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Abstracts: Antibodies are the best tools to determine the position and density of endogenous proteins in the brain. However, it is often difficult to study low copy number proteins due to limitations in antibody sensitivity and specificity. For example, presynaptic CB₁ cannabinoid receptors are visualized by most conventional antibodies on forebrain GABAergic boutons, but CB₁ expression levels are usually too low to be investigated in a quantitative manner on most other axon terminal types. To overcome this limitation, we used transgenic rabbits and mice overexpressing the neonatal Fc receptor (FcRn) to produce highly sensitive polyclonal and monoclonal antibodies against the mouse CB₁ receptor. Transgenic animals carrying and expressing extra copies of the FcRn exhibit an enhanced immune response. Accordingly, CB₁ antisera obtained from FcRn transgenic rabbits had higher titers and exhibited better dissociation kinetics compared to antisera collected from wild-type rabbits. Moreover, each antisera obtained from FcRn transgenic rabbits (n=3) revealed dense axonal CB₁-immunostaining pattern in wild-type mouse hippocampus. The specificity of this staining pattern was validated in CB₁ knockout animals. In contrast, only one antiserum collected from wild-type rabbits (n=3) could visualize CB₁ receptors. Similarly, we could successfully obtain 10 positive, high affinity anti-CB₁ IgG-expressing clones from FcRn-overexpressing mice, but none from wild-type mice. Immunostaining with monoclonal antibodies generated from these clones also revealed extensive immunolabeling of CB₁-positive GABAergic axons. To further improve the sensitivity of CB₁-immunostaining, the FcRn transgenic rabbit, producing antisera with the best dissociation constant, was further immunized and the last three bleeds were mixed and purified. Immunohistochemistry with this new antibody resulted in unprecedentedly dense CB₁-immunostaining outlining the laminar topography of glutamatergic pathways. Electron microscopic analysis demonstrated that the vast majority of a randomly selected population of excitatory terminals carried CB₁ receptors in the inner molecular layer of dentate gyrus and in the stratum oriens of CA3 subfield. STORM superresolution imaging of CA3 pyramidal cells confirmed this result at the single cell level by visualizing CB₁ in most recurrent collaterals. These findings demonstrate that FcRn-overexpressing transgenic animals are ideal tools to generate highly sensitive and specific antibodies, and were instrumental to obtain new monoclonal and polyclonal antibodies which allow quantitative investigation of CB₁ receptors in the brain.

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Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

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Topic: B.01. Neurotransmitters and Signaling Molecules

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Title: A new approach for correlated confocal and superresolution microscopy visualizes the distribution of presynaptic CB₁ cannabinoid receptors at the nanoscale

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Abstracts: How synapse-specific differences in signaling protein density determine the qualitative and quantitative properties of synaptic transmission is a fundamental question in neuroscience. However, our understanding of the molecular parameters underlying synaptic processes remains limited due to methodical limitations. Therefore, in the present study, we exploited whole-cell patch-clamp recordings, confocal microscopy and STORM superresolution microscopy to allow the integrated analysis of physiological and morphological properties of a single neuron with quantitative molecular imaging within its axon terminals. Importantly, the whole experimental and analysis process can be carried out in a few days and reveals the localization of synaptic proteins with excellent sensitivity and specificity, ultra-high spatial resolution, and in large sample sizes. We first employed methodical improvements for STORM superresolution imaging in mouse hippocampal sections, which revealed the subcellular distribution of CB₁ cannabinoid receptors with a fluorophore localization precision of 6 nm and 41 nm in the lateral and axial dimensions, respectively. The specificity of CB₁ immunolabeling in STORM images was validated by using CB₁ knockout animals. To make physiological characterization and anatomical classification possible in combination with superresolution imaging, CB₁-positive GABAergic interneurons were filled with biocytin during patch-clamp recording and their morphology was visualized by confocal microscopy. To provide a cellular context for the 3D coordinates of CB₁ localization points obtained by STORM, high magnification confocal microscopy was used to detect biocytin-labeled axon terminals and then CB₁ distribution within the selected axon terminal was imaged by STORM. To facilitate the correlative visualization and analysis of confocal and STORM microscopy data, we also developed a new software with an easy-to-use graphical user interface. A correlation analysis of

confocal and STORM images demonstrated that 3-D STORM provides a reliable quantitative readout of protein abundance in HEK cells and in GABAergic axon terminals. Finally, we applied new algorithms allowing nanoscale measurements of relative CB₁ density and intermolecular distances along the surface of GABAergic axon terminals. Taken together, our new methodology including improvements for brain tissue processing and imaging, as well as new software tools for data analysis represents a powerful approach for cell type-specific nanoscale molecular investigations in association with physiological and anatomical characterization in intact brain circuits.

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Poster

596. Synaptic Signaling: Retrograde Messengers

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Topic: B.01. Neurotransmitters and Signaling Molecules

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Title: Corticosterone-endocannabinoid signaling for memory recovery from general anesthesia

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Abstracts: The inaugural clinical practice of general anesthesia in 1840's revolutionized medicine by allowing surgery to be performed in a humane manner, and now approximately 234 million of patients each year undergo anesthesia for surgery worldwide. However, how memory recovers from anesthesia remains completely unknown. In the present study, we investigated how contextual fear memory recovers from general anesthesia with the volatile anesthetic isoflurane, a routine anesthetic in both humans and animals. We found that rats received isoflurane anesthesia for 2 h showed an approximate 25% of contextual fear memory recovery at 1 hour, 67% of memory recovery at 4 hour, and a full recovery at 12 hour after cessation of anesthesia. Contextual fear memory recovery at 1 hour after cessation of anesthesia was abolished by pretreatment of rats with the mineralocorticoid receptor (MR) antagonist RU28318 (but not by glucocorticoid receptor antagonist), calcium channel blocker, endocannabinoid