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Supplemental information

Medial orbitofrontal neurotrophin systems integrate hippocampal input into outcome-specific value representations

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Supplementary Figures, Woon et al.

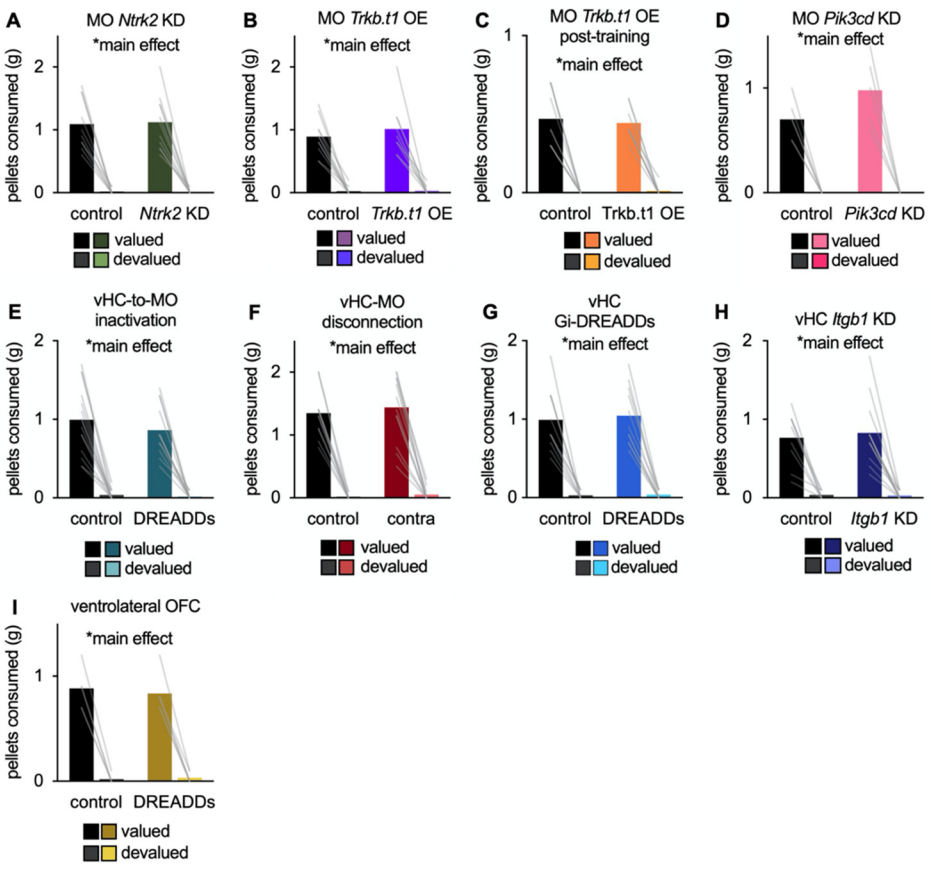

Figure S1 *Post-probe consumption tests.* (**A**) Control and *Ntrk2* KD mice preferentially consumed the valued pellet [main effect of pellet $F_{(1,19)}$=132.373, p<0.001], and no group differences were detected [no main effect of group, no interaction of pellet*group: all F<1] (*n*=10 control, *n*=11 *Ntrk2* KD). (**B**) Mice infused with control viral vectors or *Trkb.t1* in the MO pre-training preferentially consumed the valued pellet [main effect of pellet $F_{(1,18)}$=117.055, p<0.001], and no group differences were detected [no main effect of group, no interaction of pellet*group: all F<1] (*n*=11 control, *n*=9 *Trkb.t1* overexpression). (**C**) Mice infused with control viral vectors or overexpressing *Trkb.t1* in the MO prior to the choice test preferentially consumed the valued pellet [main effect of pellet $F_{(1,17)}$=163.866, p<0.001]. No group differences were detected [no main effect of group, no interaction of pellet*group: all F<1] (*n*=10 control, *n*=9 *Trkb.t1* overexpression). (**D**) Control and MO *Pik3cd* KD mice preferentially consume the valued *vs.* devalued pellet [main effect of pellet $F_{(1,9)}$=110.314, p<0.001]. No group differences were detected [no main effect of group $F_{(1,9)}$=3.064, p=0.114, no interaction of pellet*group $F_{(1,9)}$=3.604, p=0.114] (*n*=6 control, *n*=5 *Pik3cd* KD). (**E**) Control and vHC-to-MO inactivation mice preferentially consumed the valued *vs.* devalued pellet [main effect of pellet $F_{(1,27)}$=169.962, p<0.001]. No group differences were detected [no main effect

of group, no interaction of pellet*group: all F<1] (*n*=17 control, *n*=12 Gi-DREADDs). (**F**) Mice bearing ipsilateral and contralateral infusions preferentially consumed the valued *vs.* devalued pellet [main effect of pellet $F_{(1,22)}$=240.90, p<0.001]. No group differences were detected [no main effect of group, no interaction of pellet*group: all F<1] (*n*=12 control, *n*=12 contralateral). (**G**) Control and vHC Gi-DREADDs mice preferentially consumed the valued *vs.* devalued pellet [main effect of pellet $F_{(1,20)}$=151.023, p<0.001]. No group differences were detected [no main effect of group, no interaction of pellet*group: all F<1] (*n*=11 control, *n*=11 DREADDs). (**H**) Control and vHC *Itgb1* KD mice preferentially consumed the valued *vs.* devalued pellet [main effect of pellet $F_{(1,21)}$=95.915, p<0.001], and no group differences were detected [no main effect of group, no interaction of pellet*group: all F<1] (*n*=9 control, *n*=14 *Itgb1* KD). (**I**) Control mice and mice bearing Gi-DREADDs in the ventrolateral orbitofrontal cortex preferentially consumed the valued *vs.* devalued pellet [main effect of pellet $F_{(1,9)}$=242.711, p<0.001, no main effect of group, no interaction of pellet*group: F<1] (*n*=5 control, *n*=6 DREADDs). Bars and lines connecting bars = means + individual data points, *p<0.05. KD = knockdown. OE = overexpression. OFC = orbitofrontal cortex.

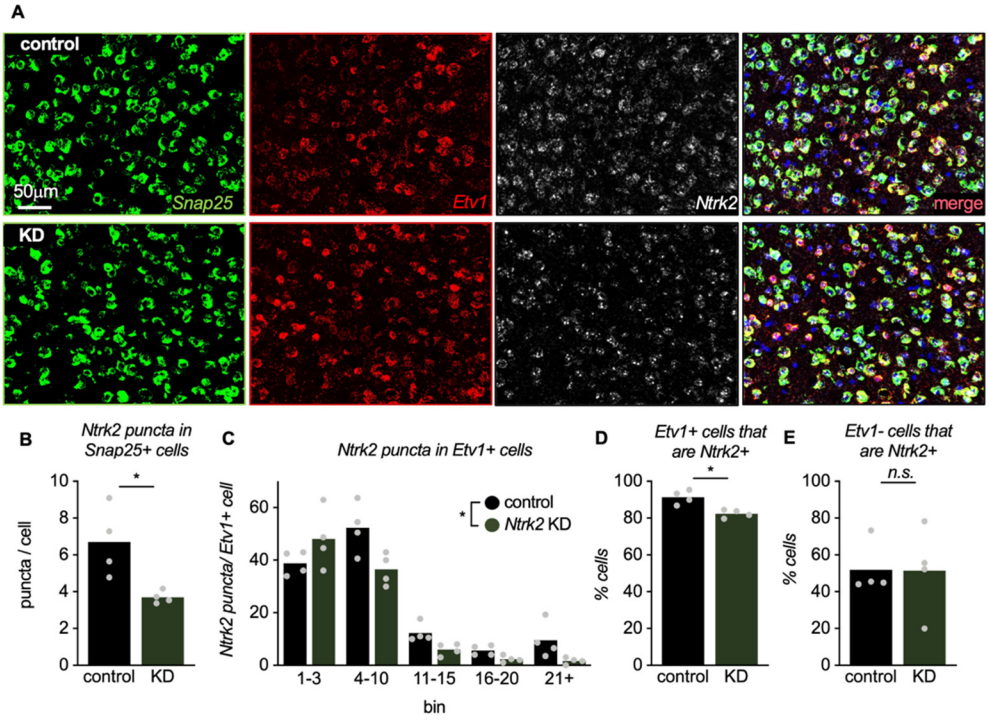

Figure S2 *Confirmation of Cre-mediated Ntrk2 knockdown, including in layer V neurons.* (**A**) Representative images of *Snap25, Etv1*, and *Ntrk2* mRNA in the MO. (**B**) The amount of *Ntrk2* puncta in *Snap25*+ cells (excitatory cells) was lower in the knockdown group [t_6=3.109, p=0.021]. (**C**) *Ntrk2* knockdown mice had fewer *Ntrk2* puncta per *Etv1*+ cell (layer V neurons) [interaction of bin*group $F_{(4,24)}$=4.682, p=0.006, main effect of group $F_{(1,6)}$=7.281, p=0.036, main effect of bin $F_{(4,24)}$=97.078, p<0.001]. (**D**) The percentage of *Etv1*+ cells that contained *any Ntrk2* puncta was lower in the knockdown group [t_6=4.089, p=0.006]. (**E**) The percentage of *Etv1*- cells that were *Ntrk2*+ did not differ between groups [t_6=0.033, p=0.974] (*n*=4/group). Bars = means + individual data points, *p<0.05. "*n.s.*" = non-significant. KD = knockdown.

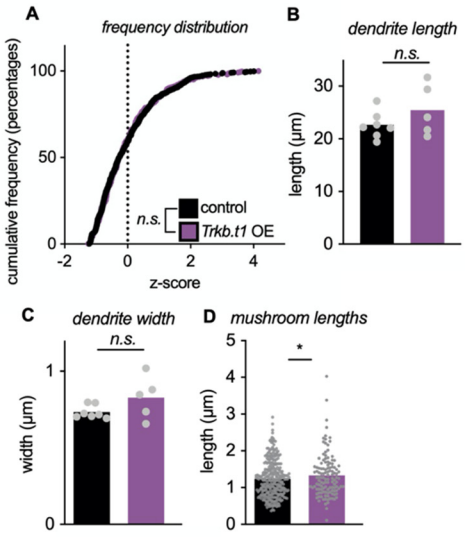

Figure S3 *Trkb.t1 overexpression did not influence spine clustering, dendritic spine length, or dendrite width on layer V neurons.* (**A**) We found no effects of *Trkb.t1* overexpression on spine clustering patterns on excitatory MO neurons [D=0.0393, p=0.8633] (*n*=243 control, *n*=105 *Trkb.t1*). (**B**) Differences in dendritic spine densities (see main text) were not biased by the lengths of sampled dendrites [$t_{(10)}$=-1.33, p=0.213]. (**C**) We also detected no evidence of dendritic blebbing (a marker of neuronal damage), as measured by dendrite diameter [$t_{(10)}$=-1.721, p=0.116] (*n*=7 control, *n*=5 *Trkb.t1*). (**D**) *Trkb.t1* overexpression lengthened mushroom-shaped dendritic spines in the MO [$t_{(422)}$=-1.745, p=0.041] (*n*=302 control, *n*=122 *Trkb.t1*). This figure is also shown in the main text in the absence of individual data points. Bars = means + individual data points, symbols = individual data points, *p<0.05. "*n.s.*" = non-significant. OE = overexpression.

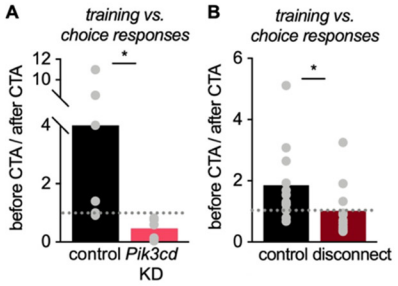

Figure S4 *Pik3cd* knockdown and *contralateral infusion mice do not update response strategies from training to test.* (**A**) Response rates for the devalued outcome during the choice test (Main Text Figure 4E) were compared to response rates generated on the same port during the last day of training. Control mice responded nearly 4 times as much for a food before it was devalued, while knockdown mice did not alter response strategies [$t_{(9)}$=2.046, p=0.036]. (**B**) Response rates for the devalued outcome during the choice test (Main Text Figure 6F) were similarly compared to responding during the last day of training. Control mice responded nearly 2 times as much for a food prior to devaluation, while disconnection mice responded equivalently [$t_{(22)}$=3.129, p=0.005]. Bars and symbols = means + individual data points, *p<0.05. *n*=6 control, *n*=5 *Pik3cd* KD, *n*=12 control, *n*=12 contralateral. KD = knockdown. "*n.s.*" = non-significant. Dotted line = 1 (equivalent responding).

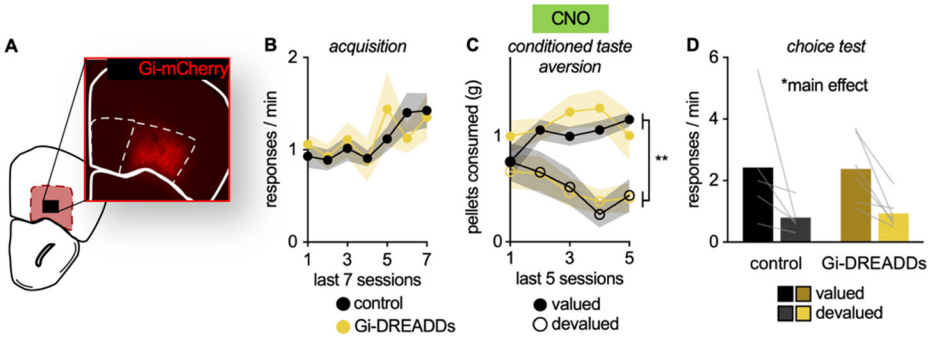

Figure S5 *Inhibiting activity of ventrolateral orbitofrontal cortex neurons during CTA does not impact value-based action.* (**A**) Representative infusion of Gi-DREADDs-mCherry into the VLO. (**B**) Mice were trained to acquire food reinforcers in the absence of CNO [main effect of session $F_{(6,54)}$=3.865, p=0.003] and no group differences detected [no main effect of group, no interaction of session*group: all F<1]. (**C**) Mice then underwent CTA. All mice were administered CNO, regardless of viral vector group, prior to sessions. Mice decreased consumption of the devalued pellet [interaction session*pellet $F_{(4,36)}$=7.64, p<0.001, main effect of pellet $F_{(1,9)}$=26.106, p<0.001, no main effect of session F<1]. No group differences were detected [no main effect of group F<1, no interaction session*group $F_{(4,36)}$=1.012, p=0.414, no interaction pellet*group F<1, no interaction session*pellet*group $F_{(4,36)}$=1.047, p=0.397]. (**D**) Mice underwent a brief choice test drug-free. Groups did not differ [main effect of pellet $F_{(1,9)}$=12.369, p=0.007, no main effect of group, no interaction of pellet*group: all F<1]. Symbols and shading = means ± SEMs, bars and lines connecting bars = means + individual data points, *p<0.05, **p<0.001. *n*=5 control, *n*=6 DREADDs.

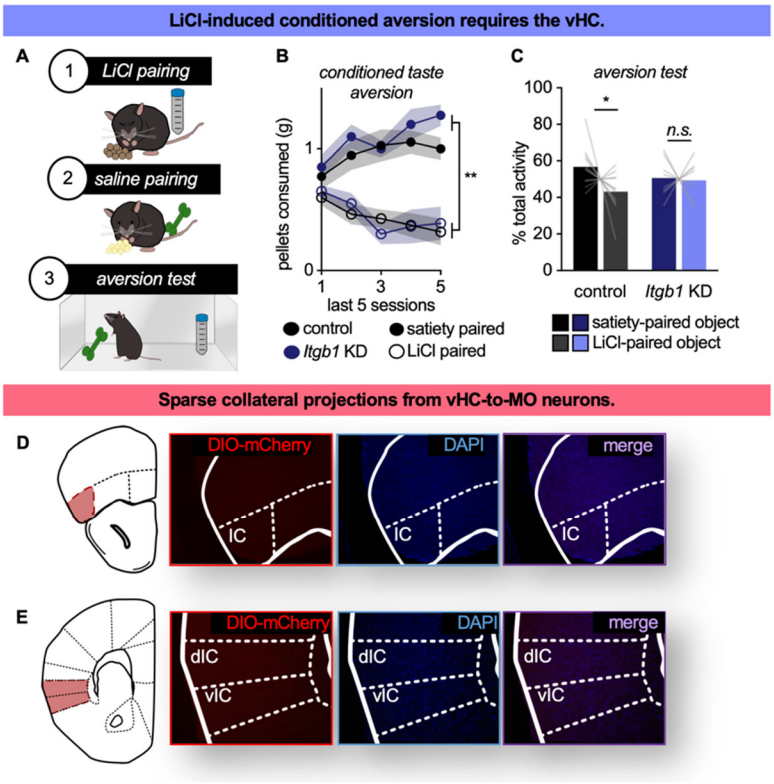

Figure S6 *LiCl-induced conditioned object aversion requires the vHC, and vHC-to-MO projections exhibit sparse collateral projections to the insular cortex (IC).* (**A**) Procedure schematic: Mice were allowed to consume one type of pellet used during training then injected with LiCl. Mice were immediately placed back in the chamber with a conical tube, such that the gastric malaise was associated with both that pellet and conical tube (LiCl-paired). Meanwhile, the other type of pellet was paired with saline and a rodent enrichment toy. Thus, the satiety sensation was associated with both the pellet and enrichment toy (saline-paired). Lastly, mice were placed in chambers equipped with beams to monitor locomotor activity. Both objects were secured to opposite ends of the chamber. If LiCl produces a more aversive response than satiety (as expected), mice will avoid interaction with the LiCl-paired object. (**B**) Food consumption during these pairing sessions did not did not differ between groups [main effect of pellet $F_{(1,17)}$=15.544, p<0.001, interaction session*pellet $F_{(1,68)}$=12.754, p<0.001, no main effect of session F<1, no main effect of group F<1, no interaction pellet*group F<1, no interaction session*group $F_{(4,68)}$=1.885, p=0.123, no interaction pellet*session*group F<1]. (**C**) Control mice spent more time in close proximity to the satiety-paired object than the LiCl-paired object [$t_{(10)}$=2.56, p=0.028]. *Itgb1* knockdown mice, however, did not preferentially interact with either object [$t_{(7)}$=0.388, p=0.71] (*n*=11 control, *n*=8 *Itgb1* KD). (**D**) Projections from the vHC to the MO can collateralize on IC neurons; however, we did not

detect fluorescence in the IC our vHC-to-MO projection-specific manipulations. Representative images of the anterior IC following viral vector infusion in the vHC. (**E**) Representative images of the dorsal IC (dIC) and the ventral IC (vIC). Symbols and shading = means ± SEMs, bars and lines connecting bars = means + individual data points, *p<0.05, **p<0.001. "*n.s.*" = non-significant. KD = knockdown. DIO = Cre-Dependent.

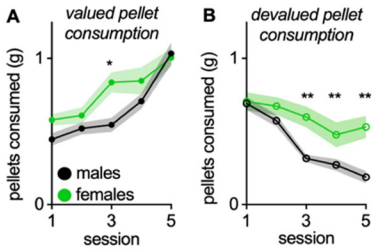

Figure S7 *Consumption during CTA differed between sexes in one experiment.* When vHC-to-MO projections were inhibited during CTA (see Main Text Figure 5), we detected a session*sex*pellet interaction [$F_{(4,160)}$=6.609, p<0.001]. Consumption of the valued pellet differed between males *vs.* females on (**A**) session 3, and for the devalued pellet (**B**) sessions 3, 4, and 5 [all: p<0.001]. Lines and shading = means + SEMs, *p<0.05, **p<0.001. *n*=24 males, *n*=19 females.

Experiment	# infusion exclusions	# post-probe consumption exclusions
MO *Ntrk2* knockdown	3	0
MO *Trkb.t1* overexpression	9	0
Post-training MO *Trkb.t1* overexpression	4	0
MO dendritic spine analyses	5	0
MO p110δ knockdown	9	1
vHC-to-MO inactivation	7	1
vHC-MO disconnection	4	0
vHC Gi-DREADD	0	1
vHC *Itgb1* knockdown	3	0
VLO Gi-DREADD	0	0

Table S1 *Summary of exclusions.* Mice with mis-targeted infusion sites were excluded from analyses. Mice that did not exhibit a preference for the valued *vs.* devalued pellet during the post-probe consumption test were also excluded from analyses. The number of mice excluded in each experiment for each reason is indicated.