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Neuropharmocological Alterations of the Aggressive Behavior of Crayfish

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Abstract

Serotonergic-related compounds often facilitate aggression in various animals, including crayfish. However to date, studies have seldom shown the mechanism by which serotonergic-related compounds alter aggressive behavior. It is assumed that serotonin changes the neurochemistry of those injected. In our study, we have attempted to report an observable mechanism by examining the communication system of crayfish. Crayfish use urine to communicate aggressive status, thus we analyzed the frequency of urine release from those injected with serotonergic-related compounds. For each trial, two size-matched crayfish, within 5% body weight, were allowed to interact after injection with serotonin, an agonist, an antagonist, or vehicle control. The concentration of all drugs was 3mM at a delivery dosage of 0.1ml/g. Aggressive interactions were recorded under black light to illuminate a fluorescein dye that was added to all injections. Urine release and aggressive behaviors were then analyzed.

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 individuals display signals and/or fight over resources, such as habitats, shelters, mates, and food sources. The primary effect of agonistic interactions is the establishment of a dominance 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 (Huber et al. 2001; Silva et al. 2013). Yet, the neural and neuroendocrine mechanisms that underlie social dominance are not well understood, largely because they include dynamic interactions within and between systems at all levels, from gene expression to complex social interactions. Despite this complexity, some of the neurochemicals that appear to play significant roles in aggression and consequently influence dominance 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 dominance behavior. The biogenic amines serotonin (Edwards and Kravitz 1997; Holmes et al. 2002), octopamine (Adamo et al. 1995, Stevenson et al. 2005; Zhou et al. 2008), norepinephrine (Barrett et al. 1990) and dopamine (Shively et al. 1997; Ryding et al. 2008) have all been strongly implicated in modifying various forms of behavior, including aggression. Dominance status and aggression are generally negatively correlated in vertebrates where serotonin and its metabolites are at higher concentrations and have prolonged effects in subordinate animals (Perreault et al. 2003; Fairbanks et al. 2004). Conversely, invertebrates exhibit a contrasting trend, where an increase in serotonin levels are closely associated with

heightened aggression or even attributed to dominant behavior (Edwards and Kravitz 1997; Sneddon et al. 2000). The hypothesized role for serotonin in aggression is that changes in social status as a result of previous social interactions alters the function of serotonin within the nervous system of invertebrates (Kravitz 2000; Moore and Bergman 2005; Johnson et al. 2009).

First, injection of serotonin 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. 1997, Pedetta et al. 2010). This second point could be summarized by stating that serotonergic function alters the "dominance decision point" of agonistic interactions by altering the probability of individuals to tailflip (Huber and Delago 1998). Those crayfish with increased serotonergic function are less likely to tailflip and thus are more likely to become dominant in social interactions. However, attention needs to be drawn to the fact that serotonin alters basic functions such as locomotion and behavioral postures in invertebrates, and consequently could be mistakenly interpreted as changes in aggression (Tierney 2001; Wu and Cooper 2012). The exact nature of the connection between serotonin and dominance remains uncertain but it is clear that serotonin and other neurotransmitters influence aggression. Moreover these chemicals have the potential to influence future social interactions.

Materials and Methods

Collection

Male crayfish, *Orconectes rusticus*, were collected from the Muskegon River near Newaygo, MI. Crayfish were socially and physically isolated in individual holding tanks for a period of 5 day to reduce the effects of prior social experience. Crayfish were size-matched within 95% for body length to reduce size influences on fights (Bergman et al. 2003).

Drug compound selection

To separate the effects of serotonin and various related compounds on aggression, we quantified the levels of aggression between crayfish injected with a serotonin-related compound and crayfish injected with control Van Harrveld's (ringer) solution. The ringer solution was made of 6.6 g NaCl, 0.15 g KCl, 0.1 g $CaCl_2$, 0.2 g $NaHCO_3$, 0.25g $MgCl_2$, 1 g fluorescein and was used for both the control injection and the vehicle for all drug treatments. The pharmacological agents to be used in the study include: 1) serotonin (5-HT); 2) tryptophan; 3) Cinanserin; 4) ringer without flourescein. Compounds 1-2 are selected because of research demonstrating a positive relationship between the compounds and aggression; a direct relationship with aggression (serotonin), and a precursor for serotonin (tryptophan). In addition we included a serotonin antagonist ant of 5-HT2β, but does not block 5-HT1α (Cinanserin) and ringer without fluorescein (Spitzer et al. 2008). Each compound was injected at a concentration of 3×10^{-3} M, a concentration sufficient to cause postural changes, with a dosage of 0.1 ml/g of crayfish. Concentrations selected are at or below concentrations that induce postural changes in crayfish.

Injection protocol

Crayfish sizes were obtained to calculate the proper amount of drug administration for the size of each crayfish, then randomly assigned to an experimental group, which will receive either an injection of normal crayfish ringer (control) (n=10 for each group). Before the injection, the crayfish were anesthetized in ice for a period of 5 min, in order to have a better efficiency with the injection due to slower heart rate. Injections will be administered over a 10s period to the second dorsal curve by injecting a 26 G needle with a BD 1 ml syringe about 1-2 mm below the exoskeleton and pushing the solution towards the heart (**Figure 1**). Previous studies demonstrated that dye injection would spread within 30 seconds to the distal regions of the ventral artery and a constant level of 5-HT remains in circulation for a minimum of 60min (Peeke et al. 2000).

Aggressive interaction protocol

For aggression experiments, crayfish were tested in pairs consisting of animals that differed by no more than 5% in body weight and 5% in carapace length. After receiving the injection, members of a matched pair were painted with different fluorescent paint and placed in the separate compartments in a fight arena and allowed to acclimate for 10 min (**Figure 2**). The fight arena was made of opaque Plexiglas (40 × 40 × 14 cm) and divided into four quadrants, separated by opaque retractable walls. After acclimation, a divider were lifted and two crayfish were allowed to interact for 15 minutes. The winner and loser of each fight were recorded under the black light as well as the temporal mechanics of the fight. Temporal mechanics included were time to different fight intensities, number of fluorescein burst (**Figure 3**) and duration of the initial encounter. All interactions were analyzed by examining the behavior of both participants and the winner was announced (**Figure 4**).

Results

- Based on the data collection, figure 5-8 were created using Microsoft Excel.
- Tryptophan is a precursor to serotonin, thus we hypothesized that its injection would increase aggression.
- Our preliminary data thus far demonstrate a potential decrease in aggression when crayfish were injected with tryptophan.
- We note that there are more burst releases of urine during the fights won by the control group, which may indicate an increase in communication during interactions.
- There was no significant difference between the ringer and fluorescein (control) fights.
- Other drugs (i.e. serotonin and cinanserin, an antagonist) are currently being analyzed using the same methodology used for tryptophan.
- In addition, we are investigating the overall intensity of fights among drug treatments, where we will also compare our results to the previous aggression study to elucidate any differences between these two studies.

Future Plans

Further and deeper analysis is currently being worked on in order to determine which crayfish was victorious in each trial, and to determine the intensity of each fight. Once this analysis is completed, we will have a stronger understanding of how serotonin influences aggression and the communication of aggressive status.

We have submitted an abstract to the Society for Integrative and Comparative Biology (SICB) in order to attend the annual conference in Portland, Oregon in January 2016. We hope to have the full analysis completed at this time when presenting at the conference. We are also striving for eventual publication in a peer-reviewed journal, once the study is fully completed.

Figures

Figure 1: An example drug injection site on the dorsal surface of the abdomen of a crayfish. Injections were done with a 26-gauge needle and a BD 1 ml syringe.

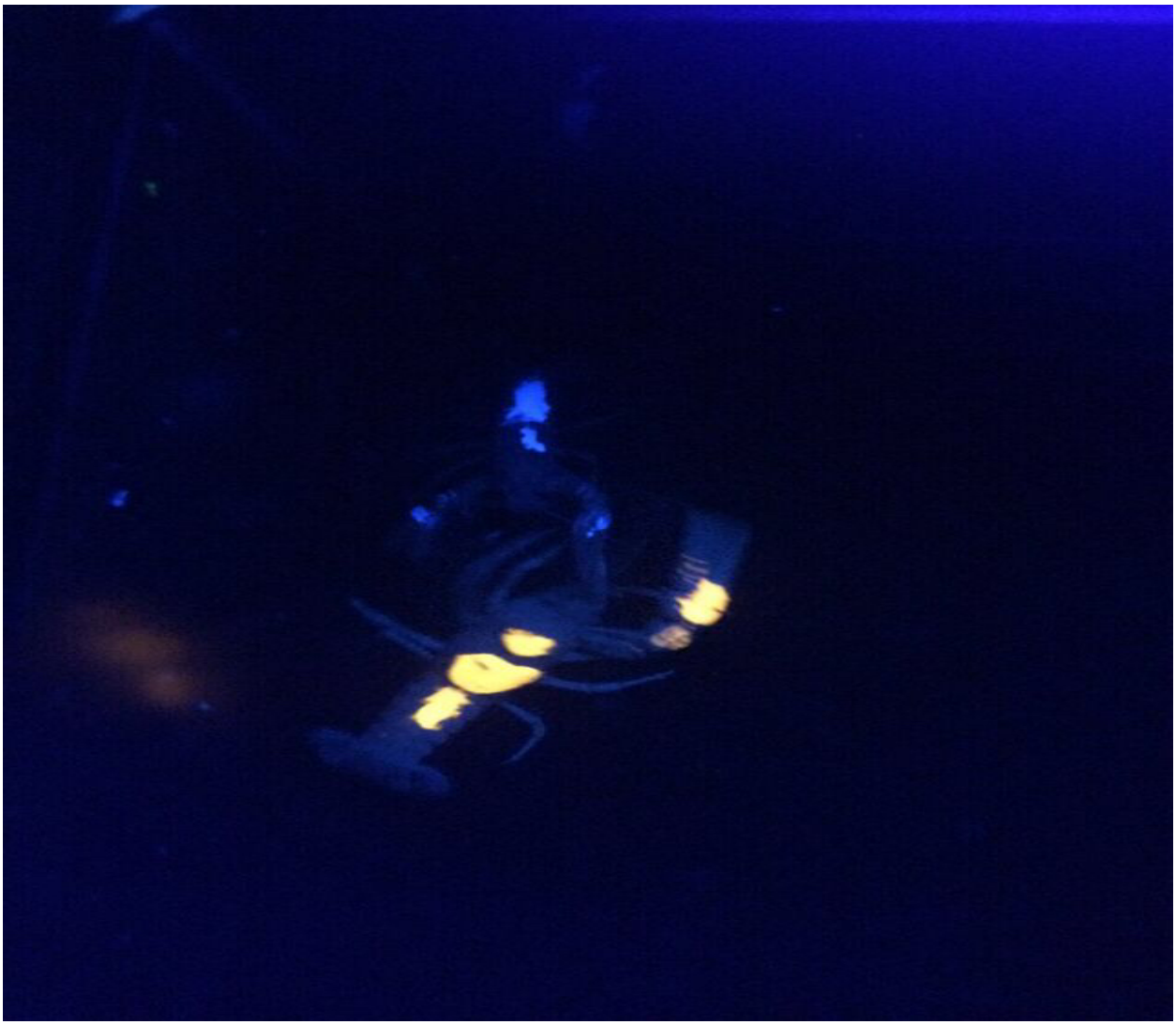

Figure 2: An example of the fights. As it is shown, each crayfish is marked using a different colored fluorescent paint and further illuminated under black light (UV-A).

Figure 3: An example of fluorescein burst. Each burst was recorded at the moment of the fight.

Intensity Level	Behavioral Description
-2	**Tail flip away from opponent or fast retreat**
-1	**Retreat by slowly backing away from opponent**
0	**Visually ignore opponent with no response or threat display**
1	**Approach without a threat display**
2	**Approach with mural spread threat display**
3	**Initial claw use by boxing, pushing and/or touching with closed claws**
4	**Active claw use by grabbing and/or holding opponent**
5	**Unrestrained fighting by pulling at opponent's claws or body parts**

Figure 4: The scoring table used for analysis of aggressive intensity of fights. Each crayfish was scored based on the table above and the winner (the one with the most points) was announced.

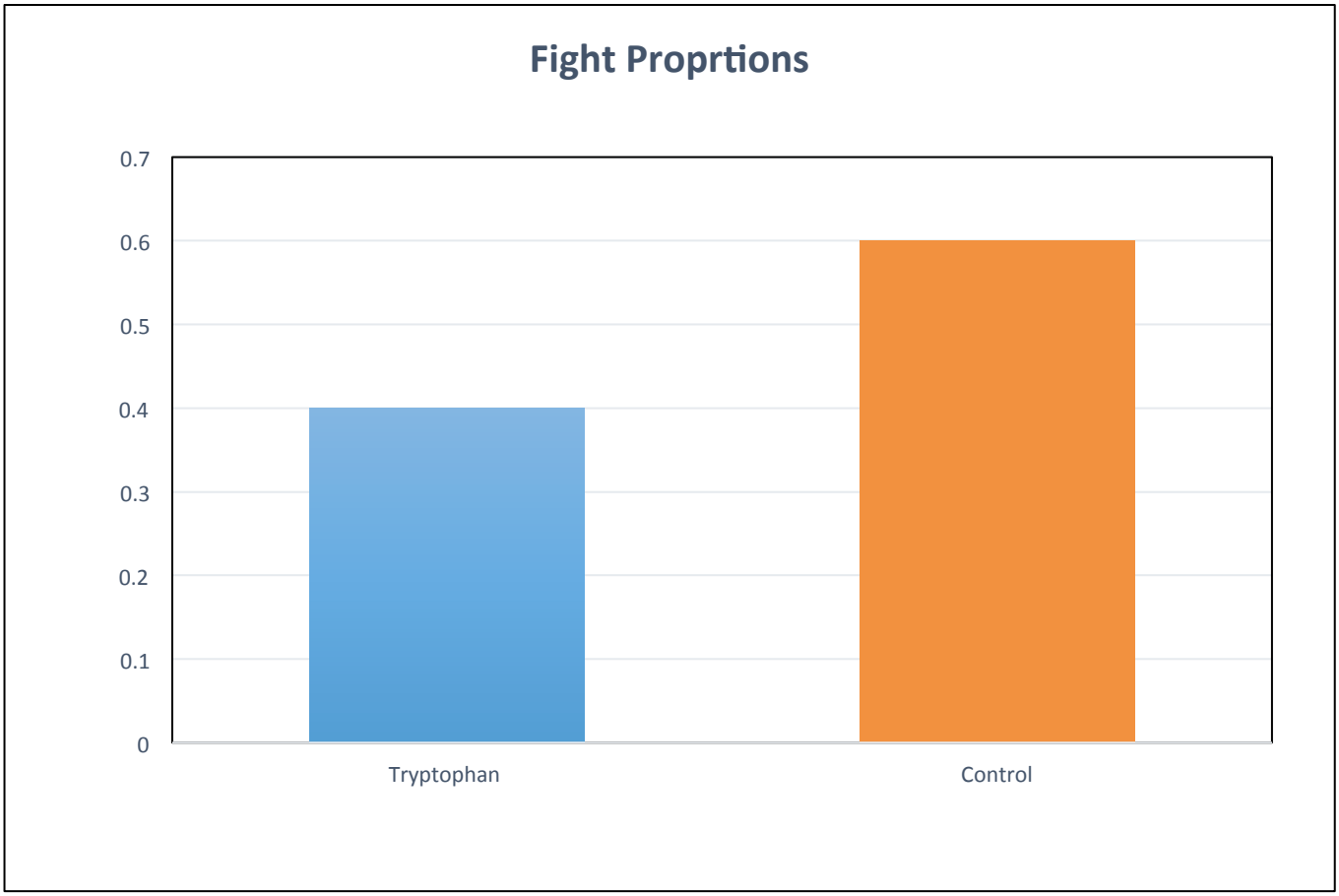

Figure 5: Proportion of fights won by male crayfish with tryptophan injection against vehicle control crayfish.

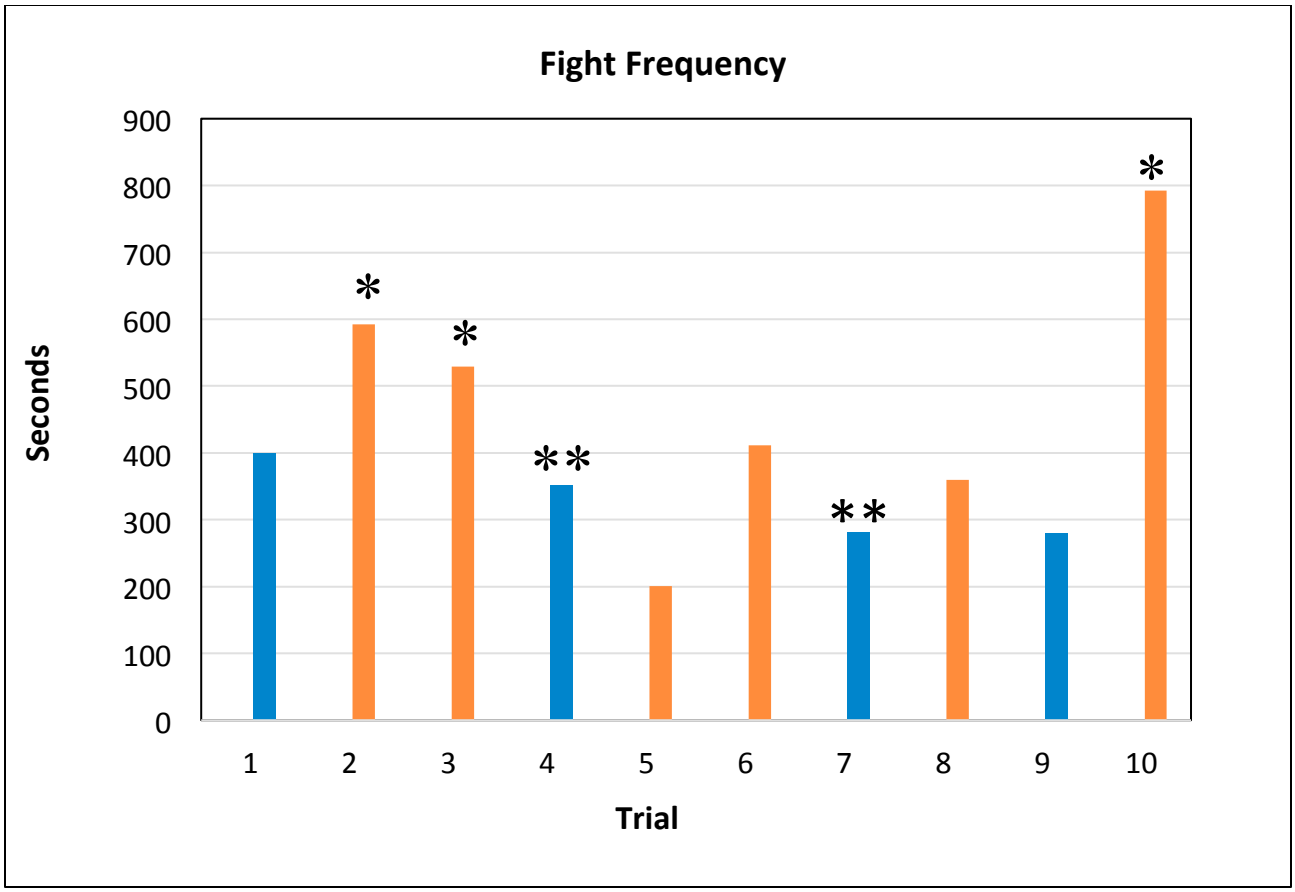

Figure 6: Fight frequency for each trial. The blue bars represent the fight won by the crayfish with tryptophan injection. The * represents the number of fluorescein bursts.

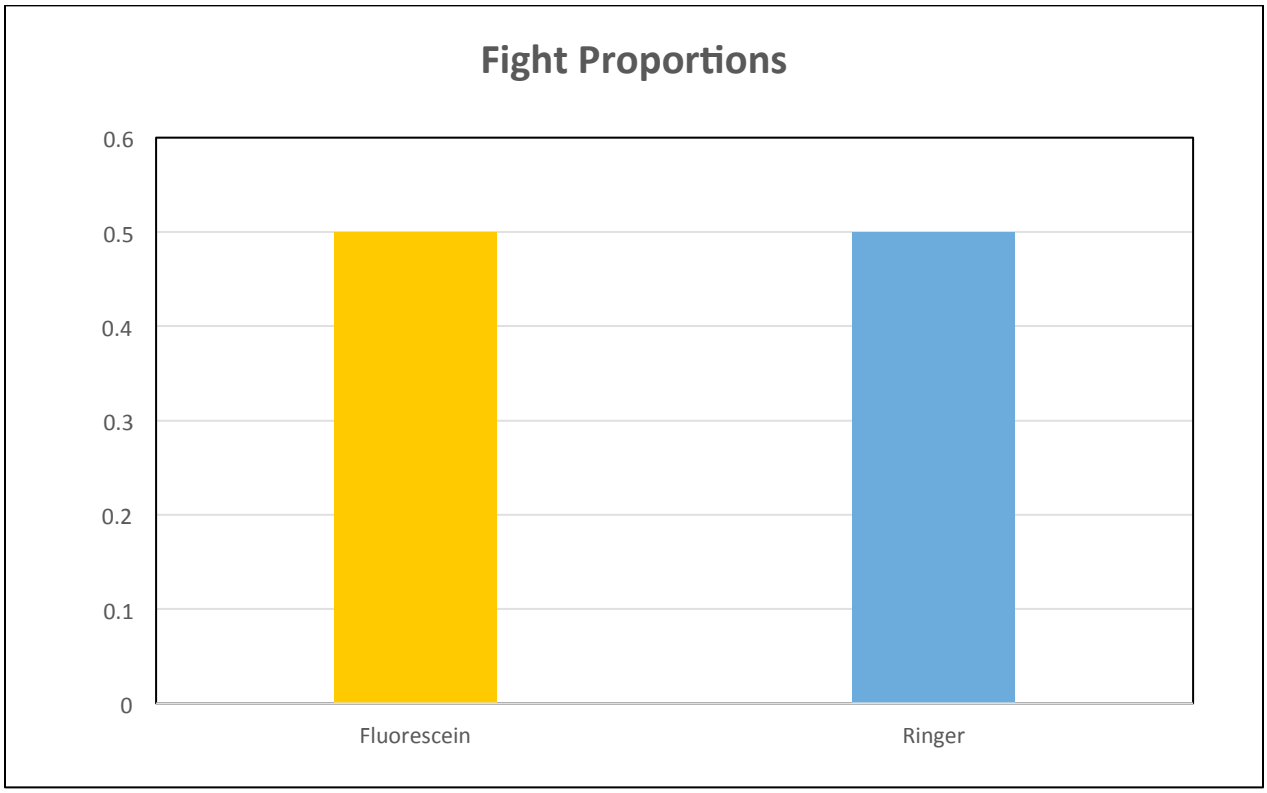

Figure 7: Proportion of fights won by male crayfish with fluorescein injection against vehicle control crayfish (ringer).

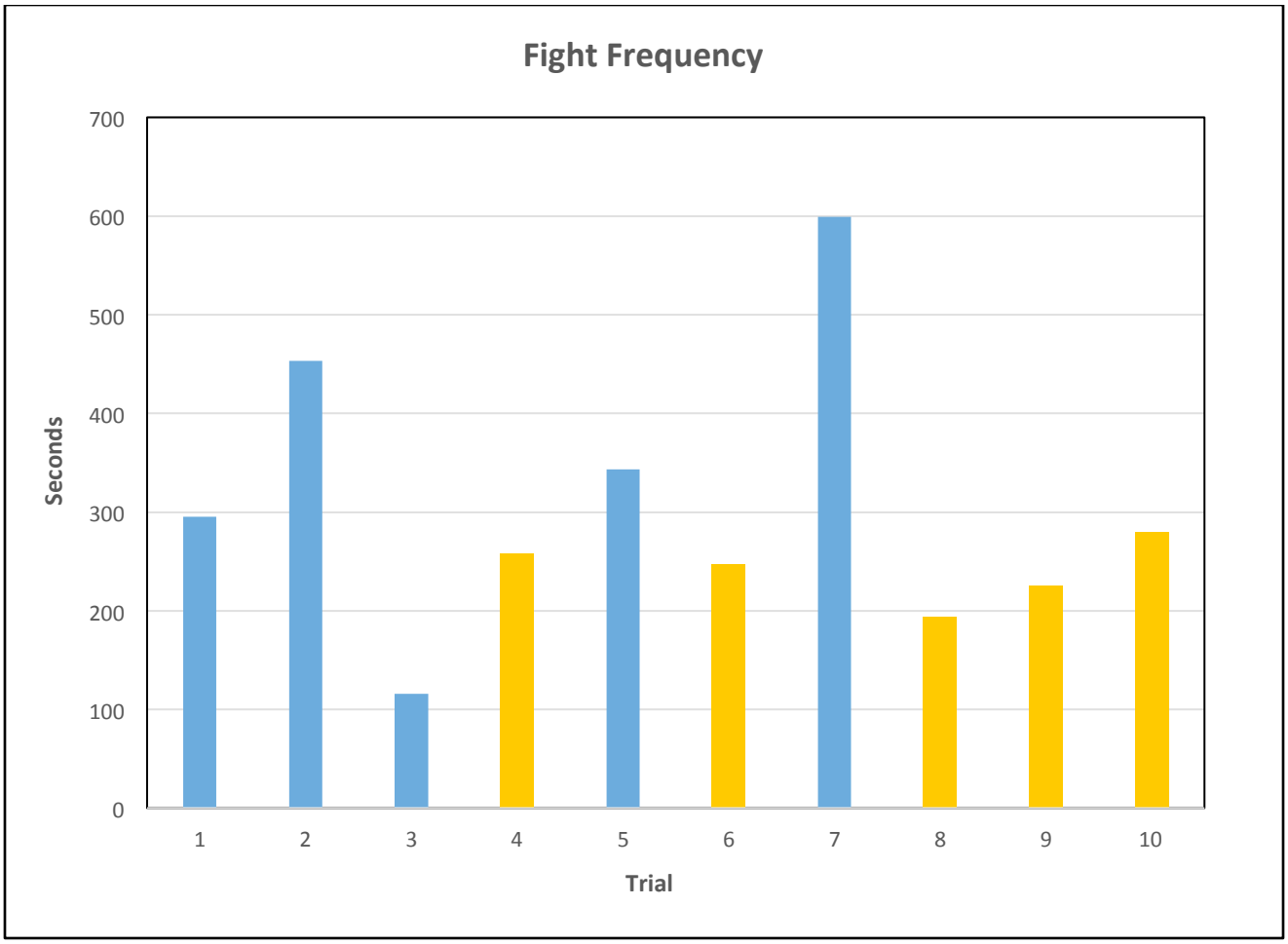

Figure 8: Overall duration of fights for each trial. The blue bars represent the fights won by the crayfish with ringer injection.

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