

Physiology 416 Neurophysiology Lab

Response Properties of *Ictaluras punctatus* Catfish
Ampullary Electroreceptors

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Abstract

*Catfish possess a passive electrosense. In order to determine some basic properties of this unusual mode of sensation, individual ampullary electroreceptors of the catfish *Ictalurus punctatus* were recorded extracellularly while under focal stimulation from a dipole stimulator. Two stimulus regimes were used: a sine wave of fixed frequency with varying amplitude, and one of fixed amplitude with varying frequency. These data enabled the determination of a firing rate versus frequency of stimulation relation, and a receptor sensitivity relation. With this information the issue of the fit of the receptors to their purported function of finding small prey just below the water substrate is addressed.*

0.1 Introduction

The skin of the catfish *Ictalurus punctatus* has numerous ampullary electroreceptors, also called small pit organs. An estimation of the number of receptors on the catfish *Ictalurus nebululosus*, based on assaying several patches of skin on the fish's body, was done by Peters *et al.* (Peters *et al.* , 1974). For one specimen the estimated number of receptors was 2,500, while for another it was 4,000. The density of receptors on the dorsal surface of the mature catfish head, the area recorded from for this experiment, was more consistent across the three subjects, at approximately 100/cm². Ampullary electroreceptors are concentrated at the anterior end of the catfish, a common pattern of electroreceptor distribution.

Passive electrosensation is defined in contradistinction to active electrosensation, whereby certain fish detect small perturbations in an autogenerated electric field to navigate and hunt (Kalmijn, 1988). Catfish, however, do not possess an electric organ. Catfish use their electroreceptors to subservise a passive electrosense, enabling them to detect the weak bioelectric fields of the small animals they prey on, which are often just below the surface of water body substrates (Peters & Bretschneider, 1972).

The impedance characteristics of the catfish electroreceptor membrane is such

that a small part of the afferent nerve extracellular current leaks through the pore of the electroreceptor (Peters *et al.* , 1988). As there is only one afferent per small pit organ, we therefore record single unit activity when monitoring one pore. In this experiment, the action potentials of small pit organ afferents located on the head of a catfish were extracellularly recorded while two different sinusoidal stimulation regimes were applied. The information gained from this protocol was used to determine the frequency response characteristics and sensitivity of the receptors. With this information, the issue of whether the animal's electrosense is apt for its purported function, the finding of small buried prey, is addressed with reference to some data on the bioelectric fields such prey emit.

0.2 Materials and Methods

The experiment was performed on immature catfish, *Ictalurus punctatus*. The exact age of the fish is unknown, but they were 15 cm in length. The fish were ordered through a local fish supplier, and were native to Illinois. Prior to the experiment the fish were kept in a large holding tank.

The experiment was performed with the fish in a 50-43-15 cm (l,w,h) Plexiglas tank. The fish was held in a harness, with a tube supplying aerated water to its mouth. Prior to placement in the tank, the fish was paralyzed with 2 μ L of Flaxedil, 10 % solution, applied intramuscularly.

For measurement of the extracellular afferent potential, an insulated tungsten electrode of 2 M Ω with an exposed tip of several μ m was used (A-M Systems 5752). The probe fed into an A-M Systems Neuroprobe differential AC amplifier, model 1600. Gain was set to 10k, with the low filter cutoff set to 100 Hz, high filter cutoff to 5 kHz, and the notch filter switched in.

Occlusion of the pores by excretions of the fish's skin prevented their clear identification under the dissection microscope, but with time a sense of pore loca-

tion developed and the majority of tries were successful. A number of pores were recorded from before a stable pore was found on the left dorsolateral aspect of the fish's head. The reference probe was placed in the tank approximately 5 cm away from the preparation, and a ground wire placed in the bath at one corner of the holding tank.

The sine wave stimuli were generated by a Wavetek arbitrary function generator, model 95. The stimuli were applied using a 1 cm custom dipole stimulator, placed approximately 1 cm away from the pore that was recorded from. Both the stimulator and the pore were approximately 3 mm under the surface of the water.

Data acquisition was with a Sun Sparc 2 with an Analyx SBUS card, running the JUDAS data acquisition system (Payne, 1995). The afferent nerve activity was captured on 2 channels, one continuous record and one that displays spikes in the recording that are over a set threshold. The stimulus from the generator was recorded on a third channel. The sampling rate for the two afferent nerve recording channels was 6,944 Hz, and the sine wave stimulus was sampled at 868 Hz.

In order to determine the frequency response relation, firing rate was estimated by dividing the measurement time at a particular stimulus setting by the total number of spikes recorded on the spike channel for that period. The recording times that were averaged over varied from 6 to 20 seconds, and the number of spikes in these intervals varied from 91 to 2,494.

In order to determine the receptor sensitivity relation, spikes per cycle of applied sine wave stimulation was calculated. Since the frequency was fixed at 8 Hz, the number of spikes in the spike channel was divided by 8 times the measurement time in seconds. Measurement times were in the region of 5 to 30 seconds, with the majority in the latter range.

0.3 Experimental Protocol

Two different stimulation regimes were used. In the first, a sine wave of 8 Hz, the optimal stimulation frequency for *Ictaluras nebululosus* ampullary electroreceptors (Peters *et al.* , 1988), was held fixed while the amplitude across the dipole stimulator was varied. In the second regime, the sine wave amplitude was fixed at some point roughly in the middle of the operating region of the electroreceptor, and the frequency was varied from 0 to 20 Hz.

Table 1 shows the full stimulus set for the two regimes. After each stimulus, the electroreceptor was allowed to recover for a minimum of 60 seconds.

Stimulus	
<i>f</i>, Hz	<i>V</i>, pp
8	0.4
8	2.0
8	2.4
8	3.0
8	4.0
8	8.0
8	10.0
0	4.0
.001	4.0
1	4.0
2	4.0
3	4.0
4	4.0
5	4.0
6	4.0
7	4.0
8	4.0
9	4.0
10	4.0
11	4.0
12	4.0
20	4.0

Table 0.1: Catfish Stimulation Protocol

0.4 Results

The spontaneous activity of the afferent was in the area of 60 Hz. A sample of three of the spikes on the continuous record channel is shown in figure 1. A sample of one half of a second of spike activity is shown in figure 2.

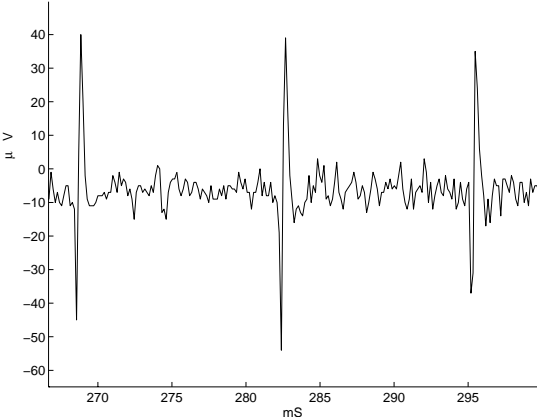


Figure 1: Sample afferent nerve spikes, spontaneous activity

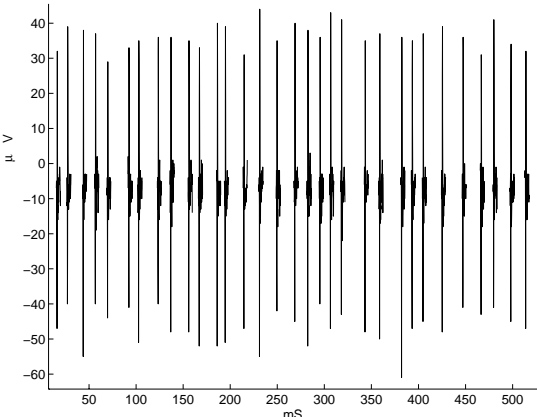


Figure 2: Spontaneous activity, spikes only

Over the recorded period of spontaneous activity, 1.226 S, there were 76 action potentials for an average firing rate of 62 Hz. A segment of afferent nerve activity

during 8 Hz stimulation at 2.4 V pp is shown in figure 3. Note that in this and all subsequent discussions of stimulation amplitude, the amplitude is as measured out of the function generator.

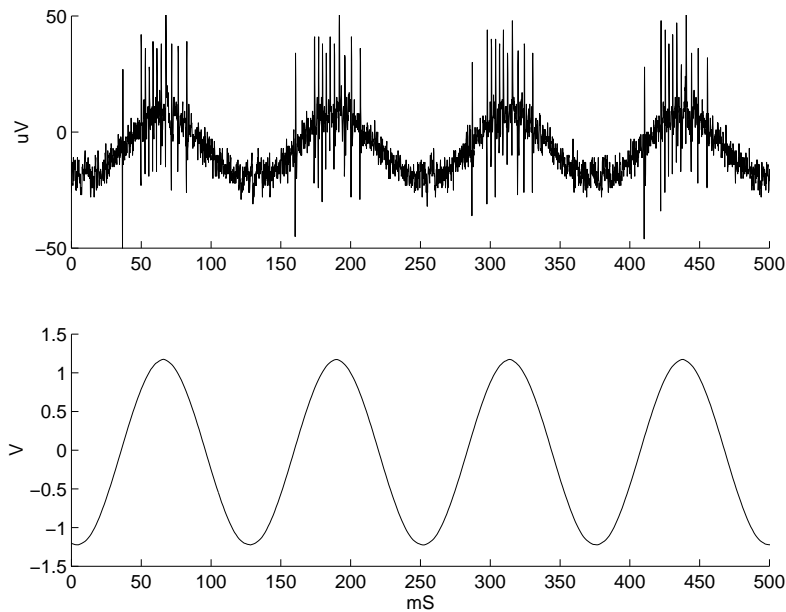


Figure 3: 8 Hz stimulation, 2.4 V pp

With the peak amplitude fixed at 4 V pp out of the generator, the receptor was stimulated at 15 different frequencies, from 0 Hz to 20 Hz, with the resulting frequency response curve shown in figure 4.

The sensitivity response characteristics were obtained by maintaining the frequency of the applied sine wave at 8 Hz, while varying the intensity from .4 to 10 volts, peak to peak. The resulting relation is shown in figure 5.

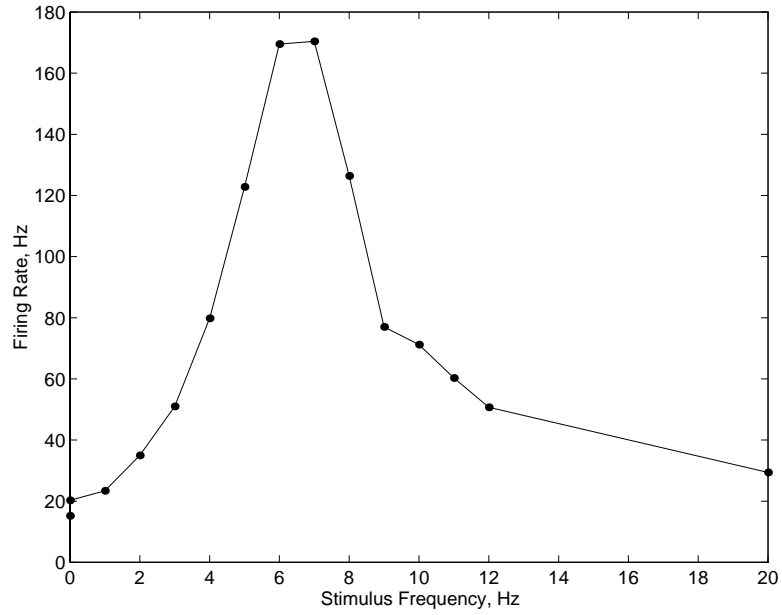


Figure 4: Frequency response relation, amplitude fixed at 4 V pp

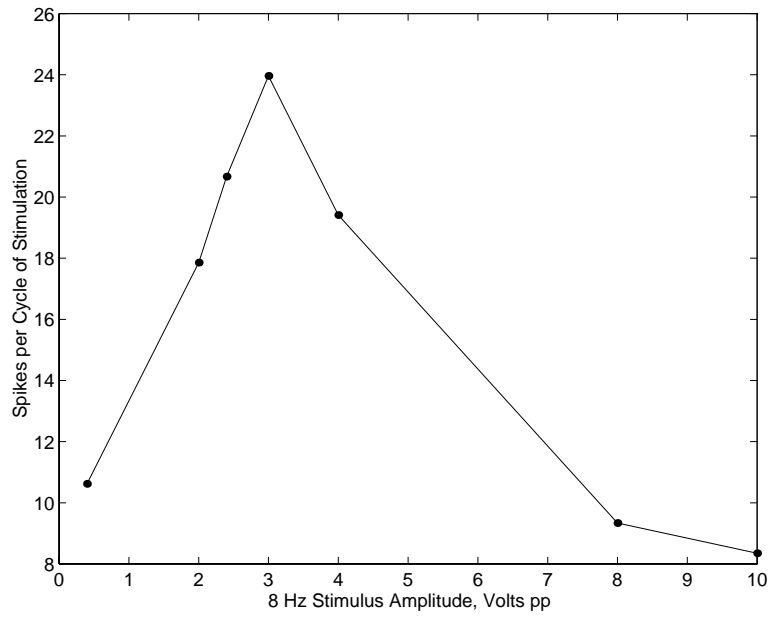


Figure 5: Sensitivity, frequency fixed at 8 Hz

0.5 Discussion

A number of problems in the collection of the data impinge on their interpretation. The signal-to-noise (S/N) ratio was poor in several of the stimulus conditions, as no preamplifier was used in the Faraday cage. Because of this, it was nearly impossible to set the spike threshold at a level where it would only capture the clear spikes; thus the number of spikes is slightly higher than actual under these conditions. The difference between the actual number of spikes and the number shown in the spike channel was checked at several points in the data with the lowest S/N, and was no more than 5%.

The single receptor that was measured during the course of the experiment was allowed a minimum of 60 seconds recovery time between stimuli. This amount of recovery time proved to be inadequate, as the spontaneous firing rate halfway through the experiment went down from 62 to 15 Hz (assuming the neuron concerned sees the same circuit when the function generator is set to 0 Hz as when the generator is switched off). A short recovery time was used because of the length of the protocol and the lack of stability in the monitoring of single pores prior to selection of the final pore.

The low filter cutoff setting of 100 Hz prevented assessment of the field seen by the pore from the applied potential from the function generator, all of which were at or below 20 Hz, although it was appropriate for recording the extracellular spikes, which had pulse widths of less than 1 mS. Thus this experiment was only able to determine a relative sensitivity relationship between the fixed-frequency, varying-amplitude stimuli. Despite the filter setting, as can be seen in figure 3 there was still some bleed-through of the 8 Hz signal in this sample from the fixed frequency regime.

The central aim of the experiment was the generation of the frequency response

curve and sensitivity relation for the *Ictalurus punctatus* ampullary electroreceptor. The frequency response curve is shown in figure 4. There are several features of the graphed relation worth noting. First, unlike *Ictalurus nebulosus* with its 8 Hz optimal frequency (Peters *et al.*, 1988), the best stimulation frequency for *Ictalurus punctatus* appears to be 7 Hz, by a wide margin over 8 Hz (average firing rates of 170 and 126 Hz, respectively).

Second, the rising edge of the response curve is much sharper than the falling edge past 9 Hz: higher frequencies off the peak of 7 Hz are less attenuated relative to lower frequencies off the peak. At 13 Hz higher than the optimal stimulation frequency, the response is still higher than only 7 Hz less, at 1 mHz. In general the response curve is that of an asymmetrical bandpass filter, with minimal attenuation at 7 Hz.

Third, there is a shift to a shallower rolloff at near 9 Hz. Peters *et al.* found that the skin resistance of *Ictalurus nebulosus* was fixed from 0 to 10 Hz, but varied with frequency over 10 Hz. As the frequency rises past 10 Hz, the skin resistance drops, becoming isoresistive with the surrounding medium in their experiment at 20 Hz, and slightly conductive at 100 Hz (Peters *et al.*, 1974). In general this effect is due to capacitive shunting of the external field to the isopotential fish body interior (Kalmijn, 1988). The frequency range of 0-20 Hz fits with the range of frequencies of bioelectric fields that are salient to catfish, which appears to be 0-25 Hz (Peters *et al.*, 1974). Since the skin resistance is presumably going down past approximately 9 Hz, the field potential seen by the receptor should also go down as it becomes progressively more shorted to the fish's body. Thus one would expect the rolloff to become steeper, not shallower. One hypothesis is that were it not for the effect of the reduced skin resistance, the rolloff would be much more shallow past 9 Hz. That this trend, presumably mediated by the biophysical properties of the receptor, would be in register with the change in skin resistance is an unlikely

coincidence, however; otherwise, we should see the shallower rolloff prior to 9 Hz.

The sensitivity response function, shown in figure 5, is somewhat less interesting, in part due to the inability to determine the absolute sensitivity as mentioned above. The optimal stimulation voltage, measured at the generator, is in the region of 3 volts peak to peak. This seems far in excess of a biologically relevant stimulus, and is troubling given that the 1 cm dipole stimulator was quite close to the animal (approximately 1 cm away). Clearly, grub beneath the water substrate will not emit a 3 V pp signal. Peters *et al.* measured a grub's potential, just below the muddy surface of a holding vessel, at 200-300 μ V, .5 cm away (Peters & Bretschneider, 1972). This was in the sensible range of *Ictaluras nebululosus*. It could be that the difficulties alluded to above, regarding spike counting and receptor exhaustion, are distorting the data here. The sensitivity function generated with a paralyzed animal using an artificial stimulus may not correspond to one using an awake behaving fish with biological stimuli. Lastly, it need not be the case that the animal be maximally sensitive to biologically realistic stimulus intensities for the sensory system to fulfill its function well. Human rhodopsin is maximally sensitive to ultraviolet light, which gets blocked by the cornea and vitreous humor, and so is of no relevance to our survival.

In conclusion, in this experiment we have uncovered some of the basic response properties of the *Ictaluras punctatus* ampullary receptor. Yet to be determined are whether the optimal stimulation frequency of 7 Hz corresponds to biologically significant stimuli, and whether the sensitivity function found here is an artifact of the experiment.

References

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