Application Note
#2009

# Webinar Recap: Nano-organization of Spontaneous GABAergic Transmission Directs its Autonomous Function in Neuronal Signaling

Super-Resolution microscopy overcomes the diffraction limit to create quantitative and high-resolution single-molecule localization data. Bruker's super-resolution fluorescence microscopy technology is utilized in various labs to generate high-quality images of a variety of specifically labeled biological specimens and structures. This application note summarizes a Bruker webinar with Natalie Guzikowski, a neuroscience student and member of the Kavalali Lab at Vanderbilt University, about their work on proteins involved with postsynaptic signaling in inhibitory synapses. Using dSTORM, a super-resolution imaging technology, and electrophysiology recording methods, their lab explores how the nano-organization of synaptic proteins governs spontaneous and evoked GABAergic transmission.

## dSTORM: A Super-Resolution Microscopy Technique

Single-molecule localization microscopy (SMLM) is a method of super-resolution imaging that overcomes the diffraction limit and achieves depths of >30 µm during imaging with the Bruker Vutara VXL. The Kavalali lab is using this fluorescence imaging approach alongside other techniques and recording methods to capture processes occurring at synapses. Specifically, they are exploring how the nano-architecture of the synaptic scaffold protein gephyrin impacts the function of the inhibitory GABAergic post-synapse.

One super-resolution microscopy technique Natalie Guzikowski and Ege Kavalali find especially helpful while investigating synaptic function in the central nervous system is dSTORM, or direct-stochastic optical reconstruction microscopy. dSTORM stochastically blinks during sample illumination with the use of photoactivatable or photoswitchable dyes activated at different wavelengths. This technique provides single-molecule localization data and can be tailored to specific research needs. The Kavalali lab processed their data by grouping single-molecule data into clusters to create heat maps, and by performing density and spatial proximity analyses.

## Nano-organization of Spontaneous GABAergic Transmission Directs Its Autonomous Function in Neuronal Signaling

To probe the nano-organization of the GABAergic synapse and the segregation of evoked and spontaneous neurotransmission, Guzikowski and Kavalali took advantage of the molecularly specific interaction of the inhibitory synapse scaffold protein gephyrin and antimalarial artemisinins, which are unique for their mammalian binding site. Using artemisinins as a probing tool, they explored the function of gephyrin during different forms of inhibitory synaptic transmission.

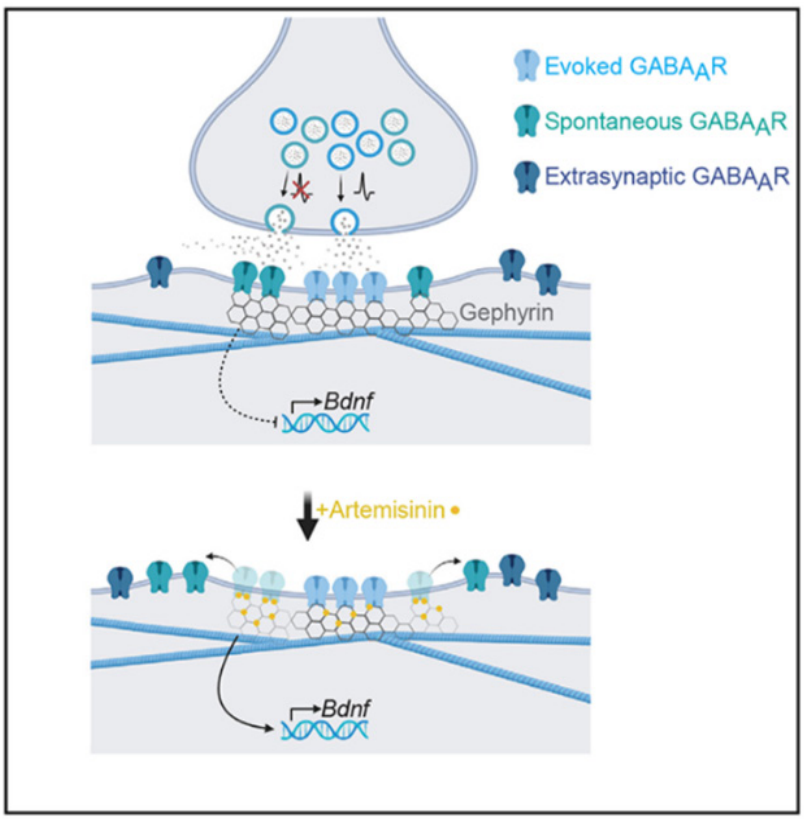


FIGURE 1
This schematic shows the inhibitory synapse of interest in the Kavalali Lab's study. Vesicles fuse at the GABA A receptors, which are anchored at the inhibitory synapse with gephyrin, the primary scaffolding protein at central inhibitory synapses.

After treating brain slice samples for one hour with artemisinin or artemether, SMLM and a density and spatial proximity analysis were performed to group single-molecule localizations to clusters, define gephyrin clusters as a synapse based on their proximity to VGAT clusters, and calculate the resulting changes in size. They found that these treatments caused a decrease in gephyrin cluster volume but not for VGAT clusters, indicating that this is a postsynaptic event due to artemisinin binding to gephyrin.

After establishing that the gephyrin volume was shrinking at individual synapses, they wanted to explore what part of the cluster was being lost. They calculated the pre-post centroid distance before and after treatment and saw no difference in distance between VGAT and gephyrin clusters. Therefore, artemisinin and artemether are not changing the center size of the cluster. Furthermore, a density analysis was performed where molecules were colored using a heat map to investigate how artemisinin is disrupting gephyrin scaffolding. Once again, quantitative analysis revealed that after treatment, the number of gephyrin molecules was reduced at the periphery of the cluster but still densely packed near the center.

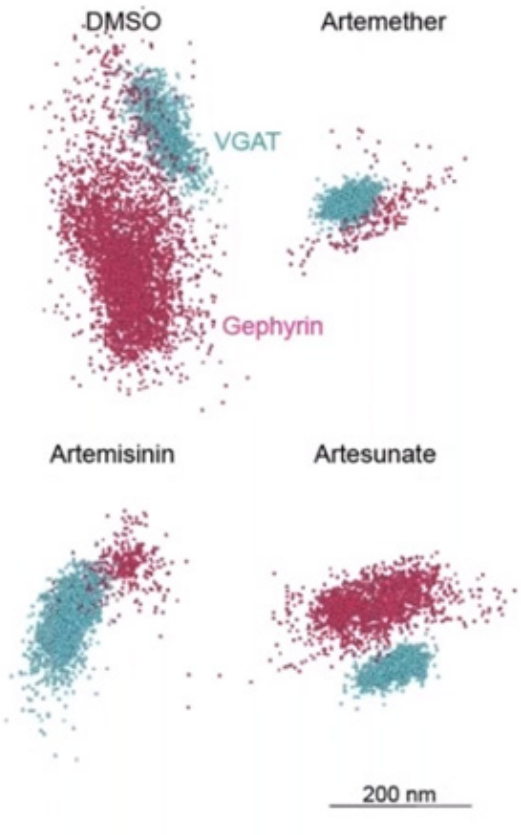


FIGURE 2
3D dSTORM reconstructions of colocalized and pre- and postsynaptic clusters with localizations color-coded by probe (vGAT, blue; gephyrin, pink).

Another prong of their research was exploring the impact of these substructure changes, specifically the selective loss of molecules at the gephyrin cluster periphery. By using patch clamp electrophysiology, they monitored neurotransmission and saw no difference in release probability after repetitive stimulation. Furthermore, they saw evoked neurotransmission is intact, but there is a selective downregulation of GABAergic spontaneous neurotransmission, which was then confirmed by measuring Bdnf activity.

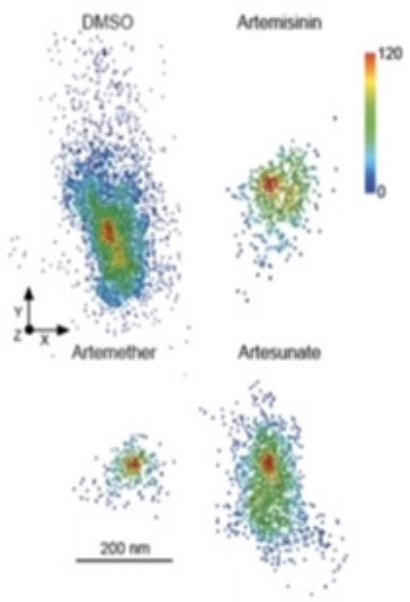


FIGURE 3
3D dSTORM reconstructions of gephyrin molecules with localizations represented by density heatmap. Red indicates that the molecules are more densely packed.

## Conclusion

The Kavalali Lab has an interest in exploring the fundamental questions: how are different modes of transmission happening (spontaneous or evoked) and where are they happening (same or different synapses)? In this webinar, Natalie Guzikowski talks about the various techniques their lab used to reach the overall conclusion that there is a center-surround organization of evoked and spontaneous neurotransmission at the GABAergic post-synapse, where gephyrin is required to anchor GABA A receptors and internal regulatory proteins. Their lab created various compelling results about GABAergic transmission with the super-resolution dSTORM technique and by combining quantitative single-molecule data with other recording and imaging methods.

## Resources

To view the entire on-demand webinar "Nano-organization of Spontaneous GABAergic Tranmission Directs its Autonomous Function in Neuronal Signaling" visit:
https://www.bruker.com/en/news-and-events/webinars/2023/nano-organization-of-spontaneous-gabaergic-transmission-directs-its-autonomous-function-in-neuronal-signaling.html

To learn more about Bruker's Vutara VXL™ super-resolution microscope visit
www.bruker.com/vutara

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