Analysis of the effect of electro-optical parameters on neurotransmitter uncaging experiments on cerebellar cells in vitro.

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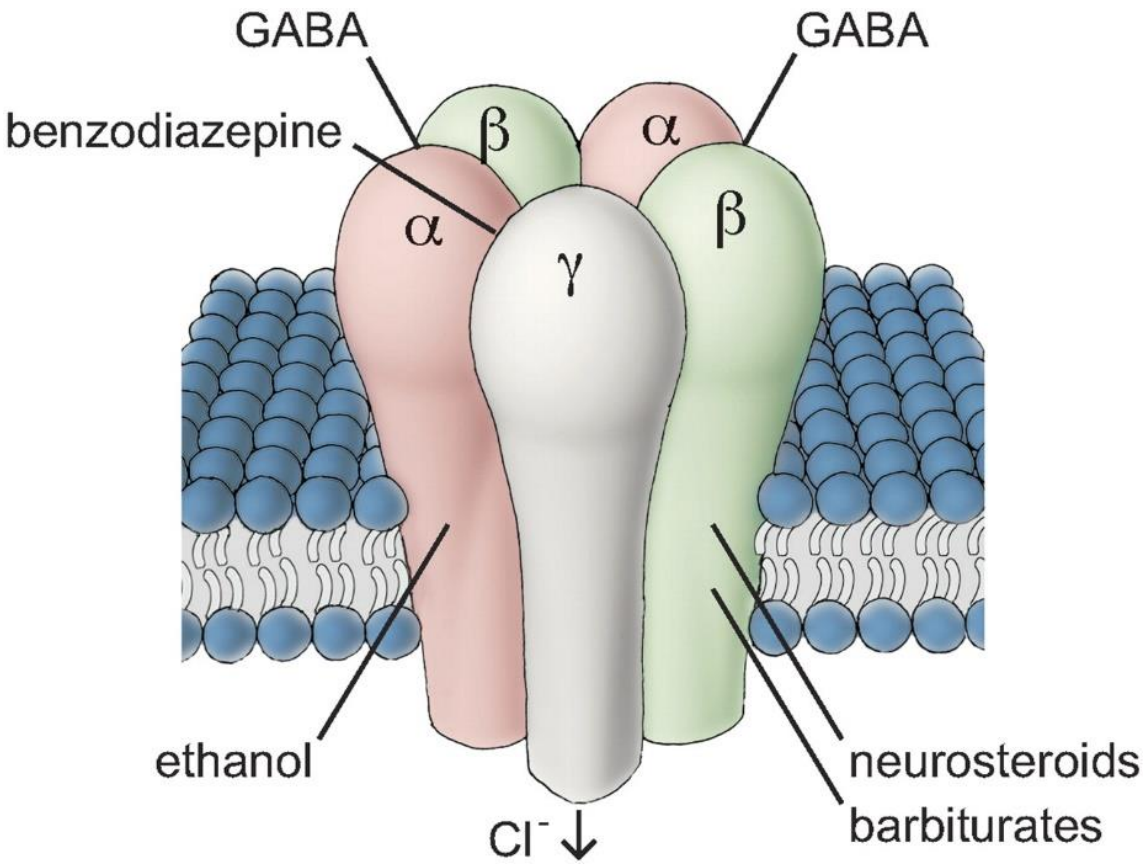
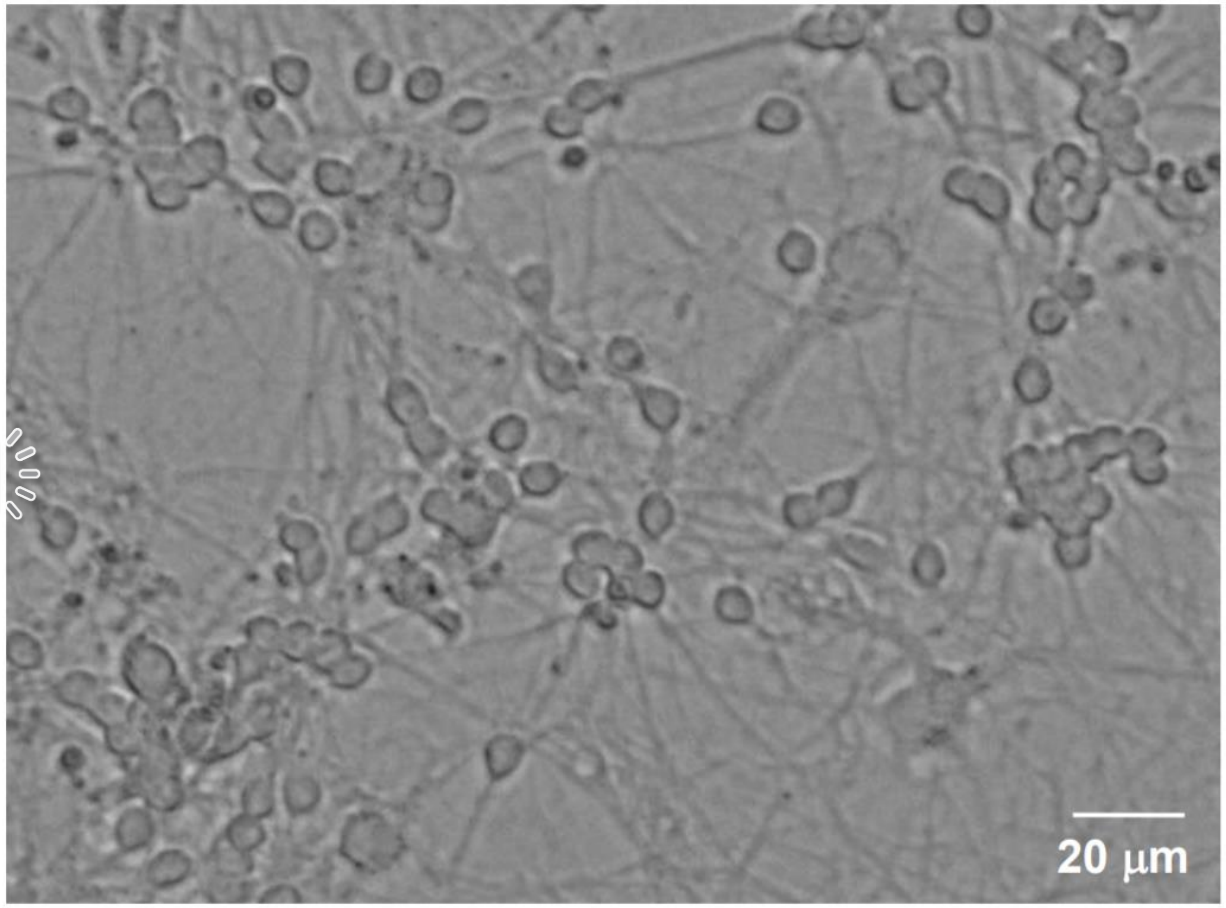
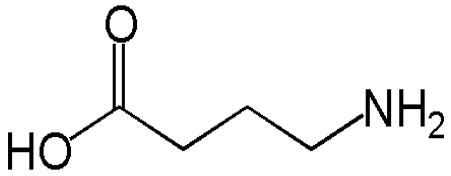
$GABA_A$ receptor



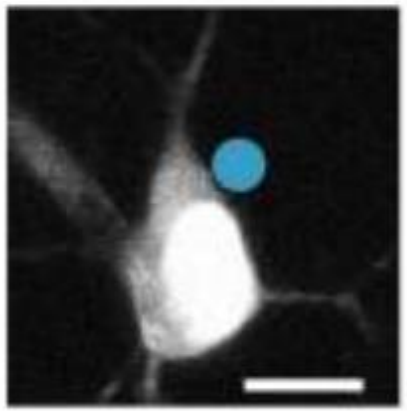


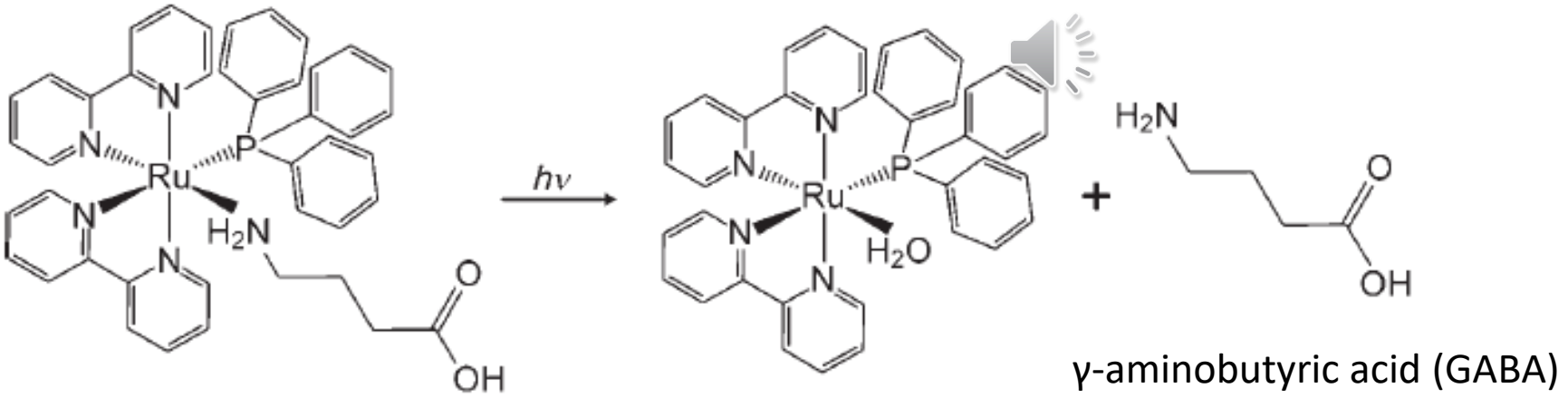
Cerebellar granule cells in vitro



γ-aminobutyric acid (GABA)

Neurotransmitter uncaging experiments



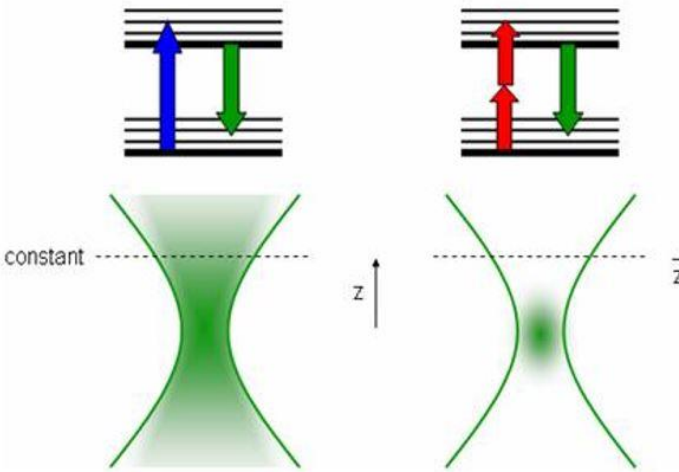


Caged neurotransmitter
RuBiGABA

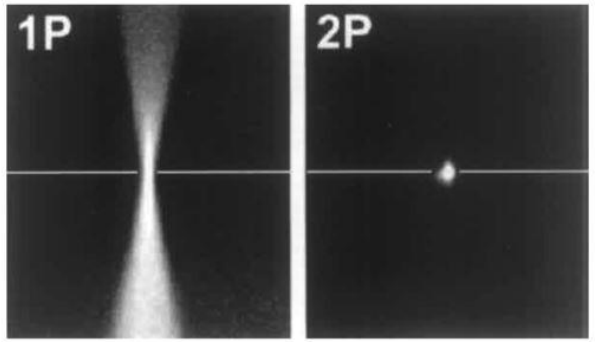
γ-aminobutyric acid (GABA)

1-photon excitation

2-photon excitation



http://biomicroscopy.bu.edu/research/nonlinear-microscopy



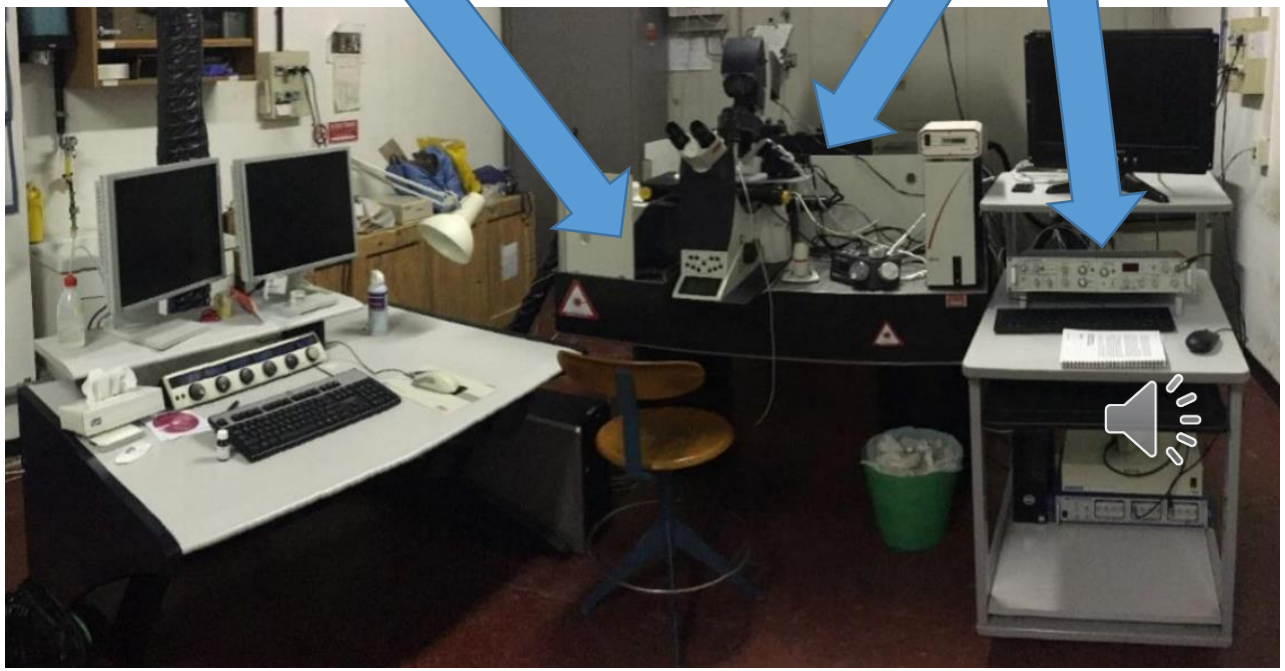
M. Rubart (2004) Two-Photon Microscopy of Cells and Tissue. Circulation Research 95:1154-1166

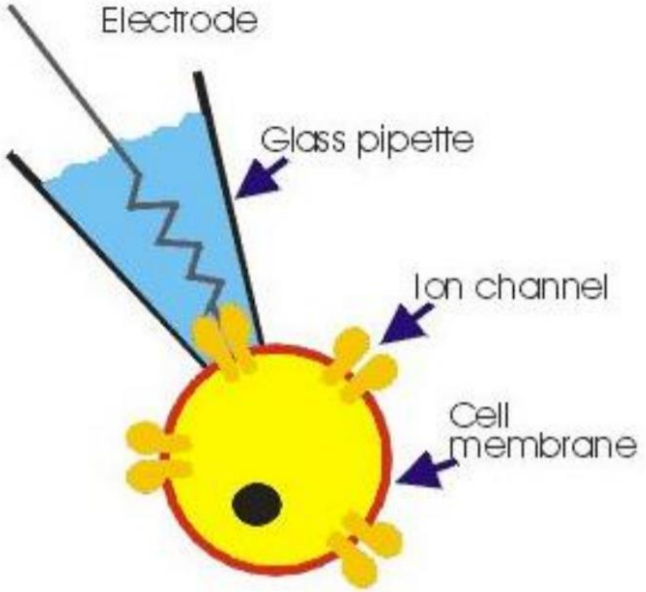
Rial Verde EM «Photorelease of GABA with Visible Light Using an Inorganic Caging Group», Front Neural Circuits. 2008;2:2. doi:10.3389/neuro.04.002.2008

Experimental set-up

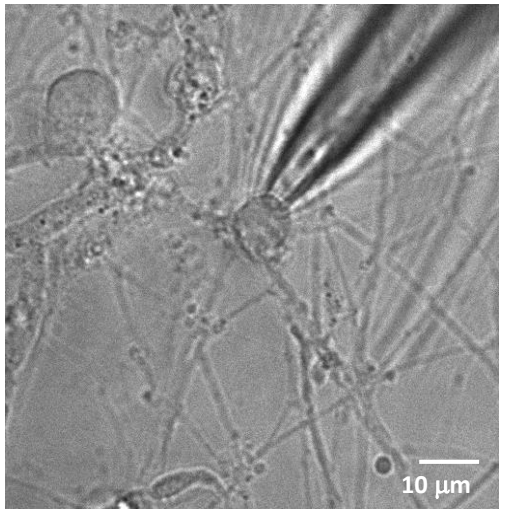
Confocal microscope

Set-up for patch-clamp





Patch-clamp technique in whole-cell configuration



Investigation of these electro-optical parameters:

laser power
exposure time
distance from cell in X-Y plane
Z-axis distance from the cell
photoactivation method.

Cozzolino et al., Sci. Rep. 2020, doi: 10.1038/s41598-020-70217-5

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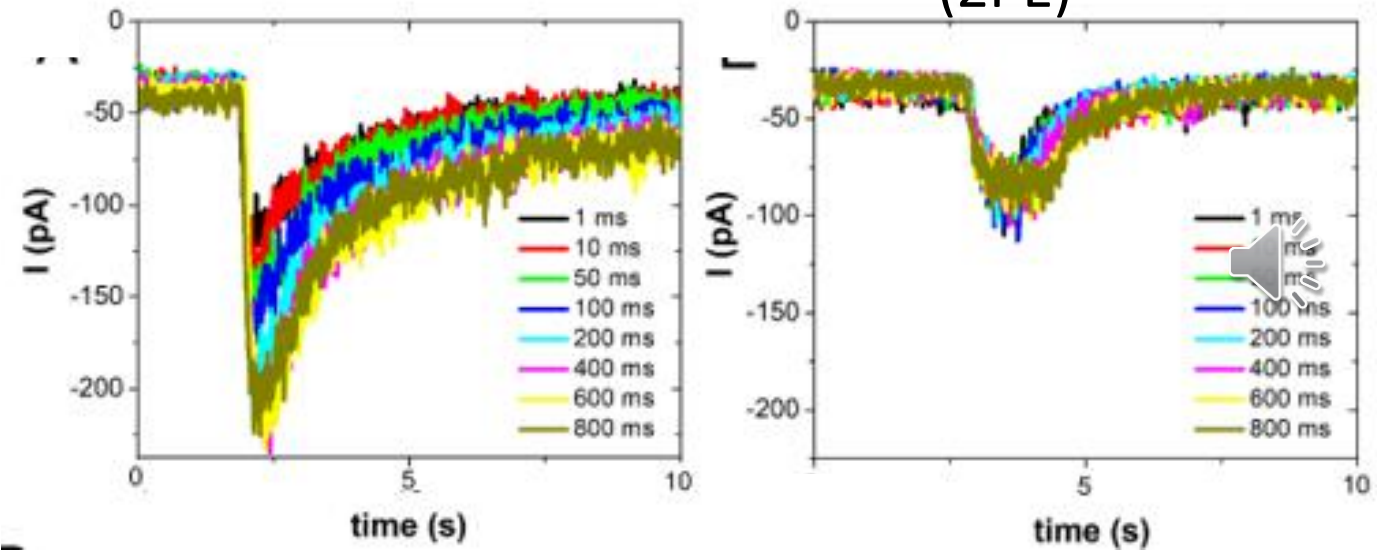
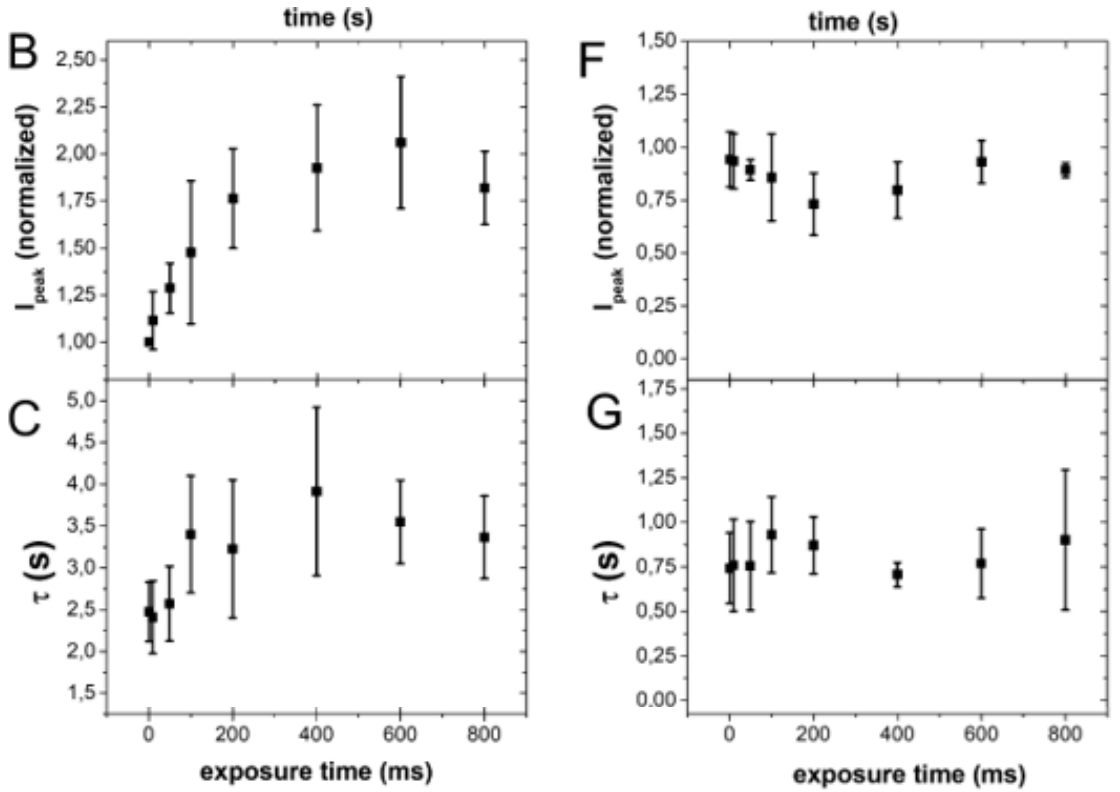
Current *versus* exposure time analysis

1-photon excitation (1PE)

2-photon excitation (2PE)



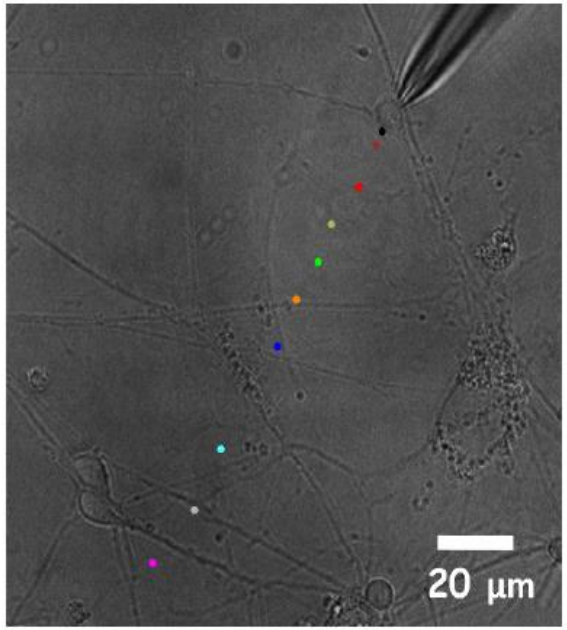
1PE 2PE

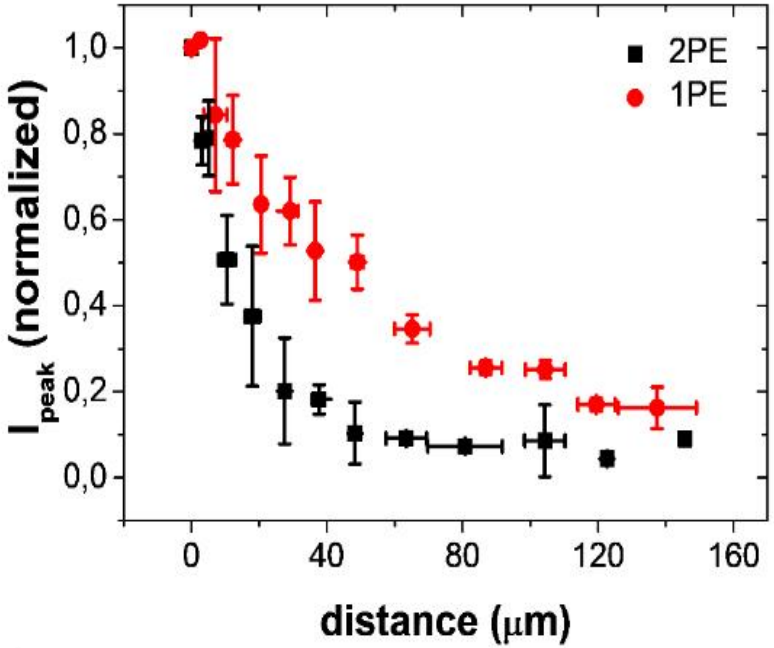


(10 µM uncaged RuBi-GABA V=-80 mV)

Cozzolino et al., Sci. Rep. 2020, doi: [10.1038/s41598-020-70217-5](https://doi.org/10.1038/s41598-020-70217-5)

X-Y plane distance from the cell

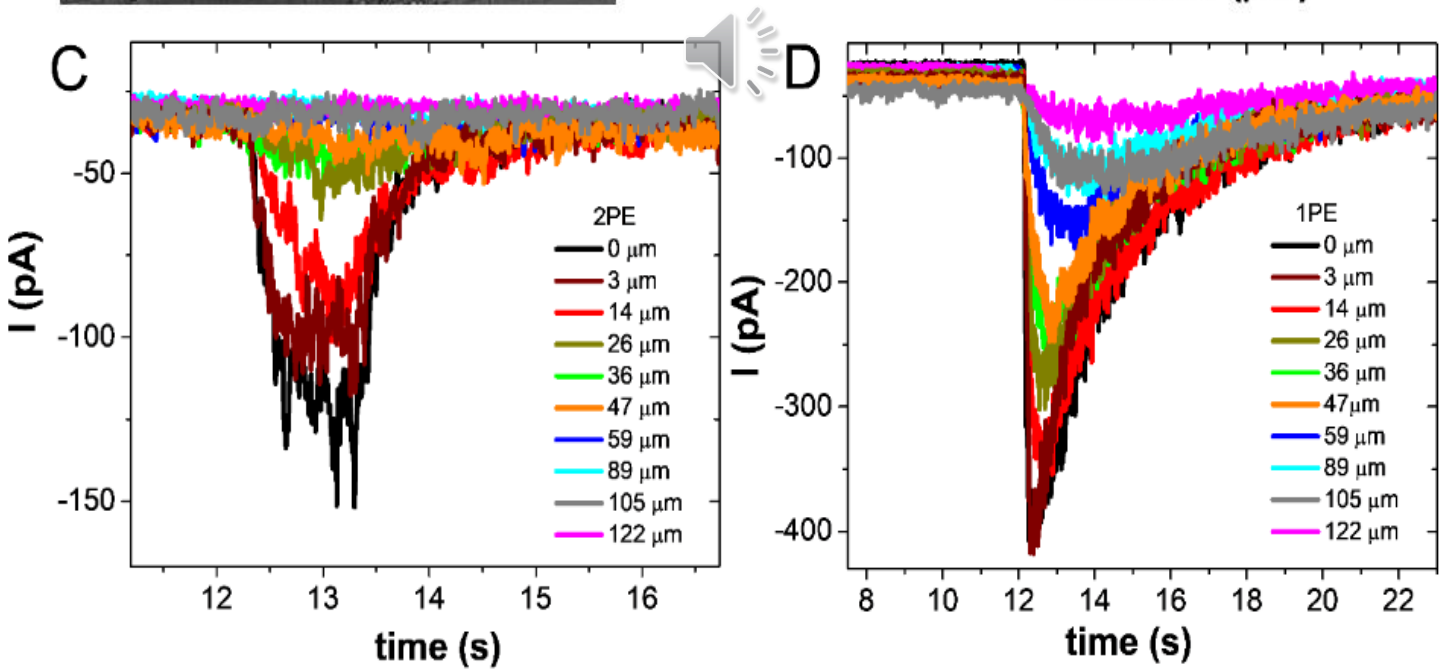




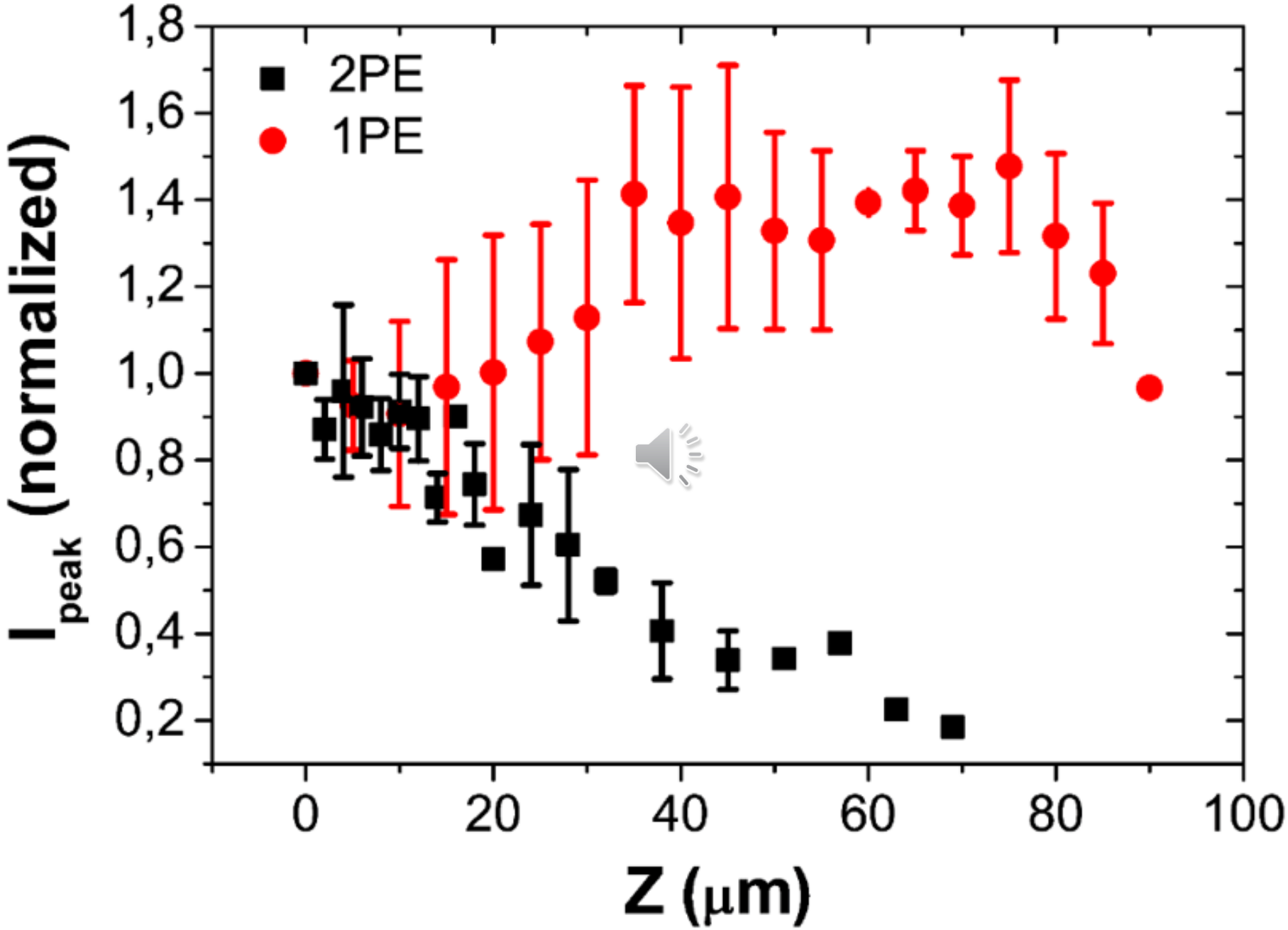
1PE (458 nm, 100 ms, 9.36 µW)

2PE (750 nm, 100 ms, 77.3 mW)

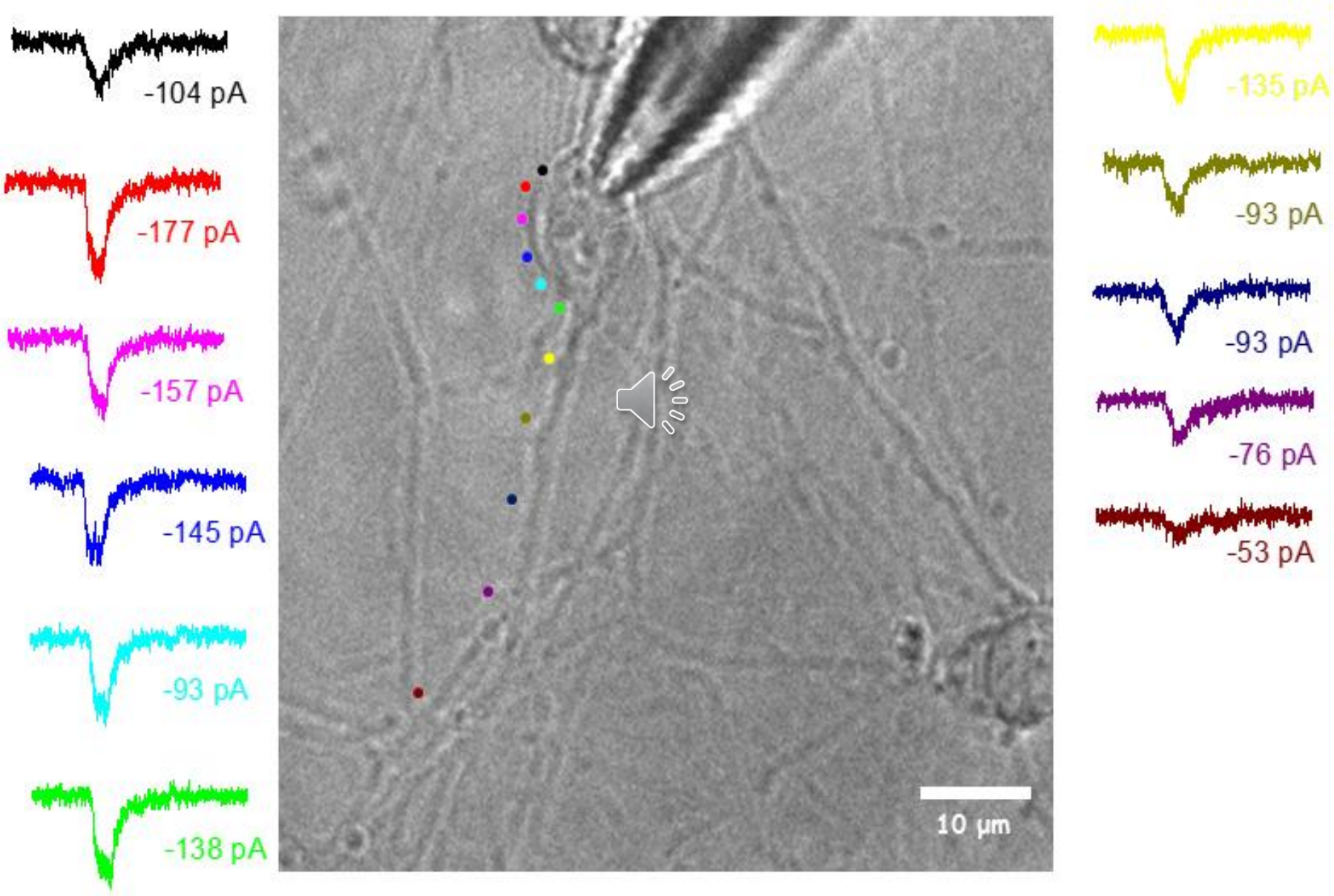
10 µM RuBi-GABA at V=-80 mV



Cozzolino et al., Sci. Rep. 2020, doi: [10.1038/s41598-020-70217-5](https://doi.org/10.1038/s41598-020-70217-5)


2PE
1PE
I_{peak} (normalized)
Z (μm)
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0
20
40
60
80
100

RuBi-GABA uncaging to map GABAergic receptors distribution



Conclusions

The combination of electro-optical techniques allows electrophysiologically examinating a confined area of the cells, enabling researchers to define detailed maps on kinetics, physiology, and pharmacology of specific molecules.

Comparison of 1PE and 2PE based uncaging processes allows refining protocols for experiments related to specific mechanisms such as to neuronal cell communications.