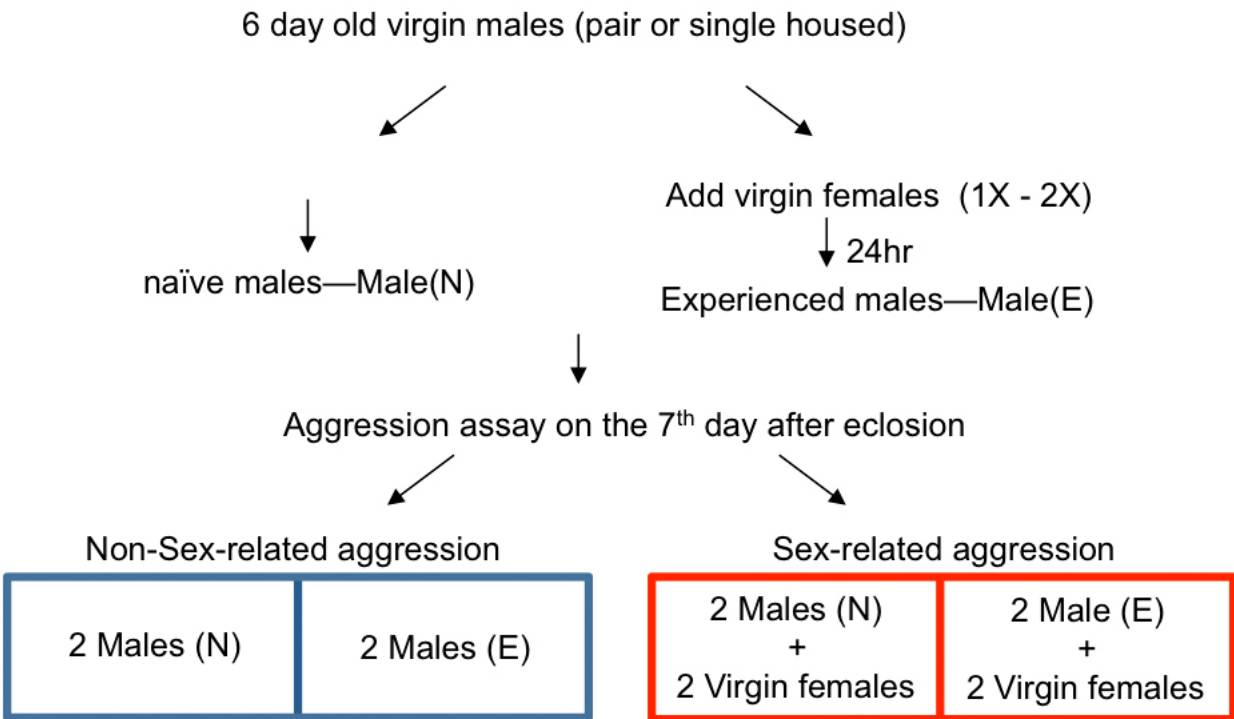


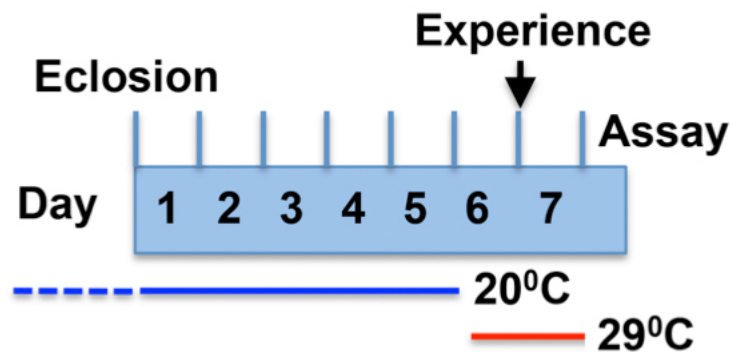
NN-A44196B Supplementary Information

Article Title:	Female contact modulates male aggression via a sexually dimorphic GABAergic circuit in <i>Drosophila</i>
Authors:	Quan Yuan, Yuanquan Song, Chung-Hui Yang, Lily Yeh Jan, Yuh Nung Jan

a Scheme for aggression behavior analysis paradigm

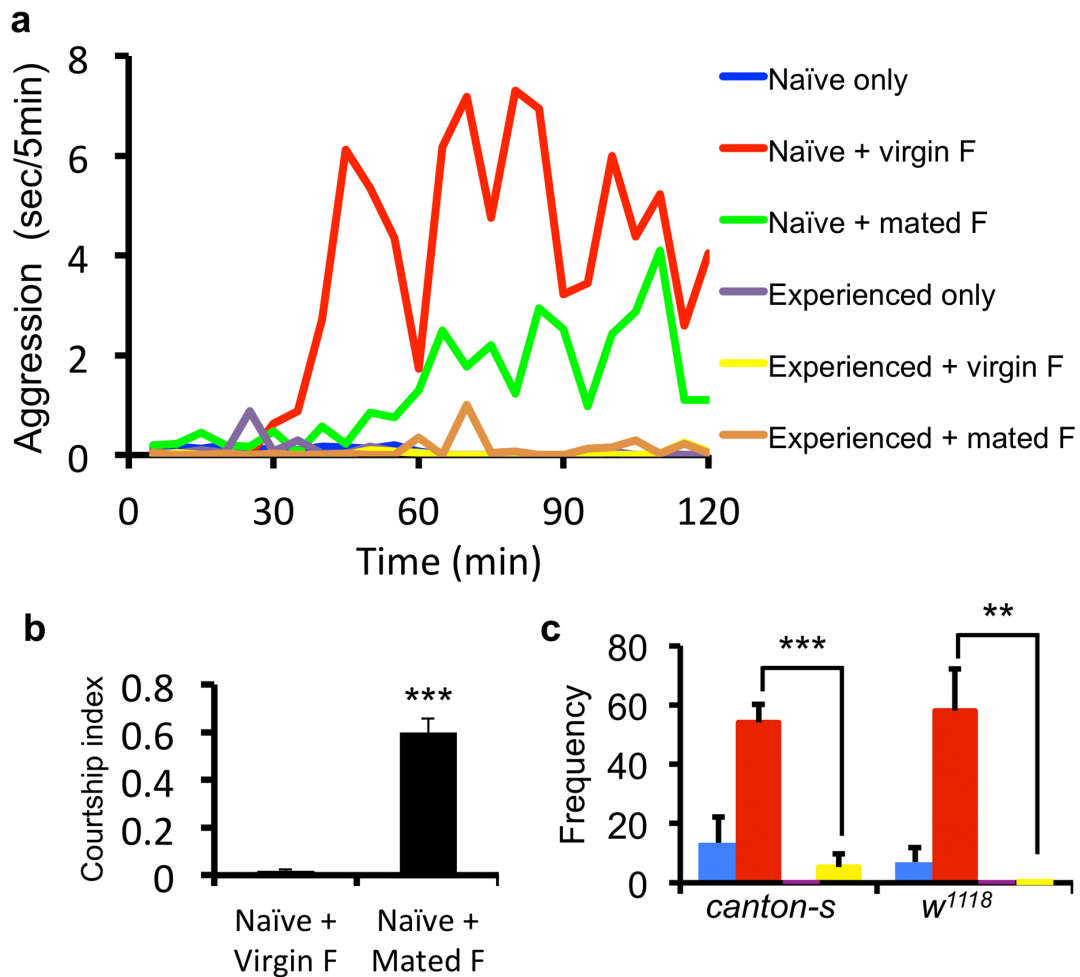


b



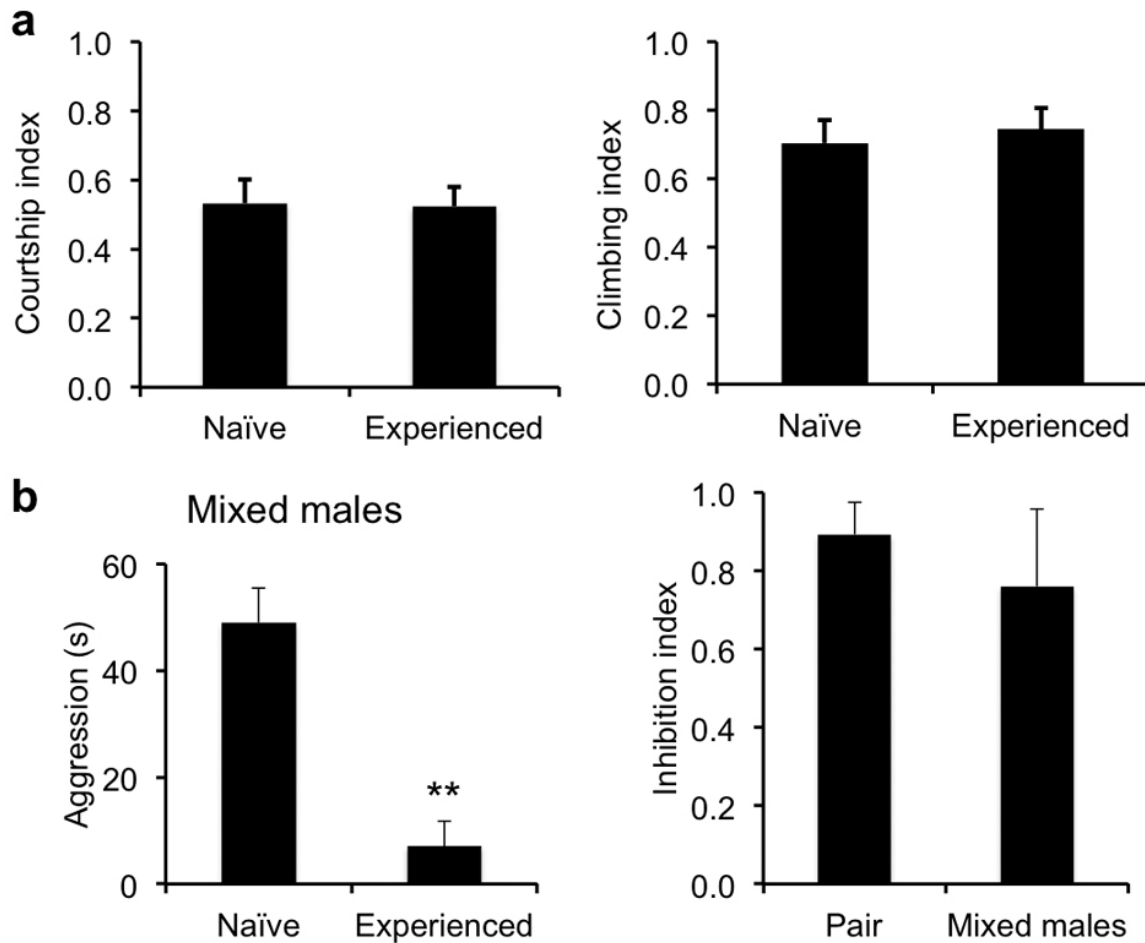
Supplementary Figure. 1 Experimental paradigms.

(a) Detailed experimental scheme for analyzing sex-related male-male aggression and its regulation by prior female experience. (b) The dis-inhibition phenotype was examined after temperature shift to induce silencing of specific group of neurons with enhancer-Gal4 driven UAS-Gal80^{ts} and UAS-Kir2.1.



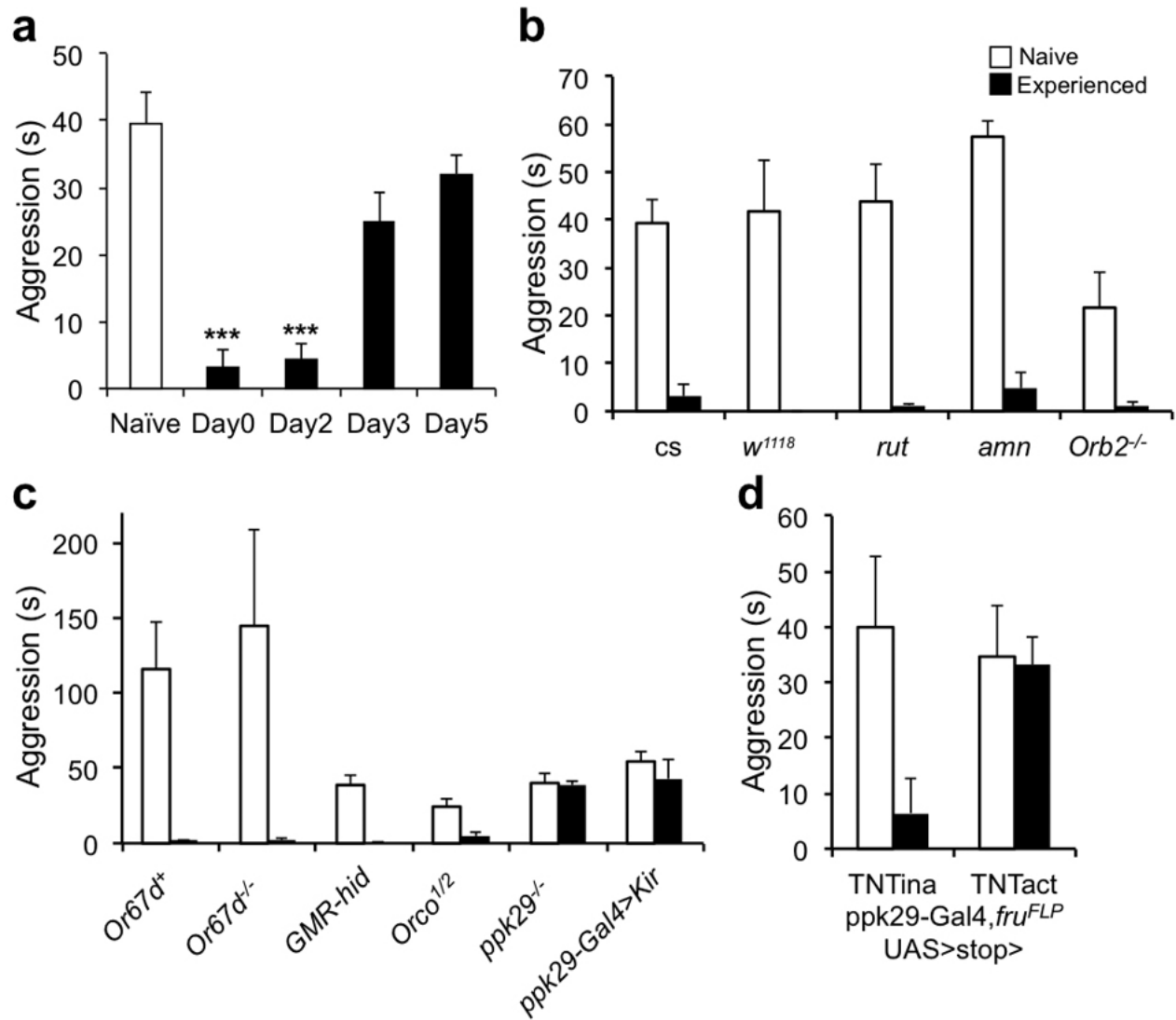
Supplementary Figure. 2 Experience with females inhibits sex-related male-male aggression.

(a) Average aggression duration of *cs* males was quantified in 5 min intervals for 2 hours for naïve male only (blue), experienced males only (purple), naïve males + virgin females (red), experienced males + virgin females (yellow), naïve males + mated females (green), experienced males + mated females (brown). (n = 4 pairs for each condition). (b) Courtship index measured at 70-75 min for naïve male + virgin female and naïve male + mated female. The latter showed significantly higher courtship index. (n = 4 for each condition). (c) Average aggression frequency of *cs* and *w¹¹¹⁸* males was quantified for the 60-90 min. No obvious difference in the inhibition of aggression is apparent between the two genotypes. (n = 11 and 6 for *cs* and *w¹¹¹⁸*). **: p<0.01, ***: p<0.001. Student's t-test. Error bars denote s.e.m.

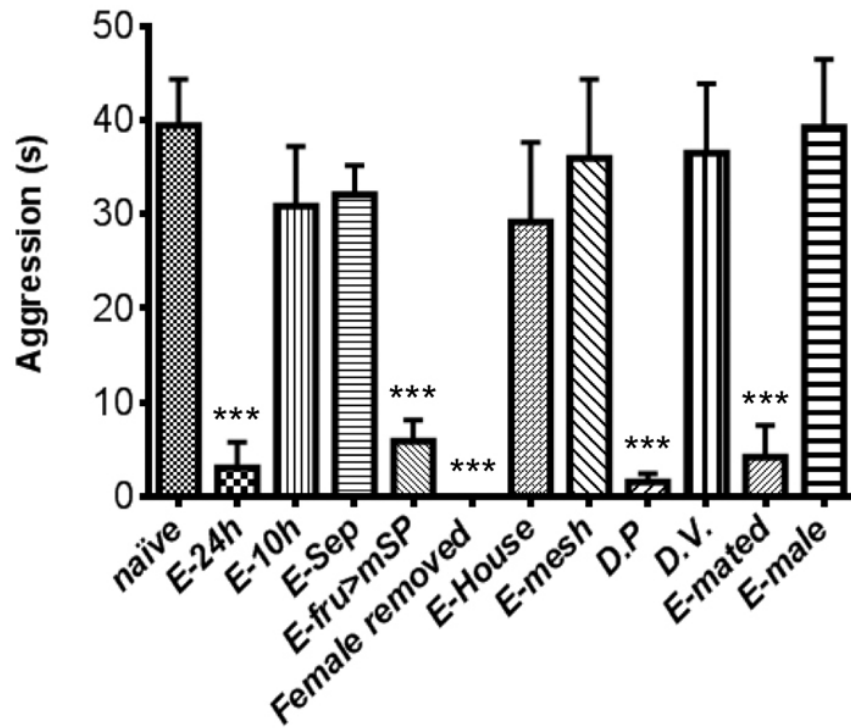


Supplementary Figure. 3 Previous female contact does not significantly affect the courtship index and locomotion of experienced males. Social hierarchy established between males housed in pairs has little to do with the female-contacts dependent inhibition of sex-related male aggression.

(a) The courtship index and climbing index of experienced vs. naïve male flies did not exhibit significant differences. ($n = 5$ for courtship index and 3 for climbing index). (b) Two male flies that were housed in pairs but not with each other (mixed group), so that they had social experiences but no established hierarchy with each other, exhibited as much contact-dependent inhibition of the sex-related aggression as did males that were raised together (pair). ($n = 5$ for each condition). Student's t-test. $p = 0.0012$ (**). Error bars denote s.e.m.



Supplementary Figure. 4 Aggression duration measurements corresponding to Fig. 2a, 2b, 2d (n = 11, 6, 7 and 5 for each condition). One-way ANOVA followed by Bonferroni's multiple comparison test. ***: p<0.001. Error bars denote s.e.m.

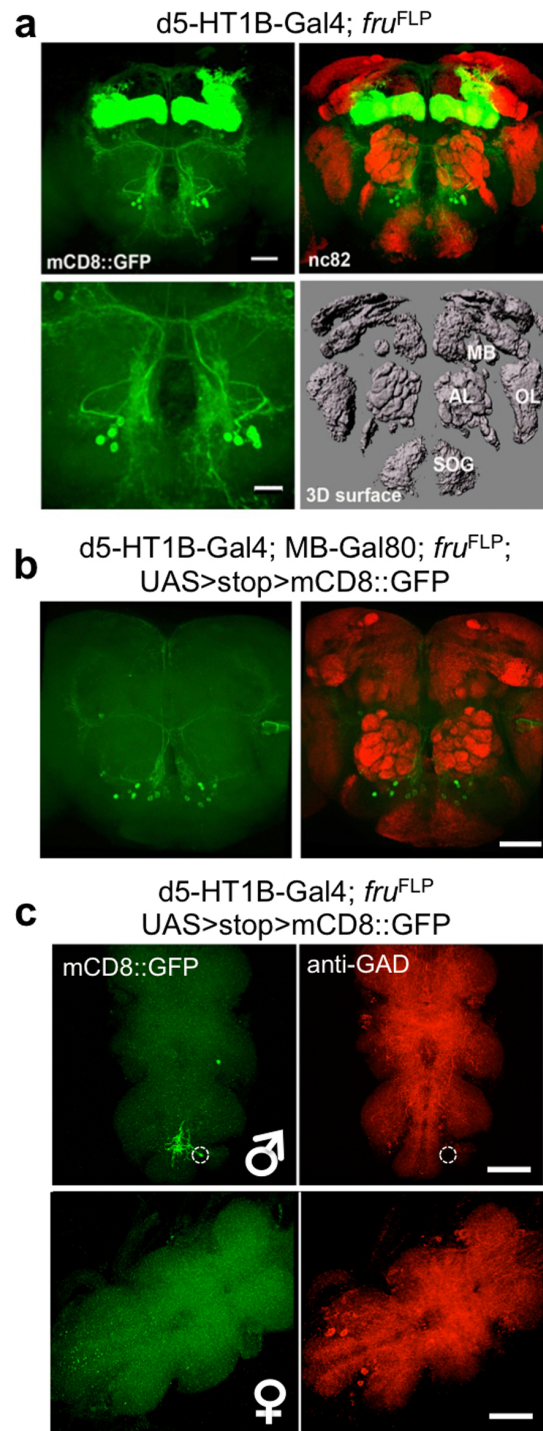


1. Naïve male, tested with virgin female
2. Experienced male with 24 hour virgin female contact, tested with virgin female
3. Experienced male with 10 hour virgin female contact, tested with virgin female
4. Experienced male mated with virgin female for about 2 hour, then separated, tested with virgin female
5. Experienced male with 24 hour virgin female expressing mSP in fru neurons, tested with virgin female
6. Naïve male with no prior female contact, tested with virgin female for about 40 minutes, then remove female after copulation
7. Experienced male with 24 hour in vials housed with female flies, tested with virgin female
8. Experienced male with 24 hour virgin female separated with nylon mesh, tested with virgin female
9. Experienced male with 24 hour virgin D.P. female contact, tested with virgin female
10. Experienced male with 24 hour virgin D.V. female contact, tested with virgin female
11. Experienced male with 24 hour mated female contact, tested with virgin female
12. Experienced male with 24 hour extra male contact, tested with virgin female

Supplementary Figure. 5 Conditioning parameters for the prior female experience dependent inhibition of aggression.

Inhibition of aggression were displayed by males conditioned with virgin females for 24 hours, with mated females, with virgin females that express membrane bound sex peptide using fru-Gal4, which would therefore reject males, and with *Drosophila pseudoobscura*. However, conditioning with virgin females for 10 hours, removing females right after mating during

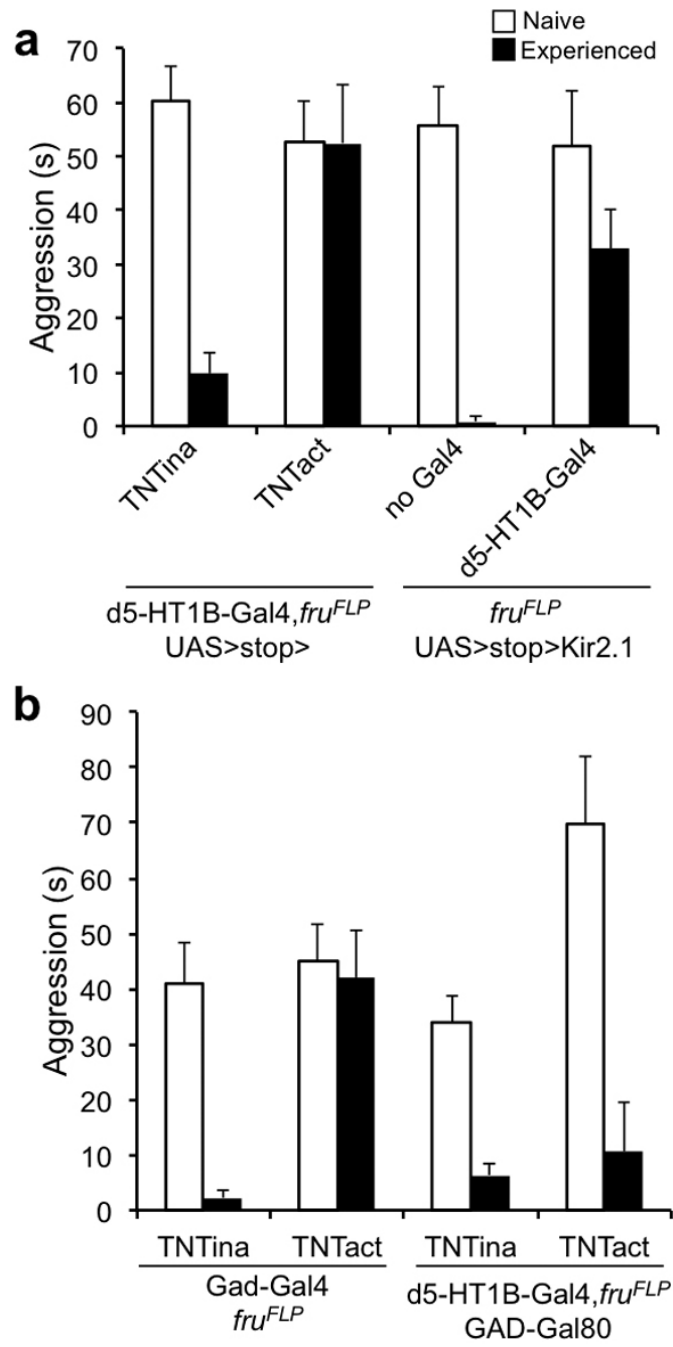
conditioning, conditioning males in vials previously housed virgin females, conditioning males with virgin females that were separated by a nylon mesh, conditioning with *Drosophila virilis*, or with extra males all failed to elicit inhibition of aggression. On the other hand, removing females after mating during testing abolished aggression, revealing the presence of females during the aggression assay is crucial. (n = 11, 11, 5, 6, 6, 6, 6, 5, 5, 5, 6 and 8 for each condition). One-way ANOVA followed by Bonferroni's multiple comparison test. ***: $p < 0.001$. Error bars denote s.e.m.



Supplementary Figure. 6 Sexual dimorphic distribution of *fru*⁺, d5-HT1B⁺ neurons in the brain and VNC of adult male and female flies.

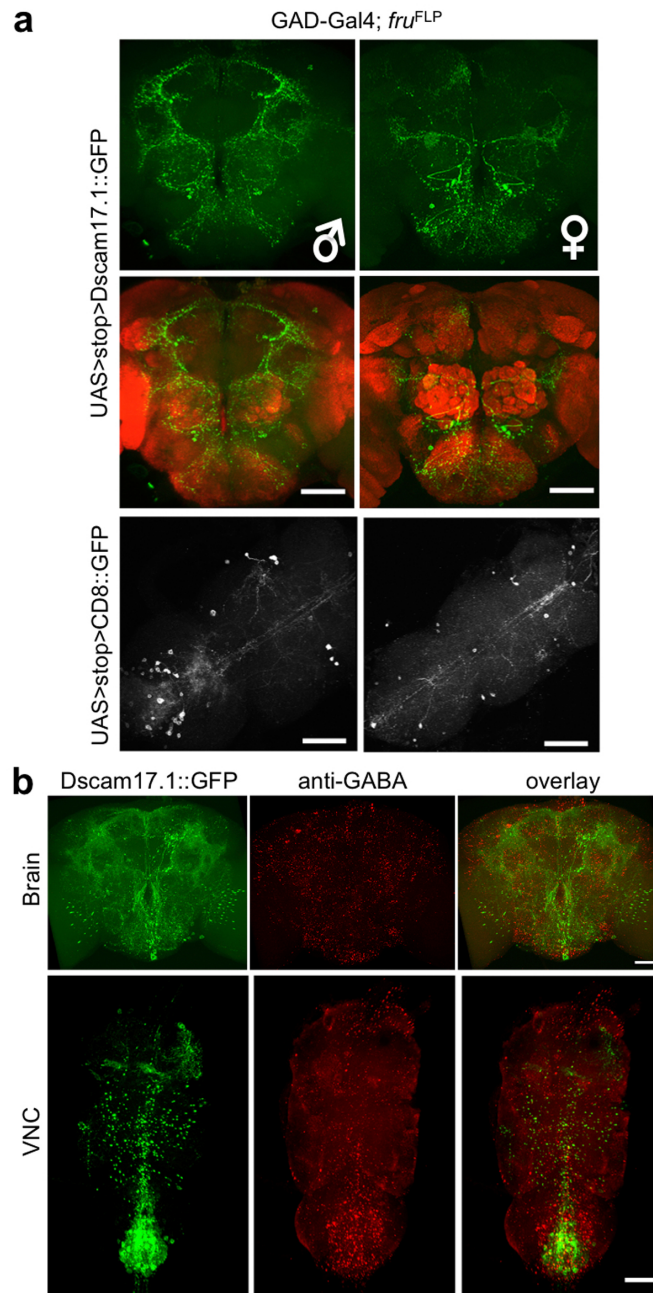
(a) *fru*⁺, d5-HT1B⁺ neurons in the adult male brain labeled by mCD8::GFP including γ -neurons of the mushroom bodies and a cluster with soma located below the antennal lobes and above the SOG region. The magnified view is shown in the lower left panel. nc82 co-staining in red shows the neuropil. A 3D rendering of the neuropil is shown in the lower right panel. (b) A

subpopulation of fru⁺, d5-HT1B⁺ neurons independent of mushroom bodies are required for the prior female-contacts dependent inhibition of aggression. MB-Gal80 co-expression excluded the mushroom body neurons from the fru⁺, d5-HT1B⁺ population, indicated by the loss of mCD8::GFP labeling. nc82 staining is in red and marks the neuropil. (c) The fru⁺, d5-HT1B⁺ neuron in the VNC is sexually dimorphic, but does not exhibit anti-GAD staining. One fru⁺, d5-HT1B⁺ neuron is evident in the male VNC and it does not overlap with anti-GAD immunostaining. No soma was labeled in female VNC. Scale bar = 40 μm.



Supplementary Figure. 7 Aggression duration measurements corresponding to Fig. 3c and Fig.

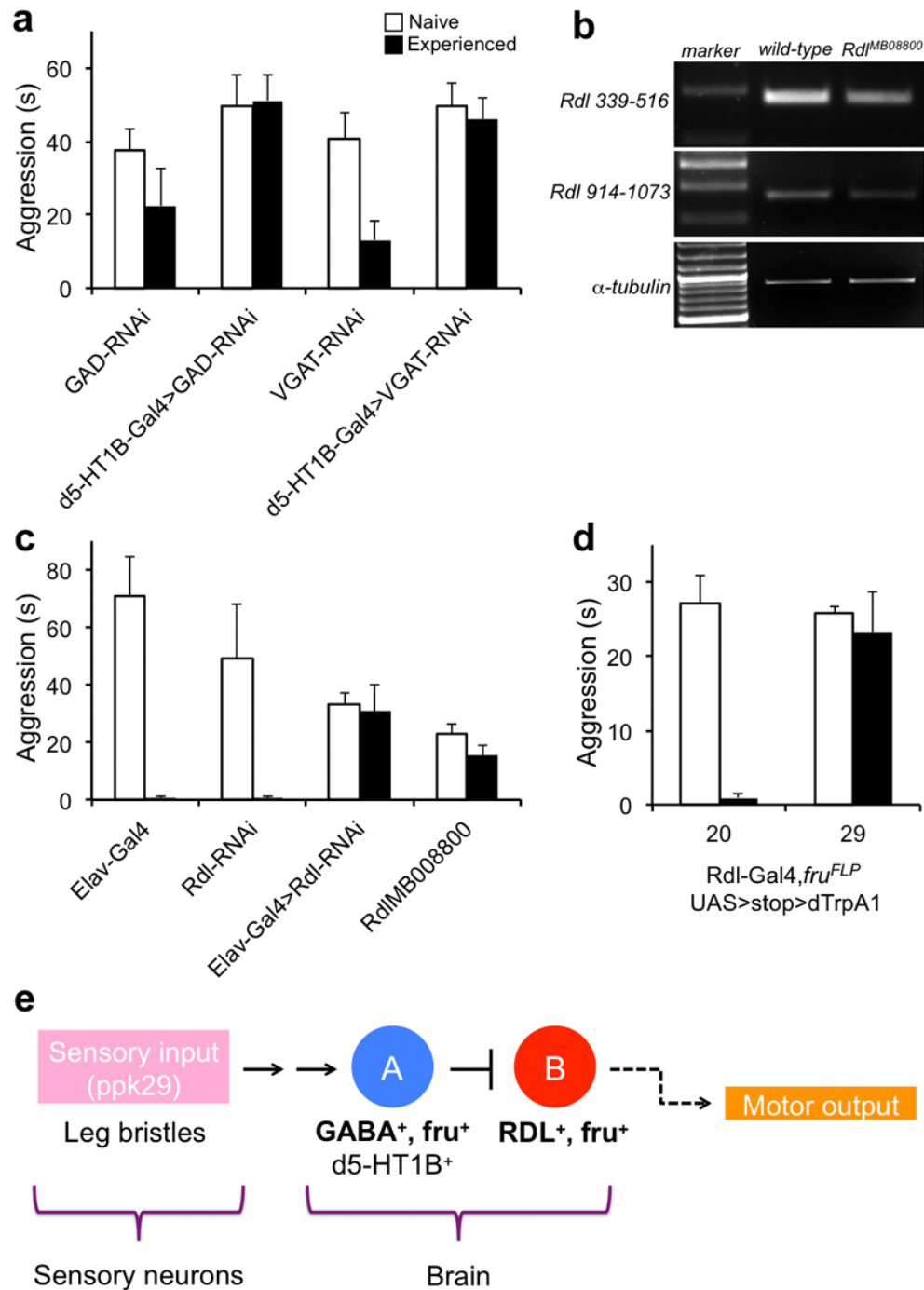
5c. Error bars denote s.e.m.



Supplementary Figure. 8 fru^+ , $GABA^+$ neurons display sexual dimorphism in the brain and VNC.

(a) fru^+ , $GABA^+$ neurons showed distinct projection patterns, as labeled by Dscam17.1::GFP, and distinct cell distribution in the VNC, as labeled by CD8::GFP between males and females.

(b) The relationship between the dendrite branching pattern of fru^+ , Rdl^+ neurons and the GABA distribution in the male fly brain and VNC. The dendritic patterning of fru^+ , Rdl^+ neurons is shown in green using Dscam17.1::GFP and the GABA distribution is shown in red by anti-GABA staining. Scale bar = 40 μ m.



Supplementary Figure. 9 Aggression duration measurements corresponding to Fig. 6a, 6b. *Rdl* transcripts are reduced in the hypomorphic *Rdl*^{MB08800} allele. Model of the GABAergic mechanisms underlying the female-contacts dependent inhibition of sex-related male-male aggression.

(a) Aggression duration measurements corresponding to Fig. 6a. (b) Semi-quantitative RT-PCR analyses showed a reduction of *Rdl* mRNA level in the *Rdl*^{MB08800} allele as compared to the wild type control, using primers targeting two regions of the transcript. The numbers indicate the base

pair location. α -tubulin mRNA, which is used as the loading control, is comparable between the two genotypes. **(c, d)** Aggression duration measurements corresponding to Fig. 6b. Error bars denote s.e.m. **(e)** Male flies, when encountering females, activated their female pheromone sensing ppk29 neurons through direct contacts, followed by the GABAergic inhibition of central neurons in the aggression circuit, which possibly express the Rdl GABA-a receptor, leading to reduced aggressive behavioral output.

Lines tested with *tub-Gal80^{ts}*; *UAS-Kir2.1*

	Labeled neurons	Baseline Aggression	Dis-inhibition	Note
<i>Trh-Gal4</i>	Serotonergic	-	-	
<i>Tdc2-Gal4</i>	Octopaminergic	ND	ND	Mating defect
<i>TH-Gal4</i>	Dopaminergic	-	-	
<i>npf-Gal4</i>	NPF/NPY	-	-	
<i>dim-Gal4</i>	Peptidergic	-	-	
<i>Ddc-Gal4</i>	Serotonergic+ Dopaminergic	reduced	-	
<i>d5-HT1B-Gal4</i>	d5-HT1B receptor	-	Yes	
<i>Cha-Gal4</i>	Cholinergic	ND	ND	Lethal
<i>fru-Gal4</i>	Fru+	ND	ND	Lethal
<i>GAD-Gal4</i>	GABAergic	-	-	
<i>V-Glut-Gal4</i>	Glutamnergic	ND	ND	Lethal

ND: not determined

-: no phenotype, similar to control

Supplementary Table Summary of the enhancer-Gal4 lines tested with *tub-Gal80^{ts}*; *UAS-Kir2.1*.

Supplementary Video 1-5

Representative one minute long videoclips extracted from the 60-90 min period. For all the videos, the configuration is as the following: top left-naive males only, top right-experienced males only, middle left-naive males + virgin females, middle right-experienced males + virgin females, bottom left-naive males + mated females, bottom right-experienced males + mated females. The genotypes are as follows:

1. *cs*
2. *cs*; UAS>stop>TNTinactive/+; *fru*^{FLP}/d5-HT1B-Gal4
3. *cs*; UAS>stop>TNTactive/+; *fru*^{FLP}/d5-HT1B-Gal4
4. *cs*; UAS>stop>dTrpA1/+; *fru*^{FLP}/d5-HT1B-Gal4 at 20°C
5. *cs*; UAS>stop>dTrpA1/+; *fru*^{FLP}/d5-HT1B-Gal4 at 29°C