3-D reconstruction of learning-induced multiple synapses using HVEM

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Structural change of synapses has long been hypothesized to modify synaptic efficacy and to be involved in learning and memory. Motor learning increases the number of synapses per Purkinje cell in the adult rat cerebellum. Interestingly, multiple synaptic varicosities (MSVs) formed between a single parallel fiber varicosity and two Purkinje spines markedly increase in number after complex acrobatic training. It should be determined, however, whether the two spines on MSVs induced by motor learning originate from the same dendrite or different ones to understand their functional implications in synaptic plasticity. Here we investigated this issue by reconstructing MSVs with serial electron microscopy following 26 days of acrobatic training. Additionally, morphological analyses of spine density, length, and shape in the Purkinje cells were performed using high voltage electron microscopy (HVEM). The results revealed that mostof MSVs in motor learning group contacted two Purkinje spines arising from the same dendrite, indicating that motor learning-induced MSVs have the potential to enhance synaptic strength. The structural analyses of dendritic spines using HVEM showed that spine density and length increased significantly in acrobat-trained animals in comparison with control animals, supporting that motor learning induces synaptogenesis in the cerebellar cortex. The spine elongation observed in the learning group might contribute to the increase in synapse number by raising the possibility to contact neighboring presynaptic varicosities. These results demonstrate that motor learning increases the number of presynaptic varicosities that contact spine pairs from a single dendrite in the cerebellum. This study suggests that the increase in synaptic strength by the generation of same dendrite MSVs during motor learning could play an important role in synaptic plasticity and motor memory formation.

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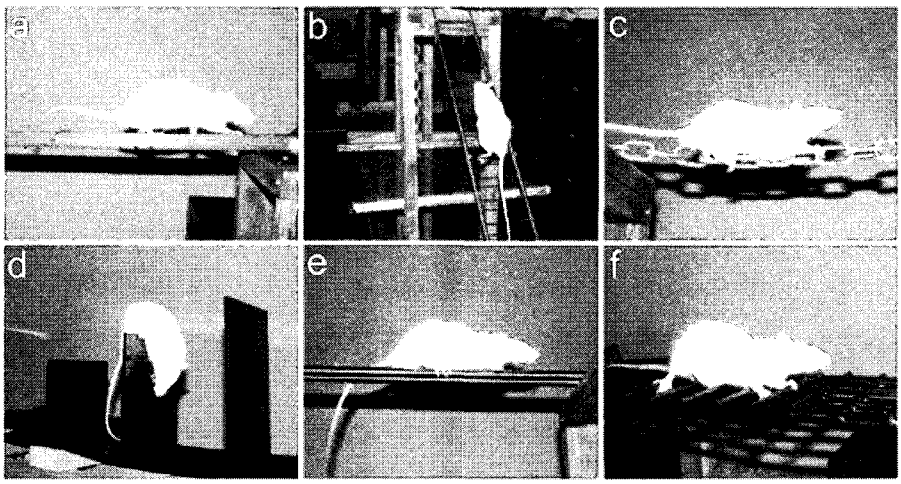

Fig. 1. The acrobat training demands a significant amount of motor coordination and balance to complete.

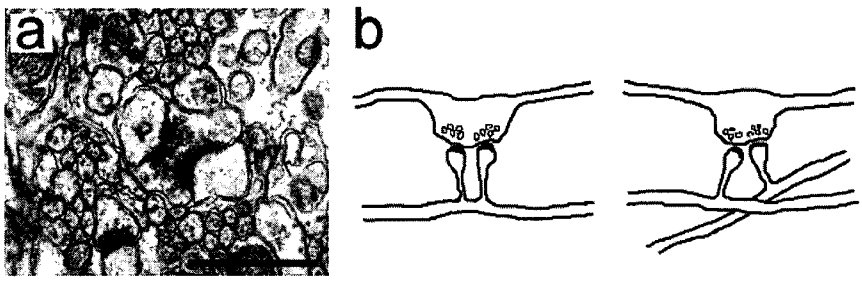

Fig. 2. A representative multiple synaptic varicosity (MSV) making contact with two Purkinje cell dendritic spines (a). Models for the origin of two dendritic spines contacting MSV (b). Scale bar, 1 μm.

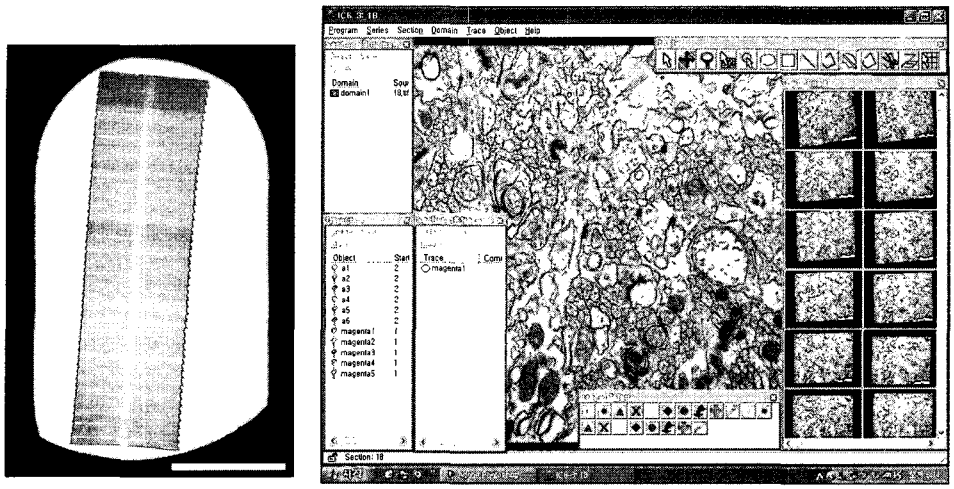

Fig. 3. Serial ultrathin sections and reconstruct software. Scale bar, 0.5mm.

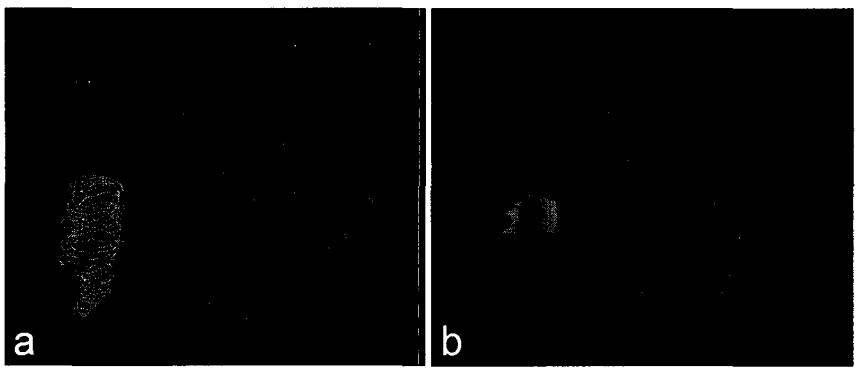

Fig. 4. A series of contours and a representative 3D reconstruction image.

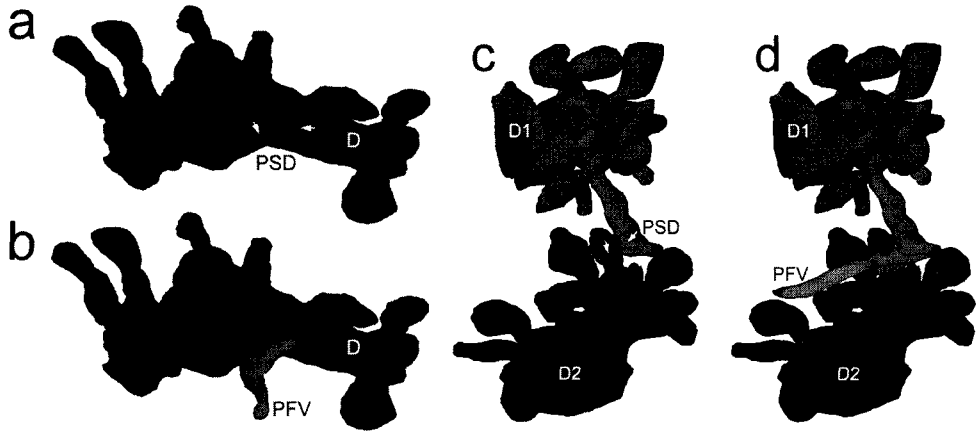

Fig. 5. Representative 3D configurations of MSVs.

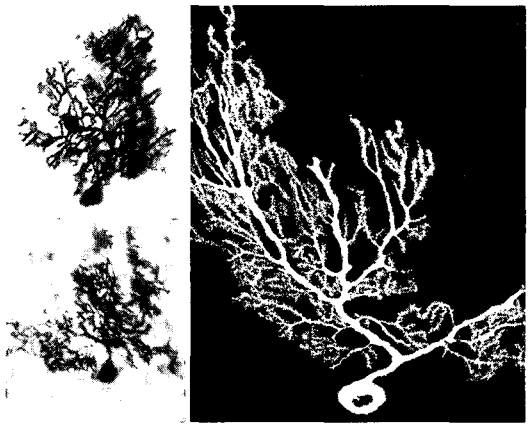

Fig. 6. Representative Golgi-impregnated Purkinje cells (a and b; LM) and a montage consisting of 7 HVEM images taken at 1,000X (c).

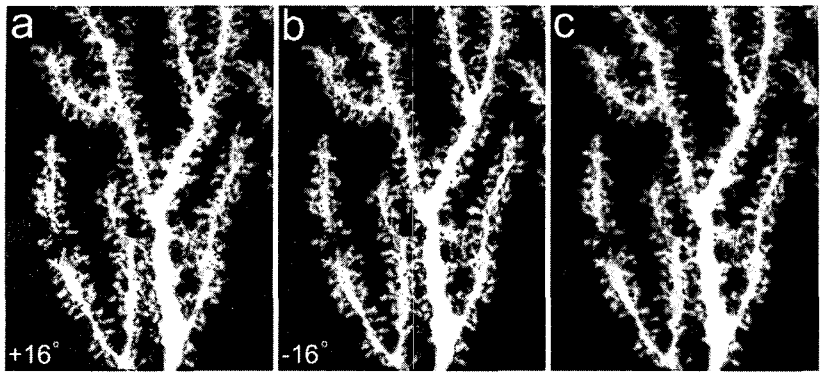

Fig. 7. The construction of anaglyphic image from a pair of tilted-HVEM images.

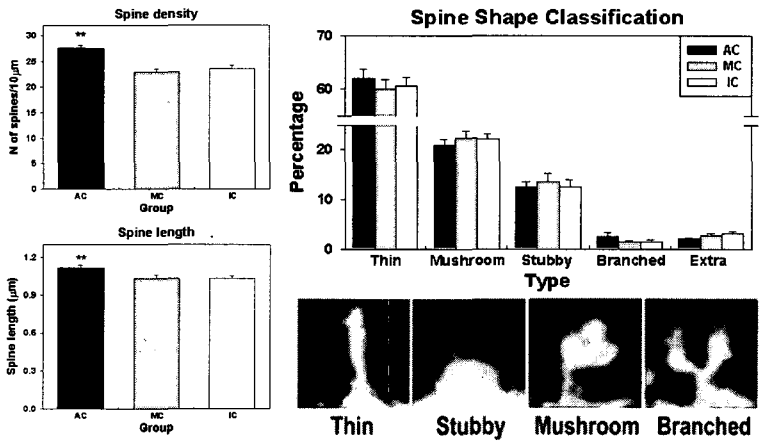

Fig. 8. Morphological analyses of spine density, length, and shapes.