTP is induced by 2 Hz stimulation for 15 min and requires the activation of postsynaptic Ni^{2+}-sensitive T-type Ca^{2+} channels for induction (Komatsu and Iwakiri, 1992; Yoshimura et al., 2008). In layer 2/3 neurons, this LTP occurs only during the critical period in normally reared cats and rats (Komatsu et al., 1988; Ohmura et al., 2003), while it can occur even in adulthood when rats are kept in darkness from birth (Yoshimura et al., 2008), just like the ocular dominance shift produced by monocular deprivation (Cynader and Mitchell, 1980; Mower et al., 1981), further supporting its involvement in the experience-dependent modification of visual responsiveness during development.

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; NMDA, N-methyl-D-aspartate; PD, postnatal day; ACSF, artificial cerebrospinal fluid; DL-APV, DL-2-amino-5-phosphonovaleric acid; TNFα, tumor necrosis factor-α; EPSC, excitatory postsynaptic current.

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It was initially considered that the effect of monocular deprivation on the ocular dominance preference of visual cortical neurons might be mediated by Hebbian synaptic plasticity, which occurs specifically at synapses, where pre and postsynaptic neurons show positively or negatively correlated activity (Stent, 1973). However, it was later proposed that the modification of synaptic strength called synaptic scaling, which occurs commonly at all synapses onto the same neurons to keep their average firing rate in an adequate level, could also contribute to this process (Kaneko et al., 2008; Turrigiano, 2008). After firing activity is lowered for a long time period, the quantal amplitude of excitatory postsynaptic currents (EPSCs) is increased (Turrigiano et al., 1998). This enhancement is mediated by tumor necrosis factor-α (TNFα), an inflammatory cytokine, and does not take place in TNFα knockout (TNFα-KO) mice (Stellwagen and Malenka, 2006). It was reported that the potentiation of nondeprived eye responses in visual cortical neurons after monocular deprivation did not occur in these mice (Kaneko et al., 2008). Based on these findings, it was suggested that the increase in quantal EPSC amplitude produced by synaptic scaling mediates the potentiation of nondeprived eye responses.

In the present study, we tested the possibility that TNFα is required for the production of T-type Ca^{2+} channel-dependent LTP. It would be concluded that this form of LTP is not involved in the effect of monocular deprivation, if it is not impaired in TNFα-KO mice. However, we found that TNFα was indispensable for the production of LTP, suggesting that either this LTP or synaptic scaling, or both contribute to the potentiation of nondeprived eye responses after monocular deprivation.

2. Materials and methods

All of the experiments were carried out under a protocol approved by the Experimental Animal Care Committee, Research Institute of Environmental Medicine, Nagoya University.

2.1. Experimental animals and slice preparations

We used pigmented (Long–Evans) rats, wild-type mice (C57BL6/J) and TNFα-KO mice (Jackson Laboratories, $Tnf^{-/-}$) at postnatal day (PD) 20–30 during the critical period of ocular dominance plasticity (Fagiolini et al., 1994; Gordon and Stryker, 1996). As described previously (Komatsu, 1994; Funahashi et al., 2013), coronal slices of primary visual cortex (300 μm thick) were prepared from rats or mice under deep anesthesia with isoflurane, and they were recovered and maintained in an interface-type chamber perfused with an artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose at 33 °C. The recording experiments were conducted in the same type of chamber as used for recovery and maintenance, which was perfused with the ACSF at 33 °C.

2.2. Analysis of LTP

Two pairs of bipolar stimulating tungsten electrodes (diameter, 100 μm; interpolar distance, 200 μm) were placed in layer 4 of the primary visual cortex, separated from each other by about 0.4 mm (Fig. 1A). A surgical cut was imposed in layers 4–5 to ensure that separate groups of presynaptic fibers were activated. One electrode was used to test the effect of conditioning stimulation and the other served as a control. Test stimulation was applied alternately to the electrodes at intervals of 5 s. As a conditioning stimulation to induce LTP, 2 Hz stimulation was applied for 15 min in most of the experiments. In some of the experiments, 2 Hz stimulation was applied for 5 min. The intensity of test stimulation was adjusted to a value eliciting about half the maximal responses. The intensity for conditioning stimulation was twice that of test stimulation. Responses evoked by electrical stimulation of presynaptic fibers were recorded extracellularly from layer 2/3 using glass microelectrodes filled with ACSF (Fig. 1A). The amplitude of the responses determined by the difference between the initial positive peak and the following negative peak was used for the assessment of LTP, because LTP of extracellular responses assessed by their amplitude was similar in time course and magnitude to LTP of intracellular excitatory postsynaptic potentials assessed by their initial slope (Yoshimura et al., 2008).

2.3. Data analysis and chemical compounds

Data was expressed as mean ± SEM and statistical analyses were performed using one-way ANOVA test, followed by Tukey's test. P values of less than 0.05 were considered significant. The drugs employed were obtained from the following sources: DL-2-amino-5-phosphonovaleric acid (DL-APV) from Tocris (Bristol, UK); monoclonal anti-TNFα antibody (clone 45418.111) from Sigma (St. Louis, MO, USA); recombinant mouse TNFα (catalog # 201-13461) and recombinant rat TNFα (catalog # 203-14261) from Wako (Osaka, Japan); anti-TNFα antibody (ab6671), control mouse IgG1 (ab18447) and control rabbit IgG (ab172569) from Abcam (Cambridge, UK); ML 218 from Almone Labs (Jerusalem, Israel); TNFα inhibitor (catalog # 654256) and TAPI-0 from Calbiochem (La Jolla, CA, USA). Bath application of antibodies, control IgG, control IgG1, TAPI-0 and a TNFα inhibitor was started at least 30 min before application of 2 Hz stimulation.

3. Results

3.1. A function neutralizing anti-TNFα antibody and a TNFα inhibitor both prevent the production of LTP in rat visual cortex

Two Hz stimulation for 15 min induces T-type Ca^{2+} channel-dependent LTP of excitatory synaptic transmission in layer 2/3 pyramidal neurons in the visual cortex of rats at PD20–30 during the critical period (Ohmura et al., 2003; Yoshimura et al., 2008). We first conducted experiments using rats, to test whether TNFα is required for the production of this form of LTP. To this end, we recorded extracellular field potentials (FPs) evoked in layer 2/3 by stimulation of presynaptic fibers using bipolar stimulation electrodes placed in layer 4 (Fig. 1A). Two Hz stimulation consistently produced LTP of FPs specifically to the activated pathway in normal ACSF (Fig. 1B), reflecting LTP of excitatory synaptic transmission in layer 2/3 pyramidal neurons, as shown in our previous studies (Ohmura et al., 2003; Yoshimura et al., 2008). When slices were perfused with ACSF containing a function neutralizing anti-TNFα antibody (20 μg/ml), 2 Hz stimulation almost always failed to induce LTP (Fig. 1C), while LTP occurred in the presence of control IgG1 (20 μg/ml) as in normal ACSF (Fig. 1D). Consistent with these observations, LTP production was prevented by a TNFα inhibitor (50 μM; Fig. 1E), which binds to the intact biologically active TNFα trimer and accelerates subunit dissociation to rapidly inactivate the cytokine (He et al., 2005). The magnitude of LTP determined at 50–60 min after 2 Hz stimulation was significantly smaller in the presence of either anti-TNFα antibody (5.3 ± 3.8% increase from baseline, $n=7$; Tukey's test, $P<0.0005$) or TNFα inhibitor (−6.3 ± 5.2% increase, $n=7$; $P<0.0005$), compared with the magnitude of control LTP (49 ± 6.7% increase, $n=9$), whereas the magnitude of LTP in the presence of control IgG1 (45 ± 7.0% increase, $n=10$; $P>0.05$) was indistinguishable from that of control LTP (Fig. 1F). These results indicate that TNFα is required for the production of LTP in rat visual cortex.

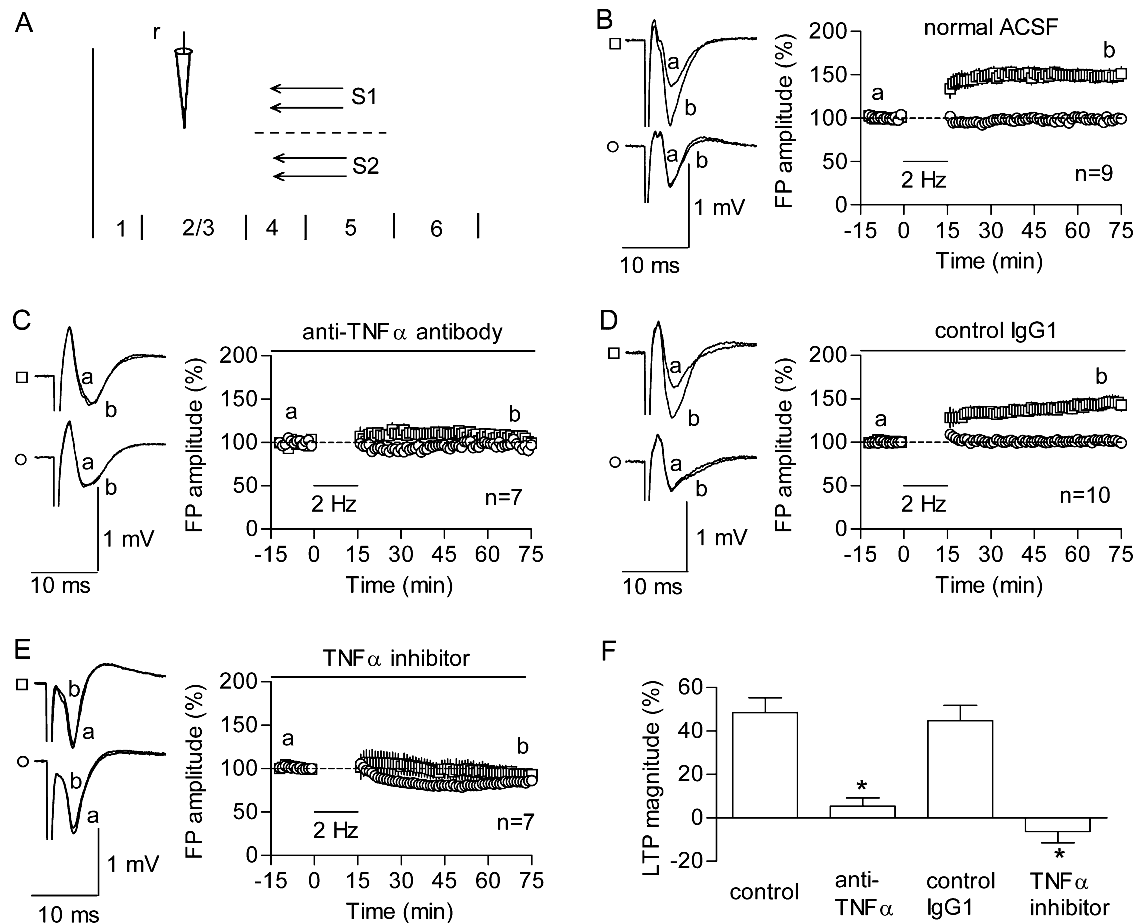

Fig. 1. TNFα is required for the production of T-type Ca^{2+} channel-dependent LTP in rat visual cortex. (A) Experimental arrangement of stimulating (S1 and S2) and recording electrodes (r). The lower figures indicate cortical layers. The dashed line indicates a surgical cut between the two stimulating electrodes. (B) Time course of LTP in normal ACSF, observed in rats at PD20–30. The amplitude of FPs (mean ± SEM, $n = 9$ slices) for the test (squares) and control pathways (circles) was expressed as the percentage of the respective baseline level determined by averaging the responses over 0–5 min before 2 Hz stimulation. The horizontal bar indicates the time period during which 2 Hz stimulation was applied. The traces on the left side show superimposed average ($n = 6$) responses sampled before and after 2 Hz stimulation for the test (upper) and control pathways (lower). The time at which responses (a and b) were sampled is indicated in the right figure. (C) Similar to B, but in the presence of an anti-TNFα antibody (20 μg/ml). The upper horizontal line indicates the time period of anti-TNFα antibody application. (D and E) Similar to C, but in the presence of a control IgG1 at 20 μg/ml (D) and a TNFα inhibitor at 50 μM (E). The number of slices was 7 (C), 10 (D) and 7 (E). (F) Summary of the magnitude of LTP (mean ± SEM) in control solution and in solutions containing an anti-TNFα antibody, a control IgG1 and a TNFα inhibitor. The asterisks indicate that the magnitude is significantly ($P < 0.05$, Tukey's test) different from that in control solution.

3.2. Exogenous TNFα facilitates the production of LTP

To test whether TNFα facilitates LTP production, we applied 2 Hz stimulation for a short period (5 min), which failed to induce LTP in normal ACSF (Fig. 2A). When 2 Hz stimulation was applied during bath application of soluble rat TNFα, LTP occurred depending on the dose used (Fig. 2B and C). The magnitude of LTP was significantly larger in the presence of TNFα at 3 μg/ml (26 ± 3.9% increase, $n = 6$; Tukey's test, $P < 0.0005$) but not at 1 μg/ml (12 ± 3.6% increase, $n = 7$; $P > 0.05$) or 0.3 μg/ml (3.7 ± 2.8% increase, $n = 8$; $P > 0.05$), compared with the magnitude of control LTP (4.0 ± 2.7% increase, $n = 11$; Fig. 2D). Therefore, we conclude that TNFα facilitates the production of LTP.

3.3. Two Hz stimulation also induces LTP in mouse visual cortex

We further examined the involvement of TNFα in LTP using TNFα-KO mice. Previously, we examined T-type Ca^{2+} channel-dependent LTP in rats and cats, but not in mice (Komatsu and Iwakiri, 1992; Yoshimura et al., 2008). Therefore, we first conducted experiments to test whether this form of LTP occurs in mouse visual cortex. In slices prepared from wild-type mice at PD20–30, 2 Hz stimulation for 15 min consistently produced LTP of FPs evoked in layer 2/3 by layer 4 stimulation (Fig. 3A). LTP occurred similarly in the presence of a high dose (100 μM) of the NMDA receptor antagonist APV (Fig. 3B). No significant difference (Tukey's test, $P > 0.05$) was found in LTP magnitude in the presence (41 ± 4.4% increase, $n = 10$) or absence of APV (41 ± 7.1% increase, $n = 9$) as summarized in Fig. 3E. LTP was not induced when 2 Hz stimulation was applied in the presence of 50 μM Ni^{2+} (−6.4 ± 4.2% increase, $n = 9$; Fig. 3C) or 10 μM ML 218 (−1.2 ± 2.9% increase, $n = 9$; Fig. 3D), a selective T-type Ca^{2+} channel blocker (Xiang et al., 2011; Horibe et al., 2014). LTP in the presence of these blockers was significantly smaller than control LTP ($P < 0.0005$; Fig. 3E). These results indicate that Ni^{2+}-sensitive T-type Ca^{2+} channel-dependent LTP occurs in mice in a way similar to that found in rats (Yoshimura et al., 2008).

3.4. TNFα is also required for the production of LTP in mice

Consistent with the results obtained from rats, LTP was substantially suppressed in the presence of a function neutralizing anti-TNFα antibody (10 μg/ml) in wild-type mice (Fig. 4A), whereas it occurred in the presence of the same dose of control IgG (Fig. 4B), as in normal ACSF (Fig. 3A). The magnitude of LTP in the presence

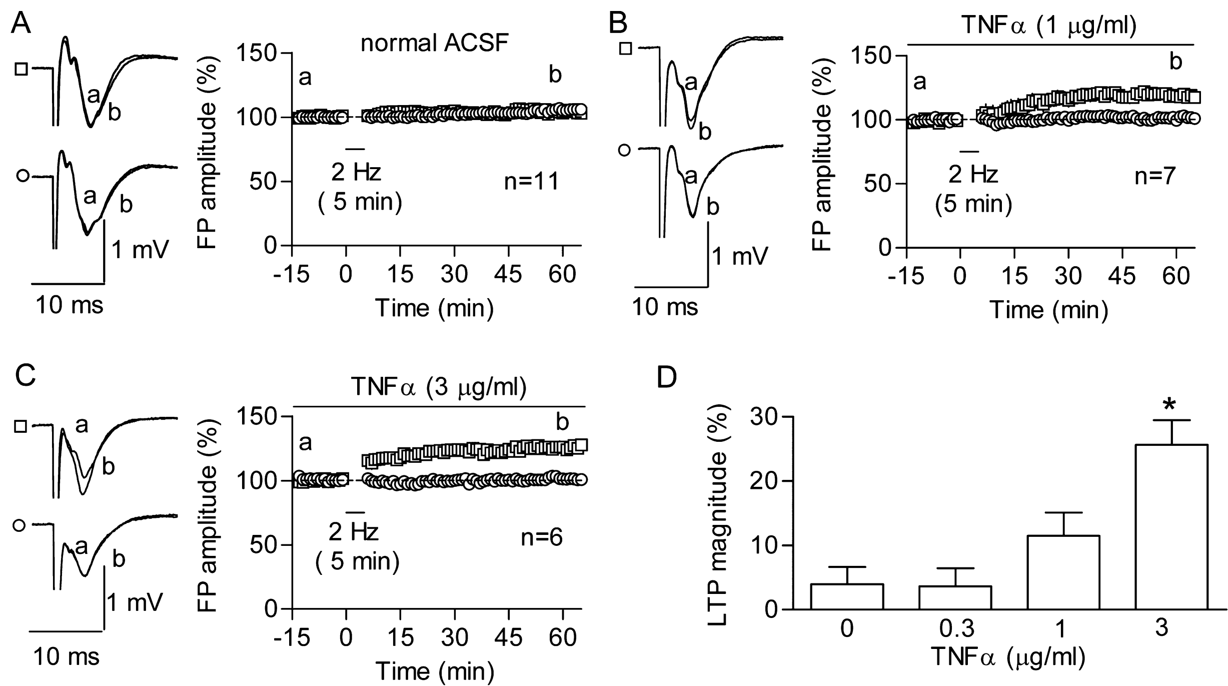

Fig. 2. Exogenous TNFα makes it possible for short 2 Hz stimulation to induce LTP in rat visual cortex. (A) Time course of LTP induced by 2 Hz stimulation for 5 min in normal ACSF observed in rats at PD20–30. The amplitude of FPs (mean ± SEM, $n = 11$ slices) for the test (squares) and control pathways (circles) was expressed as the percentage of the respective baseline level determined by averaging the responses over 0–5 min before 2 Hz stimulation. The horizontal bar indicates the time period during which 2 Hz stimulation was applied. The traces on the left side show superimposed average ($n = 6$) responses sampled before and after 2 Hz stimulation for the test (upper) and control pathways (lower). The time at which responses (a and b) were sampled is indicated in the right figure. (B and C) Similar to A, but in the presence of soluble TNFα at 1 μg/ml (B) and 3 μg/ml (C). The upper horizontal line indicates the time period of TNFα application. The number of slices was 7 (B) and 6 (C). (D) Summary of the magnitude of LTP (mean ± SEM) in the absence and presence of TNFα. The asterisk indicates that LTP magnitude is significantly ($P < 0.05$, Tukey's test) different from that in control solution.

of the antibody (4.9 ± 3.9% increase, $n = 7$; Tukey's test, $P < 0.005$) was significantly smaller than that in the presence of control IgG (40 ± 6.4% increase, $n = 9$), while the latter magnitude was indistinguishable ($P > 0.05$) from the magnitude of LTP found in normal ACSF, as shown in Fig. 4F.

Two Hz stimulation failed to induce LTP in TNFα-KO mice in normal ACSF (Fig. 4C). Bath application of TNFα (3 μg/ml) rescued the LTP (Fig. 4D). In normal ACSF, the magnitude of LTP was significantly smaller in TNFα-KO mice (5.2 ± 6.1% increase, $n = 10$; $P < 0.005$) than in wild-type mice, while the LTP magnitude in the presence of TNFα at 3 μg/ml (39 ± 11% increase, $n = 5$; $P > 0.05$), but not at 0.3 μg/ml (2.5 ± 4.7% increase, $n = 8$; $P < 0.005$), in TNFα-KO mice was almost the same as the magnitude of control LTP in wild-type mice (Fig. 4F). These results indicate that TNFα is also required for the production of LTP in mice and suggest that soluble TNFα contributes to this production. To test the involvement of soluble TNFα in LTP, we examined the effect of TAPI-0 on LTP in wild-type mice, because TAPI-0 inhibits the TNFα-converting enzyme (TACE) that converts membrane-bound TNFα to soluble TNFα (Black et al., 1997; Moss et al., 1997). Indeed, LTP in the presence of this compound was significantly smaller (9.8 ± 5.7% increase, $n = 7$; $P < 0.05$; Fig. 4E and F), compared with control LTP, suggesting that soluble TNFα is required for LTP production.

3.5. TNFα is required during a restricted time window soon after 2 Hz stimulation

Finally, we examined using TNFα-KO mice, when TNFα is required for LTP production. We applied TNFα (3 μg/ml) for 15 min at different timings relative to 2 Hz stimulation. LTP did not occur when TNFα was applied just before (Fig. 5A) or during 2 Hz stimulation (Fig. 5B). On the other hand, when TNFα was applied soon after 2 Hz stimulation, large LTP (63 ± 17% increase, $n = 8$) occurred and the potentiation developed gradually during TNFα application (Fig. 5C). However, LTP did not occur when TNFα application was started 15 min after 2 Hz stimulation, although a small, temporary increase in FP amplitude was observed in association with TNFα application (Fig. 5D). The magnitude of LTP observed when TNFα was applied soon after 2 Hz stimulation was significantly larger (Tukey's test, $P < 0.005$) than that observed when TNFα was applied at other timings, while no differences ($P > 0.05$) were found in LTP magnitude between the latter cases (Fig. 5E). These results indicate that TNFα is effective for LTP production only during a restricted period soon after 2 Hz stimulation.

4. Discussion

The present study demonstrated that T-type Ca^{2+} channel-dependent LTP occurred in mouse visual cortex, as it did in rat and cat visual cortex. Experiments using pharmacological methods and knockout mice consistently demonstrated that TNFα was required for the production of this LTP in the visual cortex of both mice and rats. These observations support our view that this form of LTP contributes to experience-dependent modification of visual responsiveness during the critical period.

4.1. Involvement of TNFα in T-type Ca^{2+} channel-dependent LTP

Function neutralizing anti-TNFα antibodies prevented the production of LTP in both rats and mice. In rats, LTP production was prevented by a TNFα inhibitor, and bath application of TNFα made it possible to induce LTP by 2 Hz stimulation applied for a short period, which was ineffective in normal ACSF. Furthermore, in TNFα-KO mice, LTP did not occur and was rescued almost completely by exogenous TNFα. These results indicate that TNFα is indispensable for the production of T-type Ca^{2+} channel-dependent LTP. It was reported that both NMDA receptor-dependent LTP and LTD

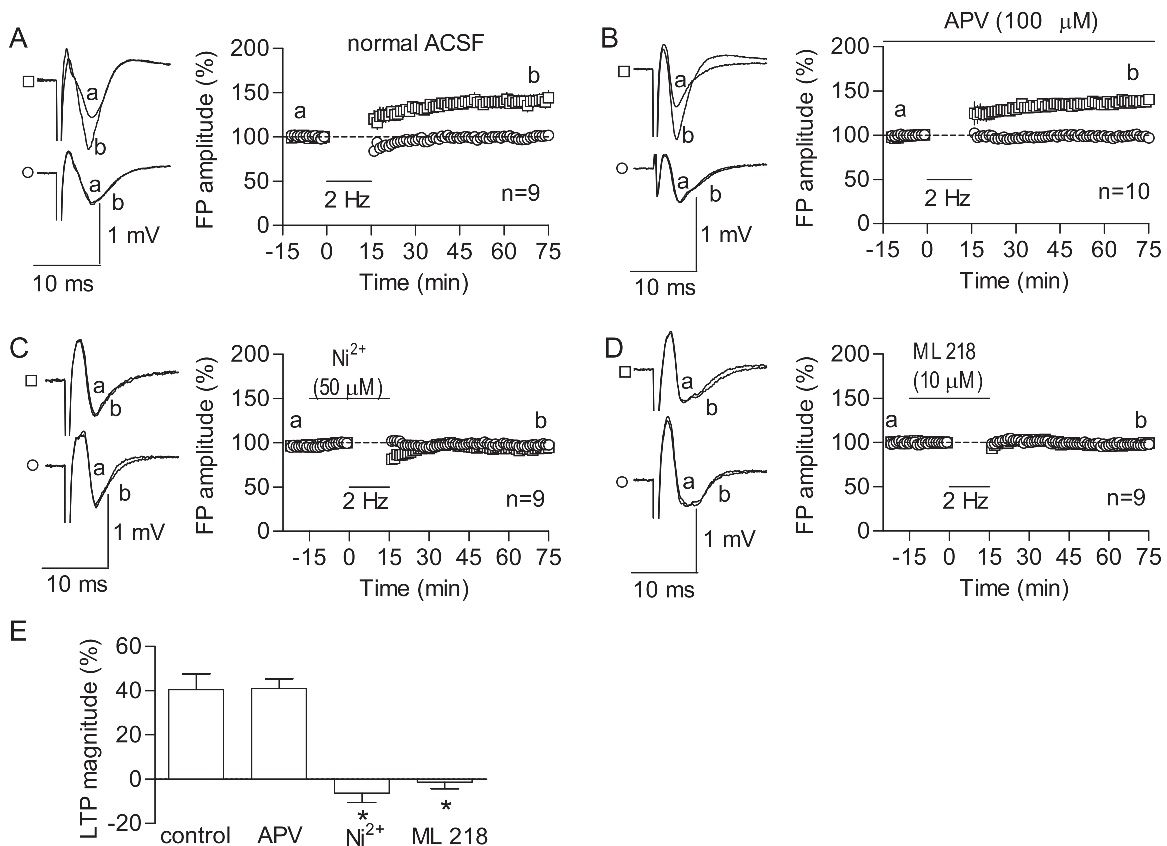

Fig. 3. Two Hz stimulation for 15 min also induces T-type Ca^{2+} channel-dependent LTP in mouse visual cortex. (A) Time course of LTP in normal ACSF observed in mice at PD20–30. The amplitude of FPs (mean ± SEM, $n = 9$ slices) for the test (squares) and control pathways (circles) was expressed as the percentage of the respective baseline level determined by averaging responses over 0–5 min before 2 Hz stimulation. The horizontal bar indicates the time period during which 2 Hz stimulation was applied. The traces on the left side show superimposed average ($n = 6$) responses sampled before and after 2 Hz stimulation for the test (upper) and control pathways (lower). The time at which responses (a and b) were sampled is indicated in the right figure. (B) Similar to A, but in the presence of 100 μM DL-APV. The upper horizontal line indicates the time period of APV application. (C and D) Similar to B, but in the presence of 50 μM Ni^{2+} (C) and 10 μM ML 218 (D). The number of slices was 10 (B), 9 (C) and 9 (D). (F) Summary of the magnitude of LTP (mean ± SEM) in control solution and in solutions containing DL-APV, Ni^{2+} and ML 218. The asterisks indicate that LTP magnitude is significantly ($P < 0.05$, Tukey's test) different from that in control solution.

in hippocampal CA1 were not affected by either TNFα application or genetic deletion of TNFα (Stellwagen and Malenka, 2006), although, in older studies, inhibitory effects of TNFα on LTP were shown in the hippocampal dentate gyrus and CA1 (Tancredi et al., 1992; Cunningham et al., 1996). The present study demonstrated for the first time a clear facilitative role of TNFα on the production of long-term synaptic modification.

The dose of exogenous TNFα required for the facilitation of rat LTP was more than 1 μg/ml and LTP was rescued by TNFα at 3 μg/ml, but not 0.3 μg/ml, in TNFα-KO mice. The effective doses are far higher than the doses (about 100 ng/ml) necessary for the quantal amplitude of excitatory and inhibitory postsynaptic currents to be maximally increased and decreased by homeostatic synaptic scaling, respectively (Beattie et al., 2002; Pribiag and Stellwagen, 2013). It is likely that TNFα is produced and released from glia rather than neurons (Poon et al., 2013; Pickering et al., 2005; Santello and Volterra, 2012), and synaptic scaling seems to be mediated by TNFα constitutively released from glia in physiological conditions (Beattie et al., 2002). It was suggested that the amount of released TNFα becomes smaller depending on the level of extracellular glutamate concentration correlating to neuronal network activity (Stellwagen and Malenka, 2006) and constitutive TNFα levels are usually low (about 2 ng/ml) in physiological conditions (Santello and Volterra, 2012). Thus, the basal amount of released TNFα in slice preparations may be far less than the amount required for LTP production. The experiments using TNFα-KO mice demonstrated that TNFα was required only during a restricted time window soon after 2 Hz stimulation for LTP production. In addition, the pharmacological inhibition of TACE suppressed the production of LTP. Therefore, soluble TNFα may be released from membrane-bound TNFα by the activity of TACE soon after 2 Hz stimulation, and five minutes may not be enough for 2 Hz stimulation to initiate the release of a necessary amount of TNFα, although it is unknown at present whether TACE can be activated by neural activity. Because the activation of postsynaptic T-type Ca^{2+} channels during 2 Hz stimulation is required for LTP induction (Yoshimura et al., 2008), the resultant postsynaptic Ca^{2+} increase might lead to the activation of TACE. Because the cellular localization of TACE is unknown at present, it is uncertain how TACE is activated. However, if the enzyme is located at the cell membrane of the postsynaptic neurons, the Ca^{2+} increase can initiate the activation of TACE through protein kinase C, because it is known that the kinase activates TACE (Murphy, 2009). If this speculation is correct, the activity of postsynaptic neurons could trigger the release of soluble TNFα from membrane bound TNFα produced by glia. An important issue that remains to be resolved is whether TNFα mediates main signals to generate LTP or only modulates it. In addition, it remains to be determined what kind of roles glia play in LTP production.

4.2. Synaptic mechanisms underlying the experience-dependent potentiation of visual responses

The age and experience dependence of T-type Ca^{2+} channel-dependent LTP is similar to that of ocular dominance plasticity

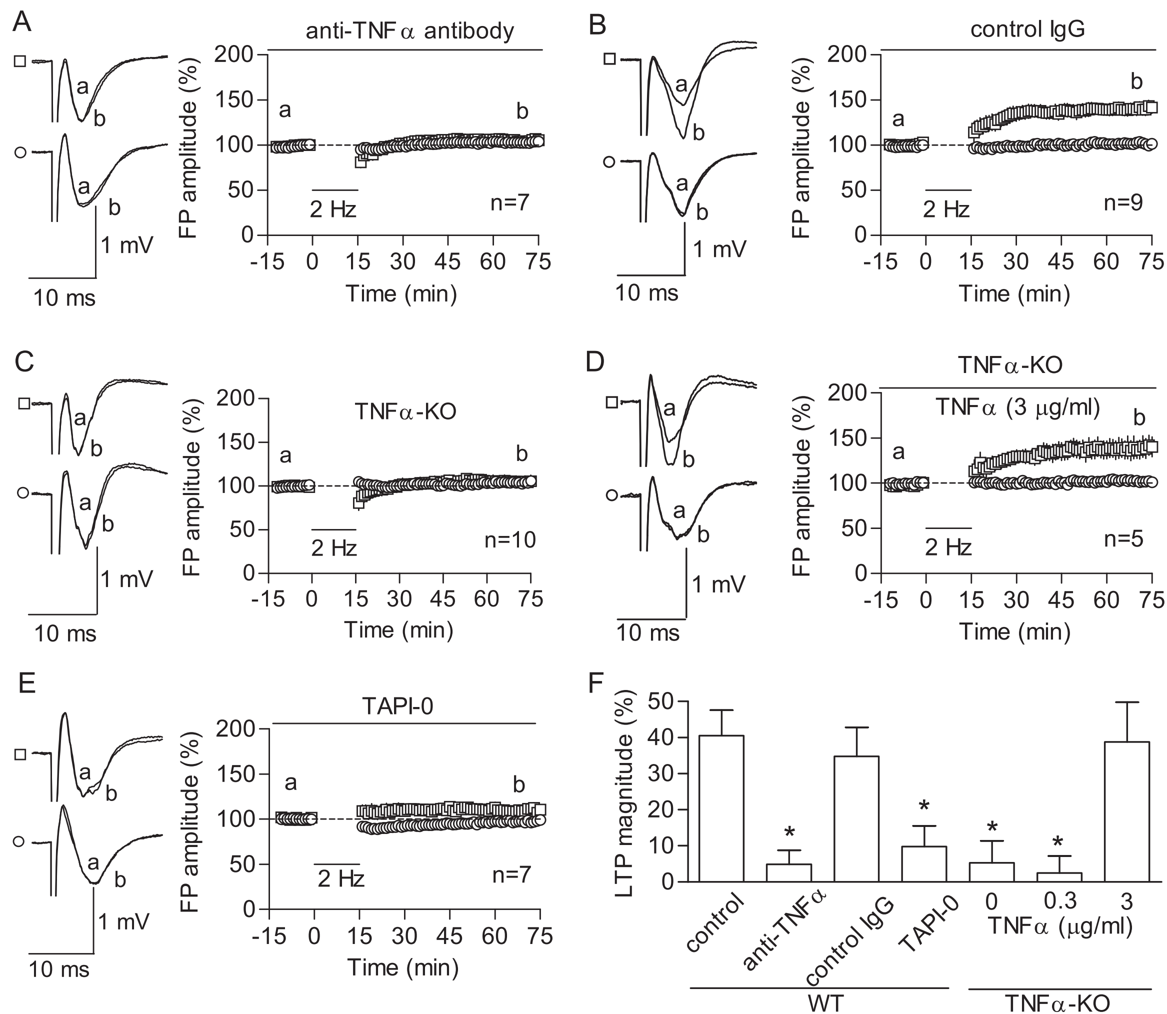

Fig. 4. TNFα is also required for the production of T-type Ca^{2+} channel-dependent LTP in mouse visual cortex. (A) Time course of LTP in ACSF containing an anti-TNFα antibody (10 μg/ml), observed in wild-type mice at PD20–30. The amplitude of FPs (mean ± SEM, $n = 7$ slices) for the test (squares) and control pathways (circles) was expressed as the percentage of the respective baseline level determined by averaging the responses over 0–5 min before 2 Hz stimulation. The horizontal bar indicates the time period during which 2 Hz stimulation was applied. The traces on the left side show superimposed average ($n = 6$) responses sampled before and after 2 Hz stimulation for the test (upper) and control pathways (lower). The time at which responses (a and b) were sampled is indicated in the right figure. The upper horizontal line indicates the time period of bath application of anti-TNFα antibody. (B) Similar to A, but in the presence of control IgG (10 μg/ml). (C–E) Similar to B, but in the absence (C) and presence of mouse soluble TNFα at 3 μg/ml (D) in TNFα-KO mice, and in the presence of 1 μM TAPI-0 in wild-type mice (E). The number of slices was 9 (B), 10 (C), 5 (D) and 7(E). (F) Summary of the magnitude of LTP (mean ± SEM) in control solution and in solutions containing anti-TNFα antibody, control IgG and TAPI-0 in wild-type mice (WT), and in the absence and presence of TNFα in TNFα-KO mice. The magnitude of LTP in control solution in wild-type mice was the same as shown in Fig. 3E. The asterisks indicate that the magnitude is significantly ($P < 0.05$, Tukey's test) different from that in control solution in wild-type mice.

(Hubel and Wiesel, 1970; Cynader and Mitchell, 1980; Mower et al., 1981), suggesting that this LTP underlies experience-dependent developmental plasticity. Our previous study using rats during the critical period showed that an infusion of a T-type Ca^{2+} channel blocker into visual cortex during monocular deprivation prevented the potentiation of visual evoked potentials (VEPs) in response to nondeprived eye stimulation, whereas it did not affect the depression of VEPs in response to deprived eye stimulation (Yoshimura et al., 2008). In addition, the infusion of the blocker prevented the potentiation of nondeprived eye responses and 2 Hz stimulation-induced LTP studied *in vivo* at the same dose. Based on these our observations, we have proposed that this form of LTP mediates the potentiation of nondeprived eye responses following monocular deprivation during the critical period. The clear age and experience-dependence of Ni^{2+}-sensitive T-type Ca^{2+} currents in layer 2/3 pyramidal neurons demonstrated in our previous study further supports this view (Horibe et al., 2014).

However, another hypothesis proposed in regard to this issue is that TNFα-dependent synaptic scaling of quantal amplitude of excitatory synaptic transmission rather than Hebbian synaptic plasticity is responsible for the potentiation of nondeprived eye responses. This hypothesis was proposed based on experiments showing that, in the visual cortex of TNFα-KO mice, the potentiation of visual responses and synaptic scaling did not occur, whereas NMDA receptor-dependent LTP normally occurred (Kaneko et al., 2008). In addition, it was demonstrated that monocular deprivation increased the quantal amplitude of EPSCs in layer 2/3 pyramidal neurons in the binocular region of rat visual cortex (Lambo and Turrigiano, 2013), although it is uncertain whether the magnitude of the increases is sufficient to explain the changes in the visual responses of cortical cells. However, the present study demonstrated that TNFα was required for the production of T-type Ca^{2+} channel-dependent LTP. The TNFα-dependent potentiation, which appeared after a longer period of monocular deprivation, seemed larger in nondeprived eye responses than in deprived eye responses (Kaneko et al., 2008), suggesting that the potentiation of nondeprived eye responses contains components mediated by LTP as well as synaptic scaling. In adult mice, monocular deprivation

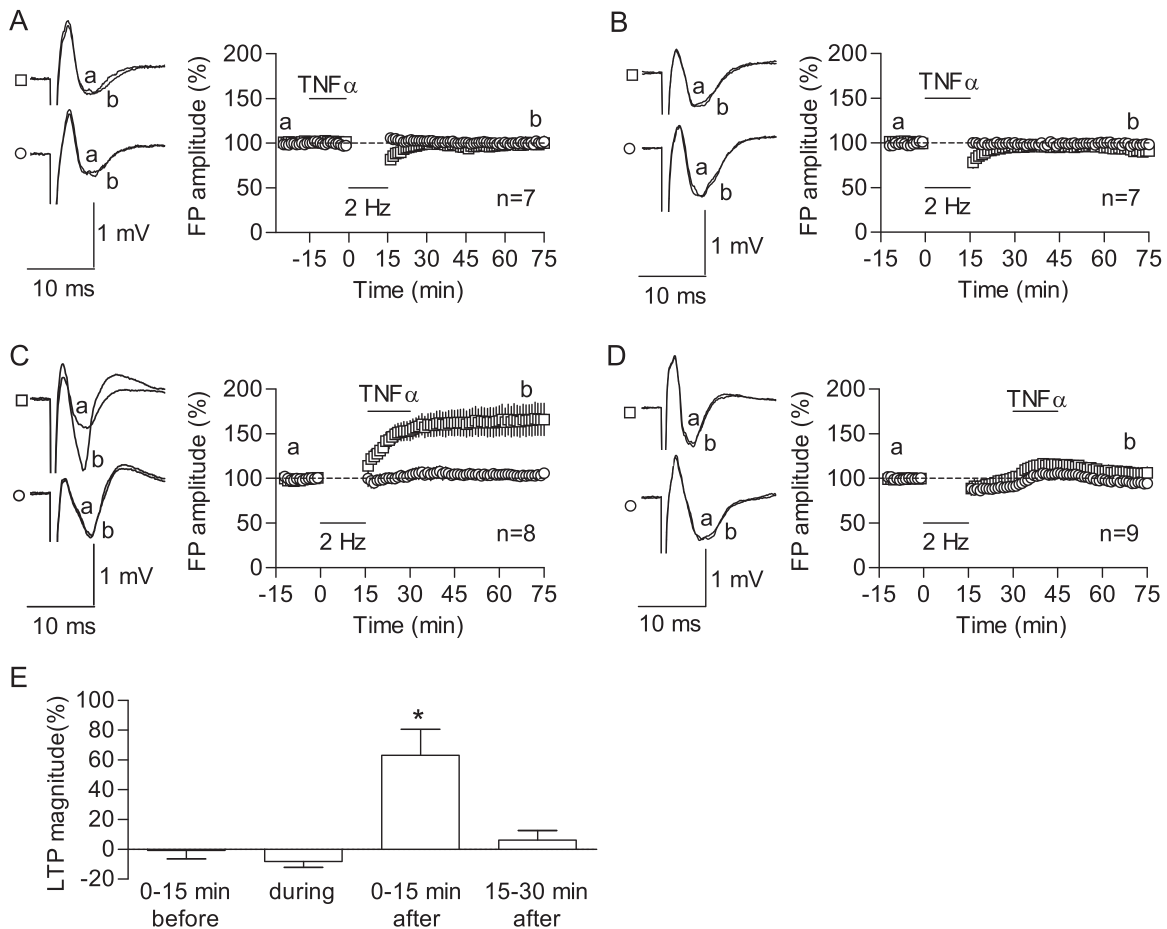

Fig. 5. The time window during which TNFα application enables LTP production in TNFα-KO mice. (A) Time course of LTP in slices prepared from TNFα-KO mice, to which TNFα (3 μg/ml) was applied for 15 min just before 2 Hz stimulation. The amplitude of FPs (mean ± SEM, $n = 7$ slices) for the test (squares) and control pathways (circles) was expressed as the percentage of the respective baseline level determined by averaging the responses over 0–5 min before 2 Hz stimulation. The lower and upper horizontal bars indicate the time period during which 2 Hz stimulation and TNFα were applied, respectively. The traces on the left side are superimposed average ($n = 6$) responses sampled before and after 2 Hz stimulation for the test (upper) and control pathways (lower). The time at which responses (a and b) were sampled is indicated in the right figure. (B–D) Similar to A, but TNFα was applied during (B), 0–15 min after (C) and 15–30 min after 2 Hz stimulation (D). The number of slices was 7 (B), 8 (C) and 9 (D). (E) Summary of the magnitude of LTP (mean ± SEM) when TNFα was applied 0–15 min before, during, 0–15 min after and 15–30 min after 2 Hz stimulation. The asterisk indicates that LTP magnitude when TNFα was applied just after 2 Hz stimulation is significantly ($P < 0.05$, Tukey's test) different from any of the other cases.

produces the potentiation of nondeprived eye responses but not the depression of deprived eye responses, and the potentiation was independent of TNFα (Ranson et al., 2012), indicating that TNFα-dependent modifications take place only during the critical period. At present, however, it is uncertain which process, LTP or homeostatic synaptic scaling, mainly contributes to the potentiation of nondeprived eye responses, although TNFα seems to play important roles in experience-dependent developmental plasticity. Further studies are necessary to resolve this issue.

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References

Bear, M.F., Cooper, L.N., Ebner, F.F., 1987. A physiological basis for a theory of synapse modification. Science 237, 42–48.

Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Zastrow, M.V., Beattie, M.S., Malenka, R.C., 2002. Control of synaptic strength by glial TNFα. Science 295, 2282–2285.

Black, R.A., Rauch, C.T., Kozlosky, C.J., Peschon, J.J., Slack, J.L., Wolfson, M.F., Canstner, B.J., Stocking, K.L., Reddy, P., Srinivasan, S., Nelson, N., Bolani, N., Schooley, K.A., Gerhart, M., Davis, R., Fitzner, J.N., Johnson, R.S., Paxton, R.J., March, C.J., Cerretti, D.P., 1997. A metalloproteinase disintegrin that releases tumor-necrosis factor-α from cells. Nature 385, 729–733.

Cunningham, A.J., Murray, C.A., O'Neill, L.A.J., Lynch, M.A., O'Connor, J.J., 1996. Interleukin-1β (IL-1β) and tumor necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neurosci. Lett. 203, 17–20.

Cynader, M., Mitchell, D.E., 1980. Prolonged sensitivity to monocular deprivation in dark-reared cats. J. Neurophysiol. 43, 1026–1040.

Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L., Maffei, L., 1994. Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. Vis. Res. 34, 709–720.

Frenkel, M.Y., Bear, M.F., 2004. How monocular deprivation shifts ocular dominance in visual cortex of young mice. Neuron 44, 917–923.

Funahashi, R., Maruyama, T., Yoshimura, Y., Komatsu, Y., 2013. Silent synapses persist into adulthood in layer 2/3 pyramidal neurons of visual cortex in dark-reared mice. J. Neurophysiol. 109, 2064–2076.

Gordon, J.A., Stryker, M.P., 1996. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. 16, 3274–3286.

He, M.M., Smith, A.S., Oslob, J.D., Flanagan, W.M., Braisted, A.C., Whitty, A., Cancilla, M.T., Wang, J., Lugovskoy, A.A., Yoburn, J.C., Fung, A.D., Farrington, G., Eldredge, J.K., Day, E.S., Cruz, L.A., Cachero, T.G., Miller, S.K., Friedman, J.E., Choong, I.C., Cunningham, B.C., 2005. Small-molecule inhibition of TNF-α. Science 310, 1022–1025.

Heynen, A.J., Yoon, B.-J., Liu, C.-H., Chung, H.J., Huganir, R.L., Bear, M.F., 2003. Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nat. Neurosci. 6, 854–862.

Horibe, S., Tarusawa, E., Komatsu, Y., Yoshimura, Y., 2014. Ni^{2+}-sensitive T-type Ca^{2+} channel currents are regulated in parallel with synaptic and visual response plasticity in visual cortex. Neurosci. Res. 87, 33–39.

Hubel, D.H., 1982. Exploration of the primary visual cortex, 1955–1978. Nature 299, 515–524.

Hubel, D.H., Wiesel, T.N., 1970. The period of susceptibility to physiological effects of unilateral eye closure in kittens. J. Physiol. (Lond.) 206, 419–436.

Kaneko, M., Stellwagen, D., Malenka, R.C., Stryker, M.P., 2008. Tumor necrosis factor-α mediates one component of competitive, experience-dependent plasticity in developing visual cortex. Neuron 58, 673–680.

Katz, L.C., Shatz, C.J., 1996. Synaptic activity and the construction of cortical circuits. Science 274, 1133–1138.

Komatsu, Y., 1994. Age-dependent long-term potentiation of inhibitory synaptic transmission in rat visual cortex. J. Neurosci. 14, 6488–6499.

Komatsu, Y., Fujii, K., Maeda, J., Sakaguchi, H., Toyama, K., 1988. Long-term potentiation of synaptic transmission in kitten visual cortex. J. Neurophysiol. 59, 124–141.

Komatsu, Y., Iwakiri, M., 1992. Low-threshold Ca^{2+} channels mediate induction of long-term potentiation in kitten visual cortex. J. Neurophysiol. 67, 401–410.

Lambo, M.E., Turrigiano, G.G., 2013. Synaptic and intrinsic homeostatic mechanisms cooperate to increase L2/3 pyramidal neuron excitability during a late phase of critical period plasticity. J. Neurosci. 33, 8810–8819.

Liu, C.-H., Heynen, A.J., Shuler, M.G.H., Bear, M.F., 2008. Cannabinoid receptor blockade reveals parallel plasticity mechanisms in different layers of mouse visual cortex. Neuron 58, 340–345.

Moss, M.L., Jin, S.-L.C., Milla, M.E., Burkhart, W., Carter, H.L., Chen, W.-J., Clay, W.C., Didsubury, J.R., Hassler, D., Hoffman, C.R., Kost, T.A., Lambert, M.H., Leesnitzer, M.A., McCauley, P., McGeehan, G., Mitchell, J., Moyer, M., Pahel, G., Rocque, W., Overton, L.K., Schoenen, F., Seaton, T., Su, J.-L., Warner, J., Willard, D., Becherer, J.D., 1997. Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α. Nature 385, 733–736.

Mower, G.D., Berry, D., Burchfiel, J.L., Duffy, F.H., 1981. Comparison of the effects of dark rearing and binocular suture on development and plasticity of cat visual cortex. Brain Res. 220, 255–267.

Murphy, G., 2009. Regulation of the proteolytic disintegrin metalloproteinases, the 'sheddases'. Semin. Cell Dev. Biol. 20, 138–145.

Ohmura, T., Ming, R., Yoshimura, Y., Komatsu, Y., 2003. Age and experience dependence of N-methyl-D-aspartate receptor-independent long-term potentiation in rat visual cortex. Neurosci. Lett. 341, 95–98.

Pickering, M., Cumiskey, D., O'Connor, J.J., 2005. Action of TNF-α on glutamatergic synaptic transmission in the central nervous system. Exp. Physiol. 90, 663–670.

Poon, V.Y., Choi, S., Park, M., 2013. Growth factors in synaptic function. Front. Synap. Neurosci. 5, 1–18.

Pribiag, H., Stellwagen, D., 2013. TNF-α downregulates inhibitory neurotransmission through protein phosphatase 1-dependent trafficking of $GABA_A$ receptors. J. Neurosci. 33, 15879–15893.

Ranson, A., Cheetham, C.E.J., Fox, K., Sengpiel, F., 2012. Homeostatic plasticity mechanisms are required for juvenile, but not adult, ocular dominance plasticity. Proc. Natl. Acad. Sci. U.S.A. 109, 1311–1316.

Santello, M., Volterra, A., 2012. TNFα in synaptic function: switching gears. Trends Neurosci. 35, 638–647.

Singer, W., 1995. Development and plasticity of cortical processing architectures. Science 270, 758–764.

Stent, G.S., 1973. A physiological mechanism for Hebb's postulate of learning. Proc. Natl. Acad. Sci. U.S.A. 70, 997–1001.

Stellwagen, D., Malenka, R.C., 2006. Synaptic scaling mediated by glial TNF-α. Nature 440, 1054–1059.

Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A., Eusebi, F., 1992. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neurosci. Lett. 146, 176–178.

Turrigiano, G.G., 2008. The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135, 422–435.

Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C., Nelson, S.B., 1998. Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391, 892–896.

Wiesel, T.N., 1982. Postnatal development of the visual cortex and the influence of environment. Nature 299, 583–591.

Xiang, Z., Thompson, A.D., Brogan, J.T., Schulte, M.L., Melancon, B.J., Mi, D., Lewis, L.M., Zou, B., Yang, L., Morrison, R., Santomango, T., Byers, F., Brewer, K., Aldrich, J.S., Yu, H., Dawson, E.S., Li, M., McManus, O., Jones, C.K., Daniels, J.S., Hopkins, C.R., Xie, X.S., Conn, P.J., Weaver, C.D., Lindsley, C.W., 2011. The discovery and characterization of ML218: a novel, centrally active T-type calcium channel inhibitor with robust effects in STN neurons and in a rodent model of Parkinson's disease. ACS Chem. Neurosci. 2, 730–742.

Yoon, B.-J., Smith, G.B., Heynen, A.J., Neve, R.L., Bear, M.F., 2009. Essential role for a long-term depression mechanism in ocluar dominance platicity. Proc. Natl. Acad. Sci. U.S.A. 106, 9860–9865.

Yoshimura, Y., Inaba, M., Yamada, K., Kurotani, T., Begum, T., Reza, F., Maruyama, T., Komatsu, Y., 2008. Involvement of T-type Ca^{2+} channels in the potentiation of synaptic and visual responses during the critical period in rat visual cortex. Eur. J. Neurosci. 28, 730–743.

Zhang, L.I., Poo, M.M., 2001. Electrical activity and development of neural circuits. Nat. Neurosci. Suppl. 4, 1207–1214.