Study of acetylcholine receptor organisation in the postsynaptic membrane of *Torpedo* electrocyte by cryoelectron tomography

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Clustering of acetylcholine receptors (AChR) at the neuromuscular junction is essential to muscle function: if it is not achieved, acetylcholine release by nerve cells cannot trigger a strong enough depolarisation to generate an action potential and muscle contraction. Rapsyn is a 43 kDa cytoplasmic protein that is necessary for the onset and the stability of AChR clusters [5]. Mutations in the gene coding for this protein are responsible for ~10% of congenital myasthenia gravis syndromes [3]. The mechanism ensuring cluster formation is not yet fully understood however. In particular, it is not clear whether rapsyn and AChRs are the only partners of the core of the cluster. Furthermore, the stoechiometry and topology of AChR:rapsyn complex are debated [1, 6, 8]. The previous studies could not resolve single molecules and only observed average behaviours of large populations of molecules.

We addressed these issues with cryoelectron tomography of *Torpedo marmorata* electric organ, a model system of the neuromuscular junction, prepared by various ways. Vitrification of native tissue by high pressure freezing turned out to be difficult. Most of the work presented was therefore performed on isolated postsynaptic membranes, which were suitable for plunge freezing. Lately, a mild purification of synaptosome, followed by high pressure freezing and vitreous sectioning has been attempted. Low-dose tilt series were acquired and tomograms were reconstructed. Individual AChRs are visible inside large clusters and other proteins are found near the cytoplasmic end of AChRs but the signal to noise ratio is too low to directly answer our questions. Subtomogram averaging and classification were therefore performed. Our results indicate that a variable number of rapsyns can bind individual AChRs.

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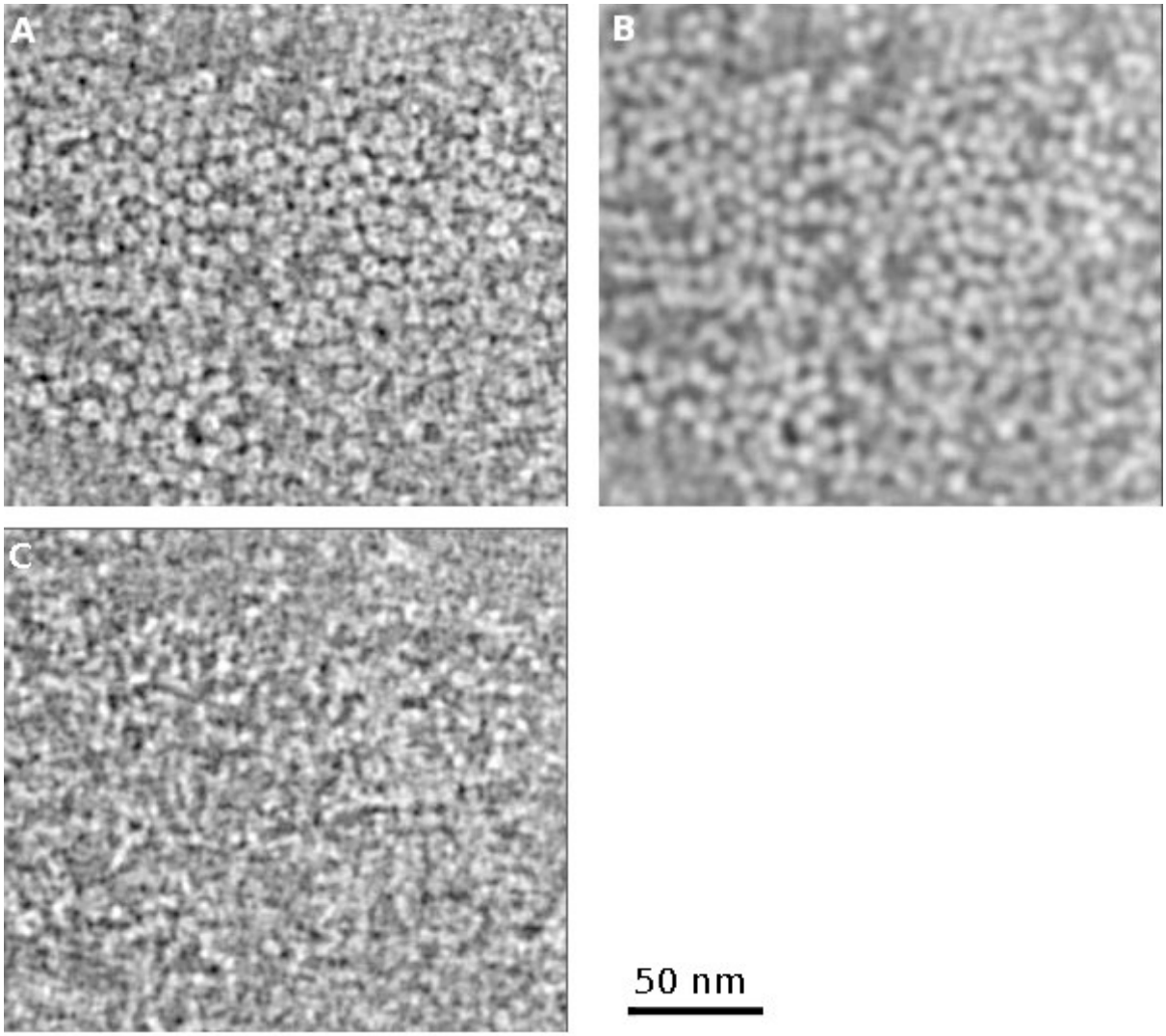

Figure 1. 7.5Å-thick slices through a tomographic reconstruction of an AChR-rich postsynaptic membrane. Raw images were aligned with IMOD [7], the tomogram was obtained by simultaneous iterative reconstruction technique implemented in Priism/IVE [2]. It was subsequently filtered with a Beltrami flow algorithm implemented in TOMOBFLOW [4]. A. Cross-section through the extracellular part of AChRs. B. Same slice as in A but filtered more strongly to facilitate the visualisation of AChR organisation. C. A slice through the cytoplasm, underneath the tip of AChRs, showing a dense protein network.

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