

Serotonin precursor (5-hydroxytryptophan) causes substantial changes in the fighting behavior of male crickets, *Gryllus bimaculatus*

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Abstract This study demonstrates that injection of the serotonin precursor 5-HTP causes substantial changes in the behavioral state, fighting behavior and ability to establish winner–loser relationships in male crickets (*Gryllus bimaculatus*). The characteristic features of 5-HTP-treated crickets include an elevated posture, enhanced general activity, longer duration of fighting, enhanced rival singing and a decreased ability to produce a clear fight loser. In addition, 5-HTP-treated males showed a slightly delayed latency to spread their mandibles, a decreased number of attacks and an equal potential to win in comparison to controls (physiological solution-treated males). The obtained results imply a significant role for serotonin in the regulation of social status-related behaviors in *G. bimaculatus*. Specifically, these data indicate that a decrease in serotonergic activity may be functionally important for the control of loser behavior and that some behavioral features of dominant male crickets are likely to be connected with the activation of the serotonergic system.

Keywords Aggression · Decision-making · Social behavior · Serotonin · Insect

Introduction

The roles of two monoamines, serotonin and octopamine, in the mechanisms that control social status-dependent behavioral changes in arthropods have been investigated since the early 80's (Livingstone et al. 1980). In crustaceans, the majority of studies has been focused on the serotonergic system (Huber et al. 1997a, b; Kravitz 2000). Serotonin has been implicated in the mechanisms underlying status-related changes in animal posture (Livingstone et al. 1980; Kravitz 1988; Cattaert et al. 2010), escape behavior (Yeh et al. 1996, 1997; Edwards et al. 2002) and aggression (Edwards and Kravitz 1997; Huber et al. 1997a, b). In another large group of arthropods, insects, the role for octopamine in the regulation of social status-related aggressiveness has been well documented (Dierick 2008; Stevenson and Rillich 2012). The activation of neuronal octopamine receptors has been demonstrated to underlie the releasing effect of flight on loser aggressiveness (Stevenson et al. 2005) as well as the enhancing effects of winning (Rillich and Stevenson 2011) and shelter occupation (Rillich et al. 2011) on cricket aggression. These findings have led to the hypothesis that insects may differ significantly from crustaceans in regards to the monoaminergic mechanisms controlling aggressive behavior (Stevenson et al. 2005). The extent of these differences have remained unclear, however, as the effects of the serotonergic system in insects have not been as thoroughly studied as they have been in crustaceans.

A growing body of evidence indicates that serotonin may play a significant role in the control of aggressive behavior in insects. In *Drosophila*, a specific group of serotonergic neurons was found to be essential for decision-making during contests (Alekseyenko et al. 2010). In addition, activation of serotonin synthesis promoted

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aggression (Dierick and Greenspan 2007). In another model insect, the cricket *Gryllus bimaculatus*, a decrease in serotonin content was demonstrated in fight losers (Murakami and Itoh 2001). Pharmacologically induced serotonin depletion also reduced the probability of winning (Dyakonova et al. 1999), enhanced the escape responses (Dyakonova et al. 1999; Stevenson et al. 2000) and decreased the duration of fights in male crickets (Stevenson et al. 2000). There are no data, however, on whether activation of the serotonergic system influences aggressive interactions in crickets, and if so, how this may occur.

The immediate metabolic precursor of serotonin, 5-hydroxytryptophan (5-HTP), has been demonstrated to be effective in the activation of the serotonergic system of various invertebrates (Sakharov 1991; Dyakonova et al. 2009), including insects (Dierick and Greenspan 2007; Anstey et al. 2009). 5-HTP that is taken up by serotonergic neurons and converted to serotonin promotes serotonin release in its natural sites of release and under physiological conditions (Lynn-Bullock et al. 2004; Dyakonova et al. 2009). In *G. bimaculatus*, 5-HTP has been demonstrated to have a strong serotonin-mediated effect on the sexual behavior of males, as it decreases the duration of the refractory postcopulatory period (Ureshi et al. 2002). In the present study, we examined the effects of 5-HTP on the aggressive behavior of preisolated adult male *G. bimaculatus*. We report that 5-HTP causes substantial changes in the behavioral state and the fighting behavior of this species. Some of these changes, such as an elevated posture and a longer duration of fighting, are similar to the effects of serotonin previously observed in crustaceans. The preliminary results of this study have been published in abstract form in the proceedings of the international meeting (Dyakonova and Krushinsky 2011).

Methods

Experimental animals

Crickets (*G. bimaculatus*) were taken from the breeding colony (28° C, 12 h:12 h light:dark cycle) approximately 2–3 weeks after their imaginal molt. All crickets were isolated for at least 12 h prior to experiments.

Application of drugs

5-Hydroxytryptophan hydrochloride (5-HTP, Sigma) was dissolved in cricket standard physiological solution to yield a final concentration of 0.1 M. The physiological solution contained (in g/L): 8.2 NaCl, 0.75 KCl, 0.4 NaHCO₃, 0.45 NaH₂PO₄·2H₂O. Experimental animals received a 100 µl injection of 5-HTP into the abdominal cavity using a

micro-syringe (Hamilton, Bonaduz, Switzerland). Control animals were injected with 100 µl physiological solution only.

Recording of behavior

Experiments were directly observed and simultaneously recorded using a Sony DCR-SR220E. All behaviors were scored using an event recorder (RealTimer Version 10, <http://www.openscience.ru>). A one-character code was assigned to each of the different components of behavior studied in the experiments, and the respective key on a computer keyboard was pressed to indicate the start and end of each behavioral sequence. This code, together with a time stamp (resolution 0.1 s), was stored for subsequent analysis.

Analysis of posture and general activity

To determine whether the serotonin precursor has any effect on posture in crickets, we placed preisolated males in individual transparent arenas (10 × 10 × 10 cm) 2–2.5 h after an injection of 5-HTP or physiological solution. Using files of video records, we evaluated the maximal distance between the lowest point of the cricket abdomen and the bottom of the arena during the 5 min of observation. The time that the cricket spent with an elevated abdomen was recorded. In addition, the time that the cricket spent actively exploring the new arena was also recorded. Control and 5-HTP-treated crickets were tested in a random order, and all behaviors were analyzed by an observer blinded to the treatment condition.

Analysis of aggressive behavior

Approximately 2–2.5 h after an injection of 5-HTP or physiological solution, two males were placed in a transparent plastic container (16 × 10 × 10 cm). One cricket was placed on each side of a centrally located movable opaque wall. Animals in each pair were matched in regards to both size and weight. In one experimental series, both crickets in a pair were treated with either 5-HTP or physiological solution. In another series, fighting pairs consisted of one control and one 5-HTP-treated animal. After a short adaptation period (10–15 min), the central wall was removed and crickets were allowed to meet.

The male fight develops in a stepwise manner with the following steps (fight levels): level 0: no interaction; level 1: pre-established dominance; level 2: bilateral antennal fencing; level 3: mandible spreading by one animal in a pair; level 4: mandible spreading by both individuals; level 5: mandible engagement; level 6: physical combat (Stevenson et al. 2000, 2005). We evaluated (1) the level of fighting at which dominant–subordinate

relationships were established, (2) total duration of the fight, (3) duration of the different steps of the fight, and (4) latency and duration of rival songs.

If no aggressive interactions were observed in the first 3 min following initial contact, the level of fight was awarded a value of zero (0). The end of a fight was determined when one of the animals stopped fighting and ran away. Winner status was given to the male that sang, chased and attacked; the subordinate male responded by fleeing or jumping. After the establishment of social rank, we recorded the behavior of each pair for 2 min to ensure that the rank had been clearly established and that the loser avoided the dominant. If additional fighting occurred during these 2 min, the behavior was recorded for an additional 2 min following the end of the second fight. If fights continued to reoccur for more than 10 min of observation, the rank was considered unestablished.

In the experimental series in which 5-HTP-treated males fought against control males, the number of attacks performed by each animal and the movement of combat into the territory of the experimental or control cricket was evaluated. In the latter case, the virtual line was drawn between the two males when they stood in front of each other with open mandibles (fight level 4), and the shift of the virtual line to the territory of the control or the experimental animal at the end of the fight was noted.

Statistical analysis of data

Significant differences between experimental and control groups were tested by the multivariate test, MANOVA. Univariate tests were applied to determine specific effects. The Kruskal–Wallis ANOVA test was applied to compare the following variables: the duration of fights, the duration of the fourth stage of the fight, the latency and duration of rival singing, the level of fight and the number of attacks. The Fisher test was applied to determine the difference in the relative frequency of the following events: the established ranks, the number of wins, the number of multiple fights (>2), and the number of fights that ended in the territory of the experimental or the control animal.

Results

Posture and general activity of preisolated male crickets treated with 5-HTP

Two and a half hours after drug or vehicle injection, 8 of 12 5-HTP-treated crickets observed in individual containers exhibited an elevated posture. This posture involved an elevated thorax and abdomen that were detached from the bottom of the container. In the control group, only 3 of 12

crickets demonstrated a similar elevated pose. When placed in the new arena, 5-HTP-treated males spent significantly more time in elevated posture: 156 ± 20 s versus 53 ± 14 s, $p = 0.001$, $H = 11$, Kruskal–Wallis ANOVA test (Fig. 1a). The average maximum distance between the lowest point of the abdomen and the ground was 2.75 ± 0.3 mm in the experimental group, compared to 1.5 ± 0.3 mm in the control group ($H = 5.2$, $p = 0.02$, Kruskal–Wallis ANOVA test; Fig. 1b). In addition, 5-HTP-treated crickets spent significantly more time (179 ± 18 s) in active exploration of the arena and walking relative to the control crickets (78 ± 14 s; $p = 0.001$, $H = 10.8$, Kruskal–Wallis ANOVA test).

Fighting between 5-HTP-treated males

The multivariate analysis of variance (MANOVA) indicated highly significant differences in the parameters characterizing the agonistic behavior between 5-HTP-treated ($n = 18$ pairs) and physiological solution-treated ($n = 17$ pairs) groups (Fig. 2). The following continuous data were included in the MANOVA test: the total duration of fight, the duration of the fourth stage of the fight and the duration of and latency to initiate rival singing. Various univariate tests were applied to determine which individual behavioral parameters differed between the control and the experimental groups.

An injection of 5-HTP resulted in significantly extended fights between males (36 ± 13 s versus control 5 ± 1 s,

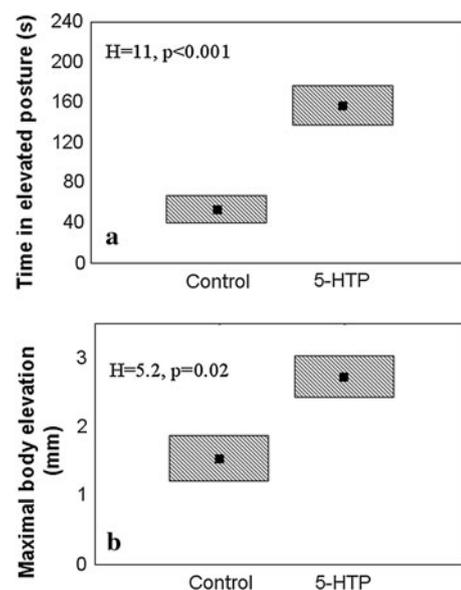


Fig. 1 The effects of 5-HTP on posture and general activity in preisolated male crickets. **a** Time (s) spent in elevated posture. **b** Maximal body elevation during the time of observation as measured by the distance between the lowest point of the abdomen and the bottom of the arena (mm). All data are presented as mean \pm standard error. H Kruskal–Wallis ANOVA test

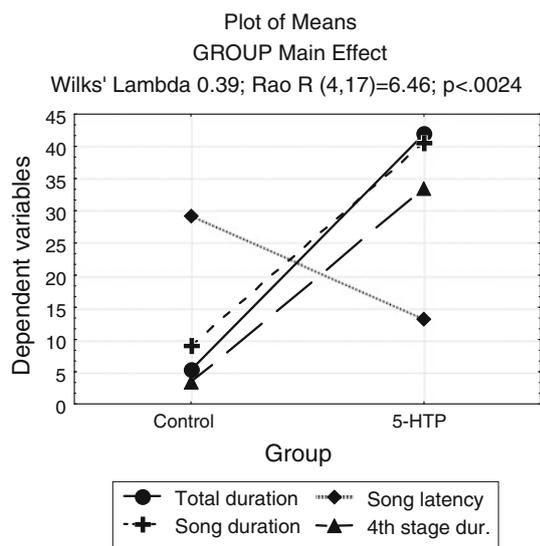


Fig. 2 The results of multivariate analysis of variance (MANOVA), plot of means (s). The following continuous data were included in the MANOVA test: the total duration of fight (marked with circles), the duration of the fourth stage of the fight (marked with triangles) and the duration of and latency to initiate rival singing (marked with crosses and rhombuses, correspondingly)

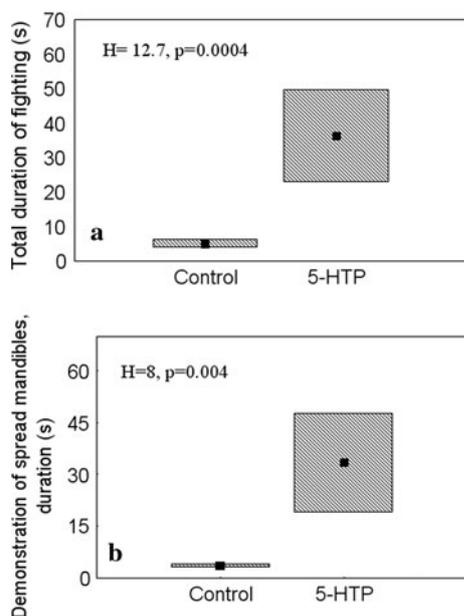


Fig. 3 The effect of 5-HTP on the duration of aggressive interactions in male crickets. **a** Total duration of fighting (s). **b** The duration (s) of stage 4 fighting, as determined by the demonstration of spread mandibles. All data are presented as mean \pm standard error. *H* Kruskal–Wallis ANOVA test

$p < 0.0005$, Fig. 3a). Multiple fights (mean = 4; greatest number in a pair = 16) were characteristic of 5-HTP-treated crickets and partially explained the increased total duration of combat. The duration of the first fight in a pair,

specifically at the fourth level (in which there was a demonstration of spread mandibles), was also significantly increased in the 5-HTP-treated group (Fig. 3b). In some experimental pairs, this demonstration of spread mandibles lasted 10–20 times longer than in the control pairs.

The intensity of aggression demonstrated by the male crickets was significantly increased by administration of 5-HTP (Fig. 4a). 5-HTP-treated pairs did not display combat at levels 0 or 1 (with a pre-established loser), whereas 12 % of control pairs displayed this low-level combat. Higher levels of aggression (5–6) also occurred more frequently in the experimental group than in the control group (76 versus 36 %, $p < 0.05$).

The probability of establishing a clear fight loser was decreased in the experimental group. In all control pairs ($n = 17$), fighting resulted in the establishment of normal winner/loser relationships. However, in 5 of 18 5-HTP-treated pairs, there was no clear loser established following multiple fights that lasted through the end of the observation period (Fig. 4b).

Demonstrative aggression was obviously enhanced in 5-HTP-treated crickets. In addition to an extended demonstration of spread mandibles (fight level 4; Fig. 3b), the total duration of rival singing was significantly higher in the 5-HTP-treated group (Fig. 5a). Although it took longer

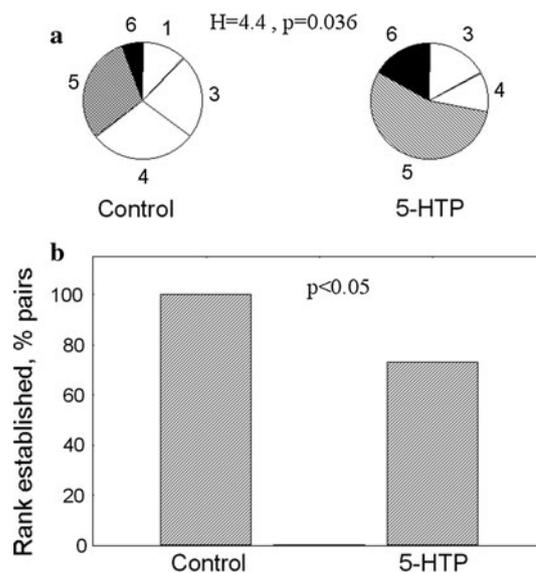


Fig. 4 The effects of 5-HTP on fight escalation and the ability to establish the loser of the fight in male crickets. **a** To represent the intensity of fighting, pie charts denote the percentage of pairs that finished fighting at levels 0–6. *Level 1* indicates pre-established dominance; *level 2* indicates bilateral antennal fencing; *level 3* indicates mandible spreading by one animal in a pair; *level 4* indicates mandible spreading by both individuals; *level 5* indicates mandible engagement; *level 6* indicates physical combat (Stevenson et al. 2000, 2005). **b** The percentage of pairs in which the fight resulted in the establishment of normal winner/loser relationships. Fisher test

to establish the winner of the fight in this group, the latency of rival singing was significantly shorter (Fig. 5b). Normally, a rival song is a characteristic feature of the aggressive behavior of a winner; however, males that had received 5-HTP sang during combat. In addition, 25 % of potential losers (males that flew from chasing dominants) also produced a rival song. This latter behavior was never observed in the control crickets.

Fighting between 5-HTP-treated and control males

Fights between 5-HTP-treated and control (physiological solution-treated) males ($n = 31$ pairs) lasted significantly longer than fights between two control males (22 ± 5 s versus 5 ± 1 s, $p < 0.01$). The average level of intensity of this fighting was 5, with 4 and 5 as the lower and upper quartiles. Level 1 fighting, which involved a pre-established loser, occurred in three pairs. In each of these three pairs, the losers were control males. Higher levels of aggression (5–6) took place in 62 % of pairs.

Multiple fights were observed significantly more often in the 5-HTP/control pairs (in 12 of 31 pairs) than in the control/control pairs (3 of 17). These multiple fights were only observed in pairs in which the 5-HTP-treated males were losing the fight. Interestingly, 6 of 15 potential losers treated with 5-HTP were singing while fleeing from the winners. In two pairs, despite the occurrence of 12–15 fights, social rank was not established at the end of the observational period, although there was a tendency of the control male to win.

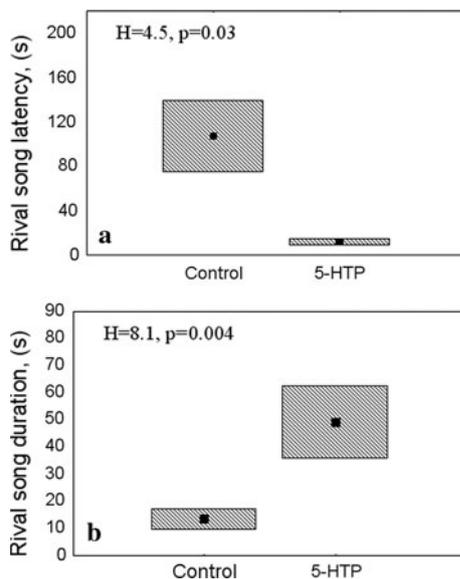


Fig. 5 The effect of 5-HTP on rival singing in male crickets. **a** Latency of singing (s). **b** Duration of rival singing (s). All data are presented as mean \pm standard error. *H* Kruskal–Wallis ANOVA test

The probability of 5-HTP-treated crickets winning against control crickets was not significantly different from chance (16 of 31). Analysis of video files revealed that 5-HTP-treated males attacked their opponents significantly less often (mean number of attacks per fight = 3 ± 0.6 versus 5.8 ± 1.3 , $p = 0.04$, Kruskal–Wallis ANOVA test, Fig. 6a). As a result, only 5 of 16 winners that had received 5-HTP won the fight within the territory of the control cricket. The remainder of wins by 5-HTP-treated crickets occurred within their own territory. In total, 23 fights were finished in the territory of 5-HTP-treated crickets and only six were completed in the territory of a control male ($p < 0.001$, Fig. 6b). Finally, in 75 % of cases, the control crickets were the first to open their mandibles during the initial contact with a 5-HTP-treated male.

Discussion

This study demonstrates that injection of the serotonin precursor 5-HTP causes substantial changes in the behavioral state, fighting behavior and ability to establish winner–loser relationships in male crickets (*G. bimaculatus*). The characteristic features of 5-HTP-treated crickets include elevated posture, enhanced general activity, longer demonstrative stages of fighting, enhanced rival singing and a decreased ability to establish a clear loser at the end

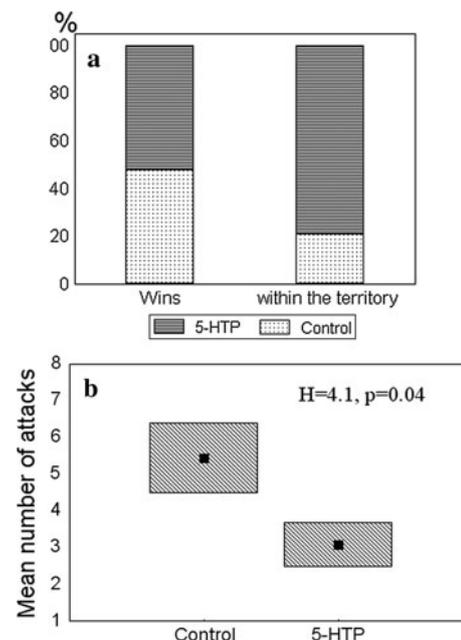


Fig. 6 Wins and attacks in 5-HTP/control pairs of male crickets. **a** The probability of winning (*first column*; no significant difference) and relative frequency of wins within the territory of 5-HTP-treated or control males (*second column*; $p < 0.001$, Fisher test); *light gray* control, *dark gray* 5-HTP. **b** Number of attacks per fight, mean with standard error. *H* Kruskal–Wallis ANOVA test

of a fight. On the other hand, 5-HTP-treated crickets showed a slightly delayed latency to spread their mandibles, a decreased number of attacks and an equal potential to win in comparison to control males. The obtained results imply a significant role of serotonin in the regulation of social status-related behaviors in *G. bimaculatus*.

The activation of serotonin synthesis hampers the establishment of a fight loser

Serotonin depletion pharmacologically induced via injection of the false serotonin precursor α -methyltryptophan (AMTP) has previously been reported to result in a reduced probability to win a fight in male crickets (*G. bimaculatus*) (Dyakonova et al. 1999). AMTP-treated males were capable of aggressive fights, but the duration of the fights was shortened (Stevenson et al. 2000, 2005). In addition, AMTP enhanced the escape responses in *G. bimaculatus* (Dyakonova et al. 1999; Stevenson et al. 2000). A decreased ability to win fights and an enhanced exhibition of escape reactions typically characterize loser behavior (Alexander 1961; Dyakonova et al. 1999). Indeed, application of high-performance liquid chromatography has also demonstrated serotonin depletion in fight losers of this species (Murakami and Itoh 2001). Thus, it has been assumed that inhibition of the serotonergic system might be connected to the control of this loser behavior (Dyakonova et al. 1999; Rillich and Stevenson 2011).

To test the effects of serotonergic system activation on male competition, we used an immediate metabolic precursor of serotonin that has been shown to effectively activate serotonin synthesis in many species, including *G. bimaculatus* (Ureshi et al. 2002). In all previous investigations, the effects of 5-HTP were fully explained by the increased synthesis of serotonin. In contrast to some other monoamine neurotransmitter precursors such as tyramine (Vierk et al. 2009) or L-DOPA (Dyakonova et al. 2009), to our knowledge, there is no evidence to date of possible direct effects of 5-HTP on either vertebrate or invertebrate nervous systems. The effects of the serotonin precursor observed here are in opposition to the earlier reported effects of AMTP (Dyakonova et al. 1999; Stevenson et al. 2000, 2005) and are in accordance with the suggestion that 5-HTP acts via an increase in serotonin production. Although the possibility that 5-HTP had direct actions or acted via nonspecific binding cannot be completely excluded, this possibility is unlikely.

Treatment with 5-HTP resulted in significantly longer fights accompanied by accelerated rival singing. This prolonged duration of fighting is most likely explained by a suppressive effect of serotonin on the decision to escape during the fight. These results are in accordance with data obtained in crustaceans demonstrating that smaller

crayfishes treated with serotonin spend an increased amount of time fighting larger opponents (Huber et al. 1997a, b). These data are also in alignment with the known suppressive effects of serotonin on the avoidance and escape behaviors of insects (Goldstein and Camhi 1991; Dyakonova et al. 1999; Stevenson et al. 2000, 2005). However, the suppressed escape behavior may not be the only factor affecting the duration of fights in 5-HTP-treated males. Several other behavioral parameters were changed by the serotonin precursor and indicated that preisolated 5-HTP-treated crickets had stronger winner-like behavior than control males. 5-HTP-treated males had significantly higher postures and demonstrated enhanced rival singing relative to control males. In addition, there were no pre-established losers (i.e., fight level 1) in the experimental group.

5-HTP-treated males tended to fight many times in a row; indeed, one pair of males participated in 16 fights during the 10-min observation period. In a significant number of cases, the loser was not established until the end of observation. Multiple fights also occurred in combats between 5-HTP-treated and control males, but only in pairs in which the 5-HTP-treated crickets were losing the fight. In a combat between a 5-HTP-treated and a control male in which the experimental cricket was winning, the winner-loser relationship was established as in a combat between two control males. We assume that these multiple fights may occur as a result of an inability of the mechanisms that underlie loser-type of behavior to function properly at a high level of serotonin. The suggestion that the 5-HTP-treated cricket losers do not “feel” like losers is also based on statistically significant occurrences of rival singing in 5-HTP-treated losers. Normally, songs are only produced by fight winners. Previous work has also shown that antagonism of serotonin autoreceptors, which should lead to a stimulation of the serotonin system, decreased the time of aggression inhibition in losing crickets (Yano et al. 2012). These results are consistent with previous data demonstrating a depletion of serotonin in subordinate males (Murakami and Itoh 2001) and suggest that suppression of the serotonergic system is functionally required for the production of clear fight losers.

On the basis of the available data on the serotonergic control of loser behavior in crickets, the following hypothesis is suggested for further verification. The serotonergic system is activated during fighting, and 5-HTP is required for the inhibition of the avoidance response or the decision to retreat. Even a transient deficiency in serotonergic modulation may result in an immediate avoidance reaction, which in turn changes the state of serotonergic system by either inhibiting it or altering the responsiveness of 5-HTP targets.

Loser behavior in crickets is manifested by the avoidance of conspecific males. Notably, in desert locusts, the

level of serotonin has been demonstrated to be crucial for the switching of the behavioral response in the presence of conspecifics from avoidance to approach (Anstey et al. 2009). In another large group of invertebrates, representing the *Lophotrochozoa* phylum, mollusca, serotonin is known to play a very similar role in approach-avoidance decision-making. Orienting behavior toward a novel object (D'yakonova and Sakharov 1995) or an unexpected touch (Yafremava et al. 2007) in both freshwater and seawater gastropods is activated by serotonin. Therefore, the role of serotonin in approach-avoidance decision-making might have some phylogenetically conserved basis in invertebrates.

5-HTP and winner-type of behavior: everything but real aggressiveness?

In *G. bimaculatus*, as in many other species, winners and losers differ in a number of behavioral characteristics. Winners are more aggressive (Rillich and Stevenson 2011), more active (Kortet and Hedrick 2007), have elevated postures, call females more frequently and have greater mating success (Simmons 1988; Dyakonova and Krushinsky 2008). Avoidance behavior is suppressed in fight winners (Dyakonova et al. 1999; Stevenson et al. 2000). A number of behavioral changes produced by 5-HTP are similar to the effects of winning. For example, 5-HTP had been shown to activate sexual behavior (Ureshi et al. 2002). Here, we demonstrated that 5-HTP-treated males were more active in the arena, had significantly higher postures and sang more intensively. In addition, there were no occurrences of opponent avoidance during the initial contact in the experimental group, and the durations of the fights were increased.

From the 5-HTP effects described above, we had expected that 5-HTP-treated males could win against control crickets. However, this turned out to be untrue. The probability of the 5-HTP crickets winning was not significantly higher than chance. Detailed analysis has revealed that 5-HTP-treated males attacked their opponents significantly less frequently, and as a result, they were more often pushed back during the fight. Paradoxically, when the 5-HTP males do win against control crickets, they most often win in their own "territory." The prolonged duration of stage 4 fighting in 5-HTP/5-HTP pairs may partially result from a suppression of attacking behavior. The effect of 5-HTP on the decision to attack seems to be specific, as there was not an overall suppressive effect of 5-HTP on the general activity of the crickets or on their ability to switch behaviors. In contrast to the effects detected on attack and avoidance behaviors, other behaviors, such as singing and exploration, were accelerated in 5-HTP-treated crickets.

5-HTP-treated crickets differ from legitimate fight winners in regards to their demonstrated suppression of aggressive attacks. Recently, it has been well documented that winning enhances the aggressiveness of crickets and that octopamine plays a crucial role in this effect (Rillich and Stevenson 2011). Therefore, although many 5-HTP-induced behavioral changes, including an increase in sexual activity (Ureshi et al. 2002), elevated postures, demonstrative aggressiveness and rival singing resemble the features of winner behavior, 5-HTP treatment does not produce a behavioral state completely identical to that of a winner in crickets.

In terms of a general potentiating effect of 5-HTP on fighting, our findings agree with data obtained in *Drosophila* (Dierick and Greenspan 2007; Alekseyenko et al. 2010). 5-HTP has been reported to promote aggressive interactions (Dierick and Greenspan 2007), and a specific group of neurons was found to be responsible for fight escalation during conflict (Alekseyenko et al. 2010). In crickets and flies, the disruption of serotonin synthesis similarly resulted in a decreased ability to fight (Dyakonova et al. 1999; Dierick and Greenspan 2007). However, there seem to be differences in the specific effects of serotonin on the speed of fight escalation between crickets and *Drosophila* (Alekseyenko et al. 2010). In contrast to the latter report, 5-HTP decreased the speed of fight escalation in crickets by making the demonstrative stages of fight last longer.

Similarities of 5-HTP effects in crickets and serotonin effects in crustaceans

The basic features of male social aggression are very similar in insects and crustaceans, two groups of arthropods. Up until now, however, there was not enough evidence to speculate on whether the basic neurochemical mechanisms underlying this behavior are conserved between insects and crustaceans. Indeed, it has even been suggested that these groups differ in basic mechanisms controlling aggression (Stevenson et al. 2005; Stevenson and Rillich 2012) and that insects are actually more closely related to vertebrates than to crustaceans in this regard. Given these data, our findings are important for comparative physiology as they note obvious similarities between insects and crustaceans in terms of the effects of serotonin.

The most peculiar similarity in the effects of serotonin observed between crickets and crustaceans is the activation of mechanisms controlling elevated posture. Serotonin has been suggested to be involved in the postural control of dominant crayfishes and lobsters because 5-HT injection caused animals to stand on the tips of their walking legs (Livingstone et al. 1980; Kravitz 1988). We observed a very similar effect of 5-HTP in our experiments using

crickets. That is, crickets treated with 5-HTP kept their body significantly higher than control males and spent significantly more time in an elevated posture. In crustaceans, the effect of serotonin on the postural network has been well-studied using *in vitro* electrophysiological experiments. The dependence of this effect on the social status of an animal has been demonstrated using both *in vivo* and *in vitro* experiments (Cattaert et al. 2010). Whether the effect of serotonin on cricket posture is similarly modulated by social status, however, remains unclear.

Another similarity between crickets and crustaceans is that animals with higher levels of serotonin are able to fight for a longer duration. In a previous crayfish experiment, when a smaller male in a fighting pair was treated with serotonin, the resulting fighting behavior lasted significantly longer relative to a fight in which a smaller male was treated with the physiological solution (Huber et al. 1997a, b). Therefore, similar effects on fight duration have been detected in insects and in crustaceans.

Finally, it is important to note that subordinate status is similarly accompanied by a significant decrease in the level of serotonin in not only crickets (Murakami and Itoh 2001) but also in some crustaceans (Sneddon et al. 2000). In one crustacean study (Sneddon et al. 2000), a significant decrease in octopamine was also observed in subordinate males.

Together, these data suggest that along with interspecific differences in serotonin neuromodulation (Antonsen and Paul 1997), there are likely common and highly conserved functions of serotonin in the control of social aggression in arthropods. Postural control and the suppression of the decision to retreat appear to be the main candidates for these conserved functions.

Serotonin and octopamine: opposite and supplementary roles in social rank establishment

Activation of neither the octopaminergic (Stevenson et al. 2005) nor the serotonergic system is sufficient to produce winner-type behavior in crickets. Octopamine enhances aggressiveness, but, unlike winner status, also activates an escape behavior and shortens the duration of fights in crickets (Dyakonova et al. 1999; Stevenson et al. 2000). Octopamine also has no effect on rival singing, another characteristic feature of winner behavior (Stevenson et al. 2005). Serotonin, as our results with 5-HTP treatment indicate, might be responsible for many behavioral features of a winner including an elevated posture, an increase in general activity, song production and the suppression of the decision to retreat during combat; however, in contrast to winning a fight, serotonin has a suppressive effect on aggressive attacks. The

known effects of these monoamines appear complementary in the modulation of the behavioral state of a winner. Thus, one may suggest that activation of both systems is necessary for the maintenance of winner behavior.

Serotonin and octopamine are often reported to function as antagonists in arthropods (Livingstone et al. 1980; Antonsen and Paul 1997; Scheiner et al. 2006; D'iakonova 2007) and it has generally been thought that neuromodulators with antagonistic functions are necessarily involved in the regulation of opposing behaviors. However, multiple sites of regulation, some synergistic and others antagonistic, of a single neuromodulator within the same functional domain have recently been discovered. This has stimulated the discussion of the possible advantages of control based on the partially antagonistic effects of a neuromodulator (Harris-Warrick and Johnson 2010). One suggested important action of these opposing modulatory actions is the ability to stabilize the modulated state of the network (Harris-Warrick and Johnson 2010). This allows to suggest that parallel activation or inhibition of two monoamine systems with partially opposing effects may regulate social status-related behaviors, a hypothesis that must be experimentally verified.

To conclude, our results imply a significant role of serotonin in the regulation of social status-related behaviors in *G. bimaculatus*. Specifically, these data indicate that a decrease in serotonergic activity may be functionally important for the control of loser behavior, whereas some behavioral features of dominant behavior (such as elevated posture, higher general activity, rival singing and a suppressed decision to retreat) are likely to be connected with the activation of the serotonergic system.

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