The role of the patch compartment and eIF2a in METH-induced habitual behaviors

Sonia Singhal, David Christy, and Ashley Horner
Department of Biomedical Sciences, Mercer University School of Medicine, Macon, GA, USA 31207

Introduction

The initial basis of the experiment centered on examining how repetitive exposure to methamphetamine impacts behavior in rat animal models. With repetitive exposure to methamphetamine, the animals can form habitual behaviors resulting from stimulus-response learning, meaning an association is formed between a stimulus and an automatic response. To ensure the repetitive methamphetamine use is a habitual behavior, the drug is paired with a negative stimulus, LiCl, to create an aversion. If the use of methamphetamines is unchanged, then the behavior is considered habitual. Further investigation into the striatum began with the consideration that enhanced activity of the patch compartment, which has a high density of mu opioid receptors (MOR), likely corresponds with habitual drug abuse and therefore, higher addiction and habit-forming behaviors. Exposing the animal models to methamphetamines, which have high affinity for mu-opioid receptors, was thought to enhance activation of the patch compartment. However, using designer receptors exclusively activated by designer drugs (DREADDS) contained within a virus, the patch compartment neurons were inhibited and addictive tendencies could potentially be controlled. The four experimental groups include vehicle infusion with saline, vehicle infusion with LiCl, virus infusion with saline, and virus infusion with LiCl. Once the experiment with the animal models was completed, the rats were sacrificed in order to use the brain tissue samples for further examination.

To further understand the neurological causes of these behaviors, neurochemical changes in the patch compartment of the striatum were examined using immunohistochemical co-localization staining. The neurons of interest in the patch compartment for this experiment include those that contain the proteins, mu opioid receptors and the eukaryotic translation initiation factor 2α, eIF2a. From prior research experiments conducted, reduced activity of eIF2a is connected to increasing synaptic connections in the brain and allowing animal models to feel greater senses of pleasure. This inherently leads to increased use of repetitive, habitual uses of drugs. It is possible the use of drugs, such as cocaine and methamphetamines, are causing eIF2a inhibition. Performing an immunohistochemical co-localization staining will correlate the eIF2a expression levels in mu opioid receptor containing neurons and contribute to the understanding of drug use in the patch compartment of the striatum.

Methods

For the behavior portion of this experiment, male rats undergo a series of surgeries and training sessions. The rats first have intracranial infusions to infuse either a vehicle solution, made from 5%DMSO in sterile saline, or DREADD virus. Then they are given 6 weeks to recover before a jugular catheterization surgery. From this surgery, the rats have an indwelling jugular catheter in order to run the self-administration METH training. The training includes three different random interval (RI) reinforcement schedules. The rats have a RI-15 second, RI-30 second, and RI-60 second schedule two days each. Once a repetitive, habitual behavior is formed from the self-administration training, the rats are given lithium chloride (LiCl) to create an aversion or saline for the control group. Lastly, the rats have an extinction test to determine the extent of the habitual behaviors. After, the rats are immediately sacrificed to retrieve the brains for immunohistochemistry staining.

A double staining of eIF2a and MOR is run on striatum samples from each rat behavior experimental group. The staining process includes a series of steps shown below:
- Incubate in blocking solution, made from 10% NDS, 0.30% Triton-X-100, for 1 hour at room temperature
- Replace with first primary antibody, eIF2a, at a 1:500 dilution and incubate overnight
- Then replace with first secondary antibody for eIF2a at a 1:250 dilution for 1 hour at room temperature
- Now replace with second primary antibody, MOR, at a 1:500 dilution and incubate overnight
- Replace with secondary antibody for MOR at a 1:1000 dilution and incubate for 1 hour at room temperature
- Finally, tissue samples are washed with TBS-Base and mounted on slides to examine under the microscope

Results & Discussion

As this is an ongoing project, there is further research needed to be completed. At this point though, from the double staining process, there are positive staining results in the experimental groups. Pictured on the right, this is a striatum double stained with eIF2a and MOR in a vehicle and saline rat experimental group. The zoomed in red picture shows the MOR staining while the green shows eIF2a staining. The bottom picture shows the two staining overlapped. In this picture, the yellow dots are the area of interest as this is where the positive double staining is. Not all of the areas of interest will be yellow as the primary antibodies are from the same species which can alter results slightly.

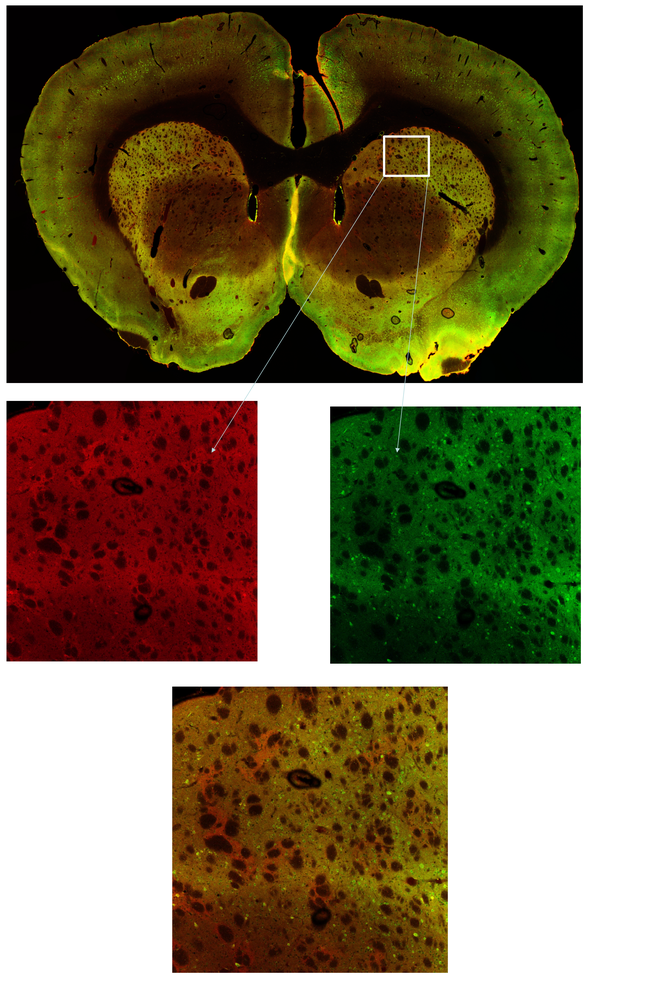

Figure 1: Top image shows a complete striatum image. Below are the MOR staining in red and eIF2α staining in green. On the bottom is the overlapping MOR and eIF2α staining.

Future plans of the study include imaging all of the behavioral groups and running them through ImageJ, a program provided by the National Institutes of Health (NIH). ImageJ can be programed with set values to measure each color. Then it will calculate all the color values within an image. This will show how much staining there is in one part of the image compared to another image. Once this is completed for each experimental group, the color values can be compared, and hopefully measurable differences in the staining will show significant results.