ARAQUE LAB RESEARCH LABORATORY

CALCIUM IMAGING PROTOCOL

- Ca^{2+} levels in astrocytes located in the stratum radiatum of the CA1 region of the hippocampus were monitored using the Ca^{2+} indicator fluo-4.
- Astrocytes were loaded with the dye by incubating the slices with fluo-4-AM (2 μM and 0.01% of pluronic) for 45-60 min at room temperature (Araque et al. 2002; Kang et al. 1998; Nemani et al. 2010; Nett et al. 2002; Navarrete and Araque 2008)
- This protocol has been proven to be suitable for measuring the calcium activity of astrocytes (Nanclares et al. 2023).
- Briefly, fluo-4-positive cells present typical electrophysiological properties for astrocytes, this is, low input resistance, a quasi-linear voltage–current relationship under voltage-clamped conditions, and absence of action potentials.
- On the contrary, recordings from fluo-4-negative cells in the *stratum radiatum* exhibit typical neuronal characteristics, such as higher input resistances, a non-linear voltage–current curve, and firing of action potentials.
- Additionally, slices incubated with fluo-4 and sulforhodamine 101 (SR101), a widely used astrocyte marker known to label astrocytes in the hippocampus (Schnell, Hagos, and Hülsmann 2012), provide good co-localization between SR101 and fluo-4-positive cells (Nanclares et al. 2023).
- Recordings were performed in the presence of a cocktail of neurotransmitter receptor antagonists to minimize neuronal contribution to astrocyte Ca^{2+} activity (CNQX 20 μM, AP5 50 μM, MPEP 50 μM, LY367385 100 μM, picrotoxin 50 μM, CGP54626 1 μM, atropine 50 μM, CPT 2 μM, suramin 100 μM, flupenthixol 30 μM, AM251 2 μM and TTX 1 μM to block glutamatergic, GABAergic, cholinergic, purinergic, endocannabinoid and dopaminergic transmission).
- Astrocytes were imaged using a custom-made confocal microscope (Thorlabs).
- Videos were obtained at 512 × 512 resolution with a sampling interval of 1 s.
- ImageJ software (NIH) was used for quantitative fluorescence measurements.
- Ca^{2+} variations were estimated as changes in the fluorescence signal over baseline ($\Delta F/F_0$), and cells were considered to show a Ca^{2+} event when $\Delta F/F_0$ increased two times the standard deviation of the baseline.
- The astrocyte Ca^{2+} signal was quantified as the number of Ca^{2+} events per min within 10 min of recording.
- Astrocyte Ca^{2+} events were obtained from 5 to 24 astrocytes in the field of view during the recording.

Araque, Alfonso, Eduardo D. Martín, Gertrudis Perea, Jon I. Arellano, and Washington Buño. 2002. “Synaptically Released Acetylcholine Evokes Ca $^{2+}$ Elevations in Astrocytes in Hippocampal Slices.” *The Journal of Neuroscience* 22 (7): 2443–50. https://doi.org/10.1523/JNEUROSCI.22-07-02443.2002.

Kang, Jian, Li Jiang, Steven A. Goldman, and Maiken Nedergaard. 1998. “Astrocyte-Mediated Potentiation of Inhibitory Synaptic Transmission.” *Nature Neuroscience* 1 (8): 683–92. https://doi.org/10.1038/3684.

Nanclares, Carmen, Jonah Poynter, Hector A. Martell-Martinez, et al. 2023. “Dysregulation of Astrocytic Ca2+ Signaling and Gliotransmitter Release in Mouse Models of α-Synucleinopathies.” *Acta Neuropathologica* 145 (5): 597–610. https://doi.org/10.1007/s00401-023-02547-3.

Navarrete, Marta, and Alfonso Araque. 2008. “Endocannabinoids Mediate Neuron-Astrocyte Communication.” *Neuron* 57 (6): 883–93. https://doi.org/10.1016/j.neuron.2008.01.029.

Nemani, Venu M., Wei Lu, Victoria Berge, et al. 2010. “Increased Expression of α-Synuclein Reduces Neurotransmitter Release by Inhibiting Synaptic Vesicle Reclustering after Endocytosis.” *Neuron* 65 (1): 66–79. https://doi.org/10.1016/j.neuron.2009.12.023.

Nett, Wolfgang J., Scott H. Oloff, and Ken D. McCarthy. 2002. “Hippocampal Astrocytes In Situ Exhibit Calcium Oscillations That Occur Independent of Neuronal Activity.” *Journal of Neurophysiology* 87 (1): 528–37. https://doi.org/10.1152/jn.00268.2001.