

Topic Introduction

Fighting Flies: Quantifying and Analyzing *Drosophila* Aggression

Maria P. Fernandez,¹ Severine Trannoy,² and Sarah J. Certel^{3,4}

¹Department of Neuroscience and Behavior, Barnard College, New York City, New York 10027, USA; ²Centre de Recherches sur la Cognition Animale (CRCA), Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31062 Toulouse, France; ³Division of Biological Sciences, University of Montana, Missoula, Montana 59812, USA

Aggression is an innate behavior that likely evolved in the framework of defending or obtaining resources. This complex social behavior is influenced by genetic, environmental, and internal factors. *Drosophila melanogaster* remains an effective and exciting model organism with which to unravel the mechanistic basis of aggression due to its small but sophisticated brain, an impressive array of neurogenetic tools, and robust stereotypical behavioral patterns. The investigations of many laboratories have led to the identification of external and internal state factors that promote aggression, sex differences in the patterns and outcome of aggression, and neurotransmitters that regulate aggression.

AGGRESSION IN *DROSOPHILA*

Aggression is an evolutionarily conserved behavior, essential for survival and genetic success, as gaining access to mates and resources and defending progeny are key favorable outcomes (Lorenz 1966; Lorenz and Leyhausen 1973; Dow and von Schilcher 1975; Hoffmann 1989; Kravitz and Huber 2003; Arnott and Elwood 2008). Aggression can be defined as a series of species-specific behaviors that include threats, attacks, and submission (Table 1; Dow and von Schilcher 1975; Jacobs 1978; Hoffmann 1987; Chen et al. 2002). Aggression appears to be prewired, as animals with no previous social experience can engage conspecifics with highly stereotypical behavior patterns. However, like other innate behaviors, aggression is also a phenotypically plastic trait that can be modified by experience and internal states (Penn et al. 2010; Baxter et al. 2015; Kilgour et al. 2020; Legros et al. 2020; Jia et al. 2021).

The study of aggressive behavior is of interest to a wide range of disciplines, including neuroscience, behavioral genetics, evolution, and medicine. Unchecked aggression is associated with psychiatric disorders, neurodegenerative disease, and injury states (Matthews et al. 2002; Hodgins 2008; Rao et al. 2009; Vermeiren et al. 2014; Liu et al. 2016; McKinlay and Albicini 2016); and thus uncovering the neural mechanisms that regulate aggression is relevant for human health. In addition to identifying factors that drive aggression to pathological levels, the study of aggression addresses fundamental questions regarding the production of a complex social behavior in any system. For example, how is sensory information processed and integrated to promote aggression? How does the nervous system decipher and act upon the dynamic information arriving from a conspecific that is also making its own

⁴Correspondence: sarah.certel@umontana.edu

From the *Drosophila* Neurobiology collection, edited by Bing Zhang, Ellie Heckscher, Alex C. Keene, and Scott Waddell.

© 2023 Cold Spring Harbor Laboratory Press

Advanced Online Article. Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top107985

TABLE 1. Aggression behavioral patterns

Behavioral pattern	Sex of flies displaying pattern	Description	Notes
Fencing	Both	Both flies extend a leg and tap the opponent's leg.	Both low- and high-posture fencing occur frequently throughout the fight.
Lunging	Males	A male rears up on his hind legs and snaps down on the other.	The key determinant of the outcome of a fight. This is seen in most male fights.
Wing threat displays	Males	One fly quickly raises both wings to a 45° angle toward the opponent.	Often displayed by the dominant male to defend his territory.
Boxing	Males	Both flies rear up on their hind legs and strike the opponent with their forelegs.	Rarely seen in fights between wild-type males under standard conditions.
Holding	Males	One fly grasps the opponent with his forelegs and tries to immobilize him.	Often exhibited after lunging.
Headbutt	Females	Female moves forward quickly, striking the second female with her head.	Common pattern in female fights.
Shove	Females	Female snaps forward with her forelegs in a level trajectory.	Lunge-like pattern seen in female fights. Higher intensity than headbutts.

Adapted from Chen et al. (2002), Nilsen et al. (2004), and Certel and Kravitz (2012).

Although some behavioral patterns such as fencing are common to males and females, most patterns used to quantify aggression are sex-specific (i.e., only exhibited by one of the sexes). This difference is not a matter of degree (i.e., in the frequency of the behavior) but an absolute difference: lunging, wing threats, boxing, and holding are only displayed by males, whereas shoves and headbutts are only displayed by females. A common source of error is mistaking lunges (male-specific) with shoves, which are female-specific and may look similar to an inexperienced observer. During shoving, females do not raise their bodies as high as males do during lunging. Another common source of error is mistaking high-posture fencing for boxing. Boxing is a high-intensity pattern, occurring rarely in ~5% of fights between wild-type males, and is often due to the male receiving a lunge now lunging in response instead of retreating.

decisions at the same time? And how does the internal state of an organism and previous aggression experience impact the motivation and persistence to fight? *Drosophila*, with its sophisticated genetic tools (Sneve and Piatkevich 1921; Zirin et al. 2022) and straightforward behavioral assays, has become a premier model system to investigate these questions and more, to ultimately understand how aggression is produced and constrained in any system (Robie et al. 2017; Lischinsky and Lin 2020). In addition, recently developed connectomics data sets are exciting new tools for understanding circuit construction within neuronal populations that drive behavior (Li et al. 2020; Scheffer et al. 2020; Sneve and Piatkevich 2021; Dorkenwald et al. 2022; Galili et al. 2022; Winding et al. 2023).

Aggression is not a singular behavioral unit but rather the result of interactions between individuals. As such, the initiation and frequency of these interactions are impacted by assay parameters including the method of loading flies into the fight chamber, the size and territorial composition of the chamber, and the animals' previous social experience. Thus, determining which assay to use depends on the overall question that is the subject of investigation. Here, we introduce three different assays, the multiwell chamber (Fernandez et al. 2010), the sliding chamber (Trannoy and Kravitz 2015; Trannoy et al. 2015a,b), and the divider chamber (Chowdhury et al. 2021), out of several described in the literature (Dierick 2007; Dankert et al. 2009; Protocol: **Scoring and Analyzing Aggression in *Drosophila*** [Certel and Kravitz 2012]; Simon and Heberlein 2020; and Protocol: **Comparing Methods for Quantifying and Analyzing *Drosophila* Aggression** [Trannoy et al. 2023]).

AGGRESSIVE BEHAVIORAL PATTERNS BETWEEN SEXES

Both male and female *Drosophila* display aggressive behavior toward individuals of their same sex, but the patterns of aggression, like those of reproductive behaviors, differ between the sexes and are therefore referred to as sexually dimorphic (Table 1; Fig. 1) (Sturtevant 1915; Manning 1959; Jacobs 1960; Ueda and Kidokoro 2002; Nilsen et al. 2004; Pandolfi et al. 2021). Sexually dimorphic patterns can be divided into two categories: true dimorphic behaviors in which males and females show nonoverlapping behaviors, and mixed dimorphic behaviors in which males and females share some behavioral actions, whereas others are sex-specific (Ueda and Kidokoro 2002; Acebes et al. 2004; Nilsen et al. 2004; Chiu et al. 2021). In the highlighted assays, both true dimorphic and shared aggressive behavioral patterns can be quantified using the different experimental chambers.

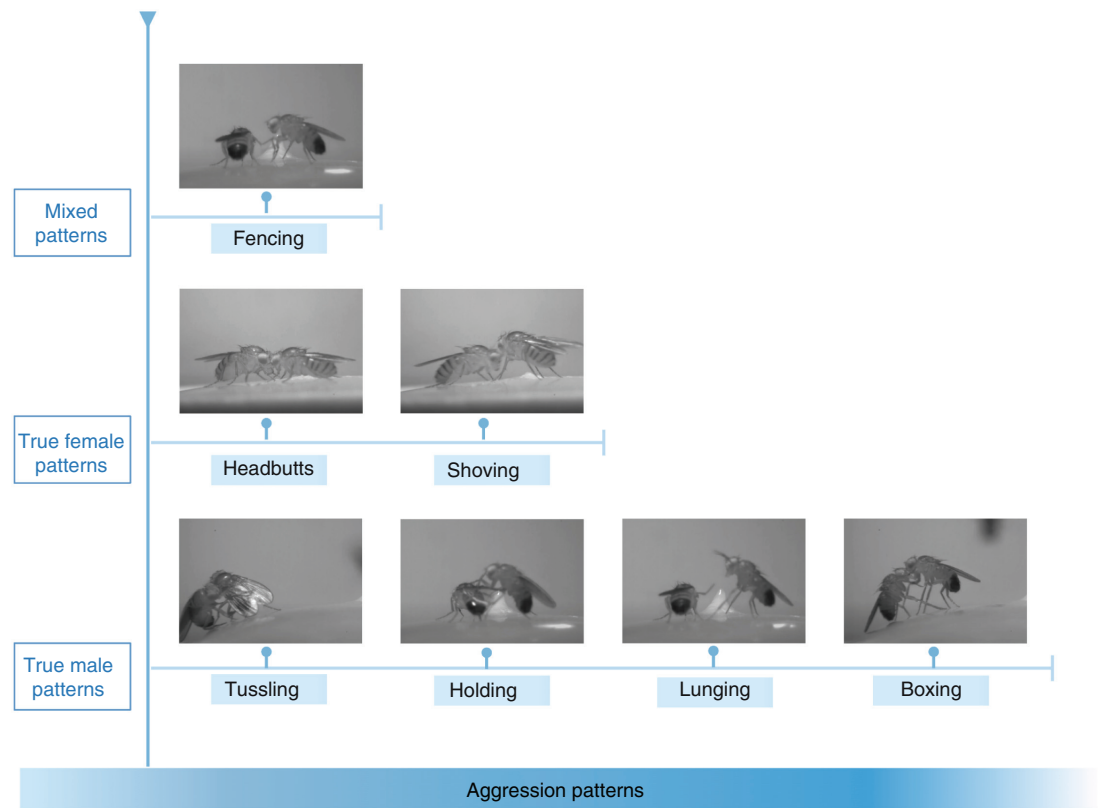


FIGURE 1. Male and female aggression patterns. Mixed patterns (*top*) are displayed by both males and females. Female-specific patterns (*middle*) include shoving and headbutts. Male-specific patterns (*bottom*) include lunging, boxing, holding, and tussling. Boxing is rarely seen in wild-type fights (~5%–10% of the pairs in a 60-min observation window) and should not be confused with high-posture fencing. (Image credit: Yick-Bun Chan. Created with www.biorender.com.)

Several comprehensive reviews of aggression discussing the molecular mechanisms and the neuronal circuits underlying sexually dimorphic behavioral patterns have been written (Kravitz and Fernandez 2015; Hoopfer 2016; Asahina 2017, 2018). Therefore, this section will only briefly describe male and female aggression. *Drosophila* fights resemble those examined in other species in that they are broken up into brief encounters (meetings between the flies) in which flies come together, interact for a period of time, and then break apart again. Early dyadic interactions between males involve orientation and approach toward the other fly, followed by an exchange of chemosensory information through fencing/touching of the forelegs and visual displays. Males show visual displays such as wing flicks and wing threats (both wings raised up to a 45° angle) (Table 1). Fights between males often escalate to physical interactions characterized by the lunge, a true dimorphic behavior. A lunge is a series of movements in which a fly (i) rears up on his hindlegs, (ii) lifts his wings to stabilize his body, and (iii) strikes down on his opponent (Jacobs 1978; Hoffmann 1987; Chen et al. 2002; Nilsen et al. 2004). As aggressive encounters continue, males modify their fighting strategies: one chases and lunges at his opponent (often in defense of a territory), whereas the second male retreats from the resource or territory (Vrontou et al. 2006). This sequence leads to the formation of a dominance relationship, which has long been evaluated based on the repeated use of lunges by one male with subsequent retreats from the territory by the second male (Hoyer et al. 2008; Penn et al. 2010). Recent studies suggest that wing threats and boxing also participate either in the establishment or in the maintenance of dominance (Legros et al. 2020; Simon and Heberlein 2020). Repetitive aggressive encounters in males can lead to wing damage or generate an internal state that displays persistence in aggression (Kim et al. 2018); both have consequences for other behaviors including courtship. Finally,

fighters can escalate to include boxing, holding, and tussling (Table 1; Fig. 1) (Nilsen et al. 2004; Dierick 2007; Dankert et al. 2009; Penn et al. 2010).

Prior fighting experience, including previous defeats or victories, influences the outcome of later contests: Losing a fight increases the probability of losing second contests, thereby revealing experience effects that involve learning and memory. Whether the question of interest is determining whether dominance is established or the memory of a fight occurs, the multiwell and sliding well procedures provide two advantages. One, a defined territory is present within the arena; and, two, flies can be separated and repaired to assess the influence of previous fighting experience. However, the sliding well assay uses negative geotaxis to introduce or reintroduce flies into the arenas, thus eliminating the handling of flies entirely (Trannoy et al. 2015). The divider assay (Chowdhury et al. 2021) does not include a specific territory or space to retreat; therefore, the criterion “three aggressive encounters with lunges/three retreats” is difficult to apply with this assay. However, a scenario with one male lunging without retaliation from the second male could be considered as a sign of dominance.

Drosophila females, as in many species, show distinct aggressive behavioral patterns (Ueda and Kidokoro 2002; Nilsen et al. 2004). Following approach and fencing, females show two possible true dimorphic attack behaviors: Either a female snaps forward with a negligible change in leg position (“headbutt”), or the female snaps forward with her forelegs in a level trajectory (“shove”). Unlike males, females alternate between competing for and sharing the resources (Nilsen et al. 2004). As in many areas of neuroscience, research on female behavior has been remarkably less extensive. However, recent progress has been made on the identification of female-specific circuitry, the intensity of female agonistic encounters, and the impact of reproductive state on female aggression (Palavicino-Maggio et al. 2019; Bath et al. 2020; Deutsch et al. 2020; Schretter et al. 2020; Chiu et al. 2021; Gaspar et al. 2022). The availability of a large portion of the female brain connectome has allowed the mapping of connections between subpopulations involved in female aggression—for example, aIPg and pC1d (Schretter 2020). In addition, a new automated tracking and classification system has been described that could improve screening for mutants and neurons that alter female-specific aggression (Bath et al. 2020).

The quantification of these true and shared aggressive behavioral patterns (Table 1; Fig. 1) can provide insight into the control of aggression by genes and circuits. Scoring visual displays (wing threats in males and wing flicking in females) and physical attacks (lunges in males and headbutts in females) may provide information on the use of low-energy threat behaviors used to discourage opponents versus the use of high-energy attack behaviors that may be required to chase a competitor from a valuable resource. However, given the time-intensive aspect of quantifying both visual and physical patterns, many studies limit quantification to the total number of physical attacks (i.e., lunges in males, headbutts in females) displayed by opponents during a fixed amount of time. Indeed, quantifying behavioral patterns by manually scoring the recorded videos is the most time-consuming aspect of the multiwell and sliding chamber assays. The 12-well chamber used in the divider assay coupled with the improved automated video analysis provide the numbers and scoring parameters needed for a high-throughput screen (Chowdhury et al. 2021). High-throughput screening approaches have been successfully used to connect sex-specific neuron classes to circuits that promote the male lunge in particular (Hoopfer et al. 2015; Watanabe et al. 2017; Wohl et al. 2020) and more recently to sexually shared neurons that control shared aggressive patterns (Chiu et al. 2021).

THE ROLE OF EXTERNAL SENSORY CUES

Although aggressive behavior between conspecifics can improve survival odds and reproductive opportunities, it is an energetically expensive behavior (Thomas et al. 2015). The decision to engage in aggression requires an assessment of the costs and benefits as well as the integration of multiple internal and external factors. External factors include the detection of pheromones from conspecifics, which is key to promote aggression as *Drosophila* males and females engage only in

intrasexual agonistic encounters (for reviews, see Billeter and Levine 2013; Fernandez and Kravitz 2013; Yew and Chung 2017)

Volatile pheromones are detected from a distance by the olfactory system, whereas nonvolatile cuticular hydrocarbon (CH) pheromones require contact detection via the gustatory system. Both are required to stimulate aggressive behavior (Amrein and Thorne 2005; Touhara and Vosshall 2009). One compound in particular, male-specific *cis*-vaccenyl acetate, promotes male aggression, and activation of the Or67d-expressing olfactory neurons that detect *cis*-vaccenyl acetate also increases male aggression (Wang and Anderson 2010; Liu et al. 2011). A second key identification stimulus for aggression is the exchange of contact pheromonal information in the form of CHs (Ferveur 1997; Amrein and Thorne 2005; Billeter and Levine 2013). *Drosophila* CH profiles are sexually dimorphic: dienes are mainly produced by females and serve as aphrodisiacs to males, whereas monoenes are produced by males and inhibit male courtship (Butterworth 1969; Brieger and Butterworth 1970; Ejima et al. 2007; Datta et al. 2008; Ronderos and Smith 2010). The exchange of male CHs during fencing is sufficient to elicit aggression, as wild-type males attack females that show a male-like pheromonal profile (Fernandez et al. 2010). Not unexpectedly, males fighting in larger chambers (multiwell and sliding well chambers) take longer to start fighting and display lower lunge numbers. The former observation may reflect an increase in the time that it takes to identify the second opponent in the chamber as a male, perhaps due to a lower concentration of pheromonal cues (Lim et al. 2014; Chowdhury et al. 2021). Latency to initiate aggression is an important behavioral parameter as it potentially reflects deficits in detecting and integrating sensory stimuli necessary for determining the sexual identity of a conspecific. As for the lower lunge number, a larger chamber size provides space for retreat, thus potentially limiting further interactions. Because chamber size directly impacts latency and lunge number, direct comparisons between data sets performed with different chambers are not possible.

Mechanosensory information also plays a role in at least male aggression. The *Drosophila* auditory organ, Johnston's organ (JO), is situated in the fly's antenna, and chordotonal receptor neurons within the JO are activated upon antennal displacement (Ishikawa and Kamikouchi 2016). Wing movements such as wing threats and flicks displayed during fight serve not only as visual signals but also as auditory signals (Jonsson et al. 2011), and the number of male aggressive encounters decreases upon disruption of JO function (Versteven et al. 2017). These results suggest that acoustic signals provide important information within the context of conspecific aggressive interactions. How hearing is integrated with other sensory modalities at the neural circuit level is an area that remains unexplored.

Movement is also required for eliciting male aggression, as flies do not attack immobilized targets. This is a clear difference from male courtship as males robustly court immobilized females. The detection of motion via vision is likely important for wild-type levels of aggression as motion-blind single-housed *ninaB*-mutant males show fewer lunges (Ramin et al. 2014); however, wild-type males are capable of intense fighting in complete darkness, both under the dark phase of a standard 12 h–12 h light–dark cycle as well as in constant darkness (Chowdhury et al. 2021), which is key to be able to study how the circadian clock regulates the timing of aggressive behavior. The divider assay is the only procedure introduced here that enables recording of behavior in the dark (Chowdhury et al. 2021). To summarize, numerous studies have shown that olfactory, gustatory, visual, and auditory cues and associated genes are required for male aggression; much remains to be studied regarding the role of these cues in female aggression.

NEUROTRANSMITTER AND INTERNAL STATE REGULATION OF AGGRESSION

As described above in the Role of External Sensory Cues section, external stimuli provide critical information that can lead to aggressive interactions between conspecifics. Internal stimuli, including hunger, mating state, or the sleep need, also influence aggression. How does the brain integrate these different inputs to set behavioral priorities and modify these priorities as contexts change? This flexibility relies on the dynamic and coordinated regulation of interconnected brain circuits and the modulation of these circuits at multiple levels, from synapses to networks.

The brain of even the “simple” fruit fly is remarkably complex. Thousands of neurons expressing canonical neurotransmitters, such as glutamate and γ -aminobutyric acid (GABA), function within excitatory or inhibitory circuits to drive behavior and cognitive processes (Bellen et al. 2010; Modi et al. 2020). For decades, researchers have taken different screening approaches using genetic tools to activate or inhibit neurons to identify neurons and their corresponding neurotransmitters, neuromodulators, or neuropeptides that promote or inhibit aggression (Martin and Alcorta 2017; Kohsaka and Nose 2021).

Forward genetic screens allow the unbiased identification of novel players, whether at the gene or neuron level. The activity of neurons or gene expression can be manipulated by transactivators including Gal4 (the yeast transcription factor that binds to upstream activating sequence (UAS) *cis*-regulatory sites), LexA (a bacterial transcription factor that binds to specific sequences called *lexA* operator [*lexO*]), and QS (QS is based on regulatory genes from the *Neurospora qa* gene cluster) that each bind to specific DNA binding sites (UAS, LexAop, and QUAS), resulting in the transcriptional activation of a downstream responder (Caygill and Brand 2016). Repressors of the transactivator and various compounds that activate or inactivate the transactivator or the repressor can be added to allow temporal or spatial control of gene expression (Venken and Bellen 2014; Caygill and Brand 2016). Downstream responders to inhibit neuronal activity include the inwardly rectifying potassium channel Kir2.1, tetanus toxin TNT, and a temperature-dependent dominant-negative form of dynamin GTPase, *Shibire*^{ts1}, whereas the NaChBac bacterial sodium channel, the temperature-dependent cation channel TrpA1, and channelrhodopsin (ChR2) are used to activate neurons (Jones 2009; Kohsaka and Nose 2021). Each method can be used with effectors such as *Shibire*^{ts1} and TrpA1 that are activated by warm temperatures. For the use of such temperature-sensitive tools, the larger chambers in the multiwell and sliding well chambers minimize the risk of condensation that can hinder a clear behavioral recording. However, the divider assay holds 12 fighting pairs per chamber and can also be behaviorally analyzed in an automated manner, which offers the possibility of a high-throughput screen using temperature to alter neuron activity.

The highly stereotypical repertoire of male and female aggressive behavioral patterns can be separated into low, mid, and high levels of intensity (Chen et al. 2002; Dierick 2007; Simon and Heberlein 2020). The intensity of aggressive encounters may reflect the value of the resource, the arousal level of the male fly, internal state signals, or other factors. In *Drosophila* males, aggression intensity is defined by lunge number as well as the usage of boxing and tussling (Chen et al. 2002; Dierick 2007; Simon and Heberlein 2020). As higher levels of aggression may reflect an increase in general activity, it is critical to quantify the locomotor activity of experimental animals and transgenic controls, which can be extrapolated from the automated behavior classifier in the divider assay as well as through independent assays (see Chiu et al. 2010; Protocol: **Locomotor Activity Level Monitoring Using the *Drosophila* Activity Monitoring (DAM) System** [Pfeiffenberger et al. 2010]; Persons et al. 2022; Sisobhan et al. 2022).

Like other animals, *Drosophila* males fight over a resource of value—that is, food, a designated territory, or a female (see Protocol: **Scoring and Analyzing Aggression in *Drosophila*** [Certel and Kravitz 2012]; Zwartz et al. 2012). Therefore, although the initiation of aggression requires male pheromonal information (Fernandez and Kravitz 2013), a nutritive resource and a territory are also relevant for the manifestation of wild-type aggression levels (Chen et al. 2002; Lim et al. 2014). The multiwell and sliding well assays have defined territories that contain a food source (Trannoy et al. 2015), whereas the divider assay chamber has a flat surface consisting of clear food medium. Furthermore, male aggression is influenced not only by the quality of the contested food resource (Lim et al. 2014), but also by dietary conditions. Males raised on sugar-containing or sugar-free diets displayed distinct fighting strategies that are dependent on these different diet-based environmental conditions (Legros et al. 2020). Diet also impacts the composition of the gut microbiome, which can modulate the internal state of an organism (Ezra-Nevo et al. 2020; Henry et al. 2020). Jia et al. determined that the production of octopamine (OA) and subsequently male aggression is dependent on gut microbiome effects set down during development (Jia et al. 2021). As alterations in the internal state of an animal, whether through directly manipulating diet or the gut microbiome,

may impact other behaviors including mating success (Jia et al. 2021) and locomotor activity (Schretter et al. 2018), assaying additional social and nonsocial behaviors is necessary to determine whether behavioral deficits are specific to aggression. Deciphering how the brain receives and integrates internal state information including the rewarding aspects of food and signals from the gut microbiome is an emerging area of interest.

Finally, neuromodulators and neuropeptides are also involved in regulating aggressive behavior. In particular, the neuropeptide Tachykinin and its receptor, *Takr86C*, play an important role in male aggression (Asahina et al. 2014). In some cases, the effects of neuromodulators and neuropeptides could be due, in part, to their interactions with other co-released neurotransmitters, such as GABA, glutamate, and acetylcholine. Monoamines and neuropeptides can be expressed in neurons that also release at least one fast-acting neurotransmitter (Nassel 2018), and, thus, the release of a combination of monoamines, neuropeptides, and neurotransmitters can provide both “classical” and “modulatory” signals, resulting in diverse and/or complementary effects on behavioral circuits (Vaaga et al. 2014; Nusbaum et al. 2017; Nassel 2018). For example, OA-producing neurons in the adult are also glutamatergic (Rodríguez-Valentín et al. 2006; Sherer et al. 2020). Both glutamate and OA are required for male aggression (Sherer et al. 2020); however, only OA is required for the suppression of intermale courtship, which suggests that glutamate uncouples aggression from OA-dependent courtship-related behavior (Sherer et al. 2020). An example of circuit regulation through multiple transmitters was uncovered by Alekseyenko et al. (2019) who reported that a subset of 5-hydroxytryptamine (5HT)-producing neurons modulates aggression via two types of downstream target neurons that express the 5HT1A receptor. One subset of 5HT1A⁺ neurons also express GABA and function to decrease aggression, whereas a separate subset of 5HT1A⁺ neurons release acetylcholine and promote aggression (Alekseyenko et al. 2019). The computational capabilities of aggression circuits could be enhanced or inhibited by the release of more than one neurotransmitter, neuromodulator, and/or neuropeptide; thus, care should be taken when assigning functional significance to an individual signaling molecule through the silencing or activation of dual-transmitting neurons until each neuroactive substance can be examined separately.



CONCLUSION

Studies in *Drosophila* are providing remarkable insight into how a myriad of contextual information influences the input–output relationship of hardwired circuits that support a complex behavioral repertoire. A comparative approach to aggressive behavior in invertebrate and vertebrate model organisms has identified many conserved molecular components that control the development of neurons required for aggression, the integration and processing of sensory stimuli, the production of neurotransmitters and neurotransmitter receptors, and complex gene–environment interactions (Thomas et al. 2015; Anderson 2016). These similarities suggest that aggression is produced and regulated by a conserved logic or framework and that results from the study of aggression in *Drosophila* will be applicable across the animal kingdom, including humans.

ACKNOWLEDGMENTS

We are grateful to Yick-Bun Chan for the images in Figure 1. We thank Herman Dierick and Lewis Sherer for helpful discussions. Work in our laboratories was supported by the National Institutes of Health (NIH) (R01 GM115510) to S.J.C. and grants from CNRS, ANR (ANR-19-CE37-0018-01), and the Fondation Fyssen (190573) to S.T., and National Science Foundation (NSF) grant IOS-2239994 to M.P.F.

REFERENCES

- Acebes A, Grosjean Y, Everaerts C, Ferveur JF. 2004. Cholinergic control of synchronized seminal emissions in *Drosophila*. *Curr Biol* 14: 704–710. doi:10.1016/j.cub.2004.04.003
- Alekseyenko OV, Chan YB, Okaty BW, Chang Y, Dymecki SM, Kravitz EA. 2019. Serotonergic modulation of aggression in *Drosophila* involves GABAergic and cholinergic opposing pathways. *Curr Biol* 29: 2145–2156.e2145. doi:10.1016/j.cub.2019.05.070
- Amrein H, Thorne N. 2005. Gustatory perception and behavior in *Drosophila melanogaster*. *Curr Biol* 15: R673–R684. doi:10.1016/j.cub.2005.08.021
- Anderson DJ. 2016. Circuit modules linking internal states and social behaviour in flies and mice. *Nat Rev Neurosci* 17: 692–704. doi:10.1038/nrn.2016.125
- Arnott G, Elwood RW. 2008. Information gathering and decision making about resource value in animal contests. *Anim Behav* 76: 529–542. doi:10.1016/j.anbehav.2008.04.019
- Asahina K. 2017. Neuromodulation and strategic action choice in *Drosophila* aggression. *Annu Rev Neurosci* 40: 51–75.
- Asahina K. 2018. Sex differences in *Drosophila* behavior: qualitative and quantitative dimorphism. *Curr Opin Physiol* 6: 35–45. doi:10.1016/j.cophys.2018.04.004
- Asahina K, Watanabe K, Duistermars BJ, Hoopfer E, Gonzalez CR, Eyjolfsson EA, Perona P, Anderson DJ. 2014. Tachykinin-expressing neurons control male-specific aggressive arousal in *Drosophila*. *Cell* 156: 221–235.
- Bath E, Biscocho ER, Easton-Calabria A, Wigby S. 2020. Temporal and genetic variation in female aggression after mating. *PLoS ONE* 15: e0229633. doi:10.1371/journal.pone.0229633
- Baxter CM, Barnett R, Dukas R. 2015. Aggression, mate guarding and fitness in male fruit flies. *Anim Behav* 109: 235–241. doi:10.1016/j.anbehav.2015.08.023
- Bellen HJ, Tong C, Tsuda H. 2010. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: a history lesson for the future. *Nat Rev Neurosci* 11: 514–522. doi:10.1038/nrn2839
- Billeter JC, Levine JD. 2013. Who is he and what is he to you? Recognition in *Drosophila melanogaster*. *Curr Opin Neurobiol* 23: 17–23. doi:10.1016/j.conb.2012.08.009
- BioRender. 2022. www.biorender.com [Accessed September 23, 2022].
- Brieger G, Butterworth FM. 1970. *Drosophila melanogaster*: identity of male lipid in reproductive system. *Science* 167: 1262. doi:10.1126/science.167.3922.1262
- Butterworth FM. 1969. Lipids of *Drosophila*: a newly detected lipid in the male. *Science* 163: 1356–1357. doi:10.1126/science.163.3873.1356
- Caygill EE, Brand AH. 2016. The GAL4 system: a versatile system for the manipulation and analysis of gene expression. *Methods Mol Biol* 1478: 33–52. doi:10.1007/978-1-4939-6371-3_2
- Certel SJ, Kravitz EA. 2012. Scoring and analyzing aggression in *Drosophila*. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot068130
- Chan YB, Kravitz EA. 2007. Specific subgroups of Fru^M neurons control sexually dimorphic patterns of aggression in *Drosophila melanogaster*. *Proc Natl Acad Sci* 104: 19577–19582. doi:10.1073/pnas.0709803104
- Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA. 2002. Fighting fruit flies: a model system for the study of aggression. *Proc Natl Acad Sci* 99: 5664–5668. doi:10.1073/pnas.082102599
- Chiu JC, Low KH, Pike DH, Yildirim E, Edery I. 2010. Assaying locomotor activity to study circadian rhythms and sleep parameters in *Drosophila*. *J Vis Exp* doi:10.3791/2157
- Chiu H, Hoopfer ED, Coughlan ML, Pavlou HJ, Goodwin SF, Anderson DJ. 2021. A circuit logic for sexually shared and dimorphic aggressive behaviors in *Drosophila*. *Cell* 184: 507–520.e516. doi:10.1016/j.cell.2020.11.048
- Chowdhury B, Wang M, Gnerer JP, Dierick HA. 2021. The Divider Assay is a high-throughput pipeline for aggression analysis in *Drosophila*. *Commun Biol* 4: 85. doi:10.1038/s42003-020-01617-6
- Dankert H, Wang L, Hoopfer ED, Anderson DJ, Perona P. 2009. Automated monitoring and analysis of social behavior in *Drosophila*. *Nat Methods* 6: 297–303. doi:10.1038/nmeth.1310
- Datta SR, Vasconcelos ML, Ruta V, Luo S, Wong A, Demir E, Flores J, Balanze K, Dickson BJ, Axel R. 2008. The *Drosophila* pheromone cVA activates a sexually dimorphic neural circuit. *Nature* 452: 473–477. doi:10.1038/nature06808
- Deutsch D, Pacheco D, Encarnacion-Rivera L, Pereira T, Fathy R, Clemens J, Girardin C, Calhoun A, Ireland E, Burke A, et al. 2020. The neural basis for a persistent internal state in *Drosophila* females. *Elife* 9: e59502. doi:10.7554/eLife.59502
- Dierick HA. 2007. A method for quantifying aggression in male *Drosophila melanogaster*. *Nat Protoc* 2: 2712–2718. doi:10.1038/nprot.2007.404
- Dorkenwald SC, McKellar E, Macrina T, Kemnitz N, Lee K, Lu R, Wu J, Popovych S, Mitchell E, Nehoran B, et al. 2022. FlyWire: online community for whole-brain connectomics. *Nat Methods* 19: 119–128.
- Dow MA, von Schilcher F. 1975. Aggression and mating success in *Drosophila melanogaster*. *Nature* 254: 511–512. doi:10.1038/254511a0
- Ejima A, Smith BP, Lucas C, van der Goes van Naters W, Miller CJ, Carlson JR, Levine JD, Griffith LC. 2007. Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. *Curr Biol* 17: 599–605. doi:10.1016/j.cub.2007.01.053
- Ezra-Nevo G, Henriques SF, Ribeiro C. 2020. The diet-microbiome tango: how nutrients lead the gut brain axis. *Curr Opin Neurobiol* 62: 122–132. doi:10.1016/j.conb.2020.02.005
- Fernandez MP, Kravitz EA. 2013. Aggression and courtship in *Drosophila*: pheromonal communication and sex recognition. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 199: 1065–1076. doi:10.1007/s00359-013-0851-5
- Fernandez MP, Chan YB, Yew JY, Billeter JC, Dreisewerd K, Levine JD, Kravitz EA. 2010. Pheromonal and behavioral cues trigger male-to-female aggression in *Drosophila*. *PLoS Biol* 8: e1000541. doi:10.1371/journal.pbio.1000541
- Ferveur JF. 1997. The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster*. *Bioessays* 19: 353–358. doi:10.1002/bies.950190413
- Galili DS, Jefferis GS, Costa M. 2022. Connectomics and the neural basis of behaviour. *Curr Opin Insect Sci* 54: 100968.
- Gaspar M, Dias S, Vasconcelos ML. 2022. Mating pair drives aggressive behavior in female *Drosophila*. *Curr Biol* 32: 4734–4742.e4.
- Henry Y, Overgaard J, Colinet H. 2020. Dietary nutrient balance shapes phenotypic traits of *Drosophila melanogaster* in interaction with gut microbiota. *Comp Biochem Physiol A Mol Integr Physiol* 241: 110626. doi:10.1016/j.cbpa.2019.110626
- Hodgins S. 2008. Violent behaviour among people with schizophrenia: a framework for investigations of causes, and effective treatment, and prevention. *Philos Trans R Soc Lond B Biol Sci* 363: 2505–2518. doi:10.1098/rstb.2008.0034
- Hoffmann AA. 1987. Territorial encounters between *Drosophila* males of different sizes. *Anim Behav* 35: 1899–1901. doi:10.1016/S0003-3472(87)80085-4
- Hoffmann AA. 1989. Geographic variation in the territorial success of *Drosophila melanogaster* males. *Behavior Genetics* 19: 241–253.
- Hoopfer ED. 2016. Neural control of aggression in *Drosophila*. *Curr Opin Neurobiol* 38: 109–118. doi:10.1016/j.conb.2016.04.007
- Hoopfer ED, Jung Y, Inagaki HK, Rubin GM, Anderson DJ. 2015. P1 interneurons promote a persistent internal state that enhances inter-male aggression in *Drosophila*. *eLife* doi:10.7554/eLife.11346
- Hoyer SC, Eckart A, Herrle A, Zars T, Fischer SA, Hardie SL, Heisenberg M. 2008. Octopamine in male aggression of *Drosophila*. *Curr Biol* 18: 159–167. doi:10.1016/j.cub.2007.12.052
- Ishikawa Y, Kamikouchi A. 2016. Auditory system of fruit flies. *Hear Res* 338: 1–8. doi:10.1016/j.heares.2015.10.017
- Jacobs ME. 1960. Influence of light on mating of *Drosophila melanogaster*. *Ecology* 41: 6. doi:10.2307/1931952
- Jacobs ME. 1978. Influence of β -alanine on mating and territorialism in *Drosophila melanogaster*. *Behav Genet* 8: 487–502. doi:10.1007/BF01067478
- Jia Y, Jin S, Hu K, Geng L, Han C, Kang R, Pang Y, Ling E, Tan EK, Pan Y, et al. 2021. Gut microbiome modulates *Drosophila* aggression through octopamine signaling. *Nat Commun* 12: 2698. doi:10.1038/s41467-021-23041-y



- Jones WD. 2009. The expanding reach of the GAL4/UAS system into the behavioral neurobiology of *Drosophila*. *BMB Rep* 42: 705–712. doi:10.5483/BMBRep.2009.42.11.705
- Jonsson T, Kravitz EA, Heinrich R. 2011. Sound production during agonistic behavior of male *Drosophila melanogaster*. *Fly (Austin)* 5: 29–38. doi:10.4161/fly.5.1.13713
- Kilgour RJ, Norris DR, McAdam AG. 2020. Carry-over effects of resource competition and social environment on aggression. *Behav Ecol* 31: 140–151. doi:10.1093/beheco/arz170
- Kim YK, Saver M, Simon J, Kent CF, Shao L, Eddison M, Agrawal P, Texada M, Truman JW, Heberlein U. 2018. Repetitive aggressive encounters generate a long-lasting internal state in *Drosophila melanogaster* males. *Proc Natl Acad Sci* 115: 1099–1104.
- Kohsaka H, Nose A. 2021. Optogenetics in *Drosophila*. *Adv Exp Med Biol* 1293: 309–320. doi:10.1007/978-981-15-8763-4_19
- Kravitz EA, Fernandez MP. 2015. Aggression in *Drosophila*. *Behav Neurosci* 129: 549–563.
- Kravitz EA, Huber R. 2003. Aggression in invertebrates. *Curr Opin Neurobiol* 13: 736–743. doi:10.1016/j.conb.2003.10.003
- Legros J, Tang G, Gautrais J, Fernandez MP, Trannoy S. 2020. Long-term dietary restriction leads to development of alternative fighting strategies. *Front Behav Neurosci* 14: 599676. doi:10.3389/fnbeh.2020.599676
- Lim RS, Eyjolfsson E, Shin E, Perona P, Anderson DJ. 2014. How food controls aggression in *Drosophila*. *PLoS ONE* 9: e105626. doi:10.1371/journal.pone.0105626
- Li F, Lindsey JW, Marin EC, Otto N, Dreher M, Dempsey G, Stark I, Bates AS, Pleijsier MW, Schlegel P, et al. 2020. The connectome of the adult *Drosophila* mushroom body provides insights into function. *eLife* doi:10.7554/eLife.62576
- Liu W, Liang X, Gong J, Yang Z, Zhang YH, Zhang JX, Rao Y. 2011. Social regulation of aggression by pheromonal activation of Or65a olfactory neurons in *Drosophila*. *Nat Neurosci* 14: 896–902. doi:10.1038/nn.2836
- Liu CS, Ruthirakuhan M, Chau SA, Herrmann N, Carvalho AF, Lanctot KL. 2016. Pharmacological management of agitation and aggression in Alzheimer's disease: a review of current and novel treatments. *Curr Alzheimer Res* 13: 1134–1144. doi:10.2174/1567205013666160502122933
- Lischinsky JE, Lin D. 2020. Neural mechanisms of aggression across species. *Nat Neurosci* 23: 1317–1328.
- Lorenz K. 1966. *On aggression*. Harcourt, Brace & World, New York.
- Lorenz K, Leyhausen P. 1973. *Motivations of human and animal behavior*. Van Nostrand Reinholds, New York.
- Manning A. 1959. The sexual behaviour of two sibling *Drosophila* species. *Behavior* 15: 123–145. doi:10.1163/156853960X00133
- Martin F, Alcorta E. 2017. Novel genetic approaches to behavior in *Drosophila*. *J Neurogenet* 31: 288–299. doi:10.1080/01677063.2017.1395875
- Matthews KL, Chen CP, Esiri MM, Keene J, Minger SL, Francis PT. 2002. Noradrenergic changes, aggressive behavior, and cognition in patients with dementia. *Biol Psychiatry* 51: 407–416. doi:10.1016/S0006-3223(01)01235-5
- McKinlay A, Albicini M. 2016. Prevalence of traumatic brain injury and mental health problems among individuals within the criminal justice system. *Concussion* 1: CNC25. doi:10.2217/cnc-2016-0011
- Modi MN, Shuai Y, Turner GC. 2020. The *Drosophila* mushroom body: from architecture to algorithm in a learning circuit. *Annu Rev Neurosci* 43: 465–484. doi:10.1146/annurev-neuro-080317-0621333
- Nassel DR. 2018. Substrates for neuronal cotransmission with neuropeptides and small molecule neurotransmitters in *Drosophila*. *Front Cell Neurosci* 12: 83. doi:10.3389/fncel.2018.00083
- Nilsen SP, Chan YB, Huber R, Kravitz EA. 2004. Gender-selective patterns of aggressive behavior in *Drosophila melanogaster*. *Proc Natl Acad Sci* 101: 12342–12347. doi:10.1073/pnas.0404693101
- Nusbaum MP, Blitz DM, Marder E. 2017. Functional consequences of neuropeptide and small-molecule co-transmission. *Nat Rev Neurosci* 18: 389–403. doi:10.1038/nrn.2017.56
- Palavicino-Maggio CB, Chan YB, McKellar C, Kravitz EA. 2019. A small number of cholinergic neurons mediate hyperaggression in female *Drosophila*. *Proc Natl Acad Sci* 116: 17029–1738.
- Pandolfi M, Scania MF, Fernandez MP. 2021. Sexual dimorphism in aggression: sex-specific fighting strategies across species. *Front Behav Neurosci* 15: 659615. doi:10.3389/fnbeh.2021.659615
- Penn JK, Zito MF, Kravitz EA. 2010. A single social defeat reduces aggression in a highly aggressive strain of *Drosophila*. *Proc Natl Acad Sci* 107: 12682–12686. doi:10.1073/pnas.1007016107
- Persons JL, Abhilash L, Lopatkin AJ, Roelofs A, Bell EV, Fernandez MP, Shafer OT. 2022. PHASE: an open-source program for the analysis of *Drosophila* phase, activity, and sleep under entrainment. *J Biol Rhythms* 37: 455–467.
- Pfeiffenberger C, Lear BC, Keegan KP, Allada R. 2010. Locomotor activity level monitoring using the *Drosophila* activity monitoring (DAM) system. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot5518
- Ramin M, Domocos C, Slawaska-Eng D, Rao Y. 2014. Aggression and social experience: genetic analysis of visual circuit activity in the control of aggressiveness in *Drosophila*. *Mol Brain* 7: 55. doi:10.1186/s13041-014-0055-0
- Rao V, Rosenberg P, Bertrand M, Salehina S, Spiro J, Vaishnavi S, Rastogi P, Noll K, Schretlen DJ, Brandt J, et al. 2009. Aggression after traumatic brain injury: prevalence and correlates. *J Neuropsychiatry Clin Neurosci* 21: 420–429. doi:10.1176/jnp.2009.21.4.420
- Robie AA, Hirokawa J, Edwards AW, Umayam LA, Lee A, Phillips ML, Card GM, Korff W, Rubin GM, Simpson JH, et al. 2017. Mapping the neural substrates of behavior. *Cell* 170: 393–406 e28.
- Rodríguez-Valentín R, López-González I, Jorquera R, Labarca P, Zurita M, Reynaud E. 2006. Oviduct contraction in *Drosophila* is modulated by a neural network that is both, octopaminergic and glutamatergic. *J Cell Physiol* 209: 183–198. doi:10.1002/jcp.20722
- Ronderos DS, Smith DP. 2010. Activation of the T1 neuronal circuit is necessary and sufficient to induce sexually dimorphic mating behavior in *Drosophila melanogaster*. *J Neurosci* 30: 2595–2599. doi:10.1523/JNEUROSCI.4819-09.2010
- Scheffer LK, Xu CS, Januszewski M, Lu Z, Takemura SY, Hayworth KJ, Huang GB, Shinomiya K, Maitlin-Shepard J, Berg S, et al. 2020. A connectome and analysis of the adult *Drosophila* central brain. *eLife* doi:10.7554/eLife.57443
- Schretter CE, Vielmetter J, Bartos I, Marka Z, Marka S, Argade S, Mazmanian SK. 2018. A gut microbial factor modulates locomotor behaviour in *Drosophila*. *Nature* 563: 402–406. doi:10.1038/s41586-018-0634-9
- Schretter CE, Aso Y, Robie AA, Dreher M, Dolan MJ, Chen N, Ito M, Yang T, Parekh R, Branson KM, et al. 2020. Cell types and neuronal circuitry underlying female aggression in *Drosophila*. *eLife* doi:10.7554/eLife.58942
- Sherer LM, Catudío Garrett E, Morgan HR, Brewer ED, Sirrs LA, Shearin HK, Williams JL, McCabe BD, Stowers RS, Certel SJ. 2020. Octopamine neuron dependent aggression requires dVGLUT from dual-transmitting neurons. *PLoS Genet* 16: e1008609. doi:10.1371/journal.pgen.1008609
- Simon JC, Heberlein U. 2020. Social hierarchy is established and maintained with distinct acts of aggression in male *Drosophila melanogaster*. *J Exp Biol* 223: jeb232439. doi:10.1242/jeb.232439
- Sisobhan S, Rosensweig C, Lear BC, Allada R. 2022. SleepMat: a new behavioral analysis software program for sleep and circadian rhythms. *Sleep* 45: zsac195.
- Sneve MA, Piatkevich KD. 2021. Towards a comprehensive optical connectome at single synapse resolution via expansion microscopy. *Front Synaptic Neurosci* 13: 754814.
- Sturtevant AH. 1915. Experiments on sex recognition and the problem of sexual selection in *Drosophila*. *J Anim Behav* 5: 351–366. doi:10.1037/h0074109
- Thomas AL, Davis SM, Dierick HA. 2015a. Of fighting flies, mice, and men: are some of the molecular and neuronal mechanisms of aggression universal in the animal kingdom? *PLoS Genet* 11: e1005416. doi:10.1371/journal.pgen.1005416
- Touhara K, Vossell LB. 2009. Sensing odorants and pheromones with chemosensory receptors. *Annu Rev Physiol* 71: 307–332. doi:10.1146/annurev.physiol.010908.163209
- Trannoy S, Kravitz EA. 2015. Learning and memory during aggression in *Drosophila*: handling affects aggression and the formation of a “loser” effect. *J Nat Sci* 1: e56.
- Trannoy S, Chowdhury B, Kravitz EA. 2015a. A new approach that eliminates handling for studying aggression and the “loser” effect in *Drosophila melanogaster*. *J Vis Exp* 2015: e53395. doi:10.3791/53395-v

M.P. Fernandez et al.

- Trannoy S, Chowdhury B, Kravitz EA. 2015b. Handling alters aggression and “loser” effect formation in *Drosophila melanogaster*. *Learn Mem* 22: 64–68.
- Trannoy S, Fernandez MP, Certel SJ. 2023. Comparing methods for quantifying and analyzing *Drosophila* aggression. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108144
- Ueda A, Kidokoro Y. 2002. Aggressive behaviours of female *Drosophila melanogaster* are influenced by their social experience and food resources. *Physiol Entomol* 27: 21–28. doi:10.1046/j.1365-3032.2002.00262.x
- Vaaga CE, Borisovska M, Westbrook GL. 2014. Dual-transmitter neurons: functional implications of co-release and co-transmission. *Curr Opin Neurobiol* 29: 25–32. doi:10.1016/j.conb.2014.04.010
- Venken KJ, Bellen HJ. 2014. Chemical mutagens, transposons, and transgenes to interrogate gene function in *Drosophila melanogaster*. *Methods* 68: 15–28. doi:10.1016/j.ymeth.2014.02.025
- Vermeiren Y, Van Dam D, Aerts T, Engelborghs S, De Deyn PP. 2014. Monoaminergic neurotransmitter alterations in postmortem brain regions of depressed and aggressive patients with Alzheimer’s disease. *Neurobiol Aging* 35: 2691–2700. doi:10.1016/j.neurobiolaging.2014.05.031
- Versteven M, Vanden Broeck L, Geurten B, Zwarts L, Decraecker L, Beelen M, Gopfert MC, Heinrich R, Callaerts P. 2017. Hearing regulates *Drosophila* aggression. *Proc Natl Acad Sci* 114: 1958–1963. doi:10.1073/pnas.1605946114
- Vrontou E, Nilsen SP, Demir E, Kravitz EA, Dickson BJ. 2006. *fruitless* regulates aggression and dominance in *Drosophila*. *Nat Neurosci* 9: 1469–1471.
- Wang L, Anderson DJ. 2010. Identification of an aggression-promoting pheromone and its receptor neurons in *Drosophila*. *Nature* 463: 227–231. doi:10.1038/nature08678
- Watanabe K, Chiu H, Pfeiffer BD, Wong AM, Hoopfer ED, Rubin GM, Anderson DJ. 2017. A circuit node that integrates convergent input from neuromodulatory and social behavior-promoting neurons to control aggression in *Drosophila*. *Neuron* 95: 1112–1128.e1117. doi:10.1016/j.neuron.2017.08.017
- Wohl M, Ishii K, Asahina K. 2020. Layered roles of fruitless isoforms in specification and function of male aggression-promoting neurons in *Drosophila*. *eLife* 9: e52702. doi:10.7554/eLife.52702
- Winding M, Pedigo BD, Barnes CL, Patsolic HG, Park Y, Kazimiers T, Fushiki A, Andrade AV, Khandelwal A, Valdes-Aleman J, et al. 2023. The connectome of an insect brain. *Science* 379: eadd9330.
- Yew JY, Chung H. 2017. *Drosophila* as a holistic model for insect pheromone signaling and processing. *Curr Opin Insect Sci* 24: 15–20. doi:10.1016/j.cois.2017.09.003
- Zirin J, Bosch J, Viswanatha R, Mohr SE, Perrimon N. 2022. State-of-the-art CRISPR for in vivo and cell-based studies in *Drosophila*. *Trends Genet* 38: 437–453.
- Zwarts L, Versteven M, Callaerts P. 2012. Genetics and neurobiology of aggression in *Drosophila*. *Fly (Austin)* 6: 35–48. doi:10.4161/fly.19249



Cold Spring Harbor Protocols

Fighting Flies: Quantifying and Analyzing *Drosophila* Aggression

Maria P. Fernandez, Severine Trannoy and Sarah J. Certel

Cold Spring Harb Protoc; doi: 10.1101/pdb.top107985; published online April 5, 2023

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

- [Behavioral Assays](#) (99 articles)
- [Drosophila](#) (272 articles)
- [Drosophila Neurobiology](#) (26 articles)
- [Drosophila Neurobiology \(2e\): A Laboratory Manual](#) (33 articles)
- [Drosophila Transgenics](#) (39 articles)
- [Imaging for Neuroscience](#) (342 articles)
- [Neuroscience, general](#) (357 articles)
- [Visualization](#) (524 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>
