THE EFFECTS OF EXOGENOUS SEROTONIN AND OCTOPAMINE ON LOCOMOTION, LEG MOVEMENT, AND TAIL CURLING IN *ORCONECTUS VIRILIS* AND *O. RUSTICUS*

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Introduction

Biogenic amines such as serotonin and octopamine may play a large role in behavioral responses such as escape behavior and physiological responses including heart rate (Listerman, 1999). For instance, serotonin acts on the lateral giant escape circuit in crayfish, which regulates escape behaviors (Glanzman and Kransne, 1983; Yeh et al., 1996, 1997). In addition, serotonin affects other behaviors such as aggression in several crayfish species (*P. clarkii, Astacus astacus* and *O. rusticus*) (Tierney et. al, 2004; Livingstone et al., 1980).

Most studies have concluded that serotonin increases aggression while octopamine suppresses it (Huber and Delago, 1998; Huber, Smith, et. al, 1997). Injection of serotonin in crayfish can induce an aggressive "5-HT posture" in which the animal is elevated above the substrate and the abdomen is flexed, as well as other postural changes such as increased leg wave (Livingston et al., 1980; Tierney et. al, 2004). Although many studies on serotonin and octopamine on aggressive behavior have been performed, few have researched the effect of either neurohormone on locomotion (Peeke et al., 2000). Those that have tested locomotion indicated variable results depending on species of crayfish (Tierney et. al, 2001, 2004).

Locomotion has also been suggested to be effected by an increased concentration of serotonin during nighttime hours (Escamilla-Chimal et. al, 2001). Because serotonin increases heart rate and heart rate and locomotion are directly correlated (Chabot et. al, 2008), we hypothesized that that serotonin would increase locomotion. Based on this hypothesis, we injected the crayfish during the day when they are naturally inactive. Since octopamine reportedly has opposite effects on other behaviors, we hypothesized it would decrease locomotion and therefore we injected it at night. This experiment aimed to determine if serotonin or octopamine had an effect on behaviors such as locomotion, flexion, or leg wave in the crayfish species *O. Rusticus* or *O. Virilis*. Specifically, long-term effects of these neurohormones on isolated crayfish were determined, which have not been examined in any previous research.

Methods

Animals and Environmental Conditions

Eight northern crayfish, four each of *Orconectes virilis* and *Orconectes rusticus*, were obtained from a commercial supplier. The animals were housed in eight separate one-gallon fish tanks fitted with visual dividers and provided with bubblers and sections of carved pipe for shelter. Both lighting and temperature were manipulated to reproduce an environment consistent with natural conditions. Florescent lighting was positioned above the animal tanks and switched on at 6 am and off at 8 pm to mimic natural day and night conditions. The temperature was maintained at 17˚C to reduce temperature fluctuations. The crayfish were fed once a week with shrimp pellets (Wardley) purchased from a local pet shop. Data were gathered for seven weeks.

Drug injections

Each crayfish was injected with 5-HT and OA (Sigma) using 1-ml 29G syringes. A low and a high dose of each neurohormone (0.3 and 3.0 mg/kg, respectively) were determined from previous studies involving the use of neurohormones on crustaceans (Listerman, et al. 2000)(Savage and Atema 2003). Each crayfish was weighed and averaged (12 grams) to determine the specific volumes of 5-HT and OA for each dosage level. These amounts were diluted to produce equal volumes of 100µl using crayfish saline of the following composition: 205.34mM NaCl; 5.36mM KCl; 13.43mM $CaCl_2{\cdot}2H_20$; 2.61mM Mg $Cl_2{\cdot}6H_20$; 9.99mM Tris, pH adjusted to 7.4. Each animal served as its own control. To account for handling, sham injections of an equal volume of 100µl crayfish saline were injected for the first week. All serotonin injections took place daily at 12:30pm. Octopamine injections were performed nightly at 8 pm. Saline injections also took place at both times. Each crayfish was uniformly handled for thirty seconds and injected with test solution into the base of third walking leg.

Recording procedures

In order to record the physical movement of each animal, the speed and total movement of each animal was recorded. This data was obtained using the "Big Brother" video surveillance system consisting of a computer, camera, infrared lights, and all necessary connections (Coulbourn Instruments, Lafayette, IN). The Big Brother system calculates the distance which the animal moves per minute. It has been set to record the position of the animal once every 5 seconds. At every 1 minute interval, Big Brother finds the total sum of the of the distance moved within that minute. The Distance is set to scale based on the width of the chamber, in this particular experiment the width is set to 15cm.

Data on posture was collected by observing each animal for 20 minutes before injection and 20 minutes after injection. A grading system was established for degree of tail curl and leg wave: no movement (0), some movement (1), and lots of movement (2).

Data Analysis

Actograms were analyzed both visually and by Lomb-Scargle periodogram analysis ($p<0.001$) to determine the significance of rhythmicity. To compare the effect of varying levels of injected neurohormones with sham injections, both ANOVA and t-test analysis were performed. The t-test analysis was used to compare the before and after activity level of the crayfish for each injection variation (saline, low, and high). P -values indicated a significant difference between control and test injections ($p<0.05$). ANOVA was also run to determine if a significant difference in locomotion occurred between the varying dosages for all tanks ($p<0.07$).

Effects of 5-HT and OA on Activity Patterns

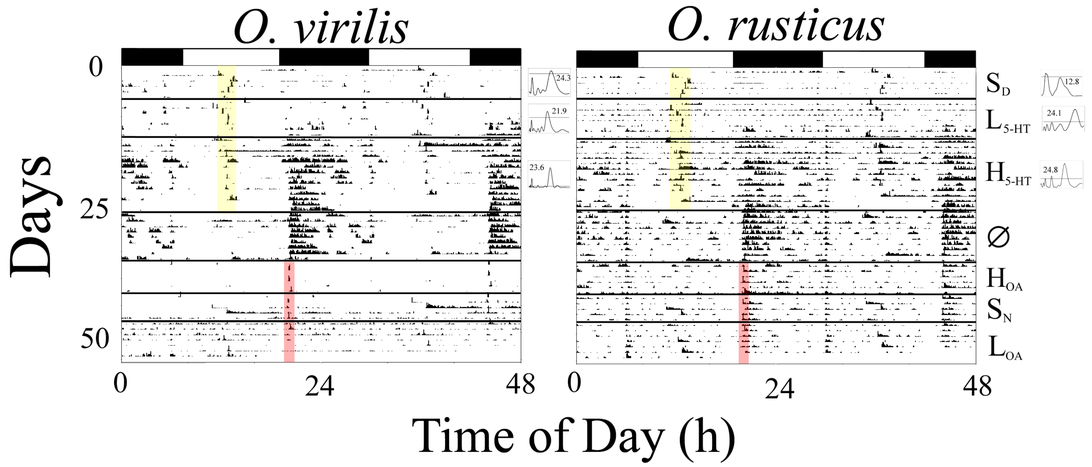

Figure 1: Representative actograms (large boxes) and periodograms (small graphs) of 5-HT and OA on *O. virilis* and *O. rusticus*. Actograms show the locomotor activity during day saline (S_D), low 5-HT ($L_{5\text{-}HT}$), high 5-HT ($H_{5\text{-}HT}$), no injection (∅), night saline (S_N), low OA (L_{OA}), and high OA (H_{OA}) periods. Spikes indicate the amount of movement. Yellow box-daytime injections; red box-nighttime injections. Periodogram analysis showed a clear change from a 24-h to 12-h cycle in 3/5 *O.rusticus* and 1/3 *O. virilis* when injected with high 5-HT. Above are two of the four actograms that appear to show a difference in movement due to OA. However, OA data was inconclusive due to the other four actograms showing no difference.

5-HT and OA have Varying Effects on Behavior

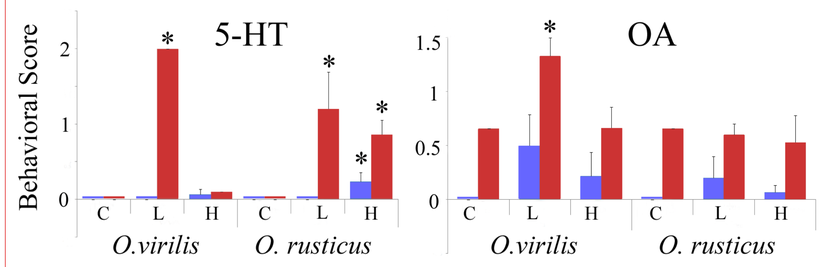

Figure 3: A comparison of the effects of 5-HT and OA on tail curling and leg wave in *O. rusticus* and *O. virilis*. Blue and red bars represent the degree of tail curl and leg wave, respectively. Bars indicate the average degree of each behavior 1-h after injections as determined by a grading scale ±SE (grading scale can be seen in methods). Included is data of control ((C) 0mg/kg)), low (L) and high (H) injections (0.3mg/kg and 3.0mg/kg, respectively). of 5-HT and OA n=5 *O. rusticus* and 3 *O. virilis*. An asterisk represents a significant difference from the saline control at the $P<0.05$ level. This figure shows that high 5-HT caused changes in behavior such as increased leg movement in both *O. rusticus* and *O. virilis* and increased tail curling in *O. rusticus*. Although OA increased leg movement in *O. virilis*, it had no effect on either tail curling or leg movement in *O. rusticus*.

OA Produces No Effects

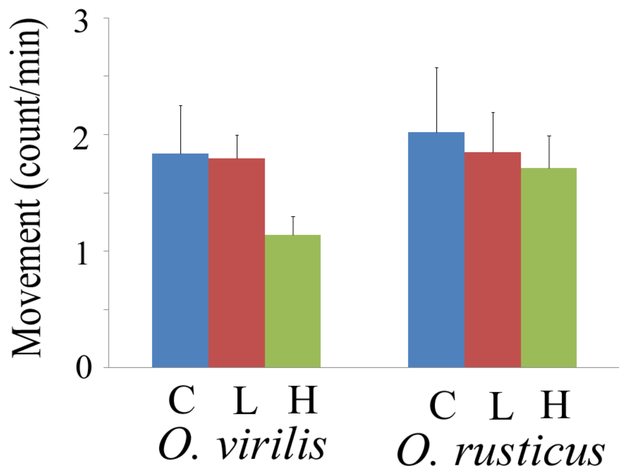

Figure 2: Effects of octopamine (OA) on *O. rusticus* and *O. virilis*. Blue bars represent the control (C), red is low dose (L), and green is high dose (H). Bars indicate mean movement (count/min) ±SE for1-h after injections across experiments of the control (0mg/kg), low OA (0.3mg/kg), and high OA (3.0mg/kg) injections. n=3 *O. virilis* and n=5 *O. rusticus*. This figure shows that neither crayfish species experienced a significant change in movement due to any of the above injections.

5-HT Increases Movement in *O. Rusticus*

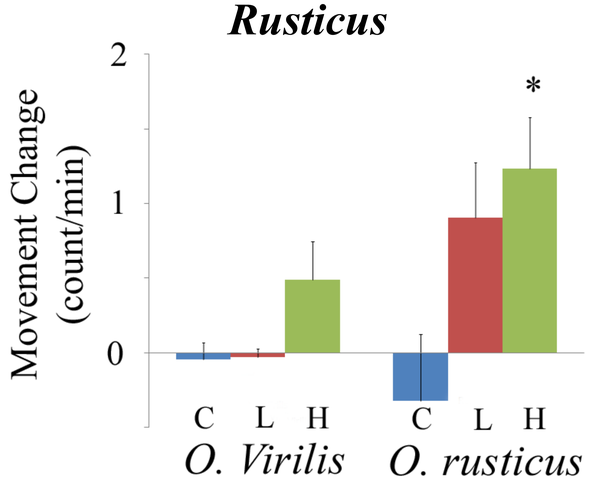

Figure 4: A comparison of the effects of serotonin (5-HT) in *O. rusticus* and *O. virilis*. Blue bars represent the control (C), red is low dose (L), and green is high dose (H). Bars indicate the average change in movement 1-h before and 1-h after injection time ±SE. Bars in the negative region indicate a decrease in movement. Included is data of control (0mg/kg), low 5-HT (0.3mg/kg), and high 5-HT (3.0mg/kg) injections. n=5 *O. rusticus* and n=3 *O. virilis*. An asterisk represents a significant difference from the saline control at the $P<0.05$ level. This figure shows that only high 5-HT in *O. rusticus* caused a significant increase in movement.

Conclusions

- Differing effects on tail curling and leg movement were observed.
- 5-HT increased locomotion on crayfish over long periods of time.
- Existence of interspecies differences in crayfish behavior as an effect of 5-HT was supported.

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