Mechanisms to separately regulate synaptic vesicle release and recycling

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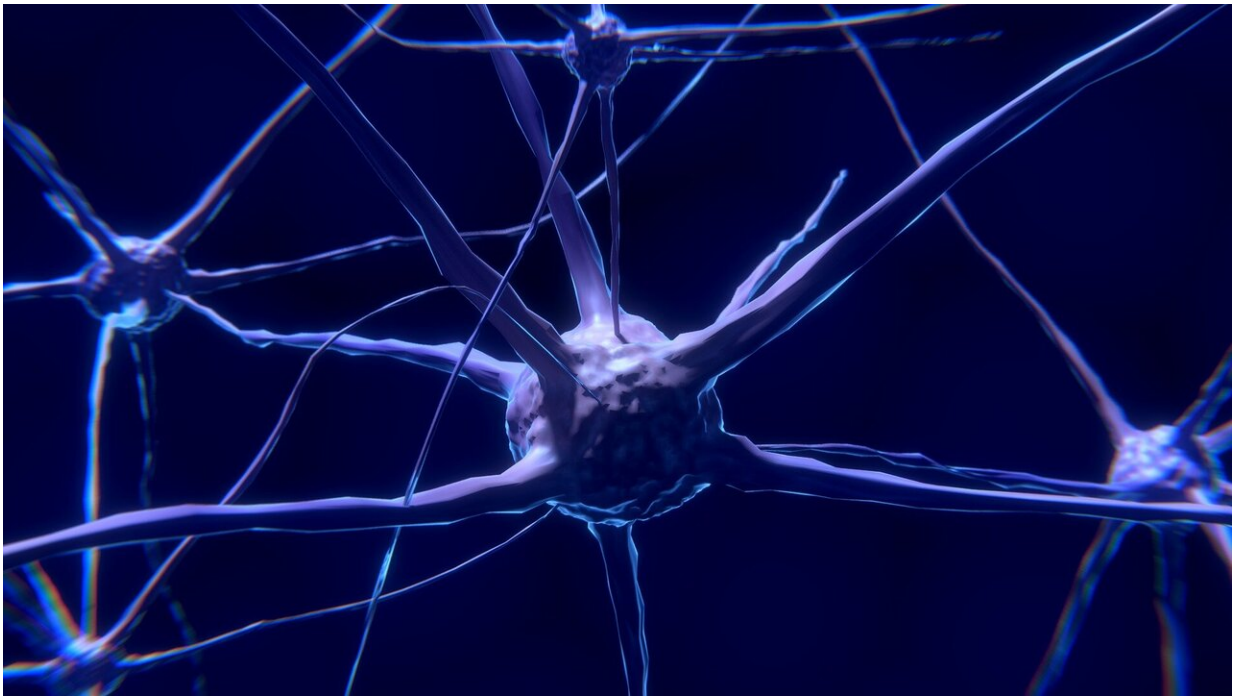

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Chemical synapses transmit information within the nervous system. When a presynaptic cell is electrically excited, synaptic vesicles fuse with the presynaptic membrane causing messenger substances within the vesicles to be released into the synaptic cleft. These then bind to receptors in the postsynaptic cell where they trigger an electrical signal once again. The temporal and spatial sequence of the incoming signals

determines how information is processed and transmitted in the brain. In order to sustain their function in the long term, chemical synapses need to recycle synaptic vesicles to make them available for renewed signal transmission. Professor Carsten Duch and Professor Martin Heine and their respective research groups at Johannes Gutenberg University Mainz (JGU) are investigating how the release and recycling of synaptic vesicles are coordinated.

"Exocytosis and endocytosis rates at chemical synapses need to be coordinated to achieve reliable signal transmission in the brain," the biologists explained. Together with Dr. Ulrich Thomas, group leader at the Leibniz Institute for Neurobiology in Magdeburg, Duch and Heine have revealed in a *PNAS* paper how spatiotemporally separated presynaptic calcium signals independently regulate exocytosis and endocytosis of synaptic vesicles, i.e., their release and recycling.

Co-existence of different types of voltage-gated calcium channels at the presynapse

At chemical synapses, incoming electrical impulses are converted into chemical signals and relayed on to the next cell. The process entails calcium ions first flowing through voltage-dependent membrane channels into the presynapse, i.e., the upstream nerve cell that transmits the signal to the postsynaptic cell. This calcium influx is tightly constrained in both time and space and results in exocytosis of synaptic vesicles from a specialized vesicle reservoir. Presynaptic calcium signals also regulate synaptic vesicle recycling, but here the temporal and spatial requirements are different. One unresolved question is how presynaptic electrical activity can lead to calcium signals with different temporal and spatial profiles in the presynaptic terminal.

By combining genetic modifications and electrophysiological and

optophysiological measurements at the neuromuscular synapse of the Drosophila melanogaster genetic model organism, the research team was able to demonstrate that the presynapse harbors two different types of voltage-gated calcium channels, Ca_v2 and Ca_v1. These, however, were found to be spatially segregated. Both types of channel open when electrical signals arrive, but only Ca_v2 channels, which are inside active zones of the presynapse, are required for exocytosis of synaptic vesicles. Ca_v1 channels are situated outside active zones and augment endocytosis of synaptic vesicles via activity-dependent calcium influx. Thus, knockdown of Ca_v2 by means of genetic manipulation prevents synaptic transmission, whereas knockdown of Ca_v1 decreases the rate of synaptic vesicle endocytosis, thereby enhancing synaptic depression during sustained activity. This is how calcium signals mediated by two different populations of largely independent voltage-gated calcium channels regulate two essential functions of the presynapse in response to neuronal activity, namely the release and recycling of synaptic vesicles.

Functional separation of Ca_v1 and Ca_v2 by means of a calcium pump

A key question was how calcium signals through different channels could be functionally separated at the nanometer scale of the presynaptic terminal, because calcium after all is a highly diffusible intracellular messenger.. According to the researchers, different vital functions of calcium signals through Ca_v1 and Ca_v2 channels are separated by a membrane-anchored calcium buffer. Ca_v2 channels are found within presynaptic active zones at distances of 70 to 140 nanometers from readily releasable synaptic vesicles. This distinct localization of Ca_v2 results in the emergence of temporally and spatially tightly regulated calcium signals within so-called nano-domains during presynaptic electrical activity, and these are essential for temporally precise synaptic transmission. Ca_v1 localizes around active zones, in theory allowing calcium influx simultaneously through both types of channels to result in

mixed signals with no measurable delay. However, mixed signals of this type are prevented by the plasma membrane calcium pump (PMCA). PMCA is located outside active zones and isolates them from the dynamic regulation of endocytosis achieved by Ca_v1-mediated calcium influx. Because Ca_v1, Ca_v2, and PMCA have been identified also at central synapses in the brains of mammals, these proteins may represent a conserved functional triad for separate activity-dependent regulation of exocytosis and endocytosis of synaptic vesicles.

Calcium channels and the regulation of essential synaptic functions

In the future, Duch's and Heine's research groups will continue to explore the interactions of calcium channels and their associated molecules at the presynapse. Calcium signals in the presynaptic terminal regulate other essential synaptic functions beyond exocytosis and endocytosis. These include the regulation of synaptic vesicle movements between distinct specialized reservoirs as well as the control of fixed synaptic transmission strengths, which are restored by compensatory mechanisms after perturbation. This homeostatic synaptic plasticity is essential for reliably processing information in the brain. As part of a project within Collaborative Research Center 1080 on Molecular and Cellular Mechanisms in Neural Homeostasis, Duch's and Heine's groups are investigating how spatiotemporally separated presynaptic calcium signals independently control exocytosis and endocytosis, the transport of vesicles between different reservoirs, and synaptic homeostasis. "Calcium signals are extremely well suited to precisely adapt a variety of vital synaptic functions to differing neuronal activities, but we are only just starting to work out the mechanisms that independently regulate these functions," Duch and Heine commented on their neurobiology research.

More information: Niklas Krick et al, Separation of presynaptic Cav2 and Cav1 channel function in synaptic vesicle exo- and endocytosis by the membrane anchored Ca2+ pump PMCA, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2106621118