# This Week in The Journal

## Effects of Shisa7 on $GABA_AR$ Channel Properties

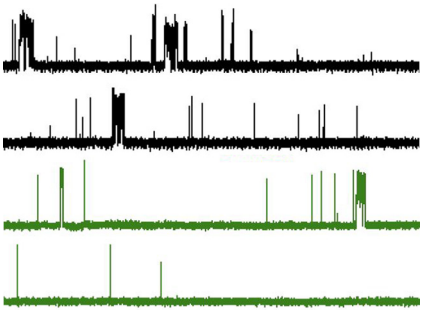
David Castellano, Kunwei Wu, Angelo Keramidas, and Wei Lu

(see pages 8758–8766)

$GABA_A$ receptors ($GABA_ARs$) are ligand-gated chloride channels that mediate most fast inhibitory transmission in the brain. How they affect the output of a given neuron depends on their subcellular localization, density, and channel properties. Subcellular localization and density are determined by interactions between $GABA_ARs$ and other proteins, including scaffolding proteins and auxiliary subunits. Channel properties (e.g., maximum conductance, open probability, and kinetics) are determined largely by which 5 of the 19 available $GABA_AR$ subunits compose the receptor. Unlike some auxiliary subunits of ionotropic glutamate receptors, no $GABA_AR$ auxiliary subunits have been reported to change single-channel properties before now. But Castellano et al. show that Shisa7 affects the open probability of $\alpha 2ß3\gamma 2L$ $GABA_ARs$.

Previous work showed that Shisa7 promotes trafficking of several subtypes of $GABA_ARs$ to the cell surface, resulting in larger whole-cell GABA currents. Shisa7 also accelerated $GABA_AR$ deactivation, but how it did so could not be determined from the whole-cell recordings performed in that study. To investigate whether Shisa7 affects single-channel properties, Castellano et al. expressed $\alpha 2ß3\gamma 2L$ $GABA_ARs$ with or without Shisa7 in HEK cells. Cell-attached single-channel recordings showed that Shisa7 did not affect the slope conductance of single $GABA_ARs$, but it decreased the frequency of channel opening. It also decreased the duration, open probability, and mean open time during bursts of channel openings and increased the mean closed time during bursts. Using an established biophysical model that describes transitions between conformational states of $GABA_ARs$, the authors determined that Shisa7 increased the time constants of the two shortest closed states, decreased the time constants of the two longest open states, and reduced the efficacy of transitions between the final closed state and the initial open state.

These results demonstrate that auxiliary subunits can affect single-channel properties of $GABA_ARs$. Specifically, Shisa7 alters the kinetics of $GABA_AR$ channel gating during bursts. This effect explains the accelerated deactivation kinetics of $GABA_ARs$ measured in previous whole-cell recordings. Together with previous work showing Shisa7 promotes surface expression of $GABA_ARs$, this study suggests that Shisa7 shapes inhibitory input to neurons by making whole-cell GABA currents larger in amplitude, but shorter in duration. This might be important for regulating spike timing.



When exposed to GABA, $\alpha 2\beta 3\gamma 2L$ $GABA_AR$ channels open more frequently when Shisa7 is not expressed (black) than when it is (green). See Castellano et al. for details.

## Polymorphism in Syngap1 3′ UTR Found in Some ALS Patients

Satoshi Yokoi, Takuji Ito, Kentaro Sahashi, Masahiro Nakatochi, Ryoichi Nakamura, et al.

(see pages 8881–8896)

Fused in sarcoma (FUS) is an RNA-binding protein whose dysfunction and/or aggregation can contribute to both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration. FUS binds to the 3′ untranslated region (UTR) of numerous target mRNAs, and it regulates mRNA transport, stability, splicing, and translation. Intriguingly, one FUS target is synaptic Ras-GTPase-activating protein 1 (Syngap1), a protein that contributes to dendritic spine maturation and has been linked to intellectual disability. Loss of FUS alters the relative levels of different alternatively spliced forms of Syngap1 and impairs maturation of dendritic spines. Whether this contributes to motor degeneration in ALS is unclear, but Yokoi et al. found a single nucleotide polymorphism (rs149438267) in the FUS-binding domain of the Syngap1 3′ UTR in a small proportion of ALS patients. They therefore investigated the effect of this variant in motor neurons derived from human induced pluripotent stem cells.

Motor neurons expressing the rs149438267 variant had fewer dendritic spines than control neurons, possibly because the polymorphism increased levels of the $\alpha 1$ isoform and reduced levels of the $\gamma$ isoform of Syngap1. Indeed, previous work has shown that overexpressing the $\alpha 1$ isoform reduces spine density, while the current study showed that overexpressing the Syngap1 $\gamma$ isoform rescued spine density in motor neurons expressing the rs149438267 variant. The change in isoform levels may have stemmed from greater binding efficacy of FUS and heterogeneous nuclear ribonucleoprotein K (HNRNPK)—an FUS-interacting protein that also regulates mRNA splicing—to the rs149438267-containing Syngap1 3′ UTR. Notably, knocking down FUS slightly decreased levels of the Syngap1 $\gamma$ isoform, whereas knocking down HNRNPK increased levels of isoform $\gamma$ and reduced levels of isoform $\alpha 1$. Finally, antisense oligonucleotides targeting the relevant portion of the Syngap1 3′ UTR reduced binding of HNRNPK and rescued spine levels in neurons carrying the Syngap1 rs149438267 variant.

These results suggest that binding of HNRNPK to the Syngap1 3′ UTR regulates splicing and thus influences dendritic spine formation and/or maintenance. In contrast, FUS binding had a relatively mild affect on splicing. Although this result somewhat weakens the hypothesis that the rs149438267 variant contributes to ALS pathology, future work should investigate this further by determining whether the variant affects motor neuron survival.

*This Week in The Journal was written by* Teresa Esch, Ph.D.

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