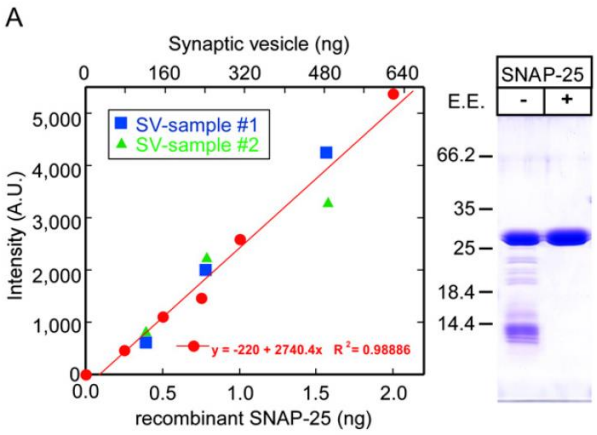
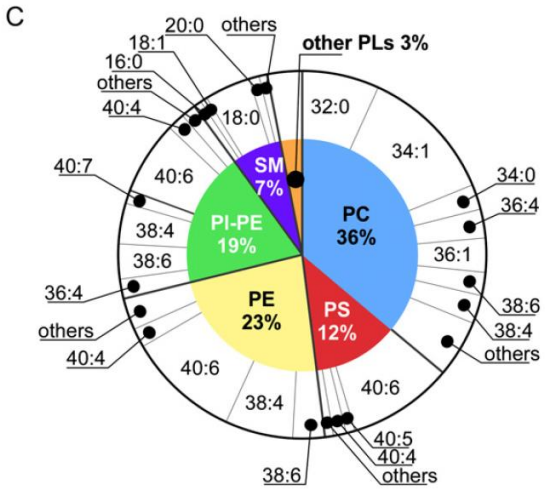


B

Protein	% of total SV proteins
Synaptophysin	10.20 ± 1.54(1,4)
Synaptobrevin 2	8.60 ± 1.55(1)
Syntaxin 1	2.00 ± 0.27(1,5)
SNAP25	0.40 ± 0.06(1,6)
Synapsins	6(2,3)
Rab3A	2.5(2)
Synaptotagmin 1	7(3)
Synaptogyrin 1	0.5(2)
SV2	1.4(2)
SCAMP	0.3(2)
CSP	0.6(2)
VGLUT1	5.36 ± 1.11(1,7)
VGLUT2	9.01 ± 2.31(1,7)
V-ATPase V1-B subunit	1.15 ± 0.21(1)



D

Membrane lipid	ng lipid/µg protein
Phosphatidylcholine	186 ± 37
Phosphatidylethanolamine	
1- ester	120 ± 18
1- ether (plasmalogen)	96 ± 14
Phosphatidylserine	68 ± 7.2
Phosphatidylinositol	9.73 - 10*
Sphingomyelin	37 ± 5.1
Cholesterol	215 ± 25
Hexylceramide	8.6 ± 4.0
Ceramide	1.2 ± 0.1
Protein : Phospholipid (w/w)	1.94 ± 0.23

Figure 1. Quantitative Measurements of SV Proteins and Lipids

(A) Left panel: Determination of the amount of SNAP-25 using quantitative western blotting of vesicle proteins in comparison to a standard curve of purified recombinant protein. All measurements within the linear range of the standard curve (0.0–2.0 ng for SNAP-25, red circles) were taken into account. Densitometric measurements from two independent SV samples (SV sample #1, blue squares; SV sample #2, green triangles) are shown (left panel). Right panel: Three micrograms of SNAP-25 standard proteins purified conventionally (left lane) and after an additional electroelution step (right lane) was subjected to SDS-PAGE followed by Coomassie blue staining.

(B) Quantities of major SV proteins. For each protein, the values are expressed as % of the specific protein related to the total SV protein. Superscripted 1 denotes data determined according to the procedure shown in (A). Superscripted 2 denotes data determined by quantitative dot blotting (Jahn et al., 1984) using purified recombinant proteins (mostly containing affinity tags) as a standard. Superscripted 3 denotes data from Chapman and Jahn (1994). Superscripted 4 denotes data for which somewhat lower values were previously reported (Navone et al., 1986). Superscripted 5 denotes values similar to those published earlier (Goelz et al., 1981; Walch-Solimena et al., 1995). Superscripted 6 denotes values lower than those published earlier by our laboratory (Walch-Solimena et al., 1995). Superscripted 7 denotes values corrected for the percentage of vesicles positive for these transporters, which was determined by immunogold labeling of negatively stained vesicle fractions.

(C and D) Quantitative analysis of lipid constituents.

(C) A pie diagram depicts the mol% of the various phospholipid species and the fatty-acid distribution.

(D) Values expressed per µg of protein. Five samples were analyzed in triplicate. The average and standard deviation are given. Asterisk indicates that only two samples were analyzed; numbers indicate range of values.

Chapman, E.R., and Jahn, R. (1994). Calcium-dependent interaction of the cytoplasmic region of synaptotagmin with membranes. Autonomous function of a single C2-homologous domain. J. Biol. Chem. *269*, 5735–5741.

Goelz, S.E., Nestler, E.J., Chehrazi, B., and Greengard, P. (1981). Distribution of protein I in mammalian brain as determined by a detergent-based radioimmunoassay. Proc. Natl. Acad. Sci. USA *78*, 2130–2134.

Jahn, R., Schiebler, W., and Greengard, P. (1984). A quantitative dot-immunobinding assay for proteins using nitrocellulose membrane filters. Proc. Natl. Acad. Sci. USA *81*, 1684–1687.

Navone, F., Jahn, R., Di Gioia, G., Stukenbrok, H., Greengard, P., and De Camilli, P. (1986). Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. J. Cell Biol. *103*, 2511–2527.

Walch-Solimena, C., Blasi, J., Edelmann, L., Chapman, E.R., Fischer von Mollard, G., and Jahn, R. (1995). The t-SNAREs syntaxin 1 and SNAP-25 are present on organelles that participate in synaptic vesicle recycling. J. Cell Biol. *128*, 637–645.