Synaptic Organization in Layer 5 of the Human Temporal Lobe: A quantitative Electron Microscopic Analysis

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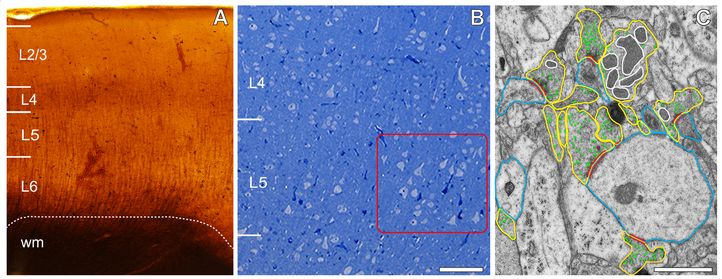
INTRODUCTION

Synapses are key elements in the induction, maintenance and termination of synaptic transmission and in the modulation of synaptic plasticity. However, relatively little is known about their quantitative geometry, particularly in the human brain. Here, we have investigated, for the first time, the synaptic organization of layer 5 (L5) in the human temporal lobe using non-affected neocortical access tissue taken during epilepsy surgery (N= 7 patients). The aim of the present study is to quantify structural parameters relevant for synaptic transmission and plasticity, namely the shape, size and distribution of active zones (AZs; equivalent to functional transmitter release sites), the size and organization of the three pools of synaptic vesicles: the readily releasable (RRP), the recycling (RP) and the reserve pool.

MATERIAL AND METHODS

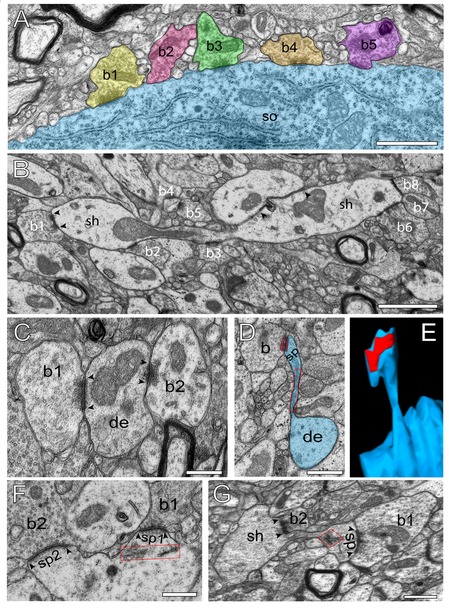
- Immersion-fixation of neocortical biopsy samples with phosphate-buffered 4% PFA and 2.5% GA for 24 - 48h
- Tissue processing and embedding in Durcupan™ for conventional electron microscopy (EM)
- Serial ultrathin sectioning (55 ± 5 nm thickness, silver to gray interference contrast; Figure 1)
- Digital images aquisition at x8000 magnification (Zeiss Libra 120 equipped with the Olympus SIS software)
- 3D-volume reconstructions and quantitative analysis of various structural parameters (see Tables 1 & 2)
- Glutamine synthetase (GS) pre-embedding immunohistochemistry to investigate astrocytic coverage of synaptic complexes

1 Semithin sectioning and computer-assisted quantitative reconstructions using OpenCAR



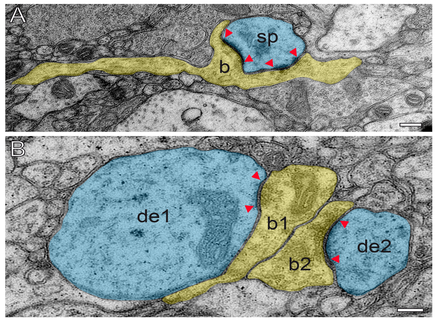
A, Low magnification light micrograph of an osmium tetroxide treated 100 µm thick vibratome section of the human temporal lobe, flat-embedded for EM. Dashed line indicates the grey/white matter border.
B, Methylene blue stained semithin section at the L4 and L5 level. Red frame: ROI. Scale bar 100 µm.
C, Computer-assisted reconstructions of synaptic structures using OpenCAR (Sätzler et al., 2002).
Color code: Synaptic boutons in yellow, dendrites and spines in blue, mitochondria in white, AZs (Pre- and postsynaptic densities) in red and synaptic vesicles in green. Scale bar 0.5 µm.

2 Multiple innervation and target specificity



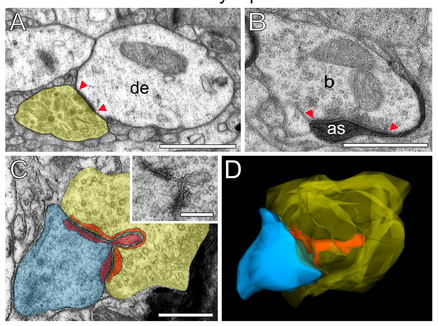
A, Row of GABAergic synaptic boutons (b1 - b5) terminating on the soma (so) of a pyramidal neuron. Scale bar 1 µm.
B, Dendritic shaft (sh) with a dense innervation of synaptic boutons (b1 - b3, b6 - b8) and two boutons (b4, b5) on the same spine. Two AZs are marked by arrowheads. Scale bar 1 µm.
C, Two synaptic boutons (b1, b2) terminating on the same dendrite (de). The AZs are marked by arrowheads. Scale bar 0.5 µm.
D, Synaptic bouton (b) terminating on the head of an elongated spine (sp, blue). The AZ is highlighted in red. Scale bar 0.5 µm.
E, 3D-volume reconstruction of the elongated spine (blue) together with the preAZ (red) on its head.
F, Two synaptic boutons (b1, b2) terminating on two stubby spines (sp1, sp2). Scale bar 0.5 µm.
G, Two synaptic boutons (b1, b2) on a spine head (sp, b1) and shaft (sh) close to the spine neck of the same dendritic shaft (sh). Scale bar 0.25 µm.
Note the presence of a spine apparati (Framed area) in **D**, **F** and **G**.

3 *En passant* vs. *endterminal* boutons



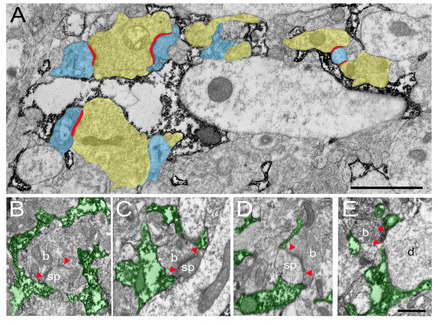
A, *En passant* bouton (b; yellow) terminating on a spine (sp; blue) forming two AZs (red arrowheads). Scale bar 0.2 µm.
B, Two *endterminal* boutons (b1, b2; yellow) on different dendrites (de1, de2; blue). AZs are marked by red arrowheads.
Scale bar 0.15 µm.

4 Structural differences of synaptic contacts



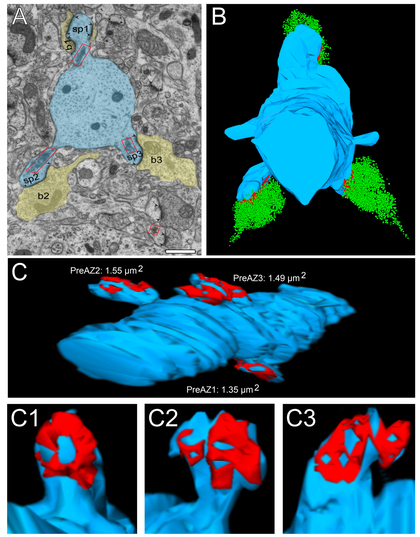
A, Degenerating synaptic bouton (yellow) identified by distorted organelles and lysosomes, on a dendrite (de). Scale bar 0.5 µm.
B, Synaptic bouton (b) establishing a contact on an astrocytic finger (as). Scale bar 0.5 µm. The AZs are indicated by red arrowheads in **A** and **B**.
C, Synaptic bouton (yellow) terminating on a spine (blue) and their respective 3D-volume reconstruction in **D**. Note the large protrusion of the membrane at the AZ (red) forming a so-called coated pit (see inset). Scale bars 0.5 µm in **C** and 0.2 µm inset.

5 Astrocytic coverage of synaptic complexes



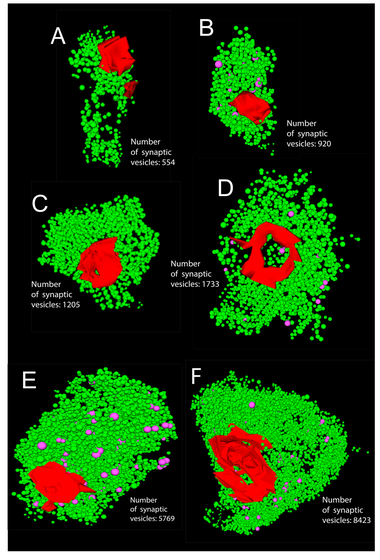
A, Astrocytic segment (dark DAB reaction product) enwrapping several synaptic complexes (boutons in yellow, target stuctures in blue and AZs in red). Scale bar 1 µm.
B - E, Synaptic complexes tightly ensheathed by fine astrocytic processes (green) reaching the AZs (red arrowheads). Scale bar 0.5 µm.

6 3D-volume reconstructions of synaptic structures



A, Synaptic boutons (b1 - b3) terminating on two mushroom-like (sp1-sp2) and a stubby (sp3) spines identified by the occurence of the spine apparati (framed areas). Scale bar 0.5 µm.
B, Corresponding 3D-volume reconstruction. Here, the outlines of the synaptic boutons are omitted to allow a direct view of the total pool of synaptic vesicles (green dots) and the preAZs (red).
C, Same dendrite as shown in **B** in a different orientation to better visualize the shape and size of the three AZs.
C1 - C3, High magnification of the three AZs. Note the perforated nature of all AZs occupying ~25 % of the total volume of the spine.

7 Total pool of synaptic vesicles at individual preAZs



A - E, 3D-volume reconstruction of individual total pools of synaptic vesicles (green dots) at AZs showing either a perforation in the pre, postsynaptic density or both. The others were not perforated. Large dense-core vesicles (magenta) were frequently observed.

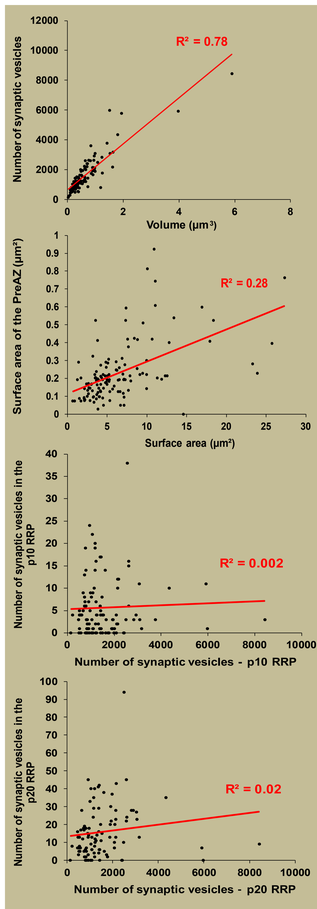
8 Quantitative analysis of structural parameters of synaptic boutons and AZs

	Synaptic boutons (N=147)		Active zones (N=140)				Mitochondria (Range 0-21)	
			Surface area±SD (µm²)		Cleft width±SD (nm)			
	Surface±SD (µm²)	Volume±SD (µm³)	Presynaptic	Postsynaptic	Lateral	Central	Volume±SD (µm³)	% to the total volume
Mean	6.09±0.92	0.63±0.18	0.23±0.05	0.27±0.12	17.24±2.39	19.05±2.93	0.12±0.10	12.04±11.89
Median	6.05	0.63	0.22	0.23	17.51	18.85	0.07	2.18
IQR	1.17	0.21	0.07	0.16	3.74	2.95	0.16	
CV	0.15	0.29	0.22	0.45	0.13	0.15	0.87	0.10

9 Quantitative analysis of synaptic vesicles

	Synaptic vesicles			Pool size of synaptic vesicles			
	Total number	Diameter (nm)	Volume (µm³)	Putative RRP		Putative RP 60-200 nm	Putative reserve pool >200 nm
				p10 nm	p20 nm		
Mean±SD	1518.52±303.18	36.69±1.71	0.05±0.02	5.42±4.09	15.21±9.02	181.86±27.05	1264.05±301.77
Median	1347.21	37.02	0.05	4.93	13.55	180.89	1150.76
IQR	541.98	3.26	0.03	6.29	16.34	47.42	540.39
CV	0.19	0.04	0.3	0.75	0.59	0.15	0.24

10 Correlation between various structural parameters of synaptic boutons



The volume of synaptic boutons vs. the total pool of synaptic vesicles.

The surface area of synaptic boutons vs. the surface area of preAZs.

The total pool of synaptic vesicles minus the RRP whithin a perimeter of 10 nm vs. RRP whithin a perimeter of 10 nm.

The total pool of synaptic vesicles minus the RRP whithin a perimeter of 20 nm vs. RRP whithin a perimeter of 20 nm

*Data points were fitted by linear regression and the R^2 is given for each correlation.