PRECISE CONTROL OF ELECTRICAL AND OPTICAL PARAMETERS IN 3D MODULATION OF $GABA_A$ RECEPTORS *IN VITRO*

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Ruthenium-bipyridinetriphenylphosphine-γ-aminobutyric acid (RuBi-GABA) is a caged compound that allows studying neuronal transmission in specific areas of the cell. This probe compound is prepared via a covalent appendage of a light-sensitive protecting group, called "cage" (RuBi), to a signaling effector (GABA) [1]. When the inhibitory neurotransmitter GABA is bound to the cage, it is inactive and does not interact with its receptor site on the cell's plasma membrane. Following linear – one-photon (1P) – and non-linear – multi-photon – absorption of light, the covalent bond with the cage breaks and GABA is released with high control in both time and space. This approach allows investigating GABA interactions with the cell in 4 dimensions (X, Y, Z, t), and it results in being advantageous to detect and map the distribution of biological targets in well-defined zones of the membrane. Using this strategy, we studied the modulation of $GABA_A$ receptors in rat cerebellar granule cells *in vitro* by coupling the photoactivation process, by confocal or two-photon excitation microscopy [2], with the electrophysiological technique of the patch-clamp in the whole-cell configuration.

We analyzed crucial physical parameters to investigate and correlate how they affect the biological response systematically. In particular, we examined the effects of photoactivation laser power, time of exposure, and distance of the uncaging point from the cell of interest in X, Y, Z spatial coordinates [3].

Our purpose was to understand how biological behavior is conditioned by the physical parameters used during the measurements to take into account and, possibly, minimize their effects. This aspect is of crucial importance when investigating the responses, generated on the model system used, i.e., granule cells, by pharmacological molecules, like benzodiazepines, in different neuronal regions (soma, cone growth, and neurites).

References:

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