The Role of Brain Estrogen Receptor Activation in Motivation for Cocaine in Pregnant Adult Sprague-Dawley Rats

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INTRODUCTION

BACKGROUND:

- Of the 5.2 million people per year who use cocaine in the United States, there are about 750,000 cocaine-exposed pregnancies (*What Are the Effects of Maternal Cocaine Use?*, 2016)
- Sex differences exist between males and females in terms of responses to cocaine, where women suffer from addiction and relapse at higher rates (Kokane & Perrotti, 2020)
- Estradiol (E2) modulates the function of the mesolimbic dopamine system (Kokane & Perrotti, 2020)
  - E2 increases cocaine-induced dopamine release in the nucleus accumbens (Yoest et al., 2019)
- E2 signaling at estrogen receptors (ER) has been shown to be involved in motivation for cocaine in nonpregnant female rats
  - The estrogen receptor antagonist ICI disrupted the formation of a conditioned place preference (CPP) for cocaine in female rats (Segarra et al., 2014)
  - Knockdown of ER-beta in the nucleus accumbens diminished a CPP for cocaine in female mice (Satta et al., 2018)

RESEARCH QUESTION:

- How does activation of brain estrogen receptors affect a pregnant rat’s motivation for cocaine?

PREDICTION:

- Administration of ICI into the lateral cerebral ventricle will disrupt the formation of a CPP in pregnant rats.

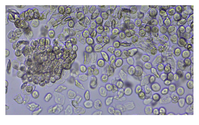
METHODS

ANIMALS: Female, 10-12 week old Sprague-Dawley rats born as the first generation offspring from breeders originally from Charles River Laboratories

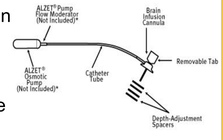
DIET: Animals were maintained on a standard diet (LabDiet 5001) with food and water available *ad libitum.*

PHARMACOLOGICAL AGENTS: Cocaine hydrochloride (Sigma-Aldrich) was dissolved in sterile saline at 10 mg/ml, and administered intraperitoneally (IP) to animals at a dose of 10 mg/kg body weight. The estradiol receptor antagonist ICI (0.5 mg/ml) or vehicle (50% DMSO, 15% ethanol, and 35% sterile water) was delivered intraventricularly at a dose of 0.075 µg/hour with a flow rate of 0.15 µl/hour.

LAVAGE AND MATING: Animals were gently restrained while a micropipet was used to flush saline into the vaginal canal of the rats. The liquid was recollected, pipetted onto a slide, and imaged from an OMAX light microscope (10x magnification) connected to a digital camera using ToupView software. The specific cell types in each sample were then characterized via visual examination of sample photomicrographs. If females were determined to be in proestrus, they were paired with a sexually-experienced male rat with mating day as day 0 of pregnancy.



STEREOTAXIC SURGERY: All animals underwent a stereotaxic procedure on day 1 of pregnancy to implant an intracerebroventricular (ICV) cannula connected to an ALZET pump for drug delivery to the brain. Animals were anesthetized using isoflurane dissolved in oxygen gas at 5% for induction and 1.5-2.5% for maintenance, and were administered the analgesic carprofen or meloxicam (5 mg/kg, SC) diluted in saline. The infusion cannula was stereotaxically targeted to the left lateral ventricle at +1.70 mm lateral and -0.50 mm posterior relative to bregma. Jeweler screws and dental cement secured the cannula to the skull, while the pump was placed subcutaneously adjacent to the thoracic spine. The incision was closed using stainless steel wound clips.



PHYSIOLOGICAL MEASURES: Animals were weighed daily. Weight data was used to track progression through pregnancy.

HISTOLOGY: At the conclusion of the experiment, animals were euthanized and their brains were collected for sectioning and staining. Brains were sectioned at 50 µm thin slices which were then cresyl violet stained. Staining allowed for the visualization of the soma of neurons in the brain tissue as a violet color. The tissue was imaged using an Olympus dissecting microscope with Toupview software. Visual inspection of photomicrographs depicting damage to the cortex allowed for determination of successful or unsuccessful entry of the tip of the cannula into the left lateral ventricle.

METHODS, CONTINUED

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 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 10 mg/kg cocaine or saline in their initially nonpreferred chamber on days 1, 3, and 5 and saline on days 2, 4, and 6 in their initially preferred chamber. After receiving their injection, animals were locked in the appropriate chamber for thirty minutes per day.

Posttest- Animals were allowed to move freely between the two chambers for 15 minutes. Their CPP score was calculated by the difference between the amount of time spent in their initially nonpreferred chamber in the posttest compared to the pretest.

RESULTS

CONDITIONED PLACE PREFERENCE (CPP) SCORES:

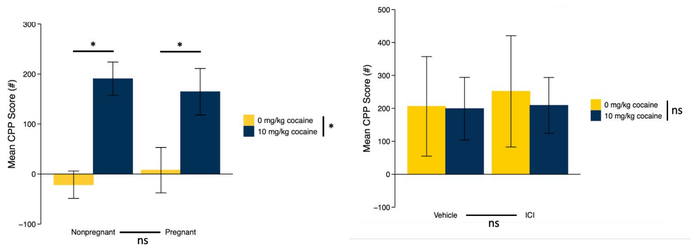


Figure 1. Mean (+/- SEM) conditioned place preference (CPP) scores for nonpregnant and pregnant animals conditioned with 0 mg/kg cocaine or 10 mg/kg cocaine. *p < 0.05 (main effect of drug treatment and simple main effect of drug treatment on CPP score at both levels of pregnancy status); ns, not significant (main effect of pregnancy status).

Figure 2. Mean (+/- SEM) conditioned place preference (CPP) scores for animals receiving hormone conditions of ICI or vehicle and conditioned with 0 mg/kg cocaine or 10 mg/kg cocaine. ns, not significant (main effects of hormone condition and drug treatment).

WEIGHT MEASURES:

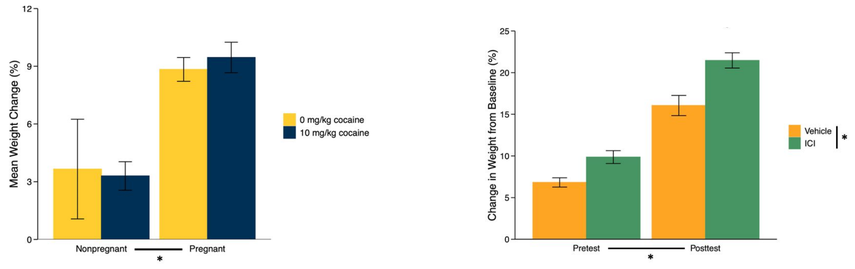


Figure 3. Mean (+/- SEM) percent weight change between the pretest and posttest for nonpregnant and pregnant animals conditioned with either 0 mg/kg cocaine or 10 mg/kg cocaine. *p < 0.05 (main effect of pregnancy status).

Figure 4. Mean (+/- SEM) percent weight change from baseline at the pretest and posttest for animals treated with vehicle or ICI. Data were collapsed across drug treatment. *p < 0.05 (main effect of time and hormone condition).

DISTANCE TRAVELED DATA:

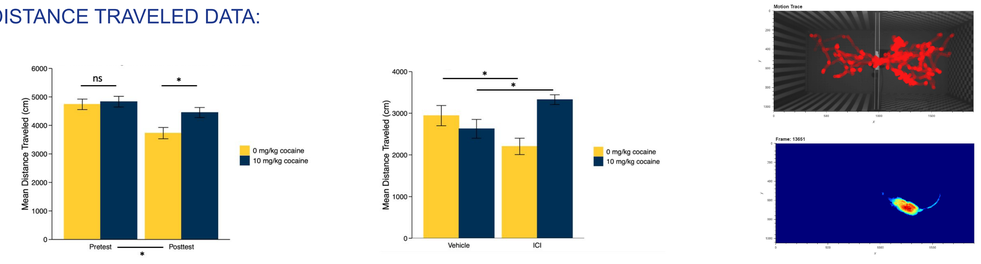
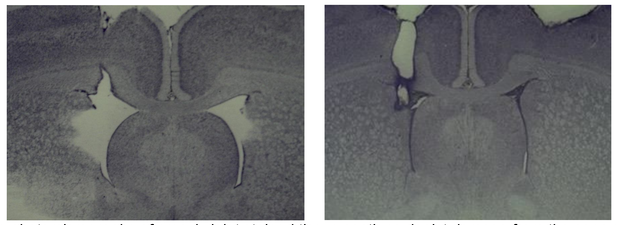


Figure 5. Mean (+/- SEM) distance traveled at the pretest and posttest for animals conditioned with 0 mg/kg cocaine or 10 mg/kg cocaine. Data were collapsed across pregnancy status. *p < 0.05 (main effect of time and simple main effect of drug treatment at posttest); ns, not significant (simple main effect of drug treatment at pretest).

Figure 6. Mean (+/- SEM) distance traveled at the posttest for animals treated with vehicle or ICI and conditioned with 0 mg/kg cocaine or 10 mg/kg cocaine. Posttest distance traveled data were analyzed through an ANCOVA with pretest distance traveled data as a covariate. *p < 0.05 (simple main effect of hormone condition on mean distance traveled at 0 mg/kg and 10 mg/kg).

RESULTS, CONTINUED

HISTOLOGY:



The photomicrographs of cresyl violet stained tissue sections depict damage from the cannula indicating successful (left) or unsuccessful (right) entry into the left lateral ventricle of the brain. These images were used to confirm that delivery of ICI or vehicle was injected into the correct location dictated by the stereotaxic apparatus. Brain sections from two animals showed unsuccessful entry. If the position of tip of the cannula was determined to be outside of the ventricle, suggesting that the animal did not receive the drug in the target location, data collected for the animal was excluded from the experiment.

SUMMARY AND DISCUSSION

- Pregnant animals did form a CPP for cocaine that was comparable to nonpregnant animals
- Estradiol’s impact on motivation for cocaine in pregnant animals could not be definitively tested given that vehicle animals did not display the expected CPP for cocaine
- Pregnant animals demonstrated an expected increase in mean body weight (Stramek et al., 2019)
- Animals treated with ICI compared to vehicle had a higher change in weight which suggests how estradiol may act in the brain to suppress appetite (Geary & Asarian, 2001)
- Animals treated with saline only traveled less during the posttest compared to the pretest due to a possible decrease in exploratory behavior after repeated exposure to the apparatus (Martinez et al., 2019)
- Saline only animals treated with ICI compared to vehicle traveled more which highlights estradiol’s role in increasing locomotion (Espinosa & Curtis, 2018)

FUTURE DIRECTIONS

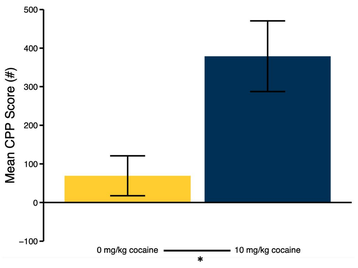


Figure 7. Mean (+/- SEM) conditioned place preference (CPP) scores for vehicle, pregnant animals conditioned with 0 mg/kg cocaine or 10 mg/kg cocaine. *p < 0.05 (main effect of drug treatment on CPP score).

- Expand sample size of pregnant animals that are pair-housed and scored using a secondary pretest
  - Account for inconsistent side preferences
  - Minimize the possible stressor of isolation

ACKNOWLEDGEMENTS

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