Research report

Heightened serotonin influences contest outcome and enhances expression of high-intensity aggressive behaviors

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HIGHLIGHTS

- We pharmacologically altered brain 5-HT levels using the precursor 5-HTP.
- We found heightened 5-HT increased the probability of winning aggressive contests.
- High-intensity behaviors and retreat threshold are influenced by 5-HT.
- Other biogenic amines including octopamine remained unaltered following fights.

ARTICLE INFO

ABSTRACT

The outcome of behavioral interactions between organisms can have significant fitness implications. Therefore, it is of great theoretical and practical importance to understand the mechanisms that modify different agonistic behaviors. Changes in central monoamines, such as serotonin (5-HT), contribute to modifying the expression of aggressive encounters in both vertebrates and invertebrates. In several invertebrate groups, neural 5-HT has been linked to heightened aggression and conflict escalation. The male stalk-eyed fly (*Teleopsis dalmanni*) competes with conspecifics daily over access to resources such as food and mates. Because encounters escalate in a stereotypical manner, stalk-eyed flies provide an excellent model system to study behavioral syndromes. We hypothesized that noninvasive, pharmacological augmentation of brain 5-HT by administration of the precursor, 5-hydroxytryptophan (5-HTP), would increase stereotypic behavioral escalation and the probability of winning a conflict over food. Size-matched male 5-HTP-treated and untreated flies were placed in a forced-fight paradigm and their aggressive behaviors scored. Individuals with higher brain 5-HT levels had a markedly higher probability of winning the contests, displayed greater levels of high-intensity aggressive behaviors and fewer retreats. Pretreatment with 5-HTP did not significantly alter octopamine or tyramine, suggesting that central 5-HT may modulate aggression in these organisms and play a role in determining reproductive success and resource attainment.

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1. Introduction

The expression of aggressive behaviors in animals is modulated and shaped by both ultimate and proximate factors [1]. Since aggression plays a vital role in the acquisition and defense of limited resources such as mates, territories, and food, the expression of aggressive behavior has clear fitness implications. For example, field crickets defending a shelter fight more fiercely and accordingly defeat intruders at a higher frequency, providing a valuable asset for mate attraction [2]. However, despite its importance, we know relatively little regarding underlying physiological and neural mechanisms of conflict resolution in insect populations.

Biogenic amines play a critical role in mediating a variety of behaviors, such as circadian rhythms, learning and memory, and social interactions, in both vertebrate and invertebrate species [3–8]. Serotonin (5-HT), in particular, has been implicated in the modulation of aggressive behaviors and is abundant across a wide range of taxa [9–12], including dipteran flies [13]. Furthermore, similarities in serotonergic neurophysiological mechanisms, including calcium-dependent release and activation of second messenger systems, 5-HT receptor subtypes, and

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synthesis from tryptophan, are shared in vertebrates and insects [7,14].

The stalk-eyed fly *Teleopsis dalmanni* (Diptera; Diopsidae) represents an exciting model for investigating proximate mechanisms underlying agonistic behaviors, because males of this sexually-dimorphic species use their eye stalks as critical aggressive signals in multiple contexts crucial for fitness [15]. Male flies aggressively defend both diurnal feeding sites [16,17] and nocturnal roosting sites that allow mating access to females [18–20]. Larger males with broader eye spans exclude smaller rivals and thus gain access to resources such as food and mates [21–23]. Agonistic encounters between males follow a stereotyped escalated progression that is terminated when one of the rivals capitulates and departs [24,25]. This predictable behavioral pattern provides a model in which any behavioral deviation can be easily scored and analyzed.

The conserved molecular and physiological processes involving monoamines that generate these complex behaviors in invertebrates and vertebrates [7], combined with the well characterized aggressive behavioral pattern exhibited in stalk-eyed flies provides a unique model system to study the potential roles of monoamines in modulating fundamental biological processes underlying aggression, escalation, and conflict resolution in insects [e.g. 26]. In order to investigate these proximate mechanisms in stalk-eyed flies, we developed a monoamine detection and quantification method that allows for non-invasive pharmacological manipulations of 5-HT alone, while simultaneously monitoring other biogenic amines and their metabolites in a single whole brain sample [27]. In this experiment, we tested the hypothesis that elevated 5-HT would alter the probability of winning in a fight. Furthermore, we investigated correlations between elevated 5-HT and specific behaviors to determine which behaviors ultimately dictated the outcome of the fights. We first treated flies with the 5-HT precursor, 5-hydroxytrptophan (5-HTP) to increase neural 5-HT concentrations, placed them in a forced-fight paradigm, and scored the behavioral interactions. We then measured whole brain monoamine levels using high performance liquid chromatography with electrochemical detection. The results of our study suggest that individuals with higher 5-HT have a significantly higher frequency of winning an aggressive interaction. Furthermore, activation of central serotonergic activity may contribute to the expression of high-intensity aggressive behaviors and decreased probability of retreat.

2. Methods and materials

2.1. Subjects

The study species used in the experiment is a sexually dimorphic stalk-eyed fly native to Malaysia (*T. dalmanni*). All individuals are descendants of pupae obtained from Dr. Gerald Wilkinson at the University of Maryland – College Park and are housed communally (~100) in cages (45 cm × 22 cm × 19 cm) with free access to food and water on a 12-h light/dark cycle between 22 and 24 °C at ~85% humidity [28]. Each cage contained string attached to the roof, simulating rootlets to encourage natural reproductive and competitive behavior. Males used in this study were at least 3 weeks post-occlusion but less than 2 months to avoid possible aging effects. Individuals were briefly anesthetized using CO$_2$ and photographed resting on their thoracic spines. Eye span was measured to the nearest 0.01 mm using Scion Image (National Institutes of Health; following [29]). The high correlation between eye span and body length makes eye span an accurate representation of body size [28,30]. Based on these measurements, we size-matched individuals to within 1% eye span difference and gave each an identifiable marking using an opaque paint pen on their thorax (mean size, 8.23 mm; range, 7.32–8.82 mm; mean difference, 0.02 mm; difference range, 0–0.11 mm [20,25]. Males were then transferred to smaller cages (14 cm × 14 cm × 14 cm) and housed with their respective treatment group (10 individuals/cage). A large food dish was placed in these smaller cages to avoid competition between the subjects over access.

2.2. Drug administration

For 4 days, flies were fed sterilized and pureed whole ear sweet corn, containing either 3 g 5-hydroxy-L-tryptophan (5-HTP)/100 mL media or vehicle/100 mL media. Specifically, control flies had a food media (100 mL) containing 1 mL methylparaben [28] as a mold inhibitor, 25 mg of ascorbic acid and a drop of food coloring to ensure sufficient mixing. Treated individuals had an additional 3 g of 5-hydroxy-L-tryptophan (H9772; Sigma, St. Louis, MO) added to the food mixture along with 25 mg of ascorbic acid to act as a stabilizer [13].

2.3. Forced-fight paradigm

After the 4 day feeding period, size-matched pairs were transferred to a fighting arena (11 cm × 6.5 cm × 5 cm) containing moist filter paper as a floor and a removable central barrier separating the individuals. The arena consisted of 3 wooden sides painted white and a forward facing glass wall for increased lighting. The top consisted of a removable piece of glass through which individuals were introduced into the chamber and video of the interaction was recorded. The two flies remained separated in the arena for a starvation period of 12 h. This phase was to encourage aggressive behavior once the barrier was removed and a single drop of corn (~4 mm in diameter) was dropped in the center of the arena. In an earlier study, we found that increased brain 5-HT concentrations were maintained in flies pretreated with a food mixture containing 3 g of 5-HTP for at least 12 h of starvation [27]. After 10 min of behavioral interactions, flies were aspirated out of the arena and their brains were rapidly dissected and stored as described below.

2.4. Dissection and sample preparation

Immediately after the removal from the fighting arena with an aspirator, flies were anesthetized using CO$_2$ and the heads were removed near the frontal part of the neck using micro-scissors under 40× magnification. Eye stalks, which contain photopigments, tissue, and nerve terminals [31] that could interfere with data acquisition, were removed ¾ of the way down; the other ¼ remained attached to the head. Previous trials conducted indicated removal of the eye stalks in this fashion resulted in much cleaner chromatographic samples while not compromising any desired monoamine concentrations from the CNS [27]. To avoid possible contamination by trapped 5-HTP treated food or other particles from entering the samples, the mouthparts were completely removed from the head. To expose the brain, micro-tweezers were inserted into the cavity, which once held the mouthparts, to split the exoskeleton. The exposed brain and remaining exoskeleton was placed in 60 μL acetate buffer containing the internal standard α-methyl-dopamine (Merck) and stored at −80 °C. Pilot studies, consistent with earlier work done in Drosophila [32], indicated that the exoskeleton in stalk-eyed flies contributes to the high levels of dopamine in the samples. Thus, dopamine was not quantified in the current study. However, a comparison of raw 5-HT, octopamine (OA) and tyramine (TA) concentrations obtained from partial exoskeleton and whole brain preparations showed no differences in values [27].

Table 1
Ethogram of behaviors exhibited by stalk-eyed flies associated with competition over a food resource.

Behavior	Description
Low-intensity	
Approach	Movement toward opponent
Line up eye stalks	Lining of eye stalks in a parallel manner
Flex forelegs	Raises and waves forelegs at opponent, no contact
High-intensity	
Jump attack	Jumps on opponents dorsal side
Strike/lunge	Swipes forelegs down on opponent making contact
Conflict resolution	
Away	Slowly turning or moving away from opponent
Pursue	Rapidly moving after opponent while no longer aligned
Retreat	Rapidly moving away from opponent while no longer aligned
End	Eye stalks no longer aligned for minimum of 3 s

(modified from [24]).

2.5. Data acquisition

Octopamine (OA), 5-hydroxytryptophan (5-HTP), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), tyramine (TA), and serotonin (5-HT) in a single whole brain sample were detected by high performance liquid chromatography with electrochemical detection as previously described [27]. After the brain samples were thawed and centrifuged at 17,000 rpm, the supernatant was removed and 45 μL of the sample was injected into the chromatographic system (Waters Associates, Inc., Milford, MA, USA). The amines were separated with a C_{18} 4 μm NOVA-PAK radial compression column (Waters Associates, Inc. Milford, MA) and detected using an LC 4 potentiostat and a glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN). The sensitivity was set at either 0.5 or 1 n/V with an applied potential of +0.9 V vs a Ag/AgCl reference electrode. The mobile phase initially was made by dissolving 8.6 g sodium acetate, 250 mg EDTA, 11 g citric acid, 330 mg octanylsulfonic acid, and 160 mL of methanol (all chemicals were obtained from Sigma–Aldrich, St. Louis, MO) in 1 L of distilled water. In order to obtain the desired separation, additional increments of octanylsulfonic acid (10 mg) and methanol (5 mL) were added to the mobile phase. Following removal of the supernatant for monoamine analysis, 60 μL of 0.4 M NaOH was added to the pellet to solubilize the remaining tissue for protein analysis [33]. The CSW32 data program (DataApex Ltd., Czech Republic), set in internal standard mode, calculated monoamine and their metabolite concentrations based on peak height values obtained from standards (all standards were obtained from Sigma–Aldrich, St. Louis, MO). The resulting amine concentrations were divided by μg protein in the sample to yield pg amine/μg protein after multiplying pg amine × 1.33 to account for the difference between injection volume (45 μL) and the preparation volume (60 μL).

2.6. Behavioral analysis

We scored behavior based on a previously developed ethogram [24]. Behaviors included in the ethogram were only those that occurred during aggressive interactions (Table 1); those that occurred as a result of other interactions were excluded. In addition, the list of behaviors included in the analysis was exhaustive and mutually exclusive, meaning every behavior performed during an aggressive interaction was accounted for with only one occurring at a specific time. The list of behaviors was broken into three categories: low-intensity behaviors, high-intensity behaviors, and conflict resolution behaviors. Each 10 min bout contained numerous aggressive interactions, or contests, initiated by an individual either approaching the other

Table 2
Brain monoamine concentrations (± SEM) for stalk-eyed flies that won or lost a contest over a food resource (pg/μg protein).

	Winners	Losers
Octopamine	380 ± 65.8 (*n* = 9)	413.2 ± 55.7 (*n* = 11)
Tyramine	25.2 ± 4.6 (*n* = 6)	21.5 ± 3.9 (*n* = 9)
5-Hydroxtryptophan	145.2 ± 37.4 (*n* = 20)	102.1 ± 49.3 (*n* = 20)

or a mutual lining up of eye stalks in a parallel manner. Within these interactions, flies will either engage in low-intensity behaviors where contact is not made with the opponent and/or high-intensity behaviors where physical contact is present. The interactions were terminated by an individual either moving away slowly, retreating, pursuing, or becoming unaligned with their opponent for a minimum of 3 s. Contests were scored using the behavior analysis program JWatcher [34]. Each fly's behavior was blindly scored, independently of its opponent, such that each fight was scored twice, once for each participant. Because each 10 min trial consisted of multiple individual contests, the overall winners and losers for each trial was determined by the sum of retreats across all of the contests within each trial. The individual with more retreats than its opponent was labeled the trial loser. Conversely, the individual with fewer retreats was designated the trial winner [17,24].

2.7. Statistical analysis

Differences in mean monoamine concentrations between single comparisons were analyzed using a two-tailed Student's *t* test. A Chi^2-test was used to examine the difference in winning frequencies between individuals with higher brain 5-HT levels and their size-matched counterpart with lower 5-HT as well as to test for differences in behavioral output between individuals with higher 5-HT than their opponent and individuals with lower 5-HT than their opponent. To test for differences in high-intensity behaviors between winners and losers, a two-tailed contingency table was constructed followed by a Fischer's exact test. A nonlinear regression model was calculated for the relationship between 5-HT concentrations and number of retreats as well as to assess total high-intensity behaviors and differences in 5-HT concentrations among opponents. For all tests, statistical significance was set at $\alpha = 0.05$.

3. Results

Oral administration of 5-HTP, the precursor of 5-HT, significantly increased the mean brain 5-HT concentration in the flies (Student's *t* test, $p < 0.001$). In contrast, pretreatment with 5-HTP did not significantly affect the concentrations of the other amines detected in the assay: OA and TA (Table 2). Dopamine concentrations are not presented due to the large contribution from the exoskeleton, resulting in an artificially large concentration. Conversely, 5-HIAA concentrations were very low and undetectable in most of the samples. Out of a total of 20 fights, 14 of the winners were in the 5-HTP-treated group (Chi^2-test, $p = 0.0736$). Previously, we reported that 85% of the winners of a contest had higher brain 5-HT concentrations regardless of pretreatment [27], suggesting the possibility that in some of the 5-HTP-treated flies, 5-HT may have decreased to control levels. Consistent with that finding, analysis of fight outcome and brain 5-HT concentrations show that winners had higher mean brain 5-HT concentrations when compared to losers regardless of pretreatment (Fig. 1, Student's *t* test, $p < 0.01$).
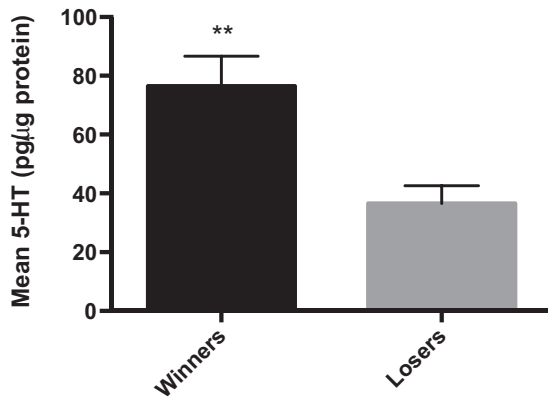
Fig. 1. Stalk-eyed flies that won a competition for a food resource (dark bar) had higher brain 5-HT concentrations than flies that lost the contest (light bar). Values are expressed as mean brain 5-HT concentrations (pg 5-HT/μg protein $\pm$ SEM; Student's t-test $p < 0.002$).
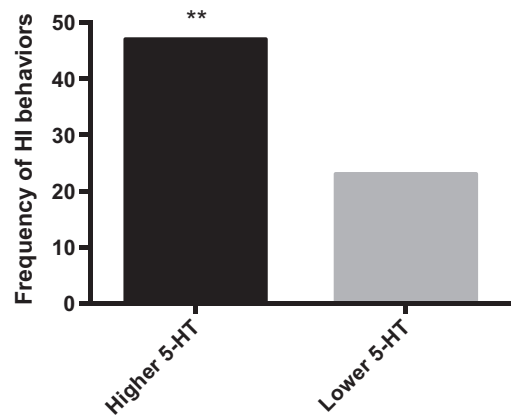

Fig. 2. Frequency of high-intensity behaviors were greater in stalk-eyed flies with higher brain 5-HT than their opponent (Chi^2-test $p < 0.005$).

The frequency of swipe/lunge and jump attack high-intensity behaviors significantly differed with 16 winners performing the behaviors compared to 5 losers (Table 3, 2-Tailed Contingency Table, Fisher's exact test, $p < 0.001$). Furthermore, the 5 losers that engaged in these behaviors performed them fewer times, with the exception of one tie, than their opponent who ultimately won the bout. Out of 70 total combined high-intensity behaviors performed in the 20 fights, 50 of them were conducted by the winners (Table 3). The high-intensity behaviors are broken down into 2 specific actions: swipe/lunge and jump attack. The swipe/lunge attacks comprised 36 of the total 50 high-intensity behaviors performed by the winners, which was more than double the frequency of swipe/lunge attacks exhibited by the losers (Table 3, Chi^2-test, $p < 0.01$). The jump attack was much more infrequent contributing only 17 of the total 70 high-intensity behaviors performed by both winners and losers (Table 3). Of these 17 jump attacks, 14 of the attacks were performed by the winners (Table 3, Chi^2-test, $p < 0.01$). In total, 11 of the 20 winners performed the jump attack while only 2 losers exhibited this high-intensity behavior (Table 3, 2-Tailed Contingency Table, Fisher's exact test, $p < 0.01$). Furthermore, we found that individuals with higher brain 5-HT than their opponent performed these high-intensity behaviors at a much higher frequency (Fig. 2, Chi^2-test, $p < 0.005$).

Table 3

High-intensity behaviors exhibited by stalk-eyed flies that either won or lost a contest over a food resource. (A) Number of individual winners and losers performing high-intensity behaviors (swipe/lunge and jump attack) ($n = 20$ fights). (B) Frequency of total behaviors performed in 20 fights.

Individuals performing	Winners	Losers
A		
High-intensity[a]	16	5
No high-intensity[a]	4	15
Swipe/lunge[b]	11	4
No swipe/lunge[b]	9	16
Jump attack[c]	11	2
No jump attack[c]	9	18
B		
Frequency of behaviors	Winners	Losers
High-intensity[***]	50	20
Swipe/lunge[**]	36	17
Jump attack[**]	14	3

[a] 2-Tailed contingency table, Fischer's exact test, $p < 0.01$.
[b] 2-Tailed contingency table, Fischer's exact test, $p < 0.05$.
[c] 2-Tailed contingency table, Fischer's exact test, $p < 0.01$.
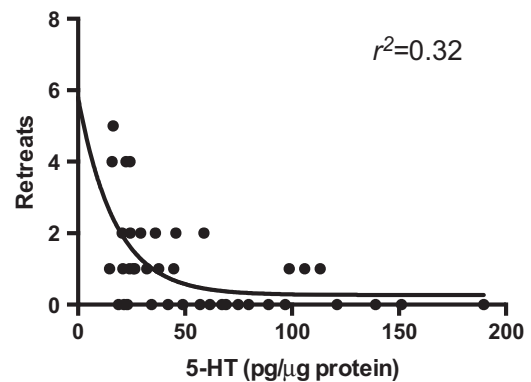[**] Chi^2-test, $p < 0.01$.
[***] Chi^2-test, $p < 0.001$.

Fig. 3. Frequency of retreats decreases as brain 5-HT concentrations increase in individual stalk-eyed flies ($p < 0.005$; nonlinear, exponential decay).

A significant negative curvilinear regression was found between number of retreats and total brain 5-HT concentrations (Fig. 3, $p < 0.005$). Thus, the higher the brain 5-HT concentration, the fewer the number of retreats an individual performed. Additionally, we observed a negative relationship between pairs who engaged in high-intensity behaviors and their mean 5-HT difference (Fig. 4, $p < 0.05$). In other words, pairs with closer 5-HT concentrations have
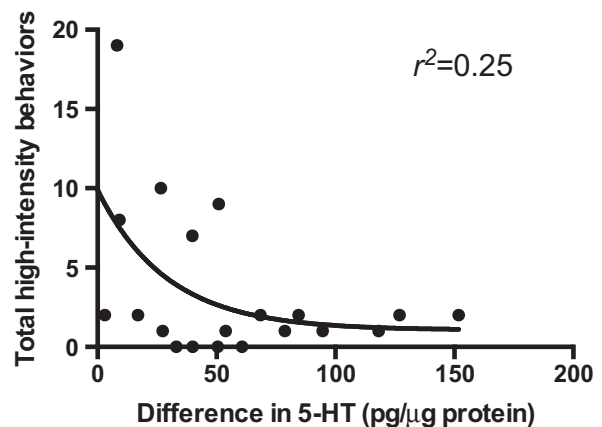

Fig. 4. Total number of high-intensity behaviors in each trial decreases as the mean difference in 5-HT between the respective paired opponents increases ($p < 0.05$; nonlinear, exponential decay).

a tendency to engage in more high-intensity behaviors during a competition than pairs with a much wider 5-HT difference.

4. Discussion

Our results suggest that high concentrations of brain 5-HT in the stalk-eyed fly have an enhancing effect on their expression of aggressive behaviors and the probability of winning a competitive bout. Using a non-invasive pharmacological manipulation of 5-HT by oral administration of its metabolic precursor, 5-HTP, we were able to pair opponents with disproportionate 5-HT concentrations and observe the behavioral outcome. In a forced-fight paradigm, males that have higher concentrations of brain 5-HT than their respective opponent had a markedly greater probability of winning a fight. These winners also engaged in high-intensity behaviors at a significantly greater frequency, indicating its role as an important factor in success. Our results extend the findings of an earlier study in *Drosophila* that showed that elevated 5-HT increased fighting and escalation frequency [13]. Furthermore, the detection of monoamine concentrations from a single individual and comparing it to their behavioral data provides an improved assessment of the functional link between monoamines and behavior. In contrast, we did not detect changes in any of the other amines measured when fight winners and losers were compared. Since 5-HTP and 5-HT increases are not limited to the CNS through oral administration of 5-HTP, potential side effects should be considered in response to treatment groups. However, physical impairments (i.e., locomotor activity) were not observed in our study but a more detailed evaluation on other physiological parameters such as feeding and metabolic rates were not available.

As a result of the oral ad libitum administration of 5-HTP, some individuals had 5-HT concentrations return to baseline levels suggesting the possibility that these individuals may have stopped eating and been starved for a longer period than 12 h implemented. In an earlier study, similar results were obtained from the evaluation of the time course for elevated brain 5-HT concentrations following consumption of food pretreated with 3 g of 5-HTP [27]. Although the mean 5-HT concentrations were elevated, a few of the individuals in the 5-HTP-treated group had brain 5-HT concentrations that were similar to values found in control insects [27]. Interestingly, in some instances treated flies that returned to baseline 5-HT concentrations had lower 5-HT than their untreated opponents. These treated individuals lost those fights, contributing to the 6 unsuccessful contests for the treated flies. Inclusion of these 5-HTP pretreated flies in the comparison of treated and control individuals suggested that the probability of a treated fly winning a fight was not significant. However, the finding that treated flies which lost their contest were defeated by control opponents with higher 5-HT concentrations strengthens the implication of 5-HT as a mediator of aggression and suggests that higher brain 5-HT concentrations represent a stronger predictor of contest outcome than treatment.

The design of our experiments allowed us to explore the proximate role of 5-HT in regulating specific behaviors associated with aggression and, by extension, the role of those behaviors in the outcome of these contests. Higher concentrations of brain 5-HT were not only associated with a higher probability of winning a contest, but also with differences in a variety of behaviors associated with aggression. Our analyses revealed a negative correlation between brain 5-HT concentrations and the number of retreats; flies with high 5-HT levels engaged in few, if any, retreat behaviors. Individuals with very high 5-HT concentrations would still continue to fight even when receiving high-intensity behaviors from their opponent, an action that caused flies with lower concentrations to retreat quite readily. When a high-intensity striking

event occurred, both opponents had the opportunity to exchange blows. Those with lower 5-HT would usually retreat after receiving the first strike, typically before they had the chance to return a hit. Additionally, in several cases flies with lower 5-HT would retreat in response to low-intensity behaviors such as flexing. The threshold at which an individual would begin to retreat rose as 5-HT levels increased. Increased fight duration along with a decrease in retreat frequency was found in previous studies involving crustaceans receiving 5-HT injections [9]. They found they could induce renewed fighting motivation in a subordinate individual by altering its decision to retreat while fight initiation, escalation, and outcome all remained unchanged [9]. This alteration in the decision to retreat could have contributed to the greater likelihood for flies with higher levels of 5-HT than their opponents to win the contests.

High concentrations of 5-HT were also positively associated with the expression of high intensity behaviors, striking with forelegs or jumping on opponent's dorsal side (Fig. 2). We demonstrated that individuals who had higher brain 5-HT concentrations than their opponent performed these high-intensity behaviors at a much higher frequency when compared to individuals with lower brain 5-HT concentrations than their opponent. Furthermore, the individuals who performed high-intensity behaviors more frequently had a much higher probability of winning the contests. The much more rare behavior of the two, jump attack, seemed to be the most energetically costly and was predominantly performed by the winners (over a 5-fold increase compared to losers; Table 3). Additionally, it is important to note that the losers that engaged in a high-intensity behavior fought an opponent that performed more or an equal number of these actions, suggesting that the frequency at which these behaviors are expressed may serve as a predictor for winning an aggressive bout and may be modulated by brain 5-HT activity.

Initially, we analyzed the absolute brain 5-HT concentrations of all individuals, predicting a positive linear relationship between high-intensity behavioral output and 5-HT levels. However, we found no significant relationship. We think this counterintuitive result can be explained when taking differences in brain 5-HT concentrations between opponents into account. When we re-analyzed the data using differences in brain 5-HT concentrations rather than absolute concentrations, there was a negative relationship in the total number of high-intensity behaviors performed in a fight and the difference in 5-HT between the opponents (Fig. 4). In other words, in cases where pairs were closer in brain 5-HT concentrations, both contestants, winners and losers, performed a higher total number of high-intensity behaviors during a contest. Whereas, in cases in which there was a large discrepancy between combatants in brain 5-HT concentrations, the bouts were much shorter, presumably because a dominant-subordinate relationship was determined early in the bout, resulting in a lower expression of high intensity behaviors overall, including by the winner. This result suggests that the display of high intensity behaviors escalated to higher levels when there was a smaller discrepancy in brain 5-HT concentrations between the two contestants. It is interesting to note that this pattern parallels the probability of escalation to higher-intensity behaviors when the difference in size between opponents decreases. These results suggest that contestants required additional information to assess their opponents prior to the contest being settled [24] and warrants a more detailed examination of the mechanisms leading to this pattern of escalation. For example, a study investigating the behavioral differences of aggressive interactions between size-matched flies in which both contestants were treated to elicit relatively high (e.g. 5-HTP pretreatment) and relatively low (e.g. inhibition of tryptophan hydroxylase by a drug such as 4-chloro-ʟ-phenylalanine) concentrations of 5-HT but having similar 5-HT concentration differences between opponents would address some of these questions.

Similarly, the differences in behavioral escalation could be tested under conditions that enhance brain 5-HT differences by matching flies pretreated with 5-HTP and flies pretreated with 4-chloro-L-phenylalanine. Results from a study of this nature could expand our understanding of proximate factors influencing conflict escalation and resolution.

Aggressive interactions usually result in an individual withdrawing from the contest following a step-wise escalation pattern matching the predictions of game theory [9,17,35]. Within these fights, opponents will gather information about the other based on physical size or fighting ability. When size discrepancies are small, behaviors will escalate to higher levels along with increased duration, in accordance with the sequential assessment model [25,35]. If the decision to withdraw from the bout is a limiting factor in ending the fights, then an element possessing the ability to alter this decision can have profound fitness implications. Our results suggest that 5-HT influences the outcomes of an aggressive contest in stalk-eyed flies, possibly by decreasing the decision to retreat and by increasing the frequency of performing high-intensity behaviors. The data suggest an overall increase in the escalation rate of behaviors for individuals with higher brain concentrations of 5-HT.

Recently, studies have found OA to also be an important factor in heightened aggression in *Drosophila* [36] and the fight or flight response for a number of insects [reviewed in [37]]. However, we did not find any relationship between aggressive behavior and brain OA concentrations among any of the fighters or as a response to altering 5-HT by pretreatment with 5-HTP. Additionally, concentrations of TA were also stable and equivalent between treated/untreated and winner/loser groups.

5. Conclusion

In summary, our results are consistent with previous work that suggests that 5-HT plays an important role in mediating aggressive behavior across a variety of species [9–13]. Our results in stalk-eyed flies agree closely to previous work involving *Drosophila* [13], which also demonstrated an increase in aggression following pharmacological elevation of 5-HT. Furthermore, our results suggest that in stalk-eyed flies, higher central serotonergic activity may be related to the expression of high-intensity behaviors and the decision to retreat that predicts the winner of a contest.

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