

E08-07-0765 Chapman

Supplemental Figures

Supplemental Figure 1. SV2C is expressed in a subpopulation of synapses in cultured hippocampal neurons and can mediate the binding of BoNT/A but not BoNT/E.

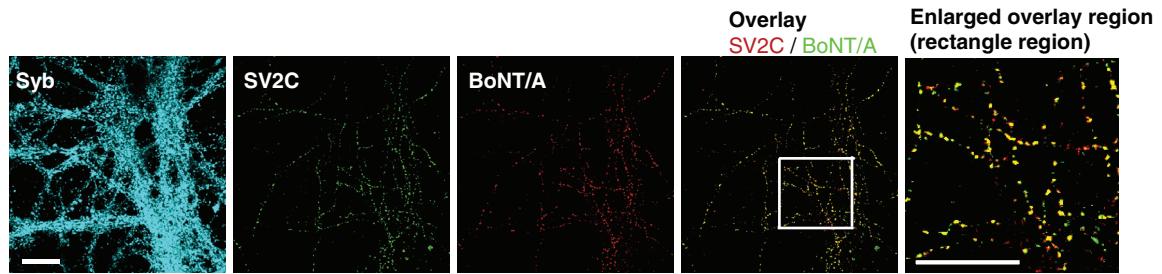
A) Cultured hippocampal neurons from SV2 A/B KO mice were exposed to BoNT/A (20 nM) for 5 min in high K⁺ buffer. Cells were washed, fixed, and immunostained using antibodies against SV2C, BoNT/A and Syb. Syb was used as a marker for synapses. SV2C was detected in a subpopulation of synapses. BoNT/A was found to bind these SV2C-positive synapses.

B) Experiments were carried out as described in panel A, except that cells were exposed to BoNT/E (30 nM). vGlut was labeled as a marker for synapses. Binding of BoNT/E to SV2C-positive cells was not detectable.

Supplemental Figure 2. SV2A(N573Q) was expressed in neurons at similar levels as WT SV2A and co-localized with the synaptic vesicle marker Syb.

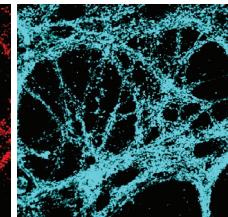
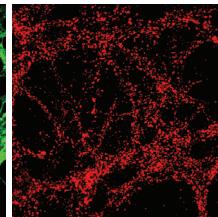
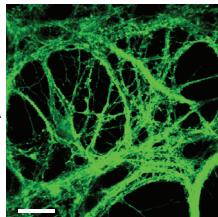
A) Cultured SV2A/B KO neurons were infected with lentiviruses that express SV2A(WT) or SV2A(N573Q), respectively. Cells were fixed and immunostained with antibodies against GFP, SV2A, and Syb. GFP is expressed under control of a separate promotor in the virus vector, and served to indicate that almost all neurons were infected. Representative images are shown.

B) Images were acquired as described in panel A. The rectangle region is enlarged to show the co-localization between Syb and SV2A(N573Q).

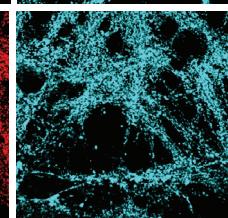
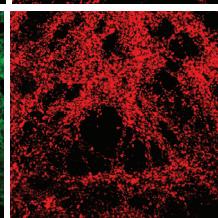
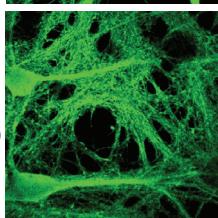
A**B**

A GFP SV2A Syb

WT SV2A

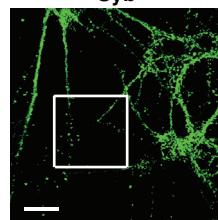


SV2A(N573Q)

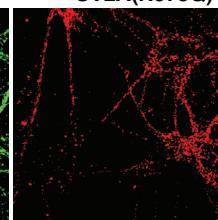


B

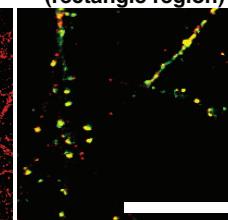
Syb



SV2A(N573Q)



Overlay
(rectangle region)



Supplemental Figure 2 (Chapman)