A REVIEW

Aggression in Drosophila

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Aggression is used by essentially all species of animals to gain access to desired resources, including territory, food, and potential mates: Fruit flies are no exception. In Drosophila, both males and females compete in same sex fights for resources, but only males establish hierarchical relationships. Many investigators now study aggression using the fruit fly model, mainly because (a) aggression in fruit flies is a quantifiable well-defined and easily evoked behavior; (b) powerful genetic methods allow investigators to manipulate genes of interest at any place or time during embryonic, larval, pupal or adult life, and while flies are behaving; (c) the growth of the relatively new field of optogenetics makes physiological studies possible at single neuron levels despite the small sizes of neurons and other types of cells in fly brains; and (d) the rearing of fly stocks with their short generation times and limited growth space requirements can easily be performed at relatively low cost in most laboratories. This review begins with an examination of the behavior, both from a historical perspective and then from the birth of the "modern" era of studies of aggression in fruit flies including its quantitative analysis. The review continues with examinations of the roles of genes, neurotransmitters and neurohormones, peptides, nutritional and metabolic status, and surface cuticular hydrocarbons in the initiation and maintenance of aggression. It concludes with suggestions for future studies with this important model system.

Keywords: aggression, Drosophila melanogaster, hierarchical relationships, learning and memory, peptides

Supplemental materials: http://dx.doi.org/10.1037/bne0000089.supp

Aggression in Drosophila: The Behavior

In an article dealing both with sex recognition and sexual selection, Sturtevant (1915) was the first to mention male–male aggression and to demonstrate a novel female–male rejection behavior in Drosophila. Using mainly D. amplipilosa for experiments, Sturtevant reported:

"If two males are courting the same female, they often grow very excited, especially if she is unwilling to stay quiet. In such cases they may sometimes be seen to spread their wings, run at each other, and apparently butt heads. One of them soon gives up and runs away. If the other then runs at him again within the next few minutes he usually makes off without showing fight." (p. 353)

This brief description suggested that competition likely exists between males for mating partners, and also that some sort of short term memory might exist in the loser fly of having recently been defeated. Females were reported to frighten away a male occasionally by charging at him with spread wings. Sturtevant was using a different species of fly than the commonly used D. melanogaster of today, and one pattern described by him involving males sounds more like the "head-butt" that is commonly seen in female fights (Nilsen, Chan, Huber, & Kravitz, 2004), but the suggestion of male–male aggression in this early work was clear.

Some 45 years later, Jacobs published a second important article on aggression (Jacobs, 1960). He referred to the Sturtevant, 1915 article but only in reference to odor perception as an important factor in courtship behavior. The discussion of this article began "The charges and tussles of the male Drosophila appear to be forms of aggression" (p. 186). Jacobs suggested further that this might be characterized as territorial behavior that was not long lasting. Their assay arenas were relatively large Petri dishes that were illuminated from above and the focus of their studies were on the effects of melanization on mating behavior. A second article by Jacobs continued the studies of melanization on mating behavior...
and aggression using ebony mutants in which β-alanine is not inserted into developing cuticles, and black variants showing reduced synthesis of β-alanine (Jacobs, 1978). Fifteen years later a more complete description of aggression and its relationship to courtship success in *D. melanogaster* was published (Dow & von Schilcher, 1975). This article made no mention of the earlier study by Jacobs, although the authors commented that there had been several descriptions of aggressive behavior in other species of flies over the previous decade. They suggested that their studies were the first observations on aggression in laboratory stocks of *D. melanogaster*. Three patterns of aggressive behavior shown by males were described: wing threat, charge, and “boxing”. The results showed wide differences between males in terms of who occupied the food surface, who attacked first, who won fights and who had success in copulation, with a tight correlation shown between the latter two. The data supported the notion that a linear hierarchy had been established between the males during the time in the arena before females were introduced, although this was not specifically discussed by the authors. Instead their focus was on fitness and the close linkage between success in aggression and mating.

The most complete descriptions of aggression in fruit flies prior to those published during the current renaissance were by Hoffmann (1987a, 1987b). Using laboratory-reared males and females of the sibling species *D. melanogaster* and *D. simulans*, Hoffmann presented a complete ethogram (word description of the patterns seen) of agonistic interactions between males of the two species. These included the following: approach, fly on, lunge, hold, tussle, on back, fall, wings erect, wing vibration, and fly off. Most of these terms had been coined in earlier studies. The adult flies used in all experiments were reared under continuous light conditions, with males collected as virgins and placed in isolation vials prior to their use in experiments. Six males and three females were placed in observation cages similar to those described by Dow and von Schilcher (1975) in the morning and all interactions between the flies were monitored for the next 8 hours via videotaping. A food cup containing fly medium coated with live yeast was placed in the center of the arena. *Winners* of encounters were defined as the male who successfully forced another male (the loser) off the food surface and remained on the cup afterwards. The percentage usage of the various behavioral patterns by the two species was described in text tables. The dynamic aspects of the interactions between both male and female flies over the extended observation period were described in the text of the article, which focused on territorial behavior, including acquisition of and defense of the territory and examination of the linkage between territoriality and success in mating behavior. The results showed that males occupying territories had greater success in mating than did males not occupying a territory, with the author reporting in a note that 80% of the matings took place on the food surface. Size and weight also influenced the outcome of the fights, a result further described in a later short article. Although an impressive description of the behavior, these articles and the interpretation of the usage of behavioral patterns suffer somewhat from the complexity of the behavioral scene. For example: in some male–male interactions hierarchical relationships might already have been established with animals recognizing familiar opponents and fighting differently against them compared with nonfamiliar opponents (Yurkovic, Wang, Basu, & Kravitz, 2006), or social interactions might change over an extended 8-hr observation period relating to less-active periods over the course of a day, rather than as a consequence of social interactions per se, or the use of mated rather than virgin females might influence courtship object choice by males and possibly also by females. To avoid such complications, authors of current studies of aggression usually pair only two males, with or without the availability of resources like food and potential mates, depending on the laboratory.

Sturtevant (1915) also was the first to mention that females sometimes charge at males as a rejection behavior, an observation that was briefly repeated in Jacobs (1960) as being directed at other flies during feeding. Other early studies of female aggression were field studies with different species of fruit flies (Pritchard, 1969) that clearly documented and partially characterized female–female aggression in relation to oviposition sites. A more recent publication, also involving field behavior studies, was by Shelly (1999) and these studies further defined behavioral patterns seen during female–female aggression (head-buttting, pushing, lunging, and wing threat) and dealt with the ecological value of such aggression during egg-laying. The first publication describing female–female aggression in a laboratory setting with *D. melanogaster* was by Ueda and Kidokoro (2002). Three behavioral patterns were described by these authors, including approach, lunge, and wings erect. The lunge described here was different from the pattern described in male aggression as a lunge in that the female does not rise above the horizontal. Moreover, the authors state several times in the article that the “behaviors are similar to those of male aggression in this species” (p. 25). The female patterns of aggression in a laboratory setting were described more fully and renamed recently, in a publication that included a quantitative analysis of female aggressive behavior (Nilsen et al., 2004). Ueda and Kidokoro (2002) reported that levels of female aggression were dependent on rearing conditions (isolated more aggressive than group housed flies) and on the food resource, with flies showing higher levels of aggression when yeast colonies were growing on the food. The latter findings were suggested as a potential link between the quality of food resources and the defense of potential future egg laying sites, a notion that has been revived in recent studies (see the following paragraphs).

### Modern Era of Studies of Aggression in *Drosophila*

Quantitative analysis of the behavior: Two articles appeared almost simultaneously in 2002 (Baier, Wittek, & Brembs, 2002; Chen, Lee, Bowens, Huber, & Kravitz, 2002), reviving interest in aggression in fruit flies after a gap of some 15 years. One analysis by Baier et al. (2002) used chambers that were the same as those used in the earlier studies, but these authors focused on exploring the roles of amines in the behavior. Pharmacological treatments were used to alter amine levels for dopamine (DA) and serotonin (5HT) but without verification of the effectiveness of the treatments. For octopamine (OA), a mutant line without the amine generated by Monasterioti et al. (Monasterio, Linn, & White, 1996), was used and a severe reduction in aggression was reported in flies without OA. However, controls were missing for mutants that expressed a wild mutation that likely interfered with vision and that also might lead to altered amine levels (Borycz, Borycz, Kubow, Lloyd, & Meinertzhagen, 2008; Sitaraman et al., 2008).
Finally, flies were placed in arenas for 2 hr before video recording was begun.

In the second article, Chen et al. (2002) designed a different chamber than those used before, aimed at observing aggression between a single pair of flies. These arenas had features built in that were modeled on real-world situations, including allowing room for losers to escape from winners by leaving the resource-containing area. A small cup containing food that the flies had been reared on was placed in the middle of the chamber, and either a headless mated female or a small dab of freshly ground yeast paste was placed on the food surface as an additional attractant (Certel & Kravitz, 2012). Other arenas that allowed for higher throughput analysis and less fly handling have been described recently (Fernandez et al., 2010), and our laboratory has just designed a new arena that requires no handling of flies prior to, during, or after fights (Trannoy, Chowdhury, & Kravitz, 2015). This new arena improves all behavioral parameters measured and in particular preserves the learning and memory that accompanies fight outcomes. Fights between pairs of flies usually are monitored by videotaping for 30 min periods after the first social interaction (called an encounter) between flies on the food surface, or after the first higher intensity encounter (lunge) is seen. Quantitative analyses of aggression between pairs of male and pairs of female flies were carried out in the Chen et al. studies using standardized ethological approaches to the study of behavior. These involved observation of fights to generate an ethogram of the behavior and then scoring all patterns and transitions between patterns during each encounter during fights. This was followed by construction of transition matrices and a graphic visualization of the matrices using a first-order Markov chain (see Figure 1). Nine offensive and four defensive patterns were listed in the male ethogram, many of which had been described in earlier studies (Hoffmann, 1987a, 1987b; see Figure 1). Female fights tend to be at lower intensity levels and some of the patterns seen during male fights also are seen during female fights: others are male or female specific. Although male fights tend to end with the establishment of a hierarchical relationship, female fights end with flies sharing the resource. Why females should expend the energy to compete in such a case is unclear. It may be that in real-world situations, preferred egg-laying sites may be scarcer. Among the male-specific offensive actions, the lunge is particularly important, as its usage predicts the outcome of a fight: the first animal to lunge, if the opponent retreats, is 16 times more likely to be the ultimate winner. Ultimate winners lunge more and more as fights progress, losers less and less, and in second fights losers are highly unlikely to lunge against familiar opponents and less likely to lunge against any opponents (Yurkovic et al., 2006). A quantitative analysis of courtship behavior in fruit using transition matrices and first-order Markov chain analyses was carried out by Markow and Hanson (1981).

Of particular interest regarding the patterns that make up the behavior, is that though they are recognizable to observers (they are stereotypical and meaningful to opponents), every time a fly utilizes a pattern it is different. A high-speed video of lunging behavior (see Movies S1 and S2 in the online supplemental materials) reveals the complexity of this key pattern of behavior. During the 30th of a second it takes to complete a lunge (Movie S1), a smooth sequential activation of different motor programs is seen (Movie S2).† Thus in a published video (Hoyer et al., 2008), the lunge fly (a) raises the anterior end of its body and forelegs, (b) lifts the wings up then down as the fly rises higher and higher above the horizontal, (c) forms a sliding tripod between the mid and hind legs and the wings as the fly approaches its maximum elevation, (d) strikes down with the forelegs so fast that at 500 frames/s the image is blurred, and (e) attempts to grab onto the opponent. If the same animal lunges again during a fight, however, several or many of the motor sequences that make up the pattern may be omitted. The ability of animals to change patterns in this way makes good sense, as the success or failure of a lunge in striking an opponent depends on what the other animal is doing. Is the other animal stationary or moving? Is it moving toward or away from the opponent? How behavioral patterns of such complexity get wired into nervous systems remains unclear (but see the work of Michael Bate; Bate, 1999; Crisp, Evers, Fiala, & Bate, 2008; Landgraf, Jeffrey, Fujioka, Jaynes, & Bate, 2003; Suster & Bate, 2002) on the formation of central motor patterns during development. Another mystery that remains to be solved asks how animals “decide” to transition from one behavioral pattern to another during fights. Finally, flies continually adjust their pattern usage directed at opponents during fights in a form of socially mediated learning (see the following section). Where and how this takes place in the nervous system and other important questions remain unanswered as investigators continue their attempts to unravel the complexity of behaviors like aggression.

The Dynamics of a Fight: Learning and Memory and “Winner” and “Loser Mentalities”

Male flies paired in arenas continue to fight for up to 5 hr, but fewer encounters are seen and the nature of the interactions differ at later compared with earlier times during fights (Yurkovic et al., 2006). Early in fights, both animals appear capable of displaying lunging and retreat behavior and may occupy the food surface. After the establishment of hierarchical relationships, losers hardly ever lunge and instead spend most of their time off the food surface, whereas winners lunge about 30% of the time and do not retreat and occupy the food surface most of the time. The numbers of encounters also drop dramatically from ca. 2/min at the start of a fight in the Canton-S strain we are using to 0.2/min at the end. When losing flies are paired in second fights 30 min after first fights; what is observed depends on the opponent. With familiar opponents, losers will not lunge and will quickly lose again. With unfamiliar opponents (naïve flies or winners of other fights), losers do occasionally lung but will be defeated again quickly. Thus in both cases, even though the losing flies fight differently depending on the opponent, they lose (or draw) fights against all opponents, developing what we call a loser mentality. Depending on the training protocol (a single long fight vs. four shorter fights in which they lose every time against different opponents over the
same time as the long fight) the duration of the loser mentality can be changed. With a single loss, the flies no longer retain a loser mentality 1 day later, with multiple losses they do. In many species, winner effects also are seen, where winning animal are more likely to win second fights against naïve opponents. Winner effects tend to last for shorter times than do loser effects, with the ethological suggestion for this being that it is risky to believe that you will always win fights. Recently, short lasting winner effects also have observed as a consequence of fighting in *Drosophila* (Trannoy et al., 2015).

**Genes and Aggression**

Several recent reviews have appeared that describe genetic studies aimed at extending our understanding of aggression (Anholt & Mackay, 2012; Zwarts, Versteven, & Callaerts, 2012). Aggression is a highly complex behavior; however, and despite evidence of important genetic contributions to the behavior, the linkages between the two remain obscure. By inbreeding winners of fights for multiple generations, lines of hyper-aggressive *Drosophila melanogaster* flies can be generated starting with a wild-type Canton-S parent strain (Dierick & Greenspan, 2006; Penn et al., 2010). Males of these strains (called bullies) (Penn et al., 2010) begin fights and start lunging sooner, retaliate against lunges from opponents more often, and win fights 94% of the time against males of the parent stock. The high levels of aggression seen in bully males is not seen in females of the bully stock. Surprisingly, when bullies are paired in fights, losers of single fights lose all competitive advantage against males from the parent stock, but these males revert to bullies a day later. *Nurture* (losing a fight) clearly triumphs against *nature* (inbreeding for aggression) in this case, but the persistence of this loser effect and how losing suppresses genetically induced high level aggression remain unknown at present. Bullies spend less time courting and show lower copulation success rates than the parent Canton-S flies (but see Dierick & Greenspan, 2006; Penn et al., 2010).

**Genome-Wide Studies**

Using a similar rearing strategy to the above over 21 generations, Dierick and Greenspan (2006) selected highly aggressive and less aggressive male flies from starting mixed populations of Canton-S flies. Via this route, they generated two lines of aggressive and two lines of less aggressive, “neutral” flies. Aggression levels were scored by measuring the latency to start fighting and the total numbers of encounters (called fights in these studies) during which fighting and higher intensity fighting were seen during a one hour time period. As described previously, aggressive fly lines showed fewer matings than did neutral lines. Microarray expression analyses on the heads of the flies demonstrated that large numbers of genes (474–775) showed differential expression levels when any two of the four lines were compared. Therefore analyses were carried out searching for gene expression patterns that changed in the same direction when brains from the aggressive lines were compared with brains of neutral flies: Some 80 genes were identified using this maneuver and most of the gene expression changes were small. Only a single gene (cytochrome P450 6a20) of those identified, when examined in a mutant line, showed effects on aggression in directions predicted by the array results. No genes directly related to amine neurons or to fruitless (see the following text) were identified in these array studies.
A series of studies from the Mackay laboratory examined transcript abundance in high- and low-aggression lines derived from laboratory stock lines and from recently isolated inbred lines in their attempts to unravel the genetic underpinnings of aggression (Edwards, Ayroles, et al., 2009; Edwards & Mackay, 2009; Edwards, Zwarts, Yamanoto, Callaerts, & Mackay, 2009; Rollmann et al., 2008). A high-throughput assay was developed for these studies, in which behaviorally heterogeneous group-raised flies were used. With flies reared in this way, low levels of aggression are seen likely relating to the prior social experience of individual flies within the population. The animals used in experiments were deprived of food for 90 min then placed in groups of 4 to 8 males (in different experiments) into chambers for 2 min to “acclimate.”

Aggression is scored during the following two minutes by counting the numbers of encounters in which any aggressive behavior is displayed by any individual fly in any of the fights taking place in the arena. This provides no information on how many flies in the arena are fighting. The very short time of scoring (2 min) and the grouping together of all aggressive behaviors as equivalent “units” of aggression ignore the dynamics of aggression and provide no information on whether or when flies use key patterns of behavior like lunges that are the predictors of the outcomes of fights. In almost all fights between male flies, the first direct contacts involve lunges that are the predictors of the outcomes of fights. In almost all this complexity, and with investigators using different methods of measuring and scoring aggression, it is no wonder that there is little agreement between investigators seeking to unravel the genetic basis of aggression, and that a very large number of genes concerned with almost all aspects of the physiology of fruit flies have been suggested to be involved in aggression thus far.

One gene, the fruitless gene of the sex determination hierarchy, has been directly related to aggression in Drosophila via a candidate gene approach (Billeter, Rideout, Dorman, & Goodwin, 2006). Male and female flies fight in same-sex pairings (Nilsen et al., 2004). This, and the observation that many of the key behavioral patterns seen during fights are sex specific (patterns of aggression are sexually dimorphic, see Figure 1) raised the possibility that, as in courtship behavior, genes of the sex determination hierarchy might be involved in aggression. This was tested directly in studies in which fruitless (fru), a gene that codes for proteins of the BTB-zinc-finger family of transcription factors, was expressed in female brains, where it ordinarily is not made, and was not expressed in male brains where at least three forms of the protein coded for by the gene normally are made (Billeter et al., 2006; Vrontou et al., 2006; Vrontou, Nilsen, Demir, Kravitz, & Dickson, 2006). Initially this was done via the constitutive expression of alleles that were spliced from transcripts of the gene derived from the P1 promoter site into male or female forms, regardless of the sex of the flies (Demir & Dickson, 2005), thereby changing the sex of brain neurons. In subsequent publications, the sex of neurons in the brain (sex is cell-specific in flies) also was changed using the Gal4 system to drive expression of UAS constructs that allow or eliminate expression of transformer, a splicing factor translated into active protein in female but not male fly brains (Chan & Kravitz, 2007; Mundiyamapurath, Chan, Leung, & Kravitz, 2009). Tra along with Tra-2 (expressed in both males and females) is required for fru, truncated transcripts are made in females, for dsx male and female forms of the protein are made. In all cases, changing the sex of brain neurons without changing the sex of the rest of the fly leads to animals fighting using patterns of aggression appropriate to the sex of the fru-expressing brain neurons and not to the genetic sex of the animals (Vrontou et al., 2006). One pattern escapes from this generalization: The extended wing threat, a threat posture ordinarily displayed only in male flies, also is seen in males with feminized brains. The development of hierarchical relationships, also is altered by these changes, which is not surprising, because flies must lunge to develop such relationships and males with feminized brain neurons no longer lunge; instead, they show the female head butt and shove patterns of aggression. A preliminary study was made on attempting to identify individual neurons involved in sex-specific circuitry related to aggression (Chan & Kravitz, 2007), but parts of the fru-related circuitries in fly brains (Cachero, Ostrovsky, Yu, Dickson, & Jefferis, 2010; Yu, Kanai, Demir, Jefferis, & Dickson, 2010) responsible for aggression, or for courtship, and how and where they intersect were unknown until recently. During the past few years, however, small sets of fru-specific and fru-neuropeptide F-expressing neurons were identified that appear to be important in male higher-level aggression (see the following Peptide section).

**Hormonal Modulation of Aggression**

As an innate behavior essential to the survival of animals in complex and often hostile environments, one anticipates that mul-
tiple sensory modalities and circuitries and numerous hormonal and neurohormonal substances will influence both the triggering and the intensity of expression of aggression. In vertebrates, aggression is modulated by amines, peptides, and steroid hormones (Montoya, Terburg, Bos, & van Honk, 2012; Morrison & Melloni, 2014). In *Drosophila*, not surprisingly, many of the same or closely related substances also influence aggression. Amines, including 5HT, OA, and DA have been the most extensively studied of these hormonal substances, and the results obtained are described in detail in the following paragraphs. More recently, peptides, including peptide F, *Drosophila* insulin-like peptides, and tachykinins also have been suggested to be involved in the modulation of aggression. Although little work has been done on steroid hormones and aggression thus far in fruit flies, one anticipates that these important hormonal substances also will be found to influence this behavior once suitable experimental protocols can be established to evaluate behavioral effects of these more difficult to work with substances.

Amines

At least five amines, all metabolites of common amino acids, exist and have been proposed to serve specific functions in the fruit fly nervous system (for a thorough review of four, see Monastirioti, 1999; Roeder, 2005; for tyramine see Bayliss, Roselli, & Evans, 2013; Roeder, 2005). Studies of amine function in relation to behavior have focused mainly on three of these: 5HT derived from tryptophan by ring hydroxylation and decarboxylation, OA synthesized by decarboxylation followed by side-chain hydroxylation from tyrosine, and DA made by ring hydroxylation and decarboxylation from tyrosine. Tyramine (TA), the direct decarboxylation product of tyrosine and the direct precursor of OA also has been proposed as a neurotransmitter/neuromodulator in *Drosophila* (Bayliss et al., 2013; Roeder, 2005). Finally, histamine (HA), synthesized from histidine by decarboxylation, has been suggested to function as a photoreceptor transmitter compound. HA and its biosynthetic enzyme have been found in small clusters of neurons in the brain and the ventral cord as well (Nassel, 1999; Roeder, 2005). Here we focus on OA, 5HT, and DA and their diverse roles in aggression in *Drosophila* as these compounds and their vertebrate counterparts norepinephrine (for OA), DA, and 5HT have been reported to influence aggression in many species up to and including humans (Yanowitch & Coccaro, 2011). As in vertebrates, only small numbers of amine neurons are found in the nervous systems of fruit flies (approximately 100 to 200 of each subtype, mostly in small clusters in the brain or ventral cord whereas some 100,000 to 200,000 total neurons are found). Again, as in vertebrates, they are involved in essentially all behaviors shown by flies (Aleksyenko, Lee, & Kravitz, 2010; A. Chen et al., 2013; Crocker, Shahidullah, Levitan, & Sehgal, 2010; Dierick & Greenspan, 2007; Gasque, Conway, Huang, Rao, & Vosschalk, 2013; Hirsh et al., 2010; Lebestky et al., 2009; Van Swinderen & Andretic, 2011; Waddell, 2013). A key question remains to be answered, however, in flies as in other species, which is “How do amines exert these myriad effects?” Several recent reviews have dealt with amine function in fruit flies (Chen et al., 2013; Foltenyi, Andretic, Newport, & Greenspan, 2007; Roeder, 2005; Van Swinderen & Andretic, 2011; Waddell, 2013; Zwarts et al., 2012). Here, the focus will be on amine effects on aggression, even though these actions overlap with effects on other behaviors like learning and memory, courtship, feeding, sleep, and so forth (Chen et al., 2013; Foltenyi et al., 2007; Roeder, 2005; Van Swinderen & Andretic, 2011; Waddell, 2013; Zwarts et al., 2012). It should be added that a growing literature demonstrates that amines also serve important roles during the development of nervous systems (Daubert & Condron, 2010), and investigators should be aware of possible developmental effects as causes of any behavioral changes induced when manipulating these systems.

OA

Throughout this review, we emphasize the difficulty of comparing behavioral results from different laboratories where investigators use different assay chambers that contain or do not contain resources, compute results using different analytical methods, utilize animals reared under different social conditions, and measure behavior over different time periods. The most difficult part of such comparisons is that under the diverse experimental paradigms, the results obtained probably are accurate. If anything, the differing results demonstrate that fruit flies, like other animals, are highly adaptable to their surroundings. How closely one comes to the normal behavior of animals in the wild may be the issue under consideration here rather than whether results are valid. A confusing story in this vein may be the multiple investigations of the role of OA in aggression. Several publications (Baier et al., 2002; Hoyer et al., 2008; Zhou, Rao, & Rao, 2008) drew the conclusion that without OA: *Drosophila* males cannot go to higher levels of aggression. More accurately, the results show that without OA, reduced or absent lunging behavior is seen. Almost all experiments examining the roles of OA in aggression have used the same or similar mutant flies, including null mutants for the biosynthetic enzyme for OA (tyramine β-hydroxylase-[Tbh]), null mutants for the enzyme that forms both TA and OA (tyrosine decarboxylase-[Tdc]-), or the Gal4 system and acute or chronic interference with transmitter release or function in neurons expressing Tdc2. The experimental protocols, however, varied widely in the different publications. In one publication (Hoyer et al., 2008), the collection of lunge data in fights was automated, lunges were the only behavioral pattern counted and data was collected 15 to 30 min after adult male flies were introduced into the fight chambers. At that time, hierarchical relationships likely would have been established in many but not all of the pairings. Thus the two flies in the chamber may no longer be behavioral equivalents because after a hierarchical relationship is established, losers behave differently than winners in traditional fight chambers. They remain off the food resource most of the time, they show few or no lunges when in contact with familiar winners, and they show dramatically reduced numbers of meetings (encounters) between themselves and the winners (Yurkovic et al., 2006). To allow automating of the counting of lunges in these studies, a chamber was designed with a relatively large center well for food in the base and in which tall chamber walls were treated with Fluon (tetrafluoroethylene) to prevent flies from landing on them. In this chamber with little possibility of escape of losers from winners, higher numbers of encounters were forced, as noted by the authors, and higher numbers of lunges were seen. Moreover, in quantifying results, these authors created a new “unit” of measurement: lunge per distance traveled. This makes the results obtained in this study difficult to
compare with others that all measure total numbers of lunges per unit of time. A second publication also reported dramatic reductions in lunge frequency with lowered or abolished OA (Zhou et al., 2008). These authors reduced the standard chamber size to increase the numbers of interactions in fights but did allow space for losers to escape from winners. They then analyzed data for the first 10 min after both flies were in the chamber, measuring the latency to the first fight (unit in which they saw physical interactions) in their chambers. They scored lunge frequency and other patterns and also showed an important change in the latency to fight in the OA mutant flies, however, lasting 2 to 6 min. The latency change, which involved a significant portion of the time (almost half) over which lunging was scored, could result in the decreased number of lunges seen. An important lengthening in the latency to engage in social behavior with OA lowering has been seen before, as such animals appear to have difficulty in recognizing whether conspecifics are courtship or fighting objects (Certel et al., 2010; Certel, Savella, Schlegel, & Kravitz, 2007), which in turn could influence aggression levels if that is the main parameter scored in male–male pairings. That is precisely the effect seen in a recent report (Andrews et al., 2014) in which an aggression vigor index (AVI = lunges from the first lunge) was computed instead of counting lunges for a single set period of time. When the AVI was measured, the total numbers of lunges did not change without OA. Their distribution, however, was altered with two groups seen: one showing more and another less than the average number of lunges per fight. This supports the notion that OA-less flies have difficulty determining the sex of a second fly and hence whether to court or to fight.

Serotonin

Although an early study in male flies reported no effects of altered 5HT levels on aggression, two subsequent articles did report actions of 5HT on aggression in flies (Alekseyenko et al., 2010; Dierick & Greenspan, 2007). Dierick and Greenspan (2007), using a combination of pharmacological treatments and genetic methods that raised or lowered 5HT levels in brains were the first to report that 5HT was not required for aggression in flies, but did increase the expression of higher level aggression. These authors also reported an effect opposite to that of 5HT by manipulation of neuropeptide F levels (see the following text). In a second set of studies, use of a Ddc-Gal4 driver line to drive expression of a temperature sensitive dynamin mutant in DA and 5HT neurons led to hyperactivity displayed as an increase in the numbers of encounters (meetings during fights) between the flies. Encounter duration was decreased and fights failed to escalate to higher intensity levels or to yield dominance relationships. Separate manipulation of serotonergic and dopaminergic neuron function; however, using Th- and dTrh-Gal4 driver lines, revealed differences between the behavioral effects of the amines (Alekseyenko et al., 2010). Reduced function of 5HT-neurons alone led to dramatic reductions in the numbers of lunges seen. A third recent publication used an intersectional genetics strategy (see Figure 2) to isolate single pairs of serotoninergic neurons to try to learn their function (Alekseyenko et al., 2014). In these studies, the authors identified one to two pairs of single serotonergic neurons from the PLP cluster (5HT-PLP neurons) that had extensive arbors of processes in the ventrolateral protocerebrum, a sensory integration center, and lesser arbors of processes near the mushroom bodies and the central complex. Silencing the 5HT-PLP neurons alone reduced the numbers of lunges seen in fights by about the same magnitude as silencing all serotonergic neurons, whereas activating the neurons enhanced aggression. Endings of these neurons are in close proximity to neurons expressing 5HT1A receptors: These reduce cAMP levels when activated in mammalian and fruit fly systems (see the following text).

The neuronal targets of serotonergic neurons in fly brains should display receptors for 5HT on their surfaces and manipulation of receptor function, therefore, might yield all or part of the phenotype displayed by altering 5HT levels. Four orthologs of mammalian receptors have been identified in Drosophila by sequence homology: d5HT1A and d5HT1B resemble mammalian 5HT1A receptors and their activation leads to decreases in cAMP synthesis, d5HT2a resemble mammalian 5HT2 receptors and influence phosphoinositide synthesis (recently a d5HT2b receptor subtype was reported with the suggestion that the original d5HT2 receptor should be called d5HT2a (Gasque et al., 2013), and d5HT7 receptors resemble the mammalian similarly named receptors and increase cAMP synthesis when activated (Blenau & Thamm, 2011). Activation or inhibition of these receptor subtypes using drugs of high specificity that were originally described in mammalian studies have yielded a variety of behavior phenotypes in flies including actions on circadian rhythms, locomotion, sleep, specific aspects of learning, and on reproductive functioning (Becnel, Johnson, Luo, Nassel, & Nichols, 2011; Johnson, Becnel, & Nichols, 2011; Nall & Sehgal, 2014). One recent report focused on effects on aggression using agonists and antagonists of various receptor subtypes (Johnson, Becnel, & Nichols, 2009). While lowered levels of 5HT itself lead to dramatic reductions in lunging behavior (see preceding text), these authors reported a variety of behavioral effects relating to aggression resulting from altering amine receptor function. The difficulties of comparing these effects with previous results, however, was that a different strain of D. melanogaster was used, different aggression chambers were used, and most important, very different definitions and hence scoring of the behavioral patterns displayed by the drug-treated flies were used. For example, these authors defined wing threat, which is as common in their experiments as fencing behavior, as when a fly “orients his body in parallel to the other and lifts one wing and vibrates it” (p. 1294). Other investigators would have called this
male–male courtship behavior because wing threat was defined previously as a rare posture with both wings erect and motionless and usually directed by a dominant animal toward an opponent. Moreover boxing is reported as very common in the recent publication, as common as fencing in fact, which is the initial contact between flies in other publications, whereas lunging is barely reported. In all other studies on aggression in wild-type fruit flies, boxing is rare and lunging is fairly common. There is little doubt that definition differences are the cause of great confusion in this case. In the intersectional genetics studies described in preceding text, it was mentioned that the arbors of the 5HT-PLP neurons involved in enhancing the usage of higher level aggression during fights overlapped with regions of high density of 5HT1A receptor expression. When the neuron pool expressing 5HT1A receptors is in turn activated, aggression is reduced. This raises the possibility that 5HT released by the 5HT-PLP neurons inhibits the firing of 5HT1A receptor-bearing neurons via a reduction of cAMP synthesis (see Figure 3) and that members of the pool of receptor expressing neurons form part of part of an inhibitory descending pathway holding aggression in check. In this case inhibition by released 5HT of a descending inhibitory pathway may be the route through which 5HT-PLP neurons enhance aggression (Alekseyenko et al., 2014).

DA

DA too is involved in multiple behaviors in flies. For example, it is reportedly involved in learning and memory, sleep, locomotor activity and stereotypical actions, and so forth (Riemensperger et al., 2011; Van Swinderen & Andretic, 2011; Waddell, 2013). In learning studies, a relatively simple hypothesis of how amines function was proposed initially by several authors. This hypothesis suggested that DA is involved in aversive and OA in appetitive learning behavior. Recently, however, this simple hypothesis was shown to be incorrect with experiments showing that DA is involved both in appetitive and aversive learning, at least in the mushroom bodies. Moreover, in this system, the OA involvement appears to be at the level of modulating DA neuron function (Burke et al., 2012). There is perhaps a lesson to be learned in these recent results: One should be cautious in proposing simple linear models for the function of amine neurons. A growing literature suggests that amine neuron function on systems require the right amount of amine to be released at the right time and in the right place in the nervous system to alter neuronal function in behavior correctly and that addition of amines to levels above or below this particular set point generates an unstable situation whereby enhanced or reduced behavioral function may result. In various test systems, U-shaped rather than simple plus or minus alterations in function have been noted on the effectiveness of amine actions on behavior (Van Swinderen & Andretic, 2011). In aggression, with an acute reduction in DA-neuron function using a Th-Gal4 driver to express the dynamint mutation (this particular Gal4 driver has been shown not to influence all DA neurons), flies fight normally at the permissive temperature but became hyperactive at the restrictive temperature, failing to even land on the food cup. In a courtship assay such hyperactive flies showed a dramatic reduction in courtship behavior as well, seeming to show no interest in normal females (Alekseyenko et al., 2010). More clear-cut effects of DA-neurons on aggression are seen by using the intersectional genetics approach that allows isolation and manipulation of single pairs of DA neurons (Alekseyenko, Chan, Li, & Kravitz, 2013). These neurons (one pair from the T1 and one pair from the PPM3 clusters of DA neurons) when altered by expression of the tetanus toxin light chain in the neurons (prevents transmitter release), display increases in the numbers of lunges during fights. Hence it appears that these neurons are involved in holding higher levels of aggression in check during fights, an effect opposite of that seen in the absence of 5HT. The two pairs of neurons identified appear to be specialist generating mainly effects on aggression and no or small effects on other behaviors. The two distinct pairs of neurons display arbors of axonal endings in different neuropil regions within the central complex where each overlap with a different subtype of DA receptor.

Peptides

Neuropeptide F (NPF). NPF is an orthologue of neuropeptide Y of vertebrate systems, sharing sequence similarity but ending in phenylalanine (F) rather than tyrosine (Y). Neuropeptide Y has been suggested to serve a modulatory role in aggression in mammalian systems (Karl et al., 2004). There are 26 NPF neurons per adult hemibrain in male fly brains found distributed in at least eight different clusters, but in females only 20 NPF-expressing neurons are found per hemibrain (Lee, Bahn, & Park, 2006). No NPF neurons are reportedly found in the ventral nerve cord. This sexually dimorphic expression pattern depends on FruM expression in the male specific NPF clusters of neurons (Lee et al., 2006). One receptor has been identified for NPF, (CG10342) NPFR1, which is

Figure 3. The 5HT-PLP neurons may function to enhance aggression by releasing 5HT onto neurons bearing 5HT1A receptors on their surfaces. 5HT1A receptors act by decreasing cAMP synthesis and inhibiting neurons expressing these receptors. Activation of neurons with 5HT1A receptors on their surfaces inhibits higher-level aggression Therefore 5HT released by activation of 5HT-PLP neurons may release higher-level aggression by inhibiting a tonically active descending inhibitory pathway.
widely distributed in the brain. NPF has been implicated in multiple behaviors in *Drosophila* including foraging and feeding, alcohol sensitivity, evening locomotor activity patterns, and a longer mating duration of male flies housed with other male flies in mating arenas (Hermann, Yoshii, Dusik, & Helfrich-Forster, 2012; Kim, Jan, & Jan, 2013; Lee et al., 2006; Nässel & Winther, 2010). As noted above, NPF also has been suggested to hold aggression in check in male flies (Dierick & Greenspan, 2007). This was demonstrated both by eliminating peptide release in NPF neurons with tetanus toxin and by feminizing the male specific NPF neurons using transformer expression. In both cases large increases in aggression were reported. The authors also point out that females, who ordinarily display low levels of aggression, express no NPF in the male specific clusters of PDF neurons yet still show low levels of aggression. This suggests that the role of NPF may be to inhibit a special aggression-promoting pathway found only in male brains.

**Tachykinins.** More recently tachykinin-expressing neurons also were reported to influence aggression and in particular to be concerned with the differences in the intensities of fighting displayed by male and female flies (Asahina et al., 2014). All tachykinins found in vertebrates share a C-terminal pentapeptide (FXGLM), whereas the tachykinin-related peptides (TKRPs) found in invertebrates have a different C-terminal pentapeptide (FX,GX,R). In *Drosophila* the TKRPs are derived from a single gene and six separate peptide isoforms have been found (Tk1–6). Two G-protein coupled receptors (Neurokinin receptor from *Drosophila* [NKD] and *Drosophila* tachykinin receptor [DTKR]) for tachykinins have been identified in *Drosophila* and the genes have been identified and characterized (Birse et al., 2006; Poels et al., 2009). There are about 150 neurons in the adult brain that express TKRPs, and these peptides also are expressed in endocrine cells of the mid-gut (Nässel & Winther, 2010). TKRPs have been shown to serve behavioral roles in fruit flies in odor detection and in control of locomotion (Winther, Acebes, & Ferrus, 2006). In a screen for peptides that might influence aggression, Asahina et al. (2014) found that TKRPs also might be involved in aggression. The behavioral screen used a variety of Gal4 driver lines for 16 different peptides (there are 5 predicted varieties of neuropeptides in the *Drosophila* genome [Nässel & Winther, 2010]) to express a TrpA1 temperature sensitive potassium channel that allows activation of neurons at elevated temperatures. In same genotype pairings of group-reared male progeny (group rearing lowers levels of aggression in controls) two of four of the different Tk-Gal4 drivers used (called Tk1- and Tk2-Gal4) yielded substantial increases in aggression during fights after temperature shifts. Only two other peptide-Gal4 drivers led to increases in aggression in the same screen: One of these was Npf-Gal4 that in these experiments led to increases rather than decreases in aggression levels (as reported in preceding paragraphs) after activation of the neurons. It is difficult to compare these results with the aforementioned studies, however, because a different Npf-Gal4 driver was used, different proteins were expressed, group-reared flies were used that could only show enhanced levels of aggression, and different behavioral assays and methods of quantifying of results were used. As with NPF, TKRPs are expressed in FRU^M^ positive and FRU^M^-negative populations of neurons and the FRU^M^-positive populations appear to be the ones important in the regulation of aggression. Deletion mutants were generated within the Tk gene locus (∆Tk1 and ∆Tk2), and when these were examined as either homozygotes or transheterozygotes in isolated male flies (aggression levels are normally elevated in these male flies) significant decreases but not elimination of lunging behavior were found. In addition when the transheterozygote males, which supposedly have no Tk peptides in the populations of neurons concerned with the aggression effects, were used in conjunction with the Tk1-Gal4 line driving TrpA1 expression, a reduction but not elimination was seen in the increased lunging behavior induced by temperature increases. These results led the authors to suggest that the residual enhanced lunging behavior in deletion mutants might result from classical transmitters still present in the involved neurons after elimination of the peptides. Experiments examining the effects of elimination of tachykinin receptors yielded confusing results. A homozygous deletion mutant of the NKD receptor gene reduced the aggression promoting effects of Tk-Gal4/UAS-TrpA1 driven enhanced aggression, supporting the suggestion that neurons releasing the DTKR^6^ peptide that selectively activates NKD receptors (Birse et al., 2006; Poels et al., 2009) might be involved in the modulation of aggression. However, rescue experiments for the NKD receptor only partially restored the Tk1-Gal4/UAS-TrpA1 enhanced aggression levels. Moreover, the same receptor mutant line had no effect on baseline aggression. An insertional mutant line for the DTKR receptor had no effect on Tk-Gal4 driven activation of the neurons or on endogenous aggression levels. The authors suggested that the receptor distribution of the two receptor types might account for these differences. Because the receptor distribution of NKD and DTKR receptors reportedly are mutually exclusive, this is a possibility. Clearly further experiments will be required to clear up some of these findings. No studies were reported aimed at identification of the DTKR-peptide or peptides involved in the aggression phenotype or on using antagonists of TK receptors like Spantides I, II, or III to see if these confirm the results of the genetic experiments.

**Nutrition, peptides, and aggression.** A rapidly growing recent literature has implicated the insulin-like peptides (ILPs) and the nutritional state of animals in the modulation of aggression. One of these articles has suggested that the quality and quantity of food available to male flies can influence aggression levels (Lim, Eijolfsdottir, Shin, Perona, & Anderson, 2014). The food effects might be mediated by the insulin-like peptide producing neurons in flies that are important contributors to the regulation of food intake and metabolism in flies, and that recently have been reported to contribute to aggression levels in male *Drosophila* as well (Luo, Lushchak, Goergen, Williams, & Nassel, 2014; Nassel, Liu, & Luo, 2015; Nassel & Williams, 2014). Of particular interest in this regard are a group of 14 neurons that are included within the median neurosecretory cell (MNC) group and that release *Drosophila* insulin-like peptides 2, 3 and 5 into the general circulation. A subgroup of these MNC neurons also secrete the *Drosophila* cholecystokin-in-like satiation hormone Drosulfakinin that has been suggested to have direct effects on hyperactivity and aggression (Nassel et al., 2015; Nassel & Williams, 2014; Williams, Goergen, Phad, Fredriksson, & Schioth, 2014). The regulation of peptide release from the MCNs is complex and includes additional peptides from the fat bodies and other neurosecretory structures, GABA, 5HT, and OA (for review, see (Nassel et al., 2015). Less complete reports have suggested that other peptides influence...
levels of aggression in *Drosophila*, including unidentified peptides whose synthesis or release from neurosecretory neurons in the brain is governed by tailless and Atrophin (Davis, Thomas, Nomie, Huang, & Dierick, 2014). Finally, certain endosymbiotic gut microbes of the Wolbachia family also have been demonstrated to contribute to various social behaviors in *Drosophila*, including aggression, by influencing the biosynthesis of OA and its biosynthetic enzymes (Rohrscheib et al., 2015).

As these studies continue, it will not be surprising to find other peptides and peptide families that influence levels of aggression, and that peptide and amine systems likely interact in complex ways to exert these influences. In this context, it is instructive to survey the literature on the modulation of the stomatogastric ganglion of decapod crustaceans (Hamood & Marder, 2014; Marder, O’Leary, & Shruti, 2014). This 30-neuron network governs the behavior of the stomach as it processes different kinds of foodstuffs by using large numbers of extrinsic (hormones, neurohormones, and synaptic contacts) and intrinsic (regulation and distribution of ion channels and receptors) elements interacting smoothly to ensure readout of a wide variety of different motor outputs from the ganglion.

**Pheromones**

*Drosophila* males normally only attack other males; therefore, displays of aggression depend on sex-recognition. In flies as in other species, this is mainly via chemosensory communication, mediated by the olfactory and gustatory systems. Volatile and semivolatile pheromones, perceived via olfactory signaling, are believed to be detected first since they act at a distance, whereas nonvolatile contact pheromones, perceived via gustatory signaling, are likely second as they require animals to touch or taste each other (Amrein & Thorne, 2005; Touhara & Vosshall, 2009). Visual and mechanosensory signals also are likely to contribute to the recognition of the presence of a conspecific, but it is less certain whether they can be used to identify the sex of an intruder. A comprehensive review of the roles of cuticular hydrocarbons in aggression and courtship has been published recently (Fernandez & Kravitz, 2013), therefore only several highlights of this topic are presented in this article.

Fights between male fruit flies typically start with low intensity interactions, like *fencing*, during which flies extend legs and contact each other. This step likely involves detection of specific cuticular hydrocarbons (CHs) or related substances that act as contact pheromones by gustatory sensilla on the legs, wings, or labium (Amrein & Thorne, 2005; Billette & Levine, 2012; Wicker-Thomas, Guenachi, & Keita, 2009). CHs are complex mixtures of long chain hydrocarbons synthesized by epidermal cells called oenocytes and transferred to the cuticle by a still poorly understood mechanism (Billette, Atallah, Krupp, Millar, & Levine, 2009; Ferveur, 1997, 2005; Jallon, Kunesch, Bricard, & Pennanche`r, 1997; Krupp et al., 2008; Wicker-Thomas et al., 2009). Subsets of CHs act as pheromones carrying information between flies: for example, dienes within the CH profile are mainly produced by females and act as aphrodisiacs, activating male courtship, and monoenes are produced by males and mainly inhibit their courtship by other males but also may trigger aggression (Amrein, 2004; Antony, Davis, Carlson, Pechine, & Jallon, 1985; Antony & Jallon, 1982; Arienti, Antony, Wicker-Thomas, Delbecque, & Jallon, 2010; Ebbs & Amrein, 2007; Foley, Che-noweth, Nuzhdin, & Blows, 2007; Jallon, 1984; Tram & Wolfner, 1998; Wicker-Thomas, 2007).

The pheromonal profiles of male and female flies can be changed to those normally found on surfaces of the opposite sex by genetic means or by a combination of genetic and chemical manipulations. For example, males can be generated that display female CHs; such flies are courted by other males (cf. Ferveur et al., 1997). To determine whether male CH profiles can trigger aggression, oenocytes were masculinized in an otherwise female fly via expression of an RNAi transgene for the sex determination gene transformer (Fernandez et al., 2010). These females displayed pheromonal profiles similar to wild type males showing high levels of monoenes and low levels of dienes. In other respects (surface markings, behavior) they were female. When such mutant females were paired with wild type males in fight arenas, males lunged at the females, suggesting that the presence of male CHs was sufficient to trigger male aggression. It is surprising to note that, however, males also attacked females with female pheromonal profiles but with a masculinized central nervous system, indicating that behavioral cues displayed by the females can override the chemical cues provided by the same females (Fernandez et al., 2010).

Flies without surface CHs also can be generated by destroying oenocytes using the proapoptotic gene (Billette et al., 2009). These “blank”, oenocyte-less females, are courted by males of their own and sibling species (Billette et al., 2009; Chertemps, Duportet, Labeur, Ueyama, & Wicker-Thomas, 2006), indicating that CHs act as a “tag” that allows males to identify conspecific females. “Blank” males are vigorously courted by wild type *D. melanogaster* males but also are attacked by them, although at reduced intensity (Fernandez et al., 2010). When oenocyte-less males are perfumed with male CH extracts, the normal aggression levels elicited from wild type males are restored (Wang et al., 2011). Blank males perfumed with the male pheromone 7-tricosene (7-T) have reduced courtship and increased aggression directed toward them from wild type males. This effect is suggested to be mediated by sensory neurons expressing the gustatory receptor Gr32a (Wang et al., 2011), a receptor subtype that had been shown previously to be involved in sensing 7-T (Lacaille et al., 2007). The same receptor has been reported to play a role in suppression of male-male courtship in experiments in which mutant males were courting immobilized wild-type males (Miyamoto & Amrein, 2008).

Much of the focus on olfactory pheromonal cues used by flies in social behavior (in courtship and in aggression) has been on the molecule cis-Vaccenyl acetate (cVA). Originally isolated over 40 years ago this substance has been reported to function as an antiaphrodisiac, an aggregation factor and most recently as an enhancer of aggression in groups of males (Bartelt, Schaner, & Jackson, 1985; J. M. Jallon, Antony, & Benamar, 1981; Wang & Anderson, 2010). In contrast to the cuticular hydrocarbons, cVA, and more recently CH503 (a second longer lasting antiaphrodisiac (Yew et al., 2009) are found in the male ejaculatory bulb and not in the oenocytes and are transferred to females during copulation resulting in a reduction in mated female attractiveness to other males (Brieger & Butterworth, 1970; Butterworth, 1969; Datta et al., 2008; Ejima et al., 2007; Guiraudie-Capraz, Pho, & Jallon, 2007; Jallon, 1984; Kurtovic, Widmer, & Dickson, 2007; Mane,
When synthetic cVA is added to chambers in aggression assays between pairs of males, levels of aggression are increased, an effect that appears to be mediated by the olfactory receptor Or67d (Wang & Anderson, 2010). In these assays, the male flies used were group reared, which lowers the aggressiveness of the flies compared to socially isolated naïve flies. A possible caveat is that hierarchical relationships already have been established among these males (Yurkovic et al., 2006). A more complicated scenario was proposed recently with the suggestion that acute application of cVA acting via Or67d might facilitate aggression while chronic application of cVA acting via Or65a receptors might decrease aggression (Liú et al., 2011). The experimental protocols and conditions, however, are so different in these two sets of experiments that it is extremely difficult to compare them. A hierarchical relationship in the aggressive responsiveness to olfactory and gustatory signals has been proposed (Wang et al., 2011) on the basis of the observation that exposure to cVA does not increase aggression in gustatory receptor Gr32a/- mutants. It remains to be determined whether cVA is sufficient to elicit or suppress male aggression, but the observation that males attack mutant females who do not synthesize cVA, suggests that this compound is not necessary for male aggression (Fernandez et al., 2010).

Summary and Conclusion

Aggression is essential for the survival and propagation of animals in the wild. Therefore it is not surprising that many substances and situations influence the behavior. These include genes and developmental factors, nutritional state of the animal, recognition of potential opponents, prior and present social experiences, hormonal and neurohormonal influences on involved circuits, and then, a complex interaction between all of these in making what appears on the surface to be simple decisions of whether or not to fight an opponent, when to switch to different intensity levels during fights, and when and if to give up in a fight. There are few, if any, single elements that control aggression as suggested in various articles, but there are myriad elements that “influence” the behavior. How then should one proceed in further exploring the roots of aggression by use of this excellent genetically tractable model organism? Following are a few possible directions in which studies with the *Drosophila* model might move.

As first steps, it seems important for investigators to attempt to standardize the conditions used to quantify the behavior. This certainly would allow for better comparisons of experimental results between laboratories. Thus, elements should be considered like the genotype and behavioral experience of the flies, how flies are introduced to fight arenas, the size and shapes of arenas, whether spaces are available for losers to retreat to, and how and for how long fights are scored. It also seems important to avoid the use of indices alone to quantify the behavior, to note whether hierarchical relationships have been formed and to provide information about fight dynamics in analyses of the behavior.

Standard binary systems in *Drosophila* (Gal4, LexA, the “Q” system) are little better than pharmacological approaches that are used to study behavior in all species, as they tend to manipulate entire populations of, for example, neurohormonal neurons. Hence these approaches often generate complicated phenotypes. A great advantage of the *Drosophila* model is that methods like intersectional genetics, split Gal4 or LexA, and MARCM methods can be used to bring the study of behavior down to single neuron levels. The first two of those methods allow readily reproducible manipulation of neurons down to single cell levels and allow definition of the precise function of those neurons in behaving animals. When combined with methods that allow labeling of synaptic inputs and outputs to those neurons using the GFP reconstitution across synaptic partners (GRASP) technique and other methods like immunocytochemistry and the more recent optogenetic procedures, circuitry can be worked out entirely at identified neuron levels.

In the future, increased usage of single neuron technology for molecular analysis should allow exploration, using RNA-seq and other analytic technologies that have now been brought to single neuron levels, to search for molecular differences that might explain functional differences between neurons. In the more distant future, it might be possible to use optogenetic techniques in combination with intersectional genetic studies to introduce molecular species to monitor neuronal activity in single identified neurons in awake behaving animals. This should be possible to do now with the transparent larvae of flies, but an ultimate goal would be to explore the possibility of doing such studies in adult flies.

The *Drosophila* model of aggression is an important and powerful one. The behavior is quantifiable and the behavioral patterns observed fall readily into the low, medium and high aggression patterns seen in other species. Decision-making results from male aggression and loser flies show dramatically altered behavioral patterns while a lesser effect is seen with winners. Perhaps the most important advantage that *Drosophila* has over other species for the study of complex behaviors like aggression is the speed, ease and low cost of genetic manipulation of the behavior. An ever-expanding wealth of genetic tools are available that allow investigators to: bring the analysis of behavior to identified neuron levels; elaborate the circuitry through which those neurons function; and that ultimately, should allow insight into how those circuits function to make the myriad decisions that animals must compute and act upon in an instant for their survival.

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Received February 19, 2015
Revision received July 7, 2015
Accepted July 7, 2015

Correction to Aydin, Frohmader, and Akil (2015)

In the article “Revealing a Latent Variable: Individual Differences in Affective Response to Repeated Injections,” by Cigdem Aydin, Karla Frohmader, and Huda Akil (Behavioral Neuroscience, Advance online publication. July 20, 2015. http://dx.doi.org/10.1016/j.bneu.20000884), there was an error in the abstract. The sentence “However, injections significantly increased time spent immobile in the forced swim test in LRs, while the identical regimen significantly decreased the same measure in HRS, compared with handled-controls.” should be “However, injections significantly increased time spent immobile in the forced swim test in HRS, while the identical regimen significantly decreased the same measure in LRS, compared with handled-controls.” All versions of this article have been corrected.

http://dx.doi.org/10.1037/bne0000099