

To court or not to court – a multimodal sensory decision in *Drosophila* males

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Abstract

When *Drosophila* males encounter another fly, they have to make a rapid assessment to ensure the appropriate response: should they court, fight or pursue a different action entirely? Previous work has focused on the significance of sensory cues detected by the male during these encounters, however recent evidence highlights the importance of the male's own internal state in shaping his responses. Additionally, once triggered, courtship is not a rigid sequence of motor actions, but rather a finely-tuned behavioural display that must continually update in response to sensory feedback. Here, we review recent findings highlighting how sensory information and internal states are integrated ensuring appropriate action selection, and how they sustain and fine-tune motor output. We further discuss recent advances in our understanding of species differences in sensory processing that may contribute to reproductive isolation.

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Highlights

- Multimodal sensory signals inform a male fly's decision to court.
- Internal states such as sleep drive and mating drive can bias this decision.
- The pC1 cluster integrates sensory input and internal states for action selection.
- Fine-tuning of specific courtship motor programs requires sensory feedback.

Introduction

Innate sex-specific behaviours in *Drosophila melanogaster* have been exploited as a powerful model system to study how the brain evaluates sensory inputs and turns them into behavioural outputs. Two key transcription factors, *doublesex* (*dsx*) and *fruitless* (*fru*), control the development of a sexually dimorphic nervous system, which underlies sex-specific behaviours, such as male courtship and aggression as well as female receptivity and postmating behaviours (reviewed in [1]). The details of female behaviour have been reviewed recently [2] and are beyond the scope of this review. Since sex-specific behaviours are hardwired into the fly nervous system via the actions of *dsx* and *fru*, the focus has been on neurons that express these transcription factors to find anatomical or molecular sex differences in neuronal populations, and to gain entry to the neural circuits underlying sex-specific behaviours. Previous studies have identified the male-specific *fru/dsx* co-expressing P1 cluster, a subset of the *dsx* expressing (*dsx*⁺) pC1 cluster, as a centre for courtship initiation [3]. This cluster, though not strictly necessary for courtship, can trigger wing extension when activated artificially or by female pheromones [3-5]. Here, we review recent evidence that P1 neurons integrate not only sensory information, but also internal states. We summarize recent work suggesting that P1 is involved in the choice between mutually exclusive behaviours, such as courtship, aggression and sleep, and therefore serves as a centre for action selection in male flies. We further present recent findings in other species which indicate that small changes in this conserved circuit can lead to reproductive isolation. Finally, we discuss how sensory feedback modulates courtship motor programs.

Sensory processing

Before a male invests time and energy into the pursuit of a potential mate, it is crucial that he accurately evaluates their suitability. To increase his chances of a successful mating and thus the production of offspring, the most relevant information he must gain during his encounter with another fly is its sex, species and mating status. In *D. melanogaster*, these traits are conveyed mainly by olfactory and gustatory pheromone signals, which have been recently reviewed in detail elsewhere [6]. Low volatility hydrocarbons such as 7,11-heptacosadiene (7,11-HD) on the female cuticle promote

courtship and are perceived by the male during the courtship ritual through tapping the female with his foreleg or licking [7]. On the other hand, male-specific gustatory and olfactory pheromones, such as 7-tricosene (7-T) and 11-*cis*-vaccenyl-acetate (cVA), inhibit male-male courtship and promote aggression [8,9]. Males also actively deposit cVA, creating 'landmarks' which are attractive to both sexes [10]. Notably, male pheromones are also transferred to females during copulation and thereby render mated females less attractive [11]. This preference for virgin females, while partly innate, also has a learned component, known as courtship conditioning [12,13].

CRISPR/Cas9 technology has made it possible to investigate the underlying circuits in other *Drosophila* species that were previously genetically intractable [14**,15*]. For example, recent work has found that pheromone-dependent male preference, rather than female choice, is the main factor that blocks inter-species courtship in *D. simulans* [14**,16]. In this species, females mainly produce 7-T and lack 7,11-HD, which is inhibitory to courtship in *D. simulans* males. Pheromone-perception circuits appear to be largely conserved, however, minor changes in the weight of excitation to inhibition onto P1 were identified as the potential basis for the switch from a stimulatory chemosensory signal in *D. melanogaster* to an inhibitory one in *D. simulans* [14**].

The role of acoustic signals in courtship has been extensively studied in females, as females evaluate male courtship song for species recognition and receptivity [17-20]. In males, the function of hearing in courtship is less clear. It has been suggested that males may use sound to localize females [21,22], and that hearing courtship or aggression song can decrease or increase aggressiveness in males, respectively [23]. A recent study looking at both males and females found that previous exposure to their own species' song led to an increase in their preference for their species-specific song, suggesting a potential learned component to song discrimination [24]. The pathways underlying song perception in males, like females, have been shown to involve the pC1 cluster [25], however, it is still unclear if this includes the P1 subset of pC1.

The role of vision in courtship has recently received increased attention. Although vision alone can trigger short episodes of courtship, more persistent courtship requires additional inputs [26-28]. Vision mainly

provides feedback for precisely controlled motor outputs, such as visual tracking [29*] and modulation of courtship song [30*,31]. A *fru*⁺ class of visual projection neurons, lobula columnar 10 (LC10), has recently been shown to regulate visual aspects of courtship behaviour [29*]. When LC10 neurons are artificially inactivated, males behave more like 'blind' flies, losing their ability to visually guide their behaviour towards the female, despite normal performance in other visual tests unrelated to courtship. Intriguingly, the anatomy and physiological responses of LC10 neurons are similar between the sexes, however, no clear behavioural function for LC10 neurons has been characterised in females [29*]. This suggests that males and females detect the same visual signals but process them differently to produce distinct behavioural responses. In a recent paper comparing gross *fru*⁺ neural anatomy between *D. melanogaster* and *D. subobscura* (a species whose courtship relies heavily on visual cues [32]), the authors uncovered a conspicuous species-specific anatomical difference in the male optic lobe, suggesting a potential correlation between *fru*⁺ visual pathway connectivity and the role of vision in courtship [15*,33].

Internal states

It has become increasingly evident that courtship is not merely a result of sensory input integration, but also depends on the internal state of the male. Although mating is a crucial behaviour for the survival of a species, competing needs of the individual, such as sleep drive or the presence of a competitor, might make it favourable to prioritise different behaviours.

A male may have very similar encounters, with comparable sensory stimuli, yet his reaction may differ depending on his motivational state, which is referred to as mating drive. Male mating drive depends on sexual experience: after several successful matings, the male's probability of initiating courtship towards an equally desirable female decreases, a behaviour that coincides with, but does not depend on, the depletion of ejaculatory fluid [34]. Recent work has identified key neurons expressing the neuromodulator dopamine as the neural basis of mating drive [34-36]. Intriguingly, it appears that dopamine directly modulates P1 neurons [34,35*], suggesting that P1 neurons might integrate internal states with sensory signals.

The competing drives of sleep and courtship have recently been explored: sleep deprivation was shown to reduce courtship [37*], conversely, a strong courtship drive was found to reduce sleep, even in sleep-deprived males [38,39*]. The decision to prioritise sleep vs. courtship is likely governed by the competition between sleep and mating drives, and depends on a *fru*⁺ cluster of clock neurons called DN1 [37*] and a newly discovered set of *fru*⁻/*dsx*⁻ octopaminergic neurons called MS1 [39*], both of which appear to be functionally connected to P1 [37*, 39*]. Consistent with this interplay between drives, P1 neuronal activation can suppress sleep [40] and DN1 plays a role in the circadian rhythmicity of male sex drive [41,42].

Similar competing drives have also been shown between aggression and courtship. Aggression arousal levels represent a long-lasting internal state which can bias the decision whether to fight or court. Like mating drive, aggression levels are partly innate [43], but also depend on experience in previous aggressive encounters [44]. Interestingly, this 'winner and loser' effect not only alters aggressive potential, but also effects courtship [45].

Command circuitry for action selection

Which centre in the brain is responsible for integrating sensory information with internal states and deciding which action to take?

In order to arrive at the correct decision, a centre for action selection should

- (i) Process sensory inputs as well as information about internal states
- (ii) Initiate or suppress motor programs depending on the outcome of the calculation

Most efforts to determine a 'decision making' centre for male courtship have focused on the pC1 cluster. Figure 1 schematically illustrates the signals that are integrated by pC1, and its *fru*⁺ subset, P1. There is clear evidence that P1 can be activated through sensory information received by gustatory pheromones [4] in addition to integrating information from other sensory modalities [28,46] (figure 1A), however, how these signals are processed by P1 is still unclear. In a recent study, Zhang et al. propose a model in which courtship initiation can be explained nearly completely by probabilistic activation of P1 neurons through tapping [35*]. In this view, motivational states mainly act by balancing excitatory and inhibitory inputs onto P1 and thereby changing the probability of its activation by a tap.

Intriguingly, most internal states that have been implicated in courtship, such as sleep [37*], mating drive [34,35*] and aggression levels [47], have also been linked to P1 neurons (figure 1B). Most conflicting behaviours are suppressed by the activation of P1, however, P1 has been shown to induce both courtship and aggression [47]. How can one cluster promote both courtship and aggression, when those two behaviours rarely occur at the same time and in the same context, and when most pheromones have opposite effects on these behaviours? It has been suggested that these mutually exclusive behaviours are triggered by different activation levels [47] or different subsets of *dsx*⁺ pC1 neurons [48], some of which are *fru*⁻ and therefore distinct from P1. In this context, it is important to note that the P1 cluster itself also constitutes a heterogeneous population of neurons [49], and a plethora of intersectional genetic techniques have been employed, which label varying subpopulations (for a detailed review, see [50]). An alternative hypothesis has been suggested by Hoopfer in a recent review [51]: P1 could simply signal the presence of a female as a limited resource, as aggressive behaviour requires the presence not only of a conspecific competitor, but also of a limited resource to fight over. Another possibility is that the differential effect is mediated by downstream targets of P1. Consistent with this hypothesis, a recently identified *fru*⁺ set of neurons, aSP2, receives convergent inputs from P1 and from octopaminergic neurons and seems to be involved in P1's aggression-promoting effect [52*]. In the case of courtship, several neurons downstream of P1 have been proposed [53], some of which also receive additional modulatory inputs [30*,54]. It is therefore tempting to speculate that these downstream targets also shape the decision to initiate specific motor programs.

Sensory modulation of courtship motor output

Several neuronal clusters have been identified which trigger courtship motor programs, and most prominently courtship song (recently reviewed in [55]). Although a recent study suggests that some motor actions, namely licking, abdominal bending and leg lifting, occur in a stereotyped sequence and are controlled by a single descending neuron [56], the courtship ritual as a whole is not a strict motor sequence, but instead is modulated by sensory feedback. Males use visual cues to modulate courtship song amplitude [31] and to choose between different song modes [30*]

dependent on the distance of the female. Moreover, the male's orientation and wing choice during song production depend on visual control [29*] and are optimized to lead to high song amplitudes at the female antennae [57]. Unsurprisingly, following behaviour also requires visual control [29*]. Furthermore, it has been shown that sustaining courtship after initiation requires several sensory inputs [26-28], as well as continued activity of P1 neurons [35*]. A dopamine-gated recurrent activation loop has been proposed as an underlying mechanism for sustaining P1 activation [35*].

Conclusion

The male's decision to court depends on sensory signals of different modalities as well as various internal states. Current evidence supports the hypothesis that most of this information is integrated in the male-specific P1 cluster, which acts as a decision-making centre for male-specific behaviours. Recent evidence suggests that this circuitry is largely conserved among *Drosophila* species. Once the decision is made, specific motor programs are initiated by downstream neurons. These motor programs are not rigid sequences, but are dynamically updated by sensory signals.

Future research aimed at uncovering the circuits underlying sensory feedback will profit from large-scale connectomics projects, such as electron microscopic imaging of the whole fly brain [58]. Single-cell RNA sequencing can be used to reveal cellular heterogeneity and accurately characterise cell markers that are unique to different cell types [59, 60]. Future studies may employ these techniques to identify functional subtypes of the pC1 cluster. Finally, CRISPR-Cas gene editing is revolutionising genetic analysis in insects, and is starting to shed light on the evolution of sexually dimorphic circuits in related *Drosophila* species [14**,15*,61].

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* of special interest

** of outstanding interest

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Figure 1: Schematic illustration of inputs to the courtship command cluster pC1/P1

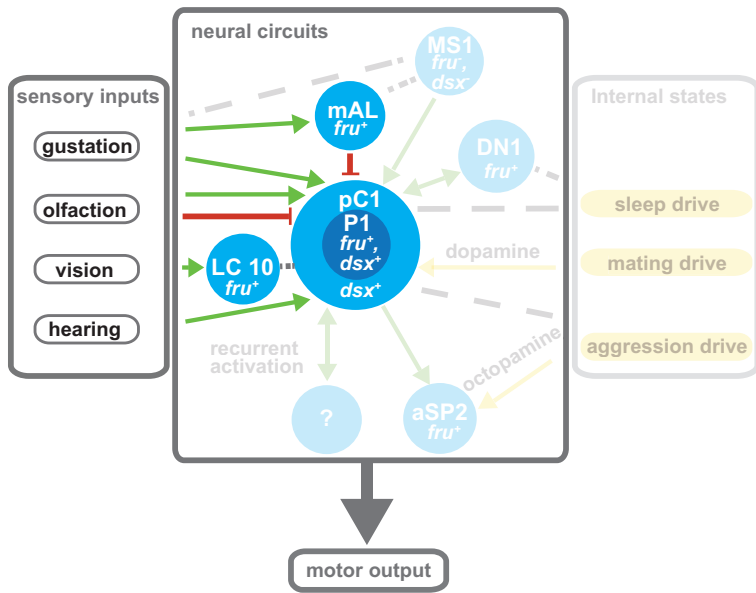
A. Sensory inputs to pC1 and its *fru*⁺ subset, P1. Excitatory connections are shown in green, inhibitory connections are depicted in red, neuromodulation is

shown in yellow. Dashed grey lines indicate putative connections that have not yet been identified.

B. Pathways signalling internal states to pC1/P1 and putative interactions with gustatory circuits. Colour coding as in A.

The illustrations are based on data from the references cited in the text.

A



B

