ELECTRICAL ACTIVITY IN THE OLFACTORY SYSTEM OF SOME FISHES

OBSERVATIONS ON THE ELECTRICAL ACTIVITY IN THE OLFACTORY EPITHELIUM AND TRACT OF SOME FISHES

By

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SCOPE AND CONTENTS:

In this investigation the electrophysiological response to stimulation of the olfactory epithelium in response to trout scent and some of its chemical components was studied.

Recordings were made both from the lamprey nasal sac and the catfish olfactory tract and the various responses described and compared with responses measured by other authors.

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I HISTORICAL SURVEY

1. Electrical activity in the olfactory system of vertebrates:

i) Olfactory epithelium

ii) Olfactory nerve

iii) Olfactory bulb

iv) Olfactory tract

v) Forebrain

Reports on investigations on electrical activity in the olfactory apparatus date from as early as 1938 (Adrian and Ludwig, 1938). In the present introduction, some of the results published are reviewed under the headings shown above, rather than in chronological order.

i) Olfactory epithelium

A resting potential has been measured across the mucous membrane of the olfactory epithelium of some fish and amphibians (Shibuya, 1960; Takagi and Shibuya, 1960) and was found to vary from 4 - 18 mV.

In response to chemical stimuli, the epithelium showed a slow potential change (Ottoson, 1954, 1955, 1956, 1958a,b,c;

Takagi and Shibuya, 1959, 1960a,b,c,d,e; Shibuya, 1960; Takagi, Shibuya, Higashino, Arai, 1960) in many species. Ottoson (1956) recorded a negative monophasic response from the frog with its amplitude being proportional to the log of the stimulus intensity. Takagi et al. in all reports, referred to similar potential changes, again from the frog, but for a prolonged stimulus these authors measured an initial increase in the response which leveled off to a plateau for the duration of the stimulus followed by a final increase in potential when the stimulus was discontinued. This the authors called an "on - off" response. Such responses were obtained separately also, but consistently only when ether was used as stimulus depending on concentration. A low concentration of ether produced an on-response, a medium concentration an off-wave, while a high concentration caused only an off-response.

Ottoson (1956) made several observations from which he concluded that this slow potential originates in the superficial parts of the sensory elements. As actual sensory part, he suggests the olfactory hairs, considering the similarity between these hairs and the outer segments of the retinal cells.

In addition to the slow potential, both Ottoson and Takagi and co-workers recorded regular oscillations with a frequency of 15 - 25 cps., superimposed upon the crest of the

slow potential. However they disagreed on whether this phenomenon was normal. Ottoson obtained the oscillations only in old or injured preparations, while Takagi et al. observed them only in fresh preparations, and found that they disappeared when the preparation had been used for a while. The experiments by Takagi showed that a certain magnitude of slow potential is needed for the oscillations to appear. Takagi suggested that the generative mechanism is probably the mutual interaction of the discharges of the olfactory nerve in the non-myelinated nerve fibres. Thus, synchronization occurred both in the olfactory epithelium and in the olfactory nerve fibres.

The above observations were measured in a large number of units in the olfactory epithelium while Shibuya and Shibuya (1963) measured single unit responses in the tortoise. They obtained spike discharges which were positive relative to the slow potential. Only a small number of spikes were present per response (4-15) and their height, frequency, and number increased with the intensity of the stimulation. They concluded that the spikes were led from the olfactory cell rather than from the axonal extension.

ii) Olfactory nerve

Ottoson (1954, 1959) made recordings from the cut end of the olfactory nerve in rabbits and frogs. He obtained a

sustained potential on which no superimposed waves were found and concluded that this potential was generated in the olfactory receptors. When the olfactory bulb was intact, different components were present due to the influence from the bulb.

Takagi et al. (1960) recorded from the olfactory nerve with the olfactory bulb intact and obtained potential oscillations with frequency and magnitude in addition to the slow potential. These oscillations followed a different sequence from those in the epithelium, but were quite similar to those in the bulb. This indicated that the oscillations in the olfactory nerve originate in the bulb rather than in the epithelium.

Simultaneous recordings from the nerve and epithelium in frogs by Mozell (1961) led him to the conclusion that there is no simple relation between their responses.

Tucker (1962) made a detailed study of the olfactory system by recording from the olfactory nerve of the tortoise under varying environmental conditions. The results of this study are unrelated to the present investigation.

iii) Olfactory bulb

In the olfactory bulb of the rabbit, Adrian (1950a) found electrical activity of two types: brief spikes and waves, 5 to 100 p.s. The brief potentials are found in the

central core of the white matter which forms the olfactory tract, mainly composed of axons of mitral cells, while the waves are recorded from the surface of the bulb. The potential waves are again divided into two groups: a) large sinusoidal oscillations, usually at a fixed frequency (65 - 10 p.s.), set up by strong olfactory stimuli and lasting only for the period of stimulation. Adrian suggests that these waves are due to a synchronized beat developing in a number of units under maximal excitation; b) smaller and often less regular waves usually associated with persistent activity in the cells of the bulb (intrinsic activity of the bulb). This activity remains after severing of the nervous connection between bulb and forebrain. In the mammal this activity is suppressed by deep anesthesia while in medium anesthesia the discharge is often so great that it was found impossible to detect any change with a weak olfactory stimulus. The frequency during deep anesthesia varies from 7 - 10 p.s. and increases with decreasing anesthesia up to 100 p.s.

Walsh (1956) measured activity from single units in the olfactory bulb of a rabbit and found that the discharge could be divided into three classes: 1) continuous with respiration. This type was absent in cannulated animals. The frequency of the impulses in the bursts was quite constant; 2) responding to stimulation by one or more odours and 3) odour specificity seemed indicated.

Both Ottoson (1959) and Adrian (1950a) mention responses in the rabbit corresponding to the above classes 1 and 3, but they seem to include class 2 with 3. They also recorded activity in response to normal breathing (air), but both mention that this activity decreases or vanishes when air is filtered through carbon. This indicates that the activity may be caused by odour elements in the ambient air.

Others who found spontaneous electrical activity in the bulb are Yamashita-Yii (1958), Hernandez - Peon et al. (1960), and Yamamoto and Iwama (1961). The former observed that the olfactory bulb showed slow waves, 6-7 cps. while fast waves of lower voltage occurred intermittently.

In 1959 Ottoson recorded from the olfactory bulb of rabbit and frog and found that stimulation of the nasal mucosa with odorized air set up a slow potential on the bulb surface which varied greatly with depth of anesthesia and from one preparation to another. A typical response consisted of a sustained potential with superimposed waves which are usually highest on top of the slow potential and decline. From his recordings, Ottoson concluded that the general properties of the slow potential change suggest that it is homologous with the dendritic portion of the cerebral cortex. The slow bulb response was found to be strikingly similar to the slow potential of the epithelium in shape and time course. However, they must be of different origin as shown by different responses to drugs and

a greater susceptibility of the former to asphyxia.

Ottoson summarizes the process as follows: "When the nasal mucosa is stimulated, the olfactory end organs develop a susteined potential that spreads along the fibres and gives rise to an impulse discharge. The afferent flow in the bulb causes a slow potential change in the glomeruli which closely follows changes in potential of the receptors. As the potential becomes larger, an increasing number of secondary neurons are thrown into synchronous action which in gross recordings is reflected as regular waves superimposed on the slow potentials. The waves are accompanied by bursts of impulses in axons passing centrally to rhinencephalic centres."

Ottoson's recordings were confirmed by Takagi and Shibuya (1960a,b,c,d,e) in frog and toad who also showed the presence of slow potentials with superimposed potential oscillations. They point out that these are of separate origin because of differences in frequency, magnitude, shape, and time of appearance. Mozell (1962) also compared the responses from the epithelium and bulb in the frog and also found the important differences.

(iv) Olfactory tract

There are only two studies of the electrical activity in the olfactory tract of the catfish: Boudreau (1962) and

Adrian and Ludwig (1938). Both of these papers studied the resting activity and the response to mechanical and chemical stimulation.

Adrian and Ludwig (l.c.) recorded a resting discharge which lasted as long as the olfactory organ survived even though only water had access to the sac. This activity disappeared when the tract was cut near the bulb and it diminished when Novocain was placed on the epithelium. Boudreau (l.c.) also found a high level of spike activity when there was no experimental stimulation of the olfactory receptors and again this activity was eliminated by the cutting of the tract between bulb and electrode and by the introduction of 2% Procaine into the sac.

Adrian and Ludwig (l.c.) point out that mechanical stimulation is the simplest way of producing a discharge and found that pressure on the roof of the sac is usually effective while touching the epithelium with a brush or moist filter paper will set up repeated discharges. However, they concluded that the effect must be due to injury of the epithelium. Boudreau repeated these experiments with similar results. He observed that the starting of a stream of water through the olfactory tract caused a drop in total activity while the cessation of the stream was followed by a profound increase in tract activity often lasting several minutes.

Adrian and Ludwig used as a main stimulus water with

decomposing animal matter and found it to be a powerful stimulus. Application of this solution to the epithelium caused an increase in quantity and size of spikes recorded. Boudreau used a variety of substances dissolved in the wash-water and found many of them to affect the spike activity in the tract. Certain chemical substances, such as butanol, acetic acid, altered the level of activity at extremely low concentration. In his experiments continuous stimulation resulted in a gradual decline of responses while a repetitive stimulus yielded responses of smaller and smaller amplitude.

Adrian and Ludwig (l.c.) found that filtering of the stimulating fluid decreased their effectiveness and contributed this to the mechanical stimulation of the particles against the folds of the epithelium, although Boudreau found the epithelium extremely sensitive to substances freely dissolved in water. Both Adrian and Ludwig, and Boudreau seemed to agree that many of the preparations showed somewhat erratic responses to chemical stimulation while mechanical stimuli provided the most consistent responses.

(v) Forebrain

Adrian and Ludwig (l.c.) observed a rhythmic electrical activity occurring in the forebrain and producing

large potential changes at 2-4 p.s. These continued after the olfactory tract had been cut. Occasionally an olfactory discharge seemed to stop or start the rhythm in the forebrain, or to upset the regularity for a few beats although as a rule the rhythm seemed to be quite independent of the olfactory system.

2. Discrimination

Very little electrophysiological work has been done on the discrimination of odours. However, a thorough investigation of specificity of units in the olfactory bulb was made by two workers: Adrian (1950 b,c, 1953) and Basavaraju, (1960).

Adrian inserted a micro-electrode into the olfactory bulb of the rabbit, recorded series of spikes of different sizes and attributed these to different mitral cells in the surroundings of the electrode. Based on these results he was able to divide olfactory receptors into four or five groups, each sensitive to a particular group of chemicals, e.g. aromatic compounds, terpenes, etc. The receptors in each group will respond to all the compounds in that group but are more sensitive to a particular chemical within the group. The specificity of a unit has never been found to be altered during an experiment.

Basavaraju (l.c.) also recorded from single units

in the olfactory bulb and found that many were specific to a particular odour or group of related substances while a few were non-specific. From this he concluded that there exists specific receptors in the olfactory epithelium which undergo functional segregation to end in synaptic connection with the mitral cells. He suggested that a single substance stimulates several different types of receptors, **e.g.** nonspecific, hydrocarbon-specific and aromatic- specific receptors. The function of the nonspecific receptors may be intensity discrimination, or the signalling of the presence and absence of odours. In addition to the above-mentioned spatial discrimination, Adrian suggests the existence of a temporal mechanism of discrimination. He found that the time course of a discharge, especially the rate of onset and rate of decline, may vary with the smell. The latency of the discharge may vary both with the velocity of the air current and with the chemical and physical properties of the stimulating molecules. Basavaraju's results agree with this and he divides the responses into three groups: discharges with 1. sudden rise and gradual drop; 2. sudden rise and sudden drop; 3. gradual rise and gradual drop. He attributed these variations, in agreement with Adrian, to the lesser or greater ease with which an odour gets into the folds of the epithelium. Mozell (1958) also worked on the electrophysiology of the olfactory

bulb of the frog and confirms Adrian's results and conclusions except for a few details.

II SCENT OF FRESH WATER FISH

A profound effect of the scent of prey on the behaviour of lamprey was described by Kleerekoper and coworkers (Kleerekoper, Taylor and Wilton, 1961). In this paper it was shown that the lamprey displays an endogenous cyclic activity. When the periodicity was gradually lost it could be reestablished by the introduction of trout scent into the tank containing the lamprey. The physiological clock could be reset by the mere addition of the scent, which, in addition, caused an immediate burst of activity.

Following the initial observations on the behavioural response of lamprey to the odour of prey a chemical study was made of the qualitative and quantitative aspects of trout scent. The total amino nitrogen was found to vary from 63-87% of total nitrogenous matter of which 98% was ammonia, and the remaining 2% consisted of amines. Ten amines were separated and assigned letters from the alphabet, A, B, ...J. Some of the amines isolated were tested in lieu of the "whole" trout scent and it was found that amines C and F had similar effects on the periodicity and activity of lamprey (Kleerekoper, 1961). A few experiments with catfish indicated similar results. The

extreme sensitivity of the sense of smell of catfish had already been indicated by Olmsted (1918).

Later observations (Kleerekoper and Mogensen, 1963) established that the perception of the prey's odour also has a directional effect on the predator.

III STATEMENT OF THE PROBLEM

The purpose of this investigation was to study the electrophysiological response to stimulation of the olfactory epithelium with "troutwater" and some of the amines isolated (Kleerekoper and Mogensen, 1961) in order to ascertain whether stimulation with these substances produces qualitatively and quantitatively different effects.

MATERIALS AND METHODS

I CHOICE OF EXPERIMENTAL ANIMALS

Two types of fish were used in these experiments: the sea lamprey, Petromyzon marinus, and several species of bullheads, Ameirus m. melas, Ameirus n. natalis, and Ictalurus l. lacustris, i.e. the Northern black bullhead, the Northern yellow bullhead and the Northern channel catfish.

In the catfish recordings were mainly taken from the olfactory tract, while in the lamprey the electrode was inserted in the nasal sac from the posterior direction and thus the recordings taken from the olfactory epithelium and the olfactory nerve.

II PREPARATION OF THE ANIMALS FOR EXPERIMENTATION

The anaesthetic found to be most useful in these experiments was urethane (Ethyl carbamate). For the bullheads a 2% solution was found most useful, although occasionally a stronger solution had to be used. For lampreys, a 1% solution was usually sufficient. The fish were anaesthetized by submersion into the solution.

Recording occurred with the fish immobilized in a horizontal position on a thick layer of wax in a rectangular

container.

III METHODS FOR RECORDING NERVE POTENTIALS

Recording was done with stainless steel electrodes on which fine points had been produced by the electrolytic method as described by Grundfest et al (1950). The needles were coated with insulating varnish (Westinghouse C.W. 8714-10, or C.W. 7826-3). The latter type was generally the most useful varnish. The electrical activity was displayed on a Dumont Dual Beam Cathode Ray Oscilloscope after having been amplified by a Grass DC Preamplifier Model P4A. The electrodes were mounted in a Brinkman micromanipulator.

A permanent record was obtained by photographing the screen of the oscilloscope with a Grass Kymograph Camera, Model C4D. In this investigation the oscilloscope beam was held stationary so that the spot moved vertically only, while the film moved continuously providing a continuous record. Clear base Linograph Ortho film was used. The oscillographic work was performed inside a large Faraday cage.

IV METHODS OF APPLYING STIMULI TO THE OLFACTORY ORGAN

Chemical stimuli were applied to the epithelium by a variety of methods, depending on the animal used and on

the desirability of establishing the exact time of stimulation.

(i) Continuous flow method

This method was used on catfish only. Apparatus was built to provide a continuous flow of distilled water from the main supply (fig. 1A). The chemical was added from a burette graduated in 1/20 ml. and was then flushed down into the main tube by a second water supply (C) which contained water from the same source as the main supply. The purpose of double supply system was to make possible the flushing of remaining chemicals in tube (D) between experiments so that no contamination of the wash-water occurred. Differences in flow rate due to the opening of the different stopcocks were not of great importance since it took about one minute before the chemical reached the epithelium by which time the flow had become steady. This time was measured by using methylene blue dye and observing the time required to reach the epithelium and that for the dye to disappear entirely from the system. The main supply tube ended in a fine tip which could be inserted into the anterior nasal opening of the catfish. The water then passed over the epithelium and flowed out of the posterior nasal opening.

(ii) Spurt method

This method was used when the time of stimulation

Fig. 1 CONTINUOUS FLOW APPARATUS

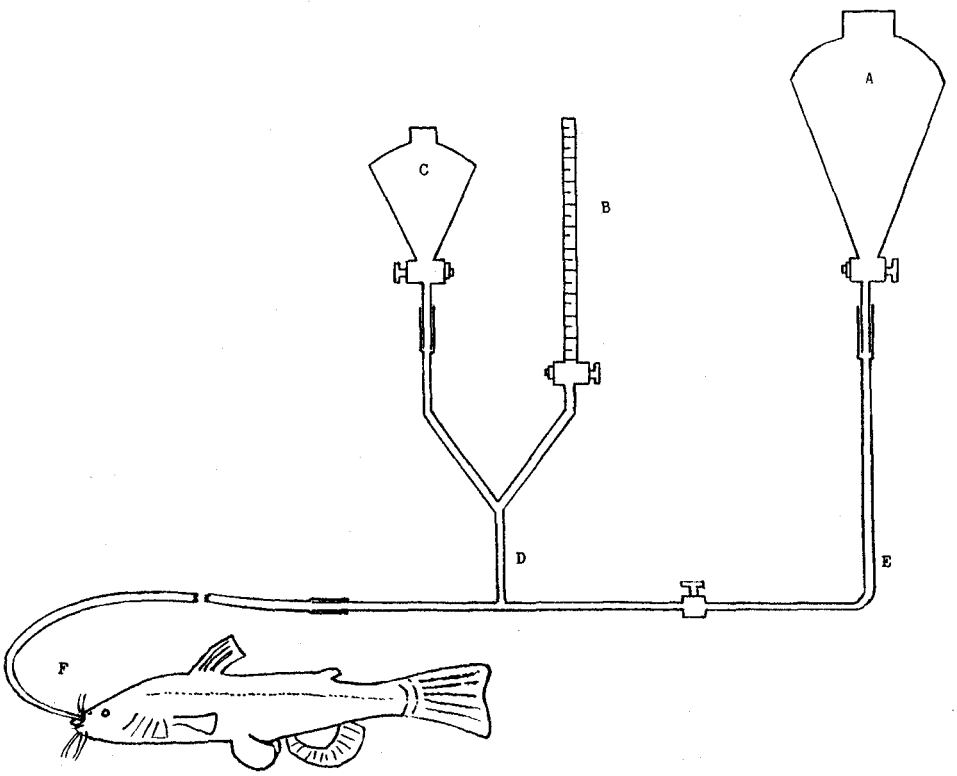
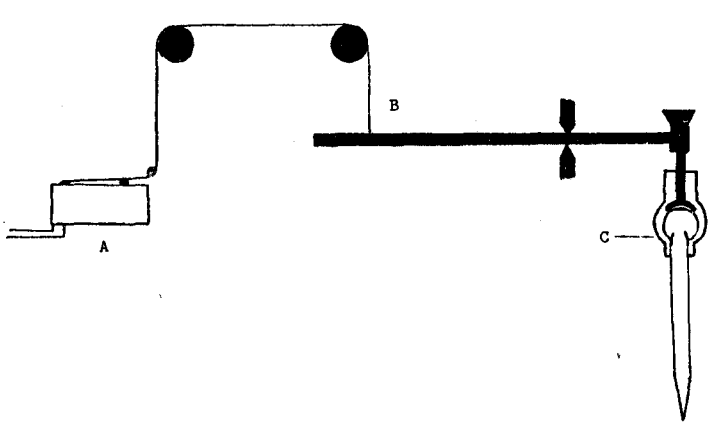


Fig. 2 DIRECT STIMULATION APPARATUS



of epithelium was to be known exactly and when it was desired to reduce the time lapse between the release of the stimulant and its actual arrival in the nasal sac. The above described apparatus was used to supply a continuous stream of water while the stimulation solution was added on top of the tube at F, so that it would flow along the tube and onto the epithelium. For this method the flap of skin over the olfactory epithelium was cut away, which cutting could be done easily without damaging the latter. The chemical was added either from a polyethylene wash bottle or from an automatic pipette through which the amounts added could be regulated precisely. By incorporating a microswitch (fig. 2A) and a lever system (B) the automatic pipette could be operated remotely while the moment of addition could be simultaneously marked on the oscilloscope screen.

(iii) Drop method

This method was used on lampreys only. Small pipettes with capillary tip were dipped into the stimulating solution. By placing the finger over the mouth of the pipette a droplet could be suspended at the capillary tip and placed upon the nasal opening of the lamprey. Some of the liquid was then carried to the nasal sac by the respiratory action of the lamprey which alternately fills and empties the sac. (Kleerekoper and van Erkel, 1960)

(iv) The following method was only used for large lampreys. Here a small funnel was constructed by drawing out a glass tube and the narrow end of the funnel was placed over or in the nasal opening of the lamprey. If any liquid was present in the tube it could be seen bobbing up and down with the respiratory movements. Thus a stimulus could be applied which was only diluted by the liquid already present in the sac.

V CHEMICAL STIMULI

The stimuli used in this investigation were natural stimuli, i.e. they could be found in the natural surroundings of the experimental animals. Troutwater, i.e. water in which trout had been swimming, was used extensively as stimulus. Also, some of the amines which were isolated from trout water (see introduction) were used as stimulus, in particular amines G and F since the others were not readily available at the time of the investigation. For control and for comparison with other investigations (Boudreau 1962, Ottoson 1956, and others) a butanol series was prepared. Each member of a series was prepared from a stock solution by dilution as follows:

solution	butanol p.p.m.	amine F p.p.m.	amine G p.p.m.
1	7.4×10^{3}	7.5×10^{3}	1×10^{4}
2	7.4×10	7.5×10	1×10^{2}
3	7.4×10^{-1}	7.5×10^{-1}	1×10^{-2}
4		7.5×10^{-3}	1×10^{-4}
5		7.5×10^{-5}	1×10^{-6}

Troutwater was obtained from an aquarium containing approximately 225 l. of water and 30 trout. Usually this aquarium had a continuous water supply but about one half hour before the troutwater was needed the supply was shut off. The amine content of the water used in the experiments will be referred to in the presentation of the results.

VI METHODS OF EVALUATION

As mentioned above the oscilloscope trace was recorded on 35 mm. film. To analyse the results the image was projected onto the screen of a microfilm reader. In this way the spikes could be counted for an arbitrary interval of approximately 1/5 second. The spikes were then divided into arbitrary classes according to size exclusively for the purpose of comparing changes in distribution, if any, in a tracing before, during, and after stimulation.

The actual values for the spikes in each class are therefore not pertinent. The total number in each class for each interval was multiplied by a weighting factor after which the products were added for each interval and then divided by the total number of spikes in the interval. Example:

class	1	2	3	4	5	6	total
number of spikes	0	2	4	5	3	0	14

Therefore the "amplitude factor" equals $\frac{2x2 + 3x4 + 4x5 + 5x3}{14} = 3.64$.

Thus a number was obtained which was representative of the average amplitude of this particular interval. This number could be used for graphing purposes and even a small change in the amplitude which could not be seen with the naked eye showed up clearly. The frequency was obtained by dividing the total number of spikes by the length of the interval. Thus, graphs could be made of frequency versus time, amplitude versus time, and frequency versus amplitude. Straight lines through the frequency versus amplitude graphs were drawn by means of the method of the "least squares" (Widder, 19) and the formula:

$$\begin{vmatrix} x & y & 1 \\ \sum_{i=1}^{n} x_i & \sum_{i=1}^{n} y_i & \sum_{i=1}^{n} 1 \\ \sum_{i=1}^{n} x_i^2 & \sum_{i=1}^{n} x_i y_i & \sum_{i=1}^{n} x_i \end{vmatrix} = 0$$

where x, y are the variables

x_i the abscissa of the i^{th} coordinate

y_i the ordinate of the i^{th} coordinate

n the number of points.

R E S U L T S

I OLFACTORY TRACT OF CATFISH

1. Resting activity

Considerable activity was recorded from the olfactory tract of the catfish even though no stimulus was applied to the epithelium. This activity may be divided into two concurrent phenomena.

(i) The first is a slow, more or less regular change in the baseline which was found very consistently in all records from the olfactory tract. (fig. 3) These are negative slow waves of 1-2½ second duration, with amplitudes varying generally from 40-50 μV although in a few cases they were found to be as high as 100 μV. The duration, amplitude and frequency of these slow waves are variable among individuals but are very consistent for any particular individual. The characteristics of shape and distribution of these waves are usually quite regular but the degree of regularity again may vary among individuals. It appeared that when the characteristics remained very similar during a tracing the animal was more responsive to odours. The waves are only occasionally affected by olfactory response. They may be obliterated during the response and reoccur afterwards with their original characteristics. Less frequently the waves will recover with the original rhythm but in a different phase.

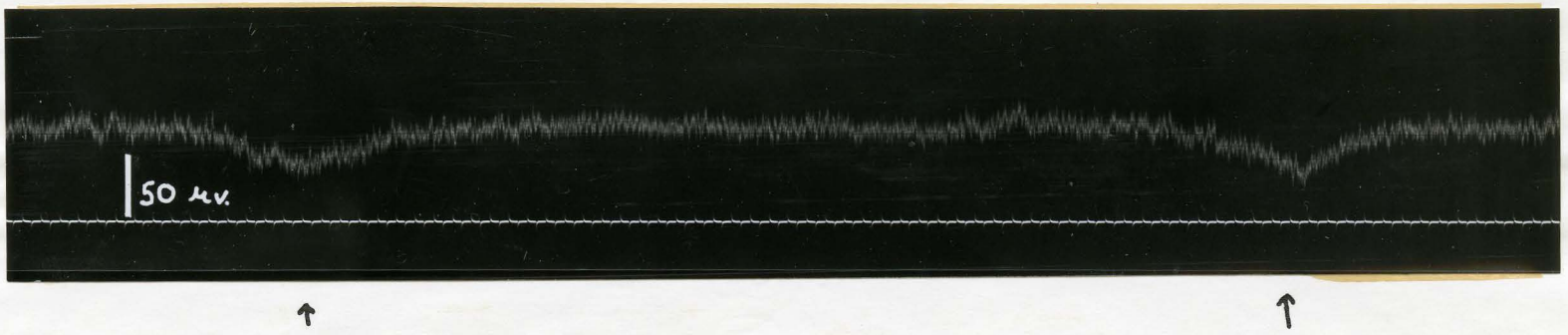


Fig. 3. Resting activity of the olfactory tract of the catfish. This record shows both the spike activity and the regular large waves (at marks). Time calibration 1/20 sec.

Between the waves is generally a plateau which may be completely straight or show some small waves at high magnification. However, at certain positions on this plateau other waves may appear which are sensitive to olfactory stimulation and will be discussed in section 3(ii). The waves remain unchanged after the olfactory tract is cut distally to the forebrain but centrally to the electrode.

(ii) Spike activity

Superimposed on the large waves is a volley of spike activity, which is present in all recordings. Even when no stimulus is applied the amplitude of the spike can be as high as 50 μV although it is most commonly less. The frequency is difficult to determine because the spikes are often overlapping in the records and should be at least 80-100 p.s. The spike activity also remained unchanged when the olfactory tract is cut between electrode and forebrain.

A typical set of graphs drawn from a record of resting activity is shown in fig. 4. The set includes a graph of frequency versus time, amplitude versus time, and a scatter plot of frequency versus amplitude. These graphs show that although both frequency and amplitude may vary with time the average level remains about the same when no stimulus is applied. The possibility of a regular re-

curring rhythm in spike activity especially in its frequency was considered. It can be shown that the values for frequency times amplitude of spikes are higher in the descending phase of the large waves than on the ascending phase.

2. Mechanical stimulation

(i) Effect of water flow

During most of the experiments a continuous flow of water was passing over the epithelium. Some records were taken before the start of the flow of water, and these were compared with the activity of the epithelium after water had been flowing for a while. Also, the effect of the starting and the cessation of the flow was observed. In some cases an interesting change in the behaviour of the large waves was noted when the records of the activity before and after flow of water were compared. After the water had been flowing for a while the rhythm appeared to have acquired an increased regularity while the magnitude of the waves was smaller than when no water was flowing. (fig.5)

Changes in spike activity were also noted when water flow was in the process of starting or stopping. (fig.6) The starting of flow causes a decrease in spike amplitude while the frequency increases slightly or remains the same. The spike activity returned to normal within a

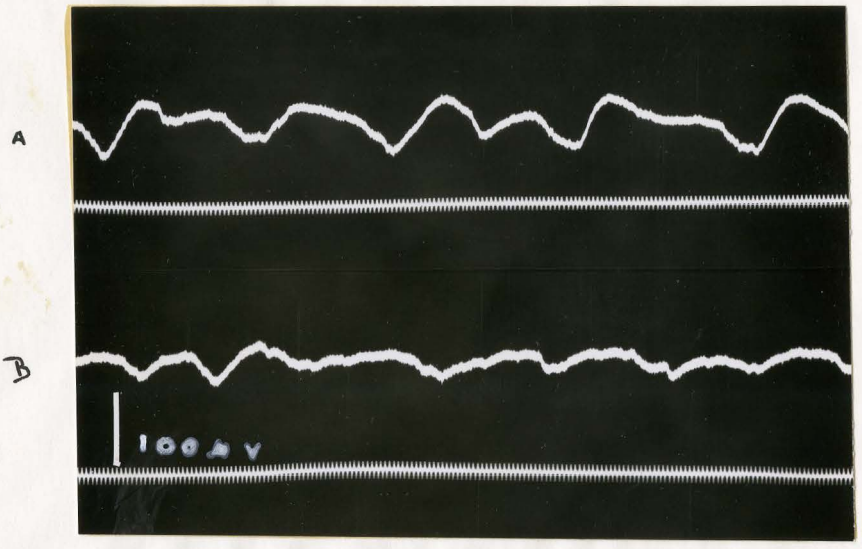
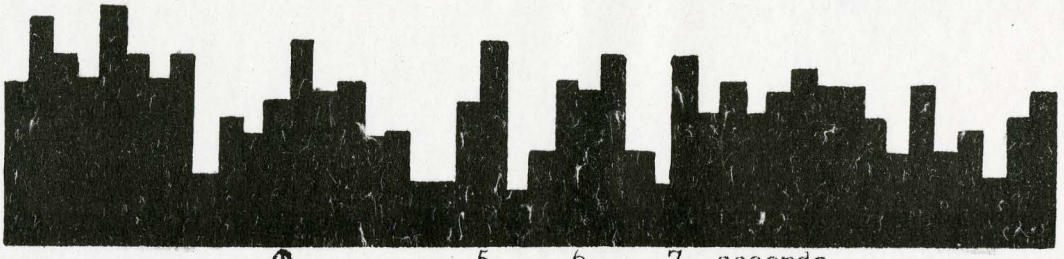


Fig. 5 Large wave resting activity

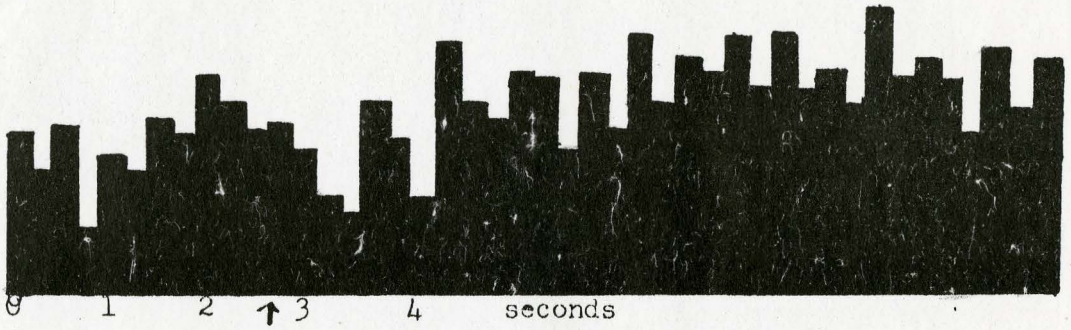
A. No water flowing

B. Water flowing

Time calibration 1/15 sec.
Vertical line, 100 μ v.



a) frequency versus time



b) amplitude versus time

Fig. 6. Spike responses to cessation of water flow.

short time. Cessation of water causes a definite increase in spike amplitude with a slight decrease in frequency. Again the spike activity returned to normal within a few seconds.

(ii) Pressure

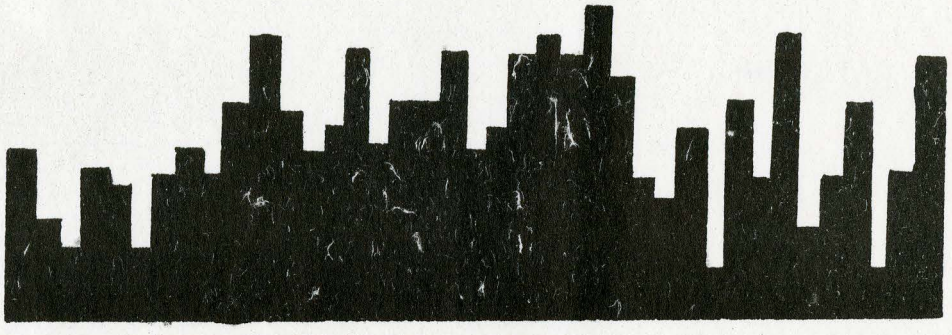
The application of pressure on the roof of the nasal sac was consistently found to have effect on the spike activity in the olfactory tract (fig.7). Throughout the period of pressure an increase in frequency was found which returned to normal immediately after the complete release of the pressure. The amplitude of the spikes decreases somewhat with the onset of the pressure. After the pressure was completely released the amplitude returned to normal within a few seconds.

A larger wave also occurs which shows the exact time of the pressure (fig. 8). This potential change is negative with the onset of pressure, returns to normal during the pressure and then becomes positive as the pressure is released.

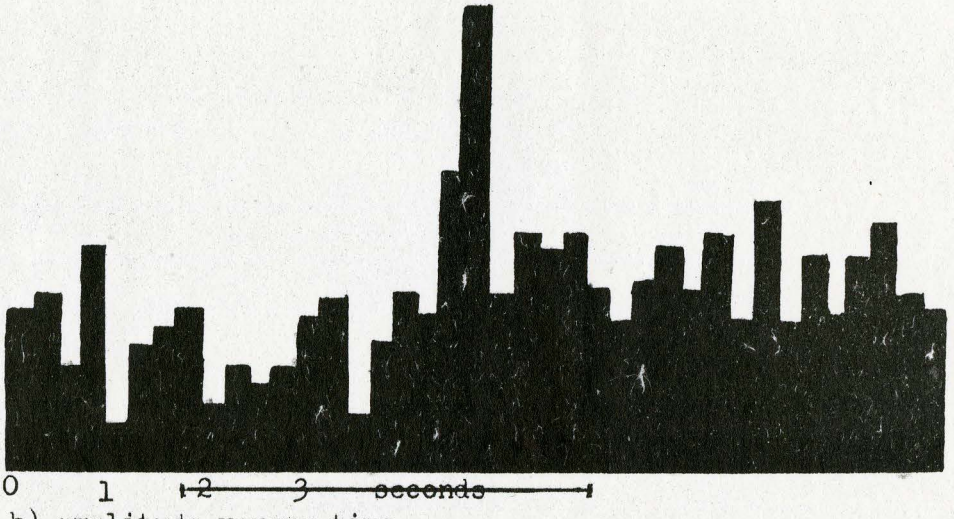
3. Chemical stimulation

(i) Response to butanol

On applying butanol to the epithelium two types of changes were noted in the activity of the olfactory tract. The most striking response was the occurrence of large



a) frequency versus time



b) amplitude versus time

Fig. 7. Spike responses to pressure on the roof of the nasal sac.

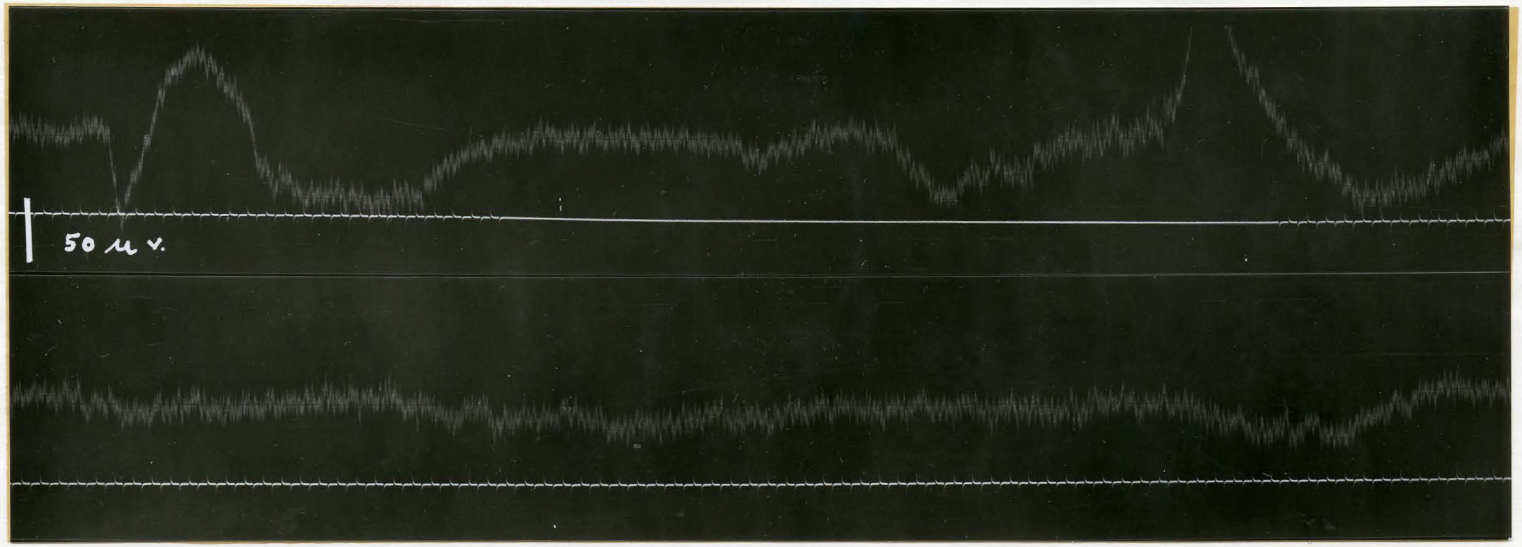


Fig. 8. Pressure on roof of nasal sac of the datfish.
The response is measured from the olfactory tract.

sinusoidal oscillations (fig.9) which varied in size and number with the strength of the stimulus. Whenever this response is produced the frequency of the oscillations is rather constant within one test and varied from 6-8 p.s. However, variations are shown in the amplitude and number of the oscillations and these depended on the strength of the stimulus. The strongest butanol solution (7.4×10^{3} p.p.m.) causes a train of 24 waves about half of which were at the maximum amplitude of 60 μV after which they slowly decreased in size until they could no longer be seen. The second strongest solution (7.4 p.p.m.) produces only 8-14 waves which, however, started out at the maximum amplitude of 75 μV (two or three waves) after which they decreased to about 30 μV. They then continue to decrease until they can no longer be seen. The weakest solution shows only a few small waves with the maximum between 20 and 30 μV while the waves themselves decrease in size very regularly.

The second type of change in the olfactory tract in response to the addition of butanol to the epithelium is seen in the spike activity (fig.10). The spike amplitude shows a definite increase in response to the stimulus while in the frequency only a slight increase was noted. An increase in strength of stimulus causes a greater increase in spike amplitude. The variation of spike amplitude with stimulus strength could not be determined ac-

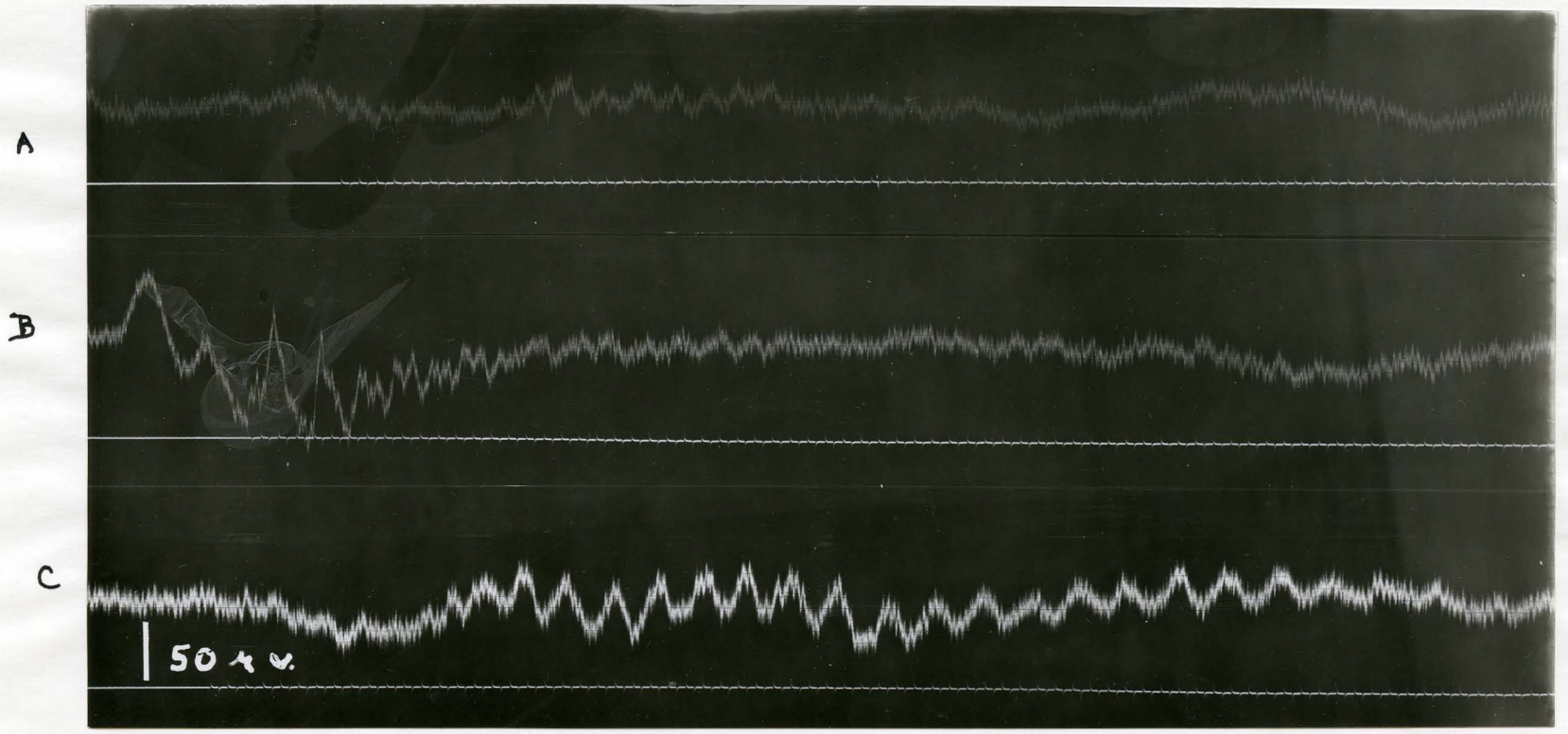
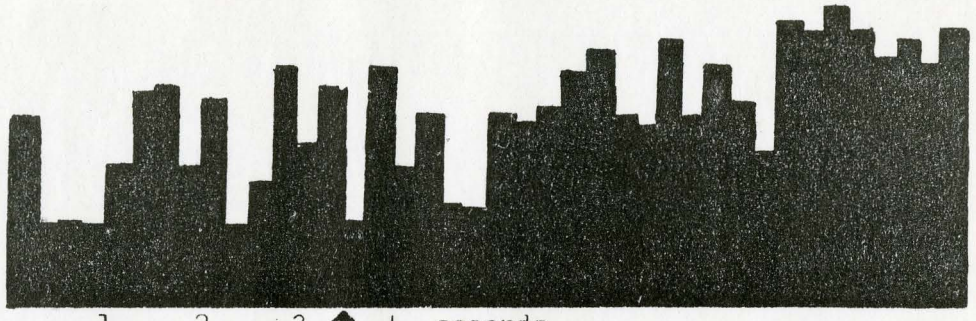
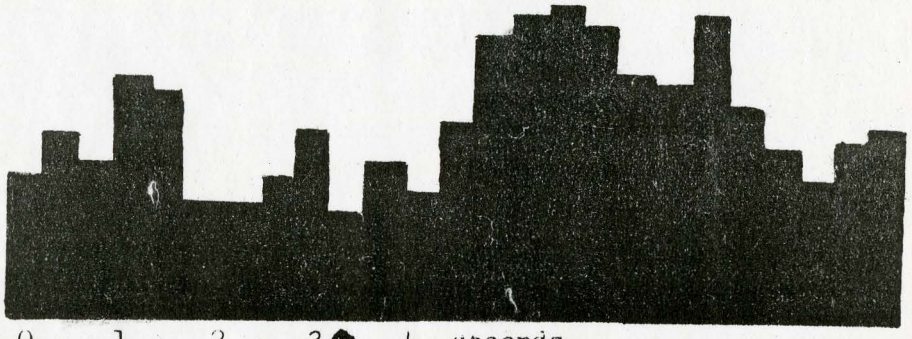


Fig. 9. Sinusoidal oscillations in response to butanol stimulation applied at the beginning of the trace.

A. Response to solution 1
B. Response to solution 2
C. Response to solution 3
Time calibration 1/20 sec.



a) amplitude versus time; solution 3.



b) amplitude versus time; solution 2.

Fig. 10. Spike responses to different solutions of butanol stimulation of catfish olfactory tract.

The arrow indicates the moment of stimulation

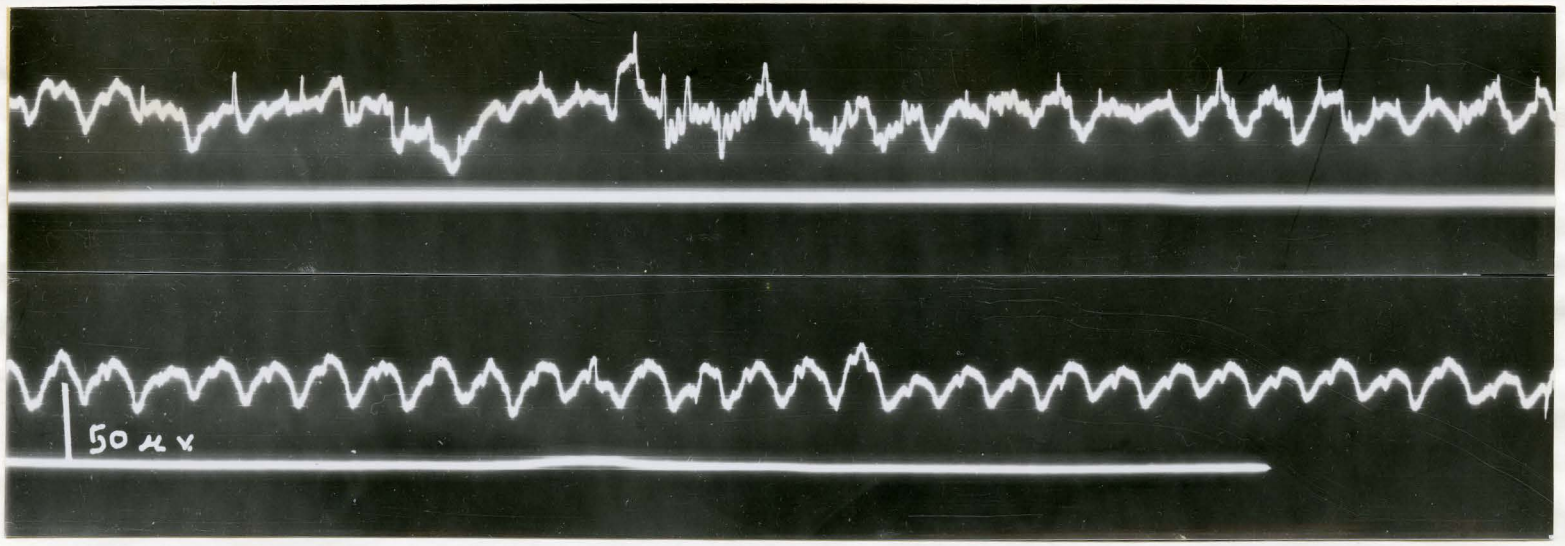


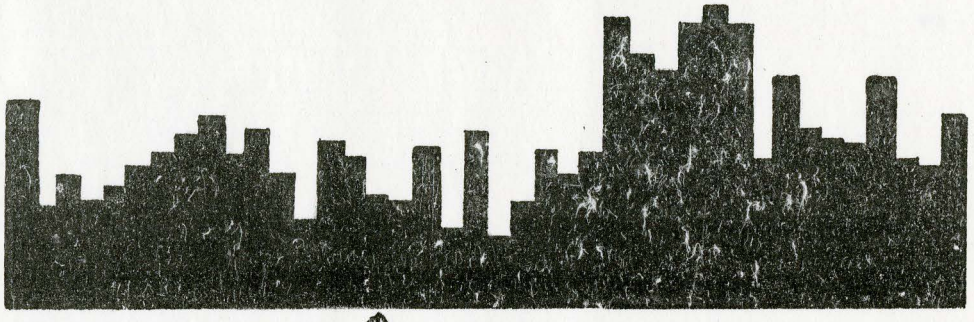
Fig. 11. Sinusoidal oscillations in response to troutwater

A. Oscillations after stimulation

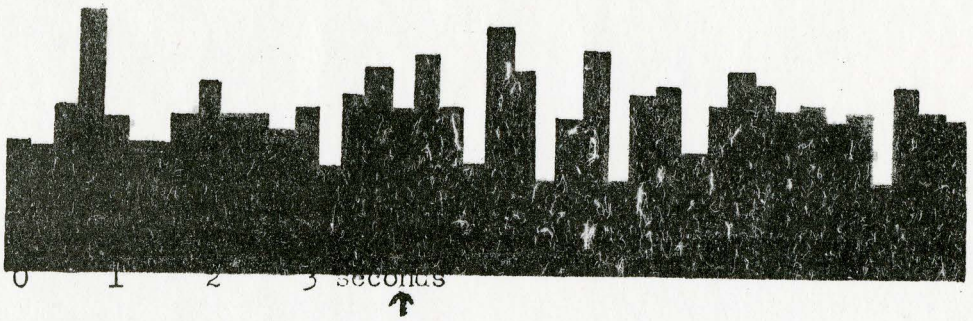
B. Standard situation, pre-stimulation

(see Methods) for small intervals for a number of trout-water tests. Fig.13 shows the data for one test which were divided into four groups: one group (I) before the response; a period (II) during the response; and two periods (III & IV) presumably after the response. The curves were fitted to 20 points by the method described (see Methods). The line representing the period of greatest response is found to have the highest position, i.e. average values of amplitude and frequency were the greatest for this group. It is of interest to see that the lines representing periods after the response are lower than the one before the response. Also the slope of the lines after the response is slightly different than the other two.

There is a third way in which response to trout-water may occur (fig.14). This figure shows the large waves for several cycles (single arrows in fig.14). These waves which have been described before are seen to be quite constant in amplitude. However, during the trout-water stimulation another potential change appears which occurs in the same position during every cycle just before the onset of the large waves (double arrow in the figure). When this additional wave appears it increases gradually with each cycle until it is larger than the regular wave. It then decreases gradually until it disappears. This



a) Amplitude versus time



b) frequency versus time

Fig. 12. Graphs of spike responses to troutwater in catfish

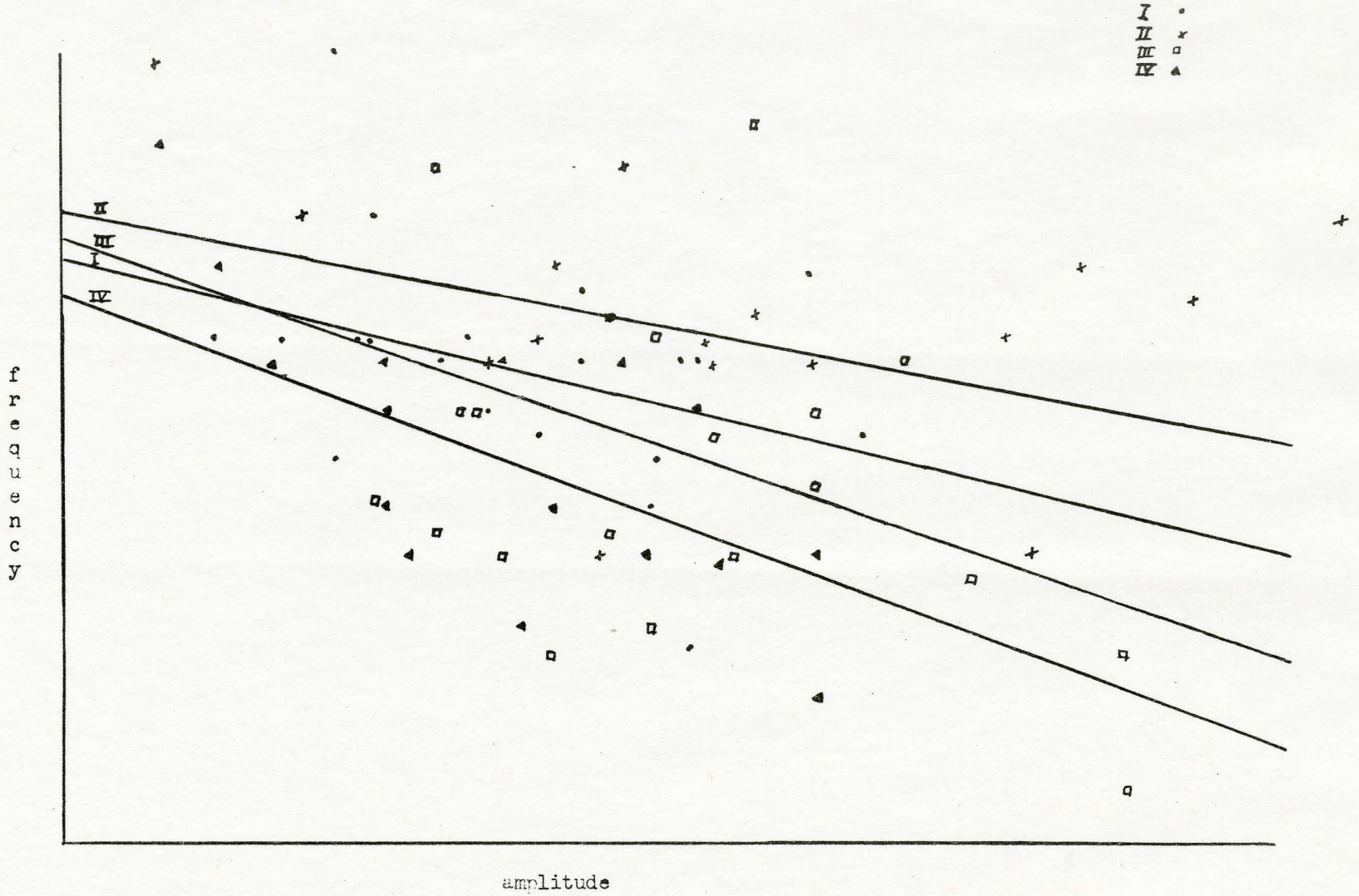


Fig. 13 Troutwater stimulation of catfish epithelium. These lines represent different periods during response as measured from the olfactory tract (see text for further explanation)

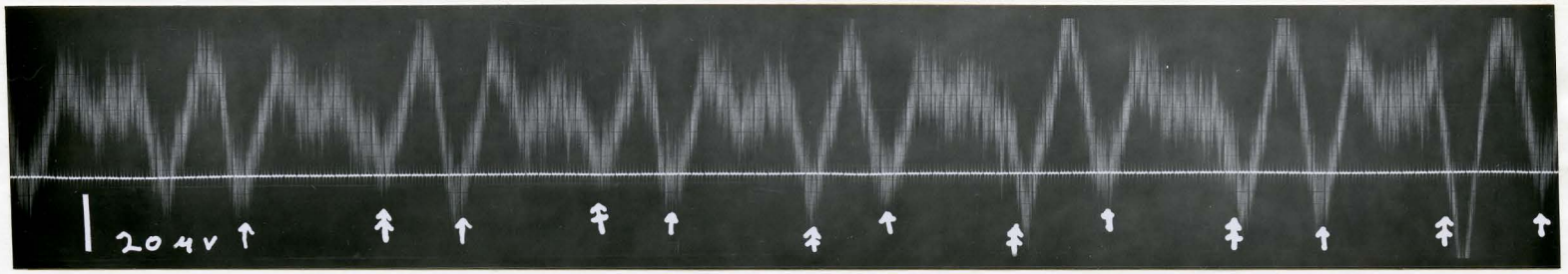


Fig. 14. Effect of troutwater stimulation. (See text).
Time calibration 1/20 sec.

response occurs when the stimulus is added by the continuous flow method.

One particular animal consistently responded to troutwater and all other stimuli with a large potential change (fig.15) which will be described in more detail in the section dealing with the amines. Before leaving the description of the response to troutwater it must be emphasized that the different types of responses may occur separately or in different combinations. The change in spike activity is always present.

(iii) Response to amines

Addition of amine F to the epithelium causes a definite increase in spike amplitude (fig.16). The increment of the amplitude increases with rising concentration of stimulus as was also observed with the butanol series. The return to normal amplitude takes longer with a higher concentration of amine F, the duration of stimulation remaining constant. There is little or no change when amine F is added to the butanol as a stimulus.

Amine G shows a similar increase in spike amplitude. In general the responses to the two amines were very similar.

As mentioned before in one individual all responses to amines and troutwater were characterized by a large positive potential with a magnitude of 100-150 μV (fig.15). A burst of large spikes (B) consistently occurred on part

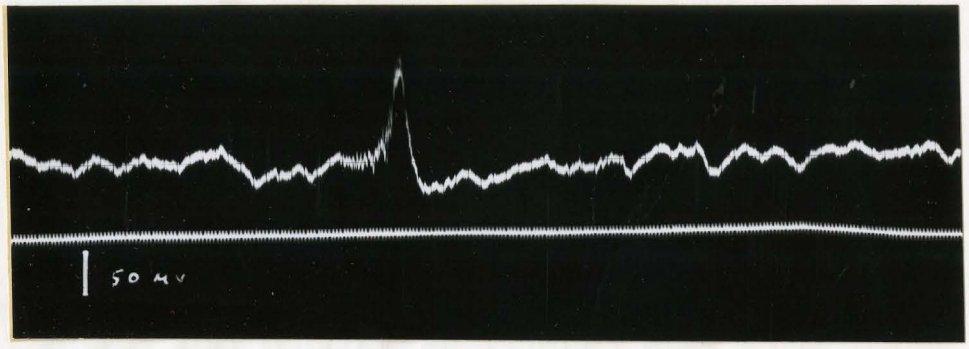


Fig. 15. Response to amine G solution 3 measured from olfactory tract of catfish.
Time calibration 1/15 sec.

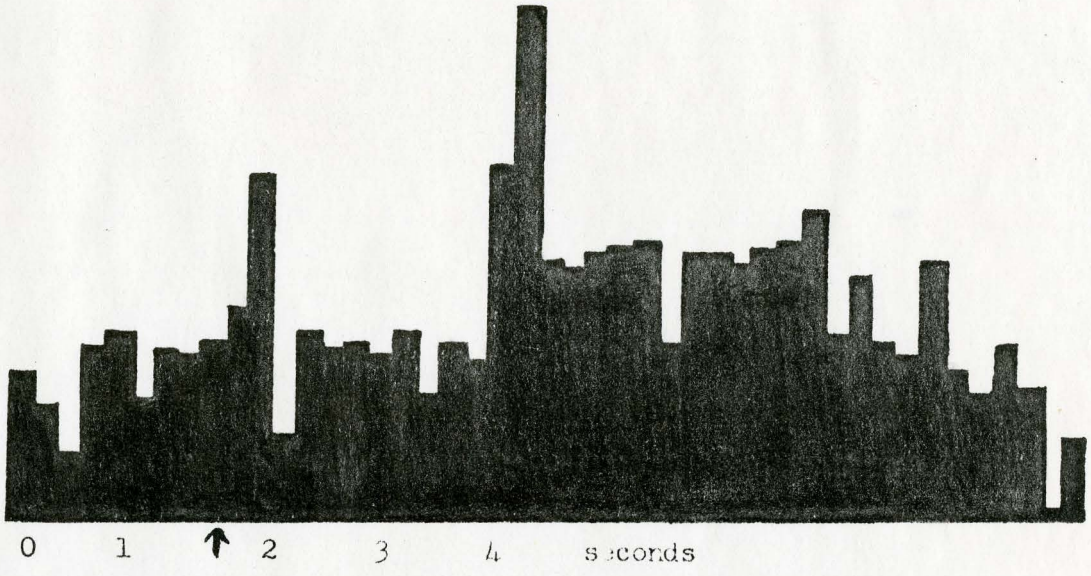


Fig. 16. Amplitude versus time. Response of the catfish olfactory tract to amine F.

of the rising phase and just before the potential (A); after this the latter increased rapidly and returned to normal level at a somewhat slower rate.

II LAMPREY OLFACTORY EPITHELIUM

1. Resting activity

The activity recorded from the epithelium, when no stimulus was applied was found to be of two types. (fig.17B, C and 18A) The first type shows a straight baseline with some spike activity superimposed. (fig.18A) Some small irregular waves could also occasionally be distinguished. The second type shows a large regular wave activity (fig.17B,C) synchronous with the respiratory movements of the gills as determined by auditory and visual inspection.

2. Chemical activity

The response to chemical stimulation also assumes two aspects which is a direct result of the two types of resting activity. When the resting activity shows no wave action (fig.18A) the addition of a drop of suitable chemical causes the start of a series of waves (fig.18 B,C) which terminate when the stimulus is washed out (fig.18D). The maximum magnitude of these waves was about 12 μV which is only attained through a number of gradually increasing waves. Once the maximum magnitude is reached it remains

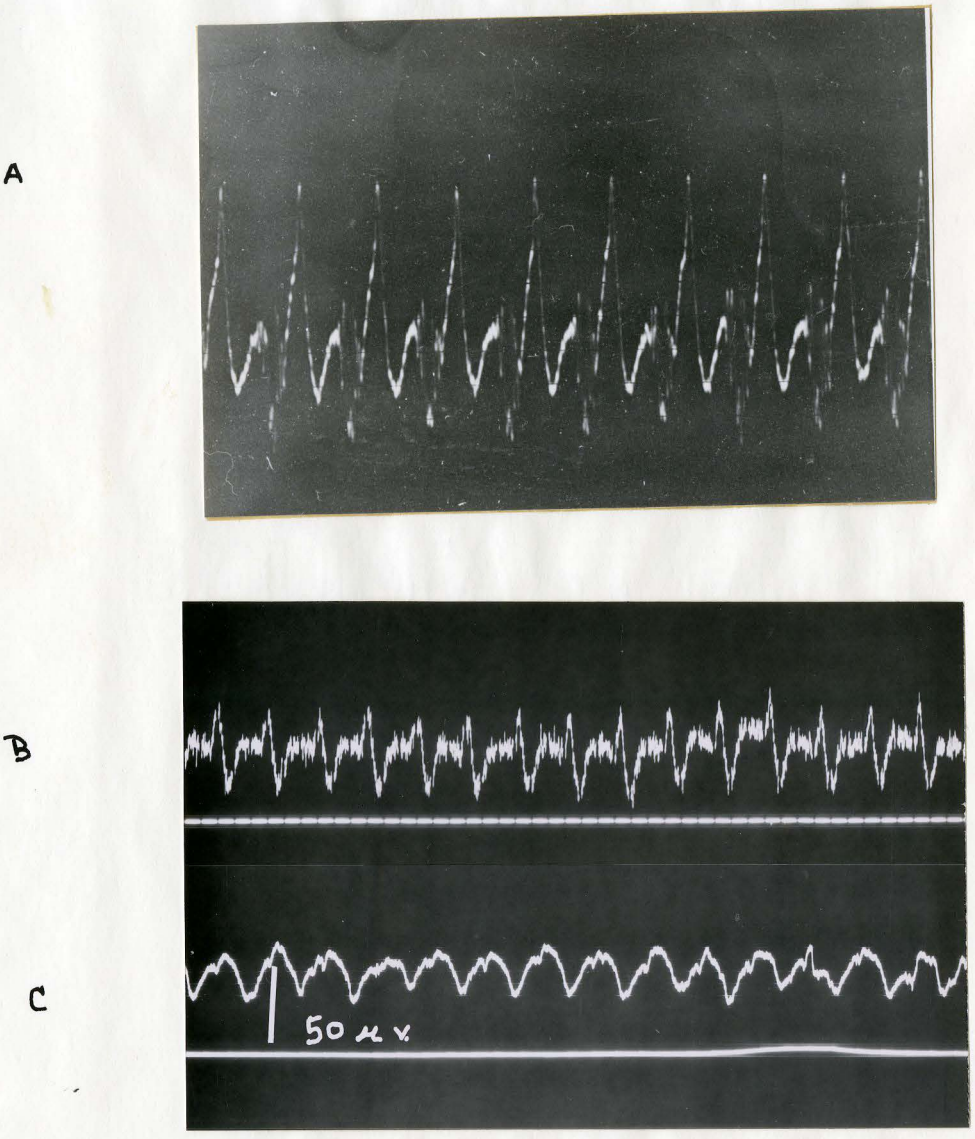


Fig. 17. Resting activity from lamprey nasal sac.

Trace A Spike potentials measured from water beside head of lamprey.
Trace B Potentials measured from nasal sac of lamprey.
Trace C As B but from different animal.

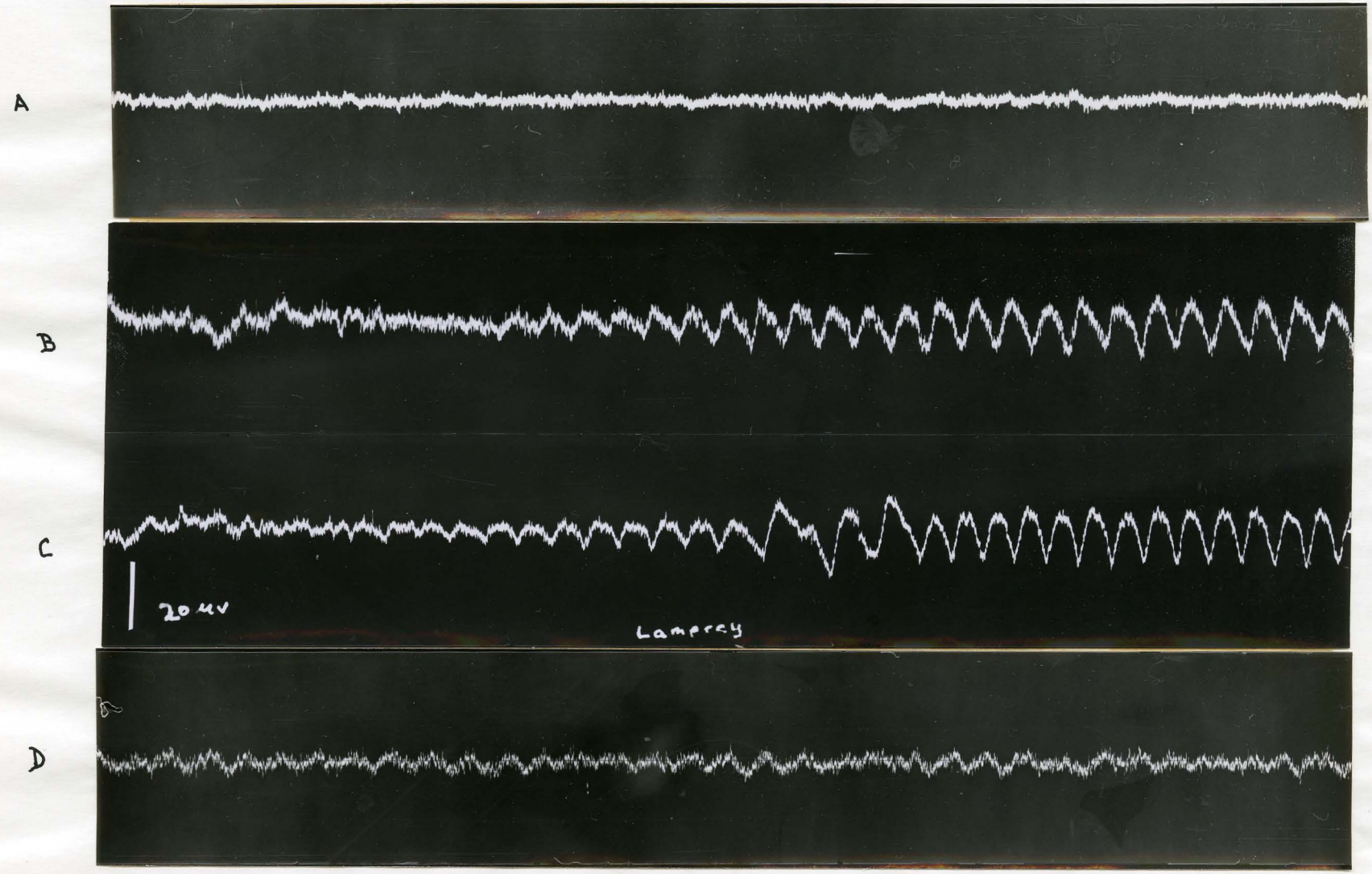


Fig. 18. Lamprey olfactory epithelium response to Amine F

A. Activity before
B. Response to solution 4
C. Response to solution 5
D. Activity after

constant until the stimulating material is removed from the nasal sac. The frequency of the waves is quite constant at almost 2 p.s. The train of waves does not start until two to three seconds after stimulation.

The other type of reaction of the olfactory to a stimulus occurs when the resting activity is characterised by waves (fig.17 B,C). In response to troutwater some potential oscillations occur (fig.19) which have a maximum amplitude of 70 μV but are generally smaller and quite irregular. Washing of the epithelium with water following stimulation produced, in this particular test a response quite different from that obtained with troutwater stimulation (fig.20). These potentials also appear at a frequency of nearly two per second but are somewhat slower than the resting waves and their bases gradually widen until they disappear completely.

Sometimes when a stimulus is applied a slow negative potential occurs followed by a slight positive potential (fig.21). The train of waves does not start until the slow potential change has disappeared. The magnitude of the slow potential in the record shown is 20 μV.

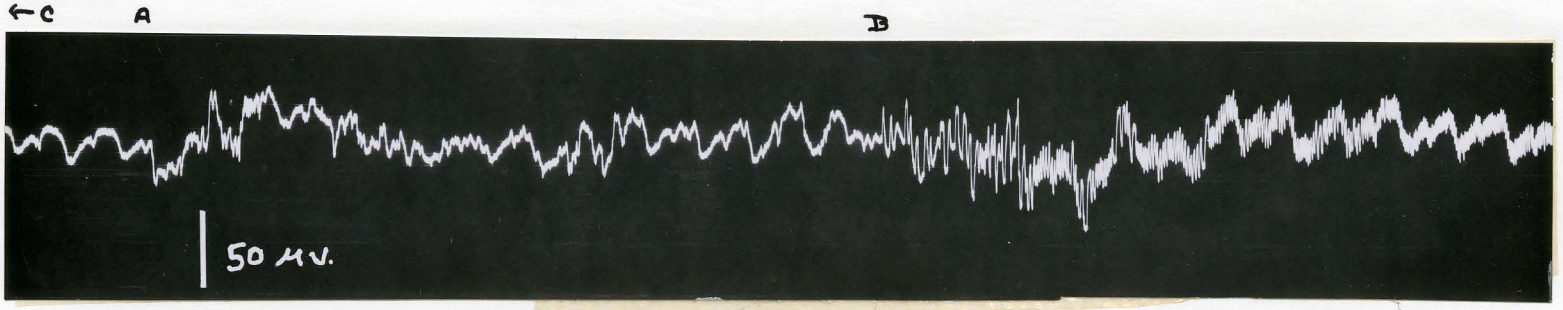


Fig. 19. Troutwater stimulation of lamprey epithelium.

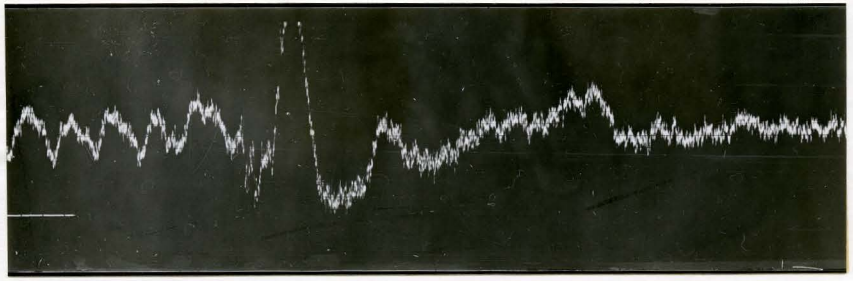


Fig. 20 Spike phenomenon on washing out troutwater.

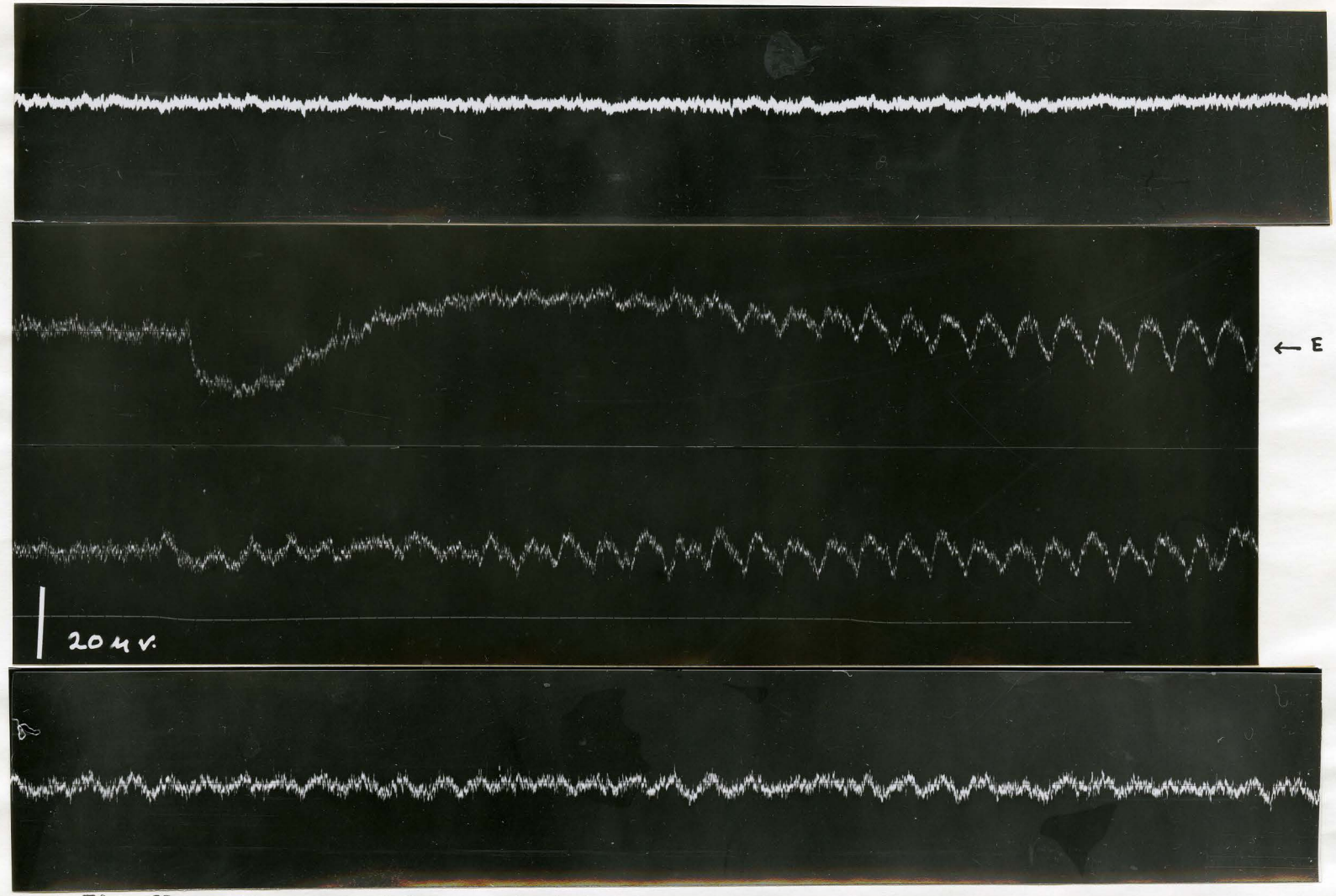


Fig. 21. Response of lamprey to Amine F.

A. Epithelium activity before response
B. Response to Amine F solution 3
C. Response to Amine F solution 1
D. Activity after response
Time calibration 1cm./sec.

D I S C U S S I O N

I OLFACTORY EPITHELIUM

(i) Resting activity

Two types of activity were observed in the olfactory epithelium of the lamprey: the straight line with some spike activity (fig.18A), and the wave activity. (fig.17B) The synchrony of the wave activity with respiration can be explained by the close relationship between the olfactory organ and the respiratory system in the lamprey.

Barring artifacts there are several physiological mechanisms by which respiration may influence the potentials in the sac. 1) Kleerekoper and van Erkel (1960) described the olfactory apparatus of Petromyzon marinus and pointed out that the nasal sac was filled by the action of the nasopharyngeal pouch. The respiratory muscles pump water in and out of this pouch and by means of the respiratory action a pulsating current of water flows over the epithelium. The mechanical stimulation of the continually flowing water could **cause** potential changes in the epithelium. 2) Another **mechanism** is the presence in the nasal sac of water contaminated with a trace of some odourous chemical. This may cause a negative potential

every time the sac is filled followed by a positive one. 3) A third possibility is suggested by the spike potentials described by Kleerekoper and Sibakin (1956) and recorded from the water surrounding the head of the lamprey. These potentials were found to be synchronous with externally visible movements of respiration and may therefore influence the recording from the nasal sac.

The second mechanism, the presence of a contaminating chemical in the nasal sac, although possible, is not likely to be the main disturbing factor in the resting activity. If a chemical caused the effect the wave form might be expected to be simpler in form and more like those in the train of waves produced by chemical stimulation (fig.18B). The same reasoning applies to the mechanical effect of water flow. One would expect a simple monophasic change in potential, varying with the pressure, rather than the more complicated shape recorded here. Also the fact that it is possible to record a resting potential without these wave forms in spite of the continuous rhythm of water flow makes this mechanism less probable.

The third possibility, concerning the influence of the spike potentials recorded about the head of the lamprey, seems the most likely. The potentials recorded from the epithelium and those recorded from the water beside the animal bear a striking resemblance to each other (fig.17A,B,C).

All the components appear the same although the relative size may vary. In both recordings the rhythmic potential changes show a quick drop from the most positive part of the cycle to the most negative of the cycle while the potential rises more slowly. About midway the rise in potential a smaller drop in potential occurs followed by increase until the maximum value is reached. Occasionally the potentials in the nasal sac have less prominent peaks and valleys with the result that a rounded-off effect occurs. However, even in this case all the components are present. This similarity between the two types of potentials measured from the two different positions suggest a common source. Kleerekoper and Sibakin (1956) showed the localization of the spike potentials to be well defined in the head region anterior of the last gill opening, i.e. the region which includes the olfactory system. This fact again makes it more likely that the spike potential influences the recording from the nasal sac.

Occasionally the animal did show normal breathing behaviour and a good response to chemical stimuli without the regular waves of resting activity. This indicates that it is possible to insert the electrode in such a manner and position that the animal's electric field does not affect the recording of the activity in the nasal sac.

(ii) Effect of chemical stimulation

No recording of responses of the olfactory to chemical stimuli in the lamprey has been recorded in the literature surveyed. It will therefore only be possible to compare the results obtained with those recorded by others in other vertebrates.

The most commonly recorded response to chemical stimuli in the epithelium by a number of authors is the slow potential (Ottoson, 1956; Takagi and Shibuya, 1960a,b,c,d). This response was occasionally recorded by the author in the lamprey, where it assumed the form of a slow negative potential change (fig.21). Ottoson (1956), in particular, found that when the electrode was inserted perpendicularly to the surface of the epithelium, in the frog, the maximum slow potential was obtained just inside the surface of the epithelium and the response decreased with further penetration of the surface. He attributed the origin of the potential to the olfactory hairs which protrude from the surface of the epithelium. In the light of these observations the fact that the slow potential was recorded only occasionally in the lamprey may be attributed to the positioning of the electrodes. Only if the tip of the electrode was in close vicinity of the extremities of the receptors could this slow potential change be expected. If one considers the structure of the nasal sac (Kleerekoper

and van Erkel, 1960) with its complicated system of folds it is not surprising that the tip of the electrodes is not always in the immediate vicinity of the olfactory hairs.

A more common response to chemical stimuli in the lamprey consists of the occurrence of oscillations, or of regular waves. Such oscillations in response to trout-water are shown at B (fig.19). They are somewhat irregular both in frequency (6-15 p.s.) and in amplitude (20-75 μV). The response of the epithelium seems to consist of a modulation of the regular waves (C in fig.19). This somewhat irregular activity then changes to the oscillations already mentioned which are superimposed on the regular waves (B in fig.21).

These oscillations are similar to those found by others. Both Ottoson (1956) and Takagi and Shibuya (1960d) recorded potentials with a frequency of 15-25 p.s. superimposed on the crest of the slow potential. The same authors also measure potential oscillations from the olfactory nerve which were only present if the olfactory bulb was intact. The two types of potential oscillations followed different sequences and the conclusion was that they had separate origins.

In the oscillations recorded in this investigation the influence of the olfactory bulb is the more likely since no slow potential was observed when the oscillations

were present while no oscillations were present when a slow potential was recorded. Again, the structure of the nasal sac with the large folds in the epithelium makes it very likely that the olfactory nerve potentials are picked up when the electrode is inserted into the sac from the posterior end as has been the procedure in these measurements.

Another type of response becomes obvious when no resting wave action was present: the addition of a chemical would start a wave action which had about the same frequency as respiration. This wave action lasted only as long as the stimulus was present in the nasal sac and discontinued when water was applied to the nostril. This wave is probably affected by respiration since its frequency is similar to that of the respiration. However, the wave shows some important differences from these measured during the resting activity. (fig.17B) In response to the chemical the ascending phase of the wave is the steeper one and the descending phase is not only less steep but may also show a small rise of potential before reaching the minimum point. This is exactly opposite to the resting wave activity. The chemical response is also of smaller magnitude which is of the order of 15 μV. This smaller magnitude and the similarity of the frequency may be the reason why this wave is not noticible when the

resting activity wave is present, although it has been observed that the addition of a chemical may have no other effect on the record than an increase in size of these potentials.

The sequences of events as represented in B, and C (fig.21) requires explanation which as yet is difficult to formulate. Adrian (1950) reports the occurrences of a train of waves in the bulb of the rabbit in response to chemical stimulation (see below) of the epithelium. The waves at E in fig.21C resemble those reported by Adrian for the bulb but are slower. It may be speculated in the absence of further data that the waves recorded from the epithelium, following the initial slow potential, originate in the bulb, while the slow potential itself finds its origin in the epithelium.

II OLFACTORY TRACT OF THE CATFISH

(i) Resting activity

In this investigation two types of resting activity were recorded from the olfactory tract of the catfish: spike activity and the large waves. The spike activity recorded here is **very** similar to that reported by both Adrian and Ludwig (1938) and Boudreau (1962). That these spikes are mostly potentials travelling in the direction of the forebrain is borne out by the fact that the cutting

of the tract between electrode and forebrain does not change the spike activity perceptibly. Both Adrian and Ludwig, and Boudreau found in addition that the cutting of the tract between bulb and electrode completely eliminated all activity; also that a local anaesthesia in the nasal sac greatly decreased the activity. From this the conclusion may be drawn that the spike activity originates distal to the olfactory tract and is under direct control of the receptors. Another paper by Adrian (1950) points to the olfactory bulb as the actual source of the spikes which in turn are affected by the epithelium.

The other type of activity which was regularly recorded from the olfactory tract in the absence of stimulation was the large regular waves (fig. 3). These large waves have not been mentioned previously in the literature. However, Adrian and Ludwig (l.c.) did mention similar rhythmic waves from the forebrain. These rhythmic waves were generally independent of olfactory stimulation although it could occasionally upset the rhythm or change the regularity for a few beats. The author observed the same behaviour with the large waves recorded from the olfactory tract. The potential changes which Adrian and Ludwig measured occurred at a frequency of 2-4 p.s. while the ones measured in this investigation occurred with intervals of 1-2½ p.s.

Adrian and Ludwig could record these potential changes only if at least one of the electrodes touched the forebrain, while in the work here reported they were measured from the olfactory stalk while neither electrode was near the forebrain. In the author's preparations recording from the tract when it was severed from the forebrain did not change the large waves. On the other hand, Adrian and Ludwig's recording from the brain remained unchanged by cutting the olfactory tract. The seemingly contradictory results suggest a fundamental difference between the two wave rhythms. Apparently the waves recorded by Adrian and Ludwig find their origin in the brain while the ones recorded by the author originated in the olfactory organ itself.

It is interesting that the appearance of the waves was more regular in shape and of smaller amplitude (fig.5) when water was flowing through the nasal sac. This may indicate that these waves pass along information about the flow, or that water as such stimulates the epithelium. In air-breathing animals, air flow seems to affect activity in the olfactory organ. Ottoson (1959) and Adrian (1950a) attribute this to the presence of weak olfactory stimuli in even the most purified air. However, Walsh (1956) assigned a separate class to neurons discharging synchronously with air flow. Since the large waves measured in this

investigation are likely due to synchronized discharge of neurons in either bulb or epithelium, they may well be analogous to neurons in air-breathing animals which are affected by air flow. It may be emphasized that the large waves are not synchronous with respiration and should not be attributed to the latter.

(ii) Mechanical stimulation

The starting of water flow causes a decrease while its cessation brings about an increase in spike amplitude. This agrees with Boudreau's observation (1962) in summating the tract activity and finding a rapid drop in activity during onset of the flow while the cessation of flow was followed by a profound increase.

Both Adrian and Ludwig (1938) and Boudreau (l.c.) found tract discharge produced by mechanical disturbance of the receptors the most reliable and reproducible event in their experiment. Pressure on the roof of the sac was found to be usually effective while the touching of the epithelium was extremely effective. In this investigation pressure on the roof of the nasal sac was consistently found to be effective and produced responses very similar to those brought about by the starting and cessation of water. Onset of pressure causes a slight decrease while the release of pressure causes an increase in spike amplitude. This indicated that the effect of water flow on

spike activity is mainly due to the change in pressure which it causes, on the receptors.

(iii) Chemical stimulation

The most consistent effect of chemical stimulation as recorded from the olfactory tract of the catfish was found to be the change in spike amplitude. The application of any chemical stimulus used in this experiment consistently caused an increase in spike amplitude. This agrees with Adrian and Ludwig's results. They observed an increase in the frequency of large spikes. In the present study it was found that the stimulus only occasionally affected the frequency of the spikes.

Adrian and Ludwig (l.c.) found a greater increase in amplitude when particles were present in the stimulating fluid. However, in this investigation, as well as that of Boudreau, an equally good result was obtained with freely dissolved substances.

In these experiments a considerable latency was found between time of application of a stimulus and time of increase in spike amplitude. Although the exact latency was not measured, time lapses of the order of one second were observed. This agrees with Adrian and Ludwig's results in finding the latency of discharge often one second and as high as five seconds.

A more striking result measured in this investigation

was the sinusoidal wave obtained in response to strong chemical stimulation (fig.9). In all observations the sinusoidal waves started before the increase in spike activity and ended before the spike activity returned to normal. The frequency of the waves was constant at 6-8 p.s. in all tests and the amplitude of the waves varied with the strength of the stimulating solution. These waves may be compared with those measured by Adrian (1950a) from the olfactory bulb of the rabbit. Adrian found sinusoidal oscillations at a fixed frequency which could be between 10-65 p.s. and lasting only for the duration of the stimulus. These oscillations were set up only by strong olfactory stimuli. This description shows a great similarity to the waves measured by this author, the only difference being that the frequency is slightly lower than the range which Adrian suggested. This low frequency may be characteristic of the catfish from which these oscillations have never before been reported. Adrian (l.c.) named as origin of these a synchronized beat developed in a number of units under maximal oscillation. In that case the difference in amplitude of these waves in response to varying concentrations may be brought about by differences in the number of units beating at the same time. The fact that troutwater which may be considered a more dilute solution has effects comparable to the strongest butanol

solution points to the great effectiveness of the stimulus.

Another response which has not before been measured from the olfactory tract is the large positive potential produced in response to both troutwater and amines in one individual. This potential is rather like the positive potential recorded from the olfactory bulb by Ottoson (1959a). He found the potential to be almost identical to the slow potential recorded from the bulb but of opposite sign. Oscillations corresponding to the spikes on the rising phase of the large potential have also been recorded by Ottoson from the olfactory bulb. The increase in spike amplitude did not occur until some time after this positive potential. Thus, it seems that both this slow potential and the sinusoidal oscillations are immediate responses to the stimulus while the spikes represent a delayed response.

S U M M A R Y

1. This thesis deals with the electrophysiological response to stimulation of the olfactory epithelium with trout scent and some of the amines isolated from the scent.

2. Recordings were taken from the catfish olfactory tract and the lamprey olfactory epithelium.

3. The resting waves measured from the lamprey olfactory epithelium probably have the same origin as the electric field around the head of the lamprey.

4. The chemical stimulation of the lamprey olfactory epithelium causes a slow potential, oscillations superimposed on the resting waves, or a train of waves (2 p.s.).

5. The resting activity in the olfactory tract of the catfish shows the presence of spike activity and large waves, both of which originate in the olfactory organ.

6. The mechanical stimulation of the olfactory epithelium causes an increase in the spike activity in the olfactory tract.

7. Chemical stimulation of the olfactory epithelium of the catfish causes an increase in spike activity, the formation of sinusoidal waves, or large positive potential. All of these probably originate in the olfactory bulb.

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