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Glua1 Ubiquitination in Synaptic Plasticity and Cognitive Functions: Implications for Neurodegeneration

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Review of Guntupalli et al.

Learning and memory rely on processes involved in synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength (Diering and Huganir, 2018). Changes in synaptic strength involve, in part, regulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs), which are the most prevalent excitatory synaptic receptor in the nervous system (Hershko and Ciechanover, 1998). This regulation includes dynamic modulation of synaptic AMPAR trafficking and expression in response to changes in synaptic transmission, which is mediated by receptor endocytosis followed by differential sorting in early endosomes between degradative and recycling pathways (Diering and Huganir, 2018).

The GluA1 subunit is an important component of AMPARs in the hippocampus and cortex and plays a significant role in synaptic plasticity and cognitive functions (Mabb, 2021). The postsynaptic density of AMPARs can be modified through protein-protein interactions and by post-translational modifications at sites on the C-terminal tail of GluA1 (Anggono and Huganir, 2012). Indeed, phosphorylation of specific residues in GluA1 have been shown to control AMPAR trafficking and gating in neurons, thereby regulating LTP and LTD and influencing synaptic plasticity and memory (Diering and Huganir, 2018). Ubiquitination, which involves the covalent attachment of a ubiquitin moiety to the C-terminal tail, is another significant process in the regulation of AMPAR surface expression and turnover (Mabb, 2021). Ubiquitination is a three-step sequence governed by E1 ubiquitin-activating enzymes (E1s), E2 ubiquitin-conjugating enzymes (E2s), and E3 ubiquitin ligases (E3s), such as Nedd4-1 (Liu et al., 2023). Ubiquitination influences receptor trafficking and synaptic strength by directing endocytosed AMPARs towards degradation pathways involving the ubiquitin proteosome system (Mabb, 2021).

Disruption of normal AMPAR trafficking can result in deficits in learning and memory (Anggono and Huganir, 2012). For example, dysregulation of glutamate receptor ubiquitination has been identified in some neurodegenerative diseases, such as Alzheimer's disease (AD). Moreover, excessive ubiquitination of GluA1 has been linked to amyloid-β (Aβ)-induced downregulation of surface AMPAR expression, leading to synaptic depression in pathological conditions (Diering and Huganir, 2018). Consequently, exploring regulation of AMPAR expression by post-translational modifications such as ubiquitination is important in understanding processes underpinning normal brain function and for identifying potential therapeutic targets of disease.

To elucidate the physiological role of GluA1 ubiquitination in synaptic plasticity, learning, and memory, Guntupalli et al. (2023) generated a knock-in mouse in which an arginine residue was substituted for lysine at the major GluA1 ubiquitination site, Lys-868. As expected, AMPA treatment failed to induce GluA1 ubiquitination in primary cortical neurons derived from knock-in mice. However, Nissl staining of the hippocampus revealed no obvious effects of the mutation on gross brain architecture in 3-month-old knock-in mice. Moreover, analysis of synaptic fractions from forebrain tissue indicated that the distribution of GluA1 was similar in wild-type and knock-in mice. This suggest that, under basal *in vivo* conditions, the ubiquitination of GluA1 is not fundamental in maintaining the steady-state levels of synaptic AMPARs.

Next, Guntupalli et al. (2023) investigated LTP and LTD in hippocampal slices from adult GluA1 K868R knock-in and wild-type mice. In the knock-in mice, input-specific LTP induced by theta-burst stimulation (TBS) was significantly enhanced compared to in wild-type littermates. Similarly, LTP induced by multiple episodes of TBS was greatly enhanced in the knock-in mice. In contrast, NMDAR- and metabotropic glutamate receptor (mGluR)-dependent LTD were impaired in slices from GluA1 K868R knock-in mice. Importantly, the enhanced LTP and deficits in LTD were not due to nonspecific effects on basal synaptic transmission, as the knock-in mice displayed normal basal synaptic properties. These findings suggest GluA1 ubiquitination is involved in bidirectional changes in synaptic strength.

Guntupalli et al. (2023) also conducted a battery of behavioral tests to assess cognitive functions in wild-type and GluA1

Received Oct. 5, 2023; revised Dec. 30, 2023; accepted Jan. 2, 2024.
The author declare no competing financial interests.
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https://doi.org/10.1523/JNEUROSCI.2018-23.2024

K868R knock-in mice. In the Y-maze novelty preference test, the GluA1 knock-in mice spent less time exploring the novel arm compared with wild-type mice, suggesting impaired short-term spatial memory. Furthermore, in the active place avoidance test, which tests hippocampal-dependent spatial memory and cognitive flexibility, the GluA1 knock-in mice showed deficits in cognitive flexibility during the reversal-learning phase.

The regulated recycling and degradation of AMPARs is essential for mediating both LTP and LTD (Anggono and Huganir, 2012). In essence, sorting of AMPARs into the recycling pathway provides a supply of receptors to maintain LTP, while trafficking AMPARs into the degradation pathway results in LTD. Guntupalli et al. (2023) propose that the absence of GluA1 ubiquitination redirected AMPAR-containing vesicles from LTD-associated degradation pathways to endosomal recycling pathways, thus facilitating LTP. The physiological importance of this was seen in the impaired performance in tests of spatial and short-term memory, which the authors postulate reflect a "saturated" LTP states that impairs the encoding or updating of new information in the short term. Additionally, the impaired LTD and deficits in behavioral flexibility emphasize the importance of GluA1 ubiquitination in cognitive processes, particularly in memory updating and cognitive flexibility.

Based on their own and previous findings, Guntupalli et al. (2023) posit that ubiquitination of GluA1 may be an important factor in AD, which is characterized by alterations in AMPAR endocytosis and a reduction in surface AMPARs. They note that the E3 ubiquitin ligase Nedd4-1 is upregulated in patients with AD and that reducing Nedd4-1 is effective in mitigating Aβ oligomer-induced removal of AMPARs. Furthermore, the introduction of Aβ oligomers has been shown to elevate GluA1 ubiquitination and reduce receptor expression. Finally, the authors have previously shown that in GluA1 K868R ubiquitin-deficient knock-in mice, the detrimental effects of Aβ on AMPAR surface expression was diminished (Guntupalli et al., 2017). Thus, inhibiting AMPAR ubiquitination may be a potential strategy to prevent cognitive impairment in AD.

Stringent orchestration of ubiquitination and deubiquitination levels, mediated by ubiquitin ligases and deubiquitinating enzymes, respectively, is essential for neuronal homeostasis, function, and survival (Liu et al., 2023). For example, the proper functioning of E3s and deubiquitinating enzymes is essential for the clearance and degradation of Aβ and tau via the proteasome, with dysregulation of these enzymes contributing to the pathology of neurodegenerative diseases (Cao et al., 2019). Furthermore, ubiquitin C-terminal hydrolase (UCH-L1), an abundant deubiquitinating enzyme, plays a central role in regulating AMPAR ubiquitination (Cao et al., 2019). Several lines of evidence suggest that UCH-L1 depletion is associated with AD pathology involving the formation of Aβ plaques and the perturbation of tau metabolism, with reduced UCH-L1 levels observed in the brains of AD patients and transgenic AD mouse models (Guglielmotto et al., 2017). Furthermore, this reduction is associated with the accumulation of ubiquitinated proteins within Aβ plaques and neurofibrillary tangles, and the co-localization of UCH-L1 with hyperphosphorylated tau. Finally, Aβ42 accumulation results in the downregulation of UCH-L1 through the activation of the NF-kB pathway (Guglielmotto et al., 2017). This disrupts brain-derived neurotrophic factor (BDNF)/neurotrophic tyrosine receptor kinase 2 (NTRK2)-mediated signaling, leading to decreased degradation of ubiquitinated and hyperphosphorylated tau and the promotion of inflammation. Thus, the role of GluA1 deubiquitination in learning and memory, as well as the development of therapeutic options for disease and injury, should be explored in future work.

Another area for future investigations is understanding the external factors that contribute to dysregulated GluA1 ubiquitination. One factor is chronic stress, which impacts glutamatergic signaling within the prefrontal cortex (PFC) and leads to a reduction in surface AMPAR subunits (Yuen et al., 2012). Chronic stress has been shown to alter Nedd4-1 signaling, with a subsequent alteration of proteasome activity (Yuen et al., 2012). It will be important for future studies to shed light on how chronic stress influences GluA1 ubiquitination and the implications for memory and cognition.

In conclusion, the study by Guntupalli et al. (2023) contributes significantly to our understanding of the role of GluA1 ubiquitination in synaptic plasticity and cognitive functions. The findings have implications for various neurobiological processes, including learning and memory, and may provide insights into potential therapeutic targets for cognitive disorders and neurodegenerative diseases.

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