Supplemental Experimental Procedures

Multiple sequence alignment and phylogenetic tree construction

To construct the phylogenetic tree of the iGluRs and IR superfamily, we used protein sequences from the *D. melanogaster* reference genome (Adams et al., 2000) and Croset *et al.* (2010) for alignment using the TCOFFEE program; the tree was made with 200 bootstraps using the PHYLIP program. Due to the high degree of sequence divergence, 10 out of 73 branch nodes have bootstrap numbers less than 80 (not shown). Nodes within the *IR20a* family have generally high boot strap numbers (156.3±8.5), with only 2 nodes below 80, indicating that this is a valid clade. Both programs were run online as part of the Bioinformatics Toolkit of the Max-Planck Institute for Developmental Biology (Biegert et al., 2006; Felsenstein, 1981; Notredame et al., 2000). The alignment was based on 33 IRs; IR56e and IR60f were omitted because they are very short polypeptides truncated by premature stop codons.

Transgenic constructs

Of the 30 *IR -GAL4* constructs, 27 were made with both 5' and 3' flanking regions of the genes of interest (Table S1). Three were made with only the 5' flanking region. Of these 30 *IR*s, *IR60c* and *IR60e* contain an internal deletion and a premature stop codon that is predicted to produce a protein lacking 21 amino acids at the C-terminus, respectively. Fragments were amplified using PfuUltra II Fusion HS DNA Polymerase (Agilent Technologies) from either CS-5 genomic DNA or tiling bacterial artificial chromosomes (BACs) corresponding to the reference genome. 5' and 3' flanking regions were chosen to include regions between the genes of interest and the next neighboring gene; the 5' flanking regions that were selected ranged from 1.1 to 9 kb, whereas the largest 3' flanking region that was selected was 12 kb and 3' flanking regions were not included for 3 constructs (Table S1). In most cases where the intergenic regions are less than 1 kb, larger regions were included. In cases where the intergenic regions exceeded the limit of PCR amplification, only the regions that are conserved among different *Drosophila* species were included. *GAL4* constructs were made in one of the following five vectors: *pG4PN+, AD1515, pCasper 5, pBDPR* and *pBGRY*. *pG4PN+, AD1515* and *pCasper 5* are *P* element transformation vectors, while *pBDPR* and *pBGRY* are made for *phiC31* integration. All vectors contain the mini-*white* gene.

pG4PN+: contains a *GAL4* gene flanked on the 5' side by restriction cloning sites and on the 3' side by the *hsp70* 3' untranslated region (Miller and Carlson, 2010).

AD1515: contains a *GAL4* ORF flanked by restriction enzyme sites on both sides (gift of Anupama Dahanukar).

pCasper 5: accepts 5' flanking region-*GAL4*-3' flanking region cassettes pre-assembled in a shuttle vector, *pC5-Kan*, via restriction enzyme sites (Le et al., 2007).

pBDPR (GenBank accession number KM016697): constructed by removing the basal promoter and *GAL4* fragments from pBPGUw (Pfeiffer et al., 2008), leaving a Gateway destination vector containing the *phiC31 attB* site.

pBGRY (GenBank accession number KM016698): derived from *pBDPR* to boost expression and reduce chromatin influence, through the addition of a pair of *Su(Hw)* insulator elements derived from the *gypsy* elements and the 3' flanking region of the *yellow* gene, respectively (Golovnin et al., 2003; Markstein et al., 2008; Parkhurst et al., 1988). 5' and 3' flanking regions and *GAL4* (or *LEXA*) cloned in Gateway *pDONR* vectors were assembled into *pBDPR* or *pBGRY* via MultiSite Gateway Pro 3-fragment recombination (Invitrogen).

For double-labeling experiments, a tandem Tomato fluorescent reporter driven by a LexA-responsive element (*LexAop*) and a *IR52a-LexA* driver were generated (see below). In addition, a transgenic line carrying multiple copies of a direct fusion construct of the *Gr66a* promoter and tandem copies of *red fluorescent protein* (*RFP*) linked by internal ribosome entry sites (*Gr66a-RFP*) was a kind gift from Jae-Young Kwon.

LexAop-m-tdTomato: *Membrane-tethered tandem Tomato* (*m-tdTomato*) is an improved version of *RFP*. The fragment was amplified from Rosa26 mT/mG obtained from Addgene (Muzumdar et al., 2007). This reporter is driven by *LexA operator* (*LexAop*) fused to a synthetic minimal promoter (DSCP) (Lai and Lee, 2006; Pfeiffer et al., 2008).

IR52a-LexA: *IR52a* 5' flanking region (Table S1) is fused to a *LexA::VP16* coding region and a *SV40* polyadenylation signal by Gateway 3-fragment recombination into a *pBGRY* vector (Lai and Lee, 2006).

RT-PCR

Proboscises (~200-400) and legs (~300-800) were dissected using forceps and placed immediately into microfuge tubes kept cold in liquid nitrogen. Tissues crushed with RNase-free plastic pestles were extracted with a QIAGEN RNeasy kit to yield total RNA. Total RNA was then enriched for mRNA using the Ambion MicroPoly(A) Purist kit. mRNAs were treated with RNAse-free DNase I (Roche #03335399001), before being used for first strand cDNA synthesis using Superscript RT III (Invitrogen), followed by PCR amplification with 32 amplification cycles. To control for contamination by genomic DNA, each batch of mRNA underwent a parallel mock reverse transcription step (no RT control) in which the reverse transcriptase was omitted, before being subjected to PCR. To provide a semi-quantitative comparison of RNA quality and quantity between samples, an RT-PCR product of *synaptotagmin I* was electrophoresed on agarose gels next to *IR* RT-PCR products. PCR primers are listed below.

IR47a forward	GCAGGACTACACCCTAAAGG
IR47a reverse	CCCACGACAACTTCCACG
IR52a forward	GCGGCAGCATGAGTTTGG
IR52a reverse	CCCGCAACGCAATATCTCC
IR52c forward	ACTAAGTACTGGATCGATCAGAGC
IR52c reverse	CAAGATAAAAAAACACAGACCCATGC
IR52d forward	CTCTTTGAGGAACATATGCTGC
IR52d reverse	ACCCTTAGCCAATTGGTTAGC
IR56b forward	CTGCGCATCGTTATCCACG
IR56b reverse	GAACAACCAGGCATCCTGG
IR56d forward	CATCCATGTGCACTTATTTGACG
IR56d reverse	CGCCTGAGAAACGATCTGG
Syt I forward	CGGATCCCTATGTCAAGGTG
Syt I reverse	TCTGGTCGTGCTTCGAGAAG

Quantitative RT-PCR

5 day old flies were used in RNA extraction as described above, except that double-stranded cDNAs were generated from DNase-treated mRNA using an iScript™ cDNA Synthesis Kit (Bio-rad 170-8890). Quantitative PCR was performed in an iQ5 real-time PCR detection system (Bio-Rad). cDNA or No-RT control were mixed with SsoFast™ EvaGreen® Supermix. The denaturation temperature was set to 95^{o}C. Annealing and extension temperatures were set to 58^{o}C. A standard curve was made to ensure optimum primer efficiency and a melting curve was generated to ensure specificity. PCR primers are listed below.

IR52c forward	ACTAAGTACTGGATCGATCAGAGC
IR52c reverse	CAAGATAAAAAAACACAGACCCATGC
IR52d forward-1	CTTGTTTTATGGTTCCCCTTCC
IR52d reverse-1	GCCACGACCTTTGCTTTATG
eIF1A forward	AGCCCACCAATATGATGTCG
eIF1A reverse	CTTCAAGGAGGACCAACAGG

Examination of *GAL4* expression patterns

To examine expression in peripheral neurons, freshly dissected tissues were used to observe GFP fluorescence. Due to the weak expression of most of the *IR-GAL4* drivers, flies with two copies of *GAL4* and two copies of *UAS-mCD8::GFP* (Lee and Luo, 1999), were used where possible (some insertions were homozygous lethal and were examined in the heterozygous condition), and were aged between 10-35 days for *GFP* to accumulate. We have examined at least five independent lines for each construct inserted via *P* elements, and two defined

integration sites, *attP2* and *attP40*, for each construct inserted via the *phiC31* method (Groth et al., 2000).

Mapping of IR-GAL4 expression to sensilla: The expression patterns represent the consensus of at least 2 transgenic lines for each *GAL4* construct (except for *IR52d-GAL4*, for which only one of two lines examined showed GFP expression). For each sensillum, the presence of one *GAL4*-positive cell in each line was given a score of 1. The scores across multiple lines were averaged and sensilla with mean scores $\geq$0.5 are indicated as "+". For each line, n$\geq$4 flies were examined, and the scores across flies were averaged in the same way. We noted that when the *phiC31* sites *attP40* and *attP2* were used, drivers inserted at *attP2* were usually stronger. Expression was observed in no more than one cell per sensillum in the labellum and legs. Although we did not observe *GAL4* expression for *IR48b*, *IR48c*, *IR54a*, *IR60c* and *IR60d*, we detected RT-PCR products of these genes in the legs (not shown).

Immunohistochemistry

Staining of CNS tissues: To observe axonal projection patterns, CNS tissues were immunostained using antibodies recognizing fluorescent reporters. Central brains and ventral ganglia were dissected in PBS and fixed in 3.7% formaldehyde in PBS with 0.4% Triton X-100 (PBS-T). After four washes with PBS-T, tissues were incubated overnight at 4^{o}C with primary antibodies in PBS-T with 5% normal goat serum (PBS-TN). Primary antibody sources and concentrations were as follows: rabbit anti-GFP (Invitrogen A6455; 1:500), mouse anti-RFP (MBL, mAb8D6, 10 μg/ml), mouse anti-Bruchpilot (Developmental Studies Hybridoma Bank, nc82 supernatant, 1:10)(Wagh et al., 2006).

Generation of anti-IR52c polyclonal antibodies: A synthetic peptide containing the sequence CKGYVGMLISQFVKKV derived from the N-terminal extracellular domain was conjugated to KLH and injected into rats using the Quick Draw protocol (Pocono Rabbit Farm and Laboratory).

Whole mount staining of legs: 20 to 100 legs were dissected and fixed in 3.7% formaldehyde in PBS-T in a microfuge tube on ice during the duration of dissection (~20-30 minutes). After completion of dissection, fixation was allowed to continue at room temperature for 15 minutes, and then the fixative was removed with two washes with PBS-T. To allow antibody access to the internal tissues, legs were subjected to two to six pulses of 10 seconds of sonication at a low setting, while keeping the microfuge tube on ice. Between each pulse of sonication, the legs were spun down in the microfuge to be checked for morphology - this sonication step breaks many of the leg bristle shafts and some of the joints between tarsal segments. The optimal number of sonication pulses required varies with the number of legs in the microfuge tube and has to be empirically determined. Sonicated legs were further permeabilized with PBS with 1% Triton X-100 overnight at 4^{o}C. This is followed by three quick washes with PBS-T and overnight

incubation with primary antibodies at 4°C. Rat anti-IR52c serum was used at a 1:750 dilution and rabbit anti-GFP antibody was used at a 1:1000 dilution (Invitrogen A6455). Antibody staining is usually optimal in the tarsal segment closest to a broken joint.

Observation of neuronal activation using a *LEXA-NFAT* fusion construct

Males of the genotype *IR52c-GAL4/LexAop-CD8-GFP-2A-CD8-GFP; 2x IR52c-GAL4/UAS-mLEXA-VP16-NFAT, LexAop-CD2-GFP* were used to drive the expression of the LEXA-NFAT fusion protein in *IR52c*$^{+}$ cells (Masuyama et al., 2012). Based on our experience with the *IR52c-GAL4* driver, we chose to age the males for 6 weeks to allow adequate expression of the LEXA-NFAT fusion protein. These males were collected within 6 hours of eclosion and housed in solitary conditions until the experiments. For males exposed to *D. melanogaster* females, 10 *Canton-S* virgins females > 7 days-old were introduced to each male each day for three days, such that there were 30 females by the third day. For males exposed to *D. simulans* females (San Diego Stock Center strain 14021-0251.006 collected from Nueva, California), 10 virgin females > 7 days-old were introduced to each male each day for three days. For males exposed to *D. melanogaster* males, each *LEXA-NFAT* male was housed with 30 *Cantonized w*^{1118} males for three days. The male forelegs were dissected and processed for immunohistochemistry as described above, except that the primary antibodies were incubated for three days at 4°C instead of one day. For the quantification of GFP and IR52c signals, we focused on the tarsal segments closest to a broken joint as the antibody staining is most reliable in such segments. More than half of the segments that were examined were tarsal segment 4, because the joint between tarsal segments 4 and 5 appears to be most easily broken during our staining protocol (see *Whole mount staining of legs*).

GFP Reconstitution Across Synaptic Partners (GRASP)

Six week-old males of the genotype *UAS-spGFP*$_{1-10}$*::Nrx/ LexAop-spGFP*$_{11}$*::CD4; 2x IR52c-GAL4/fru[P1.LEXA]* were used to drive the expression of two components of split GFP in *IR52c*$^{+}$ and *fru*$^{+}$ neurons, respectively (Fan et al., 2013). Dissected thoracic ganglia were briefly fixed for 1 minute in 1 % formaldehyde in PBS and washed before being mounted in VectorShield (Vector Lab) for visualization. Flies that underwent unilateral foreleg amputation were cultured for an additional week to allow the afferent axons to degenerate before dissection.

Image acquisition and processing

Images were acquired on a Zeiss LSM510 confocal microscope and processed using NIH ImageJ, Amira 2.2 and Adobe Photoshop.

Genomic sequence of the *IR52* cluster

During our characterization of the *IR52c*^{1} transposon line, we were interested to discover that in its genetic background, in addition to *IR52c* there are four additional genes in the *IR52* cluster rather than three as in the *Drosophila melanogaster* reference genome (Figure S4A).

Canton-S, a commonly used laboratory strain, also contains this extra gene, which we name *IR52e* due to its close homology with *IR52e* of *Drosophila simulans* (92% predicted amino acid sequence identity). Specifically, corresponding genes encoding IR52a, IR52c, and IR52d are closely related between Canton-S and the *Drosophila* reference genome (98%, 99% and 99% predicted amino acid sequence identity, respectively). Between *IR52a* and *IR52c* in the Canton-S genome lie two genes, one we refer to as *IR52b* and which is 87% identical in predicted protein sequence to IR52b in the reference genome, and *IR52e*, which is 76% identical to IR52b of the reference genome. *IR52e* is expressed in legs of Canton-S but not *Poxn* mutants, as determined by RT-PCR analysis, consistent with its expression in taste neurons (not shown). As a precaution we confirmed that *IR52e* expression does not appear affected by the $IR52c^1$ insertion or by the addition of the genomic rescue constructs (Figure S4D). We also determined by inverse PCR that the *Minos* transposon *MB02231* is inserted in *IR52e*, instead of *IR52b* as indicated on Flybase. The genomic sequence of the *IR52* cluster in the Canton-S line has been deposited in GenBank (KM016699).

Mating assays with mutations in the *IR52* cluster

$IR52c^1$ and $IR52e^1$ contain single GFP-marked Minos transposons, *MB04402* and *MB02231*, inserted into the ORFs of *IR52c* and *IR52e*, respectively (BDGP Gene Disruption Project and see above in "Genomic sequence of *IR52* cluster") (Bellen et al., 2004; Metaxakis et al., 2005). We adopted the following procedure as a precaution to avoid homozygous modifiers (O'Dell, 2003). A copy of $IR52c^1$ was outcrossed into a Berlin-K background for more than five generations and another copy was backcrossed into a CS-5 background for more than five generations. CS-5 is established from the Canton-S strain (Monte et al., 1989). To create $IR52c^1$ mutants in an outbred background, Berlinized $IR52c^1$ females were crossed to Cantonized $IR52c^1$ males. $IR52e^1$ was taken through the same procedure. The controls were generated by crossing Berlin-K females to CS-5 males. The genomic rescue constructs, *Rescue c* and *Rescue d*, were inserted into the same *attP* site on the third chromosome (*attP2*) (Groth et al., 2004), and outcrossed to CS-5 for 6 generations. In the rescue experiments, Berlinized $IR52c^1$ females were crossed to Cantonized males of either genotype: $IR52c^1$*; Rescue c* or $IR52c^1$*; Rescue d*.

The *IR52c*, *d* double mutant, *Δcd*, was generated by the imprecise excision of a copy of $IR52c^1$ that was in a Berlin-K background. To increase the probability of obtaining large deletions of more than 1 kb, excision events were induced in the genotype w^{1118}*;*$IR52c^1$*/ SM6a, P{w[+mC]=hsILMiT}2.4;* $mus309^{D2}$/$mus309^{N1}$ (Witsell et al., 2009).

Df(2R)IR52a-d was generated by inducing *hs-FLP* in the presence of *PBac{WH}f01317* and *PBac{RB}e02175*, two *FRT*-carrying piggyBacs flanking the *IR52* cluster.

Virgin females were housed in single-sex groups of five and aged for 4-6 days before being used for mating assays. Virgin males were single-housed and aged for 4-6 days unless

otherwise indicated; the single-house condition is designed to prevent alteration of male behaviors due to prior social experience (Pan and Baker, 2014). Both males and females were reared in 12/12-hour light-dark cycles.

Mating assays were performed at 25^{o}C and at 40-60% relative humidity unless otherwise indicated and were performed at least three hours before dark (Sakai and Ishida, 2001; Toda et al., 2012). One of three types of mating chambers was used:

Mating chamber type 1: For the experiments shown in Figures 7 A,C and S4E,F,H,I, a single virgin male and a single virgin female were video recorded for 10 minutes in white light in one well of a 24-well tissue culture dish (Falcon) with no fly food. To facilitate automated scoring, the wall of the well was coated with Fluon, forcing the flies to stay at the bottom of the well (2 cm^2 area). Diffused white lighting was used to minimize noise in the background. The flies were introduced, without anesthesia, into the well through a small hole in a siliconized transparent Plexiglass lid by pipetting.

Mating chamber type 2: For Figure 7B,D, a single virgin male and a single virgin female were video recorded in white light in a 7 ml chamber formed by a sandwich of two 24-well tissue culture dishes (Falcon) with no fly food, as described previously (Meissner et al., 2011); the walls were not coated with Fluon in these experiments. In the morning, single CO_2-anaesthesized CS-5 virgin females were placed in individual chambers of a 24-well tissue culture dish (Falcon). Similarly, single virgin males were placed in another 24-well dish. The two dishes were then inverted over each other with an opaque plastic film barrier separating the male and female flies. The flies were allowed to recover for 3-4 hours in a humidified chamber at 25^{o}C. The film barrier was then removed to allow the male and female to come into contact. Video recording of mating behavior was performed in white light in a 25^{o}C incubator.

Mating chamber type 3: For Figure S4J, a single virgin male and a single virgin female were filmed under infrared light in a custom-made circular Plexiglass chamber of 0.47 ml in the presence of standard corn meal fly food. Each chamber is formed from two inner and two outer slabs, as shown in Figure S5 (modified after (Drapeau and Long, 2000)). In the inner slabs, each 10 mm x 3 mm chamber opens into a small 6 mm x 3 mm chamber. On the day before the mating assay was performed, each small chamber of each inner slab was filled with melted standard corn meal fly food (see Figure S5). Upon solidification of the food, each inner slab was aligned with an outer slab and cooled on ice. The walls were not coated with Fluon in these experiments. A single CO_2-anaesthesized CS-5 virgin female is placed in each large chamber of one of the inner slabs. An opaque plastic film barrier is then used to cover the chambers with the females. Then the second inner slab is placed over the plastic film barrier and single males are placed in each large chamber of this slab; the small chambers of this slab also contain fly food. Finally, an outer slab is placed over the inner slab containing males and the sandwich is secured

with screws and nuts. The sandwich is placed in a 25°C incubator and the plastic film barrier is removed the next morning to allow the males and females to come into contact. Videorecording of mating behavior was performed under infrared light in a 25°C incubator.

Mating assay performed with flies that ectopically express NaChBac in *IR52c*$^+$ neurons

To ectopically express the bacterial depolarization-activated sodium channel NaChBac in *IR52c*$^+$ neurons, we generated male flies with two copies of *UAS-NaChBac-GFP* (Nitabach et al., 2006) and three copies of *IR52c-GAL4* (inserted in attP40 on the second chromosome and attP2 and attPVK00005 on the third). As controls, we used flies with only the *UAS-NaChBac-GFP* or the *IR52c-GAL4* insertions. All chromosomes used in this experiment were recombined into the Canton-S5 background for at least three generations. Virgin males were isolated within six hours of eclosion and aged for 5-6 weeks in a single-housed condition prior to the assay. Mating chamber type 1 (see above) was used. In each assay, a single male was introduced by pipetting into the chamber along with a five-day-old Canton-S virgin female, and their behaviors were filmed.

Automated scoring of wing extension and copulation with FlyVoyeur

Experiments in Figure 7A, C and S4 E, F, H, I were scored using an automated tool. To facilitate automated scoring, we used Fluon-coated chambers to restrict behaviors to the bottom of the chambers (see Mating chamber type 1). Movies were acquired using a video camera (Sony HDR-SR10) at 30 frames per second in uniform white light and were analyzed with FlyVoyeur, a custom-written tracking and analysis package written in MATLAB (Mathworks, Natick, MA). FlyVoyeur is freely available at http://sg-s.github.io/fly-voyeur/. The automated analysis proceeded as follows:

Videos were annotated using FlyVoyeur to indicate the position of arenas, the time at which flies were introduced into the arenas, and when to start or stop running the tracking algorithm. In each arena, the male fly and female fly were introduced sequentially, and behavior was tracked for the entire duration of the video from the moment of introduction of the second fly.

Tracking: FlyVoyeur was used to perform automated tracking on all videos. A subset of videos was manually scored to validate the automated tracking. Cross-validation with manual scoring of 46 video files showed good agreement of copulation latencies ($R^2 > 0.98$) and the time of first wing extension ($R^2 > 0.9$).

Position Estimation and Detection of Contact Between Flies: For each frame of the video, a monochrome image was extracted from one channel of the video, usually either red or blue. The image was masked to exclude parts of the image outside the arenas. Adaptive background subtraction was applied to the result using standard morphological operations. Objects were detected in this background-subtracted image, and small objects below a fixed threshold were ignored.

If the number of remaining objects matched the expected number of flies in each arena, object positions, sizes and orientations were assigned to flies based on closest distance to known positions in the previous frame. If the number of detected objects was less than two, current object sizes were compared to mean observed object sizes to check for large discrepancies, which indicated flies that were colliding, touching or otherwise in contact. If this was the case, this fused object was split into two using iterative image erosion, or, failing which, k-means clustering of identified pixel positions of the fused object.

Otherwise, a fly was assumed to be "missing", which could happen due to sudden motion of the fly or attempted flight within the arena, or, very occasionally, occlusion by the walls of the arena. The number of detected objects never exceeded the number of flies in each arena.

Detection of Orientation: Built-in object detection algorithms in MATLAB can identify the anterior-posterior axis of each fly, but cannot distinguish which end of the fly is anterior or posterior. FlyVoyeur combines the past orientation of the fly and locations of the fly's wings to accurately estimate the head and the tail end of the fly. The algorithm was insensitive to grooming by the flies, which moved the wings from default positions, and sideway and backward motions of the fly, which was common during courtship.

Detection of Wing Extension: To detect unilateral wing extensions directed at the female fly, the algorithm examines only video frames in which one fly is oriented towards the other or is otherwise in close proximity; this is determined by measurements of position and orientation of the flies. A portion of the image centered around the fly was cut out of the main image, adjusted to ensure maximal contrast, and rotated so that the fly was oriented with its body along the vertical axis. Wing resting positions were computed in normalized coordinates using the length of the fly body. Positions along the side of the fly where an extended wing would be were also computed using normalized fly length co-ordinates. A heuristic combining of the left-right asymmetry in wing resting positions, and asymmetry in wing-extended positions was used to calculate a wing asymmetry score. For example, a strong left-right asymmetry in the spaces where the resting wings are expected to reside, combined with a strong right-left asymmetry in the spaces where the extended wings are expected, would result in a large, positive wing asymmetry score.

After the completion of tracking the full video file, the absolute values of the wing asymmetry score for each fly were split into two clusters using extrema in the resultant bimodal distribution: one very low, indicating no wing extension, and another very high, indicating wing extension. In practice, wing asymmetry scores during wing extension were more than a thousand times greater than resting wing asymmetry scores, a fact that makes these two states of the wings easily distinguishable.

Implementation of the algorithm: After the initial manual annotation of the videos, all tracking code was run without user intervention. FlyVoyeur is fully parallelized and can use Graphics Processing Units (GPUs) to accelerate code. In practice, FlyVoyeur can analyze videos at >30fps on a quad-core computer.

Manual scoring of copulation and licking: Mating assays that were performed in mating chamber types 2 and 3 were scored manually for copulation. In addition, licking was scored manually in videos that were filmed from the ventral side of the flies; we defined a lick as a proboscis extension event that results in contact between the male labellum and the female abdomen. All manual scoring was done blind.

Capillary Feeder (CAFE) assay

Fifteen male flies were collected after eclosion and aged 7 days. 35 ml 2% agarose was placed in a 50 ml tube (Falcon #14-432-22) to maintain moisture. The flies were then transferred to the tube to be starved for 24 hours. Two calibrated capillaries (1-5 µl, Drummond Scientific Company #2-000-001) were presented to each tube of flies. One capillary contained 5µl 1mM sucrose, the other contained 5µl water. After 4 hours, the amount of consumption in each capillary was recorded. The consumption rate was the sum of volume consumed in both capillaries divided by the number of flies per Falcon tube and number of hours. The preference index was calculated as the volume of sucrose consumed minus that of water consumed, divided by the total volume consumed.

Climbing assay

Male flies were aged to 7 days after eclosion for climbing assay. The assay was done under safelight (Bright Lab #35010) at 25^{o}C and 50% relative humidity. Ten flies were transferred into a vial without food and gently knocked down to the bottom. The climbing score is reported as the percentage of flies that reach 5cm in 3s. The video recordings of climbing assay were visually scored by the experimenter who was blind to the genotypes of the flies.

Statistical analyses

Statistical analyses were performed using Graphpad Prism or Sigma Plot with the relevant tests indicated in figure legends. In particular, for the analysis of copulation timing and initiation of wing extension, we plotted cumulative curves using the Kaplan-Meier method under the survival analysis package and tested for statistical significance using the log-rank test.

Supplemental References

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., *et al.* (2000). The genome sequence of *Drosophila melanogaster*. Science *287*, 2185-2195.

Armstrong, N., Sun, Y., Chen, G.Q., and Gouaux, E. (1998). Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature *395*, 913-917.

Awasaki, T., and Kimura, K. (1997). *Pox-neuro* is required for development of chemosensory bristles in *Drosophila*. J Neurobiol *32*, 707-721.

Bellen, H.J., Levis, R.W., Liao, G., He, Y., Carlson, J.W., Tsang, G., Evans-Holm, M., Hiesinger, P.R., Schulze, K.L., Rubin, G.M., *et al.* (2004). The BDGP gene disruption project: single transposon insertions associated with 40% of *Drosophila* genes. Genetics *167*, 761-781.

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Variant Ionotropic Glutamate Receptors as Chemosensory Receptors in *Drosophila*. Cell *136*, 149-162.

Biegert, A., Mayer, C., Remmert, M., Soding, J., and Lupas, A. (2006). The MPI Toolkit for protein sequence analysis. Nucleic Acids Res *34*, W335-339.

Croset, V., Rytz, R., Cummins, S.F., Budd, A., Brawand, D., Kaessmann, H., Gibson, T.J., and Benton, R. (2010). Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. PLoS Genet *6*, e1001064.

Drapeau, M.D., and Long, A.D. (2000). The Copulatron, a multi-chamber apparatus for observing *Drosophila* courtship behaviors. Dros Inf Ser *83*, 194-196.

Fan, P., Manoli, D.S., Ahmed, O.M., Chen, Y., Agarwal, N., Kwong, S., Cai, A.G., Neitz, J., Renslo, A., Baker, B.S., *et al.* (2013). Genetic and neural mechanisms that inhibit *Drosophila* from mating with other species. Cell *154*, 89-102.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol *17*, 368-376.

Golovnin, A., Biryukova, I., Romanova, O., Silicheva, M., Parshikov, A., Savitskaya, E., Pirrotta, V., and Georgiev, P. (2003). An endogenous *Su(Hw)* insulator separates the yellow gene from the *Achaete-scute* gene complex in *Drosophila*. Development *130*, 3249-3258.

Groth, A.C., Fish, M., Nusse, R., and Calos, M.P. (2004). Construction of transgenic *Drosophila* by using the site-specific integrase from phage *phiC31*. Genetics *166*, 1775-1782.

Groth, A.C., Olivares, E.C., Thyagarajan, B., and Calos, M.P. (2000). A phage integrase directs efficient site-specific integration in human cells. Proc Natl Acad Sci U S A *97*, 5995-6000.

Lai, S.L., and Lee, T. (2006). Genetic mosaic with dual binary transcriptional systems in *Drosophila*. Nat Neurosci *9*, 703-709.

Le, T., Yu, M., Williams, B., Goel, S., Paul, S.M., and Beitel, G.J. (2007). *CaSpeR5*, a family of *Drosophila* transgenesis and shuttle vectors with improved multiple cloning sites. Biotechniques *42*, 164, 166.

Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron *22*, 451-461.

Ling, F., Dahanukar, A., Weiss, L.A., Kwon, J.Y., and Carlson, J.R. (2014). The Molecular and Cellular Basis of Taste Coding in the Legs of *Drosophila*. J Neurosci *34*, 7148-7164.

Markstein, M., Pitsouli, C., Villalta, C., Celniker, S.E., and Perrimon, N. (2008). Exploiting position effects and the *gypsy* retrovirus insulator to engineer precisely expressed transgenes. Nat Genet *40*, 476-483.

Masuyama, K., Zhang, Y., Rao, Y., and Wang, J.W. (2012). Mapping neural circuits with activity-dependent nuclear import of a transcription factor. Journal of neurogenetics *26*, 89-102.

Mayer, M.L., and Armstrong, N. (2004). Structure and function of glutamate receptor ion channels. Annu Rev Physiol *66*, 161-181.

McDonald, J.H., and Kreitman, M. (1991). Adaptive protein evolution at the *Adh* locus in *Drosophila*. Nature *351*, 652-654.

Meissner, G.W., Manoli, D.S., Chavez, J.F., Knapp, J.M., Lin, T.L., Stevens, R.J., Mellert, D.J., Tran, D.H., and Baker, B.S. (2011). Functional dissection of the neural substrates for sexual behaviors in *Drosophila melanogaster*. Genetics *189*, 195-211.

Metaxakis, A., Oehler, S., Klinakis, A., and Savakis, C. (2005). *Minos* as a genetic and genomic tool in *Drosophila melanogaster*. Genetics *171*, 571-581.

Meunier, N., Ferveur, J.F., and Marion-Poll, F. (2000). Sex-specific non-pheromonal taste receptors in *Drosophila*. Curr Biol *10*, 1583-1586.

Meunier, N., Marion-Poll, F., Rospars, J.P., and Tanimura, T. (2003). Peripheral coding of bitter taste in *Drosophila*. J Neurobiol *56*, 139-152.

Miller, C.J., and Carlson, J.R. (2010). Regulation of *Odor Receptor* Genes in Trichoid Sensilla of the *Drosophila* Antenna. Genetics *186*, 79-95.

Miyamoto, T., Chen, Y., Slone, J., and Amrein, H. (2013). Identification of a *Drosophila* glucose receptor using Ca2+ imaging of single chemosensory neurons. PLoS One *8*, e56304.

Miyamoto, T., Slone, J., Song, X., and Amrein, H. (2012). A Fructose Receptor Functions as a Nutrient Sensor in the *Drosophila* Brain. Cell *151*, 1113-1125.

Monte, P., Woodard, C., Ayer, R., Lilly, M., Sun, H., and Carlson, J. (1989). Characterization of the larval olfactory response in *Drosophila* and its genetic basis. Behav Genet *19*, 267-283.

Muzumdar, M.D., Tasic, B., Miyamichi, K., Li, L., and Luo, L. (2007). A global double-fluorescent *Cre* reporter mouse. Genesis *45*, 593-605.

Nitabach, M.N., Wu, Y., Sheeba, V., Lemon, W.C., Strumbos, J., Zelensky, P.K., White, B.H., and Holmes, T.C. (2006). Electrical hyperexcitation of lateral ventral pacemaker neurons desynchronizes downstream circadian oscillators in the fly circadian circuit and induces multiple behavioral periods. J Neurosci *26*, 479-489.

Notredame, C., Higgins, D.G., and Heringa, J. (2000). T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol *302*, 205-217.

O'Dell, K.M. (2003). The voyeurs' guide to *Drosophila melanogaster* courtship. Behav Processes *64*, 211-223.

Pan, Y., and Baker, Bruce S. (2014). Genetic Identification and Separation of Innate and Experience-Dependent Courtship Behaviors in *Drosophila*. Cell *156*, 236-248.

Parkhurst, S.M., Harrison, D.A., Remington, M.P., Spana, C., Kelley, R.L., Coyne, R.S., and Corces, V.G. (1988). The *Drosophila su(Hw)* gene, which controls the phenotypic effect of the *gypsy* transposable element, encodes a putative DNA-binding protein. Genes Dev *2*, 1205-1215.

Pfeiffer, B.D., Jenett, A., Hammonds, A.S., Ngo, T.T., Misra, S., Murphy, C., Scully, A., Carlson, J.W., Wan, K.H., Laverty, T.R., *et al.* (2008). Tools for neuroanatomy and neurogenetics in *Drosophila*. Proc Natl Acad Sci U S A *105*, 9715-9720.

Ramia, M., Librado, P., Casillas, S., Rozas, J., and Barbadilla, A. (2011). PopDrowser: the Population *Drosophila* Browser. Bioinformatics *28*, 595-596.

Rideout, E.J., Dornan, A.J., Neville, M.C., Eadie, S., and Goodwin, S.F. (2010). Control of sexual differentiation and behavior by the *doublesex* gene in *Drosophila melanogaster*. Nat Neurosci *13*, 458-466.

Sakai, T., and Ishida, N. (2001). Circadian rhythms of female mating activity governed by clock genes in *Drosophila*. Proc Natl Acad Sci U S A *98*, 9221-9225.

Stoletzki, N., and Eyre-Walker, A. (2011). Estimation of the neutrality index. Mol Biol Evol *28*, 63-70.

Toda, H., Zhao, X., and Dickson, B.J. (2012). The *Drosophila* Female Aphrodisiac Pheromone Activates *ppk23*(+) Sensory Neurons to Elicit Male Courtship Behavior. Cell reports *1*, 599-607.

Wagh, D.A., Rasse, T.M., Asan, E., Hofbauer, A., Schwenkert, I., Dürrbeck, H., Buchner, S., Dabauvalle, M.-C., Schmidt, M., Qin, G., *et al.* (2006). Bruchpilot, a Protein with Homology to ELKS/CAST, Is Required for Structural Integrity and Function of Synaptic Active Zones in *Drosophila*. Neuron *49*, 833-844.

Waterhouse, A.M., Procter, J.B., Martin, D.M., Clamp, M., and Barton, G.J. (2009). Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics *25*, 1189-1191.

Witsell, A., Kane, D.P., Rubin, S., and McVey, M. (2009). Removal of the bloom syndrome DNA helicase extends the utility of imprecise transposon excision for making null mutations in *Drosophila*. Genetics *183*, 1187-1193.