Role of Sensory Signals in Maintaining Dominance Hierarchies in Crayfish

Julian Vallyeason[1], Dr. Jens Herberholz[2]

[1] Thomas Jefferson High School for Science and Technology, Alexandria VA; [2] University of Maryland Department of Psychology

Introduction

The formation of social hierarchies in crayfish is mediated through a series of agonistic bouts. Once established, levels of aggression between conspecifics is drastically reduced. These hierarchies provide structure to crayfish communities, establishing mating privileges, and access to food and shelter.

The outcome of these aggressive interactions between crayfish is strongly correlated with levels of neurotransmission. Recent studies have shown that serotonin (5-HT) and dopamine (DA) increase motor activity and initiate an elevated posture in crayfish, which is correlated with dominance (Tierney et al. 2004). Octopamine (OA), an invertebrate hormone and neurotransmitter closely resembling norepinephrine, is associated with submissive behaviors in crayfish (Momohara et al. 2013).

These hierarchies are malleable, and subject to disruptions including changes in population and changing environmental factors (Aquiloni et al. 2009). However, a source of stability in these hierarchies is the winner-loser effect, where crayfish who have won previous encounters are likely to win future encounters with opponents (Issa et al. 1999).

The formation of a hierarchy is often dependent on which crayfish initiates a tail flip. The tail flip is a cardioid escape reaction in crayfish that is modulated by both giant interneurons, and non-giant neurons, as a means for protection (Edwards et al. 1999).

The hypothesis under investigation was that disruption in sensory signal transmission would alter hierarchy stability and aggression in crayfish. This was tested through measuring changes in aggression and submission across two fights, before and after treatment.

Materials and Methods

Measuring changes in crayfish aggression due to sensory stimuli takes place over two weeks, with two agonistic encounters recorded, serving as a baseline and experimental condition.

Baseline Isolation:
Two size-matched crayfish were measured, and marked with either 1 or 2 dots on their carapaces. Each was placed on one side of an opaque divider, allowing for no contact between the two. Dissolved oxygen bubblers were placed in the tanks.

Baseline Fight:
Both crayfish were removed from isolation tanks, and moved to a fight tank of similar dimensions. Each crayfish was allowed three minutes to acclimate to the new environment with a removable opaque divider placed in the middle. Upon removal of the divider, the behavior of both crayfish were recorded using a video camera for thirty minutes.

Experimental Isolation:
Crayfish were then moved to an experimental tank with either an opaque divider, a transparent (vision) divider, an olfaction divider, or a visual + olfaction (VO) divider. One food pellet was provided to each animal, and dissolved oxygen bubblers were placed on each side.

Experimental Fight:
Crayfish were removed from experimental tanks, and moved to a fight tank. After three minutes of acclimation, the agonistic bouts of both crayfish were recorded with a video camera for thirty minutes. Both crayfish were placed in a junk tank following the experimental fight.

Statistics and Analysis:
Data was analyzed using a Wilcoxon-Signed Rank test (non-parametric) for ($\alpha<0.05$) and a Kruskal-Wallis H-Test (one-way analysis of variance) with ($\alpha<0.05$) and a Friedman test (ordinal) with ($\alpha<0.05$).

Scoring Methodology and Examples

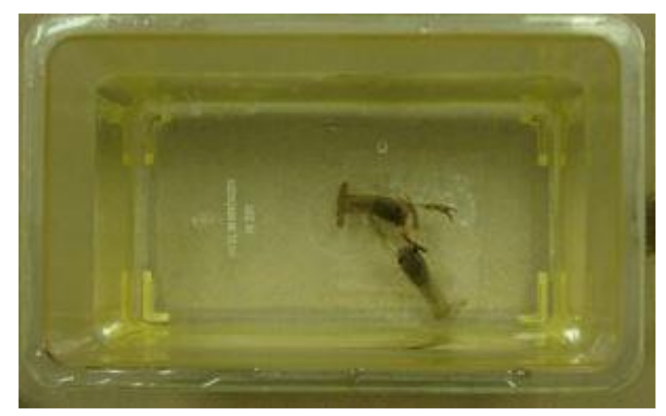

Figure 1. Two crayfish engaged in an attack maneuver. Claws remain outstretched with an elevated body position.

Figure 2. The three steps characterizing a crayfish engaged in an escape. (A) Approaching crayfish establishes physical contact with the caudal portion of the body, initiating a lateral giant (LG) tail flip. (B) Flexion production leads to the characteristic tail flip to escape the aggressor. (C) Re-extension of muscle fibers following the tail flip stabilizes the body.

Results

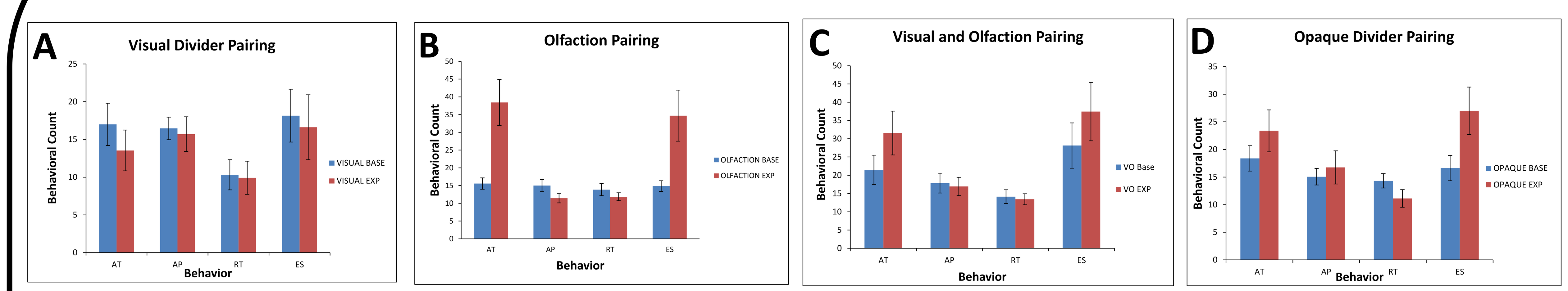

Figure 3. Mean behavioral counts for agonistic encounters in *Procambarus clarkii* during baseline and experimental fights. (A) Following visual divider pairing, all mean behavioral counts experienced a modest decline. (B) Following olfactory divider pairing, counts for attacks and escapes significantly increased (W=2<8, p=0.01<0.05) and (W=4<8, p=0.02<0.05). (C) Following visual and olfaction pairing, counts for attacks and escapes experienced a modest rise, while counts for approaches and retreats experienced modest declines. (D) Following opaque divider pairing, counts for escapes significantly increased (W=15<25, p=0.01<0.05).

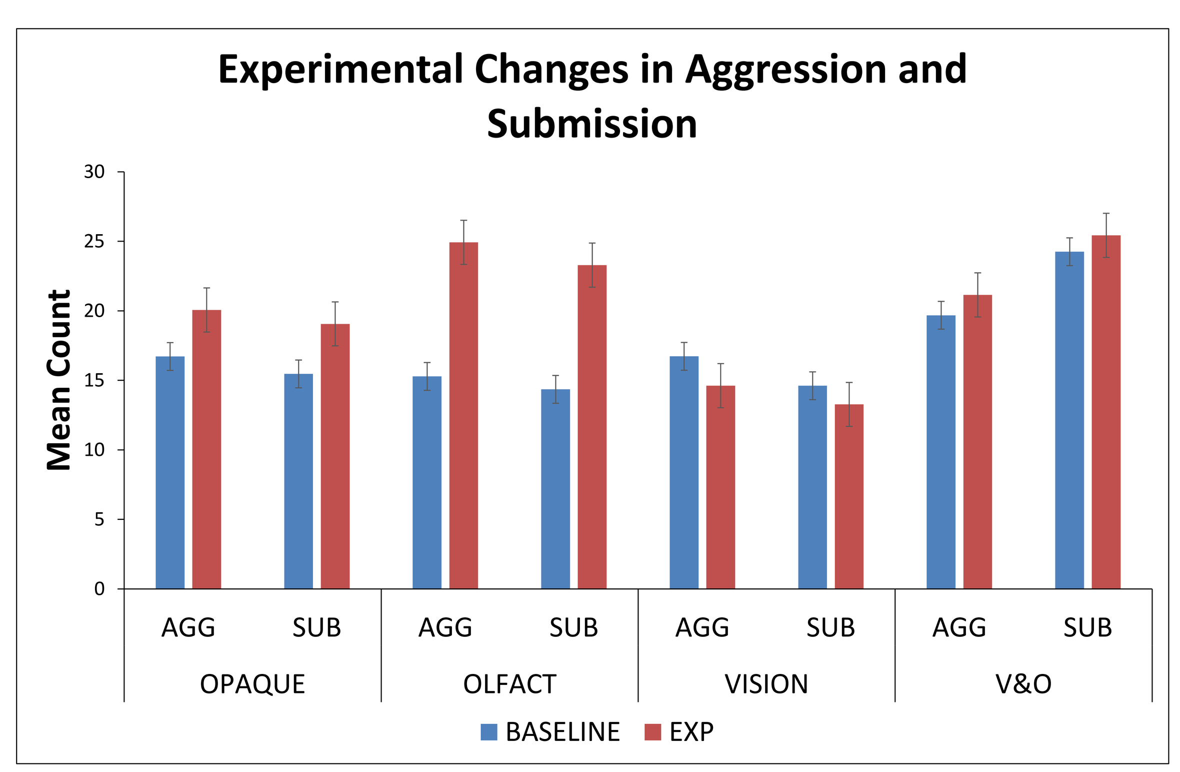

Figure 4. Calculated levels of aggression and submission in *Procambarus clarkii* following a baseline fight for each experimental condition. The olfaction condition displayed a significant increase in both aggression and submission following a baseline fight (W=3<8, p=0.01<0.05) and (W=5.5<8, p=0.025<0.05).

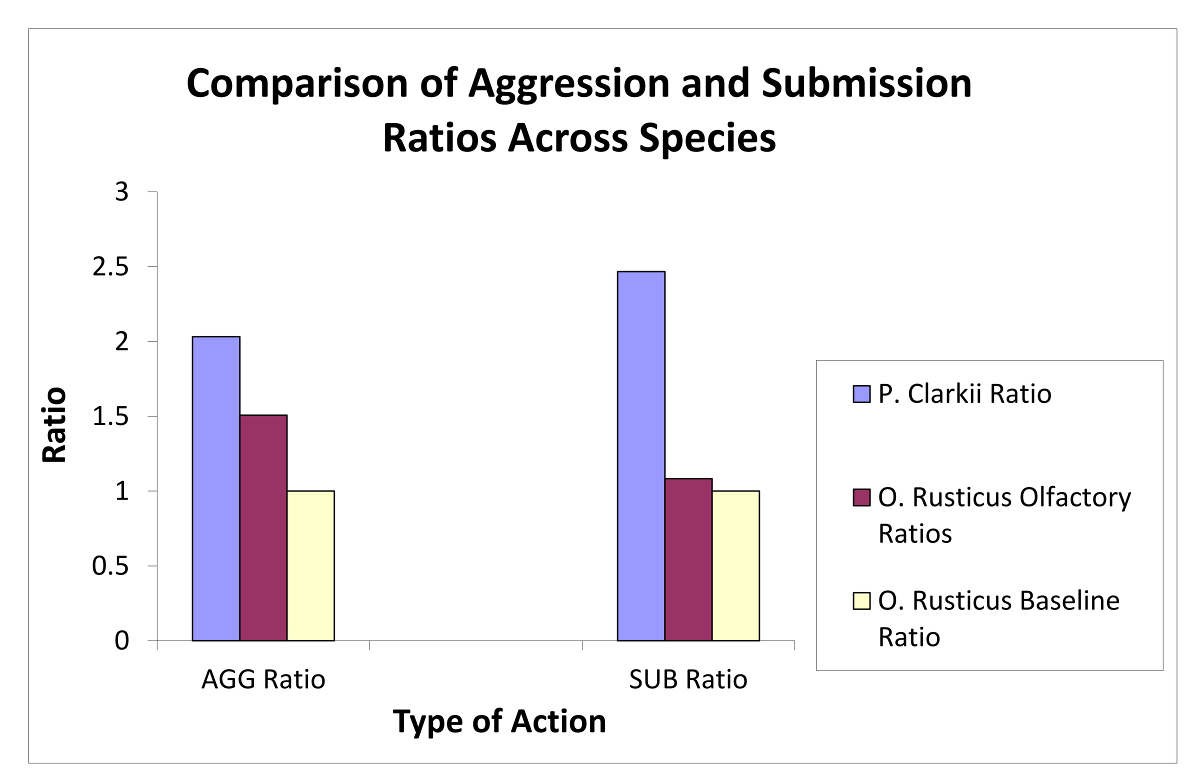

Figure 5. Ratios of aggression in *Procambarus clarkii* and *Orconectes rusticus* following baseline fights. The baseline ratio for *O. rusticus* was set to 1 to form a basis of comparison for other species and conditions.

The heightened levels of aggression and submission observed in crayfish placed under the olfactory condition indicates that disruption of sensory signal transmission affects the stability of the social hierarchies formed during the baseline fight. Visual contact appears to have the effect of stabilizing hierarchical structure due to the maintenance of aggression and submission following treatment with a transparent divider. Modest increases in aggression and submission in both the opaque and VO condition indicate that neither aggression nor submission remains constant between fights where forms of contact are allowed between animals. Compared to *Procambarus clarkii, Orconectes rusticus* has a lower overall level of aggression during both baseline and olfactory fights. However, the effect of treatment with a visual and olfactory divider remained the same across both species. This indicates that the mechanisms through which these dividers influence aggression and hierarchy stability remain relatively constant in species of crayfish.

Conclusions

The increase in both aggression and submission following olfactory contact indicates that the transfer of chemical signals using antennae and secreted urine have the ability to disrupt the stability of crayfish social hierarchies; visual contact alone appears to be sufficient in maintaining a crayfish hierarchy. The knowledge of an intruder that cannot be visually identified might cause heightened aggression due to the territorial nature of crayfish. Thus, our hypothesis was supported, as disruptions in sensory signal transmission altered levels of aggression.

Limitations in experimentation include the manual scoring of agonistic encounters and the transfer to a separate fight tank. In manually scoring behaviors, there remains room for human error. The transfer to a separate fight tank opens the plausible hypothesis that aggression levels could be modulated through transfer to a separate environment. A negative control was run with no divider following a baseline fight to determine the effect of the new environment on aggression; however, under an open condition, one crayfish was prone to killing the other.

The increase in aggression caused by the olfactory condition leads one to consider what neurotransmitters and chemical pathways are altered during the second fight. Future studies should conduct cellular physiology on crayfish following olfactory treatment. Drugs targeting specific pathways should be tested to reduce elevated aggression. Possible translational studies to humans with elevated aggression due to mental disorders could be conducted.

References

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Acknowledgements

I would like to thank the members of the University of Maryland's Herberholz Lab and Dr. Jens Herberholz for providing me with the inspiration and resources necessary to conduct the project. I would also like to thank the TJHSST Neuroscience Lab and Mr. Hannum and Dr. Locklear for providing me with the opportunity to pursue neuroscience research.