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Serotonergic-linked alterations of aggression of the crayfish

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**ABSTRACT**

Current theory suggests that aggressive behavior in the crayfish is largely modulated and regulated by the neurotransmitter serotonin (5-HT). To test this theory that links serotonin to aggression, we performed a series of drug treatments using various serotonin-related chemicals to measure their effects on subsequent aggressive behavior. Treatments included serotonin, the serotonin precursor tryptophan, agonists: 1-(3-chlorophenyl) piperazine (m-CPP) and 5-Carboxy, an antagonist: cinanserin, and a serotonin receptor specific neurotoxin: 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT). Significant increases in aggression of *Faxonius rusticus* crayfish were observed when injected with serotonin and both agonists, however no decrease in aggression occurred with the antagonist. Crayfish injected with the agonist m-CPP increased aggression but did not directly confer success in fights. Our data support the current literature that the internal aggressive state of crayfish is altered by serotonin and its agonist/antagonists, however it does not on its own improve the aggressive fighting response and/or dominance status.

**Introduction**

Aggressive encounters between animals of the same species are termed agonistic interactions to differentiate these meetings from other social relationships. Agonistic interactions occur when animals compete over resources, such as habitats, shelters, mates, and food sources. The primary effect of agonistic interactions is the establishment of a relationship that confers a pecking order for resources (Mesterton-Gibbons and Dugatkin 1995). Moreover, agonistic interactions influence neurochemistry and can have long-last effects on subsequent social interactions (Miczek et al. 1990; Brain and Haug 1992; Huber et al. 2001; Silva et al. 2013). Yet, the neural and neuroendocrine mechanisms that underlie social dominance are not fully understood, largely because they include dynamic interactions within and between systems at all levels, from gene expression to complex behavioral interactions. Despite this complexity, some of the neurochemicals that appear to play significant roles in aggression and consequently
influence agonistic behavior have been identified (Huber et al. 2001; Hrabovszky et al. 2005; Audet and Anisman 2010).

Biogenic amines are one very significant group of neural active substances that appear to play substantial roles in aggression. The biogenic amines serotonin (Olivier et al. 1995; Edwards and Kravitz 1997; Holmes et al. 2002), octopamine (Kravitz 1988; Adamo et al. 1995; Stevenson 2005; Zhou et al. 2008), norepinephrine (Thoa et al. 1972; Barrett et al. 1990) and dopamine (Rodgers et al. 1994; Shively et al. 1997; Ryding et al. 2008) have all been strongly implicated in modifying various forms of behavior. Serotonin (5-hydroxytryptamine or 5-HT) has received a disproportionate amount of attention concerning aggression research (Ferris et al. 1997; Nelson and Chiavegatto 2001; Popova 2008; Johnson et al. 2009; Hossain et al. 2020). Numerous studies have demonstrated that 5-HT plays a role in social interactions and the status of vertebrates and invertebrates (for a more complete review see Bacqué-Cazenave et al. 2020). The hypothesized role for 5-HT in aggression is that prior social interactions alters the function of 5-HT within the nervous system of invertebrates (Kravitz 2000; Moore and Bergman 2005; Johnson et al. 2009; Cattaert et al. 2010). In lobsters, crayfish, and other decapod crustaceans increases in social status can result in increases in excitability in the presence of 5-HT, which may be due in part to changes in 5-HT receptor subtype populations (Yeh et al. 1996, 1997; Krasne et al. 1997). Moreover, social status (i.e. winners and losers) has been shown to influence concentrations of neuromodulators in the hemolymph, alter the efficacy of modulators at identified synapses and that of monoamines in different nervous system regions (Yeh et al. 1996, 1997; Krasne et al. 1997; Sneddon et al. 2000). Furthermore, the different 5-HT receptor circuities appear to mediate different aspects of aggression. In Drosophila melanogaster, 5-HT2 receptor manipulation primarily alters some aggressive behaviors (i.e. lunging and boxing), whereas 5-HT1A-like receptor manipulation affects other behaviors (i.e. wing threats and fencing) (Johnson et al. 2009).

Serotonin is ubiquitous in vertebrate and invertebrate species and mediates a myriad of physiological and behavioral processes. In crustaceans, serotonin has been demonstrated to affect hormone release (Rao and Fingerman 1983; Mattson and Spaziani 1985; Sosa et al. 2004), heart rate (Florey and Rathmayer 1978; Listerman et al. 2000), and many forms of neural activation (Harris-Warrick and Kravitz 1984; Barthe et al. 1989; Ma et al. 1992; Gill and Skorupski 1996) and even neurogenesis (Zhang et al. 2011). Particularly, decapod crustaceans have been used extensively as model systems for the study of agonistic interactions and the role serotonin plays in modifying these agonistic behaviors (Edwards and Kravitz 1997; Tierney and Mangiamele 2001; Tricarico and Gherardi 2007). For example, when lobsters and crabs are injected with 5-HT, aggression toward conspecifics increases and elicits stereotyped aggressive displays (Kravitz et al. 1980; Livingstone et al. 1980; Aggio et al. 1996; Antonsen and Paul 1997). In the case of crayfish, they demonstrate similar increases in aggressive acts and sometimes posturing (Livingstone et al. 1980; Huber et al. 1997a). It has further been speculated that the behavioral plasticity associated with differences in aggression and dominance influences nervous system neurochemistry (Bergman et al. 2005; Edwards and Spitzer 2006). In other words, winning and losing fights could alter serotonin levels in the hemolymph and subsequently alter receptor sensitivity and even expression levels of various protein elements of the 5-HT system (i.e. enzymes in 5-HT metabolism, 5-HT transporters, 5-HT1A and 5-HT1B receptors) (Sneddon et al. 2000; Edwards and Spitzer 2006; Popova
These alterations in serotonin in crayfish then affect the subsequent social behavior of the crayfish by altering future levels of aggression (Yeh et al. 1997). Observations of prolonged fights of subordinate animals have led to the speculation that 5-HT reduces the subordinate animals’ willingness to retreat, and hence, that 5-HT enhances ‘aggressive motivation’ (Huber and Delago 1998). In fact, neurons within local circuits that control the tailflip response (Glanzman and Krasne 1983, 1986), exhibit reduced responsiveness in the presence of this amine (Edwards and Kravitz 1997). This reduction in tailflip frequency has been indirectly attributed to likelihood that a crustacean will become more aggressive.

The link between aggression and resulting dominance in decapod crustaceans are thought to be a direct result of 5-HT levels or activity within the nervous system. First, injection of 5-HT elicits stereotypical agonistic behaviors and posture (Tricarico and Gherardi 2007; Pedetta et al. 2010). Second, increased serotonergic function, through injections, decreases the likelihood of retreat (Huber et al. 1997b; Huber and Delago 1998; Pedetta et al. 2010), which could be summarized by stating that serotonergic function alters the fight winner decision point. Those crayfish with increased serotonergic function are less likely to retreat and thus are more likely to win agonistic interactions. However, attention needs to be drawn to the fact that 5-HT alters basic functions such as locomotion and behavioral postures in invertebrates, and consequently could be mistakenly interpreted as changes in aggression (Tierney 2001; Cattaert et al. 2010; Wu and Cooper 2012). The exact nature of the connection between 5-HT and dominance remains uncertain but it is clear that 5-HT and other neurotransmitters influence aggression, moreover these chemicals have the potential to influence future social interactions.

It is quite evident that serotonin affects aggression via behavioral changes (Huber 2005). However, the possible role that serotonin plays in modulating and regulating social status (dominance and subordination) remains somewhat elusive. The purpose of this study was to provide further evidence that 5-HT or similar amine influences fight outcome in a dyadic pair of crayfish independent of the aggressive levels exhibited. To accomplish this objective, we performed injections with various serotonin-related compounds that included 5-HT, a 5-HT precursor, 5-HT agonists, a 5-HT antagonist, and a 5-HT neurotoxin, and then subsequently measured social behavior by examining the resulting fight outcome and aggression.

**Material and methods**

Male intermolt (form I) crayfish, *Faxonius rusticus*, with fully intact appendages were socially and physically isolated in a flow-through holding tank. Crayfish were kept at a constant temperature (23°C) and light:dark cycle (14 hr:10 hr) and crayfish were isolated for a minimum of two weeks prior to experimentation to reduce the effects of prior social experience. Crayfish were fed one rabbit food pellet every other day. Crayfish were size-matched within 95% for carapace length (from rostrum to beginning of abdomen) to reduce size influences on fights (Bergman et al. 2003). Each crayfish, regardless of treatment, was used only once during the course of this study. All crayfish were marked with white correction fluid on the carapace for later identification during behavioral analysis.
Drug compound selection

To separate the effects of serotonin and various related compounds on aggression and winning, we quantified levels of aggression and the initiation and outcomes of agonistic interactions between crayfish injected with a serotonin-related compound and crayfish injected with control Van Harreveld’s saline solution. Van Harreveld’s saline was used for both the controls and the vehicle for all drug treatments. Van Harreveld’s solution consisted of 12 g of NaCl, 0.4 g of KCl, 2 g of CaCl₂, 0.5 g of MgCl₂, 0.2 g of NaHCO₃ per 1 L of H₂O with a pH of 7.4 (Tierney 2001). The pharmacological agents used in the study were acquired from the following commercial sources (Aldrich):

1. serotonin (5-HT) (N = 16)
2. tryptophan (N = 16)
3. 1-(3-chlorophenyl) piperazine dihydrochloride (mCPP) (N = 16)
4. 5-carboxamidotryptamine maleate (5-CT) (N = 16)
5. cinanserin (N = 16)
6. 5,7-DHT (N = 16)

Compounds 1–4 were chosen because each has a positive relationship between the compound and aggression; a direct relationship with aggression (serotonin), a precursor for serotonin (tryptophan), and agonists for serotonin (1-(3-chlorophenyl) piperazine [mCPP], and 5-carboxamidotryptamine maleate [5-CT]) (Livingstone et al. 1980; Issa et al. 2012; Brummer et al. 2013). In addition, we included two antagonists for serotonin – cinanserin hydrochloride and 5, 7-DHT (Fernandez Guasti et al. 1990; Sullivan et al. 2000). Each compound was injected at a concentration of 3 × 10⁻³ M, a concentration sufficient to cause postural changes (Tierney and Mangiamele 2001). mCPP is 5-HT₁₄ agonists that is less effective at 5-HT₂β (Fiorella et al. 1995; Wood et al. 2000; Spitzer et al. 2008), whereas cinanserin is an antagonist of 5-HT₂β, but does not block 5-HT₁₄ (Spitzer et al. 2008). 5-CT acts as a non-selective, high-affinity agonist at the 5-HT₁₄, 5-HT₁ β receptors, as well as at the 5-HT₂, 5-HT₃, 5-HT₆ receptors with lower affinity (Beer et al. 1992; Yamada et al. 1998; Wood et al. 2000). The 5-HT neurotoxin 5,7-dihydroxytryptamine creatinine sulfate (5,7 DHT) was used at 5 × 10⁻⁴ M concentration and was injected at a dose of 6.6 µM/g of crayfish. Concentrations were chosen to include those above and below a concentration (5 x 10⁻⁴ M) that induced postural changes in a majority of animals (Tierney and Mangiamele 2001).

Injection protocol

Crayfish sizes (mean ± SE, carapace 3.5 ± 0.1 cm, chelae 3.1 ± 0.05 cm, weight 12.9 ± 0.60 g) were obtained to calculate the proper amount of drug administration for the size of each crayfish (approximately 5 µg/g body weight). Crayfish were randomly assigned to receive either an injection of Van Harreveld’s saline (control) or one the 5-HT or related compound treatments (n = 16 for each group). Crayfish were immobilized using a harness to restrain the crayfish while injecting. Injections were administered slowly (over a 10s period) to the ventral hemolymph sinus by placing a 27 gauge needle
between the second and third walking legs. Studies by Tierney and Mangiamele (2001) demonstrated that dye injection (fast green FCF) would spread within 30 s to the distal regions of the ventral artery. Experiments with lobsters found that injected 5-HT was rapidly distributed throughout the hemolymph and that most (75%) was cleared from the hemolymph via tissue uptake and/or binding within 5 min after injection (Huber et al. 1997a). Following this initial decline, a steady level of 5-HT (or a polar 5-HT metabolite) remained in circulation for up to 60 min (Peeke et al. 2000). The crayfish were immediately removed from the harness and returned to an isolation chamber for a 10 min period for observation.

**Aggressive interaction protocol**

For aggression experiments, crayfish were tested in randomly assigned pairs consisting of animals that differed by no more than 5% in body weight and 5% in carapace length. Chelae were symmetrical and similarly sized between pairs. It has been demonstrated that these small size differences did not influence fight outcomes for *Faxonius* spp. (formerly *Orconectes*) (Bergman et al. 2003; Zulandt et al. 2008). However, it should be noted that in other species of crayfish such as *Procambarus clarkii*, similar small size differences can influence fight outcomes (Momohara et al. 2013; Bacqué-Cazenave et al. 2018). Trials were first performed to assess the effect of the injection procedure on agonistic behavior, where crayfish that received no injection interacted with crayfish that received the vehicle Van Harreveld’s saline control. No significant difference was found for the injection procedure (p > 0.05). For the pharmacological agent trials (vehicle control versus experimental), members of a matched pair were placed in the separate compartments in a fight arena and allowed to acclimate for 15 min. The fight arena was made of Plexiglas (20 × 20 × 14 cm) and was divided into halves, separated by opaque retractable walls. The arena held 10 L of dechlorinated water (filled to a depth of 4 cm from the top of the tank). After acclimation, the divider was lifted and the two crayfish were allowed to interact. An opaque curtain was drawn around fight tank to prevent external distractions. The interacting animals were videotaped for 15 minutes. After the 15-minute interaction, animals were returned to isolation tanks and not reused in future trials.

**Fight analysis**

All fights were digitally recorded from a camera positioned one meter above the test arena. An individual with no prior knowledge of which treatment or condition each crayfish was assigned to perform a blind analysis. For each agonistic encounter, the winner and loser of each fight was recorded as well as the temporal mechanics of the fight were recorded (Table 1). Temporal mechanics analyzed included time to different fight intensities and duration of the initial encounter. All interactions were analyzed by examining the behavior of both participants. To analyze data within each drug treatment, initiation and winning were analyzed using a binomial distribution test. Crayfish were classified as either having achieved the behavior (initiate or win the fight) or not achieve the behavior (probability = 0.5). The time to reach different fight intensities were not normally distributed, so all values were transformed using a square root transformation.
Table 1. Crayfish Behavioral Ethogram Codes (Used to score fight intensity levels).

<table>
<thead>
<tr>
<th>Intensity Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>−2</td>
<td>Tailflip away from opponent or fast retreat</td>
</tr>
<tr>
<td>−1</td>
<td>Retreat by slowly backing away from opponent</td>
</tr>
<tr>
<td>0</td>
<td>Visually ignore opponent with no response or threat display</td>
</tr>
<tr>
<td>1</td>
<td>Approach without a threat display; claws raised</td>
</tr>
<tr>
<td>2</td>
<td>Approach with threat display</td>
</tr>
<tr>
<td>3</td>
<td>Initial claw use by boxing, pushing and/or touching with closed claws</td>
</tr>
<tr>
<td>4</td>
<td>Active claw use by grabbing and/or holding opponent</td>
</tr>
<tr>
<td>5</td>
<td>Unrestrained fighting by pulling at opponent’s claws or body parts</td>
</tr>
</tbody>
</table>

After transformation, time to reach different fight intensities were analyzed using a one-way MANOVA followed by a Tukey-HSD test. All p-values were set at p < 0.05.

The identities of initiating and winning animals were recorded for each interaction. The crayfish that first engaged an opponent in using either a meral spread threat display or to physically contact was deemed the fight initiator. The winner was determined as the crayfish that pursued its opponent (i.e. the loser) as it retreated or tail-flipped away, or if the two crayfish adopted body postures indicating dominance. Winner crayfish tend to exhibit high body postures, extended tails and pointing chelae, whereas subordinate crayfish tend to exhibit lowered body position and tails curled under the body (Bergman et al. 2003). Prior studies indicate that 5-HT can induce an ‘aggressive posture’ that resembles a meral spread – a common display of dominant animals (Livingstone et al. 1980). Yet Tierney and Mangiamele (2001) note that the aggressive meral spread posture could involve either an elevated or depressed posture, so when observing postural effects alone status roles were not necessarily assigned.

Results

Winning and initiating agonistic interactions

The probability of winning an encounter was significantly altered by only the treatment with cinanserin (Figure 1). Cinanserin, the antagonist of 5-HT2β, significantly reduced the probability of injected crayfish to win a fight (25.0% of cinanserin-injected crayfish won; p = 0.03). All other drug treatments did not significantly differ from a random probability of 0.5. Injections with the drugs associated with aggression did not increase the probability of winning (62.5% of 5-HT injected crayfish won, p = 0.12; 56.3% of mCPP, 5-CT, and tryptophan-injected crayfish won, respectively, p = 0.17). And injection with 5,7 DHT, the 5-HT neurotoxin, did not significantly affect the probability of winning a fight (62.5% of 5,7 DHT-injected crayfish won, p = 0.12). Injection with saline did not significantly differ from equal probability (50% of saline-injected crayfish won, p = 0.20), indicating that the injection procedure did not negatively impact fight outcome.

The initiation of fights was significantly altered by the injection of serotonin and its agonists (Figure 2). The injection of serotonin, mCPP, and 5-CT significantly increased the likelihood of the crayfish injected with a pharmacological agent in initiating a fight over a non-injected crayfish (75.0% of 5-HT-injected initiated fights, p = 0.03; 81.3% of mCPP-injected initiated fights, p = 0.01; 81.3% of 5-CT-injected initiated fights, p = 0.01). Crayfish-injected with the serotonin precursor tryptophan did not show significant
differences in fight initiation (43.8% of tryptophan-injected initiated fights, $p = 0.17$). Likewise, neither of the antagonists had an effect on the likelihood of initiating a fight.
(37.5% of cinanserin-injected initiated, p = 0.12; 68.8% of 5,7 DHT-injected initiated, p = 0.06) Injection with saline did not significantly differ from equal probability (50% initiated fights, p = 0.20)

**Aggressive intensity**

The times to reach different fight intensities showed significant differences for the different drug treatments (Table 1; F12, 152 = 2.775, p < 0.03). Crayfish injected with serotonin, serotonin agonists (mCPP and 5-CT), and tryptophan reached intensity level 2 (i.e. approach with threat display) in fights significantly faster than control crayfish injected with saline (Figure 3; p < 0.05). Conversely, crayfish injected with the serotonin antagonist, cinanserin, reached level 2 intensities in their fights significantly slower than control crayfish (Figure 3; p < 0.05). Whereas, 5,7 DHT did not differ significantly in their progression to intensity 2 when compared to controls (p > 0.05). Similar significant differences were found for the times to reach intensity level 3 (i.e. initial claw use by boxing, pushing and/or touching with closed claws) for 5-HT, and its agonists, mCPP and 5-CT, as

![Graph](image-url)

**Figure 3.** Mean time (+ S.E.M.) to reach fight intensity levels 2, 3, and 4 for treatment crayfish (N = 16 trials per treatment). Asterisks signify a significant difference compared to saline-injected (control) crayfish using a one-way MANOVA with a Tukey-HSD post-hoc test (p < 0.05; n.a. indicates that no crayfish reached this level of intensity).
well as cinanserin (Figure 3; p < 0.05). Only tryptophan and 5,7 DHT injected crayfish remained non-significant when compared to saline injected crayfish. For intensity level 4 (i.e. active claw use by grabbing and/or holding opponent), crayfish injected with 5-HT and mCPP progressed significantly slower to intensity 4 than control. Crayfish injected with 5-CT and tryptophan did not achieve intensity level 4 interactions. Cinanserin and 5,7 DHT injected crayfish did not differ from saline injected. Intensity 5 behaviors (i.e. unrestrained fighting by pulling at opponent’s claws or body parts) were infrequently observed and thus not included in our analysis. Prior studies have demonstrated that *Faxonius* spp. do not typically progress to intensity level 5 in laboratory settings (Bergman et al. 2003; Bergman and Moore 2005) or even in field observations (Bergman and Moore 2003). Overall amount of time spent fighting did not significantly differ (p > 0.05).

**Discussion**

Our results indicate that serotonin and serotonin agonists influence the aggressive state of crayfish, but do not appear to directly relate to a dominance advantage (i.e. win more fights). Crayfish injected with serotonin, tryptophan, and the serotonin agonists (m-CPP and 5-CT) consistently reached heightened aggressive states faster than the other treatment groups. In fact, the quicker aggressive response seems to be an indicator of an increased aggression in the crayfish injected with 5-HT, Tryptophan, and the serotonin agonists, m-CPP and 5-CT. Our results also demonstrated a positive relationship between serotonin (+ agonists) and fight initiation behavior. Crayfish injected with 5-HT, m-CPP, and 5-CT were more likely to initiate agonistic interactions. The behavioral alterations in aggression observed in our study and that of others may be a direct result of the physiological effects of serotonin on crayfish (Livingstone et al. 1980; Tierney and Mangiamele 2001; Nagayama 2002; Le Bon-Jego et al. 2004). These studies indicate that the willingness to retreat is decreased because the animals are locked into what has been called the ‘5-HT posture.’ Notably, serotonin and the 5-HT agonist mCPP elicit a stereotyped posture characterized by increased flexion of abdomen (Tierney et al. 2004; Brummer et al. 2013).

Several studies have demonstrated that a high level of exogenous 5-HT elicits an elevated and flexed position, but not necessarily additional behaviors clearly associated with aggression. Tierney and Mangiamele (2001) indicate that 5-HT diminishes aggression between opponents by reducing walking speed thus reducing aggressive interactions by decreasing movement. And Antonsen and Paul (1997) showed dose dependent responses where decapods injected with relatively low concentrations responded with increased aggression, whereas injections with high concentrations reduced aggression. In addition, the rate at which 5-HT is infused into the crayfish will influence aggression. Notably, a slow infusion rate causes crayfish to escalate aggression more quickly, while 5-HT treatment at a faster rate results in a slower escalation (Panksepp and Huber 2002). Moreover, Lee et al. (2008) demonstrated that varying levels of serotonin can influence the efficacy of synaptic transmission to crayfish giant neurons that mediate aspects of escape. Krasne and Edwards (2002) proposed that high-serotonin depression might serve to suppress reflex escapes during fights, whereas low-serotonin facilitation might promote an enhancement of escape. Our data support these prior observations, as crayfish injected with 5-HT, tryptophan and 5-HT agonists were more aggressive in our fights.
without escape, although the injections did not give the crayfish an advantage in actual fight ability nor the eventual fight outcome.

Crayfish injected with the serotonin antagonist, cinanserin, took significantly longer periods of time to reach different levels of intensity (2 and 3) and lost significantly more fights than any other the other treatments. Cinanserin has the ability to cause a postural change where the subject’s body remains near the substrate (Livingstone et al. 1980; Fernandez Guasti et al. 1990), which may mimic a submissive posture resulting in our observed effects. By blocking the 5-HT$_2$ receptors, it is plausible that cinanserin has reduced aggression in crayfish and reduced the progression to higher fight intensities. For example, cinanserin has been used to reduce aspects of sexual behavior, which could include male aggression (Weiger 1997; Spitzer et al. 2008).

Crayfish injected with 5,7-DHT showed no significant difference to reach different levels of intensity when compared to control crayfish. 5,7-DHT is suggested to reduce the effect of serotonin in the nervous system, thus we expected reduced levels of aggression in these crayfish. 5,7-DHT competitively inhibits the uptake of serotonin and simultaneously reduces the synthesis of serotonin (Sullivan et al. 2000). It is possible that the speed of our drug delivery influenced the effectiveness of 5,7-DHT. Behaviorally, 5,7-DHT treated crayfish do not exhibit any significant differences in aggression (Panksepp and Huber 2002). Our results support this work and showed no increases in aggression when injected with 5,7-DHT.

Work on the role of serotonin and aggression in crustaceans has shown direct effects of serotonin on aggression and dominance. Serotonin plays an important role in ‘aggressive motivation’ of the crayfish by affecting the decision to retreat and risk assessment during fights (Huber et al. 1997b; Bacqué-Cazenave et al. 2018). These studies show that subordinate animals injected with serotonin have a renewed willingness to engage the dominant animal in agonistic encounters (Huber et al. 1997a, 1997b). Through multivariate analysis, these researchers dissociate duration of fight and increased levels of intensity and demonstrate that an increase in fight duration was the most important variable influenced by the amine. They proposed that the neuromechanistic pathway for the decision to retreat is altered by injection of serotonin and proposed the crucial receptors regulating this decision may be found in the deutocebral giant serotonergic neurons found in the supraesophageal ganglion of the crayfish (Huber et al. 1997a).

The prior social experience of a crayfish can modulate the functioning of serotonin in the nervous system (Horner et al. 1997; Edwards and Spitzer 2006; Issa et al. 2012). Moreover, intrinsic changes in biogenic amine neurochemistry can occur via winning or losing experiences, and these changes may then be reflected in the chemicals released into the urine of dominant and subordinate crayfish. Urine-borne chemical cues influence the progression and outcome of agonistic encounters in decapods (Breithaupt et al. 1999; Zulandt-Schneider et al. 2001; Bergman and Moore 2005). Furthermore, social status has been shown to determine the concentrations of neuromodulators in blood (Knoll and Egberink-Alink 1989), the efficacy of modulators at identified synapses (Yeh et al. 1997; Krasne et al. 1997; Issa et al. 2012) and the concentration of monoamines in different brain regions (Holladay and Edens 1987; Winberg et al. 1996). Thus, through the analysis of the urine released during a fight, we may be able to further understand the implications of urine release after a biogenic amine injection.

Serotonin and its receptors play a significant role in altering aggression in crayfish (Edwards and Spitzer 2006); however, our results indicate that it may not alone increase
the odds of winning fights. If serotonin and 5-HT related compounds influence aggression, these same compounds very likely influence urine signaling. Aggressive behavior is effective in intimidating opponents but only in conjunction with proper urine release (Breithaupt and Eger 2002). Dominant crayfish will release urine more frequently than subordinates during encounters (Bergman et al. 2005), therefore if serotonin alters urine release in an aggressive context it could have significant effects on the maintenance of hierarchies. Studying the effects of biogenic amines requires a complex analysis of the biochemical effects on the nervous system, as well as how the subject responds behaviorally when in an altered aggressive state.

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**Disclosure statement**

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