

## Cholesterol modulates acetylcholine receptor organization and stability at the cell membrane [Abstract]

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**CHOLESTEROL MODULATES ACETYLCHOLINE  
RECEPTOR ORGANIZATION AND STABILITY AT  
THE CELL MEMBRANE**

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The effect of cholesterol (Chol) on acetylcholine receptor (AChR) supramolecular organization has been studied by a combination of approaches using the clonal cell line CHO-K1/A5, a mammalian cell line stably expressing adult murine AChR. Acute (30 min, 37°C) exposure to methy-beta-cyclodextrin, commonly used as a diagnostic tool of endocytic mechanisms, dramatically accelerated AChR internalization from the cell surface. From a functional point of view, gain-of-function changes were observed in single-channel recordings upon Chol depletion. Wide-field and confocal microscopy disclosed AChR submicron-sized (240–280 nm) domains that remain stable over a period of hours at the cell membrane. Stimulated emission depletion (STED) fluorescence microscopy resolved the domains into AChR “nanoclusters” with a peak size distribution of ~55 nm. Cyclodextrin-mediated Chol depletion resulted in a smaller number of nanoclusters with larger size, and changes in nanocluster distribution on larger scales (0.5–3.5 microns). Chol content at the plasmalemma is postulated to modulate cell-surface organization and stability of receptor domains, and fine-tune

receptor channel function to temporarily compensate for acute AChR loss from the cell-surface.