Effects of Methamphetamine on the Expression of *per2* genes in the Suprachiasmatic Nucleus

Cassata D., Indalecio S., McGavern D., Clough SJ., Adamah-Biassi EB., Dubocovich ML.

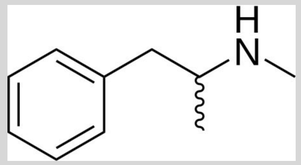
Harriet Ross Tubman Middle School, Buffalo NY. 14212, Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences University at Buffalo SUNY, Buffalo, NY 14214.

INTRODUCTION

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

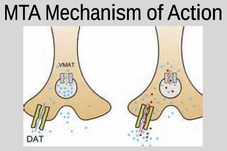
Crystal Methamphetamine

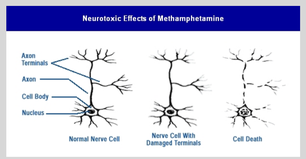
Structure of Methamphetamine



- 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 suprachiamatic 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 fist 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 suprachiamatic nucleus using immunohistochemistry.

METHODS

Brain Sectionning

- 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

Brain Sectionning Example

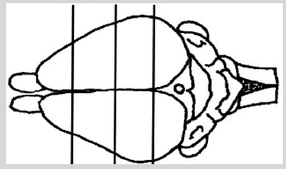
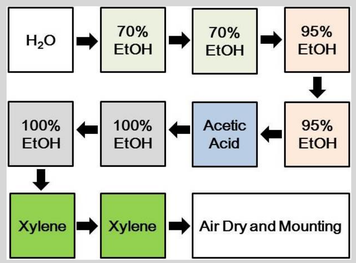


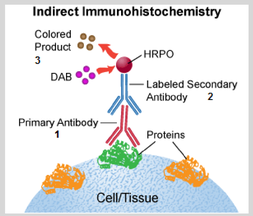
Image of the Cryostat

Crysel Violet Staining

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

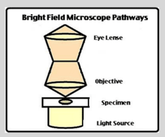


Immunohistochemistry



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

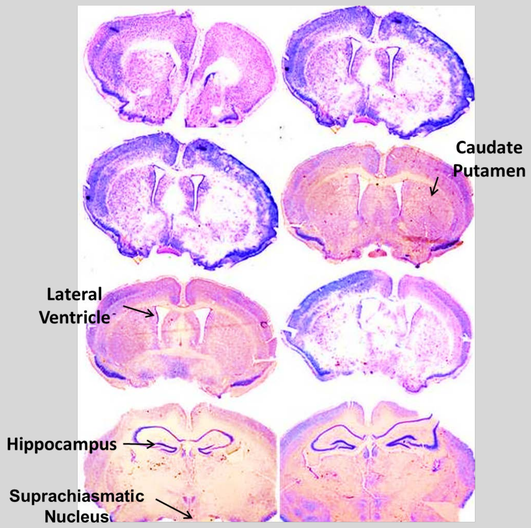
Microscopy



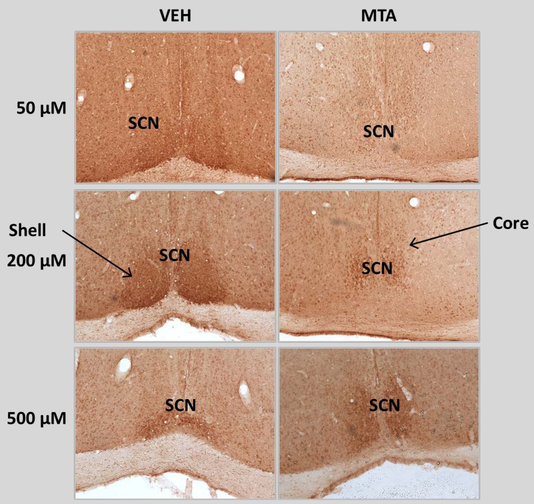
- Full Image of sections were taken with 1x objective and partial images with 10x objective

RESULTS

Mouse Brain Atlas Using Stained Sections with Crysel Violet



Expression of *Per2* gene in the Suprachiasmatic Nucleus



- 50µM primary antibody concentration produce little to no staining in the SCN
- 200µ and 500µM primary antibody concentration produced the best staining
- MTA produced staining in the core while vehicle produced staining in the shell

IMPLEMENTATIONS

- **Monthly Scientific Inquiry Projects**

Students from 7th and 8th grades will develop a research question each month and write an appropriate hypothesis. They will then design and carry out their experiment to gather data about their hypothesis. This poster and the research herein will be used as a model in teaching the purpose and process of scientific inquiry.

- **Pipette pointillism**

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.

- **Solution Chemistry with pipettes**

7th and 8th graders will use pipettes in solution preparation and projects involving dilutions. Emphasis will be made on calculations involved in preparation of solutions.

- **Metric Conversion**

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.

- **Microscopy and Staining**

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

CONCLUSION

- 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

ACKNOWLEDGEMENTS

- ISEP
- Dubocovich Lab
- NSF