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Regulation of autism-relevant behaviors by cerebellar–prefrontal cortical circuits

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Supplemental Table S1 (Statistics for Figures)**Test/Experiment****Gi mpfc rescue PC Tsc1 mice**Social Approach: Social Index (Figure 1d)
Mixed-effects model (REML)

n= GFP (VEH, CNO): 27; Gi (VEH, CNO): 30

Fixed effects (type III)

Treatment

Group

Treatment x Group

P value

F (DFn, DFd)

F (1, 107) = 10.51

F (1, 107) = 14.93

F (1, 107) = 2.634

Sidak's multiple comparisons test

Predicted (LS) mean diff.

95.00% CI of diff.

Significant?

Summary Adjusted P Value

VEH - CNO

GFP

Gi

-0.05554

-0.1660 to 0.05490

No

ns

0.4473

-0.1669

-0.2766 to -0.05722

Yes

**

0.0016

GFP - Gi

VEH

CNO

-0.07688

-0.1884 to 0.03463

No

ns

0.227

-0.1883

-0.2969 to -0.07965

Yes

0.0003

Social Novelty: Social Index (Figure 1e)

Mixed-effects model (REML)

n= GFP (VEH, CNO): 27; Gi (VEH, CNO): 30

Fixed effects (type III)

Treatment

Group

Treatment x Group

P value

F (DFn, DFd)

F (1, 106) = 3.979

F (1, 106) = 4.704

F (1, 106) = 3.334

Sidak's multiple comparisons test

Predicted (LS) mean diff.

95.00% CI of diff.

Significant?

Summary Adjusted P Value

VEH - CNO

GFP

Gi

-0.006671

-0.1345 to 0.1212

No

ns

0.9912

-0.151

-0.2768 to -0.02525

Yes

*

0.015

GFP - Gi

VEH

CNO

-0.01355

-0.1414 to 0.1143

No

ns

0.9641

-0.1579

-0.2837 to -0.03214

Yes

*

0.0105

Social Olfaction (Figure 1f)

Two-way ANOVA

n=16 per group

ANOVA table

Interaction

Scent

Group

P value

F (DFn, DFd)

P<0.0001

F (42, 900) = 3.057

P<0.0001

F (14, 900) = 27.03

P<0.0001

F (3, 900) = 21.95

Sidak Post Test

Social 1

Gi VEH vs. Gi CNO

Gi VEH vs. GFP VEH

Gi VEH vs. GFP CNO

Gi CNO vs. GFP VEH

Gi CNO vs. GFP CNO

GFP VEH vs. GFP CNO

P value

<0.0001

<0.0001

0.9391

0.0179

<0.0001

<0.0001

0.205

Social 2

Gi VEH vs. Gi CNO

Gi VEH vs. GFP VEH

Gi VEH vs. GFP CNO

Gi CNO vs. GFP VEH

Gi CNO vs. GFP CNO

GFP VEH vs. GFP CNO

<0.0001

0.9926

0.7672

<0.0001

<0.0001

0.9867

Grooming (Figure 1g)

Two-way ANOVA

n=21 per group

ANOVA table

Interaction

Treatment

Group

P value

F (DFn, DFd)

P=0.0978

F (1, 80) = 2.806

P=0.0003

F (1, 80) = 14.06

P=0.0023

F (1, 80) = 9.893

Sidak Post Test

VEH :GFP vs. VEH :Gi

VEH :GFP vs. CNO:GFP

VEH :GFP vs. CNO:Gi

VEH :Gi vs. CNO:GFP

VEH :Gi vs. CNO:Gi

CNO:GFP vs. CNO:Gi

0.884

0.6131

<0.0001

0.9987

0.0015

0.0061

Water Maze (Figure 1h)

Two-way ANOVA

n=GFP (VEH, CNO): 12; Gi (VEH, CNO): 10

ANOVA table

Interaction

Trial day

Group

P value

F (DFn, DFd)

P=0.1842

F (15, 261) = 1.329

P<0.0001

F (5, 261) = 59.95

P=0.2720

F (3, 261) = 1.309

Sidak post test

Trial 1

Gi VEH vs. Gi CNO

Gi VEH vs. GFP VEH

Gi VEH vs. GFP CNO

Gi CNO vs. GFP VEH

Gi CNO vs. GFP CNO

GFP VEH vs. GFP CNO

0.9472

>0.9999

0.9995

0.8821

0.7755

>0.9999

Trial 2

Gi VEH vs. Gi CNO

Gi VEH vs. GFP VEH

Gi VEH vs. GFP CNO

>0.9999

>0.9999

>0.9999

GI CNO vs. GFP VEH	>0.9999
GI CNO vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	>0.9999
Trial 3	
GI VEH vs. GI CNO	0.9998
GI VEH vs. GFP VEH	>0.9999
GI VEH vs. GFP CNO	0.9993
GI CNO vs. GFP VEH	0.9954
GI CNO vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	0.9917
Rev Trial 1	
GI VEH vs. GI CNO	0.037
GI VEH vs. GFP VEH	>0.9999
GI VEH vs. GFP CNO	0.9993
GI CNO vs. GFP VEH	0.0379
GI CNO vs. GFP CNO	0.013
GFP VEH vs. GFP CNO	>0.9999
Rev Trial 2	
GI VEH vs. GI CNO	0.0582
GI VEH vs. GFP VEH	0.7499
GI VEH vs. GFP CNO	0.3881
GI CNO vs. GFP VEH	0.872
GI CNO vs. GFP CNO	0.9842
GFP VEH vs. GFP CNO	0.9991
Rev Trial 3	
GI VEH vs. GI CNO	>0.9999
GI VEH vs. GFP VEH	0.9506
GI VEH vs. GFP CNO	0.9964
GI CNO vs. GFP VEH	0.9873
GI CNO vs. GFP CNO	0.9999
GFP VEH vs. GFP CNO	0.9995

mPFC Rescue of Rerus1 Gi

Social Approach: Social Index (Figure 2g)

Two-way RM ANOVA

n=Gi/GFP (VEH, CNO): 16; Gi/Gi (VEH, CNO):18

ANOVA table	P value	F (DFn, DFd)			
Treatment x Group	P=0.0002	F (1, 32) = 17.07			
Treatment	P=0.0254	F (1, 32) = 5.502			
Group	P=0.0160	F (1, 32) = 6.473			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VEH - CNO					
Gi/GFP	0.2236	0.1057 to 0.3414	Yes	***	0.0002
Gi/Gi	-0.06164	-0.1728 to 0.04948	No	ns	0.3638
Gi/GFP - Gi/Gi					
VEH	0.06387	-0.04231 to 0.1700	No	ns	0.3163
CNO	-0.2214	-0.3275 to -0.1152	Yes	****	<0.0001

Social Novelty: Social Index (Figure 2h)

Two-way RM ANOVA

n=Gi/GFP (VEH, CNO): 16; Gi/Gi (VEH, CNO):18

ANOVA table	P value	F (DFn, DFd)			
Treatment x Group	P=0.0045	F (1, 32) = 9.355			
Treatment	P=0.0126	F (1, 32) = 6.989			
Group	P=0.0552	F (1, 32) = 3.960			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VEH - CNO					
Gi/GFP	0.2154	0.08642 to 0.3444	Yes	***	0.0009
Gi/Gi	-0.01568	-0.1373 to 0.1059	No	ns	0.9444
Gi/GFP - Gi/Gi					
VEH	0.03302	-0.09544 to 0.1615	No	ns	0.8048
CNO	-0.1981	-0.3265 to -0.06960	Yes	**	0.0015

Social Olfaction (Figure 2i)

Two-way ANOVA

n=Gi/GFP(VEH, CNO): 13; Gi/Gi (VEH, CNO):18

ANOVA table	P value	F (DFn, DFd)			
Interaction	P=0.2260	F (42, 870) = 1.162			
Scent	P<0.0001	F (14, 870) = 14.24			
Group	P=0.1826	F (3, 870) = 1.622			
Sidak Post Test					
Social 1			P value		
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO			0.0006		
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH			0.9984		
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO			0.0143		
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH			0.0104		
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO			<0.0001		
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO			0.0075		
Social 2					
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO			0.9006		
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH			0.9612		
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO			0.117		
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH			>0.9999		
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO			0.6471		
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO			0.652		

Grooming (Figure 2j)

Two-way ANOVA

n=Gi/GFP (VEH, CNO): 13; Gi/Gi (VEH, CNO): 19

ANOVA table	P value	F (DFn, DFd)
Interaction	P=0.0067	F (1, 58) = 7.921
Treatment	P=0.0024	F (1, 58) = 10.05

Group	P=0.1484	F (1, 58) = 2.145			
Sidak Post Test					P value
VEH :Gi Crus1/GFP mPFC vs. VEH :Gi Rerus1/Gi mPFC					0.9202
VEH :Gi Crus1/GFP mPFC vs. CNO:Gi Crus1/GFP mPFC					0.0014
VEH :Gi Crus1/GFP mPFC vs. CNO:Gi Rerus1/Gi mPFC					0.7957
VEH :Gi Rerus1/Gi mPFC vs. CNO:Gi Crus1/GFP mPFC					0.0106
VEH :Gi Rerus1/Gi mPFC vs. CNO:Gi Rerus1/Gi mPFC					0.9999
CNO:Gi Crus1/GFP mPFC vs. CNO:Gi Rerus1/Gi mPFC					0.022
<u>Water Maze (Figure 2k)</u>	n=Gi/GFP (VEH, CNO): 8; Gi/Gi (VEH, CNO): 13				
Two-way ANOVA					
ANOVA table	P value	F (DFn, DFd)			
Interaction	P=0.1884	F (15, 228) = 1.325			
Day	P<0.0001	F (5, 228) = 25.20			
Group	P=0.0575	F (3, 228) = 2.537			
Sidak Post Test					P value
Trial 1					0.9872
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO					>0.9999
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH					0.9706
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					0.9975
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH					0.749
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO					0.9822
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					
Trial 2					>0.9999
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO					0.9955
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH					0.9998
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					0.9998
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH					>0.9999
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO					>0.9999
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					
Trial 3					>0.9999
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO					0.9768
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH					0.9526
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					0.9818
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH					0.9618
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO					>0.9999
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					
Rev Trial 1					0.9989
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO					0.9502
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH					0.0097
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					0.8141
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH					0.0359
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO					0.0039
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					
Rev Trial 2					0.9991
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO					0.8767
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH					>0.9999
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					0.9818
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH					0.9998
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO					0.9398
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					
Rev Trial 3					>0.9999
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ Gi mPFC CNO					0.9925
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC VEH					0.9982
Gi Rerus1/ Gi mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					0.9844
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC VEH					0.9997
Gi Rerus1/ Gi mPFC CNO vs. Gi Rerus1/ GFP mPFC CNO					0.9398
Gi Rerus1/ GFP mPFC VEH vs. Gi Rerus1/ GFP mPFC CNO					
<u>Dentate Gi Rescue PC Tsc1</u>					
<u>Social Approach: Social Index (Figure 3d)</u>	GFP (VEH, CNO): n=14; Gi (VEH, CNO): n=10				
Two-way RM ANOVA					
ANOVA table	P value	F (DFn, DFd)			
Treatment x Group	P=0.3707	F (1, 22) = 0.8351			
Treatment	P=0.0049	F (1, 22) = 9.801			
Group	P=0.0177	F (1, 22) = 6.579			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VEH - CNO					
GFP	-0.1013	-0.2428 to 0.04025	No	ns	0.19
Gi	-0.1848	-0.3523 to -0.01732	Yes	*	0.0292
GFP - Gi					
VEH	-0.05612	-0.1939 to 0.08170	No	ns	0.5788
CNO	-0.1396	-0.2774 to -0.001802	Yes	*	0.0466
<u>Social Novelty: Social Index (Figure 3e)</u>	GFP (VEH, CNO): n=14; Gi (VEH, CNO): n=10				
Two-way RM ANOVA					
ANOVA table	P value	F (DFn, DFd)			
Treatment x Group	P=0.7764	F (1, 22) = 0.08269			
Treatment	P=0.0172	F (1, 22) = 6.645			
Group	P=0.0804	F (1, 22) = 3.359			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VEH - CNO					
GFP	-0.09797	-0.2305 to 0.03454	No	ns	0.1717
Gi	-0.1226	-0.2794 to 0.03422	No	ns	0.1425
GFP - Gi					

VEH	-0.09875	-0.2705 to 0.07298	No	ns	0.3438
CNO	-0.1233	-0.2951 to 0.04838	No	ns	0.1961
Social Olfaction (Figure 3f)					
ANOVA table	GFP (VEH, CNO): n=14; Gi (VEH, CNO): n=15				
Interaction	P value	F (DFn, DFd)			
Scent	P<0.0001	F (70, 990) = 2.028			
Group	P<0.0001	F (14, 990) = 38.78			
	P<0.0001	F (5, 990) = 12.20			
Sidak Post Test					
Social 1	P value				
Gi VEH vs. Gi CNO	<0.0001				
Gi VEH vs. GFP CNO	0.9106				
Gi VEH vs. GFP VEH	0.9525				
Gi CNO vs. GFP CNO	<0.0001				
Gi CNO vs. GFP VEH	<0.0001				
GFP CNO vs. GFP VEH	>0.9999				
Social 2	<0.0001				
Gi VEH vs. Gi CNO	0.9514				
Gi VEH vs. GFP CNO	0.974				
Gi VEH vs. GFP VEH	<0.0001				
Gi CNO vs. GFP CNO	<0.0001				
Gi CNO vs. GFP VEH	>0.9999				
GFP CNO vs. GFP VEH	>0.9999				
Grooming (Figure 3g)					
Two-way ANOVA	n=15 per group				
ANOVA table	P value				
Interaction	P=0.9471	F (1, 56) = 0.004440	F (DFn, DFd)		
Treatment	P=0.5900	F (1, 56) = 0.2937			
Group	P=0.2986	F (1, 56) = 1.101			
Sidak Post Test					
VEH :GFP vs. VEH :Gi	P value				
VEH :GFP vs. CNO :GFP	0.9824				
VEH :GFP vs. CNO :Gi	0.9997				
VEH :Gi vs. CNO :GFP	0.8428				
VEH :Gi vs. CNO :Gi	0.9995				
CNO :GFP vs. CNO :Gi	0.9987				
	0.9669				
Water Maze (Figure 3h)					
Two-way ANOVA	n=9 per group				
ANOVA table	P value				
Interaction	P=0.8753	F (15, 192) = 0.5968	F (DFn, DFd)		
Trial Day	P<0.0001	F (5, 192) = 96.18			
Group	P=0.0017	F (3, 192) = 5.215			
Sidak Post Test					
Trial 1					
GFP VEH vs. GFP CNO	0.9938				
GFP VEH vs. Gi VEH	>0.9999				
GFP VEH vs. Gi CNO	>0.9999				
GFP CNO vs. Gi VEH	0.9938				
GFP CNO vs. Gi CNO	0.9989				
Gi VEH vs. Gi CNO	>0.9999				
Trial 2					
GFP VEH vs. GFP CNO	0.9374				
GFP VEH vs. Gi VEH	>0.9999				
GFP VEH vs. Gi CNO	>0.9999				
GFP CNO vs. Gi VEH	0.7956				
GFP CNO vs. Gi CNO	0.9689				
Gi VEH vs. Gi CNO	0.9987				
Trial 3					
GFP VEH vs. GFP CNO	0.9989				
GFP VEH vs. Gi VEH	0.9993				
GFP VEH vs. Gi CNO	0.9998				
GFP CNO vs. Gi VEH	0.9473				
GFP CNO vs. Gi CNO	0.963				
Gi VEH vs. Gi CNO	>0.9999				
Rev Trial 1					
GFP VEH vs. GFP CNO	0.9873				
GFP VEH vs. Gi VEH	0.9965				
GFP VEH vs. Gi CNO	0.4437				
GFP CNO vs. Gi VEH	>0.9999				
GFP CNO vs. Gi CNO	0.705				
Gi VEH vs. Gi CNO	0.8222				
Rev Trial 2					
GFP VEH vs. GFP CNO	0.7414				
GFP VEH vs. Gi VEH	>0.9999				
GFP VEH vs. Gi CNO	0.7897				
GFP CNO vs. Gi VEH	0.6344				
GFP CNO vs. Gi CNO	>0.9999				
Gi VEH vs. Gi CNO	0.6995				
Rev Trial 3					
GFP VEH vs. GFP CNO	0.979				
GFP VEH vs. Gi VEH	>0.9999				
GFP VEH vs. Gi CNO	>0.9999				
GFP CNO vs. Gi VEH	0.9473				
GFP CNO vs. Gi CNO	0.963				
Gi VEH vs. Gi CNO	>0.9999				
ChR2 vm thalamus-prelimbic cortex					
Social Approach: Social Index (Figure 4i)					
Mixed-effects model (REML)	GFP (Laser OFF, ON):17; ChR2 (Laser OFF, ON): 14				
Fixed effects (type III)	P value				
	F (DFn, DFd)				

Stimulation	0.0044	F (2, 65) = 5.909			
Group	<0.0001	F (1, 35) = 21.14			
Stimulation x Group	<0.0001	F (2, 65) = 11.53			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
GFP					
Off vs. 20 Hz	-0.07655	-0.2020 to 0.04894	No	ns	0.3634
Off vs. 4 Hz	0.02133	-0.1077 to 0.1503	No	ns	0.9692
20 Hz vs. 4 Hz	0.09787	-0.02761 to 0.2234	No	ns	0.1703
ChR2					
Off vs. 20 Hz	0.295	0.1527 to 0.4373	Yes	****	<0.0001
Off vs. 4 Hz	0.2246	0.08226 to 0.3669	Yes	***	0.0008
20 Hz vs. 4 Hz	-0.07048	-0.2102 to 0.06926	No	ns	0.5271
GFP - ChR2					
Off	-0.01956	-0.1638 to 0.1247	No	ns	0.983
20 Hz	0.352	0.2133 to 0.4908	Yes	****	<0.0001
4 Hz	0.1837	0.04188 to 0.3255	Yes	**	0.0065
<u>Social Novelty: Social Index (Figure 4j)</u>					
Mixed-effects model (REML)					
Fixed effects (type III)	P value	F (DFn, DFd)			
Stimulation	0.0022	F (2, 97) = 6.534			
Group	0.0002	F (1, 97) = 14.62			
Stimulation x Group	0.0091	F (2, 97) = 4.931			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
GFP					
Off vs. 20 Hz	0.02193	-0.1203 to 0.1642	No	ns	0.9753
Off vs. 4 Hz	-0.004471	-0.1485 to 0.1396	No	ns	0.9998
20 Hz vs. 4 Hz	-0.0264	-0.1667 to 0.1139	No	ns	0.9566
ChR2					
Off vs. 20 Hz	0.2965	0.1366 to 0.4564	Yes	****	<0.0001
Off vs. 4 Hz	0.1648	0.007440 to 0.3222	Yes	*	0.0371
20 Hz vs. 4 Hz	-0.1317	-0.2891 to 0.02571	No	ns	0.1285
GFP - ChR2					
Off	-0.01128	-0.1644 to 0.1418	No	ns	0.9972
20 Hz	0.2633	0.1137 to 0.4129	Yes	***	0.0001
4 Hz	0.158	0.009431 to 0.3066	Yes	*	0.0334
<u>Social Olfaction (Figure 4k)</u>					
Two-way ANOVA					
ANOVA table	P value	F (DFn, DFd)			
Interaction	P<0.0001	F (70, 810) = 4.288			
Scent	P<0.0001	F (14, 810) = 62.92			
Group	P<0.0001	F (5, 810) = 17.83			
Sidak Post Test			P value		
Social 1			<0.0001		
Laser Off vs. Laser On 20 Hz			0.8743		
Laser Off vs. Laser On GFP 20 Hz			>0.9999		
Laser Off vs. Laser Off GFP			0.9997		
Laser Off vs. Laser On GFP 4hz			<0.0001		
Laser Off vs. Laser On ChR2 4Hz			<0.0001		
Laser On 20 Hz vs. Laser On GFP 20 Hz			<0.0001		
Laser On 20 Hz vs. Laser Off GFP			<0.0001		
Laser On 20 Hz vs. Laser On GFP 4hz			<0.0001		
Laser On 20 Hz vs. Laser On ChR2 4Hz			>0.9999		
Laser On GFP 20 Hz vs. Laser Off GFP			0.8903		
Laser On GFP 20 Hz vs. Laser On GFP 4hz			0.2578		
Laser On GFP 20 Hz vs. Laser On ChR2 4Hz			<0.0001		
Laser Off GFP vs. Laser On GFP 4hz			0.9995		
Laser Off GFP vs. Laser On ChR2 4Hz			<0.0001		
Laser On GFP 4hz vs. Laser On ChR2 4Hz			<0.0001		
Social 2			0.0042		
Laser Off vs. Laser On 20 Hz			0.7218		
Laser Off vs. Laser On GFP 20 Hz			0.0005		
Laser Off vs. Laser Off GFP			<0.0001		
Laser Off vs. Laser On GFP 4hz			0.2026		
Laser Off vs. Laser On ChR2 4Hz			<0.0001		
Laser On 20 Hz vs. Laser On GFP 20 Hz			<0.0001		
Laser On 20 Hz vs. Laser Off GFP			<0.0001		
Laser On 20 Hz vs. Laser On GFP 4hz			<0.0001		
Laser On 20 Hz vs. Laser On ChR2 4Hz			0.9792		
Laser On GFP 20 Hz vs. Laser Off GFP			0.2149		
Laser On GFP 20 Hz vs. Laser On GFP 4hz			0.0246		
Laser On GFP 20 Hz vs. Laser On ChR2 4Hz			0.0005		
Laser Off GFP vs. Laser On GFP 4hz			0.9999		
Laser Off GFP vs. Laser On ChR2 4Hz			<0.0001		
Laser On GFP 4hz vs. Laser On ChR2 4Hz			<0.0001		
<u>Grooming (Figure 4l)</u>					
Two-way ANOVA					
ANOVA table	P value	F (DFn, DFd)			
Interaction	P=0.0013	F (2, 54) = 7.537			
Row Factor	P=0.0197	F (2, 54) = 4.225			
Column Factor	P=0.0002	F (1, 54) = 15.91			
Sidak Post Test			P value		
Off:ChR2 vs. Off:GFP			>0.9999		
Off:ChR2 vs. 20 Hz:ChR2			0.0152		
Off:ChR2 vs. 20 Hz:GFP			>0.9999		
Off:ChR2 vs. 4 Hz:ChR2			0.0005		
Off:ChR2 vs. 4 Hz:GFP			>0.9999		

Off:GFP vs. 20 Hz:ChR2	0.0978
Off:GFP vs. 20 Hz:GFP	>0.9999
Off:GFP vs. 4 Hz:ChR2	0.0044
Off:GFP vs. 4 Hz:GFP	0.9992
20 Hz:ChR2 vs. 20 Hz:GFP	0.0079
20 Hz:ChR2 vs. 4 Hz:ChR2	0.9948
20 Hz:ChR2 vs. 4 Hz:GFP	0.0075
20 Hz:GFP vs. 4 Hz:ChR2	0.0044
20 Hz:GFP vs. 4 Hz:GFP	0.9992
4 Hz:ChR2 vs. 4 Hz:GFP	0.0002

Arch

Social Approach: Social Index (Figure 4p)

Mixed-effects model (REML)

Fixed effects (type III)

Stimulation

Group

Stimulation x Group

GFP (Laser OFF, ON): 13; Arch (Laser OFF, ON): 17

P value	F (DFn, DFd)
0.0002	F (2, 99) = 9.110
<0.0001	F (1, 99) = 23.15
0.1821	F (2, 99) = 1.733

Sidak's multiple comparisons test

Predicted (LS) mean diff.

95.00% CI of diff.

Significant?

Summary Adjusted P Value

GFP					
Off vs. 20 Hz	-0.08233	-0.2388 to 0.07411	No	ns	0.4959
Off vs. 4 Hz	-0.1001	-0.2566 to 0.05629	No	ns	0.3259
20 Hz vs. 4 Hz	-0.01782	-0.1743 to 0.1386	No	ns	0.9897
Arch					
Off vs. 20 Hz	-0.1504	-0.2859 to -0.01489	Yes	*	0.0246
Off vs. 4 Hz	-0.2583	-0.3937 to -0.1228	Yes	****	<0.0001
20 Hz vs. 4 Hz	-0.1079	-0.2434 to 0.02759	No	ns	0.1586
GFP - Arch					
Off	-0.09197	-0.2383 to 0.05436	No	ns	0.3417
20 Hz	-0.16	-0.3063 to -0.01368	Yes	*	0.0274
4 Hz	-0.2501	-0.3964 to -0.1038	Yes	***	0.0002

Social Novelty: Social Index (Figure 4q)

Mixed-effects model (REML)

Fixed effects (type III)

Stimulation

Group

Stimulation x Group

GFP (Laser OFF, ON): 13; Arch (Laser OFF, ON): 17

P value	F (DFn, DFd)
0.0002	F (2, 58) = 10.24
<0.0001	F (1, 43) = 25.93
0.0037	F (2, 43) = 6.411

Sidak's multiple comparisons test

Predicted (LS) mean diff.

95.00% CI of diff.

Significant?

Summary Adjusted P Value

GFP					
Off vs. 20 Hz	0.009836	-0.1291 to 0.1487	No	ns	0.9975
Off vs. 4Hz	-0.08185	-0.2229 to 0.05922	No	ns	0.4115
20 Hz vs. 4Hz	-0.09169	-0.2306 to 0.04719	No	ns	0.2999
Arch					
Off vs. 20 Hz	-0.2306	-0.3531 to -0.1081	Yes	****	<0.0001
Off vs. 4Hz	-0.2815	-0.4025 to -0.1605	Yes	****	<0.0001
20 Hz vs. 4Hz	-0.05092	-0.1719 to 0.07009	No	ns	0.6706
GFP - Arch					
Off	-0.002185	-0.1292 to 0.1248	No	ns	>0.9999
20 Hz	-0.2426	-0.3671 to -0.1182	Yes	****	<0.0001
4Hz	-0.2019	-0.3278 to -0.07593	Yes	***	0.0008

Social Olfaction (Figure 4r)

Two-way ANOVA

GFP (Laser OFF, ON): n=9; Arch (Laser OFF, ON): n=12

ANOVA table

Interaction

Treatment

Group

P value	F (DFn, DFd)
P<0.0001	F (98, 1050) = 4.734
P<0.0001	F (14, 1050) = 50.51
P<0.0001	F (7, 1050) = 18.28

Sidak Post Test

Social 1

Arch Laser Off vs. Arch 20 Hz

Arch Laser Off vs. GFP 20 Hz

Arch Laser Off vs. GFP Laser Off

Arch Laser Off vs. Arch 4 Hz

Arch Laser Off vs. GFP 4 Hz

Arch 20 Hz vs. GFP 20 Hz

Arch 20 Hz vs. GFP Laser Off

Arch 20 Hz vs. Arch 4 Hz

Arch 20 Hz vs. GFP 4 Hz

GFP 20 Hz vs. GFP Laser Off

GFP 20 Hz vs. Arch 4 Hz

GFP 20 Hz vs. GFP 4 Hz

GFP Laser Off vs. Arch 4 Hz

GFP Laser Off vs. GFP 4 Hz

Arch 4 Hz vs. GFP 4 Hz

P value

<0.0001

0.4423

0.1762

<0.0001

0.1597

<0.0001

<0.0001

>0.9999

<0.0001

0.0002

<0.0001

0.0002

<0.0001

>0.9999

<0.0001

Social 2

Arch Laser Off vs. Arch 20 Hz

Arch Laser Off vs. GFP 20 Hz

Arch Laser Off vs. GFP Laser Off

Arch Laser Off vs. Arch 4 Hz

Arch Laser Off vs. GFP 4 Hz

Arch 20 Hz vs. GFP 20 Hz

Arch 20 Hz vs. GFP Laser Off

Arch 20 Hz vs. Arch 4 Hz

Arch 20 Hz vs. GFP 4 Hz

GFP 20 Hz vs. GFP Laser Off

GFP 20 Hz vs. Arch 4 Hz

GFP 20 Hz vs. GFP 4 Hz

GFP Laser Off vs. Arch 4 Hz

GFP Laser Off vs. GFP 4 Hz

<0.0001

0.0124

0.0888

<0.0001

0.0003

<0.0001

<0.0001

0.5063

<0.0001

>0.9999

<0.0001

0.9974

<0.0001

0.8589

Arch 4 Hz vs. GFP 4 Hz						<0.0001
Grooming (Figure 4s)						
Two-way ANOVA	GFP (Laser OFF, ON): n=14; Arch (Laser OFF, ON): n=11					
	Ordinary					
ANOVA table	P value		F (DFn, DFd)			
Interaction	P=0.0010		F (2, 69) = 7.683			
Treatment	P=0.0484		F (2, 69) = 3.166			
Group	P<0.0001		F (1, 69) = 69.14			
Sidak Post Test						
Laser Off :GFP vs. Laser Off :Arch						0.6929
Laser Off :GFP vs. 20 Hz:GFP						0.9898
Laser Off :GFP vs. 20 Hz:Arch						<0.0001
Laser Off :GFP vs. 4 Hz:GFP						>0.9999
Laser Off :GFP vs. 4 Hz:Arch						<0.0001
Laser Off :Arch vs. 20 Hz:GFP						0.0553
Laser Off :Arch vs. 20 Hz:Arch						0.0003
Laser Off :Arch vs. 4 Hz:GFP						0.5135
Laser Off :Arch vs. 4 Hz:Arch						0.0114
20 Hz:GFP vs. 20 Hz:Arch						<0.0001
20 Hz:GFP vs. 4 Hz:GFP						0.9988
20 Hz:GFP vs. 4 Hz:Arch						<0.0001
20 Hz:Arch vs. 4 Hz:GFP						<0.0001
20 Hz:Arch vs. 4 Hz:Arch						0.9957
4 Hz:GFP vs. 4 Hz:Arch						<0.0001
Vermis Gi						
Social Approach: Social Index (Figure 5k)						
Mixed-effects model (REML)	n=GFP (VEH, CNO):12; Gi (VEH, CNO):15					
Fixed effects (type III)	P value		F (DFn, DFd)			
Treatment	0.0017		F (1, 52) = 10.98			
Group	0.0008		F (1, 52) = 12.78			
Treatment x Group	0.0118		F (1, 52) = 6.805			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
VEH - CNO						
GFP	0.02322	-0.08999 to 0.1364	No	ns	0.8694	
Gi	0.1951	0.09409 to 0.2961	Yes	****	<0.0001	
GFP - Gi						
VEH	0.03183	-0.07970 to 0.1434	No	ns	0.7637	
CNO	0.2037	0.1009 to 0.3065	Yes	****	<0.0001	
Social Novelty: Social Index (Figure 5l)						
Mixed-effects model (REML)	n=GFP (VEH, CNO):12; Gi (VEH, CNO):15					
Fixed effects (type III)	P value		F (DFn, DFd)			
Treatment	0.0001		F (1, 54) = 16.80			
Group	<0.0001		F (1, 54) = 26.27			
Treatment x Group	0.1167		F (1, 54) = 2.541			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
VEH - CNO						
GFP	0.07429	-0.02894 to 0.1775	No	ns	0.1966	
Gi	0.1689	0.07961 to 0.2581	Yes	***	0.0001	
GFP - Gi						
VEH	0.1047	0.005588 to 0.2039	Yes	*	0.0366	
CNO	0.1993	0.1055 to 0.2931	Yes	****	<0.0001	
Grooming (Figure 5m)						
Two-way ANOVA	n= 11 per group					
	Ordinary					
ANOVA table	P value		F (DFn, DFd)			
Interaction	P=0.0029		F (1, 50) = 9.786			
Treatment	P=0.0491		F (1, 50) = 4.067			
Group	P=0.0305		F (1, 50) = 4.958			
Sidak Post Test				P value		
VEH:GFP vs. VEH:Gi				0.979		
VEH:GFP vs. CNO:GFP				0.9729		
VEH:GFP vs. CNO:Gi				0.0449		
VEH:Gi vs. CNO:GFP				>0.9999		
VEH:Gi vs. CNO:Gi				0.002		
CNO:GFP vs. CNO:Gi				0.0037		
Water Maze (Figure 5n)						
Two-way ANOVA	GFP (VEH, CNO): n=11; Gi (VEH, CNO): n=9					
ANOVA table	P value		F (DFn, DFd)			
Interaction	P<0.0001		F (15, 232) = 6.260			
Trial Day	P<0.0001		F (5, 232) = 71.63			
Group	P<0.0001		F (3, 232) = 12.49			
Sidak Post Test				P value		
Trial 1						
GFP (VEH) vs. GFP (CNO)				0.9837		
GFP (VEH) vs. Gi(VEH)				0.9305		
GFP (VEH) vs. Gi (CNO)				0.8902		
GFP (CNO) vs. Gi(VEH)				>0.9999		
GFP (CNO) vs. Gi (CNO)				0.9997		
Gi(VEH) vs. Gi (CNO)				>0.9999		
Trial 2						
GFP (VEH) vs. GFP (CNO)				0.9279		
GFP (VEH) vs. Gi(VEH)				0.998		
GFP (VEH) vs. Gi (CNO)				0.6696		
GFP (CNO) vs. Gi(VEH)				0.999		

GFP (CNO) vs. Gi (CNO)	0.9984
Gi(VEH) vs. Gi (CNO)	0.9563
Trial 3	
GFP (VEH) vs. GFP (CNO)	0.9825
GFP (VEH) vs. Gi(VEH)	0.9987
GFP (VEH) vs. Gi (CNO)	0.9628
GFP (CNO) vs. Gi(VEH)	>0.9999
GFP (CNO) vs. Gi (CNO)	>0.9999
Gi(VEH) vs. Gi (CNO)	0.9997
Rev Trial 1	
GFP (VEH) vs. GFP (CNO)	<0.0001
GFP (VEH) vs. Gi(VEH)	0.0018
GFP (VEH) vs. Gi (CNO)	<0.0001
GFP (CNO) vs. Gi(VEH)	0.9156
GFP (CNO) vs. Gi (CNO)	<0.0001
Gi(VEH) vs. Gi (CNO)	<0.0001
Rev Trial 2	
GFP (VEH) vs. GFP (CNO)	>0.9999
GFP (VEH) vs. Gi(VEH)	0.9188
GFP (VEH) vs. Gi (CNO)	0.1105
GFP (CNO) vs. Gi(VEH)	0.9746
GFP (CNO) vs. Gi (CNO)	0.2144
Gi(VEH) vs. Gi (CNO)	0.7593
Rev Trial 3	
GFP (VEH) vs. GFP (CNO)	0.9937
GFP (VEH) vs. Gi(VEH)	>0.9999
GFP (VEH) vs. Gi (CNO)	0.9785
GFP (CNO) vs. Gi(VEH)	0.9957
GFP (CNO) vs. Gi (CNO)	>0.9999
Gi(VEH) vs. Gi (CNO)	0.9853

Vermis Gq

Social Approach: Social Index (Figure 5p)
Mixed-effects model (REML)

n=GFP (VEH, CNO): 12; Gq (VEH, CNO): 18

Fixed effects (type III)	P value	F (DFn, DFd)			
Treatment	0.8065	F (1, 27) = 0.06119			
Group	0.5738	F (1, 29) = 0.3237			
Treatment x Group	0.3168	F (1, 27) = 1.040			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VEH - CNO					
GFP	0.04993	-0.09474 to 0.1946	No	ns	0.6648
Gq	-0.03044	-0.1482 to 0.08734	No	ns	0.7937
GFP - Gq					
VEH	0.01625	-0.1162 to 0.1487	No	ns	0.9512
CNO	-0.06411	-0.1965 to 0.06832	No	ns	0.4681

Social Novelty: Social Index (Figure 5q)

n=GFP (VEH, CNO): 12; Gq (VEH, CNO): 18

Mixed-effects model (REML)

Fixed effects (type III)	P value	F (DFn, DFd)			
Treatment	0.9962	F (1, 27) = 2.347e-005			
Group	0.1124	F (1, 29) = 2.680			
Treatment x Group	0.7999	F (1, 27) = 0.06554			
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
VEH - CNO					
GFP	-0.01023	-0.1600 to 0.1396	No	ns	0.9838
Gq	0.01063	-0.1110 to 0.1322	No	ns	0.9736
GFP - Gq					
VEH	0.06584	-0.07638 to 0.2081	No	ns	0.4987
CNO	0.0867	-0.05552 to 0.2289	No	ns	0.3057

Grooming (Figure 5r)

Two-way ANOVA

n= GFP (VEH, CNO): 10; Gq (VEH, CNO): 18
Ordinary

ANOVA table	P value	F (DFn, DFd)			
Interaction	P=0.0474	F (1, 54) = 4.118			
Treatment	P=0.0130	F (1, 54) = 6.593			
Group	P=0.0007	F (1, 54) = 12.89			
Sidak Post Test			P value		
VEH :GFP vs. VEH :Gq			0.8371		
VEH :GFP vs. cno :GFP			0.9996		
VEH :GFP vs. cno :Gq			0.0002		
VEH :Gq vs. cno :GFP			0.9814		
VEH :Gq vs. cno :Gq			0.0027		
cno :GFP vs. cno :Gq			0.0018		

Water Maze (Figure 5s)

Two-way ANOVA

n=9 per group

ANOVA table	P value	F (DFn, DFd)			
Interaction	P<0.0001	F (15, 198) = 5.453			
Treatment	P<0.0001	F (5, 198) = 198.6			
Group	P<0.0001	F (3, 198) = 19.03			
Sidak Post Test			P value		
Trial 1			0.9764		
GFP (VEH) vs. GFP (CNO)			>0.9999		
GFP (VEH) vs. Gq (VEH)			0.8434		
GFP (VEH) vs. Gq(CNO)					

GFP (CNO) vs. Gq (VEH)			0.9813
GFP (CNO) vs. Gq(CNO)			0.9764
Gq (VEH) vs. Gq(CNO)			0.8533
Trial 2			
GFP (VEH) vs. GFP (CNO)			0.8434
GFP (VEH) vs. Gq (VEH)			0.8899
GFP (VEH) vs. Gq(CNO)			0.9024
GFP (CNO) vs. Gq (VEH)			0.9993
GFP (CNO) vs. Gq(CNO)			0.9991
Gq (VEH) vs. Gq(CNO)			>0.9999
Trial 3			
GFP (VEH) vs. GFP (CNO)			>0.9999
GFP (VEH) vs. Gq (VEH)			>0.9999
GFP (VEH) vs. Gq(CNO)			0.9928
GFP (CNO) vs. Gq (VEH)			>0.9999
GFP (CNO) vs. Gq(CNO)			0.9928
Gq (VEH) vs. Gq(CNO)			0.9943
Rev Trial 1			
GFP (VEH) vs. GFP (CNO)			0.7721
GFP (VEH) vs. Gq (VEH)			0.0539
GFP (VEH) vs. Gq(CNO)			<0.0001
GFP (CNO) vs. Gq (VEH)			0.0027
GFP (CNO) vs. Gq(CNO)			<0.0001
Gq (VEH) vs. Gq(CNO)			<0.0001
Rev Trial 2			
GFP (VEH) vs. GFP (CNO)			0.0151
GFP (VEH) vs. Gq (VEH)			0.0845
GFP (VEH) vs. Gq(CNO)			0.0006
GFP (CNO) vs. Gq (VEH)			<0.0001
GFP (CNO) vs. Gq(CNO)			<0.0001
Gq (VEH) vs. Gq(CNO)			0.3247
Rev Trial 3			
GFP (VEH) vs. GFP (CNO)			0.9928
GFP (VEH) vs. Gq (VEH)			0.7893
GFP (VEH) vs. Gq(CNO)			0.4339
GFP (CNO) vs. Gq (VEH)			0.9163
GFP (CNO) vs. Gq(CNO)			0.6055
Gq (VEH) vs. Gq(CNO)			0.9258
<u>In vivo Single Unit Recordings</u>			
mPFC single unit frequency; Gi Rcrus1 vs GFP Rcrus1 (Figure 2a)	n= GFP: 16; Gi: 12	Mann-Whitney Test	P value
mPFC single unit frequency; <i>Tsc1</i> PC mutant vs control (Figure 1a)	n= Control: 15; Mutant: 30	Mann-Whitney Test	<0.0001
mPFC single unit frequency; <i>Tsc1</i> PC mutant with mPFC DREADDs (Figure 1c)	n= GFP: 7; Gi: 9	Mann-Whitney Test	0.0002
mPFC single unit frequency; Lateral Nucleus DREADDs (Figure 3b)	n= GFP: 6; Gi: 11	Mann-Whitney Test	0.0004
mPFC single unit Frequency; ChR2 thalamus (Figure 4h)	n= GFP: 6; ChR2: 16		
Two way ANOVA			
ANOVA table	P value	F (DFn, DFd)	
Interaction		P=0.4494 F (1, 38) = 0.5841	
Stimulation		P=0.3310 F (1, 38) = 0.9695	
Group		P=0.0010 F (1, 38) = 12.79	
Sidak Post Test	P value		
4 Hz:ChR2 vs. 4 Hz:GFP		0.0253	
4 Hz:ChR2 vs. 20 Hz:ChR2		0.5052	
4 Hz:ChR2 vs. 20 Hz:GFP		0.0168	
4 Hz:GFP vs. 20 Hz:ChR2		0.3617	
4 Hz:GFP vs. 20 Hz:GFP		>0.9999	
20 Hz:ChR2 vs. 20 Hz:GFP		0.2735	
mPFC single unit Frequency; Arch thalamus (Figure 4o)	n= GFP: 7; Arch: 9		
ANOVA table	P value	F (DFn, DFd)	
Interaction		P=0.4619 F (1, 31) = 0.5551	
Stimulation		P=0.2577 F (1, 31) = 1.330	
Group		P<0.0001 F (1, 31) = 59.47	
Sidak Post Test	P value		
20 Hz:Arch vs. 20 Hz:GFP		<0.0001	
20 Hz:Arch vs. 4 Hz:Arch		0.7019	
20 Hz:Arch vs. 4 Hz:GFP		<0.0001	
20 Hz:GFP vs. 4 Hz:Arch		0.0004	
20 Hz:GFP vs. 4 Hz:GFP		0.9999	
4 Hz:Arch vs. 4 Hz:GFP		0.0001	
Single unit activity mPFC; vermis DREADDs (Figure 5a)	n= GFP: 7; Gi: 9	Mann-Whitney Test	P value
			<0.0001

Supplemental Table S2 Extended Data (ED) Figure Statistics

Test/Experiment

<u>Awake Recorded Single Unit Frequency PRL Control vs. PC Tsc/ Mutants (ED Figure 1b)</u>	n= Control: 13; Mutant: 16
Mann-Whitney Test	p= 0.0015
<u>Awake Recorded Single Unit Frequency PRL Rcrus1 Gi VEH vs. CNO (ED Figure 3b)</u>	n= Veh: 10; CNO:14
Mann-Whitney Test	p= 0.0064
<u>Single Unit Frequency motor cortex- Rcrus1 DREADDs (ED Figure 3c)</u>	n= 9
<u>Single Unit Frequency Right PRL- Rcrus1 DREADDs (ED Figure 3d)</u>	n= 6
Mann-Whitney Test	p= 0.0565
<u>mPFC single unit frequency; Gi Rcrus1 GFP mPFC vs Gi Rcrus1 Gi mPFC (ED Figure 3e)</u>	n= 9 per group
Mann-Whitney Test	p<0.0001
<u>mEPSC Frequency (ED Figure 2b)</u>	n= 5 mice, 12-15 cells
Unpaired t- test (two tailed)	p= 0.2516
<u>mEPSC Amplitude (ED Figure 2c)</u>	n= 5 mice, 12-15 cells
Unpaired t- test (two tailed)	p= 0.702
<u>mEPSC Rise Time (ED Figure 2d)</u>	n= 5 mice, 7-10 cells
Unpaired t- test (two tailed)	p=0.1238
<u>mEPSC Decay Time (ED Figure 2e)</u>	n= 5 mice, 7-10 cells
Unpaired t- test (two tailed)	p=0.2612
<u>mIPSC Frequency (ED Figure 2g)</u>	n= 5 mice, 12-15 cells
Unpaired t- test (two tailed)	p= 0.0017
<u>mEPSC Amplitude (ED Figure 2h)</u>	n= 5 mice, 12-15 cells
Unpaired t- test (two tailed)	p= 0.1019
<u>mIPSC Rise Time (ED Figure 2i)</u>	n= 5 mice, 10-11 cells
Unpaired t- test (two tailed)	p=0.0096
<u>mIPSC Decay Time (ED Figure 2j)</u>	n= 5 mice, 10-11 cells
Unpaired t- test (two tailed)	p=0.164

mPFC Rescue Tsc/ PC Mutant

Social Approach: 3 way ANOVA (ED Figure 1c)
Mixed-effects model (REML)

n= GFP (VEH, CNO): 27; Gi (VEH, CNO): 30

Fixed effects (type III)	P value	F (Dfn, Dfd)
NA vs NO	0.0205	F (1, 118) = 5.515
GFP vs Gi	0.8289	F (1, 118) = 0.04692
VEH vs CNO	0.9837	F (1, 96) = 0.0004198
NA vs NO x GFP vs Gi	0.0149	F (1, 118) = 6.111
NA vs NO x VEH vs CNO	0.0018	F (1, 96) = 10.28
GFP vs Gi x VEH vs CNO	0.002	F (1, 96) = 10.07
NA vs NO x GFP vs Gi x VEH vs CNO	0.0229	F (1, 96) = 5.346

Sidak's multiple comparisons test

Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
NA-GFP VEH vs. NO-GFP VEH	-5.949	-42.58 to 30.68	No	ns >0.9999
NA-GFP CNO vs. NO-GFP CNO	4.221	-33.05 to 41.49	No	ns >0.9999
NA-Gi VEH vs. NO-Gi VEH	2.312	-35.55 to 40.18	No	ns >0.9999
NA-Gi CNO vs. NO-Gi CNO	65.04	29.68 to 100.4	Yes	**** <0.0001
NA-GFP VEH vs. NA-GFP CNO	13.06	-20.16 to 46.28	No	ns 0.9695
NA-Gi VEH vs. NA-Gi CNO	-49.28	-82.62 to -15.95	Yes	*** 0.0004

Social Novelty: 3 way ANOVA (ED Figure 1d)
Mixed-effects model (REML)

n= GFP (VEH, CNO): 27; Gi (VEH, CNO): 30

Fixed effects (type III)	P value	F (Dfn, Dfd)
FA vs NA	0.0767	F (1, 112) = 3.192
GFP vs Gi	0.2565	F (1, 112) = 1.301
VEH vs CNO	0.8317	F (1, 100) = 0.04539
FA vs NA x GFP vs Gi	0.0797	F (1, 112) = 3.128
FA vs NA x VEH vs CNO	0.0302	F (1, 100) = 4.837
GFP vs Gi x VEH vs CNO	0.0663	F (1, 100) = 3.447
FA vs NA x GFP vs Gi x VEH vs CNO	0.0484	F (1, 100) = 3.993

Sidak's multiple comparisons test

Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
FA-GFP VEH vs. NA-GFP VEH	1.049	-36.14 to 38.24	No	ns >0.9999
FA-GFP CNO vs. NA-GFP CNO	-1.305	-39.84 to 37.24	No	ns >0.9999
FA-Gi VEH vs. NA-Gi VEH	-0.639	-39.13 to 37.85	No	ns >0.9999
FA-Gi CNO vs. NA-Gi CNO	-49.76	-85.69 to -13.83	Yes	** 0.001
NA-GFP VEH vs. NA-GFP CNO	10.93	-23.51 to 45.38	No	ns 0.9949
NA-Gi VEH vs. NA-Gi CNO	-34.18	-68.18 to -0.1718	Yes	* 0.0479

Distance Traveled Open Field (ED Figure 1g)

Two-way ANOVA
ANOVA table
Interaction
Minute
Group

n=10 per group

P value	F (Dfn, Dfd)
P=0.7645	F (42, 540) = 0.8328
P<0.0001	F (14, 540) = 5.392
P<0.0001	F (3, 540) = 12.90

Sidak Post Test

0:00-1:00	P value
Gi CNO vs. Gi VEH	0.3048
Gi CNO vs. GFP VEH	0.6123
Gi CNO vs. GFP CNO	>0.9999
Gi VEH vs. GFP VEH	0.9985
Gi VEH vs. GFP CNO	0.4856
GFP VEH vs. GFP CNO	0.8012

1:00-2:00

Gi CNO vs. Gi VEH	0.0777
Gi CNO vs. GFP VEH	0.1057
Gi CNO vs. GFP CNO	0.3642
Gi VEH vs. GFP VEH	>0.9999
Gi VEH vs. GFP CNO	0.9834
GFP VEH vs. GFP CNO	0.9938

2:00-3:00

Gi CNO vs. Gi VEH	0.0813
Gi CNO vs. GFP VEH	0.329
Gi CNO vs. GFP CNO	0.1097
Gi VEH vs. GFP VEH	0.9907
Gi VEH vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	0.997

3:00-4:00

Gi CNO vs. Gi VEH	0.1265
Gi CNO vs. GFP VEH	0.5776
Gi CNO vs. GFP CNO	0.2495
Gi VEH vs. GFP VEH	0.9655
Gi VEH vs. GFP CNO	0.9998
GFP VEH vs. GFP CNO	0.9971

4:00-5:00

Gi CNO vs. Gi VEH	0.7715
Gi CNO vs. GFP VEH	0.7841
Gi CNO vs. GFP CNO	0.8137
Gi VEH vs. GFP VEH	>0.9999

Gi VEH vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	>0.9999
5:00-6:00	
Gi CNO vs. Gi VEH	0.9832
Gi CNO vs. GFP VEH	0.448
Gi CNO vs. GFP CNO	0.2732
Gi VEH vs. GFP VEH	0.9026
Gi VEH vs. GFP CNO	0.7503
GFP VEH vs. GFP CNO	>0.9999

6:00-7:00	
Gi CNO vs. Gi VEH	0.0633
Gi CNO vs. GFP VEH	0.7688
Gi CNO vs. GFP CNO	0.6747
Gi VEH vs. GFP VEH	0.7122
Gi VEH vs. GFP CNO	0.8019
GFP VEH vs. GFP CNO	>0.9999

7:00-8:00	
Gi CNO vs. Gi VEH	0.3471
Gi CNO vs. GFP VEH	0.4549
Gi CNO vs. GFP CNO	0.433
Gi VEH vs. GFP VEH	>0.9999
Gi VEH vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	>0.9999

8:00-9:00	
Gi CNO vs. Gi VEH	0.0869
Gi CNO vs. GFP VEH	0.9934
Gi CNO vs. GFP CNO	0.4023
Gi VEH vs. GFP VEH	0.3213
Gi VEH vs. GFP CNO	0.9814
GFP VEH vs. GFP CNO	0.8122

9:00-10:00	
Gi CNO vs. Gi VEH	0.1694
Gi CNO vs. GFP VEH	0.6702
Gi CNO vs. GFP CNO	0.1843
Gi VEH vs. GFP VEH	0.9653
Gi VEH vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	0.9725

10:00-11:00	
Gi CNO vs. Gi VEH	0.9947
Gi CNO vs. GFP VEH	>0.9999
Gi CNO vs. GFP CNO	0.8838
Gi VEH vs. GFP VEH	0.9988
Gi VEH vs. GFP CNO	0.9974
GFP VEH vs. GFP CNO	0.934

11:00-12:00	
Gi CNO vs. Gi VEH	0.8978
Gi CNO vs. GFP VEH	0.9112
Gi CNO vs. GFP CNO	0.6256
Gi VEH vs. GFP VEH	0.2596
Gi VEH vs. GFP CNO	0.9986
GFP VEH vs. GFP CNO	0.0941

12:00-13:00	
Gi CNO vs. Gi VEH	0.9527
Gi CNO vs. GFP VEH	>0.9999
Gi CNO vs. GFP CNO	0.9917
Gi VEH vs. GFP VEH	0.9708
Gi VEH vs. GFP CNO	0.6223
GFP VEH vs. GFP CNO	0.984

13:00-14:00	
Gi CNO vs. Gi VEH	0.9186
Gi CNO vs. GFP VEH	0.9166
Gi CNO vs. GFP CNO	0.9826
Gi VEH vs. GFP VEH	0.2961
Gi VEH vs. GFP CNO	0.4734
GFP VEH vs. GFP CNO	>0.9999

14:00-15:00	
Gi CNO vs. Gi VEH	0.9999
Gi CNO vs. GFP VEH	0.9998
Gi CNO vs. GFP CNO	>0.9999
Gi VEH vs. GFP VEH	0.9931
Gi VEH vs. GFP CNO	>0.9999
GFP VEH vs. GFP CNO	0.9975

Time in the Center Open Field (ED Figure 1f)

Two-way ANOVA	n=10 per group	
ANOVA table	P value	F (DFn, DFd)
Interaction	P=0.7942	F (1, 36) = 0.06903
Treatment	P=0.7338	F (1, 36) = 0.1175
Group	P=0.9691	F (1, 36) = 0.001526
Sidak Post Test	P value	
VEH :GFP vs. VEH :Gi	>0.9999	
VEH :GFP vs. CNO :GFP	>0.9999	
VEH :GFP vs. CNO :Gi	>0.9999	
VEH :Gi vs. CNO :GFP	>0.9999	
VEH :Gi vs. CNO :Gi	0.9987	
CNO :GFP vs. CNO :Gi	>0.9999	

Distance Traveled Elevated Plus Maze (ED Figure 1e)

Two-way ANOVA	n=20 per group	
ANOVA table	P value	F (DFn, DFd)
Interaction	P=0.0655	F (1, 76) = 3.491
Treatment	P=0.0025	F (1, 76) = 9.794
Group	P=0.1707	F (1, 76) = 1.913
Sidak Post Test	P value	
VEH : Gi vs. VEH :GFP	0.9406	
VEH : Gi vs. CNO : Gi	0.0123	
VEH : Gi vs. CNO :GFP	0.776	
VEH :GFP vs. CNO : Gi	0.0042	
VEH :GFP vs. CNO :GFP	0.1369	
CNO : Gi vs. CNO :GFP	0.9994	

Rotarod (ED Figure 1h)

Two-way ANOVA	n= GFP (VEH, CNO): 9; Gi (VEH, CNO): 11	
ANOVA table	P value	F (DFn, DFd)
Interaction	P=0.9994	F (12, 194) = 0.1640
Treatment	P<0.0001	F (4, 194) = 12.37
Group	P=0.3508	F (3, 194) = 1.099

Sidak Post Test	P value
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Day 1	0.9977
Mutant mPFC Gi CNO vs. Mutant, mPFC Gi VEH	0.9997
Mutant mPFC Gi CNO vs. Mutant mPFC GFP VEH	0.956
Mutant mPFC Gi CNO vs. Mutant mPFC GFP CNO	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP VEH	0.9991
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP CNO	0.9958
Mutant mPFC GFP VEH vs. Mutant mPFC GFP CNO	
Day 2	0.711
Mutant mPFC Gi CNO vs. Mutant, mPFC Gi VEH	0.8577
Mutant mPFC Gi CNO vs. Mutant mPFC GFP VEH	0.7939
Mutant mPFC Gi CNO vs. Mutant mPFC GFP CNO	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP VEH	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP CNO	>0.9999
Mutant mPFC GFP VEH vs. Mutant mPFC GFP CNO	
Day 3	0.9137
Mutant mPFC Gi CNO vs. Mutant, mPFC Gi VEH	>0.9999
Mutant mPFC Gi CNO vs. Mutant mPFC GFP VEH	0.9755
Mutant mPFC Gi CNO vs. Mutant mPFC GFP CNO	0.9077
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP VEH	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP CNO	0.9731
Mutant mPFC GFP VEH vs. Mutant mPFC GFP CNO	
Day 4	>0.9999
Mutant mPFC Gi CNO vs. Mutant, mPFC Gi VEH	>0.9999
Mutant mPFC Gi CNO vs. Mutant mPFC GFP VEH	0.9973
Mutant mPFC Gi CNO vs. Mutant mPFC GFP CNO	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP VEH	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP CNO	>0.9999
Mutant mPFC GFP VEH vs. Mutant mPFC GFP CNO	
Day 5	>0.9999
Mutant mPFC Gi CNO vs. Mutant, mPFC Gi VEH	>0.9999
Mutant mPFC Gi CNO vs. Mutant mPFC GFP VEH	0.9975
Mutant mPFC Gi CNO vs. Mutant mPFC GFP CNO	>0.9999
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP VEH	0.9935
Mutant, mPFC Gi VEH vs. Mutant mPFC GFP CNO	0.9852
Mutant mPFC GFP VEH vs. Mutant mPFC GFP CNO	

Mnfc Rescue of Recrusl Gi DREADDs

Social Approach: 3 way ANOVA (ED Figure 3f)

Three-way ANOVA

ANOVA table			F (DFn, DFd)
NA vs NO			F (1, 64) = 64.16
Gi/GFP vs Gi/Gi	P<0.0001		F (1, 64) = 1.966
VEH vs CNO	P=0.1657		F (1, 64) = 1.276
NA vs NO x Gi/GFP vs Gi/Gi	P=0.2628		F (1, 64) = 2.416
NA vs NO x VEH vs CNO	P=0.1250		F (1, 64) = 2.909
Gi/GFP vs Gi/Gi x VEH vs CNO	P=0.0930		F (1, 64) = 10.35
NA vs NO x Gi/GFP vs Gi/Gi x VEH vs CNO	P=0.0020		F (1, 64) = 11.67
	P=0.0011		

Sidak's multiple comparisons test

Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
NA:Gi/GFP VEH vs. NO:Gi/GFP VEH	93.61	44.55 to 142.7	Yes	**** <0.0001
NA:Gi/GFP CNO vs. NO:Gi/GFP CNO	2.926	-46.14 to 51.99	No	ns >0.9999
NA:Gi/Gi VEH vs. NO:Gi/Gi VEH	56.36	10.10 to 102.6	Yes	** 0.0066
NA:Gi/Gi CNO vs. NO:Gi/Gi CNO	86.66	40.40 to 132.9	Yes	**** <0.0001
NA:Gi/GFP VEH vs. NA:Gi/GFP CNO	83.82	29.83 to 137.8	Yes	*** 0.0002
NA:Gi/Gi VEH vs. NA:Gi/Gi CNO	-33.62	-84.52 to 17.28	No	ns 0.4905

Social Novelty: 3 way ANOVA (ED Figure 3g)

Three-way ANOVA

ANOVA table			F (DFn, DFd)
FA vs NA	P<0.0001		F (1, 64) = 23.36
Gi/GFP vs Gi/Gi	P=0.6957		F (1, 64) = 0.1544
VEH vs CNO	P=0.7449		F (1, 64) = 0.1068
FA vs NA x Gi/GFP vs Gi/Gi	P=0.2129		F (1, 64) = 1.583
FA vs NA x VEH vs CNO	P=0.0214		F (1, 64) = 5.566
Gi/GFP vs Gi/Gi x VEH vs CNO	P=0.3791		F (1, 64) = 0.7846
FA vs NA x Gi/GFP vs Gi/Gi x VEH vs CNO	P=0.0471		F (1, 64) = 4.100

Sidak's multiple comparisons test

Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
FA:Gi/GFP VEH vs. NA:Gi/GFP VEH	-62.91	-110.1 to -15.69	Yes	** 0.002
FA:Gi/GFP CNO vs. NA:Gi/GFP CNO	8.16	-39.06 to 55.38	No	ns >0.9999
FA:Gi/Gi VEH vs. NA:Gi/Gi VEH	-49.35	-93.87 to -4.838	Yes	* 0.019
FA:Gi/Gi CNO vs. NA:Gi/Gi CNO	-43.93	-88.45 to 0.5832	No	ns 0.0559
NA:Gi/GFP VEH vs. NA:Gi/GFP CNO	45.36	-4.073 to 94.80	No	ns 0.0964
NA:Gi/Gi VEH vs. NA:Gi/Gi CNO	-1.82	-48.43 to 44.79	No	ns >0.9999

Distance Traveled Open Field (ED Figure 3i)

Two-way ANOVA

ANOVA table			F (DFn, DFd)
Interaction	P=0.9920		F (42, 945) = 0.5459
Minute	P=0.2477		F (14, 945) = 1.229
Group	P=0.0029		F (3, 945) = 4.702

Sidak Post Test

0:00-1:00	P value
Gi/Gi VEH vs. Gi/Gi CNO	0.603
Gi/Gi VEH vs. Gi/GFP VEH	0.9825
Gi/Gi VEH vs. Gi/GFP CNO	0.9945
Gi/Gi CNO vs. Gi/GFP VEH	0.9867
Gi/Gi CNO vs. Gi/GFP CNO	0.9658
Gi/GFP VEH vs. Gi/GFP CNO	>0.9999
1:00-2:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.6567
Gi/Gi VEH vs. Gi/GFP VEH	>0.9999
Gi/Gi VEH vs. Gi/GFP CNO	0.9351
Gi/Gi CNO vs. Gi/GFP VEH	0.8219
Gi/Gi CNO vs. Gi/GFP CNO	0.1636
Gi/GFP VEH vs. Gi/GFP CNO	0.9165
2:00-3:00	
Gi/Gi VEH vs. Gi/Gi CNO	>0.9999
Gi/Gi VEH vs. Gi/GFP VEH	0.995
Gi/Gi VEH vs. Gi/GFP CNO	0.7768
Gi/Gi CNO vs. Gi/GFP VEH	0.9998
Gi/Gi CNO vs. Gi/GFP CNO	0.6117
Gi/GFP VEH vs. Gi/GFP CNO	0.4722
3:00-4:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9475
Gi/Gi VEH vs. Gi/GFP VEH	0.9945

Gi/Gi VEH vs. Gi/GFP CNO	0.8947
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	0.3616
Gi/GFP VEH vs. Gi/GFP CNO	0.6155
4:00-5:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9993
Gi/Gi VEH vs. Gi/GFP VEH	0.997
Gi/Gi VEH vs. Gi/GFP CNO	0.9931
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	>0.9999
Gi/GFP VEH vs. Gi/GFP CNO	>0.9999
5:00-6:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.6652
Gi/Gi VEH vs. Gi/GFP VEH	>0.9999
Gi/Gi VEH vs. Gi/GFP CNO	0.4608
Gi/Gi CNO vs. Gi/GFP VEH	0.7042
Gi/Gi CNO vs. Gi/GFP CNO	0.9992
Gi/GFP VEH vs. Gi/GFP CNO	0.5056
6:00-7:00	
Gi/Gi VEH vs. Gi/Gi CNO	>0.9999
Gi/Gi VEH vs. Gi/GFP VEH	0.9106
Gi/Gi VEH vs. Gi/GFP CNO	0.9995
Gi/Gi CNO vs. Gi/GFP VEH	0.9008
Gi/Gi CNO vs. Gi/GFP CNO	0.9996
Gi/GFP VEH vs. Gi/GFP CNO	0.7651
7:00-8:00	
Gi/Gi VEH vs. Gi/Gi CNO	>0.9999
Gi/Gi VEH vs. Gi/GFP VEH	>0.9999
Gi/Gi VEH vs. Gi/GFP CNO	0.9985
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	0.9961
Gi/GFP VEH vs. Gi/GFP CNO	0.998
8:00-9:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9998
Gi/Gi VEH vs. Gi/GFP VEH	>0.9999
Gi/Gi VEH vs. Gi/GFP CNO	0.9954
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	0.9622
Gi/GFP VEH vs. Gi/GFP CNO	0.9895
9:00-10:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9482
Gi/Gi VEH vs. Gi/GFP VEH	0.821
Gi/Gi VEH vs. Gi/GFP CNO	>0.9999
Gi/Gi CNO vs. Gi/GFP VEH	0.9995
Gi/Gi CNO vs. Gi/GFP CNO	0.9249
Gi/GFP VEH vs. Gi/GFP CNO	0.7892
10:00-11:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.8823
Gi/Gi VEH vs. Gi/GFP VEH	0.9271
Gi/Gi VEH vs. Gi/GFP CNO	0.9928
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	0.9994
Gi/GFP VEH vs. Gi/GFP CNO	0.9997
11:00-12:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9996
Gi/Gi VEH vs. Gi/GFP VEH	0.8889
Gi/Gi VEH vs. Gi/GFP CNO	0.9857
Gi/Gi CNO vs. Gi/GFP VEH	0.9795
Gi/Gi CNO vs. Gi/GFP CNO	0.9996
Gi/GFP VEH vs. Gi/GFP CNO	0.9997
12:00-13:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9283
Gi/Gi VEH vs. Gi/GFP VEH	0.9998
Gi/Gi VEH vs. Gi/GFP CNO	0.8675
Gi/Gi CNO vs. Gi/GFP VEH	0.9958
Gi/Gi CNO vs. Gi/GFP CNO	0.2919
Gi/GFP VEH vs. Gi/GFP CNO	0.7349
13:00-14:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.8618
Gi/Gi VEH vs. Gi/GFP VEH	0.953
Gi/Gi VEH vs. Gi/GFP CNO	0.765
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	0.1461
Gi/GFP VEH vs. Gi/GFP CNO	0.2799
14:00-15:00	
Gi/Gi VEH vs. Gi/Gi CNO	0.9747
Gi/Gi VEH vs. Gi/GFP VEH	0.9514
Gi/Gi VEH vs. Gi/GFP CNO	0.9874
Gi/Gi CNO vs. Gi/GFP VEH	>0.9999
Gi/Gi CNO vs. Gi/GFP CNO	>0.9999
Gi/GFP VEH vs. Gi/GFP CNO	>0.9999

Time in the Center Open Field (ED Figure 3i)

Two-way ANOVA

ANOVA table

Interaction

Treatment

Group

n=Gi/GFP (VEH, CNO):13; Gi/Gi (VEH, CNO): 18

P value

P=0.5888

P=0.9324

P=0.1484

F (Dfn, DFd)

F (1, 55) = 0.2956

F (1, 55) = 0.007257

F (1, 55) = 2.149

Sidak Post Test

VEH .Gi/Gi vs. VEH .Gi/GFP

VEH .Gi/Gi vs. CNO .Gi/Gi

VEH .Gi/Gi vs. CNO .Gi/GFP

VEH .Gi/GFP vs. CNO .Gi/Gi

VEH .Gi/GFP vs. CNO .Gi/GFP

CNO .Gi/Gi vs. CNO .Gi/GFP

P value

0.8598

0.9898

0.8954

0.9962

>0.9999

0.9984

Time in the Open Arm Elevated Plus Maze (ED Figure 3b)

Two-way ANOVA

ANOVA table

Interaction

Treatment

Group

n=Gi/GFP (VEH, CNO):13; Gi/Gi (VEH, CNO): 18

P value

P=0.7441

P=0.9195

P=0.4579

F (Dfn, DFd)

F (1, 38) = 0.1081

F (1, 38) = 0.01036

F (1, 38) = 0.5625

Sidak Post Test

VEH .Gi/Gi vs. VEH .Gi/GFP

VEH .Gi/Gi vs. CNO .Gi/Gi

VEH .Gi/Gi vs. CNO .Gi/GFP

VEH .Gi/GFP vs. CNO .Gi/Gi

VEH .Gi/GFP vs. CNO .Gi/GFP

P value

>0.9999

0.9989

0.9999

>0.9999

0.9993

CNO -Gi/Gi vs. CNO -Gi/GFP	0.9852			
<u>Elevated Plus Maze Distance Traveled (ED Figure 3h)</u>	n=Gi/GFP (VEH, CNO):13; Gi/Gi (VEH, CNO): 18			
Two-way ANOVA				
ANOVA table	P value	F (Dfn, DFd)		
Interaction	P=0.0604	F (1, 38) = 3.746		
Treatment	P=0.0605	F (1, 38) = 3.742		
Group	P=0.1715	F (1, 38) = 1.942		
Sidak Post Test	P value			
VEH -Gi/Gi vs. VEH -Gi/GFP	0.9995			
VEH -Gi/Gi vs. CNO -Gi/Gi	0.7836			
VEH -Gi/Gi vs. CNO -Gi/GFP	0.8015			
VEH -Gi/GFP vs. CNO -Gi/Gi	0.6031			
VEH -Gi/GFP vs. CNO -Gi/GFP	0.6311			
CNO -Gi/Gi vs. CNO -Gi/GFP	>0.9999			
<u>Rotarod (ED Figure 3k)</u>	n=10 per group			
Two-way ANOVA				
ANOVA table	P value	F (Dfn, DFd)		
Interaction	P=0.2770	F (12, 90) = 1.228		
Day	P<0.0001	F (4, 90) = 53.20		
Group	P=0.1391	F (3, 90) = 1.877		
Sidak Post Test				
Day 1	P value			
Gi/Gi VEH vs. Gi/GFP CNO	0.6696			
Gi/Gi VEH vs. Gi/Gi CNO	0.9994			
Gi/Gi VEH vs. Gi/GFP VEH	0.8059			
Gi/GFP CNO vs. Gi/Gi CNO	0.9931			
Gi/GFP CNO vs. Gi/GFP VEH	0.3941			
Gi/Gi CNO vs. Gi/GFP VEH				
Day 2	0.2754			
Gi/Gi VEH vs. Gi/GFP CNO	0.6991			
Gi/Gi VEH vs. Gi/Gi CNO	>0.9999			
Gi/Gi VEH vs. Gi/GFP VEH	0.9907			
Gi/GFP CNO vs. Gi/Gi CNO	0.1526			
Gi/GFP CNO vs. Gi/GFP VEH	0.4969			
Gi/Gi CNO vs. Gi/GFP VEH				
Day 3	0.3018			
Gi/Gi VEH vs. Gi/GFP CNO	0.2744			
Gi/Gi VEH vs. Gi/Gi CNO	0.0579			
Gi/Gi VEH vs. Gi/GFP VEH	>0.9999			
Gi/GFP CNO vs. Gi/Gi CNO	0.9813			
Gi/GFP CNO vs. Gi/GFP VEH	0.9871			
Gi/Gi CNO vs. Gi/GFP VEH				
Day 4	0.8605			
Gi/Gi VEH vs. Gi/GFP CNO	0.956			
Gi/Gi VEH vs. Gi/Gi CNO	0.8376			
Gi/Gi VEH vs. Gi/GFP VEH	0.296			
Gi/GFP CNO vs. Gi/Gi CNO	>0.9999			
Gi/GFP CNO vs. Gi/GFP VEH	0.272			
Gi/Gi CNO vs. Gi/GFP VEH				
Day 5	>0.9999			
Gi/Gi VEH vs. Gi/GFP CNO	>0.9999			
Gi/Gi VEH vs. Gi/Gi CNO	0.8922			
Gi/Gi VEH vs. Gi/GFP VEH	>0.9999			
Gi/GFP CNO vs. Gi/Gi CNO	0.9113			
Gi/GFP CNO vs. Gi/GFP VEH	0.8764			
<u>Lateral Nucleus Gi DREADDs</u>				
<u>Single Unit Frequency Lateral Nucleus DREADDs (ED Figure 4b)</u>	n= GFP: 6; Gi: 9			
Mann-Whitney Test	p = 0.0004			
<u>Social Approach: 3 way ANOVA (ED Figure 4c)</u>	n=GFP (VEH, CNO): 14; Gi (VEH, CNO): 10			
Mixed-effects model (REML)				
Fixed effects (type III)	P value	F (Dfn, DFd)		
NA vs NO	0.0954	F (1, 52) = 2.886		
GFP vs Gi	0.8022	F (1, 52) = 0.06342		
VEH vs CNO	0.338	F (1, 44) = 0.9383		
NA vs NO x GFP vs Gi	0.0174	F (1, 52) = 6.036		
NA vs NO x VEH vs CNO	0.0043	F (1, 44) = 9.073		
GFP vs Gi x VEH vs CNO	0.6679	F (1, 44) = 0.1866		
NA vs NO x GFP vs Gi x VEH vs CNO	0.3805	F (1, 44) = 0.7849		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary Adjusted P Value
NA-GFP VEH vs. NO-GFP VEH	-16.64	-55.07 to 21.78	No	ns 0.939
NA-GFP CNO vs. NO-GFP CNO	7.671	-24.81 to 40.15	No	ns 0.9997
NA-Gi VEH vs. NO-Gi VEH	2.299	-30.18 to 34.78	No	ns >0.9999
NA-Gi CNO vs. NO-Gi CNO	46.88	14.39 to 79.36	Yes	*** 0.0007
NA-GFP VEH vs. NA-GFP CNO	-15.23	-51.28 to 20.83	No	ns 0.9406
NA-Gi VEH vs. NA-Gi CNO	-30.3	-63.10 to 2.512	No	ns 0.0908
<u>Social Novelty: 3 way ANOVA (ED Figure 4d)</u>	n=GFP (VEH, CNO): 14; Gi (VEH, CNO): 10			
Mixed-effects model (REML)				
Fixed effects (type III)	P value	F (Dfn, DFd)		
FA vs NA	0.0004	F (1, 88) = 13.43		
GFP vs Gi	0.1402	F (1, 88) = 2.216		
VEH vs CNO	0.0668	F (1, 88) = 3.446		
FA vs NA x GFP vs Gi	0.043	F (1, 88) = 4.215		
FA vs NA x VEH vs CNO	0.0263	F (1, 88) = 5.107		
GFP vs Gi x VEH vs CNO	0.4733	F (1, 88) = 0.5186		
FA vs NA x GFP vs Gi x VEH vs CNO	0.7562	F (1, 88) = 0.09703		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary Adjusted P Value
FA-GFP VEH vs. NA-GFP VEH	2.163	-32.30 to 36.63	No	ns >0.9999
FA-GFP CNO vs. NA-GFP CNO	-22.91	-57.37 to 11.56	No	ns 0.4886
FA-Gi VEH vs. NA-Gi VEH	-20.25	-61.03 to 20.53	No	ns 0.8552
FA-Gi CNO vs. NA-Gi CNO	-53.33	-94.11 to -12.55	Yes	** 0.0028
NA-GFP VEH vs. NA-GFP CNO	-19.84	-54.31 to 14.62	No	ns 0.6969
NA-Gi VEH vs. NA-Gi CNO	-33.12	-73.90 to 7.660	No	ns 0.2088
<u>Distance Traveled Open Field (ED Figure 4g)</u>	n=GFP (VEH, CNO): 15; Gi (VEH, CNO): 11			
Two-way ANOVA				
ANOVA table	P value	F (Dfn, DFd)		
Interaction	P>0.9999	F (42, 810) = 0.3345		
minute	P=0.4736	F (14, 810) = 0.9784		
group	P=0.0002	F (3, 810) = 6.754		

Sidak Post Test	P value
0:00-1:00	
GFP VEH vs. GFP CNO	0.9948
GFP VEH vs. GI VEH	0.9989
GFP VEH vs. GI CNO	0.2124
GFP CNO vs. GI VEH	>0.9999
GFP CNO vs. GI CNO	0.5082
GI VEH vs. GI CNO	0.4205
1:00-2:00	
GFP VEH vs. GFP CNO	0.9999
GFP VEH vs. GI VEH	0.9998
GFP VEH vs. GI CNO	0.7087
GFP CNO vs. GI VEH	>0.9999
GFP CNO vs. GI CNO	0.8614
GI VEH vs. GI CNO	0.8687
2:00-3:00	
GFP VEH vs. GFP CNO	0.9992
GFP VEH vs. GI VEH	0.9982
GFP VEH vs. GI CNO	0.9019
GFP CNO vs. GI VEH	0.9482
GFP CNO vs. GI CNO	0.6761
GI VEH vs. GI CNO	0.9921
3:00-4:00	
GFP VEH vs. GFP CNO	0.9985
GFP VEH vs. GI VEH	0.8583
GFP VEH vs. GI CNO	0.9997
GFP CNO vs. GI VEH	0.986
GFP CNO vs. GI CNO	>0.9999
GI VEH vs. GI CNO	0.986
4:00-5:00	
GFP VEH vs. GFP CNO	0.9747
GFP VEH vs. GI VEH	0.9897
GFP VEH vs. GI CNO	0.918
GFP CNO vs. GI VEH	0.6627
GFP CNO vs. GI CNO	0.4683
GI VEH vs. GI CNO	0.9992
5:00-6:00	
GFP VEH vs. GFP CNO	0.9147
GFP VEH vs. GI VEH	0.8873
GFP VEH vs. GI CNO	0.5528
GFP CNO vs. GI VEH	>0.9999
GFP CNO vs. GI CNO	0.9847
GI VEH vs. GI CNO	0.9906
6:00-7:00	
GFP VEH vs. GFP CNO	0.9992
GFP VEH vs. GI VEH	0.7856
GFP VEH vs. GI CNO	0.7527
GFP CNO vs. GI VEH	0.9563
GFP CNO vs. GI CNO	0.931
GI VEH vs. GI CNO	>0.9999
7:00-8:00	
GFP VEH vs. GFP CNO	0.9831
GFP VEH vs. GI VEH	0.9257
GFP VEH vs. GI CNO	0.9984
GFP CNO vs. GI VEH	>0.9999
GFP CNO vs. GI CNO	>0.9999
GI VEH vs. GI CNO	0.999
8:00-9:00	
GFP VEH vs. GFP CNO	0.9929
GFP VEH vs. GI VEH	0.8593
GFP VEH vs. GI CNO	0.9825
GFP CNO vs. GI VEH	0.9964
GFP CNO vs. GI CNO	>0.9999
GI VEH vs. GI CNO	0.9998
9:00-10:00	
GFP VEH vs. GFP CNO	>0.9999
GFP VEH vs. GI VEH	0.8624
GFP VEH vs. GI CNO	0.9997
GFP CNO vs. GI VEH	0.792
GFP CNO vs. GI CNO	0.9987
GI VEH vs. GI CNO	0.9863
10:00-11:00	
GFP VEH vs. GFP CNO	0.7829
GFP VEH vs. GI VEH	0.885
GFP VEH vs. GI CNO	0.5366
GFP CNO vs. GI VEH	>0.9999
GFP CNO vs. GI CNO	0.998
GI VEH vs. GI CNO	0.9891
11:00-12:00	
GFP VEH vs. GFP CNO	>0.9999
GFP VEH vs. GI VEH	>0.9999
GFP VEH vs. GI CNO	0.9825
GFP CNO vs. GI VEH	>0.9999
GFP CNO vs. GI CNO	0.9956
GI VEH vs. GI CNO	0.9919
12:00-13:00	
GFP VEH vs. GFP CNO	0.9302
GFP VEH vs. GI VEH	0.1359
GFP VEH vs. GI CNO	0.9188
GFP CNO vs. GI VEH	0.6802
GFP CNO vs. GI CNO	>0.9999
GI VEH vs. GI CNO	0.845
13:00-14:00	
GFP VEH vs. GFP CNO	0.9582
GFP VEH vs. GI VEH	0.538
GFP VEH vs. GI CNO	0.9738
GFP CNO vs. GI VEH	0.9747
GFP CNO vs. GI CNO	>0.9999
GI VEH vs. GI CNO	0.9852
14:00-15:00	
GFP VEH vs. GFP CNO	0.9981
GFP VEH vs. GI VEH	0.8733
GFP VEH vs. GI CNO	0.9439
GFP CNO vs. GI VEH	0.9907
GFP CNO vs. GI CNO	0.9977
GI VEH vs. GI CNO	>0.9999

Time in the Center Open Field (ED Figure 4f)

Two-way ANOVA
ANOVA table
Interaction
Treatment
Group

n=GFP (VEH, CNO): 15; Gi (VEH, CNO): 11

P value
P=0.8460
P=0.4042
P=0.4185
F (Dfn, DFd)
F (1, 42) = 0.03818
F (1, 42) = 0.7100
F (1, 42) = 0.6677

Sidak Post Test

VEH:GFP vs. VEH:Gi
VEH:GFP vs. CNO:GFP
VEH:GFP vs. CNO:Gi
VEH:Gi vs. CNO:GFP
VEH:Gi vs. CNO:Gi
CNO:GFP vs. CNO:Gi

P value
0.998
0.958
>0.9999
0.8038
0.9989
0.9839

Elevated Plus Maze Time in the Open Arm (ED Figure 4e)

Two-way ANOVA
ANOVA table
Interaction
Row Factor
Column Factor

n=15 per group

P value
P=0.4303
P=0.8050
P=0.4707
F (Dfn, DFd)
F (1, 56) = 0.6312
F (1, 56) = 0.06155
F (1, 56) = 0.5274

Sidak Post Test

VEH:Gi vs. VEH:GFP
VEH:Gi vs. CNO:Gi
VEH:Gi vs. CNO:GFP
VEH:GFP vs. CNO:Gi
VEH:GFP vs. CNO:GFP
CNO:Gi vs. CNO:GFP

P value
0.8684
0.9763
0.9832
0.9997
0.9993
>0.9999

Elevated Plus Maze Distance Traveled (ED Figure 4e)

Two-way ANOVA
ANOVA table
Interaction
Treatment
Group

n=15 per group

P value
P<0.0001
P=0.9692
P=0.2359
F (Dfn, DFd)
F (1, 56) = 42.20
F (1, 56) = 0.001503
F (1, 56) = 1.435

Sidak Post Test

VEH:Gi vs. VEH:GFP
VEH:Gi vs. CNO:Gi
VEH:Gi vs. CNO:GFP
VEH:GFP vs. CNO:Gi
VEH:GFP vs. CNO:GFP
CNO:Gi vs. CNO:GFP

P value
-0.0001
0.0001
0.9462
0.9603
0.0002
0.0026

Rotarod (ED Figure 4h)

Two-way ANOVA
ANOVA table
Interaction
Trial
Group

n=10 per group

P value
P=0.7202
P<0.0001
P<0.0001
F (Dfn, DFd)
F (12, 180) = 0.7306
F (4, 180) = 13.50
F (3, 180) = 10.07

Sidak Post Test

Day 1
Gi VEH vs. Gi CNO
Gi VEH vs. GFP VEH
Gi VEH vs. GFP CNO
Gi CNO vs. GFP VEH
Gi CNO vs. GFP CNO
GFP VEH vs. GFP CNO

P value
0.9581
>0.9999
>0.9999
0.9331
0.9942
0.9996

Day 2

Gi VEH vs. Gi CNO
Gi VEH vs. GFP VEH
Gi VEH vs. GFP CNO
Gi CNO vs. GFP VEH
Gi CNO vs. GFP CNO
GFP VEH vs. GFP CNO

0.5305
0.967
0.9999
0.9671
0.3336
0.8678

Day 3

Gi VEH vs. Gi CNO
Gi VEH vs. GFP VEH
Gi VEH vs. GFP CNO
Gi CNO vs. GFP VEH
Gi CNO vs. GFP CNO
GFP VEH vs. GFP CNO

0.0283
0.9926
0.6986
0.1369
0.5705
0.9724

Day 4

Gi VEH vs. Gi CNO
Gi VEH vs. GFP VEH
Gi VEH vs. GFP CNO
Gi CNO vs. GFP VEH
Gi CNO vs. GFP CNO
GFP VEH vs. GFP CNO

0.0199
0.957
0.5449
0.1841
0.6391
0.9784

Day 5

Gi VEH vs. Gi CNO
Gi VEH vs. GFP VEH
Gi VEH vs. GFP CNO
Gi CNO vs. GFP VEH
Gi CNO vs. GFP CNO
GFP VEH vs. GFP CNO

0.0024
0.9301
0.1129
0.0471
0.7615
0.6342

ChR2 Thal-mPFCSingle Unit Frequency PAC with ChR2 mPFC Stimulation (ED Figure 6b)

Two-way ANOVA
ANOVA table
Interaction
Stimulation
Group

n= 7 per group

P value
P=0.6315
P=0.4189
P=0.5266
F (Dfn, DFd)
F (1, 24) = 0.2361
F (1, 24) = 0.6764
F (1, 24) = 0.4129

Sidak Post Test

4 Hz:GFP vs. 4 Hz:ChR2
4 Hz:GFP vs. 20 Hz:GFP
4 Hz:GFP vs. 20 Hz:ChR2
4 Hz:ChR2 vs. 20 Hz:GFP
4 Hz:ChR2 vs. 20 Hz:ChR2
20 Hz:GFP vs. 20 Hz:ChR2

P value
>0.9999
>0.9999
0.8926
>0.9999
0.9339
0.9667

Social Approach: Three way ANOVA (20 Hz) (ED Figure 6c)

Mixed-effects model (REML)

n=GFP (Laser OFF, ON):17; ChR2 (Laser OFF, ON): 14

P value
<0.0001
0.522
0.0052
0.3082
0.0033
0.6439
0.0046
F (Dfn, DFd)
F (1, 74) = 31.27
F (1, 74) = 0.4139
F (1, 60) = 8.416
F (1, 74) = 1.053
F (1, 60) = 9.387
F (1, 60) = 0.2158
F (1, 60) = 8.676

Fixed effects (type III)
NA vs NO
ChR2 vs GFP
Laser Off vs 20 Hz
NA vs NO x ChR2 vs GFP
NA vs NO x Laser Off vs 20 Hz
ChR2 vs GFP x Laser Off vs 20 Hz
NA vs NO x ChR2 vs GFP x Laser Off vs 20 Hz

Sidak's multiple comparisons test		Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
animal :Chr2 (Off) vs. object :Chr2 (Off)	83.09	32.46 to 133.7	Yes	****	<0.0001	
animal :Chr2 (20 Hz) vs. object :Chr2 (20 Hz)	0.7899	-48.36 to 49.94	No	ns	>0.9999	
animal :GFP (Off) vs. object :GFP (Off)	61.6	16.47 to 106.7	Yes	**	0.0014	
animal :GFP (20 Hz) vs. object :GFP (20 Hz)	59.98	16.84 to 103.1	Yes	**	0.0011	
animal :Chr2 (Off) vs. animal :Chr2 (20Hz)	64.2	20.67 to 107.7	Yes	***	0.0006	
animal :GFP (Off) vs. animal :GFP (20 Hz)	17.5	-20.15 to 55.14	No	ns	0.8968	
<u>Social Novelty: Three way ANOVA (20 Hz) (ED Figure 6d)</u>		n=GFP (Laser Off, ON):17; Chr2 (Laser Off, ON): 14				
Mixed-effects model (REML)						
Fixed effects (type III)	P value	F (DFn, DFd)				
FA vs NA	<0.0001	F (1, 70) = 20.06				
Chr2 vs GFP	0.7255	F (1, 70) = 0.1243				
Laser Off vs Laser On	0.0297	F (1, 60) = 4.959				
FA vs NA x Chr2 vs GFP	0.0733	F (1, 70) = 3.306				
FA vs NA x Laser Off vs Laser On	0.0001	F (1, 60) = 16.41				
Chr2 vs GFP x Laser Off vs Laser On	0.5004	F (1, 60) = 0.4596				
FA vs NA x Chr2 vs GFP x Laser Off vs Laser On	0.022	F (1, 60) = 5.525				
Sidak's multiple comparisons test		Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Familiar Animal :Chr2 (Off) vs. Novel Animal :Chr2 (Off)	-70.75	-120.9 to -20.62	Yes	***	0.0008	
Familiar Animal :Chr2 (20 Hz) vs. Novel Animal :Chr2 (20 Hz)	24.52	-24.06 to 73.10	No	ns	0.846	
Familiar Animal :GFP (Off) vs. Novel Animal :GFP (Off)	-67.36	-113.1 to -21.59	Yes	***	0.0004	
Familiar Animal :GFP (20 Hz) vs. Novel Animal :GFP (20 Hz)	-42.06	-85.54 to 1.428	No	ns	0.0659	
Novel animal :Chr2 (Off) vs. Novel animal :Chr2 (20Hz)	69.26	22.79 to 115.7	Yes	***	0.0005	
Novel animal :GFP (Off) vs. Novel animal :GFP (20 Hz)	24.18	-17.67 to 66.03	No	ns	0.6827	
<u>Social Approach: Three way ANOVA (4 Hz) (ED Figure 6c)</u>		n=GFP (Laser Off, ON):17; Chr2 (Laser Off, ON): 14				
Mixed-effects model (REML)						
Fixed effects (type III)	P value	F (DFn, DFd)				
NA vs NO	<0.0001	F (1, 72) = 22.70				
Chr2 vs GFP	0.6103	F (1, 72) = 0.2621				
Laser Off vs Laser On	0.0522	F (1, 58) = 3.930				
NA vs NO x Chr2 vs GFP	0.5115	F (1, 72) = 0.4353				
NA vs NO x Laser Off vs Laser On	0.0137	F (1, 58) = 6.461				
Chr2 vs GFP x Laser Off vs Laser On	0.5011	F (1, 58) = 0.4584				
NA vs NO x Chr2 vs GFP x Laser Off vs Laser On	0.0396	F (1, 58) = 4.431				
Sidak's multiple comparisons test		Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
animal :Chr2 (Off) vs. object :Chr2 (Off)	82.73	23.09 to 142.4	Yes	**	0.0011	
animal :Chr2 (4 Hz) vs. object :Chr2 (4 Hz)	5.489	-52.40 to 63.37	No	ns	>0.9999	
animal :GFP (Off) vs. object :GFP (Off)	61.92	9.068 to 114.8	Yes	*	0.0104	
animal :GFP (4Hz) vs. object :GFP (4Hz)	54.66	1.808 to 107.5	Yes	*	0.0372	
animal :Chr2 (Off) vs. animal : Chr2 (4Hz)	60.73	9.227 to 112.2	Yes	*	0.0105	
animal :GFP (Off) vs. animal :GFP (4Hz)	14.48	-32.84 to 61.80	No	ns	0.9958	
<u>Social Novelty: Three way ANOVA (4 Hz) (ED Figure 6d)</u>		n=GFP (Laser Off, ON):17; Chr2 (Laser Off, ON): 14				
Mixed-effects model (REML)						
Fixed effects (type III)	P value	F (DFn, DFd)				
FA vs NA	<0.0001	F (1, 70) = 22.81				
Chr2 vs GFP	0.7174	F (1, 70) = 0.1321				
Laser Off vs 20 Hz	0.0077	F (1, 56) = 7.654				
FA vs NA x Chr2 vs GFP	0.204	F (1, 70) = 1.644				
FA vs NA x Laser Off vs 20 Hz	0.0095	F (1, 56) = 7.221				
Chr2 vs GFP x Laser Off vs 20 Hz	0.1228	F (1, 56) = 2.455				
FA vs NA x Chr2 vs GFP x Laser Off vs 20 Hz	0.0652	F (1, 56) = 3.538				
Sidak's multiple comparisons test		Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Familiar Animal :Chr2 (Off) vs. Novel Animal :Chr2 (Off)	-70.61	-125.5 to -15.73	Yes	**	0.0033	
Familiar Animal :Chr2 (4 Hz) vs. Novel Animal :Chr2 (4 Hz)	1.612	-51.63 to 54.85	No	ns	>0.9999	
Familiar Animal :GFP (Off) vs. Novel Animal :GFP (Off)	-66.2	-116.2 to -16.20	Yes	**	0.0022	
Familiar Animal :GFP (4 Hz) vs. Novel Animal :GFP (4 Hz)	-53.45	-103.4 to -3.458	Yes	*	0.0272	
Novel Animal :Chr2 (Off) vs. Novel Animal : Chr2 (4Hz)	70.37	21.90 to 118.8	Yes	***	0.0008	
Novel Animal :GFP (Off) vs. Novel Animal :GFP (4Hz)	15.86	-29.85 to 61.56	No	ns	0.9875	
<u>Distance Traveled Open Field (ED Figure 6f)</u>		n=10 per group				
Two-way ANOVA						
ANOVA table	P value	F (DFn, DFd)				
Interaction	P>0.9999	F (42, 540) = 0.3635				
Minute	P=0.0792	F (14, 540) = 1.584				
Group	P<0.0001	F (3, 540) = 10.36				
Sidak Post Test		P value				
0:00-1:00						
Chr2 laser Off vs. Chr2 Laser On	0.9999					
Chr2 laser Off vs. GFP Laser Off	0.9524					
Chr2 laser Off vs. GFP Laser On	0.9262					
Chr2 Laser On vs. GFP Laser Off	0.8313					
Chr2 Laser On vs. GFP Laser On	0.7798					
GFP Laser Off vs. GFP Laser On	>0.9999					
1:00-2:00						
Chr2 laser Off vs. Chr2 Laser On	0.5178					
Chr2 laser Off vs. GFP Laser Off	>0.9999					
Chr2 laser Off vs. GFP Laser On	0.9999					
Chr2 Laser On vs. GFP Laser Off	0.7105					
Chr2 Laser On vs. GFP Laser On	0.7291					
GFP Laser Off vs. GFP Laser On	>0.9999					
2:00-3:00						
Chr2 laser Off vs. Chr2 Laser On	0.9969					
Chr2 laser Off vs. GFP Laser Off	0.7801					
Chr2 laser Off vs. GFP Laser On	0.9993					
Chr2 Laser On vs. GFP Laser Off	0.4177					
Chr2 Laser On vs. GFP Laser On	0.9417					
GFP Laser Off vs. GFP Laser On	0.9552					
3:00-4:00						
Chr2 laser Off vs. Chr2 Laser On	0.9562					
Chr2 laser Off vs. GFP Laser Off	>0.9999					
Chr2 laser Off vs. GFP Laser On	0.9986					
Chr2 Laser On vs. GFP Laser Off	0.8603					
Chr2 Laser On vs. GFP Laser On	0.7511					
GFP Laser Off vs. GFP Laser On	>0.9999					
4:00-5:00						
Chr2 laser Off vs. Chr2 Laser On	>0.9999					
Chr2 laser Off vs. GFP Laser Off	0.9934					
Chr2 laser Off vs. GFP Laser On	0.8822					

ChR2 Laser On vs. GFP Laser Off 0.9951
ChR2 Laser On vs. GFP Laser On 0.8962
GFP Laser Off vs. GFP Laser On 0.9979

5:00-6:00
ChR2 laser Off vs. ChR2 Laser On 0.9984
ChR2 laser Off vs. GFP Laser Off 0.797
ChR2 laser Off vs. GFP Laser On 0.2237
ChR2 Laser On vs. GFP Laser Off 0.9734
ChR2 Laser On vs. GFP Laser On 0.5024
GFP Laser Off vs. GFP Laser On 0.9508

6:00-7:00
ChR2 laser Off vs. ChR2 Laser On 0.9963
ChR2 laser Off vs. GFP Laser Off 0.995
ChR2 laser Off vs. GFP Laser On 0.9999
ChR2 Laser On vs. GFP Laser Off 0.8713
ChR2 Laser On vs. GFP Laser On 0.9658
GFP Laser Off vs. GFP Laser On >0.9999

7:00-8:00
ChR2 laser Off vs. ChR2 Laser On 0.7579
ChR2 laser Off vs. GFP Laser Off 0.9358
ChR2 laser Off vs. GFP Laser On >0.9999
ChR2 Laser On vs. GFP Laser Off 0.9996
ChR2 Laser On vs. GFP Laser On 0.5652
GFP Laser Off vs. GFP Laser On 0.8131

8:00-9:00
ChR2 laser Off vs. ChR2 Laser On 0.7326
ChR2 laser Off vs. GFP Laser Off >0.9999
ChR2 laser Off vs. GFP Laser On 0.925
ChR2 Laser On vs. GFP Laser Off 0.7318
ChR2 Laser On vs. GFP Laser On 0.1489
GFP Laser Off vs. GFP Laser On 0.9255

9:00-10:00
ChR2 laser Off vs. ChR2 Laser On 0.9926
ChR2 laser Off vs. GFP Laser Off 0.7508
ChR2 laser Off vs. GFP Laser On 0.1462
ChR2 Laser On vs. GFP Laser Off 0.9839
ChR2 Laser On vs. GFP Laser On 0.469
GFP Laser Off vs. GFP Laser On 0.9123

10:00-11:00
ChR2 laser Off vs. ChR2 Laser On >0.9999
ChR2 laser Off vs. GFP Laser Off 0.9942
ChR2 laser Off vs. GFP Laser On 0.3696
ChR2 Laser On vs. GFP Laser Off 0.9665
ChR2 Laser On vs. GFP Laser On 0.2376
GFP Laser Off vs. GFP Laser On 0.773

11:00-12:00
ChR2 laser Off vs. ChR2 Laser On 0.9314
ChR2 laser Off vs. GFP Laser Off >0.9999
ChR2 laser Off vs. GFP Laser On >0.9999
ChR2 Laser On vs. GFP Laser Off 0.9574
ChR2 Laser On vs. GFP Laser On 0.9645
GFP Laser Off vs. GFP Laser On >0.9999

12:00-13:00
ChR2 laser Off vs. ChR2 Laser On 0.9906
ChR2 laser Off vs. GFP Laser Off 0.9555
ChR2 laser Off vs. GFP Laser On 0.9567
ChR2 Laser On vs. GFP Laser Off 0.6196
ChR2 Laser On vs. GFP Laser On 0.623
GFP Laser Off vs. GFP Laser On >0.9999

13:00-14:00
ChR2 laser Off vs. ChR2 Laser On 0.9148
ChR2 laser Off vs. GFP Laser Off 0.9964
ChR2 laser Off vs. GFP Laser On 0.9945
ChR2 Laser On vs. GFP Laser Off 0.9983
ChR2 Laser On vs. GFP Laser On 0.5673
GFP Laser Off vs. GFP Laser On 0.869

14:00-15:00
ChR2 laser Off vs. ChR2 Laser On 0.7073
ChR2 laser Off vs. GFP Laser Off 0.9926
ChR2 laser Off vs. GFP Laser On 0.9992
ChR2 Laser On vs. GFP Laser Off 0.975
ChR2 Laser On vs. GFP Laser On 0.4162
GFP Laser Off vs. GFP Laser On 0.9073

Time in the Center Open Field (ED Figure 6g)

Two-way ANOVA	n=10 per group		
ANOVA table	P value	F (Dfn, Dfd)	
Interaction	P=0.9672	F (1, 36) = 0.001717	
Treatment	P=0.6082	F (1, 36) = 0.2674	
Group	P=0.8433	F (1, 36) = 0.03967	
Sidak Post Test	P value		
Laser Off:ChR2 vs. Laser Off:GFP	>0.9999		
Laser Off:ChR2 vs. 20 Hz:ChR2	0.9992		
Laser Off:ChR2 vs. 20 Hz:GFP	>0.9999		
Laser Off:GFP vs. 20 Hz:ChR2	0.9968		
Laser Off:GFP vs. 20 Hz:GFP	0.9997		
20 Hz:ChR2 vs. 20 Hz:GFP	>0.9999		

Elevated Plus Maze Time in the Open Arm (ED Figure 6e)

Two-way ANOVA	n=GFP (Laser OFF, ON):12; ChR2 (Laser OFF, ON): 10		
ANOVA table	P value	F (Dfn, Dfd)	
Interaction	P=0.4347	F (1, 40) = 0.6227	
Treatment	P=0.4341	F (1, 40) = 0.7813	
Group	P=0.3043	F (1, 40) = 1.083	
Sidak Post Test	P value		
Laser Off:ChR2 vs. Laser Off:GFP	0.744		
Laser Off:ChR2 vs. 20 Hz:ChR2	0.8734		
Laser Off:ChR2 vs. 20 Hz:GFP	0.7435		
Laser Off:GFP vs. 20 Hz:ChR2	>0.9999		
Laser Off:GFP vs. 20 Hz:GFP	>0.9999		
20 Hz:ChR2 vs. 20 Hz:GFP	>0.9999		

Elevated Plus Maze Total Distance Traveled (ED Figure 6e)

Two-way ANOVA	n=GFP (Laser OFF, ON):12; ChR2 (Laser OFF, ON): 10		
ANOVA table	P value	F (Dfn, Dfd)	
Interaction	P=0.9316	F (1, 40) = 0.007462	
Treatment	P=0.3820	F (1, 40) = 0.7813	
Group	P=0.6226	F (1, 40) = 0.2460	

Sidak Post Test	P value				
Laser Off:ChR2 vs. Laser Off:GFP		0.9999			
Laser Off:ChR2 vs. 20 Hz:ChR2		0.987			
Laser Off:ChR2 vs. 20 Hz:GFP		>0.9999			
Laser Off:GFP vs. 20 Hz:ChR2		0.9136			
Laser Off:GFP vs. 20 Hz:GFP		0.9925			
20 Hz:ChR2 vs. 20 Hz:GFP		0.999			
Single Unit Frequency in Parietal Association Cortex- mPFC ChR2 (ED Figure 6b)					
Two-way ANOVA	n=7 per group				
ANOVA table	P value		F (DFn, DFd)		
Interaction		P=0.6315	F (1, 24) = 0.2361		
Stimulation		P=0.4189	F (1, 24) = 0.6764		
Group		P=0.5266	F (1, 24) = 0.4129		
Sidak Post Test	P value				
20 Hz:GFP vs. 20 Hz:ChR2		0.9667			
20 Hz:GFP vs. 4 Hz:GFP		>0.9999			
20 Hz:GFP vs. 4 Hz:ChR2		>0.9999			
20 Hz:ChR2 vs. 4 Hz:GFP		0.8926			
20 Hz:ChR2 vs. 4 Hz:ChR2		0.9339			
4 Hz:GFP vs. 4 Hz:ChR2		>0.9999			
Arch Rescue Tsc1 PC Mutant					
Social Approach: 3 way ANOVA (20 Hz) (ED Figure 7a)					
Mixed-effects model (REML)	n=GFP (Laser OFF, ON):13; Arch (Laser OFF, ON): 17				
Fixed effects (type III)	P value		F (DFn, DFd)		
NA vs NO		0.3014	F (1, 132) = 1.076		
GFP vs Arch		0.2446	F (1, 132) = 1.366		
Laser Off vs Laser On		0.0515	F (1, 132) = 3.862		
NA vs NO x GFP vs Arch		0.0366	F (1, 132) = 4.461		
NA vs NO x Laser Off vs Laser On		0.0326	F (1, 132) = 4.662		
GFP vs Arch x Laser Off vs Laser On		0.0054	F (1, 132) = 8.001		
NA vs NO x GFP vs Arch x Laser Off vs Laser On		0.3521	F (1, 132) = 0.8720		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Novel Animal :GFP (Off) vs. Novel Object :GFP (Off)	-21.39	-79.21 to 36.44	No	ns	0.9819
Novel Animal :GFP (20 Hz) vs. Novel Object :GFP (20 Hz)	1.403	-56.42 to 59.23	No	ns	>0.9999
Novel Animal :Arch (Off) vs. Novel Object :Arch (Off)	0.5265	-49.55 to 50.60	No	ns	>0.9999
Novel Animal :Arch (20 Hz) vs. Novel Object :Arch (20 Hz)	58.05	7.973 to 108.1	Yes	*	0.0117
Novel Animal :GFP (Off) vs. Novel Animal :GFP (20Hz)	-3.365	-61.19 to 54.46	No	ns	>0.9999
Novel Animal : Arch (Off) vs. Novel Animal : Arch (20Hz)	-73.34	-123.4 to -23.26	Yes	***	0.0005
Social Novelty: 3 way ANOVA (20 Hz) (ED Figure 7b)					
Mixed-effects model (REML)	n=GFP (Laser OFF, ON):13; Arch (Laser OFF, ON): 17				
Fixed effects (type III)	P value		F (DFn, DFd)		
FA vs NA		0.0567	F (1, 134) = 3.694		
GFP vs Arch		0.0656	F (1, 134) = 3.446		
Laser Off vs 20 Hz		0.2553	F (1, 134) = 1.305		
FA vs NA x GFP vs Arch		0.0616	F (1, 134) = 3.552		
FA vs NA x Laser Off vs 20 Hz		0.051	F (1, 134) = 3.877		
GFP vs Arch x Laser Off vs 20 Hz		0.4318	F (1, 134) = 0.6218		
FA vs NA x GFP vs Arch x Laser Off vs 20 Hz		0.1176	F (1, 134) = 2.480		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Familiar Animal :GFP (Off) vs. Novel Animal :GFP (Off)	2.7	-44.80 to 50.20	No	ns	>0.9999
Familiar Animal :GFP (20 Hz) vs. Novel Animal :GFP (20 Hz)	-3.268	-49.26 to 42.72	No	ns	>0.9999
Familiar Animal :Arch (Off) vs. Novel Animal :Arch (Off)	-1.991	-43.12 to 39.14	No	ns	>0.9999
Familiar Animal :Arch (20 Hz) vs. Novel Animal :Arch (20 Hz)	-55.66	-96.79 to -14.52	Yes	**	0.0016
Novel Animal :GFP (Off) vs. Novel Animal :GFP (20Hz)	-5.664	-52.41 to 41.08	No	ns	>0.9999
Novel Animal : ChR2 (Off) vs. Novel Animal : ChR2 (20Hz)	-41.46	-82.59 to -0.3219	Yes	*	0.0468
Social Approach: 3 way ANOVA (4 Hz) (ED Figure 7a)					
Mixed-effects model (REML)	n=GFP (Laser OFF, ON):13; Arch (Laser OFF, ON): 17				
Fixed effects (type III)	P value		F (DFn, DFd)		
NA vs NO		0.316	F (1, 70) = 1.020		
GFP vs Arch		0.0014	F (1, 70) = 11.13		
Laser Off vs laser On		0.1089	F (1, 62) = 2.646		
NA vs NO x GFP vs Arch		0.0054	F (1, 70) = 8.250		
NA vs NO x Laser Off vs laser On		0.0092	F (1, 62) = 7.235		
GFP vs Arch x Laser Off vs laser On		0.0529	F (1, 62) = 3.894		
NA vs NO x GFP vs Arch x Laser Off vs laser On		0.1451	F (1, 62) = 2.178		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Novel Animal :GFP Off vs. Novel Object :GFP Off	-21.42	-64.36 to 21.52	No	ns	0.8561
Novel Animal :GFP 4 Hz vs. Novel Object :GFP 4 Hz	-5.414	-48.35 to 37.52	No	ns	>0.9999
Novel Animal :Arch Off vs. Novel Object :Arch Off	0.508	-36.68 to 37.69	No	ns	>0.9999
Novel Animal :Arch 4 Hz vs. Novel Object :Arch 4 Hz	55.43	18.25 to 92.62	Yes	***	0.0003
Novel Animal :GFP Off vs. Novel Animal :GFP 4 Hz	15.73	-26.11 to 57.57	No	ns	0.9767
Novel Animal :Arch Off vs. Novel Animal :Arch 4 Hz	-29.75	-65.95 to 6.456	No	ns	0.1924
Social Novelty: 3 way ANOVA (4 Hz) (ED Figure 7b)					
Mixed-effects model (REML)	n=GFP (Laser OFF, ON):13; Arch (Laser OFF, ON): 17				
Fixed effects (type III)	P value		F (DFn, DFd)		
FA vs NA		0.064	F (1, 134) = 3.487		
GFP vs Arch		0.0374	F (1, 134) = 4.422		
Laser Off vs Laser On		0.0542	F (1, 134) = 3.774		
FA vs NA x GFP vs Arch		0.0551	F (1, 134) = 3.743		
FA vs NA x Laser Off vs Laser On		0.0566	F (1, 134) = 3.699		
GFP vs Arch x Laser Off vs Laser On		0.2899	F (1, 134) = 1.129		
FA vs NA x GFP vs Arch x Laser Off vs Laser On		0.1202	F (1, 134) = 2.446		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
FA:GFP (Off) vs. NA:GFP (Off)	2.7	-36.83 to 42.23	No	ns	>0.9999
FA:GFP (4Hz) vs. NA:GFP (4Hz)	-1.847	-41.38 to 37.69	No	ns	>0.9999
FA:Arch (Off) vs. NA: Arch (Off)	-1.99	-36.23 to 32.25	No	ns	>0.9999
FA: Arch (4Hz) vs. NA: Arch (4Hz)	-46.12	-79.53 to -12.71	Yes	**	0.0012
NA: GFP (Off) vs. NA:GFP (4Hz)	16.74	-22.79 to 56.27	No	ns	0.9496
NA:Arch (Off) vs. NA: Arch (4Hz)	-16.5	-50.32 to 17.33	No	ns	0.874
Distance Traveled Open Field (ED Figure 7d)					
Two-way ANOVA	n=10 per group				
ANOVA table	P value		F (DFn, DFd)		
Interaction		P=0.9974	F (42, 540) = 0.4881		
Treatment		P=0.2119	F (14, 540) = 1.285		
Group		P<0.0001	F (3, 540) = 11.20		
Sidak Post Test	P value				

0:00-1:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	>0.9999
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.907
Mutant Arch Laser Off vs. GFP Laser Off	0.9912
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.8909
Mutant Arch 20 Hz vs. GFP Laser Off	0.988
Mutant GFP Laser On vs. GFP Laser Off	0.9994
1:00-2:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	>0.9999
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.8985
Mutant Arch Laser Off vs. GFP Laser Off	>0.9999
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.8452
Mutant Arch 20 Hz vs. GFP Laser Off	0.9995
Mutant GFP Laser On vs. GFP Laser Off	0.9733
2:00-3:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.9974
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9993
Mutant Arch Laser Off vs. GFP Laser Off	0.9164
Mutant Arch 20 Hz vs. Mutant GFP Laser On	>0.9999
Mutant Arch 20 Hz vs. GFP Laser Off	0.9976
Mutant GFP Laser On vs. GFP Laser Off	0.9933
3:00-4:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.9213
Mutant Arch Laser Off vs. Mutant GFP Laser On	>0.9999
Mutant Arch Laser Off vs. GFP Laser Off	0.6394
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.9586
Mutant Arch 20 Hz vs. GFP Laser Off	0.9976
Mutant GFP Laser On vs. GFP Laser Off	0.7286
4:00-5:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.9645
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9951
Mutant Arch Laser Off vs. GFP Laser Off	0.7922
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.6998
Mutant Arch 20 Hz vs. GFP Laser Off	0.999
Mutant GFP Laser On vs. GFP Laser Off	0.4015
5:00-6:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.8102
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9947
Mutant Arch Laser Off vs. GFP Laser Off	0.9515
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.4155
Mutant Arch 20 Hz vs. GFP Laser Off	0.9997
Mutant GFP Laser On vs. GFP Laser Off	0.6539
6:00-7:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.8744
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9959
Mutant Arch Laser Off vs. GFP Laser Off	0.1522
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.9957
Mutant Arch 20 Hz vs. GFP Laser Off	0.8162
Mutant GFP Laser On vs. GFP Laser Off	0.4375
7:00-8:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	>0.9999
Mutant Arch Laser Off vs. Mutant GFP Laser On	>0.9999
Mutant Arch Laser Off vs. GFP Laser Off	0.6991
Mutant Arch 20 Hz vs. Mutant GFP Laser On	>0.9999
Mutant Arch 20 Hz vs. GFP Laser Off	0.7923
Mutant GFP Laser On vs. GFP Laser Off	0.8487
8:00-9:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.4738
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9998
Mutant Arch Laser Off vs. GFP Laser Off	0.4766
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.2765
Mutant Arch 20 Hz vs. GFP Laser Off	>0.9999
Mutant GFP Laser On vs. GFP Laser Off	0.2786
9:00-10:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.8322
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9816
Mutant Arch Laser Off vs. GFP Laser Off	0.5149
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.9985
Mutant Arch 20 Hz vs. GFP Laser Off	0.9982
Mutant GFP Laser On vs. GFP Laser Off	0.9404
10:00-11:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.3237
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9998
Mutant Arch Laser Off vs. GFP Laser Off	0.9926
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.1675
Mutant Arch 20 Hz vs. GFP Laser Off	0.7421
Mutant GFP Laser On vs. GFP Laser Off	0.9373
11:00-12:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.428
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9997
Mutant Arch Laser Off vs. GFP Laser Off	0.7982
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.6709
Mutant Arch 20 Hz vs. GFP Laser Off	0.9964
Mutant GFP Laser On vs. GFP Laser Off	0.9475
12:00-13:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.6656
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9883
Mutant Arch Laser Off vs. GFP Laser Off	0.3687
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.974
Mutant Arch 20 Hz vs. GFP Laser Off	0.999
Mutant GFP Laser On vs. GFP Laser Off	0.8209
13:00-14:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.9919
Mutant Arch Laser Off vs. Mutant GFP Laser On	0.9994
Mutant Arch Laser Off vs. GFP Laser Off	0.9528
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.9144
Mutant Arch 20 Hz vs. GFP Laser Off	>0.9999
Mutant GFP Laser On vs. GFP Laser Off	0.785
14:00-15:00	
Mutant Arch Laser Off vs. Mutant Arch 20 Hz	0.0743
Mutant Arch Laser Off vs. Mutant GFP Laser On	>0.9999
Mutant Arch Laser Off vs. GFP Laser Off	0.9994
Mutant Arch 20 Hz vs. Mutant GFP Laser On	0.0982
Mutant Arch 20 Hz vs. GFP Laser Off	0.1889
Mutant GFP Laser On vs. GFP Laser Off	>0.9999

Time in the Center Open Field (ED Figure 7d)

n=10 per group

Two-way ANOVA					
ANOVA table	P value		F (Dfn, DFd)		
Interaction		P=0.9680	F (1, 36) = 0.001627		
Treatment		P=0.6120	F (1, 36) = 0.2618		
Group		P=0.3320	F (1, 36) = 0.9670		
Sidak Post Test	P value				
Laser Off :Arch vs. Laser Off :GFP		0.986			
Laser Off :Arch vs. 20 Hz:Arch		0.9993			
Laser Off :Arch vs. 20 Hz:GFP		0.9997			
Laser Off :GFP vs. 20 Hz:Arch		0.8798			
Laser Off :GFP vs. 20 Hz:GFP		0.9997			
20 Hz:Arch vs. 20 Hz:GFP		0.9788			
<u>Time in the Open Arm Elevated Plus Maze (ED Figure 7c)</u>	n=GFP (Laser OFF, ON):10; Arch (Laser OFF, ON): 14				
Two-way ANOVA					
ANOVA table	P value		F (Dfn, DFd)		
Interaction		P=0.5231	F (1, 44) = 0.4143		
Treatment		P=0.1081	F (1, 44) = 2.690		
Group		P=0.9886	F (1, 44) = 0.0002066		
Sidak Post Test	P value				
Laser Off :Arch vs. Laser Off :GFP		0.9984			
Laser Off :Arch vs. 20 Hz:Arch		0.4086			
Laser Off :Arch vs. 20 Hz:GFP		0.8311			
Laser Off :GFP vs. 20 Hz:Arch		0.8196			
Laser Off :GFP vs. 20 Hz:GFP		0.9874			
20 Hz:Arch vs. 20 Hz:GFP		0.998			
<u>Total Distance Traveled Elevated Plus Maze (ED Figure 7c)</u>	n=GFP (Laser OFF, ON):10; Arch (Laser OFF, ON): 14				
Two-way ANOVA					
ANOVA table	P value		F (Dfn, DFd)		
Interaction		P=0.0103	F (1, 44) = 7.194		
Treatment		P=0.1490	F (1, 44) = 2.157		
Group		P=0.0310	F (1, 44) = 4.968		
Sidak Post Test	P value				
Laser Off :Arch vs. Laser Off :GFP		0.007			
Laser Off :Arch vs. 20 Hz:Arch		0.0146			
Laser Off :Arch vs. 20 Hz:GFP		0.0709			
Laser Off :GFP vs. 20 Hz:Arch		0.9955			
Laser Off :GFP vs. 20 Hz:GFP		0.9661			
20 Hz:Arch vs. 20 Hz:GFP		0.9998			
<u>Vermis Gi</u>					
<u>Social Approach: 3 way ANOVA (ED Figure 9b)</u>	n=GFP:12; Gi:15				
Mixed-effects model (REML)					
Fixed effects (type III)	P value		F (Dfn, DFd)		
NA vs NO		<0.0001	F (1, 70) = 68.72		
GFP vs Gi		0.6843	F (1, 70) = 0.1668		
VEH vs CNO		0.0855	F (1, 38) = 3.117		
NA vs NO x GFP vs Gi		0.0108	F (1, 70) = 6.858		
NA vs NO x VEH vs CNO		0.0085	F (1, 38) = 7.703		
GFP vs Gi x VEH vs CNO		0.0895	F (1, 38) = 3.036		
NA vs NO x GFP vs Gi x VEH vs CNO		0.0684	F (1, 38) = 3.518		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Novel Animal:GFP (VEH) vs. Novel Object:GFP (VEH)	134.3	63.69 to 204.8	Yes	****	<0.0001
Novel Animal:GFP (CNO) vs. Novel Object:GFP (CNO)	116.6	53.78 to 179.4	Yes	****	<0.0001
Novel Animal:Gi (VEH) vs. Novel Object:Gi (VEH)	110.9	53.98 to 167.8	Yes	****	<0.0001
Novel Animal:Gi (CNO) vs. Novel Object:Gi (CNO)	19.51	-39.09 to 78.11	No	ns	0.9923
Novel Animal:GFP (VEH) vs. Novel Animal:GFP (CNO)	9.065	-54.85 to 72.98	No	ns	>0.9999
Novel Animal:Gi (VEH) vs. Novel Animal:Gi (CNO)	80.15	24.92 to 135.4	Yes	***	0.001
<u>Social Novelty: 3 way ANOVA (ED Figure 9c)</u>	n=GFP:12; Gi:15				
Mixed-effects model (REML)					
Fixed effects (type III)	P value		F (Dfn, DFd)		
FA vs NA		<0.0001	F (1, 74) = 63.17		
GFP vs Gi		0.2776	F (1, 74) = 1.196		
VEH vs CNO		0.0939	F (1, 40) = 2.944		
FA vs NA x GFP vs Gi		0.0016	F (1, 74) = 10.73		
FA vs NA x VEH vs CNO		0.0015	F (1, 40) = 11.55		
GFP vs Gi x VEH vs CNO		0.1034	F (1, 40) = 2.778		
FA vs NA x GFP vs Gi x VEH vs CNO		0.193	F (1, 40) = 1.753		
Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Familiar Animal:GFP (VEH) vs. Novel Animal:GFP (VEH)	-113	-169.0 to -57.10	Yes	****	<0.0001
Familiar Animal:GFP (CNO) vs. Novel Animal:GFP (CNO)	-79.6	-129.2 to -30.00	Yes	****	<0.0001
Familiar Animal:Gi (VEH) vs. Novel Animal:Gi (VEH)	-78.14	-123.1 to -33.14	Yes	****	<0.0001
Familiar Animal:Gi (CNO) vs. Novel Animal:Gi (CNO)	-2.059	-44.63 to 40.51	No	ns	>0.9999
Novel Animal:GFP (VEH) vs. Novel Animal:GFP (CNO)	17.11	-36.05 to 70.27	No	ns	0.9926
Novel Animal:Gi (VEH) vs. Novel Animal:Gi (CNO)	65.28	21.23 to 109.3	Yes	***	0.0007
<u>Distance Traveled Open Field (ED Figure 9f)</u>	n= 15 per group				
Ordinary					
ANOVA table	P value		F (Dfn, DFd)		
Interaction		P=0.9996	F (42, 900) = 0.4195		
Minute		P<0.0001	F (14, 900) = 4.756		
Group		P<0.0001	F (3, 900) = 15.90		
Sidak Post Test	P value				
0:00-1:00					
GFP (VEH) vs. GFP (CNO)		0.9998			
GFP (VEH) vs. Gi (VEH)		0.9268			
GFP (VEH) vs. Gi (CNO)		0.9957			
GFP (CNO) vs. Gi (VEH)		0.746			
GFP (CNO) vs. Gi (CNO)		>0.9999			
Gi (VEH) vs. Gi (CNO)		0.588			
1:00-2:00					
GFP (VEH) vs. GFP (CNO)		0.8296			
GFP (VEH) vs. Gi (VEH)		0.0496			
GFP (VEH) vs. Gi (CNO)		0.862			
GFP (CNO) vs. Gi (VEH)		0.5157			
GFP (CNO) vs. Gi (CNO)		>0.9999			
Gi (VEH) vs. Gi (CNO)		0.4716			
2:00-3:00					
GFP (VEH) vs. GFP (CNO)		0.9999			
GFP (VEH) vs. Gi (VEH)		0.9531			
GFP (VEH) vs. Gi (CNO)		>0.9999			
GFP (CNO) vs. Gi (VEH)		0.9921			
GFP (CNO) vs. Gi (CNO)		>0.9999			
Gi (VEH) vs. Gi (CNO)		0.9881			

3:00-4:00		
GFP (VEH) vs. GFP (CNO)	0.999	
GFP (VEH) vs. Gi (VEH)	0.9516	
GFP (VEH) vs. Gi (CNO)	0.9045	
GFP (CNO) vs. Gi (VEH)	0.9979	
GFP (CNO) vs. Gi (CNO)	0.9918	
Gi (VEH) vs. Gi (CNO)	>0.9999	
4:00-5:00		
GFP (VEH) vs. GFP (CNO)	0.9968	
GFP (VEH) vs. Gi (VEH)	0.9898	
GFP (VEH) vs. Gi (CNO)	0.9971	
GFP (CNO) vs. Gi (VEH)	0.827	
GFP (CNO) vs. Gi (CNO)	0.8877	
Gi (VEH) vs. Gi (CNO)	>0.9999	
5:00-6:00		
GFP (VEH) vs. GFP (CNO)	0.9999	
GFP (VEH) vs. Gi (VEH)	0.6718	
GFP (VEH) vs. Gi (CNO)	0.9996	
GFP (CNO) vs. Gi (VEH)	0.424	
GFP (CNO) vs. Gi (CNO)	>0.9999	
Gi (VEH) vs. Gi (CNO)	0.3861	
6:00-7:00		
GFP (VEH) vs. GFP (CNO)	0.8793	
GFP (VEH) vs. Gi (VEH)	0.2327	
GFP (VEH) vs. Gi (CNO)	0.6777	
GFP (CNO) vs. Gi (VEH)	0.8793	
GFP (CNO) vs. Gi (CNO)	0.9997	
Gi (VEH) vs. Gi (CNO)	0.9783	
7:00-8:00		
GFP (VEH) vs. GFP (CNO)	>0.9999	
GFP (VEH) vs. Gi (VEH)	0.6453	
GFP (VEH) vs. Gi (CNO)	0.9519	
GFP (CNO) vs. Gi (VEH)	0.4923	
GFP (CNO) vs. Gi (CNO)	0.8807	
Gi (VEH) vs. Gi (CNO)	0.991	
8:00-9:00		
GFP (VEH) vs. GFP (CNO)	0.9923	
GFP (VEH) vs. Gi (VEH)	0.0864	
GFP (VEH) vs. Gi (CNO)	0.1784	
GFP (CNO) vs. Gi (VEH)	0.2866	
GFP (CNO) vs. Gi (CNO)	0.4984	
Gi (VEH) vs. Gi (CNO)	0.9994	
9:00-10:00		
GFP (VEH) vs. GFP (CNO)	>0.9999	
GFP (VEH) vs. Gi (VEH)	0.2649	
GFP (VEH) vs. Gi (CNO)	0.7989	
GFP (CNO) vs. Gi (VEH)	0.1267	
GFP (CNO) vs. Gi (CNO)	0.5759	
Gi (VEH) vs. Gi (CNO)	0.9555	
10:00-11:00		
GFP (VEH) vs. GFP (CNO)	0.9352	
GFP (VEH) vs. Gi (VEH)	0.534	
GFP (VEH) vs. Gi (CNO)	0.2237	
GFP (CNO) vs. Gi (VEH)	0.9808	
GFP (CNO) vs. Gi (CNO)	0.8068	
Gi (VEH) vs. Gi (CNO)	0.9985	
11:00-12:00		
GFP (VEH) vs. GFP (CNO)	0.9554	
GFP (VEH) vs. Gi (VEH)	0.3755	
GFP (VEH) vs. Gi (CNO)	0.9742	
GFP (CNO) vs. Gi (VEH)	0.0457	
GFP (CNO) vs. Gi (CNO)	0.4802	
Gi (VEH) vs. Gi (CNO)	0.8563	
12:00-13:00		
GFP (VEH) vs. GFP (CNO)	0.9995	
GFP (VEH) vs. Gi (VEH)	0.2057	
GFP (VEH) vs. Gi (CNO)	0.7956	
GFP (CNO) vs. Gi (VEH)	0.3696	
GFP (CNO) vs. Gi (CNO)	0.9471	
Gi (VEH) vs. Gi (CNO)	0.9188	
13:00-14:00		
GFP (VEH) vs. GFP (CNO)	>0.9999	
GFP (VEH) vs. Gi (VEH)	0.6325	
GFP (VEH) vs. Gi (CNO)	0.7113	
GFP (CNO) vs. Gi (VEH)	0.74	
GFP (CNO) vs. Gi (CNO)	0.8138	
Gi (VEH) vs. Gi (CNO)	>0.9999	
14:00-15:00		
GFP (VEH) vs. GFP (CNO)	0.9722	
GFP (VEH) vs. Gi (VEH)	0.3947	
GFP (VEH) vs. Gi (CNO)	0.4642	
GFP (CNO) vs. Gi (VEH)	0.8768	
GFP (CNO) vs. Gi (CNO)	0.9279	
Gi (VEH) vs. Gi (CNO)	>0.9999	
<u>Time in the Center Open Field (ED Figure 9c)</u>		
n= 15 per group		
Two-way ANOVA		
ANOVA table	P value	F (DFn, DFd)
Interaction	P=0.5597	F (1, 60) = 0.3441
Treatment	P=0.6093	F (1, 60) = 0.2640
Group	P=0.9675	F (1, 60) = 0.001675
Sidak Post Test	P value	
VEH .GFP vs. VEH .Gi	0.9994	
VEH .GFP vs. CNO .GFP	0.969	
VEH .GFP vs. CNO .Gi	0.9997	
VEH .Gi vs. CNO .GFP	0.9992	
VEH .Gi vs. CNO .Gi	>0.9999	
CNO .GFP vs. CNO .Gi	0.9981	
<u>Elevated Plus Maze Time in the Open Arms (ED Figure 9d)</u>		
n= GFP: 15; Gi: 12		
Two-way ANOVA		
ANOVA table	P value	F (DFn, DFd)
Interaction	P=0.8692	F (1, 57) = 0.02737
Treatment	P=0.6207	F (1, 57) = 0.2475
Group	P=0.0128	F (1, 57) = 6.599
Sidak Post Test	P value	
VEH .GFP vs. VEH .Gi	0.3657	

VEH .GFP vs. CNO .GFP	>0.9999
VEH .GFP vs. CNO .Gi	0.5838
VEH .Gi vs. CNO .GFP	0.2164
VEH .Gi vs. CNO .Gi	0.9982
CNO .GFP vs. CNO .Gi	0.3743

Elevated Plus Maze Distance Traveled (ED Figure 9d)

Two-way ANOVA			
ANOVA table	P value	F (Dfn, Dfd)	
Interaction	P=0.7601	F (1, 57) = 0.09412	
Treatment	P=0.2746	F (1, 57) = 1.217	
Group	P=0.0040	F (1, 57) = 8.993	

Sidak Post Test	P value	
VEH .GFP vs. VEH .Gi	0.3836	
VEH .GFP vs. CNO .GFP	0.993	
VEH .GFP vs. CNO .Gi	0.0241	
VEH .Gi vs. CNO .GFP	0.7352	
VEH .Gi vs. CNO .Gi	0.9154	
CNO .GFP vs. CNO .Gi	0.09	

Rotarod (ED Figure 9g)

Two-way ANOVA			
ANOVA table	P value	F (Dfn, Dfd)	
Interaction	P=0.4218	F (12, 195) = 1.031	
Day	P<0.0001	F (4, 195) = 26.48	
Group	P=0.0059	F (3, 195) = 4.290	

Sidak Post Test	P value	
Day 1		
GFP VEH vs. GFP CNO	0.9996	
GFP VEH vs. Gi VEH	0.9911	
GFP VEH vs. Gi CNO	0.9992	
GFP CNO vs. Gi VEH	0.8744	
GFP CNO vs. Gi CNO	0.9595	
Gi VEH vs. Gi CNO	>0.9999	

Day 2		
GFP VEH vs. GFP CNO	0.9998	
GFP VEH vs. Gi VEH	0.967	
GFP VEH vs. Gi CNO	0.9995	
GFP CNO vs. Gi VEH	0.9907	
GFP CNO vs. Gi CNO	0.9729	
Gi VEH vs. Gi CNO	0.8135	

Day 3		
GFP VEH vs. GFP CNO	0.8819	
GFP VEH vs. Gi VEH	0.9642	
GFP VEH vs. Gi CNO	0.9686	
GFP CNO vs. Gi VEH	>0.9999	
GFP CNO vs. Gi CNO	0.2467	
Gi VEH vs. Gi CNO	0.5088	

Day 4		
GFP VEH vs. GFP CNO	0.6908	
GFP VEH vs. Gi VEH	0.9986	
GFP VEH vs. Gi CNO	0.7503	
GFP CNO vs. Gi VEH	0.9542	
GFP CNO vs. Gi CNO	0.0235	
Gi VEH vs. Gi CNO	0.4238	

Day 5		
GFP VEH vs. GFP CNO	0.1446	
GFP VEH vs. Gi VEH	0.9973	
GFP VEH vs. Gi CNO	>0.9999	
GFP CNO vs. Gi VEH	0.4528	
GFP CNO vs. Gi CNO	0.0742	
Gi VEH vs. Gi CNO	0.9875	

Vermis Gq Rescue Tsc1 PC Mutants

Social Approach: 3 way ANOVA (ED Figure 9h)

Mixed-effects model (REML)

Fixed effects (type III)	P value	F (Dfn, Dfd)	
NA vs NO	0.4851	F (1, 60) = 0.4934	
GFP vs Gq	0.6265	F (1, 60) = 0.2392	
VEH vs CNO	0.0647	F (1, 52) = 3.562	
NA vs NO x GFP vs Gq	0.635	F (1, 60) = 0.2276	
NA vs NO x VEH vs CNO	0.9354	F (1, 52) = 0.006632	
GFP vs Gq x VEH vs CNO	0.0492	F (1, 52) = 4.056	

Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
NA:GFP (VEH) vs. NO:GFP (VEH)	6.76	-40.54 to 54.06	No	ns	>0.9999
NA:GFP (CNO) vs. NO:GFP (CNO)	-3.334	-50.64 to 43.97	No	ns	>0.9999
NA:Gq (VEH) vs. NO:Gq (VEH)	5.094	-33.53 to 43.72	No	ns	>0.9999
NA:Gq (CNO) vs. NO:Gq (CNO)	12.85	-25.78 to 51.47	No	ns	0.9924
NA:GFP (VEH) vs. NA:GFP (CNO)	33.1	-14.01 to 80.22	No	ns	0.3917
NA:Gq (VEH) vs. NA:Gq (CNO)	-4.785	-43.22 to 33.65	No	ns	>0.9999

Social Novelty: 3 way ANOVA (ED Figure 9i)

Mixed-effects model (REML)

Fixed effects (type III)	P value	F (Dfn, Dfd)	
FA vs NA	0.1737	F (1, 60) = 1.896	
GFP va Gq	0.7103	F (1, 60) = 0.1392	
VEH vs CNO	0.7837	F (1, 52) = 0.07617	
FA vs NA x GFP va Gq	0.519	F (1, 60) = 0.4208	
FA vs NA x VEH vs CNO	0.7385	F (1, 52) = 0.1127	
GFP va Gq x VEH vs CNO	0.4073	F (1, 52) = 0.6979	
FA vs NA x GFP va Gq x VEH vs CNO	0.5907	F (1, 52) = 0.2929	

Sidak's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
FA:GFP (VEH) vs. NA:GFP (VEH)	-16.03	-61.64 to 29.58	No	ns	0.9878
FA:GFP (CNO) vs. NA:GFP (CNO)	-13.12	-58.73 to 32.49	No	ns	0.998
FA:Gq (VEH) vs. NA:Gq (VEH)	0.9634	-36.28 to 38.20	No	ns	>0.9999
FA:Gq (CNO) vs. NA:Gq (CNO)	-11.44	-48.68 to 25.80	No	ns	0.9962
NA:GFP (VEH) vs. NA:GFP (CNO)	5.411	-40.92 to 51.74	No	ns	>0.9999
NA:Gq (VEH) vs. NA:Gq (CNO)	-14.07	-51.89 to 23.76	No	ns	0.9776

Distance Traveled Open Field (ED Figure 9k)

Two-way ANOVA			
ANOVA table	P value	F (Dfn, Dfd)	
Interaction	P=0.9974	F (42, 915) = 0.4924	
Minute	P=0.2164	F (14, 915) = 1.275	
Group	P<0.0001	F (3, 915) = 12.41	

Sidak Post Test	P value
0:00-1:00	
GFP (VEH) vs. GFP (CNO)	0.9683
GFP (VEH) vs. Gq (VEH)	0.9898
GFP (VEH) vs. Gq (CNO)	0.7369
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	0.999
Gq (VEH) vs. Gq (CNO)	0.9868
1:00-2:00	
GFP (VEH) vs. GFP (CNO)	0.7965
GFP (VEH) vs. Gq (VEH)	0.9993
GFP (VEH) vs. Gq (CNO)	0.9968
GFP (CNO) vs. Gq (VEH)	0.9543
GFP (CNO) vs. Gq (CNO)	0.9748
Gq (VEH) vs. Gq (CNO)	>0.9999
2:00-3:00	
GFP (VEH) vs. GFP (CNO)	0.7873
GFP (VEH) vs. Gq (VEH)	0.5646
GFP (VEH) vs. Gq (CNO)	0.7768
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	>0.9999
Gq (VEH) vs. Gq (CNO)	0.9998
3:00-4:00	
GFP (VEH) vs. GFP (CNO)	0.7397
GFP (VEH) vs. Gq (VEH)	0.4839
GFP (VEH) vs. Gq (CNO)	0.7873
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	>0.9999
Gq (VEH) vs. Gq (CNO)	0.9984
4:00-5:00	
GFP (VEH) vs. GFP (CNO)	0.8532
GFP (VEH) vs. Gq (VEH)	>0.9999
GFP (VEH) vs. Gq (CNO)	0.6553
GFP (CNO) vs. Gq (VEH)	0.9465
GFP (CNO) vs. Gq (CNO)	>0.9999
Gq (VEH) vs. Gq (CNO)	0.8235
5:00-6:00	
GFP (VEH) vs. GFP (CNO)	>0.9999
GFP (VEH) vs. Gq (VEH)	0.958
GFP (VEH) vs. Gq (CNO)	0.6097
GFP (CNO) vs. Gq (VEH)	0.8946
GFP (CNO) vs. Gq (CNO)	0.8461
Gq (VEH) vs. Gq (CNO)	0.1253
6:00-7:00	
GFP (VEH) vs. GFP (CNO)	>0.9999
GFP (VEH) vs. Gq (VEH)	0.9982
GFP (VEH) vs. Gq (CNO)	0.6255
GFP (CNO) vs. Gq (VEH)	0.9965
GFP (CNO) vs. Gq (CNO)	0.7632
Gq (VEH) vs. Gq (CNO)	0.3029
7:00-8:00	
GFP (VEH) vs. GFP (CNO)	0.9957
GFP (VEH) vs. Gq (VEH)	0.9921
GFP (VEH) vs. Gq (CNO)	0.6699
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	0.9742
Gq (VEH) vs. Gq (CNO)	0.9682
8:00-9:00	
GFP (VEH) vs. GFP (CNO)	0.992
GFP (VEH) vs. Gq (VEH)	0.9704
GFP (VEH) vs. Gq (CNO)	0.3652
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	0.8675
Gq (VEH) vs. Gq (CNO)	0.891
9:00-10:00	
GFP (VEH) vs. GFP (CNO)	0.9613
GFP (VEH) vs. Gq (VEH)	0.842
GFP (VEH) vs. Gq (CNO)	0.042
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	0.4395
Gq (VEH) vs. Gq (CNO)	0.5286
10:00-11:00	
GFP (VEH) vs. GFP (CNO)	0.9075
GFP (VEH) vs. Gq (VEH)	>0.9999
GFP (VEH) vs. Gq (CNO)	0.1553
GFP (CNO) vs. Gq (VEH)	0.9115
GFP (CNO) vs. Gq (CNO)	0.8729
Gq (VEH) vs. Gq (CNO)	0.1593
11:00-12:00	
GFP (VEH) vs. GFP (CNO)	>0.9999
GFP (VEH) vs. Gq (VEH)	0.5855
GFP (VEH) vs. Gq (CNO)	0.9786
GFP (CNO) vs. Gq (VEH)	0.646
GFP (CNO) vs. Gq (CNO)	0.9877
Gq (VEH) vs. Gq (CNO)	0.1465
12:00-13:00	
GFP (VEH) vs. GFP (CNO)	>0.9999
GFP (VEH) vs. Gq (VEH)	>0.9999
GFP (VEH) vs. Gq (CNO)	0.6264
GFP (CNO) vs. Gq (VEH)	>0.9999
GFP (CNO) vs. Gq (CNO)	0.7166
Gq (VEH) vs. Gq (CNO)	0.7628
13:00-14:00	
GFP (VEH) vs. GFP (CNO)	>0.9999
GFP (VEH) vs. Gq (VEH)	0.996
GFP (VEH) vs. Gq (CNO)	0.334
GFP (CNO) vs. Gq (VEH)	0.9996
GFP (CNO) vs. Gq (CNO)	0.3276
Gq (VEH) vs. Gq (CNO)	0.1025
14:00-15:00	
GFP (VEH) vs. GFP (CNO)	0.9739
GFP (VEH) vs. Gq (VEH)	0.9993
GFP (VEH) vs. Gq (CNO)	0.5466
GFP (CNO) vs. Gq (VEH)	0.9992
GFP (CNO) vs. Gq (CNO)	0.9841
Gq (VEH) vs. Gq (CNO)	0.8203

Time in the Center Open Field (ED Figure 9k)

Two-way ANOVA
ANOVA table
Interaction
Treatment
Group

n= GFP: 13; Gq:17

P value

P=0.9955 F (Dfn, DFd)
P=0.2061 F (1, 61) = 3.191e-005
P=0.0173 F (1, 61) = 1.633
F (1, 61) = 5.987

Sidak Post Test

P value

0.3887
0.947
0.0456
0.9659
0.925
0.4637

Elevated Plus Maze Time in Open Arm (ED Figure 9i)

Two-way ANOVA
ANOVA table
Interaction
Treatment
Group

n= GFP: 10; Gq: 15

P value

P=0.6068 F (Dfn, DFd)
P=0.7507 F (1, 51) = 0.2682
P=0.0005 F (1, 51) = 0.1021
F (1, 51) = 13.73

Sidak Post Test

P value

0.1957
0.9953
0.122
0.035
>0.9999
0.0171

Elevated Plus Maze Distance Traveled (ED Figure 9j)

Two-way ANOVA
ANOVA table
Interaction
Treatment
Group

n= GFP: 10; Gq: 15

P value

P=0.5757 F (Dfn, DFd)
P=0.5566 F (1, 51) = 0.3172
P<0.0001 F (1, 51) = 0.3502
F (1, 51) = 31.72

Sidak Post Test

P value

0.0007
0.975
0.0004
0.0044
>0.9999
0.0026

Rotarod (ED Figure 9l)

Two-way ANOVA
ANOVA table
Interaction
Day
Group

n= GFP: 8; Gq:11

P value

P=0.9917 F (Dfn, DFd)
P<0.0001 F (12, 180) = 0.2807
P=0.0245 F (4, 180) = 11.50
F (3, 180) = 3.204

Sidak Post Test

P value

>0.9999
0.9855
0.9916
0.9093
0.9997
0.66

Day 2

GFP (VEH) vs. GFP (CNO)
GFP (VEH) vs. Gq (VEH)
GFP (VEH) vs. Gq (CNO)
GFP (CNO) vs. Gq (VEH)
GFP (CNO) vs. Gq (CNO)
Gq (VEH) vs. Gq (CNO)

0.8474
>0.9999
0.6958
0.6639
>0.9999
0.452

Day 3

GFP (VEH) vs. GFP (CNO)
GFP (VEH) vs. Gq (VEH)
GFP (VEH) vs. Gq (CNO)
GFP (CNO) vs. Gq (VEH)
GFP (CNO) vs. Gq (CNO)
Gq (VEH) vs. Gq (CNO)

>0.9999
0.8625
0.9998
0.6704
>0.9999
0.5708

Day 4

GFP (VEH) vs. GFP (CNO)
GFP (VEH) vs. Gq (VEH)
GFP (VEH) vs. Gq (CNO)
GFP (CNO) vs. Gq (VEH)
GFP (CNO) vs. Gq (CNO)
Gq (VEH) vs. Gq (CNO)

0.9999
0.8009
0.996
0.5525
0.9537
0.9786

Day 5

GFP (VEH) vs. GFP (CNO)
GFP (VEH) vs. Gq (VEH)
GFP (VEH) vs. Gq (CNO)
GFP (CNO) vs. Gq (VEH)
GFP (CNO) vs. Gq (CNO)
Gq (VEH) vs. Gq (CNO)

>0.9999
0.9243
0.9926
0.9697
0.999
0.9994

Single Units (Raw values)

mPFC frequency with mPFC GFP (ED Figure 10a)
mPFC frequency with mPFC Gi (ED Figure 10a)
mPFC frequency with Rerus1 GFP (ED Figure 10b)
mPFC frequency with Rerus1 Gi (ED Figure 10b)
Motor cortex frequency with Rerus1 Gi (ED Figure 10b)
mPFC frequency with Gi Rerus1 GFP mPFC (ED Figure 10c)
mPFC frequency with Gi Rerus1 Gi mPFC (ED Figure 10c)
Right mPFC frequency with Rerus1 Gi (ED Figure 10d)
LN frequency with LN GFP (ED Figure 10e)
LN frequency with LN Gi (ED Figure 10e)
mPFC frequency with LN GFP (ED Figure 10f)
mPFC frequency with LN Gi (ED Figure 10f)
mPFC frequency with GFP (4 Hz) (ED Figure 10g)
mPFC frequency with Chr2 (4 Hz) (ED Figure 10g)
mPFC frequency with GFP (20 Hz) (ED Figure 10h)
mPFC frequency with Chr2 (20 Hz) (ED Figure 10h)
Mutant mPFC frequency with GFP (4 Hz) (ED Figure 10i)
Mutant mPFC frequency with Arch (4 Hz) (ED Figure 10h)
Mutant mPFC frequency with GFP (20 Hz) (ED Figure 10j)
Mutant mPFC frequency with Arch (20 Hz) (ED Figure 10j)
PAC GFP (4 Hz) (ED Figure 10k)
PAC Chr2 (4 Hz) (ED Figure 10k)
PAC GFP (20 Hz) (ED Figure 10l)

Units

Mice

Test

P value

Wilcoxon matched-pairs signed rank 0.6875
Wilcoxon matched-pairs signed rank 0.0039
Wilcoxon matched-pairs signed rank 0.1245
Wilcoxon matched-pairs signed rank 0.1211
Wilcoxon matched-pairs signed rank 0.3438
Wilcoxon matched-pairs signed rank 0.0039
Wilcoxon matched-pairs signed rank 0.0039
Wilcoxon matched-pairs signed rank 0.2969
Wilcoxon matched-pairs signed rank 0.4063
Wilcoxon matched-pairs signed rank 0.0078
Wilcoxon matched-pairs signed rank 0.7188
Wilcoxon matched-pairs signed rank 0.001
Wilcoxon matched-pairs signed rank >0.9999
Wilcoxon matched-pairs signed rank 0.0001
Wilcoxon matched-pairs signed rank >0.9999
Wilcoxon matched-pairs signed rank <0.0001
Wilcoxon matched-pairs signed rank 0.5703
Wilcoxon matched-pairs signed rank 0.0039
Wilcoxon matched-pairs signed rank 0.6406
Wilcoxon matched-pairs signed rank 0.0039
Wilcoxon matched-pairs signed rank 0.8438
Wilcoxon matched-pairs signed rank 0.2344
Wilcoxon matched-pairs signed rank 0.8906

PAC ChR2 (20 Hz) (ED Figure 10l)	7	3	Wilcoxon matched-pairs signed rank	0.1406
mPFC with Vermis GFP(ED Figure 10m)	7	3	Wilcoxon matched-pairs signed rank	0.8125
mPFC with Vermis Gi (ED Figure 10m)	9	4	Wilcoxon matched-pairs signed rank	0.0039

Supplemental Table S3
MRI Structural Covariance

<u>All Models</u>		q values
Cerebellar Structure	Left mPFC	Left PRL
Rcrus1 (total)	0.077632862	0.019168798
Rcrus1 (gray)	0.054059331	0.013982979
Vermis 7, 8, 9	0.080404885	0.028228459
Vermis 7	0.011084808	0.005893087
Vermis 8	0.006678341	0.002963781
Vermis 9	0.575474507	0.305006934

<u>30 models (individual: p<0.05)</u>		q values
Cerebellar Structure	Left mPFC	Left PRL
Rcrus1 (total)	4.68E-05	3.79E-05
Rcrus1 (gray)	1.87E-05	1.87E-05
Vermis 7, 8, 9	6.87E-09	8.77E-09
Vermis 7	1.87E-05	3.32E-05
Vermis 8	8.77E-09	1.29E-08
Vermis 9	5.86E-07	1.30E-06

Mouse models
16p11.2 (df/+)
16p11.2 (dp/+)
Ar (12Q/+)
Ar (12Q/12Q)
Ar (21Q/21Q)
Ar (48Q/48Q)
Arid1b (+/-)
Chd7 (+/gt)
Chd8 (+/-)
Dvl1 (-/-)
Foxp1 (+/cKO)
Gtf2i (+/-)
Gtf2i (dp/dp)
Itgb3 (-/-)
Kctd13 (+/-)
Magel2 (-/-)
Mecp2 (308/Y)
Nf1 (+/-)
Nlgn3 (R451C KI/Y)
Scn1a (+/-)
Shank3 (+/-) e21
Slc6a3 (+/Val559)

Slc6a3 (Val559/Val559)
Slc6a4 (-/-)
Taok2 (-/-)
Taok2 (+/-)
Turner (XO)
Ube3a (+/-)
Ube3a-2 (TRE/+); Camk2a (+/+)
Ube3a-2 (TRE/+); Camk2a (tTA/+)
WT
L7Cre;Tsc1 (flox/flox)

Demographics

	n	(% of total)
control	1101	66.33%
mutant	559	33.67%
male	1123	67.70%
female	537	32.30%

age range PND: 30-245

Supplemental Table S4
Human MRI Structural Covariance

Brain Area		p values	q values	
mPFC		0.055	0.134	
Gyrus Rectus		0.012	0.0695	
Superior frontal (medial orbital part)		0.067	0.134	
ACC (mediorbital frontal)		0.16	0.242	
Anterior cingulate/paracingulate		0.66	0.689	
Superior parietal gyrus		0.69	0.689	
Demographics		male	female	age (mean [SD])
Total scans	902	588	314	13.2 (6.6)
ASD	425	341	84	12.8 (5.9)
Typically Developing	477	247	230	14.1 (7.9)

Supplemental Table S5

AAV8-CMV-GFP	Figure 1 b-h, Figure 2 f-k, Figure 3 b-h, Figure 4 e-s, Figure 5 a & j-s, Figure S1, Figure S3, Figure S4, Figure S6, Figure S7, Figure S10, Figure S11, Figure S12
AAV2-hSyn-hM4D(Gi)-mCherry	Figure 1 b-h Figure 2 f-k, Figure 3 b-h, Figure S1, Figure S3, Figure S4, Figure S12
AAV8-hSyn-DIO-hM4D(Gi)-mCherry (hM4Di)	Figure 2 a & f-k, Figure 5 a & j-n, Figure S3, Figure S10, Figure S12
AAV1.hSyn.Cre.WPRE.hGH	Figure 4 a-d, Figure 5 f-I, Figure S5
ChR2 (AAV5-hSyn-hChR2(H134R)-mCherry)	Figure 4 e-l, Figure S6, Figure S12
Arch 3.0 (AAV5-hSyn-eArch3.0-EYFP)	Figure 4, m-s, Figure S7, Figure S12
AAV9.hSyn.eGFP.WPRE.bGH	Figure 5 d & e, Figure S9
AAV9.hSyn.TurboRFP.WPRE.rBG	Figure 5 d & e, Figure S9
AAV8-hSyn-DIO-hM3D(Gq)-mCherry (hM3Dq)	Figure 5 o-s, Figure S10, Figure S12

All viral titers: 10^{12} vg/ml.

3-50nl virus was injected for tracing studies. 100-350nl virus was injected for behavioral and electrophysiological recording experiments.