**Application number: 324/24**

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**[currently 6.5 pages; 3.5 more pages for figures/tables and 5 more for experimental procedures etc.]**

**The neural basis of persistent internal states in *Drosophila***

**scientific abstract**

Animal behavior is shaped by a variety of ‘‘internal states’’- partially hidden variables that profoundly shape perception, cognition, and action. The neural basis of internal states, such as fear, arousal, hunger, motivation, aggression, and many others, is a prominent focus of research efforts across animal phyla. Internal states can be inferred from changes in behavior, physiology, and neural dynamics and are characterized by properties such as pleiotropy, persistence, scalability, generalizability, and valence. To date, it remains unclear how internal states and their properties are generated by nervous systems.

Recent studies in mice and flies revealed that activating small subsets of neurons drive persistent mating and aggression as well as persistent brain activity. Recently, we and other labs have found that activating a small set of sexually dimorphic cells in the brain of a female vinegar fly drives a persistent state of aggression as well as persistent neural activity in 4.3% of the central brain, including in cells who express the sex determination genes *fruitless* and *doublesex*. Both female aggression and the persistent brain activity outlasts the stimulation by minutes. While we were able to identify the spatial locations of the persistent activity throughout the female brain, the specific cell types who carry this persistent activity and the underlying mechanics are currently unknown.

Here we propose to reveal the neural and molecular mechanisms underlying the persistent internal state of aggression in the female brain, testing the hypothesis that neuromodulators are essential for this minutes-long persistent activity. We used the wiring diagram of the adult female brain and recent mapping we performed for sexually dimorphic cells in this diagram to find candidate cell types for physiological screening, and mapped candidate neuromodulators for screening of their persistent neural response and for their necessity for the persistent behavioral state.

Taken together, this proposal aims to reveal neural and molecular mechanisms underlying a minutes-long persistent internal state of aggression in a complex nervous system.

**Detailed description of the research program**

**Scientific background**

**1.1 The neural basis of persistent internal states**

Nervous systems are in a constant state of flux, with rich internal dynamics that determine how brains respond to inputs and produce outputs. The hidden processes that underlie these dynamics can be described as “internal states” and include arousal, motivation, emotion, and varying homeostatic needs. Internal states allow us to integrate information about our external environment and internal physiological conditions into centralized brain states, which shape how sensory information is processed and orchestrate appropriate behavioral and physiological responses [1,2](https://paperpile.com/c/HsD0w6/jBsV+5LHO). Although internal states are difficult to observe directly, they can be inferred from observations of an animal’s overt behavior or from within the brain, such as by investigating neuronal dynamics or perturbing neural function. For instance, an animal’s state of aggression could be inferred from observing attacks elicited by conspecifics and perturbing small groups of neurons in mice and flies drive persistent defensive or aggressive behaviors [3–6](https://paperpile.com/c/HsD0w6/bIVN+zbu3+Zrw7+Nwdz).

Several recent studies have discovered consistent changes in neuronal dynamics encompassing multiple cell types and brain systems concomitant to behavioral and/or physiological state changes [3,7–9](https://paperpile.com/c/HsD0w6/0l2z+kqJg+sOmK+bIVN). A wide variety of animals - from flies to mice to humans - appear to organize their behavior in a state-like fashion, suggesting that the neural mechanisms that underlie the generation of internal brain states are evolutionarily ancient [10](https://paperpile.com/c/HsD0w6/bEGu). In humans, changes in state representation, switching, and timing are thought to occur in many psychiatric and neurological diseases [Refs]. The ubiquity of internal states across animal species suggests that general principles found in animals will hold relevance for understanding the human condition in health and disease.

To be both flexible and stable, internal states often possess the following features: pleiotropy (each state influences multiple aspects of behavior and physiology in parallel), persistence (the ability of internal states to produce behavioral and physiological responses that outlast the termination of the stimulus that initiated the response), scalability (the ability of these responses to scale with the magnitude of the stimulus), generalizability (the degree to which an internal state can produce responses to stimuli that are distinct from the original stimulus that elicited the response), and valence (the positive or negative affect associated with that state).

Recent studies in mammals and invertebrates have begun to uncover potential mechanisms for maintaining persistent behavioral states on various timescales. These (mutually-inclusive) mechanisms can be “electronic,” such as persistent firing in certain cell types or circuits, “biochemical,” such as slow decay of second messengers or their effectors influencing neural excitability, or “systemic,” such as persistent elevation of circulating hormones or neuromodulators, and may span timescales of hundreds of milliseconds to tens of seconds to days.

Neuromodulators occupy an ideal position with respect to the control of internal states—they modulate synaptic and cellular function over long timescales because of their impact on biochemical signaling and ion channel function, they can titrate their effects via magnitude of modulator release, and they can act locally as well as send far-reaching diffuse signals across multiple brain regions [11](https://paperpile.com/c/HsD0w6/lnkA). This makes them prime candidates for the flexible, scalable, and persistent control of behavior - key requirements for an internal state [12–20](https://paperpile.com/c/HsD0w6/7BOC+qXI7+auhC+9aOG+QSQO+7QPN+dpqD+4sL0+YM3u). Both the release of slow-acting neuromodulators [19](https://paperpile.com/c/HsD0w6/4sL0), recurrent connectivity [21,22](https://paperpile.com/c/HsD0w6/P4V4+t6tR) or a combination of the two [23](https://paperpile.com/c/HsD0w6/AdYw) are often invoked to explain persistent activity .

**1.2 The control of persistent internal states of mating and aggression in flies**

Recent work in mice and flies revealed that activating small populations of neurons drive persistent mating and aggression [1](https://paperpile.com/c/HsD0w6/jBsV). In male flies, these neurons are located within the pC1 population of male-specific interneurons in the central brain. pC1 neurons in males and females express the sex determination gene *Doublesex* (dsx), and activating a male-specific pC1 subset called ‘P1’ drives persistent aggression towards male targets, and persistent courtship (specifically, singing) towards females or even with no social target at all [6](https://paperpile.com/c/HsD0w6/Nwdz). It is still unclear if there are two non-overlapping populations who drive the persistent aggression and mating states in male flies [1,6,24](https://paperpile.com/c/HsD0w6/RpUa+Nwdz+jBsV).

Most studies on mating and aggression in *Drosophila* have been focused on the male side. This is both as in *Drosophila melanogaster* males are actively courting the female, and their behaviors are quantifiable [25,26](https://paperpile.com/c/HsD0w6/R0TQ+KX6m), and also, since male flies are generally more aggressive than female flies [27](https://paperpile.com/c/HsD0w6/tNiR). However, females are also showing a number of active behaviors during mating, including vaginal plate opening, ovipositor extrusion, and possibly even singing (). Females also show aggression, both when fighting on food resources [27](https://paperpile.com/c/HsD0w6/tNiR). Post-mated female aggressive competition over food is strongly stimulated by the receipt of sperm at mating, and in part by an associated seminal fluid protein, the sex peptide [28](https://paperpile.com/c/HsD0w6/MKqN), and mated females show a series of aggressive rejecting behaviors towards a courting male [29–31](https://paperpile.com/c/HsD0w6/hzJy+QRgY+zDdh).

Recent studies revealed that artificial stimulation of pC1 neurons in females drives both persistent mating and persistent aggression that outlasts the stimulation by minutes [3](https://paperpile.com/c/HsD0w6/bIVN). Activating only a subset of pC1 neurons - pC1d and pC1e is sufficient for driving the persistent aggression (female chasing and ‘shoving’), while not affecting female receptivity. pC1d alone promotes only time-locked, but not persistent aggression, suggesting that persistent aggression promoted by pC1d+e is an emergent property of their co-activation [32](https://paperpile.com/c/HsD0w6/sCjf). Another population of cells who drive persistent female aggression and persistent brain activity is the *fruitless* expressing group called ‘aIPg’ [32,33](https://paperpile.com/c/HsD0w6/OwLJ+sCjf). Although aIPg neurons are reciprocally connected with both pC1d and pC1e cells [3,33](https://paperpile.com/c/HsD0w6/OwLJ+bIVN), and persistent activity in *fruitless* expressing cells following pC1d+e activation largely overlaps with the location of aIPg cell bodies, recent work suggests that neither aIPg nor pC1d are persistently active following aIPg or pC1d+e activation, implying that the persistent internal state is maintained by other mechanisms . A caveat to this observation is that the genetic driver used in the study (SS36564, [32](https://paperpile.com/c/HsD0w6/sCjf)) labeled only ~15 cells per hemisphere [33](https://paperpile.com/c/HsD0w6/OwLJ), while we now identified 58 cells with aIPg morphology in the female connectome (Ref to our draft on dsxfru cells). Moreover the SS36564 split line doesn’t label all the aIPg subtypes, including a sub-group we named as aIPg-a (hemibrain 1.1 type SMP555/556) [3,33](https://paperpile.com/c/HsD0w6/bIVN+OwLJ).

Which neurons carry the pC1d+e/aIPg induced long persistent aggression and persistent neural activity is currently unknown.

**1.3** **The role of neuromodulators in controlling aggression and mating in *Drosophila***

Across organisms, neuromodulators have been repeatedly identified as central elements in the generation of internal states, with a wide range of circuit organizations that deploy neuromodulators in distinct manners [12,15,17,34](https://paperpile.com/c/HsD0w6/7BOC+5EY8+9aOG+7QPN). In *Drosophila*, neuromodulators are found to play important roles in olfaction, taste, foraging, feeding, clock function/sleep, aggression, mating/reproduction, learning and other behaviors [35,36](https://paperpile.com/c/HsD0w6/mX0q+qREA). Neuromodulations play an important role in regulating social behavior in multiple insects [37](https://paperpile.com/c/HsD0w6/gnWG). In *Drosophila*, multiple neuromodulators play a role in controlling mating and aggression in behaviors. This includes the monoamines Dopamine, Octopamine and Serotonin and the following neuropeptides: Neuropeptide F (NPF), short Neuropeptide F (sNPF), SIFamide (SIFa), Drosulfakinin (DSK), Myoinhibitory peptide (MIP), the diuretic hormones Dh44 and Dh31, Tackykunin (Tk) and Natalisin (See Table 1).

**Dopamine** plays an important role in controlling mating motivation in both sexes [21,38](https://paperpile.com/c/HsD0w6/emsr+P4V4). In males, Dopaminergic activity in the anterior part of the Superior Medial Protocerebrum (SMPa) is a functional neuronal correlate of mating drive, modulating the response of P1 courtship command neurons to stimulation by female perceptual input [21](https://paperpile.com/c/HsD0w6/P4V4). Dopaminergic neurons are also involved in controlling aggression in males [(Alekseyenko et al. 2014)](https://paperpile.com/c/HsD0w6/8Kbu). **Serotonin** plays a role in controlling male and female mating behaviors [39,40](https://paperpile.com/c/HsD0w6/nXEy+6WFh). In females, Trh expressing fru-positive neurons mediate virgin female receptivity [40](https://paperpile.com/c/HsD0w6/6WFh). Serotonin also regulates male aggression [41–44](https://paperpile.com/c/HsD0w6/8Kbu+w01e+PsVt+M9XN). In females, sex peptide regulates receptivity through Serotonergic neurons [45](https://paperpile.com/c/HsD0w6/NyDi). **Octopamnie** regulates aggression in both sexes [(Zhou et al. 2008; Hoyer et al. 2008; Certel et al. 2010; Certel et al. 2007)](https://paperpile.com/c/HsD0w6/XIW7+8JZo+fvno+KFZq). **NPF** negatively modulates male aggression [44](https://paperpile.com/c/HsD0w6/M9XN), and was found to have a male-specific expression in six extra brain neurons compared to female brains [47](https://paperpile.com/c/HsD0w6/TdPc). Silencing of the NPF neurons in males leads to an increase in fighting frequencies [44](https://paperpile.com/c/HsD0w6/M9XN) while genetic ablation of NPF neurons results in decreased male courtship activity [47](https://paperpile.com/c/HsD0w6/TdPc). The same neuronal network in males may use NPF to decrease aggression levels and to increase courtship behavior [44,47](https://paperpile.com/c/HsD0w6/M9XN+TdPc). NPF regulates courtship through a male-specific fruitless expressing neural circuit [48](https://paperpile.com/c/HsD0w6/iiI3)**.** Consistently, Fru-positive NPF neurons were found to excite dopamine neurons projecting to the SMPa. Therefore a circuit including Dopamine and NPF regulate male courtship motivation [21](https://paperpile.com/c/HsD0w6/P4V4). NPF is also expressed in *fruitless*-positive neurons in the female’s central brain [49](https://paperpile.com/c/HsD0w6/UfBT). The functional role of these neurons is currently unknown. RNA profiling data indicate that aIPg neurons produce the neuropeptide **sNPF** [33](https://paperpile.com/c/HsD0w6/OwLJ), making it a strong candidate in enabling aIPg-induced persistent aggression. sNPF is also expressed in fru-positive Kanyon cells [49](https://paperpile.com/c/HsD0w6/UfBT). The neuropeptide **SIFa** modulates sexual behavior in both sexes [50](https://paperpile.com/c/HsD0w6/nEIK). Disruption of SIFa signaling results in males exhibiting remarkable promiscuity in their courtship attempts [50,51](https://paperpile.com/c/HsD0w6/nEIK+K74C). Hence, male flies with disrupted SIFamide signaling also court other males, while female flies become more receptive as the time to copulation is drastically reduced. SIFa receptor (but not SIFa) is expressed in *fruitless* neurons [49,51](https://paperpile.com/c/HsD0w6/UfBT+K74C). **DSK** controls copulation duration and sexual arousal in males [52,53](https://paperpile.com/c/HsD0w6/Ts9K+ZqHy) and was recently shown to also modulate female receptivity via DSK receptors on pC1 cells [54](https://paperpile.com/c/HsD0w6/MFHr). **MIP** is expressed in *doublesex-*positive neurons (likely in pC1a, based on the morphology in Fig 4C of [55](https://paperpile.com/c/HsD0w6/H7t9)). Fru-positive MIP expressing cells do not modulate female receptivity, and their role in mediating female aggression was not tested. Dsx-positive MIP expressing abdominal neurons control female receptivity. Another peptide implicated in aggressive behavior is **Dh44**. Knockdown of the Dh44 receptor Dh44R1 results in male flies that display increased aggressivity if they have been kept singly-housed, but are less aggressive after being kept in a group [56](https://paperpile.com/c/HsD0w6/LUy2). In females, suppressing the activity of Dh44 or its receptor Dh44R1 expedites sperm ejection [57](https://paperpile.com/c/HsD0w6/x4Bw). Dh44 is expressed in *fruitless* positive cells in the brains of adult males and females [49](https://paperpile.com/c/HsD0w6/UfBT). The specific role of these groups is currently unknown. The **Dh31** G-coupled receptor hector is required in a subset of *fruitless* neurons for male courtship behavior [58](https://paperpile.com/c/HsD0w6/GxXo)**.** Its role in controlling female social behaviors is currently unknown. A set of 4 pairs of *fruitless* positive **Tachykinin**-expressing neurons control the level of male-male aggression, but have no influence on male-female behavior [59,60](https://paperpile.com/c/HsD0w6/Pcwo+LSs2) through two separate downstream targets that are differentially modulated by tachykinin [61](https://paperpile.com/c/HsD0w6/D8Lj). Tachykinin is also expressed in *fruitless* positive neurons in the female brain [49](https://paperpile.com/c/HsD0w6/UfBT), whose function is currently unknown. Last, activation or silencing of the neural activities in the **Natalisin**-specific cells induces significant defects in the mating behaviors in both sexes [62](https://paperpile.com/c/HsD0w6/HIvl). Taken together, existing data indicates that multiple neuromodulators play critical roles in controlling social behaviors in *Drosophila melanogaster*. In most cases, their role in controlling aggression in females was not explored.

1. **Research Objectives**

The overall objective of this proposal is to reveal neural circuits and mechanisms underlying minutes-long persistent internal states. We aim to reveal which cells carry the persistent activity underlying a persistent state of aggression in adult female vinegar flies. We will test the hypothesis that neuromodulators are necessary for this persistent internal state. In **Aim 1** we will identify neuronal populations who carry the persistent activity. In **Aim 2** we will test which neuromodulators are essential for persistent aggression. In **Aim 3** we will reveal the underlying circuitry. Aims 1,2 are independent of each other and will be conducted in parallel. Aim 3 depends on the results from Aims 1,2.

**Aim 1. Reveal specific cell types who carry pC1/aIPg induced persistent activity**

We aim to reveal specific neural populations who carry minutes-long persistent activity following the activation of aIPg or pC1d+e cells in the adult female brain. We will do it by optogenetically activating aIPg or pC1d+e cells and recording Calcium activity during and following the stimulation period in specific neuronal populations, using a two-photon microscope [(Deutsch et al. 2020)](https://paperpile.com/c/HsD0w6/bIVN).

As testing all the existing cell types in the adult female brain is not experimentally feasible, we will focus on 3 specific groups: (1) Neurons who express receptors for candidate neuromodulators, using existing Gal4 lines (see Table xx). (2) Cell types who are, according to FlyWire, downstream and strongly connected to pC1d/e and aIPg (Fig. XX). (3) specific dsx- and fru- positive cell types, based on overlap with persistent activity and their connectivity with aIPg and pC1d/e. In most of the experiments we will express csChrimson using LexA-aIPg as in Chiu at al [32](https://paperpile.com/c/HsD0w6/sCjf), and express the genetically encoded Calcium indicator (GCaMP) using the Gal4 system.

\* In the case of the monoamoines – consider also ingestion (also for Aim 2). E.g., for dopamine TH inhibitors and DOPA??

**Aim 2. Determine which neuromodulators are necessary for pC1/aIPg induced persistent activity**

We aim to test the hypothesis that neuromodulators are essential for aIPg induced persistent aggression, and determine which neuromodulators are necessary for the persistent behavioral phenotype - female aggression. As in Aim 1, we will optogenetically activate aIPg neurons using csChrimson and the LexA system, and use RNA interference to knock down gene expression for specific neuromodulators or their receptors (see Table xx) using the Gal4 system. RNAi will be expressed in 3 populations: pan-neuronally, in fru-positive neurons and in dsx-positive neurons. We will stimulate aIPg neurons for 30 second [32](https://paperpile.com/c/HsD0w6/sCjf) in a solitary female. We will add a wild-type male to the arena 3 minutes after stimulus offset following our previous protocol [(Deutsch et al. 2020)](https://paperpile.com/c/HsD0w6/bIVN) and measure female aggression using an automated pipeline as before [(Deutsch et al. 2020; Pereira et al. 2022; Kabra et al. 2013)](https://paperpile.com/c/HsD0w6/bIVN+VFyB+ZHCb) . This temporal separation aims to ensure that in the behavioral experiments, as under the two-photon microscope, the persistent state doesn’t depend on ongoing sensory stimuli during the activation period.

**Aim 3. Reveal neural circuit and mechanism for persistent internal state in *Drosophila***

Given our observations in Aims 1,2, here we aim to propose neural circuits and mechanisms underlying persistent internal states of female receptivity and aggression, as well as a mathematical model that is consistent with our experimental observations.

Firsat, we will narrow down the populations of cells who carry persistent activity based on the spatial distribution of the persistent activity we found on Aim 1 and on information from the female connectome. For example, if we find persistent activity in DA cells using Th-Gal4, in aim 3 we will find - starting with existing driver lines - which specific DA population carries this persistent activity. Once we find subpopulations who carry the persistent activity we will test if killing them (using reaper) or inactivating them (using tetanus toxin) blocks aIPg- or pC1d+e- driven persistent aggression.

Last, based on the previous results, we will test if blocking specific neuromodulators in specific cell types has a significant effect on persistent aggressive behavior and on persistent brain activity. The details on the genotypes used for this and other experiments, as well as more followup experiments we will conduct in Aim 3 are described below under ‘Experimental procedures’. These include a wider set of activation stimuli to quantify how the persistent activity evolves over time in specific circuits, and - if necessary - RNAseq in specific neural populations of cells who showed persistent activity to look for specific ligands and receptors.

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**Experimental procedures – in progress…**

Including - Potential problems and alternative strategies for each aim

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Figure

Fig1 – general scheme, results from my eLife paper on persistent activity

Fig 2 – **prelim data** - fly wire. Below is one subplot but also add some more diagrams of connectivity between aIPg/pC1d+e and other cell types.

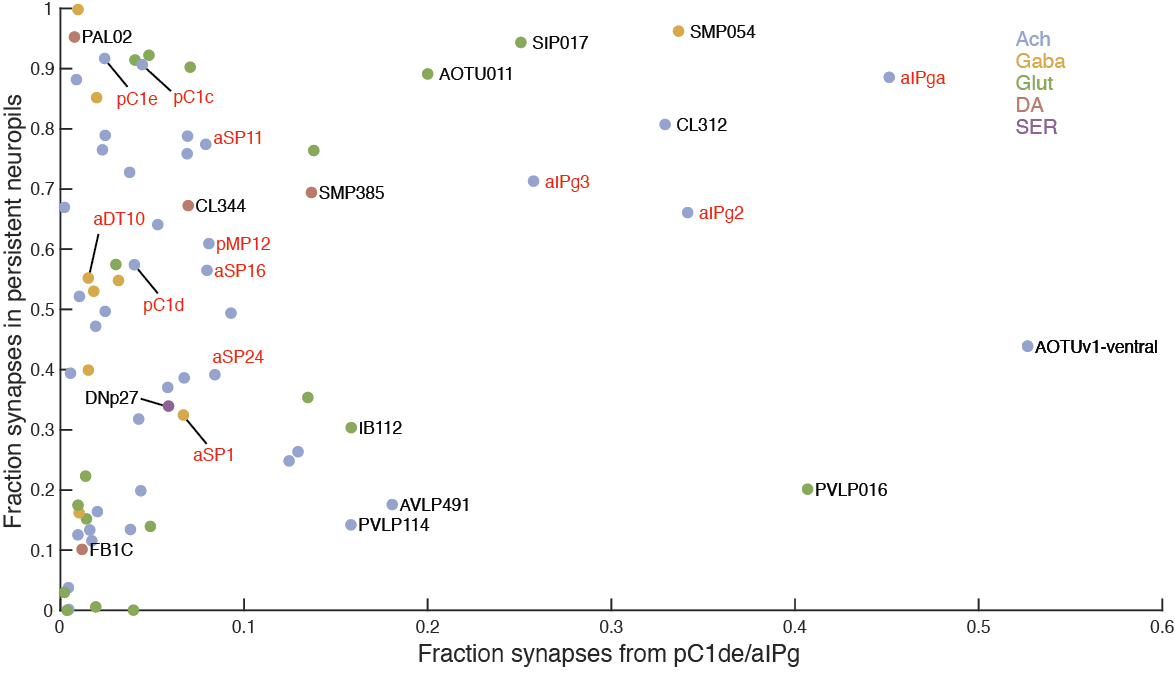


Fig 3 - **Prelim data** – imaging dsxfru (responses from fru-LexA; persistent activity in pC1 and add the connectivity with pC1c here or in fig 2). Show also the imaging setup including optogen activation.  
[perhaps show here also the fru-GcaMP following pC1d+e activation]

Fig 4 – **prelim data** - DA application drives hyperpolarization in dsx+ cell

Table xx – draft

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Modulator** | **Social phenotype (Refs)** | **Identified in flywire?** | **Gal4-modulat;Gal4-receptor;RNAi** | comments |
| **Dopamine** | **Female** receptivity DA PPM3 (not fru) [38](https://paperpile.com/c/HsD0w6/emsr) | Partially - nt detection () | (start with TH-gal4) | Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT) |
| **Serotonin** | **Male courtship**: [39](https://paperpile.com/c/HsD0w6/nXEy)  Female: Trh+ fru+ neurons (they named them ‘PLP‘) mediate virgin **female receptivity** [40](https://paperpile.com/c/HsD0w6/6WFh) | Partially - nt detection () | ;;BDSC\_33612  (Serotonin receptors DBSC 27632, 27633, 55846) | Couldn’t find them in CODEX (looked at fruitless calls in the PLP area)  Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT) |
| **Octopamine** |  | Partially - nt detection () | dTdc2-Gal4;;BDSC\_76062 | Gal4 - <https://www.frontiersin.org/articles/10.3389/fnbeh.2018.00131/full>  76062 is Tbh |
| **NPF** | **Male:** fru**+** male-specific NPF recurrently connected to P1  [48](https://paperpile.com/c/HsD0w6/iiI3)**.** Also related to ejaculation being rewarding in males[63](https://paperpile.com/c/HsD0w6/V0TL) | **yes** | BDSC\_84671 (2A-Gal4);BDSC\_84672 (2A-Gal4 - NPFR);BDSC\_27237 (RNAi for NPF)  BDSC\_25939 UAS-RNAi for NPFR | Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT) |
| **sNPF** | **Male:**  **Female**: RNA profiling data indicate that aIPg neurons produce the neuropeptide sNPF | **no?** | BDSC\_84706 (2A-Gal4);BDSC\_84691 (2A-gal4 SNPF-R);  BDSC\_25867 RNAi for sNPF  BDSC\_27507 RNAi for SNPF-R | “a large portion of the more than 4000 mushroom body Kenyon cells expresses short neuropeptide F” [64,65](https://paperpile.com/c/HsD0w6/YHId+GCfD)  Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT) but in KCs |
| **SIFa** | **Males and Females**: 4 cells; ablation or RNAi causes stronger mating drive in both sexes**.** [50](https://paperpile.com/c/HsD0w6/nEIK)  **Males:** ablation of the SIFa receptor in fru drives MM courtship. SIFR are expressed in fru but SIFa is not. [51](https://paperpile.com/c/HsD0w6/K74C) | **Yes** | SIFa receptor gal4 was made in this study[51](https://paperpile.com/c/HsD0w6/K74C)  BDSC\_84690 (2A-Gal4 SIFa);;BDSC\_29428 (RNAi for SIFa)  For SIFaR - BDSC\_76670 (trojan-Gal4)  Also BDSC\_84689 is 2A-Gal4-SIGaR  BDSC\_25831 is RNAi for SIFaR |  |
| **DSK**  **(Drosulfakinin)** | **Male:** copulation duration and sexual arousal [52,53](https://paperpile.com/c/HsD0w6/Ts9K+ZqHy)  Controls fighting downstream P1 -  <https://elifesciences.org/articles/54229>  **Female:**DSK modulates female receptivity via pC1-DSK-MP1-CCKLR-17D3 receptor expression neurons [54](https://paperpile.com/c/HsD0w6/MFHr) | **Yes, DSKMP1B** | BDSC\_84630 (2A-Gal4);no Bllomington for the receptor but - *CCKLR-17D3*GAL4 was used here- <https://elifesciences.org/articles/76025/figures#comment> (see supp table)  ;BDSC\_25869 (RNAi for DSK)  BDSC\_28333 RNAi for CCKLR\_17D3 (DS) | Ortholog of mammalian CCK.The receptor is:” CCKLR (used the GeneSwitch system: [66](https://paperpile.com/c/HsD0w6/HTNP))  Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT)  Both Gal4 and UAS are on III |
| **MIP** | **Female (VNC):** *Dsx* *+* abdominal *Mip* neurons control female receptivity [55](https://paperpile.com/c/HsD0w6/H7t9) | **no?** | **Yes - here** [55](https://paperpile.com/c/HsD0w6/H7t9)  BDSC\_84651 (2A-Gal4 Mip);  Shared receptor with sex peptide (SPR). BDSC\_84692 (T2A-Gal4 SPR)  ;BDSC\_26246 (RNAi) | Co-expressed with sex peptide (Refs in the Meet review) |
| **Dh44** | **Male:** aggression[56](https://paperpile.com/c/HsD0w6/LUy2)  **Female:** sperm ejection and storage [57](https://paperpile.com/c/HsD0w6/x4Bw)**.** | **Yes (Dh44)**  6 cells (3L/3R)  In putative fru+ IPC cells | BDSC 84627  ;  Dh44-R2 (receptor)  BDSC\_66865 (Trojan-Gal4)  ;BDSC\_25804 RNAi for Dh44  BDSC\_29610 RNAi for Dh44-R2 | Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT) |
| **Hh31 (the receptor is called ‘hector’)** | **Male:** hector increase courtship through hector receptors on fru cells  [58](https://paperpile.com/c/HsD0w6/GxXo) |  | BDSC\_84623 and BDSC\_84624 for different isofors of Dh33 (both )  hector: 4395-Gal4 was generated in Li et al 2011  BDSC\_29623  RNAi for Hector |  |
| ***Tackykinin* (*Tk*)** | Male aggression [59,60](https://paperpile.com/c/HsD0w6/Pcwo+LSs2)  Female? |  | M;BDSC\_84693  BDSC\_25800 RNAi  Two receptors: TkR86C and TkR99D  But no Galf, only -  https://www.jneurosci.org/content/43/19/3394.full#ref-67 | Intersection with fru shown in [49](https://paperpile.com/c/HsD0w6/UfBT) also for females! |
| **Natalisin**  **(NTL)** | “*Natalisin-RNAi* and the activation or silencing of the neural activities in the natalisin-specific cells in *D*. *melanogaster* induced significant defects in the mating behaviors of both males and females”[62](https://paperpile.com/c/HsD0w6/HIvl) | no | BDSC\_84668 2A-Gal4 Natalinin   \* Vienna *Drosophila* RNAi stock center transformation ID 19547 according to <https://www.pnas.org/doi/10.1073/pnas.1310676110> |  |

**References**

1. [Anderson, D. J. Circuit modules linking internal states and social behaviour in flies and mice. *Nat. Rev. Neurosci.* **17**, 692–704 (2016).](http://paperpile.com/b/HsD0w6/jBsV)

2. [Tinbergen, N. The study of instinct Clarendon Press. *Oxford* **195**, l (1951).](http://paperpile.com/b/HsD0w6/5LHO)

3. [Deutsch, D. *et al.* The neural basis for a persistent internal state in Drosophila females. *eLife* vol. 9 Preprint at https://doi.org/](http://paperpile.com/b/HsD0w6/bIVN)[10.7554/elife.59502](http://dx.doi.org/10.7554/elife.59502) [(2020).](http://paperpile.com/b/HsD0w6/bIVN)

4. [Kunwar, P. S. *et al.* Ventromedial hypothalamic neurons control a defensive emotion state. *Elife* **4**, (2015).](http://paperpile.com/b/HsD0w6/zbu3)

5. [Wang, L., Chen, I. Z. & Lin, D. Collateral pathways from the ventromedial hypothalamus mediate defensive behaviors. *Neuron* **85**, 1344–1358 (2015).](http://paperpile.com/b/HsD0w6/Zrw7)

6. [Hoopfer, E. D., Jung, Y., Inagaki, H. K., Rubin, G. M. & Anderson, D. J. P1 interneurons promote a persistent internal state that enhances inter-male aggression in Drosophila. *Elife* **4**, (2015).](http://paperpile.com/b/HsD0w6/Nwdz)

7. [Gründemann, J. *et al.* Amygdala ensembles encode behavioral states. *Science* **364**, (2019).](http://paperpile.com/b/HsD0w6/0l2z)

8. [Lovett-Barron, M. *et al.* Multiple convergent hypothalamus–brainstem circuits drive defensive behavior. *Nat. Neurosci.* **23**, 959–967 (2020).](http://paperpile.com/b/HsD0w6/kqJg)

9. [Xu, S. *et al.* Behavioral state coding by molecularly defined paraventricular hypothalamic cell type ensembles. *Science* **370**, (2020).](http://paperpile.com/b/HsD0w6/sOmK)

10. [Nath, R. D. *et al.* The Jellyfish Cassiopea Exhibits a Sleep-like State. *Curr. Biol.* **27**, 2984–2990.e3 (2017).](http://paperpile.com/b/HsD0w6/bEGu)

11. [van den Pol, A. N. Neuropeptide transmission in brain circuits. *Neuron* **76**, 98–115 (2012).](http://paperpile.com/b/HsD0w6/lnkA)

12. [Bargmann, C. I. Beyond the connectome: how neuromodulators shape neural circuits. *Bioessays* **34**, 458–465 (2012).](http://paperpile.com/b/HsD0w6/7BOC)

13. [Bargmann, C. I. & Marder, E. From the connectome to brain function. *Nat. Methods* **10**, 483–490 (2013).](http://paperpile.com/b/HsD0w6/qXI7)

14. [Flavell, S. W. *et al.* Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in C. elegans. *Cell* **154**, 1023–1035 (2013).](http://paperpile.com/b/HsD0w6/auhC)

15. [Harris-Warrick, R. M. & Marder, E. Modulation of neural networks for behavior. *Annu. Rev. Neurosci.* **14**, 39–57 (1991).](http://paperpile.com/b/HsD0w6/9aOG)

16. [Kennedy, A. *et al.* Internal States and Behavioral Decision-Making: Toward an Integration of Emotion and Cognition. *Cold Spring Harb. Symp. Quant. Biol.* **79**, 199–210 (2014).](http://paperpile.com/b/HsD0w6/QSQO)

17. [Marder, E. Neuromodulation of neuronal circuits: back to the future. *Neuron* **76**, 1–11 (2012).](http://paperpile.com/b/HsD0w6/7QPN)

18. [Nusbaum, M. P. & Blitz, D. M. Neuropeptide modulation of microcircuits. *Curr. Opin. Neurobiol.* **22**, 592–601 (2012).](http://paperpile.com/b/HsD0w6/dpqD)

19. [Taghert, P. H. & Nitabach, M. N. Peptide neuromodulation in invertebrate model systems. *Neuron* **76**, 82–97 (2012).](http://paperpile.com/b/HsD0w6/4sL0)

20. [Zelikowsky, M., Ding, K. & Anderson, D. J. Neuropeptidergic Control of an Internal Brain State Produced by Prolonged Social Isolation Stress. *Cold Spring Harb. Symp. Quant. Biol.* **83**, 97–103 (2018).](http://paperpile.com/b/HsD0w6/YM3u)

21. [Zhang, S. X., Rogulja, D. & Crickmore, M. A. Recurrent Circuitry Sustains Drosophila Courtship Drive While Priming Itself for Satiety. *Curr. Biol.* **29**, 3216–3228.e9 (2019).](http://paperpile.com/b/HsD0w6/P4V4)

22. [Joshua, M. & Lisberger, S. G. A tale of two species: Neural integration in zebrafish and monkeys. *Neuroscience* **296**, 80–91 (2015).](http://paperpile.com/b/HsD0w6/t6tR)

23. [Kennedy, A. *et al.* Stimulus-specific hypothalamic encoding of a persistent defensive state. *Nature* **586**, 730–734 (2020).](http://paperpile.com/b/HsD0w6/AdYw)

24. [Koganezawa, M., Kimura, K.-I. & Yamamoto, D. The Neural Circuitry that Functions as a Switch for Courtship versus Aggression in Drosophila Males. *Curr. Biol.* **26**, 1395–1403 (2016).](http://paperpile.com/b/HsD0w6/RpUa)

25. [Dickson, B. J. Wired for sex: the neurobiology of Drosophila mating decisions. *Science* **322**, 904–909 (2008).](http://paperpile.com/b/HsD0w6/R0TQ)

26. [Coen, P. *et al.* Dynamic sensory cues shape song structure in Drosophila. *Nature* **507**, 233–237 (2014).](http://paperpile.com/b/HsD0w6/KX6m)

27. [Nilsen, S. P., Chan, Y.-B., Huber, R. & Kravitz, E. A. Gender-selective patterns of aggressive behavior in Drosophila melanogaster. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 12342–12347 (2004).](http://paperpile.com/b/HsD0w6/tNiR)

28. [Bath, E. *et al.* Sperm and sex peptide stimulate aggression in female Drosophila. *Nat Ecol Evol* **1**, 0154 (2017).](http://paperpile.com/b/HsD0w6/MKqN)

29. [Aranha, M. M. & Vasconcelos, M. L. Deciphering Drosophila female innate behaviors. *Curr. Opin. Neurobiol.* **52**, 139–148 (2018).](http://paperpile.com/b/HsD0w6/hzJy)

30. [Cook, R. & Connolly, K. Rejection Responses By Female Drosophila Melanogaster : Their Ontogeny, Causality and Effects Upon the Behaviour of the Courting Male. *Behaviour* **44**, 142–165 (1973).](http://paperpile.com/b/HsD0w6/QRgY)

31. [Wang, F., Wang, K., Forknall, N., Parekh, R. & Dickson, B. J. Circuit and Behavioral Mechanisms of Sexual Rejection by Drosophila Females. *Curr. Biol.* **30**, 3749–3760.e3 (2020).](http://paperpile.com/b/HsD0w6/zDdh)

32. [Chiu, H. *et al.* Cell type-specific contributions to a persistent aggressive internal state in female Drosophila. *bioRxiv* 2023.06.07.543722 (2023) doi:](http://paperpile.com/b/HsD0w6/sCjf)[10.1101/2023.06.07.543722](http://dx.doi.org/10.1101/2023.06.07.543722)[.](http://paperpile.com/b/HsD0w6/sCjf)

33. [Schretter, C. E. *et al.* Cell types and neuronal circuitry underlying female aggression in Drosophila. *Elife* **9**, (2020).](http://paperpile.com/b/HsD0w6/OwLJ)

34. [Getting, P. A. Emerging principles governing the operation of neural networks. *Annu. Rev. Neurosci.* **12**, 185–204 (1989).](http://paperpile.com/b/HsD0w6/5EY8)

35. [Nässel, D. R. & Zandawala, M. Recent advances in neuropeptide signaling in Drosophila, from genes to physiology and behavior. *Prog. Neurobiol.* **179**, 101607 (2019).](http://paperpile.com/b/HsD0w6/mX0q)

36. [Kim, S. M., Su, C.-Y. & Wang, J. W. Neuromodulation of Innate Behaviors in Drosophila. *Annu. Rev. Neurosci.* **40**, 327–348 (2017).](http://paperpile.com/b/HsD0w6/qREA)

37. [Simpson, S. J. & Stevenson, P. A. 3 Neuromodulation in Insects of Social Behavior. *The Oxford Handbook of Molecular* (2015).](http://paperpile.com/b/HsD0w6/gnWG)

38. [Ishimoto, H. & Kamikouchi, A. A Feedforward Circuit Regulates Action Selection of Pre-mating Courtship Behavior in Female Drosophila. *Curr. Biol.* **30**, 396–407.e4 (2020).](http://paperpile.com/b/HsD0w6/emsr)

39. [Becnel, J., Johnson, O., Luo, J., Nässel, D. R. & Nichols, C. D. The serotonin 5-HT7Dro receptor is expressed in the brain of Drosophila, and is essential for normal courtship and mating. *PLoS One* **6**, e20800 (2011).](http://paperpile.com/b/HsD0w6/nXEy)

40. [Ma, B. *et al.* Serotonin Signaling Modulates Sexual Receptivity of Virgin Female Drosophila. *Neurosci. Bull.* **38**, 1277–1291 (2022).](http://paperpile.com/b/HsD0w6/6WFh)

41. [Alekseyenko, O. V. *et al.* Single serotonergic neurons that modulate aggression in Drosophila. *Curr. Biol.* **24**, 2700–2707 (2014).](http://paperpile.com/b/HsD0w6/8Kbu)

42. [Alekseyenko, O. V., Lee, C. & Kravitz, E. A. Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male Drosophila melanogaster. *PLoS One* **5**, e10806 (2010).](http://paperpile.com/b/HsD0w6/w01e)

43. [Johnson, O., Becnel, J. & Nichols, C. D. Serotonin 5-HT2 and 5-HT1A-like receptors differentially modulate aggressive behaviors in Drosophila melanogaster. *Neuroscience* **158**, 1292–1300 (2009).](http://paperpile.com/b/HsD0w6/PsVt)

44. [Dierick, H. A. & Greenspan, R. J. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat. Genet.* **39**, 678–682 (2007).](http://paperpile.com/b/HsD0w6/M9XN)

45. [Yang, Y. T. *et al.* Sex peptide regulates female receptivity through serotoninergic neurons in Drosophila. *iScience* **26**, 106123 (2023).](http://paperpile.com/b/HsD0w6/NyDi)

46. [Zhou, C., Rao, Y. & Rao, Y. A subset of octopaminergic neurons are important for Drosophila aggression. *Nat. Neurosci.* **11**, 1059–1067 (2008).](http://paperpile.com/b/HsD0w6/XIW7)

47. [Lee, G., Bahn, J. H. & Park, J. H. Sex- and clock-controlled expression of the neuropeptide F gene in Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 12580–12585 (2006).](http://paperpile.com/b/HsD0w6/TdPc)

48. [Liu, W. *et al.* Neuropeptide F regulates courtship in Drosophila through a male-specific neuronal circuit. *Elife* **8**, (2019).](http://paperpile.com/b/HsD0w6/iiI3)

49. [Palmateer, C. M. *et al.* Single-cell transcriptome profiles of Drosophila fruitless-expressing neurons from both sexes. *Elife* **12**, (2023).](http://paperpile.com/b/HsD0w6/UfBT)

50. [Terhzaz, S., Rosay, P., Goodwin, S. F. & Veenstra, J. A. The neuropeptide SIFamide modulates sexual behavior in Drosophila. *Biochem. Biophys. Res. Commun.* **352**, 305–310 (2007).](http://paperpile.com/b/HsD0w6/nEIK)

51. [Sellami, A. & Veenstra, J. A. SIFamide acts on fruitless neurons to modulate sexual behavior in Drosophila melanogaster. *Peptides* **74**, 50–56 (2015).](http://paperpile.com/b/HsD0w6/K74C)

52. [Wu, S. *et al.* Drosulfakinin signaling in fruitless circuitry antagonizes P1 neurons to regulate sexual arousal in Drosophila. *Nat. Commun.* **10**, 4770 (2019).](http://paperpile.com/b/HsD0w6/Ts9K)

53. [Tayler, T. D., Pacheco, D. A., Hergarden, A. C., Murthy, M. & Anderson, D. J. A neuropeptide circuit that coordinates sperm transfer and copulation duration in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 20697–20702 (2012).](http://paperpile.com/b/HsD0w6/ZqHy)

54. [Wang, T. *et al.* Drosulfakinin signaling modulates female sexual receptivity in Drosophila. *Elife* **11**, (2022).](http://paperpile.com/b/HsD0w6/MFHr)

55. [Jang, Y.-H., Chae, H.-S. & Kim, Y.-J. Female-specific myoinhibitory peptide neurons regulate mating receptivity in Drosophila melanogaster. *Nat. Commun.* **8**, 1630 (2017).](http://paperpile.com/b/HsD0w6/H7t9)

56. [Kim, Y.-K. *et al.* Repetitive aggressive encounters generate a long-lasting internal state in Drosophila melanogaster males. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 1099–1104 (2018).](http://paperpile.com/b/HsD0w6/LUy2)

57. [Lee, K.-M. *et al.* A neuronal pathway that controls sperm ejection and storage in female Drosophila. *Curr. Biol.* **25**, 790–797 (2015).](http://paperpile.com/b/HsD0w6/x4Bw)

58. [Li, Y. *et al.* The hector G-protein coupled receptor is required in a subset of fruitless neurons for male courtship behavior. *PLoS One* **6**, e28269 (2011).](http://paperpile.com/b/HsD0w6/GxXo)

59. [Asahina, K. *et al.* Tachykinin-expressing neurons control male-specific aggressive arousal in Drosophila. *Cell* **156**, 221–235 (2014).](http://paperpile.com/b/HsD0w6/Pcwo)

60. [Wohl, M., Ishii, K. & Asahina, K. Layered roles of fruitless isoforms in specification and function of male aggression-promoting neurons in Drosophila. *Elife* **9**, (2020).](http://paperpile.com/b/HsD0w6/LSs2)

61. [Wohl, M. P., Liu, J. & Asahina, K. Drosophila Tachykininergic Neurons Modulate the Activity of Two Groups of Receptor-Expressing Neurons to Regulate Aggressive Tone. *J. Neurosci.* **43**, 3394–3420 (2023).](http://paperpile.com/b/HsD0w6/D8Lj)

62. [Jiang, H. *et al.* Natalisin, a tachykinin-like signaling system, regulates sexual activity and fecundity in insects. *Proc. Natl. Acad. Sci. U. S. A.* **110**, E3526–34 (2013).](http://paperpile.com/b/HsD0w6/HIvl)

63. [Zer-Krispil, S. *et al.* Ejaculation Induced by the Activation of Crz Neurons Is Rewarding to Drosophila Males. *Curr. Biol.* **28**, 1445–1452.e3 (2018).](http://paperpile.com/b/HsD0w6/V0TL)

64. [Johard, H. A. D. *et al.* Intrinsic neurons of Drosophila mushroom bodies express short neuropeptide F: relations to extrinsic neurons expressing different neurotransmitters. *J. Comp. Neurol.* **507**, 1479–1496 (2008).](http://paperpile.com/b/HsD0w6/YHId)

65. [Nässel, D. R., Enell, L. E., Santos, J. G., Wegener, C. & Johard, H. A. D. A large population of diverse neurons in the Drosophila central nervous system expresses short neuropeptide F, suggesting multiple distributed peptide functions. *BMC Neurosci.* **9**, 90 (2008).](http://paperpile.com/b/HsD0w6/GCfD)

66. [Robles-Murguia, M., Hunt, L. C., Finkelstein, D., Fan, Y. & Demontis, F. Tissue-specific alteration of gene expression and function by RU486 and the GeneSwitch system. *NPJ Aging Mech Dis* **5**, 6 (2019).](http://paperpile.com/b/HsD0w6/HTNP)