Characterization of Mcui4 on mitochondrial calcium modulation and behavior in vivo

Elizabeth Banyas[1], Antony Abraham[2]

1. Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, 2. Center for Drug Discovery, Research Triangle Institute International

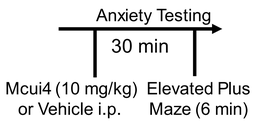
Introduction

- The mitochondrial calcium uptake 1 (*micu1*) gene encodes mitochondrial proteins that regulate calcium (Ca^{2+}) influx via a uniporter complex
- The production of mitochondrial ATP, a primary source of energy in the cell, is regulated by Ca^{2+} influx
- Repeated cocaine exposure alters the morphology and function of neuronal mitochondria
- Technology to access, observe, and manipulate mitochondria in vivo are limited
- **Aim of this experiment:** Determine the neural and behavioral effects of a Micu1-targeting drug (Mcui4) in mice
- **Overall purpose of the study:** Develop treatments for cocaine use disorder by monitoring and manipulating mitochondrial function in vivo

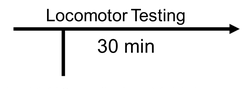
Methods

- Basic behavioral characterization following acute and repeated administration (intraperitoneal injection) of cocaine (20 mg/kg), MICU1-targeting drug (10 or 30 mg/kg), and vehicle (10% dimethylsulfoxide in saline)
- Gross locomotor observations to assess movement patterns and activity levels

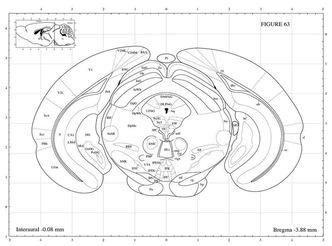
- **Evaluation of anxiety-like behavior**
  - Procedure: Testing was performed on a plus-shaped maze between 900-1700h with ~40 lux in open arms and ~10 lux in closed arms. Movement was recorded and analyzed using Ethovision software (Noldus)



- **Evaluation of locomotor behavior**
  - Procedure: Testing was performed under normal lighting conditions in a temperature-controlled room between 900-1700h. An infrared detection system monitored movement.



- **Immunohistochemistry**
  - VTA dopamine neurons were stained with 1:500 Sheep anti-Tyrosine Hydroxylase primary antibodies from Novus Biologicals (NB300-110) and 1:1000 Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 from Invitrogen (A-21448). Sections from Bregma –3.88mm were analyzed.



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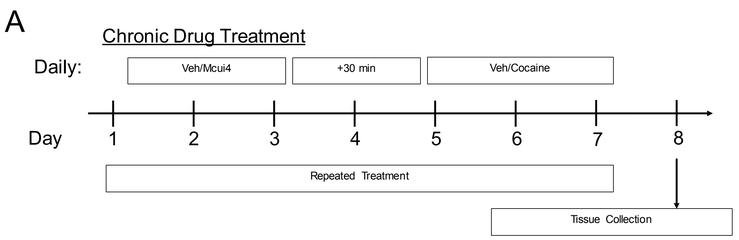
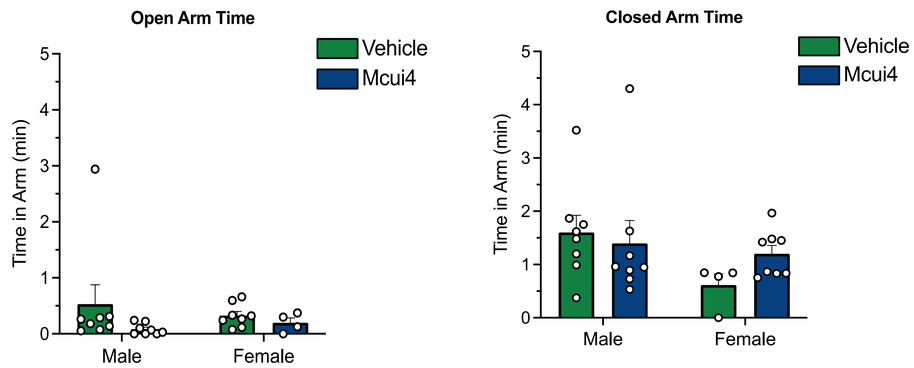
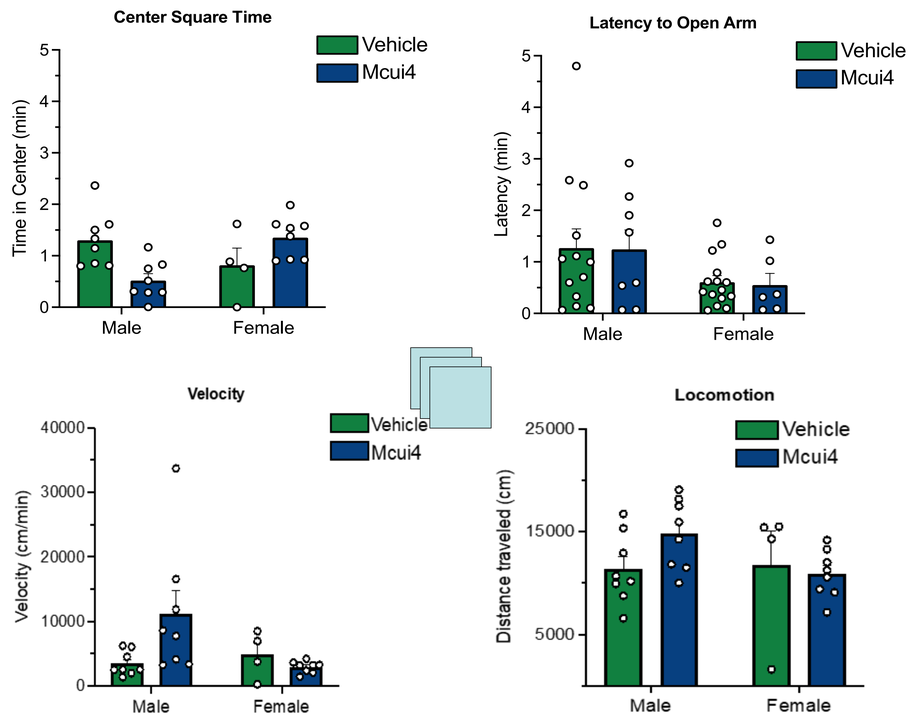


Results

B





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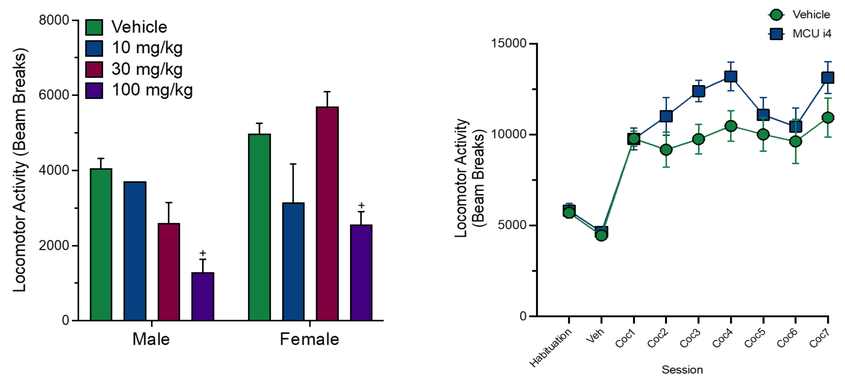

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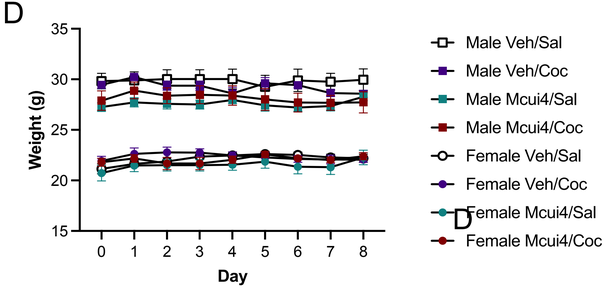

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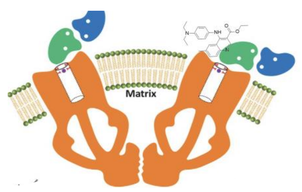

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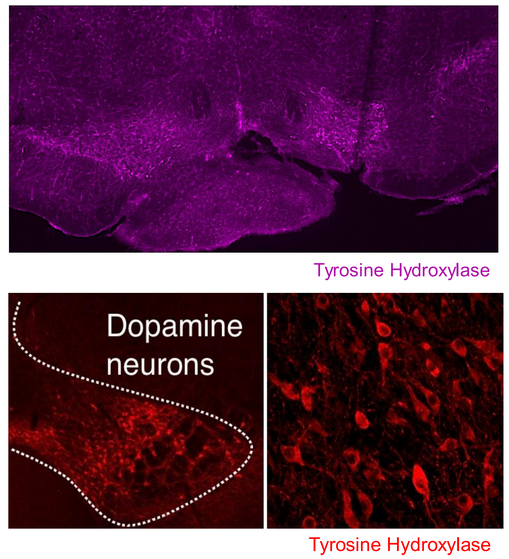

Conclusions

- Inhibition of Micu1 with Mcui4 did not alter anxiety-like or locomotor behaviors
- Males and females show similar anxiety-like and locomotor responses in the elevated plus maze
- Mcui4 did not preferentially affect males or females
- Repeated cocaine or Mcui4 treatment did not significantly affect animal weights
- Mcui4 appeared to be well-tolerated and did not produce gross locomotor or behavioral disruptions
- Preliminary data suggests that mcui4 drug might make animals more sensitive to cocaine

Future Directions

Aim to validate the direct influence of the Mcui4 drug and enhance the understanding of the molecular pathways involved in modulating neural mitochondrial activities by:

- Utilizing immunohistochemistry and photometry to visualize and quantify the effect of mitochondrial drugs within neuronal tissues
- Refining drug delivery methods to enhance the efficacy of the Micu1-targeting drug and developing novel compounds to target Micu1.
- Demonstrating the specificity of the Micu1-targeting drug through CRISPR technology
- Delineating the specific effects of Micu1 inhibition on reward learning processes by assessing additional behavioral paradigms

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