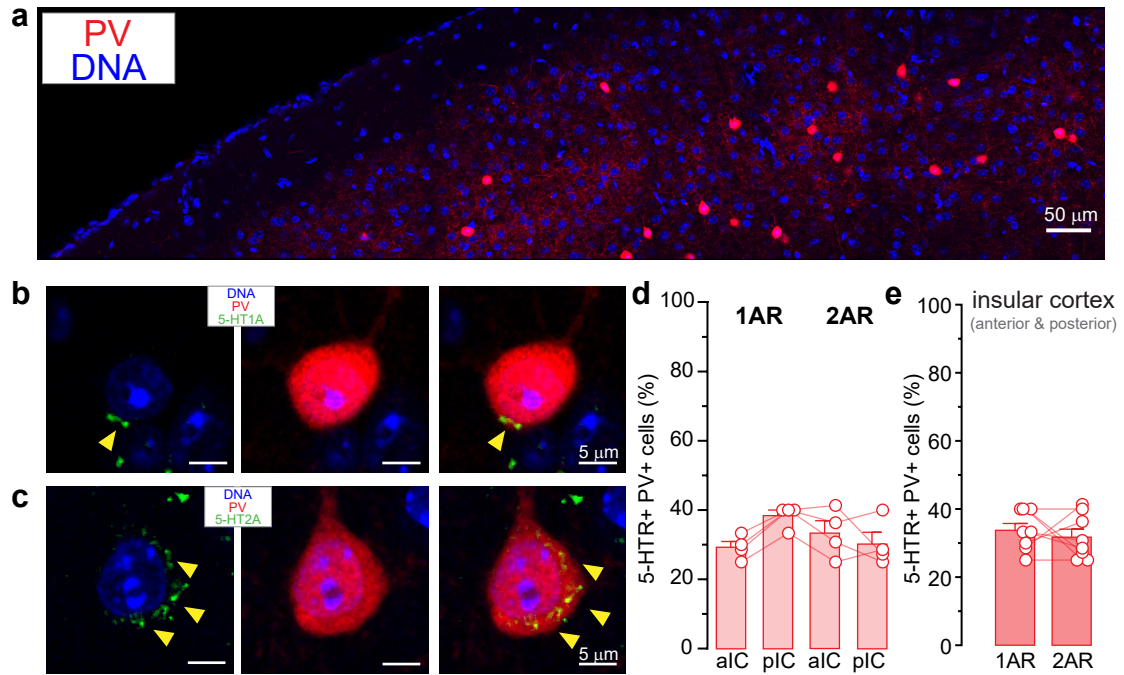


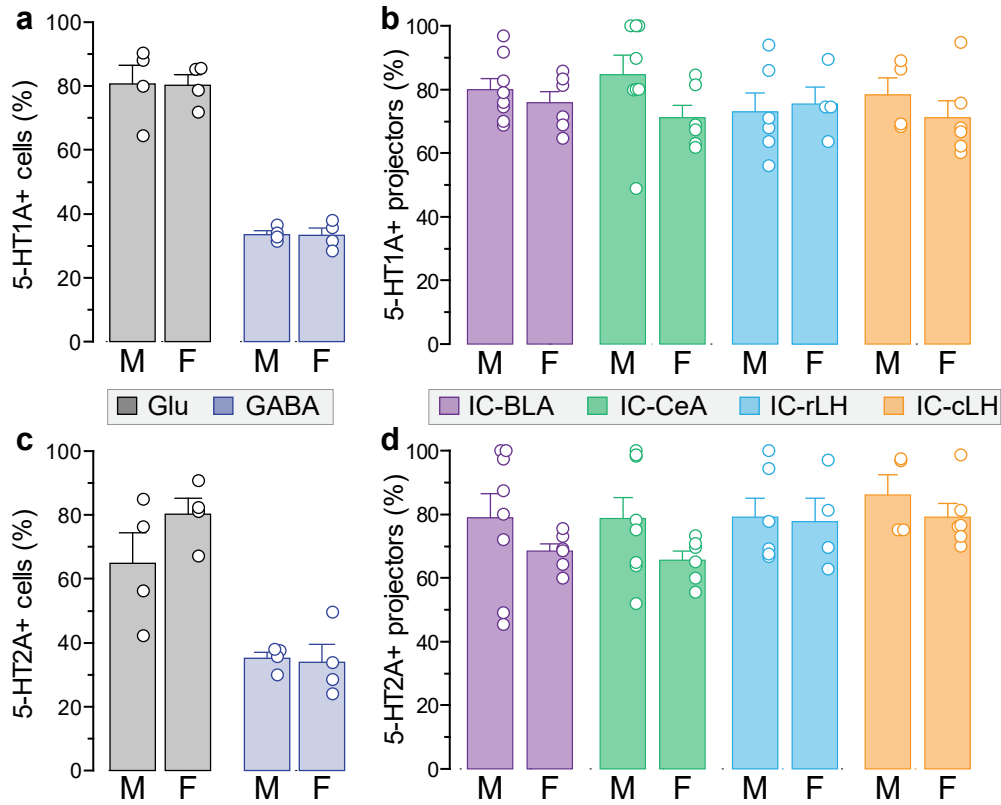
**Supplementary Figure 1.** mRNA expression level of Htr1a and Htr2a in different brain structures (data exported from Allen Brain Atlas, n=1 mouse per gene).

a-b. mRNA expression of Htr1a in isocortical (a) and subcortical regions (b).  
 c-d. mRNA expression of Htr2a in isocortical (c) and subcortical region (d).



**Supplementary Figure 2.** Expression of 5-HT1A or 2A in parvalbumin-expressing neurons in the insular cortex.

a. An image of PV labelled by tdTomato and nucleus staining in the anterior insula of PV-cre::Ai14 mouse brain.  
 b-c. Representative images of parvalbumin-expressing (PV+) neurons containing 5-HT1A (b) or 2A (c) in the insula. Yellow arrows indicate the expression of 5-HT1A or 2A.  
 d. Percentage of 5-HT1A or 2A expression in PV+ neurons in the anterior (ICa) and posterior insula (ICp). Total PV+ neurons, 1A+: ICa+ICp=91+69=160; 2A+: ICa+ICp=141+121=262; n=4 mice  
 e. 5-HT1A or 2A expressed in ~30% of PV+ neurons in the insula.



**Supplementary Figure 3.** No sex difference in 5-HT1A or 2A expression in six populations of the insular neurons.

**a and c.** Percentage of glutamatergic (Glu) and GABAergic (GABA) neurons expressing 5-HT1A (a) or 5-HT2A (c) in the male and female insula (data of Figure 1j and 5f). Total cell numbers (n=2 mice/group, data of Figure 1j and 5f):

**(a)** 5-HT1AR: Male: Glu=2051, GABA=458; Female: Glu=2274, GABA=428;

**(c)** 5-HT2AR: Male: Glu=2794, GABA=641; Female: Glu=2433, GABA=478.

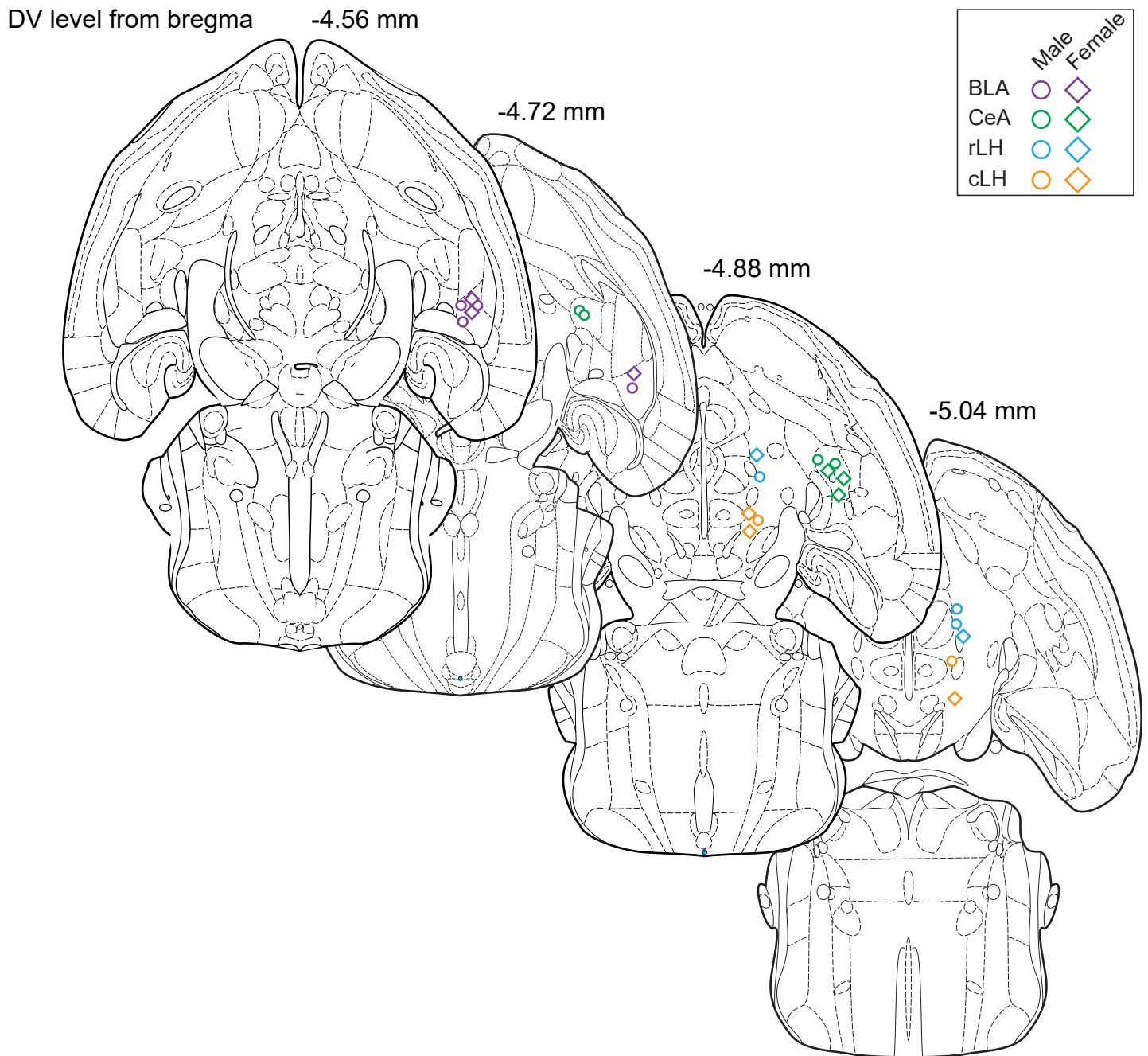
**b and d.** Percentage of 5-HT1A (b) or 5-HT2A (d) -expressing insular neurons projecting to the basolateral amygdala (IC-BLA), central amygdala (IC-CeA), and rostral or caudal parts of lateral hypothalamus (IC-rLH and IC-cLH, data of Figure 3g, 4d, 5j and 5l) in male and female brains. Total cell numbers (mice number):

**(b)** 5-HT1AR: Male: IC-BLA=852 (n=4), IC-CeA=711 (n=4), IC-rLH=402 (n=3), IC-cLH=289 (n=2);

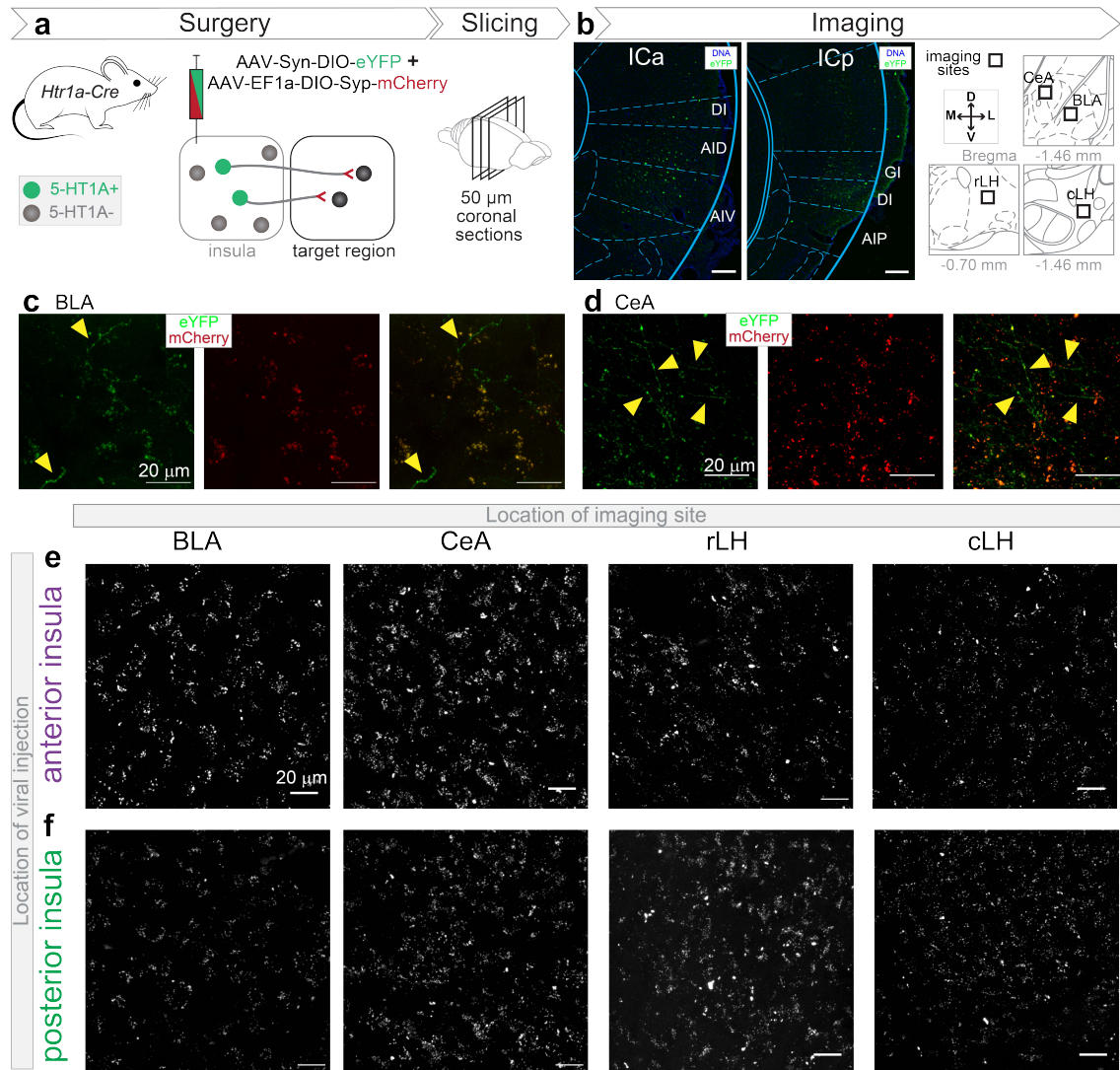
Female: IC-BLA=185 (n=3), IC-CeA=391 (n=3), IC rLH=209 (n=2), IC-cLH=377 (n=3);

**(d)** 5-HT2AR: Male: IC-BLA=370 (n=4), IC-CeA=802 (n=4), IC-rLH=368 (n=3), IC-cLH=313 (n=2),

Female: IC-BLA=378 (n=3), IC-CeA=247 (n=3), IC-rLH=492 (n=2), IC-cLH=509 (n=3).



**Supplementary Figure 4.** Map of the injection sites of the retrograde tracers (CTB), represented on horizontal brain sections of the Paxinos atlas (3d edition). To identify the injection site, we searched the horizontal sections where the CTB signal had the largest spread in the target region, and the injection point was defined at its center (in medio-lateral and antero-posterior axis).



**Supplementary Figure 5.** Evidence of synaptic contacts of 5-HT1A<sup>+</sup> insular neurons in the amygdala and lateral hypothalamus.

- a. Experimental design to detect synaptic terminals of 5-HT1A<sup>+</sup> insular neurons through cre-dependent expression of eYFP and synaptophysin-mCherry in the anterior or posterior insula of *Htr1a-Cre* mice.
- b. (Left) Confocal image of the cre-dependent viral vector injection site in a coronal section of the anterior (DI: dysgranular insula, AID: agranular insula dorsal part, AIV: agranular insula ventral part) and the posterior insula (GI: granular insula; AIP: agranular insula posterior part). Note eYFP expression in the soma of 5-HT1A<sup>+</sup> neurons. (Right) Imaging locations of synaptophysin-eYFP in the basolateral and central amygdala (BLA and CeA) as well as the rostral and caudal part of the lateral hypothalamus (rLH and cLH). Distances are in the anteroposterior axis from Bregma.
- c-d. Representative images of eYFP for labelling axonal projection and synaptophysin-mCherry for visualizing synaptic terminals in the BLA (c) and CeA (d), originating from 5-HT1A<sup>+</sup> neurons of the posterior insula. Yellow arrows indicate axonal projection labelled by eYFP.
- e-f. Confocal images of synaptophysin-mCherry in the BLA, CeA, rLH and cLH, expressed in the 5-HT1A<sup>+</sup> neurons of the anterior (e) and posterior insula (f).