“Mating proximity blinds threat perception”

Laurie Cazalé-Debat et al., *Nature*, 2024

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Agenda

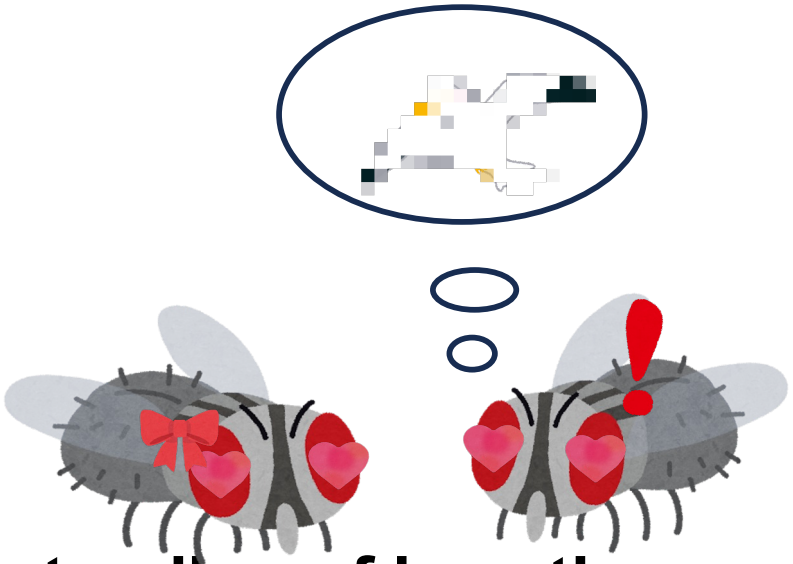
- Background
- Purpose of this Paper
- Experiment setup
- Neurons that balance risk and reward
  - LC16 neurons detect visual threat
  - 5-HT neurons send a signal
  - P1, pIP10 regulate the courtship-escape choice
- Males ignore threats late in courtship
- Dopamine ramps up during courtship
- Dopamine blocks visual threat detection
- Conclusions

Background

Animals constantly balance risks and rewards.

This trade-off has been studied in humans, rodents, and invertebrates.

- Avoiding danger increases survival but may lost mating opportunities.
- **Yet, how the brain resolves this conflict remains unclear.**



Still lacking a detailed understanding of how these competing needs are prioritized in the brain.

Purpose of this paper

Big Question

How neural networks balance the trade-off between risk and reward ?

Risk:
- Eaten by predator
- **Die**

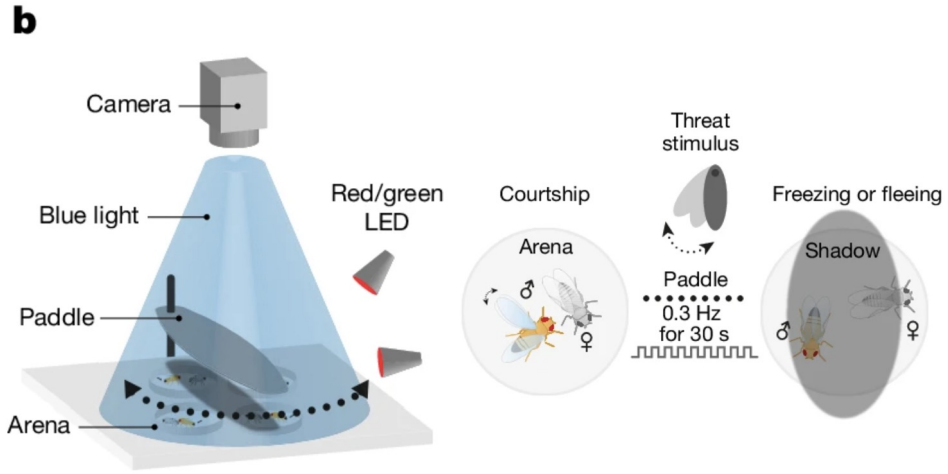
Reward:
- Courtship
- **Reproduction**

Paper's Goal

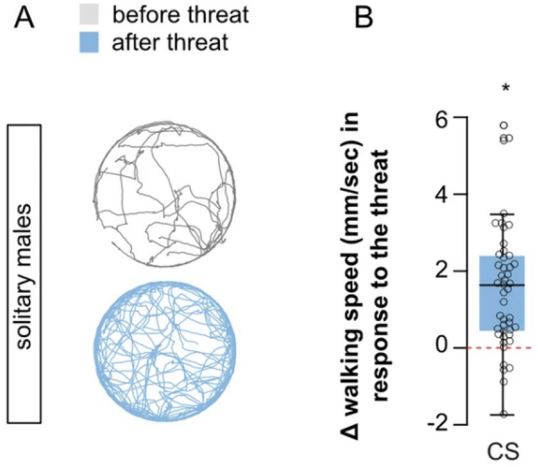
Unraveling how neural networks balance survival and reproduction.

Experiment setup

- **sex–danger conflict assay**(Fig. b).
- *Drosophila* males and virgin females were presented in arena with a visual threat: a predator-like moving shadow.



- The threat caused males to show **defensive responses** such as **running away** and **freezing**(Fig. A, B).

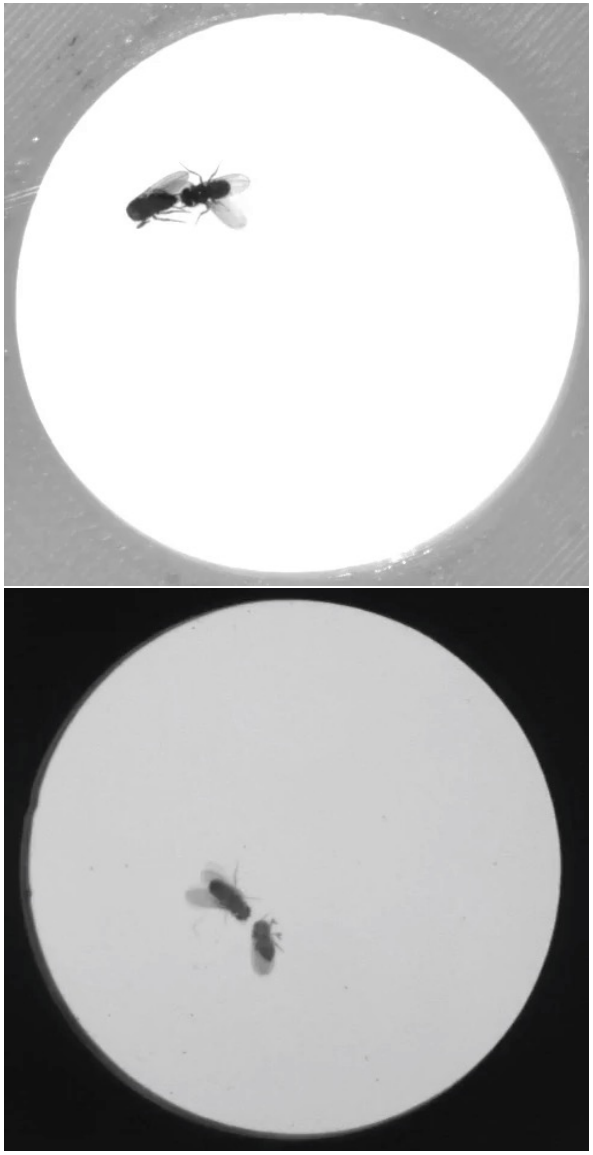


Q1: Do *Drosophila* interrupt sex when they sense a visual threat during mating?

A1: They interrupted their courtship !

- *Drosophila* males courted the females and showed low defensive behaviours (**without a visual threat**).

- *Drosophila* males immediately **quitted** courtship and engaged in defensive response (**with a visual threat**).



Q2: Then which neurons detect the visual threat?

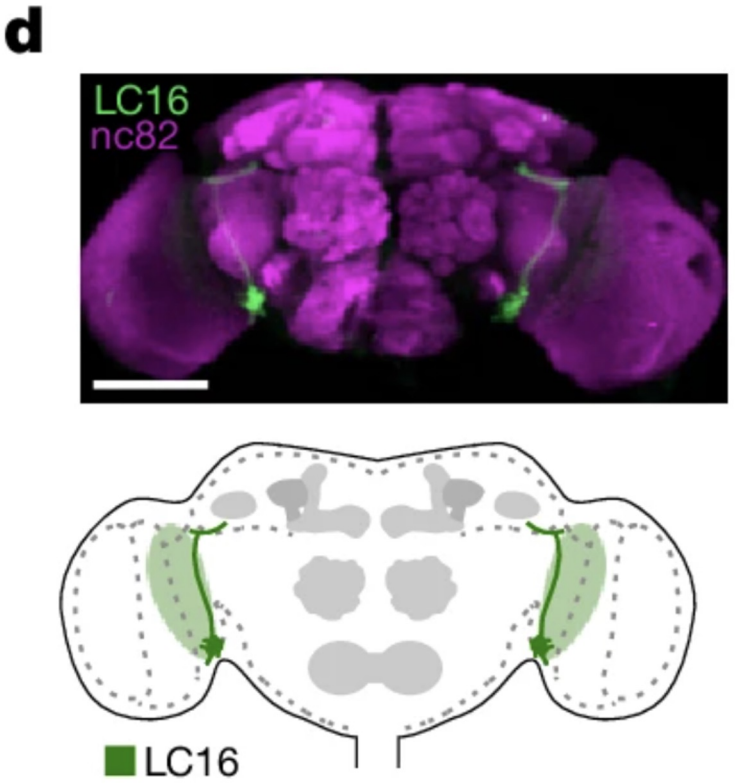
A2: LC16 neurons detect visual threats and trigger defensive actions!

What are LC neurons?

- Lobular columnar(LC) neurons: link early visual processing to brain centers, responding to motion and conspecific cues.

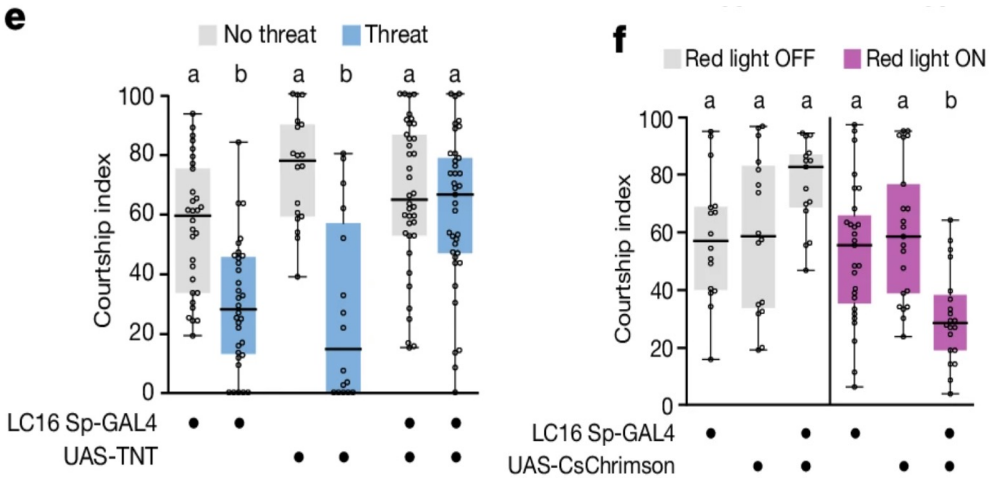
Hypothesis

Do LC neurons detect visual threats and convey to brain centres?



A2: LC16 neurons detect visual threats and trigger defensive actions!

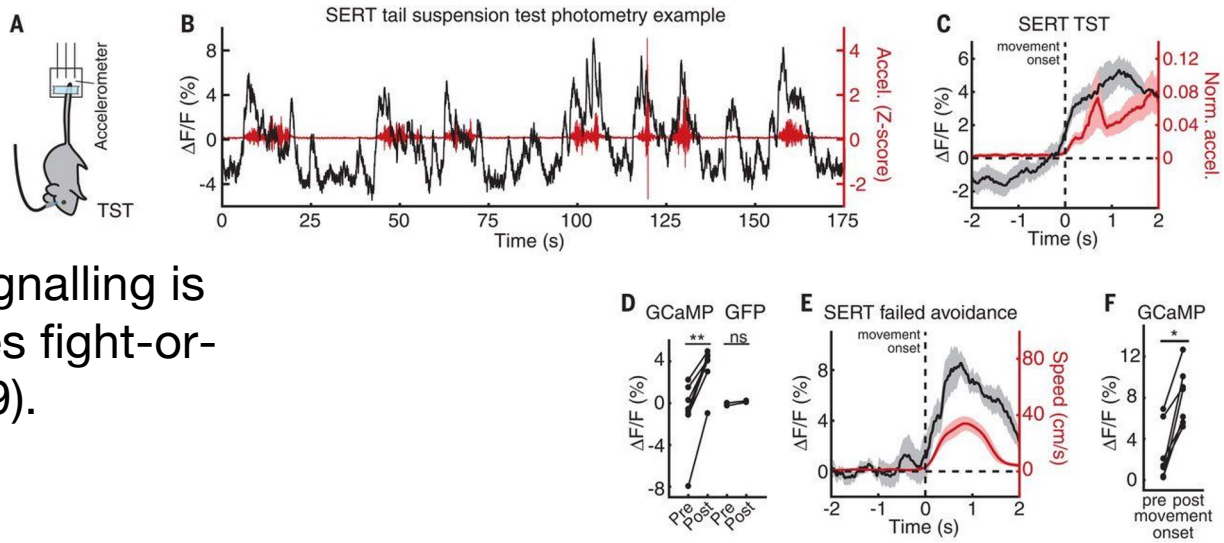
- LC16 were **silenced** by TNT.
  → males **continued** the courtship despite the threat(Fig. e).
- LC16 neurons were **activated** w/o the threat(Fig. f).
  →males **stopped** courting
- Males with **silenced** LC16 neurons.
  →showed **normal** courtship w/o the threat.



LC16 neurons detect visual threats.
If LC16 is **silenced**, males **continue** courtship even under threat.
If LC16 is **activated**, males **stop** courtship even without a real threat.

Q3: Then how LC16 deliver the visual threats?

The role of 5-HT signalling in Animals, might be also in *Drosophila*?



Research has shown that 5-HT signalling is increased by stress and modulates fight-or-flight responses(Seo C *et al,.* 2019).

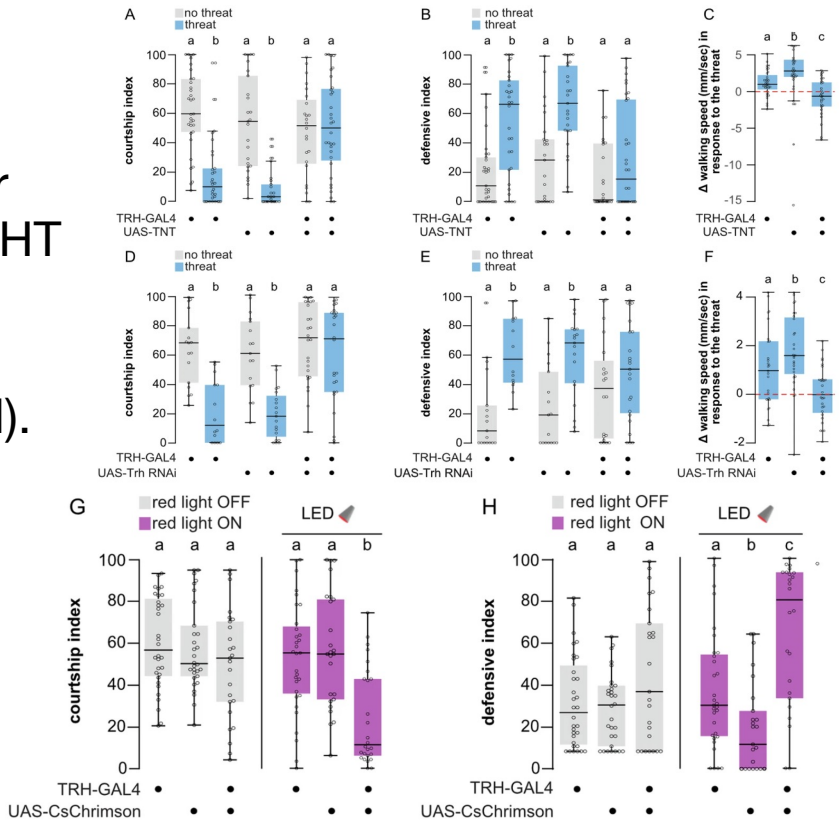
Seo C *et al,.* 2019,

Hypothesis

Could **5-HT** signaling also suppress courtship in *Drosophila*?

A3: 5-HT signalling modulates fight-or-flight responses in *Drosophila*!

- 5-HT signal is created in 5-HT neurons.
- The threat **did not increase** walking speed or suppress courtship in males with **silenced** 5-HT neurons(Fig. A~F).
- Activation of 5-HT neurons **suppressed courtship** even without a real threat(Fig, G, H).



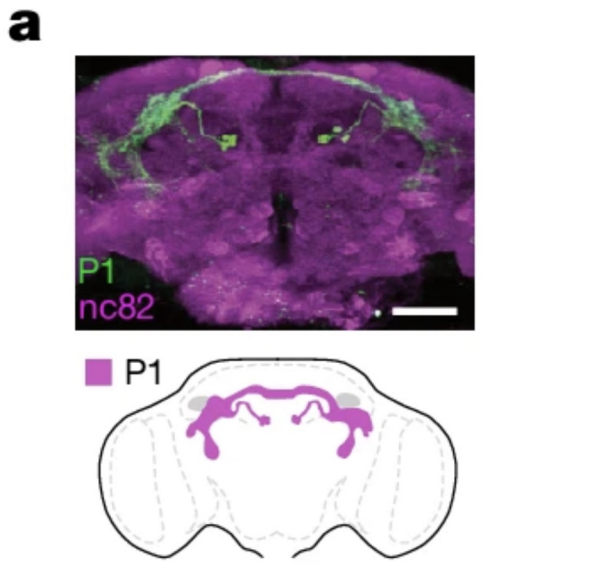
LC16 neurons deliver the threats to **5-HT neurons**:
5-HT neurons are **important** for **prioritizing escape** over courtship in response to visual threats.

Q4: Then what neurons actually regulate the courtship-escape choice?

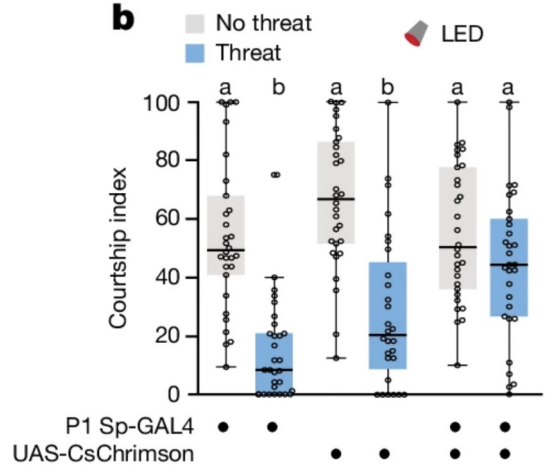
A4: P1 neurons regulate the courtship-escape choice!

What are P1 neurons?

- P1 neurons are the neuron cluster which has a role of the central mating regulation hub that internal states(Fig. a).



- **Activated** P1 neurons in males during the exposure to the visual threat(Fig. b) .
- It caused them to **continue** to court, **overriding** the threat response.



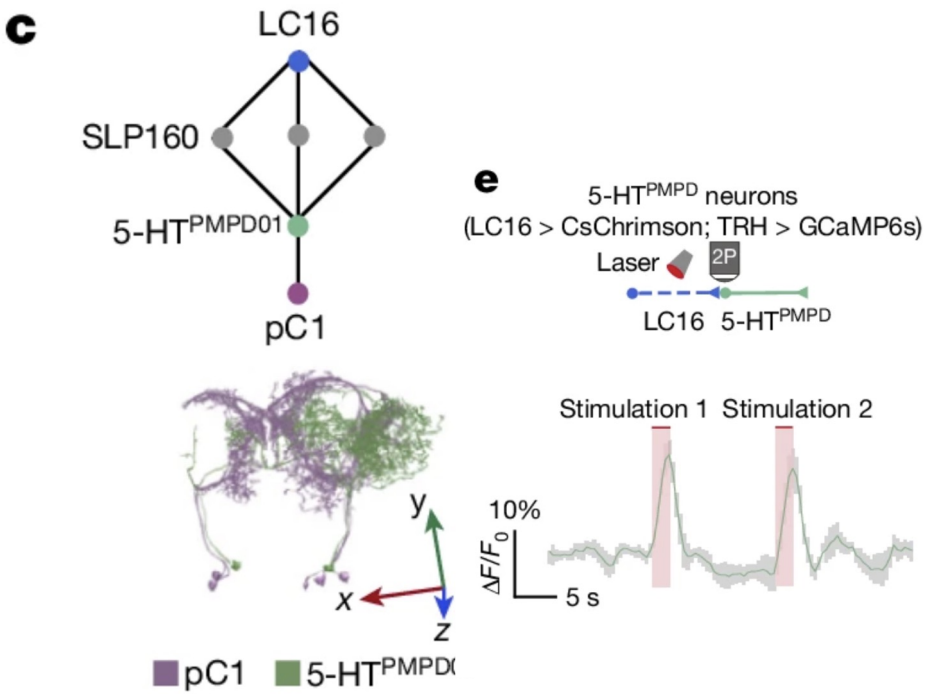
Q5: Then how LC16 neurons connect to P1 neurons?

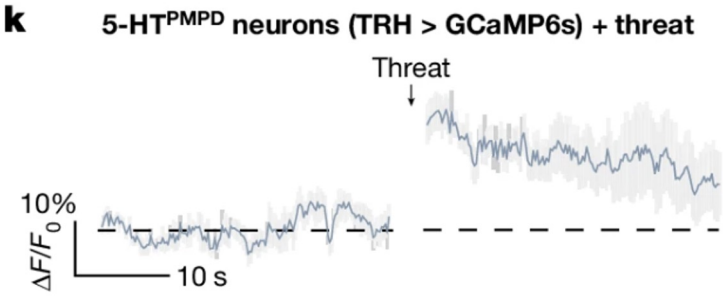
A5: 5-HT^{PMPD} neurons link LC16 and P1.

- From the female *Drosophila* connectome, a 5-HT neuron in the PMPD cluster is an attractive candidate that connects between LC16 and P1(Fig.c).

- Activated LC16 simulation **reliably increased** the signal in 5-HT^{PMPD} neurons(Fig.e).

- Threat exposure triggered significant calcium influx in 5-HT^{PMPD} neurons (Fig. k), **similar** to the threat response of LC16 neurons.





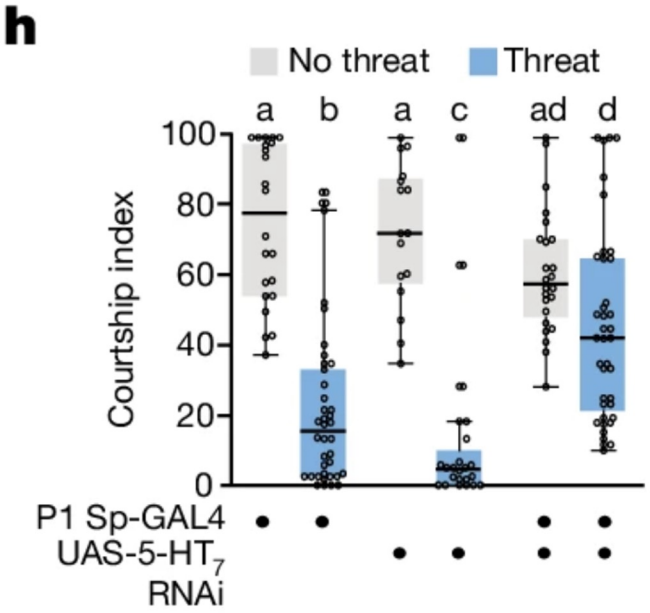
A5: 5-HT^{PMPD} neurons link LC16 and P1.

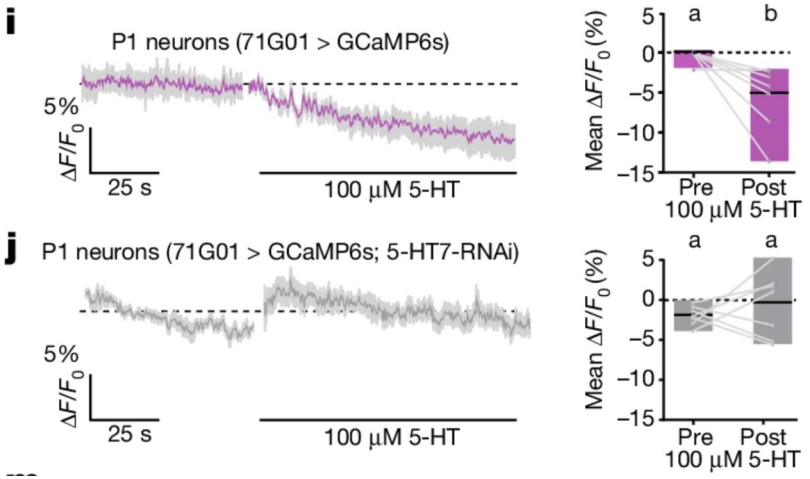
Five 5-HT G protein receptors in *Drosophila*

5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_{1B}$	5-HT$_{2B}$	**5-HT$_7$**

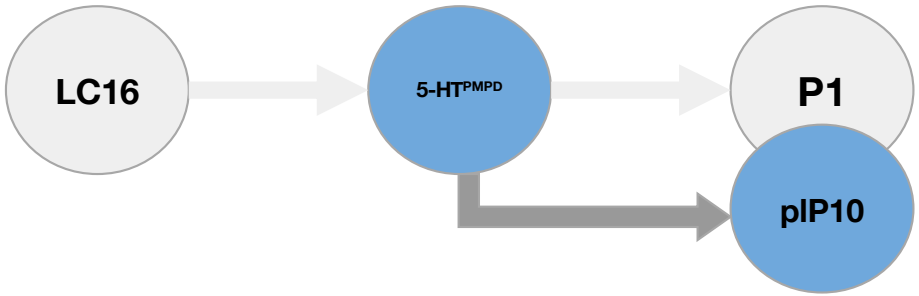
- Flies deficient in either **5-HT7** or **5-HT2B** receptors in P1 responded **less** to the **threat.**
- Knocking down 5-HT7 **increased** courtship(Fig. h).

- 5-HT **reduces** P1 activity,
  but this effect is **lost** w/o 5-HT$_7$ (Fig. i, j).



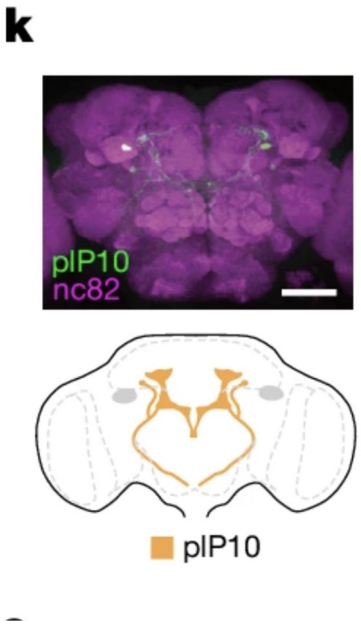


5-HT^{PMPD} also involves pIP10 neurons.

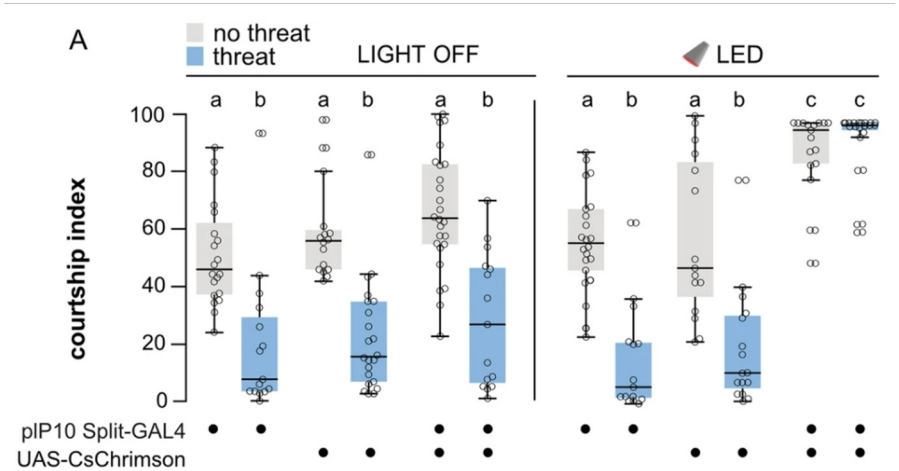


What are pIP10 neurons?

- pIP10 neurons are neurons which target the wing motor region in the ventral nerve cord and are crucial for courtship song(Fig. k).



- Activation of pIP10 resulted in high, **sustained** courtship levels throughout threat delivery (Fig. A).
- Optogenetic inhibition of pIP10 w/o the threat robustly **suppressed** courtship.



5-HT^{PMPD} also involves pIP10 neurons.

5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_{1B}$	**5-HT$_{2B}$**	5-HT$_{7}$

- 5-HT$_{2B}$ knockdown in pIP10 → Courtship **continued** despite threat(Fig. I).
- It **did not affect** defensive behaviors in solitary males.

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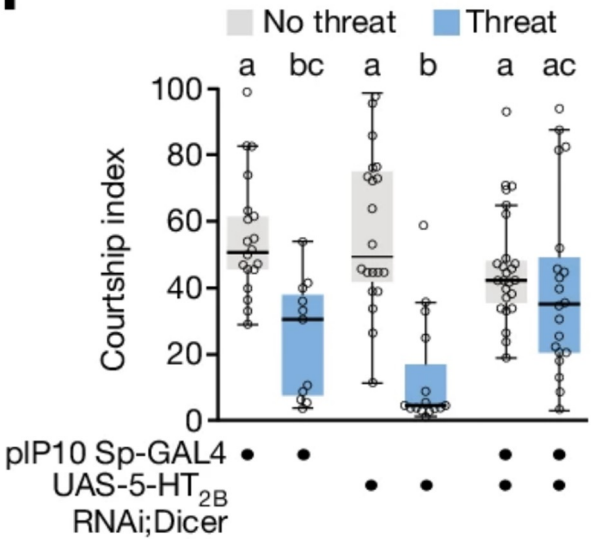


5-HT^{PMPD} neurons…
- **connect** between LC16 neurons and P1, pIP10 neurons.
- Suppress courtship by inhibiting **P1** cells via **5-HT$_7$**.
- Likely inhibit **pIP10** neurons via **5-HT$_{2B}$** to prevent courtship enduringly.

Key takeaways so far are…

Big Question

How neural networks balance the trade-off between risk and reward ?

Threat detection→**LC16 neurons** activate **5-HT^{PMPD}** neuron.
5-HT neuron suppresses **P1 & pIP10**, shifting priority to survival.
Neural networks use serotonin(**5-HT**) to **balance risk & reward**.

But...

Does this trade-off remain constant throughout courtship?

Males ignore threats late in courtship

Early

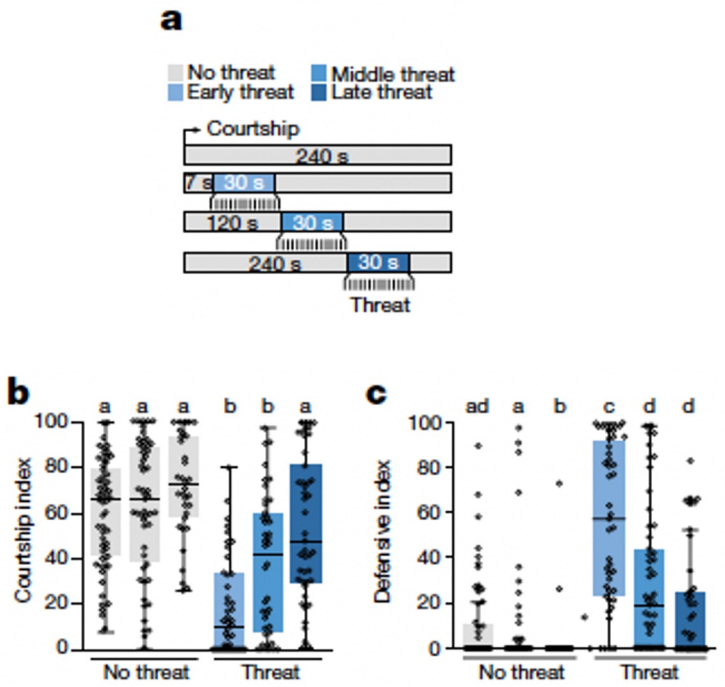
- male flies will terminate courtship when they encounter visual threats.

Middle

- males' responses to visual threats gradually weaken

Late

- less susceptible to disturbance



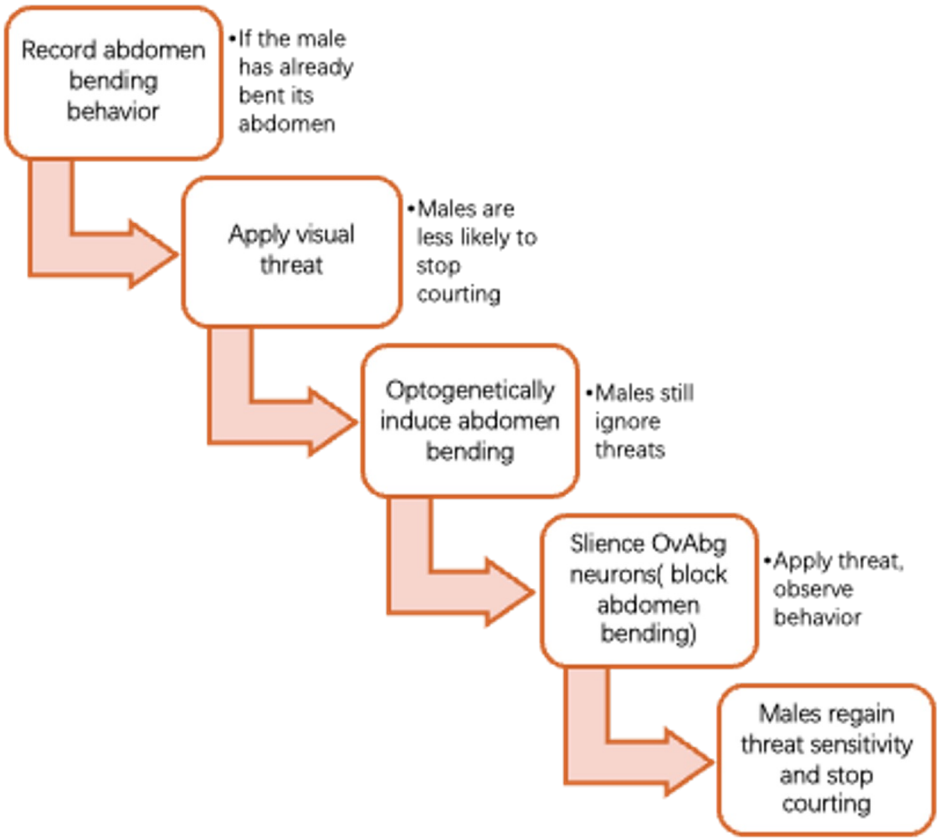
Result: As courtship advances, the male Drosophila response to visual threat diminishes.

Fig. b: courtship index
Fig. c: defense index

Males ignore threats late in courtship

Goal: Does **abdomen bending** *reduce threat sensitivity in late-stage courtship?*

Experiments:



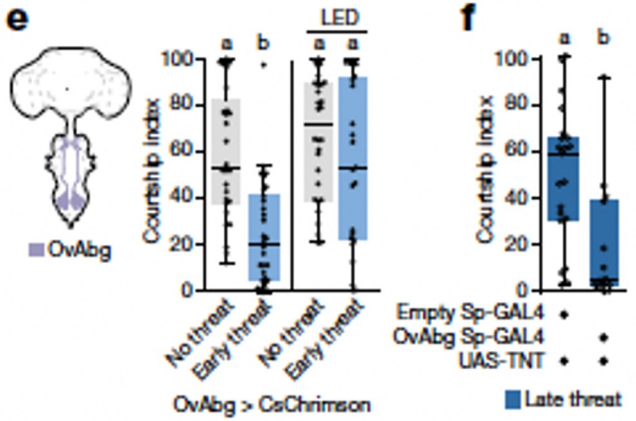


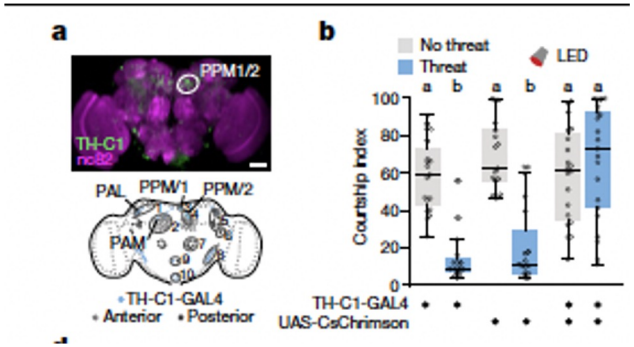
Fig. e: LED-induced abdominal flexion
Fig. f: OvAbg modulate threat perception

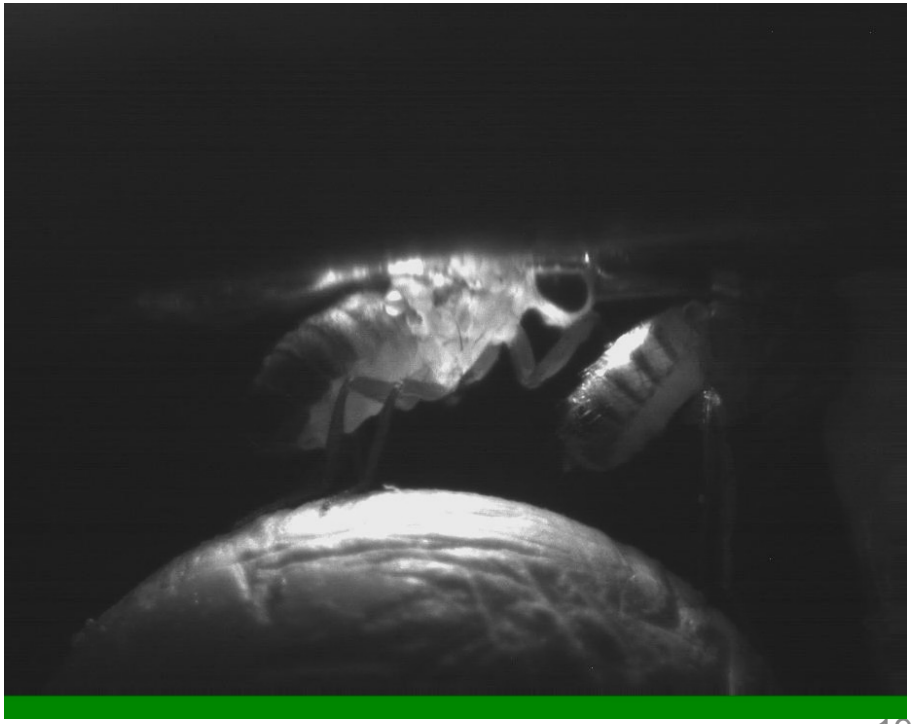
Result:
OvAbg *neurons trigger abdomen bending, which activates* ***PPM1/2*** *dopamine release, leading to reduced threat sensitivity in late-stage courtship.*

Dopamine ramps up during courtship

Goal: How does **dopamine activity** change during courtship progression?

Experiments:
Tracked **PPM1/2** activity using two-photon imaging.
Compared dopamine activity in two conditions:
• Males courting a female.
• With or without abdomen bending.

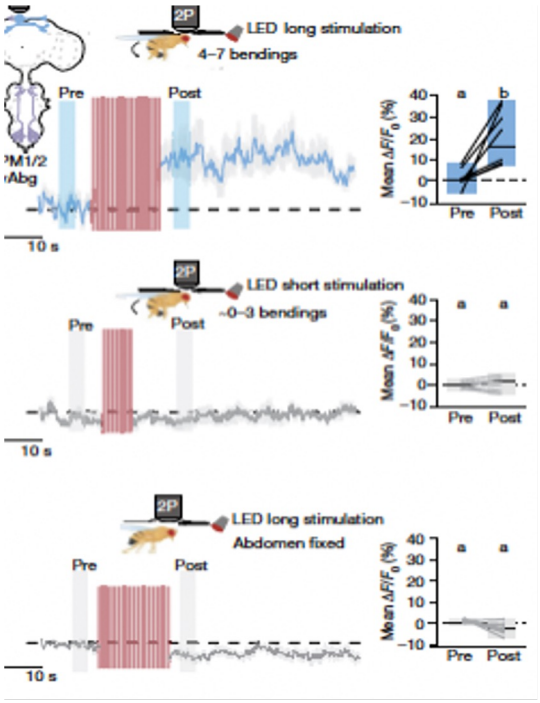




Results:
• **PPM1/2** activity increased only when courting a female.
• **Abdomen bending** was necessary for dopamine ramping.

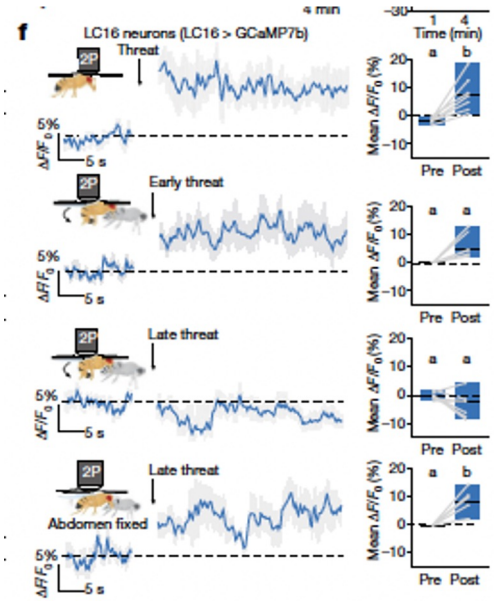
Dopamine ramps up during courtship

Goal: Does **abdomen bending** trigger dopamine ramping and suppress threat responses?



- Long duration LED stimulation (triggers 4-7 abdominal flexions).
- Short-duration LED stimulation (triggers 0-3 abdominal flexions).

Experiments (Fig. Left):
Induced **abdomen bending** (**OvAbg** activation) to test if it increases dopamine.
Mechanically blocked **abdomen bending** to test if dopamine ramping requires proprioceptive feedback.



Results (Fig. Right):
- Induced bending increased dopamine in a dose-dependent manner.
- Blocking bending prevented dopamine increase, proving reliance on proprioception feedback.
- **Late-stage bending suppressed threat responses, keeping males focused on mating.**

Dopamine blocks visual threat detection

Goal: Does **PPM1/2** inhibit **LC16** to suppress visual threat perception during courtship?

Experiments:

Neural anatomy (Fig. e)

• **PPM1/2** projects to **LC16** axon terminal Dopamine release.

• Dopamine increased during courtship with a female.

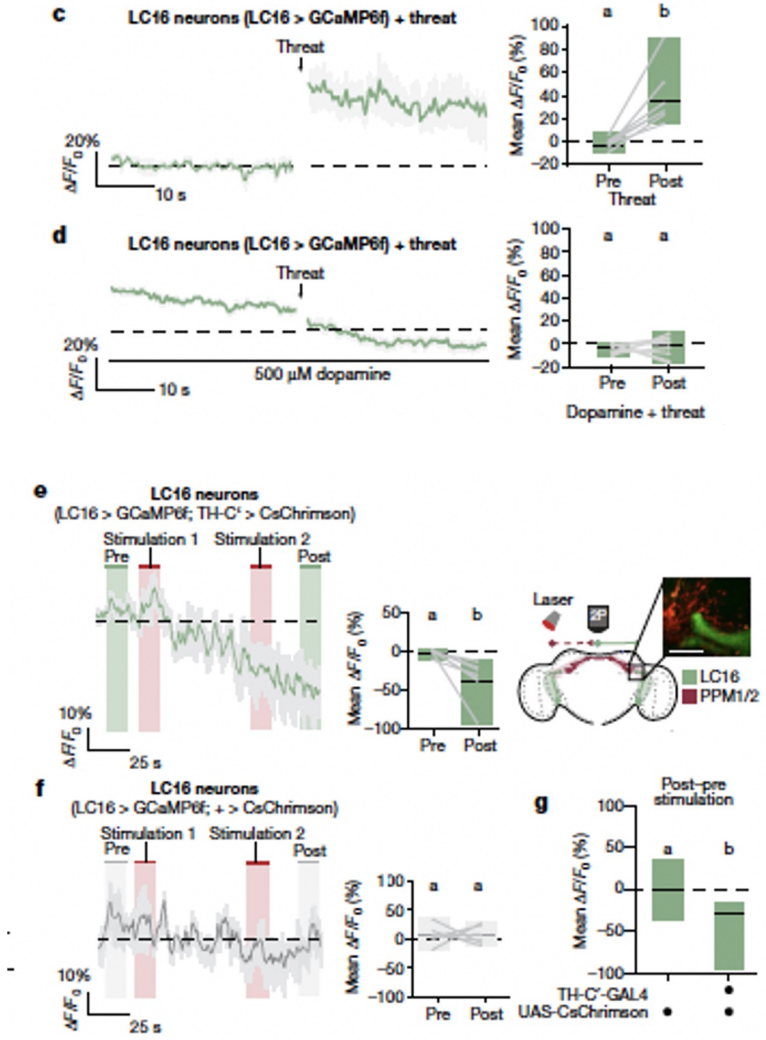
Dopamine inhibition test (Fig. d, f).

• Dopamine application & **PPM1/2** activation suppressed **LC16** activity.

What we found?:

• **PPM1/2** releases dopamine during courtship.

• Dopamine inhibits **LC16**, suppressing visual threat responses.

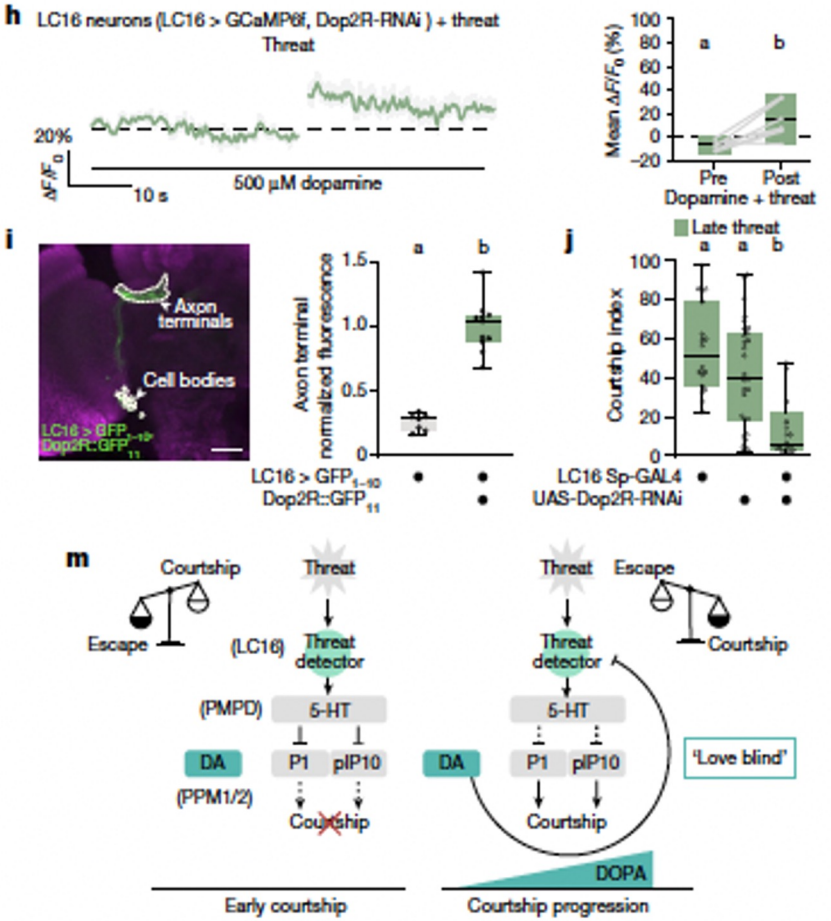


Dopamine blocks visual threat detection

Goal: Does **PPM1/2** inhibit **LC16** via **Dop2R** to regulate courtship persistence?

Experiments:
- **Dop2R** knockdown restored threat Sensitivity (Fig. h).
- Knocking down **Dop2R** restored defensive responses and reduced Courtship (Fig. j).

Results:
Dop2R suppresses LC16, ensuring courtship persistence.
Knocking down Dop2R restores threat responses and reduces courtship.



Conclusion

Big Question was...

How neural networks balance the trade-off between risk and reward ?

Early Courtship (**high** risk **low** reward)

LC16 detect visual threat

5-HT release serotonin

P1 terminate courtship

Late Courtship (**high** risk **high** reward)

PPM1/2 release dopamine

PPM 1/2 inhibit LC16

LC16 ignore threat

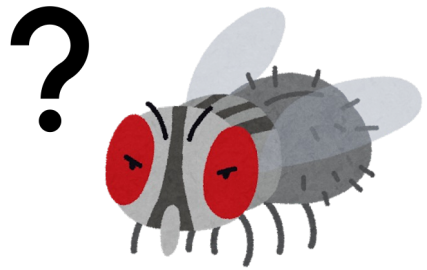
P1 continue courtship

Discussion

- Only visual threats?
  —What about olfactory or temperature cues for threat detection?

- Only males were studied?
  —What about females experience “love blindness” phenomenon?

- Only on the dopamine-inhibitory pathway?
  —What about other neurotransmitters influence courtship decisions?



EPFL

Thank you for attention!