PROJECT

Supervisor: Danilo De Gregorio

Title: Reversing fentanyl-induced reward maladaptation: circuit and molecular mechanisms of LSD action

Curriculum: Neuroscience and experimental Neurology_

Link to the personal page of the University or relevant hospital site website: **https://research.hsr.it/en/divisions/neuroscience/neuropsychopharmacology/danilo-de-gregorio.html**

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Opioid use disorder (OUD), particularly driven by synthetic opioids such as fentanyl, represents a major and escalating public health crisis. Beyond physical dependence, fentanyl induces persistent maladaptive neuroplastic changes within cortico-limbic circuits, driving compulsive drug-seeking and relapse. Current pharmacotherapies (e.g., methadone, buprenorphine) primarily target withdrawal symptoms but do not directly reverse the underlying circuit dysfunction associated with addiction.

Recent evidence shows that psychedelics such as lysergic acid diethylamide (LSD) promote robust neuroplasticity in the prefrontal cortex (PFC) and related circuits. However, whether LSD can specifically counteract opioid-induced reward learning and maladaptive plasticity remains unknown, particularly at the level of: i)reward-associated behavior (e.g., conditioned place preference); ii)PFC-dependent circuit function; iii) glutamatergic neuronal activity

This represents a critical gap in understanding the potential of psychedelics in addiction biology.

Rationale and hypothesis

We hypothesize that:

LSD reverses fentanyl-induced maladaptive reward learning by restoring PFC-dependent glutamatergic control over addiction-related circuits.

Specifically: Fentanyl induces pathological reward encoding measurable via conditioned place preference (CPP); LSD normalizes this through activity-dependent plasticity in PFC excitatory neurons (CaMKIIα+); optogenetic inhibition of PFC glutamatergic neurons will abolish LSD's anti-addictive effects, demonstrating circuit specificity. This project focuses on functional circuit mechanisms rather than descriptive morphology, representing a conceptual advancement over previous work.

Objectives and specific aims

Aim 1: To assess fentanyl-induced reward-related behavior in mice using the conditioned place preference (CPP) paradigm and evaluate the effects of LSD and ketamine on CPP acquisition, extinction, and reinstatement.

Aim 2: To investigate the role of medial prefrontal cortex (mPFC) glutamatergic neurons in mediating the behavioral effects of LSD, using optogenetic inhibition of CaMKIIα-expressing neurons during CPP paradigms.

Aim 3: To validate molecular pathways associated with LSD-mediated modulation of fentanyl reward in primary cortical neurons.. Based on the behavioral and circuit-level findings obtained in Aims 1 and 2, targeted molecular analyses will be performed in primary cortical neurons to validate candidate plasticity-related pathways associated with LSD-mediated modulation of fentanyl-induced maladaptive reward processes. Analyses will focus on selected synaptic and activity-dependent markers identified from in vivo experiments, including glutamatergic signaling proteins and immediate early genes. This aim is intended as a complementary mechanistic validation and not as a large-scale discovery-oriented in vitro screening approach.

Expected outcomes

We expect LSD and ketamine to reduce fentanyl-induced reward-related behavior, as measured by conditioned place preference, and to facilitate extinction while reducing reinstatement. Furthermore, we anticipate that inhibition of mPFC glutamatergic neurons will abolish the behavioral effects of LSD, supporting a circuit-specific mechanism of action. In vitro, both compounds are expected to normalize fentanyl-induced impairments in neuronal morphology and molecular signatures, though through partially distinct pathways. Overall, this project will clarify the therapeutic potential and mechanisms of two compounds with growing relevance for neuroplasticity-centered psychiatry. This proposal is complementary to ongoing work in the laboratory focused on direct in vitro neuroplasticity mechanisms. While previous projects primarily investigate cellular and molecular responses to psychedelics in neuronal cultures, the present proposal specifically addresses circuit-level and behavioral mechanisms underlying fentanyl-associated reward maladaptation using in vivo approaches. Any in vitro experiments

included here are limited to targeted validation directly supporting the in vivo findings, thereby minimizing overlap in activities, resource utilization, and expected outcomes.

Skills that the student should acquire (max. 600 characters including spaces):

The student will acquire expertise in addiction-related behavioral paradigms (CPP), in vivo circuit manipulation (optogenetics) and primary cortical neuron culturesAdditional skills include synaptic biology, data analysis, and comparative pharmacology of psychedelics and ketamine.

References (max. 15)

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2 Vollenweider FX, Preller KH. Psychedelic drugs: neurobiology and potential for treatment of psychiatric disorders. Nat Rev Neurosci. 2020;21(11):611–624.

3 Williams NR, Heifets BD, Blasey C, Sudheimer K, Pannu J, Pankow H, et al. Attenuation of Antidepressant Effects of Ketamine by Opioid Receptor Antagonism. Am J Psychiatry. 2018;175(12):1205–1215.

4 Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med. 2016;22(3):238–249.

5 JL, Lin WC, Cha JW, So PT, Kubota Y, Nedivi E. Structural basis for the role of inhibition in facilitating adult brain plasticity. Nat Neurosci. 2011;14(5):587–594.

6 Y, Cui Y, Sang K, Dong Y, Ni Z, Ma S, et al. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. Nature. 2018;554(7692):317–322.

7 Moliner R, Pérez-Fernández V, Monfort M, Roger-Sánchez C, Miñarro J, Rodríguez-Arias M. LSD-induced gene expression changes in the prefrontal cortex: relevance to plasticity and cognition. Neuropharmacology. 2022;207:108963.