HEARING IN FROGS

A STUDY ON HEARING IN
RANA PIPIENS AND *RANA CLAMITANS*

By

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SCOPE AND CONTENTS: This thesis deals with a study on the ability of *Rana pipiens* and *Rana clamitans* to perceive acoustic stimuli. The research has been carried out in three parts: conditioning to sound; recording of the respiratory rate in response to sound; observation on the threshold of sound perception. A review of the available literature on hearing in frogs, a description of the methods used and the results obtained are presented, followed by a general discussion and conclusions.

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I wish to express my sincere gratitude to Dr. Herman Kleerekoper, who suggested the theme for this thesis and gave guidance and encouragement during its progress.

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REVIEW OF THE LITERATURE ON HEARING IN THE FROG

I. Behaviour of the frog in response to sound in its natural habitat

When R. Yerkes (1903) studied frogs in their natural habitat he noticed that foreign sounds such as pistol shots, hand clapping, loud shouting etc. did not produce any motor response in these animals. Yerkes believed that sound only modifies the behaviour of frogs in response to stimuli other than sound.

Courtis (1907) studied the response of toads to sound stimuli during the mating season, when the males produce a characteristic sound. The other males and females respond by moving towards the sound-producing individuals. According to Courtis the females are able to locate the source of sound from a distance of 30-40 feet.

II. Direct observations on the ear and the auditory nerve

The fact that the tympana of the frogs are directly affected by sound was discovered by Wada (1924). He observed that during an acoustic stimulation the tympana began to vibrate if the range of sound was between e^2 and g^4 (659-3136 cps). Whistles and bells were used as sources of sound and produced vibrations of the tympana when the frog was in the air as well as when it was submerged under water at the depth of 1 cm. When the animal was submerged below 1.5 cm no more vibrations were registered. However the author does not state whether the source of sound was located in or outside the water. In the latter case the high impedance in the transmission of sound from air to water would have been an important factor.

In the experiments of Gruenberg (1921) and Kuroda (1926) the frogs were exposed to a) detonations and b) to a continuous action of sound during a period of 336 hours. Neither author obtained definite results. But although Gruenberg failed to observe any anatomical change in the nerve endings of the acoustic nerve he maintains that this fact does not necessarily prove the absence of the sense of hearing in the lower vertebrates.

The electrical response of the auditory mechanism of *Rana esculenta* and *Rana temporaria* was studied by Adrian, Craik and Sturdy (1938). The acoustic nerve was exposed and lifted on the electrodes. Electrical potentials in response to sounds of various pitch and intensity were recorded. It was observed that only loud sounds produced a response. *R. esculenta* showed a greater sensitivity than *R. temporaria*. A continuous note from a loudspeaker seemed to be less effective than loud speech. The range of frequency of the sounds which produced a rhythmic nerve response was found to vary with the change of temperature; an increase in temperature enlarged the range of the nerve response. At a constant temperature the threshold for the nerve response rises as the frequency is increased.

III. Observations on other physiological responsesto sound

a) Skin

The fact that the skin possesses electrical properties was discovered over one hundred years ago when Du Bois-Reymond found that by connecting two loci on the skin by means of unpolarisable electrodes with a sensitive galvanometer an electrical current can be detected (Gildemeister 1915). This was explained by the existence of electrical potentials between any two loci on the skin. These skin potentials exhibit a great variability, being greatly influenced by seasonal changes, nutritional state, the duration of the experiments and the pretreatment of the animals. Tarchanoff (1890) equalised the potentials between two loci on the skin and could then demonstrate that stimulation by light, sound and touch produced a new, momentary potential between the loci in question. This he called the G S R (galvanic skin reflex). It can be found in humans, dogs, cats, toads, frogs, fish etc.

The influence of stimulation of the various sense organs on the GSR has been studied by many investigators. Kohlrausch and Schilf (1922) found that noises like hand clapping, knocking on the floor, footsteps outside the laboratory etc., acted on frogs that were slightly curarised in the same fashion as stimulations by touch. Loud whistles of different frequencies were found to be without any influence. The croaking of another frog produced a positive reaction in only half of the animals used, the rest showed either a questionable reaction or did not react at all. No attempt has been made to determine the influence of sex or season on GSR in connection with acoustic stimulation in frogs.

The influence of various noises and sounds on the GSR of frogs was explored by Buitendijk and Berelman (1930). Their results show that violent noises like hammering on the table or vibrations of a tuning fork, caused the GSR to disappear rapidly. On the other hand, more delicate vibrations, for instance the sound produced by rubbing of paper, invariably called forth a reaction which persisted for some time. These authors repeated the experiments of Kohlrausch and Schilf with the stimulation by sound of a croaking frog with the result that the GSR appear in the majority of the animals but it could not be obtained more than once or twice. In their experiments the sound of running water seemed to be the most powerful stimulus in producing the GSR.

b) Respiration

According to Babak (1913) the respiratory movements of the floor of the mouth of frogs represent an indicator of the state of the central nervous system and can be used when studying the influence of various factors like light, sound etc. or sensitivity of these animals to temperature. The fact that changes in the environment produce modifications of the character of the breathing movements of frogs has been observed by Yerkes (1903) and he used it in his experiments concerned with hearing. When recording the respiratory movements on a smoked drum he noticed that after an auditory stimulus was given for the first time an increase in respiration rate occurred. The effect of the same stimulus however diminished after repetition and finally disappeared. The effect produced depended on the quality of the sound, for instance the sound of falling water resulted in an acceleration of the respiratory rate whereas a loud shrill whistle caused an inhibition of respiration. The ringing of a bell also produced an inhibition, a sound of a tuning fork of 500 cps brought about a slight increase of the rate of breathing. Yerkes concluded that frogs are able to hear sounds ranging from 50 cps to at least 1000 cps and that the failure of these animals to demonstrate motor responses to strong auditory stimuli is an inhibition phenomenon.

The influence of sound produced by various electric bells on the respiratory movements of frogs and toads was studied by Ryo Kuroda (1926) who found their respiratory rate to be quite irregular and rapid under the experimental conditions. The sound of one bell (no frequency given) seemed to be inhibitory but that of another bell produced a slight acceleration.

The fact that external stimulation by various factors (light, sound, changes in temperature etc.) causes a change in the respiration rate has been noticed by several other investigators. Blankenagel (1931) who studied the function of the cerebral hemispheres in *Rana temporaria* came to the conclusion that the cerebral hemispheres exert an inhibitory influence on the frequency of the movements of the floor of the mouth and control their amplitude. These respiratory movements are influenced by sudden changes in the external environment, by acoustic stimuli, sharp changes in the light intensity, etc. Observations of this nature were made by Diebschlag (1934-35), Bajandurov (1932-33) and others.

c) The effects of sound on motor responses

In the course of his studies on the behaviour of frogs Yerkes (1905) failed to obtain any motor responses to ordinary sounds in the laboratory. However in an original experiment in which a tactual stimulation accompanied or soon followed the acoustic stimulus he observed that the sound can modify the responses of a frog depending on the time interval between the auditory and the tactual stimulations. The experiments were conducted on Green, Leopard and Bull Frogs. Yerkes was able to show that a reflex motor response was reinforced by the sound of an electric bell, applied within an interval of 0.35 second before the tactual stimulation. Intervals longer than 0.35 second but shorter than 1 second weakened the response. After an interval of one second the response to the tactual stimulus was completely inhibited. Thus, Yerkes concluded, an auditory stimulus may either reinforce or inhibit a reflex motor response, depending on the time interval between the acoustic and tactual stimuli. The above experiments were performed with vibrations of 50-10,000 cps. After the removal of the tympana and the columellae sounds still modify the reactions of the frog. Cutting of the acoustic nerves caused the disappearance of these phenomena. The author observed that the frogs reacted more vigorously during the breeding season. In the winter period their sensitiveness to the auditory stimuli had diminished considerably, especially in males. No attempt was made to determine the whole range of perception beyond the frequencies mentioned above; the site of hearing in the labyrinth has not been determined either.

Bruyn and van Nifterik (1920) repeated Yerkes' experiments on *Rana clamitans* and *Bufo* and confirmed his findings. However they found that in *Bufo* the sound stimuli affected motor responses even with an interval of 10 seconds between the sound and tactual stimuli.

Using a somewhat different experimental approach Yerkes (1904) conducted further studies on this problem. The experiments proved not to be very conclusive and in Yerkes' opinion audition is of secondary importance to the frog.

d) Conditioning of frogs with sound

Although fish lend themselves quite easily to some forms of conditioning, turtles (Poljakow 1930) are able to form associations with come considerable difficulties. The frog apparently possesses the ability to learn, although the process requires a lengthy period of time. In a series of experiments by means of the maze method conducted by Yerkes (1903), it was discovered that new habits require for their formation considerable time and they can be easily inhibited by such factors as fright. He failed in the attempts to form in frogs an association between light stimuli and electric shocks and auditory stimuli and electric shock.

In the course of his investigations of the functions of the cerebral hemispheres in *Rana temporaria* F. Blankenagel (1931) made an attempt to train these frogs on sound stimuli. In order to escape an electric shock his frogs could jump into a tank filled with water. The sound was given with a whistle whose pitch could be adjusted. In spite of continuous training during a period of three and a half weeks the animals could not be made to jump into the water on sound alone. Thus the expected conditioned reflex had failed to appear. The visible reaction was restricted to occasional body movements (turning, squirming etc.) and it was noted that more frogs stayed in the basin instead of coming out onto the platform. The frogs from which the cerebral hemispheres had been removed did not show this behaviour. Blankenagel concluded that the normal frogs form associations, which, although they do not lead to flight, produce a state of increased sensitiveness or "awareness".

Bajandurov (1932-33) used acoustic and light stimulation to condition *Rana esculenta*. He designed his experiments in such a way that not only the frog's motor reactions but also its respiratory movements could be measured as they were recorded on the revolving drum of a kymograph. The animals were confined to a completely dark chamber. Prior to the training 18 frogs were tested as to their sensitivity to sound and found to be indifferent to it. However after several combinations of sound with the electrical stimulations some of the frogs began to show alteration of their respiration movements that at later stages of the experiments was followed by motor reactions. These were quite inconstant and appeared usually at the end of the day. When the sound was not reinforced with electrical stimulation all of these phenomena disappeared, which, according to Bajandurow, puts them in the class of conditioned reflexes. It is interesting to note the temporal relationship that becomes evident during this work: the formation of the conditioned reflexes was more difficult, nearly impossible if the conditioning stimulus (sound) followed the reinforcing stimulus at an interval greater than 0.5 to 2 seconds.

Diebschlag (1934-35) kept his animals in an environment as natural as possible. The terrarium contained a water basin and the rest was overgrown with moss. The author tried to condition the frogs to light and sound using weak electric shocks as a reinforcing stimulus. The buzzing tone of an inductorium was used as an auditory stimulus. The results were quite inconclusive; the author considers the conditioned reflex, obtained under such circumstances, to be very labile and easily extinguishable. In the course of the experiments the following observation was made which is of special interest: at first the tone was produced only when the frog was jumping; later the frog jumped only when the inductorium produced its characteristic sound. After a failure of the tone to appear two to five times this phenomenon disappeared.

In summarizing the literature data on hearing in frogs we find that according to these data neither in their natural habitat nor in the experimental conditions do these animals respond to sound by motor reactions. It has been observed that during the breeding season the females of some species move towards the croaking males, but it has not been definitely proven that sound was the stimulus entirely responsible for this movement.

The direct observations on the ear and the acoustic nerve indicate a) that the electric potentials from the acoustic nerves could be obtained, but mainly on "loud" sounds; b) that the tympana of the frogs vibrate in response to sound stimuli.

The occurrence of the electrical potentials depends, according to Adrian, on the frequency of the sound and on the temperature of the environment.

The results obtained by authors using the GSR method reveal that the frogs in many instances display a sensitivity to sounds in general.

The respiratory movements of the frogs are influenced by the acoustic and other environmental stimuli.

In the frog an acoustic stimulus can produce a change in a reflex motor response to another stimulus (tactual e.g.)

The experiments in which an attempt was made to condition frogs to sound and light either failed entirely or were not very convincing.

Thus no definite proof as to the ability of the frogs to perceive sound has been furnished by inducing motor responses.

Provided the indirect evidence of the sound perception in frogs is accepted, the frequency range and the intensity threshold remain undetermined. The influence of the season and sex on hearing ability are also unknown.

Direct motor responses to acoustic stimuli would prove beyond doubt that the animals perceive these acoustic stimuli. Among several methods found in the reviewed literature only the following could supply direct evidence for the hearing ability of the frogs: recording of the electrical potentials and GSR; conditioning; recording of the respiratory movements.

In the present investigation conditioning to sound and the effect of sounds on respiratory movements were studied. In addition observations were made on the threshold of perception in this species.

METHODS

I Method of conditioned reflexes

II Recording of the changes in respiratory movements

III Observation on the threshold of perceptions of sound

Method of conditioning

Two series of experiments were conducted: series I in February-March 1954 and series II in May-September 1954.

Series I

In this series of experiments four specimens of *Rana pipiens* were used. A frame covered with plastic screening cloth was placed horizontally in a terrarium about 2 inches above the bottom. The screening cloth served two purposes: firstly to permit the dampness to rise from the water on the bottom of the terrarium and prevent the dessication of the animals and secondly to hold copper wires in place, which were arranged at a distance of 1 inch from each other and which transmitted the electrical stimulus to the frogs sitting on them. These wires were connected to an inductorium, fed by two dry cells, 1.5 Volt each. An audio-generator served as a source of sound, transmitted by a telephone. The latter was placed in the terrarium at a height of about 15 inches, on one of the side walls. The frogs remained in the terrarium during the whole period of these experiments. To prevent the animals from seeing the observer the walls of the terrarium were covered with thick paper. This also kept down the intensity of the illumination inside the terrarium. The top of the terrarium was covered with a glass plate in order to prevent excessive evaporation and the escaping of the animals. A time-delay-relay of conventional circuit was used which permitted an automatic delivery of the acoustic and electric stimuli every 30 minutes. The time lag between these stimuli (the sound preceding the shock) could be varied from a fraction of a second to 3 seconds (Figure 1).

Series II

This series of experiments (May-September 1954) consisted in a) the observation of the animals in respect to their reactions to the environmental sound stimuli; b) the study of the responses of the frogs to various frequencies produced by the audio-generator and attempts of conditioning to these frequencies (200 cps and 300 cps); c) tests with an "escape board". These experiments are described below.

a) From May-June 1954 a number of frogs *Rana pipiens* and *Rana clamitans* (male and female) were kept in moist battery jars, each jar containing two animals. The motor reactions of the animals to various environmental acoustic stimuli were observed.

b) During the same period (May-June) frogs of both species were subjected to conditioning experiments. They were attempted with sound of 50, 200, 300, 500, 2000, 8000, and 10000 cps. The frogs were kept in the battery jars in pairs. Ten to fifteen minutes before the experiment each pair was transferred into the experimental terrarium where it was submitted to one particular frequency only. The conditions of the experiments were the same as described above (series I) with one modification: the interval timer was replaced by a simple hand key in order to eliminate its inherent noise and to allow for stimuli at varying intervals. (Figure 2)

Since it was felt that the handling of the animals during the transfers from the battery jars into the terraria might be detrimental to the experiment a number of observations (June-September) was made by keeping the frogs permanently in the experimental terraria. The screen floor in these terraria was replaced by a foam rubber pad onto which the copper wires were placed and kept in position by a wooden frame. This change was made after several of the experimental animals began to show abrasions with subsequent wound development in places where they came in contact with the screen and wires. The foam rubber pad prevented the appearance of this condition. Two male *Rana pipiens* were kept in one terrarium and were submitted to a frequency of 200 cps. Two to four other terraria contained a pair of *Rana clamitans* each (male and female) which underwent training with sound of 300 cps. The time intervals between the presentation of the sound-shock combinations were not kept constant in order to eliminate the possibility of conditioning of the frogs to the time factor and varied from one to 35 minutes. The interval between the sound and the electrical stimulus was 0.5-2 seconds. The electrical stimulus was discontinued simultaneously with the acoustic stimulus. The duration of the combined stimulus (sound-shock) varied between 5-10 seconds.

c) In order to enable the animals to escape the electrical shock an "escape-board" was introduced into one of the terraria (September 1954) on which they could jump. The frogs were prevented from remaining on the board during the intervals between the applications of stimuli by placing the board with the frogs on it in vertical position. Then it was restored to its former horizontal position. The sound preceded the electrical stimulus by 0.5-2 seconds; their combined presentation lasted up to 30 seconds. The electrical stimulus was applied in short shocks.

Recording of the changes in respiratory movements

The influence of sound of various frequencies on the respiratory movements as observed on the floor of the mouth of *Rana clamitans* and *Rana pipiens* (males and females) was studied in the period of March-June 1955.

Individual animals were placed in a plastic box (3.5" x 1.75" x 1.5") which was covered with dark paper to prevent the influence of visual stimuli. This box was attached to a stand with a clasp that also kept the top of the box in place and prevented the animal from escaping. A heart lever was introduced through a slit and adjusted so that the movements of the floor of the mouth could be recorded on the revolving drum of a kymograph (Figure 3). After the frog was kept in the box for about one hour and had become quiet, recordings were started.

A short recording of the "normal" environmental sounds preceded the presentation of various frequencies produced by an audio-generator. These frequencies ranged from 25 to 20,000 cps. A signal magnet marked the beginning and the end of each sound presentation. A timer was used to record the time at intervals of 1 second.

Observation on the threshold of sound perception

The relative thresholds for sound perception at various frequencies were observed by measuring the output of the audio-generator by means of a galvanometer. The threshold values were determined by observing the lowest output of current at which a response (i.e. change in the respiratory movements) could still be observed. No attempt was made to correct the data so collected with reference to the curves of response of the transducers.

RESULTS

Experiments on conditioning

The first series of experiments, conducted on *Rana pipiens* in February-March 1954 (page 11) consisted of 148 trials, i.e. 148 repetitions of the sound-shock combinations.

The sound preceded the electrical stimulus by two seconds; the interval between the presentation of the sound-shock combinations was 30 minutes, repeated 4 to 48 times during a 24-hour period. The frequency of the sound was 500 cps, in pulses of 25 seconds. Several applications at random of the acoustic stimulus alone, without the electrical stimulus, showed that no conditioned reflex had been developed. At no time could a visible motor response to sound be observed during or after the 148 trials in *Rana pipiens*.

Observations made on animals in the period of May-June 1954 a) (page 12) showed that at times male *Rana clamitans* reacted to sounds coming from common environmental sources. The sound of a croaking frog produced a croaking response in some other frogs, even when visual stimulation was prevented. In several instances such noises as produced by passing trains, motor boats, wind and waves on the adjacent marsh, loud talking and laughing caused croaking and sometimes movement of the animals. A very approximate imitation of the croaking sound had the same effect. The sounds produced by an audio-generator resulted in a change of the respiratory movements in males as well as females. In one instance, when a male *Rana clamitans* was tested with the frequency of 300 cps (the speaker was at a distance of about 20 inches), it reacted with a short croak at the onset of sound. This it occasionally repeated on later tests.

Although sounds of various frequencies were tried out in the conditioning of *Rana pipiens* and *Rana clamitans* (b page 12) in May and June, no positive results were obtained in these experiments.

In the conditioning experiments conducted in June-September 1954 (as described in c) page 13) a pair of *Rana pipiens* were subjected to 445 trials (application of the sound-shock combinations). At the end of this period the frogs showed an occasional respiratory acceleration when exposed to sound stimuli alone. No other reaction was observed in this species.

Out of a total number of 14 *Rana clamitans* used in these experiments, only four survived long enough to be subjected to more than 400 trials. The results were similar to those obtained with *Rana pipiens*, with the exception of two frogs D and L which after 193 trials showed a response by movement during the presentation of the conditioning stimulus. In frog D this response was more regular than in frog L.

Table I

Number of trial	July 9, 1954	Response	
	Time a. m.	Jumping	Accelerated Respiration
321	8:16	+	-
322	8:23	+	-
323	8:32	+	-

Table I cont'd.

324	8:37	?	?
325	8:41	0	-
326	8:42	0	+
327	8:50	0	+
328	9:00	+	-
329	9:05	+	-
330	9:12	0	+
331	9:17	0	+
332	9:20	0	+

Table I represents the results for frog D on July 9, 1954. Sound alone was first given for 2 seconds; then an electric shock was applied without interrupting the sound.

Occasionally the acoustic stimulus was presented alone without being followed by the electrical shock. For instance, on the 339th trial sound was given for 4 seconds but the electrical stimulus was omitted. Both frogs reacted on sound by jumping. Frog D jumped in the first second, frog L in the third. It is of interest to notice that out of a group of 14 frogs only the frogs D and L responded postively to sound conditioning and that only these two animals had developed in the course of experiments wounds in the region of the pelvic and pectoral girdles and on the limbs at the places of close contact with the wire floor of the terrarium. After the 356th trial the experiment was interrupted and the animals allowed to recover in a terrarium with a ground cover of plant material. Being subsequently returned to the experimental terrarium (in which the screening was substituted by foam rubber) after recovering from their wounds, and exposed to further conditioning they did not again show the above response after 337 trials on D and 512 trials on L.

Artificial infliction of wounds did not reproduce these results in two other animals.

The experiments with the use of an "escape-board" (c, page 13) were conducted on a pair of *Rana clamitans*. After a fraction of a second the sound (1000 cps) was followed by electrical shocks as described before. The animals exposed to the sound-shock combinations learned to seek refuge on the "escape-board", provided they could see it either at the start of the trial (because of the initial position of the animal) or after a number of jumps at random. It was not possible to condition the frogs to escape onto the board on sound stimulus alone. When, however, an animal was already sitting on the board it frequently responded to the sound stimulus alone by motor reactions such as jumping and climbing movements.

Changes in the respiratory movements

The results of these experiments (page 14) show a distinct change of the breathing movements in response to sound. The figures 4, 5, and 6 show different types of response. Figure 4 indicates an increase of the amplitude of the movements in "a" and decrease of the amplitude in "b". This decrease is delayed by 9 seconds. Figure 5 shows at "a" an immediate inhibition of the respiratory movement after the initiation of sound. In Figure 6 the sound inhibited the respiratory movements during 1.5 seconds ("a"), while at another time ("b") a movement of the whole frog occurred in response to sound. Such a movement of the animal has been frequently recorded under the conditions of these experiments.

At attempt was made to find a relationship between the frequency of the sound, the interval between sounds and the latent period of the reaction. The result of the plots are shown on Figures 7 and 8. Here Δt is the latent period of the reaction, f the frequency and l the period between two sound stimuli. These figures indicate the absence of any relationship between the frequency and the latent period of the reaction. However the interval between the sound stimuli affects the latent period as can be seen on Figure 8. There is a tendency for the latent period to be shorter the longer the interval between the acoustic stimuli. The dispersion of the points on the graph indicate that this relationship has the character of a trend rather than of a regular phenomenon; however it can serve as an indicator of the fact that frogs are able to perceive acoustic stimuli.

Using the same method (recording the respiratory movements) an attempt was made to establish a preliminary threshold curve for hearing in the frog. The curves were measured for frequency range of 50 to 15,000 cps in three frogs (Rana clamitans, male and female, and Rana pipiens, male). A total of 70 measurements was made. Since the intensity of sound is proportional to the output current of the oscillator (V^2), the minimum value of V^2 at which the response occurred was plotted against the frequency on the logarhythmic scale to obtain the threshold curve. The result is shown on figure 9. The relationship: $V = f(\lg f)$ has been first fitted to the measured data by the method of the least squares. It has been assumed that this relationship is parabolic. This assumption is justified since the obtained function explains about 84% of the whole variance in the measurements. The relationship obtained was:

$$V = 32.907 - 21.111\ \mathrm{lgf} + 3.551\ (\mathrm{lgf})^2$$

The relationship; $V^2 = f_1\ (\mathrm{lgf})$, required in this case, can be obtained by squaring both sides of the above equation. This last equation will represent the line in the coordinates (Figure 9) and will be a curve of the fourth order, since it will contain the lgf in the 1st, 2nd, 3rd, and fourth power. The accuracy of the fit will remain the same. The results obtained from all three frogs can be expressed by the same curve, which means that the animals have a similar threshold for sounds at these frequencies. The fact that the reactions of the frogs to various frequencies are expressed by a continuous and logical curve indicates that these results are not accidental and thus it follows that frogs can hear. The change in the respiratory movements serves as an indicator of this phenomenon.

DISCUSSION

Observations by previous authors have demonstrated that sound stimuli can affect respiratory movements in the frog *Rana clamitans*. Similar observations were made with respect to the effect of sound on the galvanic skin reflex, gastric hunger movements (Buitendijk and Zerelman, 1930; Patterson, 1920) and on biopotentials in the acoustic nerve (Adrian et al, 1938).

Among the above results, already mentioned in this thesis, no exact information is available on the frequency range of sound perception in frogs, although one author states that *Rana clamitans* is able to perceive at least sounds between 50-10,000 cps (Yerkes, 1905). On the other hand it has been stated by a number of authors that the sound produced by the croaking males of frog species during the breeding season attracts females of these species. Direct observations of similar nature on the toad have also been reported (Courtis, 1907). The literature does not reveal any results of sensory physiological experiments on the exact range and threshold of hearing of frogs. Since neither this information nor an analysis of the croaking sound was available no direct sensory physiological support for the observations in the field regarding the "breeding call" of the male frog could be obtained.

The experiments described in this thesis have provided a threshold curve of hearing in *Rana clamitans* and *Rana pipiens*, based on relative sound intensities. Observation on the biopotentials in the auditory nerve of the frog (Adrian et al, 1938) give support to our findings in the higher frequency range of the threshold curve, although these authors did not give exact information on the frequency and the sound characteristics of the sources used in their experiments.

The threshold curve presented in this thesis is limited to the range of 30 to 15,000 cps. However it may not represent the whole range of frequencies perceived by the species. The elaboration of such a complete and reliable curve would require a practical source of pure sound for the extremes of the frequency spectrum. Such a source was not available.

An analysis of the croaking sound of the male frog was not made but it is likely that at least a number of its components lie within the frequency range covered by the threshold curve presented in this thesis. Whether or not these components are effective or decisive in the "breeding call" remains to be settled and depends on an analysis of the whole frequency range of the croaking sound, and of the perception, and corresponding innate releasing mechanism of the female of the species.

It is of some interest to note that measurements of the threshold perception do not vary significantly with the sex of the animals so that the threshold curves for both sexes are essentially similar.

Previous authors reported that sounds of different acoustic characteristics may have either an inhibitory or a stimulating effect on the respiratory movements of the frog. The results presented in this thesis clearly show that sounds of the same intensity and frequency may either be inhibitory or stimulating and that it must be the physiological condition of the animal rather than the acoustic characteristic of the sound which determines the nature of the response.

Some observations made on the latent period of the response to sound seem to indicate that it is not affected by the frequency of the sound. However the intervals between successive stimuli seem to be of importance--the longer the interval the shorter the latent period.

Conditioning experiments were performed by some investigators (Yerkes, 1903; Blankenagel, 1931; Bajandurow, 1932-33; Diebschlag, 1935) on various frog species (_R.R. clamitans, temporaria, esculenta_) with either negative or unsatisfactory results as to the ability of the frog to be conditioned by sound stimuli alone. In this thesis new attempts are reported on conditioning _R.R. clamitans_ and _pipiens_ with sound. The results demonstrated that out of a total of 18 experimental animals (14 _Rana clamitans_ and 4 _Rana pipiens_) two _Rana clamitans_ became conditioned after a period of training by means of a punishment technique. It seems safe to assume that frogs can be conditioned to sound under given physiological conditions as yet undetermined.

CONCLUSIONS

1. Frogs of the species *Rana pipiens* and *Rana clamitans* were shown to be able to perceive sounds of frequencies from 25 to 15,000 cps, possibly up to 20,000 cps.
2. A threshold curve for sound perception in these species has been established for frequencies from 30 to 15,000 cps.
3. The acoustic stimulus applied at different times under similar conditions may produce either an acceleration or an inhibition of the respiratory movement or remain without any noticeable response.
4. The latent period of the response to sound does not seem to depend on the frequency of the acoustic stimulus, but rather on the length of the interval between the successive stimuli.
5. No influence of sex on the perception of pure sounds has been observed.
6. The conditioning experiments on frogs showed that they can be conditioned to sound under certain physiological conditions as yet undetermined.

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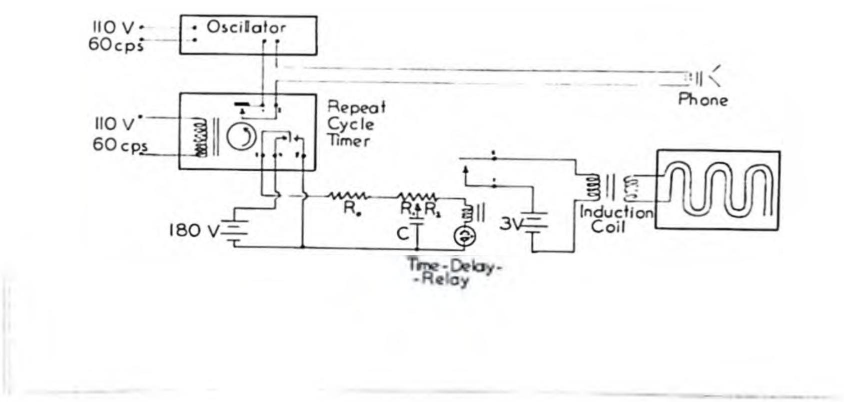


Figure 1

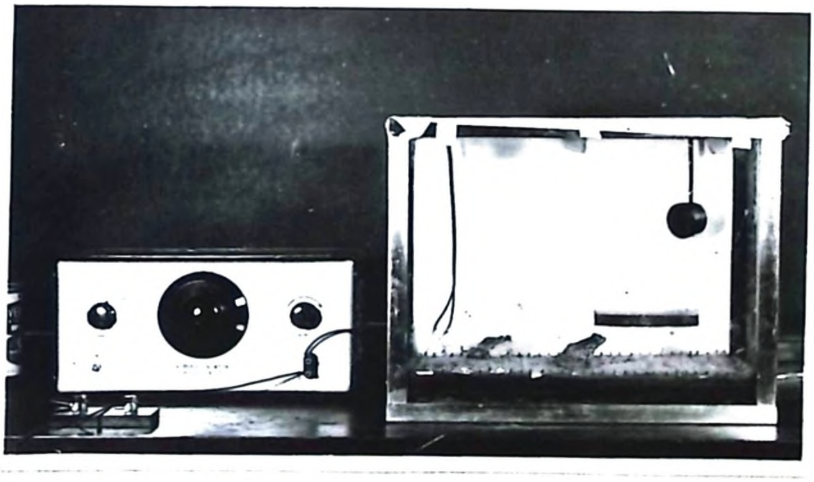
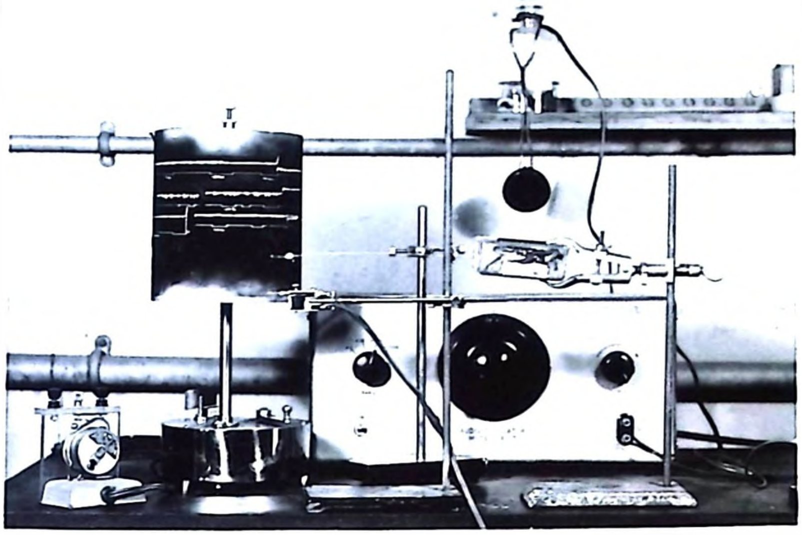


Figure 2



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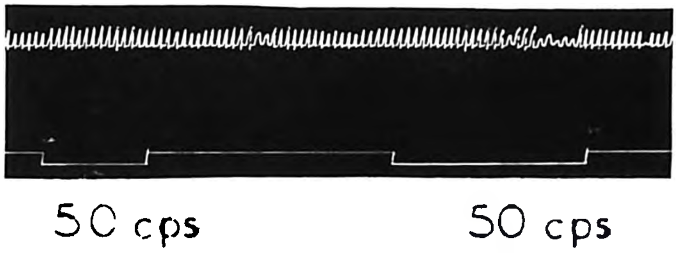
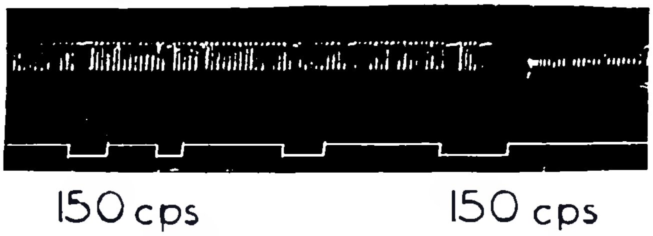


a b

Figure 4

Figure 5



a

b

Figure 6

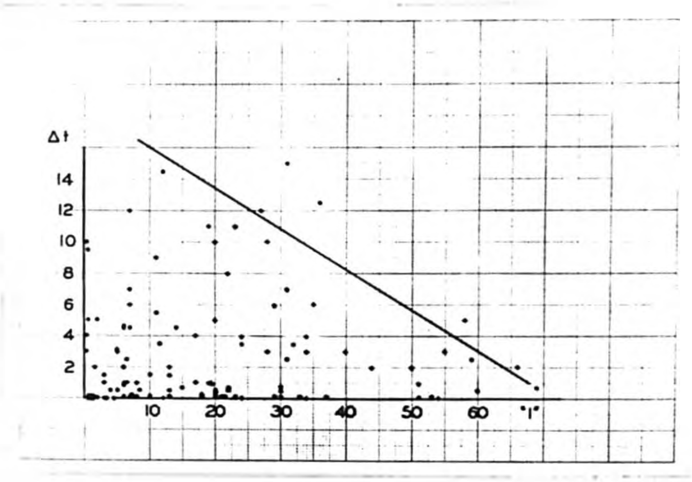


Figure 7

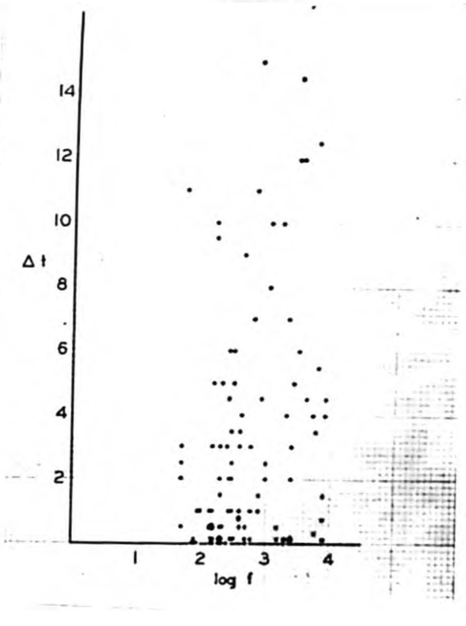


Figure 8

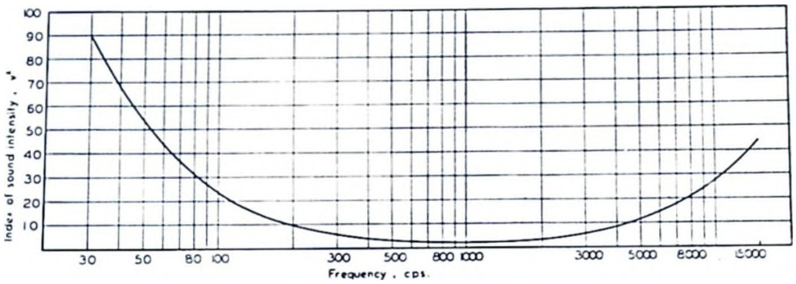

100
90
80
70
60
50
40
30
20
10
Index of sound intensity, v^2
30
50
80
100
300
500
800
1000
3000
5000
8000
15000
Frequency, cps.

Figure 9