**INTRODUCTION**

- Methamphetamine (MTA) is a psychostimulant that is used and abused recreationally for its intense rush of pleasure and/or prolonged euphoria.

Crystal Structure of Methamphetamine

- MTA functions by causing a reversal of the dopamine transporter (VMAT) leading to a surge of dopamine in the nerve terminal.
- MTA causes dopamine depletion at the nerve terminal leading to neuronal death.

MTA Mechanism of Action

The effects of MTA on the dopaminergic system circadian behaviors have been widely studied.

- However, the effects of MTA on per2 gene in the suprachiasmatic nucleus (SCN) is lacking.
- The suprachiasmatic nucleus harbors the principal pacemaker that is responsible for synchronizing circadian behaviors.
- The per2 gene encodes components of the circadian rhythms of locomotor activity, metabolism, and behavior.

**GOAL OF THE STUDY**

- Our first goal was to make an atlas of the mice brain using crysel violet staining.

- The second goal was to determine the effects of repeated methamphetamine injections on the expression of per2 gene in the suprachiasmatic nucleus using immunohistochemistry.

**METHODS**

**Brain Sectioning**

- Brain sections were obtained from mice treated with either vehicle (1% EtOH) or four consecutive injections of MTA (1.2mg/Kg).
- The brains were sectioned (30µm) from rostral to caudal using a cryostat.

**Crysel Violet Staining**

- Sections from mice treated with vehicle (1% EIOH) were stained with crysel violet.
- Pictures of the stained section were taken using light microscopy (See Below).
- A mouse brain atlas was produced.

**Immunohistochemistry**

- Step 1 – Rabbit Polyclonal anti Per 2 Antibody (50, 200, 500 µM)
- Step 3 – Biotinylated Anti-rabbit IgG
- Step 3 – Chromogen

**Microscopy**

- Full image of sections were taken with 1x objective and partial images with 10x objective

**RESULTS**

**Mouse Brain Atlas Using Stained Sections with Crysel Violet**

- Expression of Per2 gene in the Suprachiasmatic Nucleus

**ACKNOWLEDGEMENTS**

- NSF
- ISEP
- Dubocovich Lab
- Dubocovich Lab

**IMPLEMENTATIONS**

- Monthly Scientific Inquiry Projects
- Solution Chemistry with pipettes
- Pipette pointillism
- Metric Conversion
- Microscopy and Staining
- Brain sections were stained with crysel violet and allowed development of a brain atlas that will help students learn about different areas of the brain.
- Methamphetamine treatment increased Per2 gene expression in the shell.
- 200 and 500µm primary antibody produced the best staining of Per2 gene

**CONCLUSION**

- Our first goal was to make an atlas of the mice brain using crysel violet staining.

- The second goal was to determine the effects of repeated methamphetamine injections on the expression of per2 gene in the suprachiasmatic nucleus using immunohistochemistry.

- Students will learn staining techniques and microscopy with study done over the summer as templates.

- The metric system will be taught with a bigger emphasis on the microliter since this unit of volume is widely used in labs requiring micro-volumes during reagent preparation.

- Students will develop science artwork using different size pipettes to make different size dots. The goal is for the students to master the use of pipettes. 6th through 8th graders will receive small group instruction from UB students provided by the ISEP grant.

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