Reports

Duplex Nature of Reception of Simple Sounds in the Scape Moth, Ctenucha virginica

Moths of a number of families have been shown to possess tympanal organs sensitive to sounds of high frequencies which may enable the insects to escape capture by bats (*1*). Reactions to sounds are of both excitatory and inhibitory types, without obvious relationships to species or habits. Destruction of the tympanal organs abolishes most of the responses to sounds, but some individuals respond occasionally even after destruction of the organs.

In a study of reactions of the scape moth, *Ctenucha virginica* (family, Amatidae), to "pure" tones (*2*), we have found a possible explanation for responses of moths with the tympana destroyed. Briefly, *C. virginica* showed a duplex pattern of response to simple sounds: at frequencies of 150 to 15,000 cy/sec and median sound pressures of 95 to 100 db (*re* 0.0002 μbar), the reactions were generally excitatory in nature; at frequencies above approximately 15,000 cy/sec and median sound pressures of 80 to 85 db, the reactions were either inhibitory or, if excitatory, were different from those at lower frequencies. Destruction of both tympana abolished the responses to frequencies above 15,000 cy/sec, but did not affect the reactions at lower frequencies.

The methods used for testing the reactions of these moths to sounds were like those reported by us earlier (*3*). The animals were tested individually either in small cubical cages (15 cm on a side) or affixed to small wax blocks on the ends of glass rods by the dorsal wall of the thorax, with the wings free. Sounds pressure thresholds for reactions to 1- to 2-second bursts of "pure" tones of 150 to 40,000 cy/sec were determined by stimulating the insects at different sound pressures, using innate reactions to the sounds as indicators of reception. Frequencies below 150 cy/sec were produced by the equipment at intensities too low to stimulate; 40,000 cy/sec was the highest frequency that the apparatus could produce. Thus, actual frequency limits for the responses were not determined. A total of about 3000 threshold determinations were made with 21 individuals (16 males, 5 females).

At frequencies of 150 to 15,000 cy/sec, free moths usually responded by tilting the body to bring the anterior end nearer the substrate. Fixed moths jerked the antennae or legs. At 20,000 cy/sec and higher, free animals responded only occasionally and then by flicking the wings. Fixed animals, on the other hand, responded quite consistently: if not flying, they flicked the wings slightly; if flying, they usually stopped almost immediately when stimulated by the sound, as Treat (*1*) also found for this species. For 19 frequencies between 150 and 15,000 cy/sec, there were no significant differences in median sound pressure thresholds. Any sound within this wide frequency range, if it achieved sufficient intensity (above 85 to 90 db), was stimulating. At 20,000, 30,000, and 40,000 cy/sec, median sound pressure thresholds were significantly lower than at lower frequencies and essentially the same (84 to 85 db). At 15,000 cy/sec, some moths responded as at lower frequencies, others as at higher frequencies. All responded as at lower frequencies below 12,000 cy/sec and as at higher frequencies above 18,000 cy/sec. It is almost certain that frequencies above 40,000 cy/sec would stimulate the moths, for reactions occurred at that frequency at just as low intensities as at 20,000 and 30,000 cy/sec.

Bilateral destruction of the tympana abolished the responses above 15,000 cy/sec, but did not affect those at lower frequencies. With or without tympana, removal of the following parts of the body, individually or in most possible combinations, did not significantly alter the responses or thresholds at lower frequencies: antennae, legs, two-thirds of the wings, head, and abdomen. In fact, isolated heads responded by antennal movements at only slightly higher intensities than whole animals. The results, which are like those reported for the butterfly, *Cercyonis pegala* (*3*), suggest that the receptors are widespread on the body, possibly tactile hairs or chordotonal organs in the body wall.

These observations show clearly the necessity for specification of the frequencies and intensities of sounds used in studying acoustical reactions of insects. There are a number of mechanoreceptors on the insect body which are at least theoretically susceptible of stimulation by sounds. Careful control of intensities and frequencies may enable one to determine the relationships within this galaxy of receptors which enable the insect to react to its acoustic environment.

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2. This work was supported in part by research grant No. E-802 from the National Microbiological Institute, National Institutes of Health, U.S. Public Health Service.
3. H. Frings and M. Frings, *Ann. Entomol. Soc. Amer.* 49, 611 (1956). "Pure" tones were produced by a Hewlett-Packard (200-A) audio oscillator activating a Jensen (NF-101) loudspeaker for frequencies of 150 to 4000 cy/sec and an Altec (633-A) microphone for 4000 to 40,000 cy/sec. The animals were tested in a small anechoic chamber, where they were placed 15 cm from the transducers. Sound pressures at that distance were measured for 150 to 10,000 cy/sec with a calibrated Scott (410-B) sound-level meter, and for higher frequencies by a substitution method using specially calibrated equipment of the Acoustics Laboratory of Pennsylvania State University by Fujio Oda, to whom we express our appreciation.

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Chlorpromazine and Reserpine Prevention of Myocardial Damage by Histamine and Serotonin

Blood-cell disintegration during clotting is accompanied by release of serotonin (*1*) and histamine (*2*). In myocardial infarction, these two agents could become active within the musculature of the heart. In order to determine whether they are liberated in quantities sufficient to cause damage, the effect of whole blood and serum, as well as of serotonin

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All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

and histamine, was studied in the isolated and perfused rabbit heart. Reserpine (*3*) and chlorpromazine (*4*), drugs which are known to inhibit the action, respectively, of serotonin and of histamine, were investigated with respect to their usefulness in prevention of cardiac effects caused by these two agents.

For each series of experiments, five hearts of albino rabbits (weighing approximately 2.5 kg) were used. The hearts were isolated and perfused with oxygenated Locke solution (at 38°C) under constant pressure (Langendorff method). Controls were carried out (i) by briefly inserting a 27-gage needle into the left ventricular myocardium to establish the extent of trauma; (ii) by injecting 0.05 and 0.2 ml of Locke solution into two different sites of the left ventricular myocardium to determine changes, and their duration, resulting from volume effect. Five one-hundredths milliliter of whole blood, serum, or the supernatant of centrifuged blood coagula, as well as serotonin or histamine in 0.05-ml volume of Locke solution, was injected into the left ventricular wall. Care was taken to avoid damage to major arteries and veins. Two milliliters of 0.4 percent T-1824 (Evans' blue) in Locke solution were injected into the aortic cannula to facilitate macroscopic determination of the "infarct." Reserpine or chlorpromazine in amounts from 0.0001 to 1 μg/ml was added to the perfusion solution in the reservoir to determine their efficacy in preventing the effect of intramyocardially given serotonin or histamine. Serums obtained from centrifuged blood coagula (1 ml), histamine, serotonin, reserpine, and chlorpromazine in varying concentrations were injected into the aortic cannula. The effects of these agents on the functioning of the heart were kymographically recorded. Coronary flow was measured with Condon's magnet tipper. Reserpine and chlorpromazine were tested with respect to their effectiveness in altering the action of serum, histamine, and serotonin on the functioning of the heart and coronary flow.

Control experiments showed that insertion of a needle into the myocardium leaves a small opening through which minute amounts of perfusion fluid and dye escape. Small volumes of Locke solution or of whole blood injected into the ventricular wall did not interfere with the dye distribution; this fact could be established with the "intravital" staining technique.

Injection of 0.05 ml of serum (taken immediately after clot formation) into the same site prevented staining of the injected area for approximately 12 minutes, whereas serum obtained through centrifugation of a coagulum caused marked focal damage, which spread rapidly within the first 20 minutes and expanded more slowly during the following 2 hours.

Extent of the damaged area was made visible by injection of Evans' blue, which caused intense coloring of the entire myocardium, sparing only the "infarct." In each case, the "infarct" involved the entire thickness of the ventricular wall.

Injection of serotonin in amounts from 0.00000005 to 300 μg induced "infarcts," involving an area of about 1 cm in diameter, which were fully developed within 20 minutes and disappeared after 45 minutes.

Injections into the myocardium of 0.00000005 to 100 μg of histamine caused the gradual development of an "infarct," which reached its greatest extent at the end of 2 hours and was still detectable after 5 hours.

Reserpine added to the perfusion fluid in concentrations of 0.01 and 0.1 μg/ml did not significantly alter the serum- or serotonin-induced "infarct" formation and did not alter the histamine-induced "infarct" formation at all. In doses of 1 μg and more, it prevented 50 percent of "infarct" formation but at the same time markedly reduced the coronary flow.

Chlorpromazine, added to the perfusion fluid in concentrations of 0.001 μg/ml and higher, prevented "infarct" formation that otherwise followed intramyocardial injection of serum (0.1 ml), serotonin (300 μg), and histamine (100 μg). In lower concentrations, chlorpromazine did not give complete protection.

One milliliter of serum (obtained through centrifugation of blood coagula) injected into the aortic cannula did not significantly alter either the functioning of the heart or the coronary flow.

It was established that histamine (*5*) and serotonin (*6*), administered by way of the aortic cannula, have transient, strongly positive chronotropic and inotropic effects and that they increase the coronary flow by approximately 10 percent. The action of chlorpromazine and reserpine on the functioning of the heart and coronary flow are summarized and compared in Table 1.

The absence of extensive thrombosis and complete occlusion in a high percentage of myocardial infarct cases (*7*) suggested the possibility that some other factors—possibly of a chemical nature—are involved in the development of this pathology.

The foregoing study was based on the hypothesis that one or several chemical agents, liberated during the clotting process, may produce myocardial damage. Histamine and serotonin, both known to be released from certain disintegrating blood cells, were suspected of being such pathogenic agents.

To test this hypothesis, it was necessary to establish (i) whether myocardial damage could, indeed, be produced by endogenous chemicals in the absence of mechanical obstruction; (ii) whether such chemicals, in order to exert damaging effect, would have to act within the myocardium or within the coronary vessels; and (iii) whether a small blood clot would liberate such substances in quantities sufficient to become pathogenic.

The finding that chlorpromazine in very low dosage prevented the myocardiotoxic effect of serum, histamine, or serotonin suggested the possibility that chlorpromazine could be used as an adjuvant in prophylactic treatment of myocardial infarction.

Whether the results of the foregoing study constitute an exact parallelism to the conditions that prevail in myocardial infarction can be established only through quantitative determinations of histamine and serotonin in the infarcted areas of the human heart and through histopatho-

Table 1. Comparative effects of chlorpromazine and reserpine on perfused surviving rabbit heart.

Chlorpromazine dosage (μg)	Effect on functioning of heart	Effect on coronary flow	Reserpine dosage (μg)	Effect on functioning of heart	Effect on coronary flow
0.01	None	None	0.01	None	None
0.1	None	Slight*	0.1	None	Slight†
1	None	Increase	1	None	Slight
10	Slight negative chronotropic and inotropic	Increase	10	Slight negative chronotropic and inotropic	25-percent reduction
100	Toxic	Increase	100	Marked negative chronotropic and inotropic	60-percent reduction
500	Toxic	Slight†	500	Toxic	Coronary constrictors no longer effective in further reducing flow

* Increase. † Decrease.

logic comparison between spontaneously occurring and experimentally induced lesions (*8*).

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15 April 1957

Electrochemical Basis for a Contractile Mechanism and Some Related Cellular Phenomena

Most explanations of the contractile process in muscle have been based on models which assume that the imposition of a charge on some suitable side chain can effect a contraction or extension of the polypeptide backbone chain or can produce a structural alteration in one or more of the protein components (*1, 2*). It is perhaps worth while to point out that there exists an alternative electrochemical phenomenon which could produce a contraction by a relatively long-range field effect and which forms an attractive model for the explanation of many features of the contractile mechanism. Likewise, the essential characteristics of this phenomenon suggest it as a possible cause for other long-range effects in cellular behavior.

The principle of this model is analogous to that used recently by Kolin (*3*) to separate individual proteins from a mixture. Consider a macromolecular complex (such as actomyosin) which has one end, *b*, in an environment such that the *p*H is above the isoelectric point, *pI*, of the protein component in that region, and whose other terminus, *a*, is in an environment such that the *p*H is below the isoelectric point (Fig. 1). The protein at *b* will carry, therefore, a net negative charge, while that at *a* will be positively charged. Nevertheless, in these circumstances the two ends do not necessarily attract each other electrostatically, for counter-ions, indicated by charges within the squares of Fig. 1, would be gathered around each region. If, however, a suitable electric field is now imposed on this system, the fiber should contract (Fig. 1) as a result of the repulsive force of the negative end of the field on the top section of the macromolecule and of the positive pole on the bottom section of the macromolecule.

That this combination of a *p*H gradient and an electric field will produce motion of the protein molecules in the manner described is shown not only by Kolin's rapid, sharp separations of constituents from a mixture of molecules but also by a simple macroscopic experiment that was carried out in this laboratory. Small disks of gelatin ($pI = 4.9$) were soaked in an acid buffer (*p*H 2.6) and a basic buffer (*p*H 9.6), respectively. A shallow trough was cut into paraffin (as is indicated schematically in Fig. 2), a 25-percent solution of glycerol in distilled water was poured into the trough, and the acidified gelatin disk was floated at *a*, the alkaline one at *b*. On imposition of an electric field in the direction indicated, *a* moved toward the right, *b* toward the left. When the electrode polarities were reversed, the disks reversed their direction of motion. These movements are exactly what one would expect for a cationic macromolecule at *a* and an anionic one at *b*.

In muscle fibers, the geometric localization of the oxidative enzymes (*4*) and of ATP-ase activity (*2*) could provide the *p*H and electric field gradients. Likewise, in other cells, localization of metabolic activities in various regions could establish a combined *p*H and electric field which might effect other intracellular motions, such as those of the spindle. In a cell membrane, contractile (or extensile) effects of these combined fields could markedly affect permeability by the creation of "holes" in the surface structure as the protein macromolecules moved together (or apart).

It seems worth while, therefore, to consider in further detail the effects of a combined *p*H and electric field as the basis of various relatively long-range phenomena in cellular behavior.

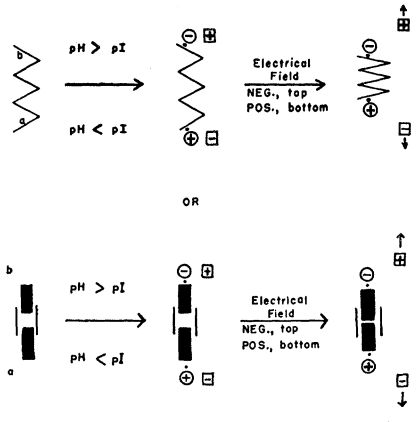

Fig. 1. Schematic diagram of contraction produced by establishment of *p*H gradient and electric field. Charges within circle represent those on protein; charges within squares represent counter-ions.

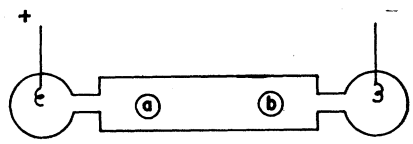

Fig. 2. Diagram of apparatus for moving gelatin disks. Disk *a* is equilibrated in acid buffer, and is therefore cationic. Disk *b* is equilibrated in basic buffer and is therefore anionic. Platinum-wire electrodes are immersed at the ends of the trough.

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Atmospheric Carbon-14

According to Libby (*1*), most of the neutrons which escape into the surrounding atmosphere from an atomic or thermonuclear explosion interact with the nitrogen of the atmosphere to produce C^{14} through the nuclear reaction

$$N^{14} + n \rightarrow C^{14} + H^{1}$$

Because of the extensive use of C^{14} dating techniques, small additions of this material to the atmosphere may be important.

Libby (*1*) further estimates that, even to double temporarily the atmospheric radiocarbon content, megatons of fission of the order of 1000 would be required. With the measurement techniques now in use, it is possible to make measurements of the present equilibrium level of C^{14} in contemporary biological materials to an accuracy of approximately 1 percent. From these considerations it seems probable that only thermonuclear explosions will produce sufficient C^{14} to give measurable increases.

On the assumption that any C^{14} formed in weapons tests would be present in the air as CO_2 (*2*), collections of this gas from the atmosphere were begun in 1952. A vacuum pump was used to draw filtered air through a solution of sodium hydroxide (80 g of NaOH in 2 gal of water) at a flow rate of 10 to 12 lit/min.