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10. To increase the motivational properties of the monetary incentives, cash (dollar bills and coins) in the amount of the cumulative total was kept on the table at which the participant was seated and was incremented after each block of trials. When trial blocks resulted in a net loss, that total was not subtracted from the take-home amount delivered at the end of the experimental session. These procedures—the presence of the cash and the steadily increasing cumulative award—were used to increase the participant's motivation to attend to the gains and losses.
11. ERP activity was quantified as the mean amplitude in the 200- to 300-ms epoch after the onset of the stimulus, relative to a 100-ms prestimulus baseline. Analyses used repeated-measures analysis of variance (ANOVA), with Greenhouse-Geisser correction applied for violations of the ANOVA assumption of sphericity (*9*).
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Control of Synaptic Strength by Glial TNFα

Eric C. Beattie,[1] David Stellwagen,[1] Wade Morishita,[1] Jacqueline C. Bresnahan,[2] Byeong Keun Ha,[2] Mark Von Zastrow,[3] Michael S. Beattie,[2]* Robert C. Malenka[1]*

Activity-dependent modulation of synaptic efficacy in the brain contributes to neural circuit development and experience-dependent plasticity. Although glia are affected by activity and ensheathe synapses, their influence on synaptic strength has largely been ignored. Here, we show that a protein produced by glia, tumor necrosis factor α (TNFα), enhances synaptic efficacy by increasing surface expression of AMPA receptors. Preventing the actions of endogenous TNFα has the opposite effects. Thus, the continual presence of TNFα is required for preservation of synaptic strength at excitatory synapses. Through its effects on AMPA receptor trafficking, TNFα may play roles in synaptic plasticity and modulating responses to neural injury.

Glia, long considered to be primarily supportive of neurons, are now thought to be more active participants in neural circuit function (*1*, *2*). Recently, it has been shown that astrocytes are required for normal synaptogenesis and synaptic stability due to the release of diffusible, extracellular signal(s) (*3*–*5*), one of which appears to be cholesterol (*6*). Whether glia are required for the rapid continual maintenance of synaptic strength is unknown. Here we present evidence that in both cultured hippocampal neurons and hippocampal slices, glial cells constitutively release the cytokine TNFα, which markedly influences synaptic strength at excitatory synapses via rapid effects on the trafficking of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPARs). That TNFα might influence surface expression of AMPARs and synaptic strength was suggested by observations that TNFα enhanced brainstem neuron responses to excitatory afferent inputs (*7*) and potentiated the cell death induced by injection of the excitotoxin kainate into the spinal cord, an effect that was blocked by an AMPAR antagonist (*8*). Furthermore, several reports have suggested that TNFα may influence synaptic function (*9*–*11*).

To determine if TNFα increases AMPAR surface expression, we exposed cultured hippocampal neurons to TNFα (0.6 to 60 nM for 15 min) (*12*). This caused a twofold increase in the levels of surface AMPARs in the plasma membrane (Fig. 1, A and B). Because the media contained antagonists of all subtypes of glutamate receptors (*12*), this action of TNFα was not due to an indirect effect of TNFα on astrocyte-mediated glutamate release (*13*), which might affect AMPAR trafficking (*14*, *15*). To determine whether the effect of TNFα on AMPAR surface expression was due to an increase in the delivery of new surface AMPARs, we visualized only those AMPARs that appeared in the plasma membrane during the TNFα treatment (*16*) (Fig. 1C). TNFα treatment (6 nM for 10 min) caused a marked increase in the delivery of new AMPARs to the plasma membrane compared to untreated cells (Fig. 1, C and D).

To address whether the TNFα-induced increase in AMPAR surface expression happens at synapses and thereby modifies synaptic strength, we compared the percentage of synapses, identified by synaptophysin staining, that contained detectable levels of AMPARs in untreated and TNFα-treated cells (*17*). TNFα caused a significant increase in this measure (Fig. 1, E and F). We also examined whether TNFα af-

[1]Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, CA 94304, USA. [2]Department of Neuroscience, The Ohio State University Medical Center, Columbus, OH 43210, USA. [3]Departments of Psychiatry and Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94143, USA.

*To whom correspondence should be addressed. E-mail: beattie.2@osu.edu (M.S.B.); malenka@stanford.edu (R.C.M.)

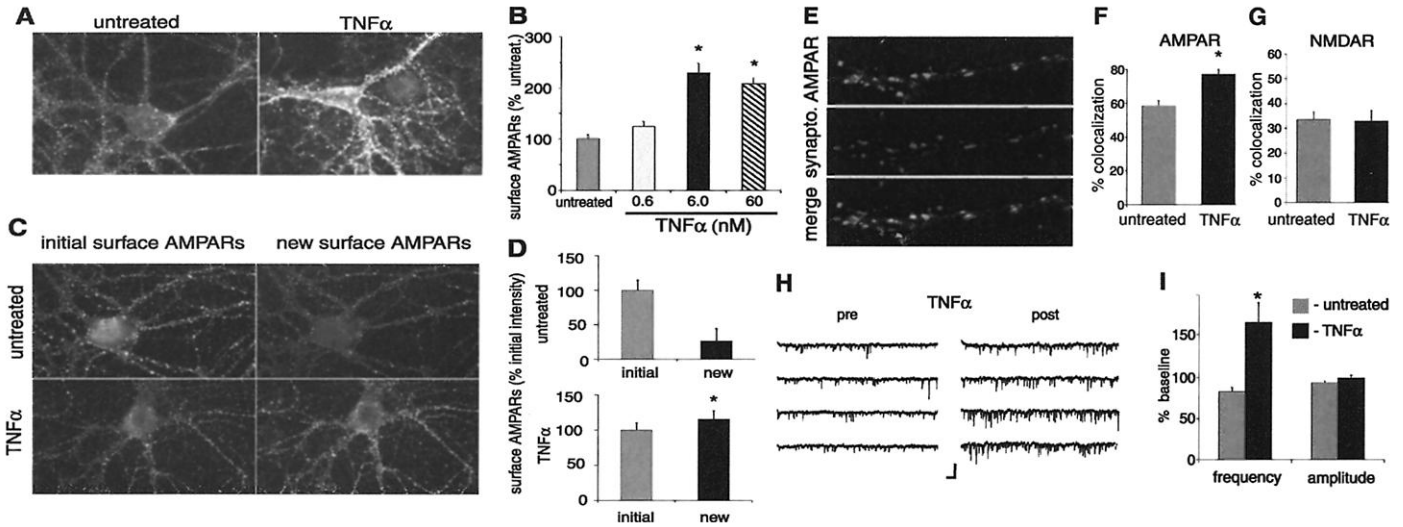

Fig. 1. TNFα increases surface expression of AMPARs at synapses. (**A**) Examples of surface AMPAR staining in untreated and TNFα-treated neurons. (**B**) Quantitation of effects of TNFα on surface AMPAR staining ($n = 30$ to 50 for each group; $^*P < 0.01$; untreated, 100 ± 9%; 600 pM, 126 ± 10%; 6 nM, 231 ± 19%; 60 nM, 209 ± 10%). (**C**) Examples of staining for initial and new surface AMPARs in untreated and TNFα-treated neurons. (**D**) Quantitation of effects of TNFα on delivery of new surface AMPARs ($n = 24$ for each group; $^*P < 0.01$ comparing untreated and TNFα-treated new surface expression; untreated initial, 100 ± 10%; untreated new, 26 ± 18%; TNFα initial, 100 ± 10%; TNFα new, 115 ± 12%). (**E**) Example of colocalization of AMPARs with synaptophysin on a dendritic process. (**F**) Quantitation of the percent of total synaptophysin staining that overlaps with AMPAR staining. ($n = 18$ for each group; $^*P < 0.01$; untreated, 58 ± 3%; TNFα, 77 ± 3%). (**G**) Quantitation of percent of total synaptophysin staining that overlaps with NMDAR staining ($n = 20$ for each group; untreated, 34 ± 3%; TNFα, 33 ± 4%). (**H**) Examples of mEPSCs recorded before and after application of TNFα (calibration bars: 20 pA, 500 ms). (**I**) Quantitation of percent change in mEPSC frequency and amplitude in untreated and TNFα-treated neurons ($n = 11$ for each group; $^*P < 0.01$; percent initial mEPSC frequency: TNFα treatment, 167 ± 23%; control treatment, 83 ± 4%; percent initial mEPSC amplitude: TNFα treatment, 99 ± 4%; control treatment, 94 ± 2%).

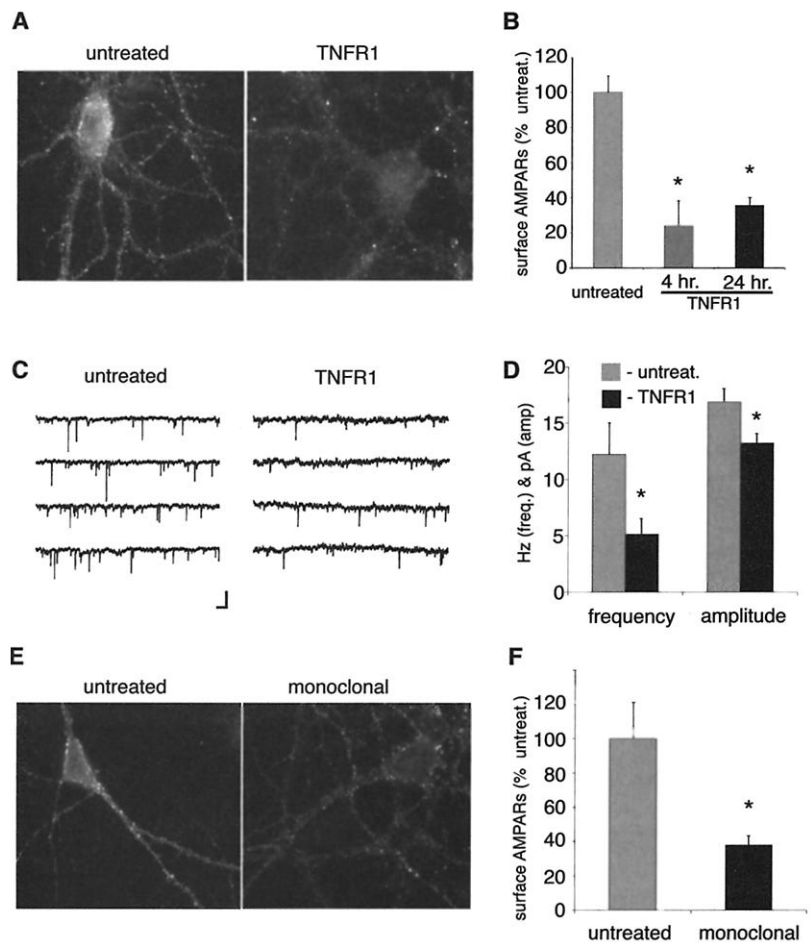

Fig. 2. Blocking TNFα action decreases surface AMPARs and synaptic strength. (**A**) Examples of surface AMPAR staining in untreated and TNFR1-treated cells. (**B**) Quantitation of effects of TNFR1 on surface AMPAR staining. [$n = 25$ to 30 for each group; $^*P < 0.01$; untreated, 100 ± 8%; TNFR1 (4 hours), 24 ± 14%; TNFR1 (24 hours), 35 ± 4%]. (**C**) Examples of mEPSCs recorded from untreated and TNFR1-treated cells (calibration bars: 20 pA, 500 ms). (**D**) Mean mEPSC frequency and amplitude in untreated and TNFR1-treated neurons. ($^*P < 0.01$; TNFR1-treated cells: 5.1 ± 1.4 Hz, 13.3 ± 0.8 pA, $n = 16$; untreated cells: 12.2 ± 2.8 Hz, 16.9 ± 1.2 pA, $n = 15$) (**E**) Examples of surface AMPAR staining in untreated and anti-TNFα- treated cells. (**F**) Quantitation of effects of anti-TNFα on surface AMPARs ($n = 25$ for each group; $^*P < 0.01$; untreated, 100 ± 21%; monoclonal antibody, 36 ± 5%).

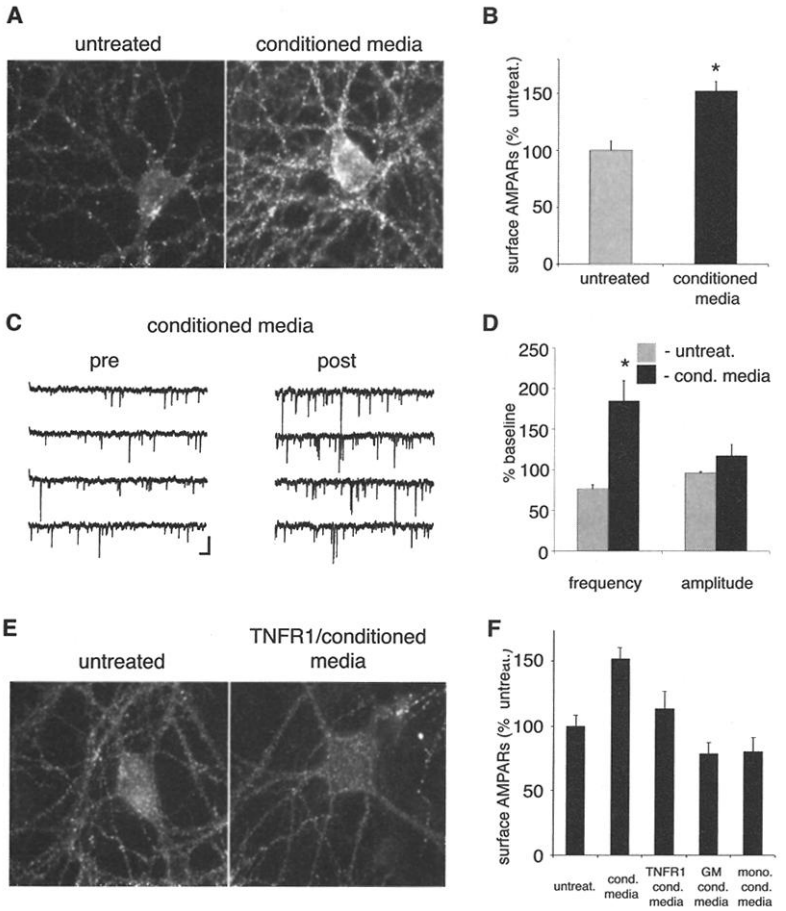

Fig. 3. Astrocyte-conditioned media increases surface expression of AMPARs and synaptic strength via TNFα. **(A)** Examples of surface AMPAR staining in untreated and conditioned media–treated neurons. **(B)** Quantitation of effects of conditioned media on surface AMPAR staining (*$P < 0.01$; untreated, 100 ± 9%, $n = 45$; conditioned media, 152 ± 9%, $n = 37$). **(C)** Examples of mEPSCs before and after application of conditioned media (calibration bars: 20 pA, 500 ms). **(D)** Mean percent change in mEPSC frequency and amplitude in cells treated with normal or conditioned media [$n = 7$ cells (normal media), $n = 8$ cells (conditioned media); *$P < 0.01$; percent initial mEPSC frequency: conditioned media, 185 ± 25%; normal media, 76 ± 5%; percent initial mEPSC amplitude: conditioned media, 117 ± 14%; normal media, 96 ± 2%]. **(E)** Examples of surface AMPAR staining in an untreated cell and a cell treated with conditioned media containing TNFR1. **(F)** Quantitation of effects of conditioned media versus conditioned media containing either TNFR1, anti-TNFα, or the matrix metalloproteinase inhibitor GM 6001 [$n = 31$ to 45 for each group; untreated, 100 ± 9%; conditioned (cond.) media, 151 ± 9%; TNFR1 and conditioned media, 113 ± 13%; anti-TNFα and conditioned media, 80 ± 10%; GM 6001 and conditioned media, 78 ± 9%]. Experiments were performed in parallel using the same conditioned media.

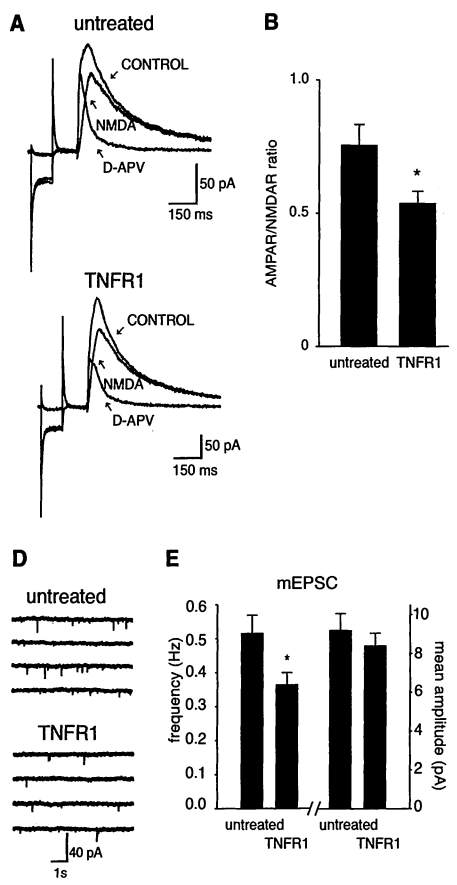

Fig. 4. Blocking TNFα action in hippocampal slices decreases synaptic strength. **(A)** Dual-component (AMPAR and NMDAR) EPSCs recorded at +40 mV from untreated and TNFR1-treated hippocampal slices. The NMDAR antagonist, D-APV, was applied to isolate the AMPAR EPSC, which was then subtracted from the dual-component EPSC to yield an NMDAR EPSC. **(B)** Quantitation of the AMPAR/NMDAR EPSC ratio in untreated and TNFR1-treated slices ($n = 10$ each group, *$P < 0.05$; control slices, 0.75 ± 0.08; TNFR1-treated slices, 0.54 ± 0.05). **(C)** Magnitude of paired-pulse facilitation at various interstimulus intervals in untreated and TNFR1-treated slices ($n = 22$ each group). **(D)** Examples of mEPSCs recorded from untreated and TNFR1-treated slices. **(E)** Mean mEPSC frequency and amplitude from untreated and TNFR1-treated slices ($n = 8$ each group, *$P < 0.05$; control slices, 0.52 ± 0.05 Hz, 9.2 ± 0.9 pA; TNFR1-treated slices, 0.36 ± 0.04 Hz, 8.4 ± 0.7 pA).

fected the synaptic localization of *N*-methyl-D-aspartate receptors (NMDARs) (*17*), but we observed no effect (Fig. 1G), a result consistent with the suggestion that synaptic NMDARs are less mobile than AMPARs (*18*). To test if the AMPARs delivered to synapses because of TNFα treatment are functional and modify synaptic strength, we recorded miniature excitatory postsynaptic currents (mEPSCs) before and after TNFα application (*19*). Within 10 min of its application, TNFα caused a significant increase in the mean frequency, but not in the mean peak amplitude, of mEPSCs (Fig. 1, H and I) (*20*).

TNFα is expressed in situ by glia and neurons (*21*), raising the possibility that endogenous TNFα influences AMPAR surface expression and synaptic transmission. To test this hypothesis, we examined the effects of treating cultures with a soluble form of the TNF receptor 1 (TNFR1), which functions as a TNFα antagonist (*13, 21*). Treatment with TNFR1 (10 μg/ml) for periods as short as 4 hours caused a clear (>60%) decrease in the surface expression of AMPARs (Fig. 2, A and B) (*22*). To determine whether endogenous TNFα also influences synaptic strength, we recorded mEPSCs from TNFR1-treated preparations (4 to 24 hours) and observed significant

decreases in both the mean frequency and amplitude of mEPSCs (Fig. 2, C and D) (*19*). As an additional test for the effects of endogenous TNFα, we treated cultures with an antibody to TNFα (anti-TNFα) (50 μg/ml), which functions as a TNFα antagonist (*13, 21*). This treatment also caused a decrease in AMPAR surface expression (Fig. 2, E and F).

The effects of TNFR1 and anti-TNFα indicate that endogenous TNFα influences AMPAR surface expression and synaptic strength. Where is this TNFα produced? To test if astrocytes were a major source of TNFα, we prepared astrocyte-conditioned media and examined its effects on AMPAR surface expression and mEPSCs (*23*). Application of conditioned media (for 15 min) caused a significant increase in the surface expression of AMPARs (Fig. 3, A and B) and also an increase in the mean frequency of mEPSCs when compared to the application of control media (Fig. 3, C and D). Thus, the effects of the conditioned media mimicked those of TNFα. To test whether the effects of the conditioned media required TNFα, we added one of three reagents to the media before its application: TNFR1, anti-TNFα, or the matrix metalloproteinase inhibitor GM 6001, which will prevent the release of TNFα (*24*). Each one of these reagents eliminated the effects of the conditioned media on AMPAR surface expression (Fig. 3, E and F), demonstrating that the presence of TNFα in the conditioned media is required for its enhancing action.

Although neuronal cultures are commonly used to examine the functions of glia (*1–6*), the interactions between glia and neurons in culture may not exactly replicate what happens in situ. To test whether endogenous TNFα also affects excitatory synapses in more-intact brain tissue, we incubated acutely prepared hippocampal slices in TNFR1 and then assayed synaptic strength by measuring the ratio of AMPAR- to NMDAR-mediated synaptic currents (*25*). Consistent with endogenous TNFα acting to influence synapses in a manner similar to that observed in culture, the AMPAR/NMDAR ratio was significantly smaller in treated than in untreated slices (Fig. 4, A and B). To assess whether TNFR1 incubation had an effect on presynaptic function, we examined paired-pulse facilitation, which is inversely correlated with the probability of neurotransmitter release (*26*). Incubation with TNFR1 had no effect on this form of short-term synaptic plasticity (Fig. 4C). Finally, we also examined mEPSCs and found that TNFR1 pretreatment caused a significant decrease in the frequency, although not in the mean amplitude, of mEPSCs (Fig. 4, D and E).

These results provide support for a novel role for glia in the rapid control of synaptic strength at excitatory synapses, as well as identify a protein, TNFα, that is necessary for fulfilling this function. The close apposition of astrocytes with excitatory synapses (*27*) provides a clear morphological substrate for facilitating this glial-neuronal communication. Our findings suggest possible novel roles for glial-released TNFα in normal and pathological brain function. For example, if neural activity influences TNFα production, this may contribute to the changes in synaptic strength that occur during various forms of synaptic plasticity, such as NMDAR-dependent long-term potentiation and long-term depression, which involve AMPAR trafficking (*14, 15*). TNFα also may contribute to neural injury (*21*), in part by increasing the surface expression of AMPARs; this hypothesis has therapeutic implications (*28*).

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12. Mixed neuronal-glial hippocampal cultures were prepared as described (*29*) and contained ~50% neurons and ~50% astrocytes (±20%) (as well as small numbers of microglia) as estimated by staining for the neuronal-specific marker MAP-2 and the astrocyte-specific marker glial fibrillary acidic protein. Surface AMPARs were visualized in 18- to 22-day-old cultures, by using an antibody to the NH_2-terminus of GluR1 after treatment with vehicle or TNFα at 37°C. Immediately before treatments, the glutamate receptor antagonists CNQX (20 μM), D-APV (50 μM), and LY31149 (50 μM) were applied to block possible activation of AMPARs, NMDARs, and mGluRs, respectively. Images were obtained with a 63× objective mounted on a Zeiss Axioskop, digitized with a cooled CCD camera (Hammamatsu, Bridgewater, NJ), and analyzed with Metamorph software (Universal Imaging, West Chester, PA). Each experimental manipulation was repeated a minimum of three times each with different culture preparations. *N* values in the text represent the number of microscope fields examined. Each field contained 1 to 4 neurons. Statistical significance was determined using Student's *t* -test. Errors in text and figures represent SEM Details regarding culture preparation, AMPAR staining, and image analysis can be found in (*30*).
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17. Cells were treated with vehicle or 3.5 to 6 nM of TNFα for 15 min at 37°C, chilled on ice, and washed three times with cold phosphate-buffered saline before fixation with 4% formaldehyde. Staining for GluR1 or the NMDAR subunit NR1 and synaptophysin was performed simultaneously. Images were collected as described (*12*), using a Zeiss Axiovert microscope connected to a Zeiss LSM 510 confocal system. Colocalization was calculated by measuring the magnitude of GluR1 or NR1 signal overlap with synaptophysin signal, using the "measure colocalization" mode of Metamorph Analysis. Detailed methods can be found in (*30*).
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19. Recordings of mEPSCs were made from 18- to 24-day-old cultures as described (*29*). TNFα (60 nM) and conditioned media were applied directly to cells through a large-bore pipette, by using a picospritzer (8 psi; 10 ms) every 10 s for 5 min. Control cells were administered vehicle (either Ringer's or culture media) in an identical fashion. On each experimental day, application of control or experimental (TNFα, conditioned media) solutions were interleaved and applied to cells from the same culture preparation. To examine the effects of TNFR1, we treated cultures with TNFR1 (10 μg/ml) and compared them with untreated sister cultures on the same day. Detailed methods can be found in (*30*).
20. Several lines of evidence [presented in (*30*)] suggest that the TNFα-induced increase in mEPSC frequency is due, at least in part, to an increase in synaptic AMPARs. Our results, however, do not rule out additional presynaptic effects of TNFα that may contribute to the observed increase in mEPSC frequency.
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23. Astrocyte-conditioned media was prepared as described (*31*). We also examined the cellular source of TNFα by staining cultures with a TNFα antibody and found that >95% of astrocytes exhibited TNFα immunoreactivity. Detailed methods can be found in (*30*).
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25. Transverse hippocampal slices (400 μm) were prepared from 2- to 4-week-old Sprague-Dawley rats, and standard recording techniques were used as described (*32*). Details of electrophysiological procedures can be found in (*30*).
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