Investigating altered lipid raft dynamics following seizures at hippocampal synapses with AFM

Place of work/: Epilepsy and Aging Team, BioISI – GER and BioISI – BiopHysNano, FCUL.

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Lipid rafts are membrane nanodomains (100–300 nm), not detectable by light microscopy, involved in synaptic receptor (R) clustering, synaptic signaling, synaptic vesicle recycling, neurotransmitter release and synaptic plasticity, a cellular process involved in learning and memory (*1*). Recently we showed that classic raft-associated proteins like caveolin-1 and flotillin-1 are dramatically reduced at synapses after seizure-like events (*2*). Likewise, postsynaptic proteins like PSD-95, an NMDA/AMPA R anchoring protein present at glutamatergic synapses, and gephyrin, a $GABA_A$ R anchoring protein present at GABAergic synapses, are markedly altered following *in vitro* seizure-like activity (*2*), suggesting altered lipid raft stability may be crucial to deficits in synaptic plasticity in epilepsy.

This work will use atomic force microscopy (*3*, *4*)(AFM) to unravel changes in synaptic and neurite membrane structure and lipid raft size and distribution in 1) synaptosomes obtained from hippocampal slices subjected to seizure-like activity and 2) hippocampal neuron neurites in culture exposed to similar seizure-like activity.

To unravel the subsynaptic location of the main lipid domains affected by seizures, we will use immunogold electron microscopy detection of caveolin-1 and flotillin-1 in the synaptosome preparations and neuronal cultures used in AFM studies.

Experimental work will be conducted with the support of the BioISI cell culture and FCUL microscopy facilities.

References:

1. D. Cunha-Reis, A. Caulino-Rocha, *Front. Cell. Neurosci.* **14**, 153 (2020).
2. J. Carvalho-Rosa, N. Rodrigues, A. Silva-Cruz, S. Vaz, D. Cunha-Reis, *bioRxiv*, in press, doi:10.1101/2022.12.06.519267.
3. A. P. Carapeto *et al.*, *Int. J. Mol. Sci.* **21**, 2916 (2020).
4. J. S. Cristóvão *et al.*, *ACS Chem. Neurosci.* **11**, 2753–2760 (2020).

Alternative topics: (please contact the supervisor)**:**

- A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?.
- Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels.