Cellular-resolution two-photon microscopy uncovers the spatio-temporal organization of the cerebellar granular layer activity

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The cerebellar granular layer network
Detection of multiple signals

Dynamic processes occurring at multiple sites in living samples requires high throughput parallel acquisition of rapidly time-varying signals.

- Optical techniques
- Two-photon microscopy
  - Localized excitation (femtoliter)
  - High signal to noise ratio
- Laser beam-splitting

**Submicrometer resolution**

**Simultaneous excitation of multiple sites**
The SLM-2PM

- simultaneous excitation and recording from multiple sites with single cell resolution
- high throughput parallel acquisition of rapidly time-varying signals
- spatial and temporal resolution determined by digital camera performance

Gandolfi et al., 2014; Pozzi et al., 2015
SLM-2PM image of acute parasagittal cerebellar slice (220 µm thick) bulk loaded with Fura-2AM (50µg/2mL), 40X magnification.
Fast recording of calcium signals from multiple neurons

SLM-2PM image → Multi-spot illumination

acute cerebellar slice bulk loaded with Fura-2 AM (50µg/2mL)
40X magnification

selection of neurons for subsequent activity recordings
GrCs calcium responses elicited by an electrical stimulation of the mossy fibers
(10 pulses – 50 Hz repeated 4 times at 0.1 Hz)

$[\text{Ca}^{2+}]_i < \text{fluorescence}$
The GrCs activity is organized in **center-surrounds units**, with excitation prevailing in the core and inhibition in the surround.
Spatial organization of long-term synaptic plasticity

Control

Gabazine (10 µM)

CaR-P : 99.8 ± 6.5%, n=26 cells, p<0.01
CaR-D : -35.1 ± 3.9%, n=67 cells, p<0.01
No change : -3.7 ± 1.6%, n=47 cells, p=0.1

CaR-P : 86.8 ± 4.7%, n=217 cells, p<0.01
CaR-D : -48.6 ± 2.3%, n=51 cells, p<0.01
No change : -3.1 ± 1.7%, n=108 cells, p=0.8
Modeling the granular layer activity

800 x 800 x 150 mm³; 484,000 granule cells (GrCs); 914 Golgi cells (GoCs); 29500 Glomeruli (GLOMs).
The network activity was elicited by stimulating the GLOMs.

Python-NEURON.

E-I balance

Long-term plasticity

https://www.biorxiv.org/content/10.1101/2020.03.14.991794v1#xref-fn-1-1
Thanks for your attention!

For more questions, contact me at: marialuisa.tognolina@unipv.it

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