



Some (Syn)Gaps are Worse than Others: Deciphering The Role of Syngap Isoforms in Excitatory Synaptic Function

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Endogenous Syngap Alpha Splice Forms Promote Cognitive Function and Seizure Protection

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Loss-of-function variants in SYNGAP cause a developmental encephalopathy defined by cognitive impairment, autistic features, and epilepsy. SYNGAP splicing leads to expression of distinct functional protein isoforms. Splicing imparts multiple cellular functions of SynGAP proteins through coding of distinct C-terminal motifs. However, it remains unknown how these different splice sequences function in vivo to regulate neuronal function and behavior. Reduced expression of SynGAP- α 1/2 C-terminal splice variants in mice caused severe phenotypes, including reduced survival, impaired learning, and reduced seizure latency. In contrast, upregulation of α 1/2 expression improved learning and increased seizure latency. Mice expressing α 1-specific mutations, which disrupted SynGAP cellular functions without altering protein expression, promoted seizure, disrupted synapse plasticity, and impaired learning. These findings demonstrate that endogenous SynGAP isoforms with α 1/2 spliced sequences promote cognitive function and impart seizure protection. Regulation of SynGAP- α expression or function may be a viable therapeutic strategy to broadly improve cognitive function and mitigate seizure.

Commentary

Neurons communicate with each other via synapses, where neurotransmitters released from the axon terminals of one neuron activate receptors on the postsynaptic membrane of dendrites of another neuron. A critical aspect of postsynaptic function is to relay neurotransmitter-mediated receptor activation to intracellular signaling cascades that will generate a change in neuronal function. If this finely balanced mechanism is disturbed, neurons will not respond adequately to stimuli, which can lead to brain dysfunction and epilepsy.

The postsynaptic density, an electron-dense structure just below the postsynaptic membrane composed of a network of scaffolding and signaling molecules, plays an important role in mediating neurotransmitter signaling at excitatory synapses and is essential for proper neuronal communication. Indeed, haploinsufficiency of one of the most abundant proteins within the postsynaptic density, **Synaptic Ras GTPase Activating Protein** (SynGAP) inevitably leads to a neurodevelopmental disorder (Intellectual developmental disorder, autosomal dominant 5; MRD5; OMIM #612621).¹ SynGAP disorders are characterized by mild to severe intellectual disability often associated with

autism. Most individuals with SynGAP mutations develop epilepsy, including epileptic encephalopathies.² Thus, the phenotypic spectrum of SynGAP disorders is broad, and epilepsy, which is often pharmacoresistant, is a major issue in managing the disease.³

Although the SynGAP protein has been studied for over 20 years, there are still no disease-targeted treatments for SynGAP-associated disorders.⁴ SynGAP plays a crucial role in regulating NMDA receptor activity and AMPA receptor trafficking, which is believed to contribute to the increased excitability of SynGAP-deficient neurons;⁵ however, critical details about the underlying mechanisms are still unknown. SynGAP functions as a GTPase activating protein (GAP) but several protein binding domains in its sequence also make it an important scaffolding protein independent of its enzymatic function. Conserved protein binding domains form the basis for the postsynaptic scaffold and can also mediate activity-dependent changes in the protein network via posttranslational modifications.⁶ It is not well understood which of the two functions of SynGAP, enzymatic or scaffolding, are most

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important for controlling neuronal excitability and ultimately underly epilepsy and cognitive impairment in its absence. A new study by Kilinc, Rumbaugh and colleagues published in *eLife* provides crucial insight into this question by assessing isoform-specific roles of SynGAP.⁷

Kilinc and colleagues made the interesting observation that, in addition to SynGAP's role as a GTPase and a scaffold to maintain the larger postsynaptic protein network, the activity-dependent regulation of its localization within the postsynaptic density is critical for a neuroprotective role. Notably, this function is mediated through a short C-terminal "PDZ-domain" binding motif present in only one of the four C-terminal isoforms of SynGAP ($\alpha 1/2$, β , γ), the $\alpha 1$ isoform. PDZ domains, named after three proteins in which they were discovered first, post synaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (ZO-1), are conserved protein binding domains. Present in many synaptic proteins, they bind to specific C-terminal sequences and are crucial for the assembly of large protein networks, including the postsynaptic density.⁸

Using an array of different transgenic mouse lines that expressed varying levels of the four C-terminal isoforms of SynGAP, the authors showed that proper expression of the isoform $\alpha 1$ is essential for activity-regulated postsynaptic function. Mice with reduced $\alpha 1/2$ isoforms had cognitive impairments and higher susceptibility to flurothyl-induced seizures, whereas a mouse model with slightly increased $\alpha 1$ and $\alpha 2$ expression improved spatial memory and reduced seizure susceptibility. The strongest evidence in favor of a critical role of the $\alpha 1$ isoform was provided by showing that a transgenic mouse line with a mutation just in the PDZ-binding motif of the $\alpha 1$ isoform had impaired cognition and increased seizure susceptibility. This mutation led to reduced levels of SynGAP in the postsynaptic density, which could be reversed by blocking steady state activity in cultured neurons, supporting a specific role in activity-dependent changes at the postsynaptic membrane. Thus, the $\alpha 1$ isoform with its unique capability to bind to PDZ domain-containing proteins in an activity-dependent manner may be an important contributor to intellectual disability and seizure susceptibility associated with SynGAP deficiency.


Although the study by Kilinc et al. used mouse models and was not set up to test novel therapies, the results should be considered for future treatment development. First, they underscore the importance of SynGAP's function as an activity-dependent scaffold protein. The enzymatic function of SynGAP is to activate small GTPases, which is important for proper relay of neuronal signaling; however, enhancing just the GTPase activating function of SynGAP may not be sufficient to treat the disorder. Instead, the enzymatic function along with the activity-dependent scaffolding properties of the $\alpha 1$ isoform have to be restored for improvement of symptoms. Second, the results suggest that phenotypes relevant to SynGAP disorders are responsive to $\alpha 1/2$ gene dosage. Moderately increased expression of $\alpha 1/2$ isoforms reduces neuronal excitability and delays

latency to flurothyl-induced seizures compared with wild type mice or SynGAP heterozygous mice. This suggests augmenting isoform-specific SynGAP expression, potentially even in other epilepsy disorders characterized by increased excitatory neuron activity, could be therapeutic.

Several caveats and open questions remain. For example, seizure susceptibility was only scored based on behavior. Future studies using long-term EEG recordings are needed to assess if seizures induced by flurothyl or other pro-convulsants are changed in severity and duration, and to test if the C-terminus of the $\alpha 1$ isoform mediates the altered basal EEG activity observed in the SynGAP mouse model.⁹ Most of the transgenic mouse lines used in this study showed changes in the expression of the β isoform, in addition to $\alpha 1$ and $\alpha 2$. While the authors provide evidence that altered expression of the β isoform may not mediate the behavioral and hyperexcitability phenotypes, it could still contribute, which warrants future investigation. Lastly, to further elucidate how SynGAP deficiency leads to intellectual disability and epilepsy, it will be important to understand the temporal and spatial regulation of SynGAP isoforms. SynGAP isoforms are differentially expressed during development¹⁰ and while SynGAP is mostly confined to glutamatergic neurons, it is also detectable in specific populations of inhibitory neurons.⁴ Revealing the isoform-specific SynGAP expression in different cell types and brain regions across development, together with a better understanding of the factors affecting SYNGAP splicing will be essential to reveal the brain circuits most vulnerable to SynGAP haploinsufficiency.

SynGAP mutations are estimated to underlie approximately 1% of all intellectual disabilities and are associated with epileptic encephalopathies. Given the important role of a single SynGAP isoform for neuronal function and excitability, it will be interesting to examine if so far unrecognized SynGAP disorders caused by mutations in genes influencing its splicing may further increase the proportion of intellectual disabilities and epilepsy disorders caused by SynGAP dysfunction.

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