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Neuronal and synaptic ultra-structural organization in layer 5 of the human gyrus temporalis

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OBJECTIVES

The aim of this study is to realize quantitative 3D reconstructions of layer 5 (L5) synaptic boutons and their target structures, in the human gyrus temporalis using biopsy material from patients that underwent tumor- but in most cases- epileptical brain surgery, in order to directly compare structural and functional aspects of synaptic transmission and plasticity.

METHODS

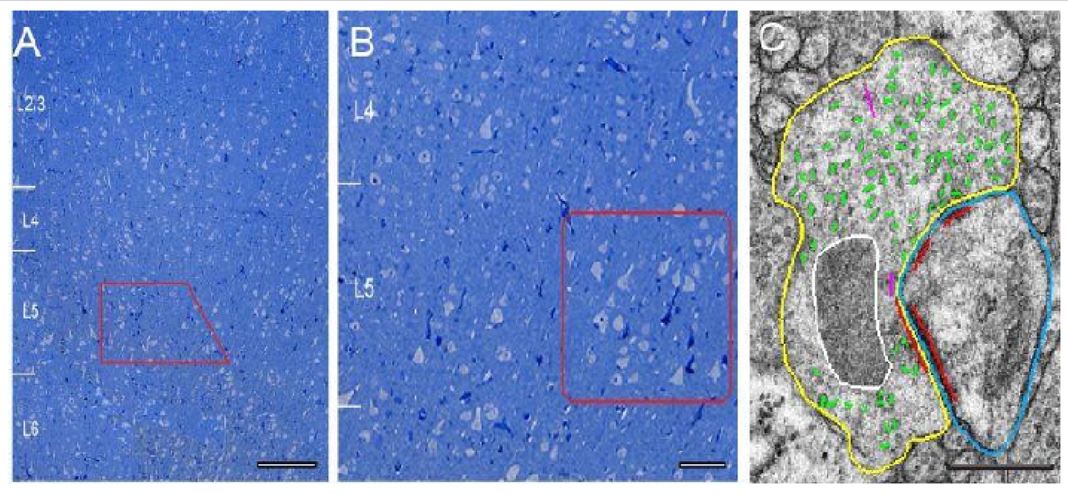

Figure 1: Localization of the region of interest on semithin sections
A, Semithin toluidine blue-stained section through the neocortical layers 2/3 to 6. **B,** High power magnification at the level of layers 4 and 5. Scale bars 100 µm. **C,** Computer-assisted quantitative reconstructions of synaptic structures using OpenCAR (Sätzler et al., 2002). Color code: Synaptic bouton in yellow, dendrite and spine in blue, mitochondrion in white, active zones (Azs) (pre- and postsynaptic densities) in red and synaptic vesicles in green. Scale bar 0.5 µm.

RESULTS

3.1 General description of L5 cortical synapses

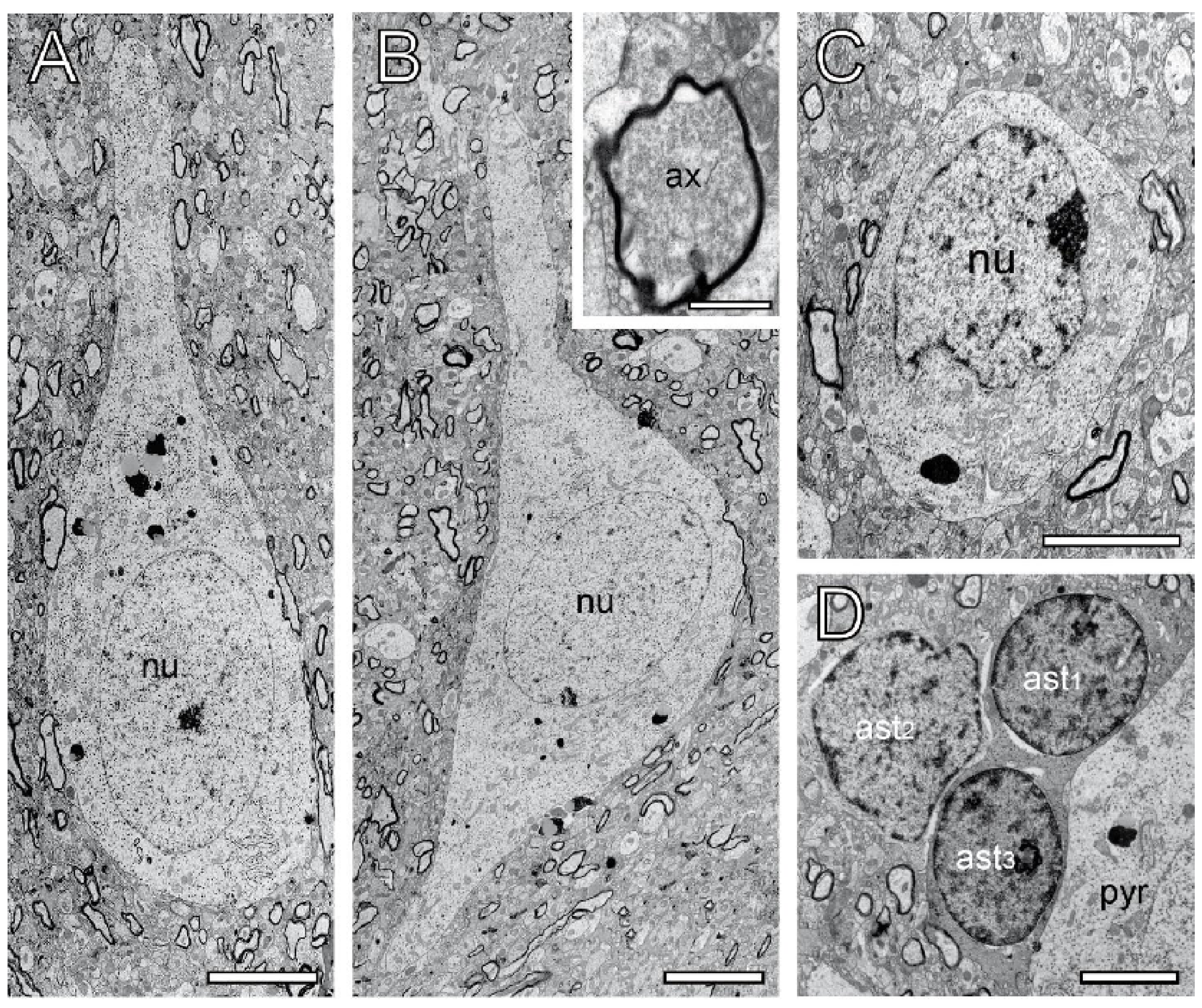

Figure 2: Neuronal composition of layer 5 in the human Gyrus temporalis

A, B, Two typical examples of large pyramidal neurons in L5 of the human neocortex. Scale bars A, B: 5 µm. Myelinated axons of different caliber were observed, some of which contain synaptic vesicles (Inset, Scale bar: 0.5 µm); **C,** GABAergic interneuron. Note the difference in size when compared with the pyramidal cells. Scale bar in C: 2 µm; **D,** Cluster of astrocytes in close proximity to a pyramidal neuron. Scale bar D: 1 µm.

3. 2 Glial coverage of L5 synaptic boutons

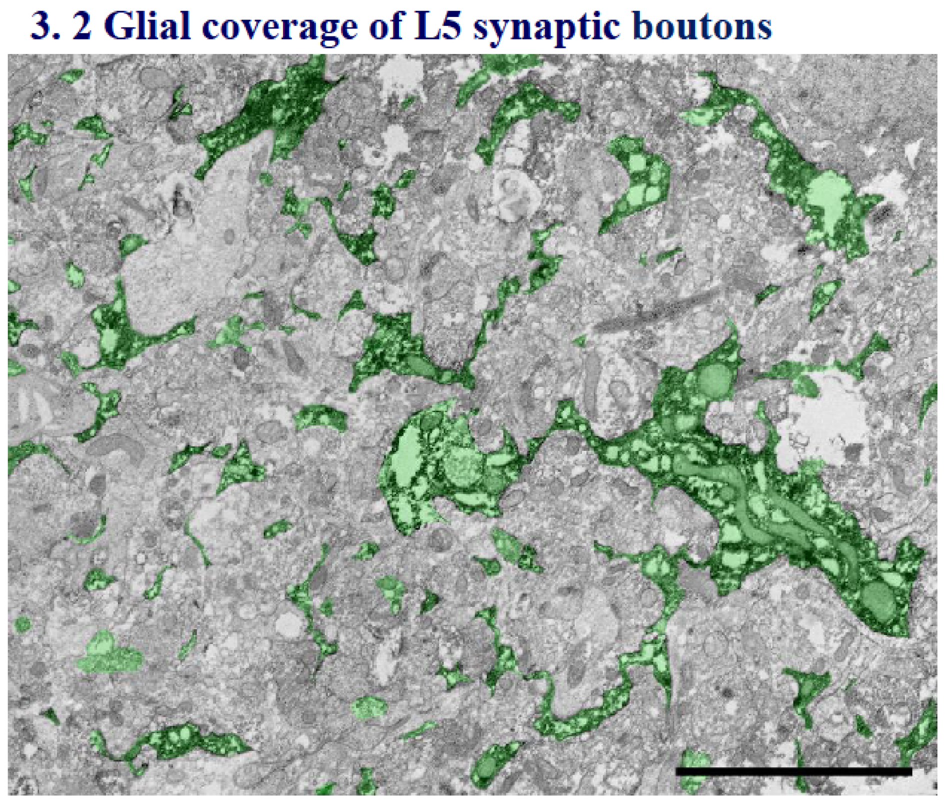

Figure 3: Astrocytic coverage of synaptic complexes in L5: Low magnification electron micrograph showing the dense network of astrocytes and their fine processes (highlighted in transparent green) as revealed by glutamine synthetase immunohistochemistry (dark DAB reaction product). Scale bar 2 µm.

3.3 Synaptic organization of L5

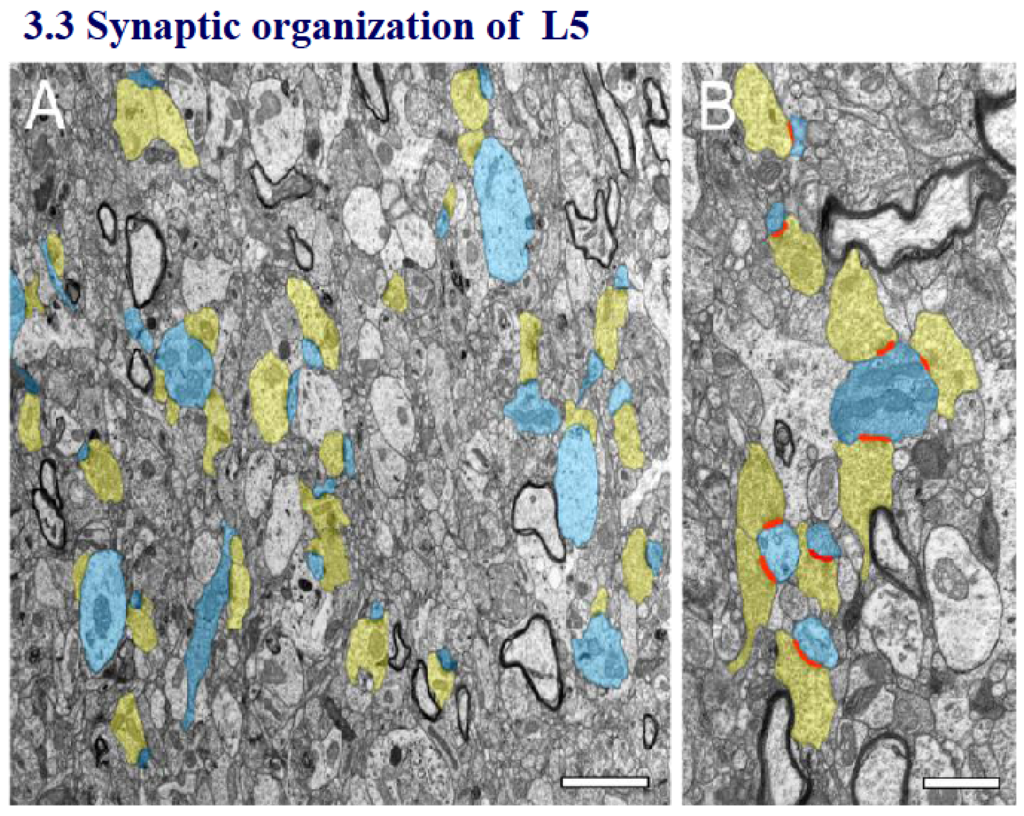

Figure 4: Neuropil and synaptic organization of L5
A, Low magnification electron micrograph showing a dense network of synapses and their target structures in L5. Scale bar 2 µm. **B,** Higher magnification electron micrograph with several synaptic complexes given in transparent yellow and blue. The Azs are given in red. the target structures, shafts or dendritic spines, are innervated by either a single or multiple synaptic boutons. Scale bar 0.5 µm.

3.4 3D volume reconstructions

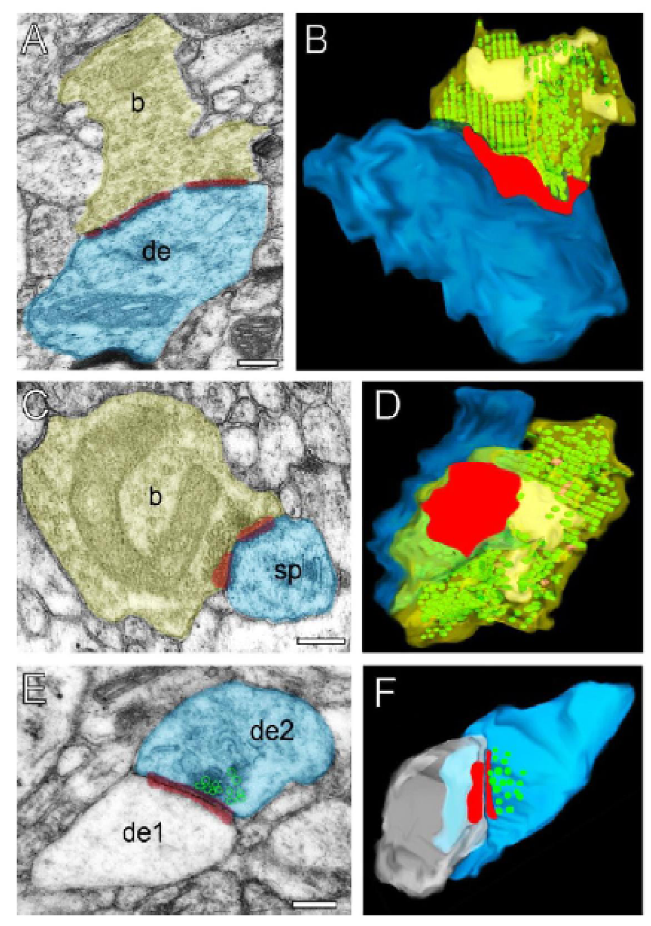

Figure 5a: Individual synaptic complexes
A, C, Two synaptic complexes with a bouton (transparent yellow) and either a dendrite (de) (A) or a spine (C) (transparent blue) and their corresponding 3D reconstructions (B, D). **E,** Dendro-dendritic synapse (de1, de2); with de1 in transparent white. and the corresponding 3D reconstruction in **F.** AZs in red, synaptic vesicles (open green circles). Scale bars A, B 0.5 µm; C 1 µm.

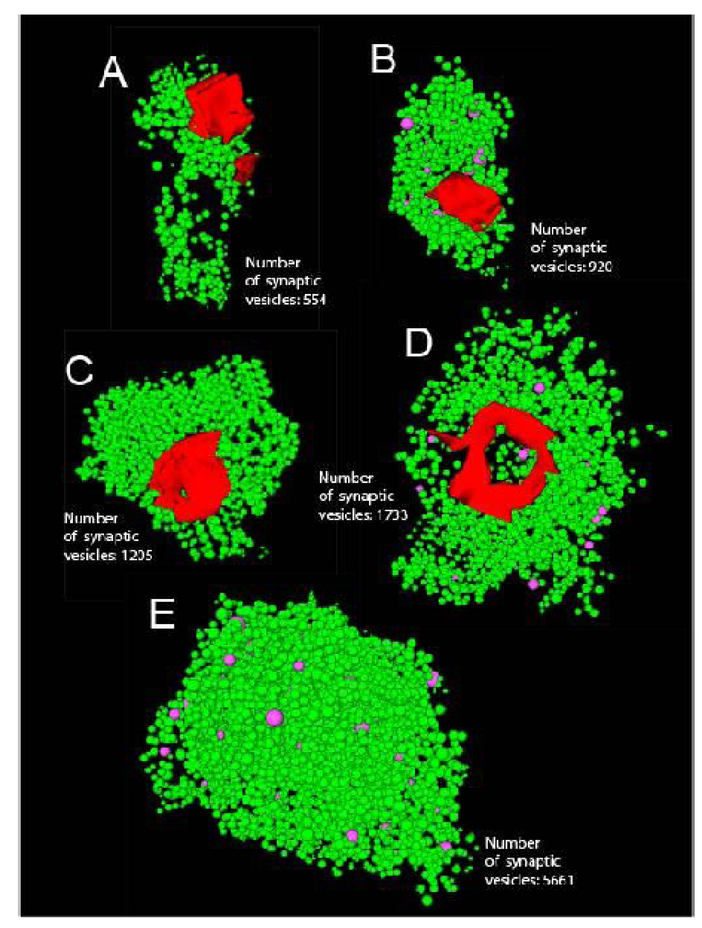

Figure 5b: Total pool of synaptic vesicles at individual L5 synaptic boutons
A-E, 3D volume reconstructions of individual total pools of synaptic vesicles at AZs that showed either a perforation in the pre- and postsynaptic density or both, the others were non-perforated. Large dense core vesicles (magenta) were frequently observed.

3. 6 Quantitative analysis

Table 1: Quantitative analysis of structural parameters of L5 human synaptic boutons vs L4 adult rat

	Synaptic Boutons		Synaptic Vesicles		Dense-Core Vesicles	
	N° of analyzed boutons	Volume ± SD (µm³)	Mean number ± SD	Mean diameter (nm) ± SD	Mean number ± SD	Mean diameter (nm) ±SD
L5 human neocortex	70	0.63±0.09	1671.57±391.55	31.99 ± 0.87	9.78 ±5.74	66.21±1.27
L4 in the 'barrel field' of adult rat (Rollenhagen et al., 2014)	252	0.20±0.07	561 ± 108	29.85 ± 4.63		

CONCLUSIONS

We have investigated -for the first time- the cortical synapses of L5 in human neocortex. This layer is characterized by large pyramidal neurons representing (~85%) of the neurons. The others were various types of GABAergic interneurons much smaller in size. Astrocytes, the non-neuronal cells, form a dense, lattice-like network showing, sometimes, cluster-like arrangements around neurons. ~85% of spines contain a spine apparatus, a specialized form of the endoplasmic reticulum, which makes them more mobile. They are thought to modulate short- and long-term synaptic plasticity (Gray 1959a, b; Deller et al., 2003; Konur and Yuste, 2004; Holtmaat et al., 2005; Umeda et al., 2005). Both pre- and postsynaptic densities are often perforated with periodic interruptions of the protein matrix. Also non-perforated ones were frequently found. Furthermore, we observed multi-vesicular bodies, i.e., endosomal organelles involved in endocytosis and trafficking functions. Synaptic boutons are relatively large and the size of the total pool of synaptic vesicles was nearly 3-fold larger when compared with layer 4 of the 'barrel' field in the adult rat somatosensory cortex (Rollenhagen et al., 2014).

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