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 speaker concealed beneath the center barrier. A level of time (usually five or ten seconds), the animal pulses of alternating electrical current. When the after being shocked, the sound (conditioned; (unconditioned stimulus or US) were stopped. This an “escape.” If, however, the subject crossed their started but before the shock came on, the sound was delivered. This is termed an “avoidance. level of 90 percent avoidances was achieved at rate of 25 trials per day. An avoidance was, well-known audiometer. The trials are scheduled; positive response, the stimulus level is lowered response, the level is raised by the same amount consists of a zig-zag line, and a threshold of positive responses is high. The statistical point usually chosen as the stimulus level of positive responses is .50 (Stevens, 1961; This point can be determined by a series of trial levels are presented, and there are numerous available for making such a determination (Guil in recent years has come into prominence and technique is particularly efficient in locating completely unknown, and this was the technique we of the audition of fishes.

We started with the simplest possible instrument was observed by means of a mirror suspended on the audio signal (CS) and the secound on per second to deliver the shock pulse. A block diagram of this apparatus. The audio level was with a small hydrophone, calibrated preamplifier.

There were several difficulties encountered with it demonstrated the feasibility of the study. However, since the judgment of a positive response had the timing of the CS-US interval and shock pulse a stop watch. Generally it took two observers to a single fish, and the entire process was clearly cut time-consuming. The intertrial intervals were varied from becoming conditioned to the timing of trials, the determination might take half a day. It was evident procedure was necessary.
sensory capacities of animals, there are two basic properties to its normal habitat, then a behavioral response is observed. This response may be unconditional or conditional. Often, of course, both methods are used to approach this problem, the animal is exposed to the stimulus of sound, and an investigation of sound production in fish, it became clear that measurement was needed on auditory reception in these species. The auditory capacities of fish, the relation of the response of the entire organism and with the auditory environment on hearing, was determined by Contract No. 552 (06) between the Office of Science Foundation.

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 was pressed on the audio signal (CS) and the second 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 operators 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).

Audio-Ichthyotron Mark III

This control system was designed and constructed of the Courant Institute of Mathematical Sciences: Biometrics Laboratory, Brooklyn State Hospital. The design of this model was that solid-state circuits all mechanical relays were eliminated, reducing minimum. Six test tanks were controlled so the s tandem, consequently eliminating the waiting per sequence of operations began by engaging the Start four functions: (1) It turned on a gating circuit signal to go through from the oscillator to the speaker; (2) it reset and started a clock circuit response time; (3) it reset an intertrial counter for test tank; (4) it started a delay circuit (CS-US interval) shock pulsing gate after an appropriate delay. waiters from 0 to 99 seconds, but in practice a five-or ten-second delay was needed. When the animal crossed the barrier, a photocell turned off the audio gating circuit, stopped the clock circuit. A cable from the control center supplied each of which was equipped with a speaker, photocell, a selector switch could activate any one of the tester tank. The alternating signal, CS. For intensity discrimination, the two oscillators

Audio-Ichthyotron Mark IV

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The 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

signal could represent "false positive responses" and a record of these was needed.
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 Biophysics 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 Bekesy 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 a block diagram of this apparatus. A variety of auditory as studies of conditioning and learning, can be investigated.

Beginning with a simple, jury-rigged system, we developed a complex, efficient data-gathering machine. But a word must be introduced. The behavior of the animal must still be unusual for the animal to inform the experimenter if the conditioning properly. It is not enough to treat the subject as a box. A given animal is the product of a long evolutionary, a developmental history in which maturation and learning, and its behavior must be analyzed and interpreted with the animal's level of integration.

References


ram of the Audio-Ichthyotron Mark III, designed by Robert Tavolga and Wollinsky (1965).

different stimulus intensity levels, and for frequency differences, the same intensity but different frequencies. Instrumentation is currently under way at the Bio-Department of Animal Behavior at the American Museum. Results thus far are preliminary, but indications show at least three or four decibels in sound pressure difference in frequency.

Audio-Ichthyotron Mark V

Separate and new apparatus that is still to be thoroughly developed is the automatic features of the von Bekesy audiometer. Objective in determining the stimulus level at the next trial is automatically programmed. The data, printed on paper, include the response time, the number of trials and the setting on the automatic attenuator. In the future, to use a punched or magnetic-tape data read-out the results can be done by a computer. FIGURE 4 shows

Tavolga: The Audio-Ichthyotron

FIGURE 4. Block diagram of the Audio-Ichthyotron Mark V, designed by Robert Laugelser 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 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.

References


SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

INTRODUCTION TO A PROGRAM DEVELOPED TO STUDY THE INTERACTION OF MAMMALIAN CELLS

Dorris J. Hutchison, Moderator
Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York University Medical College, New York

This symposium was organized by Allan R. Gold, Cornell University. He deserves a word of gratitude for his effort. It is intended to be the moderator of the meeting, especially since the idea for the symposium came from him many years ago. This evening some of the other early workers will have a chance to review past and present studies on the uptake of DNA by mammalian cells.

Research Aims

The aims and objectives of our various research projects are to understand mammalian cells either in vivo or in vitro to examine the phenomenon. There are two major problems to be solved, and both of these aims can be forthcoming. The first problem is how to maintain a biologically active state for a period sufficient for the introduction of genetic markers into the genome of the receptor molecule. The second problem is the necessity for an assay system to measure the transformation.

Summation

The studies of Bensch point up the need for methods to identify the DNA. The work of Benesch's group at Yale has demonstrated that certain cocarcinogens can transform DNA-protein by L-cells. The two major problems are that transformation could not occur and that it was not demonstrated.

Kay, using Ehrlich-Lettre carcinoma, has shown that labeled DNA into DNA of the tumor cells. His results strongly suggest that DNA is highly polymerized during incorporation into DNA and that there is no evidence of some breakdown of DNA during prolonged incubation.

*This paper, presented as an Introduction to the three papers, was read at a meeting of the Section on March 8, 1966.
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