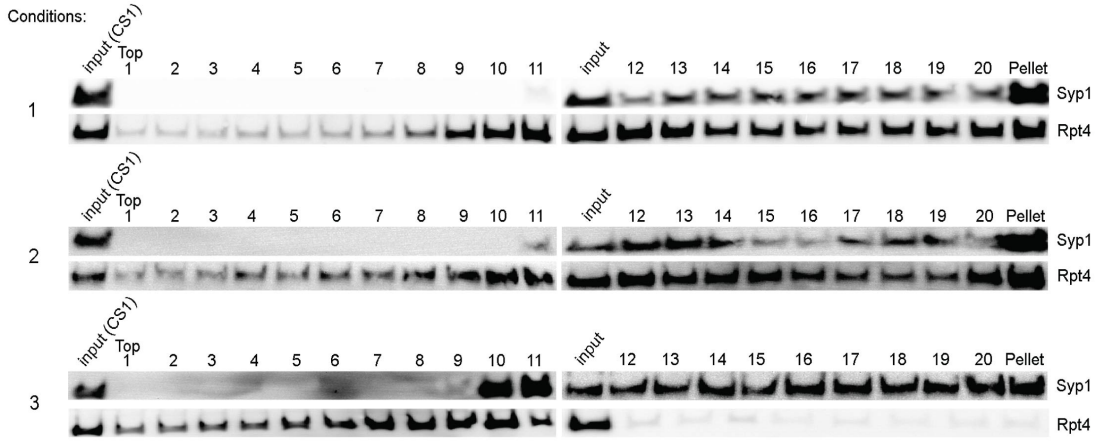

Optimisation of the sucrose cushion step.
Representative immunoblots of fractions taken from the sucrose cushion, following centrifugation under different conditions. Fractions were tested for the presence of synaptophysin (synaptic vesicle marker) or Rpt4 (proteasome marker); in our experience, proteasomes are the major contaminant of the synaptic vesicle preparation. Different relative volumes of the input fraction (CS1) to 0.7M sucrose cushion were used. Centrifugation forces and times for a 70.1 Ti rotor were also systematically varied (although only data for 5ml CS1 on a 5ml cushion centrifuged at 65,000 rpm (400,000 g_{max}; condition 1), 54,000 rpm (270,000 g_{max}; condition 2) and 38,000 rpm (133,000 g_{max}; condition 3) are shown). Note the differential separation of Rpt4 from synaptophysin under the various conditions. Best separation was found using a 0.7M sucrose cushion centrifuged at 38,000 rpm for 1h.