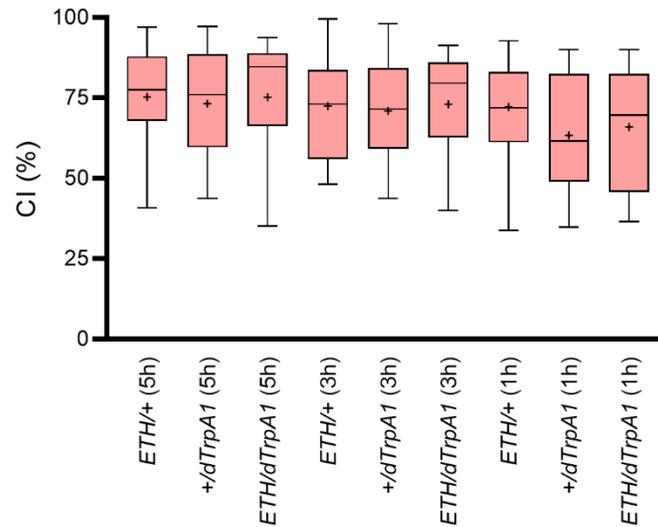
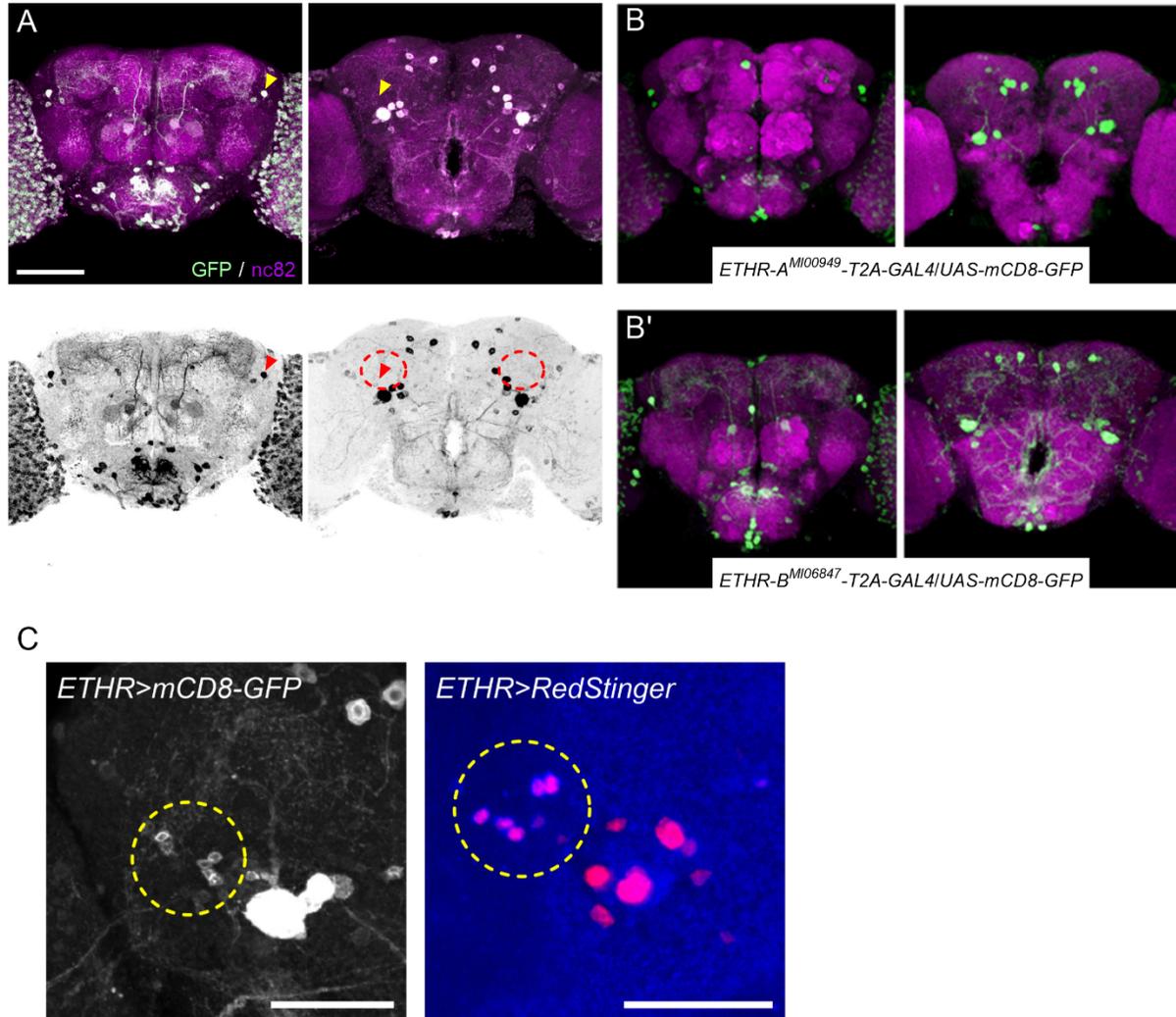


Supplementary Material



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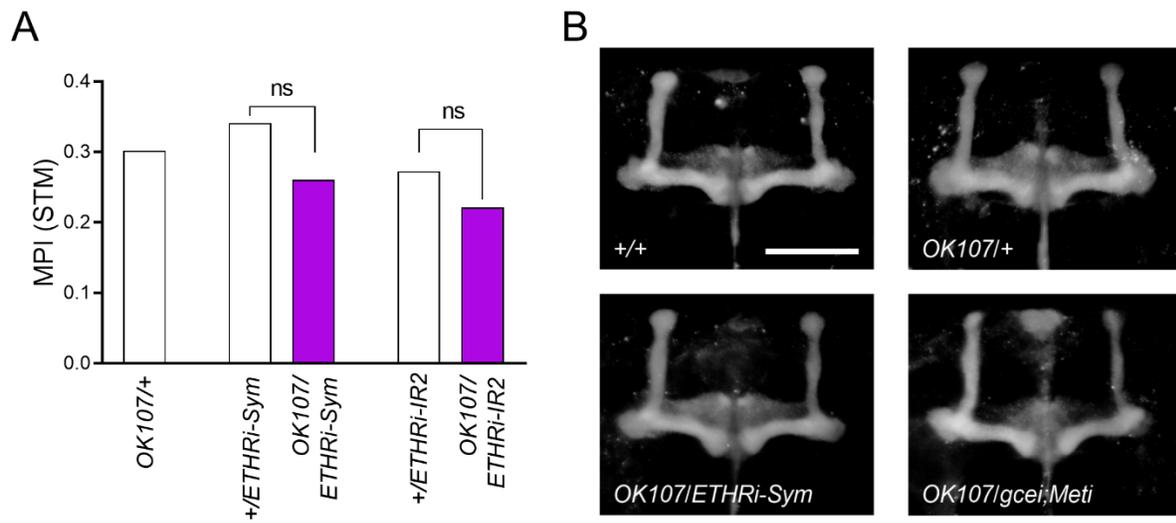
6 **Figure S1.** Activation of Inka cells during training sessions (5-, 3-, and 1-hour heat activation)
7 does not alter basal courtship activity of sham-trained males toward immobilized virgin females
8 during 10-min testing sessions. No significant change in courtship index (CI) was observed
9 compared to genetic control groups under identical experimental conditions (n = 33-40). Mann-
10 Whitney *U* Test.



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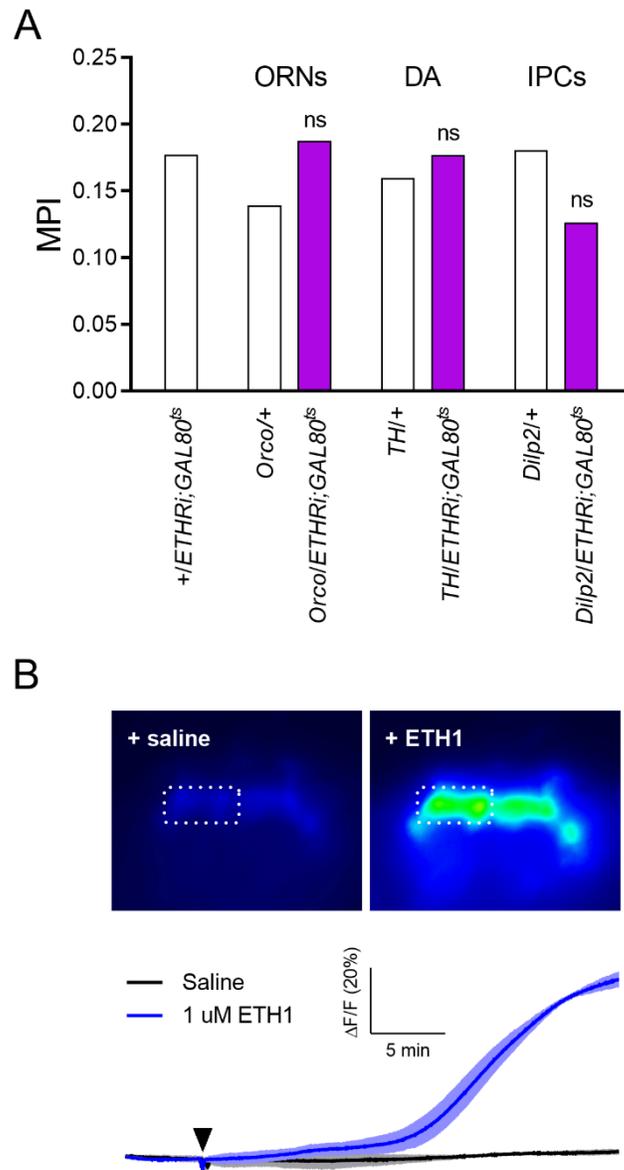
14 **Figure S2.** ETHR is expressed in broad areas of adult male brain. (A) Day-4 male brain of an
 15 *ETHR-GAL4* line (*MH1506*) expressing GFP (*UAS-mCD8-GFP*). This line drives to cells
 16 expressing both ETHR-A and ETHR-B subtypes (Diao et al., 2016). Anterior (left) and posterior
 17 (right). Arrowheads indicate putative DAL neuron (left) and Kenyon cell areas (right). A
 18 putative calyx area is indicated as dashed circles. Scale bar represents 100 μm . (B and B')
 19 Expression of ETHR subtypes in day-4 male brain. ETHR-A (B) and ETHR-B (B') expression in
 20 anterior (left) and posterior (right) brain sides. (C) An *ETHR-GAL4* driver line (*MH1506*) labels
 21 a small population of Kenyon cell-like neurons. Left: posterior view of the *ETHR-GAL4/UAS-*
 22 *mCD8-GFP* male; right, posterior view of an *ETHR-GAL4/UAS-RedStinger* male. Yellow circles
 23 represent area of interest. Scale bars = 20 μm .



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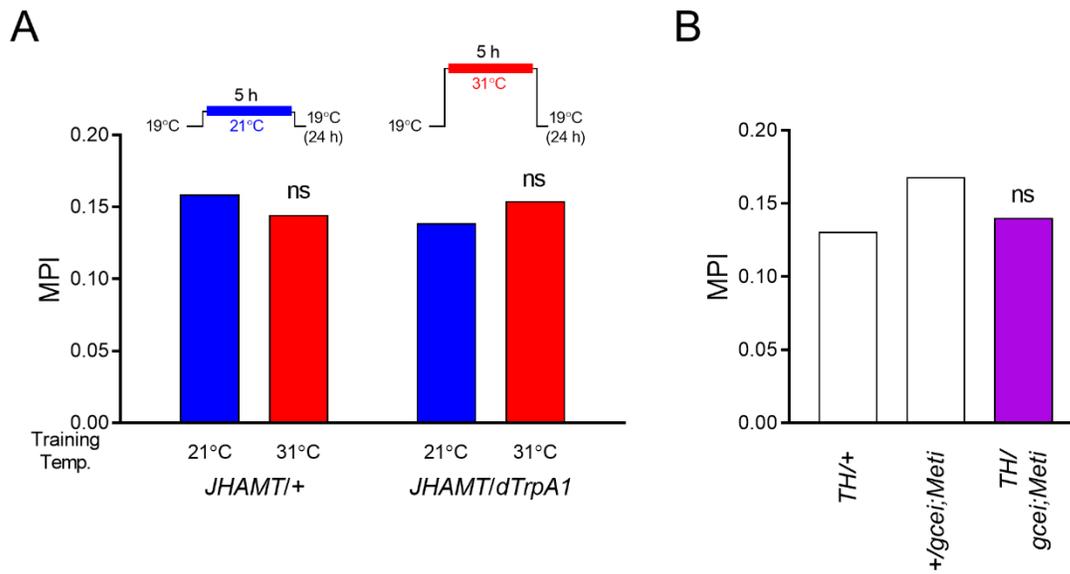
27 **Figure S3. (A)** The role of ETHR expression in the MB for STM was tested by silencing ETHR
 28 genes in the entire MB (*OK107/UAS-ETHR RNAi-Sym* and *OK107/UAS-ETHR RNAi-*
 29 *IR2*). A male was paired with a mated female for 1 hour. Following one 10-min interval,
 30 memory performance was tested by pairing the conditioned male with an immobilized virgin
 31 female for 10 minutes (n = 50-54). Random Permutation Test, ns, no significance. **(B)** RNA
 32 knockdown of ETHR or JH receptors in the MB (*OK107*, entire MB) does not induce gross
 33 morphological changes. Brains of day 4-5 posteclosion males were stained against Fas-II to
 34 visualize the MB. Scale bar represents 50 μ m.



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38 **Figure S4. (A)** Conditional ETHR silencing in ORNs, TH-positive DA neurons, and IPCs in the
 39 brain does not impair courtship LTM. Training was conducted by housing individual males at
 40 31°C during adulthood except during training and testing sessions ($n = 54-60$). Random
 41 Permutation Test, ns, no significance. **(B)** ETH mobilizes Ca^{2+} in MB lateral neuropils: above,
 42 Ca^{2+} -dependent fluorescence in MB lateral neuropil area of a *OK107/UAS-GCaMP5* male brain
 43 in response to 1 μ M DmETH1 in the absence (left) and presence (right) of ETH; bottom,
 44 averaged traces of percent $\Delta F/F_0$ following saline control (black) and 1 μ M DmETH1 (blue)
 45 treatments. Arrowhead represents saline or ETH application. Darker lines represent mean, while
 46 the light envelopes represent SEM ($n = 3$ for each).



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50 **Figure S5. (A)** CA stimulation during courtship conditioning does not affect LTM performance.
 51 Individual males were placed at 21°C or 31°C for 5 hours with a mated female and tested for
 52 courtship suppression after a 24-hour interval at 21°C compared to CI of sham-trained males (n =
 53 54-64). **(B)** RNA knockdown of both JH receptors (MET and GCE) in TH-positive neurons does
 54 not affect 24-hour lasting memory following 5-hour training with a mated female (n = 52-60).
 55 Random Permutation Test, ns, no significance.