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Live-cell imaging of serotonin receptors reveals molecular mechanisms of psychedelic signalling

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A. Background and rationale

Depression and anxiety are widespread affective disorders that impose a significant global health burden[1]. However, first-line pharmacological treatments such as selective serotonin reuptake inhibitors are only modestly effective and typically require several weeks before therapeutic onset[2,3]. Psychedelic drugs, including psilocin and lysergic acid diethylamide (LSD), have emerged as promising therapeutic agents due to their rapid and sustained antidepressant effects[4,5]. These compounds primarily exert their effects through activation of the serotonin 2A receptor (5-HT_{2A})[6], yet the molecular basis for their distinct effects compared to the endogenous ligand serotonin remains unclear. Upon activation, G protein-coupled receptors such as 5-HT_{2A} undergo endocytosis to regulate signalling, trafficking, and desensitisation. Recent studies have suggested that psychedelics may engage intracellular pools of 5-HT_{2A}, raising the possibility that signalling from distinct subcellular compartments contributes to their unique pharmacology[7]. Because intracellular compartments contain distinct signalling effectors, receptor localisation may influence downstream signalling outcomes[8,9]. Thus, whether psychedelics differentially access intracellular 5-HT_{2A}, modify its subcellular localisation, or engage distinct downstream signalling pathways compared with serotonin remains an important unresolved question.

B. Hypothesis / Aims

We hypothesised that psychedelic drugs differentially regulate the trafficking and signalling of 5-HT_{2A} compared with serotonin, potentially through engagement of intracellular receptor pools. Therefore, we aimed to define how psychedelics influence 5-HT_{2A} localisation, intracellular ligand access, and effector recruitment.

C. Methodology

Several imaging approaches were used to examine 5-HT_{2A} trafficking, effector recruitment, and ligand localisation. 5-HT_{2A} was tagged with an ALFA epitope, enabling fluorescent nanobody-based visualisation of *de novo* receptor internalisation in live cells. Subcellular trafficking was quantified using fluorescent markers of endosomal compartments to resolve receptor localisation. Biosensors, including mini-Gαq, PKC, and Ca^{2+} reporters, were used to assess canonical signalling pathways downstream of 5-HT_{2A} activation. Multiphoton imaging was additionally employed to directly track intracellular localisation dynamics of LSD and the fluorescent serotonin analogue FFN246 over time.

D. Results

We first examined whether psychedelics altered the trafficking profile of 5-HT_{2A}. Surprisingly, 5-HT_{2A} was constitutively endocytosed irrespective of ligand treatment, with receptors predominantly trafficking to late endosomal compartments under both vehicle- and drug-treated conditions. These findings suggest that receptor internalisation is an intrinsic feature of 5-HT_{2A} regulation rather than a process uniquely regulated by psychedelics.

We next investigated whether psychedelics differentially regulated Gαq coupling and downstream signalling at the plasma membrane. Compared with serotonin stimulation, the prototypical psychedelics LSD and psilocin exhibited reduced recruitment of Gαq to 5-HT_{2A} at the plasma membrane, accompanied by weaker

activation of downstream PKC and Ca^{2+} signalling pathways, consistent with their characterisation as partial 5-HT_{2A} agonists.

To determine whether intracellular receptor access may contribute to psychedelic signalling, multiphoton imaging was used to visualise LSD and the fluorescent serotonin analogue FFN246 directly. Both compounds entered cells following addition but displayed distinct localisation profiles. LSD rapidly colocalised with intracellular pools of 5-HT_{2A} within 5 minutes, appearing as punctate intracellular structures, whereas FFN246 showed comparatively diffuse distribution with limited enrichment at 5-HT_{2A} (**Figure 1**). These data indicate rapid intracellular access of psychedelics to receptor-containing compartments.

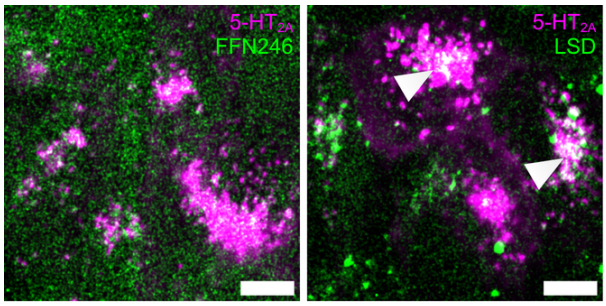

Figure 1. Representative multiphoton imaging comparing intracellular localisation of the fluorescent serotonin analogue, FFN246, and LSD at 5 min post-addition. FFN246 (left) displayed limited colocalisation with intracellular 5-HT_{2A} and was predominantly distributed diffusely throughout the cytoplasm. In contrast, LSD (right) rapidly entered cells and was enriched on intracellular 5-HT_{2A}, appearing as punctate intracellular structures (arrows). Scale bar: 10 μm.

Finally, we examined Gαq recruitment at intracellular 5-HT_{2A}. Serotonin induced robust recruitment of Gαq to 5-HT_{2A}-positive endosomes, whereas psychedelics led to lower levels of endosomal Gαq recruitment despite rapidly localising to these compartments. These data indicate that intracellular access of psychedelics does not translate into canonical Gαq activation, suggesting that psychedelics engage alternative signalling mechanisms at intracellular 5-HT_{2A}.

E. Conclusions

Our data indicate that subcellular localisation of 5-HT_{2A} does not alone account for differences between serotonergic and psychedelic signalling. Although LSD rapidly accesses intracellular receptor pools, it does not induce robust intracellular Gαq recruitment, in contrast to serotonin. Together, these findings suggest that psychedelic signalling at intracellular 5-HT_{2A} is mediated through mechanisms distinct from canonical Gαq activation and highlight new mechanistic insights into psychedelic action.

F. Innovation and Significance

This study integrates complementary live-cell imaging approaches to dissect 5-HT_{2A} signalling. We use an ALFA-tag/nanobody system to visualise receptor trafficking in real time, fluorescent biosensors to quantify effector recruitment, and multiphoton imaging to directly track intracellular ligand access. This work represents the first applications of direct imaging of psychedelic compounds alongside analysis of receptor localisation and downstream signalling within the same experimental framework.

Rather than relying on single downstream readouts of receptor activation, this approach enables simultaneous interrogation of ligand access, receptor trafficking, and effector coupling at the subcellular level. This provides a more integrated perspective for studying 5-HT_{2A} pharmacology and highlights the importance of combining spatial and signalling information to understand ligand-specific receptor behaviour.

G. Student's Role

The nominating student has played a central role in the experimental design and execution of this project. The student established the ALFA-tagged 5-HT_{2A} construct and developed the associated live-cell imaging workflow used to investigate receptor trafficking and signalling. The student performed most of the experimental work, including live-cell imaging, biosensor assays, and multiphoton imaging of ligand localisation.

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Declaration

We declare that this document was prepared primarily by the nominating student and has undergone minimal corrections by the supervisor or any other author on the accompanying abstract.

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Date: 10/06/2026

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