The Effect of Chemogenetic DREADD Manipulation in the Ventral Dentate Gyrus of Chronically Stressed Females on the Behavioral Antidepressant Response

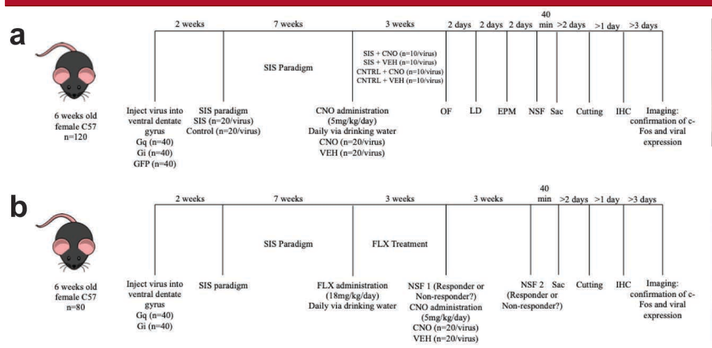
Allyson Bazer, Christine Yohn, and Dr. Benjamin Samuels
Department of Psychology, Rutgers University

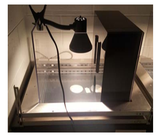
RUTGERS
Aresty Research Center for Undergraduates

Background

- The most widely prescribed class of antidepressants are selective serotonin reuptake inhibitors (SSRIs), which increase serotonin, a mood-regulating monoamine neurotransmitter, by blocking reuptake. One type of SSRI is fluoxetine (FLX), also known as Prozac. Despite the popularity of SSRIs, approximately out of 3 patients do not remit after treatment (Rush et. al., 2006).
- Mice that retain a long latency to eat in the NSF after chronic FLX treatment are considered non-responders, whereas mice with shorter latencies after chronic FLX treatment are considered responders. Therefore, NSF permits the grouping of FLX-treated animals into responders and non-responders to SSRI treatment (Samuels & Hen, 2011).
- Inhibition of the ventral dentate gyrus is important for the behavioral response to antidepressant treatment (Bagot et. al., 2015; Kheirbek et. al., 2013). Deletion of 5-HT_{1A} receptors (Gi-coupled heteroreceptors) from granule cells (GCs) in the DG abolishes the behavioral effects of SSRIs (Samuels et. al., 2015).
- Unpublished results from Samuels lab observed that responders to FLX have less DG cFos expression than non-responders to FLX and stress controls after completion of the NSF. Given that cFos is an indicator of neuronal activity within an area, this data suggests that response to FLX is related to a decrease in activation of the DG GCs.
- DREADDs are a chemogenetic method of cell manipulation used to influence levels of cell activity. Different DREADDs increase Gq- or Gi-mediated signaling to alter membrane potential, either increasing (Gq) or decreasing (Gi) the probability that an action potential will occur (Roth, 2016).
- Our current hypothesis is that Gi-DREADD-mediated inhibition of ventral DG GCs will mimic an antidepressant response, while Gq-DREADD-mediated activation of ventral DG GCs will induce anxiogenic behavioral responses. We hypothesize that Gi-DREADD-mediated inhibition of DG GCs can convert female FLX non-responders into responders. We hypothesize that Gq-DREADD-mediated stimulation of DG GCs can convert female FLX responders into non-responders.

Methods

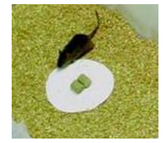




Light Dark (LD)

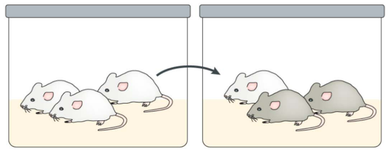
Elevated Plus Maze (EPM)

Open Field (OF)



Novelty Suppressed Feeding (NSF)

Figure 1. (a) Timeline of Gi-DREADD and Gq-DREADD-mediated manipulation in SIS (b) Timeline of Treatment Reversal for Responders and Non-Responders



Many pre-clinical models exclude the use of female subjects, despite the fact that nearly twice the number of women suffer from MDD than men. This exclusion is historically due to antiquated ideas of behavioral variations from the hormonal fluctuations of the estrous cycle (Shansky, 2019). Social instability stress (SIS) is a chronic stress paradigm, effective in both males and females. SIS involves frequent changes in the housing environment, in which the introduction of novel cage mates repeatedly over the course of several weeks creates a social imbalance.

Results: Confirmation of DREADD Activation

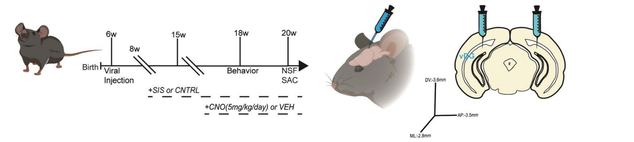


Figure 2. Mice were bilaterally injected with either AAV8-CamKIIa-hm4D(Gi)-mCherry, AAV8-CamKIIa-hm3D(Gq)-mCherry, or AAV8-CamKIIa-EGFP in the ventral DG: 3.5mm, +/-2.8mm relative to the bregma line and midline respectively at a depth of 3.6 mm from the skull.

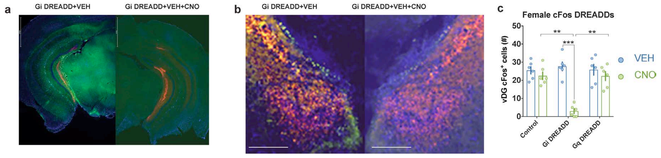


Figure 3. (a) Confirmation of CNO activation of the DREADD at 4x magnification. (b) Confirmation of CNO activation of the DREADD at 20x magnification. (c) The number of vDG cFos+ cells in Gi-DREADD+CNO mice is significantly lower than Gi-DREADD+VEH, Gq-DREADD+CNO, and GFP+CNO mice.

Results: Ventral DG DREADD-mediated Regulation of Behavior

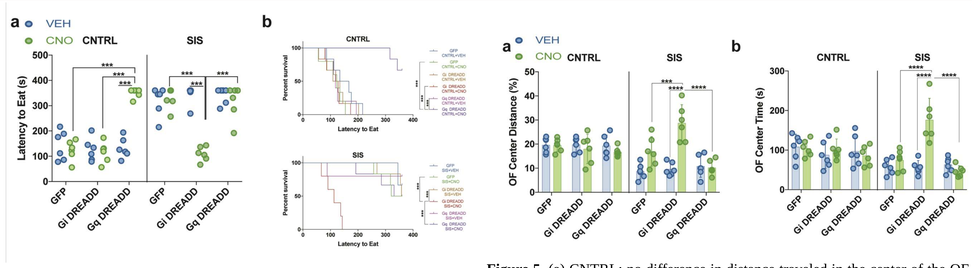


Figure 4. (a&b) CNTRL: Gq-DREADD+CNO mice had a significantly longer latency to eat than Gq-DREADD+VEH, Gi-DREADD+CNO, and GFP+CNO mice. SIS: Gi-DREADD+CNO mice had a significantly shorter latency to eat than Gi-DREADD+VEH, GFP+CNO, and Gq-DREADD+CNO mice.

Figure 5. (a) CNTRL: no difference in distance traveled in the center of the OF. SIS: Gi-DREADD+CNO mice traveled more in the center of the OF than Gi-DREADD+VEH, Gq-DREADD+CNO, and GFP+CNO mice. (b) CNTRL: there was no difference in time spent in the center of the OF. SIS: Gi-DREADD+CNO mice spent more time in the center of the OF than Gi-DREADD+VEH, Gq-DREADD+CNO, and GFP+CNO mice.

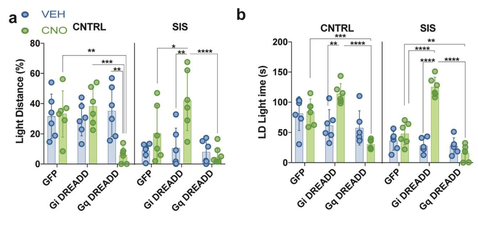


Figure 6. (a) CNTRL: Gq DREADD+CNO mice traveled less in the light than Gi DREADD+CNO, GFP+CNO, and Gq DREADD+VEH mice. SIS: Gi-DREADD+CNO mice traveled more in the light than Gi-DREADD+VEH, GFP+CNO, and Gq-DREADD+CNO. (b) CNTRL: Gq-DREADD+CNO mice spent less time in the light than Gi-DREADD+CNO, GFP+CNO, and Gq DREADD+VEH mice; Gi-DREADD+CNO mice spent more time in the light than Gi-DREADD+VEH. SIS: Gi-DREADD+CNO mice spent more time in the light than Gi-DREADD+VEH, GFP+CNO, and Gq-DREADD+CNO; Gq-DREADD+CNO mice spent less time in the light than GFP+CNO mice.

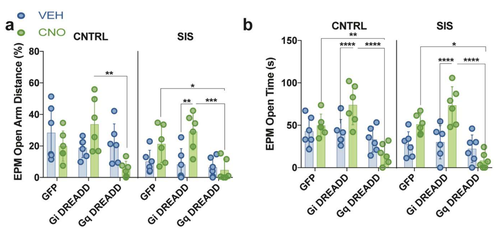


Figure 7. (a) CNTRL: Gi-DREADD+CNO mice traveled more in the open arms than Gq-DREADD+CNO mice. SIS: Gi-DREADD+CNO mice traveled more in the open arms than Gi-DREADD+VEH and Gq-DREADD+CNO mice; Gq-DREADD+CNO mice traveled less in the open arms than GFP+CNO mice. (b) CNTRL: Gi-DREADD+CNO mice spent more time in the open arms than Gi-DREADD+VEH and Gq-DREADD+CNO mice; Gq-DREADD+CNO mice spend less time in the open arms than GFP+CNO mice. SIS: Gi-DREADD+CNO mice spent more time in the open arms than Gi-DREADD+VEH and Gq-DREADD+CNO mice; Gq-DREADD+CNO mice spent less time in the open arms than GFP+CNO mice.

Results: Treatment Reversal

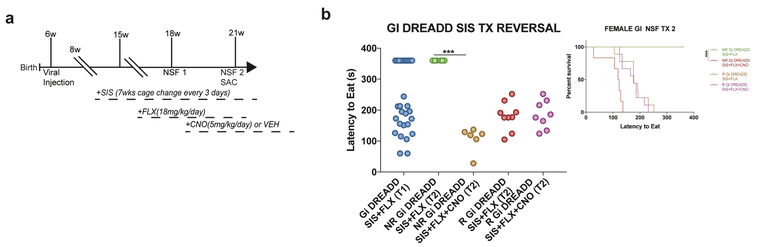


Figure 8. (a) Experimental timeline for viral surgeries, SIS paradigm, FLX administration, CNO administration, and NSF. (b) NR-SIS+FLX+CNO mice had a significantly shorter latency to eat than NR-SIS+FLX+VEH mice.

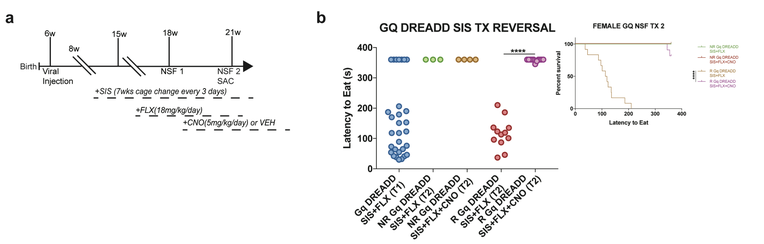


Figure 9. (a) Experimental timeline for viral surgeries, SIS paradigm, FLX administration, CNO administration, and NSF. (b) R-SIS+FLX+CNO mice had a significantly longer latency to eat than R-SIS+FLX+VEH mice

Discussion

- Behavioral response to FLX is associated with a decrease in DG GC activity, with chronic inhibition of the vDG via DREADDs mounting antidepressant-like behavioral responses in both stress and non-stress backgrounds
- Chronic stimulation of the vDG via DREADDs mounts an anxiogenic response in non-stress backgrounds and a minimal anxiogenic response in stress backgrounds
- Behavioral non-responders to FLX can be converted into behavioral responders following chronic inhibition of the vDG via DREADDs
- Behavioral FLX responders are converted into non-responders following chronic chemogenic stimulation of the vDG
- This study, along with unpublished data from Samuels lab investigating DREADD-mediated inhibition and stimulation in the vDG of males stressed by chronic corticosterone (a stress hormone) administration suggests that DREADD-mediated modulation of the DG is independent of stressor
- Due to the complex etiology of MDD, further investigation of treatment resistant depression and independence from the type of stressor, can help novel research focus on differences in physiology that lead to differences in response to antidepressant treatment
- It is apparent that more research into the neural circuitry that underlies the behavioral response to antidepressant treatment is necessary in order to develop novel pharmacotherapies; however, our results demonstrate that the vDG plays an essential role in this response

References

Bagot, R. C., Parise, E. M., Pena, C. J., Zhang, H. X., Maze, I., Chaudhury, D., . . . Nestler, E. J. (2015). Corrigendum: Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nat Commun*, *6*, 7626. doi:10.1038/ncomms8626

Kheirbek, M. A., Drew, L. J., Burghardt, N. S., Costantini, D. O., Tannenholz, L., Ahmari, S. E., ... & Hen, R. (2013). Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron*, *77*(5), 955-968.

Roth, B. L. (2016). DREADDs for neuroscientists. *Neuron*, *89*(4), 683-694.

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., ... & McGrath, P. J. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR* D report. *American Journal of Psychiatry*, *163*(11), 1905-1917.

Samuels, B. A., & Hen, R. (2011). Novelty-suppressed feeding in the mouse. In *Mood and anxiety related phenotypes in mice* (pp. 107-121). Humana Press.

Samuels, B. A., Anacker, C., Hu, A., Levinstein, M. R., Pickenhagen, A., Tsetsenis, T., ... & Gross, C.T. (2015). 5-HT1A receptors on mature dentate gyrus granule cell are critical for the antidepressant response. *Nature Neuroscience*, *18*(11), 1606-1616. doi:10.1038/nn.4116

Shansky, R. M. (2019). Are hormones a "female problem" for animal research?. *Science*, *364*(6443), 825-826.

Acknowledgements

I would like to thank my faculty mentor, Dr. Benjamin Samuels, and my graduate mentor, Christine Yohn, for their guidance and support. I would like to extend my gratitude to the undergraduate research assistants in Dr. Samuels's lab for their help, especially Sandra Ashamalla and Debbie Ma.

RUTGERS