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Sex Influences Behaviors and Neurochemistry of the Neonatal Clomipramine Model of Obsessive-Compulsive Disorder (OCD)

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INTRODUCTION

- *Obsessive-compulsive disorder (OCD)*
  - Affects 2-3% of the U.S. population and is characterized by intrusive thoughts leading to repetitive behaviors[1]
  - Current treatment, serotonin reuptake inhibitors (SRIs), are ineffective for 40-60% of patients[2]
- *Animal models in psychiatric research*
  - Easier to study than humans in a lab setting
  - Evaluated by face and construct validities[3]
- *Neonatal clomipramine (neoCLOM) animal model of OCD*[4]
  - Disrupts hypothalamic-pituitary-adrenal (HPA) axis during critical period[5]
  - Generates permanent OCD-like behaviors in rats
- *Brain structures and neurotransmitters*
  - Orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) are involved in behavioral expression[6]
  - The OFC and ACC are targets of the cortico-basal ganglia-thalamic circuit – a circuit shown to be abnormal in OCD[7]
  - Dysfunctional neurotransmission of dopamine (DA) and norepinephrine (NE) in this circuit underlies psychological and neurological disorders
- *Behavioral and neurochemical assessment*
  - Elevated-plus maze (EPM)[8]
  - OFC and ACC tissue samples analyzed for neurotransmitters using high-performance liquid chromatography after behavioral trials

Our objective was to evaluate the face and construct validities of the neoCLOM model of OCD by assessing behaviors in the Elevated Plus Maze and neurochemical levels of dopamine and norepinephrine in the Orbitofrontal and Anterior Cingulate cortices of male and female rats.

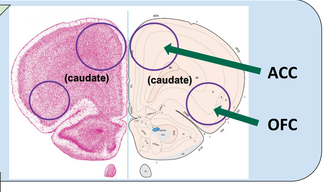
METHODS

Neonatal Treatment
Subjects were Sprague Dawley rats. On Days 9-16, rat pups received intraperitoneal injections of either 15 mg/kg clomipramine (neoCLOM) or 0.9% saline vehicle (neoSAL), delivered as 1 mL/kg.

	Male	Female
neoSAL	19	17
neoCLOM	17	19

Behavioral Trials
5 minute trials were conducted during adulthood (Days 83-92). Open and closed arm entries and time spent in arms were recorded. An arm entry was scored when a rat placed three of its paws inside the arm.

Tissue Extraction
Following rapid decapitation (Days 87-93), punches were obtained at the 12.20 mm interaural coordinate of both OFC (4.0 mm ML; 5.6 mm DV) and ACC (1.0 mm ML; 2.6 mm DV)[9]. ACC and OFC samples were homogenized and centrifuged and then supernatants extracted.

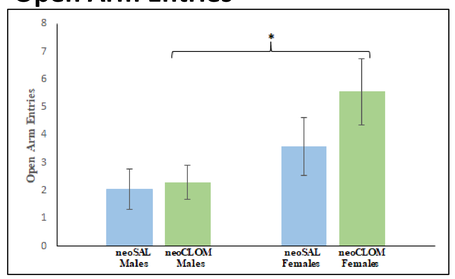


HPLC analysis
Supernatants were analyzed for NE and DA levels. Peak areas were calculated by Eicom software.

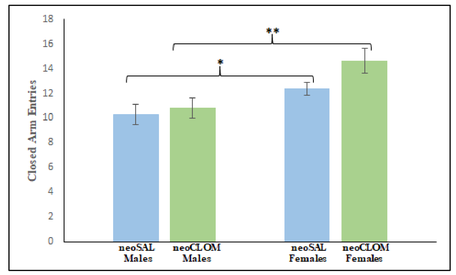
Data Analysis
SPSS was used for 2-Factor ANOVA analysis of data. Student's two-tailed t-tests were used for pairwise comparisons (* indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Data are expressed as mean ± 1 standard error of the mean (SEM). Outliers (± 2.5 SD) were removed.

RESULTS

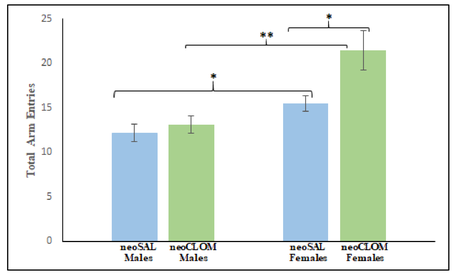
Open Arm Entries



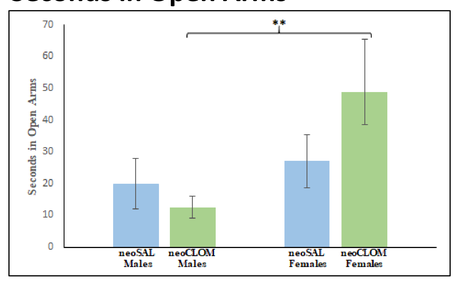
Closed Arm Entries



Total Arm Entries



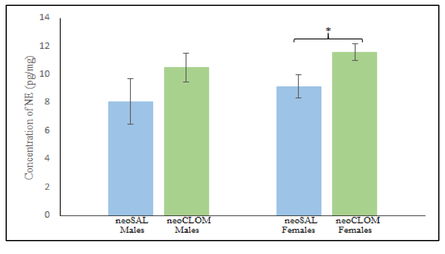
Seconds in Open Arms



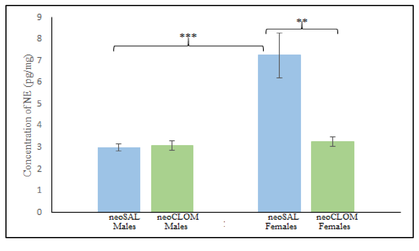
➢ **Neonatal treatment differences**
Total arm entries: neoCLOM females > neoSAL females

➢ **Sex differences**
Open arm entries: neoCLOM females > neoCLOM males
Closed arm entries: female groups > male groups
Total arm entries: female groups > male groups
Seconds in open arms: neoCLOM females > neoCLOM males

Norepinephrine in the OFC



Norepinephrine in the ACC

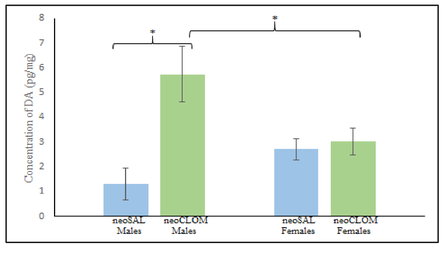


➢ **Norepinephrine in OFC**
neoCLOM females > neoSAL females

➢ **Norepinephrine in ACC**
neoCLOM females < neoSAL females
neoSAL females > neoSAL males

➢ **Dopamine in OFC**
neoCLOM males > neoSAL males
neoCLOM females < neoCLOM males

Dopamine in the OFC



CONCLUSIONS

➢ Behaviors in the Elevated Plus Maze do not support the face validity of the neoCLOM model.

➢ Female groups had higher levels of locomotion than male groups.

➢ Although the EPM has been used as a reliable assay for anxiety in male rats, results suggest that it may not be appropriate for assessing anxiety in female rats.

➢ Among females, increased NE levels in the Orbitofrontal Cortex and decreased levels in the Anterior Cingulate Cortex were associated with hyperlocomotion in the EPM.

Promise for the construct validity of the neoCLOM model is suggested by the increased levels of orbitofrontal cortical NE in females and of DA in males

NEXT STEPS

➢ Evaluating predictive validity of the neoCLOM model using known pharmaceutical treatments of OCD
➢ Evaluate alternative behavioral assays for female rats
➢ Investigate other brain structures associated with OCD
➢ Further evaluation of female models (effect of estrous cycle)

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