# Correlating Fluorescence and High-Resolution Scanning Electron Microscopy (HRSEM) for the study of $GABA_A$ receptor clustering induced by inhibitory synaptic plasticity

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## Supplementary figures

**Fig. S1**

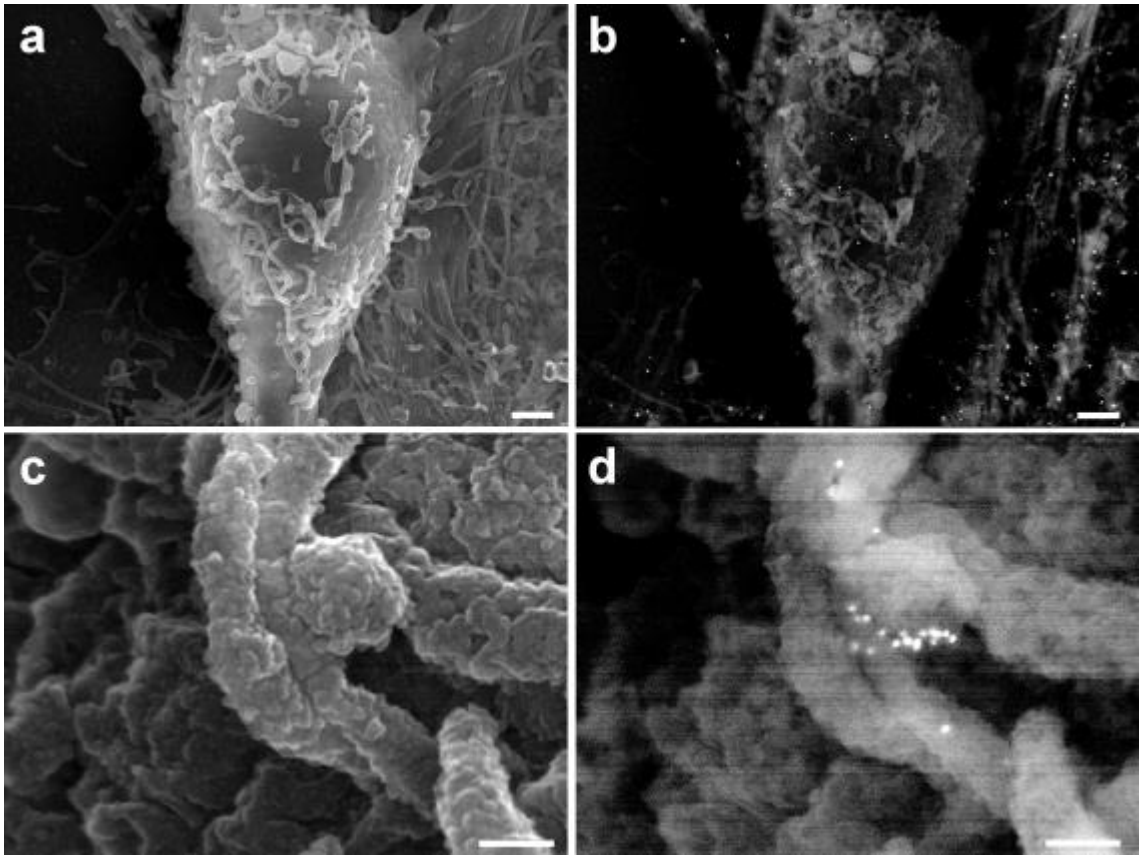


**Fig. S1. SE coupled with BSE images of $GABA_AR\alpha1$ immunolabeled primary mouse hippocampal neurons using FluoroNanogold™ (a and b) and 10 nm colloidal gold particle-conjugated secondary antibody (c and d).** a: low magnification SE image of a neuron cell body. b: BSE image of the same field of view shown in a. The white spots are the enhanced gold nanoparticles. c: high magnification SE image of a contact region between neuritis, i.e. *bona fide* synaptic contact. d: BSE image of the same field of view shown in d. The white spots are the 10 nm gold nanoparticles. Scale bars:1 µm in a and b; 0.1 µm in c and d.

**Fig. S2**

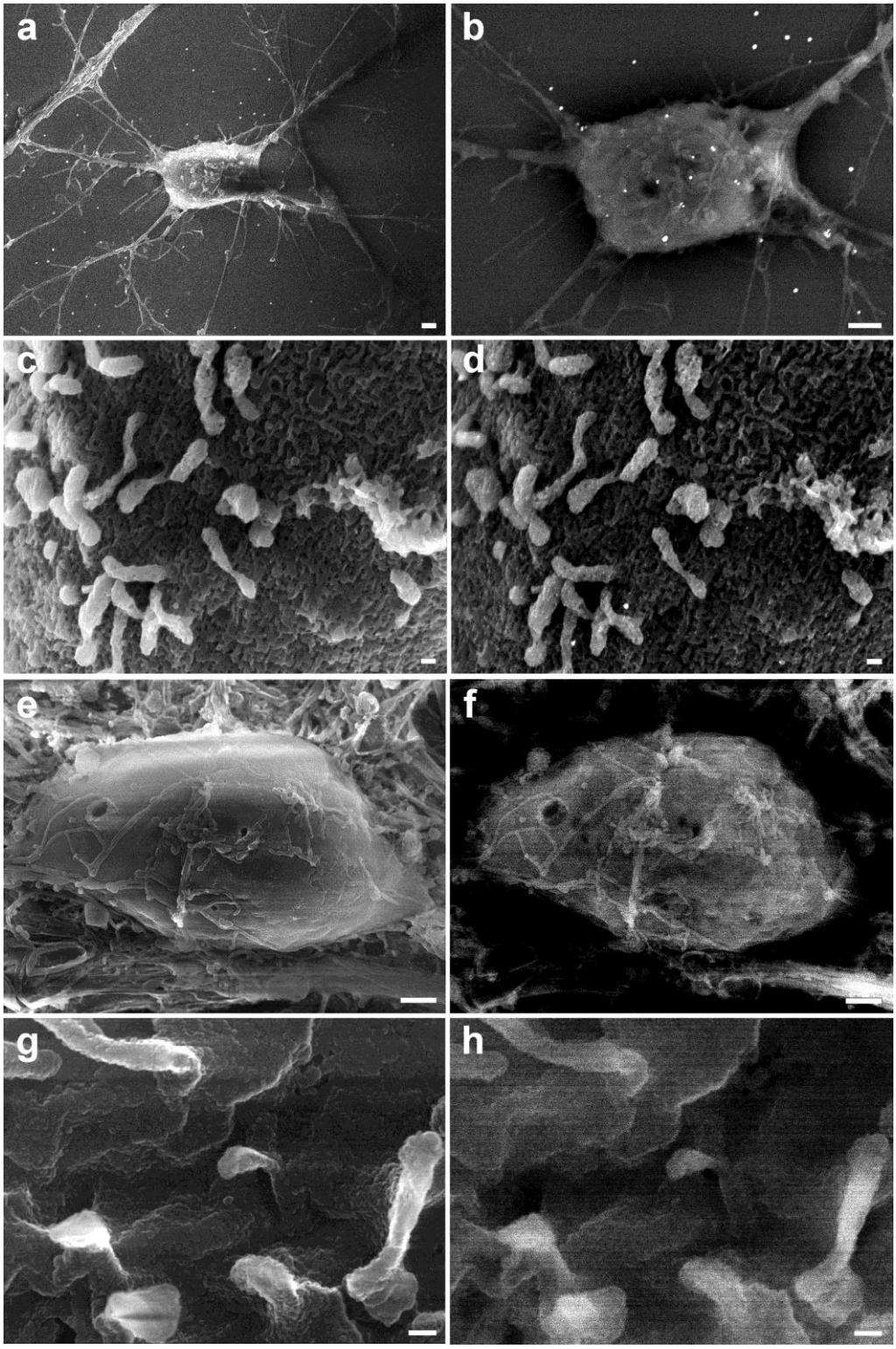


**Fig. S2. SE coupled with BSE images of negative control primary mouse hippocampal neurons using FluoroNanogold™ followed by gold enhancement (a-d) and 10 nm colloidal gold particle-conjugated secondary antibody (e-h).** a: low magnification SE image of a neuron cell body. b: BSE image of the same field of view shown in a. The large white spots on the coverslip and on the neuron are FluoroNanogoldTM non-specific labelling. c: high magnification SE image of a cell body region. d: BSE image of the same field of view shown in c. The small white spots on the background are non-specific labelling produced by the gold enhancement reaction. The small white spots are the 10 nm gold nanoparticles. e: low magnification SE image of a neuron cell body. f: BSE image of the same field of view shown in e. f: high magnification SE image of a cell body region. d: BSE image of the same field of view shown in d. Scale bars:1 µm in a, b and e, f; 0.1 µm in c, d and g, h.

**Table S1.** Average cluster density, average cluster size (n gold particles/cluster) and average number of clusters formed by n≤5 and n>5 gold particles on the cell membrane of cell bodies and neurites of CTRL and NMDA stimulated hippocampal neurons. $n_i$=image number; $n_c$=cluster number. Values are average ± s.e.m.

| | *Cluster average density ± s.e.m. (n/ $\mu m^2$)* | | *Clusters size ± s.e.m.* | | *Average number of clusters formed by n gold particles ± s.e.m.* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | ***n ≤5*** | ***n>5*** | ***Total*** |
| ***CTRL neurons*** | 16.9 ± 3.6 | $n_i$=19 | 3.6 ± 0.2 | $n_c$=584 | | 49.8 ± 17.1 | 10.4 ± 4.2 | $n_c$=662 |
| | | | | | | | | |
| ***NMDA neurons*** | 29.4 ± 5.1 | $n_i$=44 | 6.2 ± 1.2 | $n_c$=1660 | | 76.5 ± 14.4 | 31.0 ± 7.1 | $n_c$=4169 |

**Table S2.** Average post-synaptic membrane surface, average number of gold clusters *per* synapse and average gold cluster volume *per* synapse and post-synaptic area on CTRL and NMDA mice hippocampal neurons measured on tomograms. Values are average ± s.e.m.

| | *Average post-synaptic membrane surface ($\mu m^2$) ± s.e.m.* | | *Average number of gold clusters/synapse ± s.e.m.* | | *Average gold cluster volume/synapse (µm3) ± s.e.m.* | | *Average gold cluster volume/post synaptic area (µm) ± s.e.m.* | |
|---|---|---|---|---|---|---|---|---|
| ***CTRL neurons*** | 0.74 ± 0.11 | n=14 | 2.6 ± 0.3 | n=14 | 0.001 ± 0.0002 | n=14 | 1.8 ± 0.3 | n=14 |
| | | | | | | | | |
| ***NMDA neurons*** | 0.70 ± 0.15 | n=12 | 4.0 ± 0.6 | n=12 | 0.002± 0.0005 | n=12 | 3.2 ± 0.9 | n=12 |

**Movie S1.** HAADF STEM WBP tomogram and relative 3D model of an inhibitory synapse immunolabeled for the $GABA_AR\alpha1$ in a CTRL hippocampal neuron as shown in Figure 4A.

**Movie S2.** HAADF STEM WBP tomogram and relative 3D model of an inhibitory synapse immunolabeled for the $GABA_AR\alpha1$ in a NMDA stimulated hippocampal neuron as shown in Figure 4B.