

administration of FLX in adulthood did not improve motivational deficits and did not alter hedonic perception. Chronic administration of tianeptine (TIA) slightly improved motivational behavior without altering hedonic perception. In contrast, chronic administration of the mu opioid antagonist methocinnamox (MCAM) markedly improved motivational deficits in mice, even while blunting hedonic perception. The ability of MCAM to enhance motivation was selective to early-FLX exposed mice. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our results reveal that unexpectedly opioid receptor antagonism is effective at improving motivation in mice exposed to SSRIs in early life. This suggests potential novel treatment approaches in individuals with motivational impairments and a history of in utero exposure to SSRIs.

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### **Xylazine quantitation in Puerto Rican drug users and cardiotoxic protein markers profile expression analysis**

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**OBJECTIVES/GOALS:** Our research addresses critical gaps by examining the prevalence, blood concentrations, and health implications of xylazine abuse, with a focus on its cardiotoxic effects. We aim to map the geographic distribution of xylazine use across Puerto Rico and characterize its impact on cardiomyocytes at relevant exposure levels. **METHODS/STUDY POPULATION:** To accurately detect and quantify xylazine in blood samples, we will employ chromatographic techniques coupled with mass spectrometry (UPLC/MS or GC/MS). The xylazine prevalence across the island will be mapped based on health system classifications. Samples will be categorized according to the eight healthcare regions of Puerto Rico, as defined by the "Administración de Seguros de Salud" (ASES), ensuring comprehensive geographic representation. We will investigate the expression profiles of proteins associated with cardiac injury and dysfunction in human cardiomyocytes and in the blood of drug users. Blood samples will be provided by "Iniciativa Comunitaria." We will assess the xylazine effects on human cardiomyocyte viability and identify key biomarkers of cardiotoxicity induced by xylazine exposure. **RESULTS/ANTICIPATED RESULTS:** In previous research, we demonstrated that xylazine induces cell death in endothelial cells through both extrinsic and intrinsic pathways. We also observed an increase in reactive oxygen species (ROS) levels after drug exposure, indicating oxidative stress as a potential mechanism of toxicity. Additionally, DNA damage was detected. Given the known relationship between endothelial damage and cardiomyocyte dysfunction in drug-induced cardiotoxicity, we hypothesize that xylazine concentrations vary regionally within Puerto Rico and that chronic xylazine abuse will elevate markers of cardiac injury and dysfunction at common user doses. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The increasing xylazine abuse, particularly in Puerto Rico, represents a critical public health challenge. Our study will fill a knowledge gap by providing crucial data on xylazine's cardiotoxicity and mapping its geographic prevalence, with the potential to advise healthcare approaches and improve care for drug-using Hispanic populations.

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### **Elucidating the role of the rete ovarii in fertility and progesterone signaling**

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**OBJECTIVES/GOALS:** The goal of this study is to determine the function of the rete ovarii (RO), an uncharacterized secretory epithelial appendage to the ovary. I am testing the hypothesis that the RO is critical for the maintenance of the ovarian reserve and fertility, and progesterone signaling plays a role in the function of the RO. **METHODS/STUDY POPULATION:** For this project, I am utilizing a mouse model. To visualize the rete ovarii (RO) in vivo, I am using a Pax8rtTA; TRE-H2B-Gfp (PTG) reporter mouse, which expresses green fluorescent protein in the RO. To determine the role of the RO in fertility and maintenance of the ovarian reserve, I will surgically ablate the RO or perform a sham surgery on adult female PTG mice. Then, I will follow-up with quantification of the ovarian reserve and long-term fertility tracking studies. To determine how the RO responds to progesterone, the RO will be cultured ex vivo in the presence and absence of progesterone. I will perform a morphometric analysis of the RO, as well as collect secreted proteins from the media for proteomic analysis. **RESULTS/ANTICIPATED RESULTS:** If the RO is important for ovarian homeostasis, I expect that in the absence of the RO, ovarian functions such as maintenance of the ovarian reserve and fertility will be impaired. Additionally, because the RO expresses progesterone receptors, I anticipate that the RO will be responsive to progesterone as shown in changes in the morphometric analysis and in changes in secreted proteins in the presence of progesterone. **DISCUSSION/SIGNIFICANCE OF IMPACT:** A major gap in knowledge regarding female physiology and reproductive health is the role of the RO. We expect this work to reveal that progesterone signaling in the RO is important for regulating ovarian functions and to show that the RO is a critical modulator of female fertility and reproductive function.

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### **Biomarkers for HIV neutralization breadth development in early life**

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**OBJECTIVES/GOALS:** A key strategy in generating a protective HIV vaccine is the elicitation of broadly neutralizing antibodies (bnAbs), capable of neutralizing a large diversity of HIV-1 isolates. The goal of this study is to identify molecular signatures of HIV bnAb development early in life, to guide the development of a successful pediatric HIV vaccine strategy. **METHODS/STUDY POPULATION:** We previously defined HIV neutralization breadth in 40 ART-naïve children living with HIV. Single-cell RNAseq was performed utilizing peripheral blood mononuclear cells (PBMCs) from the top 5 children with highest neutralization breadth scores and compared their transcriptome to that of PBMCs from 5 children that did not develop neutralization breadth within the first three years of life. Additionally, we incorporated analysis of PBMCs from 5 healthy uninfected children, matched to our experimental groups by race,