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A Role for Retinoic Acid in Homeostatic Plasticity

Rachel D. Groth[1,*] and **Richard W. Tsien**[1,*]
[1]Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305, USA

Abstract

Prolonged changes in neuronal activity trigger compensatory modifications in synaptic function to restore firing rates to normal levels. In this issue of *Neuron*, Aoto et al. demonstrate that synthesis of retinoic acid offsets chronic network inactivity by increasing synaptic strength through upregulation of GluR1 receptors.

Modulation of synaptic transmission in response to specific patterns of neuronal firing underlies the storage of memory traces in the mammalian CNS. Thus, understanding how synaptic connections are preserved and modified is of particular importance. Interestingly, decreasing activity in a neuron *before* synapse formation leads to a reduction in functional synaptic inputs to that neuron. Reducing activity in a single neuron *after* synapses are established, however, leads to a compensatory increase in synaptic inputs that tends to restore the neuronal firing to "normal" levels (Burrone et al., 2002). Keeping neuronal activity within an optimal range is thought to be necessary for safeguarding information processing capabilities.

Homeostatic plasticity has often been studied by means of pharmacological manipulations that block neuronal firing and/or synaptic transmission in cultured neurons (Turrigiano et al., 1998). Chronically decreasing the activity of a network triggers a *quantitative* change in synaptic strength, typically involving an increase in the size of the unitary synaptic current produced by a single quantum of released transmitter. This quantal amplitude is reflected by the amplitude of spontaneous miniature excitatory postsynaptic currents (mEPSCs) and has been attributed to an increased number of AMPA receptors in the postsynaptic membrane. To a first approximation, the regulation can be conceptualized as a uniform multiplication of the strength of individual synapses, which would preserve the relative strengths of synapses while scaling the intensity of the overall synaptic input to within useful bounds (Turrigiano and Nelson, 1998).

Adaptation to inactivity can also involve *qualitative* changes in the properties of synaptic transmission that could fundamentally alter the way that neurons perform computations. In addition to increasing the total number of postsynaptic AMPA-type glutamate receptors, prolonged blockade of activity can also trigger an adaptive switchover from GluR2-containing (Ca^{2+}-impermeable) receptors to homomeric GluR1 (Ca^{2+}-permeable) receptors (Thiagarajan et al., 2007). Changing the relationship between glutamate release and Ca^{2+} influx will in turn alter the neuron's ability to undergo further synaptic plasticity, a phenomenon known as metaplasticity. Expression of homeostatic synaptic adaptation is not the exclusive monopoly of postsynaptic elements. For example, presynaptic changes in the probability of vesicular release can also occur as a consequence of activity blockade (see Thiagarajan et al., 2007 for references).

*Correspondence: E-mail: rwtsien@stanford.edu (R.W.T.), E-mail: rgroth@stanford.edu (R.D.G.).

Three signaling molecules have recently gained attention for their role in expression of homeostatic plasticity following activity blockade: the β isoform of Ca^{2+} and calmodulin-dependent protein kinase II (β-CaMKII), the immediate early gene product Arc/Arg3.1, and the cytokine tumor necrosis factor-α (TNFα) (Thiagarajan et al., 2007; Shepherd et al., 2006; Stellwagen and Malenka, 2006). Blockade of neuronal activity increases the expression of βCaMKII within neurons (Thiagarajan et al., 2007) and TNFα within glial cells (Stellwagen and Malenka, 2006). Individually, β-CaMKII and TNFα both have been shown to increase synaptic strength by increasing surface expression of GluR2-lacking AMPA receptors (M. Lindskog, T.C. Thiagrajan, and R.W.T., unpublished data; Stellwagen and Malenka, 2006). Expression of Arc/Arg3.1 is dramatically *decreased* by activity blockade, thereby dampening its ability to trigger endocytosis of AMPA receptors, with the overall effect of increasing surface GluR1 receptors (Shepherd et al., 2006). Despite the aforementioned progress, a comprehensive understanding of this kind of synaptic regulation remains elusive.

In this issue of *Neuron*, Aoto et al. (2008) provide compelling evidence for a novel pathway in homeostatic adaptation to inactivity involving retinoic acid (RA), the biologically active derivative of vitamin A (retinol), and its receptor RARα. Best known for its role in regulating neural development, RA has recently emerged as an important signaling molecule and regulator of synaptic plasticity in the adult CNS as well. For example, vitamin A deprivation prevents long-term potentiation (LTP) and reduces long-term depression (LTD) in the hippocampus (Maden, 2002). Adding to this growing literature, Aoto and colleagues (2008) found that acute application of RA increased mEPSC amplitude in both cultured hippocampal slices and dissociated hippocampal cultures, suggesting that RA may be involved in homeostatic plasticity. Because RA is a lipophilic molecule that can directly pass through cell membranes, regulation of its synthesis is all-important for its signaling function. RA is derived from vitamin A in two oxidative steps: first, the conversion of retinol to retinal by retinol dehydrogenases (RoDHs), and second, the oxidation of retinal to RA by retinal dehydrogenases (RALDHs). Blocking RA synthesis with inhibitors of either RoDH or RALDH prevented the inactivity-induced increase in mEPSC amplitude elicited by 24 hr blockade of excitability and NMDA receptor function. Furthermore, such activity blockade occluded the ability of RA to increase mEPSC amplitude, implicating endogenous RA in the adaptation to inactivity.

To determine whether blocking neuronal activity increases RA synthesis, Aoto et al. (2008) turned to a reporter construct that reads out RA activation of nuclear RA receptors (RARs) by putting multiple RA response elements in control of the transcription of GFP. They found that blocking neuronal activity increased GFP expression in transfected neurons, implying that inactivity triggers synthesis of RA. Furthermore, when HEK293 cells transfected with the GFP-based reporter were plated with activity-deprived neurons, increased expression of GFP was also observed, confirming that RA can act as a membrane-permeant messenger. Aoto and colleagues (2008) attributed the RA production to neurons (rather than glia) because they found strong RALDH1 immunoreactivity in neurons, extending out into the neurites.

To explore the role of RA in adaptation to inactivity, Aoto et al. (2008) sought to determine how RA increased mEPSC amplitude. They demonstrated that RA increased surface GluR1 expression and that a blocker of GluR2-lacking AMPA receptors abolished the RA-induced increase in mEPSC amplitude. Interestingly, RA was able to increase surface GluR1 expression in the presence of a transcriptional inhibitor, but not a protein synthesis inhibitor, suggesting that RA may trigger local translation of GluR1. Consistent with this notion, Aoto and colleagues (2008) demonstrated that RA increased GluR1 protein levels in synaptoneurosomes, a preparation that lacks cell nuclei.

In lieu of the canonical RA regulation of transcription, Aoto and colleagues (2008) looked for a nonconventional pathway for homeostatic compensation. Immunostaining showed that the

RA receptor RARα was localized within dendrites as well as the nucleus (see also Chen and Napoli, 2008). To test whether RARα mediates the acute effects of RA on synaptic transmission, Aoto et al. (2008) used an shRNA strategy to reduce the levels of RARα. RARα knockdown prevented RA from increasing GluR1 expression and mEPSC amplitude, while the effects were rescued by expression of an shRNA-resistant RARα. In addition, application of a selective RARα agonist recapitulated the effects of RA. Accordingly, Aoto and colleagues (2008) propose that RARα senses the inactivity-generated RA, causing in turn an upregulation of GluR1 receptors and increased synaptic strength.

It is often the case that experiments that point to an unprecedented role for a signaling molecule end up raising as many questions as they answer. This study by Aoto et al. (2008) is no exception. The following is a brief discussion of the most intriguing issues.

How Does Inactivity Increase the Level of RA?

The findings of Aoto et al. (2008) focus attention on the control points for RA synthesis in neurons. To explain the low ambient level of RA within neurons, indicated by their GFP reporter data, Aoto and colleagues (2008) focused on RALDH1, the enzyme that makes RA, as the target of modulation by activity-deprivation. However, the available evidence is also compatible with regulation of RoDH, since this upstream enzyme is also critical for the pathway leading to RA production. Control of RoDH would be consistent with (1) the ability of the RoDH inhibitor citral to prevent the synaptic adaptation and (2) the already high basal levels of RALDH1 protein, which suggests that RALDH1 might not be the biochemical bottleneck.

Whichever enzyme is rate limiting, a pressing question is how its activity might be linked to the function of NMDA receptors. Given that NMDAR blockade by APV (in addition to impulse blockade by TTX) was necessary to trigger an increase in RA, the key enzyme might be negatively regulated by NMDAR-mediated Ca^{2+} entry in dendritic spines. This would fit nicely with the finding that NMDA receptors activated by spontaneous synaptic transmission suffice to suppress local GluR1 synthesis (Sutton et al., 2006). Finally, the activity of the RA-metabolizing enzyme, CYP26A1 (cytochrome p450, 26) should be examined to complete the picture of how RA is regulated.

Where Is RA Produced and Where Does It Act?

Given that RA is a membrane-permeable signaling molecule, it could potentially act in a paracrine or autocrine manner, depending on where it is synthesized and where its target is located. RA-mediated paracrine communication between cells would mesh well with mounting evidence that adaptation to inactivity is not purely cell autonomous but can involve *trans*-synaptic signaling. For example, decreasing activity in a postsynaptic cell through expression of an inwardly-rectifying potassium channel (Kir2.1) causes changes in presynaptic vesicle turnover (Burrone et al., 2002). Conversely, suppression of presynaptic activity by use of the same K channel increasespostsynapticGluR1expression (Hou et al., 2008). Both findings illustrate how a sensor of activity on one side of the synapse may drive compensatory changes expressed on the other side. Clearly, such effects require cell-cell communication, but the nature of the message remains obscure. Whether RA or some other diffusible messenger participates in *trans*-synaptic crosstalk between neurons remains to be tested.

The data of Aoto et al. (2008) seem more consistent with an autocrine action of RA. The most obvious site of RA production is the postsynaptic cell itself, where the signal from NMDA receptors originates. Likewise, the RA-induced increases in surface GluR1 immunoreactivity and mEPSC amplitude all fit with a simple postsynaptic target mechanism—a local action of RA within the same cell where it was produced. It might seem ironic if a potentially powerful trans-membrane signaling molecule was simply used as a classical intracellular messenger.

However, there is a precedent for this. Nitric oxide generated in response to NMDA receptor activation regulates AMPA receptor trafficking via local cyclases and kinases (Serulle et al., 2007): thus, a membrane-permeant agent may act on an identified target within the same cell in which it is synthesized.

At present, a combination of autocrine and paracrine actions cannot be excluded. The use of RA over different ranges may be dependent on the nature of the change in activity levels as well as the identity of the synapses involved in the adaptation. For example, RA is a candidate retrograde messenger at hippocampal synapses where adaptation to inactivity involves clear presynaptic modifications, such as the mossy fiber-CA3 synapse (Kim and Tsien, 2008). Determining the origin and target of RA signaling will be essential to further understanding of its functional significance. The recruitment of a membrane-permeable signaling molecule carries with it intriguing questions about the local or global nature of the signal. It will be interesting to work out the consequences of a readily diffusible messenger that drives local protein translation and what this implies for synapse and dendritic specificity.

How Does RARα Signal to Increase Translation of GluR1?

It is now well accepted that dendrites have the capacity for local synthesis of proteins. Aoto and colleagues (2008) have presented convincing evidence that RARα triggers local translation of GluR1, but the mechanism by which this occurs is unclear. Although the classical role of RARα is that of a transcription factor, its implication in rapid, nongenomic signaling should come as no surprise. Indeed, many other members of the nuclear hormone receptor superfamily, including steroid hormone receptors, have been shown to mediate rapid actions, independent of gene expression regulation, via conventional second messenger cascades. It remains to be determined whether RARα induces GluR1 synthesis by co-opting a signaling cascade already known to regulate translation or through a novel mechanism. Intriguingly, RARα has been detected within RNA granules (Chen et al., 2008), suggesting that an even more unconventional mechanism might be at play.

How Does RA Fit in with Other Candidates for Molecular Players in Adaptation to Inactivity?

Given that RA, βCaMKII, and TNFα each induce upregulation of GluR1 postsynaptically (and consequently increased quantal amplitude), we appear to have an embarrassment of riches in the form of multiple signaling candidates. Thorny issues regarding differences in induction protocols, timescale, and source(s) of the various messengers may help account for the lack of an obvious link between RA, β-CaMKII, and TNFα. For example, while blocking action potentials with TTX is sufficient to increase expression of β-CaMKII and TNFα, upregulation of RA requires the additional blockade of NMDA responses with APV. Further, while expression of β-CaMKII and RA is increased within 24 hr, upregulation of TNFα requires activity-deprivation for at least 48 hr. On the other hand, potential overlaps do exist. TNFα triggers insertion of GluR1 in the membrane through activation of the phosphoinositide 3-kinase (PI3K) signaling pathway. Likewise, RA can activate PI3K signaling (for example, see Masiá et al., 2007), so activation of this pathway may be a shared target. Delving into the details of the respective mechanisms may yet reveal additional ways that these signaling pathways may synergize with each other. In conclusion, Aoto et al. (2008) have presented strong evidence that RA and RARα, agents previously known for biological actions of a very different kind are critical for synaptic adaptation to inactivity. Understanding the logic of their involvement will be advanced by focusing on how NMDARs generate RA production, how RARα activation is linked to delivery of GluR1, and whether a potentially diffusible messenger must cross cell membranes to carry out its job.

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