Variant Ionotropic Glutamate Receptors as Chemosensory Receptors in *Drosophila*

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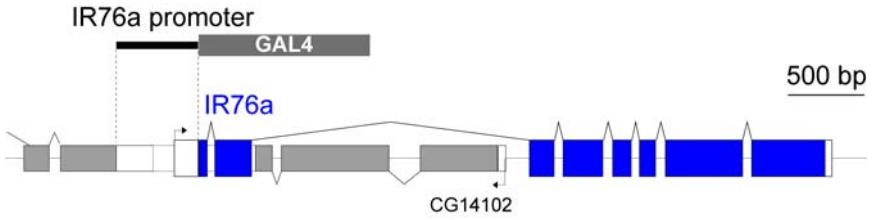
Supplemental Experimental Procedures

Transgene construction

For all transgenes, Oregon-R genomic DNA or cDNA was amplified using the Expand High Fidelity PLUS PCR system (Roche), cloned into pGEM-T Easy (Promega), sequenced, and subcloned with restriction enzymes whose sites were incorporated into the PCR primers into appropriate vectors as described below.

IR76a promoter-GAL4: we first determined the transcription start site of *IR76a* using the SMART 5'RACE kit (Clontech) and OR antennal cDNA. This revealed that the *IR76a* transcript includes exons computationally predicted to be in an upstream gene, *CG34257*, spanning another predicted transcript on the opposite strand (*CG14102*). The sequence of this extended predicted full-length *IR76a* ORF has been deposited in Genbank (Accession number FJ495546). The *IR76a* promoter, corresponding to nucleotides 19791840-19792377 in AE014296 (*Drosophila melanogaster* chromosome 3L) was subcloned *NotI/BglII* into pCaSpeR-AUG-*Gal4* (Vosshall et al., 2000). This promoter region contains a 27 bp deletion (nucleotides 19792232-19792258 in AE014296) that was confirmed as a true genomic polymorphism by sequencing multiple independent PCR products. A schematic of the *IR76a* locus, indicating the genomic region used to generate the *IR76a* promoter-GAL4 driver transgene is provided below. Exons from the *IR76a* locus are indicated in blue and exons from *CG14102*, a gene transcribed from the opposite strand within the second intron of *IR76a*, are in gray.



IR25a gene targeting construct: 5' and 3' homologous arms (4825968- 4830967 and 4834318- 4839317 of AE014134 （chromosome 2L) were subcloned as *AvrII* and *NotI* fragments respectively to flank the *white* reporter gene in CMC105 (Larsson et al., 2004).

UAS-IR84a: the predicted full-length ORF (Accession number NM_141463), with a Kozak consensus sequence upstream of the initiation codon, was subcloned as a *XhoI* fragment into pUAST-attB (Bischof et al., 2007).

UAS-IR76a: the predicted full-length ORF with Kozak consensus sequence was subcloned as a *MfeI/SalI* fragment into pUAST (Brand and Perrimon, 1993).

UAS-IR75d: the predicted full-length coding region with Kozak consensus sequence corresponding to 18904138-18901644 in AE014296 (chromosome 3L) was subcloned as an *EcoRI/SalI* fragment into pUAST.

UAS-IR92a: the predicted full-length coding region with Kozak consensus sequence corresponding to 16164695-16169624 in AE014297 (chromosome 3R) was subcloned as an *EcoRI/BglII* fragment into pUAST.

UAS-IR31a: the predicted full-length coding region with Kozak consensus sequence corresponding to 10405752-10408011 in AE014134 (chromosome 2L) was subcloned as an *EcoRI/SpeI* fragment into pUAST.

Sources of *Drosophila* mutant alleles and transgenic lines

amos^{1} (zur Lage et al., 2003), *atonal*^{1} (Jarman et al., 1994), *Df(2L)M36F-S6*, *Df(3R)p13* (Bloomington *Drosophila* Stock Center), *70FLP,70I-SceI/Cyo* and *70FLP* (Rong and Golic, 2000), *UAS-mCD8:GFP* (Lee and Luo, 1999), *OR35a-GAL4* (Fishilevich and Vosshall, 2005).

Sources of antibodies

Primary antibodies: mouse monoclonal nc82 1:10 (provided by R. Stocker), mouse monoclonal 21A6 (Developmental Studies Hybridoma Bank), rabbit α-GFP 1:1000 (Molecular Probes; Jackson Immunoresearch).

Secondary antibodies: Alexa488- and Cy3-conjugated α-mouse IgG or α-rabbit IgG 1:100 or 1:1000 for whole mount brains and antennal sections, respectively (Molecular Probes; Jackson Immunoresearch).

Electrophysiological recordings and odor delivery

Extracellular recordings in single sensilla of 2-8 day old flies were performed as described (Benton et al., 2007). For odor delivery, 10 µl odorant dilution was added to a 6 mm filter paper disk (Whatman) placed inside a 1 ml tuberculin syringe (Becton, Dickinson and Company). Charcoal-filtered airflow (35 ml s^{-1}) was used to deliver odors to the preparation through a 10 ml serological pipette trimmed to remove the tapered tip, and the cut end positioned 15 mm away from the preparation. Half this airflow was diverted through the odor syringe during the odor stimulation period (1 s) under the control of the Syntech CS-55 Stimulus controller.

SUPPLEMENTAL REFERENCES

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