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Identifying Temporal Differences in Vibration Receptor Activation in the Sand Fiddler Crab, Uca Pugilator

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Abstract:

Substrate vibration is used in numerous organisms as a means of communication. Herein we explore the role of substrate vibration in the process of localization in Uca Pugilator, the sand fiddler crab, by tracking temporal differences in receptor activation. Extracellular recordings of receptor activity were amplified and analyzed. Analogous systems are outlined and applied to our case, including relevant information from studies on scorpions. It seems the fiddler crab uses a spatial array of vibration-sensitive receptors to localize sound sources. Our behavioral studies, along with others', show that fiddler crabs are able to process these time differences on a millisecond scale. Studies are ongoing to determine how central neural pathways process this vital information.

Introduction:

The fiddler crab is well known for its sexually dimorphic claws. The males possess one larger claw or chela and one small claw, while females have two small claws. During the daytime, males occasionally utilize their major claws to fight off other males in competition for females. Furthermore, males use this large claw in a waving display to attract potential mates. However, waving displays are poorly visible at night so crabs must rely on other means of communication. When night falls, male fiddler crabs use their large chela to send vibrational signals to potential mates or competitors (1). These signals are called raps and are created by fiddler crabs drumming the sand outside their burrows. These vibrational signals are detected by Barth's Myochordotonal Organ (MCO), which functions as a tympanic membrane with the capacity to convert mechanical disturbances into electrophysiological pulses which can be processed and refined by the crab's neural network (1 & 2). Barth's Myochordotonal Organ is located in the upper portion of each walking leg, known as the merus. It is believed that the lower portions of the leg transmits vibrational stimuli to the MCO which then activates vibration sensitive (VS) neurons in the central nervous system (CNS) (3).

Vibrational stimuli are not always intentional, and animals frequently create incidental vibrations that can attune others to their locations. These vibrations, produced unwittingly by the movement of predators or prey are also received by the MCO, unrestricting the confines of vibrational relevant information to just mating behaviors. This extra sensory detail provides the animal with a better understanding of its environment heightening both its predator avoidance and overall fitness (4).

Communication can be described as transferring information from a sender to a receiver so that the receiver's behavior is modified in a predictable fashion with adaptive value for the sender or receiver, or perhaps both (4 & 5). The fiddler crab and numerous other organisms (outlined below) utilize

specific waveforms to communicate and interact. The waves pertaining to *Uca Pugilator* include compressional P (primary) waves and love waves, which have propagation velocities of approximately 100 m/s and 40 m/s, respectively.
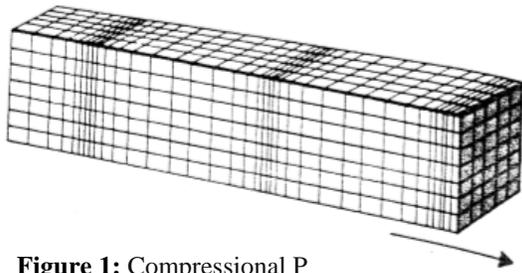

Figure 1: Compressional P waves can be categorized by direction, speed of propagation, and attenuation.
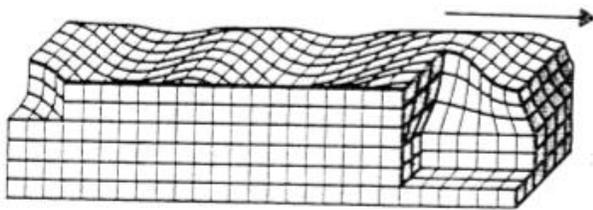

Figure 2: Love waves or transverse surface waves.

These values are well within the range of resolution for other arthropods such as scorpions, and can be altered naturally depending on factors such as soil compactness or sand moisture content (6).

Analogous Systems:

The detection of vibrationally relevant information is found throughout the animal kingdom with numerous species from insects to elephants utilizing this form of communication (7). Perhaps the best studied of these animals is the nocturnal scorpion, *Paruroctonus mesaensis*. At the end of each walking leg of the scorpion there are two sense organs, the basitarsal compound slit sensilla (BCSS) and tarsal sensory hairs, which are excited by substrate vibrations that are likewise conducted through the sand (8). The scorpion's eight legs form a spatial array which can detect slight differences in arrival time of a substrate derived signal, indicating which direction the scorpion should orientate itself if it need flee or attack. Some spiders use similar localizations techniques as described above with very comparable organs between the two (8 & 9). Similarly, elephants may be able to detect substrate-borne vibrations due at least in part to "acoustic fat" located in their feet. The animals take on a unique posture upon "listening" where more pressure is placed on the front feet and the ears are aligned, with the feet, perhaps heightening their attentiveness to these detectable vibrations (10). Even humans are known to maintain a level of substrate-borne vibration discrimination, expanding this communication techniques applicability. Congenitally deaf human subjects were able to discriminate frequency differences between vibrational stimuli delivered to the left hand (11). Such widespread use of substrate-borne vibrational signaling, especially those examples from morphologically similar animals, make this form of communication a likely candidate in the case of the fiddler crab *Uca Pugilator*. Though it is probable the fiddler crab uses this medium, the question remains as to how various receptors in the merus are activated and how the animal uses this information to quantify temporal cues available for localization. Herein, I address the above question in hopes of understanding how the crab utilizes these inputs to detect actions in its immediate environment.

Materials and Methods:

Subjects:

Male and female *Uca Pugilator* fiddler crabs (**Figure 3)** were collected from either Folly Beach, South Carolina or purchased from Gulf Specimen Marine Laboratory located in Panacea, Florida. The specimens were immediately placed into a tank filled with sand and circulating seawater upon arrival at the University of Tennessee, Knoxville.

Surgical procedure and positioning of the animal:

Both males and females with a full set of legs were used in the procedure. Currently molting crabs were avoided due to their softer than normal shell. Initially, the two claws were removed via tweezers. A straight pin was then used to create a small puncture in the dorsal carapace where a ground wire would later be inserted. The specimen was suspended over a sand-filled arena that was previously dampened to mimic natural conditions. The crab was attached to a Plexiglas rod via a dab of superglue; this rod, with the crab attached, was then fastened to a ring

Figure 3: A male *Uca Pugilator*

stand so that all eight of the crab's legs could touch the moistened sand, yet the crab could not flee. Once again, a pin was used to create a tiny puncture in the second walking leg on both the right and left sides of the animal. These puncture wounds were created in the ischium of the crab's leg to not damage the receptor located in the merus. In these three puncture sites (two in the legs and one in the back carapus) a ten-micrometer silver wire was inserted to detect electrical signals. These silver wires were connected to amplifiers capable of better visualizing the outputs (**Figure 4)**.

Recording techniques:

The sand arena was previously marked at specific locations approximately 10 cm from the animal to the right, left, front, and back. During times of minimal crab movement, vibrational stimuli were generated at

Figure 4: Experimental setup with animal suspended over sand-filled arena

these four locations via a forty-gram metal ball that was dropped approximately ten times at each location. Electrical signals due to receptor activation were received via the silver wires and amplified. These amplified signals were visualized and stored for off-line analysis using Labchart.

Results:

Data was obtained from 15 mature *Uca Pugilators*. For each crab, an average of forty data points was collected and stored to later be analyzed. These raw recordings demonstrate a vague pattern but upon magnifying the recordings a trend was much more evident. In general, when the stimulus was presented from the rear or front of the animal, there was little if any difference in receptor activation times. However, when the stimulus was presented from either the right or the left side, a clear difference in time of receptor activation could usually be identified. An example of one of the many recordings is presented in **Figure 5**; as evident in the figure, there is little difference when the stimulus was presented from the front, but a clear difference when it was presented from the left. As expected, the receptors on the crab's left were stimulated first followed by the receptors on the crab's right. The review of the individual recordings was followed by a statistical analysis of each data set. For crabs that had over ten recording at each of the four positions (right, left, front, and back) a one-way ANOVA (Dunn test) was run on the time differences. An example corresponding to animal number ten is present in **Figure 6 and 7**. As predicted, the only groups that were not significantly different are the F vs. B and the R vs. L, indicating that discrimination between these values was negligible.
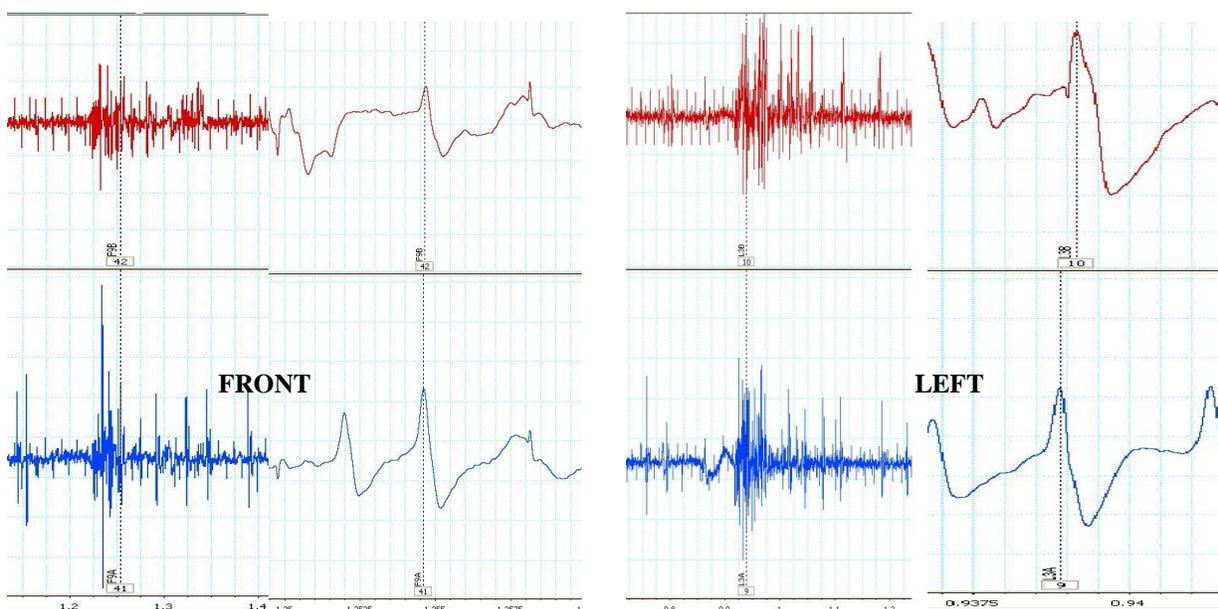

Figure 5: The raw recordings are shown on the left of each couple, while the magnified version of the same recording is depicted on the right. The blue plot translates to the left receptor; the red plot is the right receptor.

Average Time Difference of Receptor Activation

Stimulus Position	Mean with Standard Error (ms)
LEFT	0.47 ± 0.10
RIGHT	0.54 ± 0.09
FRONT	0.21 ± 0.05
BACK	0.11 ± 0.02

Figure 6: Data from Uca 10 (width approximately 3.6 cm). As predicted the front and back values are smaller than the left and right values.

One Way ANOVA/ Dunn Test

Groups	Diff of Means	T	P	P<.050
R vs. B	.426	4.258	<.001	Yes
L vs. B	.351	3.509	.006	Yes
R vs. F	.334	3.176	.012	Yes
L vs. F	.259	2.464	.0555	No
F vs. B	.0919	.892	.613	No
R vs. L	.0750	.732	.469	No

Figure 7: The means from figure 6 were used to run a Dunn Test. The F vs. B and R vs. L groups should be similar because they correspond to the same stimulation from the same distance just at different sides of the animal. The L vs. F group is addressed in the text.

Conclusion & Discussion:

The above results make sense if the stimulus was presented equidistant from the crab in each respective direction. Furthermore, when R vs. B was compared their respective values were very different, giving weight to the idea that these vibrational stimuli can be captured and interpreted in an appropriate time scale. It should be noted that hypothetically L vs. F should have a p value below .05 (actual value is .0555), but this minute mishap is due to a statistical cut off point rather than a significant finding.

In summary, the goal of the study was to better understand how fiddler crabs use substrate-borne vibrations governing localization. Receptors are activated in the merus as the legs receive useful vibrational signals. The combination of these signals is integrated and used to track the location of a mate/predator. If the signal is received on the crabs right initially then received on the left side, it becomes clear that the signal is passing from right to left (**Figure 8**). This flow of information could serve fundamental purposes for the animal, allowing it to localize vibrational stimuli. Our study indicates that fiddler crabs process these time difference on a millisecond scale based on spatial distribution of the legs. This time scale is fitting for other crab species. These findings point to the idea that fiddler crabs employ a spatial array for localization of vibration signals. This parallels the findings of Brownell and Farley in scorpions. In which, they found the basitarsal compound slit sensilla (BCSS) appeared to detect surface waves and convey information to the scorpion regarding direction of the vibrational source (8).

The underlying mechanism of how the animal processes this information remains to be elucidated. The central neural pathway is essential in transforming these time differences into usable information that can help localize "sounds". Questions remain as to how crabs isolate meaningful vibrations from useless ones or how the animals operate in such narrow time windows. Furthermore, can these animals use this spatial array to detect moving sounds? It would be interesting to repeat the above experiment with a vibration source that was capable of moving. Additionally, one could selectively ablate receptors on one side and most likely observe a loss of this localization phenomenon.
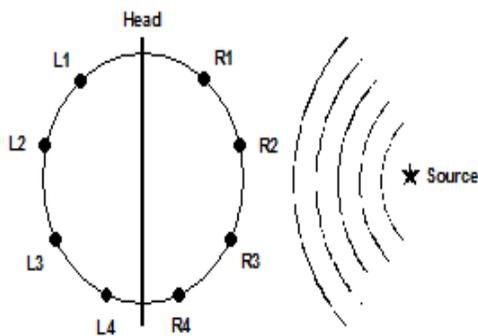

Figure 8: A signal traveling from right to left would contact R2 and R3 first, and eventually L3 and L2. This information is useful for sound localization.

Acknowledgements:

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