

# **Genetic and neuronal modulation of *D. melanogaster* female receptivity**

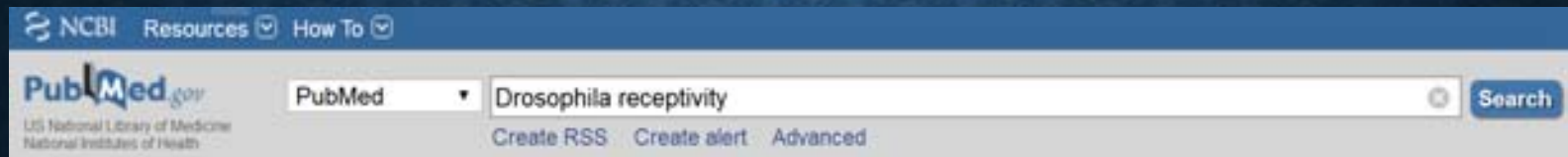
Organizer: Peng Qionglin

Invited members: Su Xiangbin, Pan Yufeng

2018-9-28

# How do we specify the topic?

- 199 items were found when searching keywords, *Drosophila receptivity*, in NCBI.



- The full texts of 136 articles were downloaded by Su Xiangbin.
- General topics, such as post-mating behavior, were drafted out after quickly read through those articles by Peng Qionglin.
- Prof. Pan Yufeng joined in the group.

# How do we cooperate in a group?

## **The first discussion:**

determining an appropriate topic and divide it into three small specific topics

- Part I Overview, virgin female receptivity (Peng Qionglin)
- Part II Sex-peptide (SP) signaling in post-mating switch (Su Xiangbin)
- Part III Neuronal modulation of post-mating behaviors (Pan Yufeng)

## **The second discussion:**

Combining three parts and rehearsing the journal report.

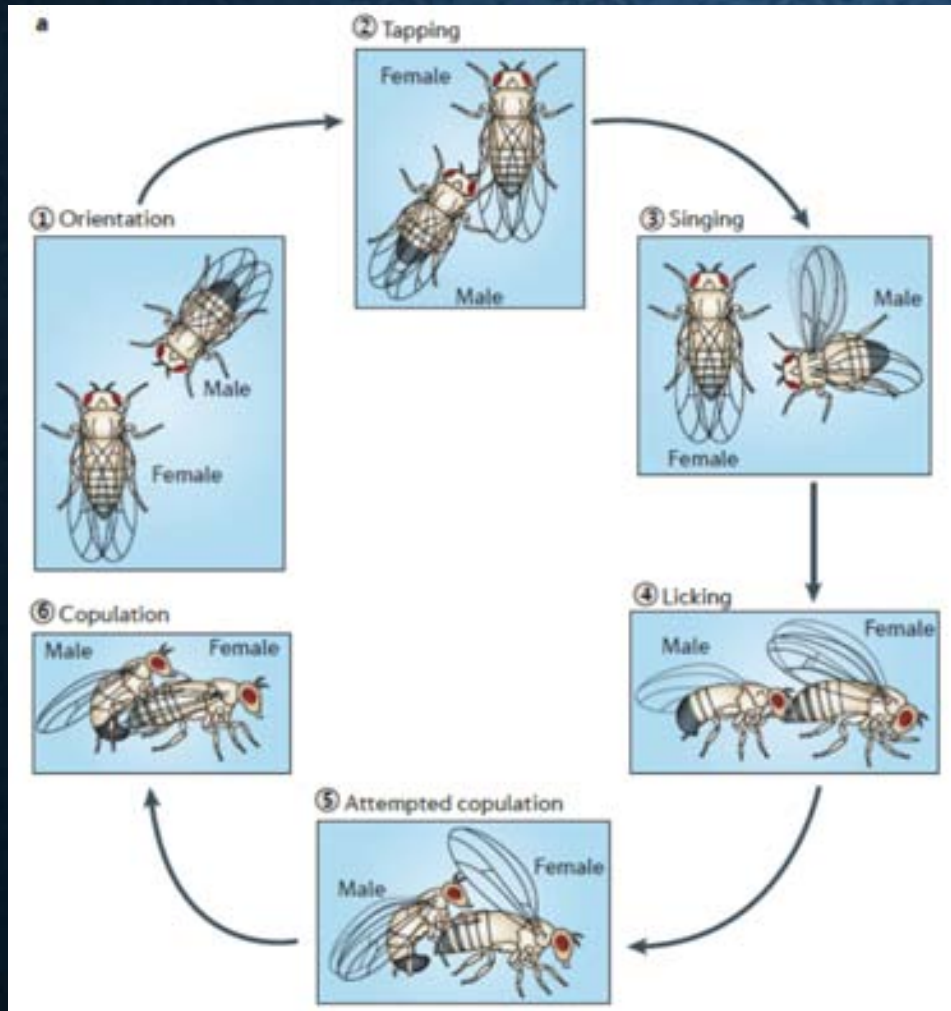
**Genetic and neuronal modulation  
of *D. melanogaster* female receptivity**



# CONTENT

- Part I Overview, virgin female receptivity
- Part II Sex-peptide (SP) signaling in post-mating switch
- Part III Neuronal modulation of post-mating behaviors

# Courtship in *Drosophila melanogaster*



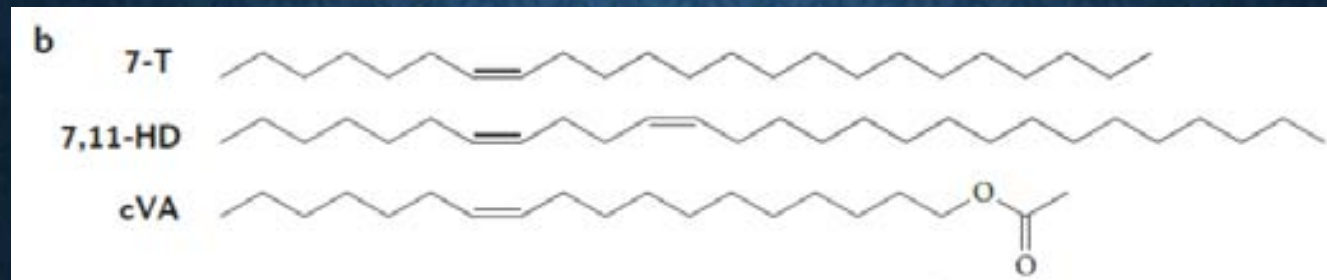
## Discrete steps of male mating behavior:

1. Orientation towards and following the female
2. Touching abdomen with the foreleg
3. Wing extension and vibration
4. Licking of the female's genitalia
5. Attempted copulation
6. copulation

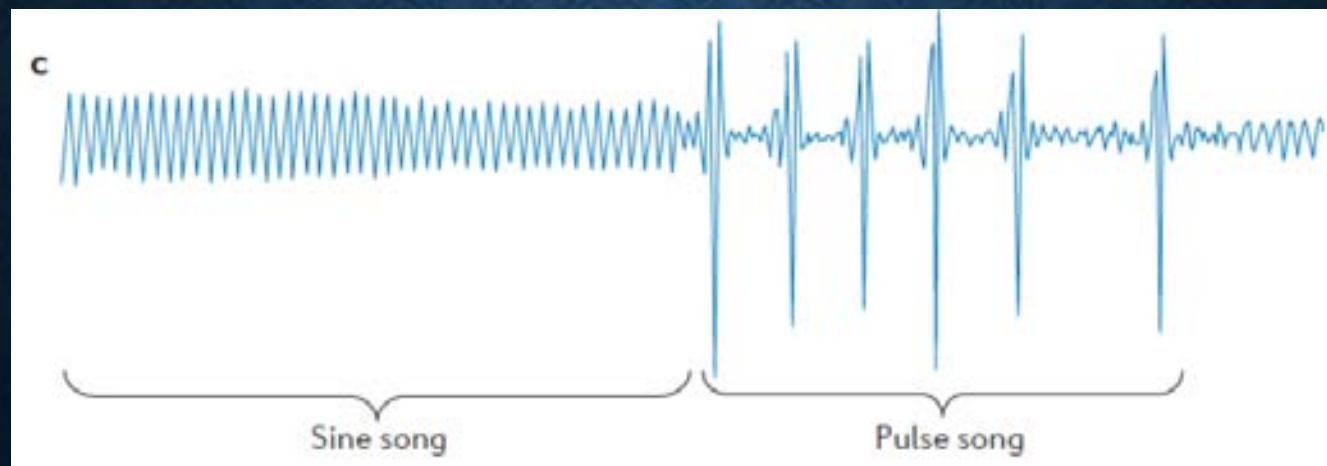
# How does a female decide to copulate with a male?

## -Two import male stimuli

Sex pheromones

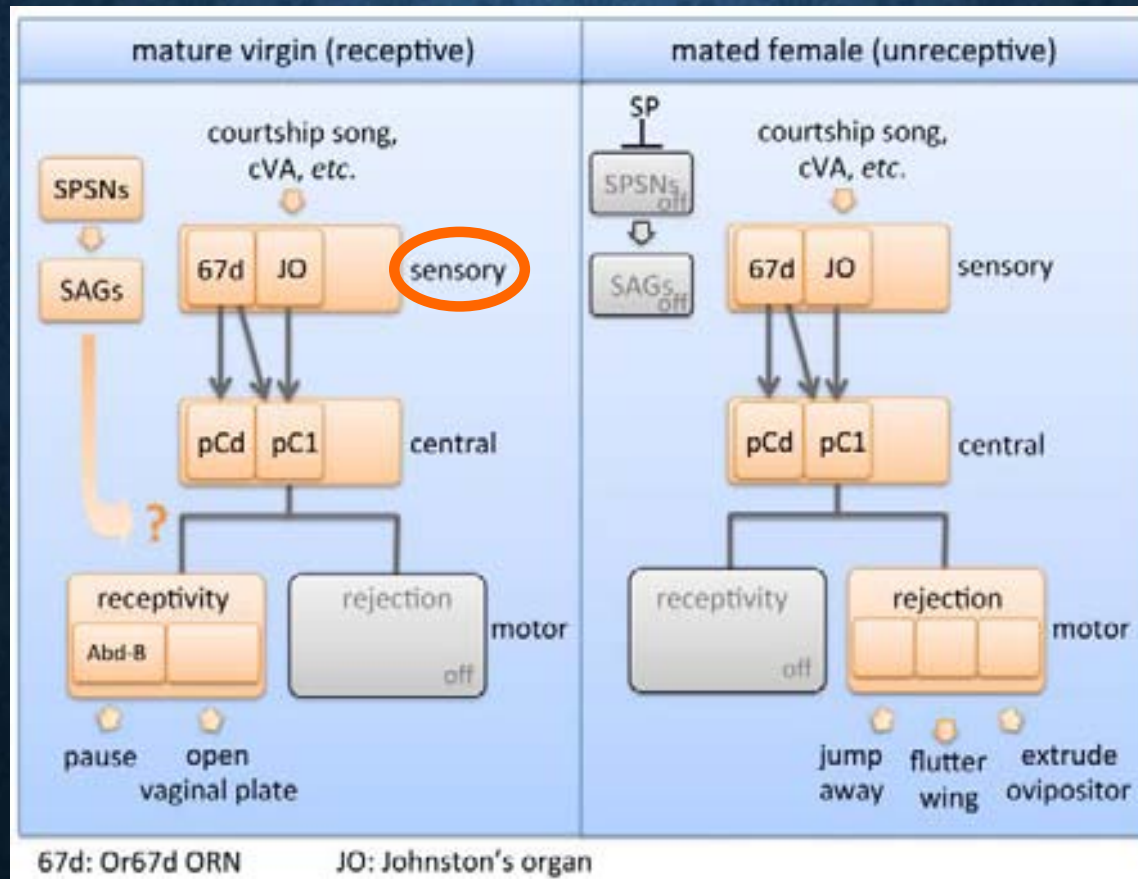


Courtship songs



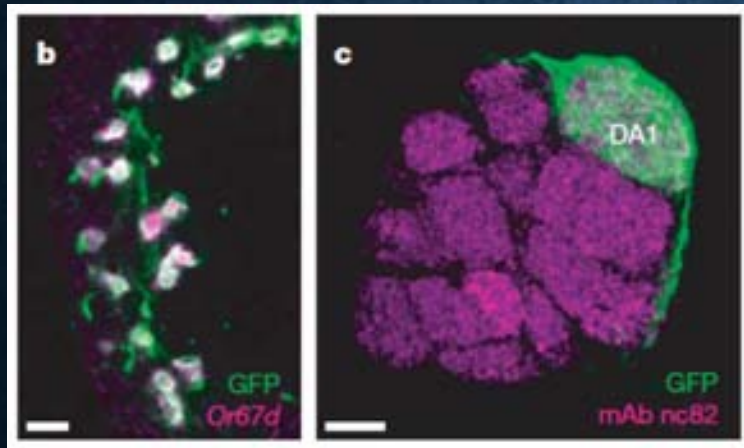
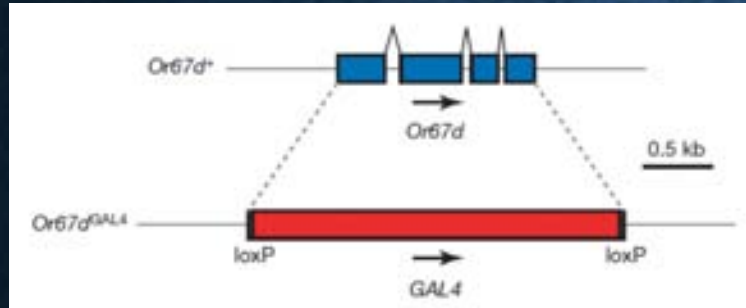
# How does a female decide to copulate with a male?

## -Receptivity circuit inside female

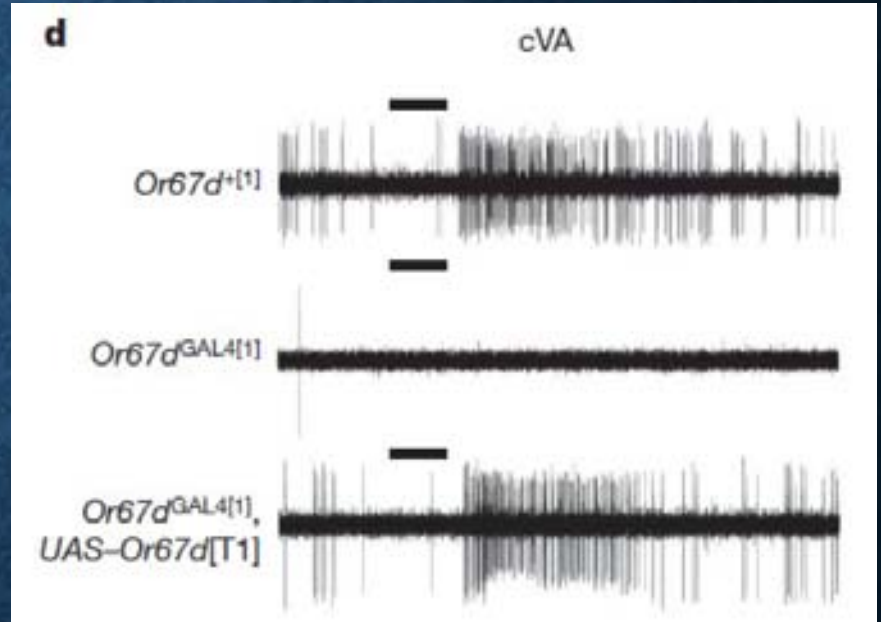




# Or67d mediates physiological responses to cVA



Or67d<sup>GAL4</sup>/UAS-mCD8-GFP



single-sensillum recording

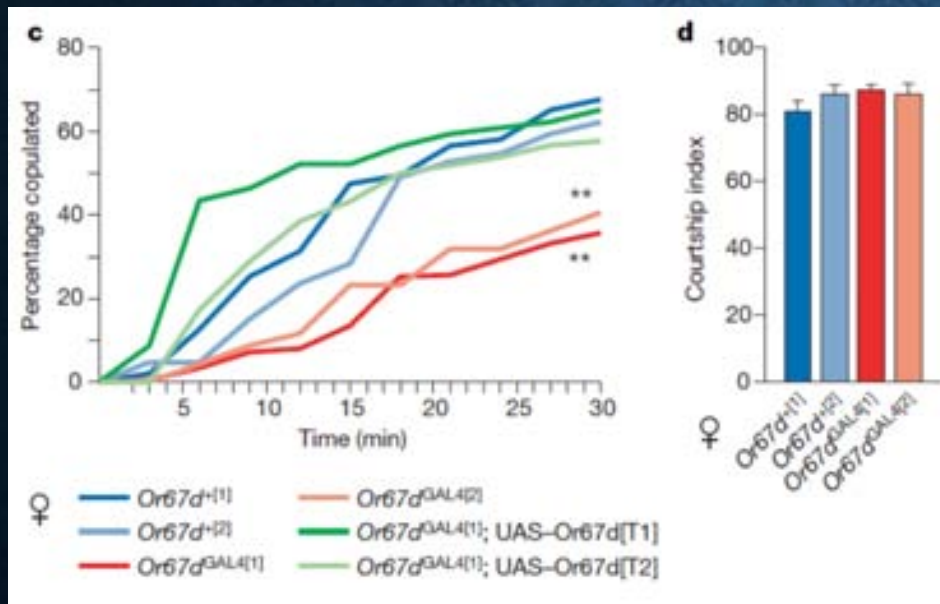
The responses to cVA were quantitatively indistinguishable in males and females.

Kurtovic, A., et al. (2007)

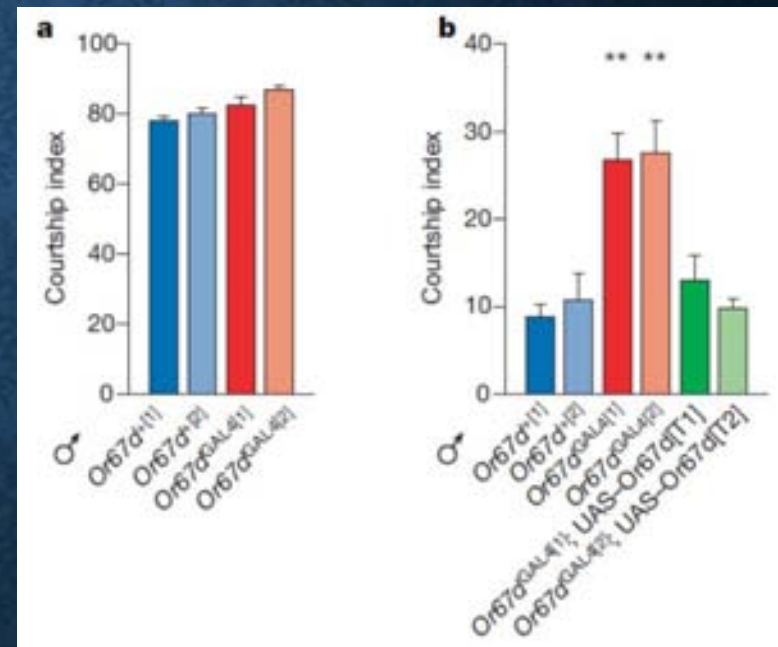
# Or67d functions in male and female mating behaviors

Or67d<sup>Gal4</sup> mutant females display reduced receptivity

Or67d<sup>Gal4</sup> mutant males display male-male courtship



female  
WT male

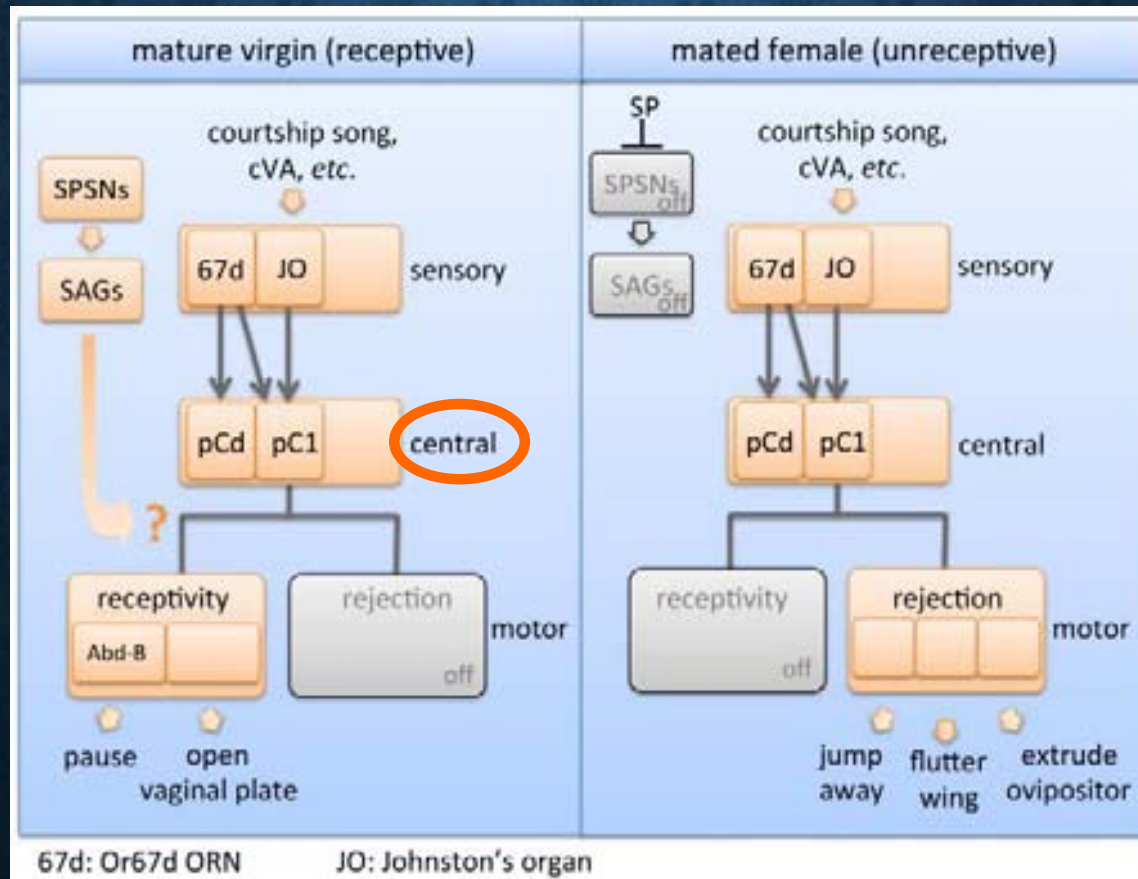


male  
WT virgin female

male  
WT male

# How does a female decide to copulate with a male?

## -Receptivity circuit inside female



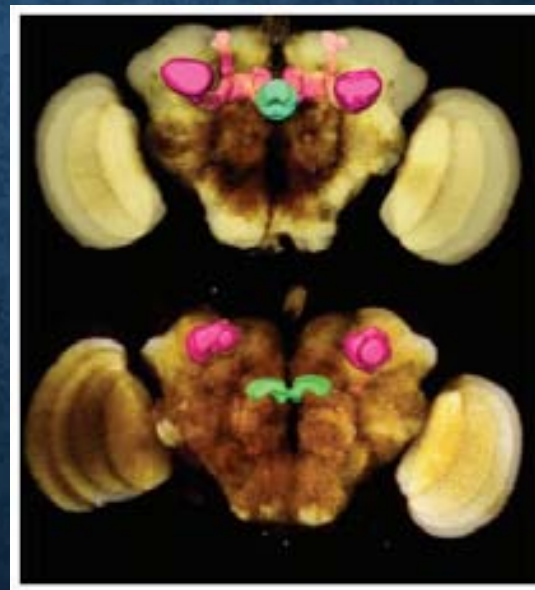


# MB and EB maybe responsible for female receptivity

(a) Receptivity of female controls

Female	Mating/tested	Percent mating
Wild-type	225/231	97
<i>icebox</i> /wild-type	218/251	86
<i>icebox</i> / <i>icebox</i>	63/268	23

Female receptivity phenotype of ***icebox* (*ibx*)** mutants caused by a mutation in the L1-type cell adhesion molecule ***neuroglian* (*Nrg*)**.



CS

*ibx* mutant

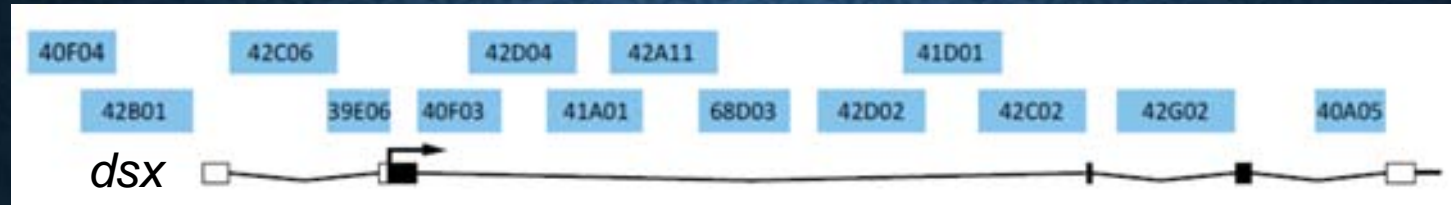
MBs (magenta) and EBs (green)

Carhan, A., et al. (2005)

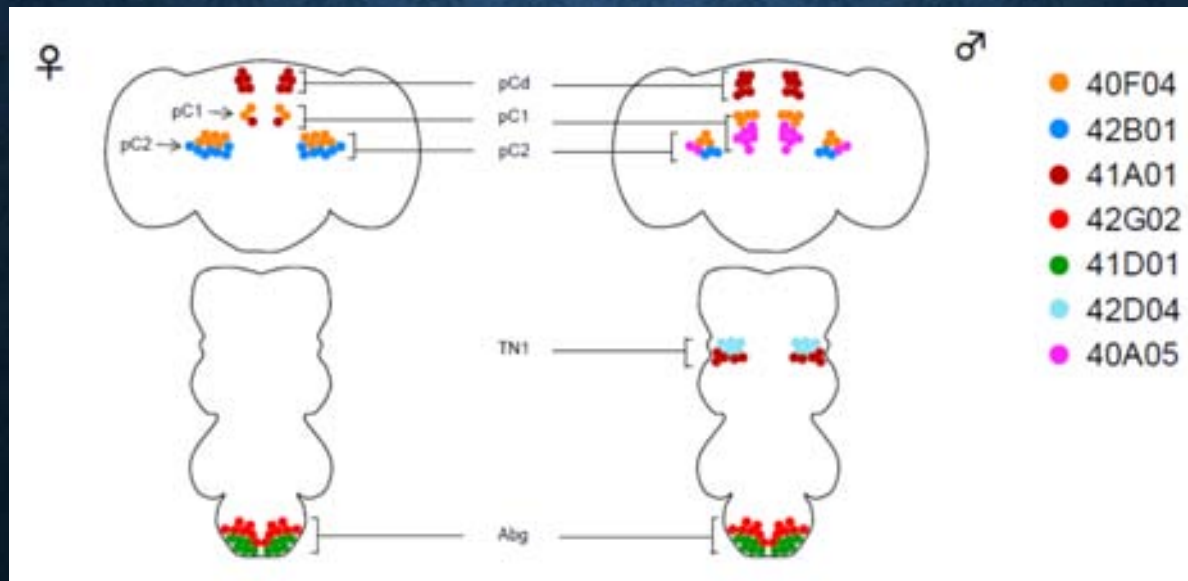


# Genetic subdivision of *dsx* neurons

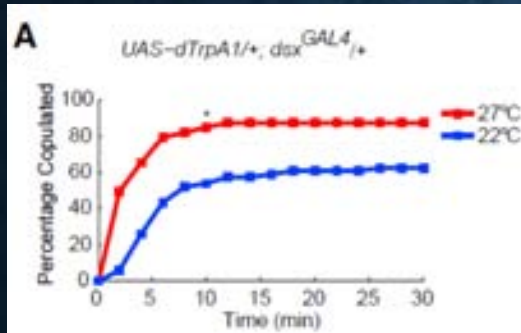
Different *dsx* genomic fragments



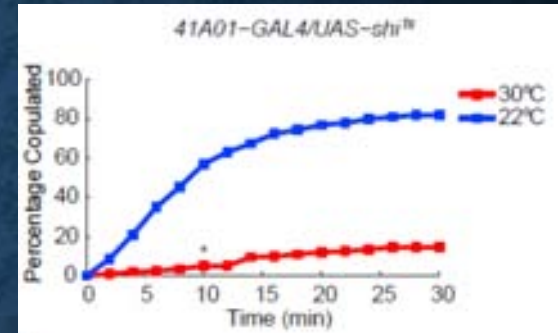
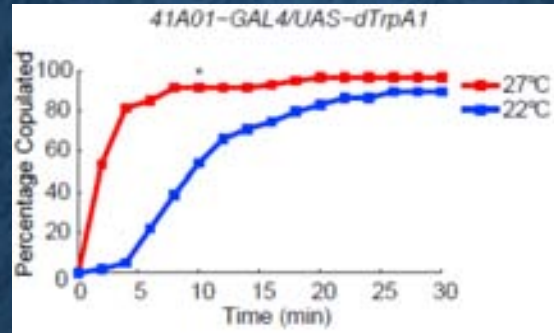
*dsx* fragment-GAL4 expression patterns



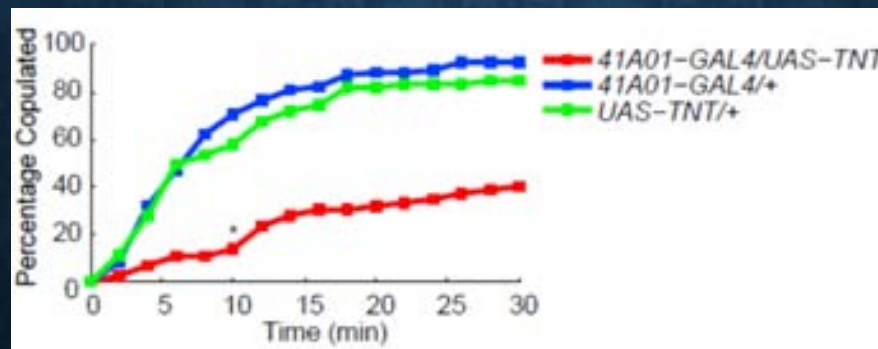
# *dsx* neurons and 41A01-GAL4 neurons are important for female receptivity



Activation-Enhances female receptivity

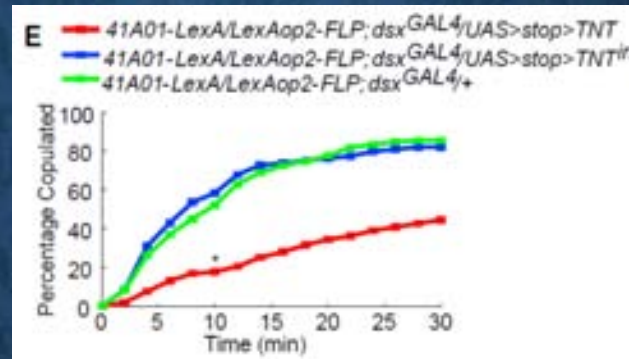
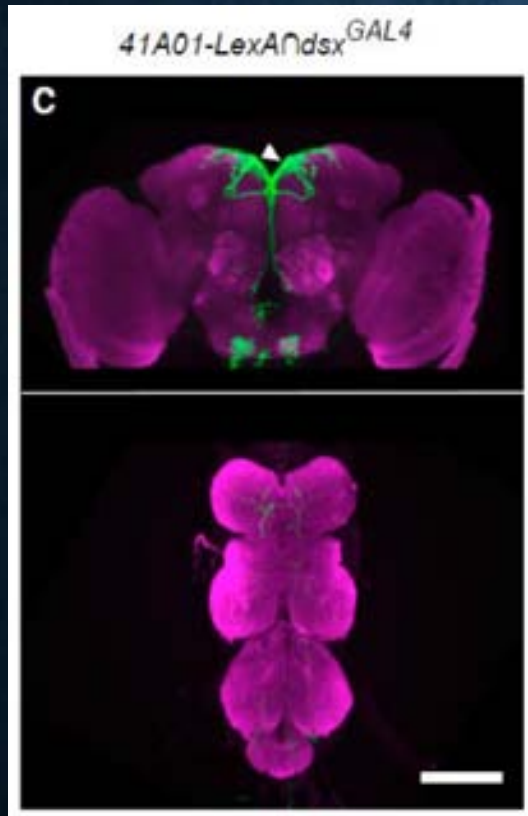


Silencing-Decreases female receptivity

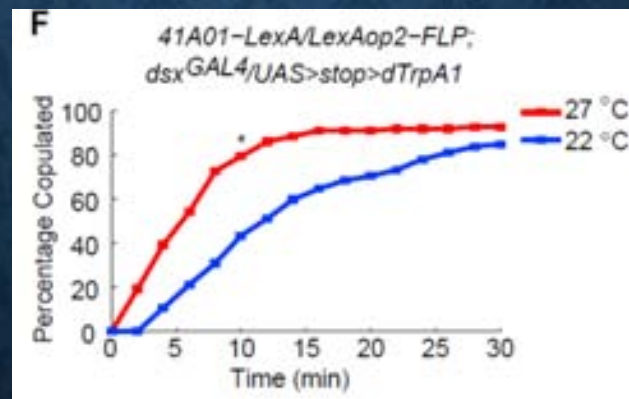


Silencing-Decreases female receptivity

# pCd neurons are important for female receptivity



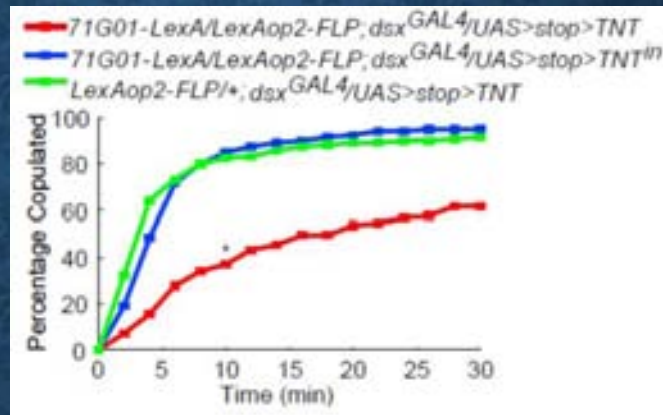
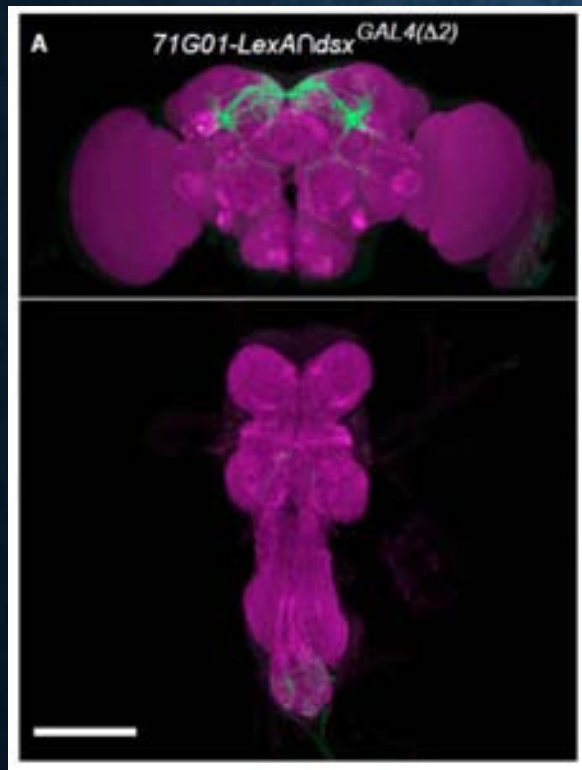
Silencing-Decreases  
female receptivity



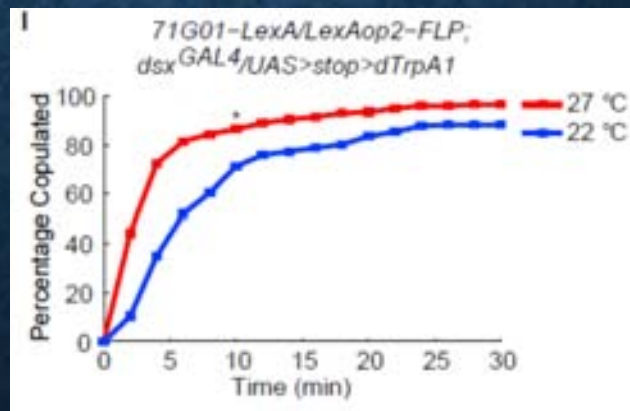
Activation-Enhances  
female receptivity



# pC1 neurons are important for female receptivity



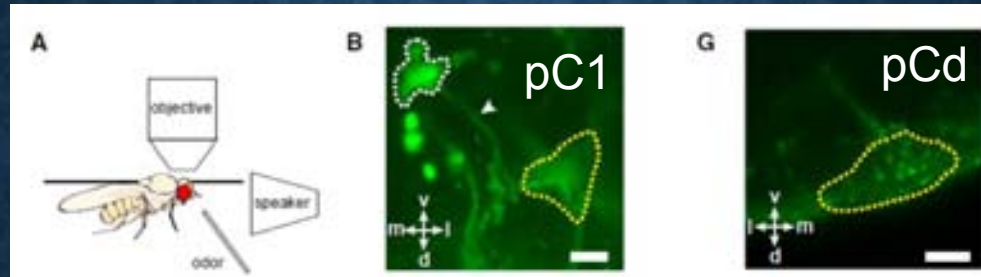
Silencing-Decreases female receptivity



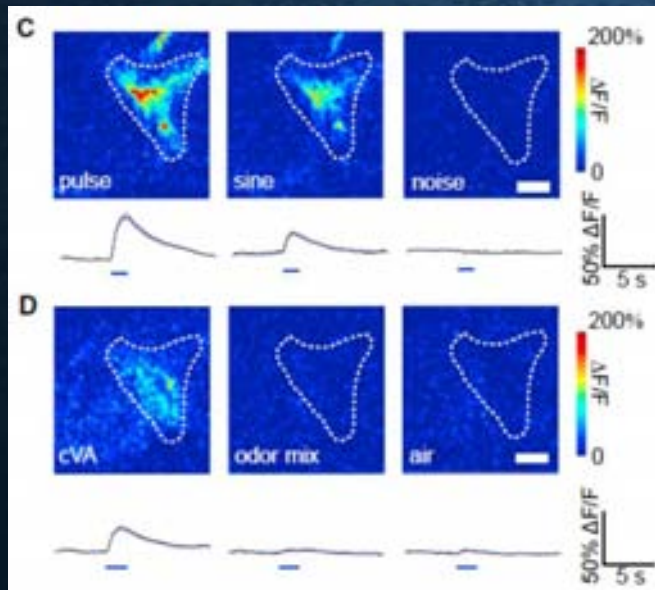
Activation-Enhances female receptivity



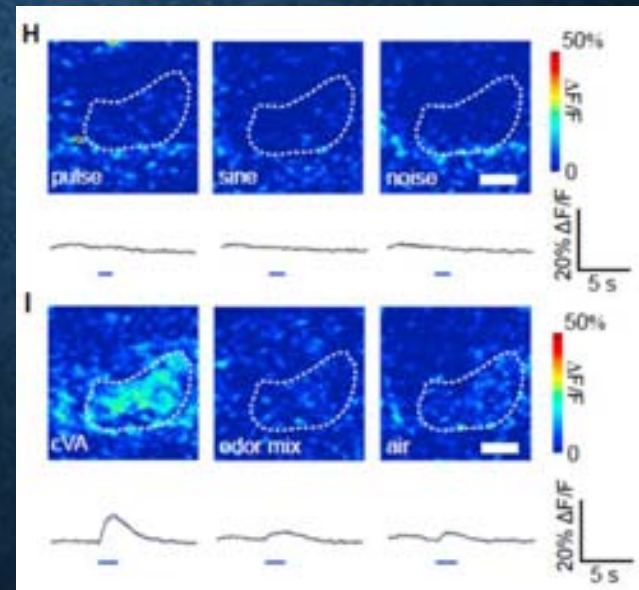
# Calcium responses of female pC1 and pCd neurons to courtship song and cVA



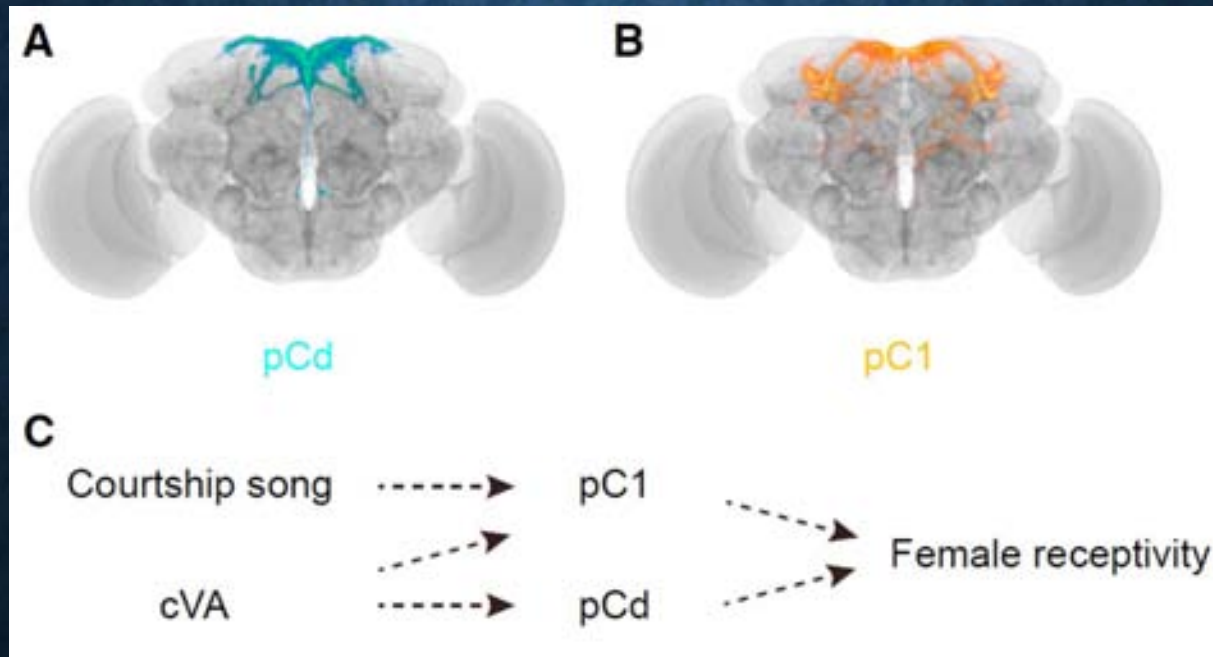
## pC1 Neuron responses



## pCd Neuron responses

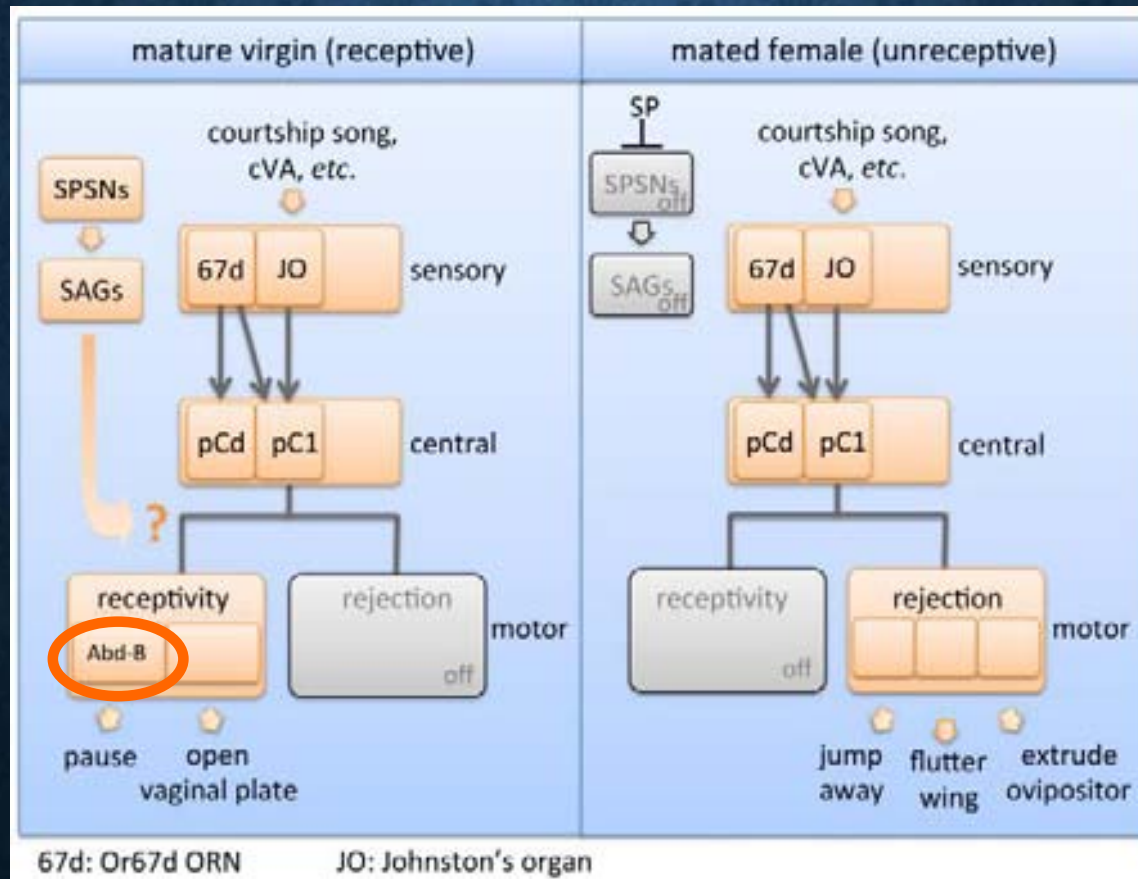


# pCd and pC1 Neurons function in female receptivity



# How does a female decide to copulate with a male?

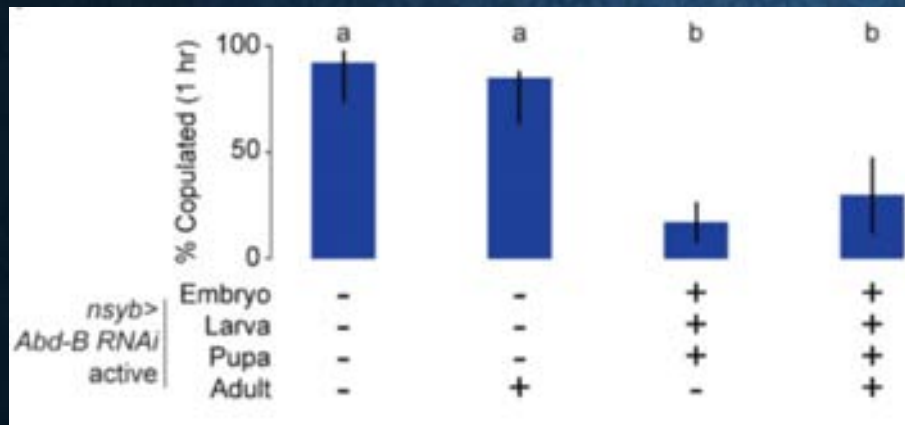
## -Receptivity circuit inside female





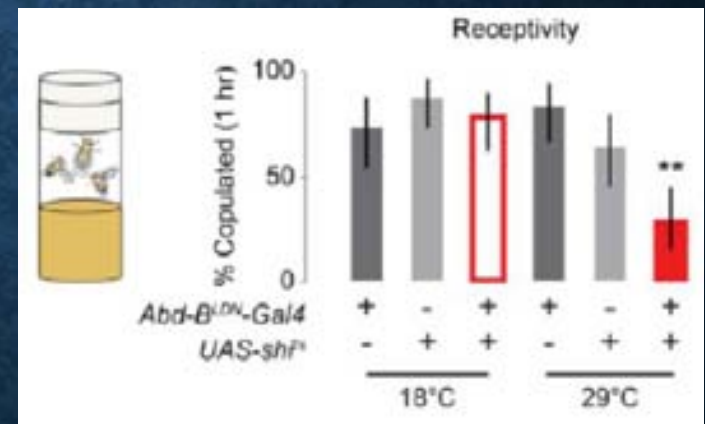
# Abd-B are required for virgin female receptivity

*Abd-B* plays a developmental role in forming the female receptivity circuit



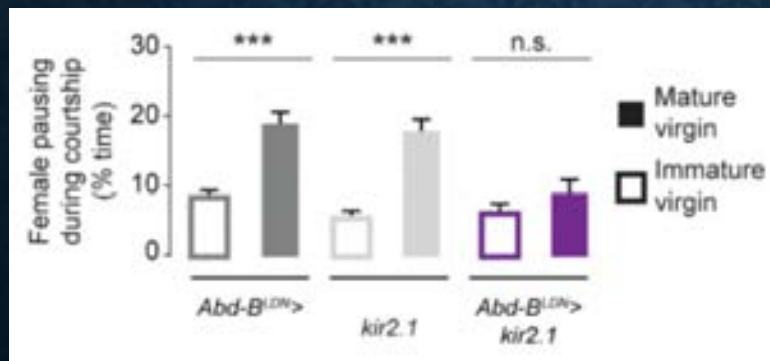
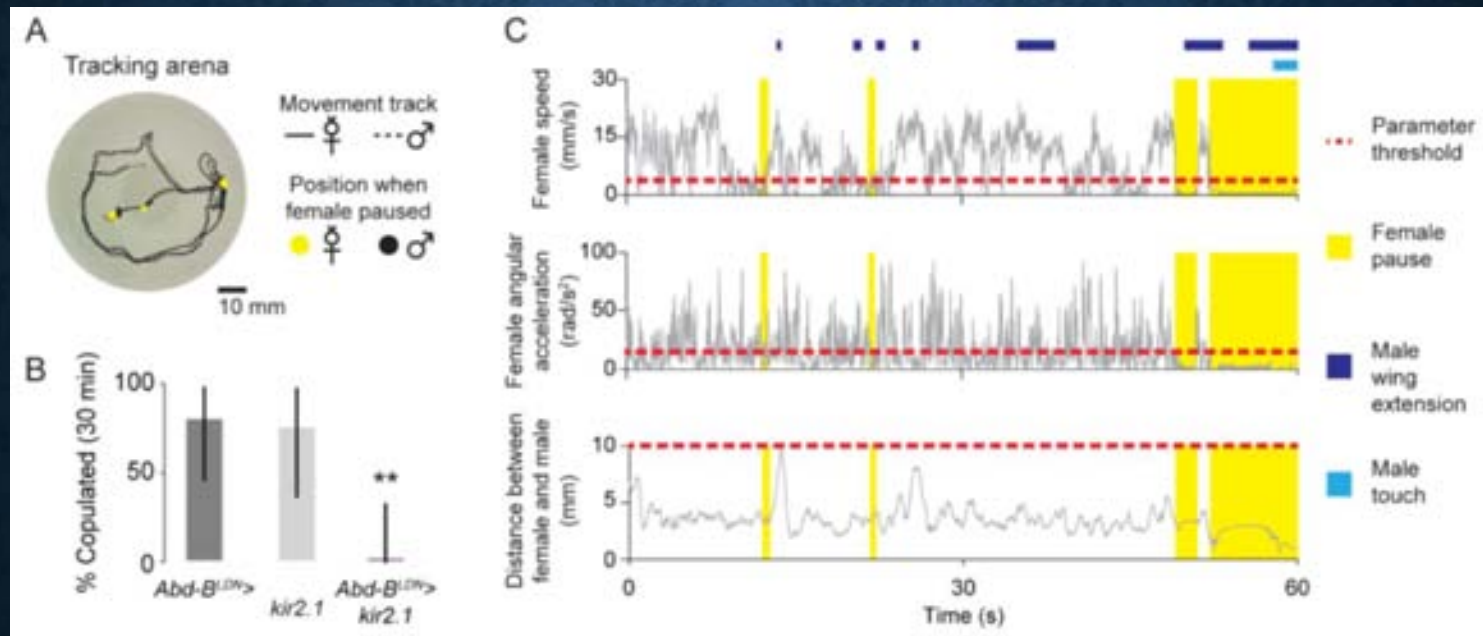
Abd-B , Abdominal-B  
homeobox (Hox) transcription factor

*Abd-B* neurons is functionally required for virgin female receptivity



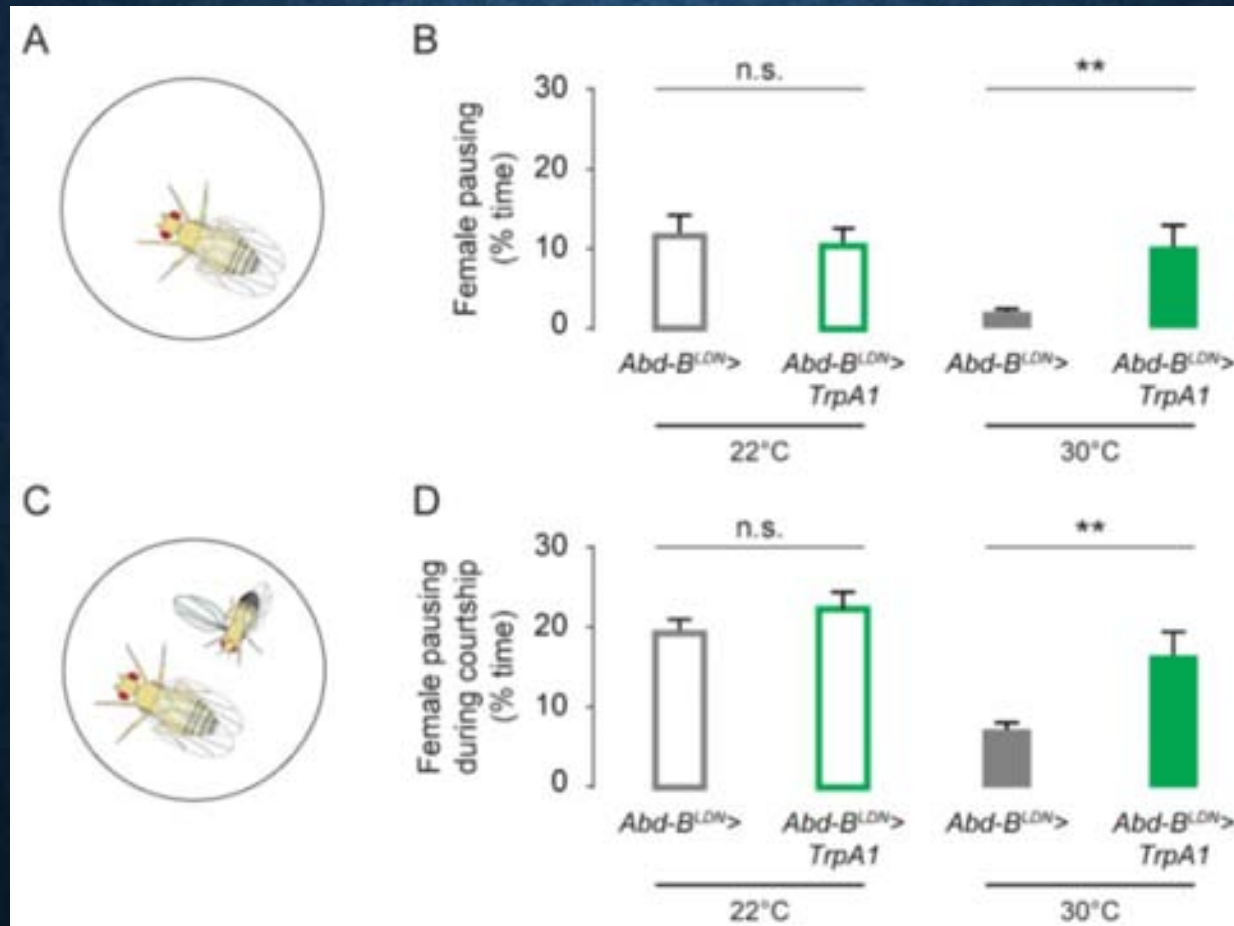


# Silencing *Abd-B* neurons decreases pausing during courtship

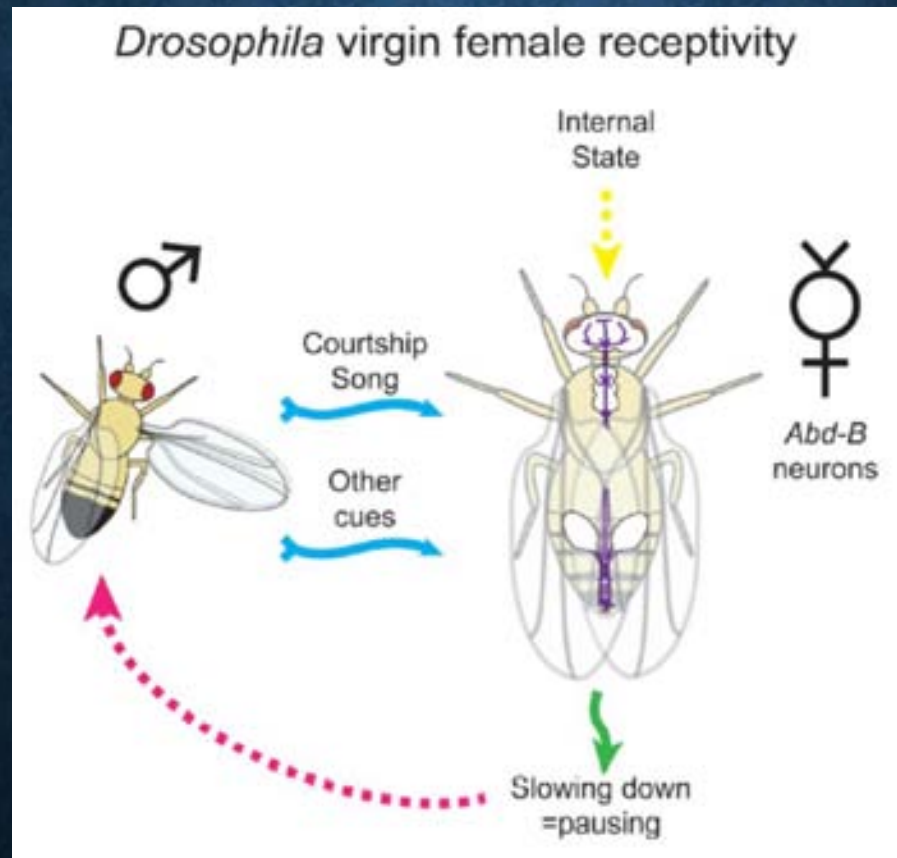


Bussell, J. J., et al. (2014)

# Activating *Abd-B* neurons induces pausing

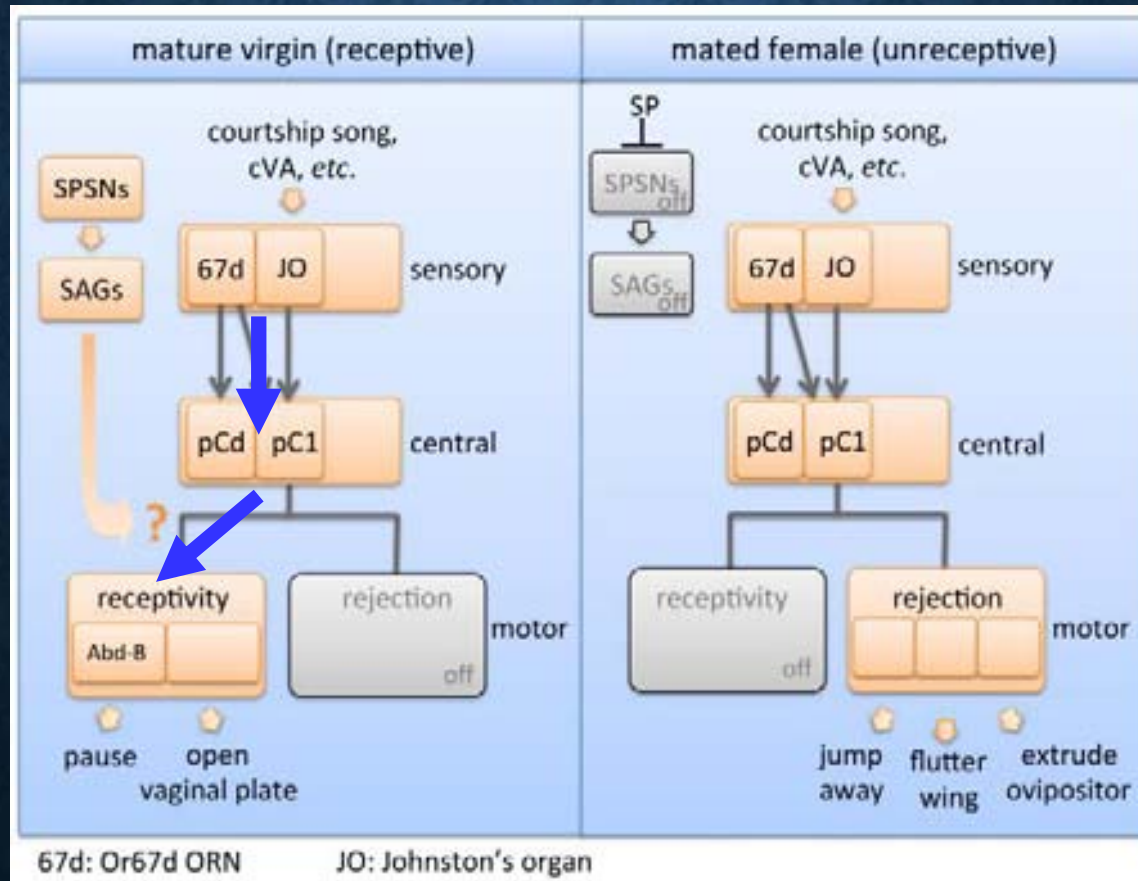


# Abd-B neurons are required for virgin female receptivity



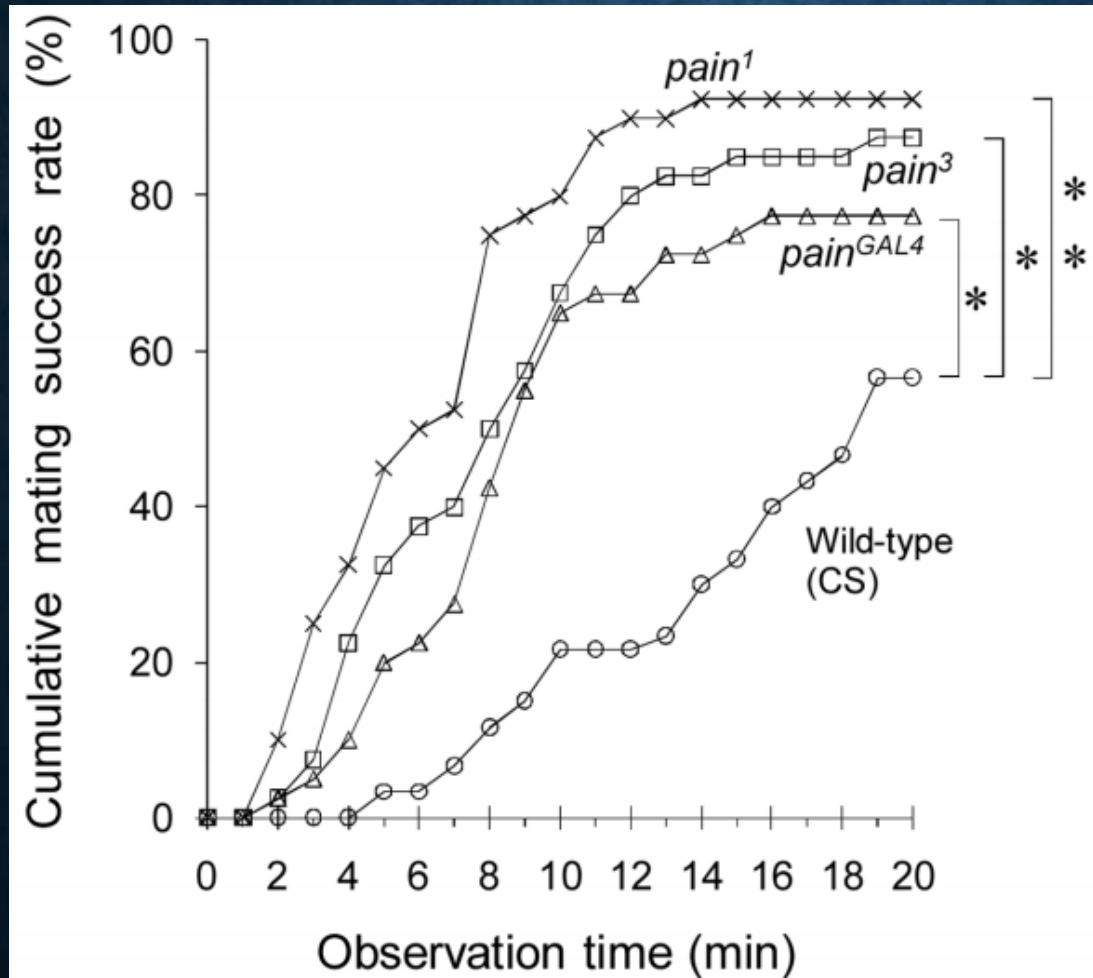
# How does a female decide to copulate with a male?

## -Receptivity circuit inside female



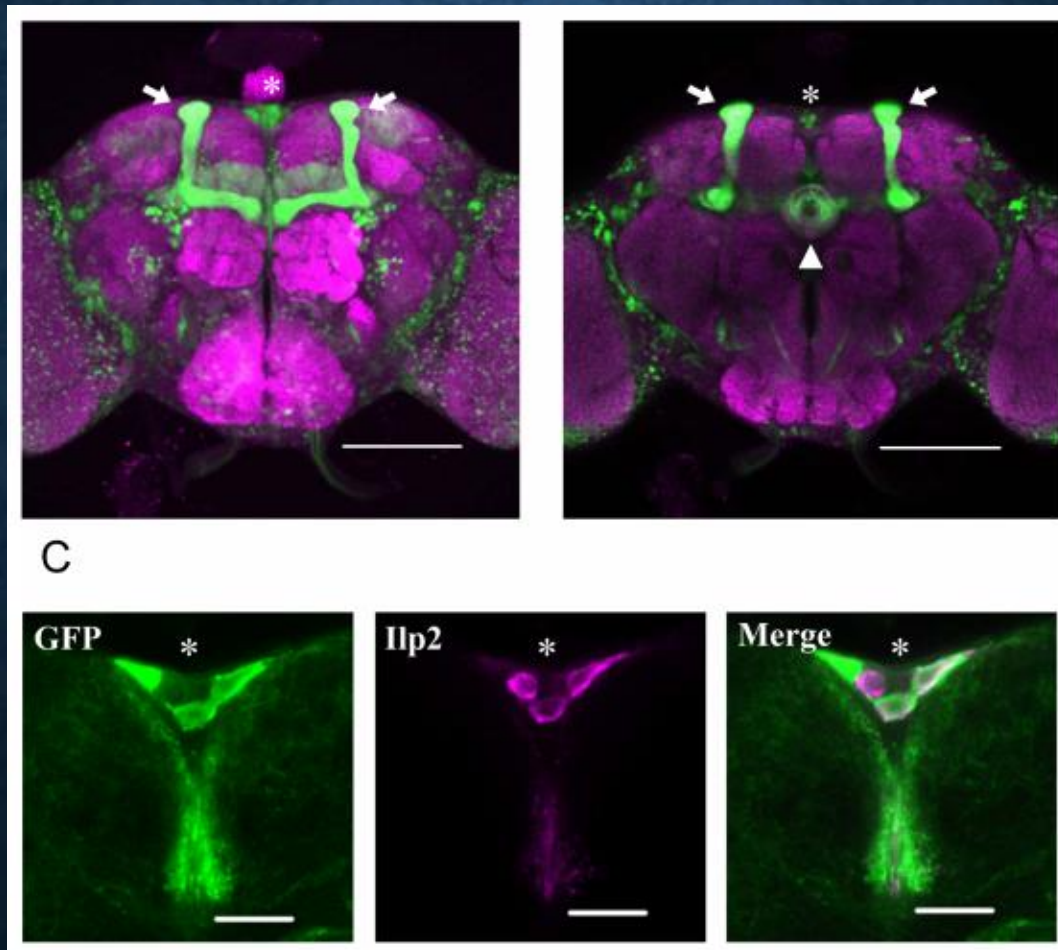


# *pain* (*painless*) mutant females copulate earlier than wild-type females

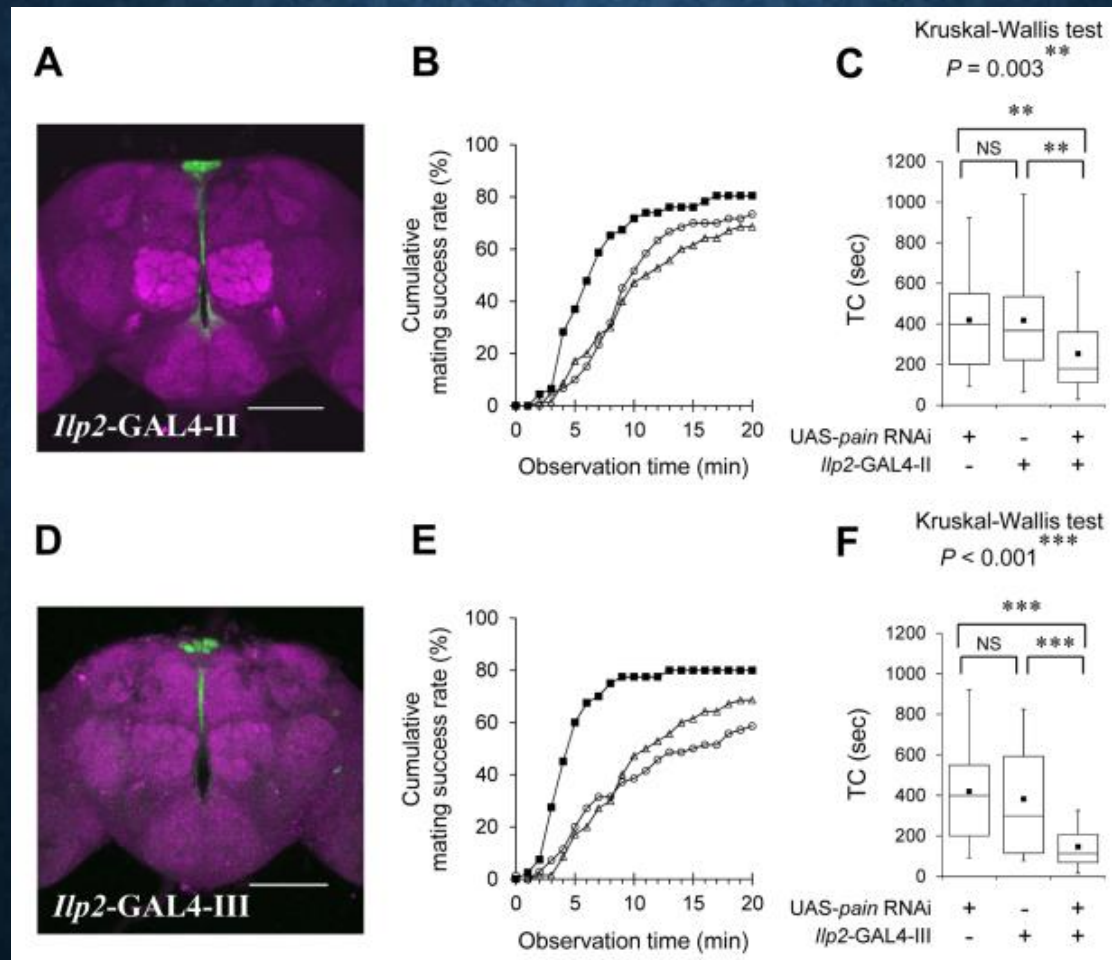


*pain*:  
a homolog of the  
mammalian  
*TRPA1/ANKTM1*

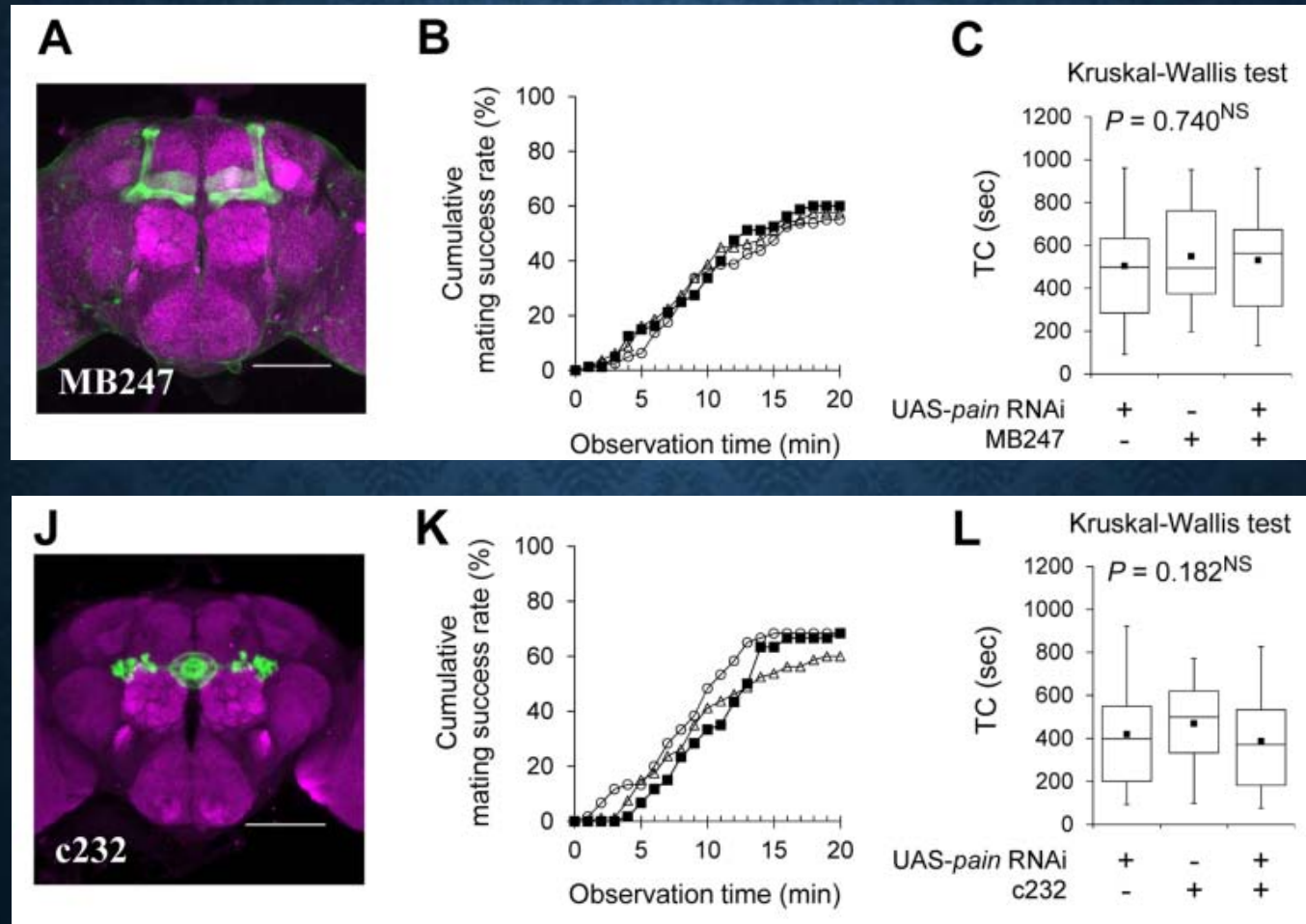
# *pain*-GAL4 drives GFP reporter expression in the female brain



# Knockdown of *pain* expression in IPCs enhances female sexual receptivity



# Knockdown of *pain* expression in the MBs or EB do not affect female sexual receptivity

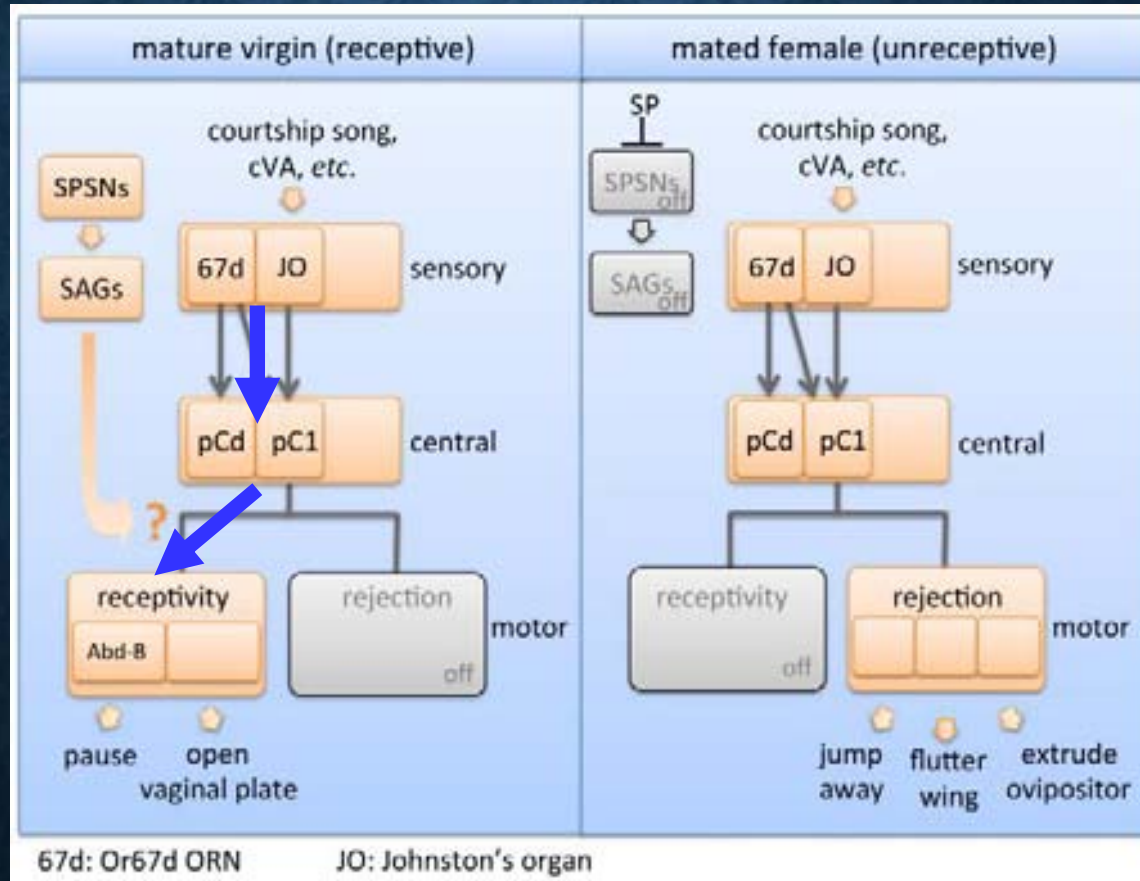




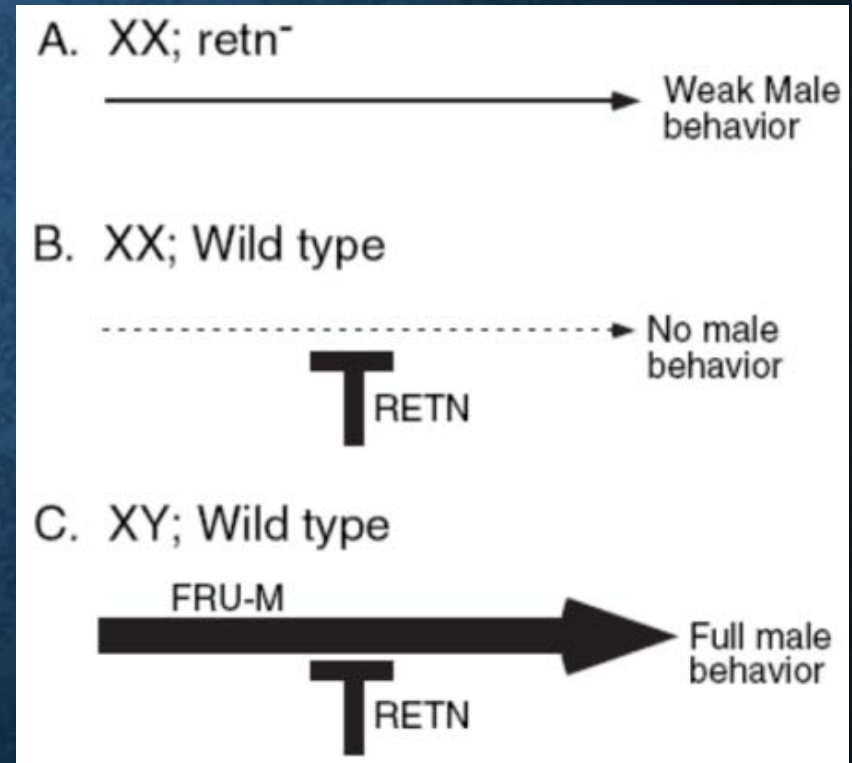
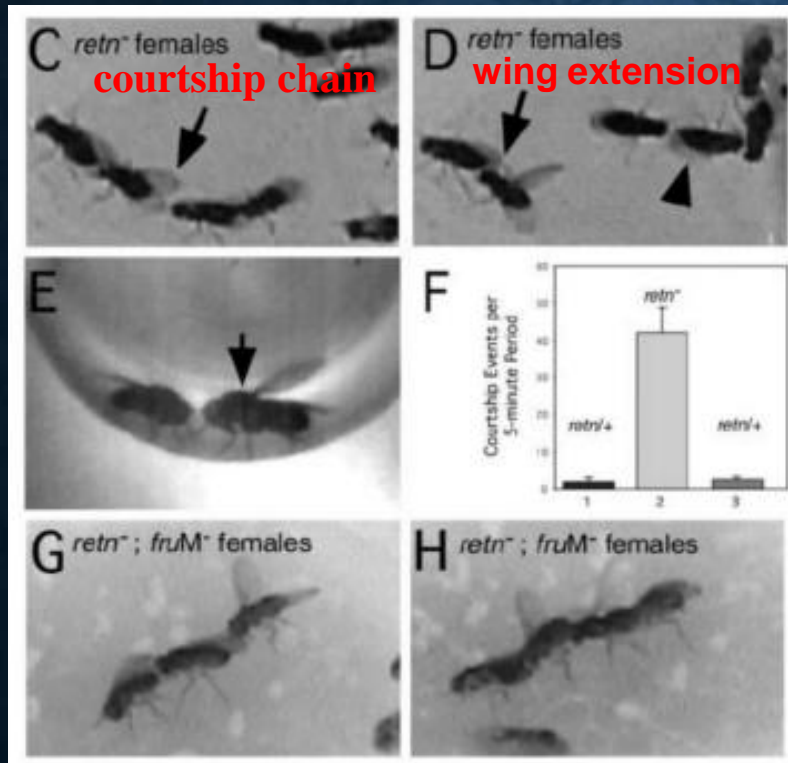
# How does a female decide to copulate with a male?

## -Receptivity circuit inside female

Other genes ?



# *retn* (*retained/dead ringer*) female behavior

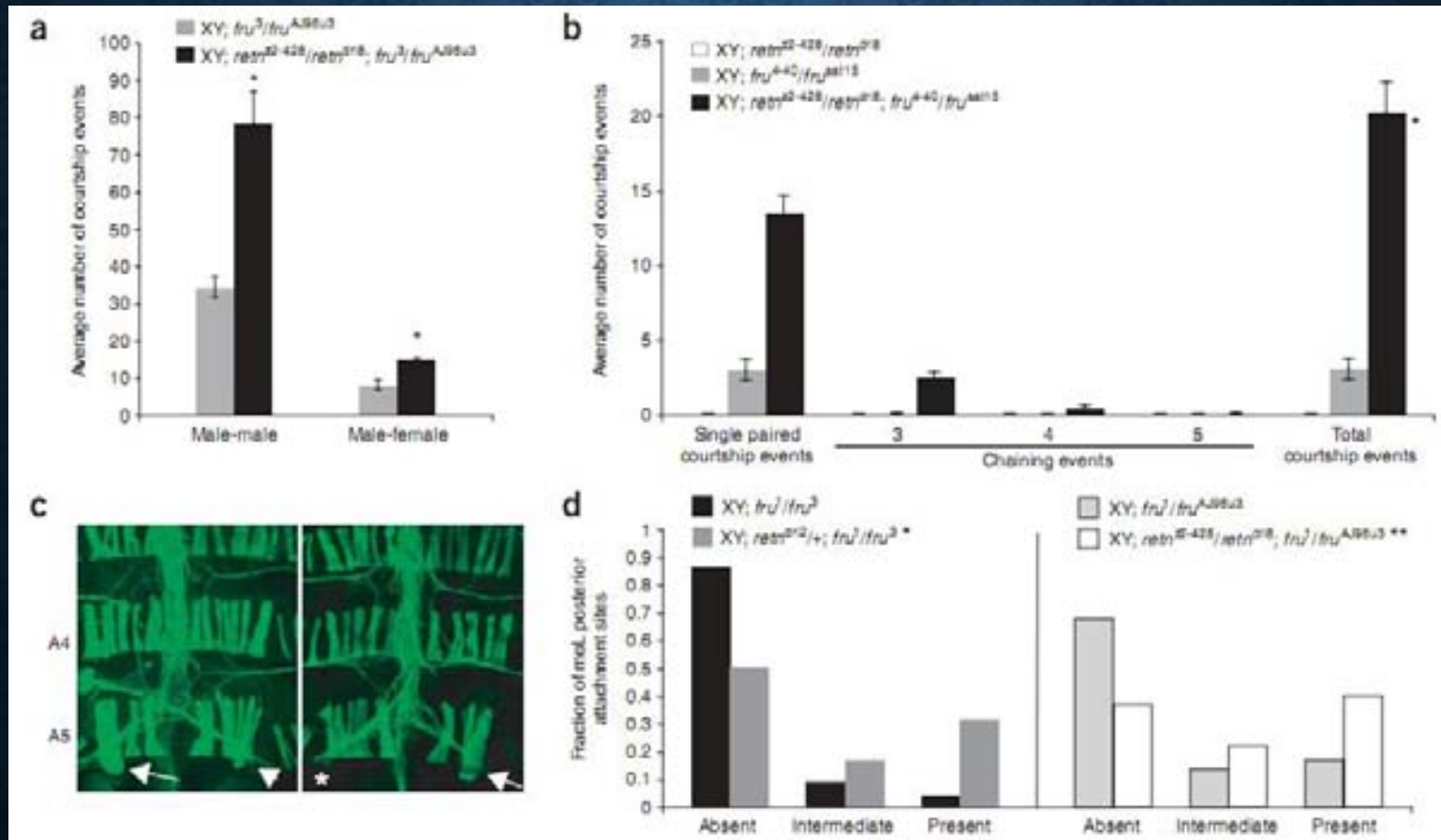


**RETN: A-T Rich Interaction Domain (ARID) transcription factor**

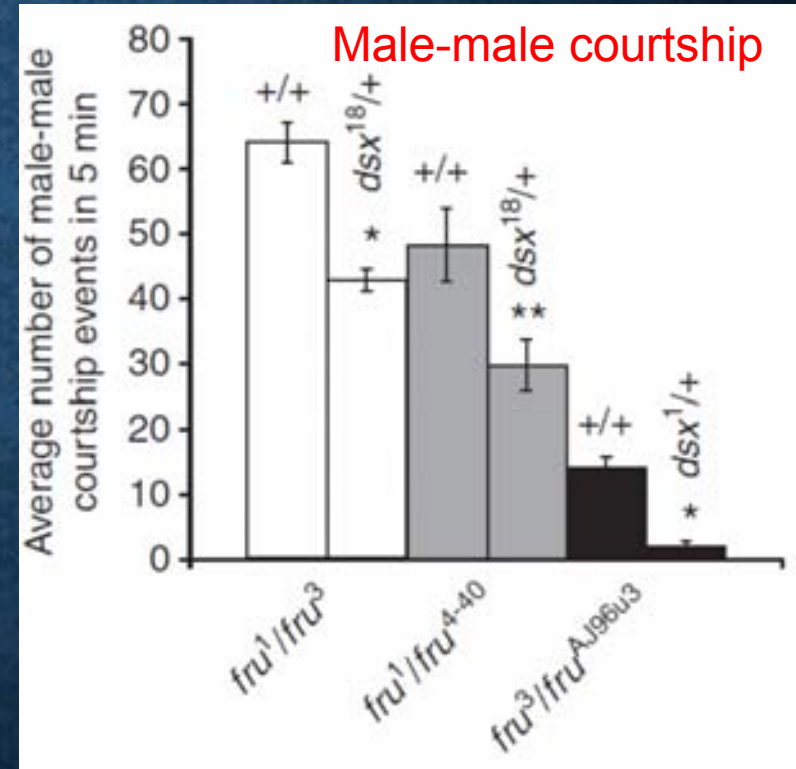
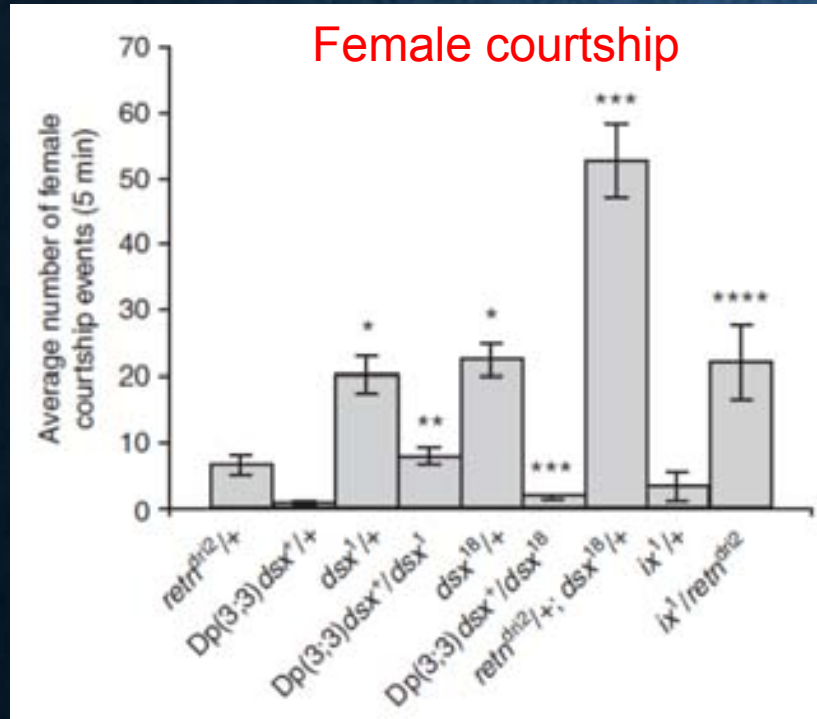
Ditch, L. M., et al. (2005)

# *retn* is antagonistic to *fru*<sup>M</sup> in production of courtship and development of the mol

*fru*<sup>M</sup>-males gain courtship activity with reduced *retn* function

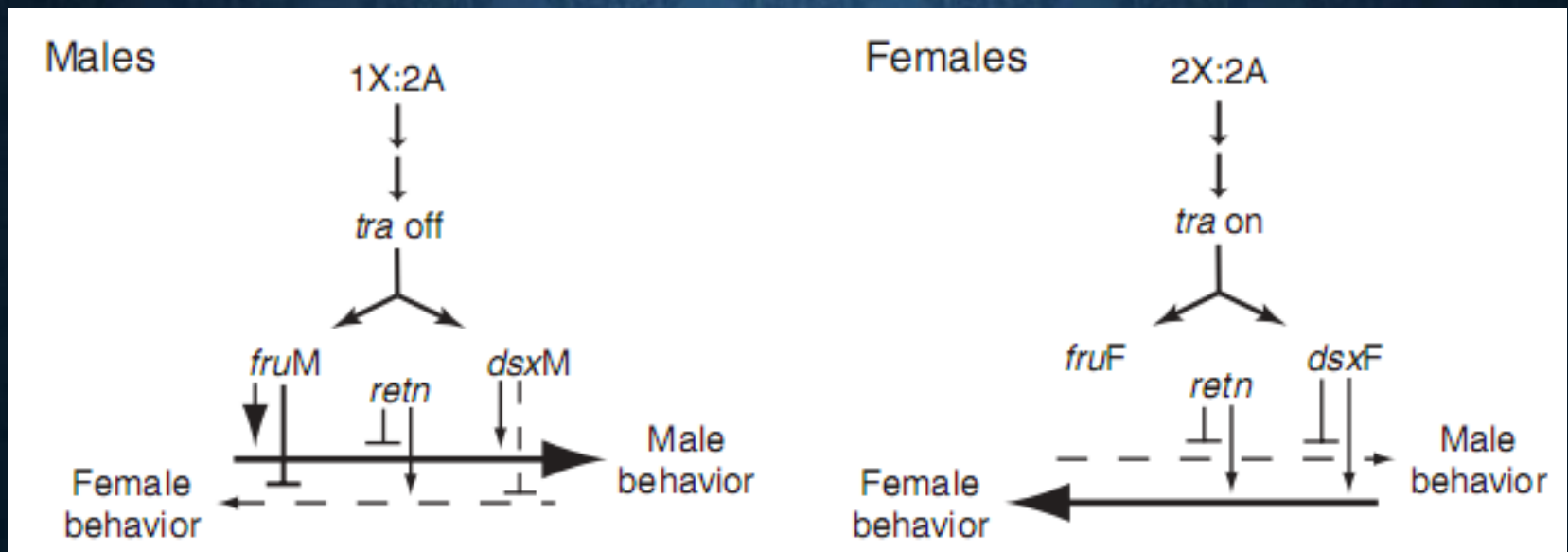


# *dsx* controls sexual behavior



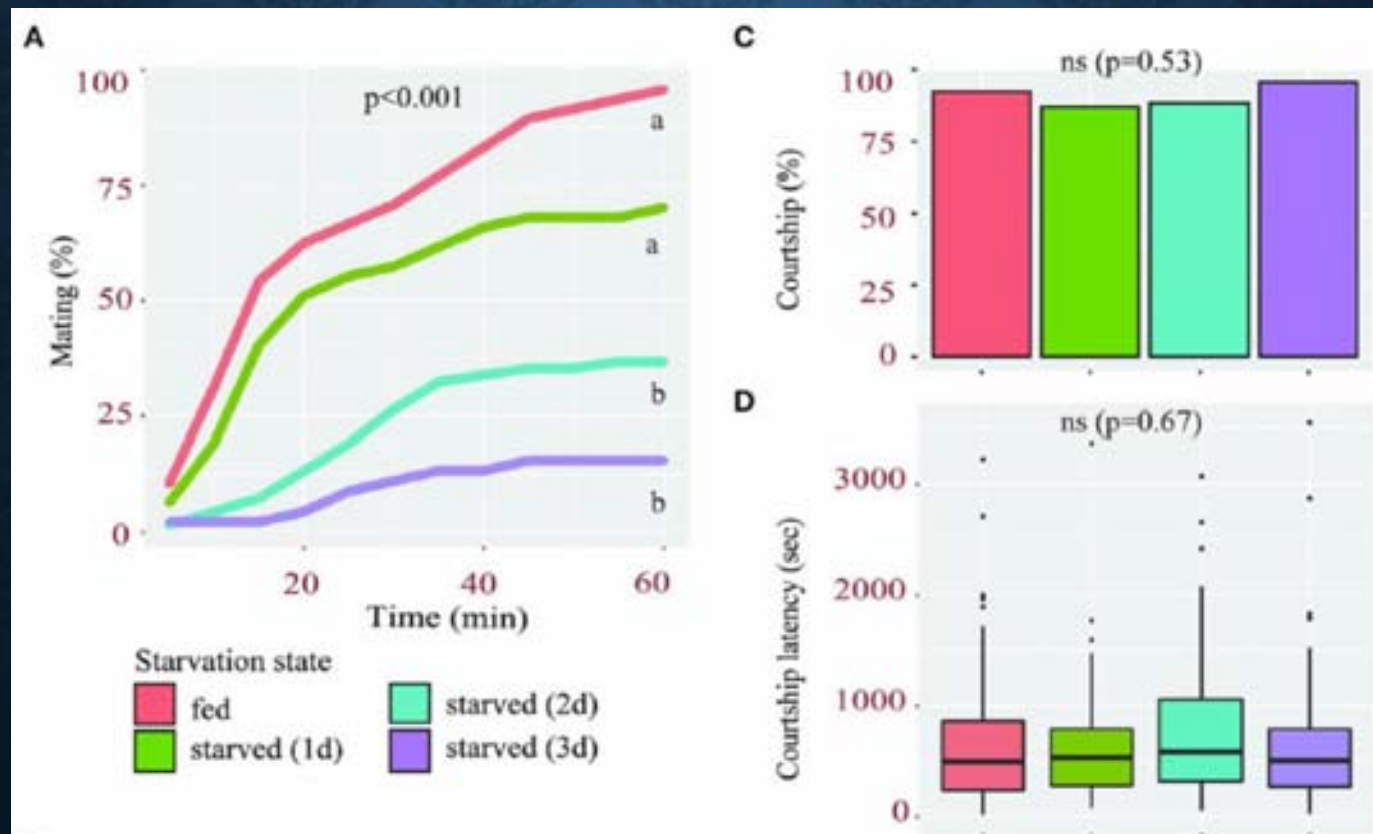


# *fru* and *dsx* act as parts of a 'switch' system controlling sexual behavior

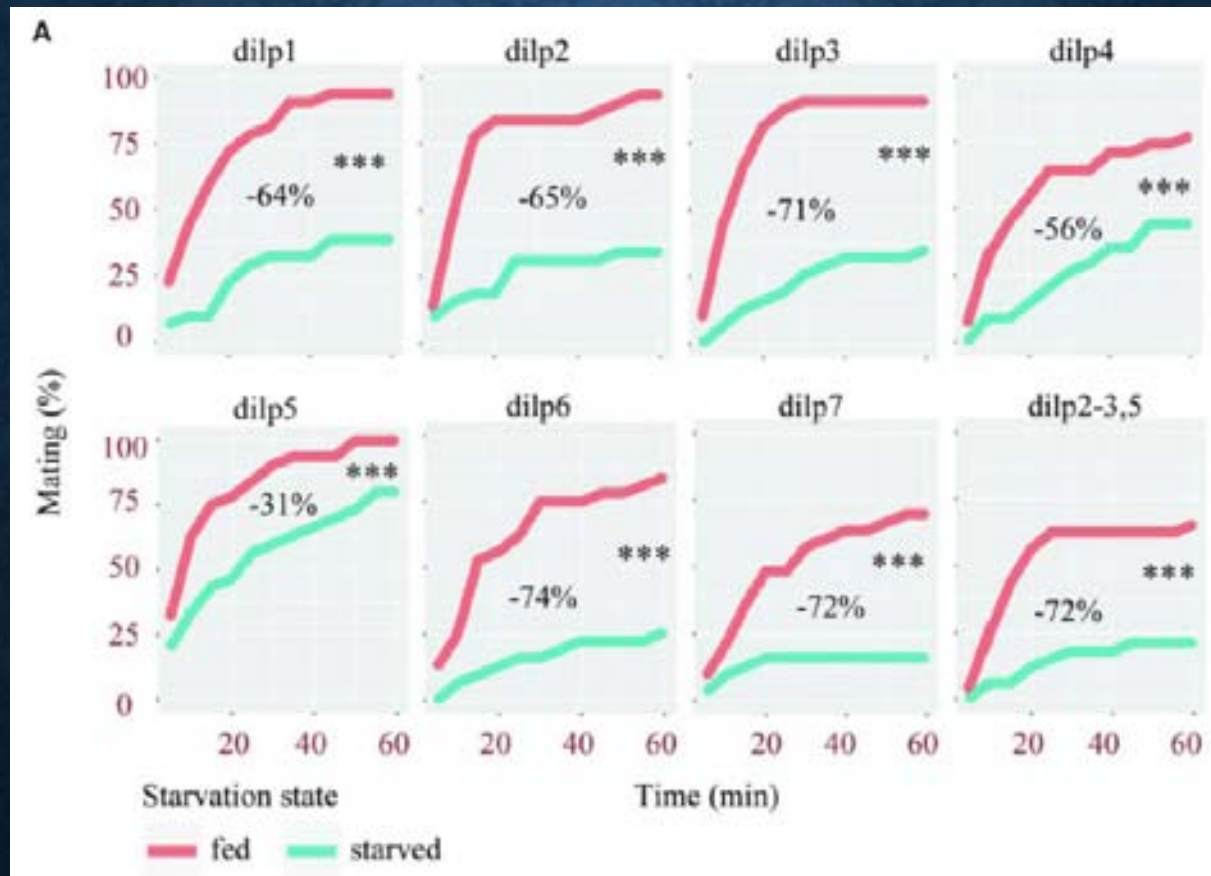


# Insulin signaling regulates female sexual receptivity during starvation in *Drosophila*

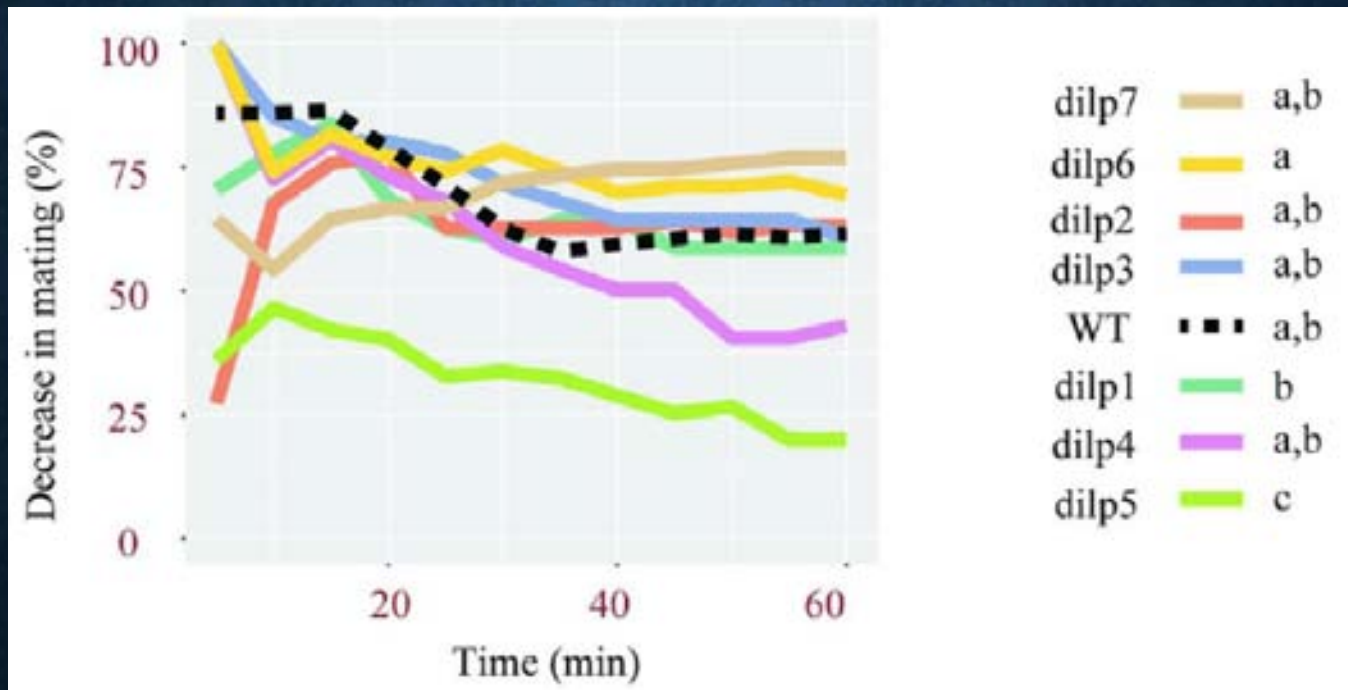
## Starvation regulates female sexual receptivity



# *dilp* genes showed significantly reduced sexual receptivity after starvation

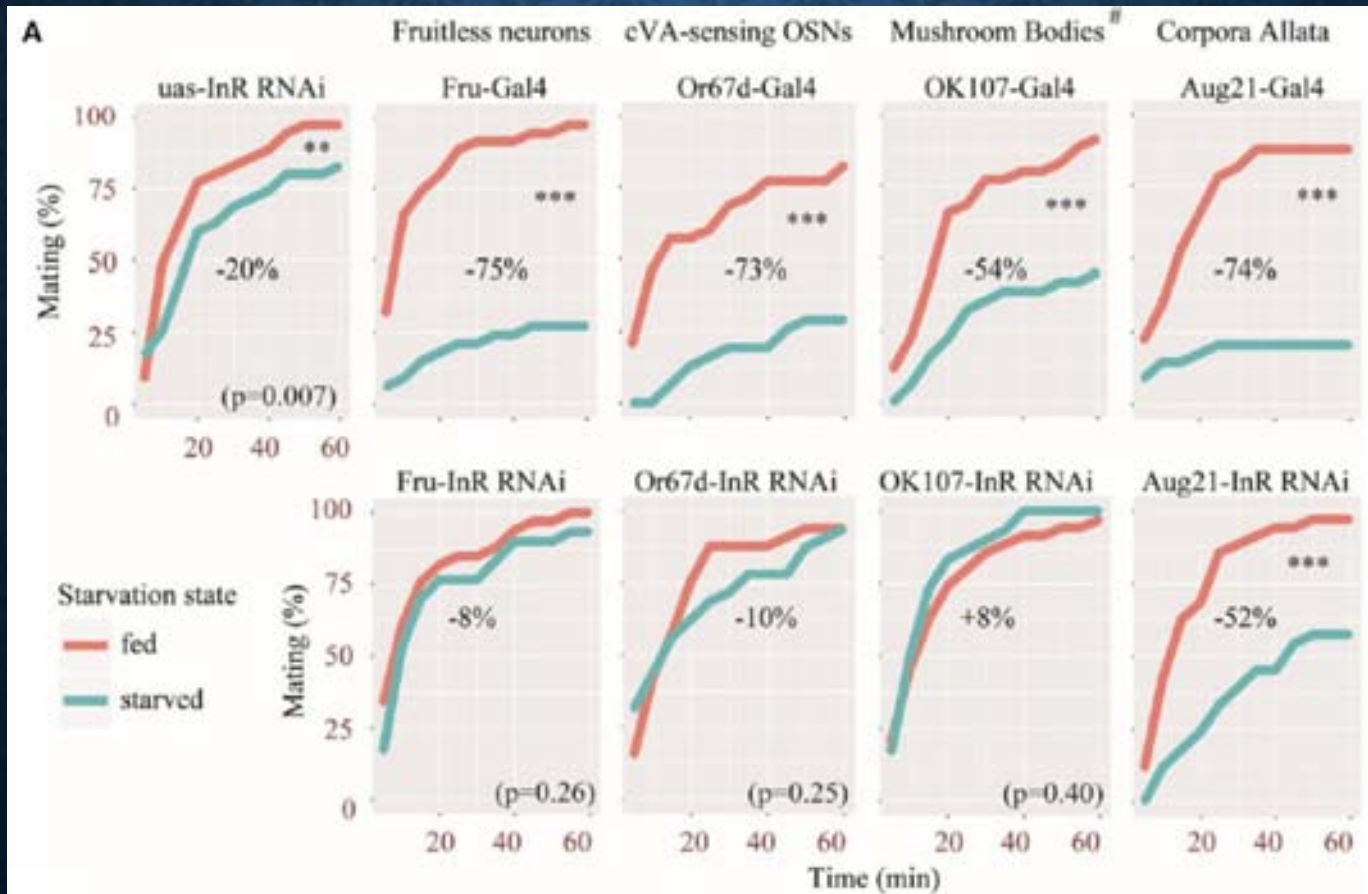


## Mutations of single *dilps* differentially affect female receptivity





# Disrupting the insulin signaling in specific neuronal circuitries inhibits the effect of starvation on sexual receptivity



Lebreton, S., et al. (2017)

# SUMMARY

- Whether virgin females accept males depends on multiple facts, courtship song, cVA, sensory neurons, CNS, transcription factors and so on.
- The mind of females would change after copulation, so called post-mating switch.

# References

- Yamamoto, D. and M. Koganezawa (2013). "Genes and circuits of courtship behaviour in *Drosophila* males." Nat Rev Neurosci **14**(10): 681-692.
- Liu, Y. and C. H. Yang (2014). "Unveiling the secrets to her heart." Neuron **83**(1): 3-5.
- Kurtovic, A., et al. (2007). "A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone." *Nature* 446(7135): 542-546.
- Carhan, A., et al. (2005). "Female receptivity phenotype of icebox mutants caused by a mutation in the L1-type cell adhesion molecule neuroglian." *Genes Brain Behav* 4(8): 449-465.
- Zhou, C., et al. (2014). "Central brain neurons expressing doublesex regulate female receptivity in *Drosophila*." *Neuron* 83(1): 149-163.
- Bussell, J. J., et al. (2014). "Abdominal-B neurons control *Drosophila* virgin female receptivity." *Curr Biol* 24(14): 1584-1595.



# References

- Sakai, T., et al. (2009). "The *Drosophila* TRPA channel, Painless, regulates sexual receptivity in virgin females." *Genes Brain Behav* 8(5): 546-557.
- Sakai, T., et al. (2014). "Insulin-producing cells regulate the sexual receptivity through the painless TRP channel in *Drosophila* virgin females." *PLoS One* 9(2): e88175.
- Ditch, L. M., et al. (2005). "*Drosophila* retained/dead ringer is necessary for neuronal pathfinding, female receptivity and repression of fruitless independent male courtship behaviors." *Development* 132(1): 155-164.
- Shirangi, T. R., et al. (2006). "A double-switch system regulates male courtship behavior in male and female *Drosophila melanogaster*." *Nat Genet* 38(12): 1435-1439.
- Lebreton, S., et al. (2017). "Insulin Signaling in the Peripheral and Central Nervous System Regulates Female Sexual Receptivity during Starvation in *Drosophila*." *Front Physiol* 8: 685.



# CONTENT

- Part I Overview, virgin female receptivity
- Part II Sex-peptide (SP) signaling in post-mating switch
- Part III Neuronal modulation of post-mating behaviors

## Overview:

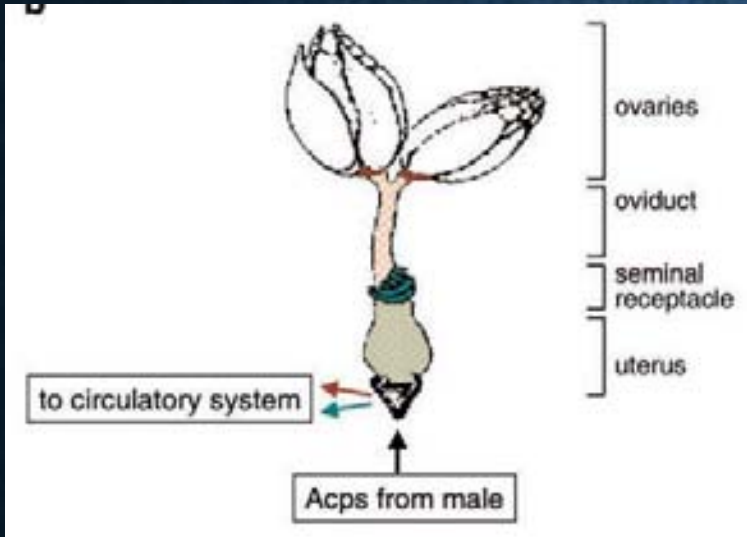


Fig. Female genital tract simplified map:

From:MF wolfneer. 2002. The gifts that keep on giving physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*.

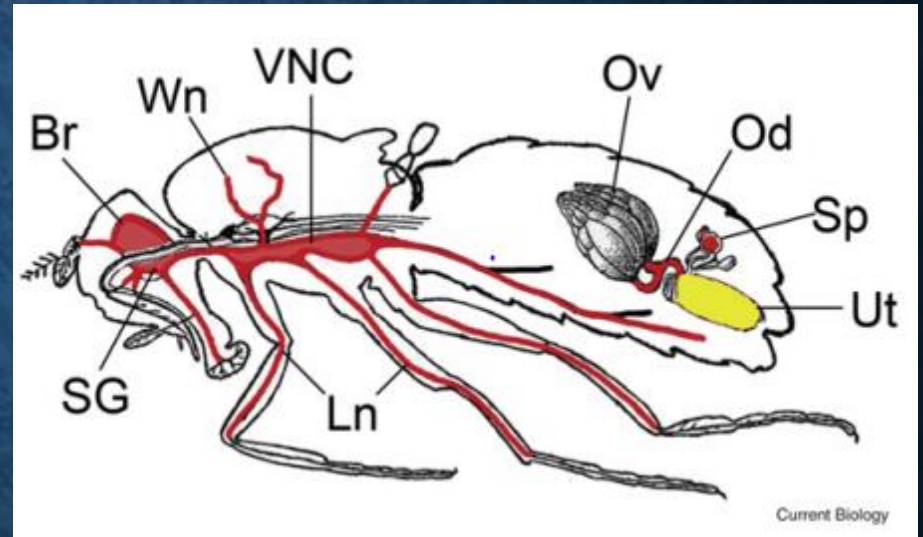
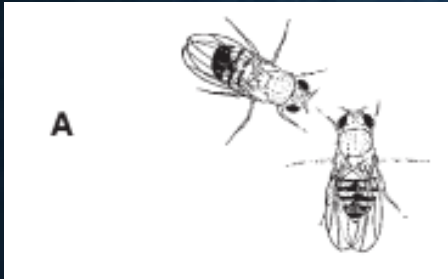


Fig. Distribution of sex Peptide Receptor and sp Binding Patterns in Adult Female *Drosophila*:

From:E. Kubli. 2008. Sexual behaviour a receptor for sex control in *Drosophila* females.

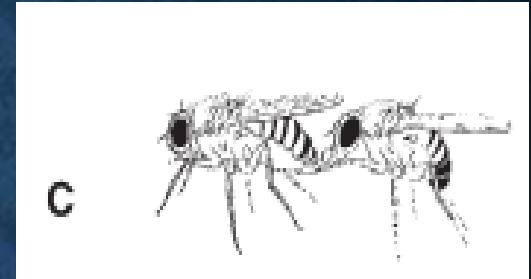
## Mating behavior:



A. Orientation of the male towards the female.



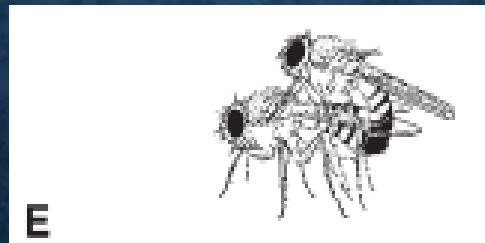
B. Love song of the male.



C. The male licks the genitalia of the female.



D. Attempted copulation.



E. Copulation.



F. A rejection response by the female.

## Female Post-mating behavior in *drosophila*:

- 1、 Stimulate female egg production and ovulation.
- 2、 Reduce their receptivity to mating.
- 3、 Mediate sperm storage.
- 4、 A mated female has a short lifespan.



seminal fluid



## The function of sperm and seminal fluid proteins:

<b>sperm</b>	<b>Fertilization</b>
	<b>Increase egg production rate</b>
	<b>Store sperm to maintain the state of intersection</b>
	<b>Sperm competition</b>
<b>seminal fluid proteins</b>	<b>Increase egg production rate</b>
	<b>Reduce female lifespan</b>
	<b>Reduce their receptivity to mating</b>
	<b>Mediate sperm storage</b>

## Accessory glands proteins:

### Acps: accessory gland proteins

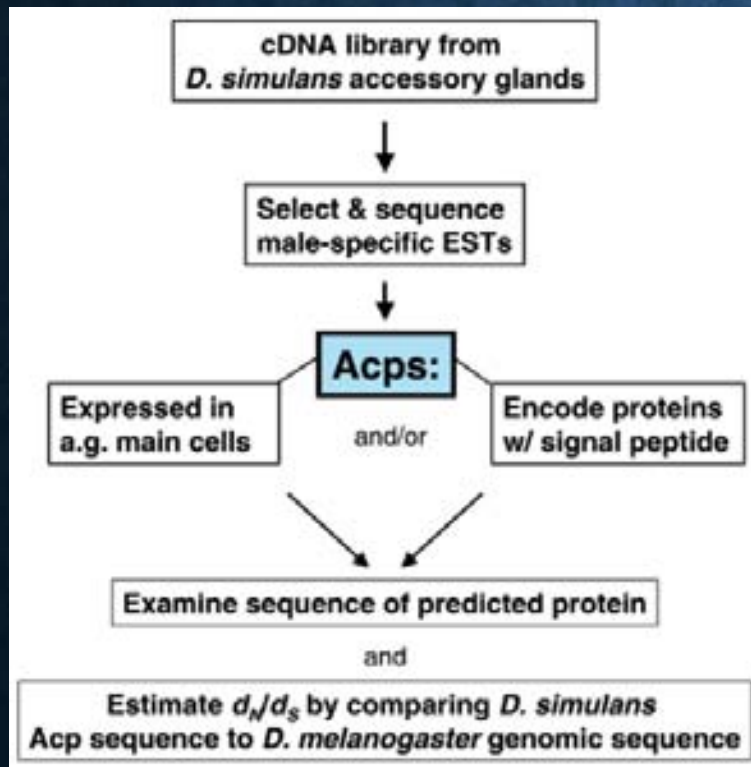


Fig. The “evolutionary EST screen” that identified Acp genes.

### The 83 predicted Acps include:

- **Peptides (25) or prohormone precursors**  
e.g. the ovulation hormone Acp26Aa (ovulin)  
the ‘sex peptide’ Acp70A
- **Glycoproteins**  
e.g. the sperm storage protein Acp36DE
- **Modifying enzymes**  
Proteases (9)  
Protease inhibitors (8)  
e.g. the trypsin inhibitor Acp62F  
Lipases (6)
- **Many novel proteins**
- **~ 11% with signs of rapid evolution**

From:MF wolfneer. 2002. The gifts that keep on giving physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*.

# Sites of synthesis of seminal fluid proteins:

## Accessory glands proteins:

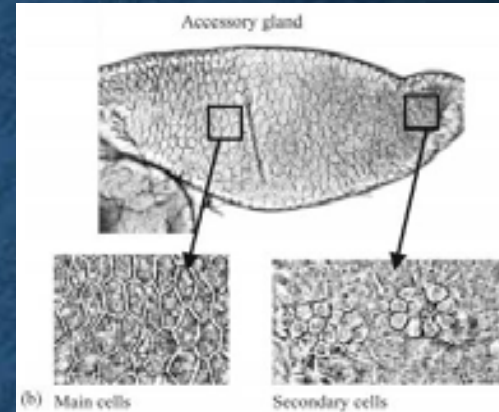
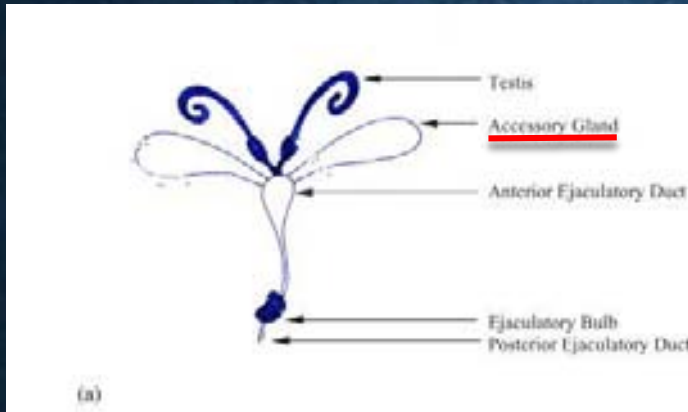


Table 1  
Sites of synthesis of seminal fluid proteins

Site of synthesis	Nature of secreted substances	References
Accessory gland main cells	An estimated 83 accessory gland proteins, many with unknown functions. Acps include peptides, prohormones, glycoproteins, enzymes (putative proteases, protease inhibitors, lipases) and antibacterial peptides	[29,34,36,89,91,100,107,117]
Accessory gland secondary cells	Filaments of unknown constituents	[5,83]
Ejaculatory duct	Dup 99B (peptide) Esterase-6 (enzyme) Glucose dehydrogenase (enzyme) Andropin (peptide) Drosomycin (peptide)	[90] [44,71] [17,18] [66,89] [39]
Ejaculatory bulb	PEB-me (protein) <i>cis</i> -Vacacenyl acetate (lipid) Esterase-6 (enzyme) Drosomycin (peptide)	[63,67] [14,16] [99,105] [39]

From: Tracey Chapman. 2004. Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies.

Table 2

Summary of known functions of seminal fluid peptides

Trait	Acp(s) responsible	References
Egg-laying	Acp70A Acp26Aa Dup99B <sup>a</sup>	[21,29,62] [22,50–52] [90]
Receptivity	Acp70A Dup99B <sup>a</sup>	[21,29,62] [90]
Sperm storage	Main cell Acps Acp36DE	[56,112] [12,78]
Sperm displacement	Main cell Acps	[49,86]
Sperm competition	Acp26Aa, Acp29AB, Acp36DE, Acp53Ea Acp36DE	[32] [24]
Protection against bacterial infections	Andropin <sup>b</sup> Two unidentified Acps <sup>b</sup>	[89] [66]
Protection against proteolysis	Acp62F <sup>c</sup> Acp76A <sup>c</sup>	[64] [34,117]
Protection against fungal infections	Drosomycin <sup>c</sup>	[39]

<sup>a</sup> Phenotype is observed in response to ectopic injections, no effects of Dup99B are seen when females are mated to Dup99B-deficient males.

<sup>b</sup> In in vitro assays.

<sup>c</sup> Putative functions which need further confirmation, e.g. in assays with Acp-deficient males.

➤ Egg-laying: Acp70A (sex peptide)、Acp26Aa、Dup99B.

➤ Receptivity: Acp70A、Dup99B.

➤ Sperm storage: Acp36DE.

From: Tracey Chapman. 2004. Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies.



## Summary:

- 1、 Male *drosophila* provide information on post-mating behavior.
- 2、 Multiple seminal fluid proteins work together on post-mating behavior.
- 3、 Typical postmating behavior is mainly caused by sex peptide.



Sex-peptide controls female post-mating behavior in *Drosophila*.

## Structure and function analysis in Sex peptide:

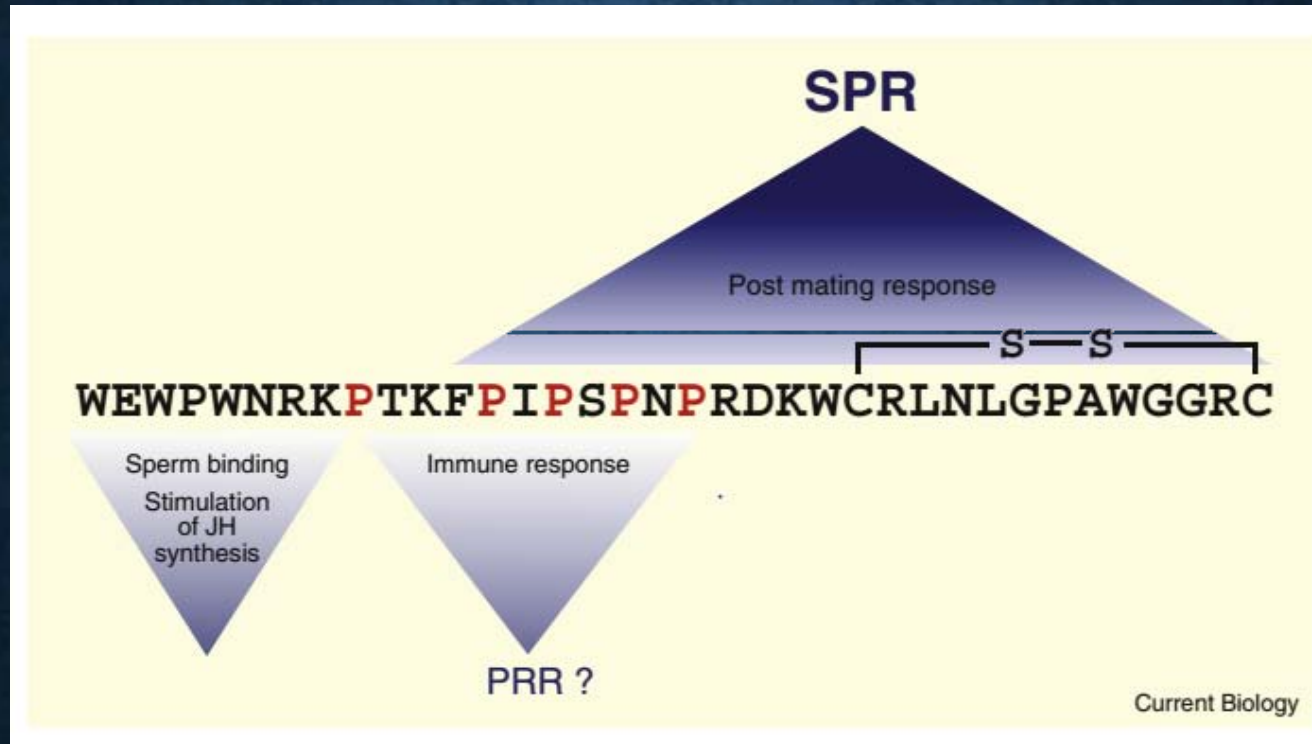


Fig. Structure–function relationship in Sex-peptide.

Several receptors may interact with SP. SP receptor very likely interacts with the carboxy-terminal part of SP known to be essential for eliciting the post-mating responses. The prolines indicated in red are hydroxylated and may interact with pattern recognition receptors (PRR) and thus induce antimicrobial peptide synthesis. The amino-terminal part of SP is essential for inducing the synthesis of juvenile hormone (JH) and for binding to sperm.

From: E. Kubli. 2008. Sexual behaviour a receptor for sex control in *Drosophila* females.

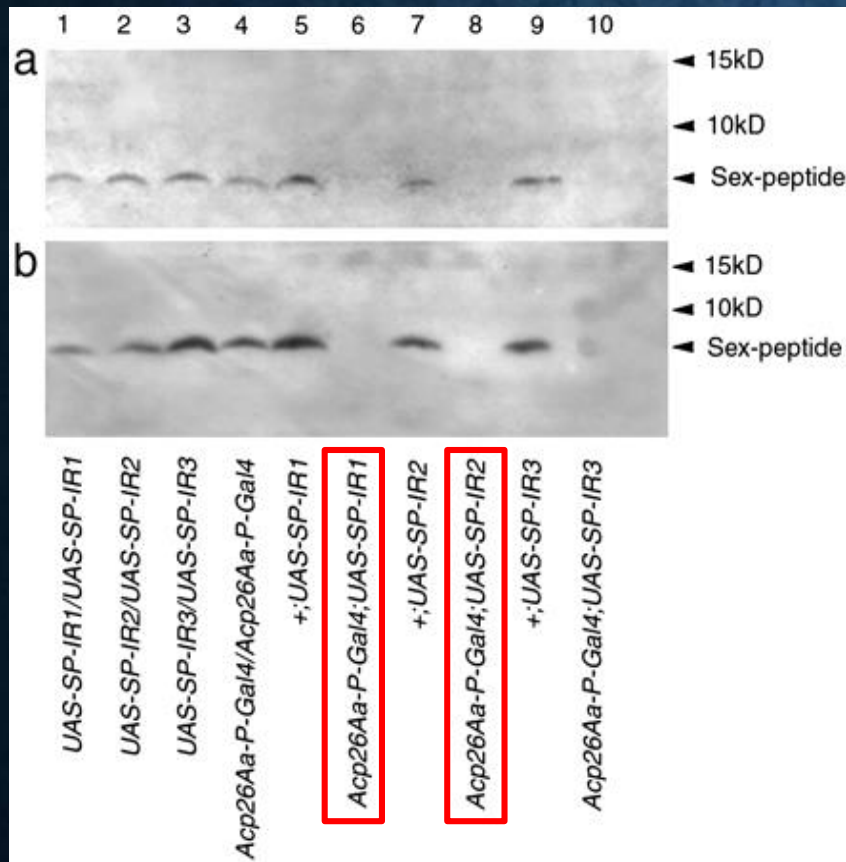


Fig. Female post-mating responses analyzed by using RNA interference:

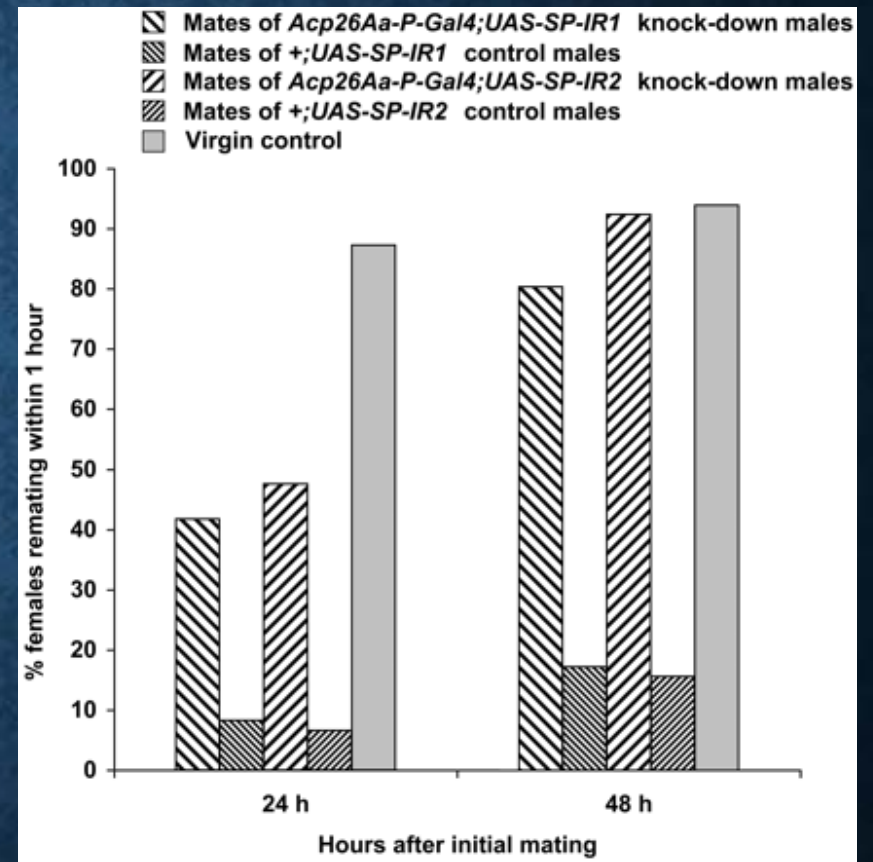


Fig. Effect of SP on female receptivity:



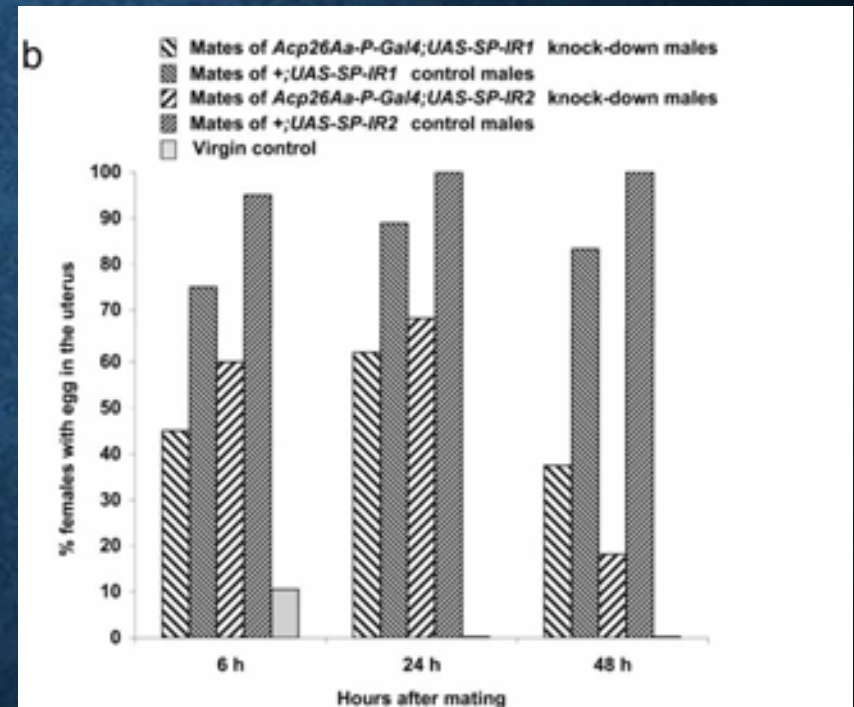
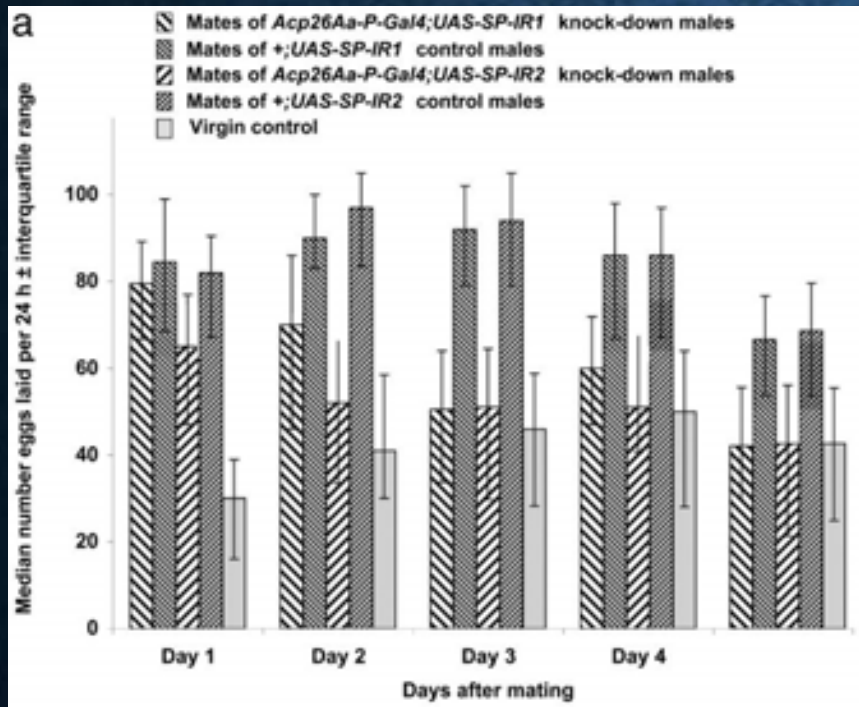


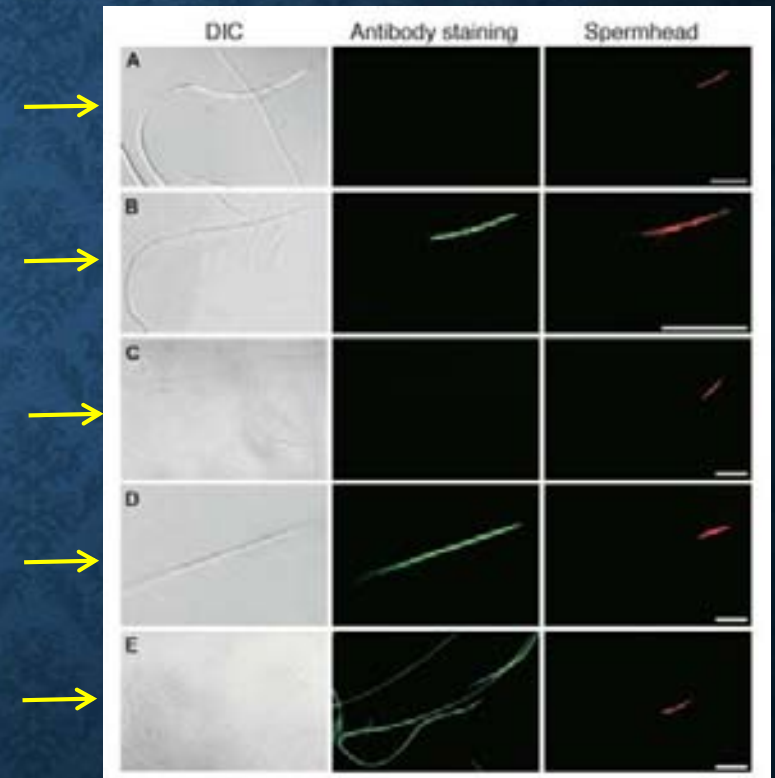
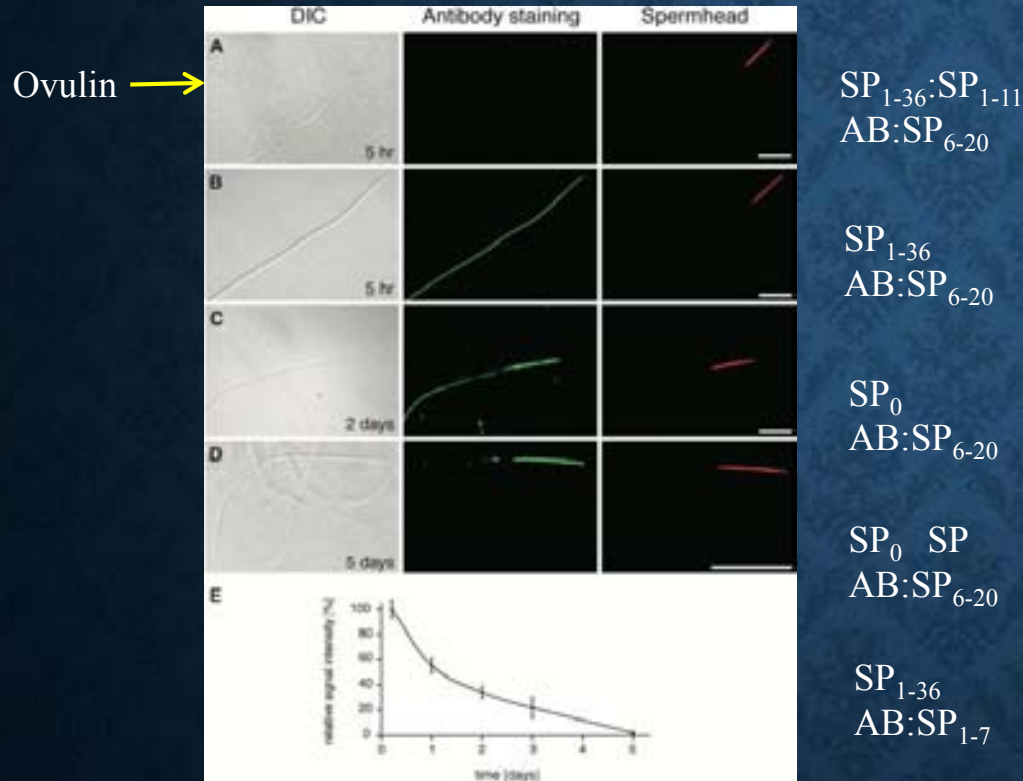
Fig a and b. Effect of SP on oviposition and ovulation:

From: Tracey Chapman. 2003. The sex peptide of *Drosophila melanogaster* female post-mating responses analyzed by using RNA interference.

## Molecular mechanisms of sex peptides and sex peptide receptors in post-mating response:

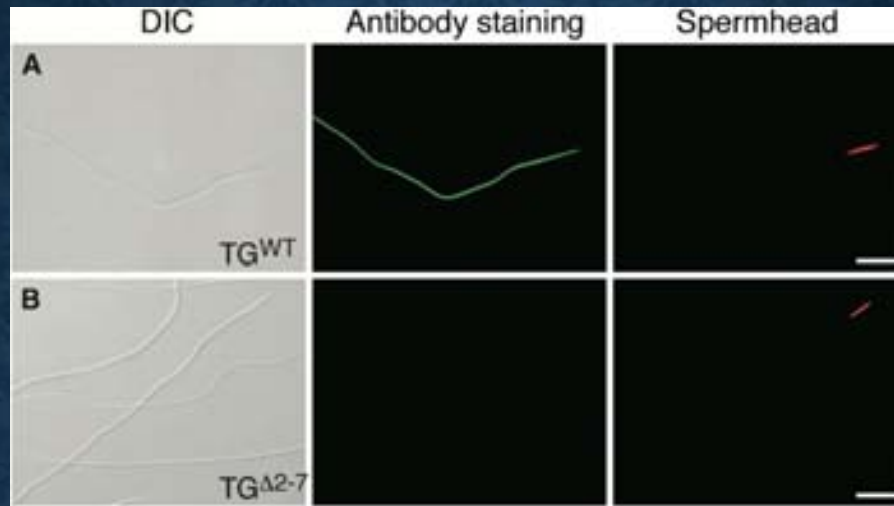
- 1、 Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*.
- 2、 Sex peptide receptor mediates the post-mating switch in *Drosophila* reproductive behaviour.
- 3、 SPR acts in nervous system.

## Sex peptide regulates the gradual release of sperm in female drosophila:



From: Jing Peng. 2005. Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*.

Unmodified SP →



SP lacking the N-terminal →

Fig. Sex-Peptide Lacking the N-Terminal End Cannot Bind to Sperm.

Unmodified SP →



A modified trypsin cleavage site. →

Fig. SP Containing a Modified Trypsin Cleavage Site Binds to Sperm Permanently and Cannot Be Cleaved.

From: Jing Peng. 2005. Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*.



## Conclusions:

In sum, the PMR of *D. melanogaster* females can be divided into two phases: the short-term PMR and the long-term PMR, respectively. The short-term PMR are induced immediately after mating mainly by free SP, the long-term PMR, lasting about one week, by the C-terminal SP fragment cleaved from SP bound to the sperm tail. Both responses likely elicit the PMR by binding of SP to specific sites in the central and peripheral nervous systems.

## Molecular mechanisms of sex peptides in post-mating responses:

- 1、 Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*.
- 2、 Sex peptide receptor mediates the post-mating switch in *Drosophila* reproductive behaviour.
- 3、 SPR acts in nervous system.

SPR is required for the post-mating switch induced by SP.

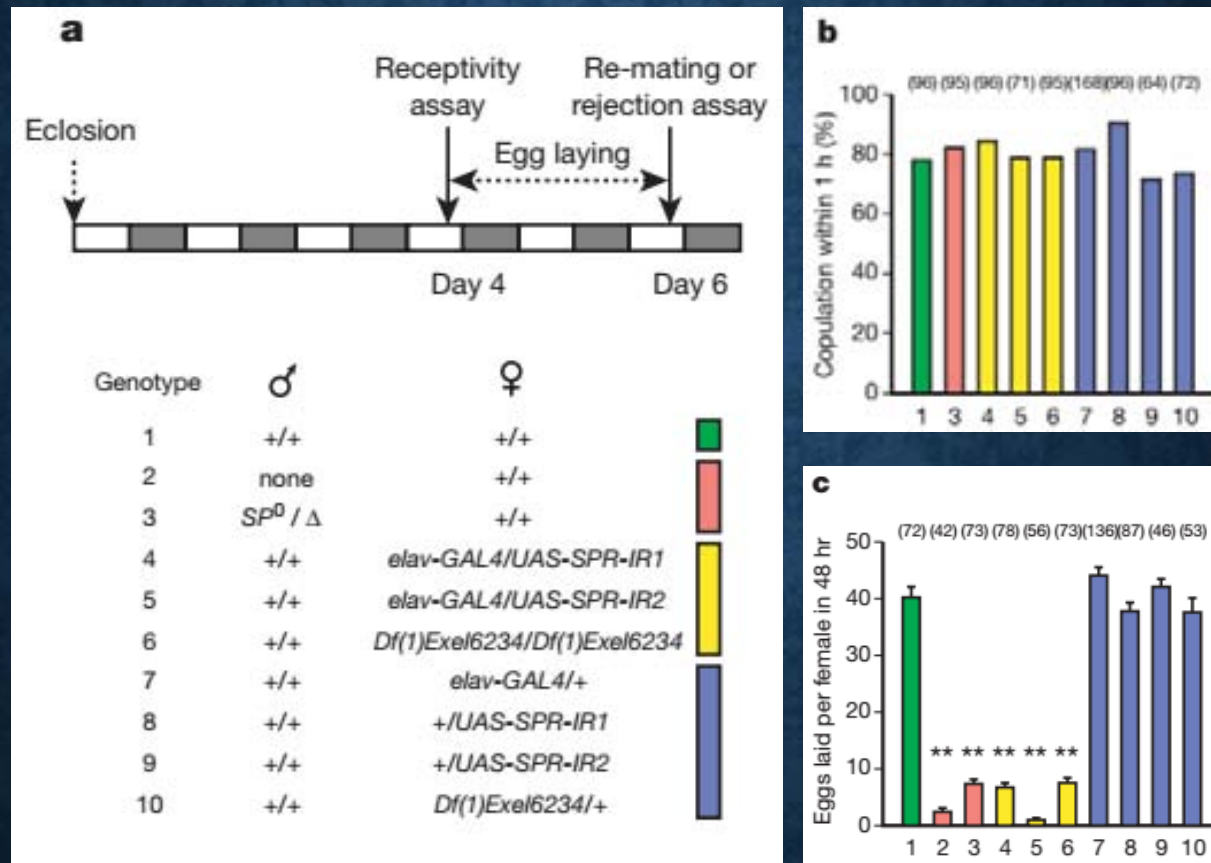


Fig a. Protocol for behavioural experiments in b–e. b, Receptivity of virgin females. c, Number of eggs laid per female

From: Nilay Yapici. 2008. A receptor that mediates the postmating switch in drosophila reproductive behavior.

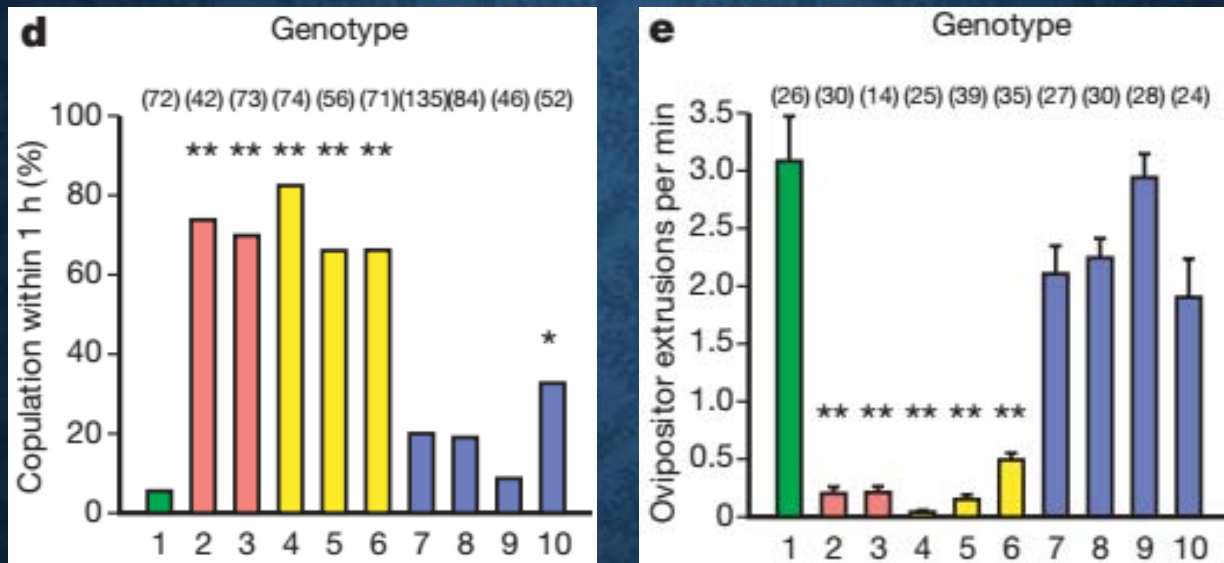


Fig d, Re-mating frequency. e, Ovipositor extrusions per minute during a ten-min courtship assay with a naive wild-type male.

From: Nilay Yapici. 2008. A receptor that mediates the postmating switch in drosophila reproductive behavior.





## HHS Public Access

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Published in final edited form as:

*J Insect Physiol.* 2015 May ; 76: 1–6. doi:10.1016/j.jinsphys.2015.03.006.

### **Sex Peptide Receptor is required for the release of stored sperm by mated *Drosophila melanogaster* females**

**Frank W. Avila<sup>\*</sup>, Alexandra L. Mattei<sup>\*</sup>, and Mariana F. Wolfner**

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA

Frank W. Avila<sup>\*</sup>, Alexandra L. Mattei<sup>\*</sup>, and Mariana F. Wolfner

**by mated *Drosophila melanogaster* females**

**sex peptide receptor is required for the release of stored sperm**

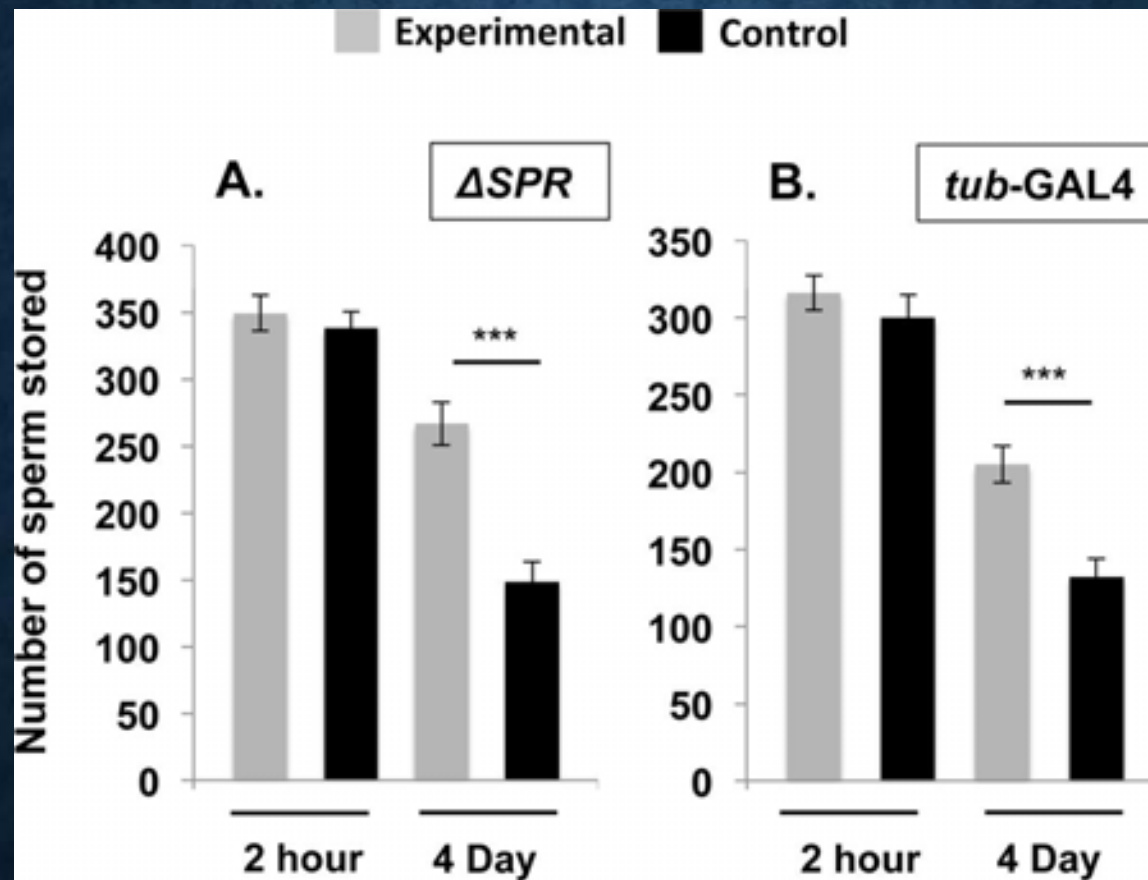


Fig. SPR is required to mediate SP' s effect on sperm release from storage.

From: Nilay Yapici. 2008. A receptor that mediates the postmating switch in drosophila reproductive behavior.

## Molecular mechanisms of sex peptides in post-mating responses

- 1、 Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*.
- 2、 Sex peptide receptor mediates the post-mating switch in *Drosophila* reproductive behaviour.
- 3、 SPR acts in nervous system.

SPR is expressed in the female reproductive organs and nervous system.

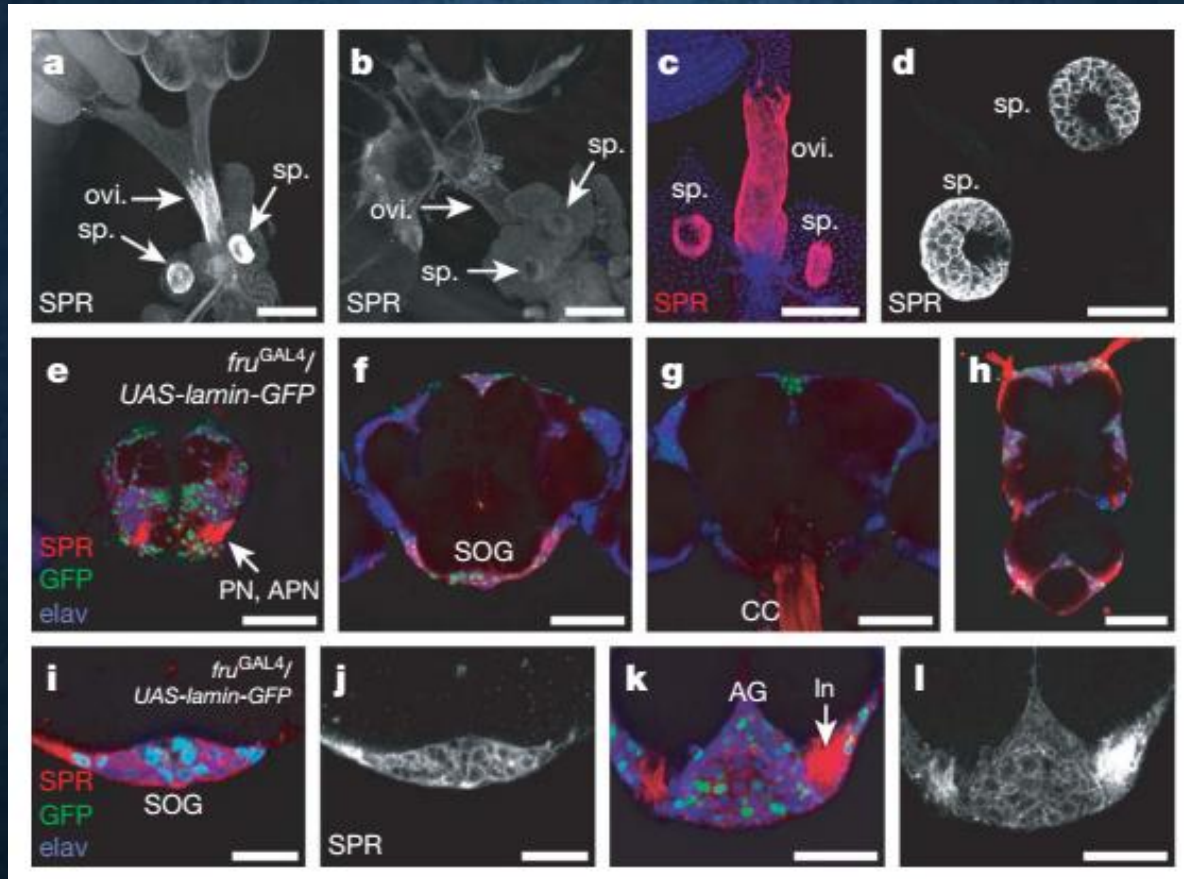


Fig. SPR is expressed in the female reproductive organs and nervous system.

From: Nilay Yapici. 2008. A receptor that mediates the postmating switch in drosophila reproductive behavior.



## SPR acts in fru neurons.

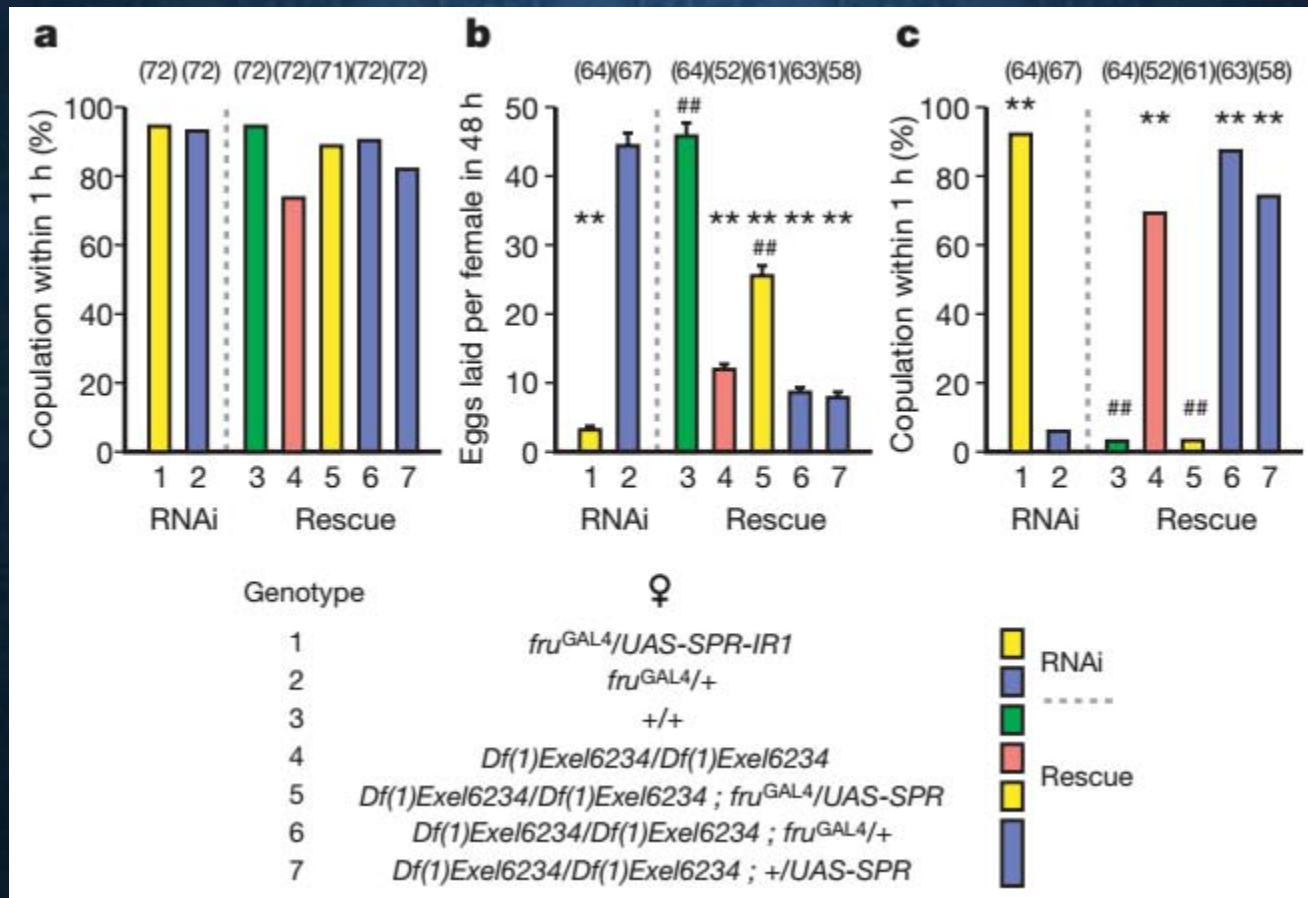
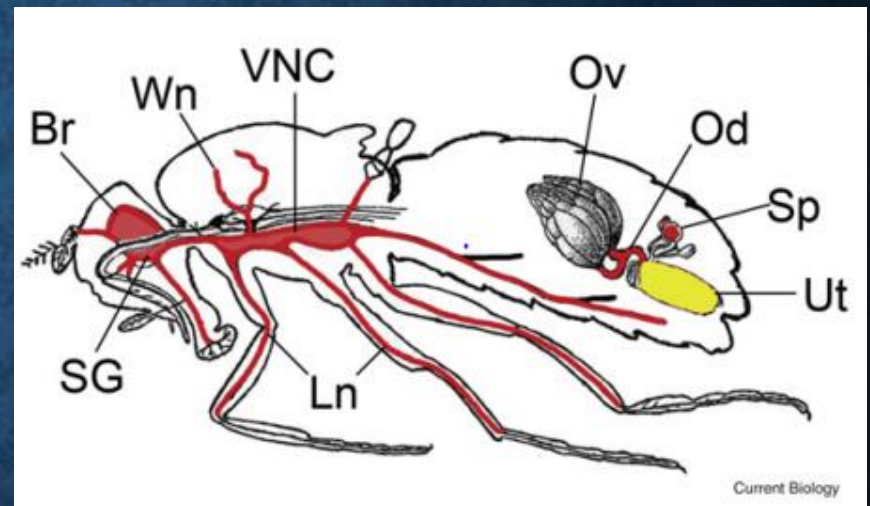
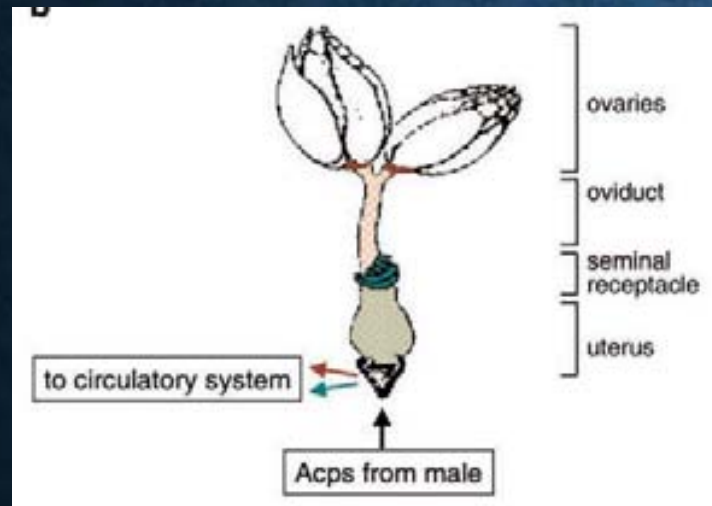


Fig: SPR acts in fru neurons. a, b, c, Receptivity (a), egg-laying (b) and re-mating (c) assays for females of the indicated genotype, mated with wild-type males

From: Nilay Yapici. 2008. A receptor that mediates the postmating switch in drosophila reproductive behavior.

## Conclusions:



## References:

MF wolfneer, et al. (2002). The gifts that keep on giving physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* (Edinb); 88(2):85-93.

E. Kubli, et al. (2008). Sexual behaviour a receptor for sex control in *Drosophila* females. *Curr Biol* ;18(5):R210-2.

E. Kubli, et al. (2003). Sex peptides seminal peptides of the *Drosophila* male. *Cell Mol Life Sci*. 60(8):1689-704.

MF wolfneer, et al. (2002). The gifts that keep on giving physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* (Edinb). 88(2):85-93.

Tracey Chapman, et al. (2004). Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides*; 25(9):1477-90.



## References:

Nilay Yapici, et al. (2008). A receptor that mediates the postmating switch in drosophila reproductive behavior. *Nature*. ;451(7174):33-7.

Tracey Chapman, et al. (2003). The sex peptide of *Drosophila melanogaster* female post-mating responses analyzed by using RNA interference. *Proc Natl Acad Sci U S A*;100(17):9923-8.

Jing Peng, et al. (2005). Gradual release of sperm bound sex-peptide controls female postmating behavior in *Drosophila*. *Curr Biol* 8;15(3):207-13.

Nilay Yapici, et al. (2008). A receptor that mediates the postmating switch in drosophila reproductive behavior. *Curr Biol*. 10;22(13):1155-65. doi: 10.1016/j.cub.2012.04.062.

Peng J, et al. (2015). Sex peptide receptor is required for the release of stored sperm by mated *Drosophila melanogaster* females. *Curr Biol*. ;15(3):207-13.



# CONTENT

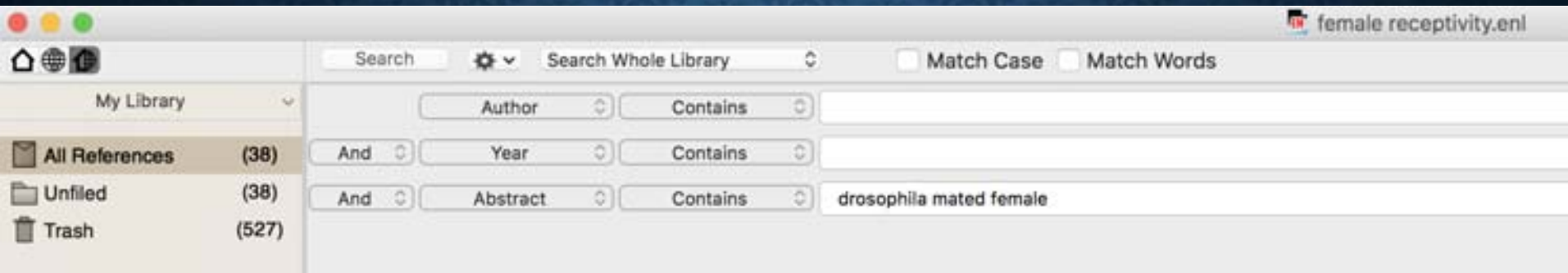
- Part I Overview, virgin female receptivity
- Part II Sex-peptide (SP) signaling in post-mating switch
- Part III Neuronal modulation of post-mating behaviors

**Neuronal modulation  
of post-mating behaviors in  
*D. melanogaster* females**

Pan Yufeng

2018-9-28

# How do I find related papers?



500+ papers: read titles and journal names;

38 papers : read abstract or more;

22 papers: this presentation.


# What happens to female during mating?

- Transfer of sperm
- Transfer of seminal fluids including sex-peptide (SP)
- Transfer of male-specific pheromones (e.g. cVA)
- Physical contact/stimulation



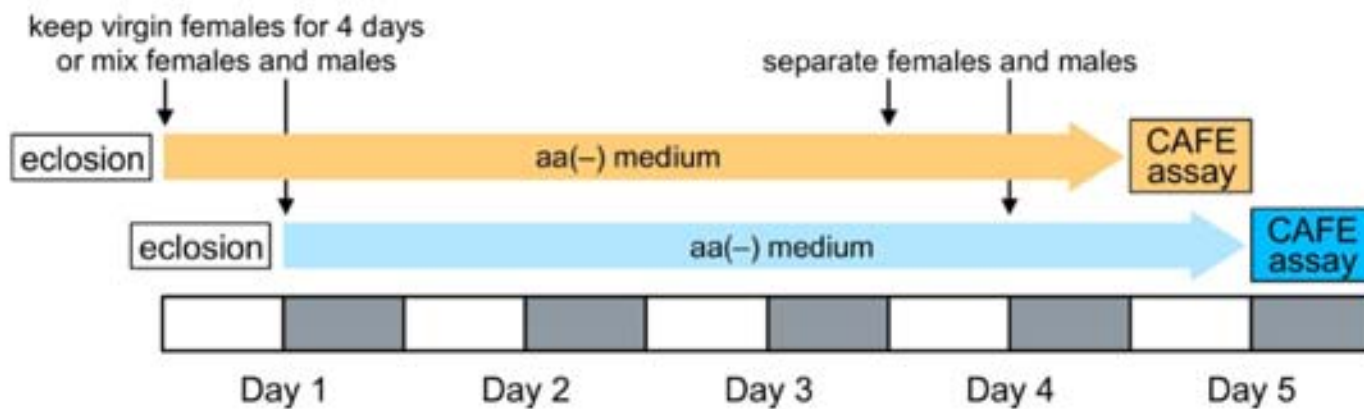
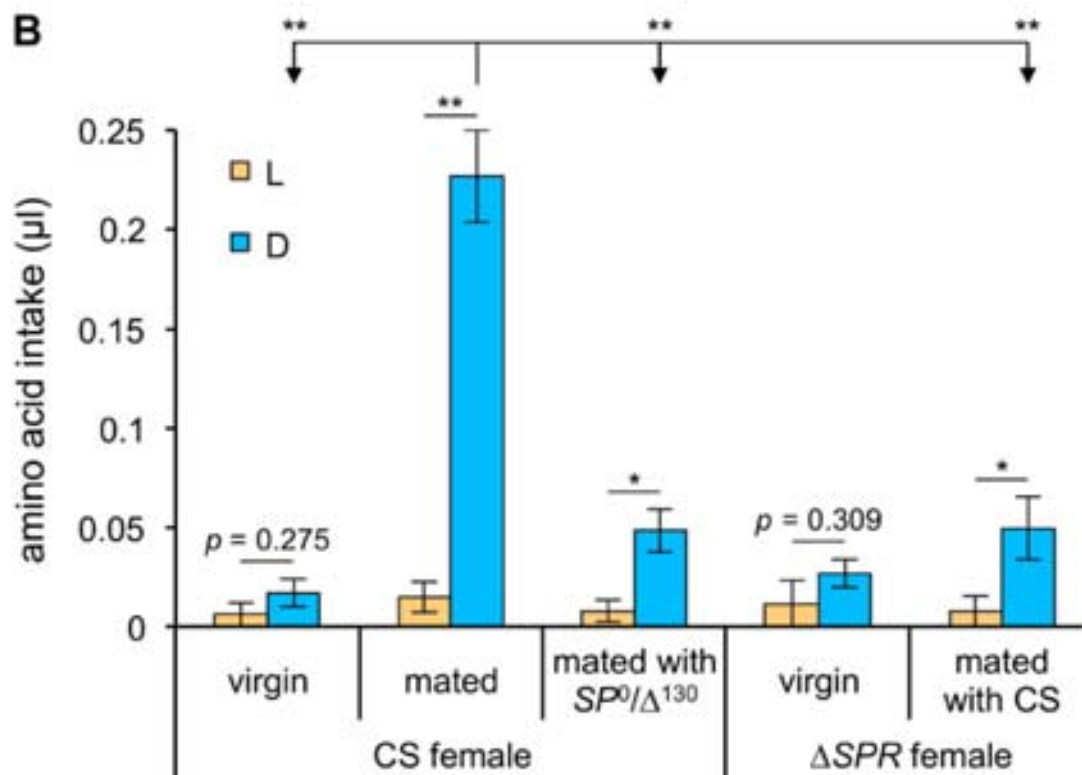
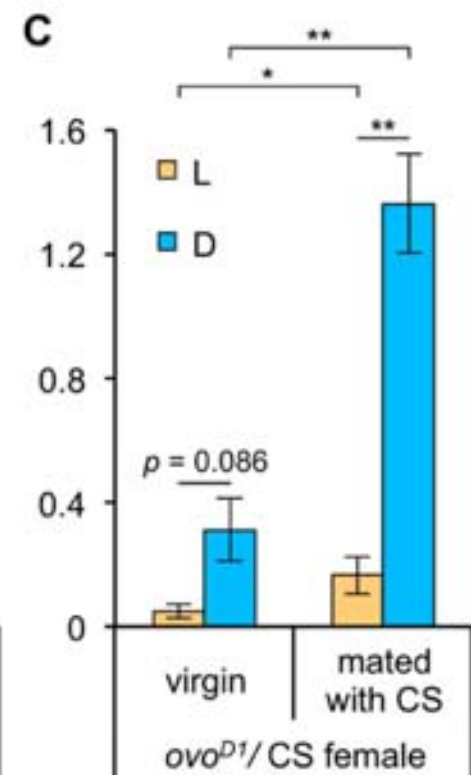


# What happens to female during mating?

- Transfer of sperm
  - Transfer of seminal fluids including sex-peptide (SP)
  - Transfer of male-specific pheromones (e.g. cVA)
  - Physical contact/stimulation
- 
- Fertilization
  - SP-induced post-mating behaviors
  - Reduce female attractiveness;
  - Unknown

# **Question 1: how do mated females behave differently?**

- Mated females consume more amino acids

**A****B****C**

**Fig 4. A post-mating signal elevates amino acid consumption during the dark phase.** (A) The experimental scheme for the CAFE assays. Each L phase is shown by a white box and each D phase by a gray box. (B and C) Amino acid consumption during L (orange bars) and D (blue bars) phases was quantified using no-choice CAFE assays with the following strains: virgin CS females and CS females mated with CS or  $SP^0/\Delta^{130}$  males (B;

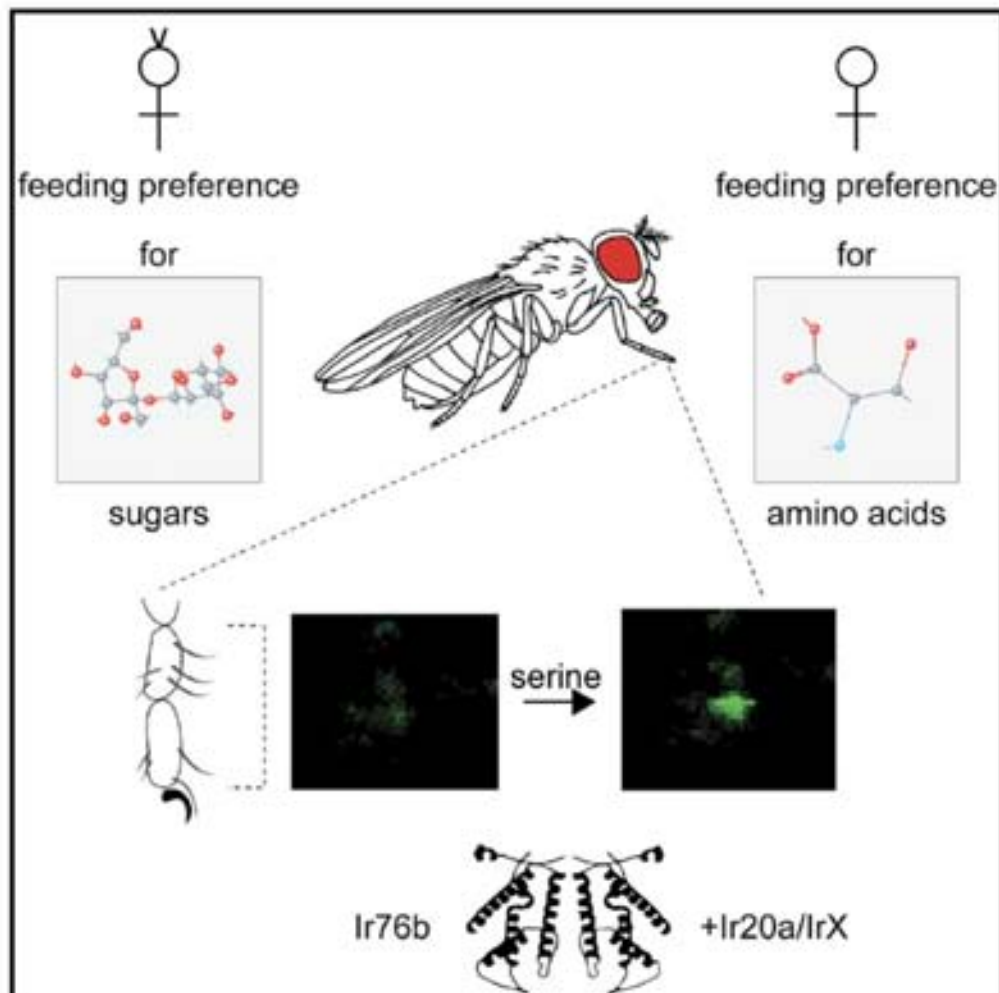
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# Cell Reports

## A Molecular and Cellular Context-Dependent Role for Ir76b in Detection of Amino Acid Taste

### Graphical Abstract



### Authors

Anindya Ganguly, Lisa Pang,  
Vi-Khoi Duong, Angelina Lee,  
Hanni Schoniger, Erika Varady,  
Anupama Dahanukar

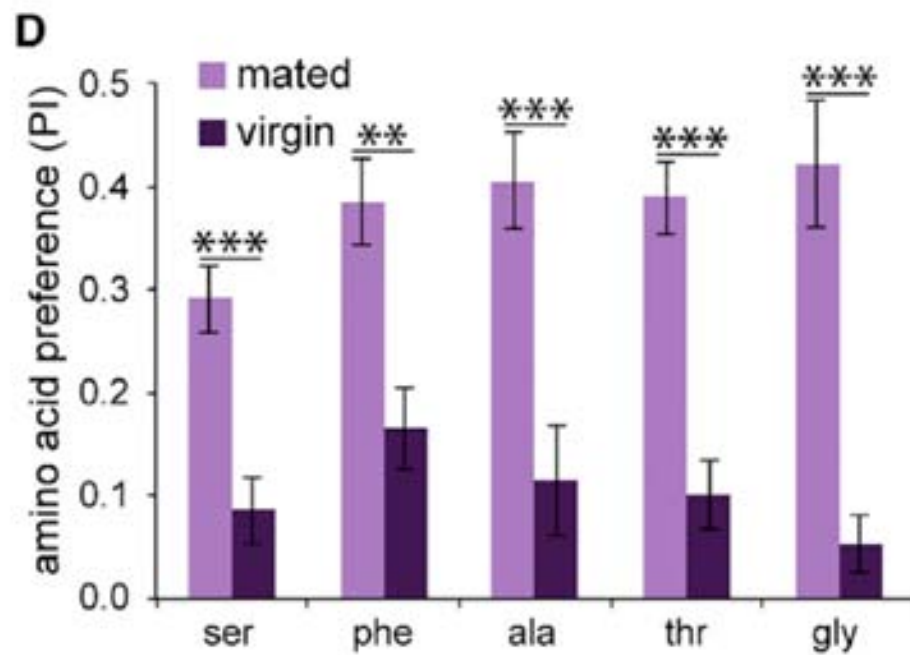
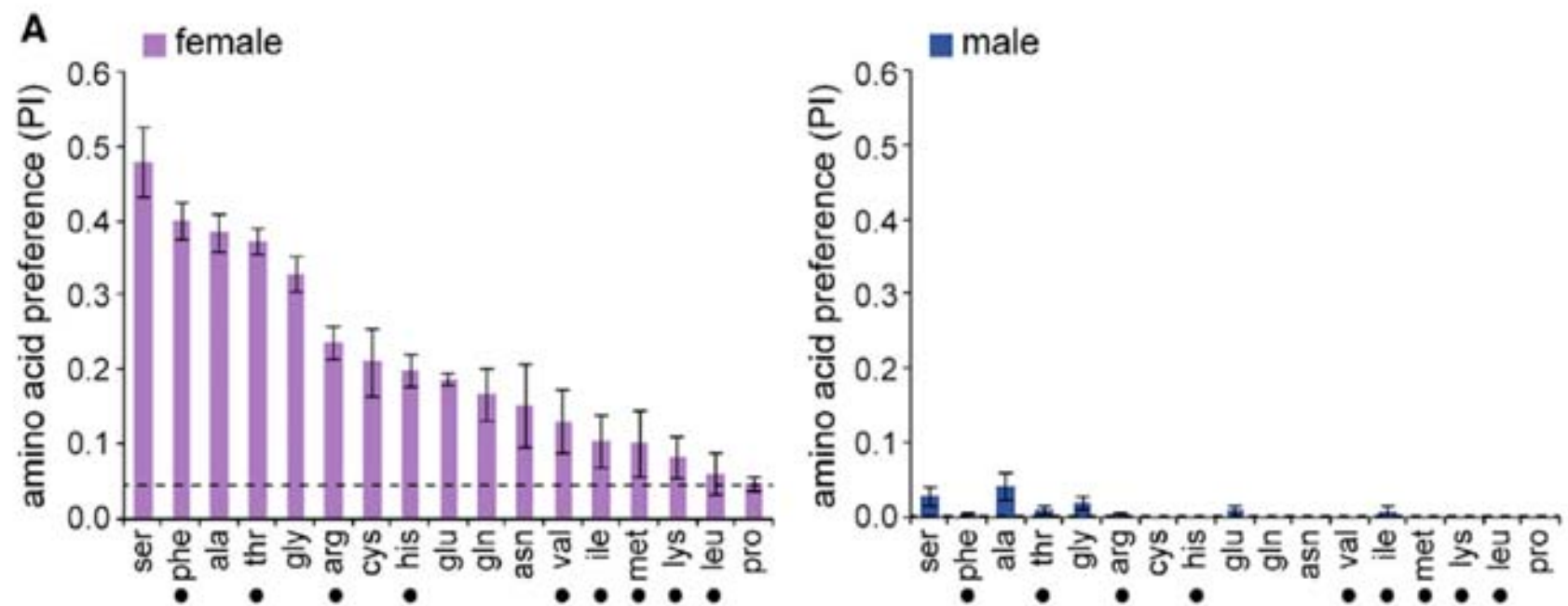
### Correspondence

anupama.dahanukar@ucr.edu

### In Brief

Ganguly et al. demonstrate that Ir76b mediates cellular and behavioral responses to amino acids that underlie post-mating yeast and amino acid feeding preferences of *Drosophila* females. Ir20a, possibly one among many factors, plays a role in changing Ir76b activity from an ungated salt receptor to an amino-acid-gated receptor.





# Another study shows that mating has no influence on amino acids consumption

Cell Research

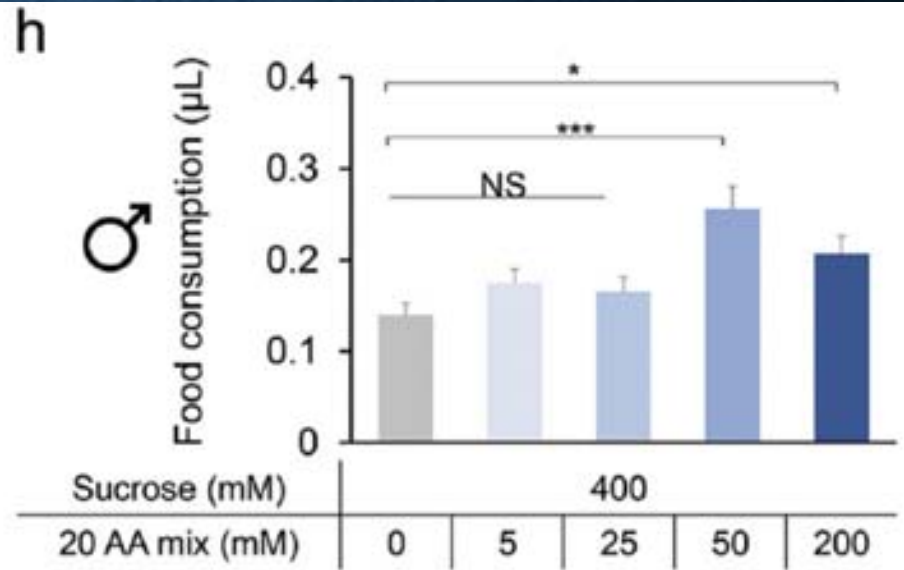
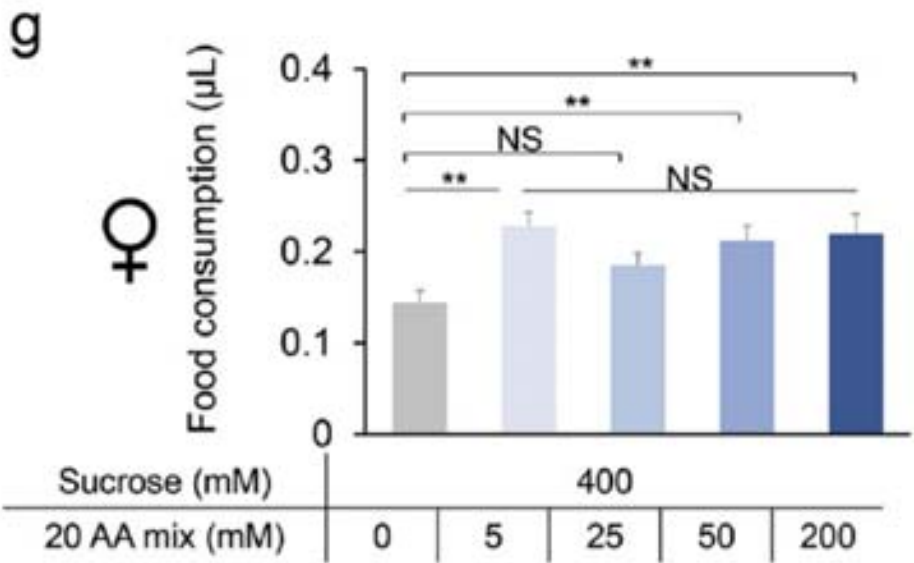
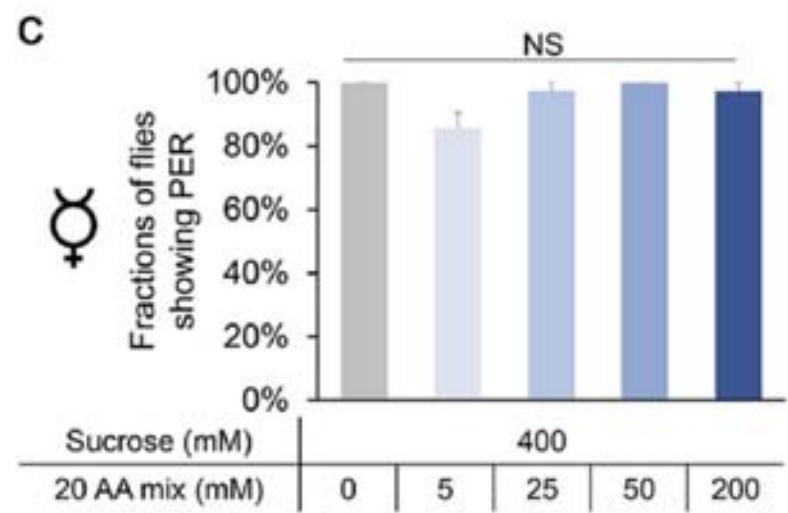
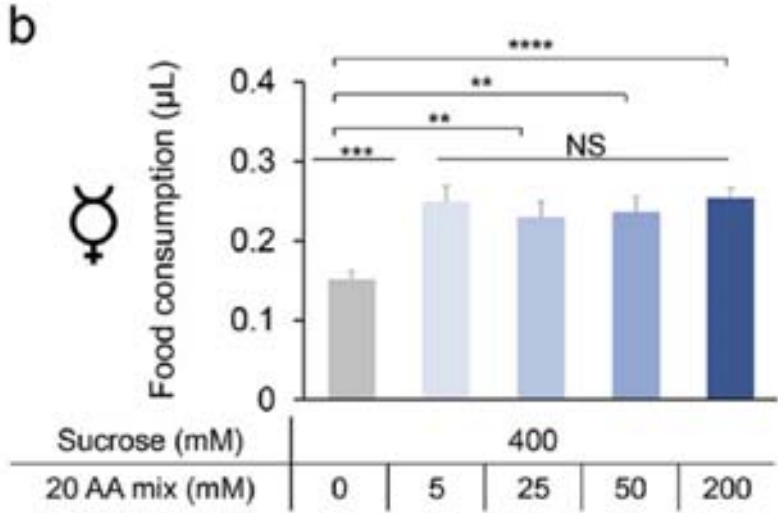
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[www.cell-research.com](http://www.cell-research.com)



ARTICLE OPEN

## A post-ingestive amino acid sensor promotes food consumption in *Drosophila*

Zhe Yang<sup>1,2</sup>, Rui Huang<sup>3,4</sup>, Xin Fu<sup>5,6,7</sup>, Gaohang Wang<sup>1,2</sup>, Wei Qi<sup>1,2</sup>, Decai Mao<sup>8</sup>, Zhaomei Shi<sup>5</sup>, Wei L. Shen<sup>5</sup> and Liming Wang<sup>1,2</sup>



# **Question 1: how do mated females behave differently?**

- Mated females consume more amino acids
- Mated females are more aggressive



Article | Published: 15 May 2017

# Sperm and sex peptide stimulate aggression in female *Drosophila*

Eleanor Bath , Samuel Bowden, Carla Peters, Anjali Reddy, Joseph A. Tobias, Evan Easton-Calabria, Nathalie Seddon, Stephen F. Goodwin & Stuart Wigby

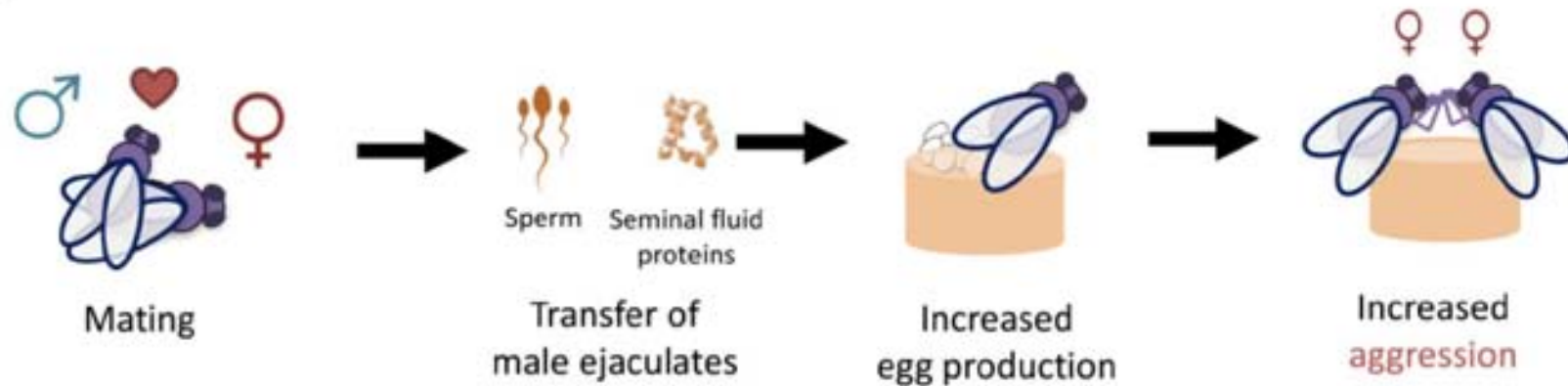
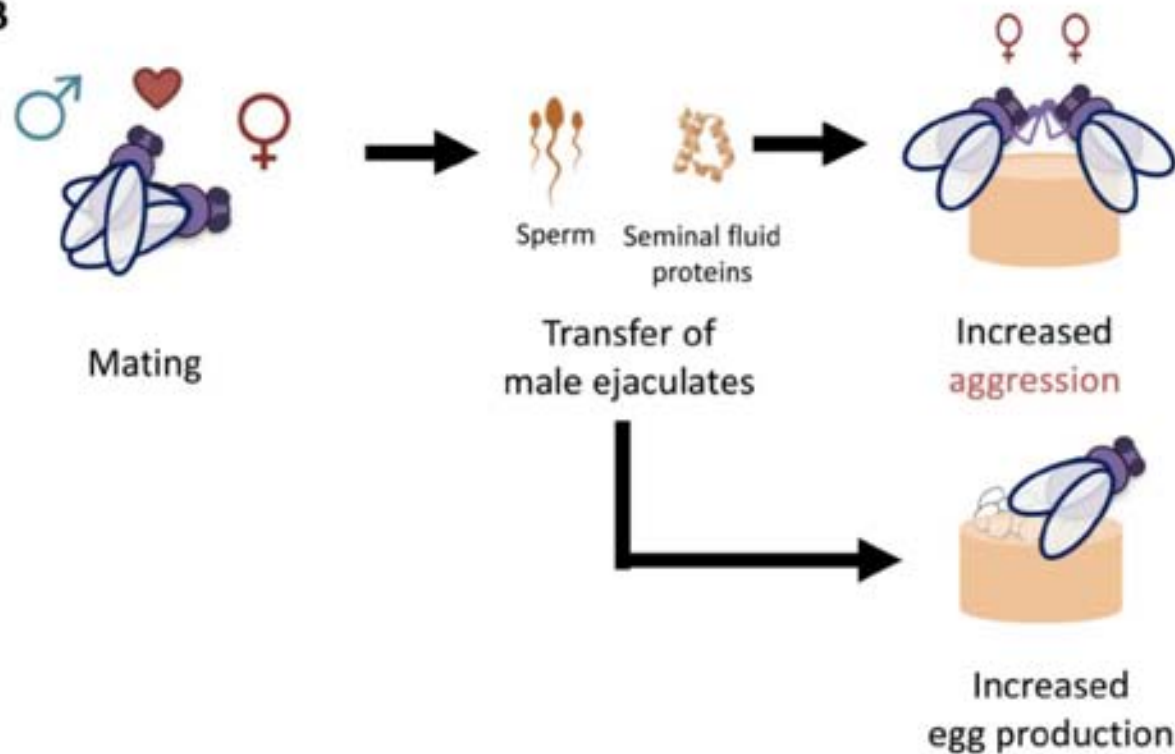
**A****B**

Figure 1. Two proposed pathways for mating-induced female aggression

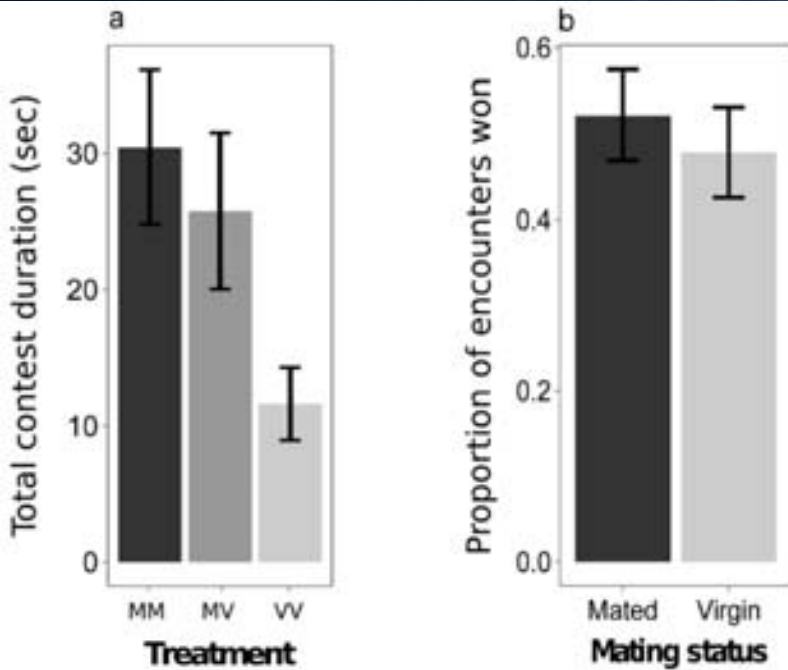


Figure 2. Mated females spend more time fighting than virgins but do not win more fights.

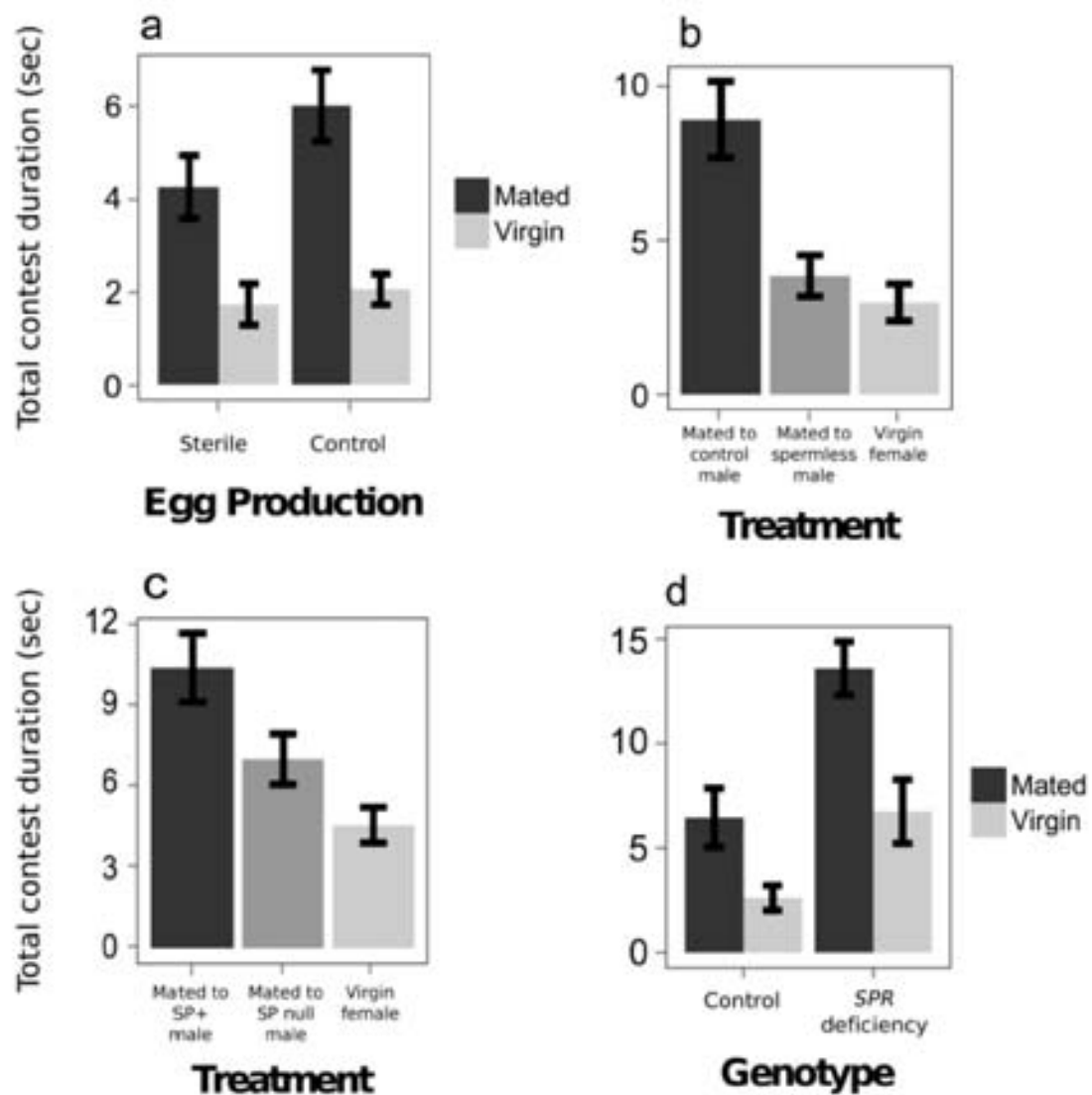


Figure 3. Effects of male ejaculate components, and female egg-production and sex peptide receptor on female total contest duration.

This is all the data this paper has.

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Scientific blogs (4)

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Breitbart News Network

[Butting heads: sex enrages female fruit flies](#)

Yahoo! News

[Sex enrages female fruit flies – Reports](#)

Uncova

[Frisky Female Fruit Flies Become More Aggressive Towards Each Other After Sex](#)

Science Newsline

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Yahoo! News

[Butting heads: sex enrages female fruit flies \(AFP\)](#)

Yahoo! News

[Make love, then war: Study shows sex enrages female fruit flies](#)

The Malay Mail Online

[Frisky female fruit flies become more aggressive towards each other after sex](#)

Phys.org

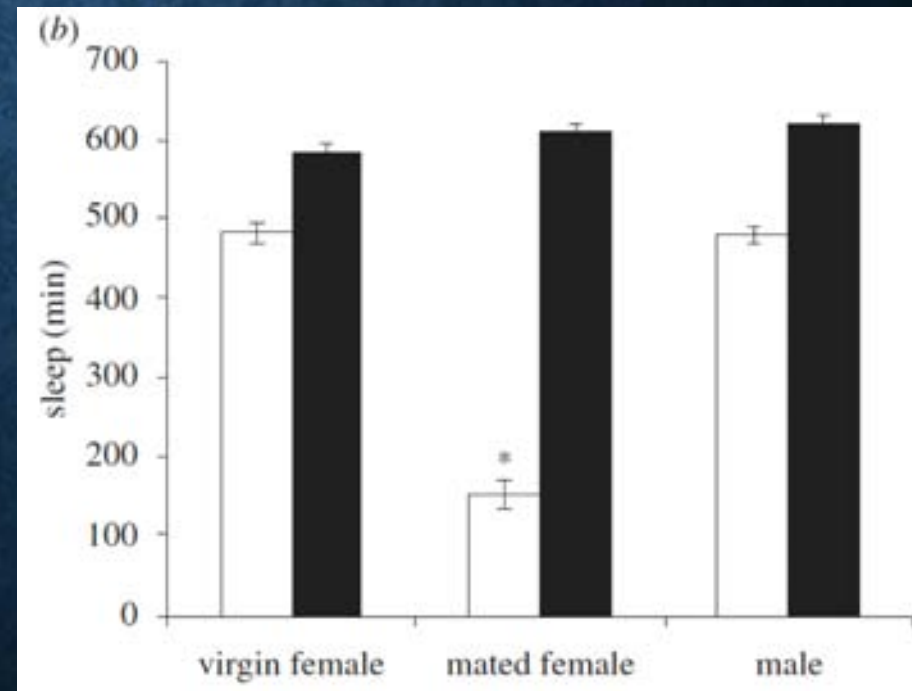
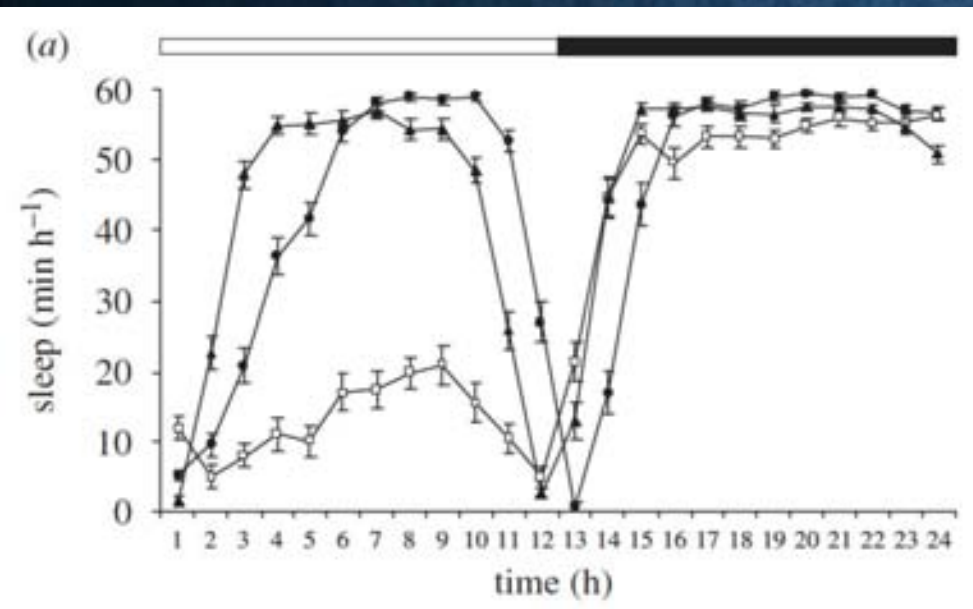


# **Question 1: how do mated females behave differently?**

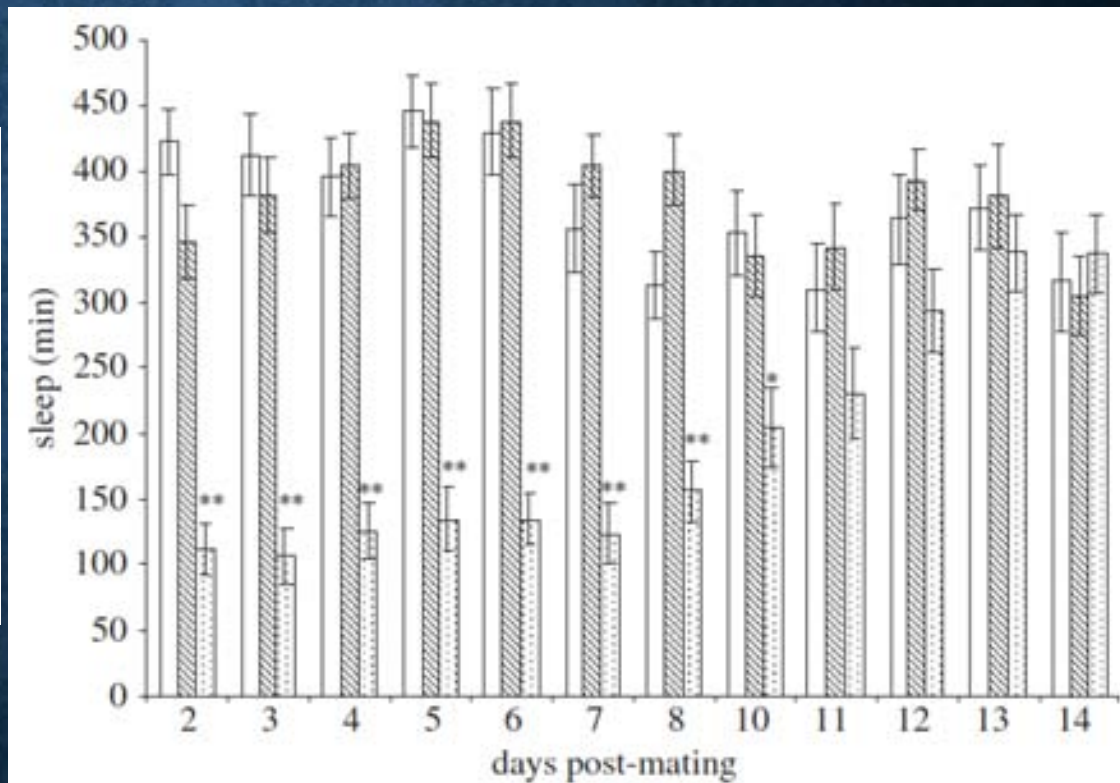
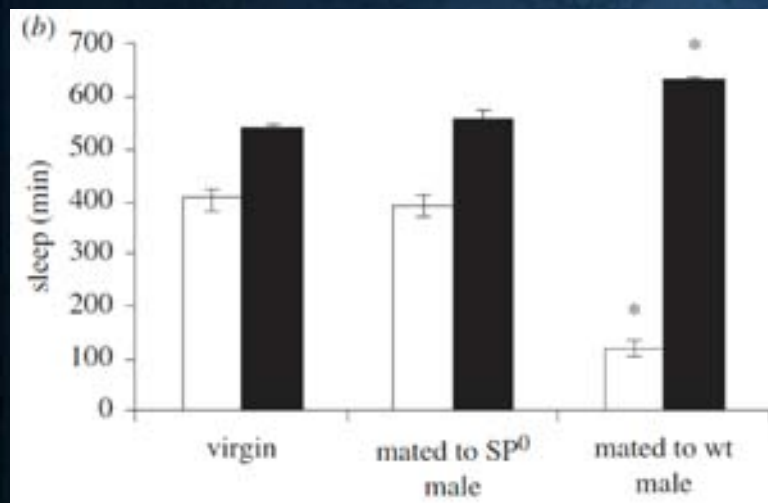
- Mated females consume more amino acids
- Mated females are more aggressive
- Mated females have increased locomotion activity

# ***Drosophila* male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female**

R. Elwyn Isaac<sup>1,\*</sup>, Chenxi Li<sup>1</sup>, Amy E. Leedale<sup>1</sup> and Alan D. Shirras<sup>2</sup>



(filled circle, virgin female; open square, mated female; filled triangle, male)



open bar, virgin;  
hatched bar, mated to SP0 male;  
dotted bar, mated to wt male.

# **Question 1: how do mated females behave differently?**

- Mated females consume more amino acids
- Mated females are more aggressive
- Mated females have increased locomotion activity
- Mated females have increased starvation resistance

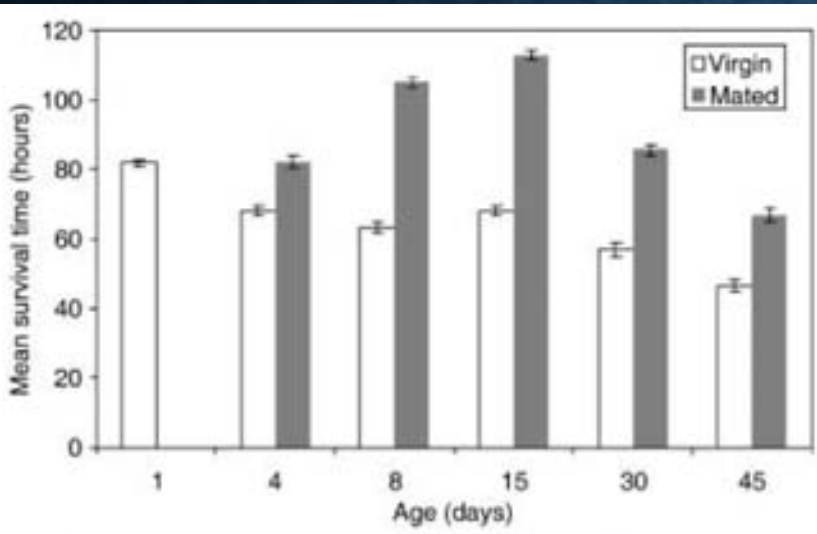


SHORT TAKE

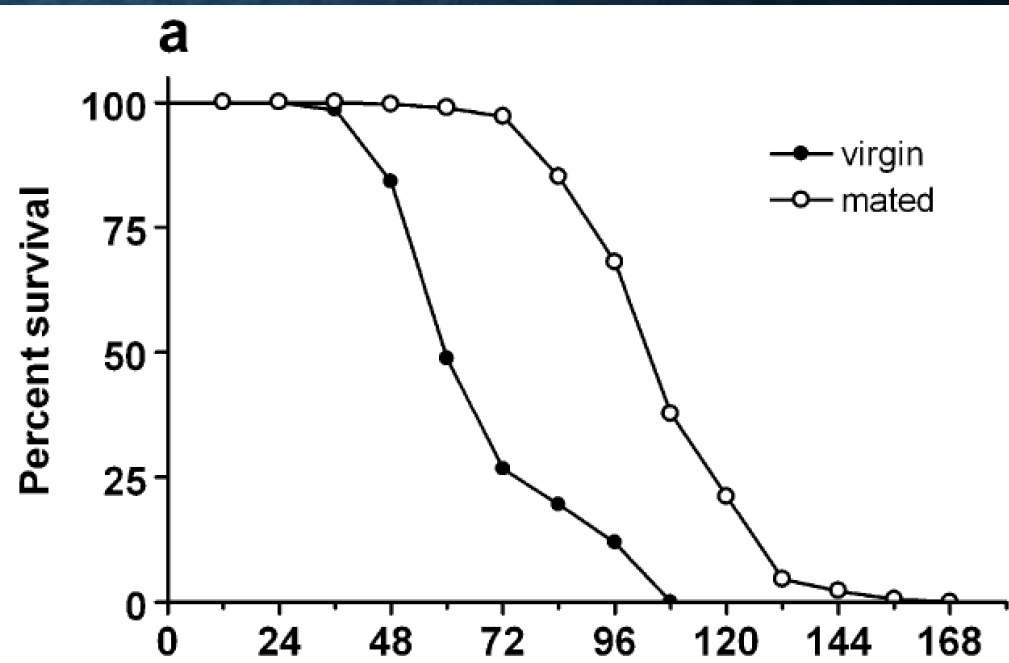
# Mating increases starvation resistance and decreases oxidative stress resistance in *Drosophila melanogaster* females

Brandy Rush,<sup>1</sup> Sarah Sandver,<sup>1</sup> Jessica Bruer,<sup>1</sup> Robin Roche,<sup>2</sup> Michael Wells<sup>2</sup> and Jadwiga Giebultowicz<sup>1</sup>

2002; Kubli, 2003). Mated females show reduced sexual receptivity and a substantial increase in egg production. Mating also



**Fig. 1** Age-dependent profiles of starvation resistance in virgin and mated Canton-S females reared on molasses/cornmeal medium containing 7% yeast. Bars represent mean ( $\pm$  SEM) survival time, which was significantly longer for mated than for virgin females at every age tested (t-test:  $P < 0.01$



# Increased starvation resistance in mated females may depends on selective accumulation of lipids

**Table 1** Fat, protein and glycogen content in mated and virgin females of *Drosophila melanogaster* subjected to starvation

Nutrient	Before starvation		Starved 36 h		Starved 60 h	
	Virgin	Mated	Virgin	Mated	Virgin	Mated
Triacylglycerols	55.2 ± 1	108.4 ± 10*	21.2 ± 0.7	69.8 ± 4.6*	11.1 ± 2	45.2 ± 6*
Proteins	199.0 ± 31	178.6 ± 34	202.9 ± 24	165.0 ± 30	223.4 ± 34	176.2 ± 39
Glycogen	18.2 ± 1	19.0 ± 2	2.1 ± 0.3	1.3 ± 0.6	0.6 ± 0.5	0.4 ± 0.2

All values are expressed in micrograms/fly; the data are means ± SEM values ( $n = 5$ , except  $n = 3$  for virgins starved for 60 h).

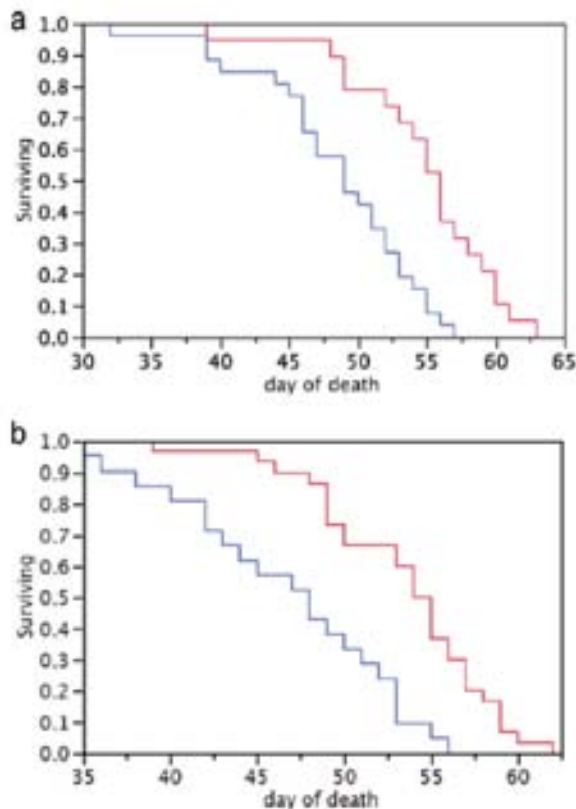
The data are pooled from two experiments, in which either 15 or 20 flies were analyzed per sample.

Significant differences ( $P < 0.01$ ) between the values for virgin and mated females obtained by unpaired t-test are indicated by \*.

## "Cost" of virginity in wild *Drosophila melanogaster* females

Therese Ann Markow

Division of Biological Sciences, University of California, La Jolla, San Diego, California



**Figure 1.** (A, B) Female survival after collection in the field for Replications 1 (top) and 2 (bottom). Virgin females are indicated by the blue line, randomly collected mated females are indicated by red. For Replication

In the first replication, there were 19 virgin and 26 mated females and the mated females lived an average of approximately 6 days longer

Rather than a “cost of mating,” there appears to be a “cost of virginity” to female *D. melanogaster* in the wild.



# **Question 1: how do mated females behave differently?**

- Mated females consume more amino acids
- Mated females are more aggressive
- Mated females have increased locomotion activity
- Mated females have increased starvation resistance
- Mated females lay eggs and reject males

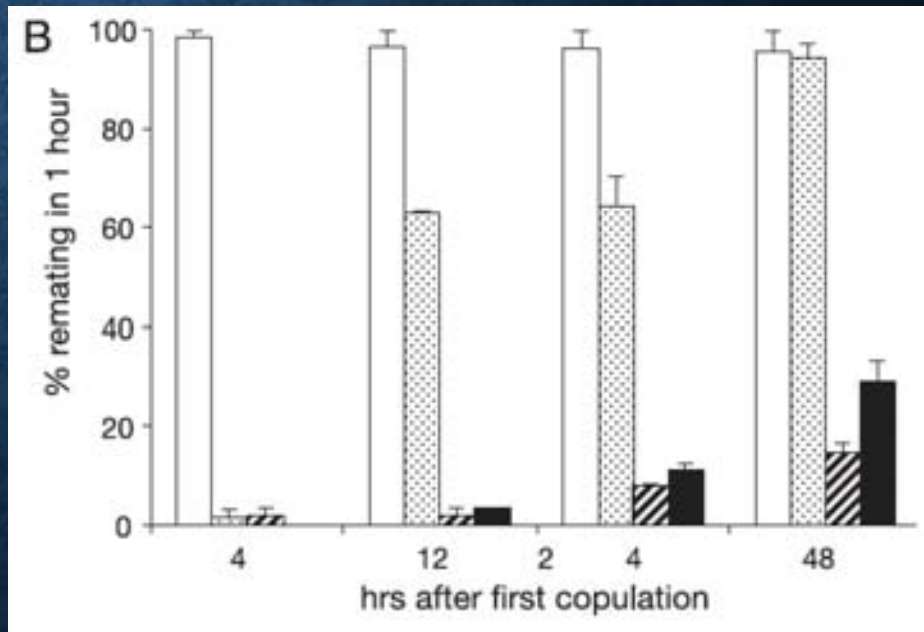
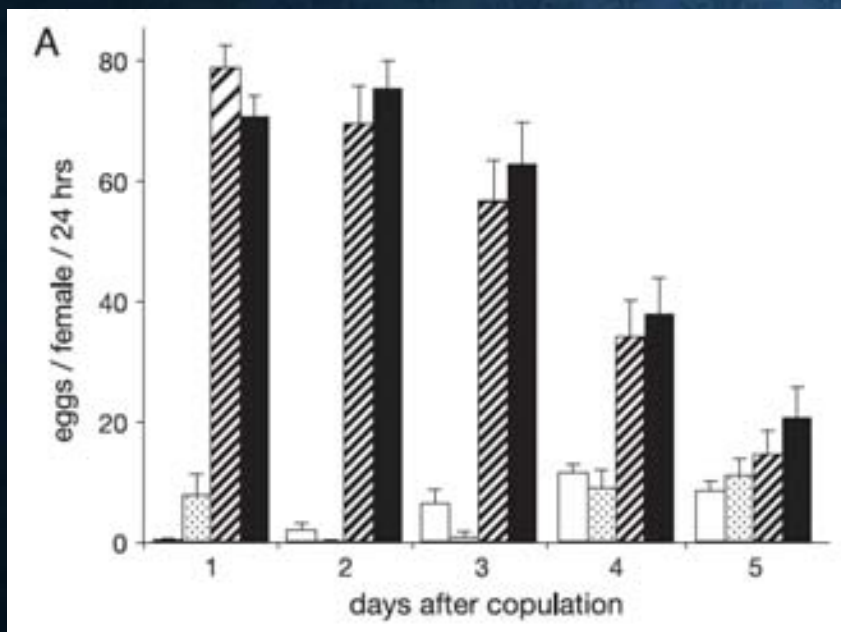


# Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*

Huanfa Liu and Eric Kubli\*

Zoological Institute, University of Zurich-Irchel, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Edited by Wendell Roelofs, Cornell University, Geneva, NY, and approved May 21, 2003 (received for review March 25, 2003)



VC ♀

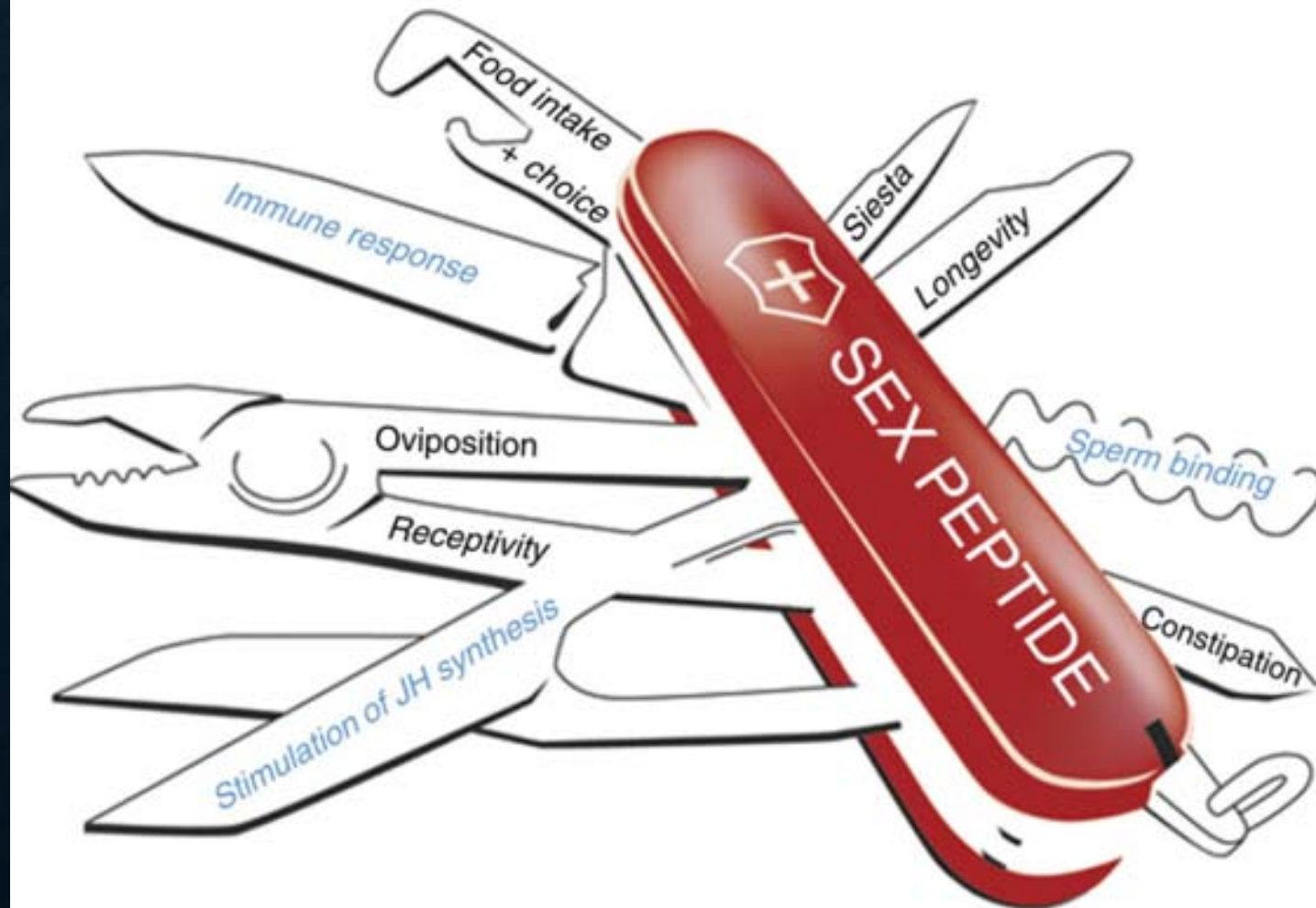
VC ♀ x SP<sup>0</sup>/SP<sup>Δ</sup> ♂

VC ♀ x WT ♂

VC ♀ x SP<sup>+</sup>/SP<sup>Δ</sup> ♂

Dispatches

## Sexual Behavior: How Sex Peptide Flips the Postmating Switch of Female Flies



## Question 2: what's the neural basis for post-mating behaviors

- *fruGAL4* neurons are involved;



# Shared neural circuitry for female and male sexual behaviours in *Drosophila*

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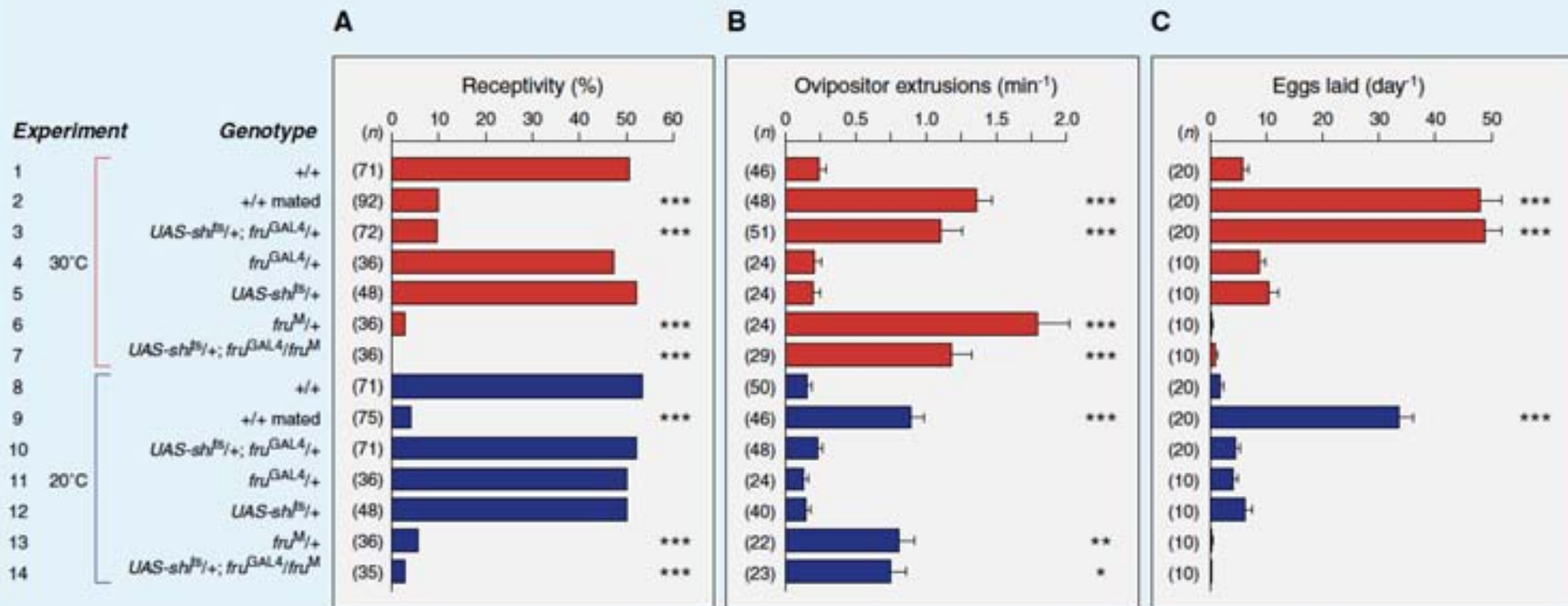


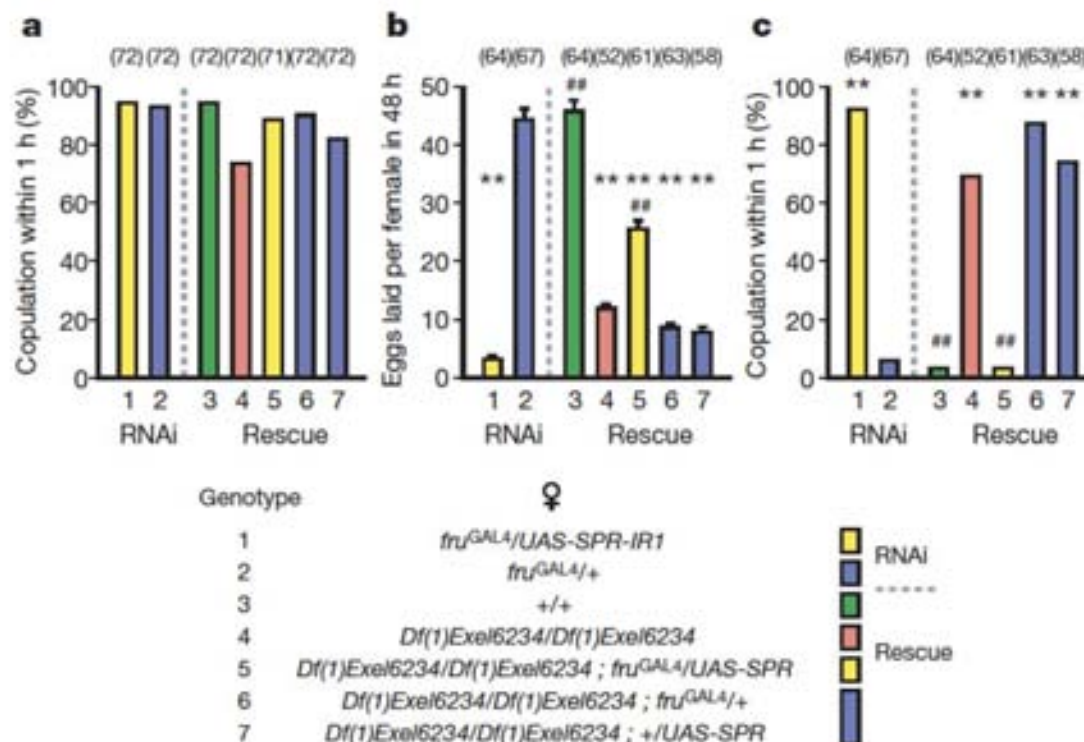
Figure 1. *fru<sup>GAL4</sup>* neurons mediate female reproductive behaviours.



## ARTICLES

# A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour

Nilay Yapici<sup>1\*</sup>, Young-Joon Kim<sup>1\*</sup>, Carlos Ribeiro<sup>1</sup> & Barry J. Dickson<sup>1</sup>

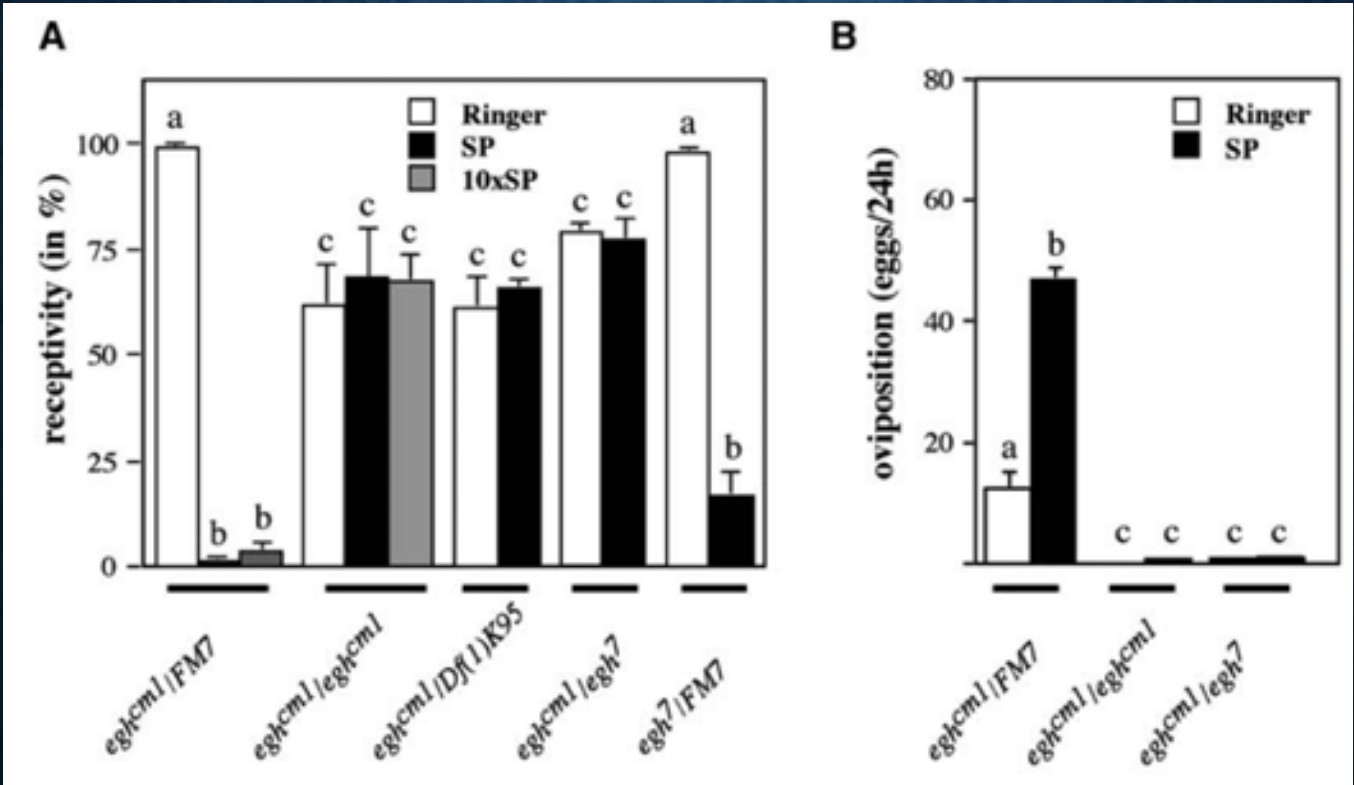


**Figure 4 | SPR acts in *fru* neurons. a, b, c, Receptivity (a), egg-laying (b) and re-mating (c) assays for females of the indicated genotype, mated with wild-type males and assayed according to the protocol of Fig. 1a. For the RNAi**

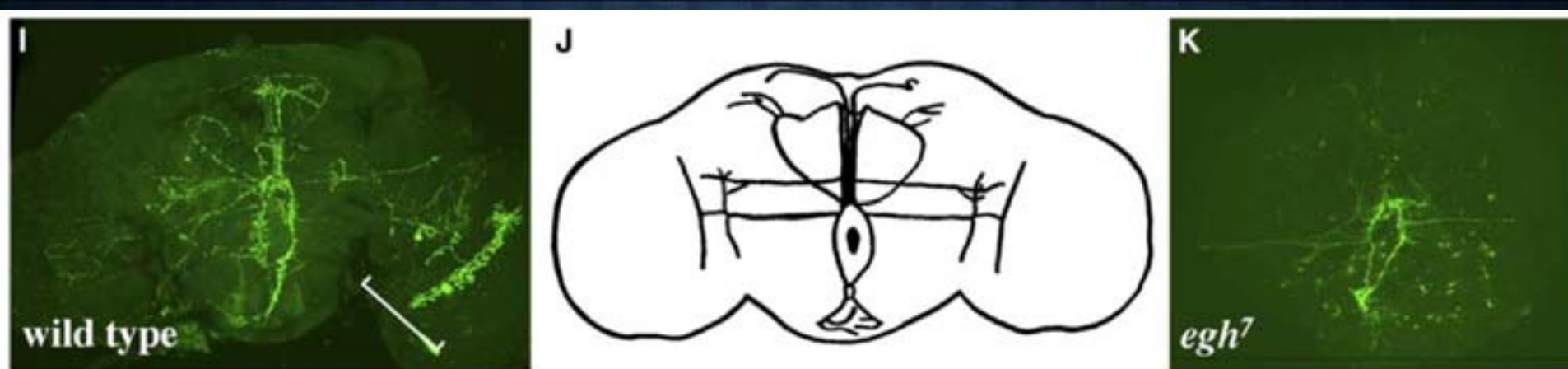
## Question 2: what's the neural basis for post-mating behaviors

- *fruGAL4* neurons are involved;
- Subsets of ascending VNC neurons;

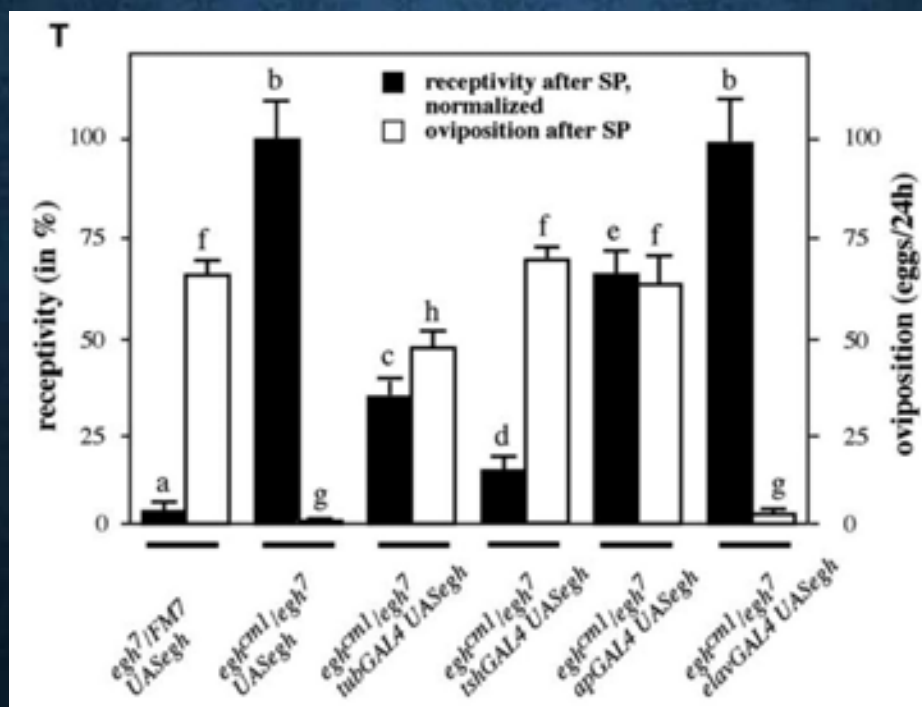
# Sex-Peptide-Regulated Female Sexual Behavior Requires a Subset of Ascending Ventral Nerve Cord Neurons



The *egghead* gene involved in glycosphingolipid biosynthesis provides an essential component to the SP response.



Clonal analysis of VNC Ap neuronal projections to the central brain.



*egh* expression in VNC neurons (*tshGAL4*) rescues mutant phenotype.

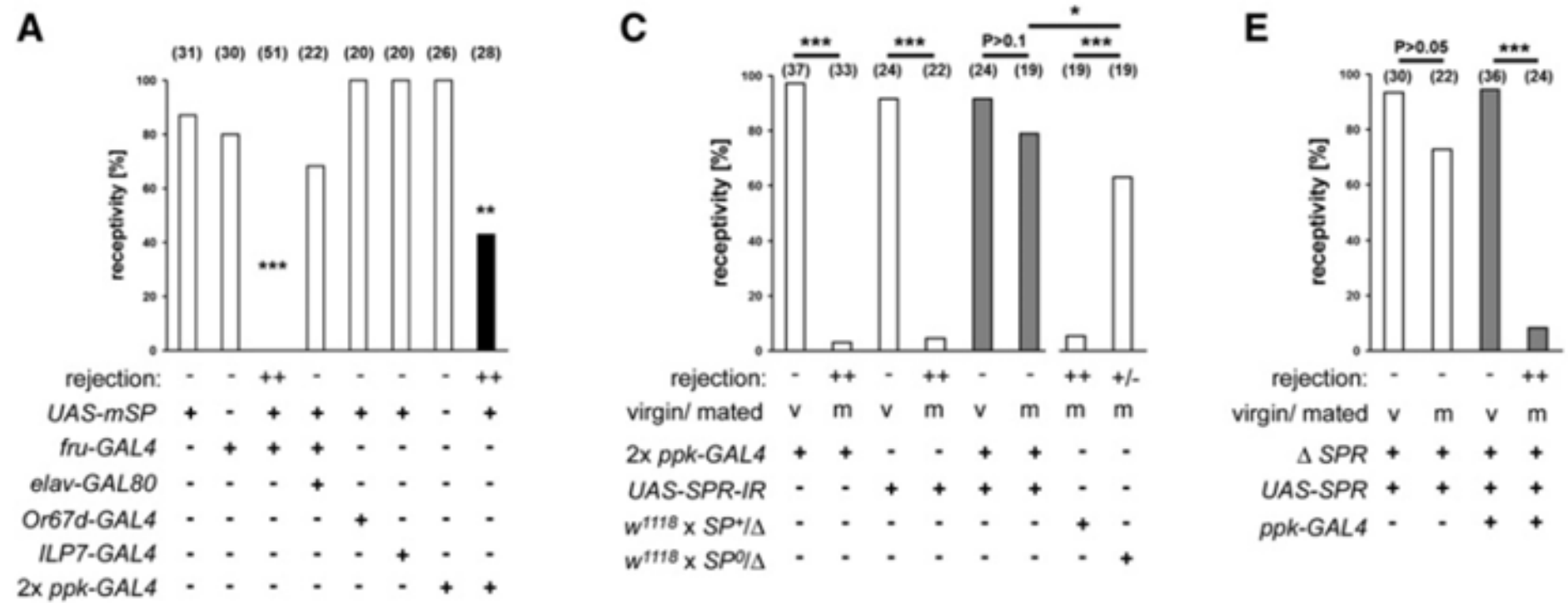


## Question 2: what's the neural basis for post-mating behaviors

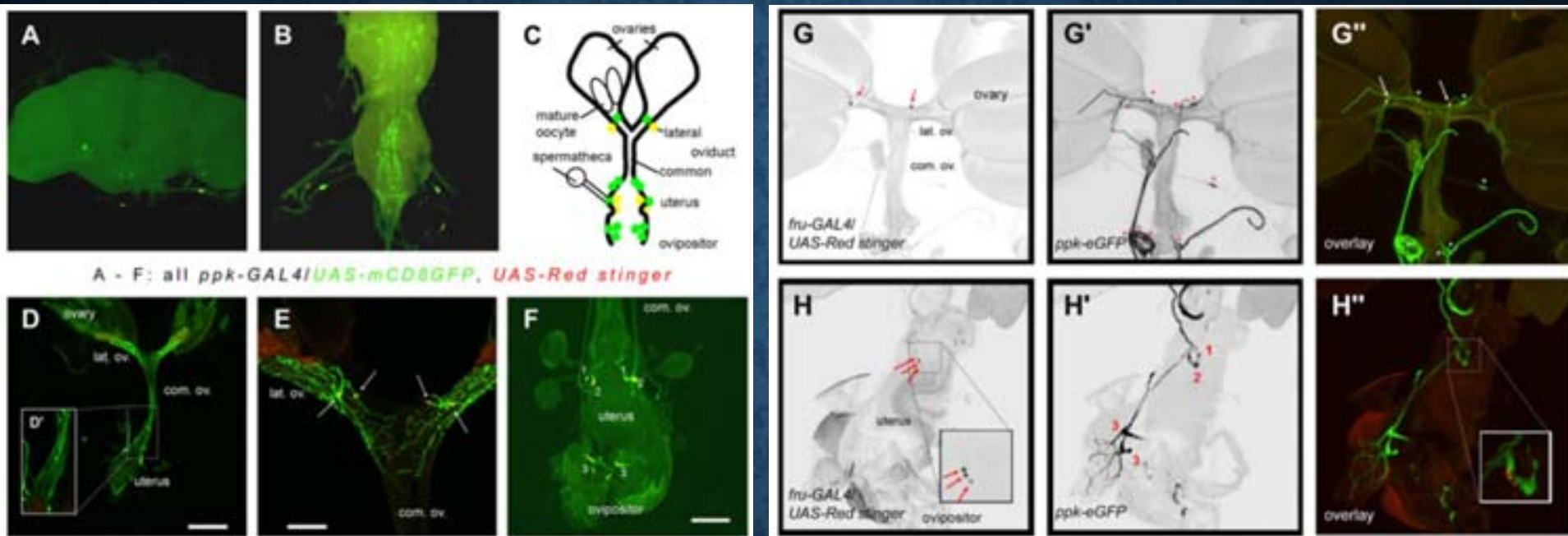
- *fruGAL4* neurons are involved;
- Subsets of ascending VNC neurons;
- *fru* and *ppk*-positive sensory neurons in the genital tract;

# Control of the Postmating Behavioral Switch in *Drosophila* Females by Internal Sensory Neurons

Chung-hui Yang,<sup>1,3</sup> Sebastian Rumpf,<sup>1,3</sup> Yang Xiang,<sup>1</sup> Michael D. Gordon,<sup>2</sup> Wei Song,<sup>1</sup> Lily Y. Jan,<sup>1</sup> and Yuh-Nung Jan<sup>1,\*</sup>



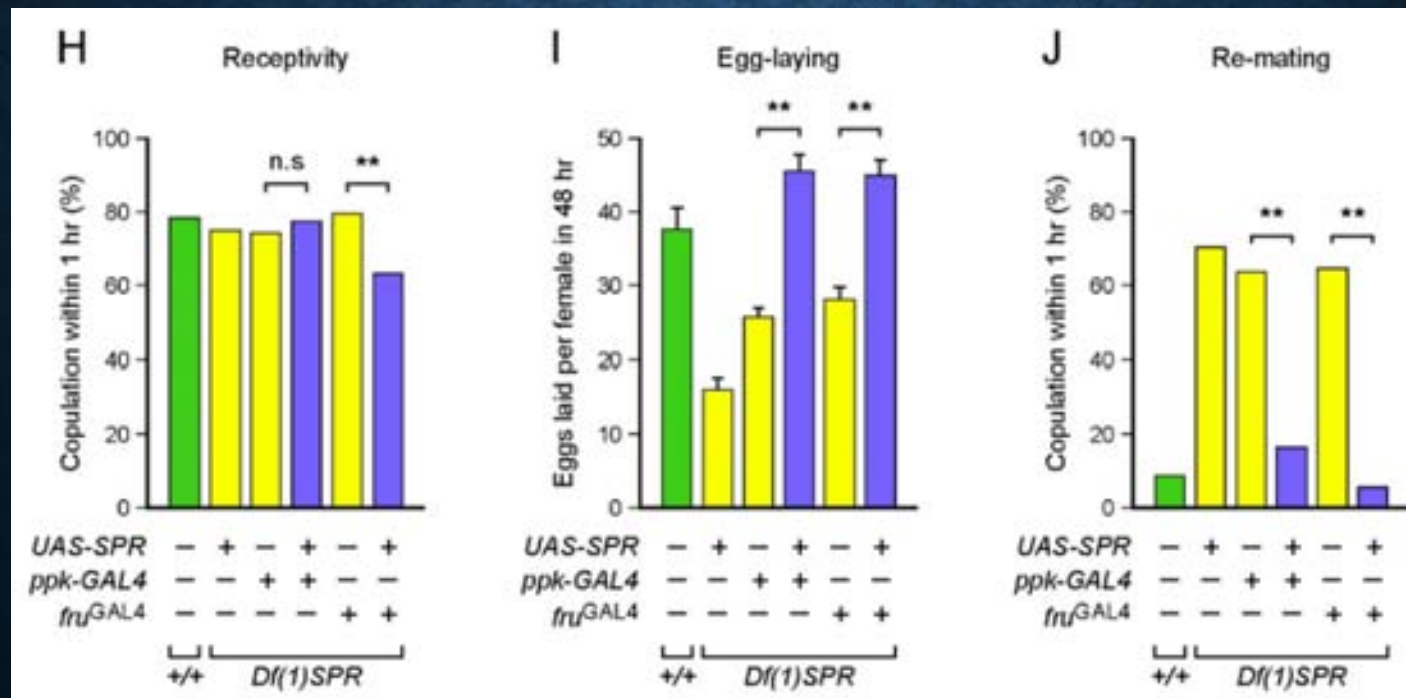
ppk-GAL4 Labels SP-Responsive Neurons



*ppk-GAL4* and *fru-GAL4* Expression Overlap in Sensory Neurons on the Female Reproductive Tract

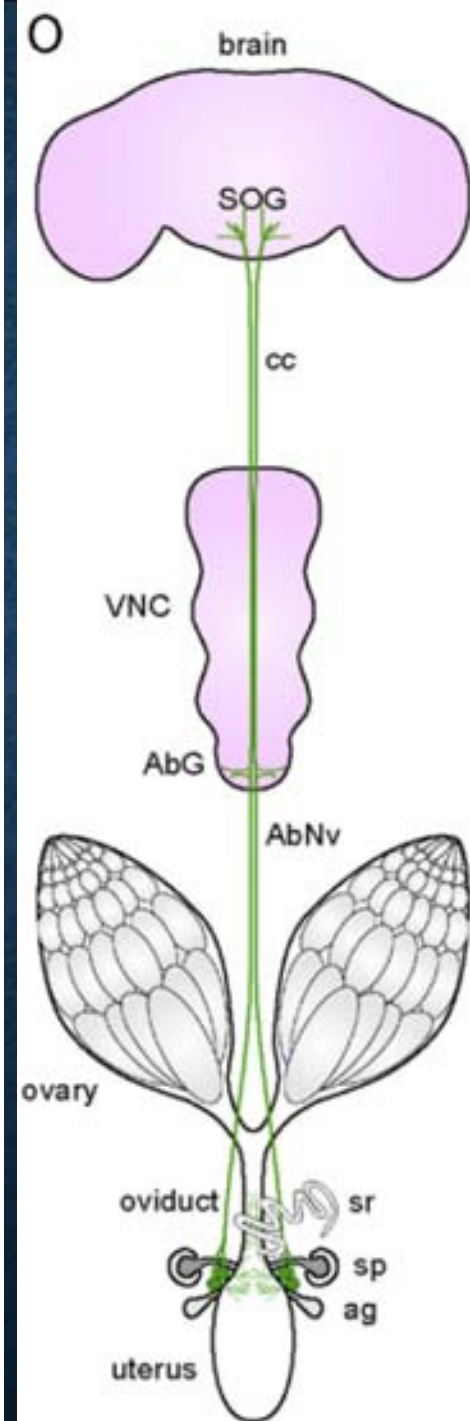
# Sensory Neurons in the *Drosophila* Genital Tract Regulate Female Reproductive Behavior

Martin Häsemeyer,<sup>1</sup> Nilay Yapici,<sup>1</sup> Ulrike Heberlein,<sup>2</sup> and Barry J. Dickson<sup>1,\*</sup>



SPR Acts in *ppk*<sup>+</sup> *fru*<sup>+</sup> Sensory Neurons



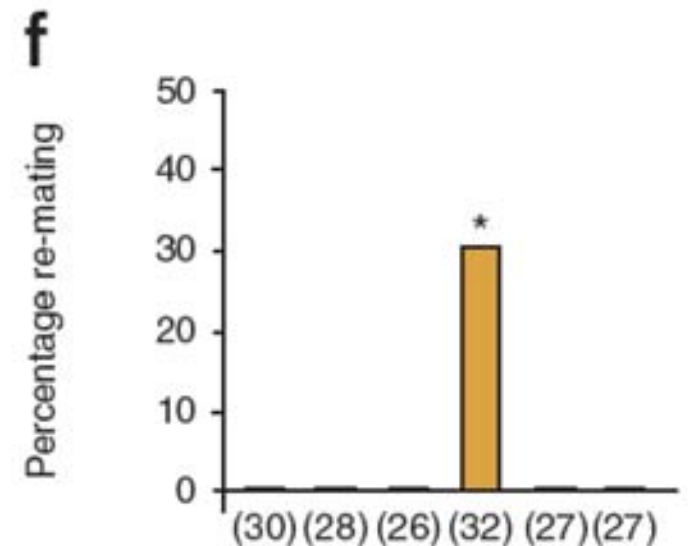
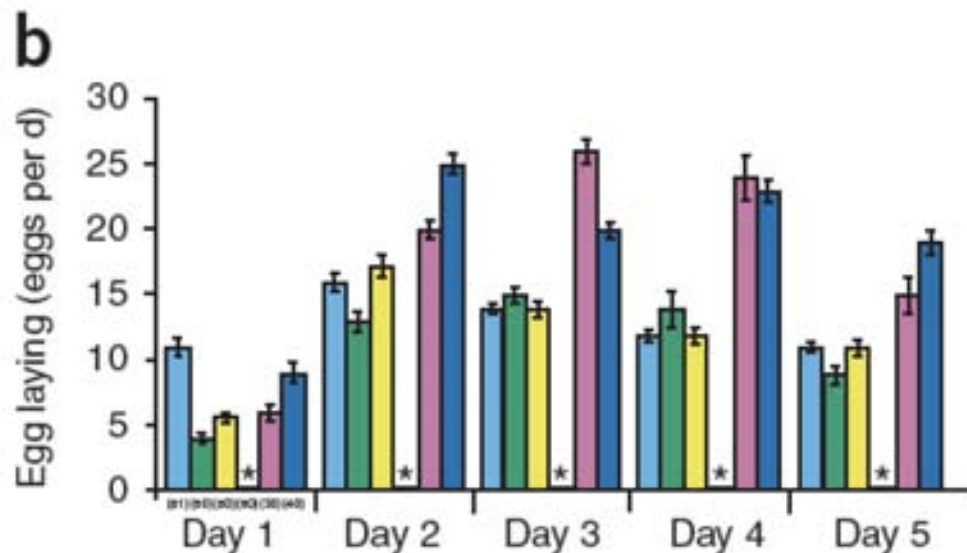


## Question 2: what's the neural basis for post-mating behaviors

- *fruGAL4* neurons are involved;
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- *fru* and *ppk*-positive sensory neurons in the genital tract;
- *dsx* neurons are involved (several sub-types, **including *ppk*-positive sensory neurons, octopaminergic neurons, SAG neurons and Mip neurons**);

# Control of sexual differentiation and behavior by the *doublesex* gene in *Drosophila melanogaster*

Elizabeth J Rideout<sup>1,3,4</sup>, Anthony J Dornan<sup>1,4</sup>, Megan C Neville<sup>1,2,4</sup>, Suzanne Eadie<sup>1</sup> & Stephen F Goodwin<sup>1,2</sup>



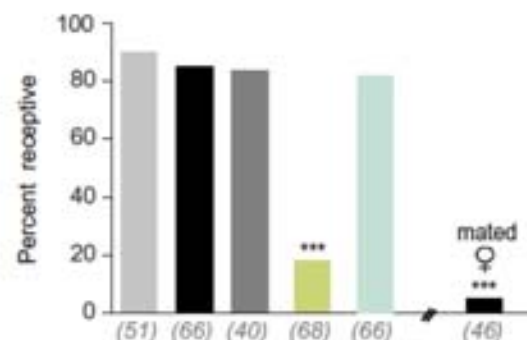


# Neural Circuitry Underlying *Drosophila* Female Postmating Behavioral Responses

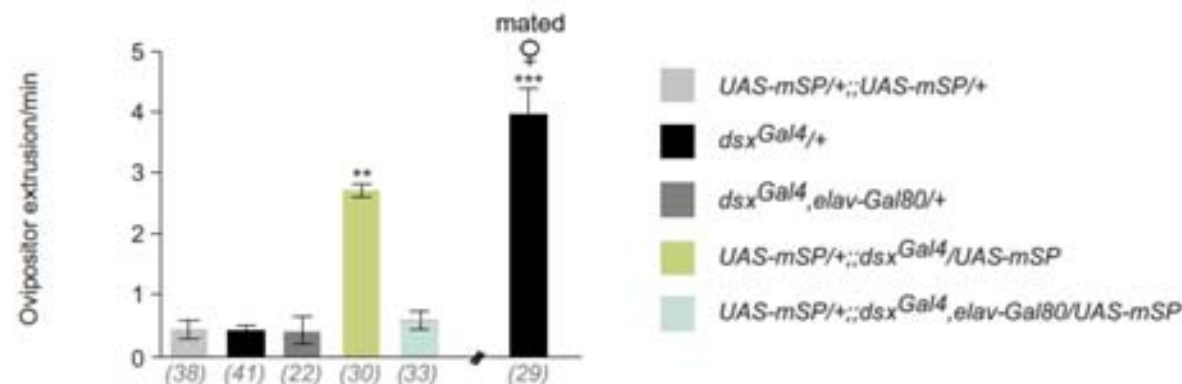
Carolina Rezával,<sup>1</sup> Hania J. Pavlou,<sup>1</sup> Anthony J. Dornan,<sup>2</sup>  
Yick-Bun Chan,<sup>3</sup> Edward A. Kravitz,<sup>3</sup>  
and Stephen F. Goodwin<sup>1,\*</sup>

type and fitness before sanctioning mating [1]. An unreceptive female exhibits rejection behaviors such as kicking and ovipositor extrusion [1, 6–8]. A receptive female will facilitate copula-

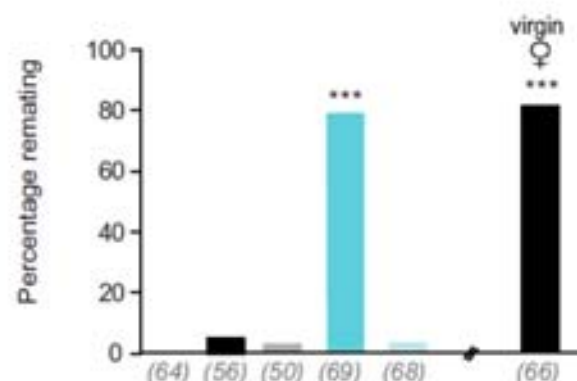
A



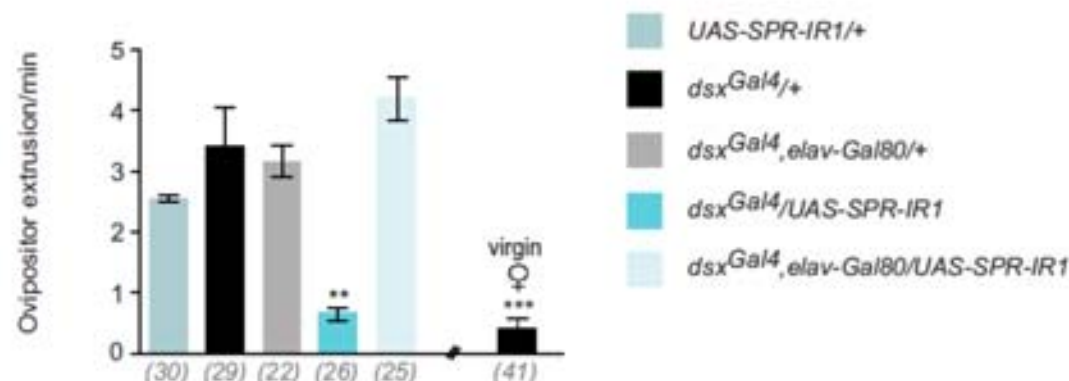
B



A

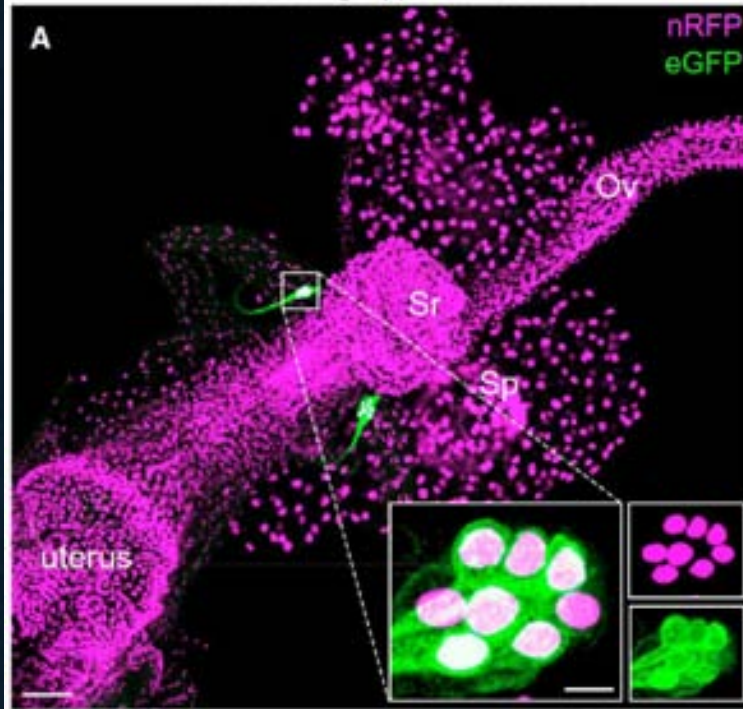


B

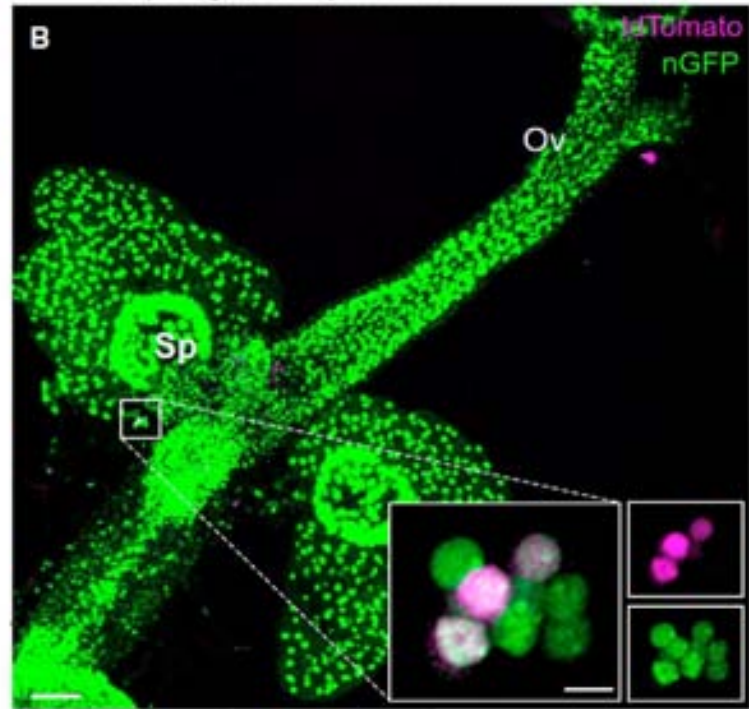




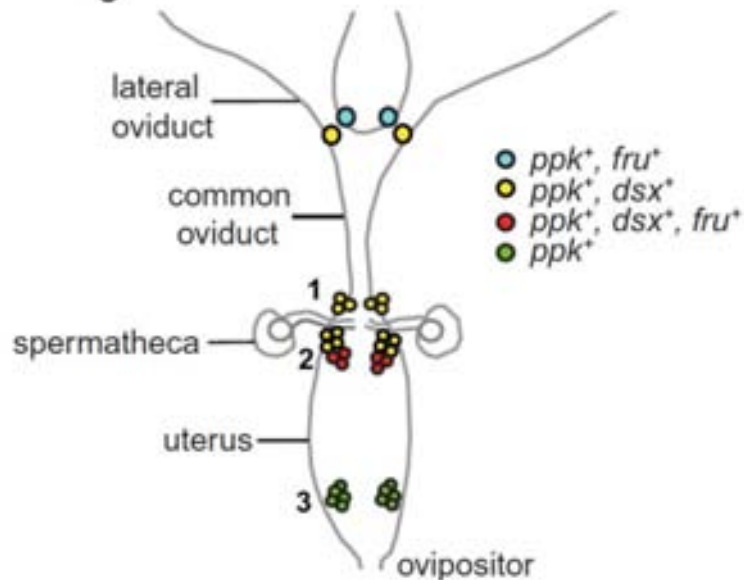
UAS-red Stinger/ppk-eGFP;dsx<sup>Gal4</sup>/+



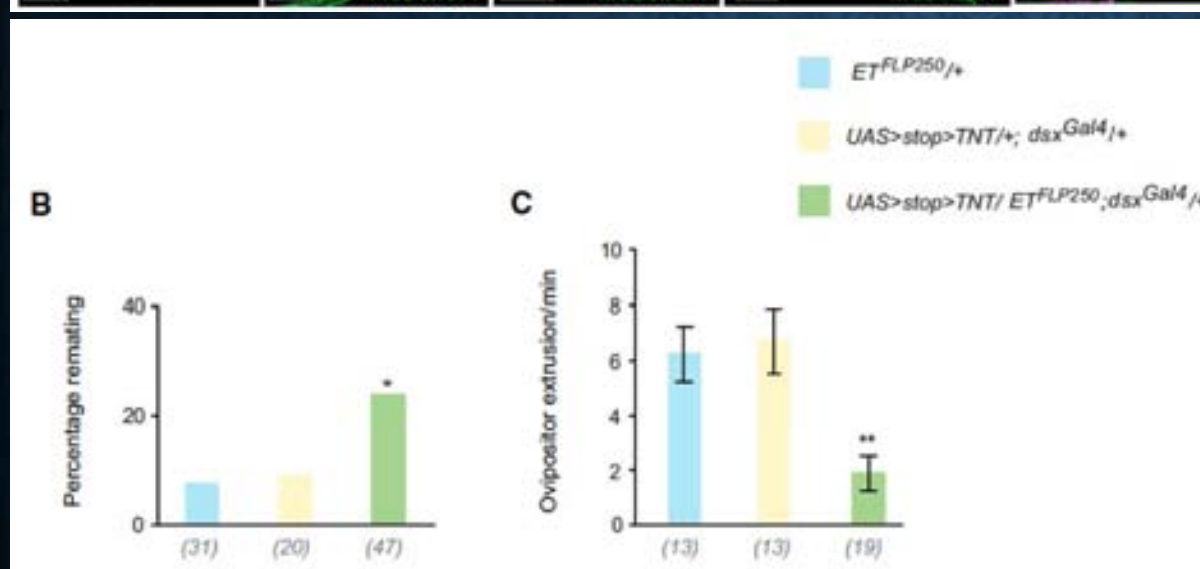
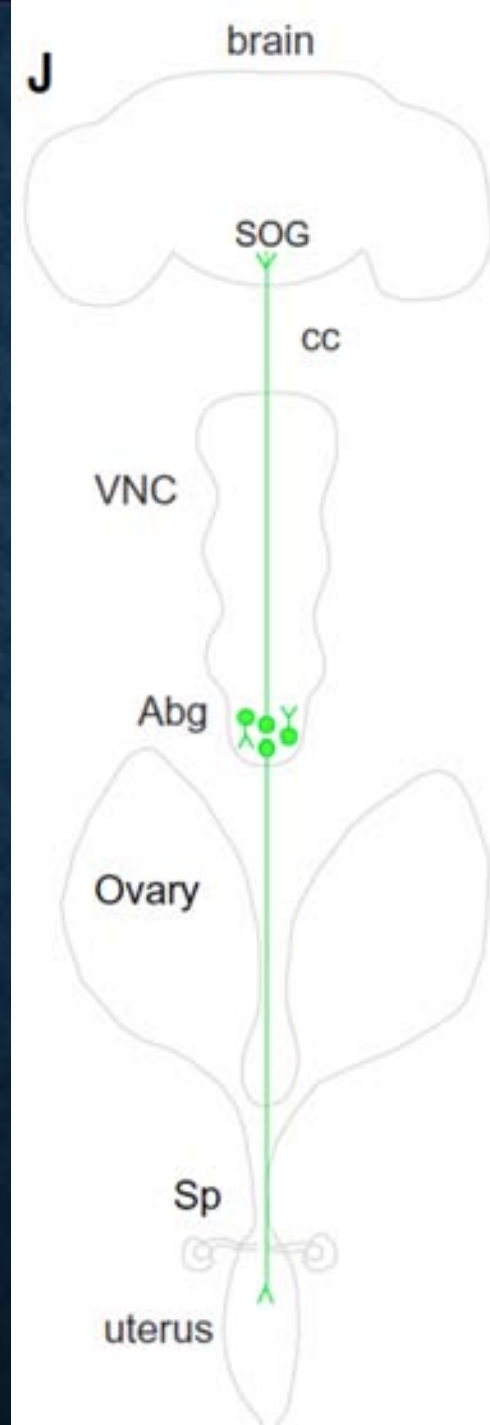
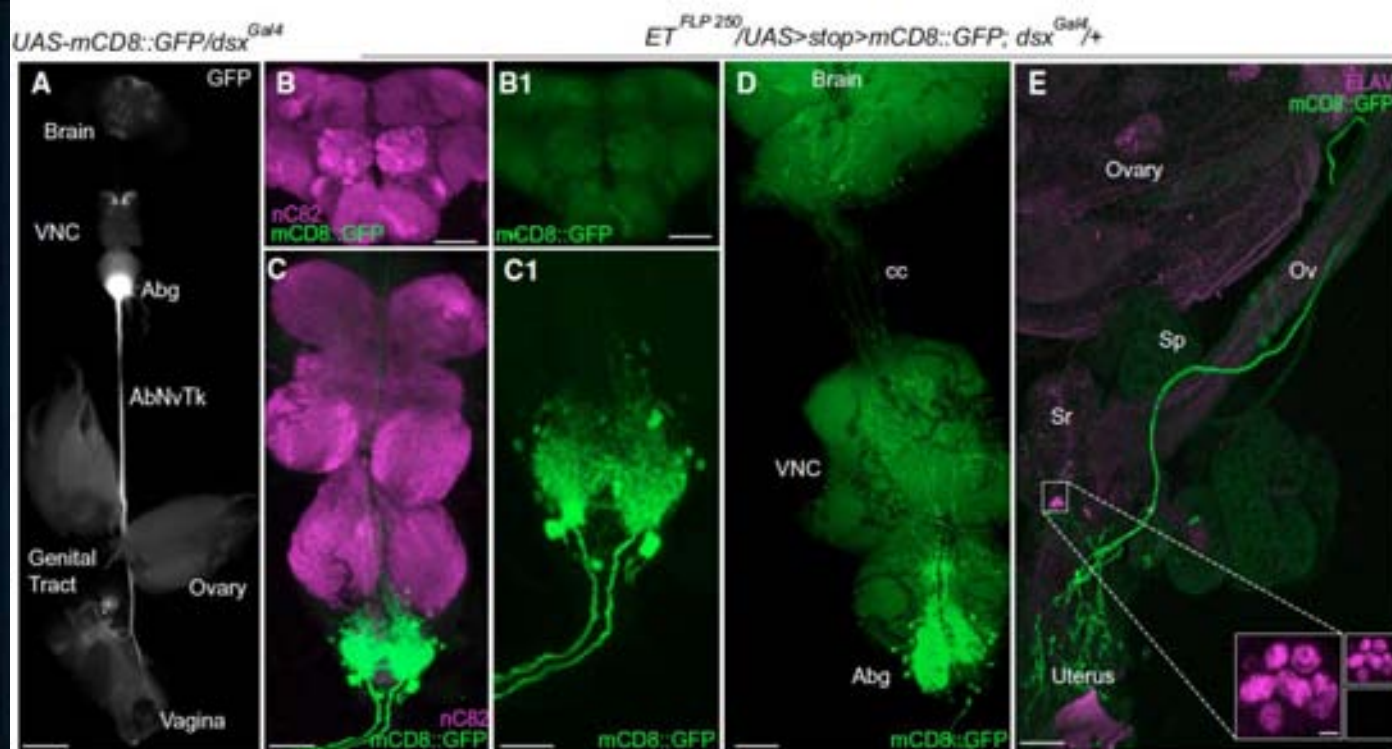
UAS-pStinger, LexAop-IdTomato::nls; fru<sup>P1LexA</sup>/dsx<sup>Gal4</sup>



**C**



*dsx*, *fru* and *ppk* sensory neurons  
in the reproductive tract



*dsx* neurons in Abg involved in post-mating behaviors

## Question 2: what's the neural basis for post-mating behaviors

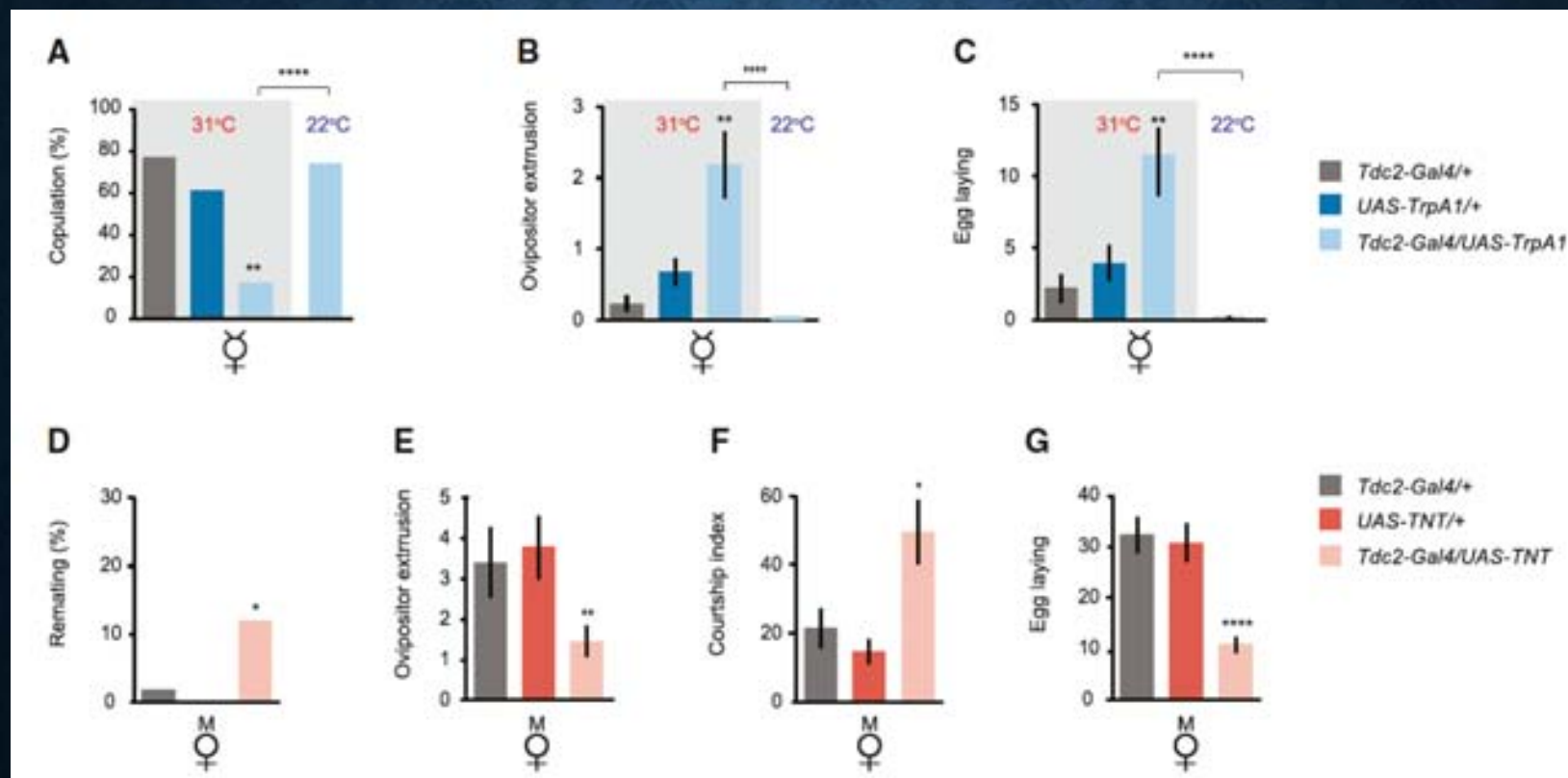
- *fruGAL4* neurons are involved;
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# Sexually Dimorphic Octopaminergic Neurons Modulate Female Postmating Behaviors in *Drosophila*

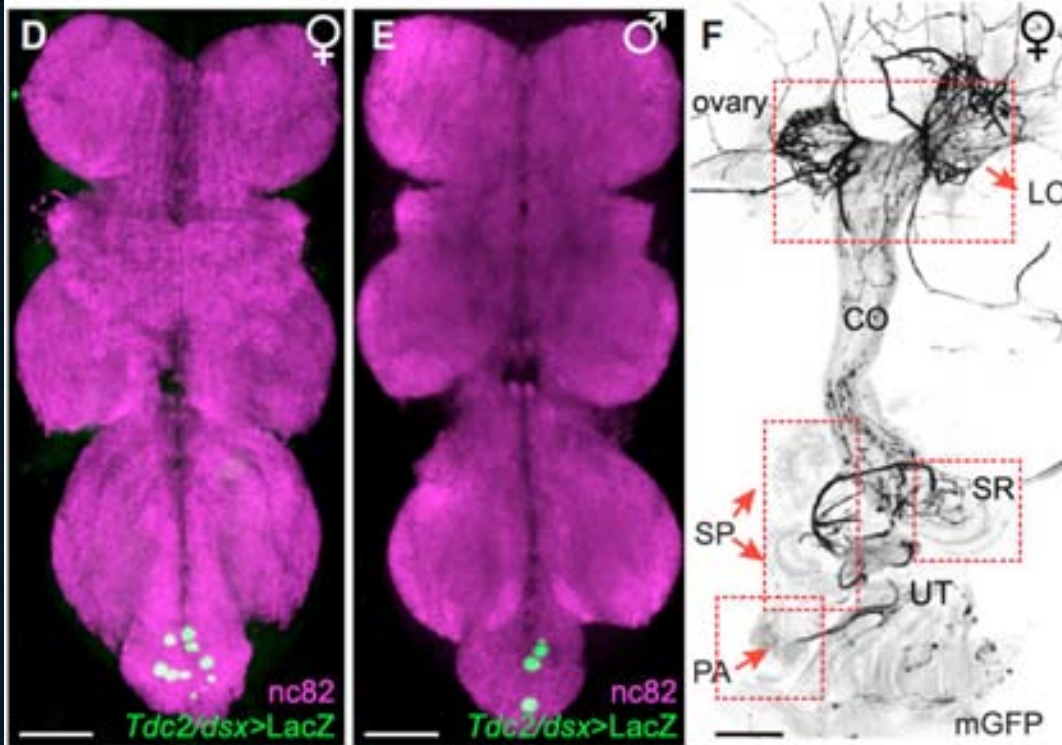
Carolina Rezával,<sup>1,\*</sup> Tetsuya Nojima,<sup>1</sup> Megan C. Neville,<sup>1</sup>  
Andrew C. Lin,<sup>2</sup> and Stephen F. Goodwin<sup>1,\*</sup>

We first evaluated the effects of depleting OA on female behavior by testing a null mutation in the gene that encodes

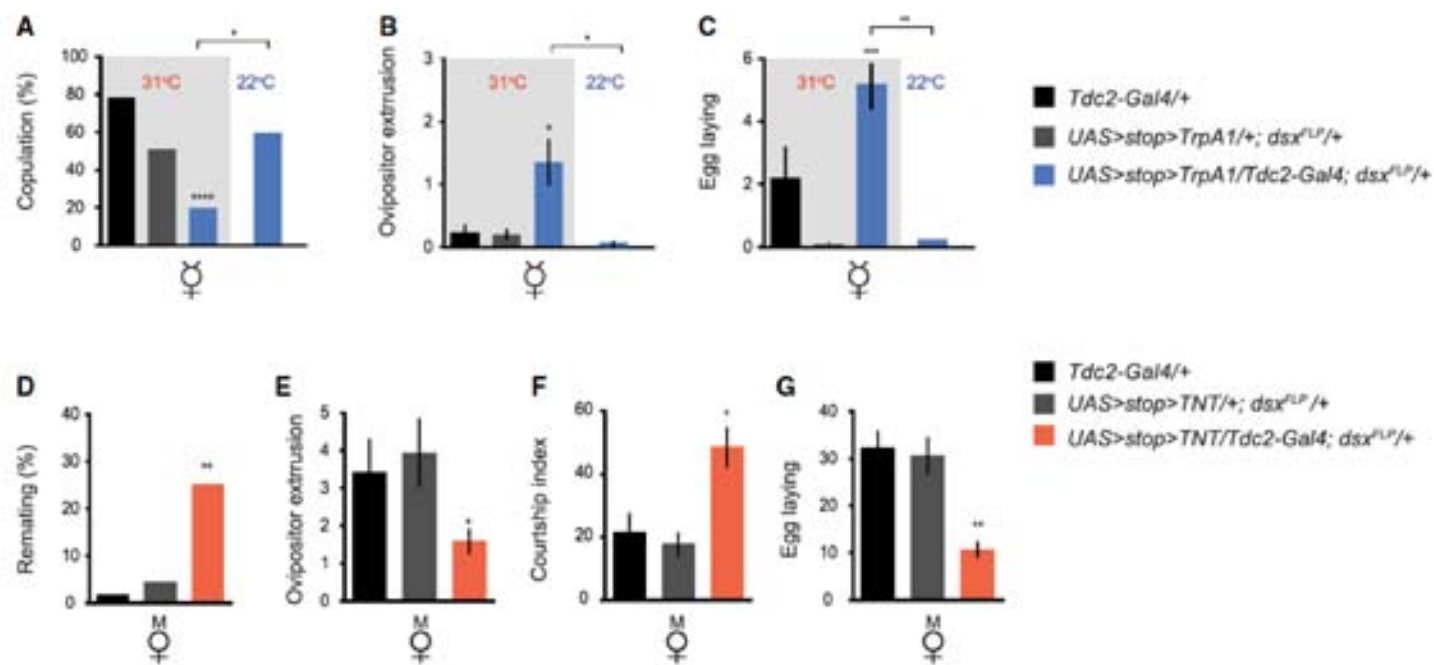


*Tdc2*<sup>+</sup> Neurons Are Involved in Post-mating Behaviors





Sexually Dimorphic  
*Tdc2/dsx+* Abg neurons  
 involved post-mating  
 behaviors

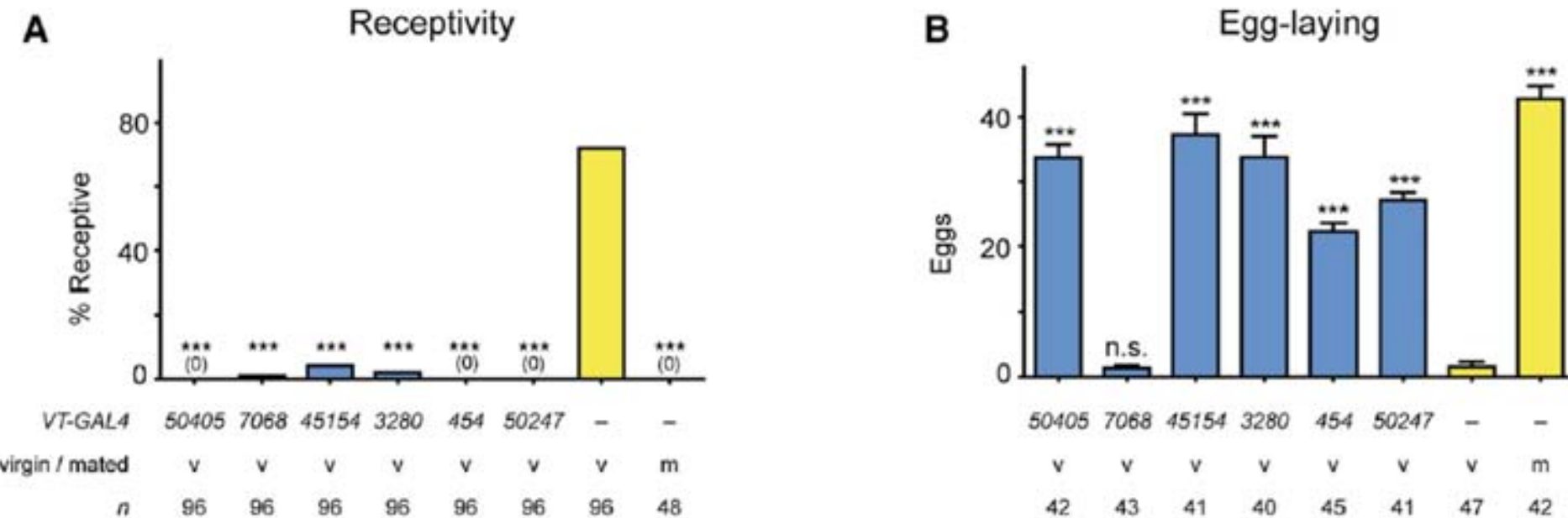


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# Ascending SAG Neurons Control Sexual Receptivity of *Drosophila* Females

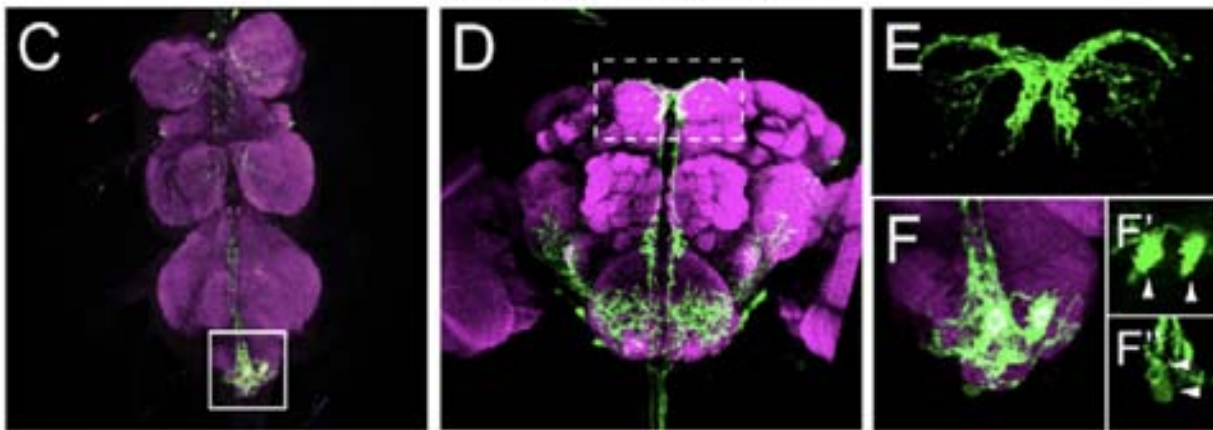
Kai Feng,<sup>1,2,5</sup> Mark T. Palfreyman,<sup>1,5,\*</sup> Martin Häsemeyer,<sup>1,3</sup> Aaron Talsma,<sup>1</sup> and Barry J. Dickson<sup>1,4,\*</sup>



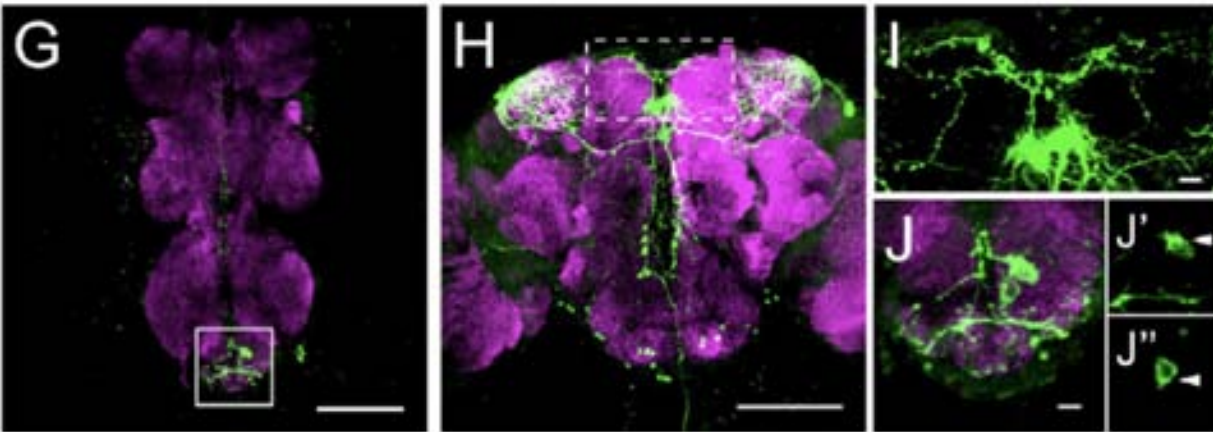
Identification of VT lines that induce post-mating behaviors when driving UAS-kir2.1



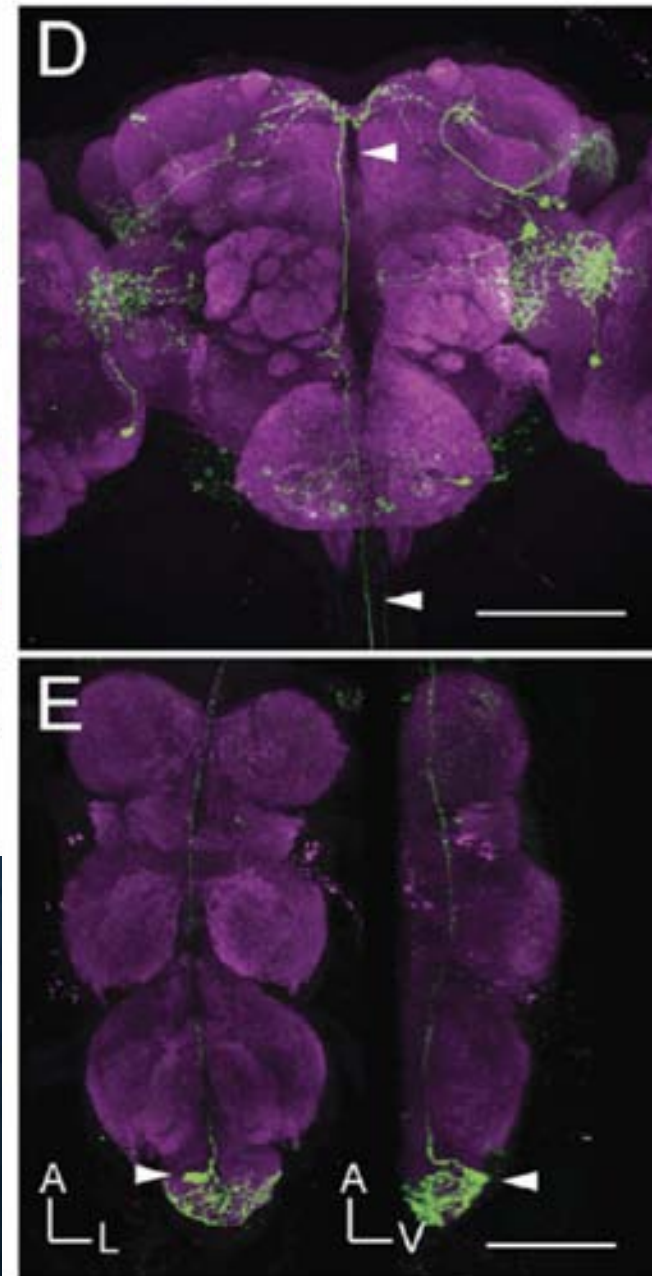
SAG-1, UAS-mCD8-GFP



SAG-2, UAS-mCD8-GFP



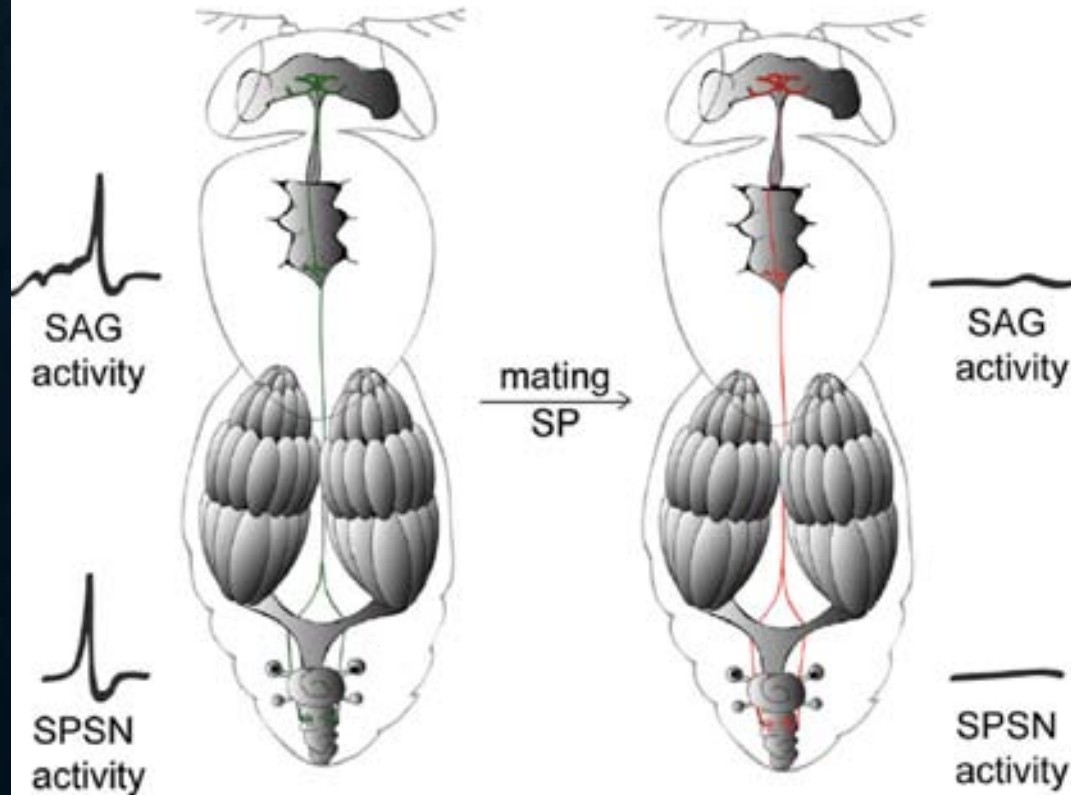
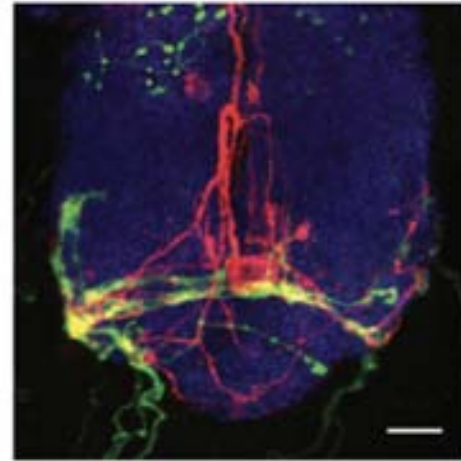
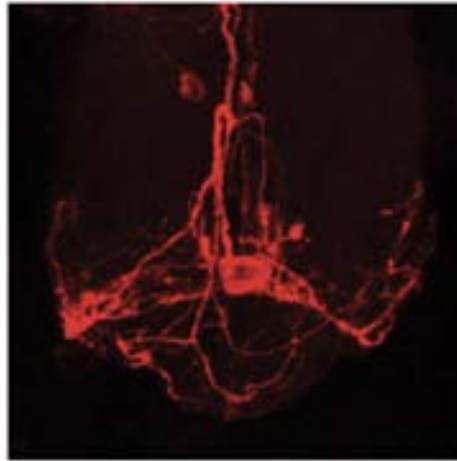
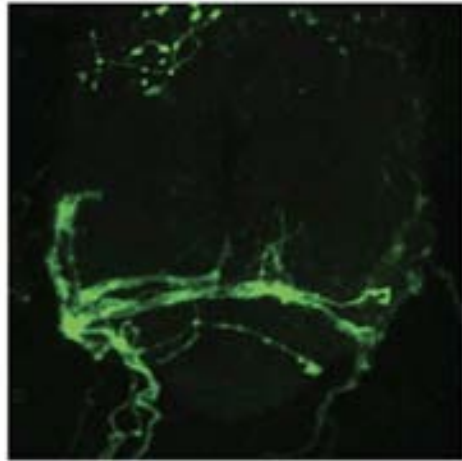
VT50405-GAL4  
UAS-mCD8-GFP  
(stochastic labeling)



SAG neurons

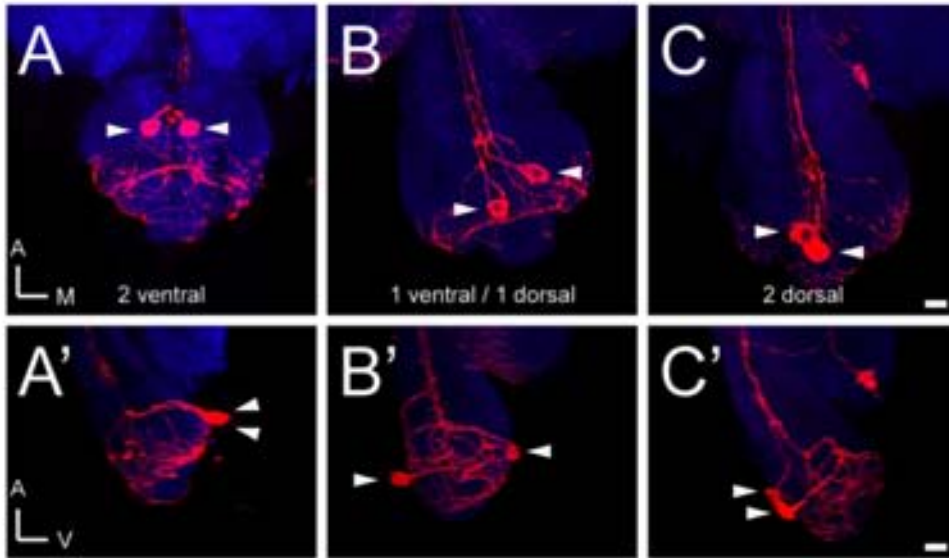


SPSN (VT3280-*lexA3*, *lexAop-myrGFP*)  
 SAG (SAG-2-GAL4, UAS-mCD8-*tdTomato*)

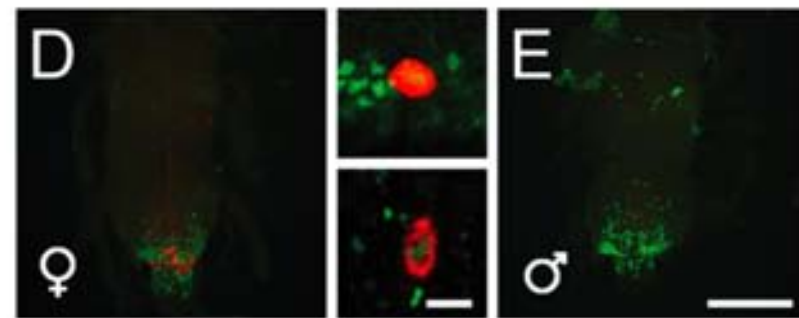


SAG neurons are downstream of *ppk*, *fru* and/or *dsx* sensory neurons in the reproductive tract.

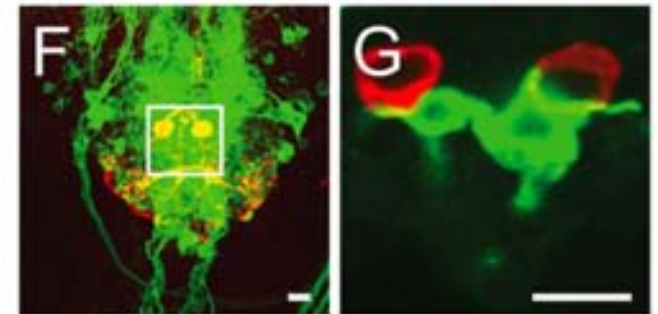
SAG-2, UAS-mCD8-*tdTomato*



SAG-2, UAS-mCD8-*tdTomato* anti-Dsx



*fruP1.lexA*, *lexAop-CD2-GFP*  
SAG-2, UAS-mCD8-*tdTomato*



SAG neurons are *dsx*-positive, but *fru*-negative

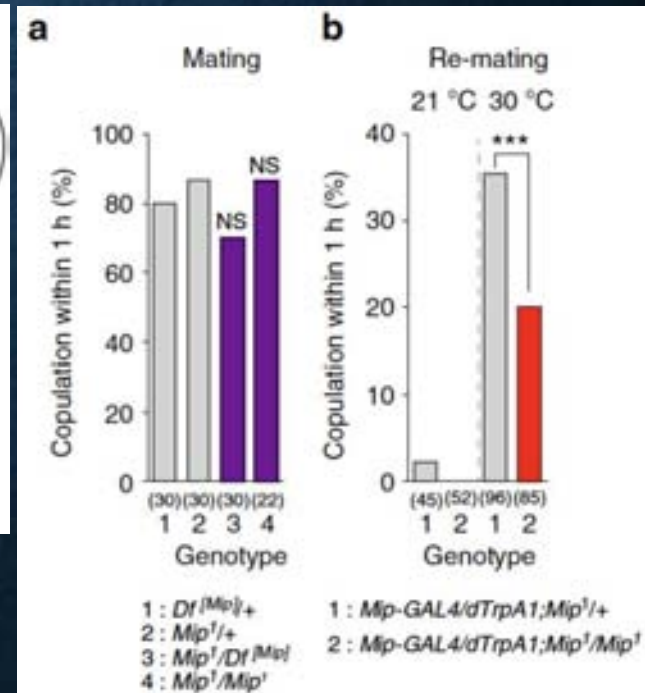
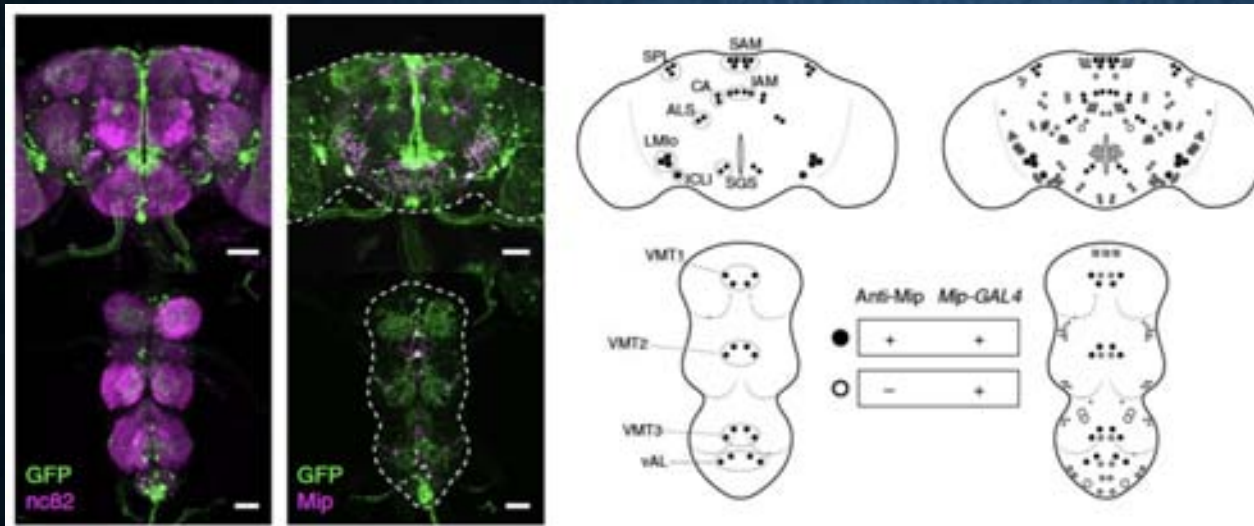
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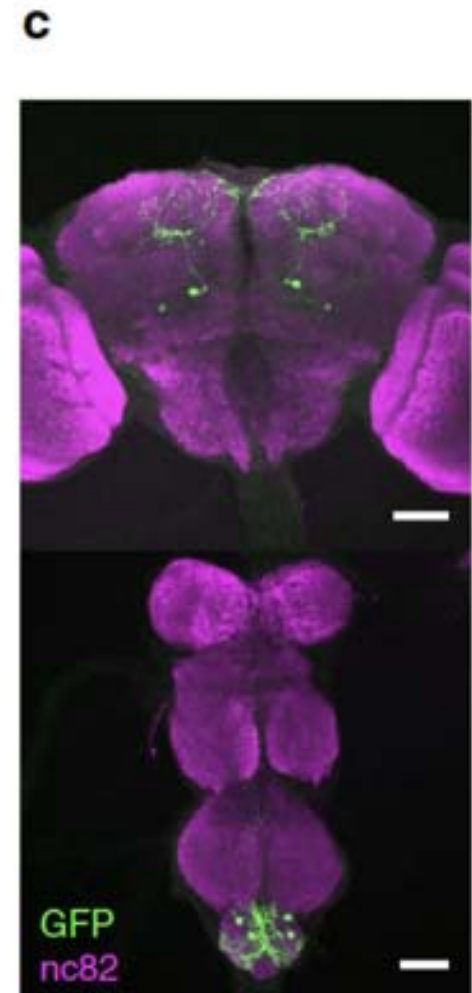
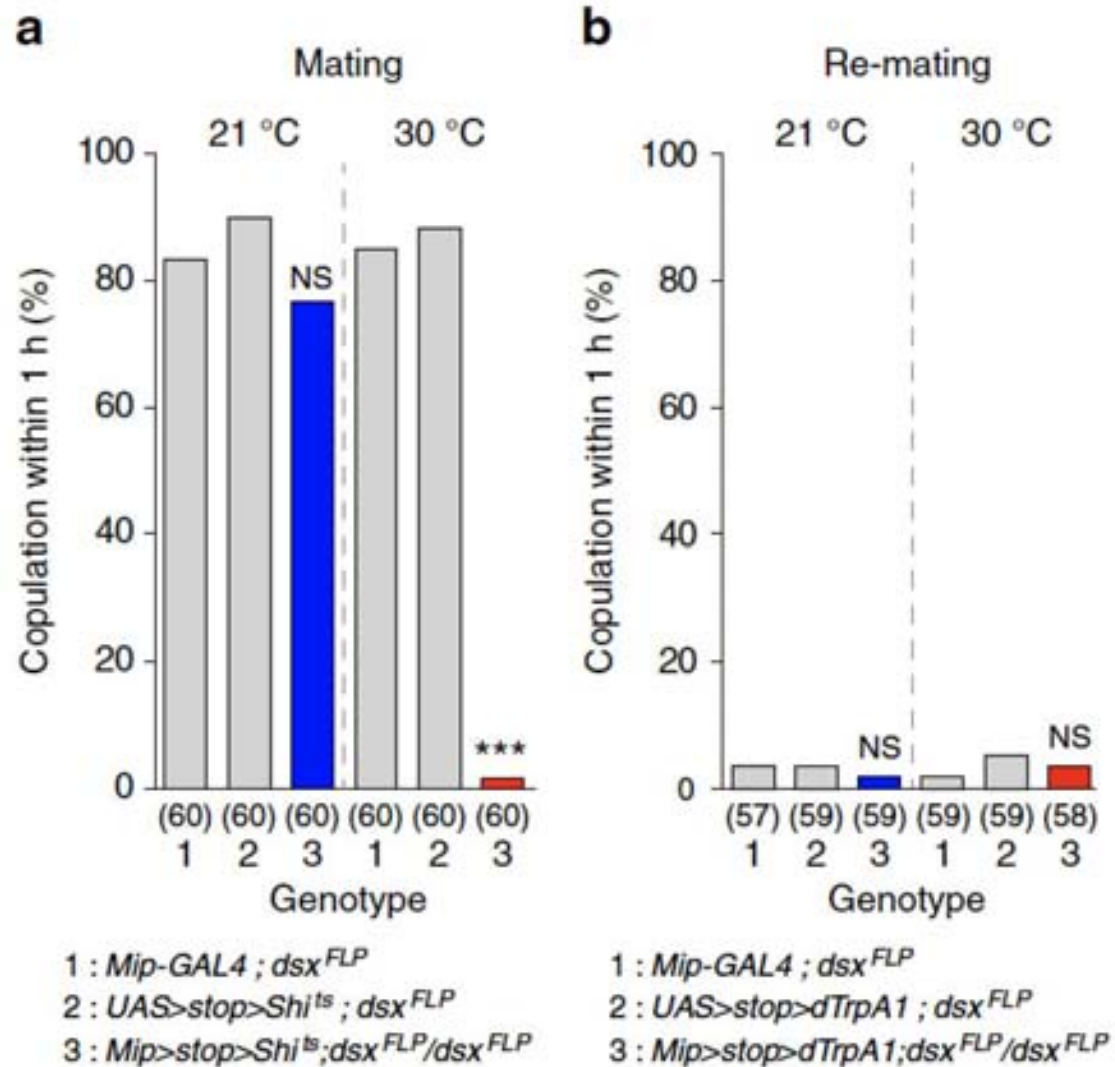
# Female-specific myoinhibitory peptide neurons regulate mating receptivity in *Drosophila melanogaster*

Yong-Hoon Jang<sup>1</sup>, Hyo-Seok Chae<sup>1</sup> & Young-Joon Kim<sup>1</sup>

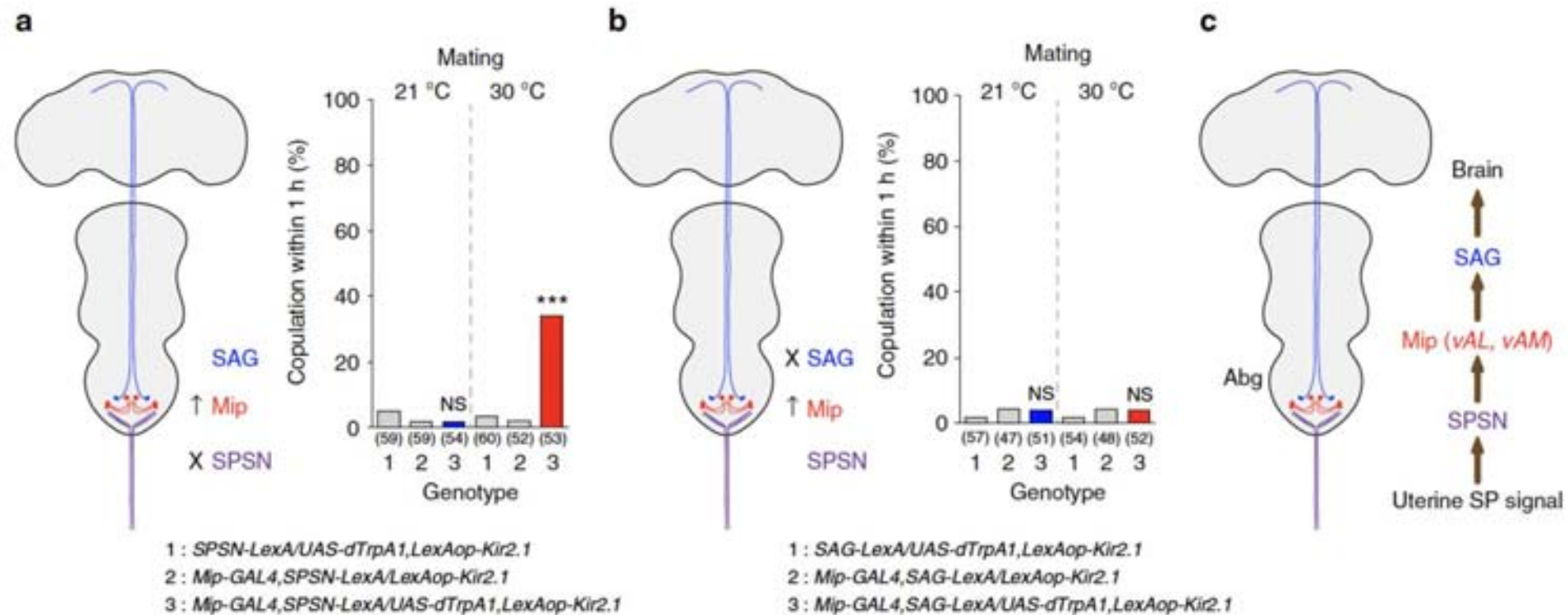


Mip-neurons are involved in post-mating behaviors





*dsx*-positive Mip neurons are involved in receptivity



Mip neurons may position within the SPSN and SAG signaling axis

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