

Leica EM HPM100

Freeze-fracture replication of pyramidal cells

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Living up to Life

Leica EM HPM100

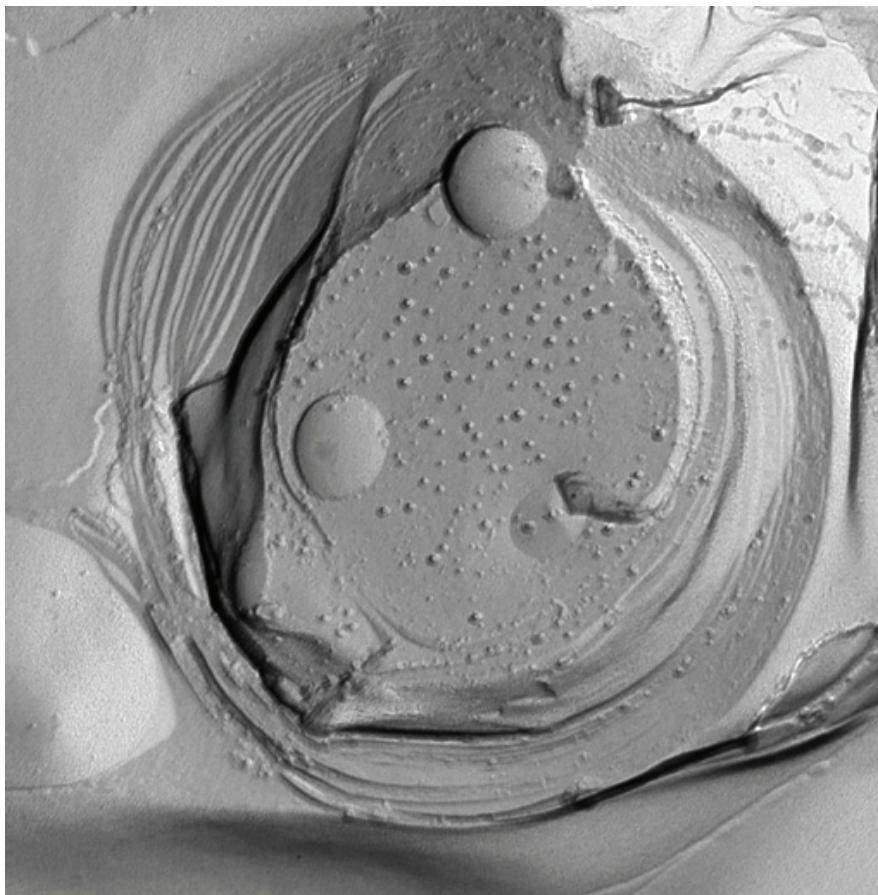
Freeze-fracture replication of pyramidal cells

Method:

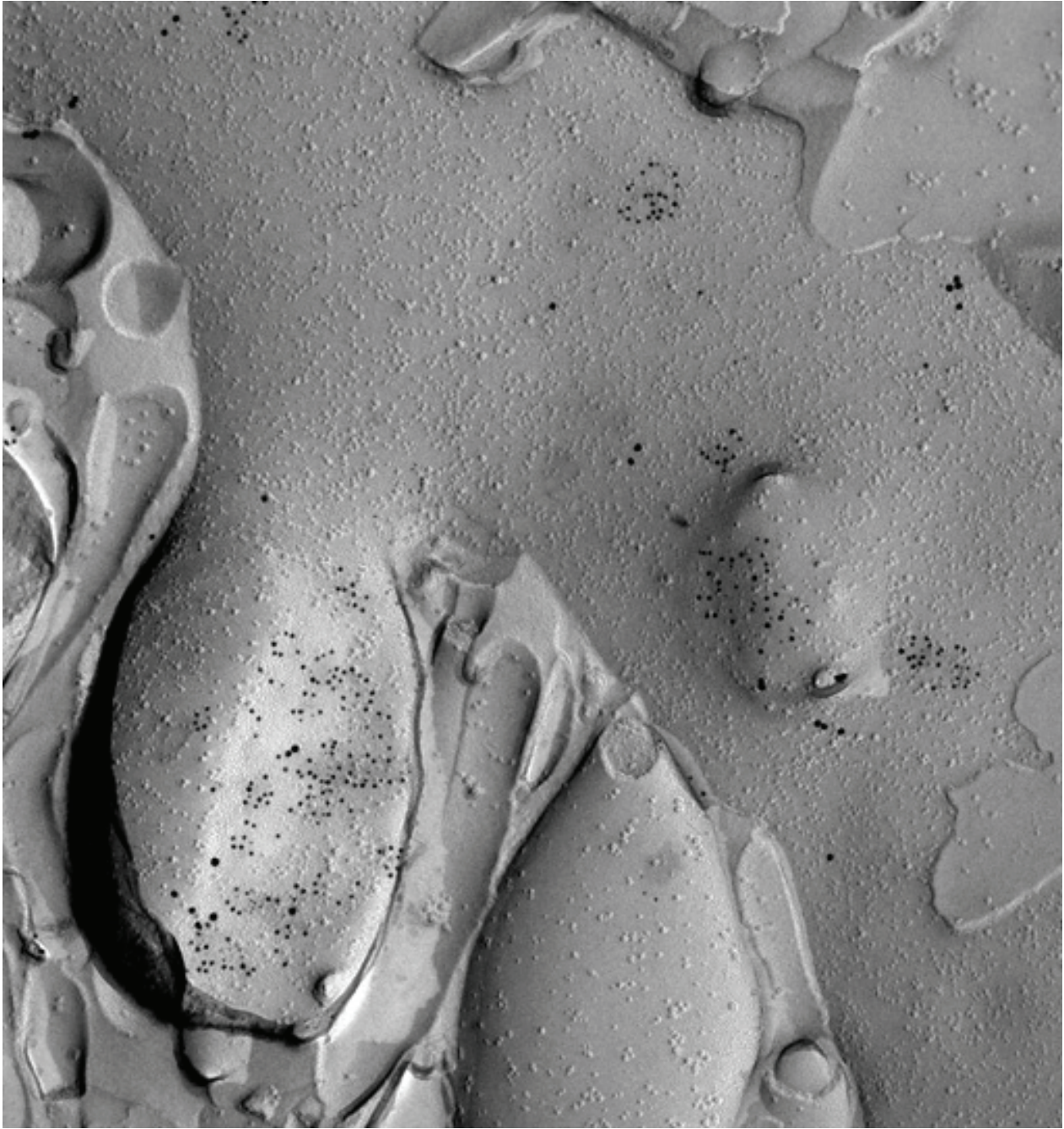
Frozen samples (90 μ m thick slices frozen by HPM100) were inserted into a double replica table and then fractured into two pieces at -130°C (after insertion of the tissue into BAF 060 the samples should be left in the chamber for 20 min to reach the -130°C).

Fractured faces were replicated by (i) deposition of carbon (5 nm) at 45° angle with sample rotation, (ii) platinum/carbon (2 nm) at 60° angle without sample rotation, (iii) carbon (15-20 nm) at 45° angle with sample rotation.

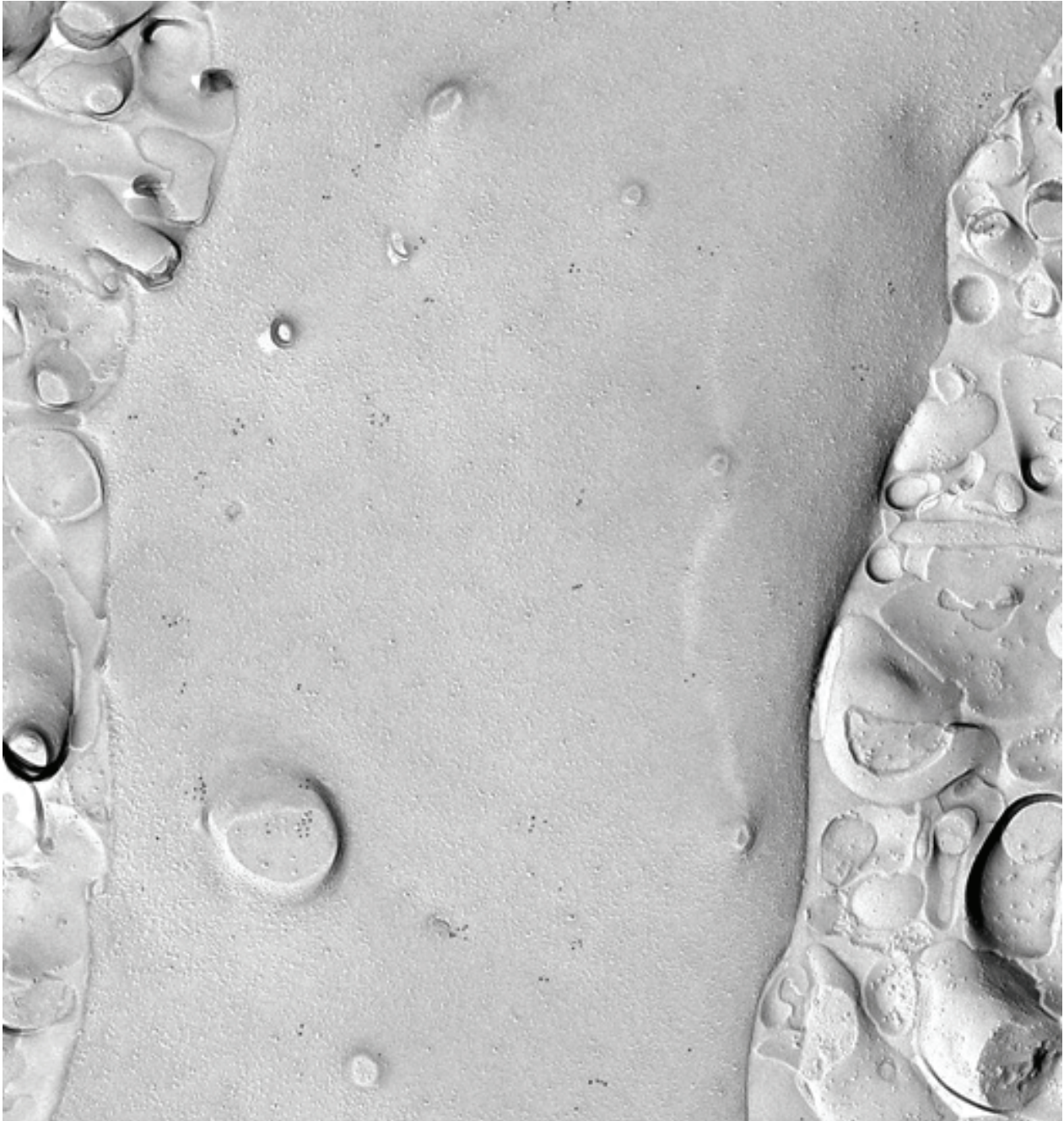
Replicas are transferred into 2.5% SDS solution and samples are incubated at $+80^{\circ}\text{C}$ for 18 hours with continuous shaking.



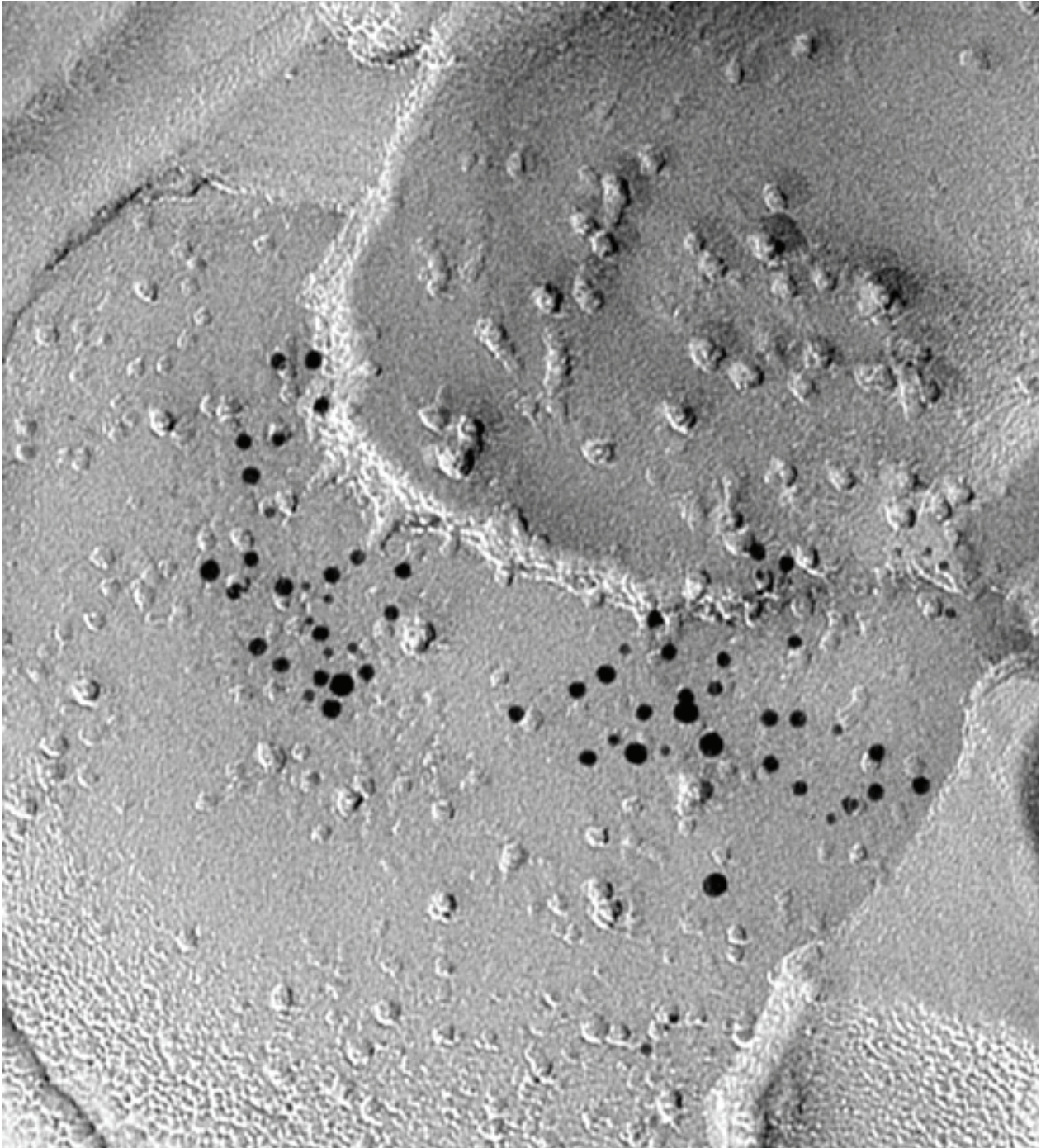
Myelinated axons



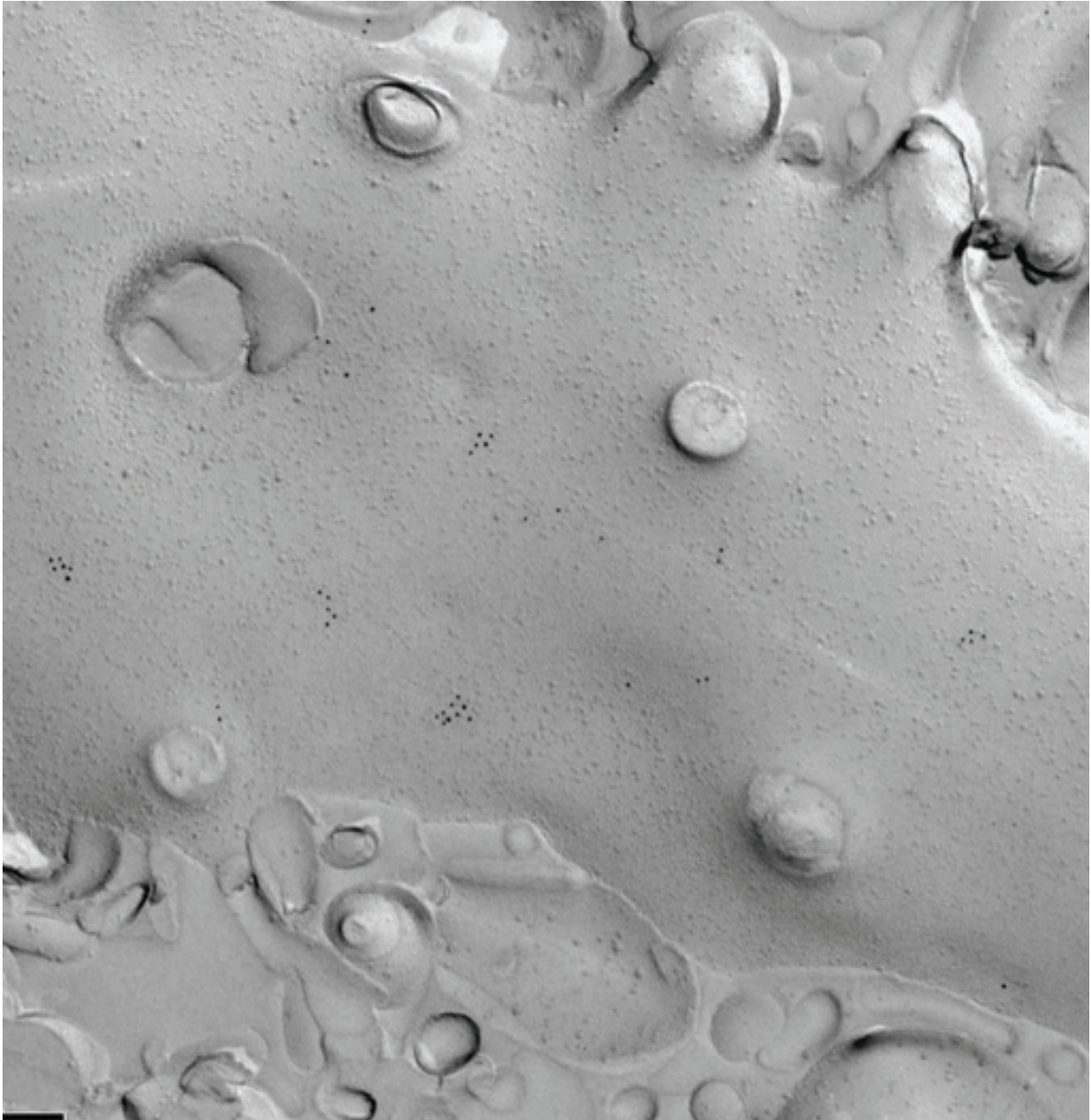
Synaptic connection between a bouton (P-face) and a dendritic spine (E-face) of a pyramidal cell



Dendritic shafts of presumed CA1 pyramidal cells showing immunoreactivity for KCTD12 that is an auxiliary subunit of the GABA(B) receptor.



Synaptic connection between an axon terminal (unlabeled) and a presumed dendritic spine showing immunoreactivity for PSD95 (15nm), KCTD12 (10nm), and GABA(B1) (5nm). The image is obtained from the CA1 area of the hippocampus.



Dendritic shafts of presumed CA1 pyramidal cells showing immunoreactivity for KCTD12 that is an auxiliary subunit of the GABA(B) receptor.