CELLULAR DYNAMICS OF A FLUORESCENT G-PROTEIN COUPLED RECEPTOR UNDER PHYSIOLOGICAL CONDITIONS.

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Opioid receptors belong to the G protein-coupled receptor family. Together with endogenous opioid peptides, they form a neuromodulatory system that plays a major role in nociception, modulates affective behavior and is the target of exogenous drugs of abuse. Knock-in mice expressing the delta opioid receptor in fusion with the green fluorescent protein (DOR-eGFP) have been generated in the laboratory and have enabled visualization of the receptor with subcellular resolution both in vivo and ex vivo in primary neuronal cultures [1]. Our recent work also correlated drug internalizing properties and receptor localization in neurons to pain-relieving effects [2,3]. The fluorescent receptor fusion also enables to overcome the lack of specific antibodies to detect the delta receptor.

Current work addresses receptor dynamics in response to endogenous peptide release under physiological conditions both in vivo and ex vivo. Using DOR-eGFP fluorescent knock-in mice, we developed a new protocol in which drug-free animals were tested in a context previously paired with chronic morphine administration. Receptor internalization under physiological conditions was visualized for the first time reflecting endogenous peptide release in the hippocampus when drug and context were associated. Confocal imaging revealed an internalization profile different from the one previously described upon pharmacological activation. Using a correlative light-electron microscopy approach, we are currently analyzing DOR-eGFP fine intracellular distribution following systemic administration of exogenous ligands or upon activation by endogenous peptide release.

In parallel, we develop ex vivo approaches using acute brain slices. We address real-time receptor dynamics in a context that maintains neuronal connectivity to determine how receptor activation and trafficking affect neuronal activity and plasticity. We have optimized acute hippocampal slice preparation to visualize DOR-eGFP localization with subcellular resolution. We are currently developing local agonist delivery to characterize receptor activation and trafficking in response to a pharmacological stimulation by exogenous opiate drugs. In addition, we are also setting up a protocol of electrical stimulation to address receptor activation and trafficking in response to a physiological stimulation that releases endogenous opioid peptides.