GluA3 shapes synaptic plasticity and neuronal structure

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AMPA-type glutamate receptors (AMPARs) are composed of GluA1–GluA4 subunits, which mediate fast synaptic transmission. Among AMPAR subunits, the physiopathological role of GluA3 remains largely unclear. Here, to gain insight into its function, we downregulated GluA3 through an shRNA-based approach in both in vitro and in vivo models.

Primary rat hippocampal neurons were transduced with either shRNA-GRIA3 or scrambled RNA viral vectors at DIV10, and at DIV20 we performed biochemical and molecular analyses. Dendritic arbor complexity of GluA3-silenced neurons was significantly reduced, and, when examining dendritic spine morphology, we observed an increased percentage of thin spines. At the molecular level, GluA3 downregulation selectively reduced the surface insertion of GluA1-containing AMPARs, GluN2B-, and GluN2A-containing NMDARs. Live calcium imaging revealed a significant reduction in NMDAR-mediated spontaneous synaptic calcium transients, which was independent of glutamate release, as indicated by live glutamate imaging analyses. In addition, repeated single-spine stimulation led to impaired spine-size enlargement and aberrant calcium accumulation in dendritic spines. This could be at least partially explained by the impaired recruitment of GluA1-containing AMPARs to the postsynaptic membrane, as indicated by SEP-GluA1 knock-in fluorescence at the spine following single-spine stimulation. Transcriptomic analysis via RNA-seq showed dysregulated expression of key activity-related genes (e.g., CAMK2A, MAPK1), as well as pathways related to protein trafficking and neuronal development.

Notably, the impact of GluA3 silencing was time-dependent: earlier silencing (DIV3) produced stronger effects than silencing at DIV10, whereas later silencing (DIV14) was no longer detrimental. To further validate the contribution of GluA3 to brain function, we are currently extending our investigation to an in vivo model. Considering the time-dependent role of GluA3 in primary hippocampal neurons, we downregulated its expression in the dorsal CA1 (dCA1) region of the hippocampus in both adolescent and adult mice. Analysis of dendritic architecture revealed alterations similar to those observed in our in vitro model, thus strengthening the hypothesis that GluA3 acts as an upstream regulator of

neuronal architecture. Finally, we are conducting behavioral experiments to determine whether the absence of GluA3 leads to the onset of cognitive deficits in both adult and adolescent mice.