Supplementary Material for

Projecting neurons from lateral entorhinal cortex to basolateral amygdala mediate the encoding of incidental odor-taste associations

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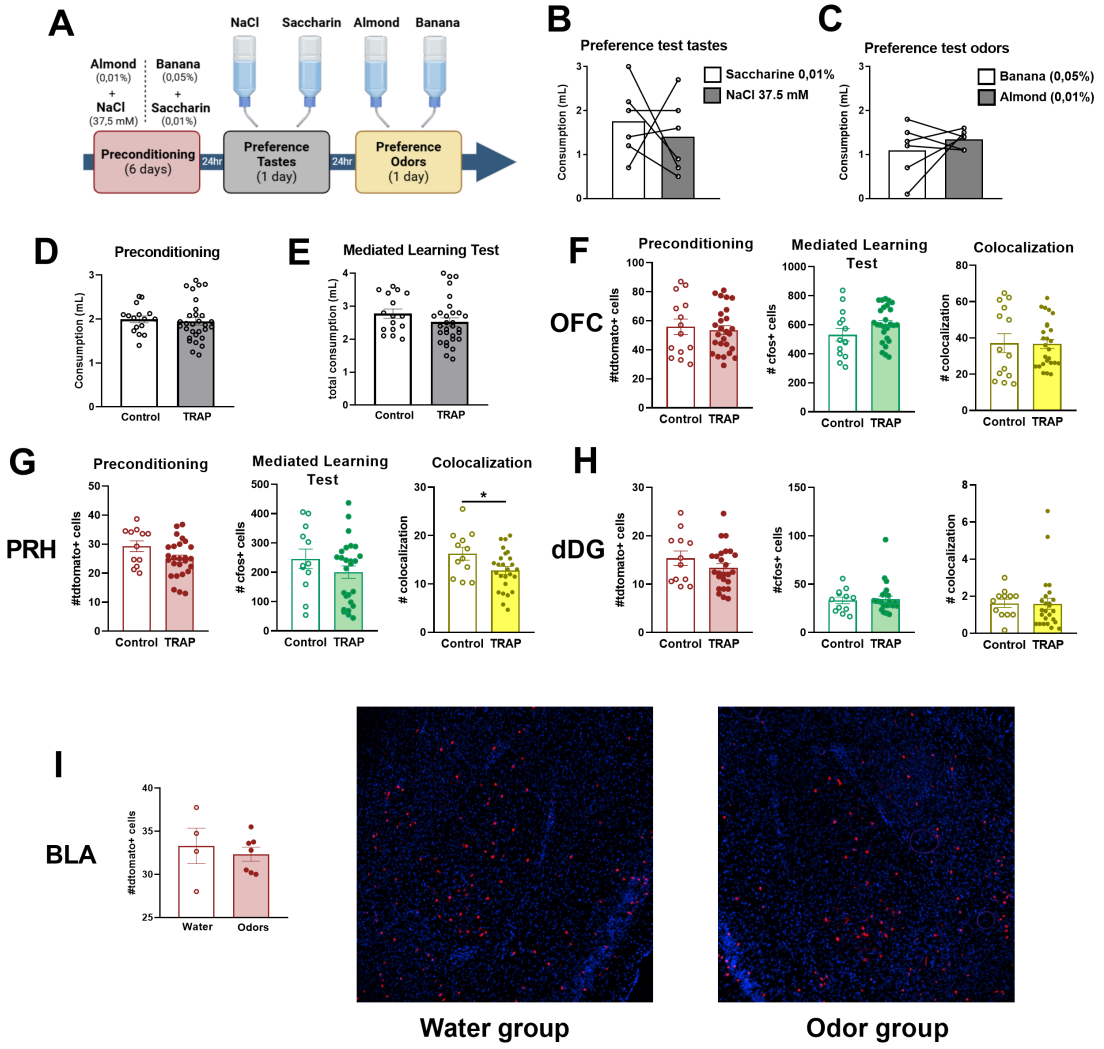

Fig S1. Increase activity of BLA during odor-taste incidental associations. (A) Schematic illustration of the procedure used to assess potential innate preferences for odors and tastes. Liquid consumption during taste preference test comparing saccharin and NaCl (B) and odor preference test comparing almond and banana (C). Average liquid consumption during the preconditioning phase (D) and total liquid consumption during mediated learning between TRAP and Control groups. Quantification of tdTomato-positive cells, c-Fos-positive cells, and colocalization of tdTomato/c-Fos-positive cells in the orbitofrontal cortex (F), perirhinal cortex (G), and dorsal dentate gyrus (H) of TRAP2:Ai14 mice. (I) Representative images and quantification of tdTomato-positive cells in TRAP2:Ai14 mice exposed to either water or odors. Data are represented as mean ± SEM. For statistical details and n, see Supplementary Table 2. *p<0.05. OFC: Orbitofrontal cortex; PRH: Perirhinal cortex; dDG: dorsal Dentate Gyrus; BLA: Basolateral Amygdala

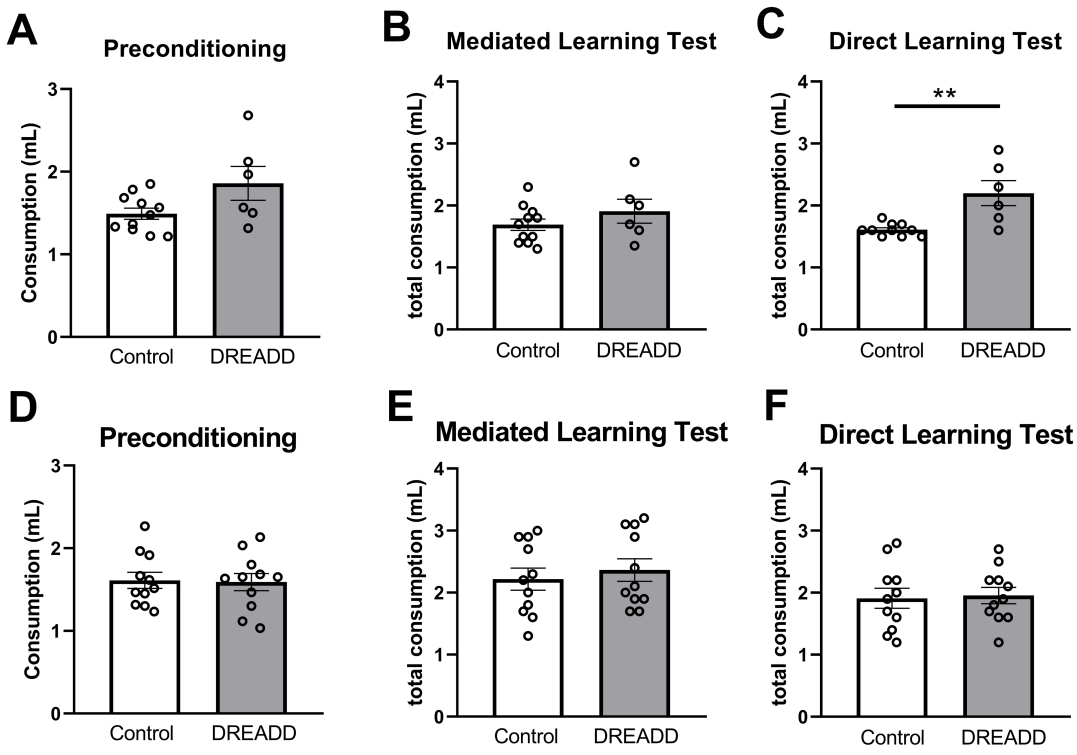

Fig S2. BLA is necessary for the encoding of incidental associations during odor-taste sensory preconditioning. (A) Average liquid consumption during the preconditioning phase in Control and DREADD groups injected with J60. (B) Total liquid consumption between groups during mediated learning (B) and direct learning (C) in C57BL/6J mice. Average of liquid consumption between Control and DREADD group during preconditioning (D) and total liquid consumption between groups during mediated learning (E) and direct learning (F) in TRAP2:Ai14 mice. Data are represented as mean ± SEM. For statistical details and n, see Supplementary Table 2. ** $p<0.01$ (Control vs. DREADD).

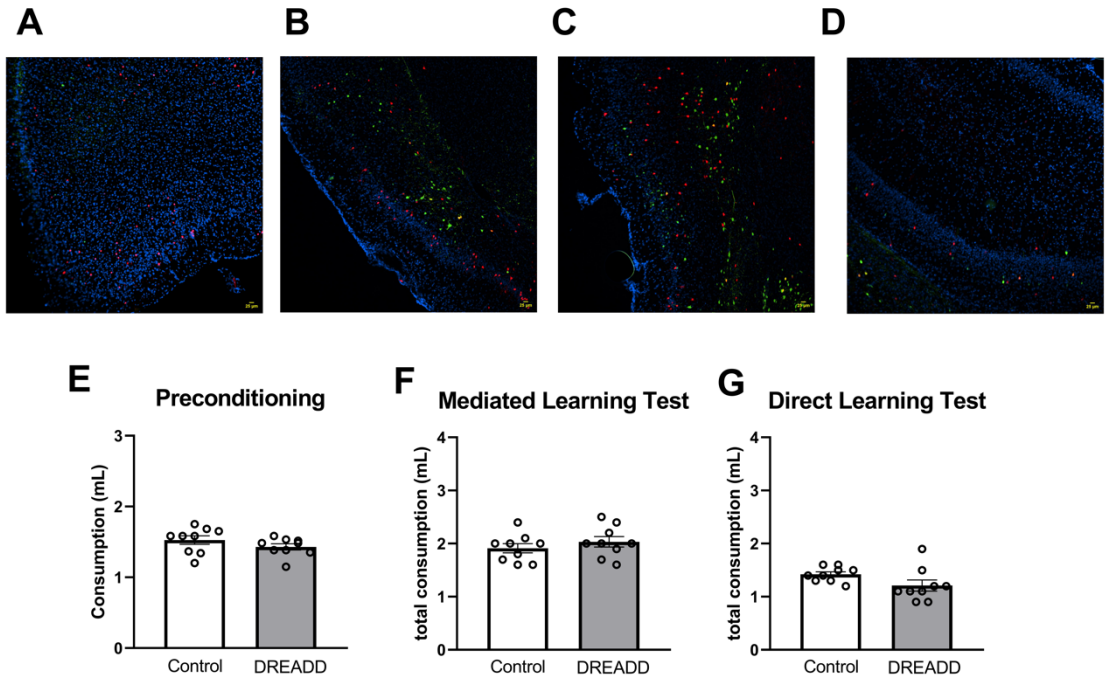

Fig S3. Projections from LEnt to BLA are important for the encoding of incidental associations. Representative images of activated neurons during incidental associations that project to the basolateral amygdala (BLA) from the orbitofrontal cortex (A), piriform cortex (B), perirhinal cortex (C), and ventral CA1 (D). Average liquid consumption during the preconditioning phase between Control and DREADD groups injected with J60 (E), and total liquid consumption between groups during the mediated learning (F) and direct learning (G) tests in C57BL/6J mice (related to Fig. F-I). Data are presented as mean ± SEM. For statistical details and n, see Supplementary Table 2. Scale bar: 25 µm.

Figure	*Experiment*	*Sample size per group*	*Shapiro-Wilk Normality Test* *Passed normality test?*	*Statistical test*	*Statistical value*
Figure 1	(B) Mediated learning in C57BL/6J mice	n=9	Yes	Paired t-test	**t=2,712, df=8, p<0.05**
	(C) Direct Learning in C57BL/6J mice	n=9	Yes	Paired t-test	**t=8,008, df=8, p<0.001**
	(E) Mediated learning in TRAP2 mice	n=30	Yes	Paired t-test	**t=7,826, df=29, p<0.001**
	(F) tdtom+ cells in preconditioning phase	n=15-23	Yes	Unpaired t-test	**t=2,047, df=36**
	(G) cfos+ cells in mediated learning test	n=15-23	Yes	Unpaired t-test	**t=0,087, df=36, p=ns**
	(H) colocalization+ cells	n=15-23	Yes	Unpaired t-test	**t=1,274, df=36, p=ns**
Figure 2	(C) Mediated Learning in C57BL/6J BLA inhibition preconditioning	n=6-11	Yes	2 way ANOVA repeated measures (Bonferroni)	**Interaction: F (1, 15) = 8,227, p<0.01**
					mCS+ vs mCS- (Control) p<0.05
	(D) Direct Learning in C57BL/6J BLA inhibition preconditioning	n=6-11	Yes	2 way ANOVA repeated measures	**CS+ vs CS: p<0.001**
	(G) Mediated Learning in TRAP2 preconditioning BLA neuronal ensembles	n=11	Yes	2 way ANOVA repeated measures	**mCS+ vs mCS: p<0.001**
					Control vs TRAP: p<0.001
	(H) Direct Learning in TRAP2 preconditioning BLA neuronal ensembles	n=11	Yes	2 way ANOVA repeated measures	**CS+ vs CS: p<0.001**
Figure 3	(B) Mediated learning in TRAP2 mice injected with retro DIO-GFP	n=7	Yes	Paired t-test	**t=10,48, df=6, p<0.001**
	(C) Activated areas during preconditioning projecting to BLA	n=5-8	Yes	1 way ANOVA	**F(20,24), p<0.001**
	(H) Mediated Learning in C57BL/6J LEnt-BLA inhibition preconditioning	n=9	Yes	2 way ANOVA repeated measures (Bonferroni)	**Interaction: F (1, 16) = 5,976, p<0.05**
					mCS+ vs mCS- (Control) p<0.05
	(I) Direct Learning in C57BL/6J LEnt-BLA inhibition preconditioning	n=9	Yes	2 way ANOVA repeated measures	**CS+ vs CS: p<0.001**

Table 1. Statistical analysis. Related to Main Figures 1-3

Figure	Experiment	Sample size per group	Shapiro-Wilk Normality Test Passed normality test?	Statistical test	Statistical value
Suppl 1	(B) Tastes preference test	n=6	Yes	Paired t-test	**t=0,5840, df=5, p=ns**
	(C) Odors preference test	n=6	Yes	Paired t-test	**t=0,8669, df=5, p=ns**
	(D) Preconditioning consumption TRAP2	n=15-30	No	Mann Whitney test	**p=ns**
	(E) Total mediated learning consumption TRAP2	n=15-30	Yes	Unpaired t-test	**t=1,261, df=44, p=ns**
	(F) tdtom+ cells in OFC	n=14-26	Yes	Unpaired t-test	**t=0,3913, df=38, p=ns**
	(F) cfos+ cells in OFC	n=14-26	Yes	Unpaired t-test	**t=1,625, df=38, p=ns**
	(F) colocalization+ cells in OFC	n=14-26	No	Mann Whitney test	**p=ns**
	(G) tdtom+ cells in PRH	n=12-26	Yes	Unpaired t-test	**t=1,934, df=36, p=ns**
	(G) cfos+ cells in PRH	n=12-26	Yes	Unpaired t-test	**t=1,180, df=36, p=ns**
	(G) coloc+ cells in PRH	n=12-26	Yes	Unpaired t-test	**t=2,292, df=36, p<0.05**
	(H) tdtom+ cells in dDG	n=12-25	Yes	Unpaired t-test	**t=1,203, df=35, p=ns**
	(H) cfos+ cells in dDG	n=12-25	No	Mann Whitney test	**p=ns**
	(H) coloc+ cells in dDG	n=12-25	No	Mann Whitney test	**p=ns**
	(I) tdtom+ cells in water vs odor presentations	n=4-7	Yes	Unpaired t-test	**t=0,5194, df=9, p=ns**
Suppl 2	(A) Preconditioning consumption in C57BL/6J Gi DREADDs BLA	n=6-11	Yes	Unpaired t-test	**t=2,107, df=15, p=ns**
	(B) Total mediated learning consumption in C57BL/6J Gi DREADDs BLA	n=6-11	Yes	Unpaired t-test	**t=1,159, df=15, p=ns**
	(C) Total direct learning consumption in C57BL/6J Gi DREADDs BLA	n=6-11	Yes	Unpaired t-test	**t=3,736, df=14, p<0.01**
	(D) Preconditioning consumption in TRAP2 DIO Gi DREADDs BLA	n=11	Yes	Unpaired t-test	**t=0,1386, df=20, p=ns**
	(E) Total mediated learning consumption in TRAP2 DIO Gi DREADDs BLA	n=11	No	Mann Whitney test	**p=ns**
	(F) Total direct learning consumption in TRAP2 DIO Gi DREADDs BLA	n=11	Yes	Unpaired t-test	**t=0,2181, df=20, p=ns**
Suppl 3	(E) Preconditioning consumption in C57BL/6J LEnt-BLA Gi DREADDs	n=9	Yes	Unpaired t-test	**t=1,297, df=16, p=ns**
	(F) Total mediated learning consumption in C57BL/6J LEnt-BLA Gi DREADDs	n=9	Yes	Unpaired t-test	**t=0,9280, df=16, p=ns**
	(G) Total direct learning consumption in C57BL/6J LEnt-BLA Gi DREADDs	n=9	Yes	Unpaired t-test	**t=1,843, df=16, p=ns**

Table 2. Statistical analysis. Related to Figures S1-3