



# Impact of a Ketogenic Diet on the Reinforcing Properties of Varying Doses of Cocaine

## in Young Adult Sprague-Dawley Rats

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### INTRODUCTION

#### BACKGROUND:

- Among the 5.5 million cocaine users ages 12 and older in the United States, 1.0 million suffer from cocaine use disorder which leads to a spiraling effect of health, social, and financial consequences (Lipari, 2019).
- A ketogenic diet (KD) is composed of a high fat, low carbohydrate, moderate protein diet
  - In the absence of glucose, the body enters ketosis and ketone bodies are produced to serve as the body's main energy source (Tillery et al., 2021).
  - The increased ketone availability in the brain decreases oxidative stress, increases ATP production, and decreases neuroinflammation which counteracts the effects of substance use disorder.
- Based on the lab's previous work, a KD demonstrated potential in drug addiction therapy where a KD reduced the reinforcing properties of 10 mg/kg cocaine compared to standard diet male and female rats (Huston et al., in prep).

#### RESEARCH QUESTION:

- Does a ketogenic diet alter the sensitivity of rats to the reinforcing properties of cocaine?

#### PREDICTION:

- Animals will be less sensitive to cocaine at doses lower than 10 mg/kg compared to doses higher than 10 mg/kg

### METHODS

**ANIMALS:** Fifty-seven young adult Sprague-Dawley male and female rats born as the first generation offspring from breeders originally from Charles River Laboratories

**DIET:** At 5 weeks of age, the animals began on a KD composed of 75.1% fat, 8.6% protein and 3.2% carbohydrates (Bio-Serv F3666) and continued on the diet throughout the entirety of the study.

**PHARMACOLOGICAL AGENTS:** Cocaine hydrochloride (Sigma-Aldrich) was dissolved in sterile saline at 5 mg/ml, 10 mg/ml, and 15 mg/ml. Animals were given injection volumes of 1 mL per 1 kg of body weight.

**BEHAVIORAL TESTING:** Animals underwent conditioned place preference (CPP) testing to assess their ability to associate stimuli from a particular chamber in a two-chamber apparatus with the rewarding effects of varying doses of cocaine.

**Pretest:** Animals were allowed to move freely between the two chambers of the apparatus for fifteen minutes to assess their time spent in each chamber and locomotor activity.

**Conditioning:** Animals received intraperitoneal injections of 0, 5, 10, or 15 mg/kg cocaine in their initially nonpreferred chamber on days 1, 3, and 5 and saline on days 2, 4, and 6 in their initially preferred chamber for thirty minutes per day.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
0 mg/kg cocaine 5 mg/kg cocaine 10 mg/kg cocaine 15 mg/kg cocaine	Saline	0 mg/kg cocaine 5 mg/kg cocaine 10 mg/kg cocaine 15 mg/kg cocaine	Saline	0 mg/kg cocaine 5 mg/kg cocaine 10 mg/kg cocaine 15 mg/kg cocaine	Saline
Initially nonpreferred chamber	Initially preferred chamber	Initially nonpreferred chamber	Initially preferred chamber	Initially nonpreferred chamber	Initially preferred chamber
12-13 animals per treatment group	All animals	12-13 animals per treatment group	All animals	12-13 animals per treatment group	All animals

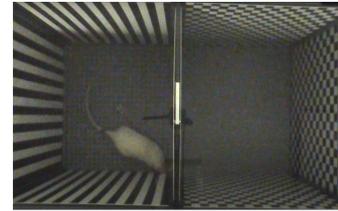
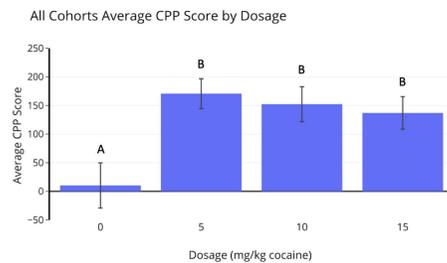
**Posttest:** Animals were allowed to move freely between the two chambers for 15 minutes to assess the difference between the amount of time spent in their initially nonpreferred chamber in the posttest compared to the pretest to calculate their CPP score.

**PHYSIOLOGICAL MEASURES:** Animals were weighed daily including baseline, pretest, and posttest weights. Immediately following euthanization, trunk blood samples were collected to measure blood glucose and ketone levels using Precision Xtra meters.

### RESULTS

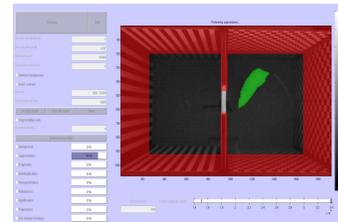
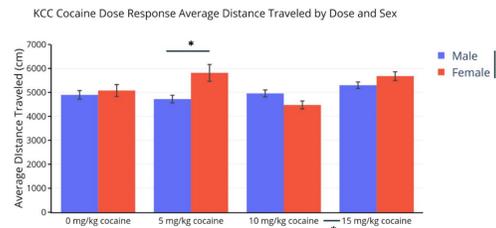
#### BEHAVIORAL:

##### Conditioned Place Preference (CPP) Scores



Mean (SEM +/-) CPP score by cohort and dosage. When examining the combined data across all cohorts, there was a statistically significant main effect of dosage on CPP scores ( $p = 0.001$ ). Post hoc Tukey tests revealed that there were significantly higher CPP scores at 5 mg/kg ( $p = 0.003$ ), 10 mg/kg ( $p = .018$ ) and 15 mg/kg ( $p = 0.025$ ) compared to 0 mg/kg cocaine, but not between 5 mg/kg and 10 mg/kg, 10 mg/kg and 15 mg/kg, or 5 mg/kg and 15 mg/kg. Dissimilar letters in the graph reflect statistically significant pairwise comparisons between cocaine dose treatment groups.

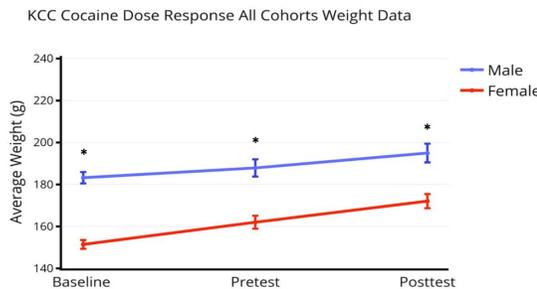
##### Average Distance Traveled



Mean (SEM +/-) average distance traveled by sex and dosage. There was a statistically significant main effect of sex on average distance traveled ( $p = .033$ ) where females traveled a greater average distance than males. There was also a significant main effect of dosage of cocaine on average distance traveled ( $p = 0.006$ ). Post hoc Tukey tests revealed that there was a significantly higher distance traveled for animals who received 15 mg/kg compared to 10 mg/kg cocaine ( $p = 0.011$ ). There was also a significant dose by sex interaction ( $p = 0.007$ ) where at a dosage of 5 mg/kg cocaine, there was a significantly greater average distance traveled for females compared to males ( $p = 5.445E-4$ ). There was also a statistically significant main effect of time on distance traveled ( $p = 2.641E-4$ ) where animals traveled more during the pretest compared to posttest (data not shown).

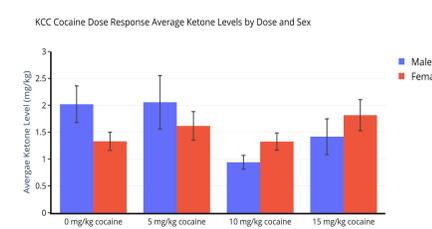
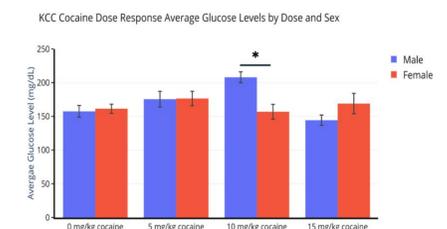
#### PHYSIOLOGICAL:

##### Weight Measures



Mean (SEM +/-) for weight at baseline, pretest, and posttest by sex. There was a significant interaction between time and sex on weight ( $p = 0.009$ ). After running simple main effect comparisons, it was found that male animals had significantly higher weights compared to female animals at all three timepoints of the baseline ( $p = 4.129E-9$ ), pretest ( $p = 2.914E-7$ ), and posttest ( $p = 3.294E-6$ ). Star symbols in the graph reflect timepoints where the weight differences of females versus males was statistically significant.

##### Blood Glucose and Ketone Levels



Mean (SEM +/-) blood glucose and ketone levels by sex and dosage. There was a significant interaction between dosage of cocaine and sex on average glucose levels where male animals who received 10 mg/kg cocaine had significantly higher blood glucose levels compared to females in this same drug treatment group ( $p = 0.002$ ). There were no significant main effects of drug dose or sex on ketone levels.

### SUMMARY AND DISCUSSION

- Physiological findings:
  - Blood glucose and ketones levels as well as weights were in the expected range for animals on a KD based on the lab's previous work
- Behavioral findings:
  - Rats on a KD maintained sensitivity to both low and high doses of cocaine
    - KD animals formed a CPP at 5, 10 and 15 mg/kg of cocaine with no significant difference when compared to the saline-only group, which does not support the initial prediction that animals would have a lower sensitivity to cocaine at lower doses compared to higher doses
    - These findings fail to support our prediction that administration of a KD would make rats less sensitive to the rewarding properties of cocaine suggesting that a KD may have limited therapeutic effects in cases of drug addiction

### FUTURE DIRECTIONS

- How might a KD be used as a therapeutic intervention for drug addiction reinstatement?
  - Animals will undergo an extinction/reinstatement model of CPP where they will form a CPP for one of two sides of a chamber in which they associate particular cues with the rewarding effects of cocaine
  - During the extinction phase, animals will spend time in the chambers without cocaine injections to extinguish any side preferences
  - A KD will be introduced during the extinction phase to assess its effect on the reinstatement of an animal's preference for the chamber in which they received cocaine
  - During the reinstatement phase, animals will receive cocaine and be reintroduced to the chamber to test for side preferences

### ACKNOWLEDGEMENTS

The authors would like to thank Gracie Chouinard, Kylie Brandt, Sarah Kouchak-Eftekhar and Jenny Nord for their assistance with data collection and animal care.

### REFERENCES

1. Lipari, R. N. (2019). Key Substance Use and Mental Health Indicators in the United States: Results from the 2019 National Survey on Drug Use and Health (p. 114).
2. Tillery, E. E., Ellis, K. D., Threatt, T. B., Reyes, H. A., Plummer, C. S., & Barney, L. R. (2021). The use of the ketogenic diet in the treatment of psychiatric disorders. *Mental Health Clinician*, 11(3), 211–219.