



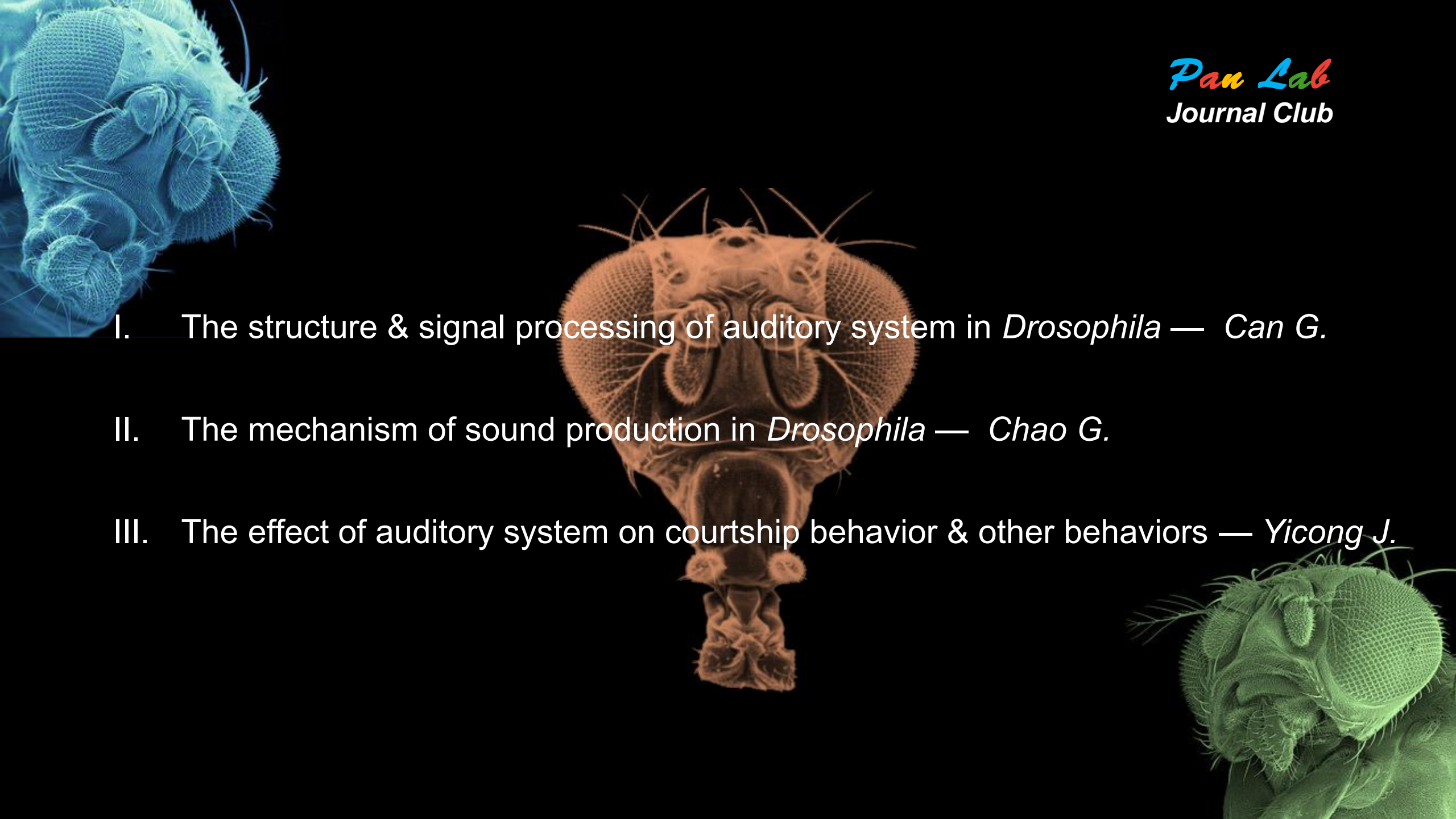
Pan Lab  
Journal Club



# Auditory system of *Drosophila* — *Listening to singing*

Can G. Chao G. Yicong J.

2019-4-1

- 
- The background of the slide features three scanning electron micrographs (SEMs) of Drosophila heads, highlighting the auditory system. In the top left, a blue-tinted SEM shows a head in profile, focusing on the large, textured compound eye and the surrounding sensory structures. In the center, an orange-tinted SEM provides a frontal view of a head, clearly showing the two large, oval-shaped auditory organs (macrotrichia) on the vertex. In the bottom right, a green-tinted SEM shows another head in profile, similar to the top-left image but with a different color scheme. The text of the list is overlaid on the central orange-tinted image.
- I. The structure & signal processing of auditory system in *Drosophila* — Can G.
  - II. The mechanism of sound production in *Drosophila* — Chao G.
  - III. The effect of auditory system on courtship behavior & other behaviors — Yicong J.



# The Structure and Signal Processing of Auditory System in *Drosophila*

Gao Can

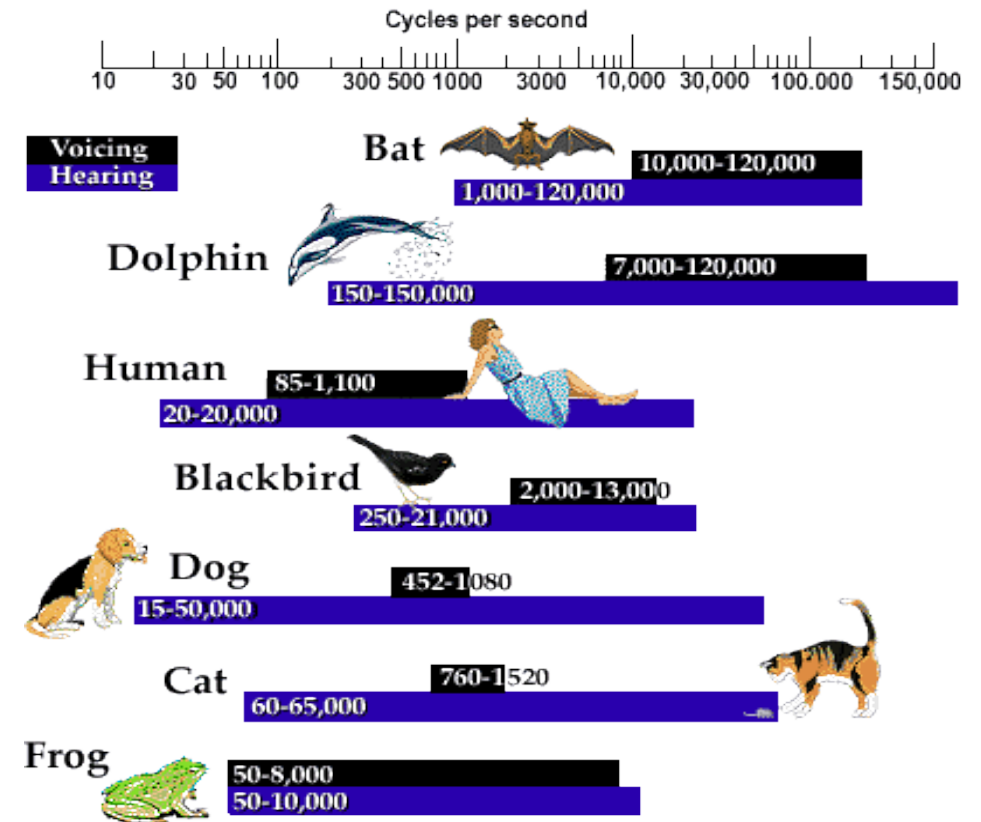
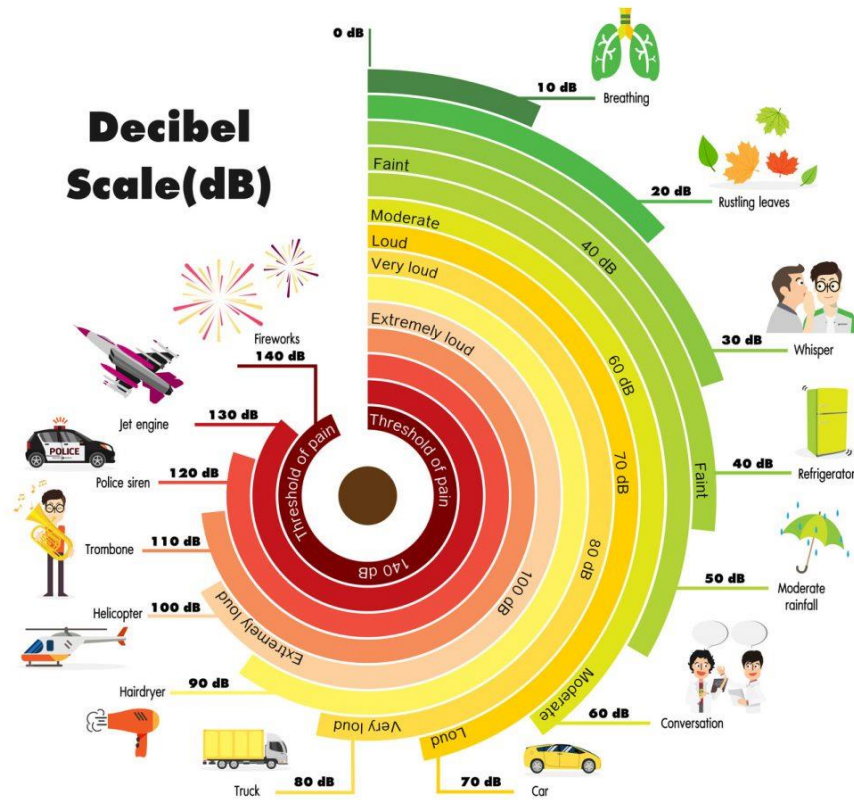
高灿

# Three elements of sound

Volume (dB)

Pitch (Hz)

Timbre





A video in collaboration between the Association of  
American Medical Colleges and Khan Academy

# Outline

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- The structure of *Drosophila* hearing organ—Johnston's organ
- Mechano-electrical transduction of auditory system
- Active amplification of auditory system
- The main auditory circuit in *Drosophila*

## Why so many studies on auditory system in *Drosophila*

- The structure of central projections from the fly ear to the brain is similar to that of its mammalian counterparts;

Many properties of the hearing system of flies parallel those in the vertebrate auditory system;

The *Drosophila* auditory organ and vertebrate hair cells are both specified by *atonal* family genes;

Genes such as *spalt*, *Distal-less*, and *crinkled*, whose mammalian homologs are linked to various human deafness syndromes.

- Johnston's organ serves as an excellent model

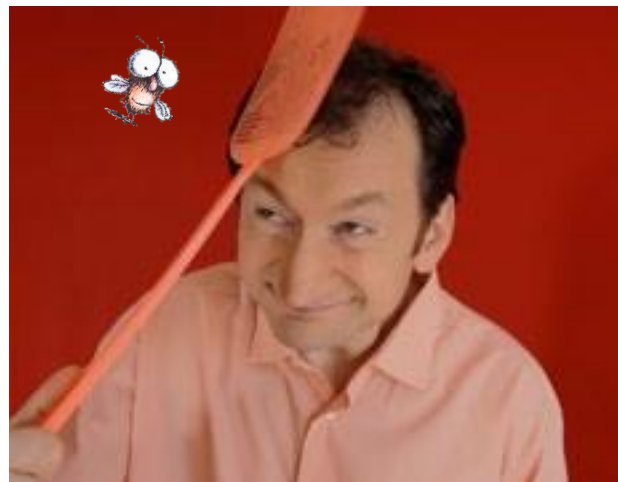


# The effects of insects auditory system

Intraspecific communication

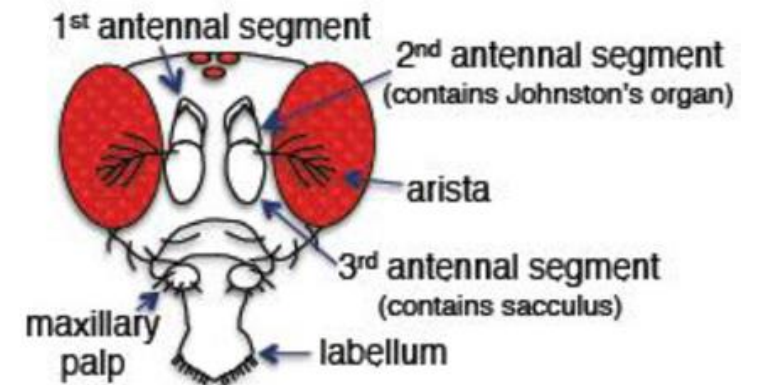
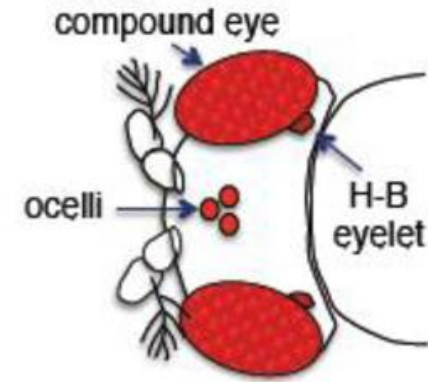
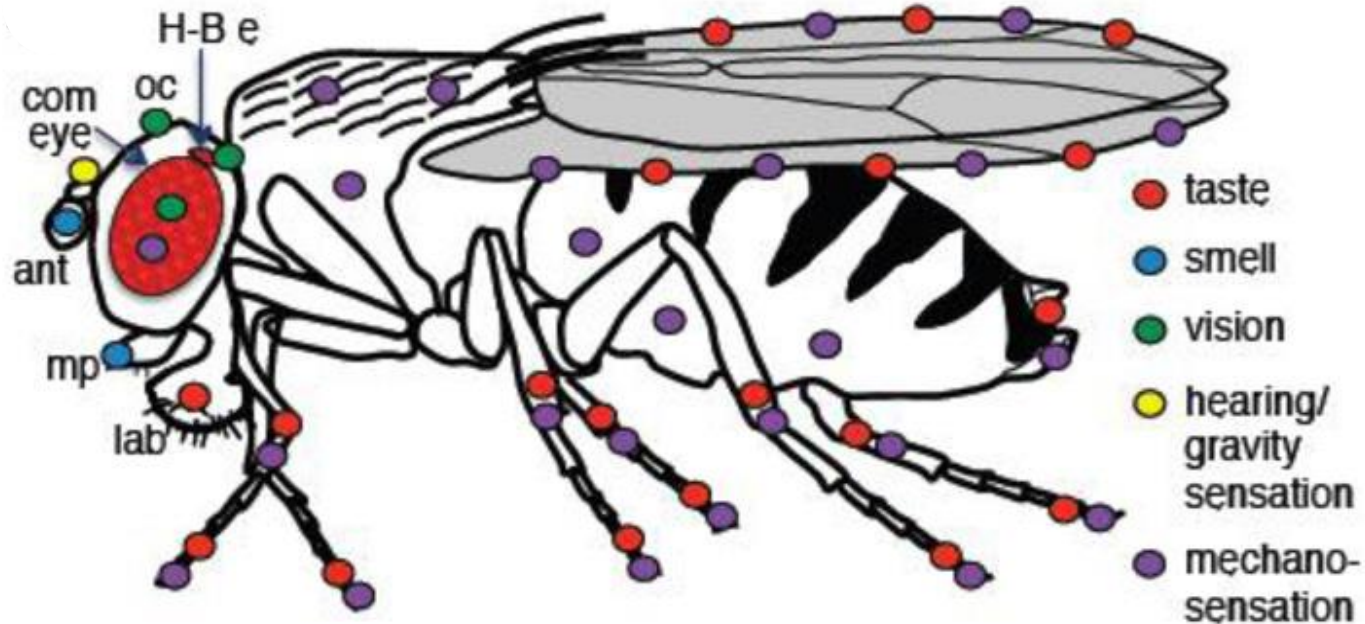


Predator detection



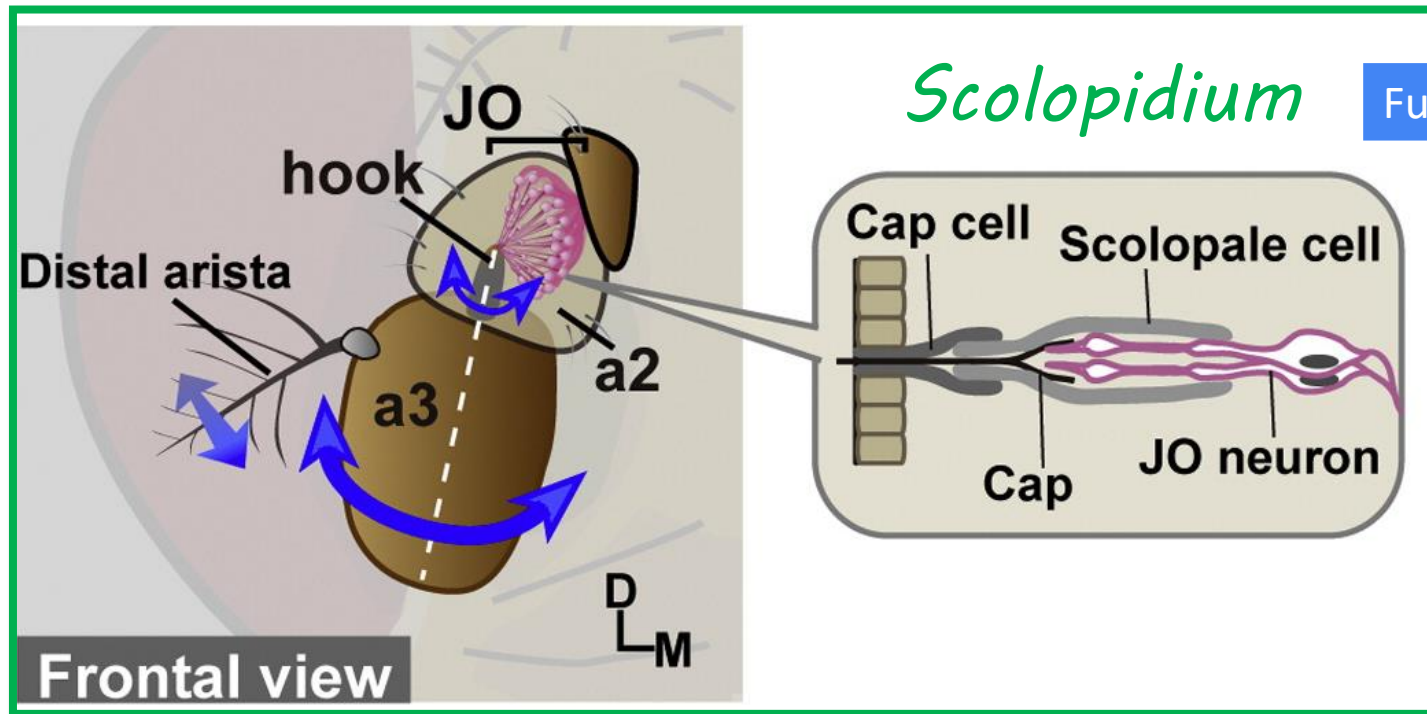
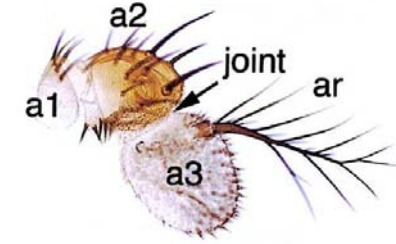


# Sensory organs of adult *Drosophila*



# Johnston's organ (JO)—the main auditory organ of *Drosophila*

- **Location** the second antennal segment (a2)
- **Structure & Components**

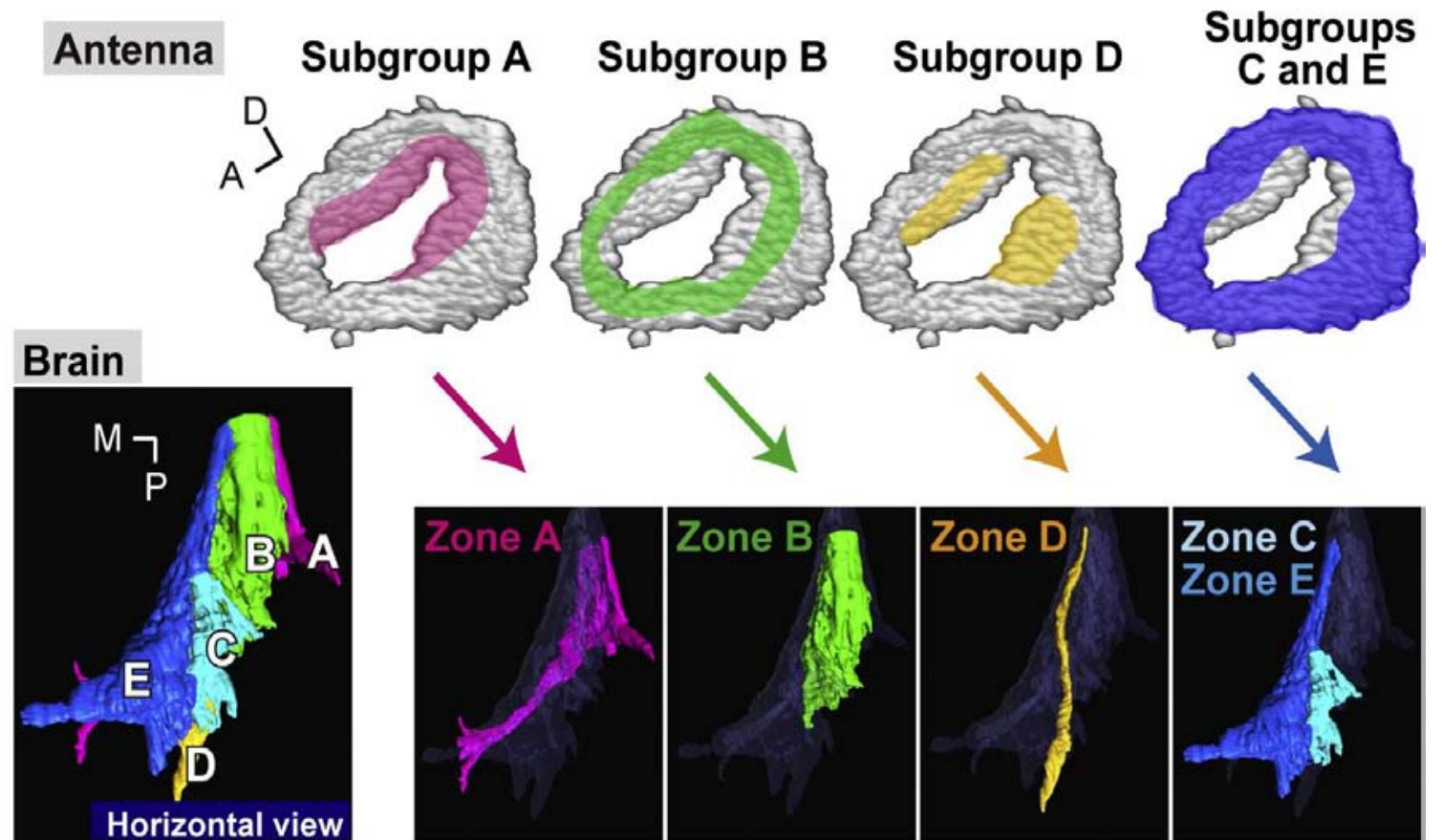
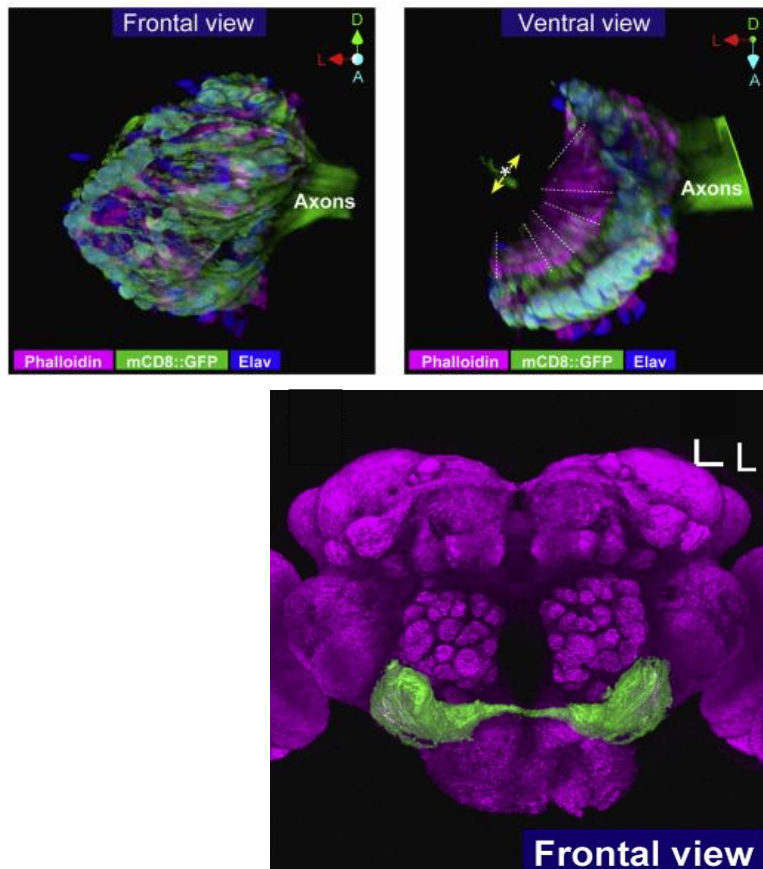


Functional sensory units of JO

JO  $\approx$  Scolopidium x 200

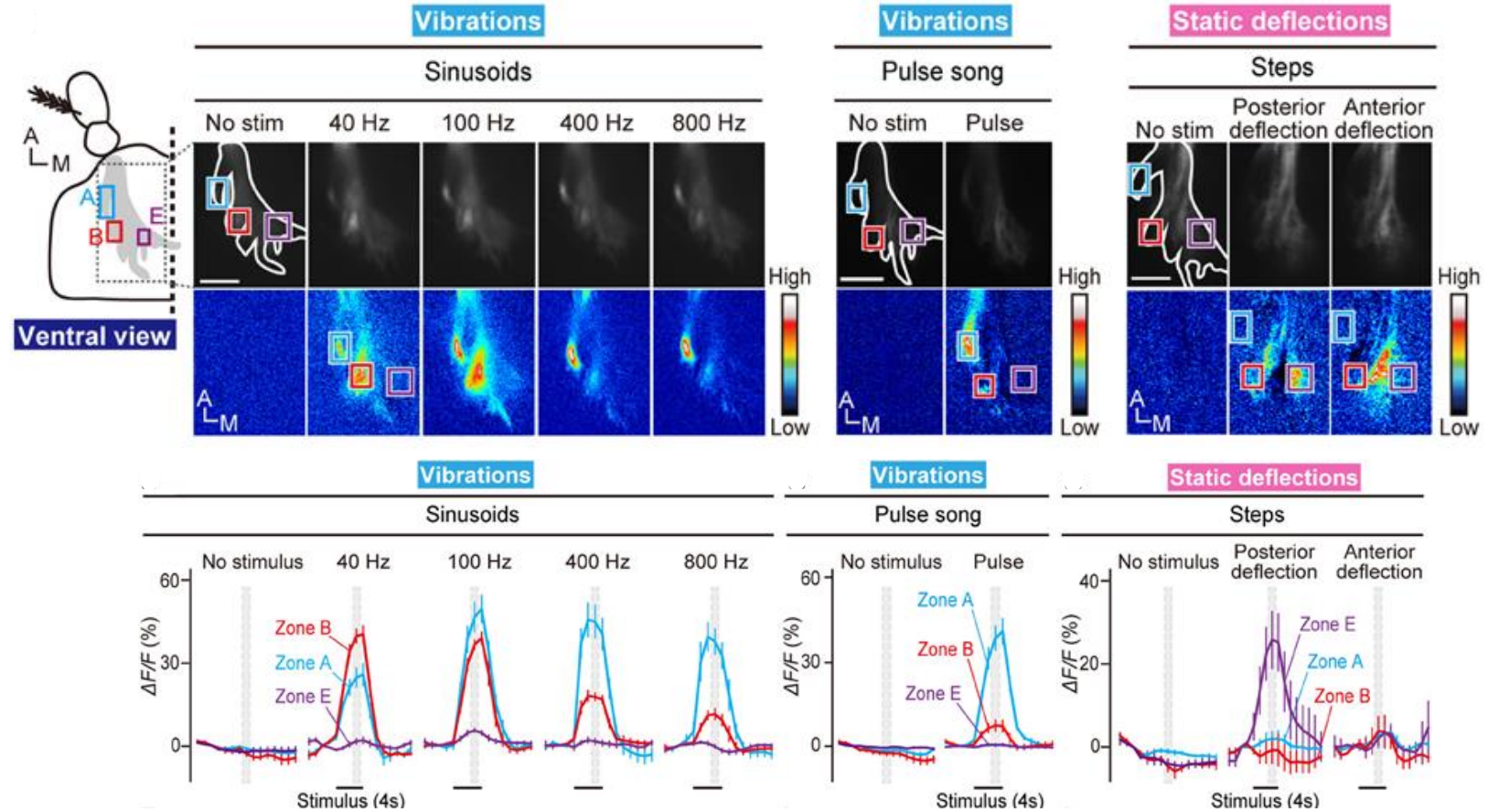
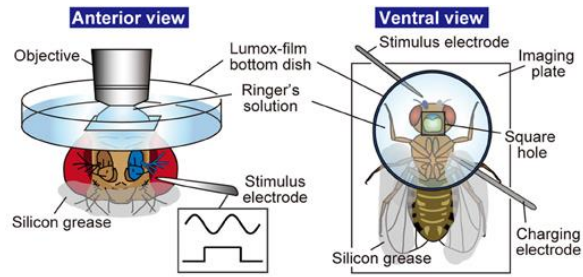
bipolar JO neurons in each unit

# Projection and classification of JO neurons

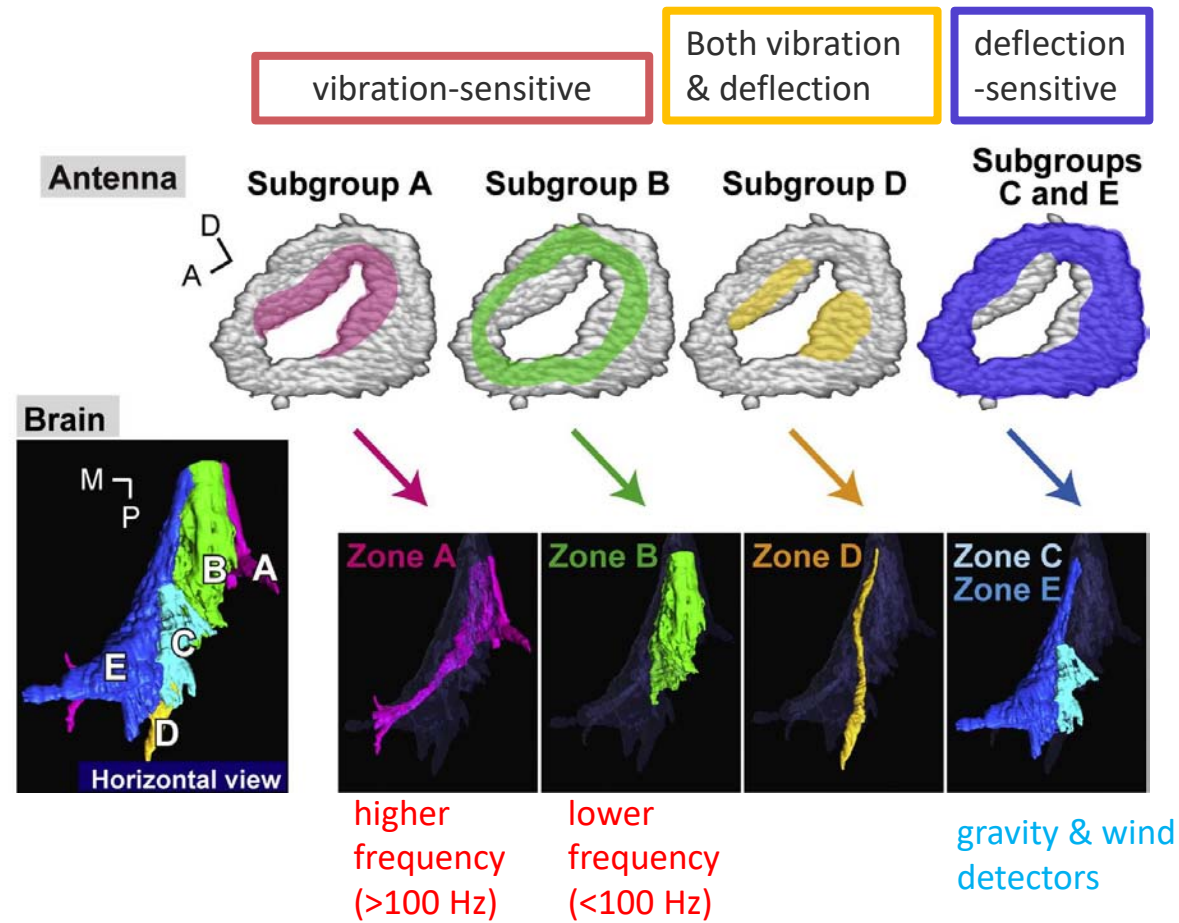




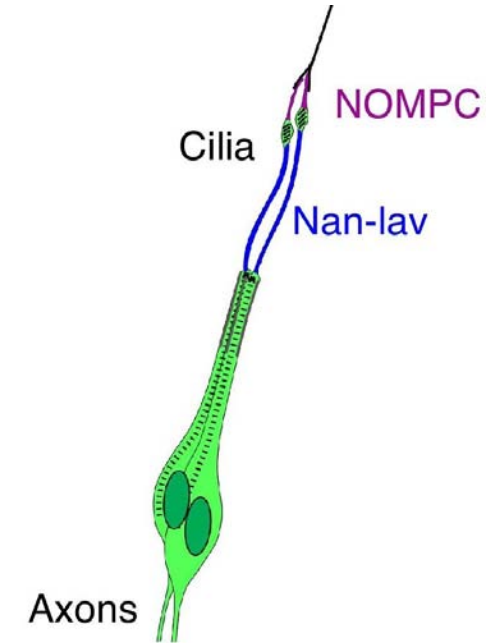
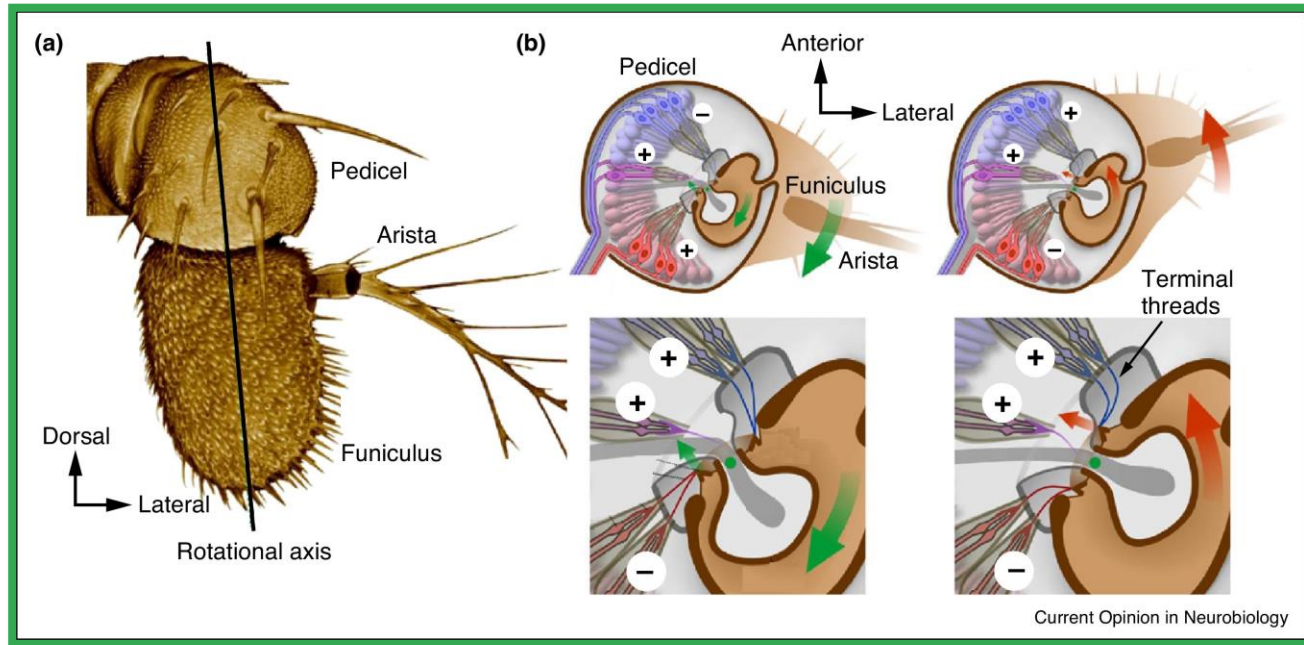
# Different subtypes of JONs responsible for different function



# Different subtypes of JONs responsible for different function



# Mechano-electrical transduction of auditory system

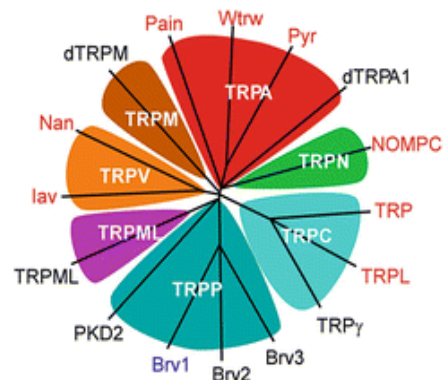


**NompC** (No Mechanoreceptor Potential C)

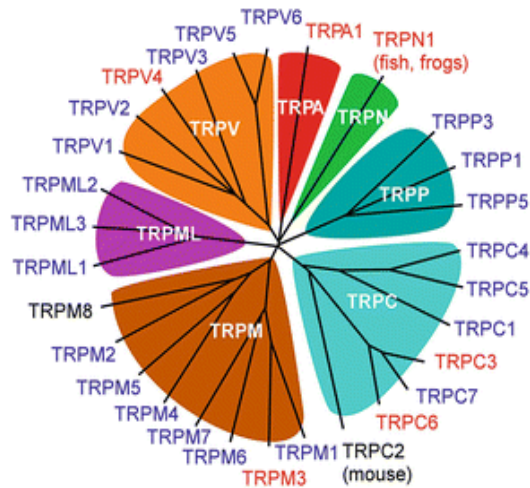
**Nan-lav** (Nanchung-Inactive)

Both are transient receptor potential (TRP) channels (瞬时受体电位通道) expressed in JO neurons

# TRP Channels



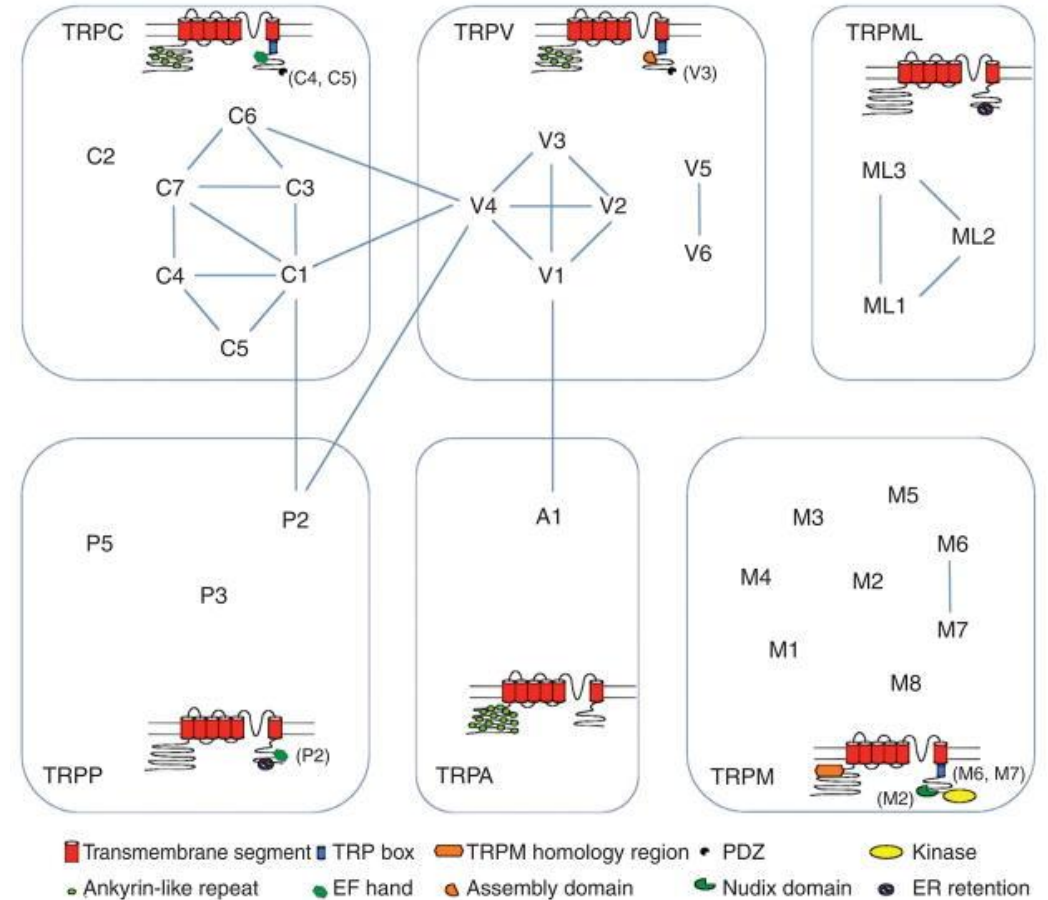
Drosophila



Vertebrate

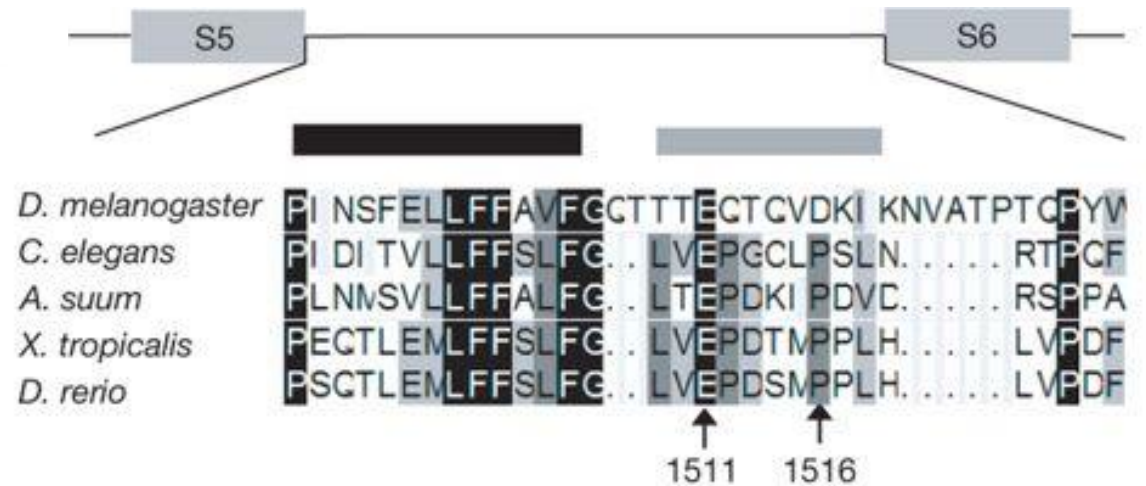
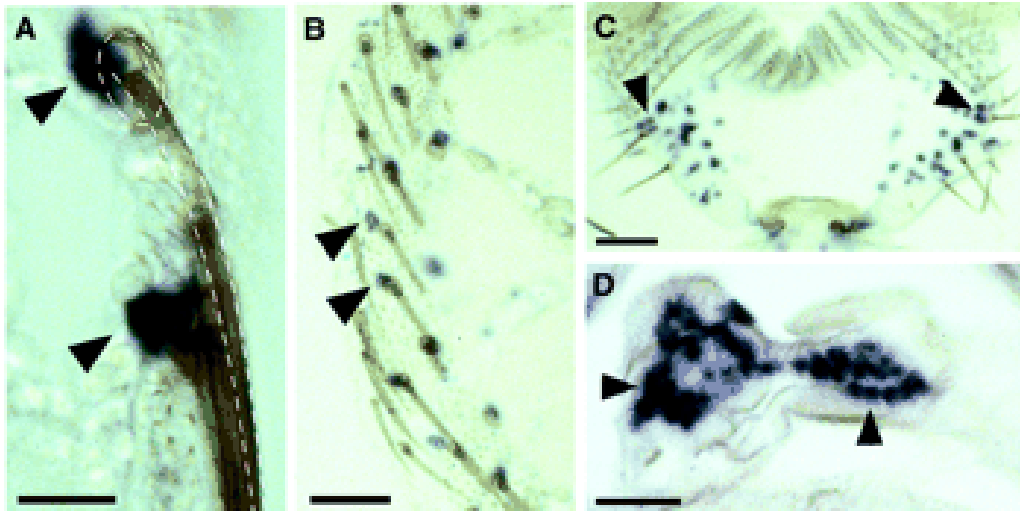


Insect TRP channels as targets for insecticides and repellents

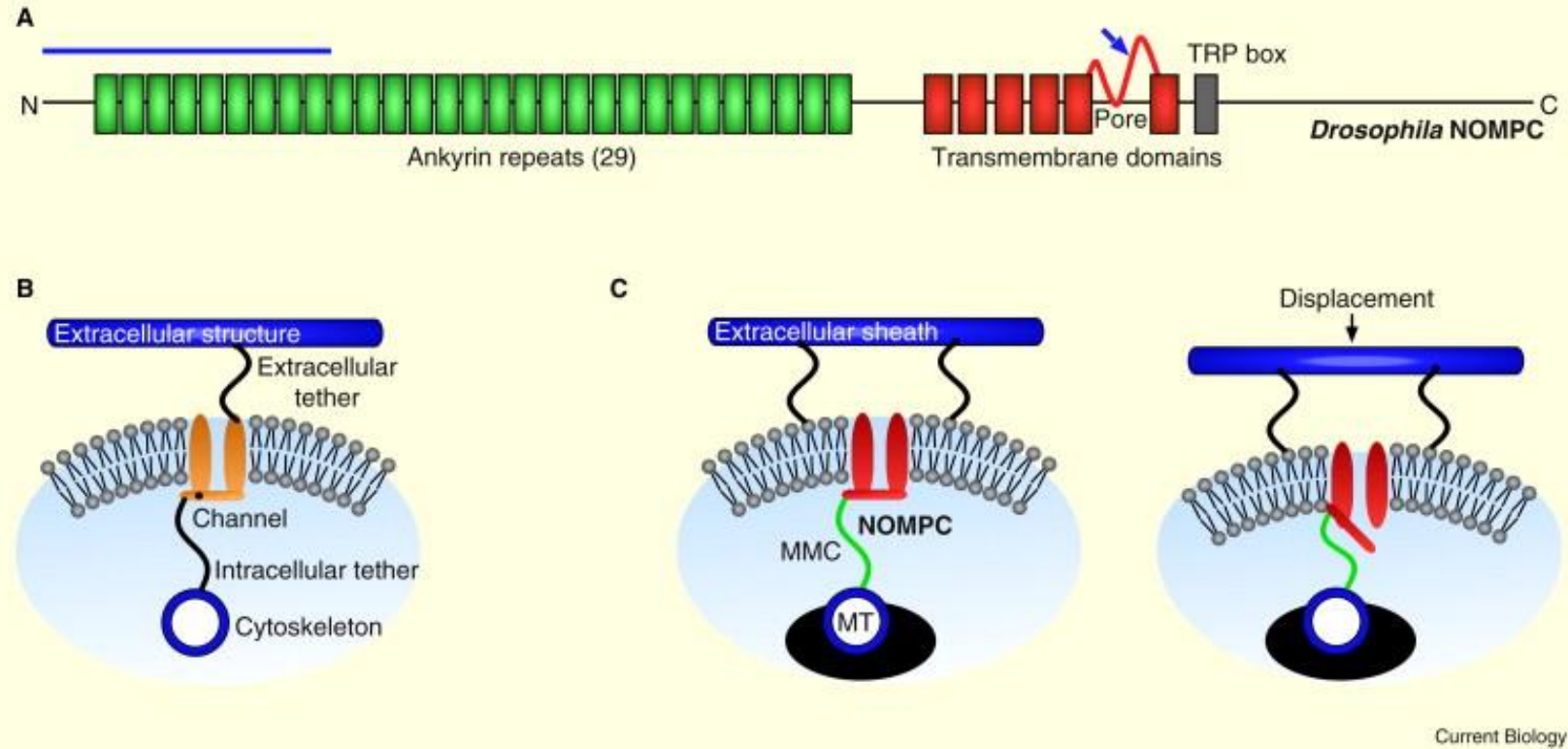




*nompC* is specifically expressed in mechanosensory organs and conserved in different species

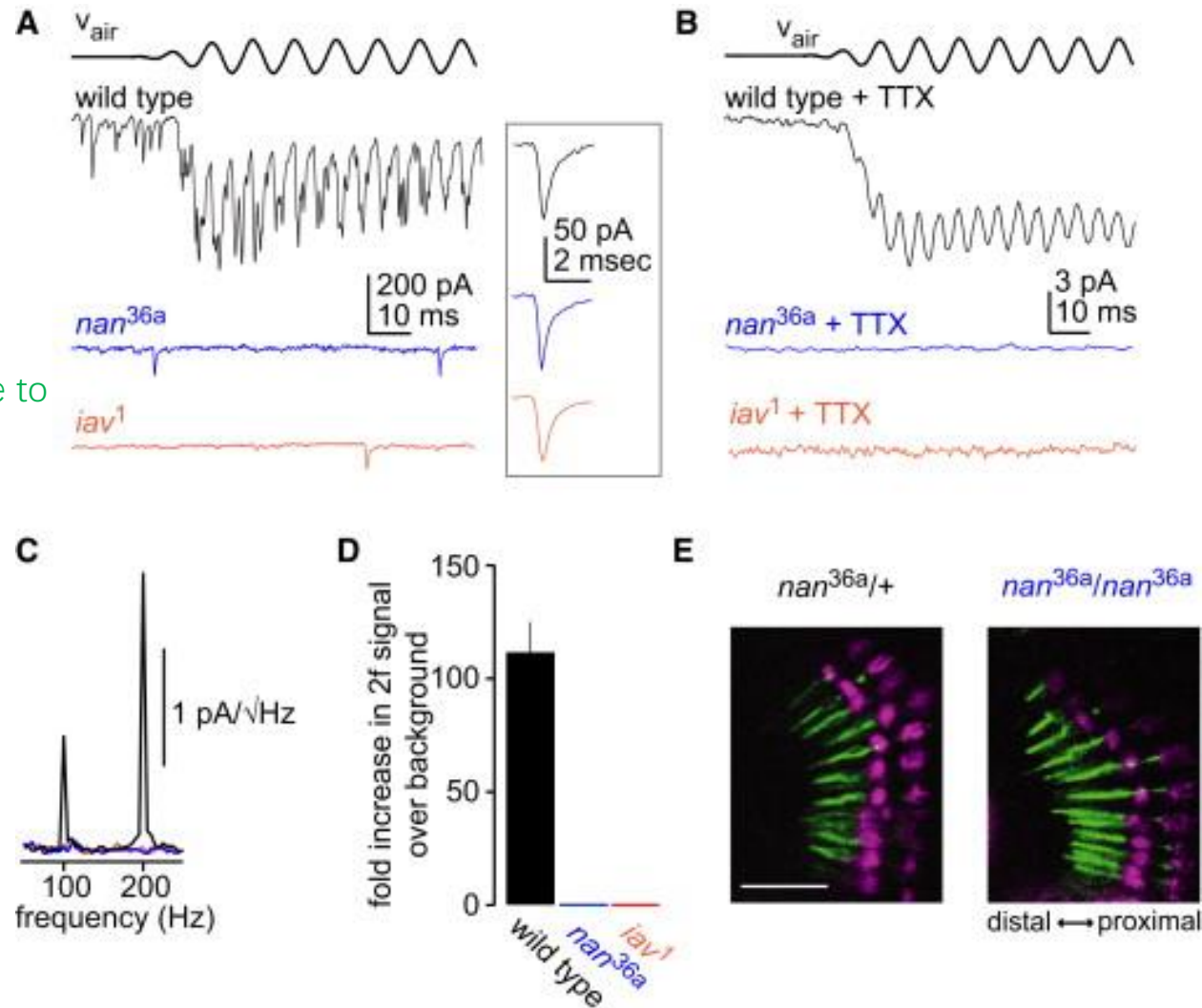


# Sensing mechanical stimuli with NOMPC

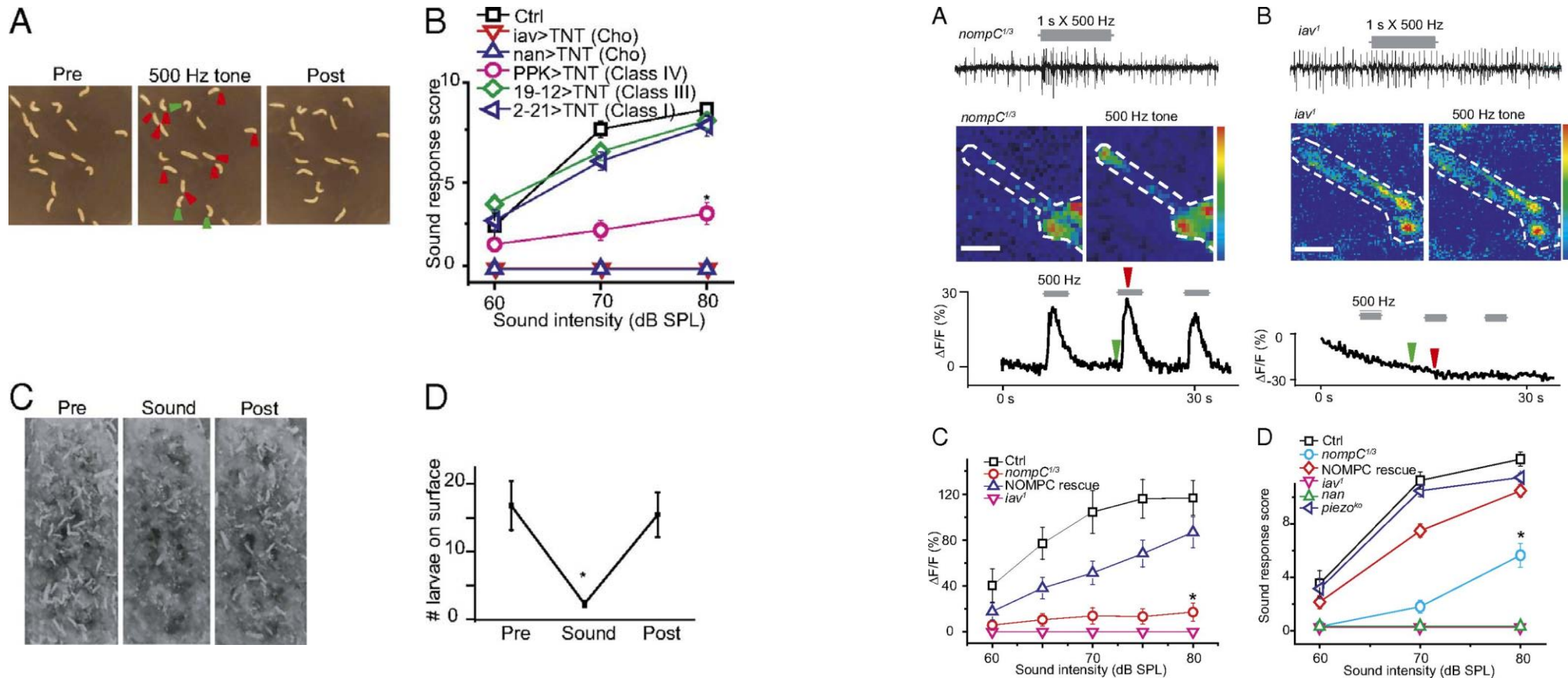


# Loss of Nanchung or Inactive Completely Abolishes Generator Currents

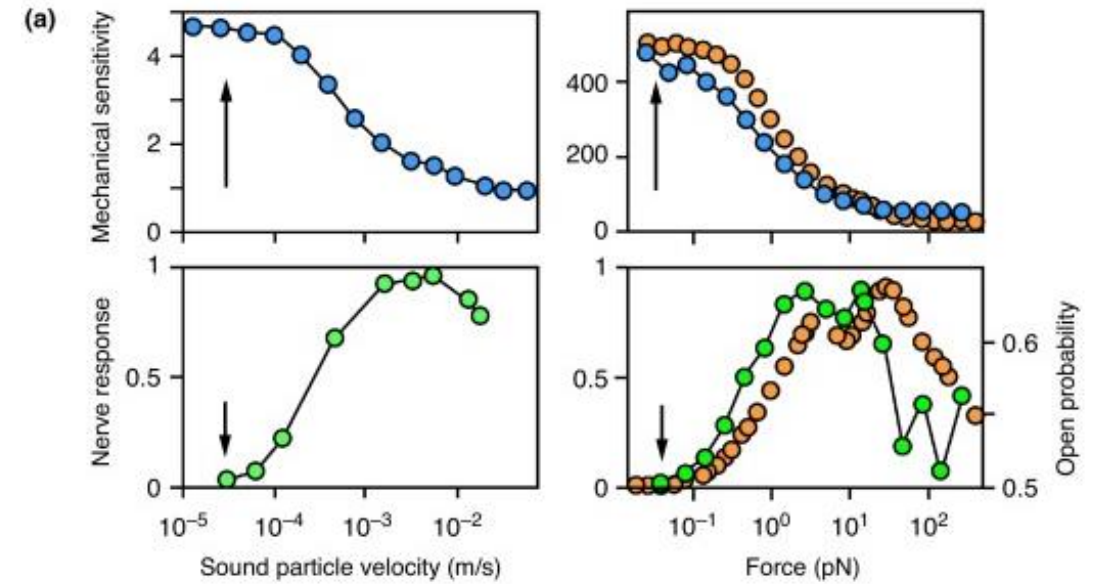
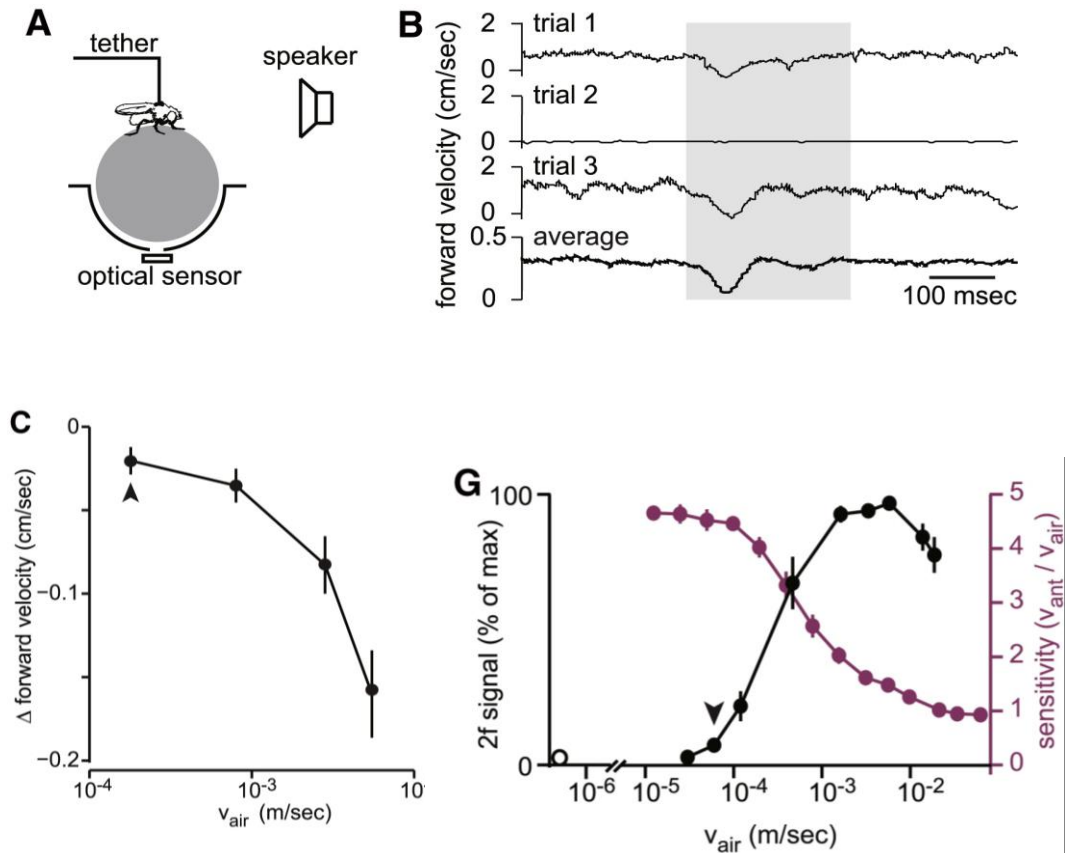
record in the GFN in response to a 100 Hz tone (0.0044 m/s).



# Sound response mediated by the TRP channels NOMPC, NANCHUNG, and INACTIVE in chordotonal organs of *Drosophila* larvae

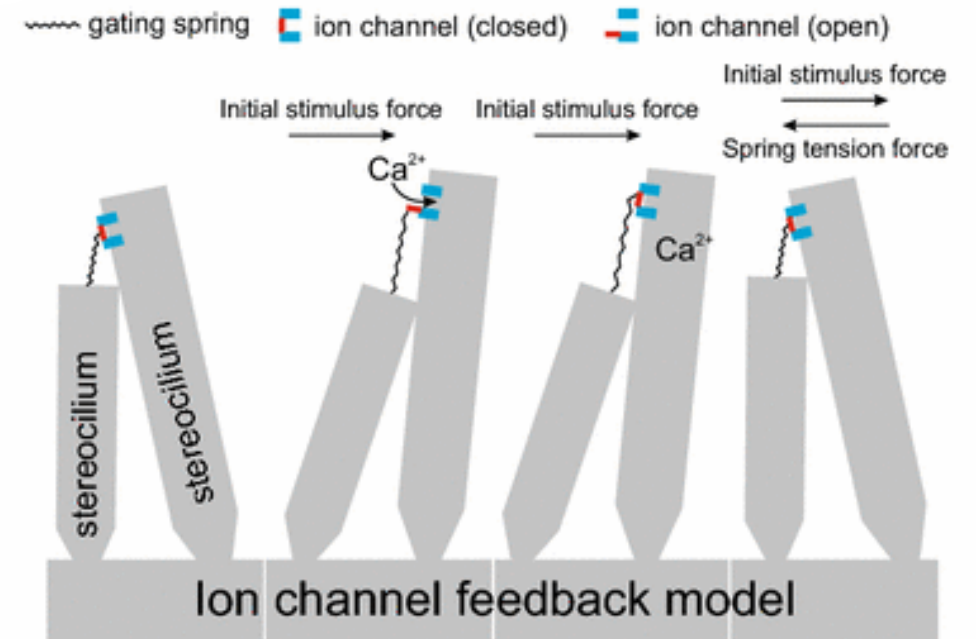
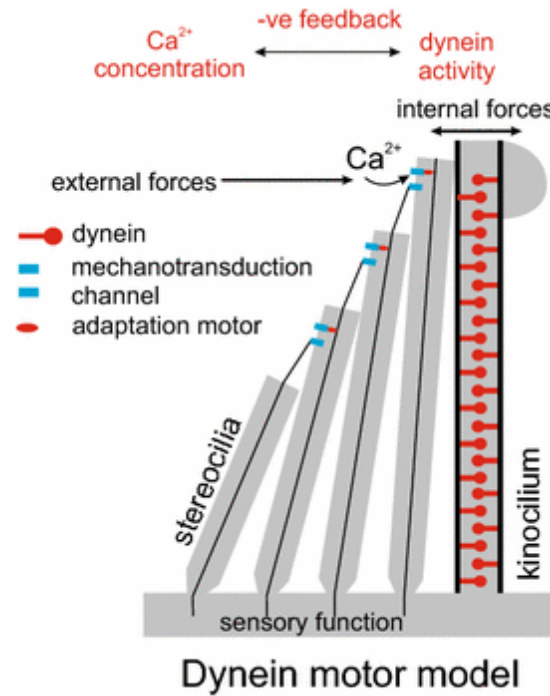
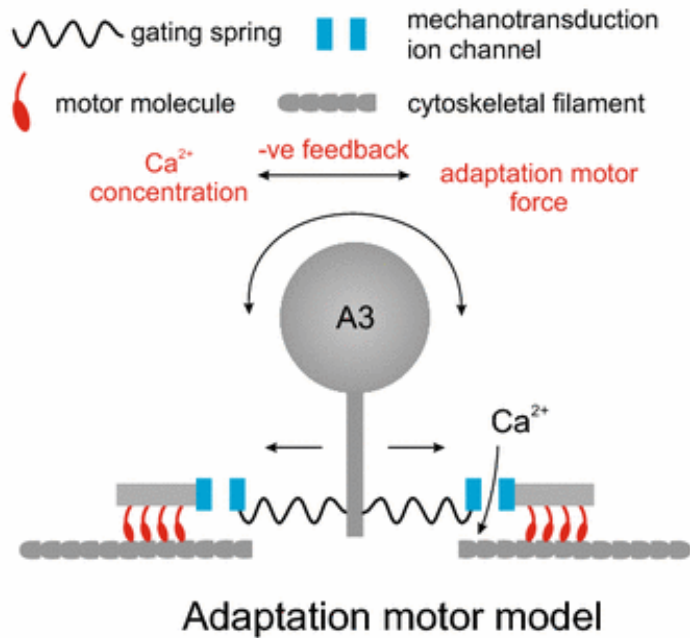


# *Drosophila* hearing is sensitive to low-intensity sounds

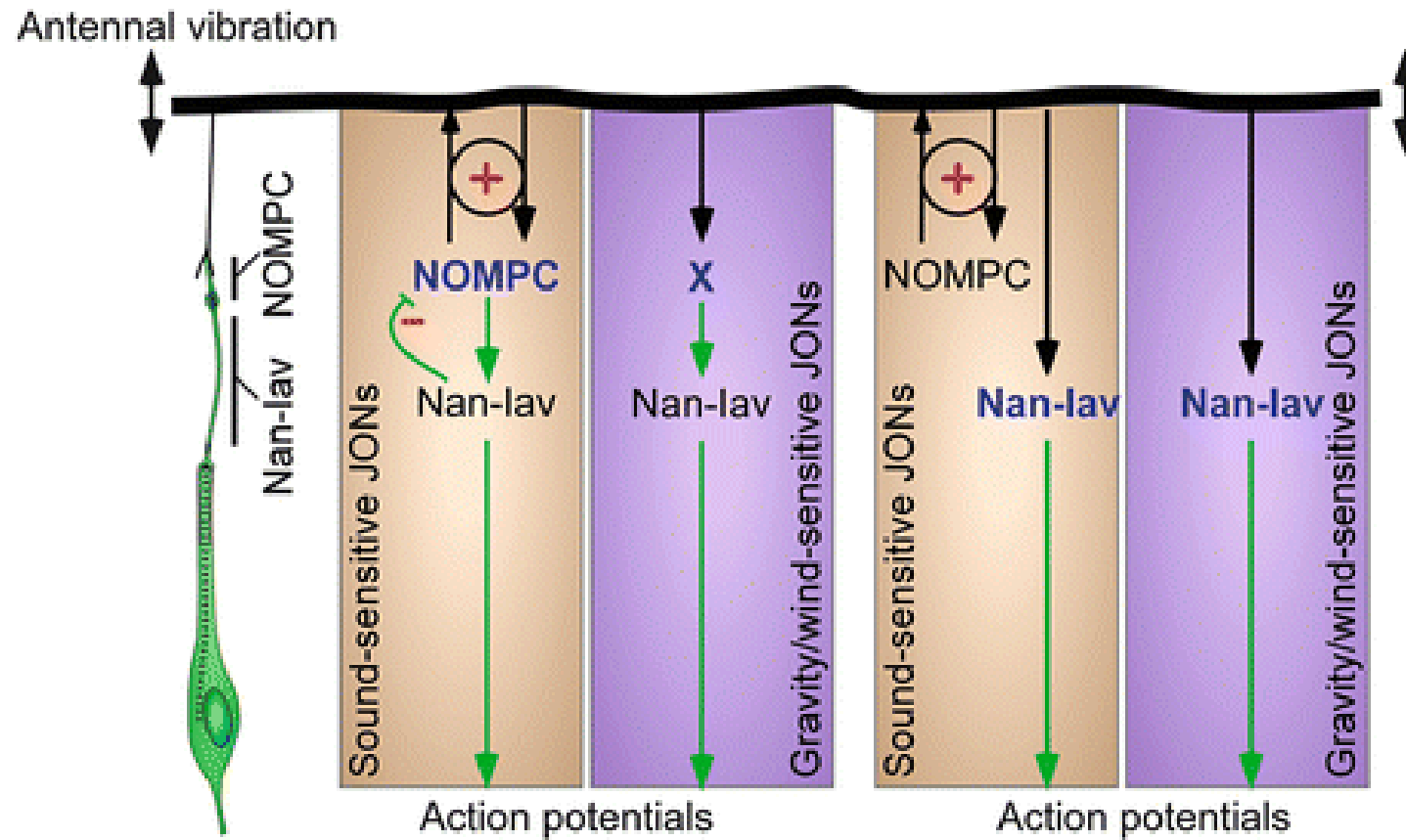




# Active amplification models

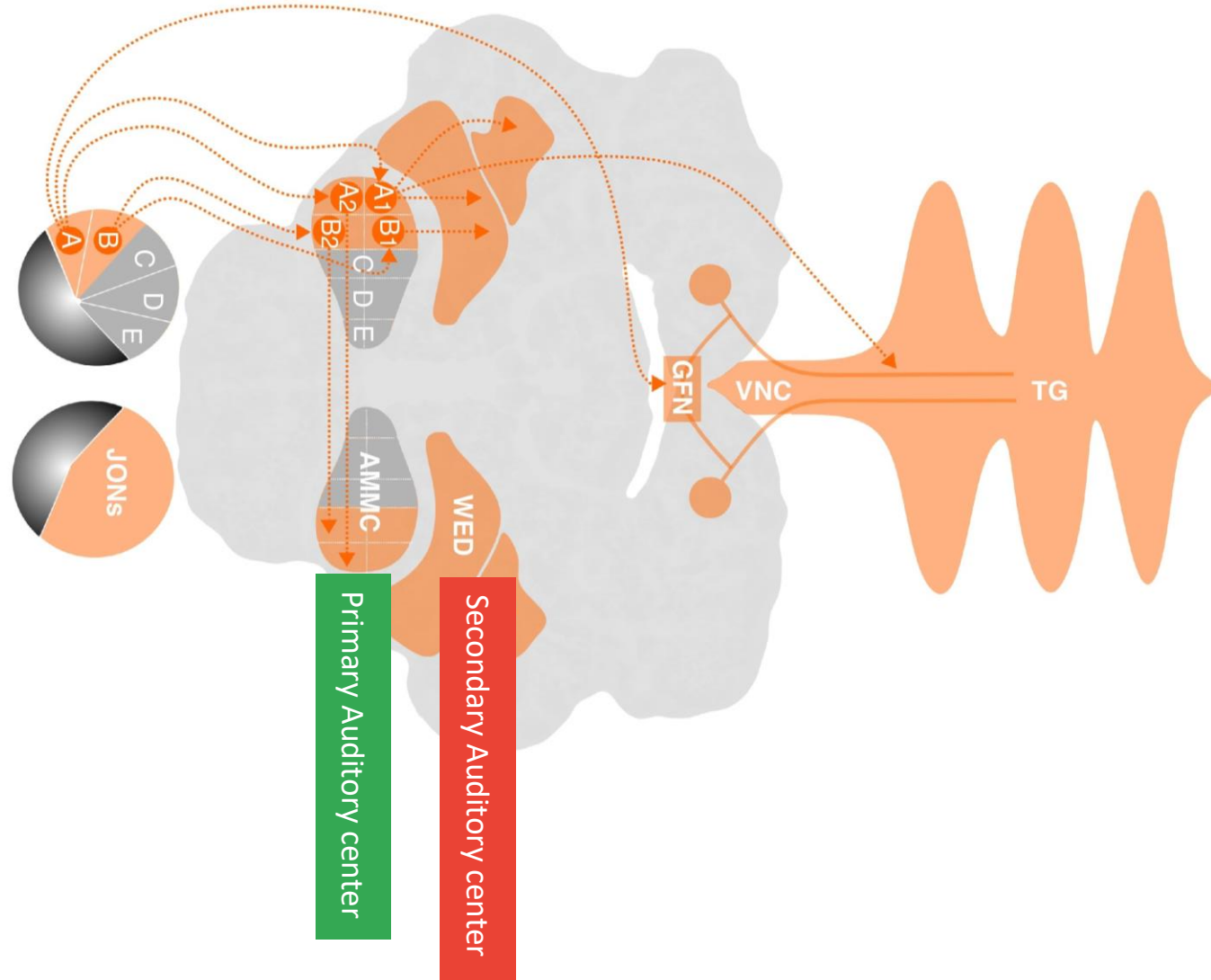


## Two models for Transduction machinery

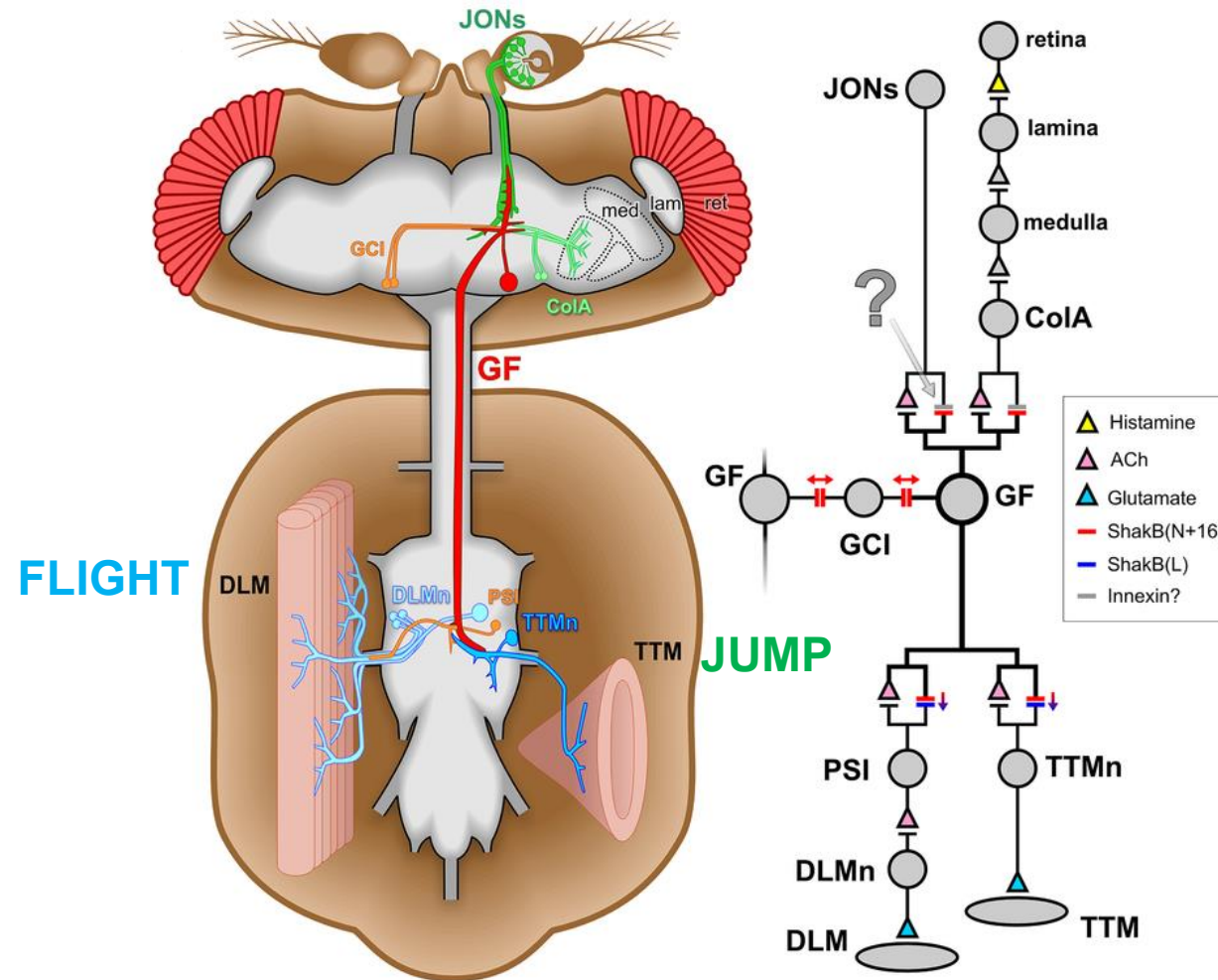




# The main auditory circuit in *Drosophila*



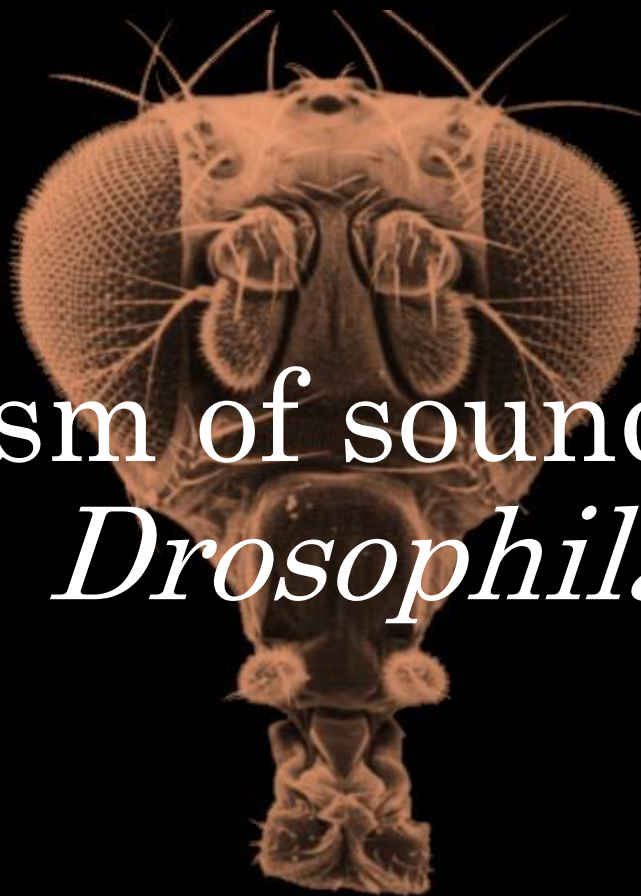
# The Drosophila giant fiber (GF) escape circuit



tergotrochanteral motoneurons (TTMn)  
 tergotrochanteral jump muscles (TTM)  
 dorsal longitudinal motoneurons (DLMn)  
 dorsolongitudinal flight muscles (DLM)

Welcome to part II





# The mechanism of sound production in *Drosophila*

Guo Chao

郭超

# Outline

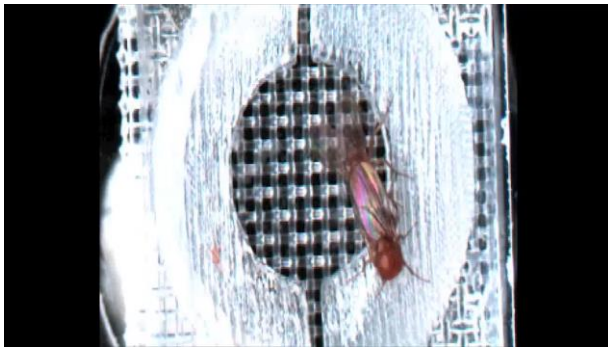
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- The sound produced by *Drosophila*
- Genetic of courtship song
- Neuronal control of courtship song
- Motor control of courtship song
- Sensory modulation of courtship song

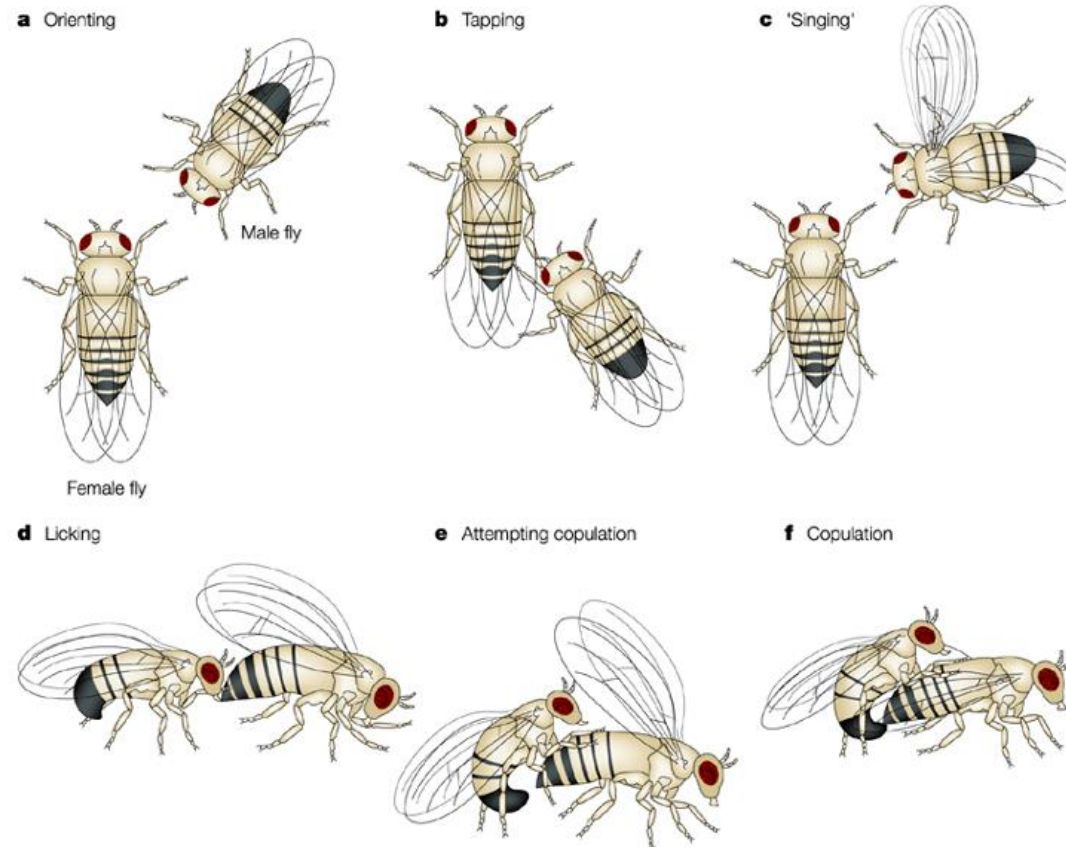


# Sound in nature

## Chirping insects



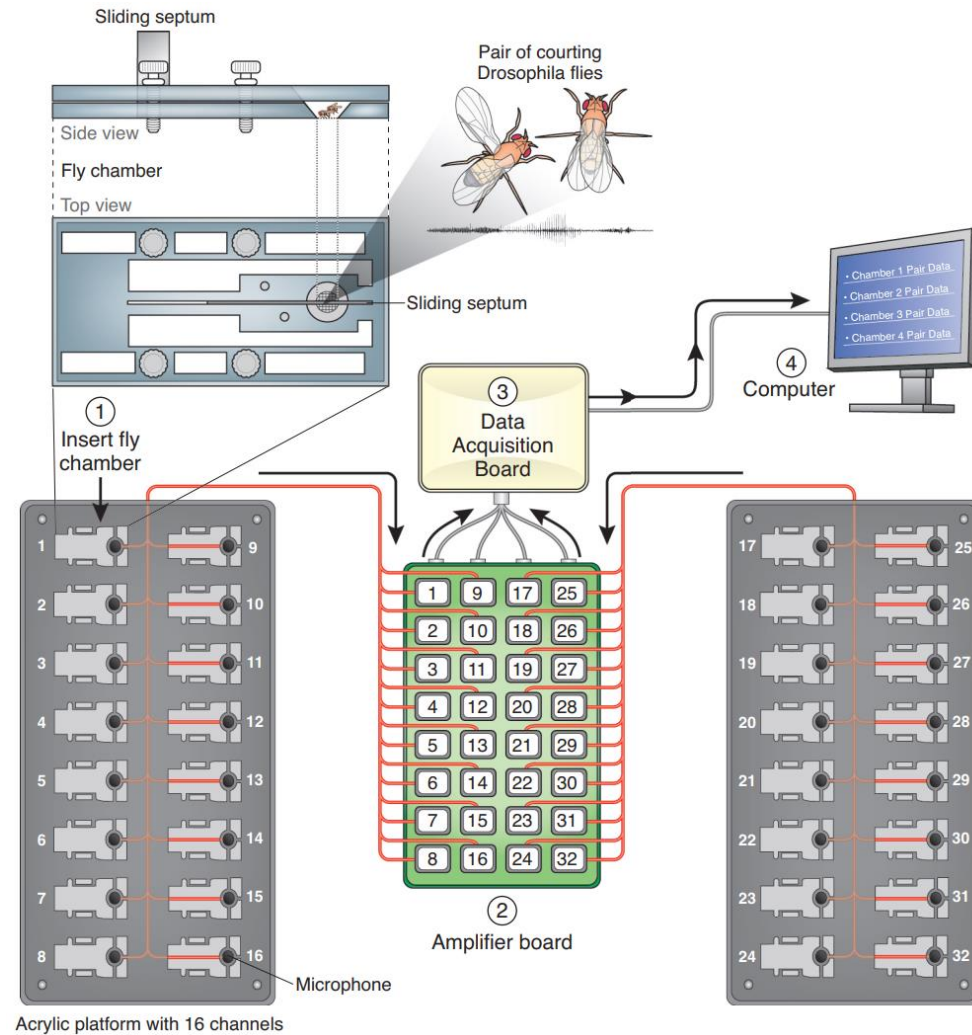
# *Drosophila* courtship and the courtship song



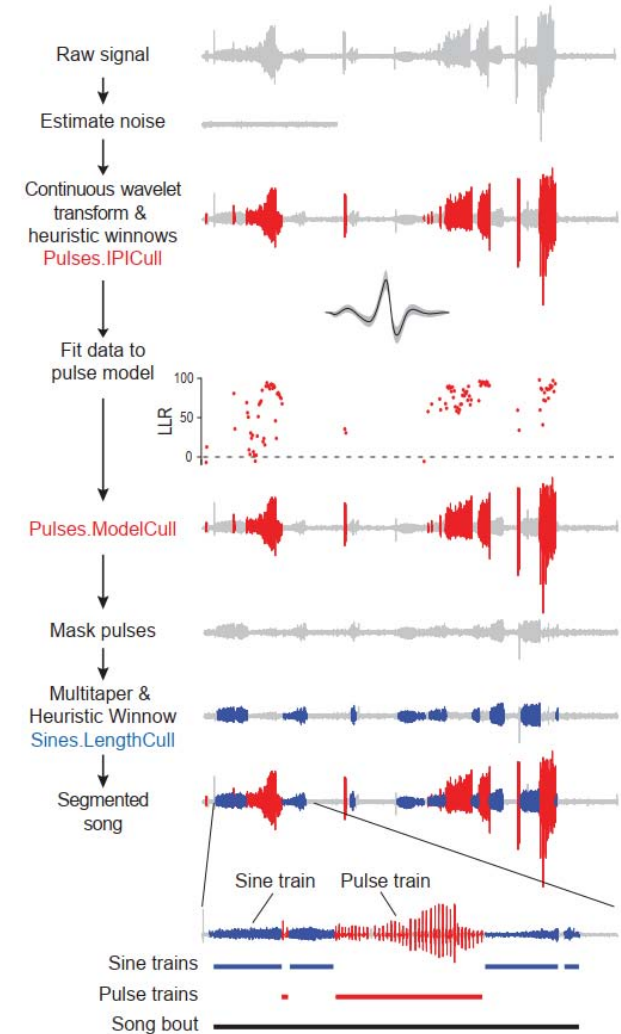
Nature Reviews | Genetics



# Sound recording & analysis

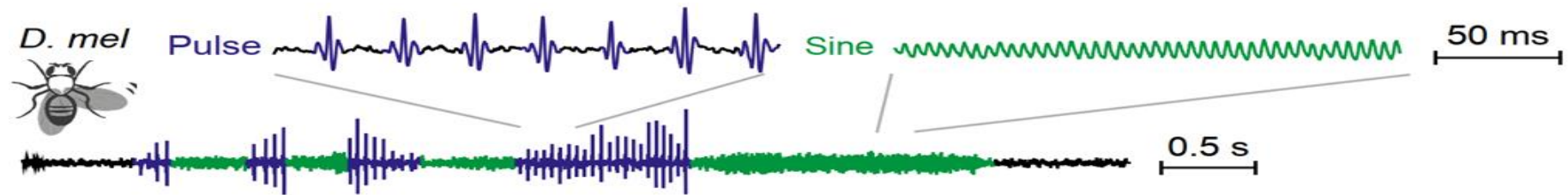


32-channel courtship song recording apparatus

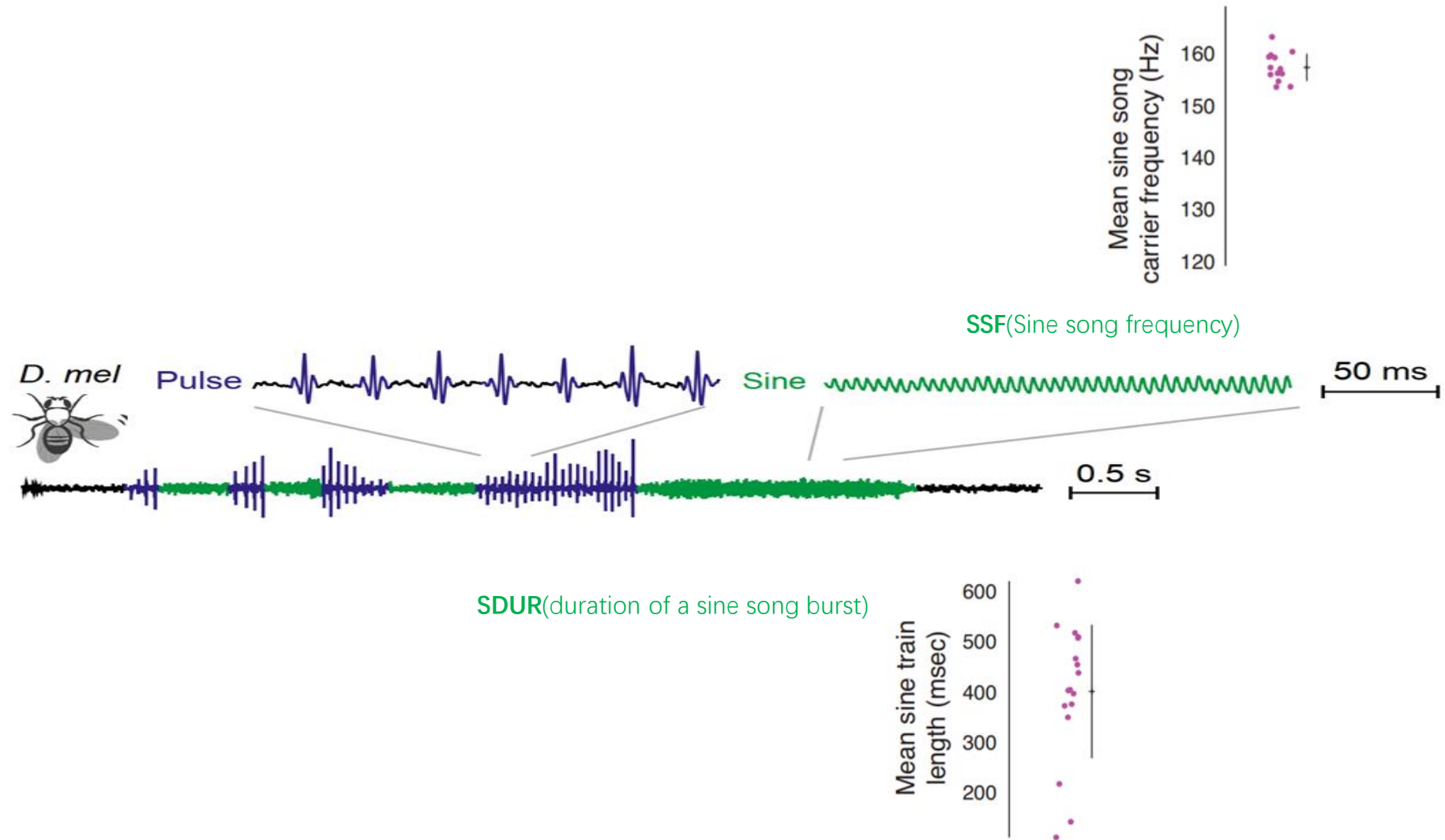


Computational analysis by FlySongSegmenter

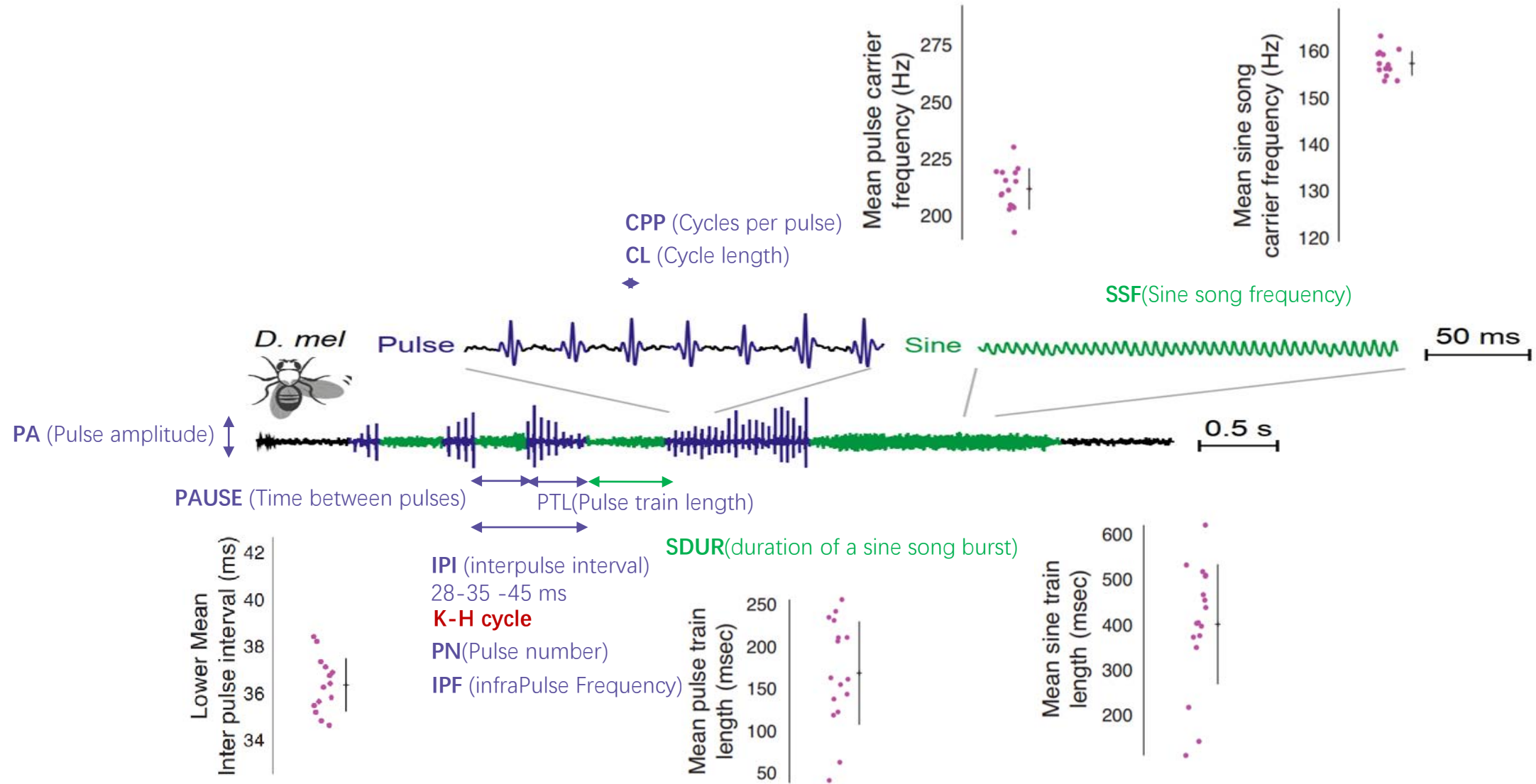
# Courtship song of *Drosophila melanogaster*



# Courtship song of *Drosophila melanogaster*

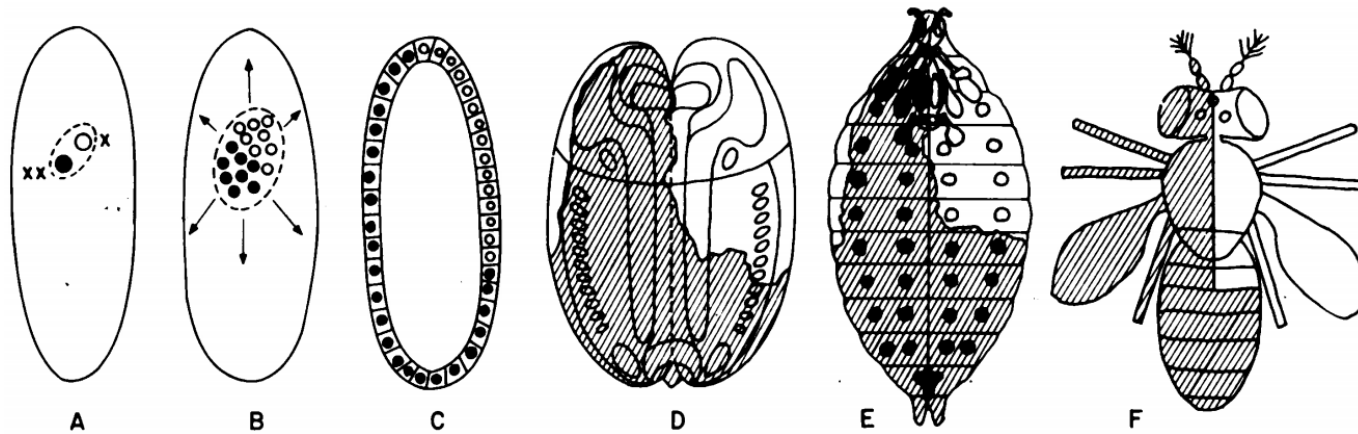


# Courtship song of *Drosophila melanogaster*

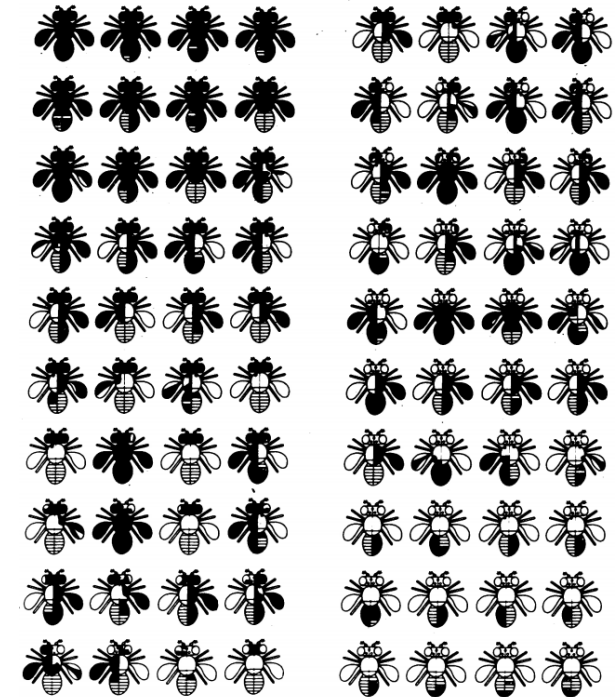


Foray into loci of courtship song by sex mosaics

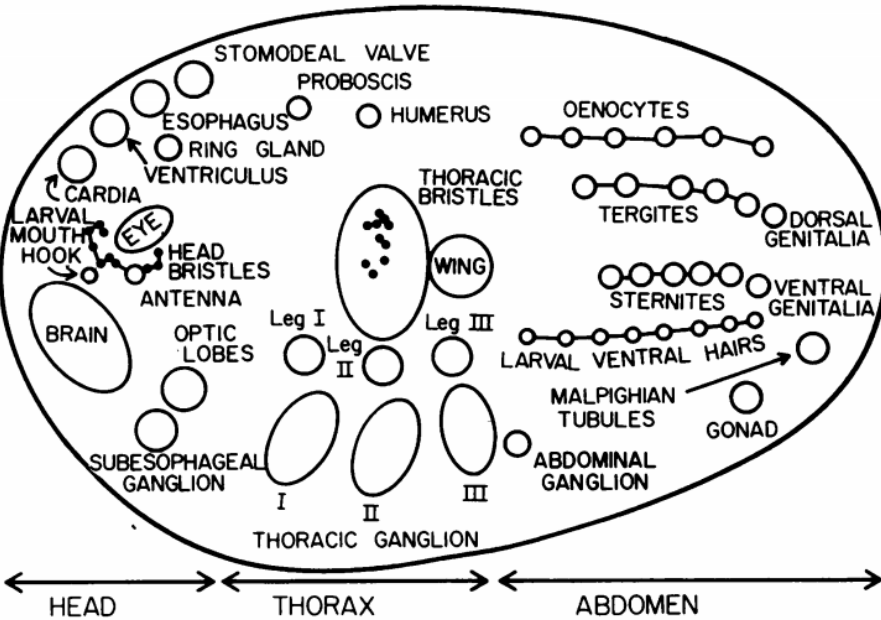
# Genetic dissection of the *Drosophila* nervous system by means of mosaics



Schematic diagram illustrating formation of a gynandromorph (sex mosaics).



# Sex-specific foci for sequential action patterns



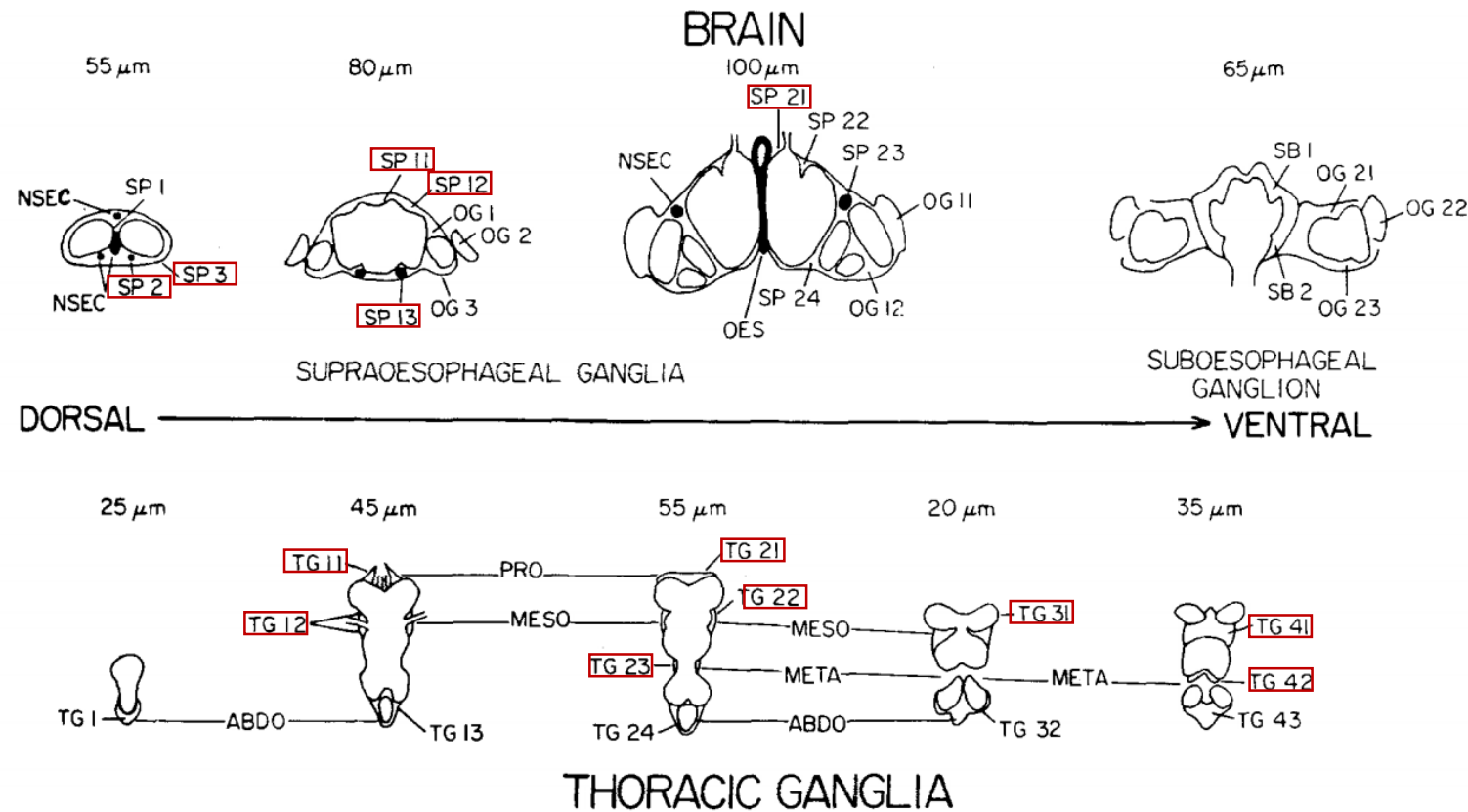
Fate map of *Drosophila* embryo,

Table 1. Male wing vibration compared with sex of cuticle

	Head		Thorax		Abdomen	
Vibrate	Male	Female	Male	Female	Male	Female
Yes	162	2	91	11	22	26
No	5	133	33	47	36	19



# Mosaicism in the central nervous systems of male-behaving mosaics

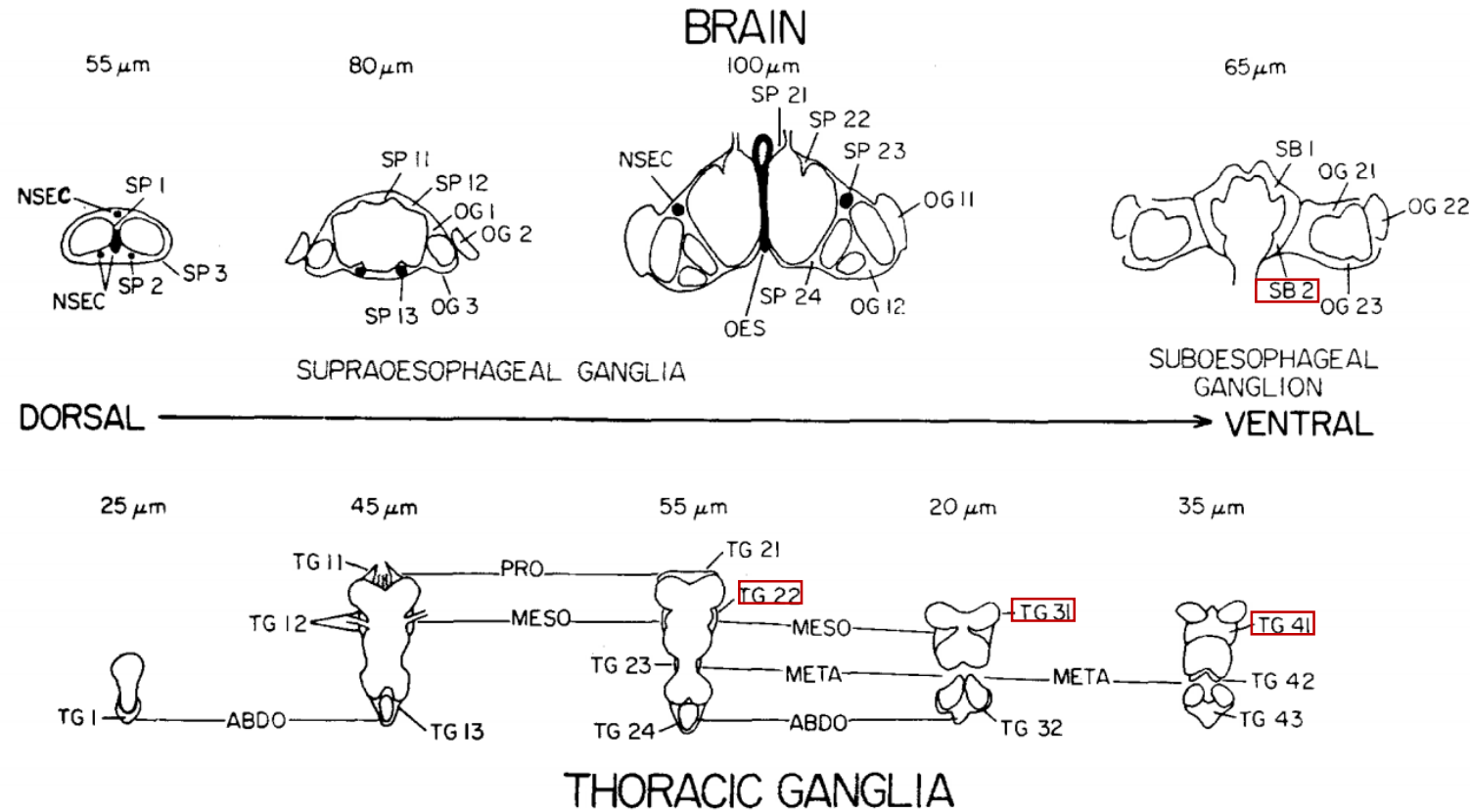


Brain	♀♀	♂♀ +♂	♂♂	P (♀♀)
SP 1	9	37	43	0.10
SP 2	0	42	49	0.00
SP 3	0	41	52	0.00
SP 11	2	45	48	0.02
SP 12	4	52	37	0.04
SP 13	3	47	41	0.03
OG 1	9	31	56	0.09
OG 2	7	30	59	0.07
OG 3	7	30	58	0.07
SP 21	3	40	51	0.03
SP 22	6	48	41	0.06
SP 23	10	46	40	0.10
SP 24	4	41	48	0.04
OG 11	7	29	60	0.07
OG 12	7	28	61	0.07
SB 1	11	42	37	0.12
SB 2	9	52	29	0.10
OG 21	9	26	59	0.10
OG 22	7	27	61	0.07
OG 23	7	28	60	0.07

Thoracic ganglion	♀♀	♂♀ +♂	♂♂	P
TG 1	46	37	8	0.51
TG 11	13	58	21	0.14
TG 12	13	56	17	0.15
TG 13	44	38	9	0.48
TG 21	13	54	17	0.16
TG 22	13	44	13	0.19
TG 23	15	56	18	0.17
TG 24	45	37	11	0.49
TG 31	16	43	13	0.22
TG 32	43	33	11	0.49
TG 41	19	44	12	0.25
TG 42	20	49	18	0.23
TG 43	43	34	19	0.50

# Internal mosaicism in gynandromorphs with different courtship songs



	Defective song			Normal song			Chi-square
	♀♀	♂♀ ♀♂	♂♂	♀♀	♂♀ ♀♂	♂♂	
Brain							
SP 1	4	12	11	5	25	32	1.38
SP 2	0	14	14	0	28	35	0.24
SP 3	0	15	14	0	26	38	1.00
SP 11	0	16	13	2	29	35	1.68
SP 12	0	16	12	4	36	25	1.83
SP 13	1	15	11	2	32	30	0.29
OG 1	2	9	18	7	22	38	0.39
OG 2	2	9	18	5	21	41	0.01
OG 3	2	9	17	5	21	41	0.01
SP 21	2	11	16	1	29	35	2.02
SP 22	4	14	10	2	34	31	4.50
SP 23	4	14	11	6	32	29	0.60
SP 24	2	14	13	2	27	35	1.20
OG 11	2	9	18	5	20	42	0.02
OG 12	2	9	18	5	19	43	0.02
SB 1	6	11	10	5	31	27	3.60
SB 2	6	13	7	3	39	22	6.95*
OG 21	4	7	18	5	19	41	0.97
OG 22	3	8	18	4	19	43	0.54
OG 23	3	8	18	4	20	42	0.56
Thoracic ganglion							
TG 1	21	7	0	25	30	8	10.78**
TG 11	10	15	3	3	43	18	16.43***
TG 12	11	13	1	2	43	16	24.82***
TG 13	20	8	0	24	30	9	10.14**
TG 21	10	12	2	3	42	15	18.31***
TG 22	12	4	0	1	40	13	44.14***
TG 23	12	14	0	3	42	18	26.62***
TG 24	23	5	0	22	32	11	19.01***
TG 31	13	3	0	3	40	13	41.75***
TG 32	21	5	0	22	28	11	15.48***
TG 41	15	4	0	4	40	12	39.08***
TG 42	16	9	0	4	40	18	35.50***
TG 43	21	5	0	22	29	9	14.84***

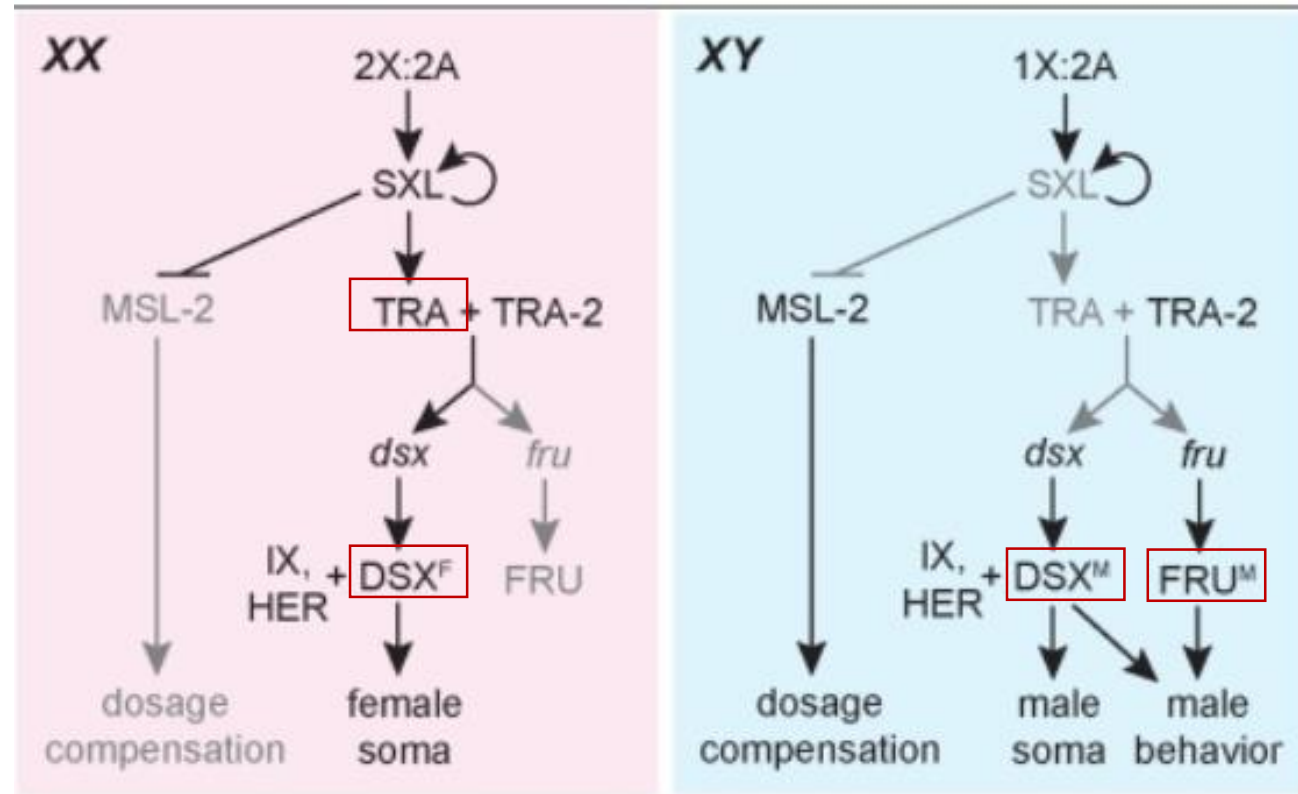
\*  $P < 0.05$   
 \*\*  $P < 0.01$   
 \*\*\*  $P < 0.001$

# Genetic dissection of courtship song

**Table II.** Genes with Effects on Courtship Song in *Drosophila melanogaster*

Locus	Traits affected <sup>a</sup>	Molecular characterization <sup>b</sup>	Chromosome
<i>period (per)</i>	K& H cycle	Transcription corepressor	X
<i>cacophony (cac)</i>	CPP, FFT, IPI, PA, sine song, PA, FFT	Voltage-gated calcium channel	X
<i>no-on transient A (nonA)</i>	CPP, FFT	RNA binding protein	X
<i>paralytic (para)</i>	IPI	Voltage-gated sodium channel	X
<i>ariadne (ari-1)</i>	no song	Ubiquitin protein ligase	X
<i>touch-insensitive-larva-B (tilB)</i>	AMP-RT, SSF	Unknown	X
<i>beethoven (btv)</i>	AMP-RT, CPP, PD, SSF	ATPase activity	2L
<i>maleless (mle)</i>	IPI, enhances <i>cac</i> IPI defects	Double stranded RNA binding domain	2R
<i>croaker</i>	CPP, IPI	Unknown	2R
<i>temperature-induced-paralytic-E (tipE)</i>	IPF, PA	Voltage-gated sodium channel	3L
<i>Cysteine string protein (Csp)</i>	CPP, IPF, PA	ATPase activity	3R
<i>transformer (tra)</i>	CPP, FFT	Pre-mRNA splicing factor	3R
<i>doublesex (dsx)</i>	IPI, sine song	Transcription factor	3R
<i>atonal (ato)</i>	AMP-RT, CPP, PL, PN	Transcription factor	3R
<i>fruitless (fru)</i>	FFT, IPI, song production	RNA polymerase II transcription factor	3R
<i>ebony (e)</i>	IPF, sine song	Beta-alanyl-dopamine synthase	3R
<i>slowpoke (slo)</i>	CPP, IPF, IPI, PA	Calcium activated potassium channel	3R

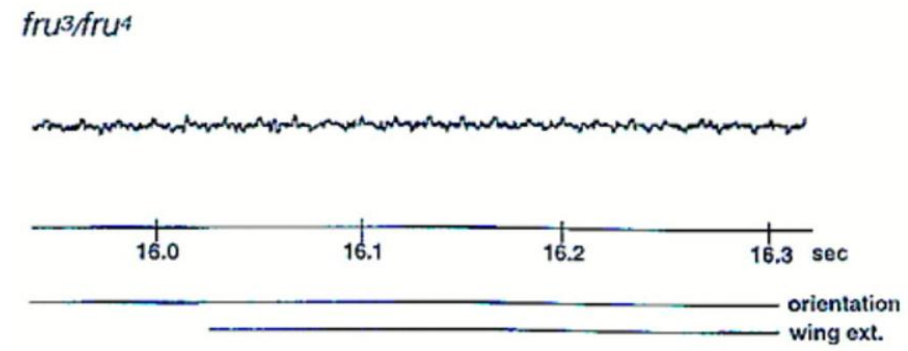
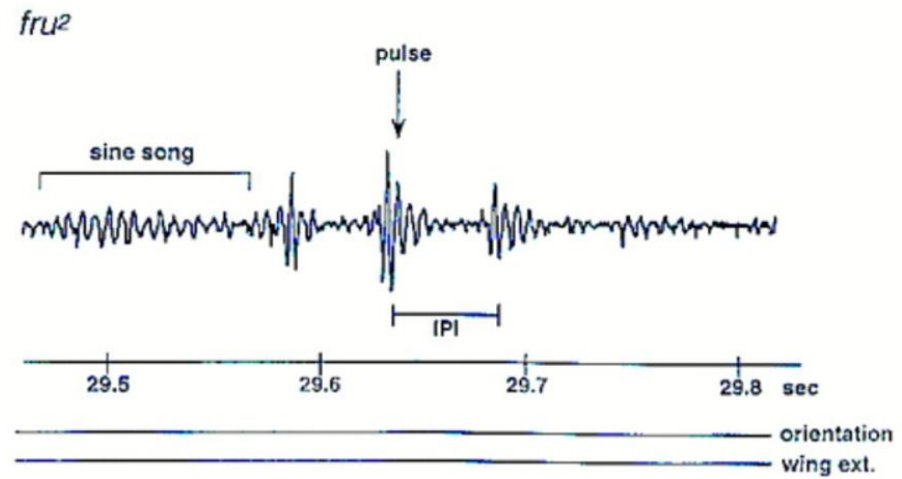
# The *Drosophila* sex hierarchy



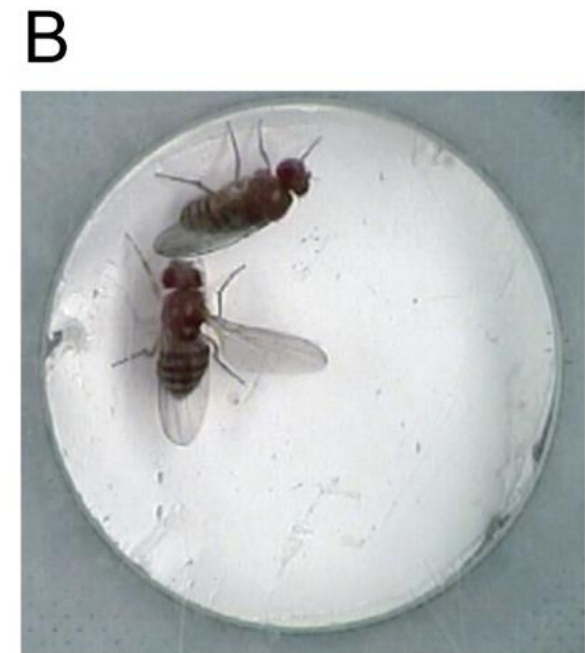
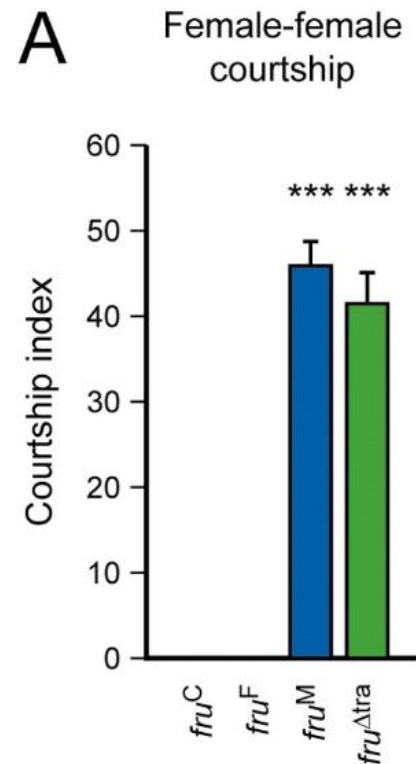
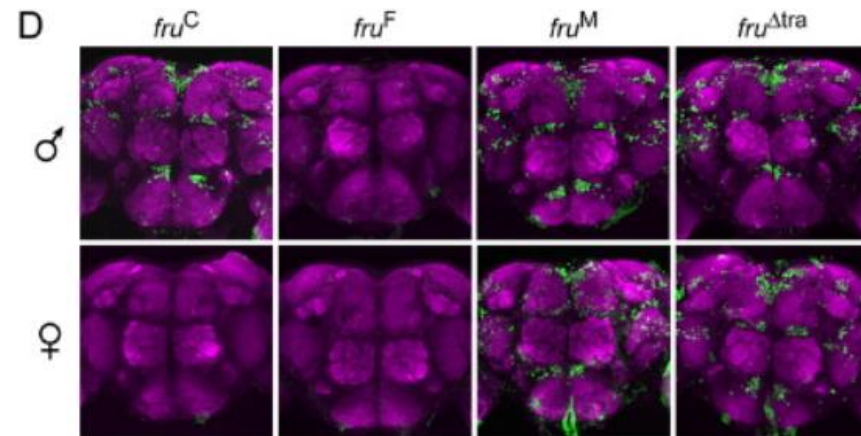
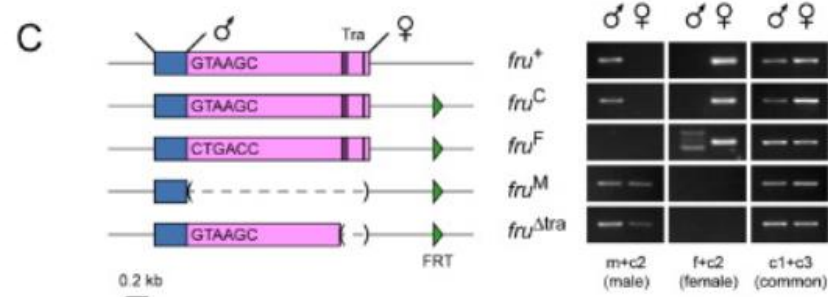
Robinett, C. C. et al. 2010



# $fru^M$ mutant males lack pulse song



# Male splicing of *fru* is sufficient for male courtship behavior



## Fru<sup>M</sup> is not sufficient for courtship song

Table S1. Song Analysis of Wild-Type and Mutant Flies.

Genotype	n	WEI	SI	SBPM	PTPM	MPPT	IPI (ms)
<i>Canton S</i> (XY)	15	38.3 ± 2.8	87.9 ± 2.4	18.6 ± 2.2	19.9 ± 1.4	8.1 ± 0.3	31.7 ± 3
XY;; <i>fru</i> <sup>M</sup> / <i>Df</i> (3R) <i>fru</i> <sup>4-40</sup>	15	53.0 ± 2.9	90.3 ± 2.0	23.1 ± 1.9	28.5 ± 2.3	9.1 ± 0.3	32.0 ± 3
XY;; <i>fru</i> <sup>Δtra</sup> / <i>Df</i> (3R) <i>fru</i> <sup>4-40</sup>	18	54.6 ± 2.8	85.3 ± 2.2	20.6 ± 1.8	29.3 ± 1.8	9.6 ± 0.4	31.7 ± 4
XX;; <i>fru</i> <sup>M</sup> / <i>Df</i> (3R) <i>fru</i> <sup>4-40</sup>	16	37.6 ± 3.3	44.8 ± 4.7*	0*	7.1 ± 1.5*	3.0 ± 0.1*	26.5 ± 7*
XX;; <i>fru</i> <sup>Δtra</sup> / <i>Df</i> (3R) <i>fru</i> <sup>4-40</sup>	13	31.4 ± 2.9	60.1 ± 5.6*	0*	9.4 ± 1.4*	2.9 ± 0.1*	24.3 ± 5*
XX;; <i>tra</i> <sup>1</sup> / <i>Df</i> (3L) <i>st-J7</i>	10	46.3 ± 4.5	89.7 ± 2.9	23.2 ± 1.8	19.2 ± 1.4	9.0 ± 0.2	33.0 ± 3
XY;; <i>ln</i> (3R) <i>dsx</i> <sup>23</sup> , <i>fru</i> <sup>3</sup> / <i>Df</i> (3R) <i>dsx</i> <sup>15</sup> , <i>fru</i> <sup>3</sup>	11	0*	0*	N.D.	N.D.	N.D.	N.D.

WEI: wing extension index

SI: song index

SBPM: sine bouts per minute

PTPM: pulse trains per minute

MPPT: mean pulses per train

IPI: interpulse interval

*dsx* mutant males has abnormal courtship song

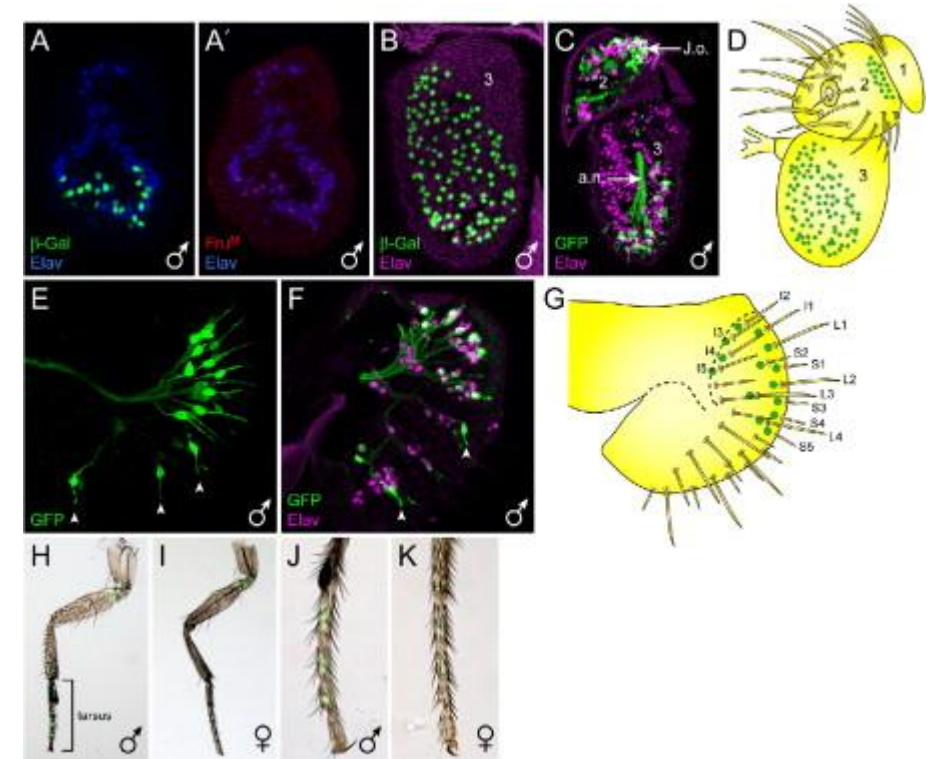
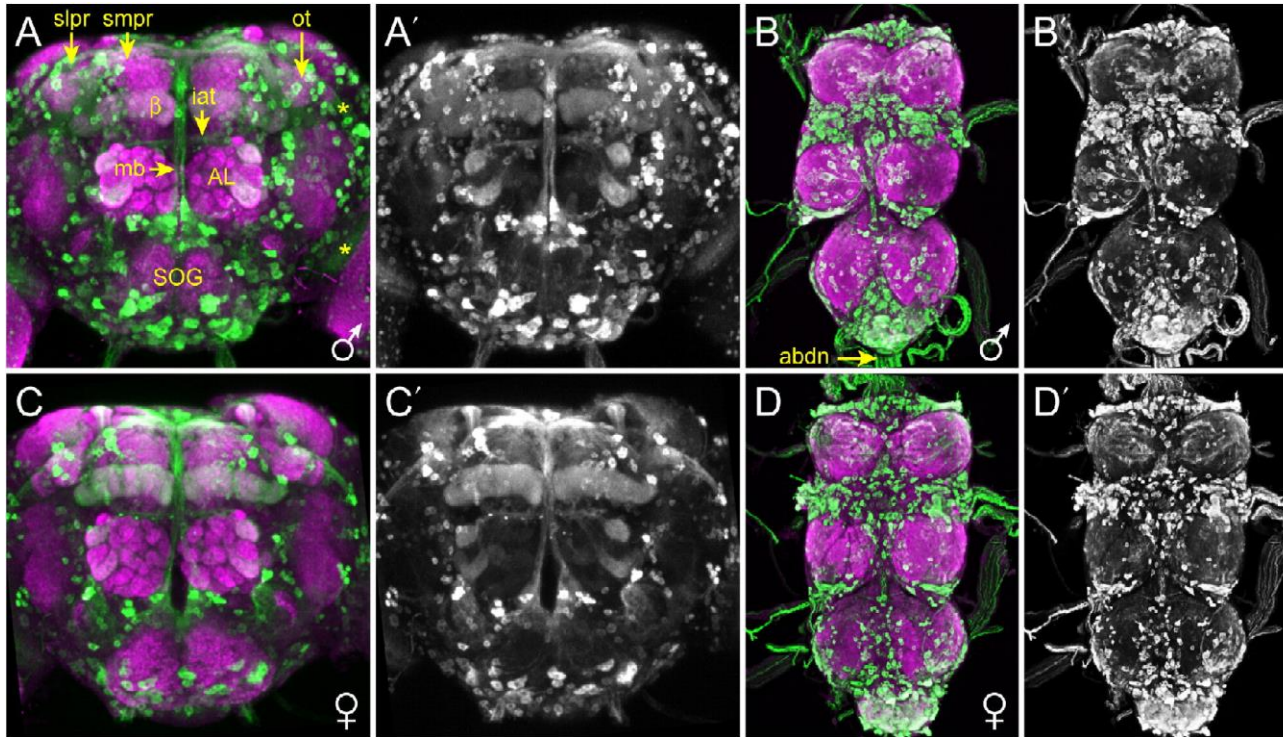
**TABLE 1. Courtship Songs of *dsx*<sup>-</sup> Males\***

	<i>dsx</i> <sup>-</sup> (n = 4)	Controls (n) (wild-type/ <i>/B<sup>S</sup></i> )
Basic song performance		
Trains/min	10 ± 3	26 ± 3 (3)/ /14 ± 3 (4)
Pulses/min	138 ± 43	338 ± 20 (3)/ /210 ± 46 (4)
Train length (no. pulses)	11.8 ± 0.3	10.3 ± 0.3 (3)/ /11.6 ± 0.5 (4)
Song-pulse parameters		
Interpulse interval (ms)	38 ± 1	35 ± 1 (5) <sup>a</sup>
Cycles/pulse	2.48 ± 0.5	3.13 ± 0.4 (13) <sup>b</sup>
Intrapulse frequency(Hz)	223 ± 8	222 ± 13 (5) <sup>a</sup>
No. FFT peaks	1.6 ± 0.2	1.1 ± 0.0 (6) <sup>c</sup>
FFT peak width (Hz)	121 ± 12	174 ± 6 (13) <sup>b</sup>

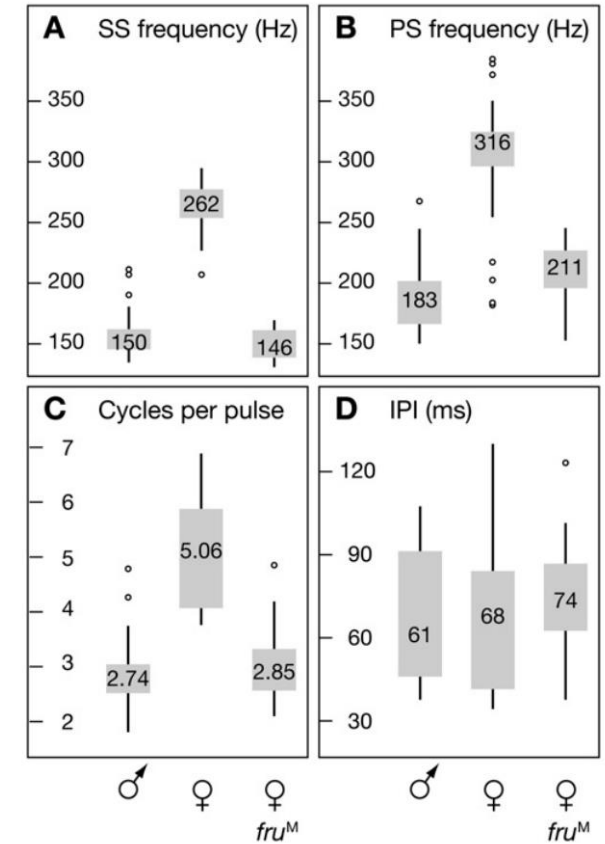
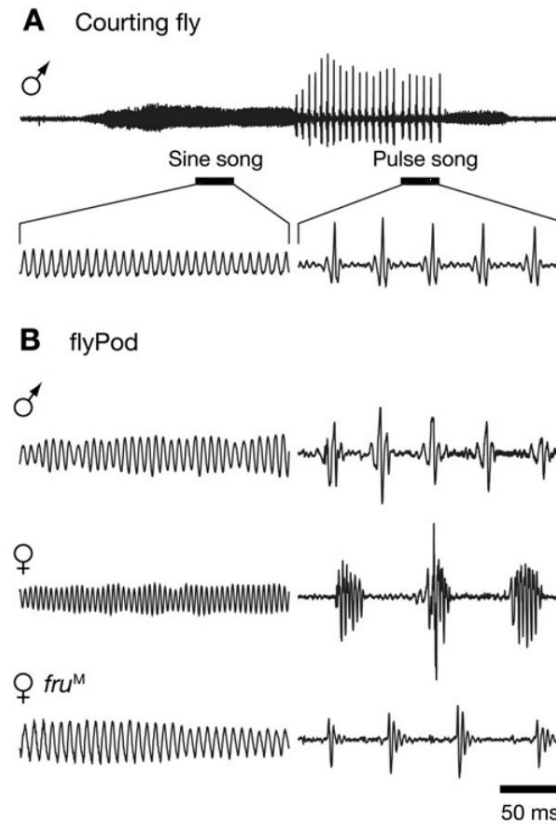
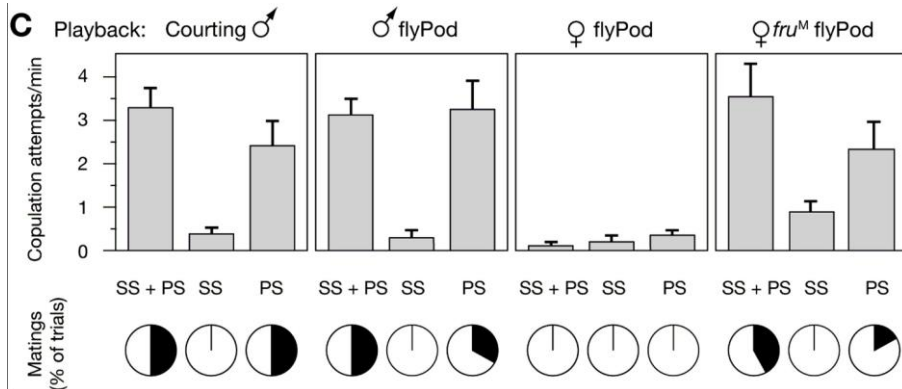
# Neuronal control of courtship song



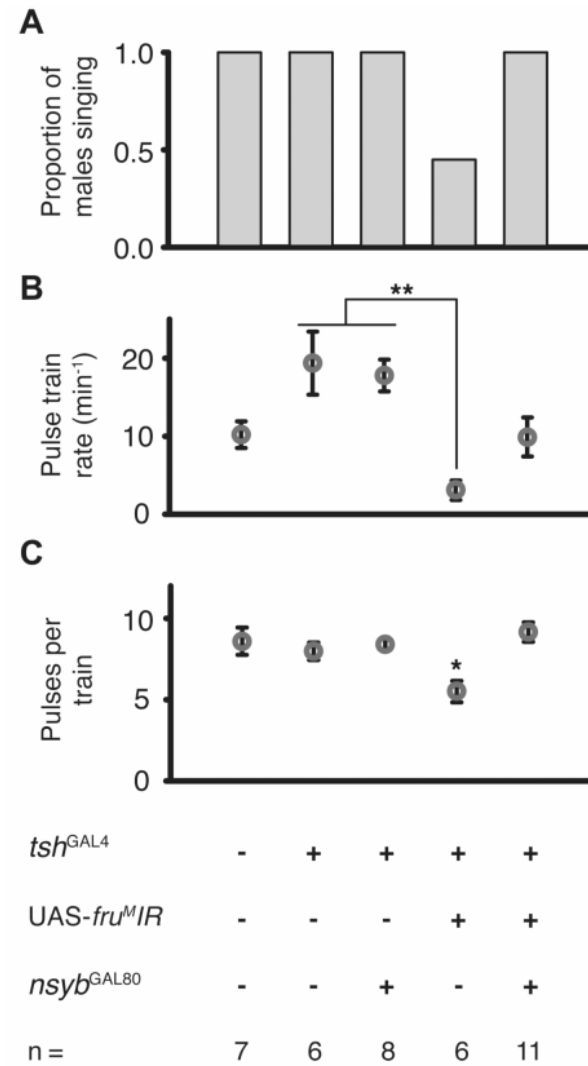
# fruGAL4 projections in the CNS and PNS



# The light-activation of *fru* neurons in beheaded flies generate courtship song

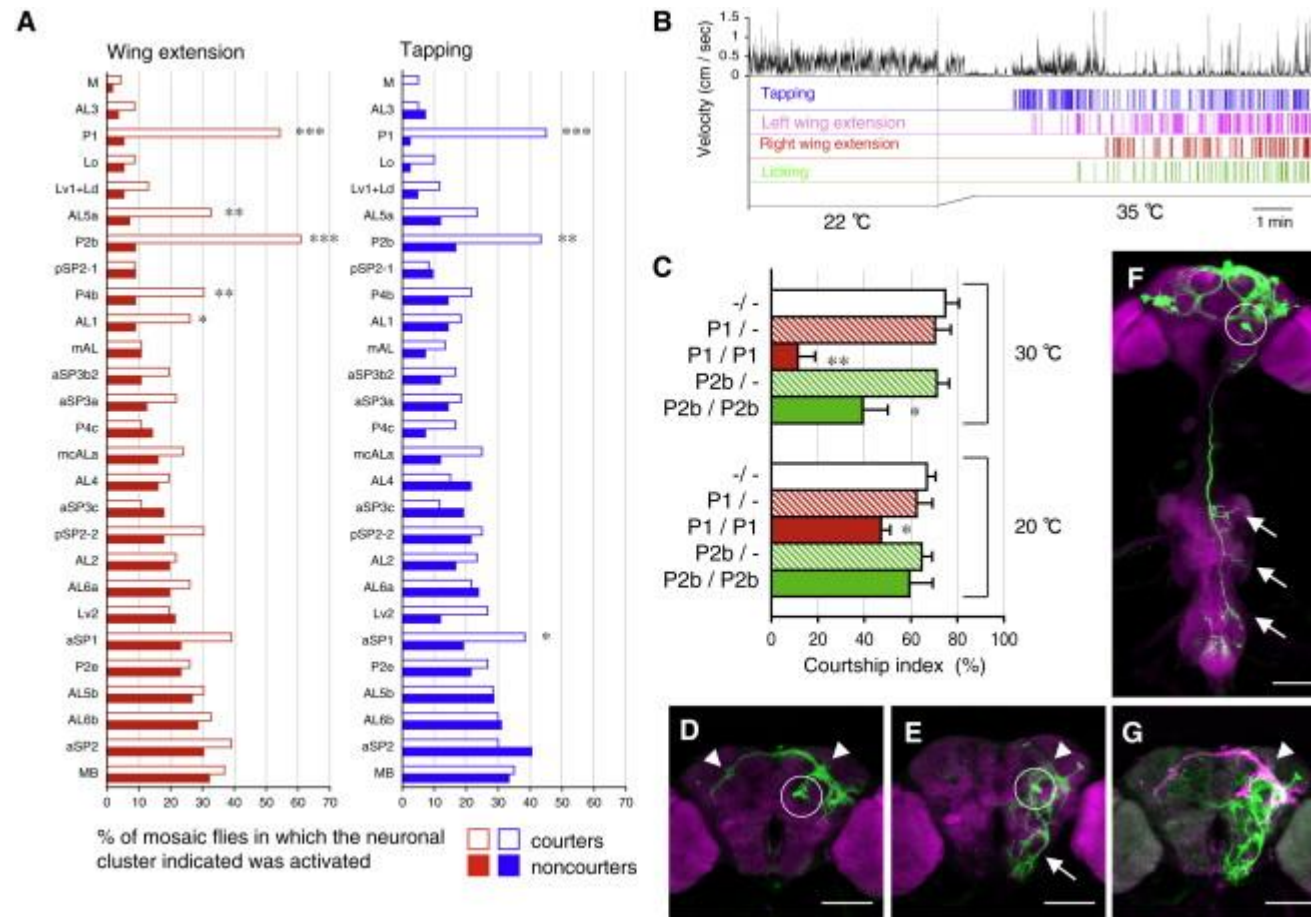


# Expression of fru<sup>MIR</sup> in tsh-specific pattern reduces amount of courtship song

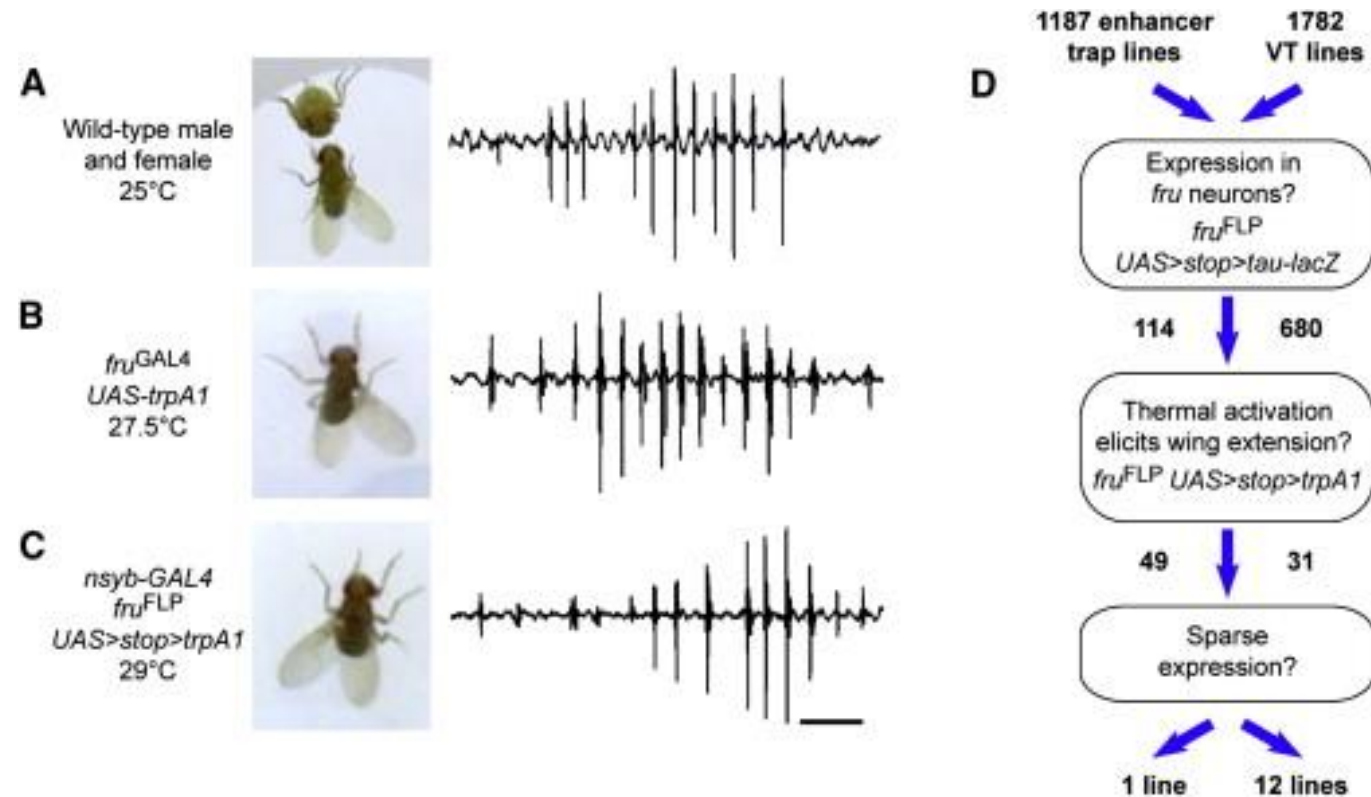


# Identification of neurons, the activation of which is correlated with the generation of courtship

*y hs-flp; FRTG13 tub-Gal80/FRTG13 UAS-mCD8::GFP; fru<sup>NP21</sup>/UAS-dTrpA1*

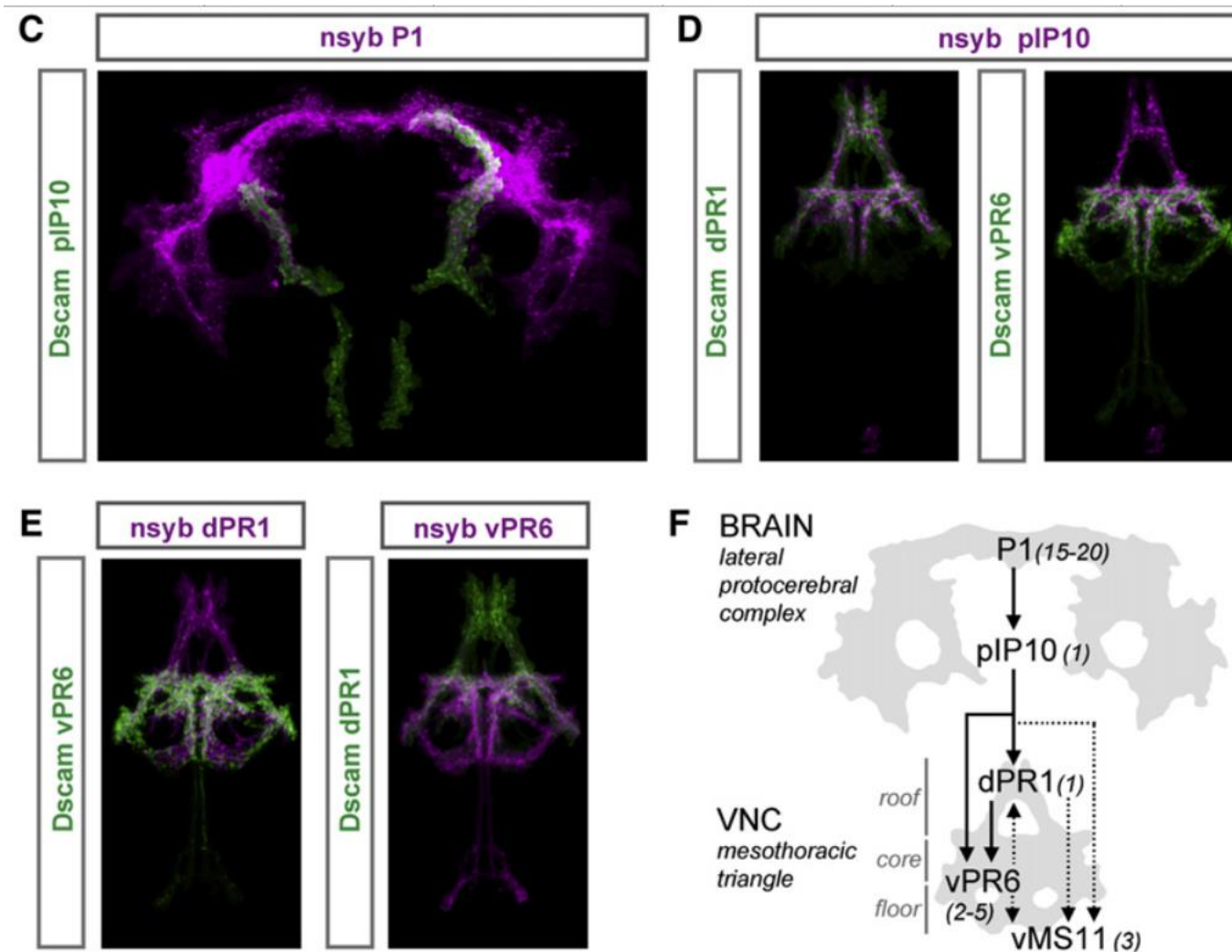


# Thermal activation of fru neurons with TrpA1 evokes courtship song

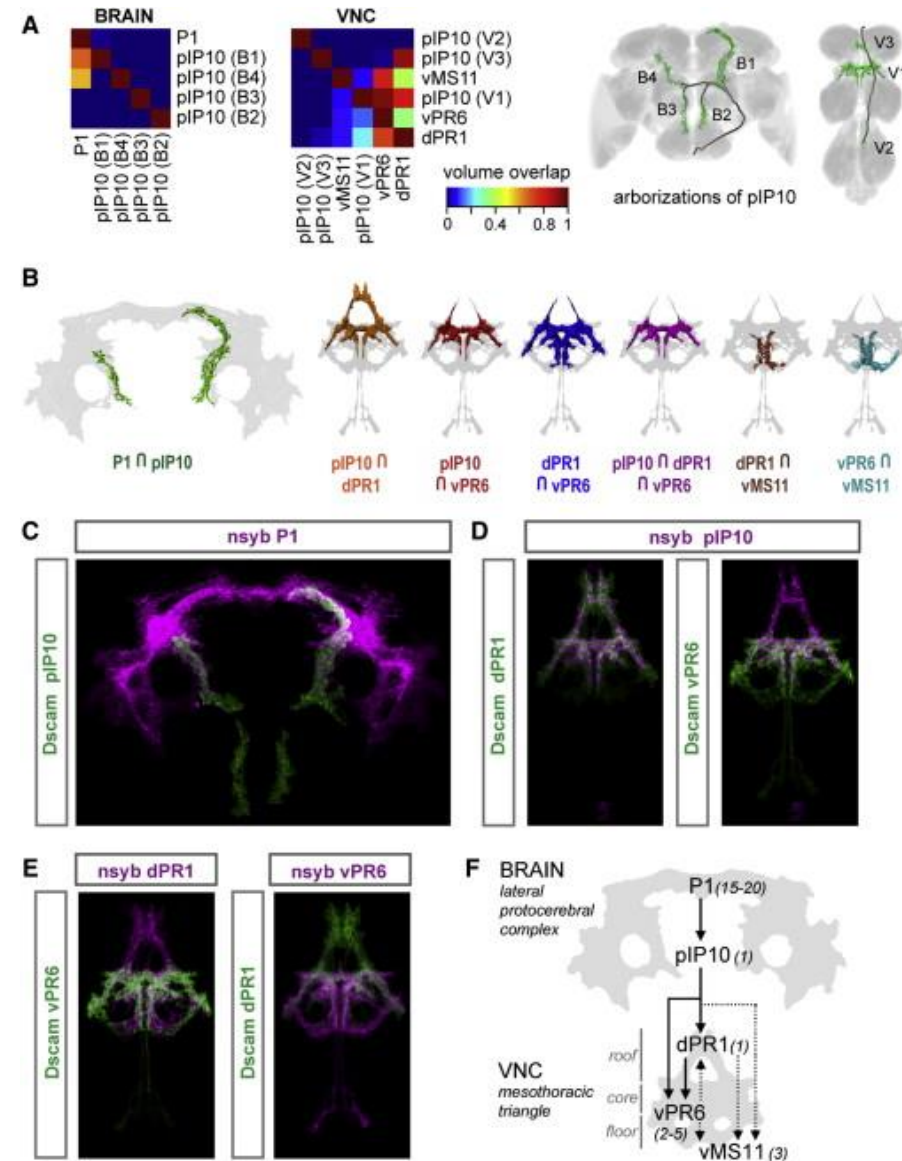




# Neuronal control of *Drosophila* courtship song



# A putative neuronal circuit for pulse song



Triggers Pulse Song

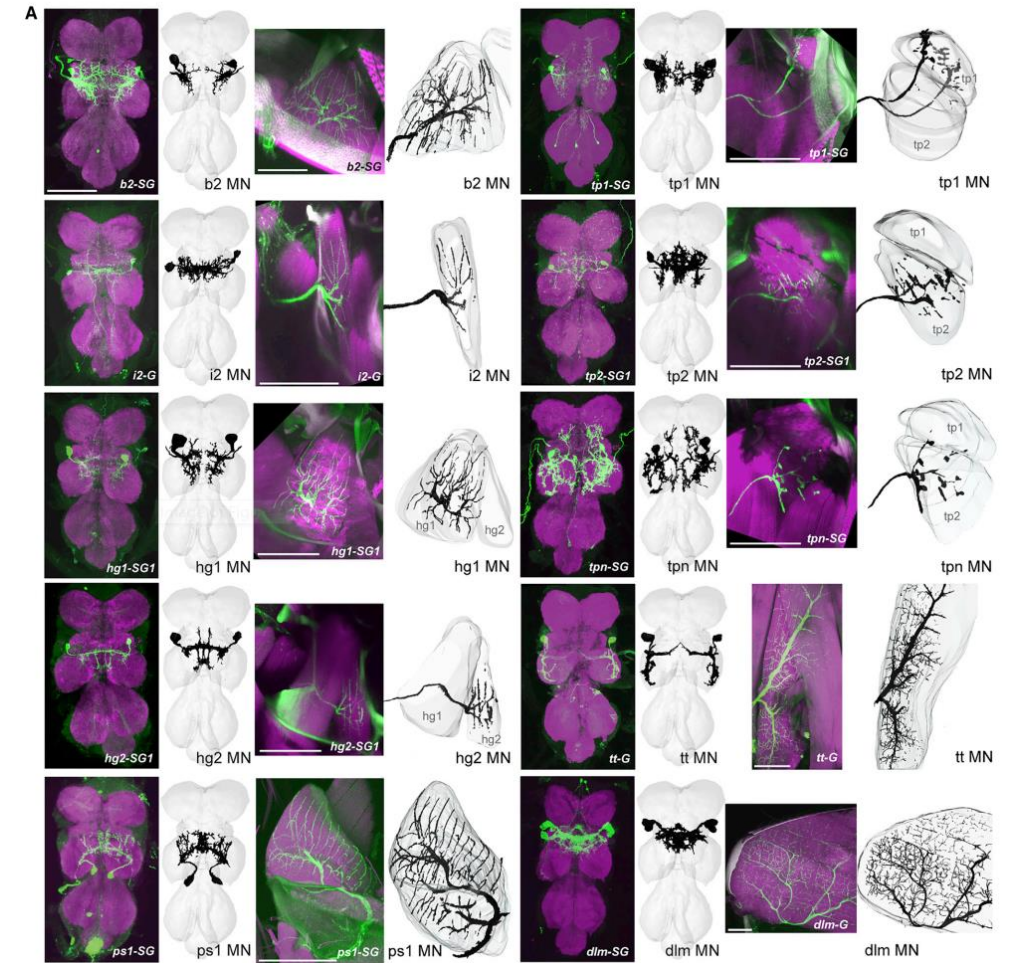
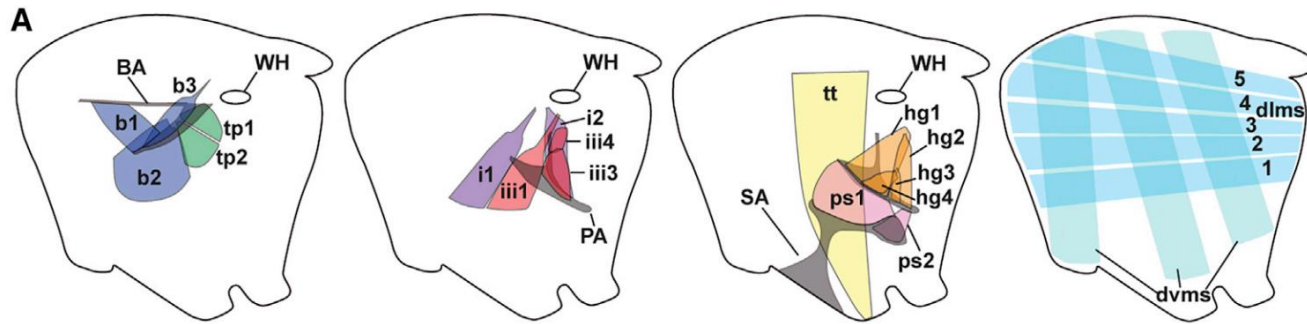
A Descending Command Neuron for Pulse Song

A Prothoracic Song Neuron

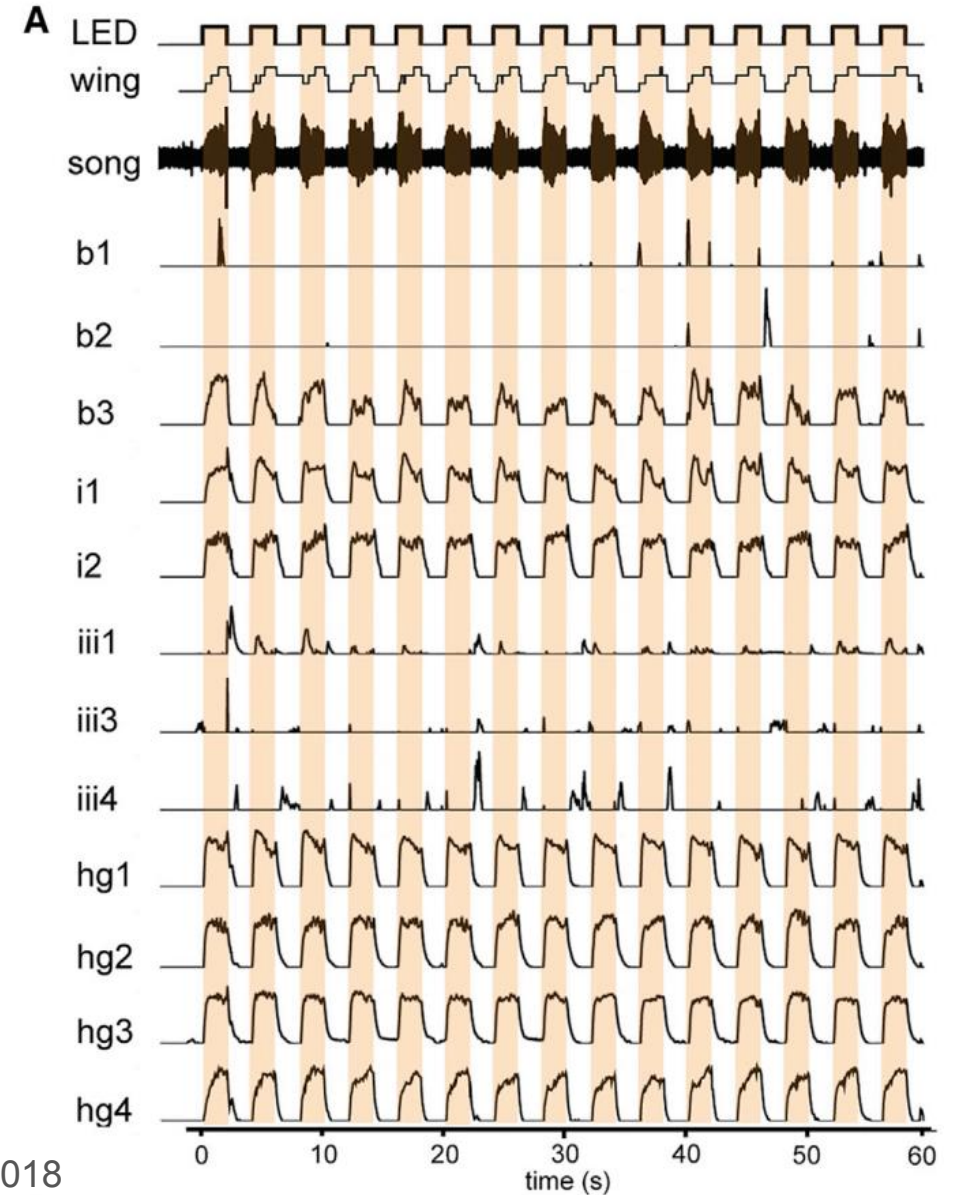
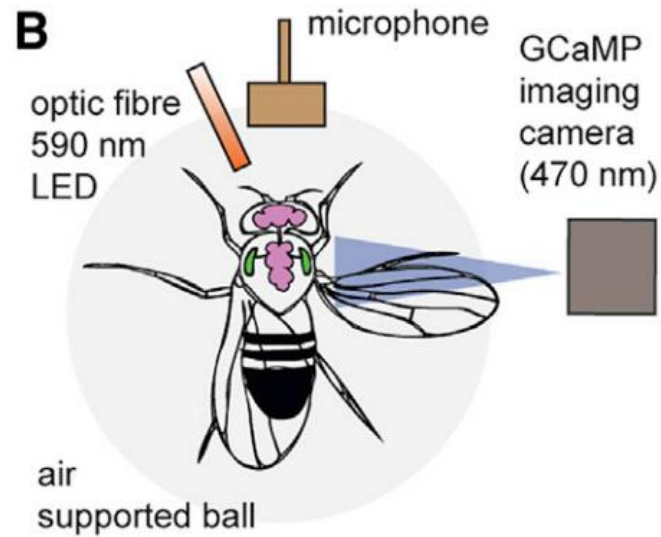
A Thoracic Neuron that Influences the IPI  
A Mesothoracic Neuron that Controls Wing Extension

Motor control of courtship song

# An Anatomical Map of Motor Neurons Innervating Wing Muscles

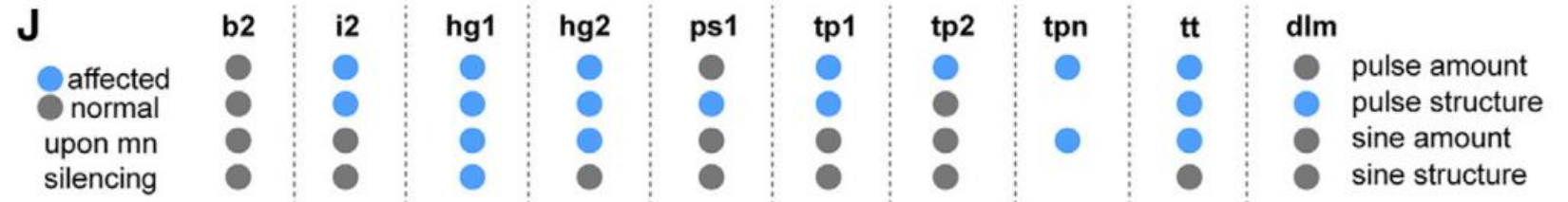
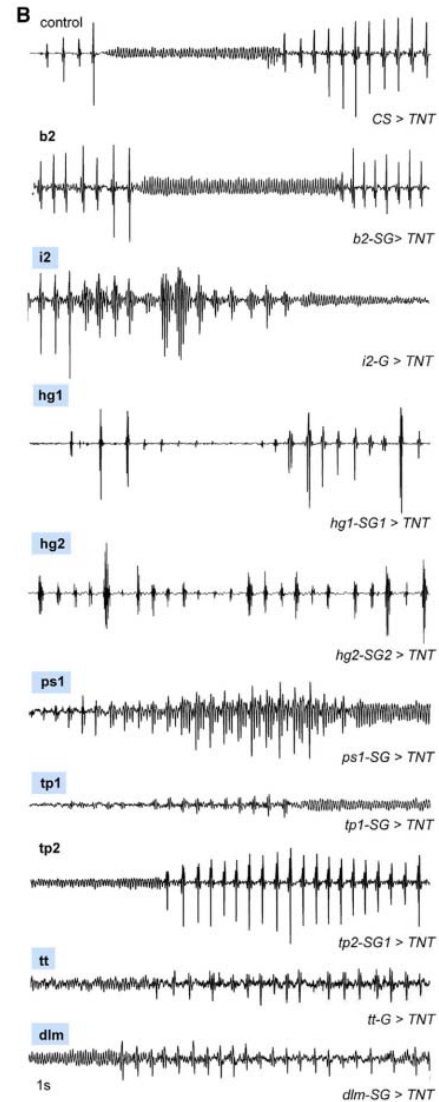


# Imaging the motor neurons while singing induced by optogenetic activation of P1P10

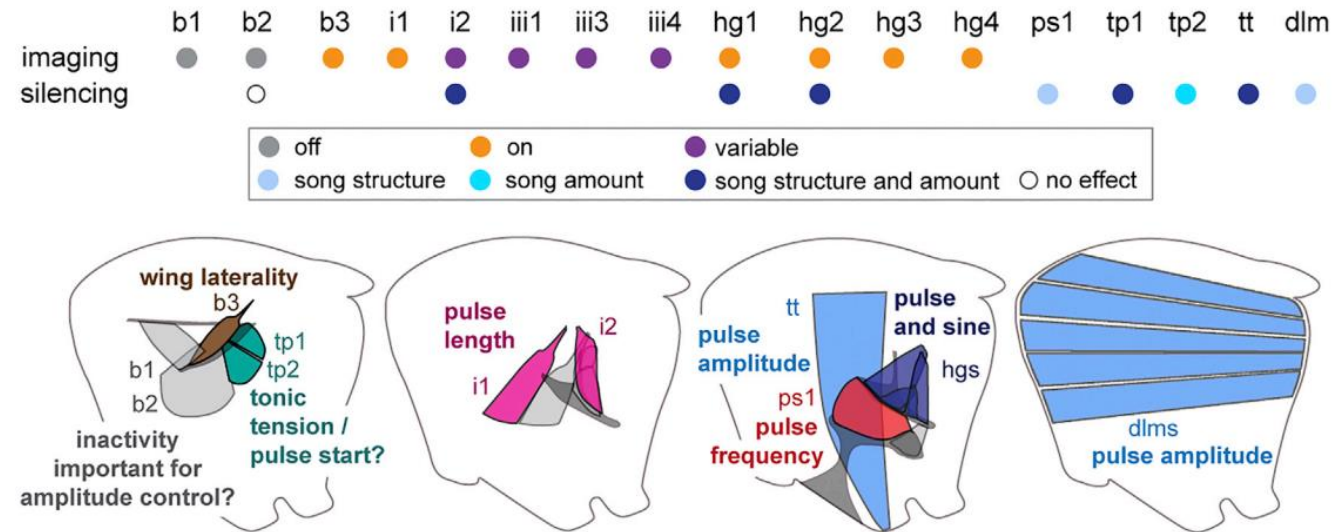




# Wing motor neurons differentially pattern courtship song

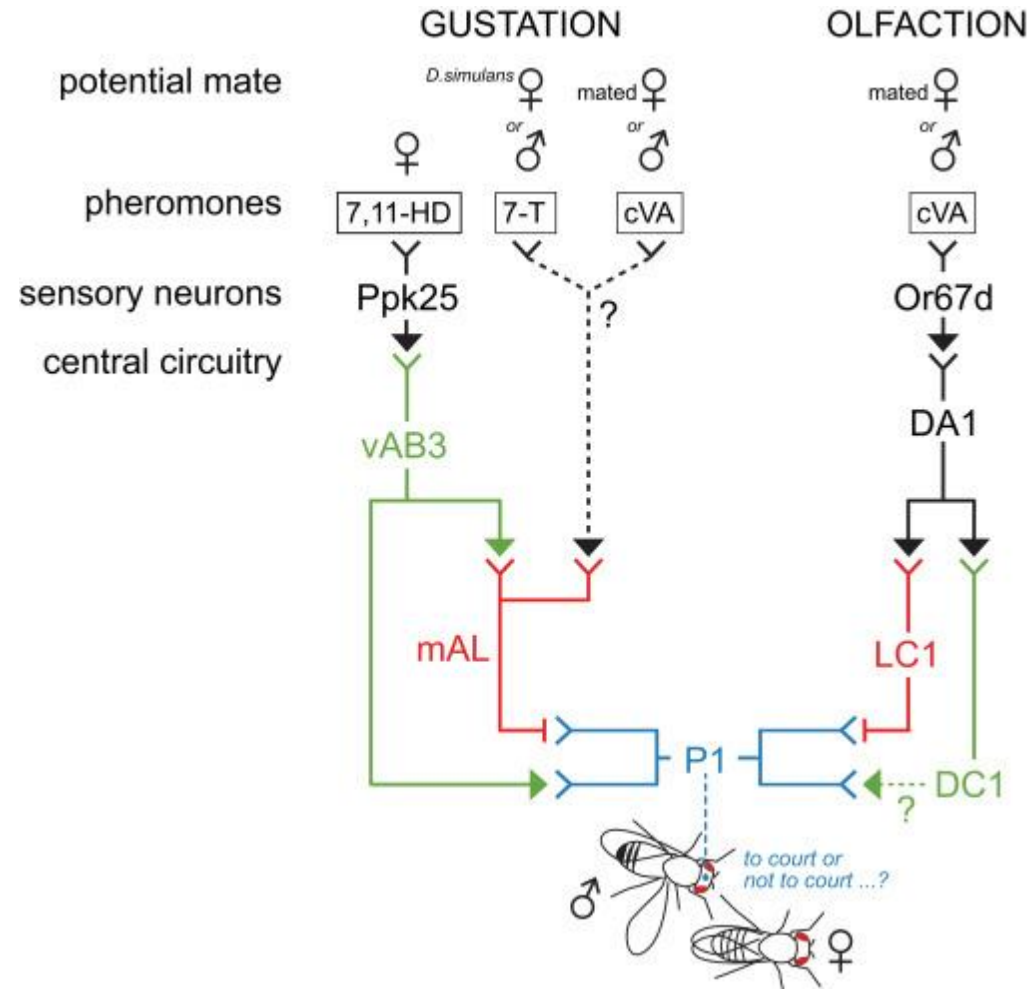


# Modes of wing motor control for song

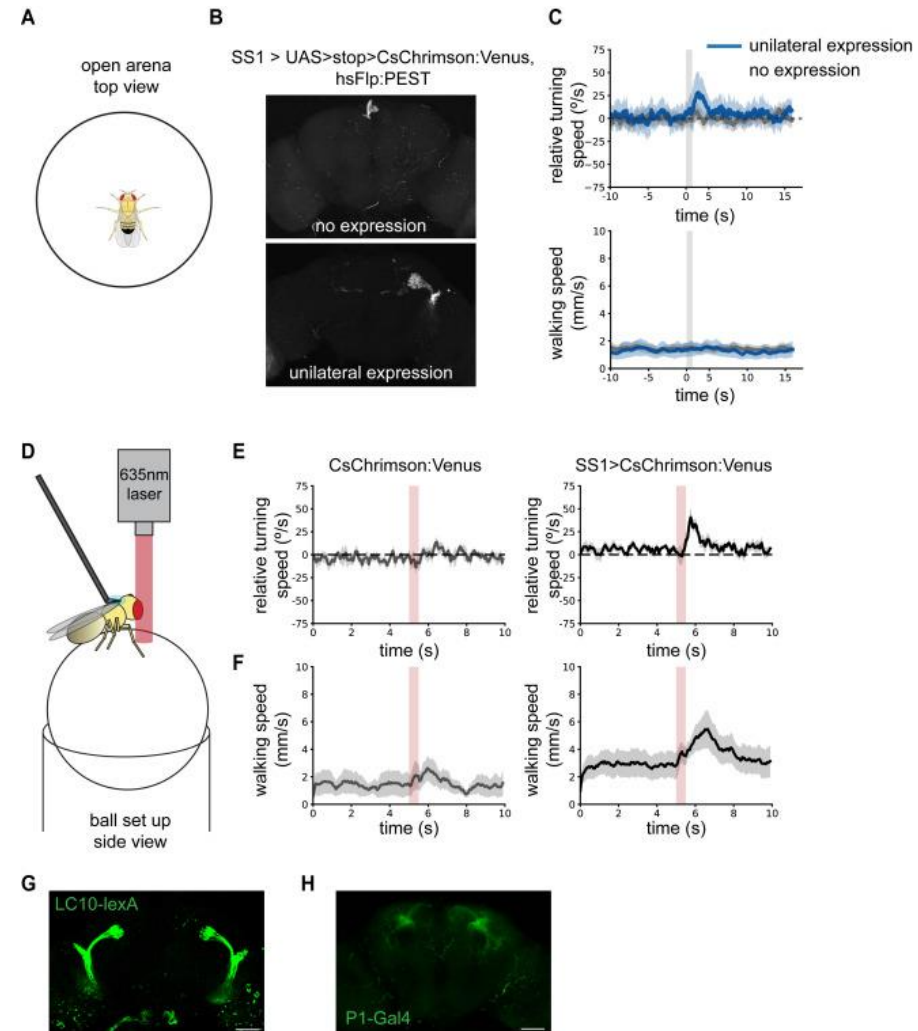


# Sensory modulation of courtship song

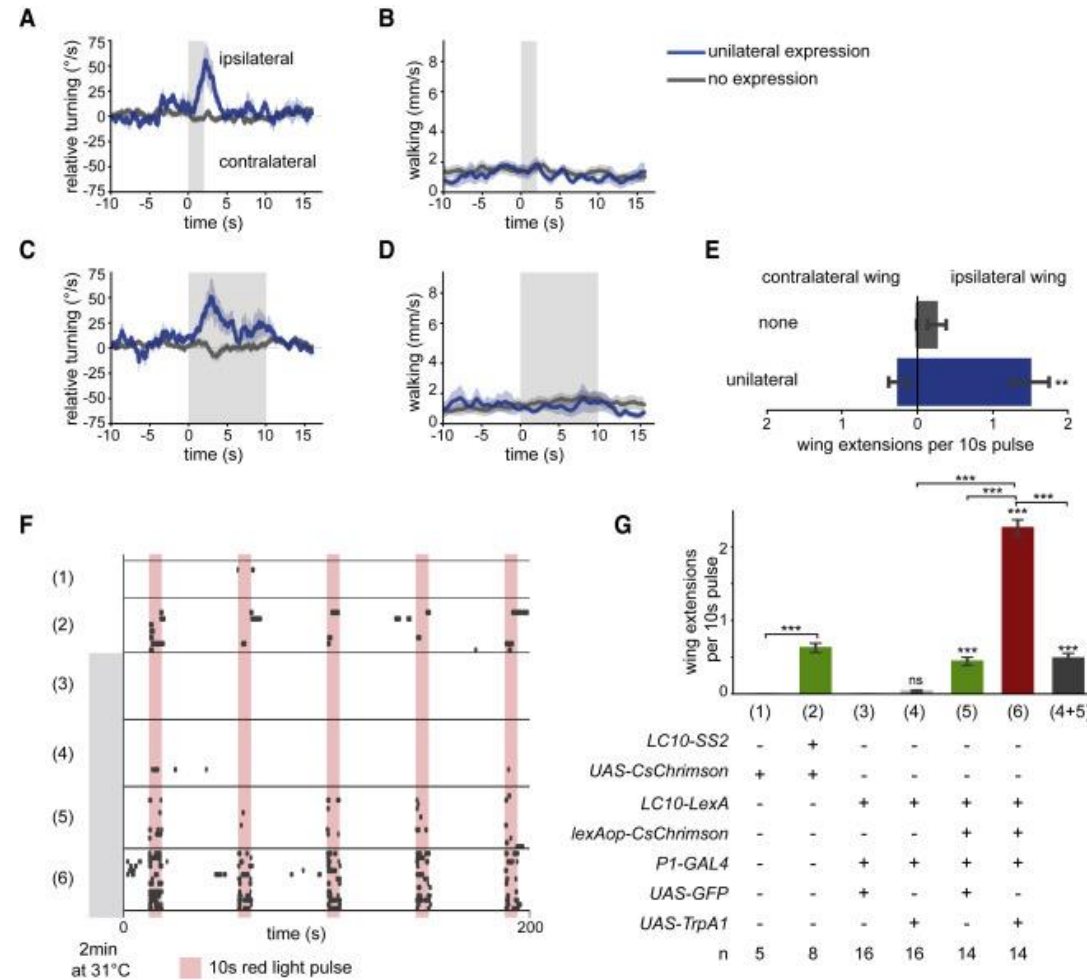
the gustatory and olfactory pathways in male *Drosophila melanogaster* controlling the decision to court



# Unilateral activation of LC10 elicits ipsilateral turning

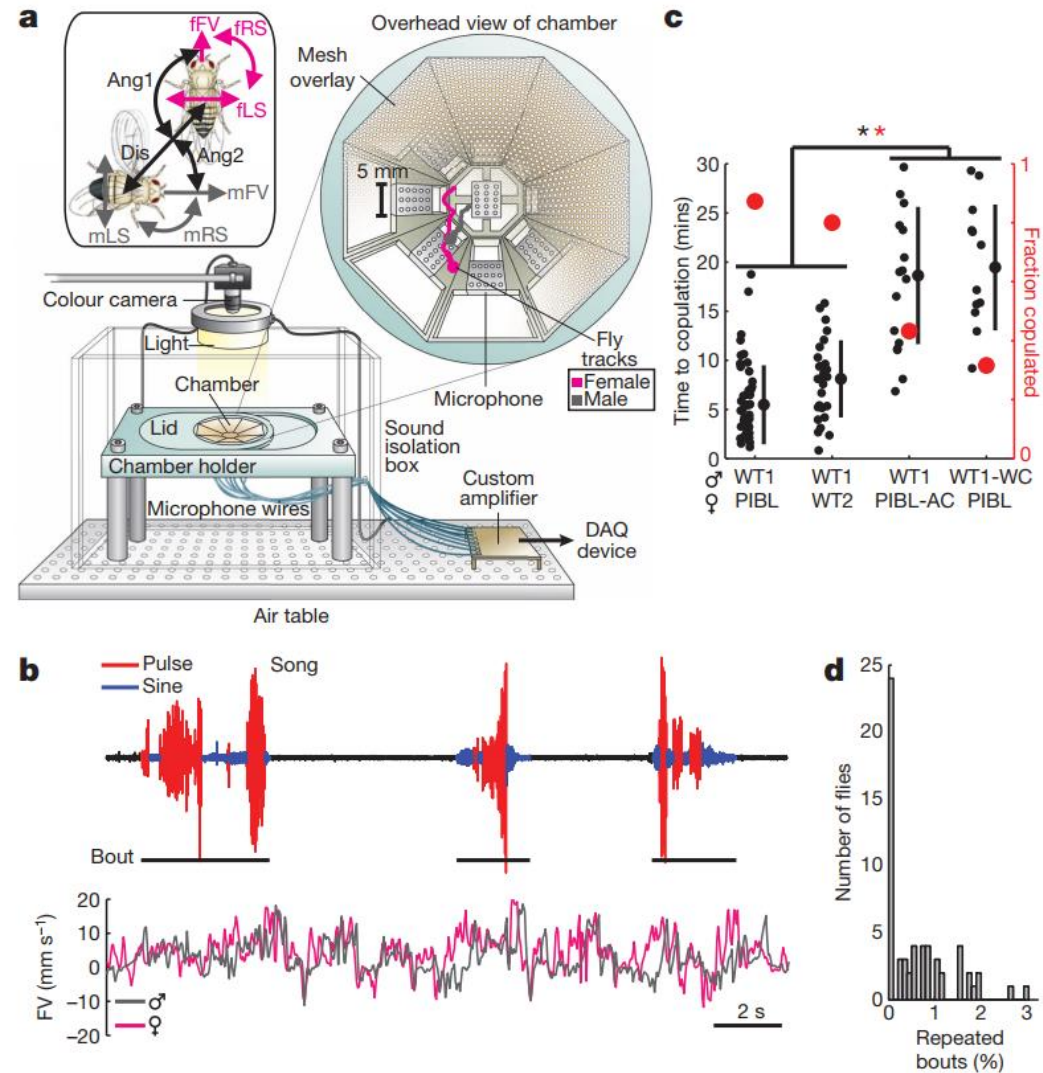


LC10 activation elicits ipsilateral turning and short wing extensions that are potentiated by pre-activation of P1 neurons

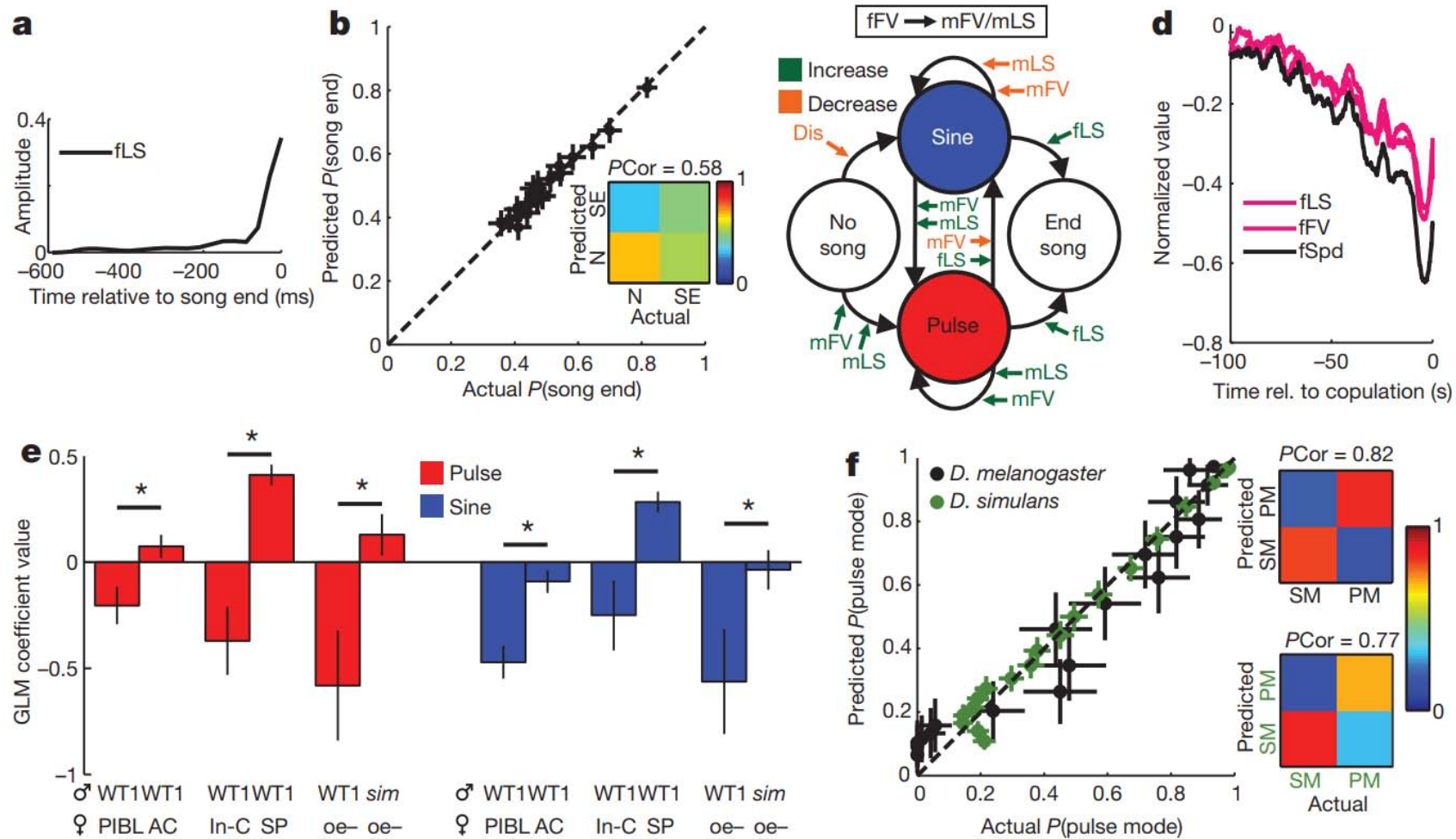




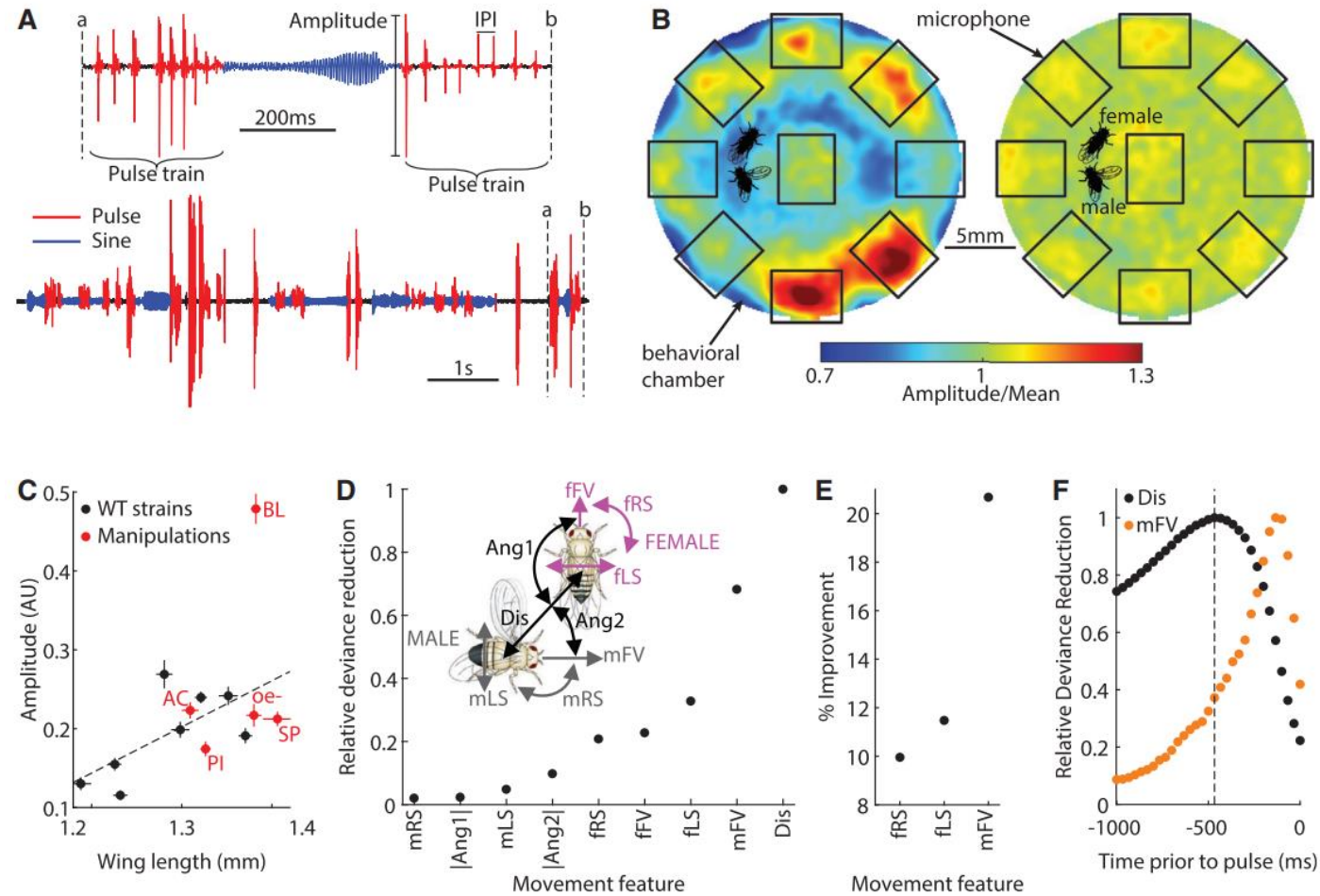
# A novel assay to study *Drosophila* song behavior



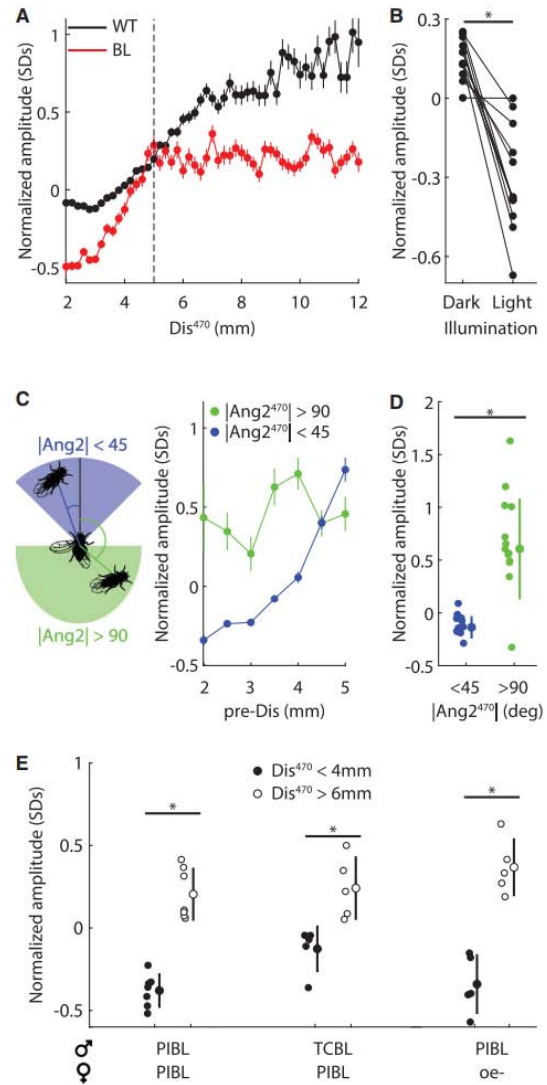
# Song patterning decisions and female responses



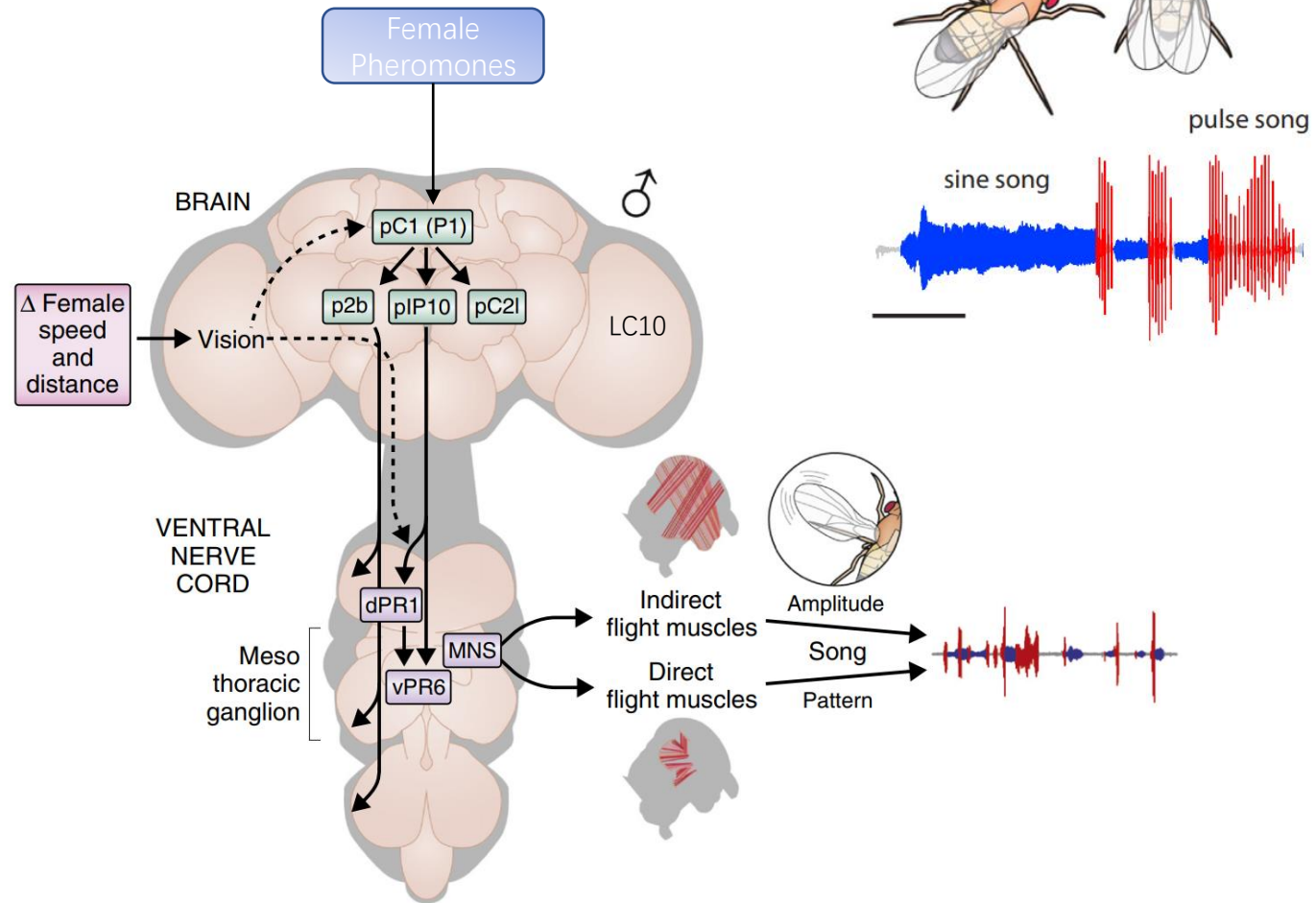
# Song amplitude modulation with distance in *Drosophila*



# The role of vision in AMD

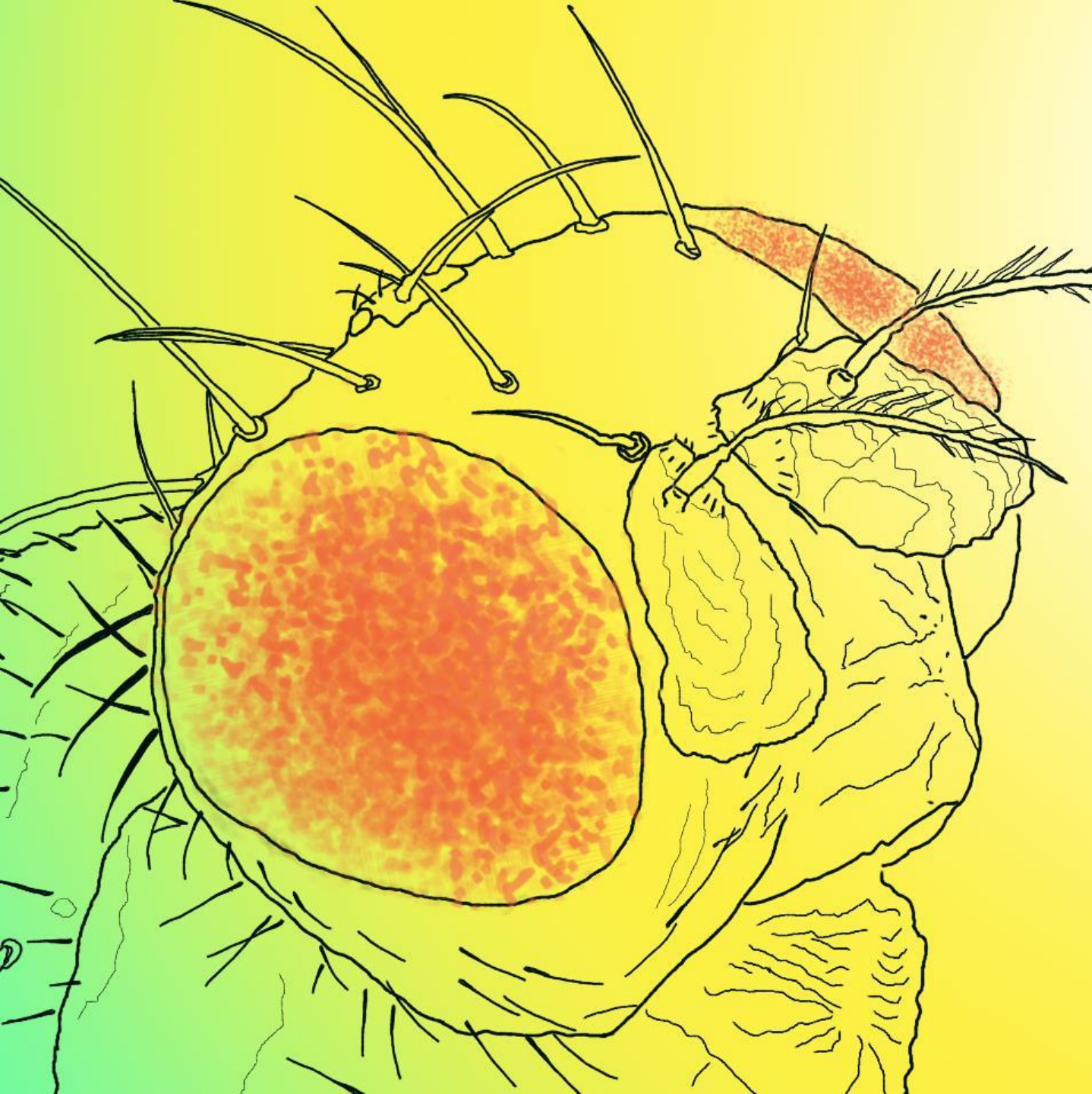


# Conclusion



Adapted from Coen, P. et al. 2016





Welcome to part III

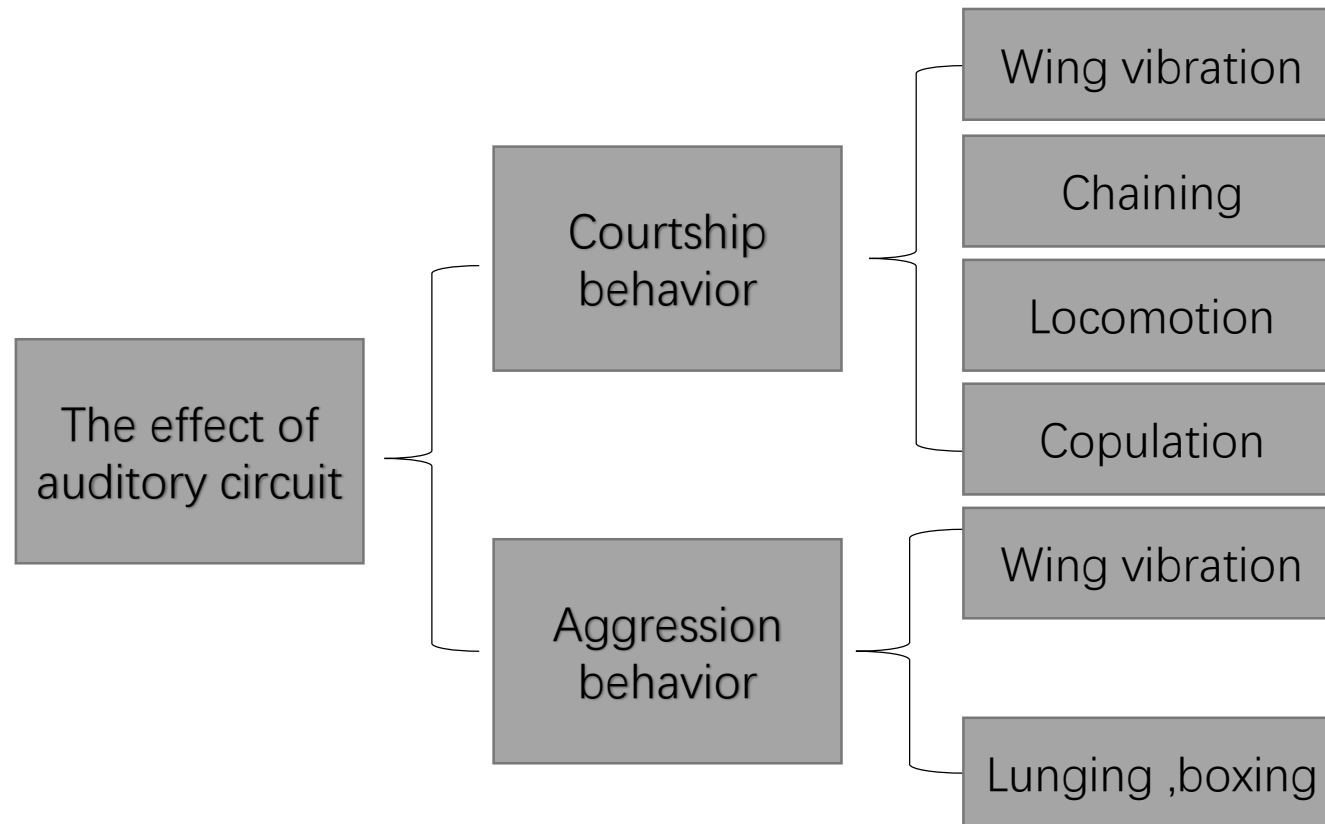
# The effect of auditory system on courtship behavior & other behaviors

*Jia Yi Cong*  
賈懿聰

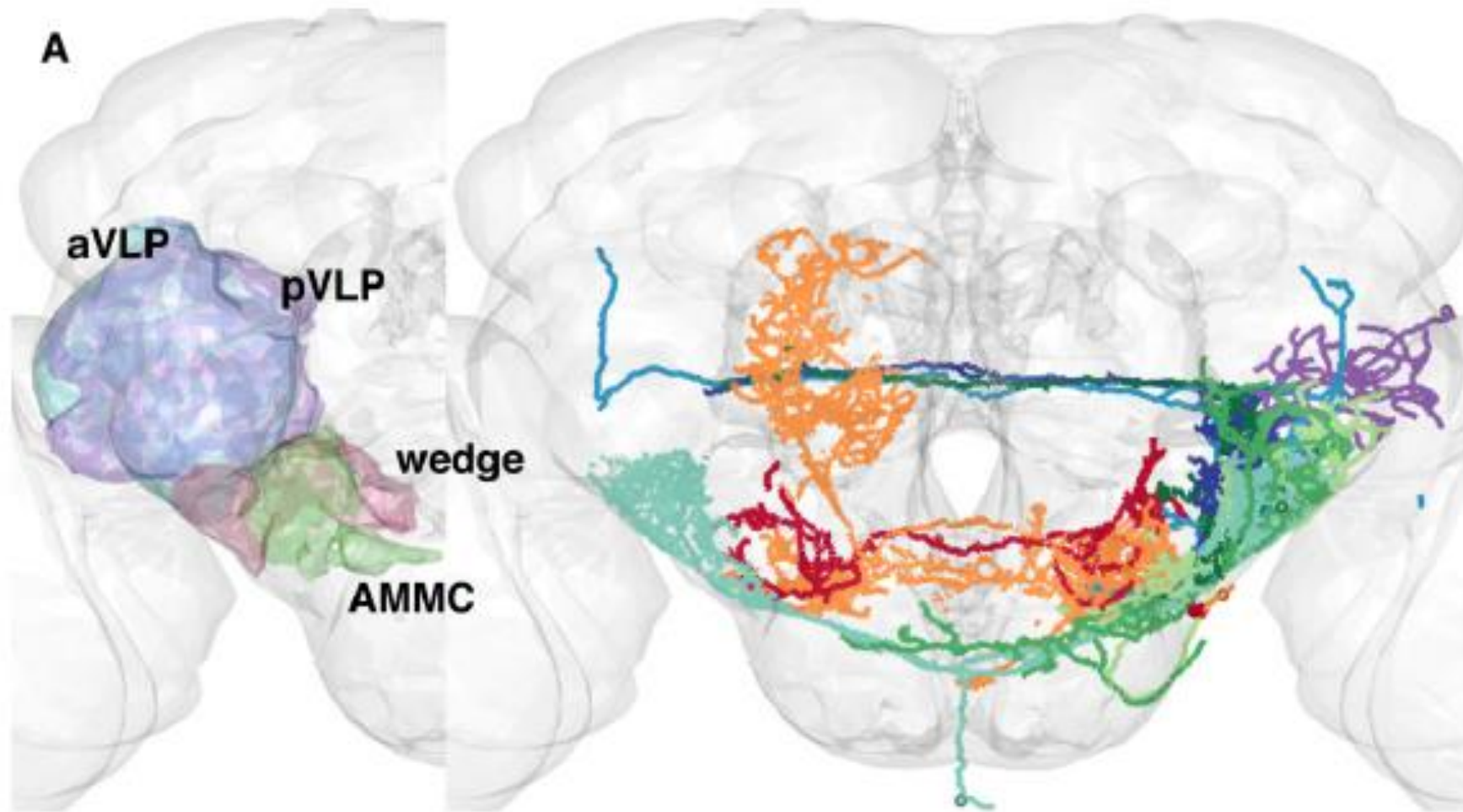


# Outline

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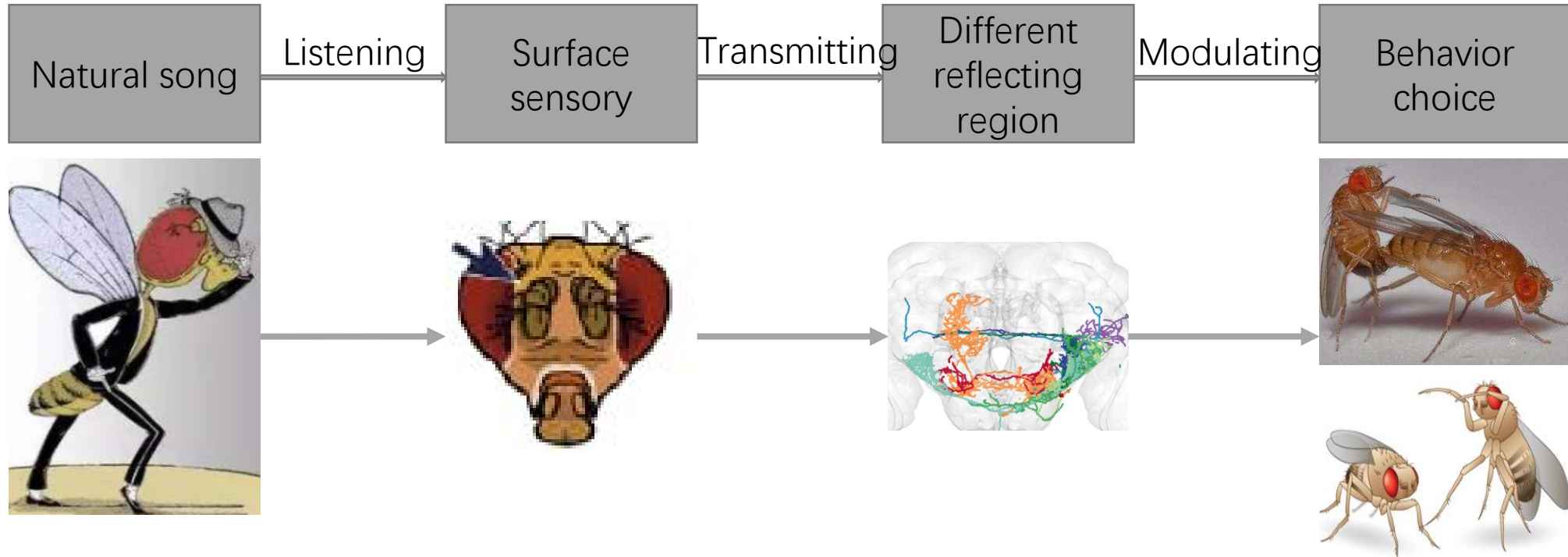


## The auditory reflex region in brain





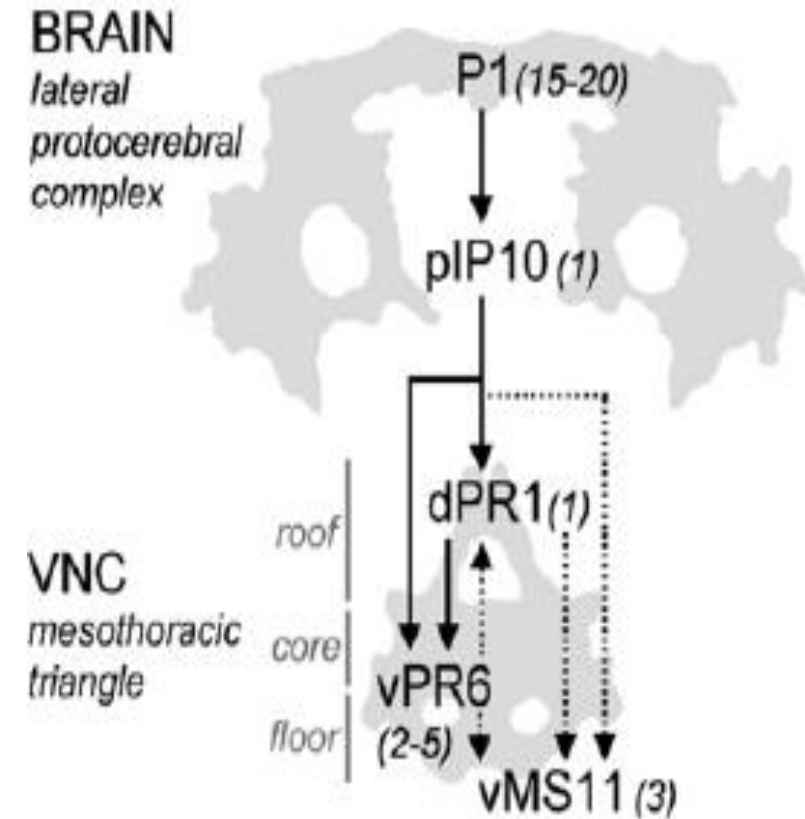
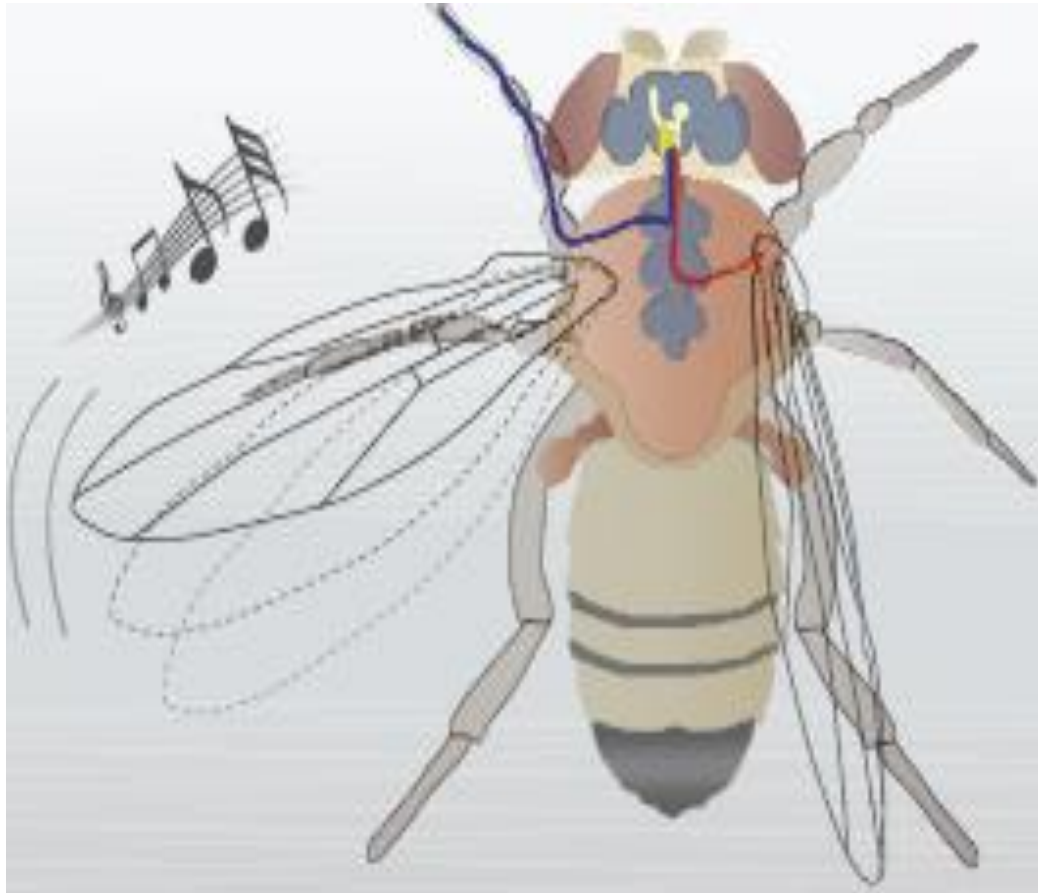
# The model of song response





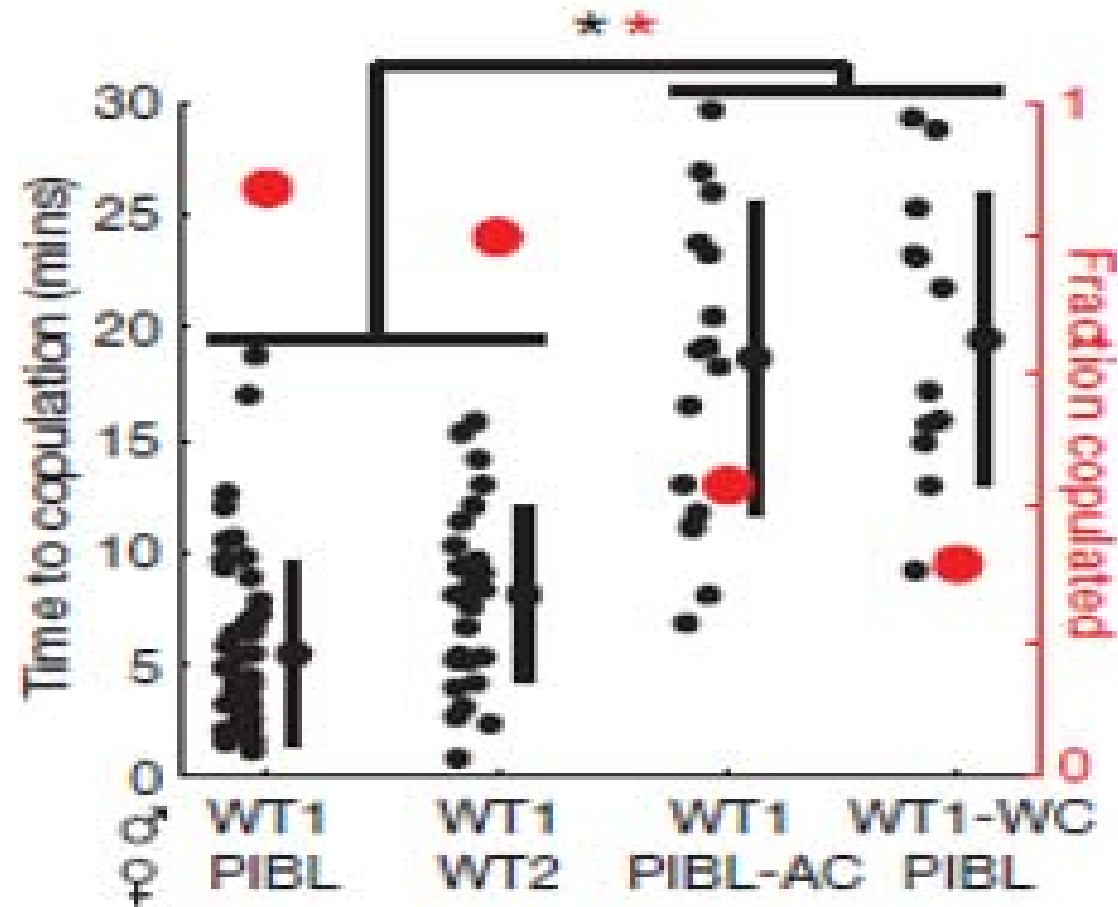
The effect of auditory system in courtship behavior

# Pheromone input received at the foreleg in the courting male shapes courtship song pattern

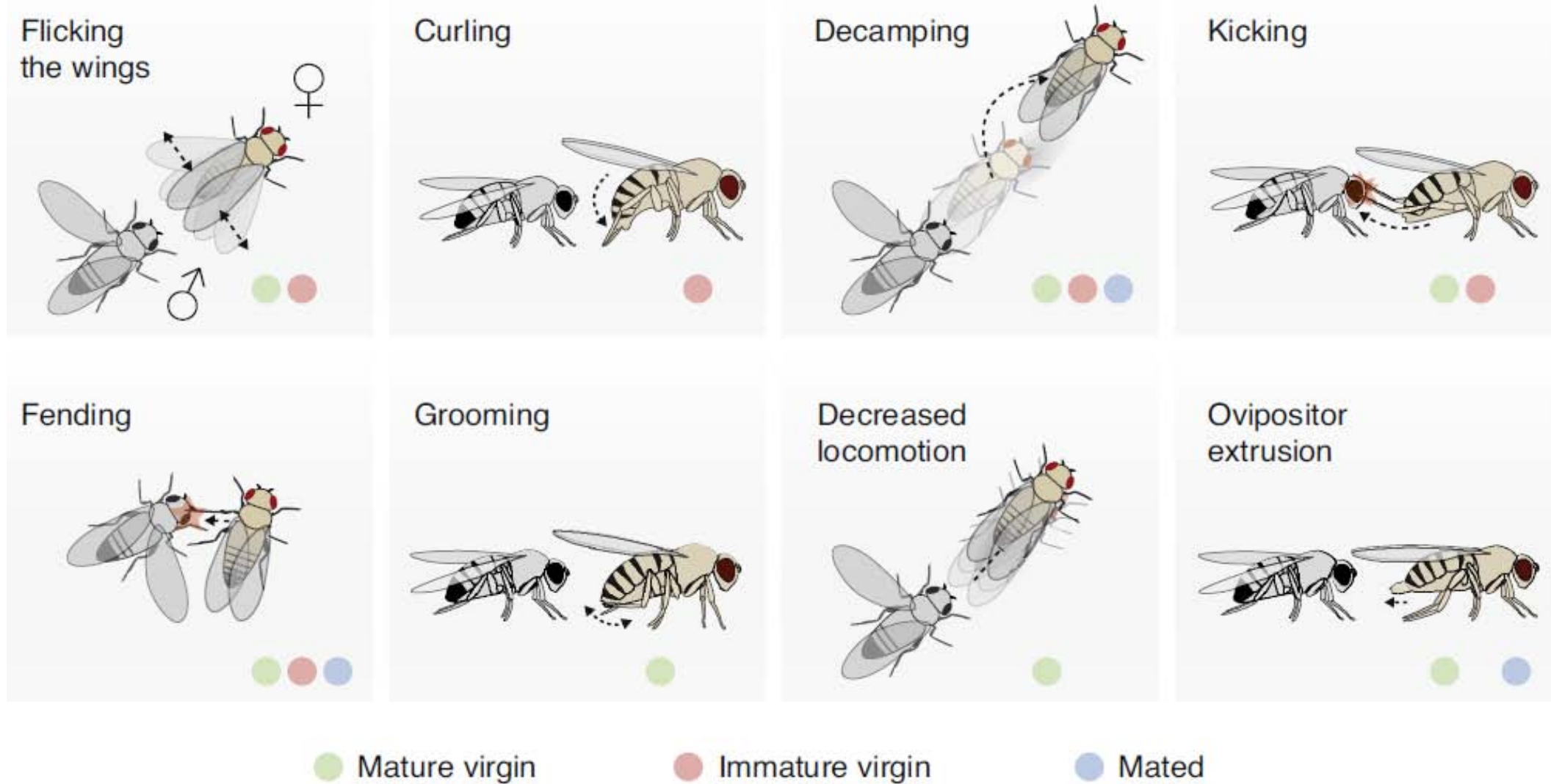


The effect of auditory system in female receptivity

## Courtship song is important for copulation



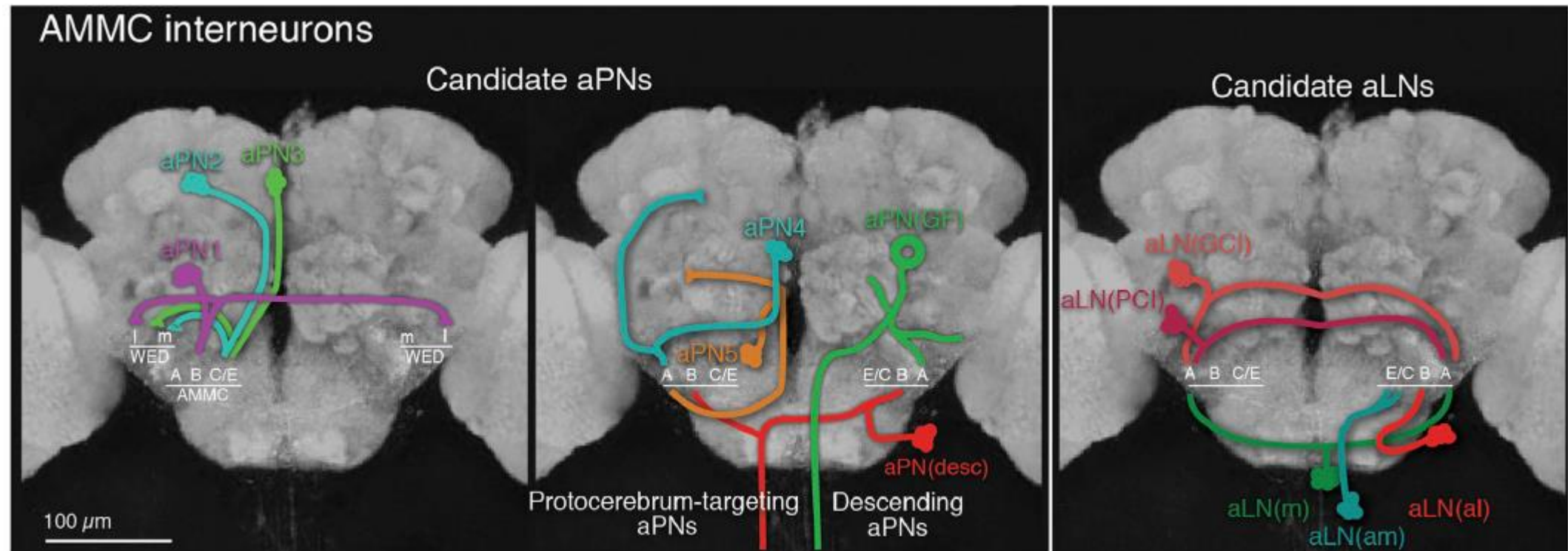
# Female response to courtship song





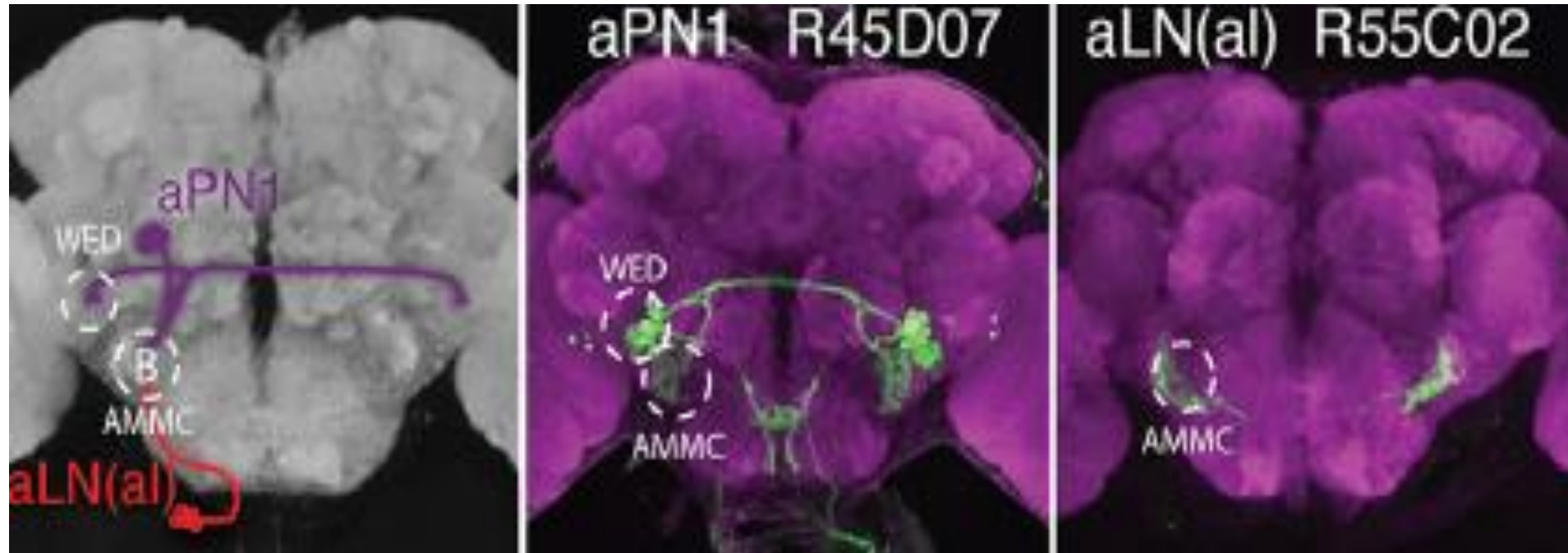
The aPN1 and aLN influence female receptivity

## Schematic projections of twelve identified classes of AMMC



Vaughan, et al. Current Biology, 2014.

## The Immunofluorescence of aPN1 and aLN



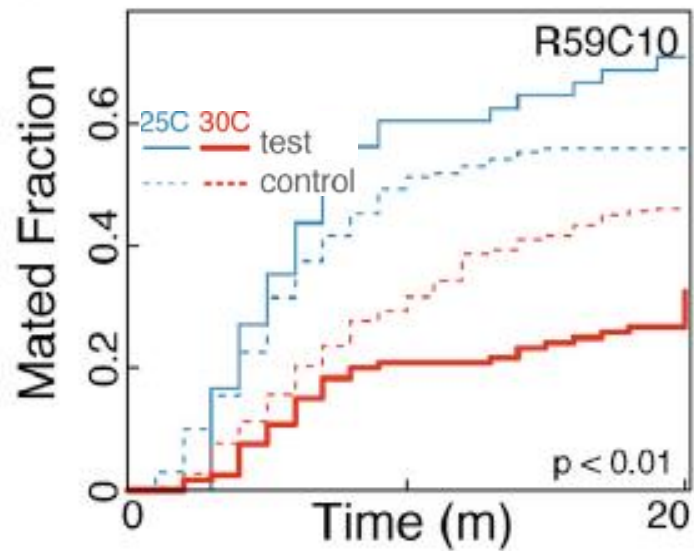
Vaughan, et al. Current Biology, 2014.

# Neuronal silencing of aPN1 and aLN(al) disrupts courtship hearing

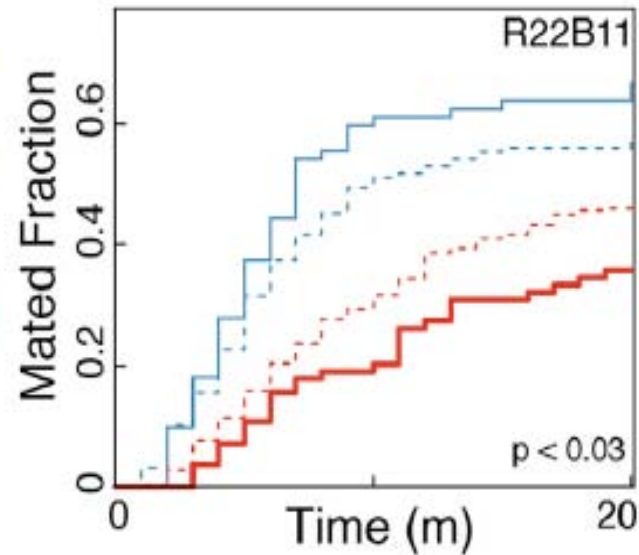


## Female Receptivity

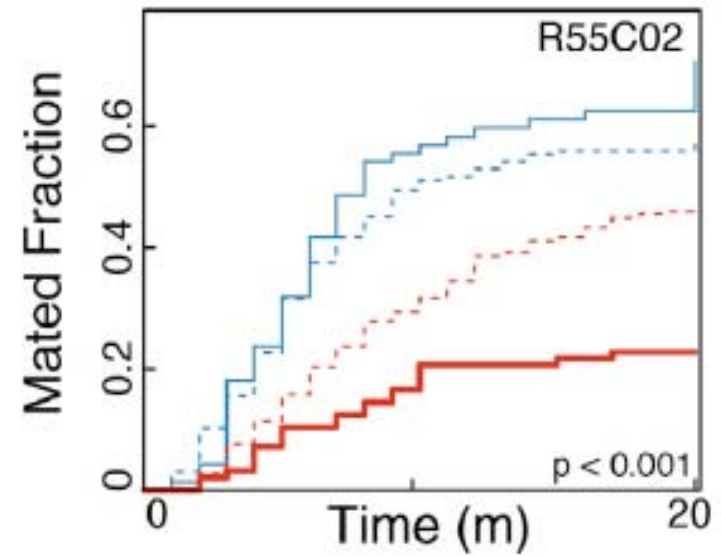
**C**



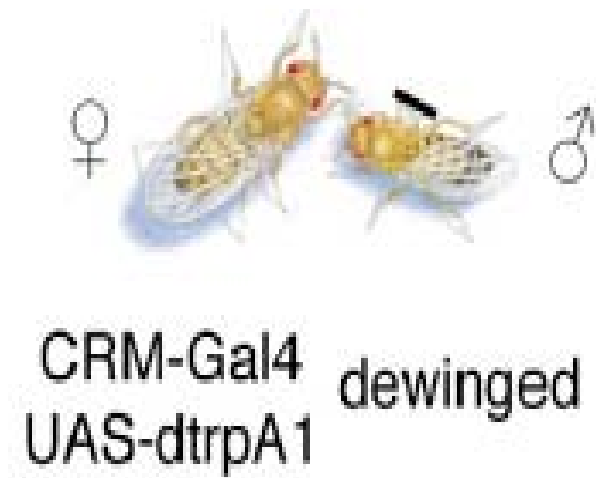
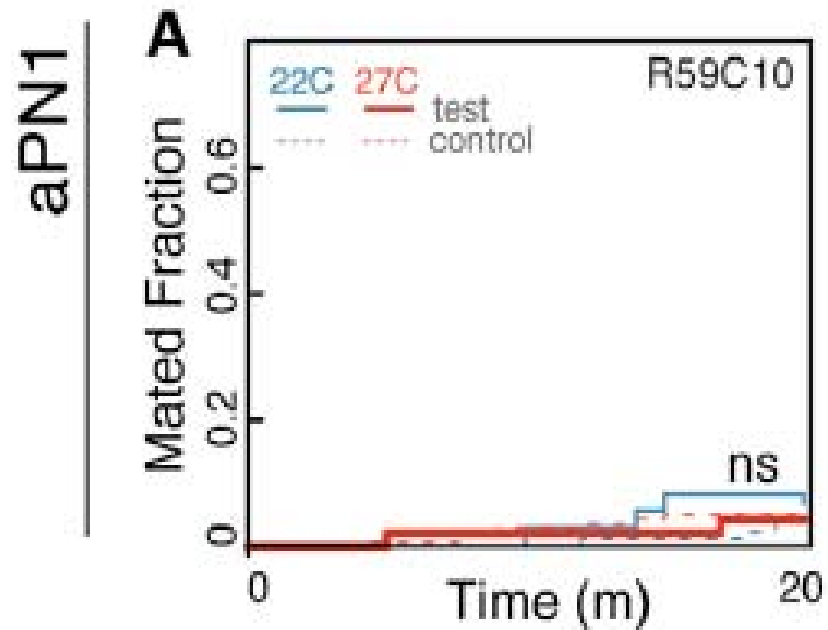
**E**



**G**



The aPN hyperactivation fails to rescue female receptivity to mute males

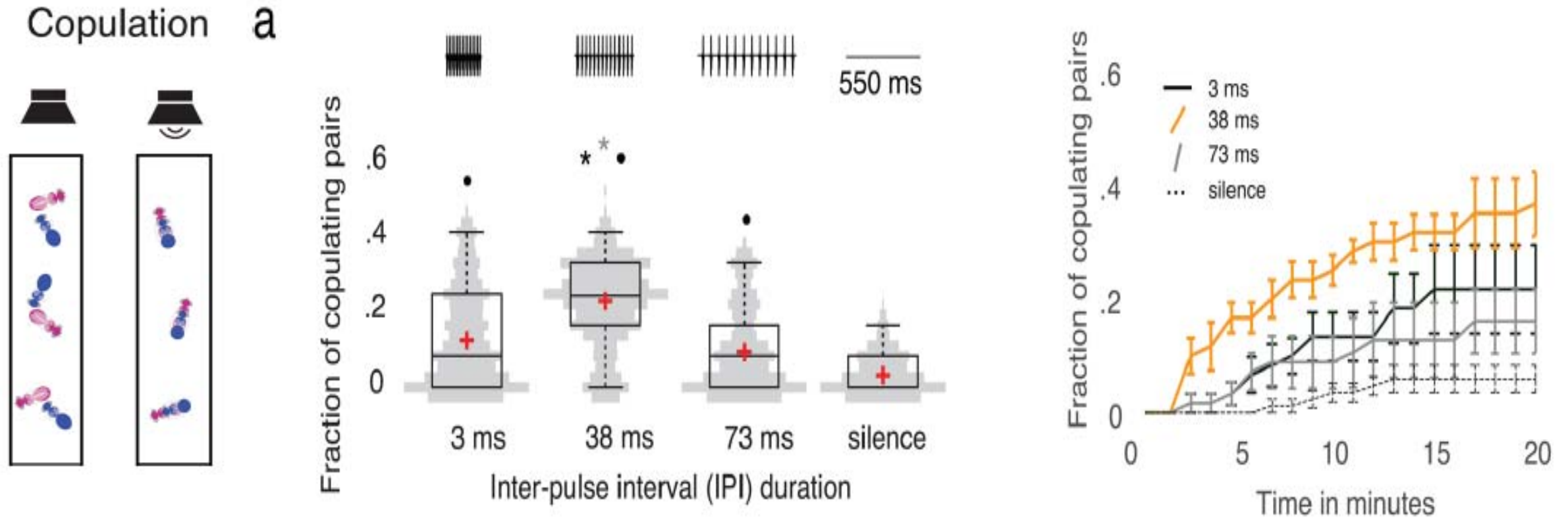


Vaughan, et al. Current Biology, 2014.

The pC1 modulates female receptivity

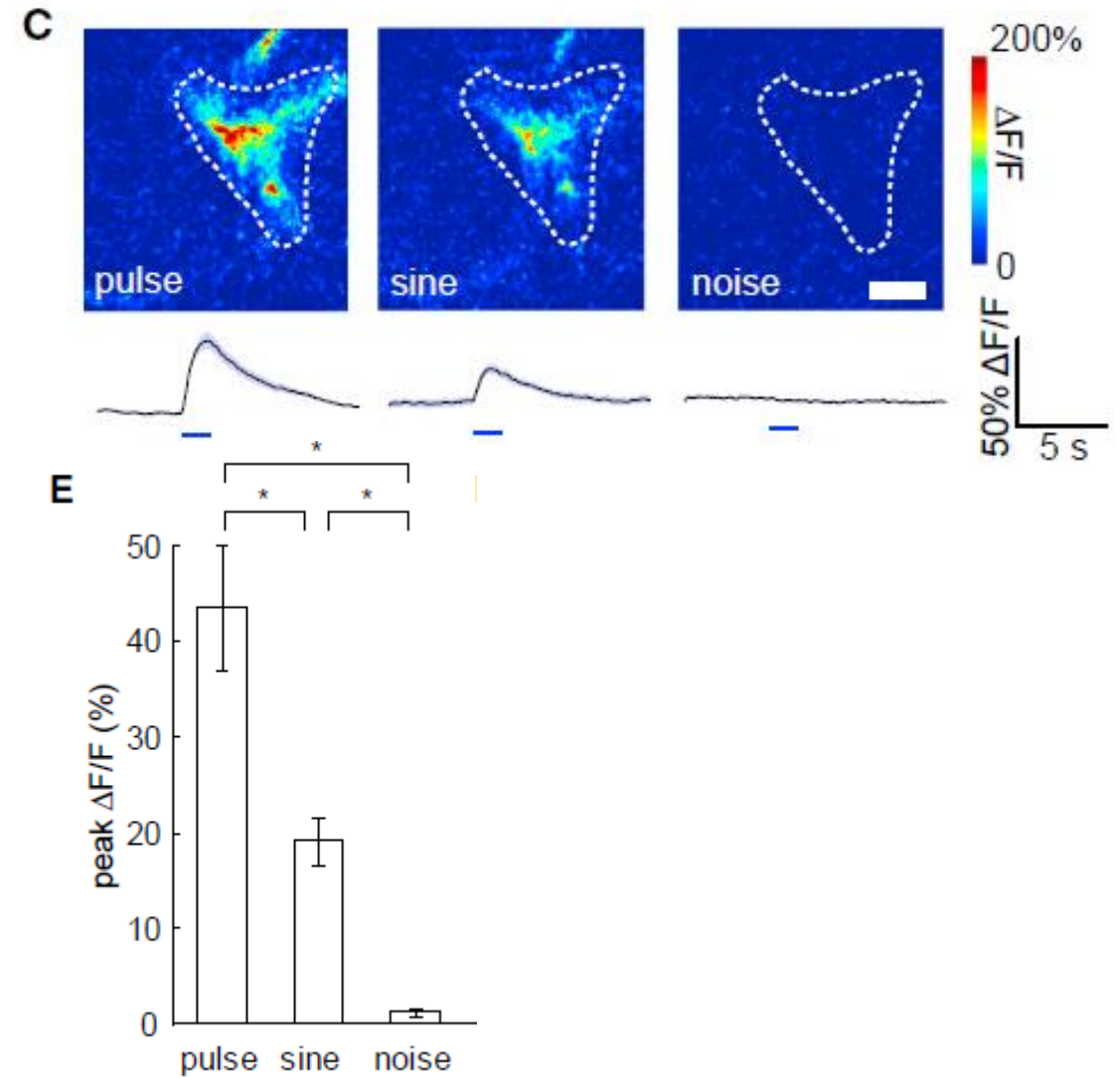
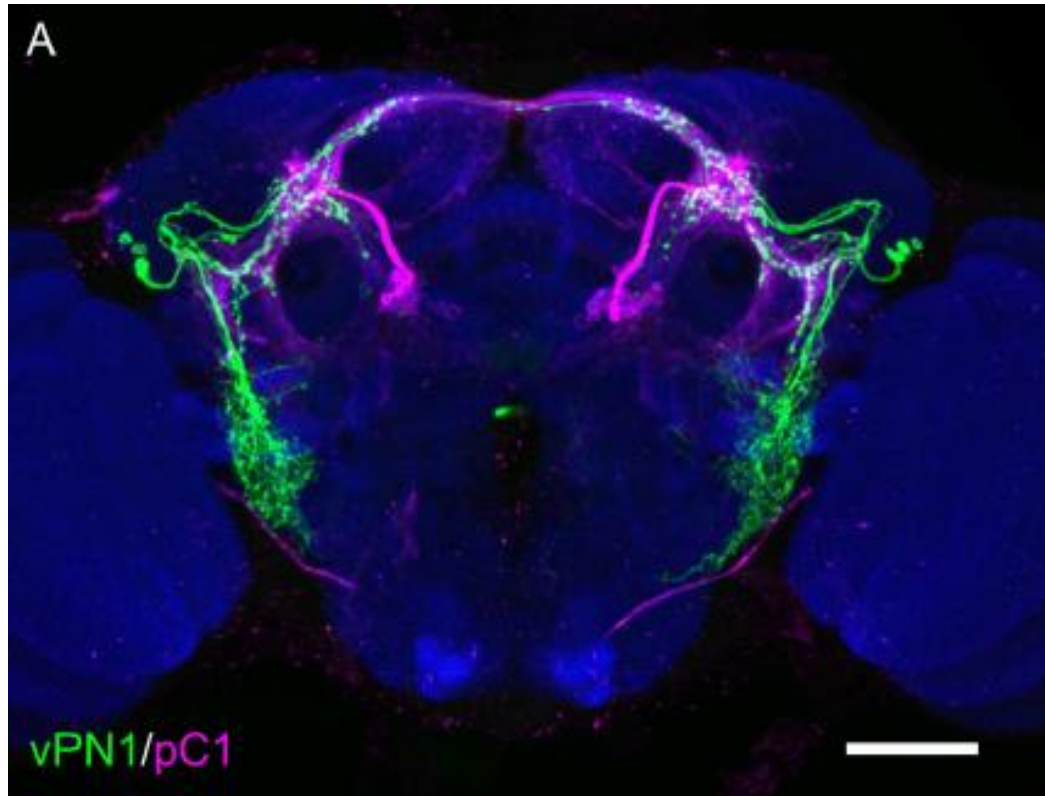


# Courtship song promoted copulation of blinded and silenced males



# pC1 neurons response to courtship song

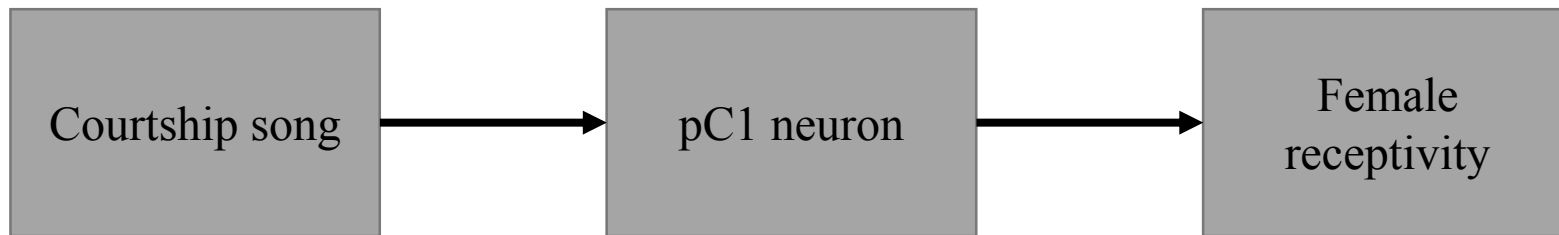
pC1 Neuron responses



Zhou, et al. Neuron, 2014.

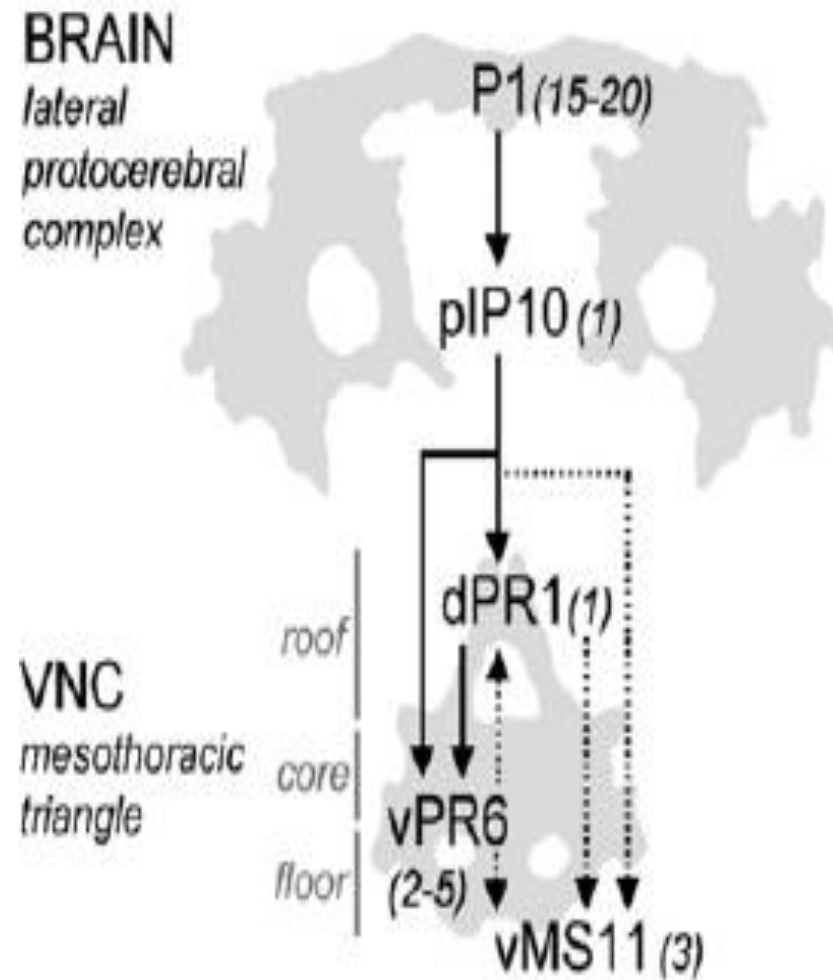
Zhou, et al. eLife, 2015.

## pC1 neurons respond to courtship song

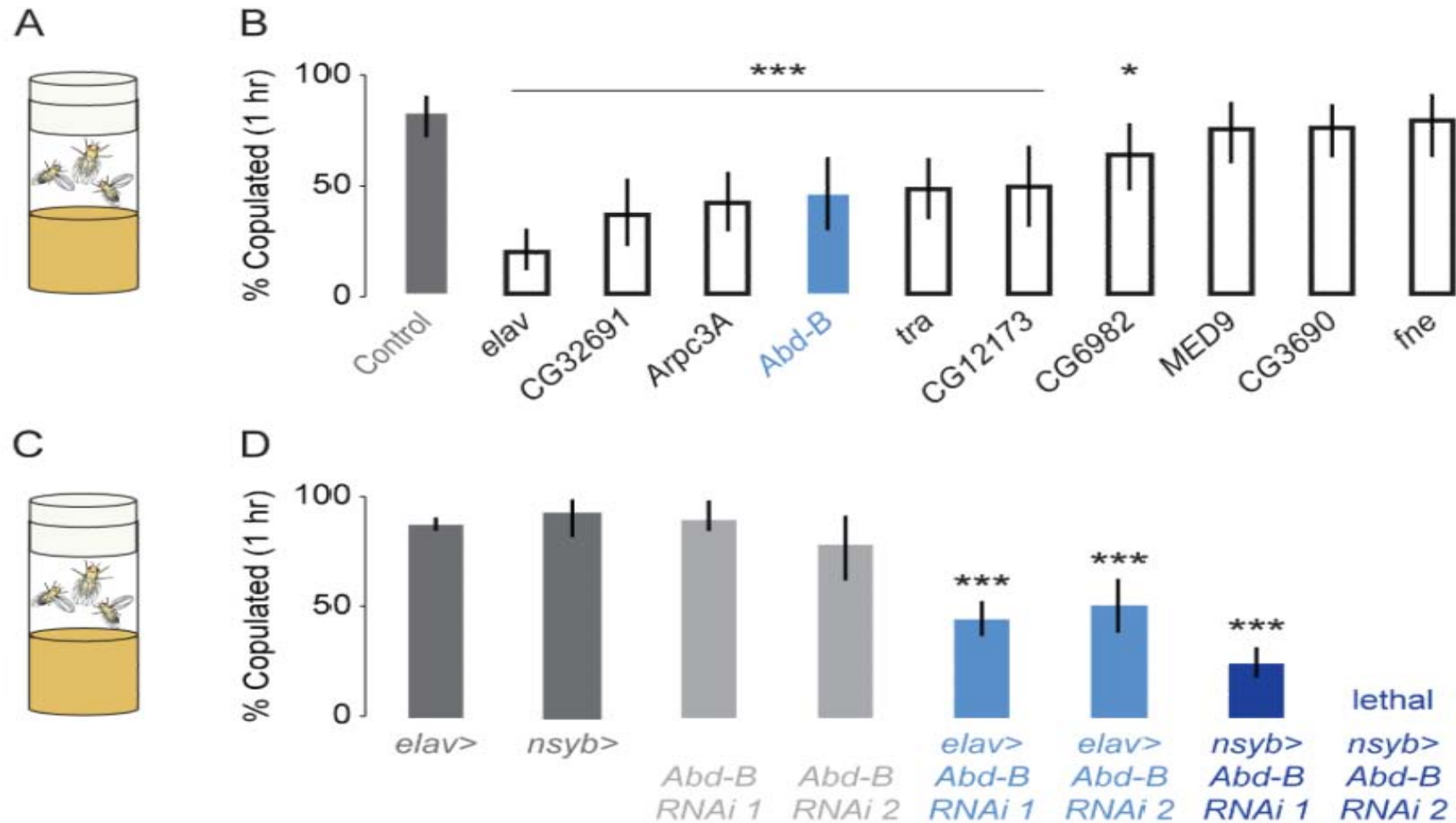


VNC neurons control of *Drosophila* courtship behavior

## VNC neurons control of *Drosophila* courtship behavior

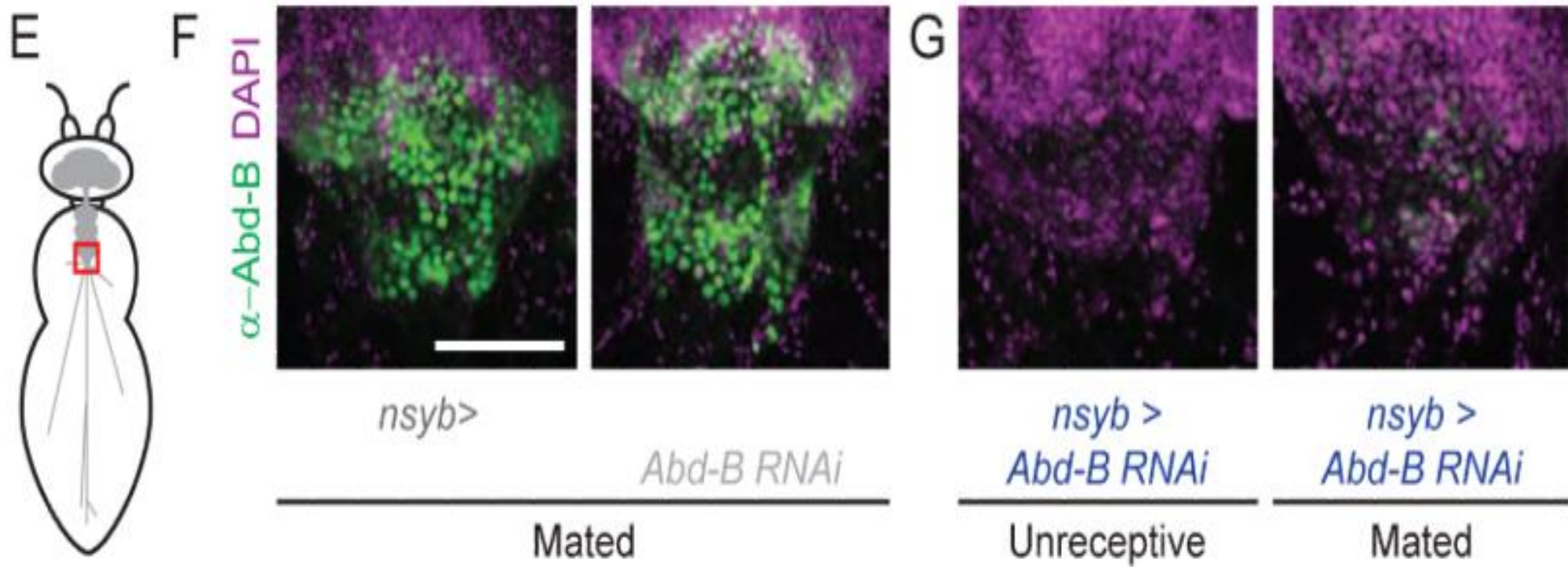


## Abd-B is required in neurons for female receptivity



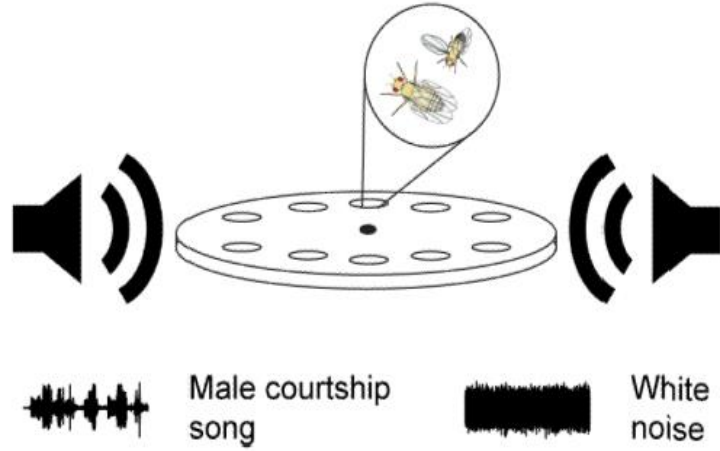


## Immunofluorescence of Abd-B

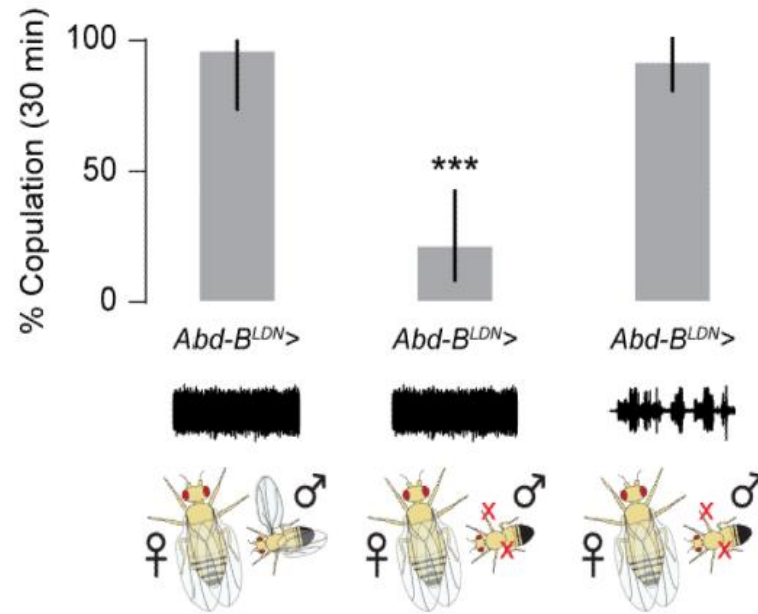


## Abd-B responses to courtship song

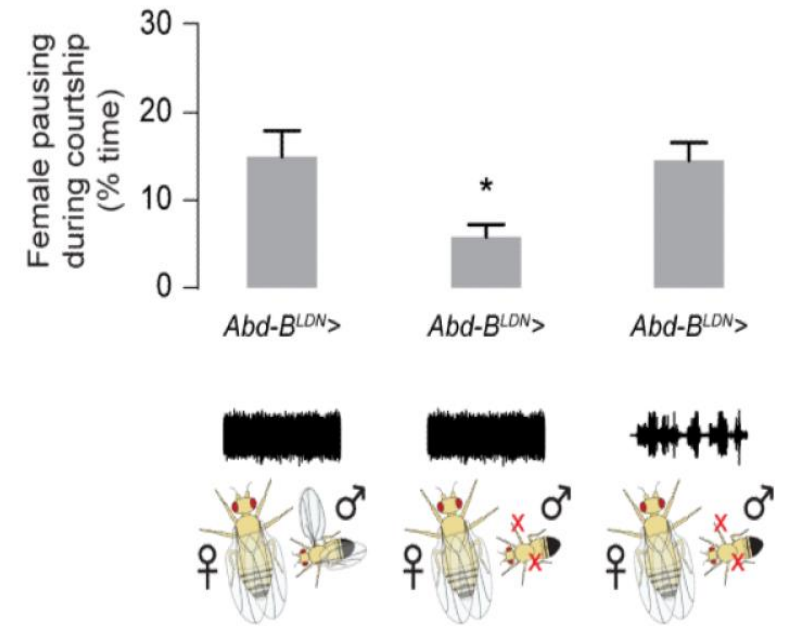
B



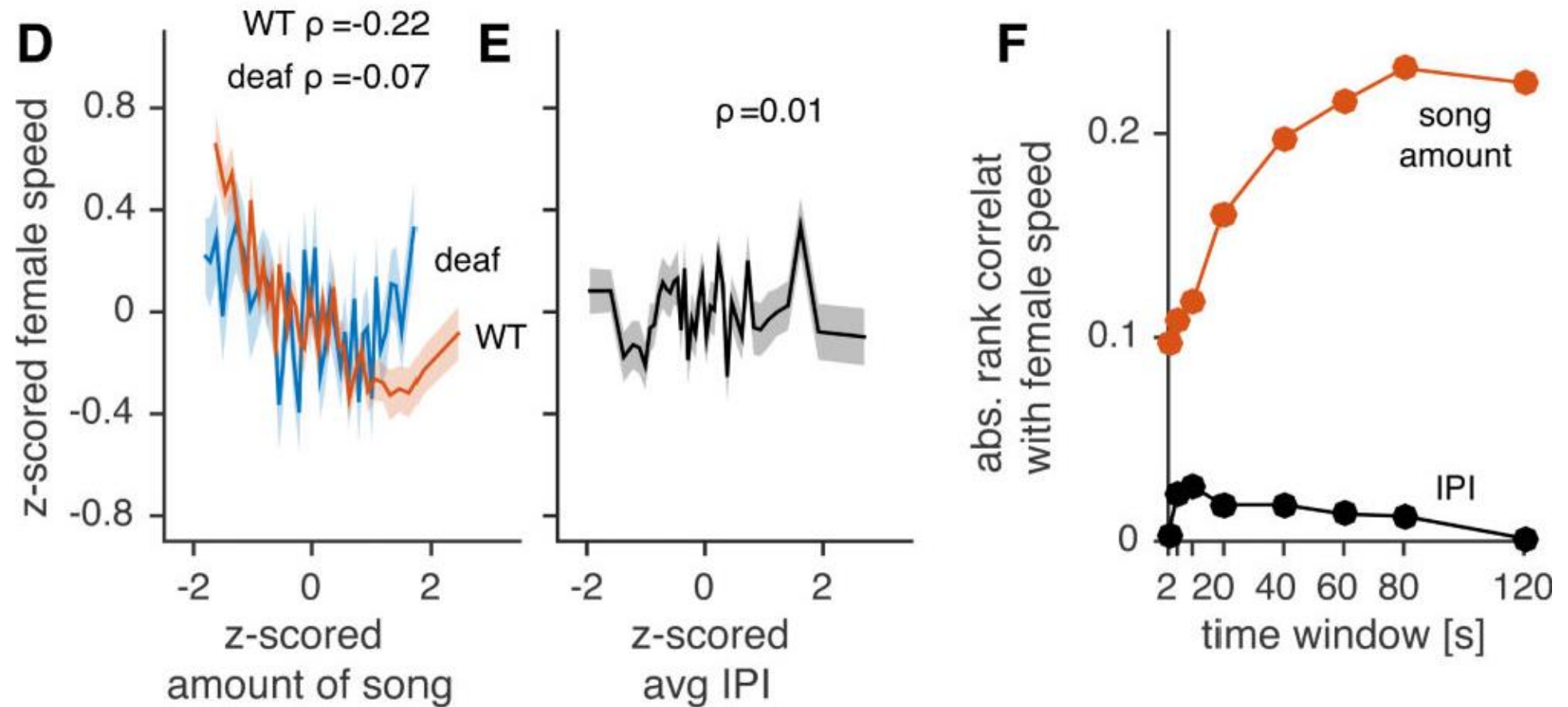
C



D

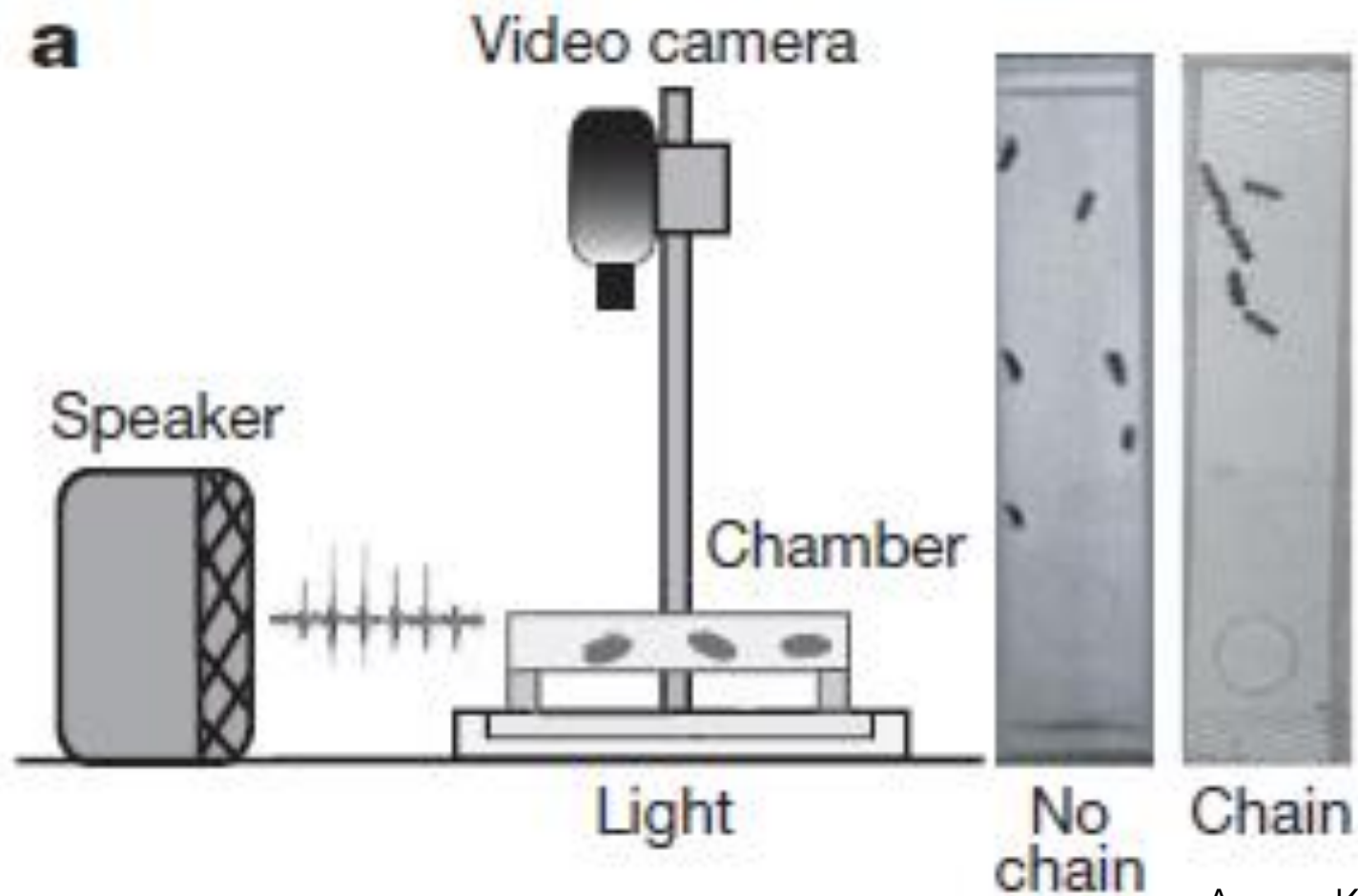


## Female locomotion responses to courtship song

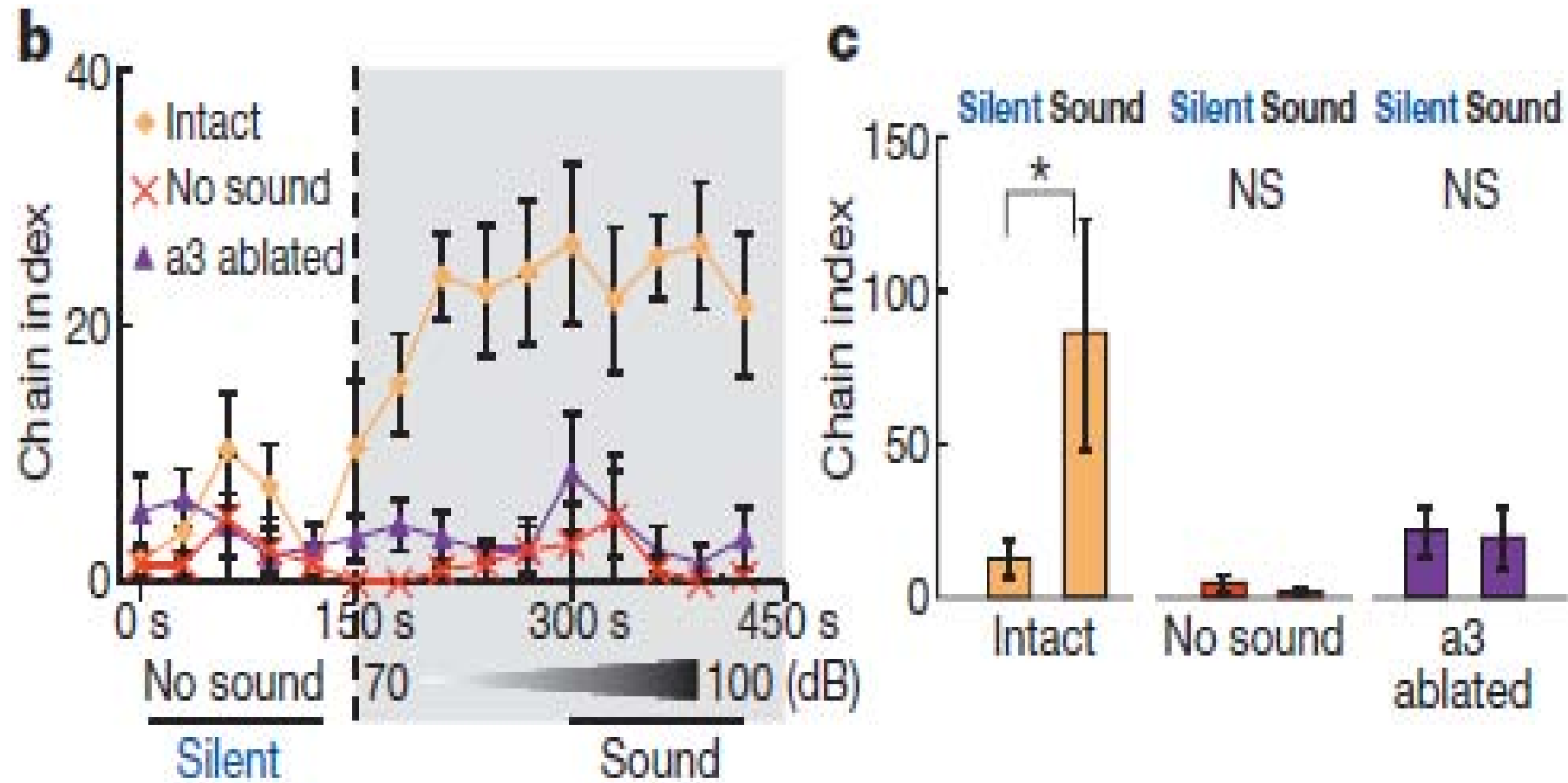


The pulse promoted male chaining behavior

## The pulse component of courtship song promoted male chaining behavior

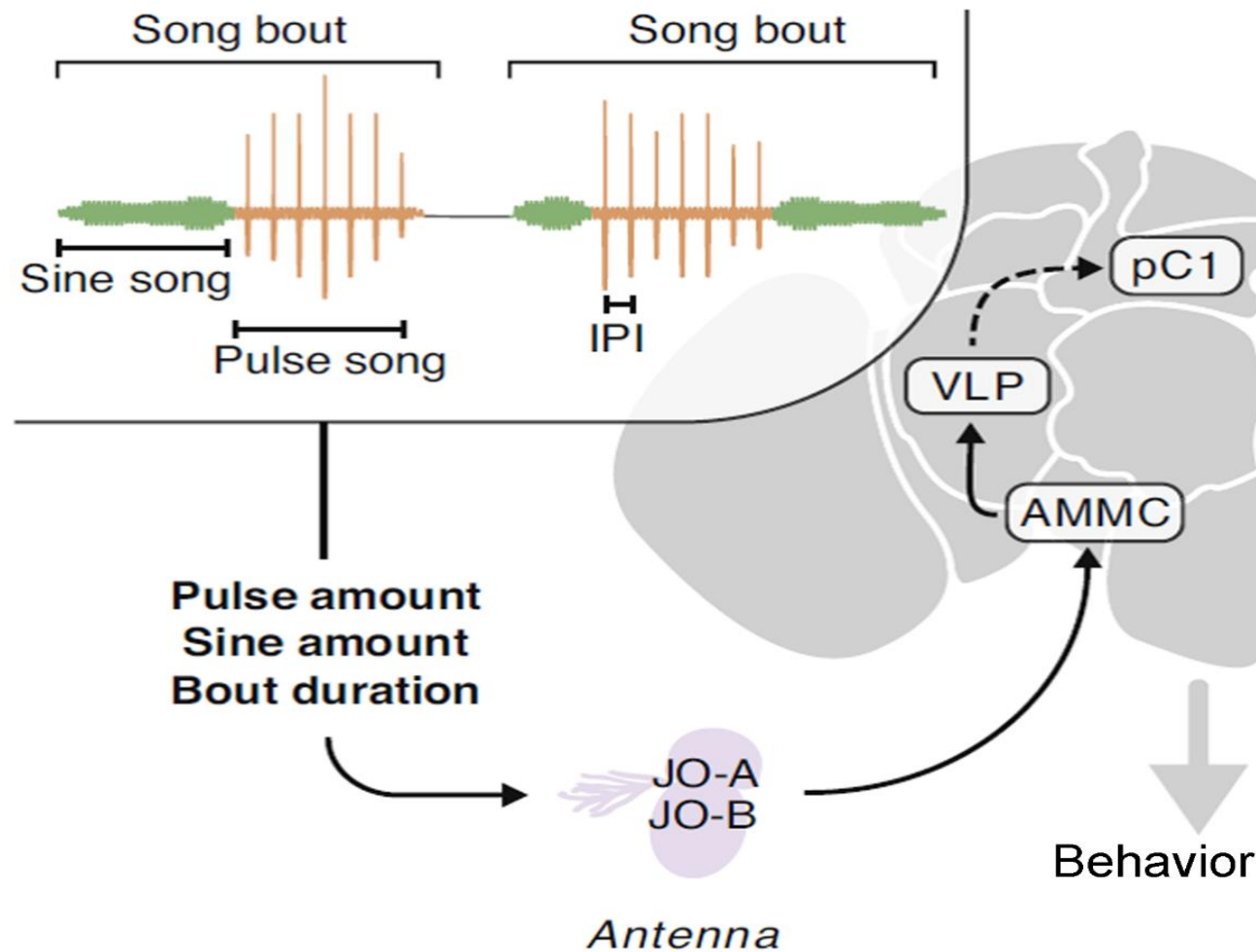


## Courtship song is necessary for chaining behavior



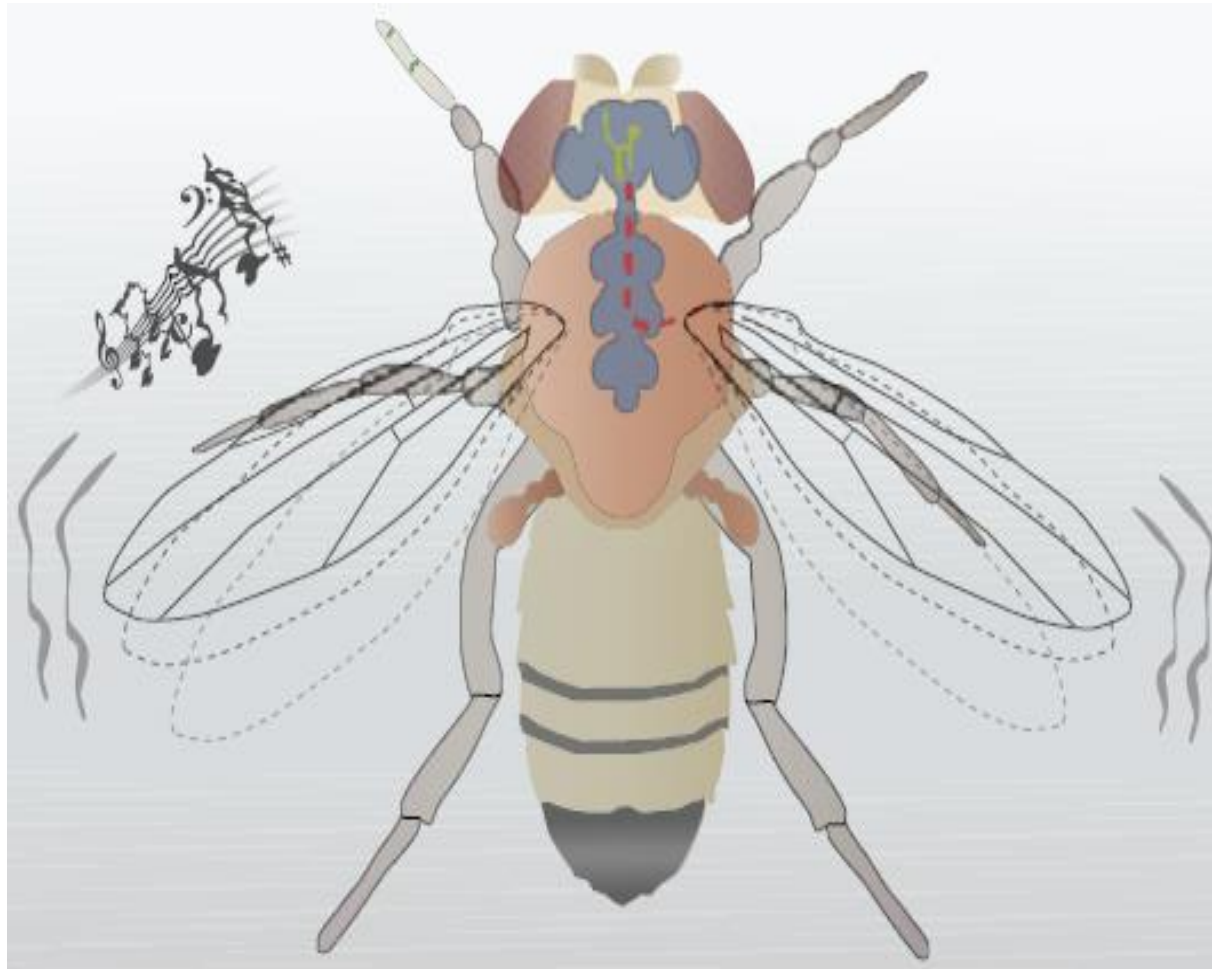


## Song is a major determinant of courtship

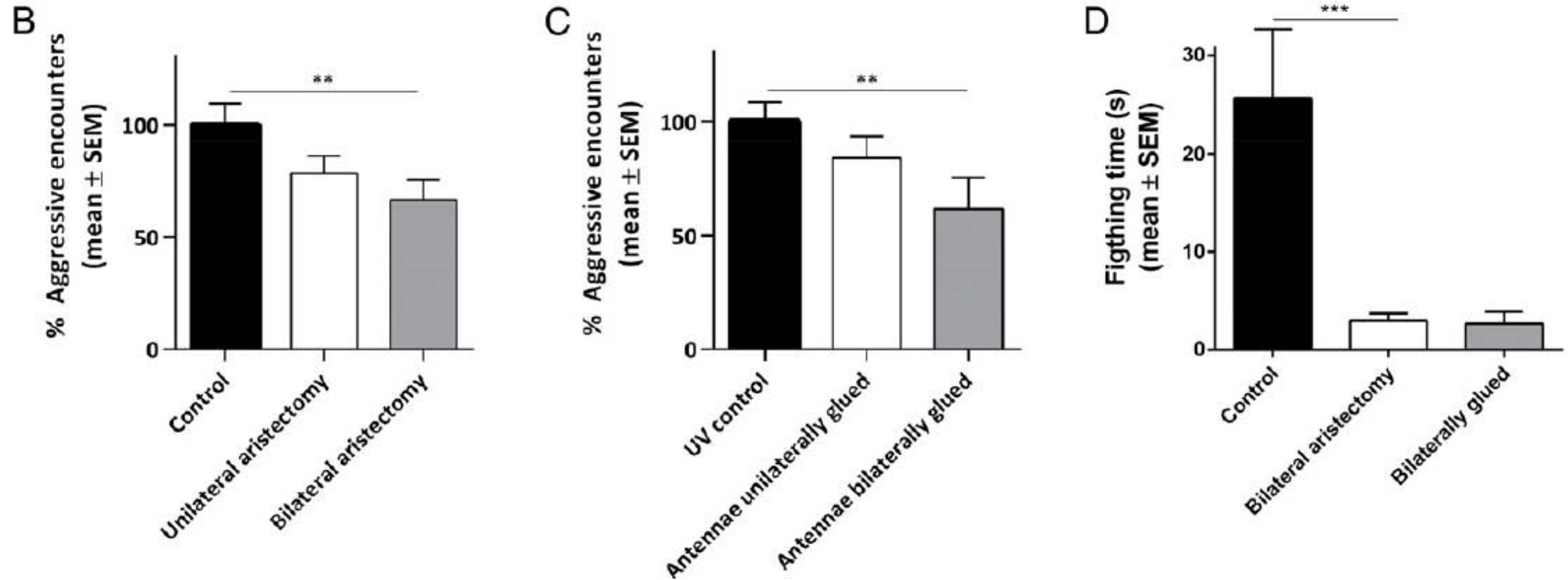


The effect of auditory system in aggression

Male forms offensive posture when feeling other males



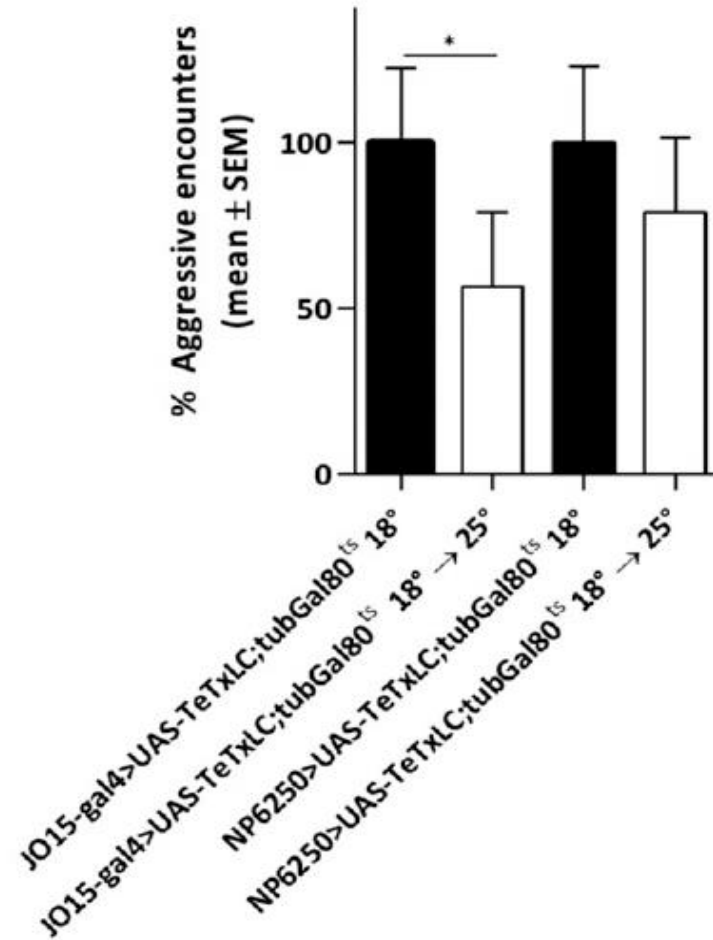
## Mechanical disruption of hearing modulates aggression



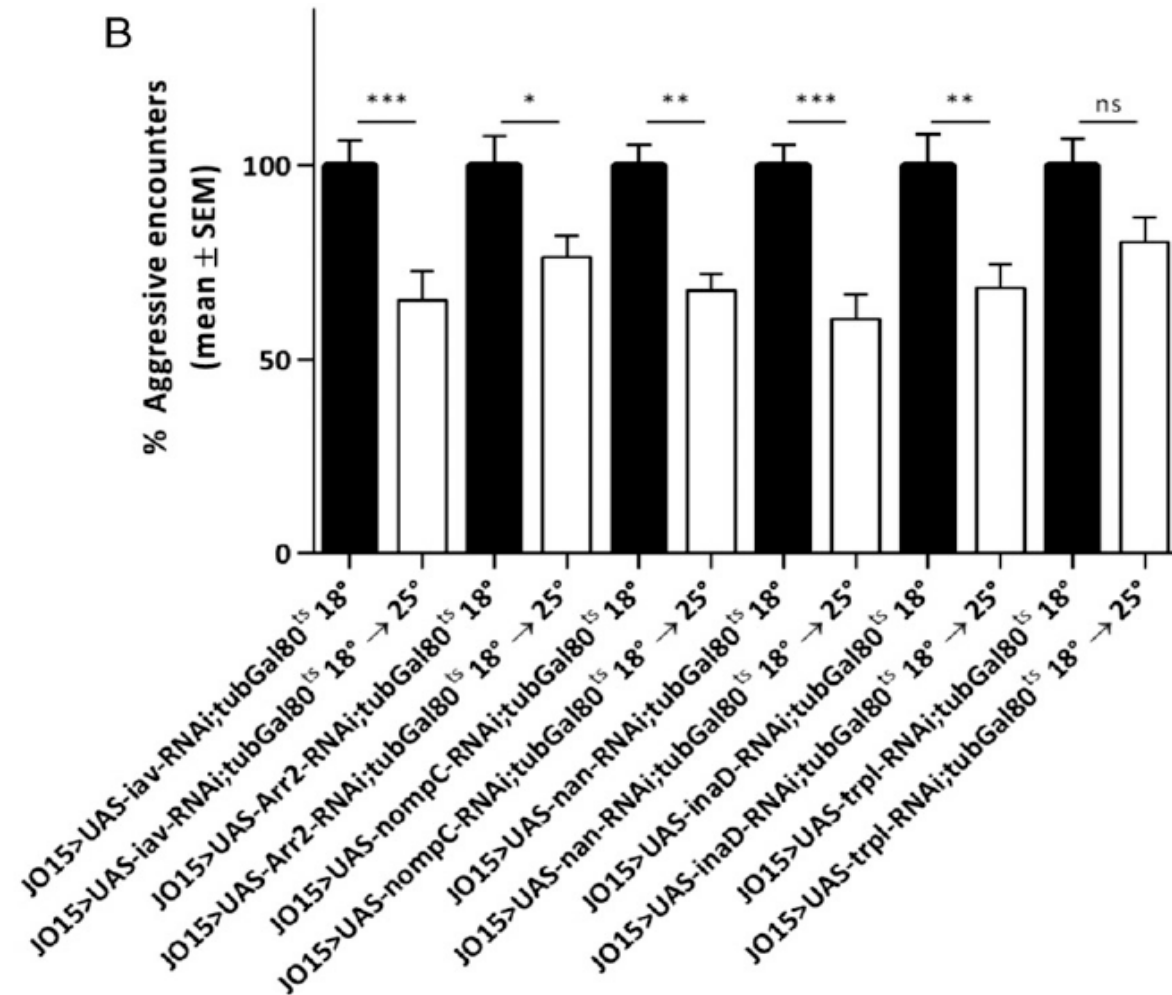
Versteven, et al. PNAS, 2017.

# Johnston's organ neurons modulates aggression

A

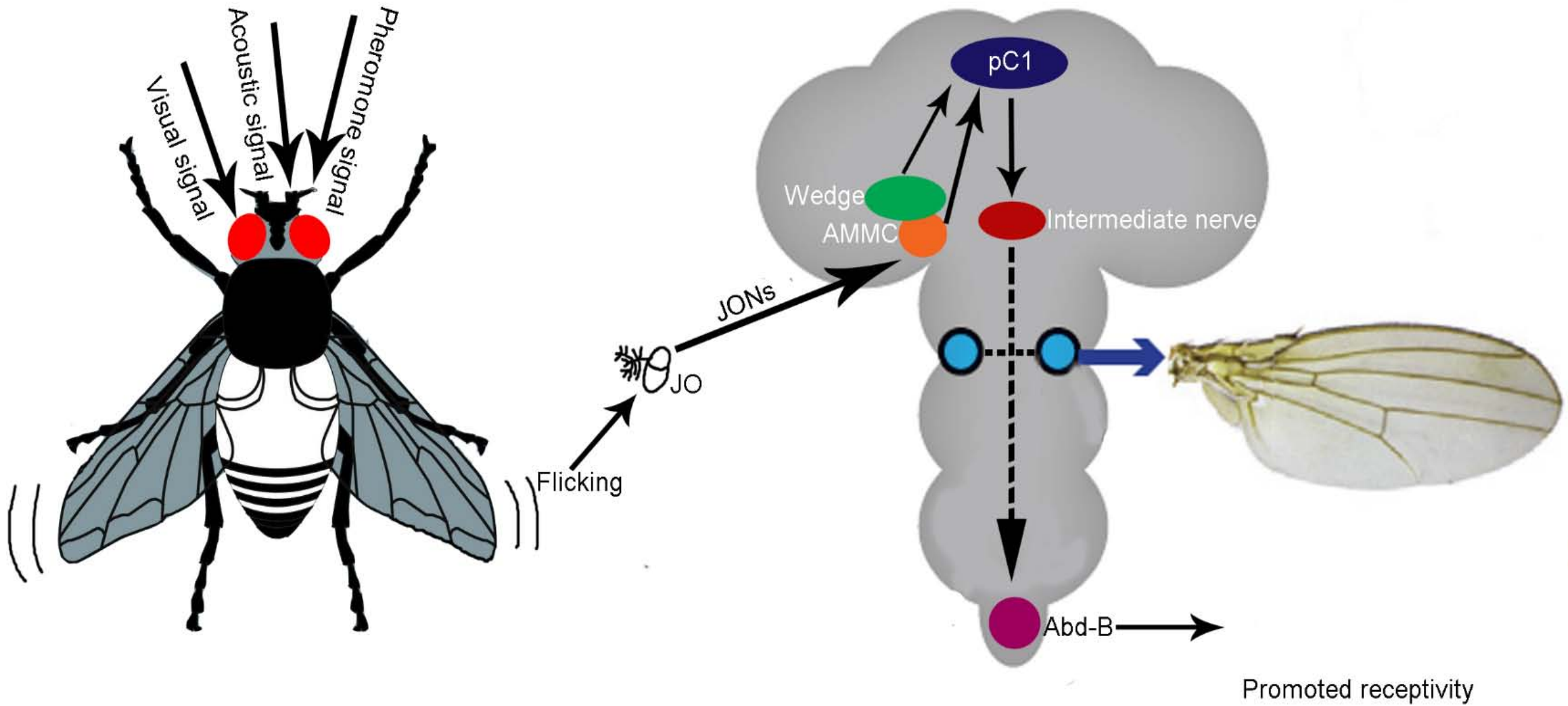


# RNAi-mediated knockdown in the hearing genes modulate aggression





# Conclusion





Thank you