Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels

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

Supervisor(s): Diana Cunha Reis, PhD (dcreis@fc.ul.pt) ORCID: 0000-0002-0900-9306.

Synaptic remodelling is believed to contribute to altered cognition and synaptic plasticity during postnatal development and upon ageing. Recently, we have shown that hippocampal LTP, a crucial cellular mechanism for learning and memory, undergoes postweaning developmental maturation (*1*). LTP also changes substantially with ageing (*2*). Hippocampal CA1 LTP depends on rapid events like phosphorylation of AMPA receptors, the autophosphorylation of CaMKII and other protein kinases like PKM (or PKCzeta). As such, the basal phosphorylation status of these proteins may be a strong conditioning for LTP expression. It has not been determined how the basal levels and phosphorylation status of these proteins change during postweaning development or during aging. Modulation of hippocampal LTP by the GABAergic-associated neuropeptide vasoactive intestinal peptide (VIP) also undergoes postweaning developmental changes (*3*, *4*).

This project aims to study the changes in enzymes and channels involved in synaptic plasticity as well as VIP and VIP receptors that are associated with postweaning development and ageing. This will be evaluated by western blot analysis in total hippocampal membranes and/or Percoll-purified hippocampal synaptosomes(*1*, *5*) obtained from:

a) 3-12-week-old rats (postweaning development).

b) 4-21-month-old rats (throughout aging).

to evaluate phosphorylation levels of AMPA receptor subunit GluA1 (associated with enhanced LTP), CaMKII and PKM. In addition, membrane levels of NMDA receptor subunits GRIN1, GRIN2 and AMPA GluA2 subunit (associated with pathological synaptic mechanisms) will also be evaluated. The expression of VIP and VIP $VPAC_1$ and $VPAC_2$ receptors and synaptic GABAergic markers may also be investigated.

For targets showing enhanced membrane levels, PCR will be performed to determine changes in gene expression (as opposed to membrane recruitment from intracellular stores).

Functional studies in hippocampal synaptosomes may be additionally performed using FM1-43 imaging *in vitro*.

References:

1. N. C. Rodrigues *et al.*, *Eur. J. Neurosci.* **54**, 5272–5292 (2021).
2. E. S. Rosenzweig, C. A. Barnes, *Impact of aging on hippocampal function: Plasticity, network dynamics, and cognition* (Elsevier Ltd, 2003), vol. 69.
3. D. Cunha-Reis, A. Caulino-Rocha, *Front. Cell. Neurosci.* **14**, 153 (2020).
4. A. Caulino-Rocha, N. C. Rodrigues, J. A. Ribeiro, D. Cunha-Reis, *Biol. 2022, Vol. 11, Page 627*. **11**, 627 (2022).
5. D. Cunha-Reis, J. A. Ribeiro, R. F. M. de Almeida, A. M. Sebastião, *Br. J. Pharmacol.* **174**, 4725–4737 (2017).

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

- A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?
- Investigating altered lipid raft dynamics following seizures at hippocampal synapses with AFM.