# Distinct cortical-amygdala projections drive reward value encoding and retrieval

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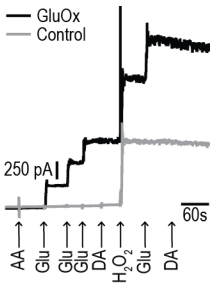
**Supplemental Material:**
Supplemental Tables: 2
Supplemental Figures: 18

| Variable | Average glutamate (μM) | Peak glutamate (μM) |
|---|---|---|
| All trials | $r_{12}$=0.151, *P*=0.640 | $r_{12}$=0.243, *P*=0.446 |
| Early trials | $r_{12}$=0.326, *P*=0.302 | $r_{12}$=0.389, *P*=0.106 |

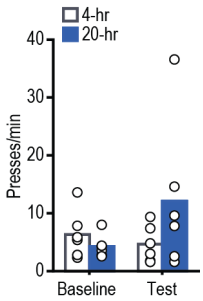
**Table S1. Summary of correlation between glutamate concentration during non-contingent sucrose exposure and subsequent rate of lever pressing.** Pearson correlations (*N*=12 biologically-independent glutamate recordings) showed there was no relationship, either over all re-exposure trials or only early re-exposure trials, between average or peak glutamate concentration change following reward delivery and subsequent lever pressing rate.

| Exposure Group | Number of bouts (per 5-min test) | Average presses per bout | Average bout duration (s) |
|---|---|---|---|
| 4-hr | 9.33±1.26 | 2.35±0.33 | 3.24±0.76 |
| 20-hr | 9.67±1.69 | 5.86±2.35 | 6.10±2.41 |

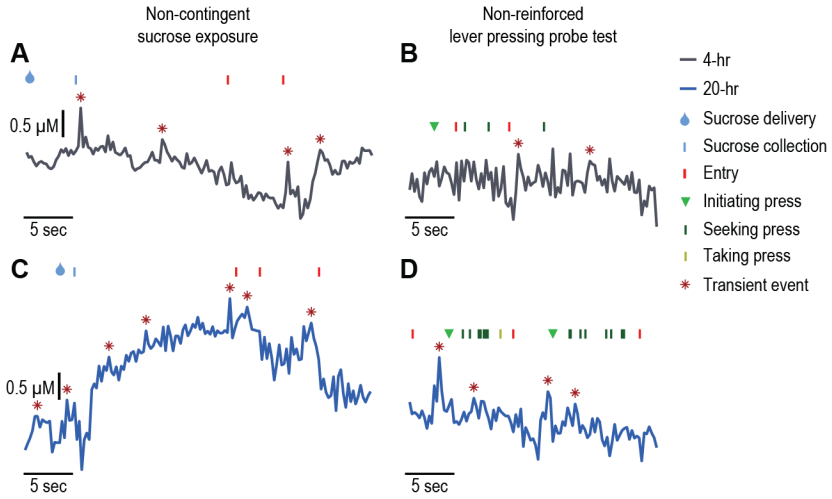
**Table S2. Summary of seeking lever press bouts during non-reinforced, lever-pressing probe test.** Data presented as average ± s.e.m. 4-hr group, *N*=6 rats; 20-hr group, *N*=6 rats.



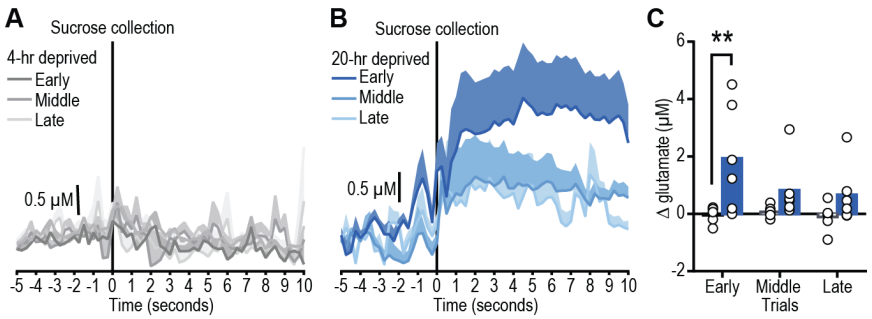

**Supplement Figure 1. Representative calibration of a microelectrode array glutamate biosensor.** Silicon-wafer-based platinum microelectrode array (MEA) probes were modified for glutamate detection as we have described previously [1-3]. Glutamate oxidase (GluOx) serves as the biological recognition. Electro-oxidation, by constant potential amperommetry, of the enzymatically-generated hydrogen peroxide reporter molecule provides the signal. Selectivity against both cations and anions is achieved by the addition of polymer coatings (see Methods). Control electrodes are identically coated with the exception that GluOx is omitted. These sensors have a subsecond response time [1-3]. To test for sensitivity and selectivity of glutamate measurement, all biosensors were calibrated *in vitro* by sequential addition of ascorbic acid (AA; 250μM), glutamate (Glu; 20 μM), dopamine (DA; 5 μM), hydrogen peroxide ($H_2O_2$; 20 μM), Glu (40 μM), and DA (10 μM), in stirred PBS at 37 $^{o}$C. The *in vitro* glutamate current response was used to determine the electrode-specific calibration factor, which averaged 135.98 μM/nA for the sensors used in these studies. The sensitivity to peroxide between glutamate oxidase coated (GluOx) and control sites did not differ more than 10% ($t_{42}$=0.32, p=0.75). The average *in vivo* limit of glutamate detection of the sensors used in this study was 0.36 μM (sem=0.03, range 0.13-0.67 μM; *N*= 22 individual biosensors).



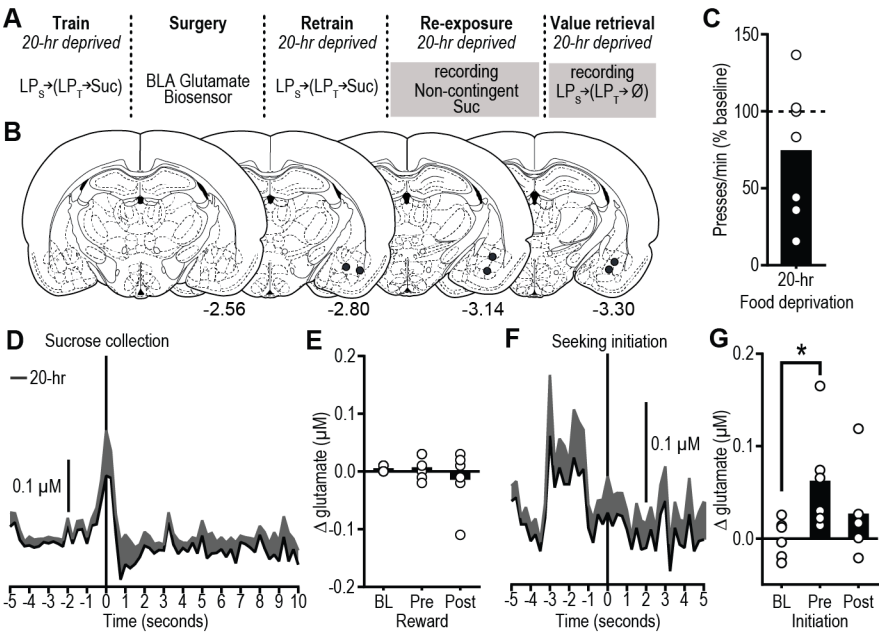

**Supplement Figure 2. Effect of incentive learning on reward seeking- raw press rates.** Reward-seeking press rate (seeking presses/min) during baseline (average of last-two training sessions in 4-hr food-deprived state prior to test) and non-reinforced, lever-pressing probe test in the hungry state (analyzed using two-way ANOVA; Test: $F_{1,10}$=3.1.577, $P$=0.24; Deprivation: $F_{1,10}$=0.71, $P$=0.42; Test x Deprivation: $F_{1,10}$=3.73, $P$=0.08) for rats given prior non-contingent sucrose exposure in control sated (4-hr food-deprived, $N$=6 rats; no value encoding) or hungry (20-hr deprived, $N$=6 rats; value encoding opportunity) state. Data presented as mean + scatter.



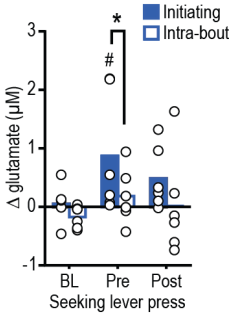

**Supplement Figure 3. Representative BLA glutamate v. time traces during reward value encoding and retrieval.** Representative, single-trial BLA glutamate concentration v. time traces from a rat that received non-contingent sucrose re-exposure in (**a-b**) the control, familiar sated (4-hr), or (**c-d**) the novel hungry (20-hr; positive value encoding opportunity) state around (**a, c**) sucrose collection during the non-contingent re-exposure and (**b, d**) the subsequent lever-pressing activity in the non-reinforced probe test conducted in the hungry state. The experiments in **a-b** and **c-d** were each repeated independently in 6 rats with similar results.



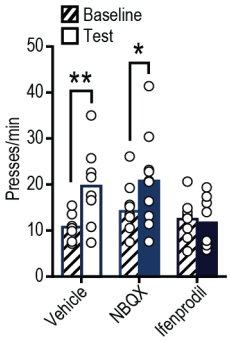

**Supplement Figure 4. BLA glutamate release during non-contingent reward re-exposure - binned.** (**a-b**) Trial-averaged, BLA glutamate v. time trace (shading reflects between-subject s.e.m) around sucrose collection and (**c**) quantification (mean + scatter) of average glutamate immediately post reward consumption during early (1-10), middle (11-20), or late (21-30) reward-delivery trials (**a**) in the familiar sated (4-hr food deprived, *N*=6 rats) state or (**b**) novel hungry (20-hr food-deprived, incentive learning opportunity, *N*=6 rats) state (two-way ANOVA: Trial bin: $F_{2,20}$=3.70, *P*=0.04; Deprivation: $F_{1,10}$=5.52, *P*=0.04; Trial bin x Deprivation: $F_{2,20}$=3.81, *P*=0.04, Bonferroni corrected *post hoc*, between groups: ***P*=0.005). Sucrose-evoked glutamate release is largest early in the re-exposure session, when incentive learning is expected to be the highest.



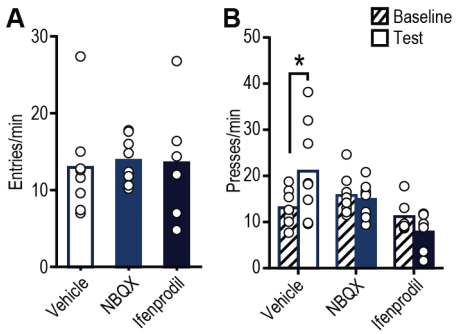

**Supplement Figure 5. BLA glutamate release during reward value encoding and retrieval in familiar hungry state.** (**a**) Procedure schematic (LPs, seeking lever press; LPt, taking lever press; Suc, sucrose; Ø, no sucrose delivered). Rats were trained while hungry (20-hr food-deprived) to press on the seeking-taking chain to earn sucrose. Biosensor glutamate recordings were made during non-contingent re-exposure to the sucrose in the familiar hungry state and during a lever-pressing probe test, also in the hungry state. (**b**) Placement of the microelectrode array biosensor tips in BLA. Numbers represent anterior-posterior distance (mm) from bregma. (**c**) Reward-seeking press rate (seeking presses/min; mean + scatter), relative to baseline press rate (dashed line), during non-reinforced, lever-pressing probe test in the hungry (20-hr food-deprived) state ($N$=6 rats; analyzed using one-sample $t$ test; $t_6$=1.59, $P$=0.16). (**d**) Trial-averaged BLA glutamate concentration v. time trace (shading reflects between-subject s.e.m.) and (**e**) quantification (mean + scatter) of average glutamate concentration change prior to (pre) and following (post) sucrose collection/consumption (occurring at time 0 s), or equivalent baseline periods (BL) during non-contingent sucrose re-exposure in familiar hungry state ($N$=6 biologically independent glutamate recordings; analyzed using one-way ANOVA; $F_{2,10}$=0.86, $P$=0.409). (**f**) Trial-averaged BLA glutamate concentration v. time trace (shading reflects between-subject s.e.m.) and (**g**) quantification of average glutamate concentration change around bout-initiating reward-seeking presses during the lever-pressing probe test in the hungry state ($N$=6 biologically independent glutamate recordings; one-way ANOVA: $F_{2,10}$=4.13, $P$=0.049; Bonferroni corrected *post hoc* test, relative to baseline: *$P$=0.017). Reward experience in the hungry state does not increase BLA glutamate concentration in the absence of encoding new information about the value of the reward in that state.



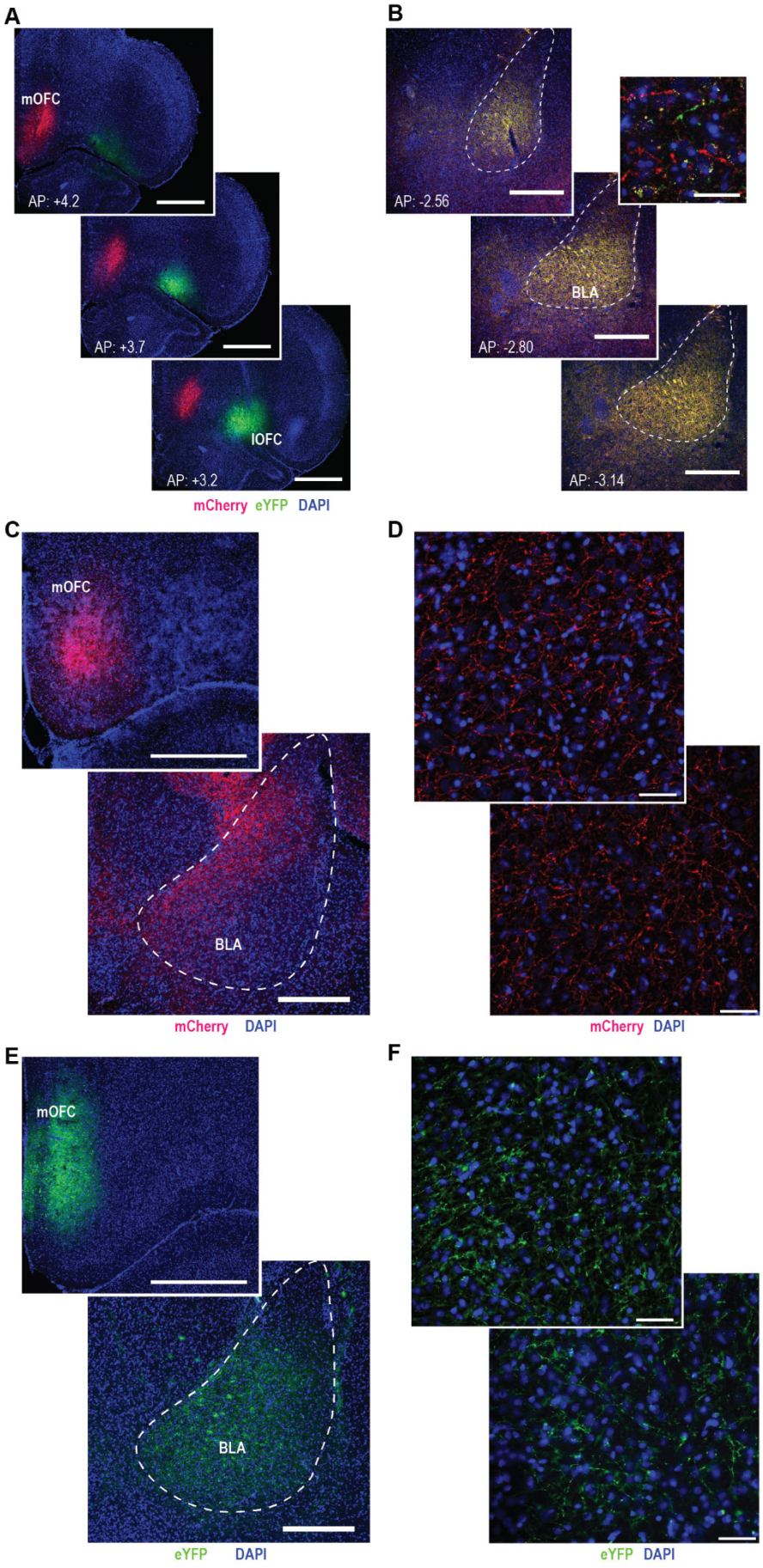

**Supplement Figure 6. BLA glutamate concentration around all reward-seeking presses.** Quantification of average glutamate concentration change around all seeking presses, separating intra-bout presses from bout-initiating seeking presses, during the lever-pressing probe test in the hungry state for subjects that had prior incentive learning experience with the sucrose in the hungry state (*N*=6 rats; one-way ANOVA: Time: $F_{2,10}$=3.07, *P*=0.09; Press type: $F_{1,5}$=8.15, *P*=0.04; Time x Press type: $F_{2,10}$=0.96, *P*=0.42; Bonferroni corrected *post hoc:* $^{*}P$=0.046, between groups; $^{\#}P$=0.012, relative to baseline). Data presented as mean + scatter. Glutamate transients do not precede each individual press but rather only precede bout-initiating reward-seeking presses.




**Supplement Figure 7. Effect of glutamate receptor antagonist on value encoding - raw press rates.** Reward-seeking press rate (seeking presses/min) during baseline and drug-free non-reinforced lever-pressing probe test in the hungry state (Vehicle, *N*=8 rats; AMPA, *N*=10 rats; NMDA, *N*=9 rats; two-way ANOVA: Test: $F_{1,23}$=12.57, *P*=0.002; Treatment: $F_{2,23}$=2.01, *P*=0.16; Test x Treatment: $F_{2,23}$=4.31, *P*=0.03; Bonferroni corrected *post hoc*: Vehicle, ***p*=0.005; NBQX, **p*=0.02) in rats that received BLA microinfusion of vehicle, AMPA, or NMDA antagonist during prior non-contingent sucrose exposure in hungry (20-hr food-deprived) state. Data presented as mean + scatter.




**Supplement Figure 8. Effect of glutamate receptor antagonists on reward checking and reward seeking - raw entry/press rates.** Following non-contingent sucrose exposure in hungry (20-hr food-deprived) state, rats received intra-BLA of Vehicle (*N*=8 rats), AMPA (*N*=8 rats), or NMDA (*N*=7 rats) antagonist prior to a non-reinforced, lever-pressing probe test in the hungry state. (**a**) Food-port entry rate (entries/min; one-way ANOVA: $F_{2,19}$=0.06, *P*=0.95) during this test. Neither treatment affected this reward-checking measure. (**b**) Reward-seeking press rate (seeking presses/min) during baseline and the on-drug post-re-exposure, non-reinforced, lever-pressing probe test (two-way ANOVA: Test: $F_{1,19}$=0.69, *P*=0.42; Treatment: $F_{2,19}$=4.95, *P*=0.02; Test x Treatment: $F_{2,19}$=5.44, *P*=0.01; Bonferroni *post hoc*: **P*=0.013, relative to baseline). Data presented as mean + scatter.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AP: +3.7
lOFC
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mCherry eYFP DAPI
B
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AP: -3.14
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mOFC
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D
mCherry DAPI
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mOFC
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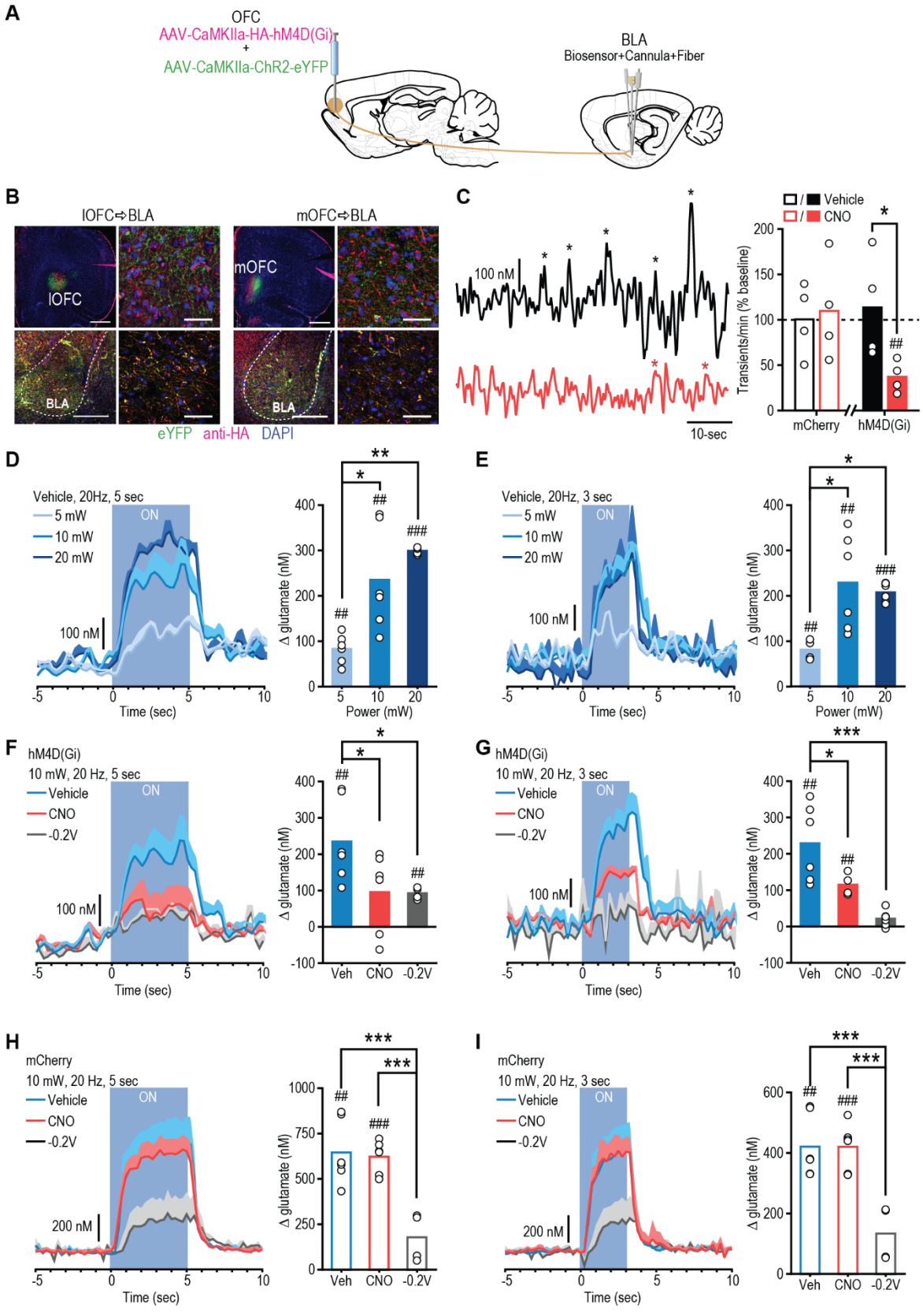
**Supplement Figure 9. lOFC and mOFC projections to the BLA.** (**a-b**) AAV5-CaMKIIa-mCherry was infused in the mOFC and AAV5-CaMKIIa-eYFP was infused into the lOFC and allowed to express for 8 weeks, to ensure terminal expression, prior to histological assessment. (**a**) Representative expression (scale bars represent 1 mm) of mCherry and eYFP in the mOFC and lOFC, respectively. (**b**) Expression of mCherry and eYFP restricted to fibers in the BLA (scale bars represent 500 μm). Inset scale bar represents 50 μm. These data provide anatomical evidence of intermingled projections from both the ventrolateral OFC and medial OFC to the BLA. (**c-f**) AAV5-CamKIIa-ChR2-eYFP or AAV8-hSyn-hM4D(Gi)-mCherry was infused into the mOFC and allowed to express for 8 weeks. (**c, e**) Representative expression of eYFP (**c**) or mCherry (**e**) in the mOFC (scale bars represent 1 mm) infusion location and the BLA terminal field (scale bars represent 500 μm). (**d, f**) Expression of eYFP (**e**) or mCherry (**f**) restricted to fibers in the BLA (scale bars represent 50 μm). Experiments in **a-f** were repeated independently in 4 rats with similar results.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BLA
BLA
eYFP anti-HA DAPI
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Vehicle
CNO
Transients/min (% baseline)
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hM4D(Gi)
D
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Time (sec)
Δ glutamate (nM)
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Vehicle, 20Hz, 3 sec
F
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Vehicle
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G
hM4D(Gi)
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H
mCherry
10 mW, 20 Hz, 5 sec
200 nM
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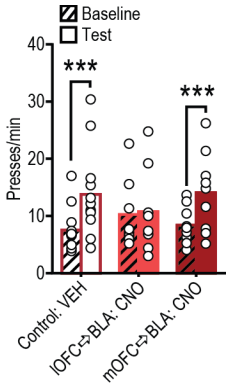
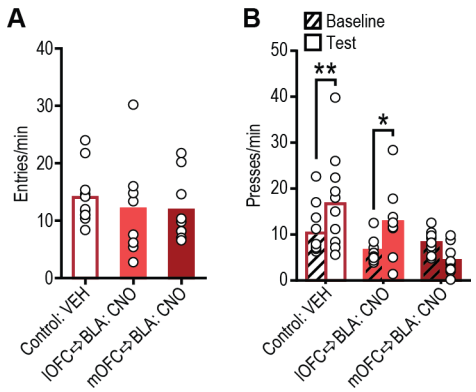
mCherry
10 mW, 20 Hz, 3 sec

**Supplement Figure 10. Validation of chemogenetic and optogenetic manipulation of OFC terminals in BLA.** (**a**) Procedure schematic. hM4d(Gi) and ChR2 were co-expressed in either the lOFC or mOFC. Following 8 weeks for terminal expression, we measured spontaneous and optically-evoked glutamate release events in the BLA of anesthetized rats prior to and following CNO infusion. (**b**) Representative immunofluorescent images of HA-tagged hM4D(Gi) and eYFP-tagged ChR2 expression in lOFC (lOFC→BLA panel: *top left,* scale bar represents 1 mm; *top right,* scale bar represents 50 µm) or mOFC (mOFC→BLA panel: top *left,* scale bar represents 1 mm; *top right,* scale bar represent 50 µm) and BLA terminal expression (lOFC→BLA panel: *bottom left,* scale bar represents 500 µm; *bottom right,* scale bar represents 50 µm; mOFC→BLA panel: *bottom left,* scale bar represents 500 µm; *bottom right,* scale bar represents 50 µm). This experiment was repeated independently in 4 rats with similar results. Data from lOFC and mOFC subjects was collapsed following evidence of no statistically significant differences between these groups. (**c**) Representative glutamate concentration v. time trace showing spontaneous, transient glutamate release events following vehicle or CNO (1 mM/0.5 µl) treatment and quantification of glutamate transient rate (transients/min) normalized to pre-infusion baseline rate (dashed line) (mCherry, $N$=4 independent glutamate recordings; hM4D(Gi), $N$=4 independent glutamate recordings; two-way ANOVA: Drug: $F_{1,6}$=4.45, $P$=0.079; Virus: $F_{1,6}$=1.14, $P$=0.33; Drug x Virus: $F_{1,6}$=7.24, $P$=0.036; Bonferroni *post hoc*: $^{*}P$=0.029, compared to Vehicle; One sample $t$ test: $t_3$=7.26, $^{\#\#}P$=0.005, relative to baseline). These data indicate that chemogenetic inhibition of OFC terminals can decrease spontaneous glutamate release events in the BLA. Importantly, however, this should not be taken as evidence that the OFC contributes to ~50% of spontaneous BLA activity, because such activity is dependent on a variety of BLA inputs and interneurons that are likely differentially sensitive to anesthesia and the animal's current state. (**d-e**) Optically-evoked BLA glutamate concentration v. time trace (shading reflects s.e.m.) and quantification (mean + scatter) of optically-evoked glutamate concentration changes. Blue light delivery for (**d**) 5 s (5 mW, $N$=6 independent glutamate recordings; 10 mW, $N$=6 independent glutamate recordings; 20 mW, $N$=4 independent glutamate recordings; one-way ANOVA: $F_{2,13}$=11.65, $P$=0.001; Bonferroni corrected *post hoc*, between groups: $^{*}P$=0.011, $^{**}P$=0.002; One sample $t$ test, relative to baseline: 5 mW, $t_5$=6.42, $^{\#\#}P$=0.001; 10 mW, $t_5$=5.01, $^{\#\#}P$=0.004; 20 mW, $t_3$=75.48, $^{\#\#\#}P$<0.001) or (**e**) 3 s (5 mW, $N$=4 independent glutamate recordings; 10 mW, $N$=6 independent glutamate recordings; 20 mW, $N$=6 independent glutamate recordings; one-way ANOVA: $F_{2,13}$=6.34, $P$=0.01; Bonferroni corrected *post hoc*: 5 mW v. 10 mW, $^{*}P$=0.014, 5 mW v. 20 mW, $^{*}P$=0.037; One sample $t$ test: 5 mW, $t_3$=7.29, $^{\#\#}P$=0.005; 10 mW, $t_5$=5.32, $^{\#\#}P$=0.003; 20 mW, $t_5$=24.40, $^{\#\#\#}P$<0.001) over OFC terminals in the BLA power-dependently evoked a glutamate concentration change. (**f-g**) Glutamate concentration v. time trace around (**f**) 5 s (Veh, $N$=6 independent glutamate recordings; CNO, $N$=6 independent glutamate recordings; -0.2 V, $N$=4 independent glutamate recordings; one-way ANOVA: $F_{2,13}$=3.77, $P$=0.05; Bonferroni corrected *post hoc*, between groups: Veh v. CNO, $^{*}P$=0.030, Veh v. -0.2 V, $^{*}P$=0.044; One sample $t$ test, relative to baseline: Veh, $t_5$=5.01, $^{\#\#}P$=0.004; CNO, $t_5$=2.13, $P$=0.09; -0.2 V, $t_3$=11.19, $^{\#\#}P$=0.002) or (**g**) 3 s (Veh, $N$=6 independent glutamate recordings; CNO, $N$=4 independent glutamate recordings; -0.2V, $N$=6 independent glutamate recordings; one-way ANOVA: $F_{2,13}$=13.80, $P$<0.001; Bonferroni corrected *post hoc*, between groups: Veh v. CNO, $^{*}P$=0.023, Veh v. -0.2 V, $^{***}P$=0.0002; One sample $t$ test, relative to baseline: Veh, $t_5$=5.32, $^{\#\#}P$=0.003; CNO, $t_3$=7.60, $^{\#\#}P$=0.005; -0.2V, $t_5$=2.42, $P$=0.060) optical stimulation of OFC terminals in BLA following intra-BLA Vehicle or CNO infusion and quantification. Optically-evoked response following CNO did not differ from current changes detected below the $H_2O_2$ (glutamate reporter molecule) oxidizing potential (0.2 V). (**h-i**) In a separate group of subjects, ChR2 was co-expressed with mCherry to control for non-specific effects of CNO in the absence of the hM4D(Gi) transgene. Glutamate concentration v. time trace around (**h**) 5 s (Veh, $N$=6 independent glutamate recordings; CNO, $N$=6 independent glutamate recordings; -0.2 V, $N$=4 independent glutamate recordings; one-way ANOVA: $F_{2,13}$=16.16, $P$=0.0003; Bonferroni corrected *post hoc*, between groups: Veh v. -0.2 V, $^{***}P$=0.0005; CNO v. -0.2 V, $^{***}P$=0.0008; One sample $t$ test, relative to baseline: Veh, $t_5$=8.94,

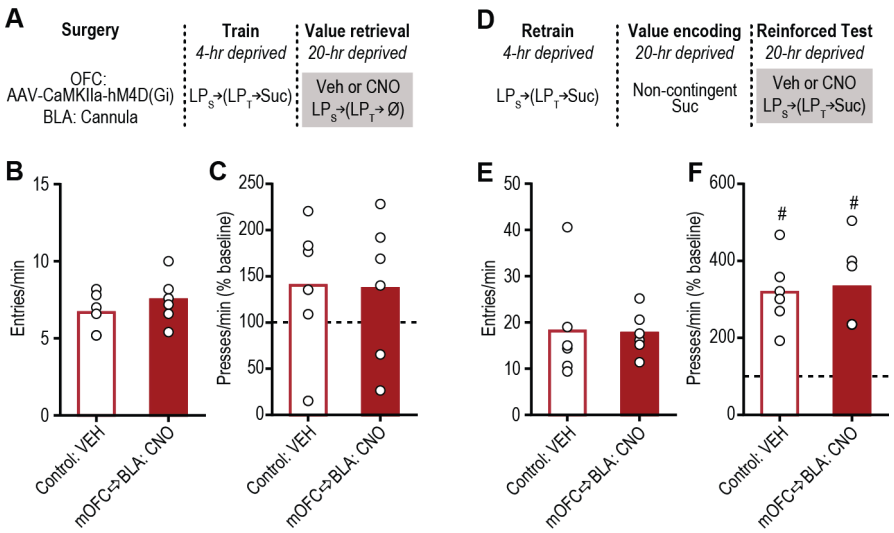
$^{\#\#}P$=0.0003; CNO, $t_5$=16.77, $^{\#\#\#}P$<0.0001; -0.2 V, $t_3$=2.69, $P$=0.074) or (**i**) 3 s (Veh, *N=6* independent glutamate recordings; CNO, *N=6* independent glutamate recordings; -0.2 V, *N=4* independent glutamate recordings; one-way ANOVA: $F_{2,13}$=14.91, $P$=0.0004; Bonferroni corrected *post hoc*: Veh v. -0.2 V, ***$P$=0.0008; CNO v/ -0.2 V, ***$P$=0.0008; One sample *t* test: Veh, $t_5$=9.94, $^{\#\#}P$=0.0002; CNO, $t_5$=13.39, $^{\#\#\#}P$<0.0001; -0.2V, $t_3$=2.96, $P$=0.060) optical stimulation of OFC terminals in BLA following intra-BLA Vehicle or CNO infusion and quantification. Optically-evoked response following CNO did not differ from current changes detected following vehicle infusion in subjects lacking hM4D(Gi). See also [4] for additional validation of OFC→BLA chemogenetic and optogenetic terminal manipulations with *ex vivo* electrophysiology.



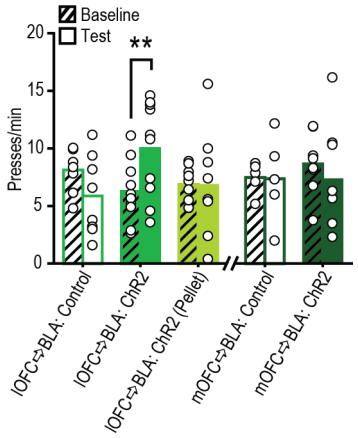

**Supplement Figure 11. Effect of inactivation of lOFC or mOFC terminals in the BLA on reward value encoding - raw press rates.** Reward-seeking press rate (seeking presses/min) during baseline and drug-free, non-reinforced lever-pressing probe test in the hungry state (Control:VEH, *N*=12 rats, ½ mOFC hM4D(Gi), ½ lOFC hM4D(Gi); lOFC→BLA:CNO, *N*=8 rats; mOFC→BLA:CNO, *N*=9 rats; two-way ANOVA: Test: $F_{1,26}$=22.94, *P*<0.0001; Treatment: $F_{2,26}$=0.04, *P*=0.96; Test x Treatment: $F_{2,26}$=4.21, *P*=0.03; Bonferroni *post hoc* relative to baseline: Control:Veh, *P*<0.0001; mOFC→BLA:CNO, ****P*=0.0006) for rats that received BLA microinfusion of Vehicle or CNO during the non-contingent sucrose re-exposure in the hungry (20-hr food-deprived) state. Data presented as mean + scatter.



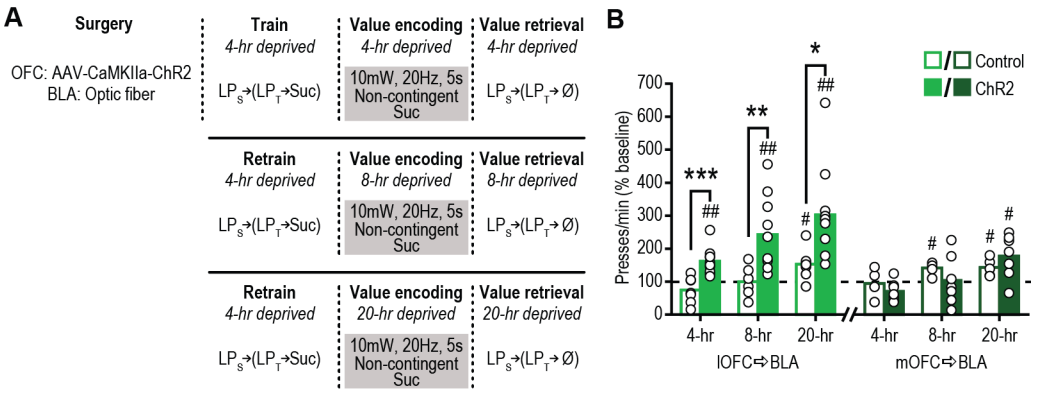

**Supplement Figure 12. Effect of inactivation of lOFC or mOFC terminals in the BLA on reward-checking and reward seeking - raw entry/press rates.** Following non-contingent sucrose exposure in hungry (20-hr food-deprived) state, rats received BLA microinfusion of vehicle or CNO (Control:Veh, *N*=11 rats, lOFC→BLA:CNO, *N*=8 rats; mOFC→BLA:CNO, *N*=9 rats) prior to a non-reinforced lever-pressing probe test in the hungry state. (**a**) Food-port entry rate (entries/min) was not altered by inactivation of either lOFC or mOFC terminals in the BLA during this test (one-way ANOVA: $F_{2,25}$=0.36, *P*=0.70). (**b**) Reward-seeking press rate (seeking presses/min) during baseline and the on-drug post-re-exposure, non-reinforced, lever-pressing probe test (two-way ANOVA: Test: $F_{1,25}$=6.54, *P*=0.02; Treatment: $F_{2,25}$=4.30, *P*=0.02; Test x Treatment: $F_{2,25}$=8.94, *P*=0.001; Bonferroni *post hoc*, relative to baseline: Control:VEH, ***P*=0.003; lOFC→BLA:CNO, **P*=0.016). Data presented as mean + scatter.



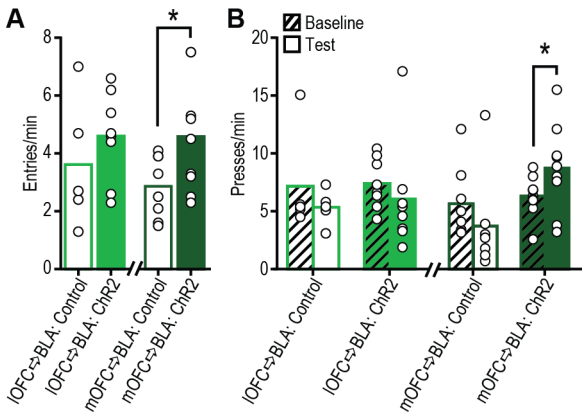

**Supplement Figure 13. Inactivation of mOFC terminals in the BLA does not disrupt reward seeking when reward value is not being retrieved from memory.** (**a**) Procedure schematic. Rats were trained while sated to lever press on the seeking-taking chain to earn sucrose. Following training, they were given two non-reinforced, lever-pressing, probe tests in the hungry state- one each following intra-BLA vehicle or CNO infusion. (LPs, seeking lever press; LPt, taking lever press; Suc, sucrose; Ø, no sucrose delivered; Veh, vehicle; CNO; Clozapine N-oxide) (**b**) Food-port entry rate (entries/min) (two-tailed paired *t* test: $t_5$=1.01, $p$=0.36) and (**c**) reward-seeking press rate (seeking presses/min), normalized to baseline press rate (dashed line), during the non-reinforced lever-pressing probe test in the hungry state following BLA microinfusion of Vehicle or CNO ($N$=6 rats). mOFC→BLA terminal inactivation was ineffective at altering reward-seeking activity in the absence of prior hunger-induced incentive learning (two-tailed paired *t* test: $t_5$=0.09, $p$=0.93). (**d**) Procedure schematic. Following retraining in the sated state, rats were given non-contingent re-exposure to the sucrose in the hungry state (the incentive learning opportunity) and then were given two reinforced lever-pressing tests in the hungry state, one each following BLA vehicle or CNO infusion (order counterbalanced). (**e**) Food-port entry rate (entries/min) (two-tailed paired *t* test: $t_5$=0.15, $p$=0.89) and (**f**) reward-seeking press rate (seeking presses/min), relative to baseline press rate (dashed line). ($N$=6 rats) mOFC→BLA terminal inactivation was ineffective at altering reward-seeking activity if reward value had been encoded, but did not have to be retrieved because the reward was present at test (two-tailed paired *t* test: $t_5$=0.34, $p$=0.75; one sample *t* test, relative to baseline: Control:VEH, $t_5$=5.81, $^{\#}P$=0.002, mOFC→BLA:CNO, $t_5$=4.98, $^{\#}P$=0.004). Data presented as mean + scatter.



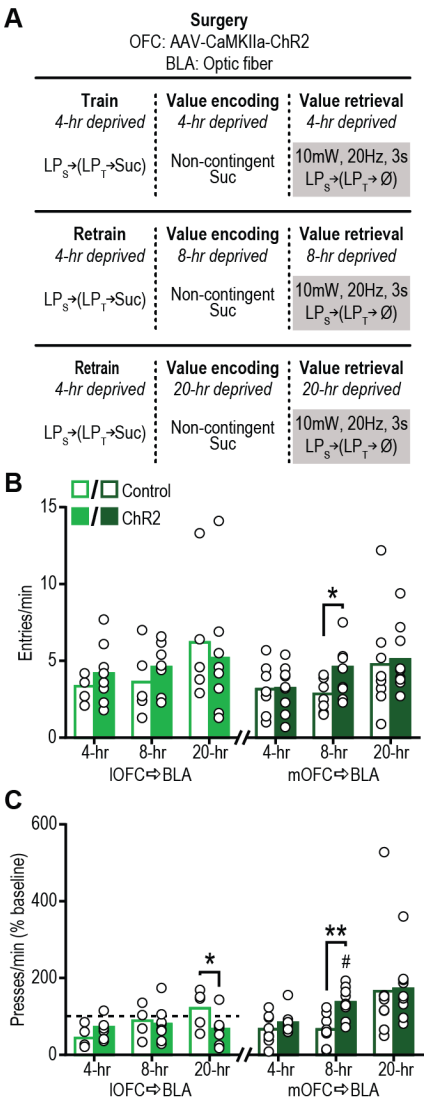

**Supplement Figure 14. Effect of optical stimulation of lOFC→BLA or mOFC→BLA projections on value encoding - raw press rates.** Reward-seeking press rate (seeking presses/min) during baseline and the post-re-exposure, manipulation-free, non-reinforced, lever-pressing probe test in the sated state in rats that received prior non-contingent sucrose or task-irrelevant (Pellet) exposure concurrent with light delivery during in sated state. lOFC→BLA:Control, *N*=8 rats; lOFC→BLA:ChR2, *N*=10 rats; lOFC→BLA:ChR2 (Pellet), *N*=9 rats; mOFC→BLA:Control, *N*=5 rats; mOFC→BLA:ChR2, *N*=7 rats. Two-way ANOVA, lOFC→BLA: Test: $F_{1,24}$=0.54, *P*=0.47; Group: $F_{2,24}$=0.60, *P*=0.55; Test x Group: $F_{2,24}$=7.89, *P*=0.002; Bonferroni corrected *post hoc*, relative to baseline: ***P*=0.004; two-way ANOVA, mOFC→BLA: Test: $F_{1,11}$=0.49, *P*=0.50; Group: $F_{1,11}$=0.11, *P*=0.74; Test x Group: $F_{1,11}$=0.36, *P*=0.56. Data presented as mean + scatter.




**Supplement Figure 15. Activation of lOFC, but not mOFC, to BLA projections concurrent with sucrose experience is sufficient to enhance value assignment across escalating food-deprivation states.** (**a**) Procedure schematic. Rats received 3 test sets in which they first received non-contingent re-exposure to the sucrose with concurrent optical activation of lOFC or mOFC terminals in BLA (ChR2 + 473 nm, 10 mW, 20 Hz, 5 s) or control light delivery (control group consisted of half eYFP + 473 nm and half ChR2 + 589 nm light delivery), and then, the next day, received a non-reinforced, lever-pressing probe test in the same deprivation state. Rats were tested at escalating food-deprivation levels (control, familiar 4-hr food-deprived state, moderate 8-hr food-deprived state, and hungry 20-hr food-deprived state). (**b**) Reward-seeking press rate (seeking presses/min), relative to baseline press rate (dashed line), during the non-reinforced, lever-pressing probe test conducted the day following non-contingent sucrose re-exposure. At each deprivation state tested, activation of lOFC terminals in the BLA concurrent with non-contingent sucrose-experience caused a subsequent upshift in reward-seeking activity (lOFC→BLA:Control, $N$=7 rats; lOFC→BLA:ChR2, $N$=10 rats; two-way ANOVA: Group: $F_{1,15}$=20.74, $P$=0.0004; deprivation: $F_{2,30}$=7.46, $P$=0.002; Group x deprivation: $F_{2,30}$=0.73, $P$=0.49; planned comparisons made using two-tailed unpaired $t$ test, between groups: 4-hr, $t_{15}$=4.42, $P$=0.0005; 8-hr, $t_{15}$=3.29, $P$=0.006; 20-hr, $t_{15}$=2.66, $P$=0.018; one sample $t$ test, relative to baseline: lOFC→BLA:ChR2: 4-hr, $t_9$=4.84, $P$=0.0009; 8-hr, $t_9$=4.06, $P$=0.003; 20-hr, $t_9$=4.49, $P$=0.002). Activation of mOFC terminals in the BLA concurrent with non-contingent sucrose-experience did not alter subsequent reward seeking, compared to controls (mOFC→BLA:Control, $N$=5 rats; mOFC→BLA:ChR2, $N$=7 rats; two-way ANOVA: Group: $F_{1,10}$=0.32, $P$=0.59; deprivation: $F_{2,20}$=6.61, $P$=0.006; Group x deprivation: $F_{2,20}$=1.62, $P$=0.22; one sample $t$ test, relative to baseline: mOFC→BLA:Control: 8-hr, $t_4$=4.86, $P$=0.008; 20-hr, $t_4$=3.55, $P$=0.024; mOFC→BLA:ChR2: 20-hr, $t_6$=3.11, $P$=0.021 ). Data presented as mean + scatter.

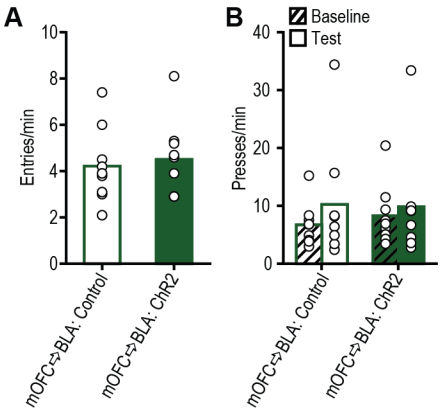



**Supplement Figure 16. Effect of optical stimulation of lOFC or mOFC terminals in the BLA on reward checking and reward seeking - raw entry/press rates.** Following non-contingent sucrose exposure in moderate hunger (8-hr food deprived) state, rats received optical stimulation of lOFC or mOFC terminals in BLA during a non-reinforced, lever-pressing probe test in that moderate hunger state. lOFC→BLA:Control, *N*=5 rats; lOFC→BLA:ChR2, *N*=8 rats; mOFC→BLA:Control, *N*=8 rats; mOFC→BLA:ChR2, *N*=9 rats. (**a**) Food-port entry rate (entries/min; two-tailed unpaired *t* test, between groups: lOFC→BLA: $t_{11}$=0.94, *P*=0.37; mOFC→BLA: $t_{15}$=2.20, **P*=0.04) and (**b**) reward-seeking press rate (seeking presses/min) during baseline and the 8-hr food-deprived non-reinforced lever-pressing probe test with optical stimulation of lOFC (two-way ANOVA: Test: $F_{1,11}$=1.68, *P*=0.22; Group: $F_{1,11}$=0.09, *P*=0.77; Test x Group: $F_{1,11}$=0.03, *P*=0.86) or mOFC terminals in BLA (two-way ANOVA: Test: $F_{1,15}$=0.12, *P*=0.73; Group: $F_{1,15}$=3.96, *P*=0.075; Test x Group: $F_{1,15}$=9.74, *P*=0.007; Bonferroni corrected *post hoc*, relative to baseline: mOFC→BLA:ChR2, **P*=0.046). Data presented as mean + scatter.




**Supplement Figure 17. Activation of mOFC, but not lOFC, terminals in the BLA during reward-seeking tests is only sufficient to enhance reward seeking in moderate deprivation state.** (**a**) Procedure schematic. Rats received 3 test sets in which they first received non-contingent re-exposure to the sucrose and then, the next day, received a non-reinforced, lever-pressing probe test in the same deprivation state. Light (473 nm, 10 mW, 20 Hz, 3 s, once/min) was delivered during the lever-pressing test. The control groups consisted of half eYFP + 473 nm and half ChR2 + 589 nm light delivery. Rats were tested at escalating food-deprivation levels (control, familiar 4-hr food-deprived state, subthreshold 8-hr food-deprived state, and hungry 20-hr food-deprived state). lOFC→BLA:Control, *N*=5 rats; lOFC→BLA:ChR2, *N*=8 rats; mOFC→BLA:Control, *N*=8 rats; mOFC→BLA:ChR2, *N*=9 rats (**b**) Food-port entry rate (entries/min; two-way ANOVA: lOFC→BLA: Group: $F_{1,11}$=0.07, *P*=0.79; Deprivation: $F_{2,22}$=1.82, *P*=0.19; Group x Deprivation: $F_{2,22}$=0.54, *P*=0.58; mOFC→BLA: Group: $F_{1,15}$=0.99, *P*=0.34; Deprivation: $F_{2,30}$=4.19, *P*=0.03; Group x Deprivation: $F_{2,30}$=1.07, *P*=0.36; planned comparisons using two-tailed unpaired *t* test, between groups: 20-hr, $t_{15}$=2.20, **P*=0.044) and (**c**) Reward-seeking press rate (seeking presses/min), relative to baseline press rate (dashed line), during the probe test with optical activation of lOFC (lOFC→BLA: two-way ANOVA: Group: $F_{1,11}$=0.46, *P*=0.51; Deprivation: $F_{2,22}$=4.68, *P*=0.02; Group x Deprivation: $F_{2,22}$=5.80, *P*=0.009; planned comparisons using two-tailed unpaired *t* test, between groups: 20-hr, $t_{11}$=2.24, **P*=0.047) or mOFC (mOFC→BLA: two-way ANOVA: Group: $F_{1,15}$=1.83,

*P*=0.20; Deprivation: $F_{2,30}$=7.81, *P*=0.002; Group x Deprivation: $F_{2,30}$=0.99, *P*=0.38; planned comparisons using two-tailed unpaired *t* test, between groups: 8-hr, $t_{15}$=3.62, ***P*=0.003) terminals in the BLA across escalating food deprivation states. Based on the initial findings that inactivation of mOFC→BLA projection activity only disrupted reward seeking when reward value was being retrieved from memory, we hypothesized that stimulation of these projections would not itself trigger reward-seeking activity, but would rather only facilitate the retrieval of reward value following a sub-threshold value encoding opportunity. Based on this *a priori* hypothesis, we used planned comparisons between control and ChR2 subjects at each deprivation state. Stimulation of lOFC terminals in BLA was found to disrupt value guided reward seeking following incentive learning. We think this is because stimulating inputs that are not necessary for value retrieval provided a reward value signal unlinked to reward or action, which could have resulted in a contingency degradation-like effect. Data presented as mean + scatter. Optical stimulation of mOFC→BLA projections only enhanced reward-seeking activity following subthreshold incentive learning.




**Supplement Figure 18. Effect of optical stimulation of mOFC terminals in the BLA on reward checking and reward seeking (no incentive learning) - raw entry/press rates.** Following non-contingent sucrose exposure in sated (4-hr food deprived) state, rats received optical stimulation of mOFC terminals in BLA during a non-reinforced, lever-pressing probe test in the moderate (8-hr food deprived) hunger state (mOFC→BLA, *N*=9 rats). Within-subject control consisted of 589 nm light delivery (test order counterbalanced). (**a**) Food-port entry rate (entries/min) (two-tailed paired *t* test: $t_8$=0.47, *p*=0.65) and (**b**) reward-seeking press rate (seeking presses/min) during baseline and the 8-hr food-deprived non-reinforced lever-pressing probe test with optical stimulation of mOFC terminals in BLA. Two-way ANOVA: Test: $F_{1,8}$=1.98, *P*=0.20; Group: $F_{1,8}$=0.66, *P*=0.44; Test x Group: $F_{1,8}$=2.29, *P*=0.17. Data presented as mean + scatter.

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