

Response of Gpm6b Expression in Mouse Nucleus Accumbens after Cocaine Conditioned Place Preference

INTRODUCTION

Substance use disorder (SUD) is classified as a neuropsychiatric disorder characterized by increased use and dependence on licit and illicit drugs, regardless of the negative consequences that may ensue¹. For individuals with SUD, the personal effects are devastating because daily living becomes difficult and the risk of other mental health illnesses such as depression and anxiety becomes higher². SUD costs the United States financially and sociologically in medical services, legal expenses, unemployment, homelessness, crime, domestic abuse, and premature deaths^{2,3}. Current treatments for addiction such as abstinence and rehabilitation do not effectively prevent future relapse, which can ultimately lead to tragic premature deaths from overdose^{3,4}. Research has shown that SUD causes acute and long-term alteration to multiple brain regions^{5,6}, including changes in gene expression⁷. It is important to study genes involved in the acquisition of drug-seeking behavior in order to understand the neurobiological mechanisms underlying SUD.

Gpm6b is a protein-coding gene that makes the Gpm6b protein, a neuronal tetraspan protein belonging to a family of proteolipids that are widely expressed in the brain in neurons and oligodendrocytes⁸. This protein is involved in neuronal differentiation and myelination. Research has shown that in Gpm6b deficiencies, sensorimotor gating is impaired, which is often associated with neuropsychiatric disorders⁸. Additionally, Gpm6b deficiencies have also been associated with claustrophobia and diminished responses in serotonin 5-HT_{2A/C} receptors⁸. 5-HT_{2A/C} receptors are cell surface receptors that are widely expressed in the central nervous system, particularly in brain regions associated with learning and cognition⁷.

Recent work in the lab identified Gpm6b as differentially expressed in the medial habenula after the reinstatement of cocaine self-administration. Given that Gpm6b has been associated with learning and cognition⁷ and is highly expressed in the nucleus accumbens, I propose to study the role of Gpm6b in addiction behavior. I will be testing the hypothesis that the Gpm6b gene is differentially expressed in the nucleus accumbens after the acquisition of drug-seeking behavior.

MATERIALS AND METHODS

Subjects

24 60-day-old male mice will be purchased from Jackson Laboratories. Mice will be housed in a 12:12 hour light/dark cycle room in their homecage with food and water provided as necessary.

Homecages are temperature controlled at 70°F. Behavioral testing will be performed during the light cycle.

Conditioned Place Preference (CPP)

The mice will perform a series of experiments in CPP chambers. CPP chambers consist of a checkered compartment, a white compartment, and a center room that allows mice to move between the two compartments. Mice will undergo 3 consecutive days of handling for 5 minutes per session. On the fourth day, mice will experience a 15-minute pre-test in which they will be free to explore both chambers. This will determine if the mice have an innate preference for a specific compartment before conditioning. If mice display an innate preference (defined as more than 200 seconds spent in a chamber), mice will be conditioned in the opposite compartment to eliminate biases in conditioning outcomes⁹.

On days 5-8 (4 days in total), mice will be conditioned in 30-minute sessions. On days 5 and 7, cocaine-conditioned mice will be administered a 10 mg/kg/IP dose of cocaine via a 1 mL syringe, and alternately on days 6 and 8, they will be administered saline. Mice of the saline control group will be administered saline on all 4 days of conditioning. To ensure counterbalance, half the mice will have cocaine paired with the checkered compartment and the other half of the mice will have cocaine paired with the white compartment.

On day 9, mice will be tested for the acquisition of conditioned place preference in a 15-minute post-test in which they will be allowed to explore all three parts of the chamber with the doors open. Behavioral tests will be analyzed with the software *Ethovision XT*. During the post-test, the cocaine-conditioned mice are expected to have a preference for the compartment in which they were placed on cocaine-conditioning days (days 5 and 7). Alternately, the saline control mice will be expected to have no preference between the two compartments.

Preparation of Neuronal Tissue for Analysis

After 1 hour following the post-test, mice will be euthanized and extracted brains flash frozen in dry ice-chilled isopentane. 0.5 mm punches from 500 µm coronal slices of the nucleus accumbens will be collected via a cryostat and stored in a -80 freezer.

RT qPCR

mRNA will be isolated from tissue punches using RNeasy Mini Kit (Qiagen) and then reverse transcribed into cDNA. The resulting cDNA will be used to perform qPCR using the Roche Light Cycler. Gene expression of GPM6B will be analyzed from qPCR data.

RESPONSIBILITY

As an undergraduate researcher in my lab for the past year, I have developed the skills necessary to independently perform behavioral tests and molecular lab techniques for this study. I have experience in handling mice, assisting in CPP, extracting, slicing, and punching neuronal tissue, and RT qPCR.

INTENDED OUTCOME

Following CPP, I expect the cocaine-conditioned mice to display a preference for the compartment in which they were placed on cocaine-conditioning days because they will learn to associate the compartment with a drug reward. Additionally, I will expect the saline control mice to have no compartment preference because they were not provided with a drug reward. Following RT qPCR, I expect there to be a prominent amount of Gpm6b expression in the nucleus accumbens tissue punches from the saline control mice since previous studies have shown that the Gpm6b gene is highly expressed in the nucleus accumbens. In the nucleus accumbens tissue punches from the cocaine-conditioned mice, I expect there to be a significant change in Gpm6b expression compared to the saline control mice punches since the nucleus accumbens is a key structure in motivation and addiction behavior.

POTENTIAL LIMITATIONS AND ALTERNATIVE APPROACHES

There have been limited studies on the Gpm6b gene and it is unknown whether it will be involved in the acquisition of drug-seeking behavior. Ultimately, if there is no differential expression of the gene after CPP, the remaining cDNA collected from neuronal tissue will be probed for other target genes – Eef1a1 and Garnl3. These genes were found in previous studies to be differentially expressed in the medial habenula after reinstatement and are highly expressed in the nucleus accumbens thus, they would make good candidates for alternate explorations. An additional 4-5 weeks would need to be spent on RT qPCR and analysis for these alternative approaches.

LAB SAFETY

To ensure safe conduct during the study, proper safety and sanitation measures will be taken. Proper handwashing will be performed before conducting any task. CPP will be self-contained, only 1 person will be allowed in the room at a time and 70% ethanol solution will be used to clean the chambers before and after the experiments. Personal protective equipment (PPE) will be worn both in the lab and in the vivarium at all times including lab coats, nitrile gloves, and surgical masks.

TIMELINE

- Week 1: Preparation of cocaine solution and RT qPCR reagents

- Weeks 2-3: Perform CPP for cocaine-conditioned mice, followed by extraction
- Weeks 3-4: Perform CPP on saline control mice, followed by extraction
- Week 5: Collect neuronal tissue punches on the cryostat from the nucleus accumbens
- Weeks 6-8: RT qPCR – mRNA isolation, cDNA synthesis, qPCR
- Weeks 9-10: Gene expression analysis

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