

Synaptic Ultrastructural Alterations in Human Focal Cortical Dysplasia



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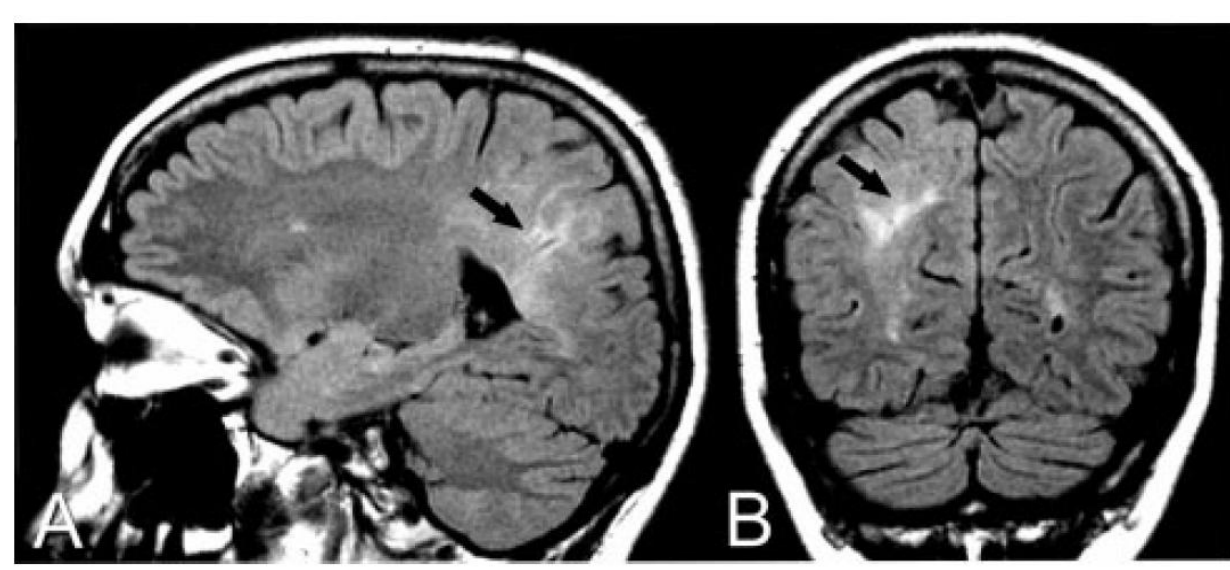
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Abstract

Focal cortical dysplasia (FCD) is a developmental disorder of the cerebral cortex and a leading cause of intractable epilepsy in children. A disrupted excitation-inhibition balance is a hallmark of neuronal hyperexcitability in FCD, yet the underlying ultrastructural mechanisms remain poorly understood. Using volume electron microscopy, we performed a morphological assessment of synapses and organelle distribution in the temporal cortical layer III of an FCD patient. Notably, the dysplastic region displayed fewer excitatory synapses but contained abnormally enlarged synapses with more synaptic vesicles. Additionally, inhibitory synapses were located further away from the nearest excitatory synapses, possibly weakening the effectiveness of inhibition in the dysplastic area. There was an increase in mitochondrial density and altered mitochondrial morphology within presynaptic boutons, along with a reduced proportion of postsynaptic protrusions containing a spine apparatus, indicating deficits in intracellular calcium handling and synaptic plasticity. These findings suggest that synaptic architectural modifications may contribute to neuronal hyperexcitability associated with epilepsy in FCD.

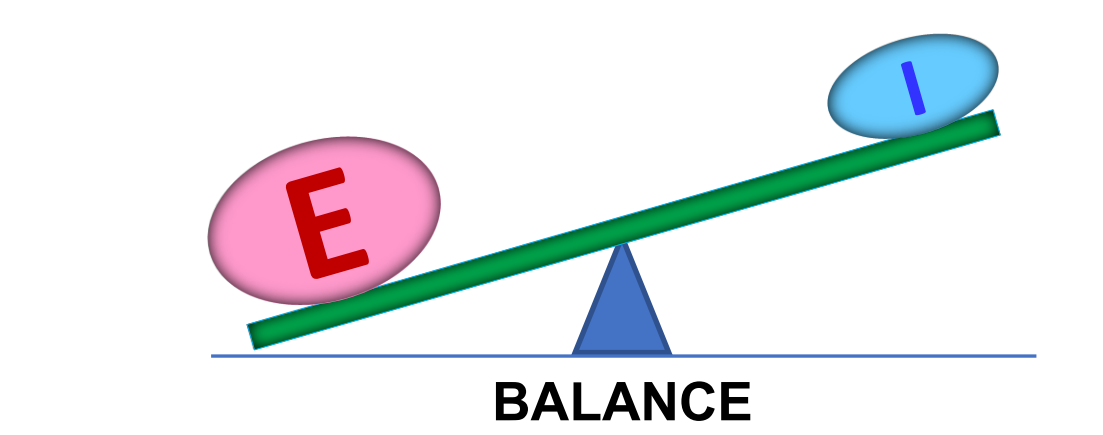
Introduction



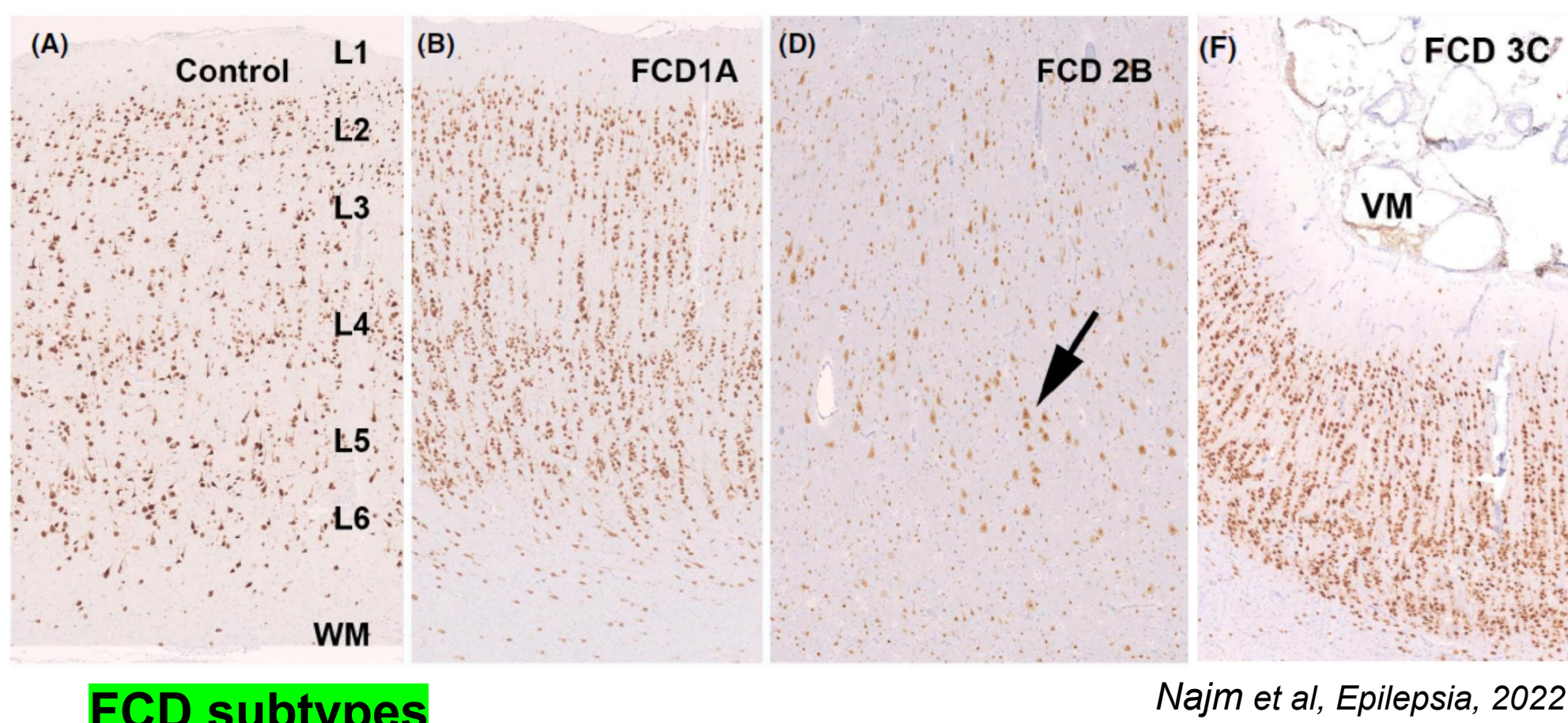
MRI of a patient with right parietal FCD
Alonso-Nanclares et al, Brain, 2005

Focal Cortical Dysplasia (FCD)

- A developmental disorder of the cerebral cortex and a leading cause of drug-resistant epilepsy
- Treatment: anti-epileptic drugs, DBS, VNS, surgery



Simple prediction:
Excitation ↑ or Inhibition ↓



FCD subtypes

- Type 1: vertical/horizontal dyslamination, microcolumns
- Type 2: dysmorphic neurons, balloon cells
- Type 3: cortical dyslamination with other lesions

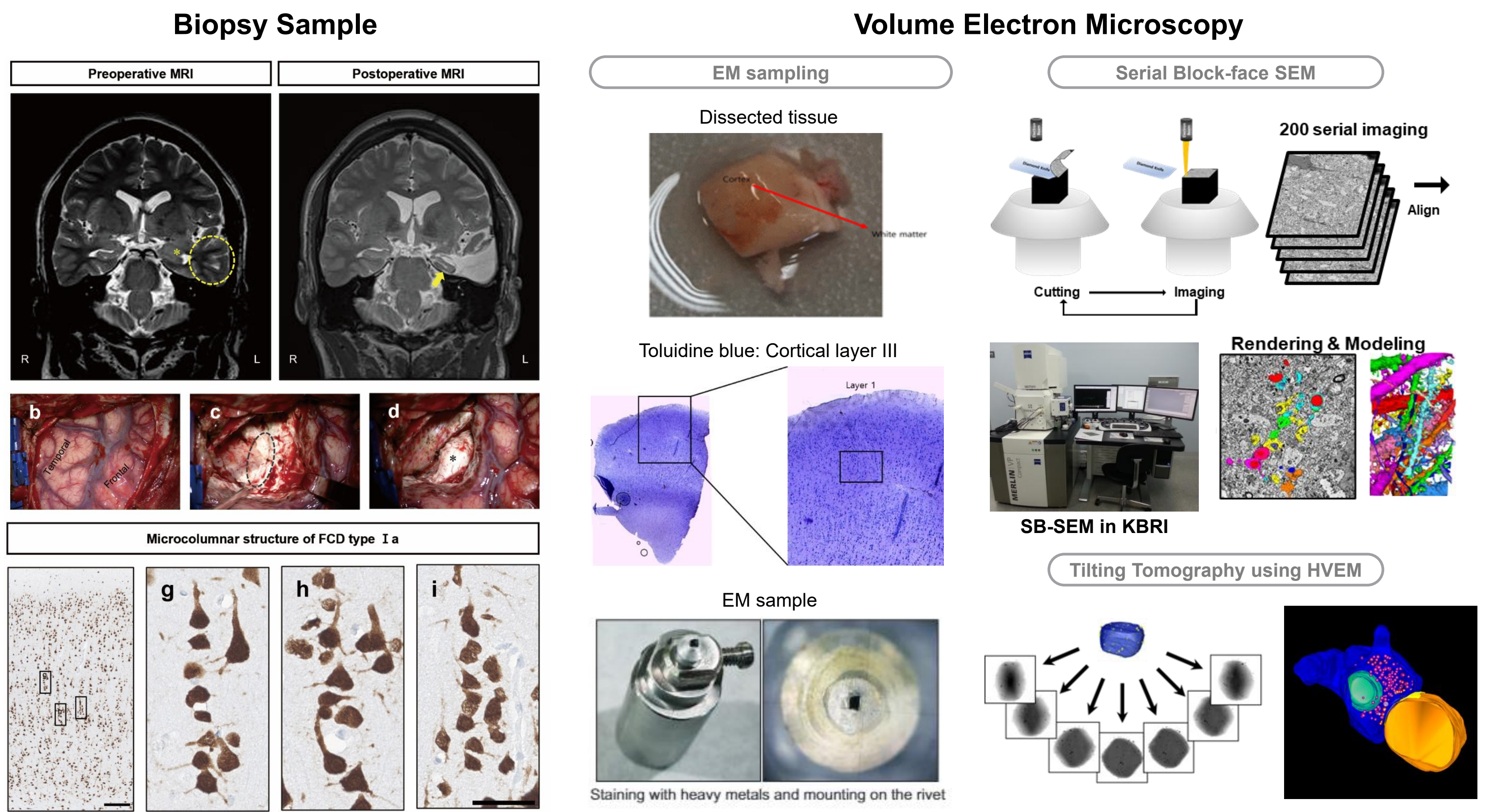
Basket and chandelier cells ↓
(Ribak, 1985; Houser et al., 1986; DeFelipe et al., 1993)

iPSC frequency for pyramidal neurons ↓
(Calcagnotto et al., 2005)

Excitatory synapse numbers ↓
(DeFelipe, 1994; Alonso-Nanclares et al., 2005; Rossini et al., 2021)

Changes at the individual synapse level?

Methods



Results

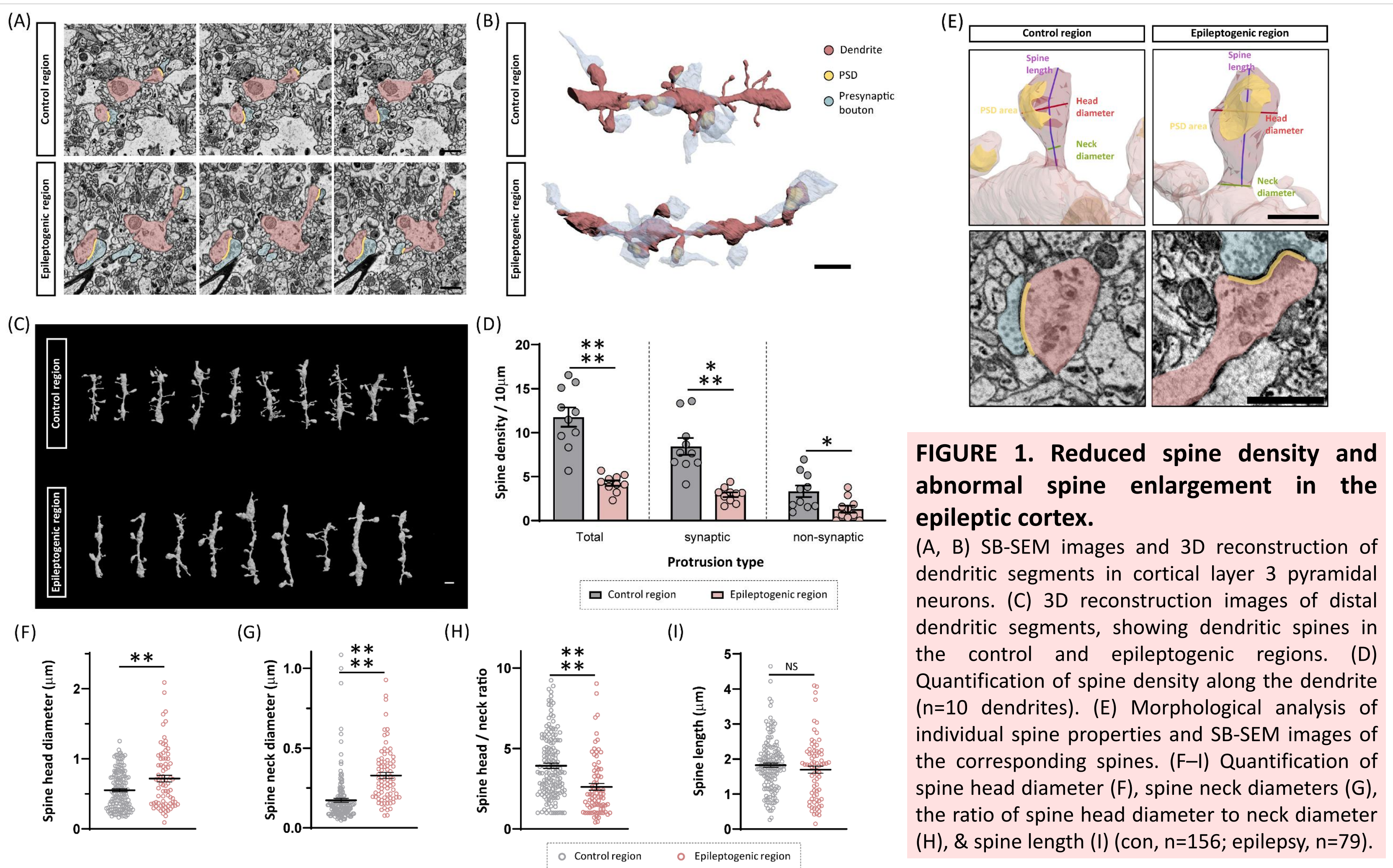


FIGURE 1. Reduced spine density and abnormal spine enlargement in the epileptic cortex.
(A, B) SB-SEM images and 3D reconstruction of dendritic segments in cortical layer 3 pyramidal neurons. (C) Quantification of spine density along the dendrite (n=10 dendrites). (D) Morphological analysis of the corresponding spines. (E-F-I) Quantification of spine head diameter (F), spine neck diameter (G), the ratio of spine head diameter to neck diameter (H), and spine length (I) (con, n=156; epilepsy, n=79).

Results

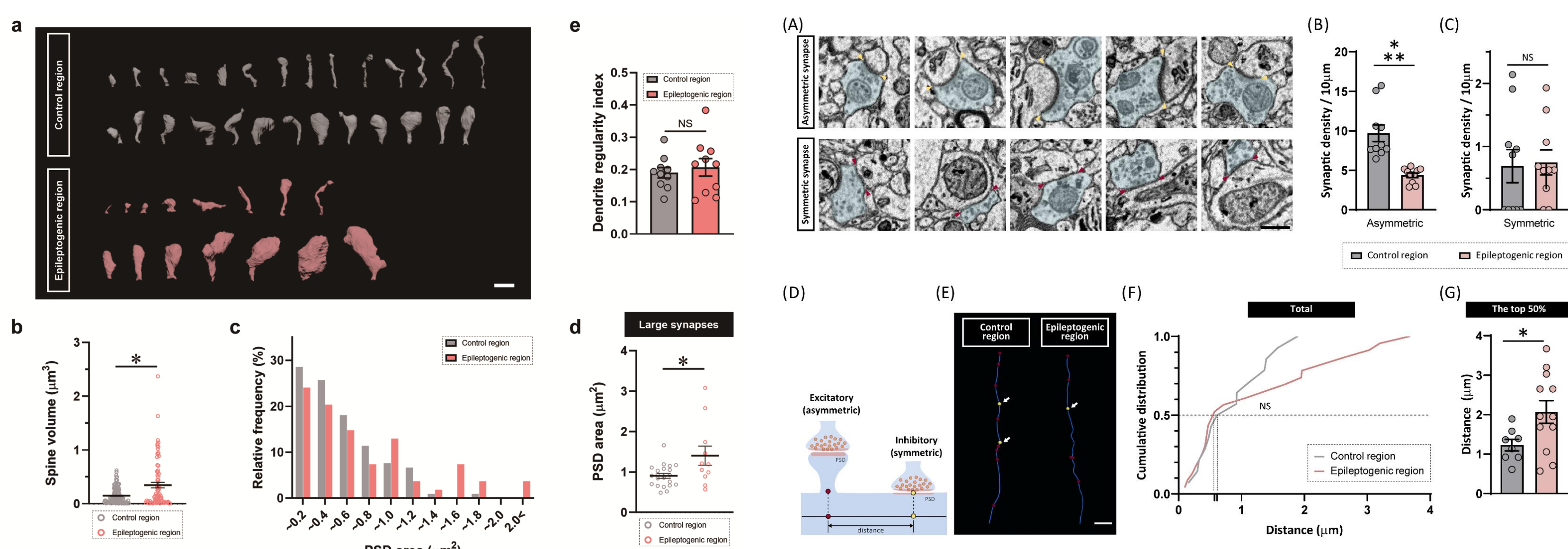


FIGURE 2. Abnormal enlargement of dendritic spines in the epileptogenic region.
(A) Representative image of dendritic spine shapes in the control (gray) and epileptogenic (pink) regions. (B) Mean spine volumes measured from the dendrite surface to the spine tip (control, n=150; epilepsy, n=76). (C) Cumulative distribution of PSD areas (control, n=105; epilepsy, n=54). (D) Average PSD areas in the top 20% of the population in C (control, n=21; epilepsy, n=11). (E) Quantification of dendritic regularity in the control and epileptogenic regions (n=10 dendrites).

FIGURE 3. Spatial distribution of excitatory and inhibitory synapses along distal dendrites.
(A) EM images of asymmetric (excitatory, E) and symmetric (inhibitory, I) synapses formed on spines or directly onto the shaft. (B, C) Quantification of E and I synaptic density (n=10 dendrites). (D) Schematic of measuring the distance between E and I synapses. (E) Skeletonized dendritic traces with annotated synapses. (F) Cumulative distribution of distance measurements between E and I synapses (con, n=14; epilepsy, n=23). (G) Average distance between E and I synapses in the top 50% of the population in F (con, n=8; epilepsy, n=12).

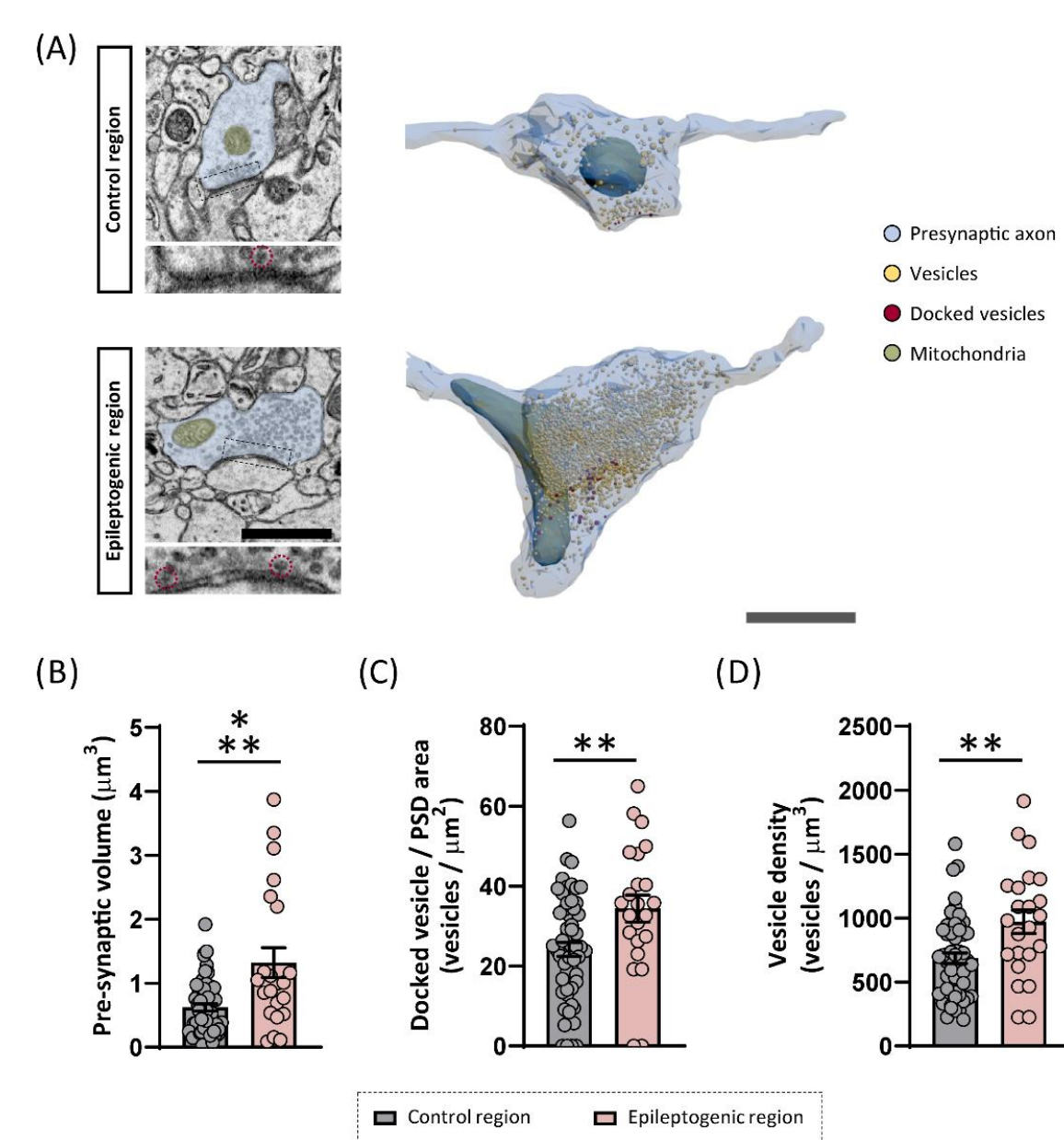


FIGURE 4. Enlarged excitatory synapses with more vesicles in the epileptogenic area.
(A) SB-SEM images and reconstruction of presynaptic boutons showing synaptic vesicles and mitochondria. (B) Quantification of presynaptic bouton volume. (C) Quantification of docked vesicle density normalized to PSD area. (D) Quantification of total vesicle density normalized to presynaptic volume.

FIGURE 5. Increased presynaptic vesicle numbers per bouton in the epileptogenic region.
(A) Schematic of a synapse imaged using HVEM tilting tomography (KBRI, Dr. Yang Hoon Huh). Each series consists of 61 images captured every 2° from -60° to +60°. (B) Representative 3D reconstructions of excitatory synapses, showing the spine head (pink), PSD (yellow), and presynaptic bouton (light blue) containing docked vesicles (red), reserve pool vesicles (orange), and mitochondria (green).

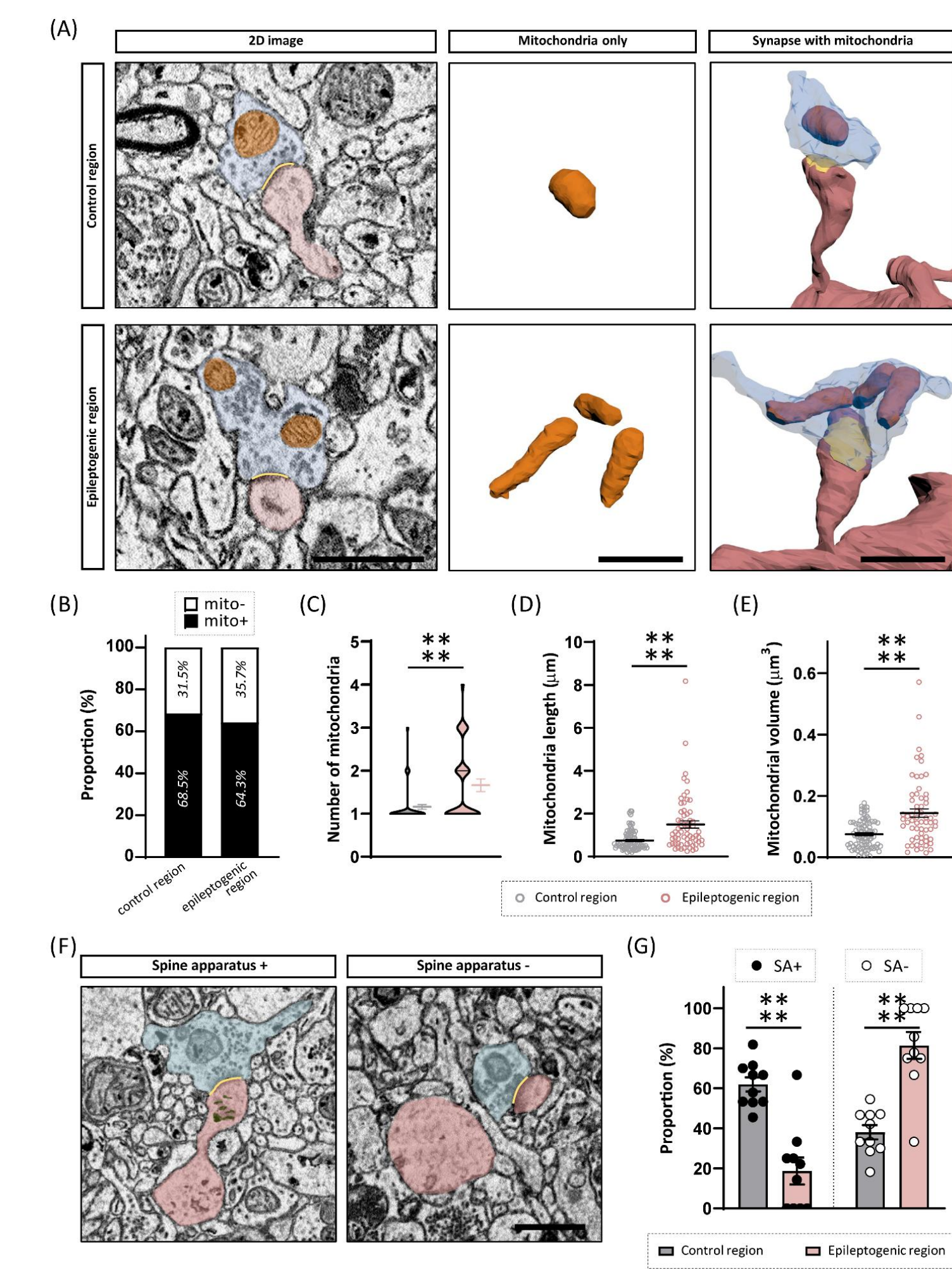


FIGURE 6. Altered presynaptic mitochondria and spine apparatus in the epileptogenic cortex.
(A) EM images of synapses containing presynaptic mitochondria (left panels). 3D reconstructions of the corresponding mitochondria and synaptic components (middle and right panels). (B) Quantification of fraction of synapses with or without presynaptic mitochondria (n=10 dendrites). (C) Quantification of individual mitochondrial numbers per bouton (con, n=64; epilepsy, n=35). (D) Average length of individual presynaptic mitochondria (con, n=81; epilepsy, n=63). (E) EM images of postsynaptic protrusions with and without spine apparatus. (F) Percentage of spines containing or lacking spine apparatus (n=10 dendrites).

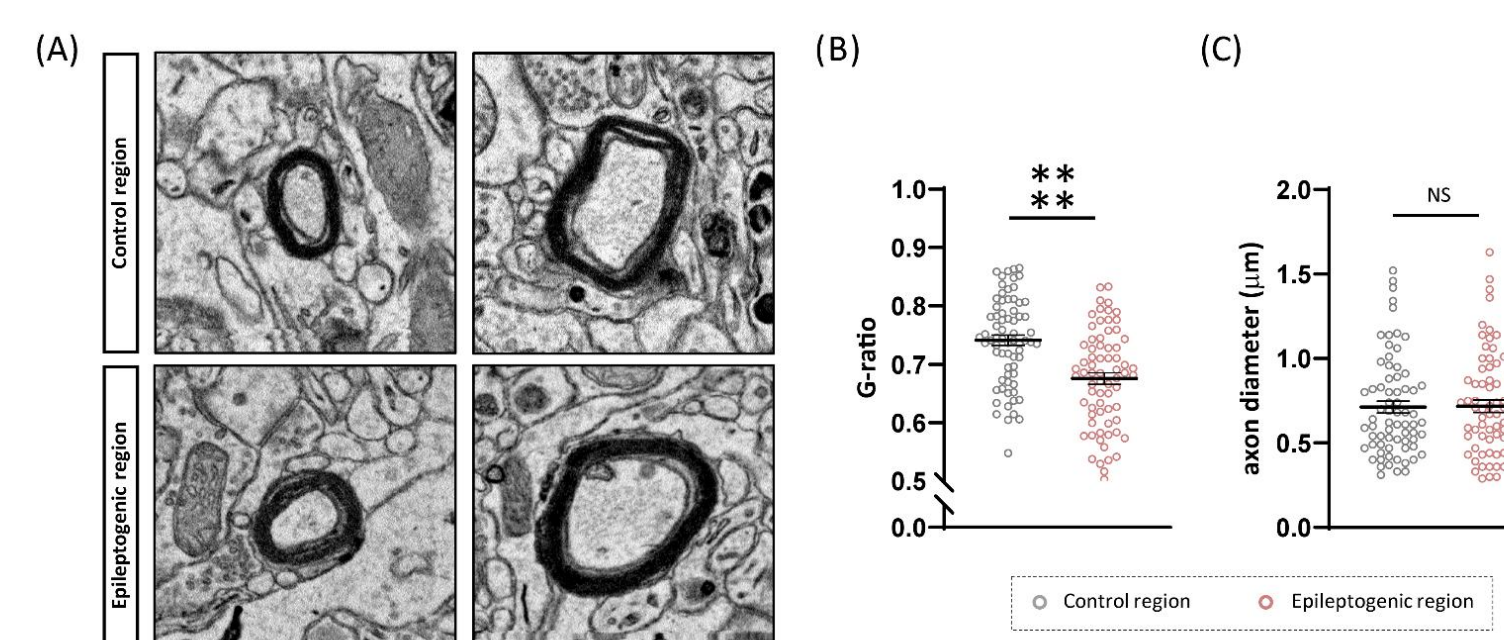


FIGURE 7. Maladaptive myelination in the epileptic cortex.
(A) EM images of myelinated axons in the control and epileptogenic areas. (B, C) Quantification of G-ratio values (B) (inner axonal diameter divided by total fiber diameter, including the axon and myelin sheath) and axon diameter (C) in control and epileptogenic regions (n=70).

Summary

- Volume electron microscopy** revealed ultrastructural alterations in synapses and organelles in the cortex of a patient with FCD.
- The dysplastic region showed **fewer excitatory synapses** on distal dendrites, with **some abnormally enlarged and vesicle-rich**.
- Inhibitory synapses** were positioned farther from excitatory synapses, potentially reducing inhibitory control in the epileptic region.
- Presynaptic boutons displayed **altered mitochondrial density and morphology**, and **fewer postsynaptic spines** contained a spine apparatus, suggesting disrupted calcium regulation and synaptic plasticity.

Acknowledgments

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