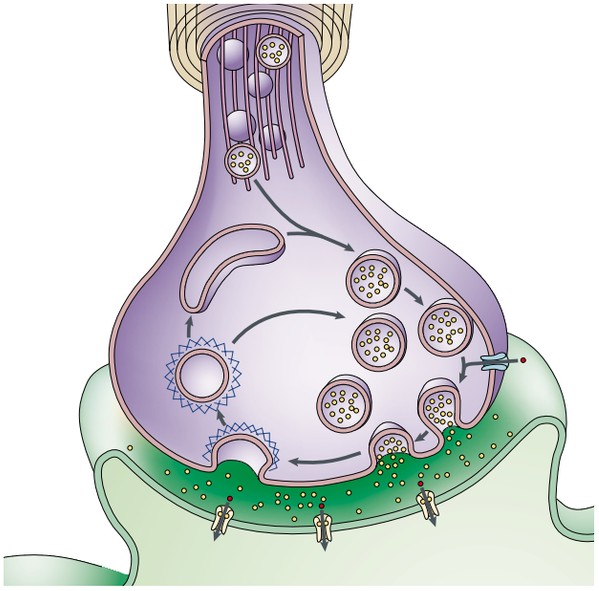

SYNAPTIC PHYSIOLOGY

It's all in the rhythm

How do exo- and endocytosis interact at the presynaptic terminal? Intuitively, we might think that the more synaptic vesicles fuse, the more endocytosis will take place to restock them. A recent paper challenges this idea by showing that endocytosis is actually reduced if release is increased.

Sun *et al.* recorded exo- and endocytosis at the calyx of Held — a giant brainstem synapse — by measuring presynaptic variations in membrane capacitance (C_m), which changes as a function of membrane area; a fast increase in C_m indicates fusion and a slower decay corresponds to membrane retrieval. They evoked fusion at different stimulation frequencies while measuring C_m. Higher stimulation frequencies led to slower endocytosis, and the net increase in C_m (and therefore in synaptic membrane area) paralleled this effect. So, the number of fused vesicles that are still part of the membrane might directly regulate the endocytic rate. The mechanism by which this regulation occurs is unknown, but this means of controlling vesicle turnover might help to prevent overactivation of the postsynaptic cell.

In a related paper, Waters and Smith reported a similar relationship between vesicle traffic and stimulation frequency at another central synapse. They measured synaptic uptake and release of the fluorescent dye FM1-43 in cultured hippocampal neurons, and found that, when stimulating at 10 Hz, fusion rate was related to the number of labelled (recycling) vesicles at the terminal. By contrast, they failed to see such a direct relationship with a stimulation frequency of 1 Hz. These observations argue that the conventional 'scaling model', which states that synaptic properties such as vesicle number and release probability scale together, might not apply at low firing frequencies.

Although we still lack an understanding of the functional implications of these results, both studies highlight the relevance of vesicle recycling to synaptic efficacy, a field that remains to be explored in detail.

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NEUROTECHNIQUE

Pure and simple

Embryonic stem (ES) cells should be able to generate any cell type, but directing their differentiation *in vitro* has proved to be far from easy. For example, ES cells that are grown in aggregates (embryoid bodies, or EBs) will generate large numbers of neurons, particularly if the EBs are exposed to retinoic acid. However, this process is relatively uncontrolled, and the EBs are highly disorganized, so the neuronal precursors might be exposed to signals that they would not normally encounter in the embryo. Perhaps because of this, neuronal precursors that are derived from EBs can only generate a limited range of neuronal cell types. Now, however, Rathjen *et al.* seem to have circumvented this problem by developing a new protocol that enables them to bypass the EB stage to generate a virtually pure neuroectoderm from mouse ES cells.

The authors previously generated a conditioned medium called MEDII, which can convert ES cells in adherent culture into a homogeneous population of primitive-ectoderm-like cells. In their new study, they cultured ES cells in suspension in the presence of MEDII. The cells formed aggregates that superficially resembled EBs, although their subsequent differentiation was much more ordered. Initially, the aggregates formed vesicles that were surrounded by a stratified epithelium, but after further culture in a different medium, over 95% of the cells took on a columnar appearance and became organized into a layer that closely resembled neuroectoderm. The cells also expressed neuroectodermal markers, including *Sox1*, *Sox2* and nestin, and on further differentiation, they gave rise to all the expected neuroectodermal derivatives, including neurons, glia and even neural crest.

The authors speculate that their technique will not only help them to generate pure neuronal populations for therapeutic purposes, but will also provide a valuable model to study neural induction and neuronal differentiation. Importantly, the aggregates generated by this technique lack visceral endoderm and other endodermal and mesodermal lineages, so the cells are not exposed to the signals that direct neuroectodermal patterning and differentiation *in vivo*. Consequently, the tissue is essentially naive, and it has no positional identity, so it could be used to test putative neural-tube patterning molecules. Also, by identifying the active components of MEDII, it should be possible to precisely define the conditions that are required to direct ES cells into the neural lineage in culture.

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