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Supplementary Materials for

Internal state configures olfactory behavior and early sensory processing in *Drosophila* larvae

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Supplementary Tables and Figures

Table S1. Fly lines used in this study.

Line	Source
Orco^{1}	BDSC #23129
GH146-GAL4	BDSC #30026
GMR32E03-GAL4	BDSC #49716
UAS-Kir2.1	BDSC #6596
Orco-GAL4	BDSC #23292
Or82a-GAL4	BDSC #23125
Or45a-GAL4	BDSC #9975
UAS-GCaMP6m; Orco::RFP	This study (*UAS-GCaMP6m*, BDSC #42748; *Orco::RFP*, BDSC #63045)
UAS-GluC1α-RNAi (TRiP HMC03585)	BDSC #53356
pLN1-GAL4 (split)	This study (*GMR21D06-AD*, BDSC #70117; *GMR50A06-DBD*, BDSC #68988)
pLN3-GAL4 (split; SS004499)	J. Truman (*GMR42E06-AD*, BDSC #71054; *GMR12C03-DBD*, BDSC #70429)
pLN4-GAL4 (split; SS001730)	J. Truman (*GMR21D06-AD*, BDSC #70117; *GMR12C03-DBD*, BDSC #70429)
UAS-mCD8::GFP; Orco::RFP	BDSC #63045
5-HT7–GAL4	M. Pankratz (*58*)
UAS-gRNA–5-HT7; UAS-Cas9	This study (Rosbash lab)
GMR60F02-GAL4	BDSC #48228
UAS-Trh-RNAi (TRiP JF01863)	BDSC #25842
UAS-CsChrimson::mVenus	BDSC #55136
Trh-GAL4	BDSC #38389
DDC-GAL4	BDSC #7009
Trh^{c01440}	BDSC #10531
UAS-SerT	BDSC #24464
UAS-SerT-RNAi (TRiP HMJ30062)	BDSC #62985
5-HT1A–GAL4	M. Pankratz (*59*)
UAS-gRNA–5-HT1A; UAS-Cas9	This study (Rosbash lab)
y^{1} *w*^{67c23}*; P{CaryP}attP1*	BDSC #8621
w^{1118}*; sna*^{Sco} *It*^{1}/CyO; MKRS/TM6B	BDSC #3703

Table S2. Guide RNA sequences for CRISPR knockout.

gRNA	Sequence
5-HT1A guide 1	TAGCGAACAGCATGAATGAC
5-HT1A guide 2	TGTCATAGCGGCCATTATCC
5-HT1A guide 3	ACGACCGCGACCCGTCGATG
5-HT7 guide 1	CACAGAAACCACAGAACCCA
5-HT7 guide 2	GCATCACCAGCAGCAATTT
5-HT7 guide 3	GGATCTCTGTGTGGCTCTTC

Table S3. Connection weights for circuit model. Includes synaptic and non-synaptic interactions (W, top five rows) and ORN/basal input to each neuron (x_{in} and x_0, respectively; bottom two rows).

	pLN0	pLN1/4	uPN	mPN	CSD
pLN0	0	-1	$-w$	$-w$	$-w$
pLN1/4	$-w$	0	$-w$	-1	0
uPN	0	0	0	0	1
mPN	0	0	0	0	0
CSD	$-\beta$	$-w$	{0, 2}	0	0
ORN	1	1	1	w	w
basal	0	0	0	α	0

Table S4. Summarized neural activities in the fed and food-deprived states.

	pLN0	pLN1/4	uPN	mPN	CSD
fed	1	0	1	1	1
food-deprived	0	1	2	0	2

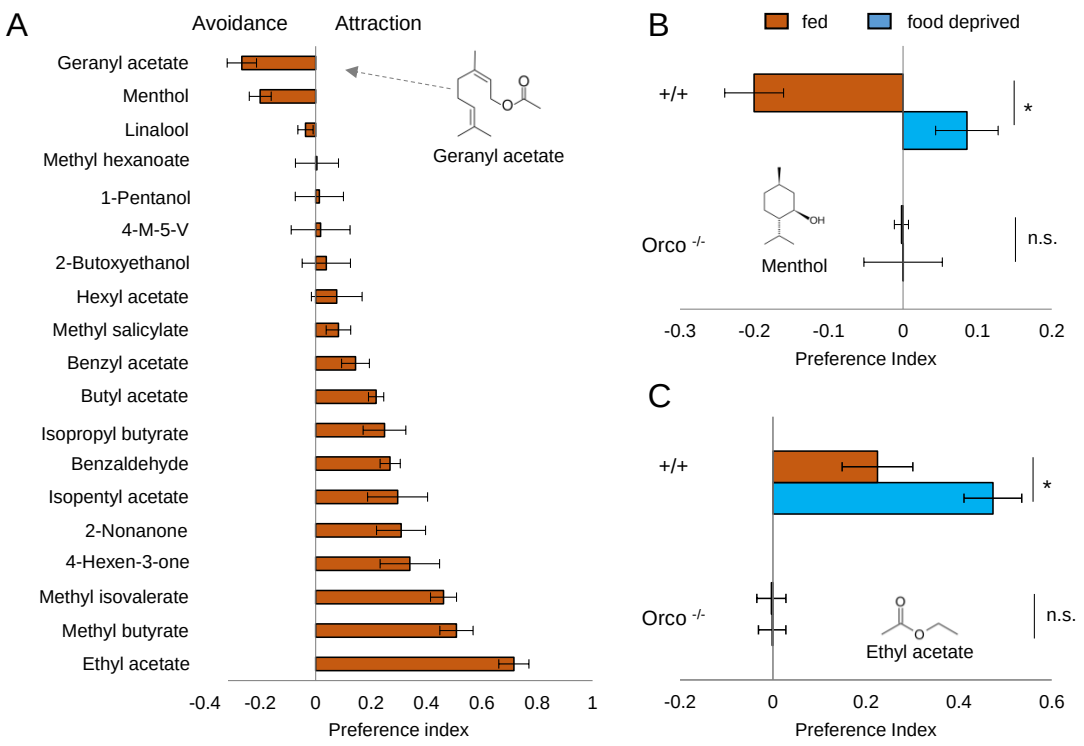


Fig. S1. Food deprivation induces a change in olfactory decision making across odorants and is ORN dependent. (**A**) Odorant screening in fed larvae. Only menthol and geranyl acetate induced aversion, strongest attraction was elicited by ethyl acetate. All odors were tested at a dilution of 10^{-4}, except menthol (10^{-3}). (**B**) After food deprivation, larvae change their response to menthol (10^{-3}) from avoidance to attraction (two-sample t-test, $p < 0.001$). Mutant larvae (*Orco*$^{-/-}$) that lack functional ORNs do not show any significant response to the odor in the fed and food deprived state (one-sample t-test, $p > 0.05$) ($N = 8$). (**C**) Larvae show increased attraction to ethyl acetate (10^{-6}) after food deprivation (two-sample t-test, $p < 0.05$). Mutant larvae (*Orco*$^{-/-}$) that lack functional ORNs do not show any significant response to the odor in the fed and food deprived state (one-sample t-test, $p > 0.05$) ($N = 6$–8).

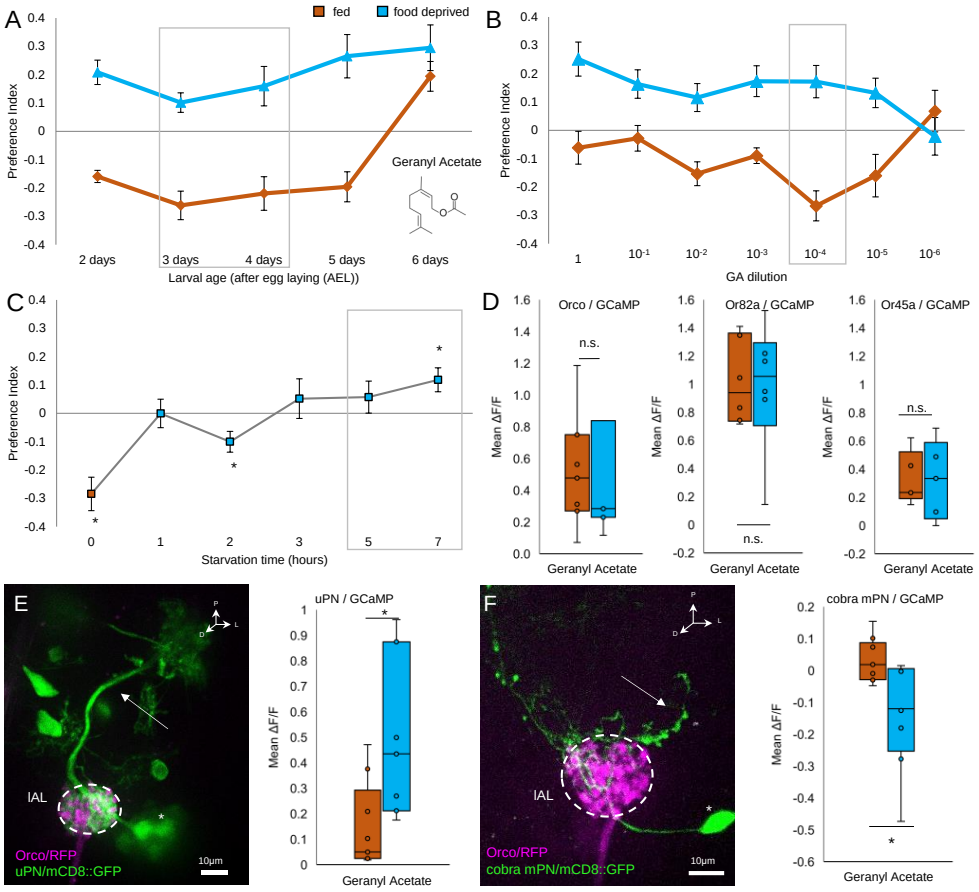


Fig. S2. GA behavior is stable across test parameters. (**A**) The switch in GA 10^{-4} response is present over all larval feeding stages (2–5 days A.E.L., two-sample t-test, $p < 0.001$). Larvae entering the wandering state show attraction already when fed (6 days A.E.L., two-sample t-test, $p > 0.05$) ($N = 8–10$). (**B**) For each GA dilution tested, except the lowest (two-sample t-test, $p > 0.05$), behavior switches significantly between states (two-sample t-test, $p < 0.05$, strongest effect for 10^{-4}: two-sample t-test, $p < 0.001$) ($N = 6–14$). (**C**) GA (10^{-4}) avoidance switches to attraction after food deprivation (one-way ANOVA, $p < 0.001$). Fed larvae avoid GA (one sample t-test, $p < 0.001$). After short food deprivation larvae lose GA avoidance (1 h, 3 h, 5 h, one sample t-test, $p > 0.05$) or only slightly avoid it (2 h, one sample t-test, $p < 0.05$). After 7 hours of food deprivation, larvae show GA attraction (one sample t-test, $p < 0.05$) ($N = 12–16$). (**D**) ORN calcium responses to GA. Mean response change normalized to baseline. ORNs exhibit same responses to GA (10^{-8}) in fed and food deprived state (two-sample t-test, $p > 0.05$) ($N = 7$). ORN-OR82a exhibits same GA (10^{-6}) response in both states (two-sample t-test, $p > 0.05$) ($N = 6$). ORN-OR45a exhibits same GA response (10^{-6}) in both states (two-sample t-test, $p > 0.05$) ($N = 5$). (**E**) *GH146-GAL4* labels uniglomerular projection neurons (uPN GAL4-line). Arrow indicates axonal projection. Asterisk indicates cell bodies. Calcium responses in uPNs to GA (10^{-6}). Mean response change normalized to baseline. Following food deprivation, uPNs respond stronger to GA (Mann-Whitney U-test, $p < 0.05$) ($N = 7–9$). (**F**) *GMR32E03-GAL4* labels the cobra mPN. Arrow indicates axonal projection. Asterisk indicates cell body. Calcium response in cobra mPN to GA (10^{-6}). Mean response change normalized to baseline. Following food deprivation, the cobra mPN is inhibited by GA (two-sample t-test, $p < 0.05$) ($N = 8–9$). Grey boxes indicate parameters used throughout the study. Data points in (**A–C**) represent pooled data from 5–15 min during testing (mean ± SEM). lAL = larval antennal lobe.

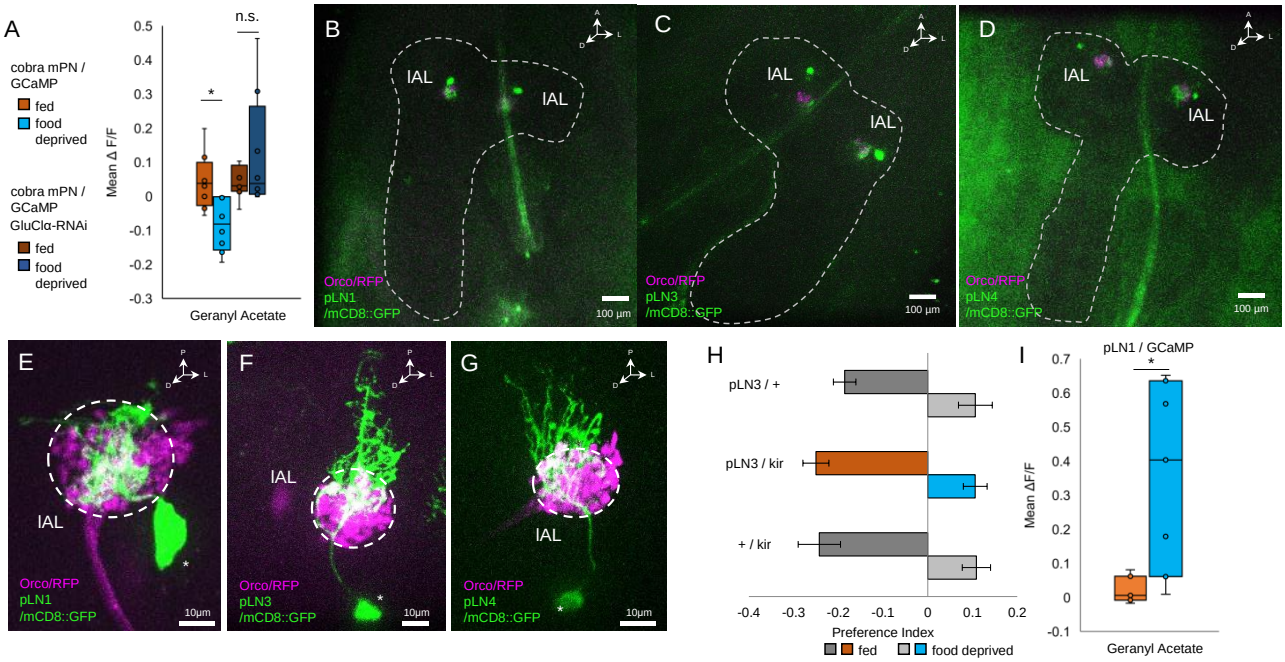


Fig. S3. Cobra mPN is inhibited by glutamate released from pLN1,4, but not pLN3. (**A**) Calcium response in the cobra mPN to GA (10^{-6}). Mean response change normalized to baseline. Light colors: The cobra mPN is inhibited by GA in the food deprived state (two-sample t-test, $p < 0.01$). Dark colors: Upon GluClα-receptor knockdown the cobra mPN shows same response in both states (Mann-Whitney U-test, $p > 0.05$) ($N = 8$). (**B–D**) Whole brain expression patterns of pLN 1, pLN3 and pLN 4 Split-GAL4 lines. Dashed line = larval brain outline. lAL = larval antennal lobe. (**E–G**) Expression patterns of pLN 1, pLN3 and pLN 4 Split-GAL4 lines in the lAL. Asterisks indicate cell bodies. lAL = larval antennal lobe. (**H**) Silencing pLN3 does not impair GA responses in both states (one-way ANOVA, $p > 0.05$) ($N = 8–10$) (**I**) Calcium response in pLN1 to GA (10^{-6}). Mean response change normalized to baseline. The pLN1 does not respond to GA in the fed state but shows increased response after food deprivation (two-sample t-test, $p < 0.05$) ($N = 7$).

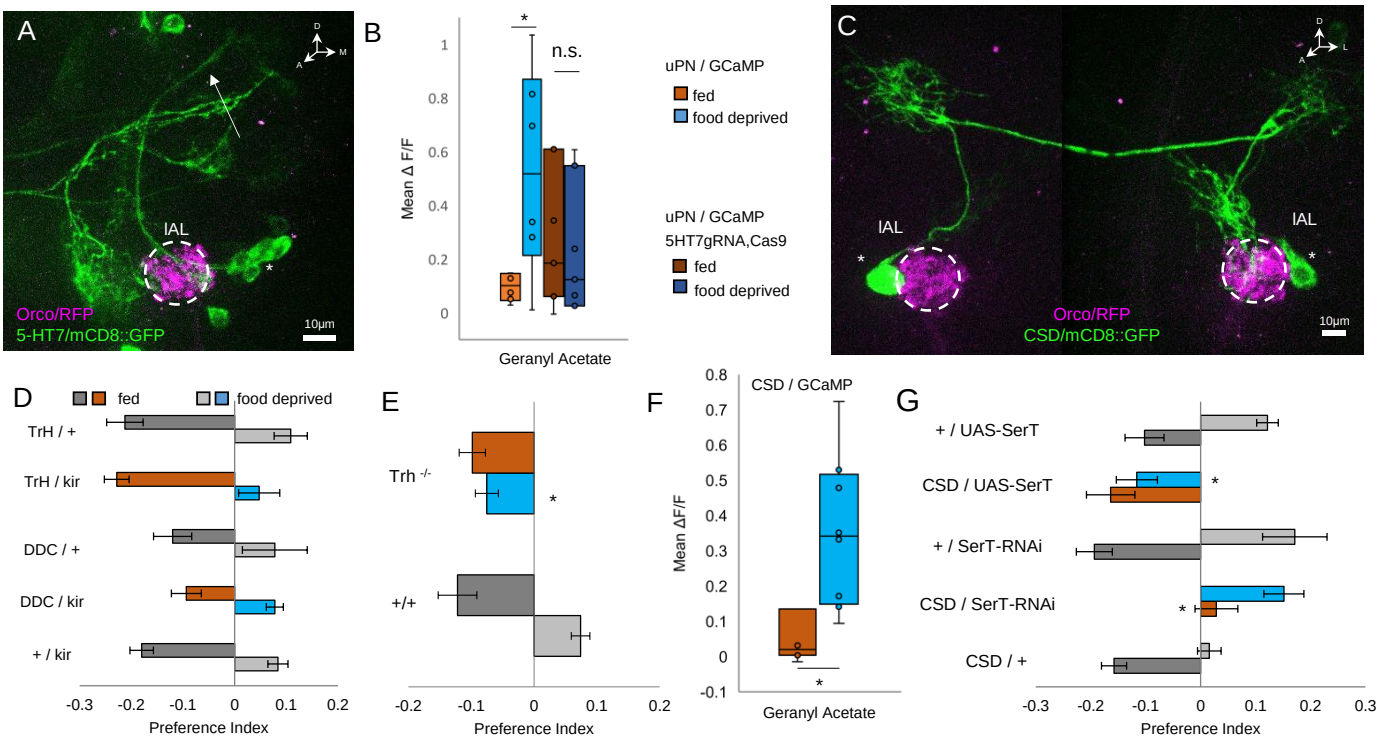
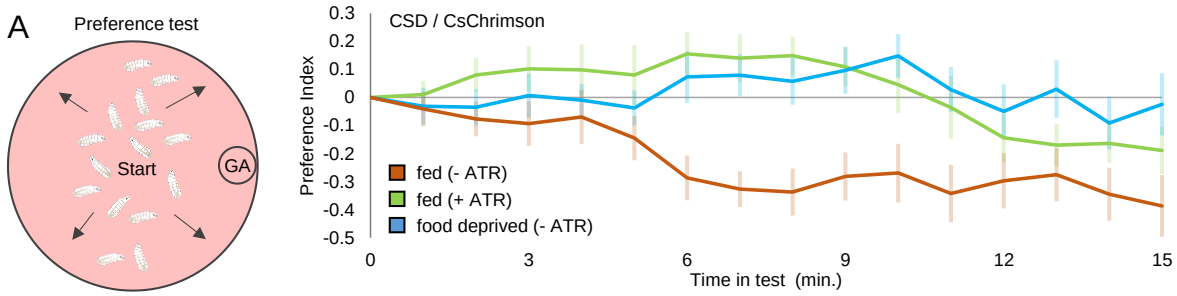


Fig. S4. Serotonin from CSD excites uPNs. (**A**) *5-HT7-GAL4* expression pattern, including uPNs. Arrow indicates axonal projections. Asterisk indicates cell bodies. (**B**) Calcium response in uPNs to GA (10^{-6}). Mean response change normalized to baseline. Light colors: uPNs show increased GA response after food-deprivation (two-sample t-test, $p < 0.05$). Dark colors: Upon 5-HT7 receptor knockout in uPNs, they show same response in both states (two-sample t-test, $p > 0.05$) ($N = 6–7$). (**C**) *GMR60F02-GAL4* labels the CSD neuron. Asterisks indicates cell bodies. (**D**) Silencing neurons labeled by *Trh-GAL4* does not affect GA response (one-way ANOVA, $p > 0.05$) ($N = 6–10$). Silencing neurons labeled by *DDC-GAL4* does not affect GA response (one-way ANOVA, $p > 0.05$) ($N = 6–14$). (**E**) Mutant larvae ($Trh^{-/-}$) unable to synthesize serotonin exhibit GA avoidance in fed state (Mann-Whitney U-test, $p > 0.05$), however do not switch to GA attraction after food deprivation (two-sample t-test, $p < 0.01$) ($N = 6–8$). (**F**) Calcium response in CSD to GA (10^{-8}). Mean response change normalized to baseline. CSD responds stronger to GA upon food-deprivation (two-sample t-test, $p < 0.05$) ($N = 8–10$). (**G**) Overexpression of the serotonin transporter in CSD using *UAS-SerT* only affects food-deprived GA attraction (one-way ANOVA, $p < 0.01$) ($N = 8$). RNAi-knockdown of SerT in CSD only impairs fed GA avoidance (one-way ANOVA, $p < 0.01$) ($N = 8–10$). lAL = larval antennal lobe.



B	CSD/ Chrimson	CSD/ Chrimson	CSD/ Chrimson	+/Chrimson	CSD/+
ATR	-	-	+	+	+
State	fed	food deprived	fed	fed	fed
GA response	Avoidance	Approach	Approach	Avoidance	Avoidance
Run length	●	●	●	●	●
Run speed	●	●	●	●	●
Run duration	●	●	●	●	●

● unchanged ● increased ● decreased

Fig. S5. CSD activation affects locomotion parameters differently than food deprivation. (**A**) Activation of the CSD neuron by expressing *UAS-CsChrimson* in fed larvae mimics the food deprived GA response behavior. (**B**) Locomotion parameters of larvae. Food deprived larvae show increased run length due to increased run speed and run duration. Artificial activation of CSD only induces an increase in run duration.

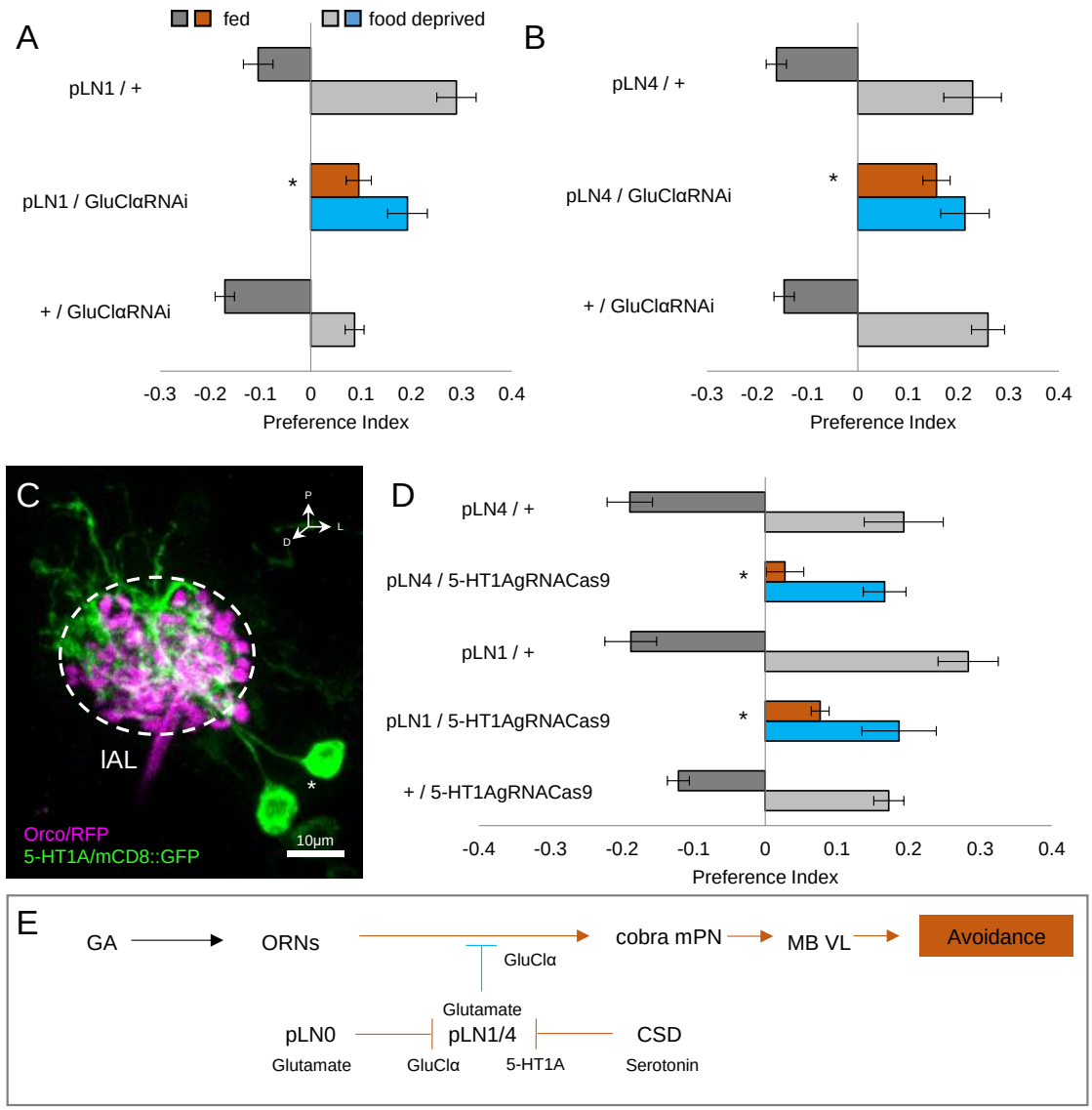


Fig. S6. pLN1/4 receive glutamatergic and serotonergic inhibition in the fed state. (**A**) Knockdown of the GluClα-receptor in pLN1 only impairs fed GA avoidance (one-way ANOVA, $p < 0.001$) ($N = 8–12$). (**B**) Knockdown of the GluClα-receptor in pLN4 only impairs fed GA avoidance (one-way ANOVA, $p < 0.001$) ($N = 7–8$). (**C**) Expression pattern of *5-HT1A-GAL4*, labelling two local lAL neurons. Asterisk indicates cell bodies. lAL = larval antennal lobe. (**D**) Knockdown of the 5-HT1A receptor in pLNs only impairs fed GA avoidance (one-way ANOVA, $p < 0.001$) ($N = 6–16$). (**E**) In the fed state, pLN1/4 are inhibited by a glutamatergic neuron (presumably pLN0) and the serotonergic CSD neuron, overall reducing inhibition onto cobra mPN.

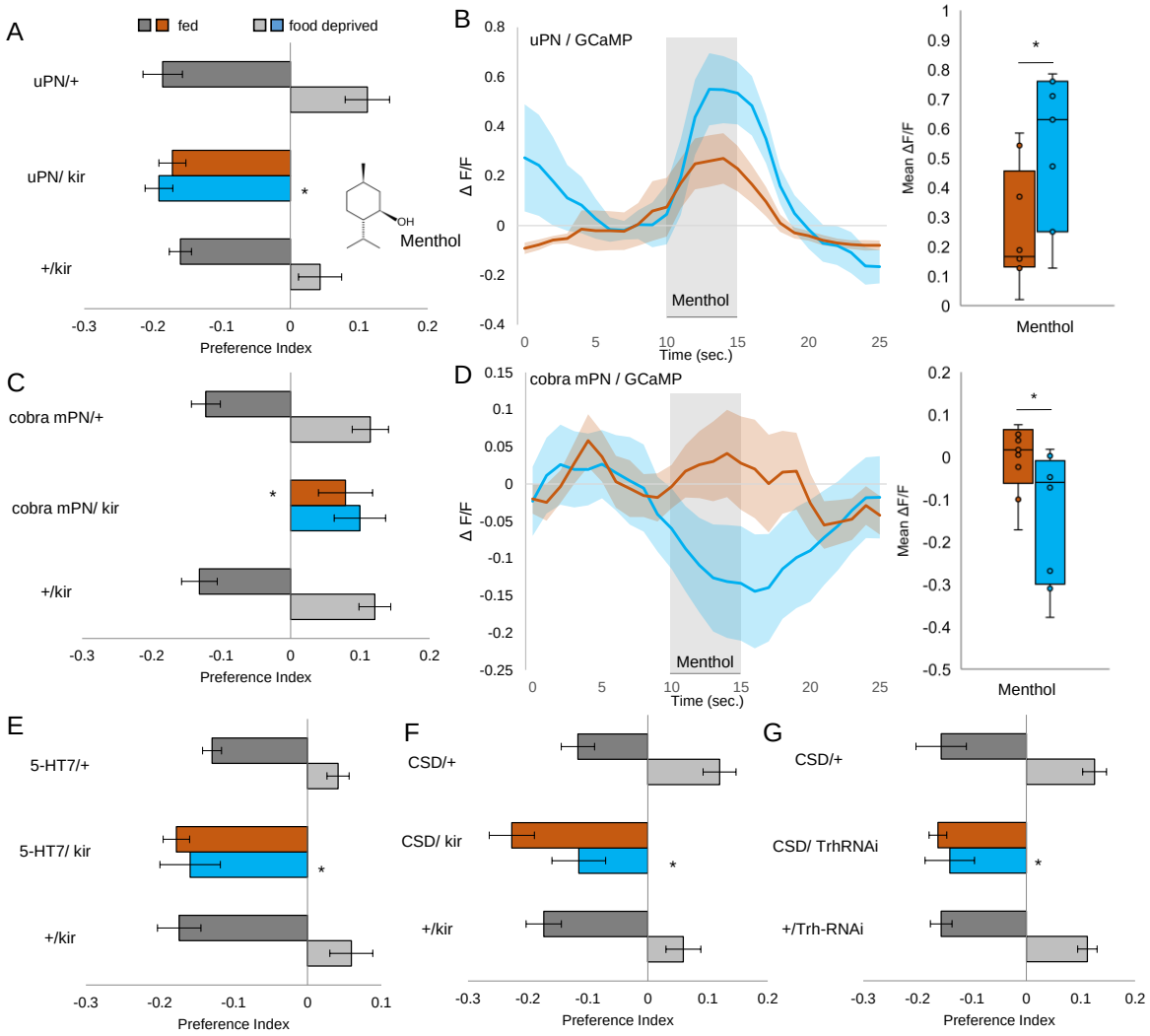


Fig. S7. State dependent olfactory behavior in response to the odorant menthol requires the same neural circuit as for GA. (**A**) Silencing uPNs (*GH146-GAL4*) only abolishes attraction to menthol in food-deprived larvae (one-way ANOVA, $p < 0.001$) ($N = 4–6$). (**B**) Food deprivation increases uPN responses to menthol (two-sample t-test, $p < 0.05$) ($N = 7–9$). (**C**) Silencing cobra mPN (*GMR32E03-GAL4*) only abolishes menthol avoidance in the fed state (one-way ANOVA, $p < 0.01$) ($N = 6$). (**D**) Food deprivation decreases mPN response to menthol (two-sample t-test, $p < 0.05$) ($N = 8–9$). (**E**) Silencing *5-HT7-GAL4* labelled neurons abolishes attraction to menthol in the food-deprived state (one-way ANOVA, $p < 0.01$) ($N = 8–10$). (**F**) Silencing CSD (*GMR60F02-GAL4*) only abolishes attraction to menthol after food-deprivation (one-way ANOVA, $p < 0.01$) ($N = 8–10$). (**G**) Knockdown of serotonin synthesis in CSD only abolishes attraction to menthol after food-deprivation (one-way ANOVA, $p < 0.01$) ($N = 8$).

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