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Title: Genetically Encoded Biosensors for Rapidly Acting Antidepressants In a Zebrafish Model

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Abstract: Ketamine has been shown to act as an antidepressant for patients with treatment-resistant depression (TRD) and major depressive disorder (MDD) with suicidal ideation. However, the mechanism by which ketamine exerts such antidepressant effects is unclear, both in terms of which pharmacological targets are important and if the metabolites of ketamine are responsible for the drug's efficacy. Because of ketamine's ability to induce symptoms of dissociation even in healthy controls, and because of the approval in 2019 of esketamine (the S(+) enantiomer) in an intranasal formulation, more investigation is warranted to determine the neural concentrations of ketamine (or its metabolites) which confer therapeutic benefit while minimizing side effects.

Due to their physiological and anatomical homology with mammals (including humans), zebrafish are a popular model organism used to study both neurophysiology as well as the effects of novel pharmaceutical compounds. Although relatively simple compared to mammalian models, zebrafish behavioral assays are a powerful high-throughput means by which behavioral responses to various perturbations (e.g. drug exposure, mutational phenotypes) can be quantified. The effects of ketamine and other antidepressants on zebrafish behavior have only recently begun to be investigated, but the broad neural effects of ketamine leave open many questions regarding its behavioral and neurophysiological mechanisms of action. Over the past decade, a variety of protein-based sensing fluorescent reporters (SnFRs) have been developed, allowing for the cellular and organellar expression of biosensors responsive to both endogenous and exogenous compounds in live tissue. The ability to use fluorescence reporting as a measure of intracellular or plasma drug concentration would help establish a new research paradigm by which behavioral changes could be mapped to specific concentrations of pharmaceutical compounds.

Our sensors utilize OpuBC, a monomeric bacterial periplasmic binding protein (PBP), which contains (a) a binding site for amines including a cation-π box, and (b) a ligand-induced "Venus flytrap" conformational change invoked by the binding of a target ligand. We inserted circularly permuted "superfolder" GFP (cpGFP), flanked by several-residue linkers, within inter-domain hinge regions and applied directed evolution, including X-ray crystallography, to optimize sensing for each drug of interest. Our iDrugSnFRs ("intensity-based Drug-Sensing Fluorescent Reporters") can detect their drug partner with responses of $\Delta F/F0 > 1$ at the half-maximal effective concentration. Using targeting and retention sequences we can direct the constructs to various organelles. These tools allow for insights into pharmacokinetics, organellar sequestration of drugs by acid trapping, protein trafficking, and upregulation—all crucial facets in the expanded understanding of "inside-out" neuropharmacology of neural drugs.