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LETTERS

Synaptic scaling mediated by glial TNF-α

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Two general forms of synaptic plasticity that operate on different timescales are thought to contribute to the activity-dependent refinement of neural circuitry during development: (1) long-term potentiation (LTP) and long-term depression (LTD), which involve rapid adjustments in the strengths of individual synapses in response to specific patterns of correlated synaptic activity, and (2) homeostatic synaptic scaling, which entails uniform adjustments in the strength of all synapses on a cell in response to prolonged changes in the cell's electrical activity[1,2]. Without homeostatic synaptic scaling, neural networks can become unstable and perform suboptimally[1–3]. Although much is known about the mechanisms underlying LTP and LTD[4], little is known about the mechanisms responsible for synaptic scaling except that such scaling is due, at least in part, to alterations in receptor content at synapses[5–7]. Here we show that synaptic scaling in response to prolonged blockade of activity is mediated by the pro-inflammatory cytokine tumour-necrosis factor-α (TNF-α). Using mixtures of wild-type and TNF-α-deficient neurons and glia, we also show that glia are the source of the TNF-α that is required for this form of synaptic scaling. We suggest that by modulating TNF-α levels, glia actively participate in the homeostatic activity-dependent regulation of synaptic connectivity.

TNF-α increases the cell-surface expression of AMPA receptors (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors, or AMPARs) in cultured hippocampal neurons and influences synaptic strength in both cultures and hippocampal slices[8,9]. These findings raise the possibility that TNF-α might have an important role in NMDA (*N*-methyl-D-aspartate) receptor-dependent LTP and LTD, which similarly involve regulation of synaptic AMPARs[4]. To investigate whether TNF-α has a role in these forms of synaptic plasticity, we incubated acute rat hippocampal slices in high, saturating levels of TNF-α. Consistent with its effects in cultured neurons, incubating slices with TNF-α caused an increase in the ratio of AMPAR- to NMDAR-mediated synaptic currents (Fig. 1a). However, this same pretreatment did not affect the magnitude of LTP, nor did TNF-α treatment affect LTD (Fig. 1b) or the agonist-induced endocytosis of AMPARs in dissociated cultures (data not shown), a culture model for LTD[10].

To further examine the requirement of TNF-α in rapidly induced, long-lasting synaptic plasticity, we assayed LTP and LTD in hippocampal slices prepared from mice lacking TNF-α (*Tnf* knockout mice)[11]. Both LTP and LTD were normal in the absence of TNF-α-mediated signalling (Fig. 1c). Finally, we examined hippocampal slices from knockout mice lacking both TNF-α receptors, TNFR1 (*Tnfrsf1a*) and TNFR2 (*Tnfrsf1b*) (ref. 12). Similar to the *Tnf* knockout mice, hippocampal slices prepared from these mice expressed LTP and LTD equivalent to that observed in slices from wild-type mice (Fig. 1d).

Although these data indicate that TNF-α is not a requisite mediator of rapid forms of long-term synaptic plasticity, it influences AMPAR and $GABA_A$ (γ-aminobutyric acid) receptor trafficking in a manner that shares key features with the homeostatic scaling of excitatory and inhibitory synapses in response to prolonged blockade of activity[1,5–7,13]. If a soluble protein such as TNF-α has a critical role in synaptic scaling, it should accumulate in the extracellular medium of hippocampal cultures subject to chronic activity blockade. To test this prediction, we treated naive cultures with media collected from sister cultures treated with tetrototoxin for 48 h (TTX, which blocks voltage-gated Na^+ channels and action potential generation) and then measured cell-surface levels of AMPARs. Consistent with previous results[5,6,8], both acute treatment with TNF-α and chronic treatment with TTX substantially increased AMPAR surface expression (Fig. 2a).

Notably, acute treatment of cultures with conditioned medium from the TTX-treated cultures was similarly effective at increasing cell-surface AMPAR levels (Fig. 2b). To test whether the effects of the conditioned medium on AMPARs required TNF-α, we added a soluble form of the TNFR1 receptor (sTNFR), which scavenges TNF-α[8,9,14], to the medium before applying it to naive cultures. In the presence of sTNFR, conditioned medium from TTX-treated cultures no longer increased surface expression of AMPARs (Fig. 2b). Acute treatment of cultures with TTX alone had no effect on cell-surface AMPAR levels ($n = 66$; data not shown); 24-h TTX treatment was also insufficient to increase surface AMPARs ($n = 60$), as was the conditioned media from 24-h-treated cultures ($n = 60$; data not shown). These results suggest that TNF-α release is not rapidly modulated by activity, but that chronic activity blockade causes release of sufficient amounts of TNF-α to acutely increase surface AMPAR expression.

To test whether the observed increases in surface AMPAR levels occur at synapses in a manner that influences synaptic strength, we recorded miniature excitatory postsynaptic currents (mEPSCs) from presumptive pyramidal cells. In agreement with published results[5,13,15], chronic activity blockade led to an increase in mEPSC amplitude (Fig. 2c). Consistent with our immunocytochemical results, acute application of conditioned medium from TTX-treated cultures to naive sister cultures also caused an increase in mEPSC amplitude (Fig. 2c), an effect that was prevented by pretreating the conditioned medium with sTNFR (Fig. 2c). On average, the frequency of mEPSCs also increased after acute treatment with conditioned medium (data not shown), consistent with the effects of acute applications of TNF-α[8]. However, this change was temporary, as no chronic manipulation had a significant effect on mEPSC frequency. These results suggest that chronic activity blockade causes the release of soluble TNF-α that is necessary for the increases in surface AMPAR levels and synaptic strength caused by conditioned medium from TTX-treated cultures. Consistent with this hypothesis, an L929 cell death assay[16] revealed that the level of TNF-α in conditioned media from activity-blockaded cultures was significantly higher than in media from control cultures (Fig. 2d).

To test directly whether TNF-α is required for homeostatic synaptic scaling in response to activity blockade, and is not an incidental byproduct of manipulating activity, we interfered with TNF-α signalling during activity blockade by adding sTNFR during

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the second day of the two-day TTX treatment. This prevented any increase in mEPSC amplitude due to the activity blockade while, as expected, causing a modest decrease in the control mEPSC amplitude[8] (Fig. 2e). In addition to increasing excitatory synaptic strength, chronic activity blockade and acute application of TNF-α also decrease inhibitory synaptic strength[7,9]. To test whether TNF-α is required for the decrease in the amplitude of miniature inhibitory postsynaptic currents (mIPSCs) caused by activity blockade[7], we again added sTNFR during the second day of TTX treatment. This prevented the decrease in mIPSC amplitude caused by TTX treatment alone (Fig. 2f).

Our results thus far provide strong evidence that TNF-α is required for the scaling of both excitatory and inhibitory synapses in response to activity blockade. However, it is possible that a related ligand that binds TNF-α receptors is the actual signalling molecule required for this form of synaptic scaling. To test this hypothesis, we examined synaptic scaling in *Tnf* knockout ($Tnf^{-/-}$) mice. Although robust synaptic scaling was observed in cultures from wild-type mice, an increase in the amplitudes of mEPSCs in response to chronic activity blockade did not occur in hippocampal cultures from $Tnf^{-/-}$ mice (Fig. 3a, b).

Synaptic scaling is a bidirectional phenomenon in which excitatory synapses scale up in response to activity reduction, but scale down in response to increases in activity[1,2,5,15,17]. Although our data indicate that scaling up of excitatory synapses requires TNF-α signalling, scaling down could result either from a reduction in constitutively produced TNF-α, which actively maintains surface levels of AMPARs[8], or by signals that regulate synapses inversely to TNF-α. To determine whether signals other than TNF-α are responsible for homeostatic weakening of excitatory synapses[1,2,5,15], we treated $Tnf^{-/-}$ mouse cultures for two days with the $GABA_A$ receptor antagonist picrotoxin (PTX, 50 μM), because $GABA_A$ receptor currents are largely inhibitory at the culture age we studied[18]. PTX-treated $Tnf^{-/-}$ cultures showed a reduction in mEPSC amplitudes equivalent to that observed in PTX-treated, wild-type mouse cultures (Fig. 3c, d). These results suggest that synaptic strength is homeostatically regulated by at least two opposing signals, with TNF-α shifting neurons towards more excitation and less inhibition, and other signals (perhaps such as brain-derived neurotrophic factor[19]) pushing neurons towards less excitation and more inhibition.

Virtually all previous work on synaptic scaling has been done in dissociated cultures, raising the possibility that the phenomenon is an artefact of the culture system. To address this concern, we studied the effects of activity deprivation on hippocampal slice cultures, a preparation that expresses normal forms of LTP and LTD and seems to traffic AMPARs in a manner indistinguishable from that seen in acute slice preparations[20–22]. Similar to dissociated cultures, blocking activity in cultured slices from wild-type mice for two days caused an increase in the amplitude of mEPSCs recorded from CA1 pyramidal

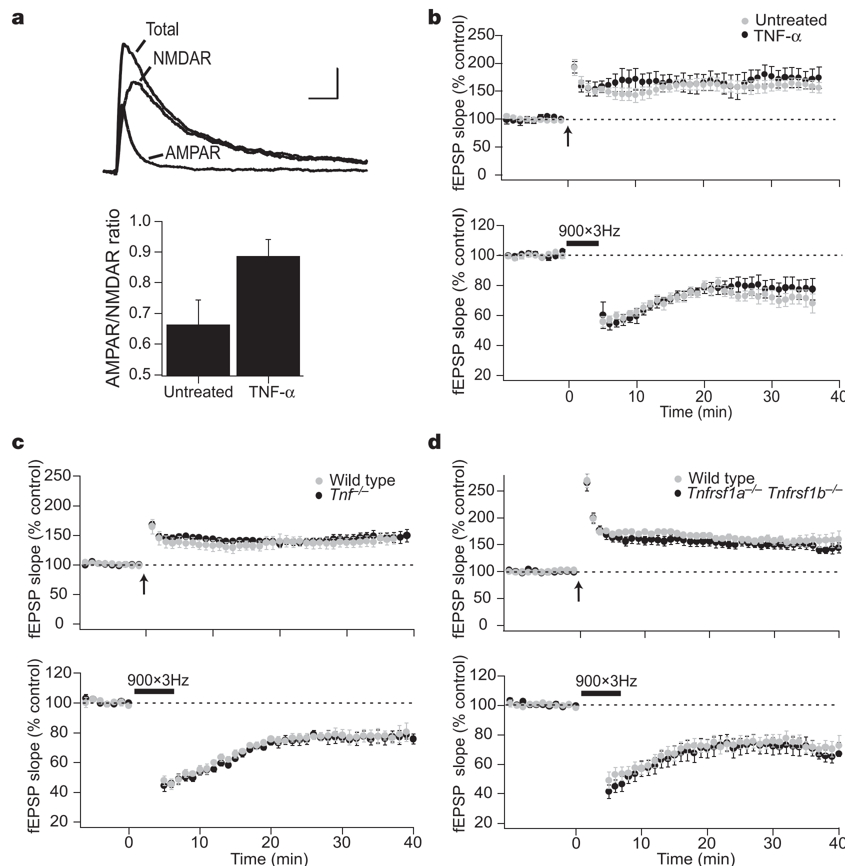

Figure 1 | TNF-α signalling is not required for CA1 hippocampal LTP or LTD. a, TNF-α treatment increases the ratio of AMPAR to NMDAR currents. Top, representative traces from whole-cell voltage-clamp recording at +40 mV, showing total current and isolated AMPAR and NMDAR currents (scale bars 25 pA, 50 ms). Bottom, group data showing that the AMPAR-to-NMDAR ratio is increased in cells from acute hippocampal slices treated with TNF-α (0.89 ± 0.05, $n = 8$), compared with control slices from the same animals (0.66 ± 0.08, $n = 7$, $P < 0.05$). **b**, Summary graphs showing LTP (top) (untreated, 162 ± 10%, $n = 5$; TNF-α, 173 ± 16%, $n = 4$) and LTD (bottom) (untreated, 71 ± 5%, $n = 8$; TNF-α, 78 ± 6%, $n = 8$) of field EPSPs in slices treated with TNF-α or untreated slices from the same animals. **c**, LTP and LTD in hippocampal slices from $Tnf^{-/-}$ mice (LTP, 139 ± 9%, $n = 5$; LTD, 76 ± 4%, $n = 6$) and wild-type mice (LTP, 145 ± 8%, $n = 4$; LTD, 78 ± 4%, $n = 3$). **d**, LTP and LTD in hippocampal slices from $Tnfrsf1a^{-/-}$ $Tnfrsf1b^{-/-}$ double knockout mice (LTP, 150 ± 8%, $n = 9$; LTD, 72 ± 6%, $n = 12$) and wild-type mice (LTP, 157 ± 8%, $n = 11$; LTD, 75 ± 4%, $n = 15$). Error bars show s.e.m.

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cells (Fig. 3e, f). However, slices prepared from $Tnf^{-/-}$ mice did not show synaptic scaling (Fig. 3e, f). These data suggest that synaptic scaling requiring TNF-α is a general phenomenon of neuronal circuits, and is not restricted to dissociated culture preparations. Consistent with this proposal, basal synaptic strength in acute hippocampal slices is influenced by constitutively released TNF-α[8].

Previous work has implied that neurons themselves sense their overall level of activity and generate the signals responsible for synaptic scaling[1,2,17]. However, we have found that TNF-α is constitutively produced by glia[8], suggesting that they, not neurons, might be the source of the TNF-α that is required for synaptic scaling in response to activity blockade. Glia are well placed to sense the overall activity of a network[23,24] through glutamate spillover[25], direct synaptic contact[26] or from other activity-regulated signals, and then provide a feedback signal to regulate network behaviour. To test directly the hypothesis that the TNF-α required for homeostatic synaptic strengthening is produced by glia, we plated either wild-type or $Tnf^{-/-}$ neurons onto confluent beds of glia derived from either wild-type or $Tnf^{-/-}$ hippocampi (Fig. 4a). Consistent with our earlier findings, $Tnf^{-/-}$ neurons plated on $Tnf^{-/-}$ glia did not show synaptic scaling in response to TTX treatment (Fig. 4c, d). However, $Tnf^{-/-}$ neurons from the same culture preparation plated onto wild-type glia showed robust increases in mEPSC amplitude after the same TTX treatment (Fig. 4c, d). This strongly suggests that glial-derived TNF-α is sufficient to induce synaptic scaling in response to activity deprivation.

As neurons can also produce TNF-α, we then tested whether glial-derived TNF-α is required for the scaling up of excitatory synaptic

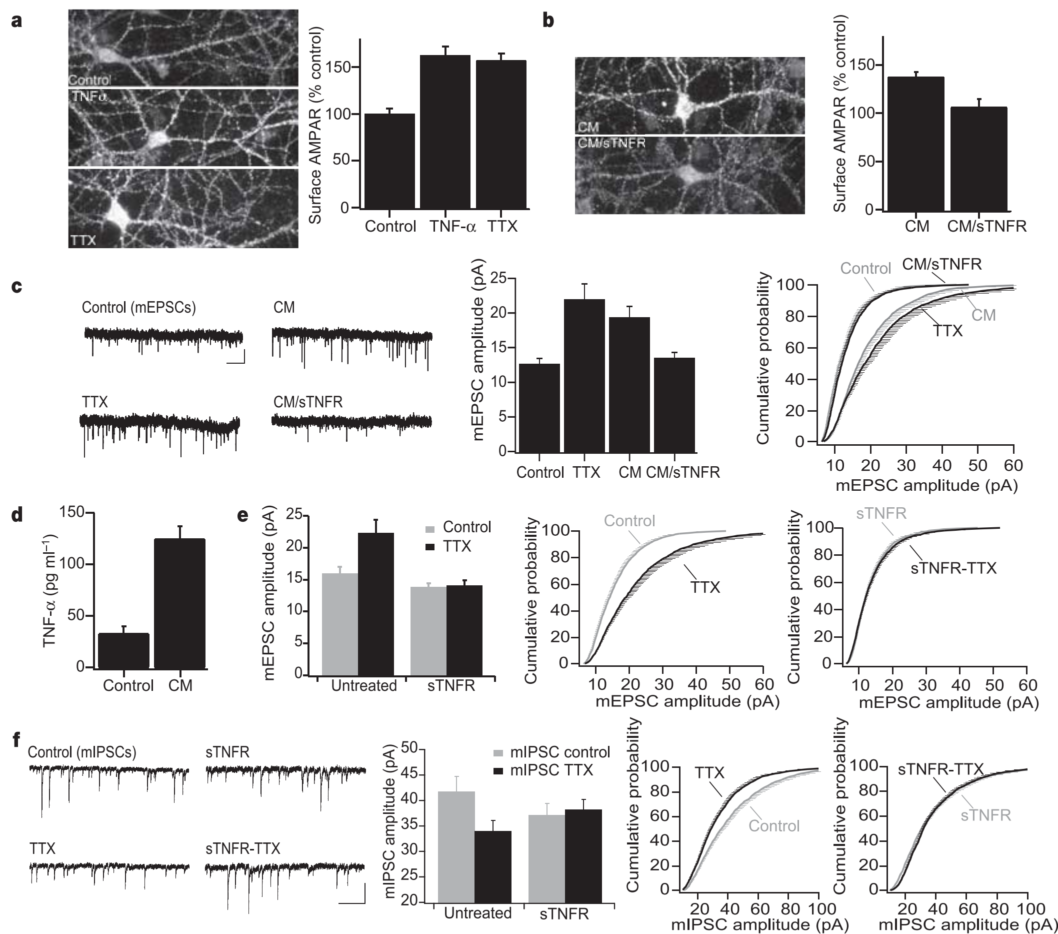

Figure 2 | TNF-α mediates synaptic scaling during activity blockade. **a**, Representative micrographs and group data showing that acute TNF-α application or two days of TTX treatment increase AMPAR surface expression in cultured hippocampal neurons relative to untreated neurons (TNF-α, 162 ± 10% of control levels, $P < 0.0001$, $n = 72$; TTX, 157 ± 7%, $P < 0.005$, $n = 90$). **b**, Sample micrographs and group data showing that acute treatment with conditioned medium (CM) from cultures treated for two days with TTX increases AMPAR surface expression compared with untreated neurons (CM, 138 ± 5%, $P < 0.0001$, $n = 174$). However, conditioned medium incubated with sTNFR does not increase AMPAR surface expression (CM/sTNFR, 106 ± 8%, $P > 0.9$, $n = 75$). **c**, Left, examples of mEPSCs recorded from cultured neurons, either untreated (control), treated for two days with TTX, acutely treated with conditioned medium from the TTX-treated cultures (CM), or acutely treated with conditioned medium incubated with sTNFR (CM/sTNFR) (scale bars 20 pA, 1 s). Middle, histogram of average mEPSC amplitudes showing that TTX (22.0 ± 2.2 pA, $n = 11$ cells, $P < 0.0001$) and CM (19.4 ± 1.5 pA, $P < 0.002$, $n = 14$) but not CM/sTNFR (13.6 ± 0.7 pA, $P > 0.9$, $n = 11$) significantly increase mEPSC amplitude compared with control cells (12.7 ± 0.7 pA, $n = 14$). Right, cumulative distributions of mEPSC amplitudes from cells shown in the histogram. **d**, TNF-α levels measured in media from control and TTX-treated cultures (control, 33 ± 7 pg ml^{-1}; CM, 125 ± 12 pg ml^{-1}; $n = 9$; $P < 0.0001$). **e**, Group data showing that addition of sTNFR during TTX treatment prevents the increase in mEPSC amplitude seen in TTX-treated sister cultures (control, 16.0 ± 1.0 pA, $n = 17$; TTX, 22.4 ± 2.0 pA, $n = 12$; sTNFR, 13.9 ± 0.6 pA, $n = 15$, $P < 0.05$; sTNFR-TTX, 14.1 ± 0.8 pA; $n = 16$, $P > 0.7$). Cumulative amplitude distributions of control (grey) and TTX-treated (black) cells, showing a rightward shift in mEPSC amplitudes attributable to TTX, and no shift in distribution of sTNFR-treated and sTNFR-TTX-treated cells. **f**, Sample recordings, average mIPSC amplitudes and cumulative amplitude distributions of mIPSCs from cells chronically treated with TTX, sTNFR or both, showing that inclusion of sTNFR also prevents the decrease in mIPSC amplitude observed after TTX treatment (TTX, 34.0 ± 2.0 pA; control, 41.8 ± 3.0 pA, $P < 0.05$, $n = 12$–14; sTNFR-TTX, 38.2 ± 2.1 pA; sTNFR, 37.1 ± 2.3 pA, $P > 0.9$, $n = 12$–14; scale bars 50 pA, 1 s). Error bars show s.e.m.

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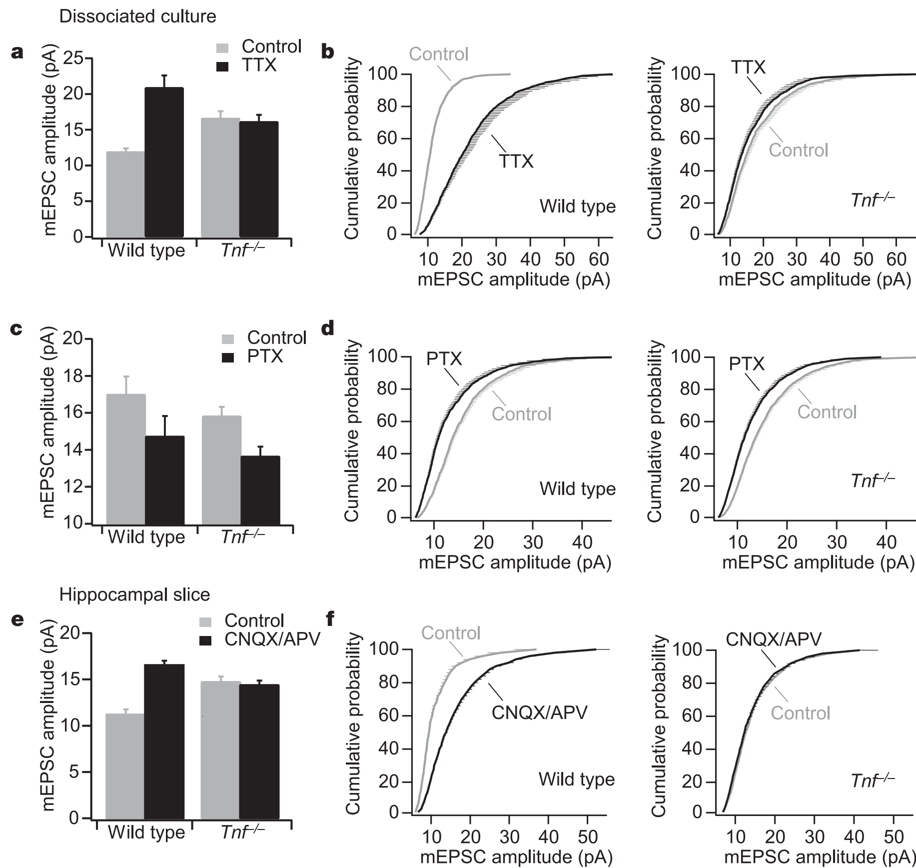

Figure 3 | *Tnf*$^{-/-}$ neurons scale down but not up following activity manipulations. **a**, Mean mEPSC amplitude of control and TTX-treated neurons from wild-type mouse cultures (TTX, 20.9 ± 1.7 pA, $n = 12$; control, 11.9 ± 0.5 pA, $n = 12$; $P < 0.0001$) and *Tnf*$^{-/-}$ cultures (TTX, 16.1 ± 0.9 pA, $n = 17$; control, 16.6 ± 1.0 pA, $n = 15$; $P > 0.7$). **b**, Cumulative mEPSC amplitude distributions of wild-type and *Tnf*$^{-/-}$ mouse cultures for control (grey) or TTX-treated (black) cells. **c**, Mean mEPSC amplitude from control and PTX-treated neurons from wild-type cultures (PTX, 14.7 ± 1.0 pA, $n = 13$; control, 17.0 ± 0.9 pA, $n = 12$; $P < 0.03$) and *Tnf*$^{-/-}$ mouse cultures (PTX, 13.7 ± 0.5 pA, $n = 20$; control, 15.7 ± 0.6 pA, $n = 17$; $P < 0.002$). **d**, Cumulative mEPSC amplitude distributions from wild-type and *Tnf*$^{-/-}$ cultures. **e**, Mean mEPSC amplitudes from hippocampal slice cultures of untreated neurons and neurons treated with the glutamate receptor antagonists CNQX and APV, in wild-type mice (CNQX/APV, 16.5 ± 0.5 pA, $n = 15$; control, 11.2 ± 0.6 pA, $n = 15$; $P < 0.0001$) and *Tnf*$^{-/-}$ mice (CNQX/APV, 14.4 ± 0.5 pA, $n = 18$; control, 14.7 ± 0.6 pA, $n = 17$; $P > 0.6$). **f**, Cumulative mEPSC amplitude distributions from wild-type and *Tnf*$^{-/-}$ mouse slice cultures. Error bars show s.e.m.

responses. As expected, wild-type neurons plated onto wild-type glia showed robust synaptic scaling (Fig. 4e, f). However, wild-type neurons from the same culture preparation plated onto *Tnf*$^{-/-}$ glia no longer showed an increase in mEPSC amplitude in response to chronic activity blockade (Fig. 4e, f). Together, these data indicate that glial-derived TNF-α is both necessary and sufficient to induce the increase in mEPSC amplitude that results from chronic activity deprivation, and we therefore suggest that glial TNF-α is a critical signalling component for this form of synaptic scaling.

How might glia sense the overall activity of an adjacent neuronal network? A straightforward hypothesis is that glia are sensitive to glutamate, the level of which would correlate with local excitatory synaptic activity. As no neurons are present in glial-only cultures (which release significant levels of TNF-α[8]), we reasoned that these cultures represent a zero-activity state. Using changes in the surface expression of AMPARs as a bioassay for TNF-α, we compared the effects of conditioned medium from glial cultures (GCM) to conditioned medium from sister glial cultures treated with glutamate (100 μM) for the duration of the conditioning (48 h), a manipulation used to mimic exposure of glia to active neural networks. As we expected[8], treatment of neuronal cultures with GCM increased cell-surface AMPAR levels (Fig. 4g). However, GCM from glutamate-treated glia was significantly less effective at increasing surface AMPAR levels (Fig. 4g). The amount of TNF-α in GCM was variable across preparations (340 ± 182 pg ml^{-1}, $n = 6$; data not shown), as measured by an L929 cell death assay[16], but again glutamate treatment consistently reduced TNF-α levels from individual preparations by about 50% (Fig. 4h). Thus, ambient glutamate might be one mechanism by which glia sense the level of neuronal activity and adjust TNF-α production.

Recent evidence suggests that glia act, at least in part, through the release of soluble factors to promote the formation of excitatory synapses during early development, and may also be important for their maintenance[27,28]. Our data extend these observations by showing that glia are actively involved in mediating an important form of activity-dependent plasticity at established functioning synapses. Thus, through their release of different factors at different developmental stages (for example, thrombospondin release early during development[27] and TNF-α release at later times), glia may be actively involved in both the establishment of neural circuits as well as their subsequent activity-dependent regulation. Although we have not identified the subtype of glia that is responsible for TNF-α production, two likely sources include astrocytes and microglia (which typically make up <5% of the cells in the glial feeder layer in our cultures).

Combined with recent work on the neuronal role of MHC (major histocompatibility complex) class I molecules[29], our results showing a previously unrecognized neuronal function for constitutively released TNF-α suggest that, perhaps owing to the relative isolation of the nervous and immune systems, traditional immune signalling

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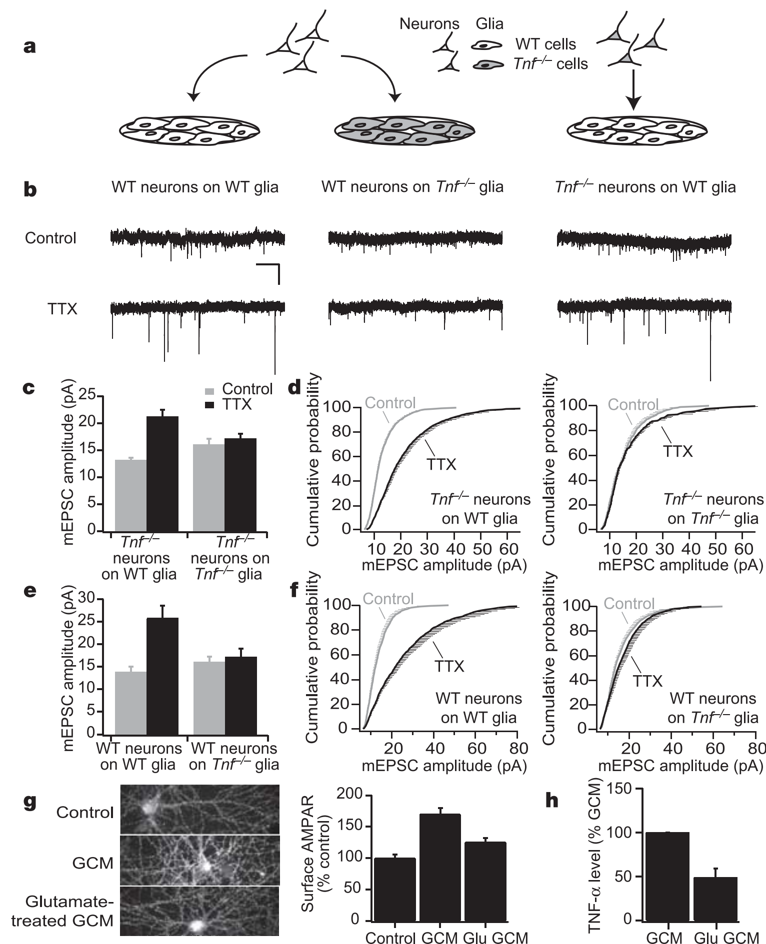

Figure 4 | Glia are the source of TNF-α required for synaptic scaling during activity blockade. a, Diagram illustrating how wild-type (WT) mouse neurons (white) were plated onto beds of either wild-type (white) or $Tnf^{-/-}$ (grey) mouse glia. $Tnf^{-/-}$ neurons (grey) were plated onto wild-type mouse glia. **b**, Representative recording of mEPSCs from control and TTX-treated cells, from neurons grown on different types of glia (scale bars 20 pA, 1 s). **c**, Mean amplitudes of mEPSCs recorded from $Tnf^{-/-}$ neurons showing that TTX-induced increases in mEPSC amplitude occur when $Tnf^{-/-}$ neurons are plated on wild-type glia (TTX, 21.2 ± 1.2 pA, $n = 17$; control, 13.2 ± 0.4 pA, $n = 15$; $P < 0.0001$) but not on $Tnf^{-/-}$ glia (TTX, 17.2 ± 0.8 pA, $n = 6$; control, 16.0 ± 1.1 pA, $n = 6$, $P > 0.4$). **d**, Cumulative distribution of mEPSC amplitudes for $Tnf^{-/-}$ neurons grown on wild-type or $Tnf^{-/-}$ glia. **e**, Mean mEPSC amplitudes recorded from wild-type neurons, showing that TTX-induced increases in mEPSC amplitude occur when wild-type neurons are plated on wild-type glia (TTX, 25.7 ± 2.8 pA, $n = 10$; control, 13.9 ± 1.1 pA, $n = 9$; $P < 0.002$) but not on $Tnf^{-/-}$ glia (TTX, 17.3 ± 1.8 pA, $n = 14$; control, 16.1 ± 1.1 pA, $n = 12$; $P > 0.6$). **f**, Cumulative mEPSC amplitude distributions for control and TTX-treated wild-type neurons plated on wild-type or $Tnf^{-/-}$ glia. **g**, Micrographs and group data showing that conditioned medium from glutamate-treated glia (glu GCM) is less effective than conditioned medium from untreated glia (GCM) at increasing cell-surface expression of AMPARs (glu GCM, 125 ± 7% of control cells; GCM, 170 ± 10% of control cells; $n = 90$; $P < 0.01$). **h**, Glutamate treatment also decreases TNF-α levels measured in GCM using an L929 cell death assay (49 ± 10% of untreated GCM, $n = 4$, $P < 0.01$). Error bars show s.e.m.

molecules have been adapted to serve different roles in nervous system function. Our data also suggest that changes in the levels of immune molecules in the brain during disease or in response to physical insults may lead to unexpected dysfunctions, because of both their direct neural actions and the disruption of their normal adaptive roles in the brain.

METHODS

See Supplementary Information for detailed methods.

Immunostaining and electrophysiology in cultures. Postnatal mixed hippocampal cultures were prepared as previously described[9], and assayed at 13–15 days *in vitro*. For some experiments, mouse neurons were plated onto previously prepared confluent beds of 2–4-week-old mouse glia. FUDR (5-fluoro-2′-deoxyuridine) was added shortly after plating to prevent the proliferation of new glia. Surface AMPARs were visualized as described[9]. *n* values in the text represent the number of microscope fields examined. Statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's *t*-tests. Whole-cell patch-clamp recordings of mEPSCs were made essentially as described[10]. A positive control for synaptic scaling was performed on every culture batch. Cumulative distributions were generated using 200 consecutive mEPSCs from each cell, averaged across all cells, and compared using a Kolmogorov–Smirnov two-sample test.

Slice electrophysiology. Acute transverse hippocampal slices (400 μm) were prepared from 2–4-week-old Sprague-Dawley rats or mice as previously described[9]. LTP was induced with 1-s, 100-Hz trains, repeated three times at 20-s intervals. LTD was induced using 900 pulses at 3 Hz. Hippocampal slice cultures were prepared from postnatal day (P) 5–6 mice essentially as described[30], as modified by O. Schluter and W. Xu (personal communication). After 6–9 days *in vitro*, whole-cell recordings of mEPSCs from CA1 pyramidal cells were made, with the addition of TTX (500 nM) and sucrose (20 mM) to the external solution.

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