Tavolga, W. N. (1966). "The audio-ichthyotron--the evolution of an instrument for testing the auditory capacities of fishes." Transactions of the New York Academy of Sciences **28**(6): 706-712.

*DIVISION OF INSTRUMENTATION*

# THE AUDIO-ICHTHYOTRON—THE EVOLUTION OF AN INSTRUMENT FOR TESTING THE AUDITORY CAPACITIES OF FISHES*

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In studies on the sensory capacities of animals, there are two basic approaches possible. One is to detect electrical changes in the sense organs or the nerves leading from them. This technique is most appropriate where the investigator is interested primarily in the function of the receptor. If one is concerned with the response of the entire organism and with the relation of its sensory properties to its normal habitat, then a behavioral approach is more suitable. Often, of course, both methods are used to complement each other.

In a behavioral approach to this problem, the animal is exposed to the stimulus and some response is observed. This response may be unconditioned or conditioned, but the latter is generally preferred because of the tighter control the experimenter has upon the behavior of the subject. In any case, the response must be clearly positive or negative, since it is a well-known fact that as the stimulus intensity approaches a minimal, i.e. threshold value, the responses of the subject become erratic and indecisive.

In the course of an investigation of sound production in fish, it became clear that accurate information was needed on auditory reception in these animals. Previous work was not considered adequate because of the lack of proper control and measurement of the acoustic stimulus and of a sufficiently objective testing technique.

Our studies concerned the auditory capacities of fish, the relation of fish sounds to behavior, and the effect of the acoustic environment on hearing in fish. As a first step, we decided to measure the acoustic sensitivity of a variety of marine species, i.e. to determine their auditory thresholds throughout their entire spectrum (Tavolga & Wodinsky, 1963). For this study we used avoidance conditioning. This is a form of instrumental conditioning in which the subject must perform some response in order to prevent the onset of a noxious stimulus. In this instance, a test aquarium was divided into two compartments separated by a hump. This barrier was covered by a depth of water just adequate for the fish to swim across but not remain there. A trial was begun with the onset of the sound signal, delivered by an underwater

*This paper was presented at a meeting of the Division on March 8, 1966. The project reported here was supported in part by Contract No. 552 (06) between the Office of Naval Research and the American Museum of Natural History, and in part by Grant GB-1574 from the National Science Foundation.

speaker concealed beneath the center barrier. After a predetermined period of time (usually five or ten seconds), the animal was given a series of short pulses of alternating electrical current. When the subject crossed the barrier after being shocked, the sound (conditioned stimulus, or CS) and shock (unconditioned stimulus or US) were stopped. This type of response was called an "escape." If, however, the subject crossed the barrier after the signal was started but before the shock came on, the sound was stopped and no shock was delivered. This is termed an "avoidance." In most species tested, a level of 90 percent avoidances was achieved within a week of training at the rate of 25 trials per day. An avoidance was, therefore, an objective index of the fact that the fish did indeed hear the sound, and by varying the intensity and frequency of the CS the entire audiogram could be determined.

The determination of a threshold is somewhat of a problem in itself, because in reality there is no such value in an all-or-none sense. There is, however, a level of stimulus intensity in a given experimental situation below which the probability of positive responses is low and above which the probability of positive responses is high. The threshold, therefore, is a statistical point usually chosen as the stimulus level at which the probability of positive responses is .50 (Stevens, 1961; Swets, 1961; Pollack, 1961). This point can be determined by a series of trials in which various stimulus levels are presented, and there are numerous psychophysical techniques available for making such a determination (Guilford, 1954). A technique that in recent years has come into prominence and wide usage is the so-called staircase or up-down method, as developed by von Békésy (1947) for his well-known audiometer. The trials are scheduled so that following each positive response, the stimulus level is lowered, while after each negative response, the level is raised by the same amount. A record is generated that consists of a zig-zag line, and a threshold value can be calculated. This technique is particularly efficient in locating a threshold whose value is completely unknown, and this was the technique we used for our measurements of the audition of fishes.

## *Audio-Ichthyotron Mark I*

We started with the simplest possible instrumentation in which the subject was observed by means of a mirror suspended over the test tank. One key switched on the audio signal (CS) and the second key was tapped at the rate of about once per second to deliver the shock pulse (US). FIGURE 1 shows a block diagram of this apparatus. The audio level was monitored and measured with a small hydrophone, calibrated preamplifier, and decibel meter.

There were several difficulties encountered with this technique, although it demonstrated the feasibility of the study. Human error was a problem, since the judgment of a positive response had to be made visually and the timing of the CS-US interval and shock pulses was done with the aid of a stop watch. Generally it took two observers to run a series of trials on a single fish, and the entire process was clearly cumbersome, inefficient, and time-consuming. The intertrial intervals were varied to prevent the subject from becoming conditioned to the timing of trials, and thus a single threshold determination might take half a day. It was evident that a more efficient procedure was necessary.

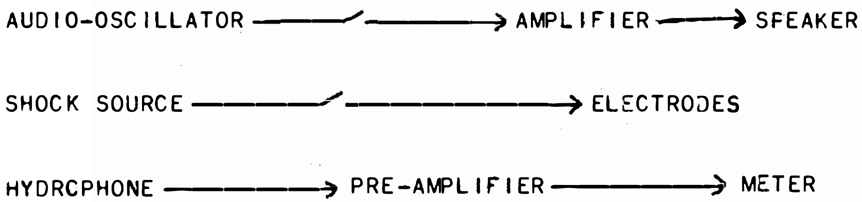


FIGURE 1. Block diagram of the manually operated initial design of the Audio-Ichthyotron.

*Audio-Ichthyotron Mark II*

The first improvement in this instrumentation was the introduction of a photocell placed at the barrier, so that when the fish swam across, a light beam was interrupted and an objective index of a response could be detected. In addition, an electrically driven clock was used to measure the response time and, simultaneously, to control the CS-US interval. This was essentially the apparatus described and used by Behrend and Bitterman (1962); Horner, Longo and Bitterman (1961); and Wodinsky, Behrend and Bitterman (1962). A block diagram of this instrumentation is shown in FIGURE 2, and this apparatus was used to determine audiograms for nine species of marine fishes (Tavolga and Wodinsky, 1963).

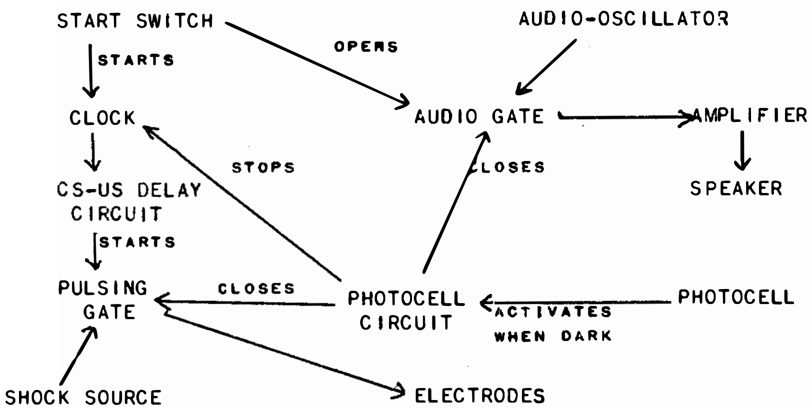


FIGURE 2. Block diagram of the Audio-Ichthyotron Mark II, based upon instrumentation described by Horner, Longo and Bitterman (1961).

In attempting to do a statistical study of threshold variability, however, this apparatus was found to be inefficient because only a single animal could be tested at one time, and much time was consumed in waiting out the intertrial period. In addition, it became necessary to observe the activity of the animals between trials; the barrier crossings during a period of no signal could represent "false positive responses" and a record of these was needed.

### *Audio-Ichthyotron Mark III*

This control system was designed and constructed by Robert Laupheimer of the Courant Institute of Mathematical Sciences and Raymond Simon of the Biometrics Laboratory, Brooklyn State Hospital. One major feature in the design of this model was that solid-state circuits were used throughout and all mechanical relays were eliminated, reducing switching transients to a minimum. Six test tanks were controlled so the subjects could be tested in tandem, consequently eliminating the waiting period between trials. The sequence of operations began by engaging the Start switch. This switch served four functions: (1) It turned on a gating circuit which permitted the audio signal to go through from the oscillator to the attenuator, amplifier, and speaker; (2) it reset and started a clock circuit to measure the subject's response time; (3) it reset an intertrial counter connected to the appropriate test tank; (4) it started a delay circuit (CS-US interval) which activated the shock pulsing gate after an appropriate delay. This delay could be set at from 0 to 99 seconds, but in practice a five-or ten-second delay was used. When the animal crossed the barrier, a photocell circuit was activated that turned off the audio gating circuit, stopped the clock, and turned off the shock circuit. A cable from the control center supplied six experimental tanks, each of which was equipped with a speaker, photocell, and shocking electrodes; a selector switch could activate any one of these tanks for a test. This selector switch selected the proper speaker to deliver the audio signal, selected the appropriate photocell circuit to be activated, disabled the intertrial counter connected to that test tank, and selected the appropriate shocking electrodes. While one tank was activated for testing, the others were in their intertrial interval, during which time the photocell circuits operated appropriate counters so that intertrial barrier crossings could be recorded. FIGURE 3 gives a block diagram of this control system, which was used in an intensive study of threshold variability in which over 400 threshold determinations were made for a single species of marine fish (Tavolga & Wodinsky, 1965).

### *Audio-Ichthyotron Mark IV*

Several minor modifications and improvements were introduced into the control apparatus; for example, the facility for delivering a pulsed instead of a steady sound was added. A major alteration in the circuitry was made in order that frequency and intensity discrimination could be tested. In this situation, a pulsed sound was fed into all six test tanks simultaneously. This was delivered through a gating circuit into an amplifier and served as the standard signal. Upon activation of the Start switch, the circuit was switched during the silent period between pulses to a second gating circuit. This gate supplied audio pulses, which alternated between two oscillators, to the test tank selected for the trial. The alternating signal, therefore, served as the CS. For intensity discrimination, the two oscillators were set at the same

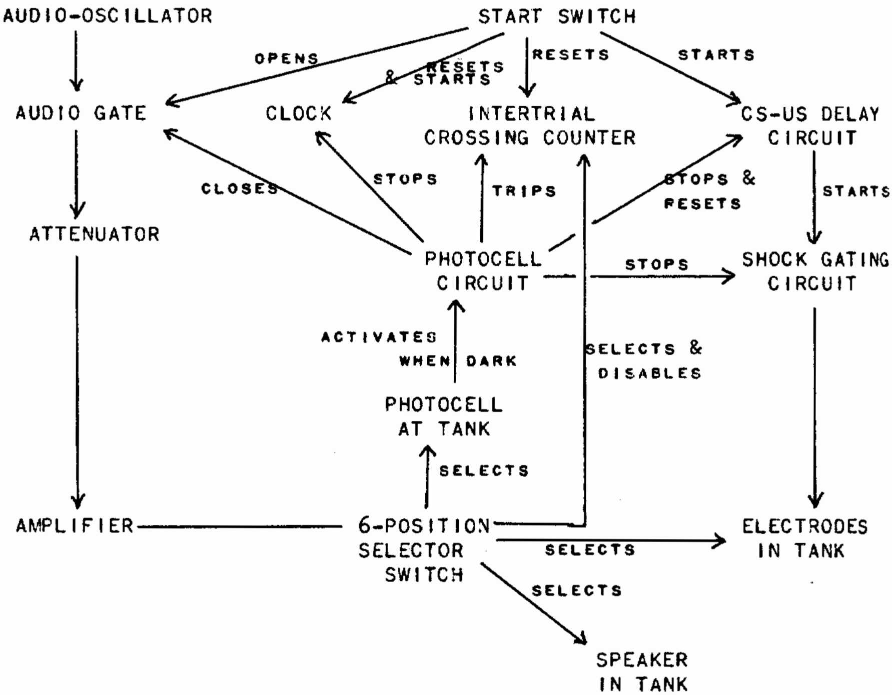


FIGURE 3. Block diagram of the Audio-Ichthyotron Mark III, designed by Robert Laupheimer and used by Tavolga and Wodinsky (1965).

frequency but at different stimulus intensity levels, and for frequency discrimination, they were set for the same intensity but different frequencies.

A study using this instrumentation is currently under way at the Bio-Acoustics Laboratory of the Department of Animal Behavior at the American Museum of Natural History. Results thus far are preliminary, but indications are that fish can discriminate at least three or four decibels in sound pressure and at least a 5 percent difference in frequency.

### *Audio-Ichthyotron Mark V*

This is an entirely separate and new apparatus that is still to be thoroughly tested in practice. Although it will only test a single subject at a time, it incorporates some of the automatic features of the von Békésy audiometer. The response of the subject determines the stimulus level at the next trial and the schedule of trials is automatically programmed. The data, printed out on adding-machine paper, include the response time, the number of intertrial crossings, and the setting on the automatic attenuator. In the future it will be possible to use a punched or magnetic-tape data read-out so that analysis of the results can be done by a computer. FIGURE 4 shows

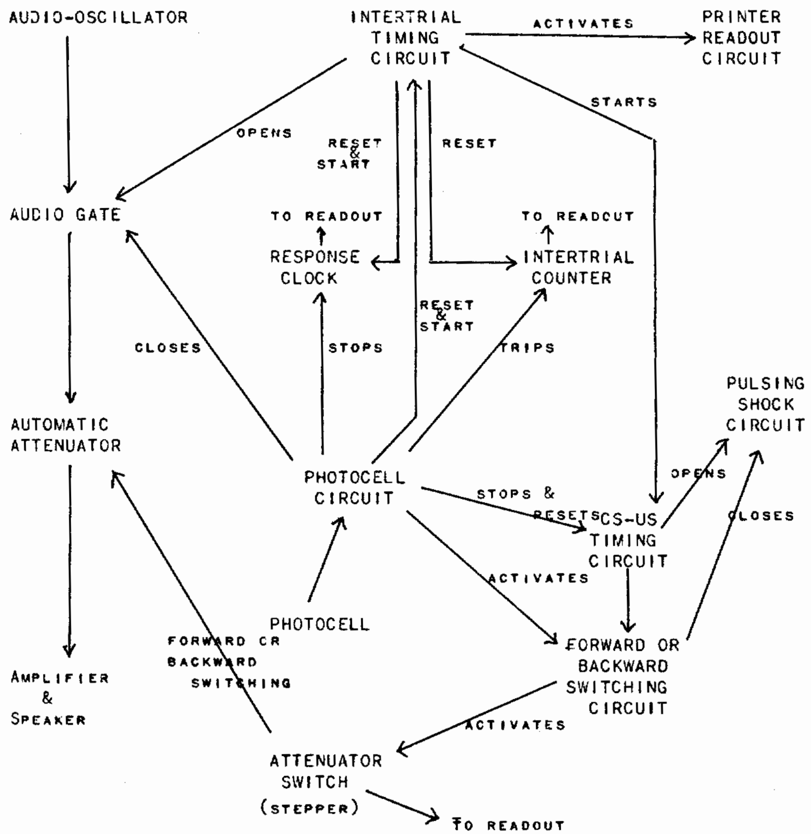


FIGURE 4. Block diagram of the Audio-Ichthyotron Mark V, designed by Robert Laupheimer and currently in use at the American Museum of Natural History.

a block diagram of this apparatus. A variety of auditory capacities, as well as studies of conditioning and learning, can be investigated with this machine.

Beginning with a simple, jury-rigged system, we have now developed a complex, efficient data-gathering machine. But a word of caution should be introduced. The behavior of the animal must still be watched, and it is not unusual for the animal to inform the experimenter if his equipment is functioning properly. It is not enough to treat the subject as some sort of "black box." A given animal is the product of a long evolutionary history and of a developmental history in which maturation and experience are coalesced, and its behavior must be analyzed and interpreted in accordance with the animal's level of integration.

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