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More than a sidekick: glia and homeostatic synaptic plasticity

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Homeostatic synaptic plasticity is thought to have a crucial role in stabilizing the activity of neurons and networks, but the mechanisms are poorly understood. In a recent study, Stellwagen and Malenka have shown that synaptic scaling can be induced by activity-dependent changes in release of the cytokine tumor necrosis factor-α (TNF-α) and, surprisingly, that the source of TNF-α is glia rather than neurons. In addition to provide insight into the mechanisms of homeostatic plasticity, these data argue for the first time for an equal partnership between glial cells and neurons in the generation of an important form of synaptic plasticity.

Introduction

Neuroscientists (not surprisingly) tend to have a neuron-centric view of the cellular processes that drive nervous-system function. It is a shock to be reminded that there are roughly ten glial cells for every neuron in the CNS, which would seem to belie the view that they are merely a passive support system for the important work of the neurons. Indeed, it is becoming increasingly clear that neuron–glial signaling is complex and can modulate the rapid electrical signaling properties of neuronal connections [1], but the nature and extent of this partnership is still largely mysterious. Are glial cells forever doomed to be Watson to the neuron's Holmes, or do they on occasion get to call the shots? A recent report by Stellwagen and Malenka [2] has raised the possibility of a much more equal partnership between neurons and glial cells in the generation of an important form of synaptic plasticity – synaptic scaling. This report strongly suggests that a factor released by glia, tumor necrosis factor-α (TNF-α), is part of the core signaling pathway that homeostatically regulates neuronal synaptic strength in response to a prolonged (several days) drop in neuronal activity.

TNF-α and glutamate-receptor trafficking

TNF-α (a cytokine) was first characterized as an immune molecule that is part of the inflammatory response to pathological states such as injury, and can initiate cell death [3]. Surprisingly, TNF-α is produced within the CNS and is constitutively released by glial cells, and can increase accumulation of a subtype of glutamate receptor, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, on neuronal cell membranes [4,5]. AMPA receptors carry the majority of excitatory synaptic currents in the CNS; thus, increasing surface expression of AMPA receptors should make neurons more sensitive to released glutamate and might contribute to the ability of TNF-α to promote excitotoxicity. Interestingly, many forms of synaptic plasticity operate through changes in the number of AMPA receptors in the synaptic membrane [6,7], raising the intriguing possibility that TNF-α might participate in the generation of some forms of synaptic plasticity – a possibility that has been confirmed in the study by Stellwagen and Malenka [2].

Role for released TNF-α in synaptic scaling

There are many forms of synaptic plasticity present at central synapses [8]: two important and well-characterized forms are long-term potentiation (LTP) [6] and synaptic scaling [7], both of which are generated, in part, through changes in synaptic AMPA receptor number. LTP is associative in nature and is thought to generate synapse-specific changes in synaptic weights that might be used to store information. Synaptic scaling, in contrast, is non-associative and works in a homeostatic manner to promote stability in neuronal firing rates by adjusting the strength of all synapses of a neuron to keep firing rates roughly constant. Without

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such homeostatic adjustment of neuronal excitability, the activity of neurons and networks is easily destabilized by changes in synapse number or strength (including those induced by LTP) [7].

The synaptic-scaling field is young and the molecular-signaling pathways involved are still almost completely unknown. Most work on synaptic scaling has been carried out *in vitro* using culture systems derived from mixed astrocytes and central neurons (cortical, spinal or hippocampal in origin). The basic observation in all of these systems is that long-lasting modulation of ongoing activity induces compensatory changes in synaptic strengths: if activity is blocked [generally by blocking spiking with tetrodotoxin (TTX)], all excitatory synaptic strengths of a neuron are scaled up in amplitude, and vice versa [9–11]. This keeps the activity of these *in vitro* networks relatively constant. Synaptic scaling also operates *in vivo* [7] where it might have an important role in balancing excitation and inhibition during periods of intense change in synapse number and strength (e.g. during certain periods of development), and preventing pathological states such as epilepsy. Given the probable importance of this form of plasticity in normal brain function, understanding which molecular signaling pathways – and which cell types – are involved is of major interest.

Fair progress has been made towards understanding the expression mechanism of synaptic scaling. There is clear evidence (from all studies using central neuronal cultures as a model system) that a major contributor to the changes in synaptic strength that underlies synaptic scaling is the change in the number of AMPA receptors that are clustered in the synaptic membrane [9–13]. Much less clear has been the route through which changes in activity are translated into changes in receptor number. Through elegant experiments, Stellwagen and Malenka [2] have suggested that a drop in neuronal activity triggers release of TNF-α, which then regulates the cell-surface level of AMPA receptor number in a compensatory direction. Acute application of TNF-α increases synaptic strength in a way that is similar to prolonged activity blockade (with TTX), and acute application of conditioned medium from activity-blocked cultures (blocked for at least 48 h) also increased synaptic strengths, indicating that a soluble factor was involved. If the conditioned medium was first treated with a soluble form of the TNF-α receptor (sTNFR) to sop up TNF-α, the ability of the conditioned medium to enhance synaptic strength was blocked. Finally, the ability of TTX to increase synaptic strength was also blocked by sTNFR. All this suggests that prolonged TTX treatment leads to TNF-α release, which then increases cell-surface AMPA receptor number and, thus, synaptic strength. Interestingly, TNF-α does not modulate hippocampal LTP indicating that, although both LTP and synaptic scaling result from changes in synaptic AMPA receptor numbers, the regulatory pathways that underlie them are distinct.

Source of TNF-α: neurons or glial cells?

If TNF-α mediates the synaptic scaling that is induced by prolonged activity blockade, where does it come from?

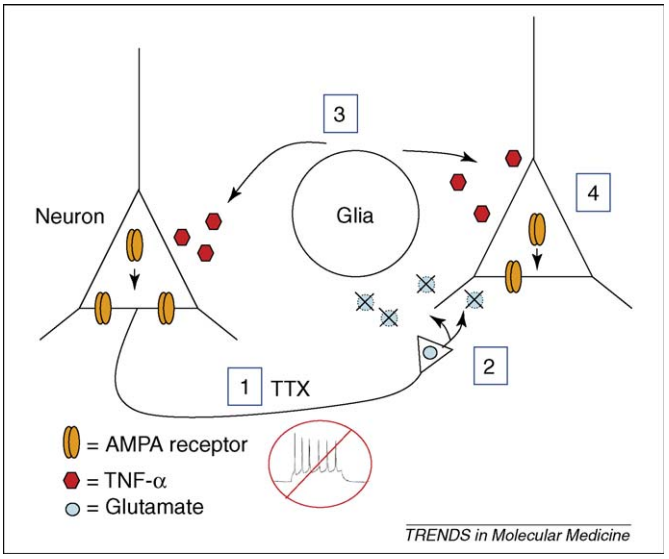

Figure 1. Model of neuron–glia–neuron interactions involved in synaptic scaling. When neuronal spiking is blocked (1), glutamate that is released from axon terminals decreases (2); this acts on glial cells to increase TNF-α release (3). TNF-α then acts on the neurons to enhance trafficking of AMPA-type glutamate receptors to the neuronal membrane (4).

Because synaptic scaling can be induced by hyperpolarization of individual neurons and independently of glutamate-receptor signaling [14,15], it has been believed to be a cell-autonomous process. Surprisingly, cultures made from TNF-α knockout mice in which wild-type neurons were plated on knockout glia showed that TNF-α is derived from glial cells rather than neurons. This leads to the unexpected conclusion that blockade of neuronal activity by TTX is sensed by glia (possibly through a reduction in neuronal glutamate release), and then glia signal back to the neurons through release of TNF-α (Figure 1). These data suggest that glial cells are an integral and necessary part of the signaling pathway mediating synaptic scaling.

Unresolved issues

I suspect that synaptic plasticity mechanisms only appear simple and straightforward before much has been published on them. This study is intriguing and convincing, but is not the entire story and is, in some respects, difficult to reconcile with earlier studies. Notably, synaptic scaling is a graded process in which synaptic strength builds up as a function of length of deprivation so that a change in synaptic strength becomes measurable after about 12 h, and after 24 h is almost as large as that produced by 48 h of blockade [9,13]. However, Stellwegan and Malenka have shown that conditioned medium from cultures that are treated with TTX for 24 h does not increase synaptic strength – it requires 48 h before this effect is produced. This discrepancy is puzzling and raises the possibility that synaptic scaling induced by shorter periods of deprivation is not mediated by TNF-α. Perhaps glial TNF-α is necessary for long-term maintenance of synaptic scaling during prolonged (>48 h) periods of deprivation, in which case this might be an important mechanism that mediates compensation to pathological

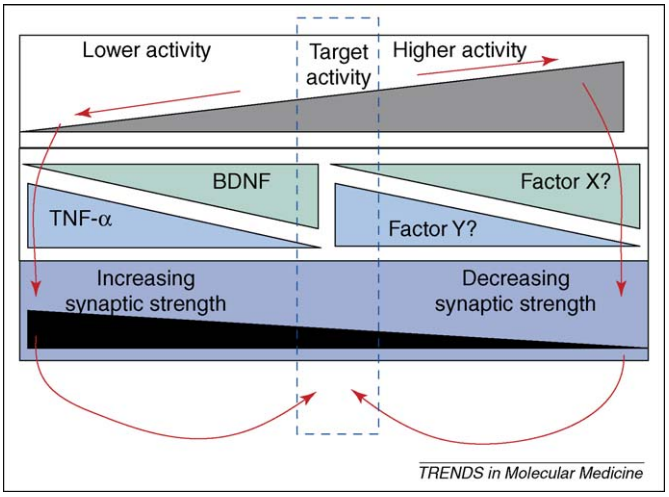

Figure 2. Activity-dependent regulation of multiple factors might contribute to synaptic scaling. When activity falls below some target range (indicated by the area outlined by the dashed line), this reduces release of BDNF and increases release of TNF-α. Both of these changes enhance excitatory synaptic strengths, which will move activity back towards the target range. Conversely, if activity rises too high, synaptic strengths are reduced; the factor(s) responsible for synaptic scaling in this direction have not been identified.

conditions such as denervation following stroke or other insults.

Another issue is that synaptic scaling is a bi-directional process; however, Stellwagen and Malenka have shown that a drop in TNF-α does not mediate the reduction in synaptic strength produced by increased neuronal firing. This raises the possibility of the existence of multiple factors acting in a push–pull fashion to regulate synaptic strengths over the entire range of activity regimes that a neuron might experience. For example, an earlier study [16] showed that addition of brain-derived neurotrophic factor (BDNF), which is released by neurons in an activity-dependent manner, to the culture medium can reverse TTX-induced synaptic scaling in a dose-dependent way, and that blocking BDNF signaling (using a soluble form of the BDNF receptor, TrkB) mimics the effects of TTX. Thus, BDNF and TNF-α have opposing effects on synaptic strengths (Figure 2), but how signals from these two pathways are integrated to mediate synaptic scaling is unclear. Finally, similar to TNF-α, BDNF signaling cannot account for the reduction in synaptic strengths produced by increased neuronal firing, indicating that there is some additional factor (or factors) waiting to be discovered that mediate the activity-dependent scaling down of synaptic strengths (Figure 2).

Concluding remarks

If the LTP field is any guide, sorting all this out is likely to take some time [17]. Nonetheless, the study by Stellwegan and Malenka [2] is a major step in the right direction, and raises some intriguing therapeutic possibilities for the treatment of disorders such as trauma-induced epilepsy. If part of the response to injury-induced denervation is a TNF-α-induced increase in excitatory synaptic transmission, this might prove a promising target pathway for therapeutic interventions. It remains something of a mystery why glia should be involved at all. Why not utilize a simple(r) cell-autonomous process that requires only a means for individual neurons to sense their own activity levels? Under certain pathological conditions, entire networks are silenced or hyperactive, and glial cells are well-placed to sense such extreme network perturbations [1]. It is tempting to speculate that TNF-α-induced synaptic scaling is a kind of 'panic button' triggered by prolonged and extreme drops in network activity. However, whether there are multiple signaling pathways that cooperate to regulate synaptic scaling over different time scales and activity regimes remains to be determined. Finally, perhaps the most interesting aspect of this study is its revision of our thinking about the nature of the neuron–glial partnership. Rather than the usual 'Batman and Robin' view of neuron–glial interactions, this seems to be a true partnership – more akin to that of 'Thelma and Louise' or 'Butch Cassidy and the Sundance Kid' – but (one hopes) without the nasty ending.

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