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**Heterogenous distribution of VACHT and VGLUT3 in striatal cholinergic varicosities  
revealed by a nanoscopic analysis**

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**Aim**

Striatal cholinergic interneurons (CINs) use both acetylcholine (ACh) and glutamate (Glut) to regulate the striatal network. CINs express vesicular transporters for acetylcholine (VACHT) and Glut (VGLUT3) with a high degree of coexpression in cholinergic varicosities. Whether VACHT and VGLUT3 are present on the same or on different pools of synaptic vesicles (SVs) remains an open question. This is an important issue since the vesicular sorting of both transporters could lead to simultaneous and/or independent release of ACh and/or Glut from CINs.

**Methods**

We used super-resolution STimulated Emission Depletion microscopy to characterize and quantify the distribution of VACHT and VGLUT3 in CINs SVs.

**Results**

Nearest-neighbor distances analysis between VACHT and VGLUT3-immunofluorescent spots revealed that 34% of cholinergic SVs contain both VACHT and VGLUT3. 40% of CINS SVs express only VACHT, while 26% contain only VGLUT3. Thus, we propose that CINs terminals have the potential to store and release simultaneously or independently ACh and/or Glut. We also determined the distribution in VACHT and/or VGLUT3 expressing SVs, of vesicular associated membrane proteins (VAMPs), proteins of the SNARE complex involved in neurotransmitter release. Fluorescent in situ hybridization demonstrated that only VAMP2 and VAMP7 are expressed by CINS. We observed that the canonical VAMP2 is expressed in a large part of VACHT and VGLUT3 striatal SVs (63% and 86%). Interestingly, in the shell part of nucleus accumbens, 25% of VACHT and VGLUT3-immunopositive SVs are also VAMP7-positive.

**Conclusion**

Our results advocate for an unexpected level of anatomical and functional heterogeneity of SVs in CINs varicosities.