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Exploring the Role of CaMKIV in Homeostatic Plasticity

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Review of Joseph and Turrigiano

Neural circuits must be able to appropriately react to chronic changes in activity to prevent excessive or insufficient activity. Hyperactivity and hypoactivity are implicated in a wide range of neurological disorders, including epilepsy, schizophrenia, and Fragile X syndrome (Wondolowski and Dickman, 2013). In the absence of other methods of regulating firing rates, activity-dependent mechanisms of Hebbian synaptic plasticity, LTP and LTD, would tend to drive neurons toward their maximum firing rates or to silence. Previous studies have shown that homeostatic mechanisms, including global synaptic scaling and regulation of intrinsic excitability, are used to keep neurons firing in the middle of their dynamic range (Desai et al., 1999; Turrigiano and Nelson, 2004; Watt and Desai, 2010). Synaptic scaling is the process through which total synaptic strength of a neuron is increased following prolonged postsynaptic activity blockade or decreased following periods of increased activity. Whereas LTP and LTD are input-specific, synaptic scaling typically affects a broad range of synapses by upregulating or downregulating expression of AMPA receptors. Homeostatic regulation of intrinsic excitability describes the nonsynaptic changes in a neuron's electrical properties, which occur through alterations in expression and distribution of voltage-gated ion channels and affect the likelihood that a neuron will produce an action potential in response to synaptic input of a given strength. Although these mechanisms have been well characterized at the cellular level, the specific molecular pathways that govern homeostatic plasticity are less well known. A recent study by Joseph and Turrigiano (2017) takes a closer look at these mechanisms by investigating the upstream signaling pathways that govern synaptic scaling and intrinsic plasticity.

Recent work has shown that calcium/calmodulin-dependent protein kinase type IV (CaMKIV), which is activated by calcium influx and initiates transcription by activating CREB, is involved in excitatory synaptic scaling (Ibata et al., 2008; Pratt et al., 2011), consistent with computational models that suggest that calcium triggers synaptic scaling (Turrigiano, 2008). Given the established cooperation between homeostatic plasticity mechanisms (Pratt and Aizenman, 2007; Lambo and Turrigiano, 2013; Cannon and Miller, 2016), Joseph and Turrigiano (2017) asked whether CaMKIV signaling also regulates inhibitory synaptic scaling and intrinsic excitability. To do so, they transfected cultured neocortical pyramidal neurons with either a kinase-dead, unphosphorylatable, dominant-negative CaMKIV construct or a constitutively active CaMKIV construct to reduce and increase, respectively, CaMKIV activity.

Although previous work had shown that CaMKIV regulates excitatory synaptic strength, these studies used manipulations that were not limited to the nucleus (Ibata et al., 2008; Pratt et al., 2011). To rule out the possibility that regulation of excitatory synaptic strength is mediated by CaMKIV in the cytoplasm, where it is initially activated, Joseph and Turrigiano (2017) used a nuclear localization signal to target CaMKIV constructs to the nucleus. They confirmed that the nuclear-localized constitutively active form increased activation of CREB and the nuclear-localized dominant-negative form reduced activation of CREB. Using whole-cell voltage-clamp recordings of neurons transfected with the constructs, the authors measured AMPA-mediated mEPSCs while blocking synaptic release, network activity, and mIPSCs. They found that the amplitude of mEPSCs was greater than controls in dnCaMKIV-expressing neurons and smaller in caCaMKIV-expressing neurons. As expression of these constructs did not affect passive properties or firing frequencies, the authors conclude that, as expected, nuclear CaMKIV activity modulates excitatory synaptic strength.

To determine whether CaMKIV signaling has the opposite effect on inhibition, the authors measured mIPSC amplitude. Using whole-cell voltage-clamp recordings of transfected neurons with blocked synaptic transmission, network activity, and mEPSCs, they found no significant differences in mIPSC amplitude in neurons expressing different constructs. The authors conclude that cell-autonomous changes in CaMKIV are not responsible for regulation of inhibitory synaptic strength and that there must

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therefore exist another upstream signaling mechanism with this function.

However, using dominant-negative and constitutively active constructs may obscure the full picture when dealing with a pathway that is coupled to the dynamic and tightly regulated nuclear calcium signal. Although pCREB intensity in dnCaMKIV neurons was significantly lower than in controls, it is possible that inhibitory scaling can still operate with very low levels of CaMKIV (and pCREB). In addition to measuring pCREB levels, it would be useful to measure general and active CaMKIV levels with CaMKIV and anti-pT196 CaMKIV antibodies. Furthermore, although this experiment shows that CaMKIV signaling is insufficient to induce scaling at inhibitory synapses by itself, it remains possible that CaMKIV signaling is necessary in combination with other processes to induce scaling. Attempting to induce inhibitory synaptic scaling using pharmacological agents, such as tetrodotoxin and bicuculline methobromide, which respectively, decrease and increase global activity, in neurons with negligible CaMKIV levels, could be used to determine whether nuclear CaMKIV signaling is a key player in inhibitory synaptic scaling. If the authors' conclusion is true, these manipulations, which normally modulate inhibitory synaptic scaling, should not show this effect.

As Joseph and Turrigiano (2017) mention, whether previous studies achieved cell-autonomous inhibitory synaptic scaling depended highly on the particular experimental setup and manipulations (Hartman et al., 2006; Peng et al., 2010; Xue et al., 2014). In this regard, the authors' decision to perform these experiments in excitatory pyramidal neurons could be key to their findings. Inhibitory interneurons also exhibit evidence of homeostatic regulation, such as activity-dependent regulation of intrinsic properties (Gibson et al. 2006). Excitatory synapses in neocortical excitatory neurons and inhibitory interneurons have been shown to respond oppositely to brain-derived neurotrophic factor, which mediates synaptic scaling in response to activity blockade (Rutherford et al., 1998). In addition, the two types of neurons have starkly different CaMKIV levels and inhibitory neurons are deficient in CaMKII. In inhibitory neurons, CaMKIV is a rate-limiting factor in CREB phosphorylation because its low endogenous levels of CaMKIV tightly regulate CREB phosphorylation, rapidly switching from minimal to maximal activation of CREB. In contrast, in excitatory neurons, basal CaMKIV levels are much higher, allowing CREB activation to proceed linearly (Cohen et al., 2016). Moreover, while CaMKIV activates CREB by phosphorylating it at Ser-133, CaMKII additionally phosphorylates CREB at an inhibitory site, Ser-142, blocking its activation by Ser-133 phosphorylation (Sun et al., 1994; Corcoran and Means, 2001; Wu and McMurray, 2001). In inhibitory interneurons, which have very low levels of CaMKII, this block would not occur.

Another main goal of the study by Joseph and Turrigiano (2017) was to determine whether the same upstream pathways regulate intrinsic plasticity and synaptic scaling. To determine whether CaMKIV regulates intrinsic excitability as well as excitatory synaptic scaling, the authors blocked synaptic transmission in each type of transfected neurons and measured firing frequency induced by various current steps. They found that dnCaMKIV-expressing neurons exhibited increased firing rates and caCaMKIV-expressing neurons exhibited decreased firing rates, suggesting that intrinsic excitability, such as excitatory synaptic scaling, is bidirectionally regulated by CaMKIV signaling. Given the corresponding CaMKIV-regulated changes in intrinsic excitability and excitatory but not inhibitory synaptic scaling, Joseph and Turrigiano (2017) predicted, and found, that CaMKIV signaling influences basal firing rates; neurons expressing dnCaMKIV exhibited increased spontaneous firing rates and neurons expressing caCaMKIV exhibited decreased spontaneous firing rates.

The confluence of evidence from Joseph and Turrigiano (2017) and other recent studies that have investigated CaMKII and CaMKIV signaling in excitatory neurons and inhibitory interneurons suggests that homeostatic regulation of excitation and inhibition may be closely tied to the levels of the two kinases. It is possible that CaMKIV and CaMKII mediate inhibitory synaptic scaling in concert in excitatory neurons, given that the additional phosphorylation of Ser-142 by CaMKII might cause the CREB activation curve to become more similar to what is observed in inhibitory neurons. Manipulating CaMKII in conjunction with CaMKIV or monitoring P-Ser-142 in response to intracellular calcium release while attempting to evoke inhibitory scaling could help reveal whether and how these kinases work together to achieve synaptic scaling. Performing experiments similar to those in this study in inhibitory neurons could also provide valuable insight regarding the degree of coordination in the upstream mechanisms of excitatory and inhibitory scaling in achieving the shared goal of homeostatic plasticity.

Taken together, the results of the study by Joseph and Turrigiano (2017) significantly contribute to our knowledge of the upstream pathways that regulate transcription in homeostatic plasticity. This is critical to understanding how neural networks maintain relatively stable firing rates despite significant activity-dependent changes. Although excitatory synaptic scaling and intrinsic plasticity operate with different proximate mechanisms, they work together to regulate neural activity in response to chronic changes. This study reinforces the mechanistic relationship between excitatory synaptic scaling and intrinsic plasticity by showing that they also share a reliance on the upstream CaMKIV signaling pathway. Establishing this connection invites future study of molecular factors that are known to activate or be activated by CaMKIV, further elaborating these mechanisms. This work paves the way for future studies, which will further clarify the specific role of CaMKIV and other kinases in homeostatic plasticity mechanisms in both excitatory and inhibitory neurons.

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