DREADD-ing Stress: Using Chemogenetics to Bypass Variability and Go Right to the Source with CRF Activation

Kristen R. Montgomery, Morgan S. Bridi, Lillian M. Folts, Ruth Marx-Rattner, Hannah C. Zierden, and Tracy L. Bale

Neuroscience Graduate Program, University of Colorado Anschutz Medical Campus, Aurora, CO
Department of Psychiatry, University of Colorado Anschutz Medical Campus, Aurora, CO

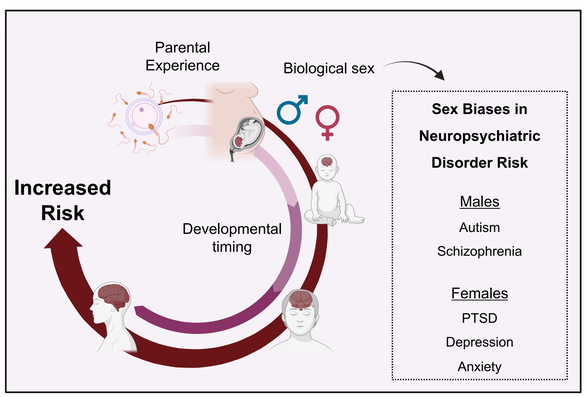
University of Colorado Anschutz Medical Campus

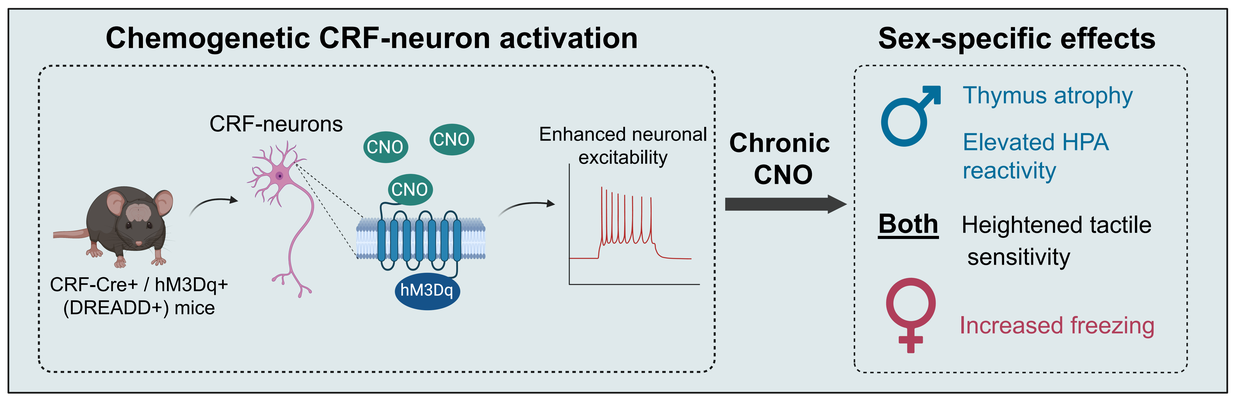
Neuroscience Graduate Program

Summary

Chronic stress across the lifespan is one of the strongest predictors of developing neuropsychiatric disorders. Risk is compounded by additional biological and environmental factors, including sex.

Existing stress models vary widely across research groups in stressor choice and exposure timing. Current paradigms use sensory stressors and require perception and experience of the stress. We hypothesized that repeated chemogenetic activation of CRF-neurons would mimic the physiological effects of chronic stress while bypassing the variability of experience and perception. We found that chronic CRF activation has sex-specific outcomes with males having increased sensitivity to the physiological effects and females showing greater limbic disruption.





Conclusions and Future Directions

- *Outcomes resulting from chronic stress are not solely due to increased glucocorticoid exposure.*
- *CRF-neurons in the PVN and CeA may be differentially sensitive to both chronic activation and sex, which could underlie the sex-biased presentation of many stress-related neuropsychiatric disorders.*
- *Future studies will examine the mechanisms underlying the sex-specificity and their developmental origins.*

Methods

Animals: Homozygous CRF-Cre (Jax, stock #012704) mice were bred with heterozygous hM3Dq-DREADD (Jax, stock #026220) mice to generate CRF-Cre+/hM3Dq- (DREADD-) or CRF-Cre+/hM3Dq+ (DREADD+) offspring. Adult (10-18 week) animals were used for all studies. Mice were singly housed for the duration of experiments. **Clozapine-N-oxide (CNO):** CNO (HelloBio) was prepared at 1 mM in 0.9% normal saline and administered via intraperitoneal injection or cookie dough treat. **Hypothalamic-pituitary-adrenal (HPA) axis assessment**: Tail blood was collected from a small nick at the end of the tail at the specified timepoints. Plasma corticosterone levels were measured by radioimmunoassay (MP Biomedicals). **Immunohistochemistry:** Animals were transcardially perfused with ice-cold phosphate buffered saline followed by ice-cold 4% paraformaldehyde. Brains were stored in 4% paraformaldehyde for 24 hours then transferred to 30% sucrose for 24 hours at 4C. Brains were frozen on dry-ice and stored at -80C. 40 um sections were incubated overnight with anti-c-Fos (1:2500, Synaptic Systems) followed by goat-anti-guinea pig-Alexa Fluor 588 (1:200, Invitrogen) for 1 hour. **Imaging:** Imaging was performed using an ECHO-Revolve fluorescent microscope at 10X magnification. Images were analyzed using FIJI. **Von Frey Filament Test:** Following a 10-minute habituation, mice were placed on a suspended wire mesh and monofilaments of increasing diameter with forces ranging from 0.008 to 11.0 g (NC Medical) were pressed against the hind paw skin. Responses were recorded until the foot was withdrawn for 5 consecutive trials. **Fear Conditioning:** On day 1, mice were habituated to a chamber with anon-restrictive acrylic cylinder in a sound-attenuated box (context A, SR-LAB-Startle Response System, San Diego Instruments) for 10 minutes followed by the chamber in context B (original chamber modified with blue light covers, a black and white checkered pattern covering all walls, and 5% vinegar to clean surfaces) for 10 minutes. On day 2, the animals were placed in context A for 5 minutes and a 30 second baseline was collected with a 65 dB tone. The conditioned stimulus (CS; tone: 80 dB) was presented for 30 seconds followed by the CS paired with an unconditioned stimulus (US) of a 1s 0.6 mA foot shock 3 times. Fear memory extinction was measured on days 3-7. Mice were placed in context B and following a 5-minute acclimation period, a 30s baseline with the background tone was collected. The CS tone was presented for 30 seconds and the baseline–CS tone presentation was repeated for 15 trials with 30s intertrial intervals. Movement was measured using a piezoelectric accelerometer and recorded with SR-Lab software.

Figures were made with BioRender.com and GraphPad Prism. Statistical analysis was performed with GraphPad Prism.

National Institute of Mental Health

Eunice Kennedy Shriver National Institute of Child Health and Human Development

These studies were supported by NIH HD097093 and MH108286 to TLB.

Results

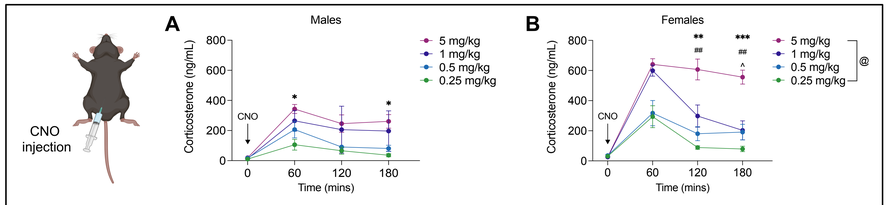


Figure 1. Female DREADD+ mice have a higher corticosterone response to CNO than males. A) Male plasma corticosterone levels and **B)** female plasma corticosterone levels following CNO injection at 4 different doses. ((n=3-5); ***p<0.001, **p<0.01, ##p<0.01, *p<0.05, ^p<0.05, main effect of time **(A,B)**, @main effect of CNO dose).

Figure 2. Male DREADD+ mice display physiological features of a chronic stress state following 5 weeks of CNO. A) Male DREADD+ mice lost body weight across 5 weeks of CNO and **B)** had decreased thymus weights compared to controls. **C)** Female DREADD+ mice did not have changes in body weight or **D)** changes in thymus weight compared to controls (n=3-7; *p<0.05, main effect of time **(A,C)**).

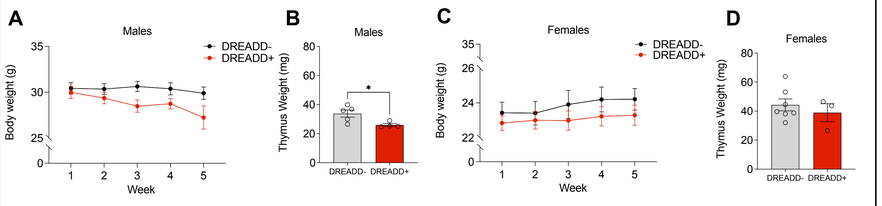
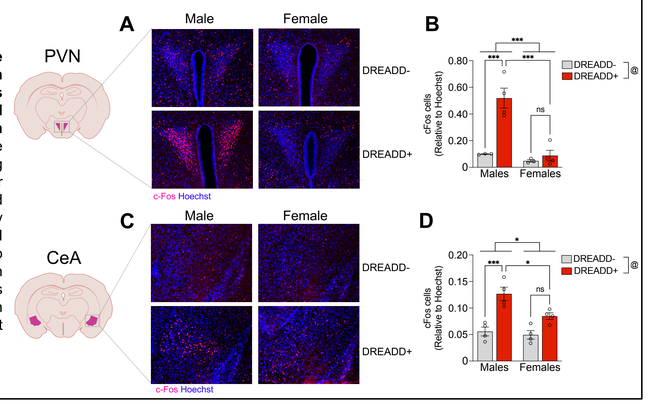


Figure 3. DREADD+ males have longer CRF-neuron activation in the paraventricular nucleus of the hypothalamus (PVN) and central amygdala (CeA) than females. A,C) Representative images of c-Fos immunostaining in the PVN and CeA 3 hours after CNO. **B,D)** DREADD+ males had increased c-Fos immunoreactivity compared to controls in the PVN and CeA while there was no significant difference between DREADD+ females and controls (n=3-4; ***p<0.001, *p<0.05, main effect of sex **(B,D)**, @main effect of genotype).



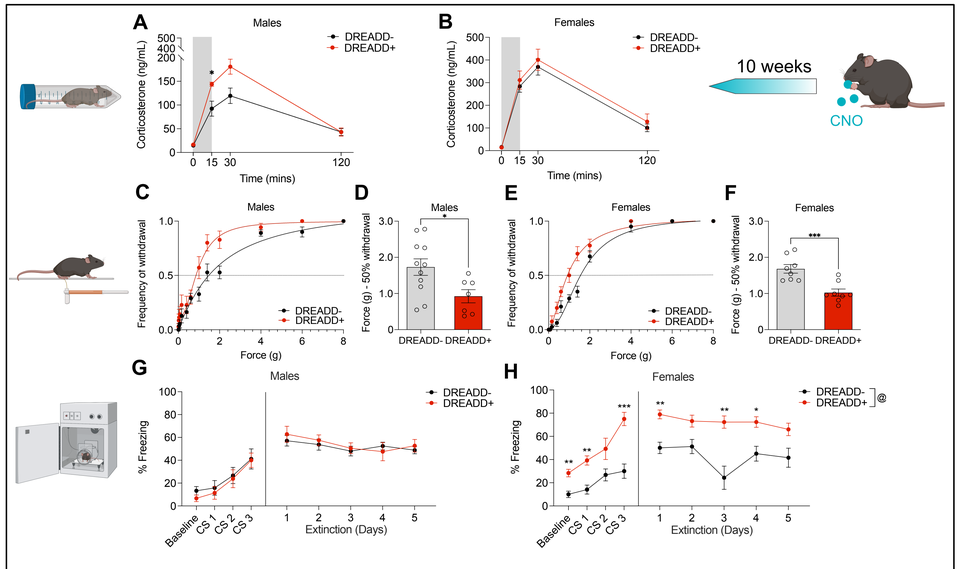


Figure 4. Male DREADD+ mice have elevated HPA responses and female DREADD+ mice have heightened fear responses following chronic CNO. A) DREADD+ males had a heightened HPA response to acute restraint following chronic CNO, while **B)** DREADD+ females had no change in their HPA response compared to controls. **C,E)** Both male and female DREADD+ mice displayed increased tactile sensitivity and **D,F)** required lower force for 50% paw withdrawal compared to controls following chronic CNO. **G)** Freezing behavior in DREADD+ males was not different compared to controls, while **H)** DREADD+ females had significantly higher freezing compared to controls. (n=7-11; ***p<0.001, **p<0.01, *p<0.05, main effect of time **(A, B, G, H),** @main effect of genotype).

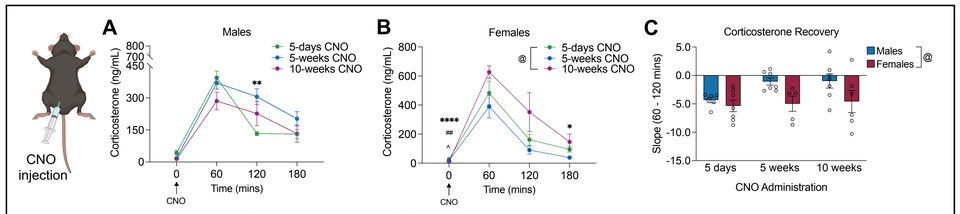


Figure 5. Chronic CNO impairs HPA recovery in DREADD+ males. A,C) DREADD+ males had delayed HPA recovery to an acute CNO injection following 5- or 10-weeks of chronic CNO. **B,C)** DREADD+ females had no change in their HPA recovery rates following chronic CNO. (n=6-7; ****p<0.0001, **p<0.01, ##p<0.01, *p<0.05, ^p<0.05, main effect of time **(A,B)**, @main effect of CNO chronicity **(B)** or main effect of sex **(C)**).