Supplementary Material for Johnson et al. (2021) Assessment of Mapping the Brain, a Novel Research and Neurotechnology Based Approach for the Modern Neuroscience Classroom. J Undergrad Neurosci Educ 19(2):A226-A259.

Lab Protocol 4

Analyzing the behavioral effects of stimulating LC-*En1* neurons *in vivo*

Background

Prior to tissue sample collection, your animals were treated with either vehicle or CNO (1 mg/kg) and tested in three anxiety-related behavioral paradigms: the **elevated plus maze test**, the **open field test**, and the **light dark test**. In addition, littermate controls that do not express the DREADD receptor were also tested. Our collaborators at the National Institute of Environmental Health Sciences (NIEHS) performed these experiments. Provided below are tables containing the raw data from these behavioral tests. ***It is important to note that the data is unpublished, and therefore you are not permitted to share this data outside of this class***!

1. Graph the data below using excel. **Create only 1 graph for each behavioral paradigm (note there are 2 tables for each paradigm-three graphs total).** Think carefully about the best way to graph the data to analyze the behavioral consequences of stimulating LC-*En1* neurons *in vivo*. You will have to calculate the **average and standard error** for each group and plot on the same graph.

2. Use a t-test to compare groups. You should compare the DREADD expressing animals treated with CNO versus vehicle. You should also use a t-test to compare the CNO treatment in DREADD expressing and non-DREADD expressing animals. Are there any other important groups to compare with a t-test?

Elevated Plus Maze

VEHICLE			
En1^{cre};Dbh^{Flpo};RC::hM3Dq	**# of open arm entries**	***En1^{cre};RC::hM3Dq***	**# of open arm entries**
C001	3	D001	4
C002	3	D002	5
C003	1	D003	7
C004	3	D004	2
C005	4	D005	2
C006	3	D006	0
C007	4	D007	3
C008	2	D008	2
C009	5	D009	6
C010	10	D010	7
C011	3	D011	3
C012	10	D012	2
C013		D013	4
C014	0	D014	0
C015	4	D015	3
C016	2	D016	2
C017	1	D017	2
C018	3	D018	3
C019	3	D019	1
C020	1	D020	
C021		D021	

CNO 1mg/kg			
En1^{cre};Dbh^{Flpo};RC::hM3Dq	**# of open arm entries**	***En1^{cre};RC::hM3Dq***	**# of open arm entries**
C001	0	D001	10
C002	0	D002	4
C003	1	D003	2
C004	2	D004	5
C005	2	D005	2
C006	1	D006	2
C007	1	D007	8
C008	1	D008	4
C009	5	D009	6
C010	3	D010	7
C011	4	D011	3
C012	8	D012	5
C013	4	D013	2
C014	1	D014	4
C015	3	D015	
C016	0	D016	3
C017	1	D017	2
C018	1	D018	0
C019	2	D019	1
C020	2	D020	6
C021		D021	2

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Light Dark Box

VEHICLE			
En1^{cre};Dbh^{Flpo};RC::hM3Dq	**light side ambulation time**	***En1^{cre};RC::hM3Dq***	**light side ambulation time**
C001	3.55	D001	7.7
C002	8.05	D002	1.75
C003	3.25	D003	3.05
C004	3.45	D004	8.75
C005	4.1	D005	3.4
C006	12.4	D006	3.5
C007	6.25	D007	1.6
C008	6.35	D008	2.75
C009	3	D009	1.7
C010	10.35	D010	0
C011	2.15	D011	8.35
C012	3.45	D012	0
C013	4.85	D013	9.55
C014	0.1	D014	0.85
C015	7.25	D015	3.45
C016	10	D016	4
C017	1.65	D017	8.5
C018	0.5	D018	3.45
C019	2.6	D019	
C020	0.25	D020	
C021		D021	

CNO 1mg/kg			
En1^{cre};Dbh^{Flpo};RC::hM3Dq	**light side ambulation time**	***En1^{cre};RC::hM3Dq***	**light side ambulation time**
C001	2	D001	3.55
C002	2.1	D002	3.9
C003	0	D003	12.3
C004	6.6	D004	2.8
C005	2.3	D005	0
C006	0.7	D006	4.7
C007	5.4	D007	15.65
C008	2.3	D008	2.35
C009	0.6	D009	6.45
C010	5.25	D010	6.2
C011	4.3	D011	2.95
C012	1.9	D012	1.15
C013	5.75	D013	4.65
C014	5.8	D014	1.65
C015	6.55	D015	10.55
C016	2	D016	6.9
C017	0.05	D017	5.65
C018	3	D018	0
C019	5.05	D019	3.85
C020	2.9	D020	
C021		D021	

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Open Field

VEHICLE			
En1^{cre};Dbh^{Flpo};RC::hM3Dq	**time in center (s)**	***En1^{cre};RC::hM3Dq***	**time in center (s)**
C001	84.25	D001	106.35
C002	108.55	D002	39.65
C003	30.3	D003	61.75
C004	62.15	D004	61.35
C005	69.95	D005	68.05
C006	110.95	D006	129.5
C007	134.35	D007	328.9
C008	106.45	D008	132.15
C009	138.8	D009	165
C010	171.4	D010	97.45
C011	81.4	D011	131.1
C012	179.2	D012	83.55
C013	118.2	D013	117.55
C014	106.4	D014	180.95
C015	113.5	D015	222.9
C016	172	D016	169.15
C017	121.65	D017	109.9
C018	74.15	D018	161.85
C019		D019	153.65
C020		D020	121.9
C021		D021	

CNO 1mg/kg			
En1^{cre};Dbh^{Flpo};RC::hM3Dq	**time in center (s)**	***En1^{cre};RC::hM3Dq***	**time in center (s)**
C001	39.65	D001	78.25
C002	61.6	D002	144
C003	83.05	D003	99.5
C004	88.1	D004	33.35
C005	53.55	D005	48.45
C006	54.15	D006	116
C007	56.35	D007	162.55
C008	117.05	D008	107.9
C009	76	D009	209.05
C010	70.7	D010	137.25
C011	130.95	D011	147.75
C012	49.4	D012	60.55
C013	144.5	D013	93.85
C014	115.15	D014	58.25
C015	132.25	D015	133.45
C016	68.5	D016	157.65
C017	85.15	D017	78.45
C018	83.1	D018	73.2
C019		D019	83.35
C020		D020	111.8
C021		D021	

Discussion Questions

1. Why do you think these 3 anxiety-related behavioral paradigms were chosen? What other behavioral paradigms might be interesting to explore? (*hint*: consider the behavioral and physiological processes that NE neurons are known to be involved in)

2. Do you think stimulation of mCherry-hM3Dq in our animals is impacting behavior in these 3 paradigms? Why or why not? Are more experiments needed?