TELEOST RESPIRATION

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SOME OBSERVATIONS ON TELEOST RESPIRATION WITH EMPHASIS ON THE GILL FILAMENT MUSCULATURE AND THE RESPIRATORY CENTRES OF THE BRAIN

By

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E R R A T U M

After this thesis had been typed it was discovered that the word "transection" had been misspelled.

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SCOPE AND CONTENTS:

Two aspects of teleost respiration have been emphasised in this investigation. Firstly the gill filaments themselves were observed in order to determine whether or not they played an active role in the ventilation of the gill lamellae. The musculature of the filaments was studied, using visual and electronic methods, and continual muscular activity was noted. Two possible functions of these muscles of the gill filaments have been proposed.

Secondly, the brain centres which initiate and maintain the respiratory rhythm were investigated. The neural mechanism was found to be much more complex than had hitherto been suspected in the literature.

P R E F A C E

This study of teleost respiration was divided into two parts.

a) The role of the gill filaments and its musculature in the ventilation mechanism.

b) The localisation of neural centres responsible for the initiation and maintenance of the respiratory rhythm.

In a wide survey of this kind, almost every aspect of the respiratory system is of importance and must be considered. These related topics are dealt with in the introductory chapters in order that the true significance of the present investigations may be appreciated.

The Introduction is arranged in five sections. The first section deals with the way in which the stream of water is presented to the respiratory surface. This section includes much of the earliest work on fish respiration. Until modern methods could elucidate the complex pumping system of the respiratory apparatus, there were many misconceptions about the interdependence of the various structures.

The second section introduces the minute structure of the gills. Included are details of their skeleton, musculature, blood circulation and respiratory surfaces.

Next, a survey of the existing literature about the role of the filament musculature is given. Three hypotheses have been evolved. Some authors thought that the filaments were continually moving to and

fro in order to increase the contact of the respiratory surface with the water. Others considered the musculature was important in assisting the blood flow through the filaments. The most recent papers denied the musculature any function at all in normal breathing.

To introduce the present investigations on respiratory control, the fourth section deals with the early hypotheses on this subject. Early authors sought for a self-regulating, peripheral mechanism which would produce the complex series of events in the respiratory cycle. Hypothetical receptors, influenced by water flow, by gas concentrations of the water or by the movements of the apparatus itself, were all put forward as being responsible.

Modern work tends towards laying more emphasis on the inherent rhythmicity of the central nervous system. The last section of the introduction includes all the work on the respiratory centre of the fish brain.

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I N T R O D U C T I O N

I HISTORICAL SURVEY

1. The mechanism of gill ventilation

Much of the work on teleost respiration in the nineteenth and early twentieth centuries concerned itself with the sequence of events in the respiratory cycle and the direction of water flow through the respiratory channels (Duverney, 1701; Dumeril, 1807; Flourens, 1830; Duvernoy, 1839; Bert, 1870; Kuiper, 1906; Francois-Frank, 1906; Van Rynberk, 1906; Baglioni, 1907). This work was reviewed by Babak (1921). The conclusion was reached that the cycle of opening and closing of the operculum overlaps the cycle of mouth opening and closing, but that the opercular movements lag slightly behind the corresponding movements of the mouth.

Woskoboinikoff and Balabai (1937), measured the variations in water pressure occurring in the respiratory chambers. They found that in the lateral gill cavities, at certain phases of the respiratory cycle, the hydrostatic pressure drops below the resting level. This decrease in pressure is much greater than the drop in pressure occurring synchronously in the mouth cavity. At other phases of the breathing cycle, hydrostatic pressure in the mouth cavity increases considerably, and this rise in pressure is much more intense than the rise occurring in the branchial chambers at that time. In other words, there is usually a

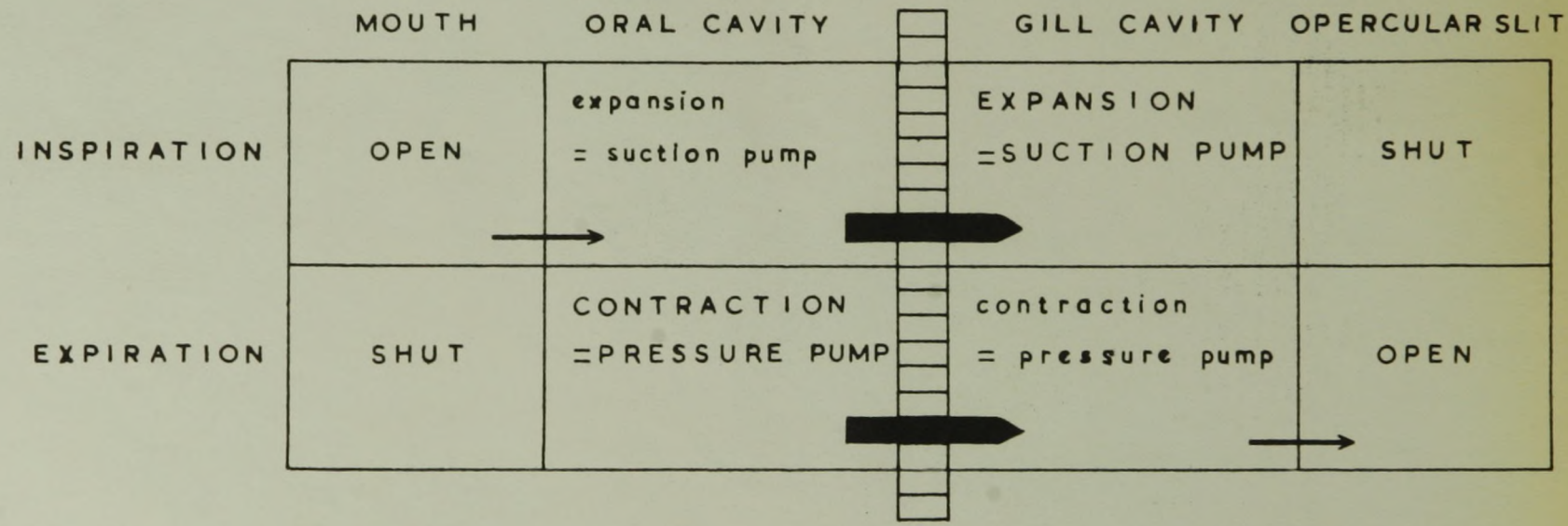


Fig. 1. A. Scheme of gill ventilation proposed by Henschel.

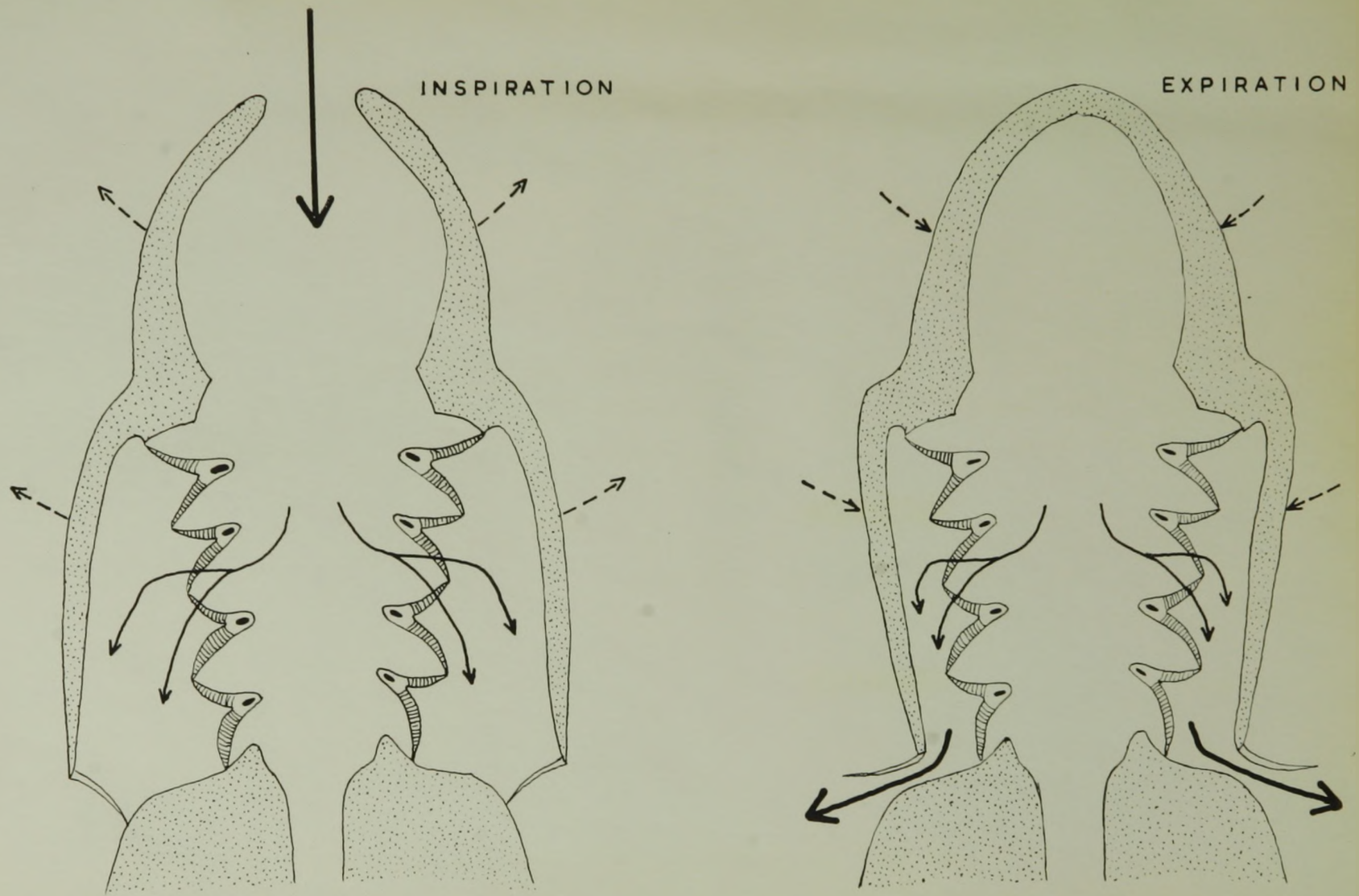


Fig. 1. B. Diagram illustrating the modern hypotheses on gill ventilation. Solid arrows denote water currents. Broken arrows show movement of respiratory apparatus.

lower pressure in the branchial cavities than in the mouth cavity. They concluded that water passes over the gills by means of a combination of two kinds of pump, a suction pump and a pressure pump. Drawing from earlier comparative anatomical studies (Woskoboinikoff, 1932), they put forward the theory that, in teleosts, the suction pump has become the most important component, and that the pressure pump is an almost vestigial system, inherited from the cartilaginous fish, with their separate gill pouches. According to this hypothesis the branchial apparatus is the most important structure, and the gill filaments have taken on the added function of a valve mechanism preventing a backflow of water. Leiner (1938) supports this theory.

Van Dam (1938) was the first to realise that, although the passage of water through the external apertures was intermittent, there was an uninterrupted flow of water over the gills themselves. His view is supported by the work mentioned above of Woskoboinikoff and Balabai. The pressure gradients between the mouth cavity and the branchial cavities are such that, at all times, there is a force driving water through the gill filaments.

Henschel (1938, 1941) made a thorough study of the muscular apparatus necessary for all these movements, and arrived at the same conclusions as Van Dam regarding the flow of water (fig. 1). The suction pump set up by the operculum and branchiostegal apparatus is the most important one, but the mouth cavity is also quite efficient. The floor of the buccal cavity can lower, thus creating a larger volume inside, without the mouth's opening to the same extent, thus a strong negative pressure is set up within the buccal cavity.

Hughes and Shelton (1958) were unaware of the work of Woskoboinikoff and Balabai when they began their experiments on the pressure changes inside the respiratory apparatus, and it was only when their work was almost completed that they saw the earlier publications. For the most part, they confirmed the earlier work, but they discovered a much more complex relationship than could have been revealed by the older methods, in which considerable inertia of the recording apparatus hampered precise observations. Hughes and Shelton found, as did the earlier workers, that both the maximum and minimum pressures recorded in the buccal cavity were higher than those of the branchial cavities. They found, however, that the time relationships were such, that at a certain short phase, the pressure was higher in the branchial cavity. On the basis of their work on the trout, they divided the breathing cycle into four phases.

1) Opercular suction pump predominant.

The operculum is abducting, but the opercular valve is closed, so that a negative pressure is produced which is greater than that in the buccal cavity. Here the negative pressure, built up as a result of the increase in volume, drops, because the mouth is reaching its fully opened position, and is even beginning to close. Thus water is pulled through the gills into the opercular cavity.

2) Transition, with a fall in the differential pressure.

The mouth continues to close, the buccal cavity diminishes in size, and, at first, some of the water is pressed out through the still open mouth, so that the pressure rises only a little. Soon, however, the mouth valves close, and prevent this outflow of water so that the pressure

rises steeply. At the same time the pressure in the opercular cavity is becoming less negative on account of the water's reaching it through the gill curtain. At the end of this phase, the operculum reaches its maximum abduction and the valve opens when the pressures on each side of it are equal.

3) Buccal pressure pump predominant.

The operculum begins to adduct, ejecting water to the exterior, therefore the pressure in the cavity is only a little higher than the outside medium. In the buccal cavity, however, as the volume continues to decrease, the pressure rises sharply, forcing more water through the gills.

4) Transition, with reversal of differential pressure.

At this stage, because the mouth movements precede those of the operculum, the mouth is beginning to open, while the operculum is still adducting. In the mouth, therefore, the pressure falls rapidly, because the buccal cavity is enlarging at a faster rate than water can enter to refill it through the half-open mouth. Simultaneously, the pressure in the opercular cavity rises, because the flexible valve meets the body wall along most of its length well before the bony part of the operculum ceases moving inwards. So, as no more water is able to leave through the slit, the flow of water is momentarily reversed through the gills. This phase occupies only one tenth of the whole breathing cycle.

This work confirms that of earlier authors concerning two active pumping systems driving the water over the gill filaments. The significance of the reversal of water flow was not explained. The earlier investigators, notably Baglioni, considered the mouth and branchial cavities to be essentially one single pump mechanism, and overlooked entirely the importance of the gill curtain.

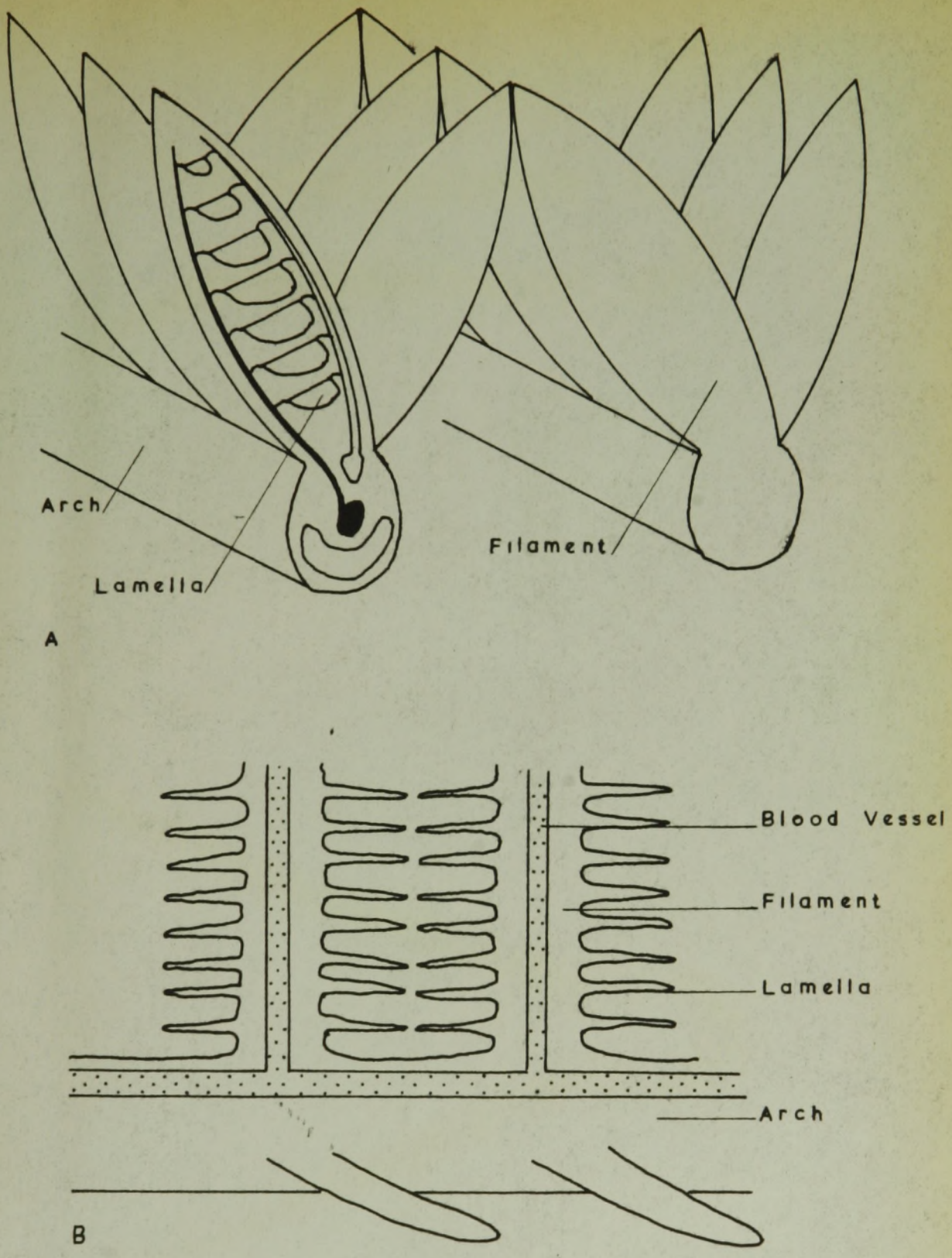


Fig. 2. The arrangement of the filaments upon the gill arch.

A. Sections of two adjacent arches showing the contact between the tips of the filaments. Redrawn after Bijtel.

B. One arch, drawn from the oral surface, showing the arrangement of the lamellae.

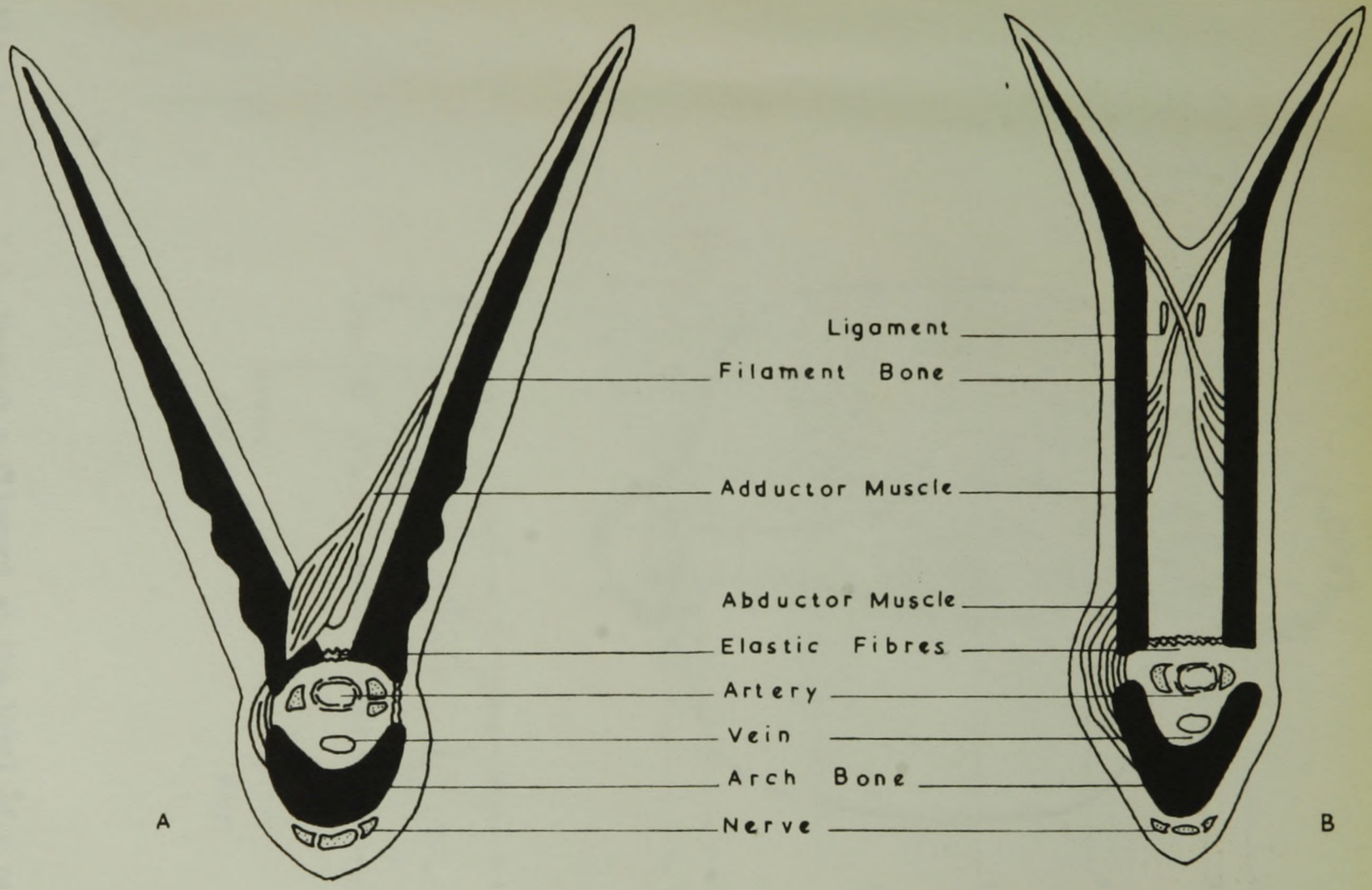


Fig. 3. T.S. through the gill arch. A. Type I arrangement of the filaments (Bijtel's classification, 1943.) B. Type II arrangement.

2. The structure of the gills

In teleosts, the trend towards fewer, more efficient gills, which has been noticeable throughout the evolution of fish, has finally reduced the number of gill arches to four. Of these, the most caudal one is generally closely connected with the wall of the branchial cavity, and has only one hemibranch. All the others have two hemibranchs. The group of arches on each side of the head is closely integrated by a liberal muscle supply into a very mobile unit capable of movement in all directions. For details of this musculature see Takakasi (1925).

The filaments, arranged in two longitudinal rows along the length of the arch, are finger-shaped feathery structures which are elliptical in cross section. The long axis of the ellipse is at right angles to the arch. Each filament is supported by a cartilaginous filament rod running almost the length of the filament, which is connected to the gill arch bone by elastic fibres (fig. 2 and 3).

The gas exchange itself takes place through the gill lamellae. These lamellae are borne on the flat, transverse surfaces of the filaments and consist of two layers of extremely thin, simple, pavement epithelium, kept apart, and supported, by special pillar cells (Reiss, 1881; Faussek, 1902). These pillar cells are hyperboloid, and have large spaces in between them through which the red blood corpuscles wander (fig. 4). This arrangement brings the blood into the closest possible contact with the outside medium, a situation favouring the exchange of gases and ions. The total lamellas surface presented to the water is vast. For example, the Perch has 133,188 - 208,080 lamellae (Byczkowska-Smyk, 1958).

The arrangement of the circulatory system also assists in gas

exchange, since the blood flows everywhere counter to the direction of the water current, Van Dam (1938). A branch from the afferent branchial artery runs up the inner surface of the filament giving off a capillary to each lamellae. The blood wanders freely throughout the cavernous tissue of the lamellae, but there are main channels around the edges of the lamellae which help to distribute the blood cells rapidly over the respiratory area, and collect them up again, ending in the filament veins (Byczkowska-Smyk, 1958). Efferent vessels run back to the arch along the outer surface of the filaments.

The filaments are constantly lubricated by mucus, which is produced by the copious goblet cells situated in the rounded surfaces of the filaments. Other secretory cells are found between the bases of the lamellae. They are very large cells, with a variable distribution among the species, and there is some evidence for believing that they are chloride secreting cells (Keys and Willmer, 1932). Thyroid secreting cells are also found here in some species.

The gill arches and rakers are abundantly supplied with taste buds but, apart from these, there is no mention in the literature of any sensory ending situated in the gills.

3. The musculature of the gill filaments

Most of the early investigators thought of the gill filaments as being completely passive structures hanging freely in the respiratory current: Baglioni's diagrams show this clearly (1907). One author did notice that they were capable of some active movement. Flourens (1830) cut off one or both opercula and observed that the two hemibranchs of one arch are widely divergent, and are continually moving back and forth so

that slits appear between them. "The gills are continually moving with a double action of alternately opening and shutting... The gill filaments part from one another, and then draw near again until they touch."

Musculature of the filaments was first mentioned by Wahlbaum (1832) but no details were given and he did not mention which species he was studying. Duvernoy (1839) found that the gill filaments are not free along their whole length, but are bound together by a sheet of firm fibro-elastic tissue. This connects adjacent filaments of the same hemibranchs, as well as corresponding filaments of the two hemibranchs of an arch. All the filaments are thus connected by their inner edges for as much as three quarters of their total length. The author noticed that there were variations as to the arrangement of the filament connections: in some fish, for example the conger eel, the fibro-elastic bands, or 'diaphragms' as he called them, were very narrow, while in others, like the sturgeon, they were very wide. All these 'diaphragms' contain, or are associated with, muscles which generally arise from the base of one filament, cross within the diaphragm, and end in a tendon on the corresponding filament of the opposite hemibranch.

Of the function of the filament muscles, Duvernoy says, "These muscles are obviously the antagonists of those forces which pass water into the gill cavities, and even of the water itself, which has the effect of parting the branchial filaments one from another." In connection with the sturgeon and eel, he goes on to say, "Their action serves to produce numerous movements of the gills...which by renewing and multiplying the points of contact between the respiratory surface and the water,

are very important." Later, Schottle (1932) describes 'fanning' movements of the filaments in Gobiiformes.

Early authors mentioned another possible function for the filament muscles. Droscher (1882) arrived at the conclusion that, when the muscles of the 'diaphragm' contracted, the blood vessels, lying between the muscle and the cartilaginous filament rod, would be compressed, thus pushing the blood forward into the cavernous tissue of the lamellae. Woskoboinikoff (1923) seemed unaware of Droscher's studies, but he put forward exactly the same hypothesis, and in the literature is generally credited with initiating it.

Reiss (1881) made a very detailed study of the filament musculature. He noticed that there were two sets of muscles activating the filaments, which he thought were antagonistic. He called the ones within the 'diaphragm' adductor muscles, and the others, at the bases of the filaments, running over the oral surface of the arches, abductor muscles. He found he could divide the teleosts into two groups on the basis of the arrangement of their filament muscles. In one group he put such fish as Esox and Perca. In these fish, when the adductor muscles cause the filaments of the pairs of hemi branchs to come closer to one another, the point around which each filament pivots is situated at the broadened convex end of the filament rod where it joins the gill arch. In the second group of fish, for example the Cyprinids and Salmo, the diaphragm connects the filaments for the basal half of their height, so the pivoting cannot be so extensive. The axis of movement lies within the diaphragm.

Bijtel (1943; 1947; 1949) confirmed the findings of Reiss in dividing teleost into two groups. She refers to fish like Esox, Perca and

Gasterosteus with free filaments as 'Type I' and those like Tinca, Cyprinus and Salmo with filaments bound to a wide diaphragm, as 'Type II'. This classification will be adhered to throughout this work. In Type I fish the adductor muscles have their origin between the basal extremities of the filaments of one hemibranch, and cross over to end on the filaments of the opposite hemibranch. There is one muscle for each filament which arises from the pair immediately opposite. Their contraction causes a pivoting of the filament at the syndesmosis between the filament bone and the arch bone. In Type II fish the muscles are not set basally, but have their origin half way along the filaments. The muscle fibres are short and soon terminate in a tendon, which crosses over to the corresponding filament of the other hemibranch. The tendons from both sides are connected, where they cross over, by the longitudinal ligament, which runs along the extreme edge of the 'diaphragm'. See fig. 3 for the arrangement of these muscles.

Rauther (1925) and Bijtel (1943; 1947; 1949) were of the opinion that during normal breathing, the hemibranchs of one arch are always in the abducted state, so that the tips of filaments from adjacent arches are always in contact (fig. 2). Bijtel bases her denial of any activity of the adductor muscles during normal breathing on unpublished work of Hofdijk - Enklaar, who introduced a celluloid window into the operculum of the fish, so that she could observe the gill filaments under normal conditions. She arrived at the conclusion that they only adduct actively at a cough. (We are indebted to Professor Baerends for the use of these unpublished manuscripts.) Bijtel thought that the parting of the tips, seen frequently when the operculum has been removed, is passive caused by

the disturbances in the normal pressure relationships operating within the respiratory channels. According to her, all the water must pass through the interlamellar spaces, and not through any gaps between the filament tips, in order to account for the high percentage of oxygen uptake measured by Van Dam (1933).

Bijtel does not agree with earlier writers about the function of the so-called abductor muscles in widening the 'V' between the hemibranchs. As she observed, they are not found in all fish, and where they are present they are often small with few fibres. In Type I fish, these muscles are inserted so close to the fulcrum of the rotation movements between filaments and arch that they would be most inefficient in pulling the filaments outward. In Type II fish, there is no hinge at the base of the filaments at all. It is much more likely that in the former group the elastic fibres connecting the bases of the rods resist both the activity of the abductor muscles and the flow of water, and keep the filaments apart. In the latter group, the cartilaginous filament rods have an elliptical cross section and longitudinal ridges on the outside. This would tend to oppose the deformation induced by the adductor muscles so that the filaments would tend to spring back immediately the muscles relaxed. Since Bijtel believes that the normal position of the filaments is the abducted one, she maintains that it would entail long contractions of the abductor muscles if they alone were responsible for holding the filaments apart. This would require such an inefficient expenditure of energy that she thinks it a very unlikely condition. Her observations led her to the conclusion that the abductor muscles have a positional effect during the abnormal pressure relationships which occur during coughing. Those would

tend to move the hemibranchs caudally, thus closing the gill slits, unless the contraction of the abductor muscles prevented this movement. They contract at the same time as the adductor muscles, only for the short duration of the cough, and are therefore relaxed during normal breathing.

An entirely new approach to the study of these muscles was introduced by Konishi (1957). He placed electrodes on the gills and recorded volleys of action potentials whose frequency had a respiratory rhythm. He concluded that the action potentials were of muscular origin, and he ascribed them to the filament muscles. He found that there were two distinct kinds of discharge, one positive and the other negative, which corresponded to the phases of inspiration and expiration respectively.

4. Hypotheses on respiratory control

Throughout the literature on the control of the respiratory rhythm in fish, there has been a controversy as to whether the rhythm is maintained by reflex means, or whether it is due to an intrinsic rhythmicity inherent in the central nervous system.

There have been three schools of thought concerning the kind of reflexes involved in the origin and maintenance of the respiratory rhythm. The flow of water itself through the respiratory channels, the movements of the parts of the respiratory apparatus, and the levels of carbon dioxide and oxygen in the bloodstream, have all been proposed as the stimuli of the controlling reflexes.

Water was supposed by some to be a specific stimulus for normal breathing (Grehant and Picard, 1873; Baglioni, 1907). However, Flourens (1930), Van Rynbeck (1906), and Lombroso (1907), all showed that fish could respire normally in other liquids such as wine, milk and oil.

Bethe (1903) maintained that the contact of liquid with the mucous membrane lining the respiratory chambers was essential for normal breathing. He thought he had proved this by immersing the fish in cocaine solution, after which breathing stopped in 15 - 20 seconds. He interpreted this as being due to a lack of stimuli reaching the brain from the anaesthetised receptors in the mucous membrane. However, these results have never been repeated, although many have used the same technique as Bethe (Van Rynbeck, 1906; Isihara, 1906; Westerlund, 1906; and Babkin and M'Gonigle, 1931).

Babkin and M'Gonigle (1931) observed that when fish are exposed to air, there is temporary inhibition or dyspnoea. These phenomena are suppressed if the fish is previously treated with cocaine. They believed that there are some receptors in the mucous membrane, sensitive to water deprivation, which are anaesthetised by cocaine, thus blocking the inhibitory reflex. They stress the fact that in the normal fish, the inhibition after water deprivation is of short duration, and regular breathing will return while the fish is in air. This behaviour shows much variation from species to species.

Lutz (1930) found that in elasmobranchs, the frequency of respiration and heart beat varied directly with the rate of water flow in perfused fish.

Many have thought that the movements of the respiratory apparatus itself provides a constant stream of stimuli. Such a kinaesthetic sense was first mentioned by Kuiper (1906), and was used by Lombroso (1907) to explain the results of a series of experiments, where one or more of the respiratory openings was immobilised. Lombroso (1907), Schönlein and

Willem (1895), Deganello (1908), and Bert (1869) found that the other parts of the apparatus performed regulatory movements which they interpreted as compensation for the inactive part. For example during mouth stenosis, the opercular movements were exaggerated, causing the water to flow in and out through the remaining openings. These compensatory movements, Lombroso thought, occurred quickly enough to indicate direct reflexes, not mediated through any centrally occurring blood stimuli. The regulation also takes place when the fish is in air.

Deganello (1908) made a study of the effects on respiration sectioning of the varying cranial nerves. He came to the conclusion that centripetal impulses carried by the N. maxillaris superior V, and by the various branches of the vagus, exert a tonic effect on the respiratory centre. There was a marked depression of breathing after sectioning these nerves.

Satchell (1959) discovered that if the pharynx of a dogfish is forcibly distended, by inflating a rubber balloon within the cavity, there is a noticeable reduction in the frequency and amplitude of respiration. This change is accompanied by a decrease in spike discharge in the motor nerves, and bursts of centripetal activity in the branches of the vagi. This would seem to indicate a regular reflex associated with the distention of the chambers.

Powers and Clark (1942) were of the opinion that breathing was completely inhibited if all the branches of IX and X were cut on both sides. This claim has never been substantiated. Occasionally a change in either frequency or amplitude can be noted, but never complete inhibition (Satchell, 1959; Shelton, 1959).

Baglioni (1907) emphasized that the level of oxygen and carbon dioxide in the bloodstream is very important. Results of experiments of this nature are very conflicting, which Olthof (1937) believed arose from the fact that, in many cases, the experimental animals used were already in a dyspnoic state, due to the immobilising apparatus and handling. He found distinct signs of dyspnoea in fish exposed to carbon dioxide, at concentrations of 1 - 4% of an atmosphere. Kouliabko (1907) isolated the head of a sturgeon from the rest of the body, maintaining the circulation by perfusing the heart with Locke solution. When this perfusate was of high carbon dioxide concentration, convulsive breathing was induced even though the fish were in a state of apnoea previously.

Black reviews many of the experiments on the influence of carbon dioxide and oxygen. It appears that the levels of these gases in the bloodstream are indeed important in the regulation of breathing as they are in other phyla. It was once thought that small fluctuations of carbon dioxide during each breathing cycle directly initiated a new breath; this is not likely. Probably the stimulation caused by the excess carbon dioxide provides a general background for the activity of the central respiratory centre (Babak, 1929).

While it is conceivable that many of the stimuli discussed above are important as regulators of breathing, they cannot possibly be responsible for generating the respiratory rhythm. Babak (1921) was one of the first authors to formulate the idea of an autochthonous automaticity of the respiratory centre. He thought, in common with many modern authors, that the centre emits a rhythmical discharge to the respiratory muscles quite independently of any extrinsic influences. This primary

rhythmical activity could then be modified secondarily by any number of stimuli. He based his hypothesis on evidence found in fish embryos. He noticed that in the embryonic stages, before the branchial apparatus was active, there were constant oscillations of the tail and fins, whose frequency and amplitude were directly dependent upon the oxygen content of the medium. Polimanti (1911) also noticed similar oscillations, and found that when respiratory movements began, they had the same frequency. von Holst (1934) observed that under certain conditions of oxygen depletion or partial anaesthesia, the adult fish will show a synchronisation of the rhythms of breathing and tail and fin oscillations. He thought that under these conditions, all peripheral receptors were inactivated, and only a basic rhythm alone was operative and affecting all moving parts.

5. The respiratory centre

Flourens, (1830), Vulpian (1866), and Steiner (1885) all looked for the anatomical localisation of the respiratory centre in fish. The latter believed it to be situated in the band of tissue bridging the duct between the IIIrd and IVth ventricles at the anterior end of the medulla. He found that isolating this dorsal part, which bears the lobus impar, caused permanent inhibition of breathing in the teleost Squalius cephalus, while disconnecting it one side led to inhibition of the ipsilateral respiratory apparatus only.

The first detailed search for the respiratory centre was made by Hyde (1904) using the skate. She made various lesions through the brain tissue, and found that there was no one spot where destruction of the tissue led to a cessation of respiration. Respiratory activity was

linked with the lobus vagi, and the motor column below. This fairly extensive area could be completely isolated anteriorly and posteriorly from the rest of the central nervous system, and would continue functioning normally. Hyde found that lesions made medially or transversely through the medulla did not destroy the activity, but that the isolated parts, after the inevitable period of shock, developed their own rhythms. She found no respiratory significance for the secondary vagal nuclei. She concluded that in the skate, the medulla was to a large extent still segmentally arranged, with independent ganglia controlling the respiratory muscles with which they are segmentally linked.

Shelton (1959) has recently carried out a series of experiments, on the tench, similar to those of Hyde. He made a series of transsections of the brain, and noticed the corresponding changes in breathing rhythm. He concluded that the area responsible for the rhythmical respiration must lie between the levels of emergence of the V and VII cranial nerves and the posterior border of the lobus impar.

One of the earliest papers on the electrical activity of the brain of fish came from Adrian and Buytendijk (1931). In the vagal lobes of the extirpated brain of the goldfish, they recorded slow rhythmical potential waves, whose frequency had the same range as that of respiration in the intact fish. Sometimes they saw irregularities in the smooth rhythm, which mirrored the coughing actions of normal fish. They concluded therefore, that the waves originated in the respiratory centre. Since the waves occurred in the completely isolated brain, the basic automaticity of the centre is clearly demonstrated.

The respiratory centre was not reexamined until 1951 when Woldring and Dirken published a paper on the unit activity in the brain of the carp. With their more sensitive equipment, they recorded not waves but volleys of regular spikes in respiratory rhythm. The brain was left *in situ* and the opercular movements were recorded synchronously. The highest frequency of discharge occurred at the point of maximal opercular adduction, and there were no spikes at all when it was maximally adducted (closed). This activity can be picked up from a fairly extensive region, .5mm on each side of the midline, extending 1.0 - 1.5mm in an antero-posterior direction, at the frontal border of the facial lobe 1.25mm below the surface. Occasionally, they found similar volleys in the brain stem ventral to the point of attachment of the cerebellum.

Hukuhara and Okada (1956) appear to be unaware of the work of Woldring and Dirken but have produced very similar results. They found spikes in approximately the same region in both carp and catfish. They describe two kinds of volleys, one occurring at abduction and one at adduction of the operculum. These could be recorded from closely contiguous areas, and presumably the inspiratory and expiratory neurones are here closely intermingled. They describe this area as being dorso-medial to the spinal tract of V and dorsal to the Substantia-gelatinosa Rolandi, a region traversed by the decussating acoustico-lateral fibres. They recorded these volleys from a brain from which every part rostral to the medulla had been removed. Then they completely denervated the brain, and, although all respiratory movement stopped, the rhythm of the volleys continued for several minutes. The deterioration after 30 minutes they ascribe to circulatory failure.

II SELECTION OF THE PROBLEM

From this survey of the existing work on fish respiration, several points emerged which prompted further investigation. Preliminary experiments indicated that the filaments did not play a passive role during normal breathing as Bijtel (1949) had stated. The work of Konishi (1957) on the spike potentials originating from the filaments seemed to us to support this tentative hypothesis. Konishi described the characteristics of the spikes, and thought they were of muscular origin, but gave no details whatsoever of the nature of the muscles which were active. Hughes and Shelton (1958) in discussing their findings about the pressure changes in the respiratory chambers, spoke of a mechanism for varying the resistance to water flow which must be operative in the gill filaments. It seemed to us that some synthesis of all these ideas was needed, and it was decided to make a thorough investigation of the gill filaments and their musculature.

Surprisingly little work has been done on the neural mechanisms responsible for respiration in fish. None of the schemes for peripheral control seem at all feasible in the light of modern findings, so this makes the basic automaticity of a brain centre seem the most likely source of the respiratory rhythm. At the time when this work was begun, no one had made a study of the teleost brain comparable to that made by Hyde (1904) on the skate. Since then, Shelton (1959) has published the results of his work on the tench, but they are of a relatively tentative nature. Similarly, the papers of Woldring and Dirken (1951) and Hukuhara and Okada (1956) only begin to investigate the

obvious complexities of the system. This present investigation was undertaken in an attempt to extend our understanding of the neural mechanisms responsible for respiration.

M A T E R I A L S A N D M E T H O D S

I CHOICE OF EXPERIMENTAL ANIMALS

The animals used were chosen so as to provide a variability of gill structure according to Bijtel's classification (1949). Many of the experiments required the fish to be exposed to air for long periods and it was found that bullheads and carp withstood this treatment better than any other species which were available. The bullhead is a Type I fish and the carp is Type II (Bijtel, 1949). A complete list of all species used is given below in order of frequency.

Type	Common Name	Taxonomic Name
I	bullhead	Ictalurus nebulosus (Le Sueur, Scott) (Amaiurus nebulosus, Le Sueur)
II	carp	Cyprinus carpio (Linnaeus)
I	rock bass	Ambloplites rupestris rupestris (Rafinesque)
I	largemouth bass	Micropterus salmoides (Lacepede)
I	common bluegill	Lepomis macrochirus macrochirus (Rafinesque)
I	pumpkinseed	Lepomis gibbosus (Linnaeus)
I	yellow perch	Perca flavescens (Mitchill)
I	black crappie	Pomoxis nigromaculatus (Le Sueur)

II PREPARATION OF THE ANIMAL FOR EXPERIMENTATION

The anaesthetic used in most of the experiments was urethane

(Ethyl Carbamate). MS222 was considered, but it was found that the level of anaesthesia at which body movement, but not respiration, was depressed, was much more critical than with urethane. Some of our experiments had to be conducted for periods of up to six hours, and it proved impossible to keep the fish at this critical level unless it were continuously perfused with MS222.

The fish was immersed in 1½% urethane solution until it no longer struggled when handled. It was allowed to recover a normal respiratory rhythm before any experimentation was begun. This technique did not depress respiration noticeably. Hughes and Shelton (1958) also maintained that respiratory depression is negligible with threshold doses of urethane.

In work on the brain, the spinal cord was severed, and the fish perfused with water until behaviour was completely normal, as evidenced by eye movements. This ensured that the optimal excitability of the brain cells was preserved.

Two types of stands were constructed to keep the fish immobile. Where the gill filaments were under examination, the fish was laid on a horizontal stand which was covered with a thick layer of wax. This was contoured to give maximum support to the head, while not interfering with the activity of the branchial apparatus. When the fish was needed in a vertical position, a plexiglass stand was used. It had upright parallel walls which were flexible enough to grip the body of the fish firmly, and accommodated various sizes of fish easily. In the brain experiments the head was clamped with a wound retractor, whose hooks grasped the cut edges of the skull.

Wherever possible, the fish was submerged in cold, running tap water (approximately 12°C). Where necessary, water was introduced into the mouth through a fine plastic tubing, which did not interfere with the respiratory movements.

III METHODS OF EXAMINING THE GILL FILAMENTS

In most cases, one operculum was removed in order to expose the gill filaments. This involved removal of the opercular and subopercular bones and clipping of the branchiostegal rays.

In order to examine the filaments under conditions of normal hydrostatic pressures, a transparent window was inserted into the operculum on one side. This method was also used by Hofdijk - Enklaar (Bijtel, 1949). Few cements will adhere to the damp surface of the operculum, and it was necessary to scrape the bone free from soft tissue, and cauterise the whole surface to clot all the blood. After careful drying, a piece of celluloid was fastened over a circular hole in the opercular bone, using Pliobond and staples. Such a method produced a watertight, fairly durable window into the branchial chamber and did not appear to hamper the fish at all.

Where the movements of the respiratory apparatus were too rapid to be followed by eye, cinematography was used. Both camera and projector could be operated at differing speeds thus allowing for detailed analysis of the complex movements of the filaments.

In conjunction with visual methods, the filament muscles were studied electrically. Fine electrodes were placed on the muscle surface

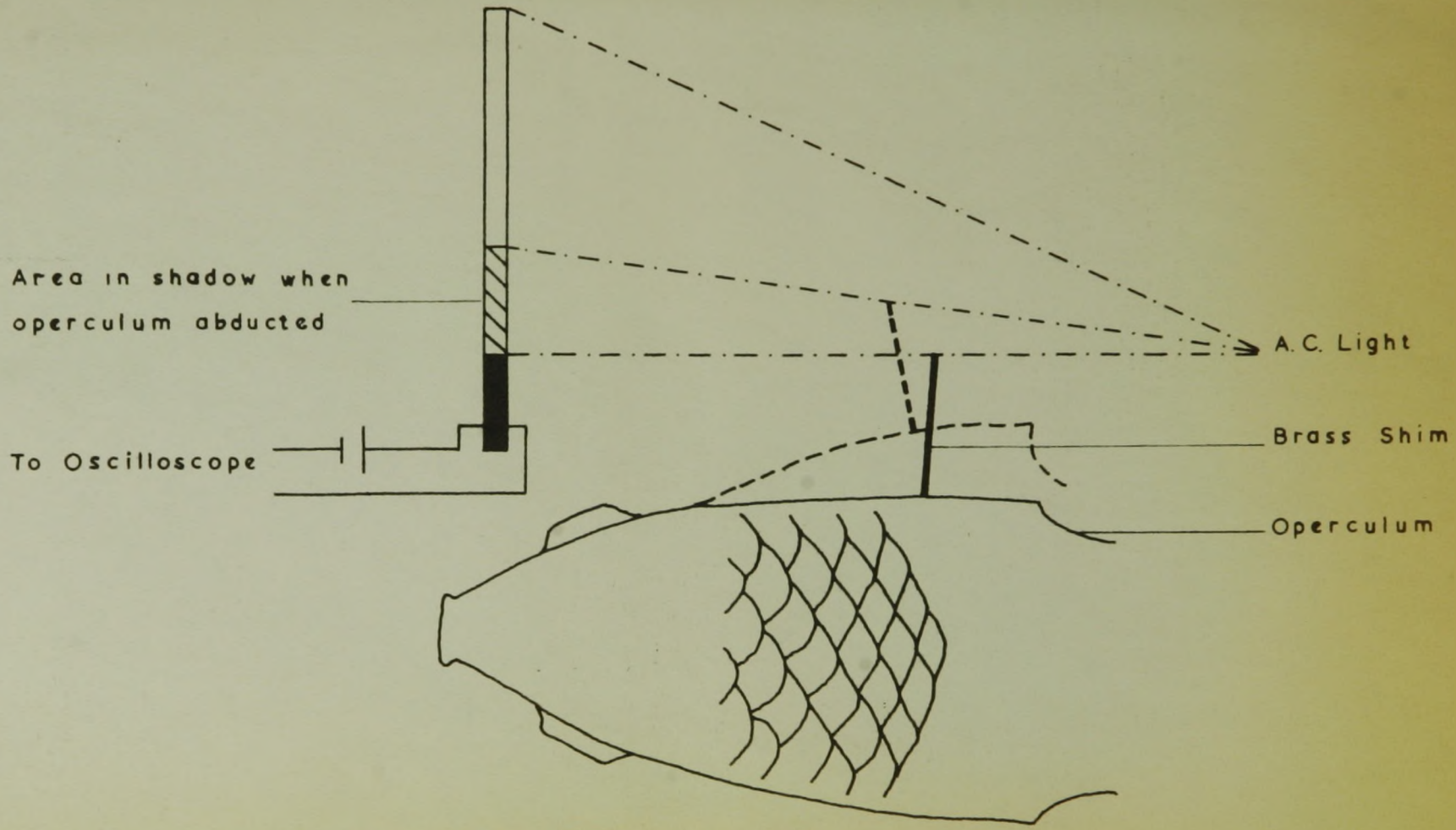


Fig. 5. Diagram illustrating the photocell method of recording opercular movement.

and their action potentials were projected upon an oscilloscope screen. A Dumont Dual Beam Cathode Ray Oscillograph, Type 322A, was used with a Grass D.C. Preamplifier, Model P4A. This system could be used in either push-pull or single-sided recordings. The background noise level of this system never amounted to more than 8 μv.

For coarse recording, steel insect pins were used as electrodes, but for more accurate work fine points were produced on stainless steel wires by the electrolytic method described by Grundfest et al. (1950). In all cases the needles were coated with insulating varnish except at the extreme tips (Westinghouse CM 28762).

Sometimes it was necessary to be able to correlate spike activity with the movements of the breathing apparatus. None of the standard physiological techniques for indicating movements was applicable here, because it was important not to interfere with the movements at all. The amplitude of these movements was very variable, and, when the conditions were best for recording the spikes, often extremely slight. The weight of any lever would completely overcome the muscle action. In addition, most mechanical methods, and many electrical ones, have a rather long latent period.

In some cases, when the opercular movements were almost normal, it was possible to use a photocell system. The fish waslaid on its side, and a piece of very light brass shim was attached to the upper operculum, so that it projected at right angles to the surface of the fish. As the operculum abducted, it raised the shim, which interrupted an a.c.-fed light beam falling on a rectangular selenium photoconductive cell (fig.5). The light beam produced an oscillation in the resistance of the photocell

of the same frequency as the a.c. source. The area of the shadow cast on the cell by the shim was proportional to the displacement, and modified the amplitude of the sine waves. The resulting modulated signal was amplified and projected onto the screen of the oscillograph. Any time lag between the movements of the operculum and the variations in the amplitude of the sine wave could be disregarded. The oscillations were photographed at the same time as the muscle spike potentials which were recorded on the other channel of the oscilloscope.

All photography of the oscilloscope screen was done with a Grass Kymograph Camera, Model C4D. The system preferred in the present investigation was to have a stationary beam, and to use continuously moving film. Clear base Linograph Ortho film was used.

This oscillographic work was performed inside a large Faraday Cage. The entire room was lined throughout with a double layer of copper gauze.

IV METHODS OF EXAMINING THE BRAIN

The work on the brain can be grouped into three sections: a) sections and extirpations, b) recording of electrical activity and c) electrical stimulation.

a) Owing to the soft nature of the brain tissue, removal by suction proved to be the most useful method. By means of an aspirator, regulated suction was applied to localised areas through a fine capillary tube. Occasionally cautery was also used. A fine platinum tip, 2mm. in diameter, allowed small burns to be made.

b) The same recording system was used as was described in the work on the filaments. A single steel electrode gave the best results, with the trunk of the fish grounded.

c) Repetitive electrical stimuli of 400 - 500 msec. duration and variable frequency and voltage were obtained from a Harvard Stimulator, Type 935A. The stimuli were delivered through bipolar electrodes consisting of fine nichrome wires, insulated except at the extreme tips. The total diameter of the pair was less than 1mm. The electrode was rigidly mounted in a Brinkman Micromanipulator whose vernier scales allowed an accurate mapping of the brain.

The responses of the respiratory apparatus to these brain experiments were very varied. Different parts of the apparatus would become active at certain stages in the experiment, and there was no way of predicting which part would show activity, or what would be the range of amplitude. Under these conditions, automatic recording devices were of no value, and the data is compiled from visual observations supported by continual measurements of the frequency of breathing.

V HISTOLOGICAL METHODS

A cursory histological study of the gills was made in order to display any nerve endings or special end organs. Where the ossification of the gill arch bone was quite advanced, the tissue was decalcified after fixation with formalin. The nitric acid method quoted in Romeis (1948) was used in the decalcification. Paraffin sections were made. Many staining techniques were used on the gill tissue, including several silver methods.

Those giving the best results are listed below.

Stain	Source	Use
Mallory's Triple Stain	Gray's Microscopists Manual	General purpose
Bethe's Methylene Blue	" " "	Nerve endings (Vital stain)
Palmgren's Silver Stain	Palmgren (1948)	Nerve endings and striated muscle and elastic fibres.

In the work on the brain the most useful stain was found to be Heidenhain's haemotoxylin (Romeis, 1948).

VI PHARMACOLOGICAL PREPARATIONS

Two chemical preparations were used extensively in this work: d-Tubocurarine Chloride pentahydrate, Brent Laboratories; Novocaine with Suprarenin, Solution 'E', Winthrop Chemical Co. Inc.

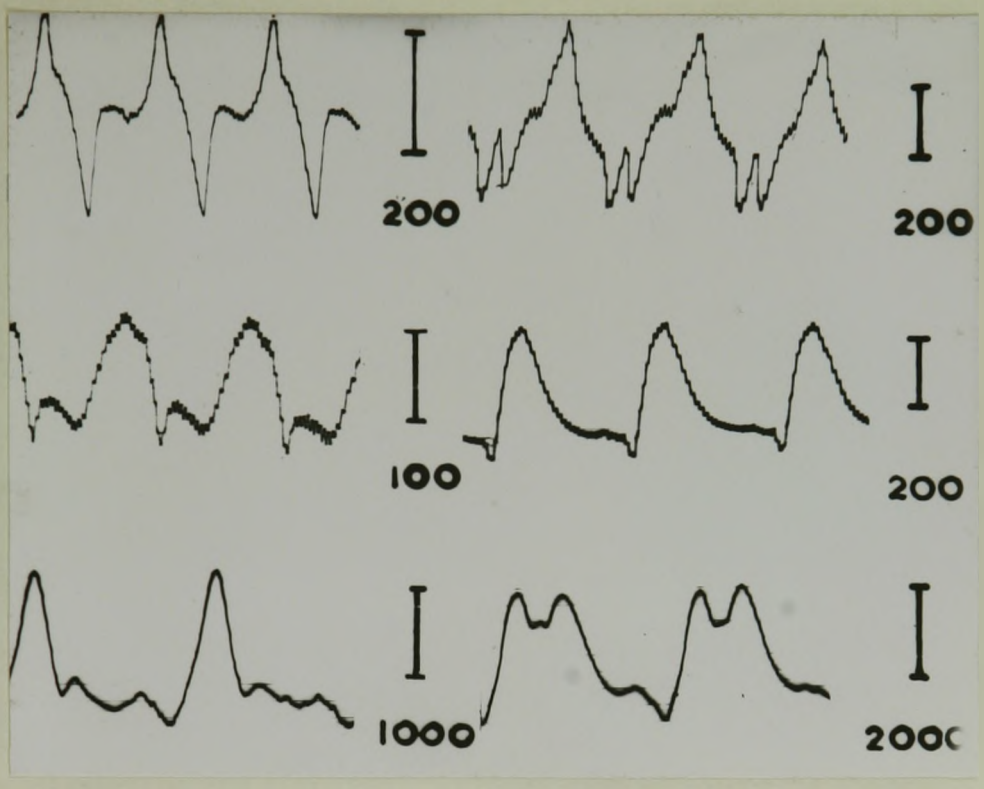


Fig. 6. Examples of wave activity recorded from an electrode placed in the water near the head of a rock bass. Calibration in figs. 6, 7, 8, 9 & 17 in μv.

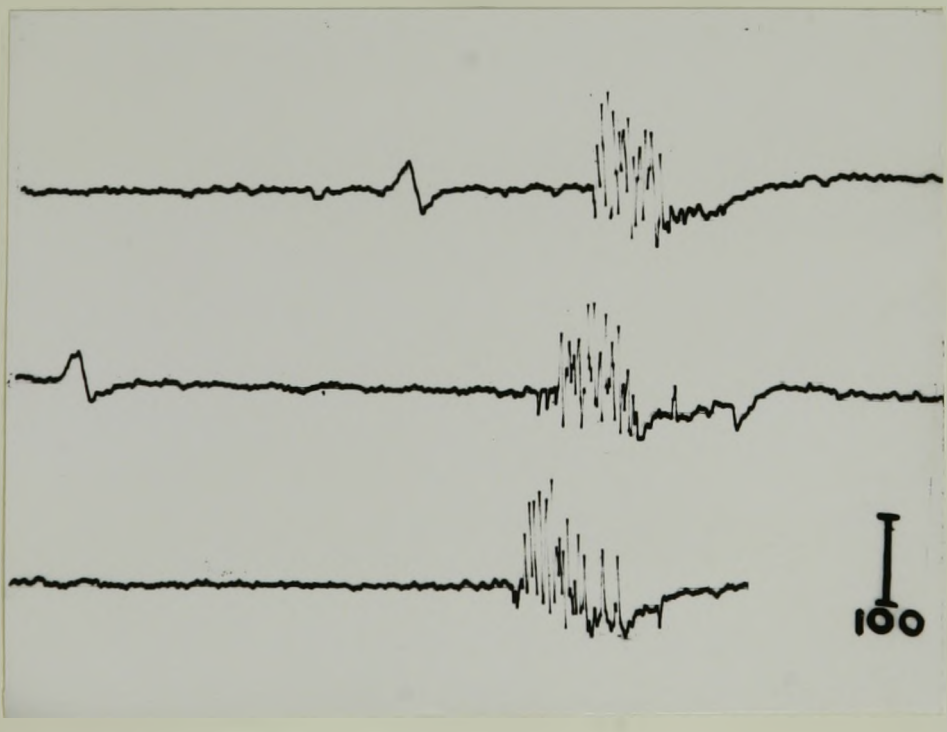


Fig. 7. Action potentials from adductor muscles; the three lines are continuous. Rock bass 8/04

R E S U L T S

I FILAMENT MUSCULATURE

1. Electrical activity recorded from the gill filaments

The water currents produced by the respiratory movements of the fish are associated with a rhythmical pattern of potential changes, which can be recorded by placing an electrode in the water near the head. An irregular, sinusoidal wave pattern can be demonstrated on the oscilloscope screen, which varies in amplitude from 100 μv - 10 mv depending upon the extent of the water flow (fig. 6).

If, however, the fish is respiring out of water, another potential pattern can be recorded: with the recording electrode placed on the filaments, a burst of spike potentials can be observed for every breathing cycle. These were first found in the rock bass (a Type I fish). The spikes here were approximately 50 μv in amplitude (fig. 7). Since the amplitude of these spikes is so much less than that of the large wave pattern, due to the water flow, the bursts were completely masked when recordings were made from the fish in water.

By plotting the whole of the gill surface with the recording electrode, it became apparent that the spikes originated from the region of the adductor muscles. In the carp, a representative of Type II, recording was complicated by vigorous movements of the gill arches, and a deeper level of anaesthesia had to be used in order to eliminate these

rocking movements. Spikes of an identical nature were found in carp also, by placing the electrode over the position of the adductor muscles. With this type of gill structure, the abductor muscles are large and active, and these are associated with another burst of spike potentials. Often, this pattern dominated the adductor muscle pattern completely, but, by using two electrodes, a few records were obtained showing both spike patterns simultaneously (fig. 8). In fish of Type I, it was exceptional to find any spike activity from the region of the abductor muscles.

If the fish coughed while recordings were being made, bursts were produced, in which the spikes were identical with those of the volleys associated with normal breaths. The bursts produced by the coughs were, however, of longer duration, and contained more spikes.

Fig. 9 shows a normal burst from the abductor muscle of carp taken at the higher film speed of 10cm./sec. The spikes vary from 6.3 - 26.7 msecs. in duration, and their frequency and amplitude are irregular. Those spikes originating from the adductor muscles had identical characteristics.

In 17 experiments, dilute solutions of curare were dropped onto the muscles of the arch and filaments. In all cases, the muscular contractions were seen to cease, and the amplitude of the spikes gradually decreased, until they were suppressed completely.

In 5 experiments, Novocaine was applied to the gills externally. In 4 of these cases, the spike activity continued unchanged.

Whenever the nerve supply to the arch was severed, all spike activity ceased in that particular arch.

2. Visual observations of muscular activity associated with the filaments

When the fish is taken out of water, the surface tension of the mucus,

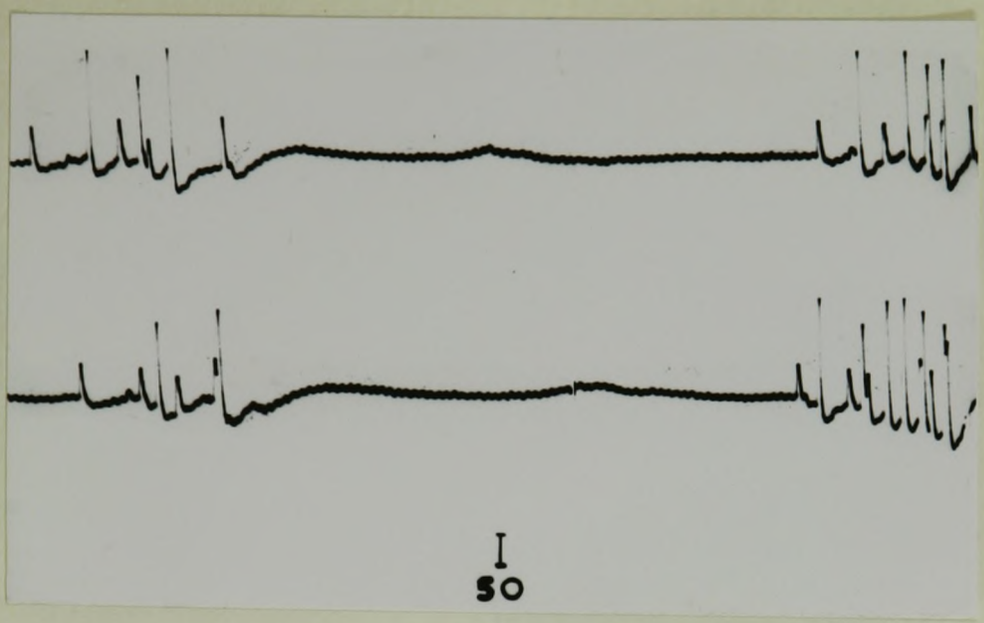


Fig. 8 (a) Action potentials from abductor muscle. Carp 10/03

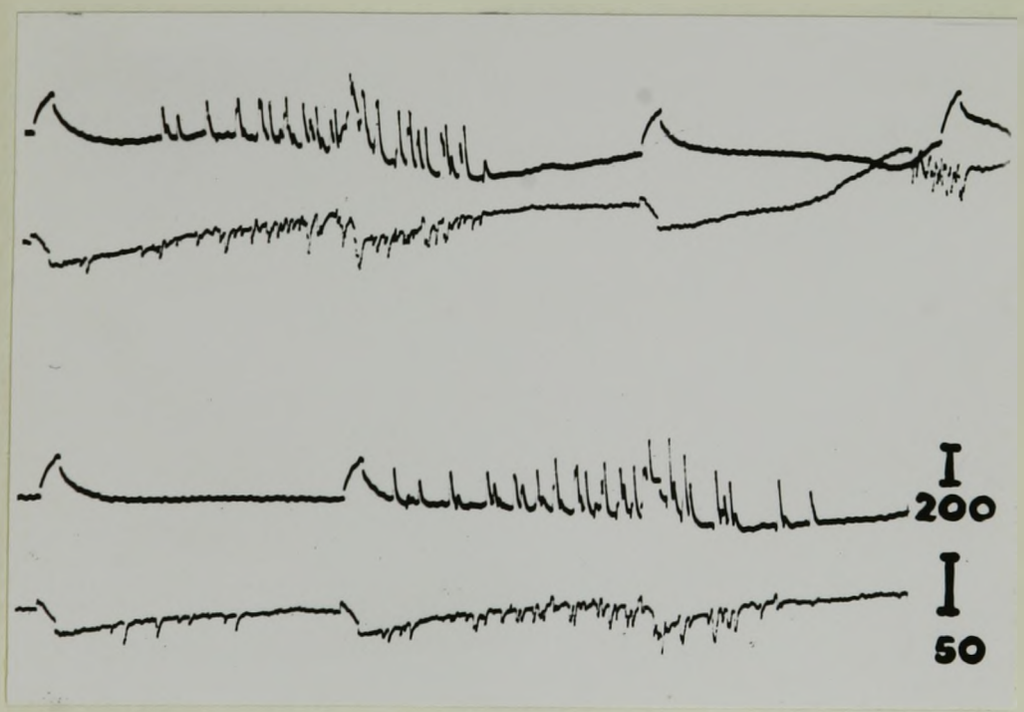


Fig. 8 (b) Action potentials recorded simultaneously from adductor and abductor muscles. Upper line: abductor muscle. Lower line: adductor muscle. Carp 21/09B

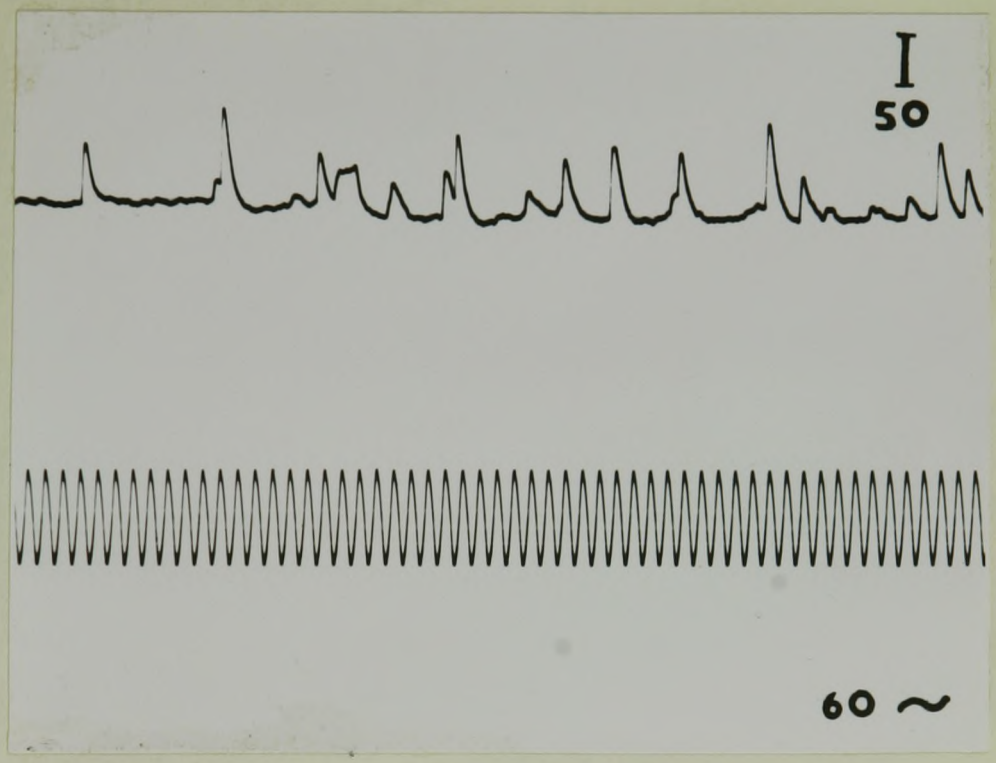


Fig. 9 (a) Action potentials from abductor muscles, filmed at double the speed of previous records. Time signal 60 c.p.s.

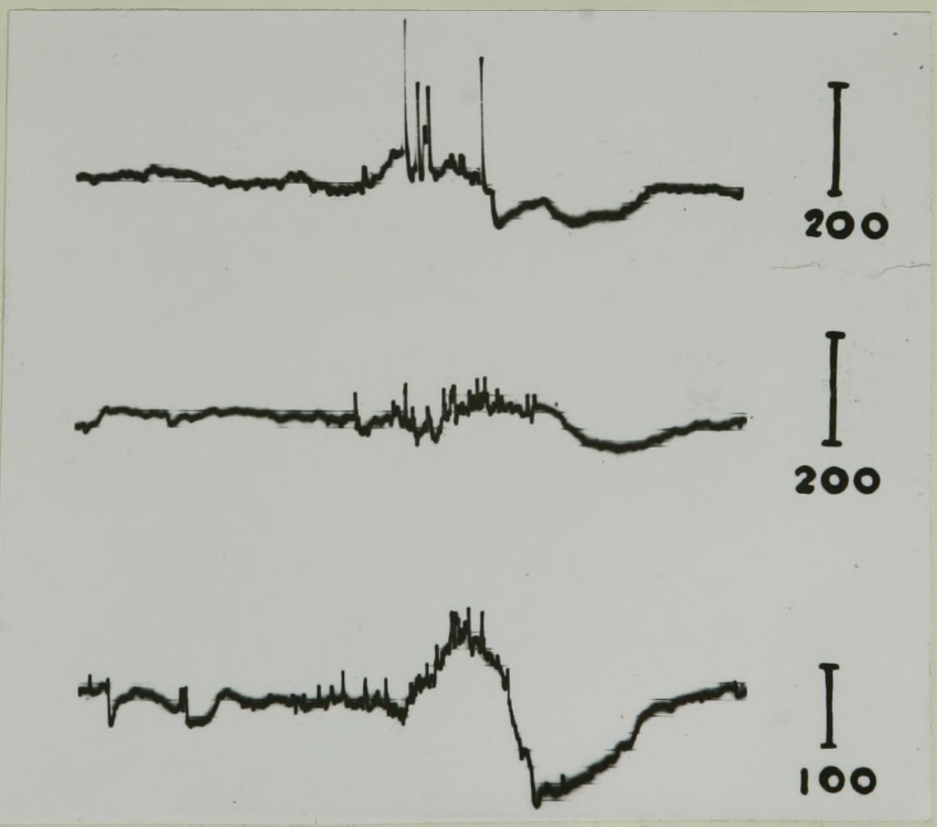


Fig. 9 (b) Depressant action of curare on action potentials recorded from adductor muscles. Top line: before application of curare. Middle line: 15 mins after i.v. injection .02ccs. curare soln. Bottom line: 30 mins. after injection. .06ccs. depressed spikes completely.

covering the gills, generally holds all the filaments tightly together. However, a group of filaments could be lifted away from the others, and, once the forces of surface tension were overcome, the natural elasticity of the connection between these filaments and the arch kept them in their normal position relative to the filaments of the other hemibranch. (That is, with a wide 'V' in between the two rows of filaments.) Under these conditions, adductor movements of the group were often observed. The adductor muscles were seen to be contracting and pulling the filaments towards the other hemibranch. The muscles are more clearly visible in Type I fish than in Type II, where the adductor muscles lie within the filament 'diaphragm'. However, even here the movement of the muscles could be seen, under high magnification, contracting below the surface of the epithelium of the 'diaphragm'. The abductor muscles are the ones most easily viewed in Type II fish, and these were also seen to be active once during every breathing cycle.

In all cases, the muscular activity was observed to be correlated with the spike activity picked up by the electrodes. Not only did the two phenomena occur in the same phase of the breathing cycle, but, if the muscle contraction was long, so was the burst of spikes; if the contraction was short, the burst was equally brief.

It was much more difficult to observe the muscle activity when the fish was in water. Even when the operculum was removed, the position of the arches made both the adductor and abductor muscles inaccessible to view. However, by tipping the fish at the appropriate angle, it was possible to make some observations and, here again, contractions of the muscles were seen during every breathing cycle. In most cases, active movement of the

filaments, and in particular, parting of the filament tips, was taken as the criterion for muscular activity.

When the operculum is removed, a major component of the branchial suction pump apparatus is eliminated, the hydrostatic pressure in the branchial chamber is the same as the external pressure, and so the pressure differential across the gill curtain is greatly exaggerated. To circumvent these artefacts, a transparent celluloid window was inserted into the opercular bone, in order to observe the filaments under normal conditions. In most fish, the anatomy of the branchial apparatus makes it impossible to insert a window large enough to view the filament tips themselves at the correct angle for discerning the parting. A fish was needed with a very shallow branchial cavity and a convex operculum, where the opercular bone approaches the very edge of the 'flap'. Such a fish is the callosoma, a harmless relative of the Amazon fish, the piranha. When the window was inserted in this species, parting of the tips was seen.

However, even with the operculum removed, many instances of filament activity were seen, where the conditions of flow made it impossible for the movements to be artefacts. Frequently it was seen that, during deep anaesthesia, the breathing movements of most of the apparatus becomes very shallow, so that there was not sufficient force to expel water through the gill filaments. Yet filament movement continued. The water was coloured with nigrosine, and no water was seen flowing through the filaments, but they were moving regularly. During the experiments on the brain, which will be discussed below, several instances of independent activity of the filaments were noticed. In some cases most of the respiratory apparatus was paralysed by destruction of the central motor nuclei, but the filament

muscles continued contracting rhythmically. On other occasions, the central co-ordination mechanism was disrupted by sectioning the brain, and the filaments showed a different rhythm from the other moving parts.

3. Variability in the amplitude of muscle contraction

In all these experiments, much variability was noticed in the extent of the movements of the filaments. When the fish coughs, the adductor muscles often contract maximally, so that the two hemibranchs on one arch come together completely. This is very rarely seen in normal breathing. However, after anaesthetising, very vigorous parting of the tips was frequently noticed with almost complete adductions. The respirations showed no other resemblance to coughs, and must be considered as normal cycles, though hyperpnoic, and they occurred in groups containing up to thirty breaths. Sometimes every alternate cycle contained such a large adduction, although the other parts of the apparatus showed normal behaviour.

Even apart from these abnormal rhythms, there was variability in the extent of muscle contraction during normal quiet breathing. It ranged from a definite parting of the filaments with every respiration, to a rocking movement of the whole filament bunch, with no actual parting at all. This latter activity involved a folding of the whole gill curtain inwards, towards the medial plane of the body, and a relaxation outwards again. If now the three arches nearest the body wall were gently depressed with a spatula, or film of plastic, leaving one arch in isolation, it could be seen that the contribution of each pair of hemibranchs to the corporate rocking movement was an adductory one. The arches were also rocking under the influence of the arcuales muscles attaching the gill arches to the skull, but in addition, the hemibranch rows were adducting. This adductory

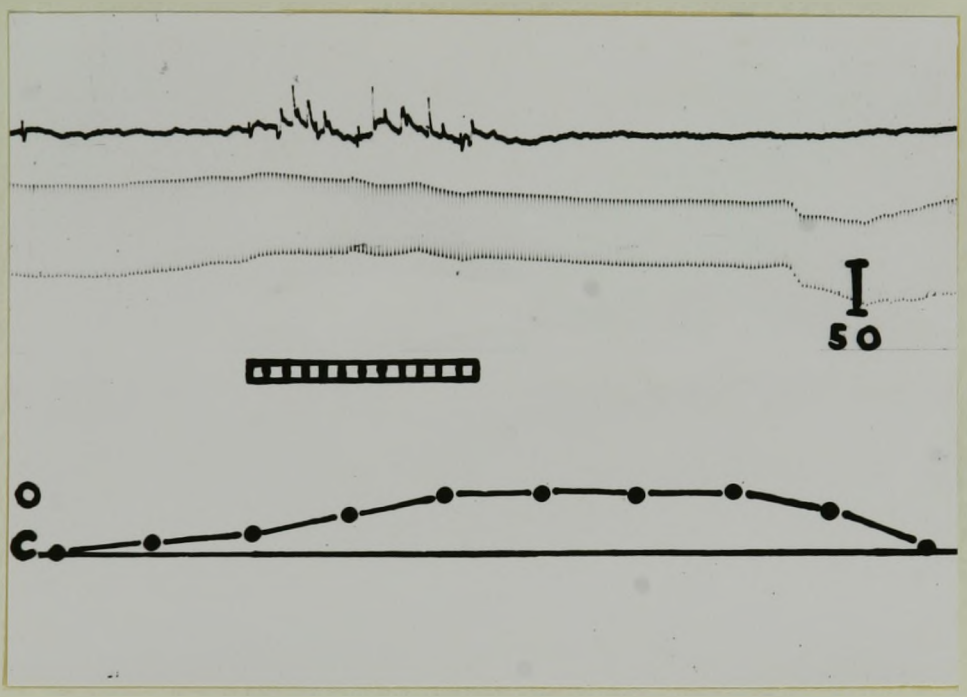


Fig. 11. Correlation of adductor muscle spike activity with opercular movement using photocell method. 1st line: adductor muscle spike potentials. 2nd line: modulated 60 c.p.s. oscillation produced by photocell. 3rd line: duration of active period of adductor muscle. 4th line: a diagram of opercular movement. O, abducted position of operculum. C, closed position of operculum. Distance between points represents one fifth sec. Ordinates inversely proportional to the amplitude of the modulated signal.

movement is very slight, and is indistinguishable when all the arches are in contact, but the muscle is continually active, even though no parting of the tips is seen. Thus, good spikes may often be obtained from a fish in air, but, if the fish is then immersed in water, no parting of the tips is visible.

4. Variability in the temporal pattern of muscular activity

In order to ascertain at what phase of the breathing cycle the muscle contractions occurred, numerous observations were recorded of the correlation between opercular movements, filament movements and the spike potentials. Many of these were purely visual observations. Others were assisted by the photo-cell method. Fig. 11 shows an example of the records obtained by the latter method. Table I summarizes all the observations. It can be seen that while the abductor muscle is active always during opercular opening, the adductor muscle shows much variation. Three patterns of activity of the filaments have been observed in any one of the species studied (fig. 12).

Type A

Filament tips part once during the phase of opercular abduction. The movement is generally quite wide, creating a large gap between the arches.

Type B

Filament tips part twice; once during opercular abduction and again during opercular adduction. The former is often a wider parting than the latter.

Type C

Filament tips part once during the phase of opercular adduction.

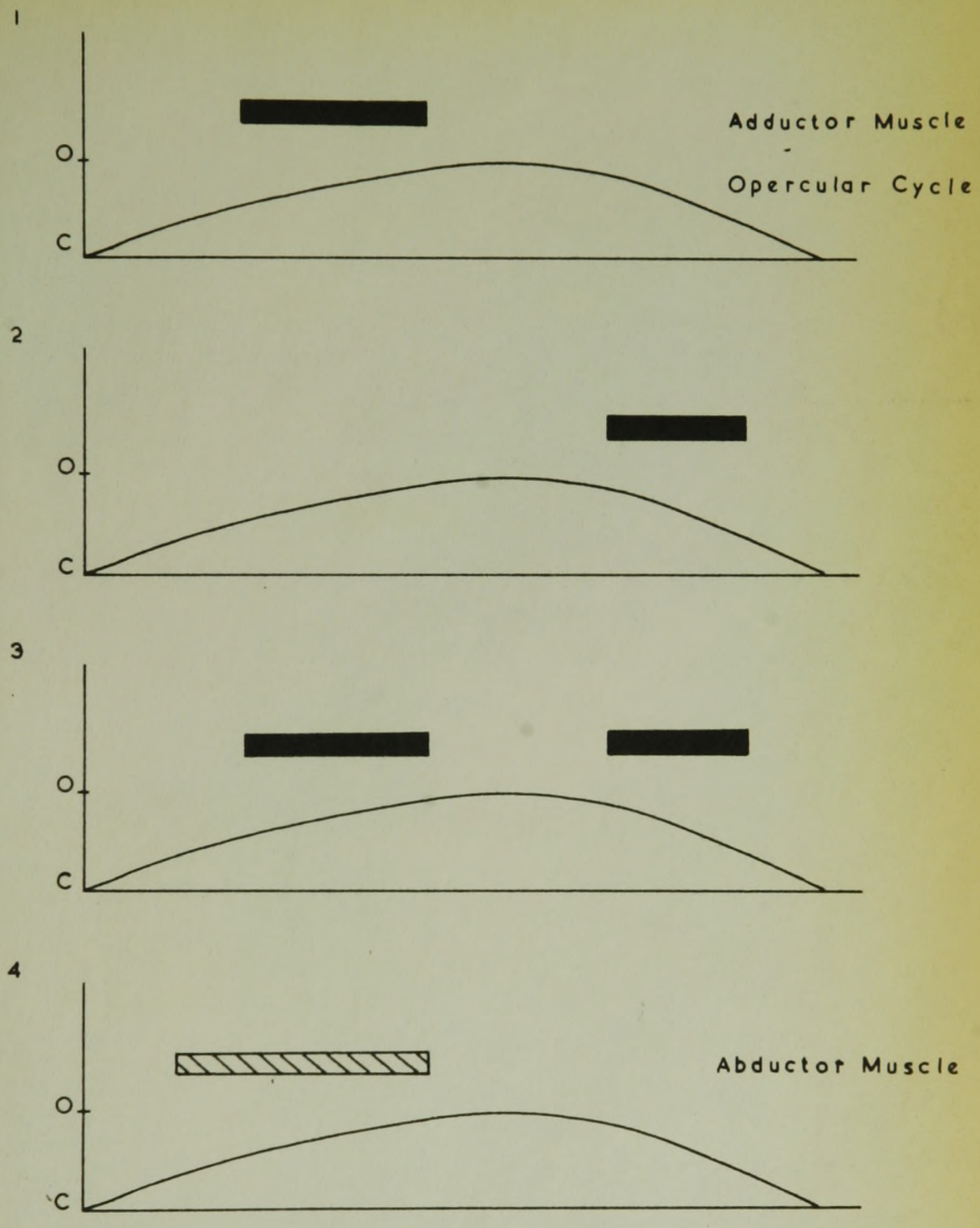


Fig. 12. Diagrams showing the different patterns of adductor and abductor muscle activity. 1. Type a 2. Type c 3. Type b 4. Abductor contraction. O. Operculum open. C. Operculum closed.

Type A parting was usually observed immediately after anaesthesia, or after long periods of air exposure, and was the pattern very often seen when recording the spikes in air. In general, it seems to be associated with hyperpnoea. Type B breathing generally followed Type A, but this type of breathing was transitory and was never maintained over long periods. Type C breathing was the most common.

TABLE I

CONDITIONS UNDER WHICH THE DIFFERENT TYPES OF ADDUCTOR MUSCLE ACTIVITY WERE OBSERVED IN *Cyprinus carpio* AND *Ictalurus nebulosus*

Type	Light anaesthesia		Deep anaesthesia		Initial respiratory movements after apnoea
	In air	In water	In air	In water	
A	4	2	6	15	4
B	-	-	-	7	2
C	8	*	-	11	6

* This was the most common type of breathing, seen in unanaesthetised and lightly anaesthetised fish. Separate observations of this nature were not recorded.

One series of observations made on carp is given in fig. 13. The fish had been immersed in 1% urethane until all breathing ceased, and was transferred to running tap water at 12°C. When breathing began, the first movements were of Type A, then these were superseded by brief periods of

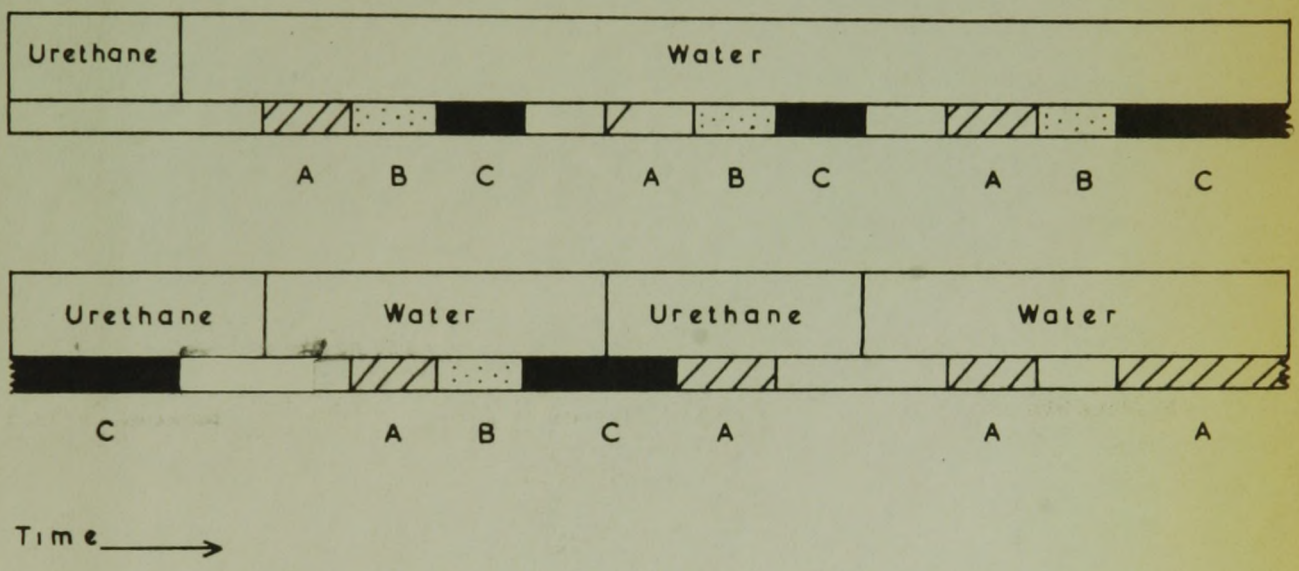


Fig. 13. Pattern of breathing observed in carp during recovery from Urethane anaesthesia, showing three types of filament behaviour.

Type B and Type C breathing, consisting of 7 - 25 breaths. The Type C breathing was terminated by a spontaneous return to the apnoeic condition. This pattern of A - B - C was repeated twice more, each time interspersed with short periods of apnoea lasting from 1 - 3 mins. Finally, the breathing became more normal and persisted as Type C. After 5 mins. of this normal behaviour, the fish was replaced in urethane until once again it stopped breathing. In water, breathing returned and underwent exactly the same pattern. However, it quickly recovered from the urethane, so the fish was anaestheised once more. Now the breathing as a whole was becoming weak and the recovery pattern changed. Finally, breathing consisted of a series of feeble Type A breaths separated by pauses of 34 - 45 secs. This was the best example of the relationship between the three types of breathing. Other records showed the same general trends, but the pattern was not repeated so regularly.

5. Electrical stimulation of the gill muscles

Electrical stimulation of the basal parts of the filaments induced contraction of the adductor muscles. The two hemibranch rows of an arch were pulled close together until they touched. When stimulating electrodes were put on the arch, the abductor muscles contracted. Their effect was to draw the two hemibranchs nearer to the oral surface of the arch, bringing about a rotation between the filaments and the arch bone.

6. Histological studies

Histological examination of the filament musculature supported the findings of earlier authors. Fish have poorly developed muscle spindles, and no specialised nerve endings were seen in the adductor or abductor

muscles. The only sensory endings seen were taste buds, which were found in great density in the epithelium of the gill arches.

II EXPERIMENTS ON THE CENTRAL NERVOUS MECHANISMS

The work on the respiratory areas of the brain, carried out primarily on the bullhead, can be divided into three sections. First, sections and extirpations were made, in an effort to assess the relationships between the parts of the brain, their relative importance and, if possible, their function with respect to respiration. Then, recording apparatus was applied to investigate the electrical activity of the respiratory areas. Finally, the brain was electrically stimulated in order to ascertain some of the functions of those parts which were found to be important in the previous experiments.

Since it was found, during the course of these investigations, that the entry of water into the brain cavity had little effect upon the activity of the brain, as far as this work was concerned, it was possible to immerse the whole of the branchial apparatus in water while the observations were being made. The fish was only lifted up into the air for the brief duration of the actual brain operations.

1. Sections and extirpations

a) Cranial nerve sections

Firstly, it was considered important to ascertain the effect of sectioning the cranial nerves supplying the respiratory muscles. It is impossible to separate the sensory and motor components, as they both emerge in the dorsal roots and run together in the post-trematic rami.

However, two pieces of information can still be obtained by sectioning the whole trunk; the significance of the centripetal sensory impulses for the maintenance of the respiratory rhythm, and the effects of a particular motor nucleus.

TABLE II

EFFECTS OF CUTTING CRANIAL NERVES

Nerve	Unilateral Section	Bilateral Section
IX & X	(1)	(3) + (1) -
V & VII	(2)	(2) +

\+ Paralysis of denervated muscle, normal respiratory movements in the remainder of the apparatus

\- Cessation of all respiratory movement

Number of experiments in brackets

Apart from the period of inhibitory shock, which was seen in varying degrees in all experiments involving damage to the central nervous system, there were, with one exception, no visible deleterious effects to the respiratory rhythm after sectioning V, VII, IX and X bilaterally (Table II). In all cases, the motor paralysis of one part produced no effects on the activity of the remaining parts of the respiratory apparatus.

After any one, or a combination, of these sections, the fish was still capable of 'coughing'.

TABLE III

EFFECTS OF TRANSSECTING THE MEDULLA

Level	Expt. No.	Sectioning affects motor function of:					Sequence of motor activity and observations on its nature	Frequency of Rhythm	Duration of Activity
		Mouth	Operculum	Tentacles	Filaments	B.S. apparatus			
A	33a	x	x	x	x	x	Normal breathing	66/min.	
B	33b	x	-	-	-	-	Isolated and erratic movements		7 mins.
C	7	-	-	x	-	-	Twitching continually	80/min.	7 mins.
		-	-	x	-	-	Periodic rhythm	40/min.	40 mins.
		-	-	x	-	-	Regular rhythm	40/min.	15 mins.
		x	x	x	-	-	Regular rhythm	40/min.	1 hr.10 mins.
		x	x	x	x	x	Vigorous regular rhythm	22/min.	
D	4	-	-	-	-	x	Irregular rhythm	44/min.	7 mins.
		-	x	-	-	x	Groups of opercular movements		20 mins.
		x	-	-	-	-	Periodic rhythm		10 mins.
		x	x	-	-	-	Regular rhythm	60/min.	9 mins.
		x	x	-	-	-	Became periodic, then ceased		
D	6	-	-	x	-	-	Rhythmical movements		10 mins.
		-	x	x	-	-	Erratic movements of L. operculum and tentacles		10 mins. *

TABLE III (Contd.)

Level	Expt. No.	Sectioning affects motor function of:					Sequence of motor activity and observations on its nature	Frequency of Rhythm	Duration of Activity
		Mouth	Operculum	Tentacles	Filaments	B.S. apparatus			
D	8	-	-	x	-	-	Rhythmical movements	34/min.	
		x	x	x	-	-	Regular rhythm	40/min.	
D	57	-	-	x	-	-	Rhythmical movements	26/min.	11 mins.
		x	x	x	-	-	Regular rhythm	30-40/min.	
D	58	-	-	x	-	-	Rhythmical movements	24-40/min.	11 mins.
		-	-	x	-	-	Irregular and rapid movements		40 mins. *
D	63	-	-	x	-	-	Irregular movements		5 mins.
		x	x	x	-	x	Regular rhythm	50/min.	
D	64	-	x	-	-	x	Momentary mechanical stimulation produced groups of normal rhythmical movements in operculum and B.S.		38 mins.
		x	x	-	-	x	Regular rhythm		
E	18	-	-	x	-	-	Twitching movements		28 mins.
		x	-	x	-	-	Regular rhythm	21/min.	20 mins.
		x	-	-	-	-	Regular rhythm. Gill arches also	28/min.	

TABLE III (Contd.)

Level	Expt. No.	Sectioning affects motor function of:					Sequence of motor activity and observations on its nature	Frequency of Rhythm	Duration of Activity
		Mouth	Operculum	Tentacles	Filaments	B.S. apparatus			
F	11	x	-	x	-	-	Tentacles quivering. Isolated movements of jaw		14 mins.
		-	-	x	-	-	Rhythmical movements	84-40/min.	10 mins.
		-	-	-	-	-	All movement stopped		1 hr.48 mins.
		-	-	-	x	-	Rhythmical movements of filaments of 1st hemibranch	32-26/min.	
F	26	-	-	-	x	-	Groups of filaments rhythmical	120-98/min.	1 hr. *
F	27	-	-	-	x	-	Quivering		14 mins.
		-	-	-	x	-	Irregular rhythm. Filaments of 2nd hemibranch	46/min.	24 mins.
		x	-	-	-	-	Regular rhythm	26/min.	

x Movement of a part of apparatus after sectioning brain.

\- No movement of a part of apparatus after sectioning brain.

* Death of fish.

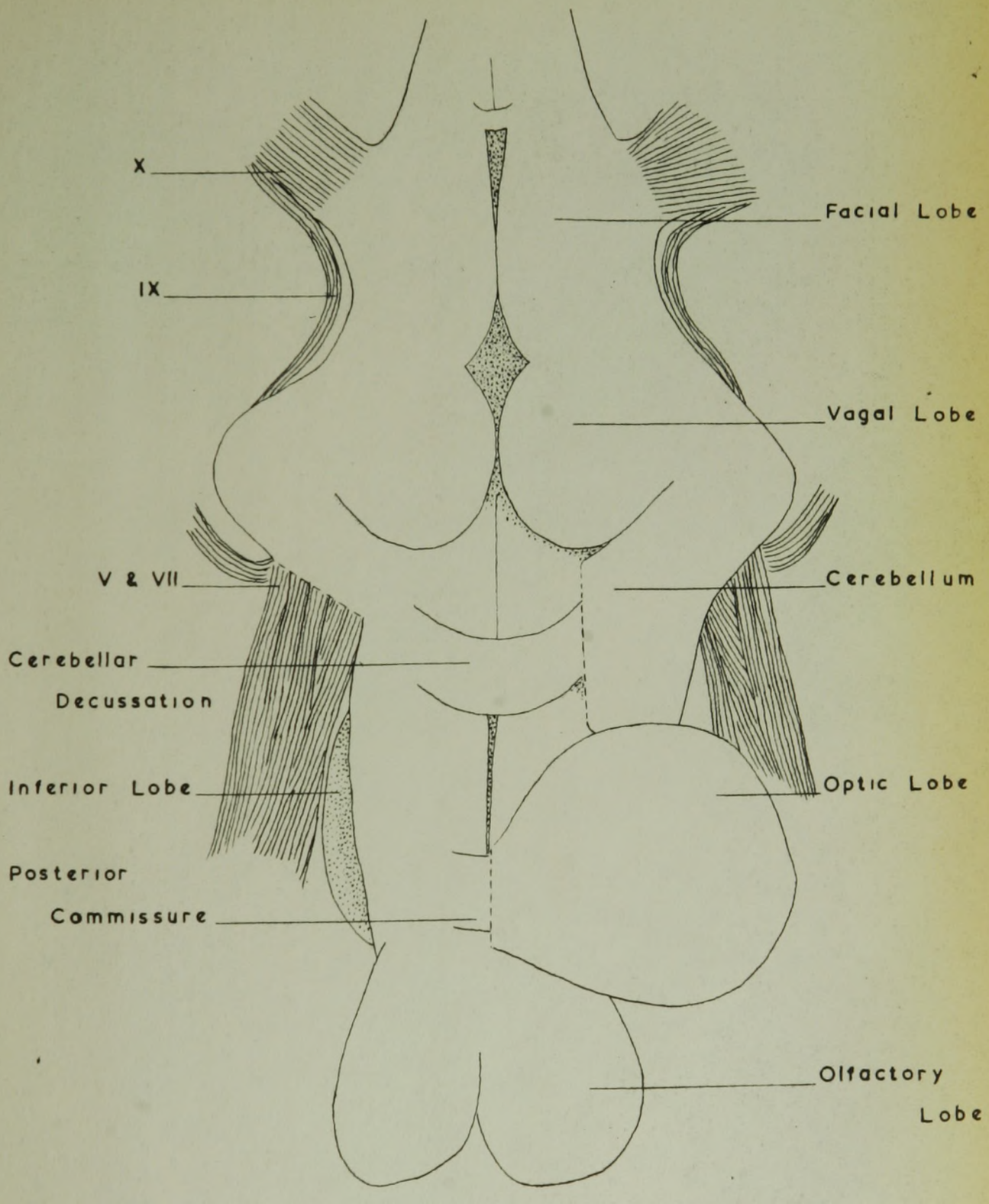


Fig. 14. The brain of the bullhead.

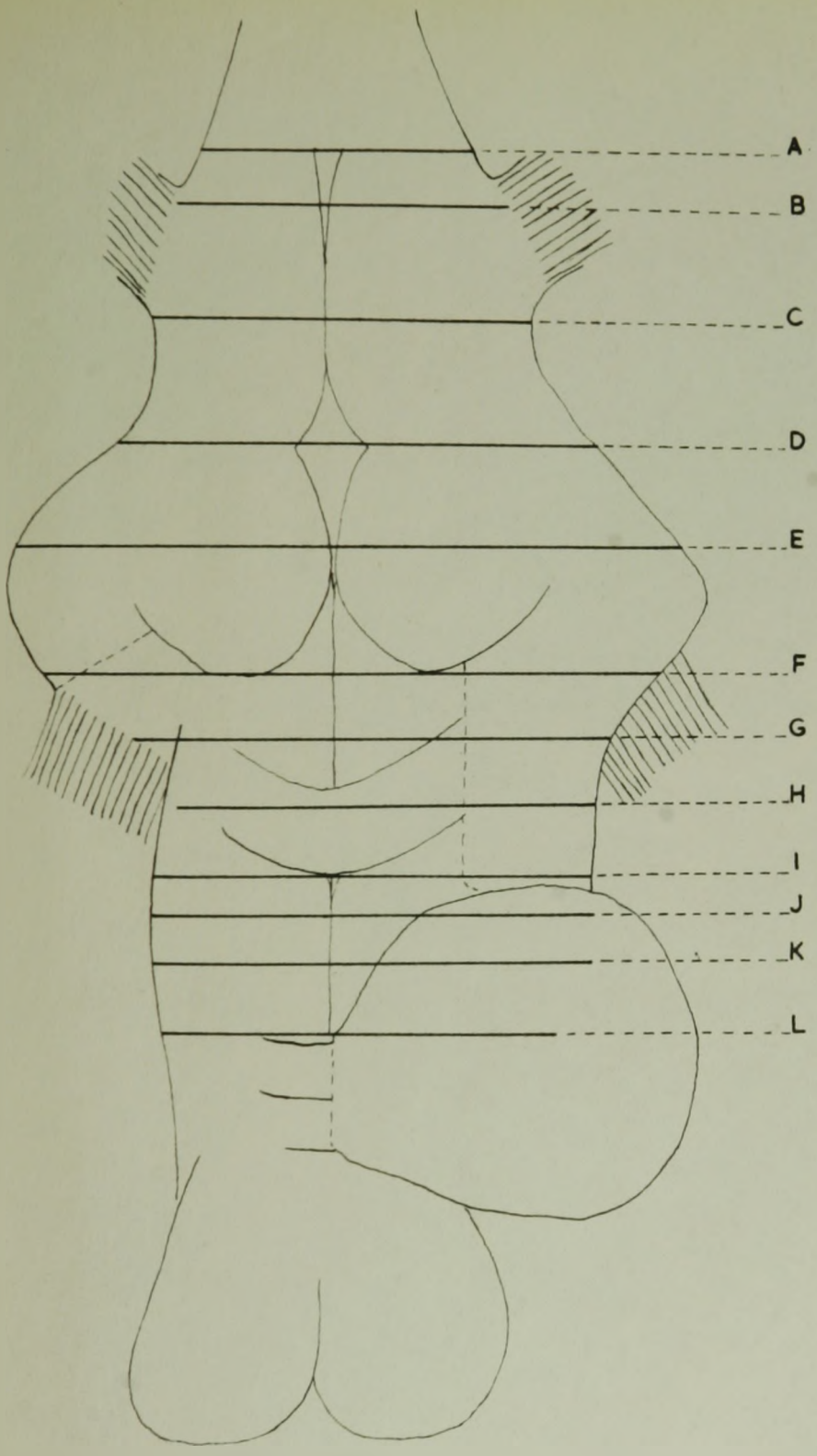


Fig. 15. Diagram showing the levels at which brain transsections were made.

b) Medullary sections

i) Transsections

Complete transsections of the medulla were made at different levels. The positions of these levels are shown in fig. 15 and the results are summarised in Table III. Refer to fig. 14 for anatomical details of brain.

It is important to note that only in one case did a transsection of the medulla lead to a complete cessation of rhythmical activity. The exception was at level B. However, as the fish died within 7 mins., we feel that little significance can be attached to this particular experiment.

With the exception of transsections at levels A and C, all other transsections led to some motor paralysis. This did not seem to affect the activity of the other parts of the respiratory apparatus, which resumed rhythmical movement. Contractions of the filament muscles were taken to be indicative of activity within the vagal motor nucleus which is associated with nerves IX and X. It can be seen that these muscles were paralysed by transsections at levels D and E. After transsections at F only certain groups of filaments showed any activity.

All the other parts of the respiratory mechanism, mouth, opercula and branchiostegal apparatus, are innervated by nerves V and VII. However, not all these parts were paralysed by transsections at the same level. The opercula and branchiostegal apparatus were paralysed as a result of sections at E and F, but the mouth remained active after all medullary sections.

These experiments revealed that the mouth tentacles, or barbels,

perform active rhythmical movements with a respiratory rhythm. It was commonly observed that the tentacles were the first parts to begin moving after the operative shock. The adductor and abductor tentaculus muscles at first quivered irregularly, producing twitching movements of the tentacles, but usually the activity became regular with a normal respiratory frequency, even though the lower jaw was still immobile. None of the medullary transsections led to permanent paralysis of the tentacles.

ii) Longitudinal sections

Longitudinal medial sections through the medulla had little effect on breathing. When all the tissue was left *in situ*, both sides of the apparatus regained normal activity. In one case, the two sides appeared to be slightly out of phase for thirty minutes, but this was corrected spontaneously. In four experiments, one side of the medulla was completely removed. Normal activity was quickly regained by the apparatus on the intact side.

iii) Removal of sensory regions

In nine experiments, *all* the sensory areas of the medulla, dorsal to the floor of the IVth ventricle, were removed. That is, in the bullhead, the facial and vagal lobes were removed, and in the carp, the lobus impar and the vagal lobes. In all cases, normal respiration was regained.

c) Supra-medullary sections

In order to expose the ventral brain stem for experimentation, it was necessary to remove the cerebellum and optic lobes. As seen below, these procedures have no effect upon the breathing rhythm.

TABLE IV

EFFECTS OF REMOVING PARTS OF BRAIN

Part	Effect upon respiration
Olfactory lobes	(5) +
Optic tecti	(3) +
Complete optic lobes	(5) + (1) -
Inferior lobes	(8) +
Cerebellum	(3) +
Cerebellar decussation	(5) +

\+ Normal breathing returned after inhibition of 30 secs - 15 mins.

\- Ceased breathing after 10 mins.

Numbers in brackets indicate number of observations.

Removal of all brain tissue dorsal to the aqueductus cerebelli and IIIrd ventricle became standard technique. Only the cerebellar decussation and the posterior commissure were left intact to serve as points of reference (see fig. 14).

The levels of transsection are given in fig. 15 and the results are summarised in Table V. Sections G - K all led to the death of the fish in less than thirty minutes. Transsections at levels G, H and J were followed by series of irregular, uncoordinated and isolated movements in all, or some of, the parts of the apparatus. Rhythmicity was lost,

but there was little permanent motor paralysis. Transsection at level I, on the other hand, induced an initial loss of rhythm, which was later regained, first of all by the mouth, and then, when that ceased, by the left branchiostegal membrane.

At level K, transsection was not followed by a loss of rhythmicity, and there were some low amplitude, regular movements of the opercula and tentacles. However, the fish died after 23 minutes.

In two out of three transsections made at level L, normal breathing was uninterrupted by the operation. In the third case there was some loss of coordination, with long periods of inactivity shown by the mouth and opercula, but some rhythmicity was demonstrated by the fish 36 hours after the operation.

d) Extirpation by cauterisation

Results of the cauterisation experiments are summarised in Table VI. Shallow cautery of the whole of the floor of the IVth ventricle, to a depth of one millimetre, had no effect on respiration. Deep cautery, approximately two millimetres in diameter at the posterior end only of the ventricle did not prevent rhythmical movements in any part of the respiratory apparatus except the mouth, which remained closed. However, although the left operculum remained active continuously, the right operculum showed long periods of inactivity, and occasionally exhibited a different rhythm from that of the rest of the apparatus. This different behaviour was peculiar to the right operculum, and the filaments on the right side retained their synchrony with the rest of the apparatus. Deep cautery at the anterior end of the ventricle induced violent coughing movements but, between the coughs, the basic rhythm was unchanged.

TABLE V

EFFECTS OF TRANSSECTION IN SUPRA-MEDULLARY REGIONS

Level	Expt. No.	Sectioning affects motor function of: Mouth	Operculum	Tentacles	Filaments	B.S. apparatus	Sequence of motor activity and observations on its nature	Frequency of Rhythm	Duration of Activity
G	23	x	x	x	x	x	5 isolated inspirations		8 mins. *
G	25	x	x	x	x	x	Slow coughing rhythm		14 mins.*
H	22	-	x	-	-	-	R. cheek active	18/min.	3 mins.
		x	x	x	x	x	Isolated gulps		
		-	-	-	x	-	Filaments of III arch	80/min.	5 mins. *
H	43	-	-	-	-	x	Irregular quivering of membrane		20 mins.
		x	-	-	-	-	Occasional mouth movements		*
I	42	x	x	x	x	x	Quivering of all parts		1 min.
		x	x	-	-	-	L. operc. and mouth quivering	90/min.	8 mins.
		x	-	-	-	-	Rhythmical movements	76/min.	1 min.
		-	-	-	-	x	L. B.S. apparatus rhythmical	56/min.	20 mins.*
J	45	-	-	-	-	x	Feeble irregular movements of L. B.S. membrane		4 mins. *

TABLE V (Contd.)

Level	Expt. No.	Sectioning affects motor function of:					Sequence of motor activity and observations on its nature	Frequency of Rhythm	Duration of Activity
		Mouth	Operculum	Tentacles	Filaments	B.S. apparatus			
K	65	-	x	x	-	-	Rhythmical movements. Small amplitude	30 to 10/min.	3 mins.
		-	x	x	x	-	Occasional coughs		9 mins.
		-	-	-	x	-	Rhythmical movements	96/min.	11 mins.*
L	44	-	x	-	-	x	Rhythmical B.S. apparatus	70/min.	
							Irregular operc. movements	12/min.	25 mins.
		x	-	-	x	-	Rhythmical filament movements	40/min.	
							Occasional mouth movements		40 mins.
		-	x	-	x	x	Rhythmical filaments and B.S. Occasional operc. movements		
		-	x	-	-	x	B.S. apparatus	80/min.	After 12 hrs.
							Operc. rhythmical	8/min.	
		x	x	-	-	-	Operc. rhythmical	10/min.	After 21 hrs.
							Occasional mouth movements		
L	47	x	x	x	x	x	Normal breathing	30/min.	
L	68	x	x	x	x	x	Normal breathing	40/min.	

TABLE VI

RESPONSES OF RESPIRATORY SYSTEM TO LOCALISED CAUTERY OF BRAIN

Expt. No.	Area cauterised	Depth	Response
C28 & C29	Floor of IVth Ventricle	1mm.	No change in breathing
C28	Anterior end of ventricle	D-V	Temporary inhibition of mouth and operculum, filaments and arches active. Regular rhythm of mouth interspersed with convulsive movements of all parts.
C29	Posterior end of ventricle	D-V	No inhibition. Mouth shut permanently. R. operculum capable of rhythmicity but had long periods of inactivity. R. filaments maintained activity synchronously with L. side.
B72	Becus of cerebellar decussation	D-V	Rhythmical movements of all parts. Abduction exaggerated.
B74	2mm. caudal to posterior commissure	D-V	Breathing unchanged.
B78	i As 74	1mm.	Breathing slightly irregular.
	ii Posterior commissure to anterier edge of facial lobe.	1mm.	Apparatus widely abducted. Regular oscillations about this position.
	iii As ii	2mm.	Abductions exaggerated. Violent coughs preceded by groups of small breaths.

D-V Burn extended from dorsal to ventral surfaces of brain stem.

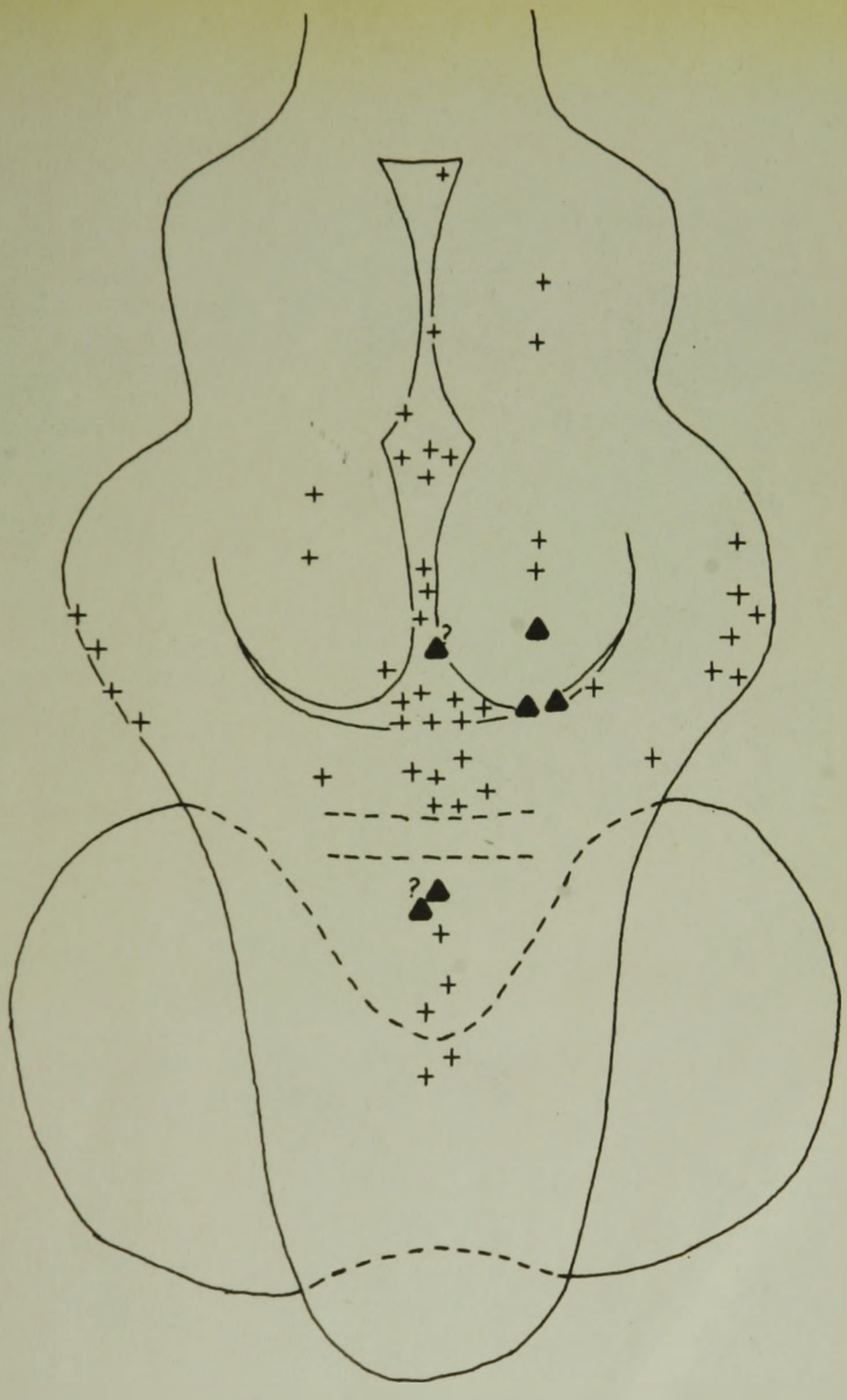


Fig. 16. The distribution of spike activity in the brain.

▲ Spikes grouped into volleys with a typical respiratory frequency.

\+ Continuous train of spikes.

Cautery in the area between the facial lobe and the cerebellar decussation, in two experiments, produced exaggerated abduction of the apparatus. In one experiment the basic rhythm remained unchanged, while in the other, the exaggerated abduction was accompanied by an abnormal periodic rhythm.

2. Recording of spike activity

Spike activity could be recorded from a large area of the brains of both bullhead and carp (fig. 16). The only reliable criterion for identifying electrical activity in the brain with respiratory function, is to determine whether the frequency of the spikes varies with the same temporal pattern as the phases of the breathing cycle. (Salmoiraghi and Burns, 1960).

Three non-respiratory sources of spike activity could be recognised. The first was an artefact. In many areas of the brain, when the needle was first introduced, spikes were recorded of increased frequency and amplitude. This discharge died away very rapidly, and was probably due to the mechanical stimulation given to the neurones by the penetration of the needle (fig. 17). A similarly large discharge was found in the vestibular areas which did not die away. This discharge was probably caused by sensory influx from the lateral line systems. The third non-respiratory source of spikes was the optic tectum. Here spikes were found whose frequency was dependent upon the illumination.

Spikes grouped in the respiratory rhythm were found at the anterior end of the medulla. The best example of this was recorded from a curarised carp. All muscular movement of the fish had ceased,

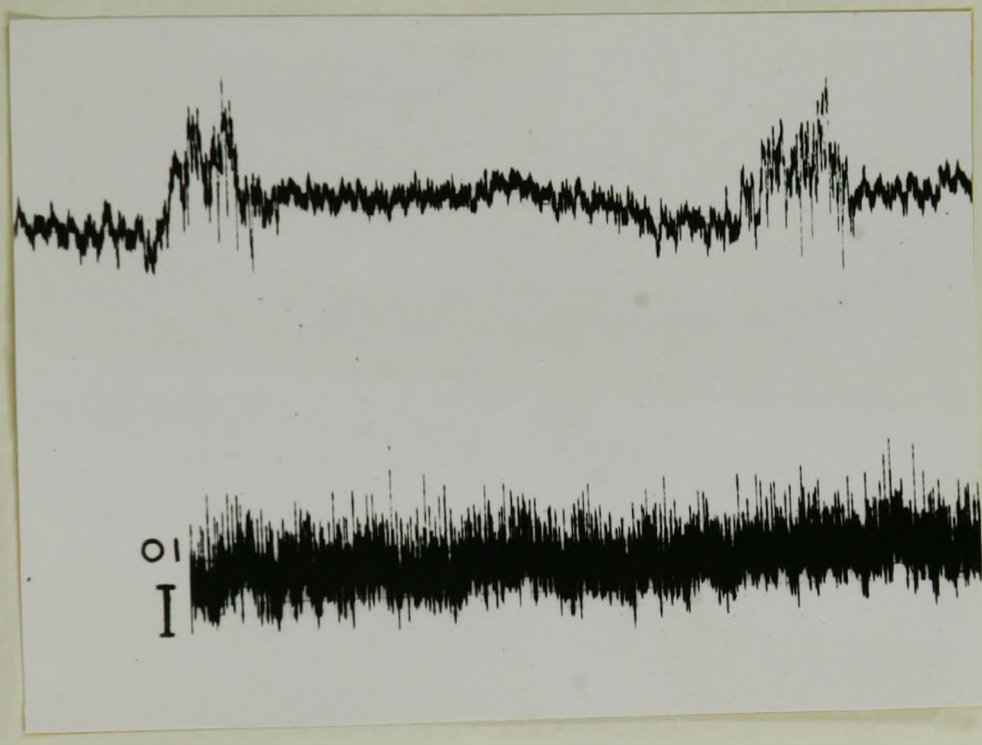


Fig. 17. Spike activity recorded from respiratory area of medulla. Upper line: injury discharge produced by penetration of recording electrode. Lower line: regular bursts of spikes correlated with respiratory rhythm. Same position of electrode tip and same amplification as in upper line.

following an intravenous injection of 1.5mg curare. The introduction of the electrode into the brain stem below the lobus impar, to the right of the midline, produced, at first, the usual rapid sequence of spikes due to the mechanical stimulation. This gradually died away, and was followed by a series of spikes in regular volleys, at a frequency of 50/min. This is a characteristic respiratory rhythm in this species (fig. 17).

Many spikes were also found in the floor of the IIIrd ventricle which outlasted the injury discharge. On rare occasions they gave some indication of being grouped into a respiratory rhythm.

Sensory areas, vagal and facial lobes, known to be connected physiologically with the respiratory system were, on the whole, silent. Only the ventral regions of the brain gave any respiratory discharge.

3. Brain stimulation

Ten bullheads and two carp were used in the brain stimulation experiments, and a total of 169 observations were made. The loci stimulated were mapped on squared paper by means of vernier readings on the electrode holder, and by the topography of the brain. A composite diagram of the results of all the experiments was also made (fig. 18). The loci were determined on this diagram as accurately as was possible with respect to the anatomy, allowing for variations in brain size. It must be remembered that the diameter of the stimulating electrodes was approximately one millimetre, and the points entered on the diagrams only indicate the centre of the circular area stimulated. These points may not, therefore, represent the exact positions of the neurones stimulated.

In these diagrams, all the points have been projected onto one

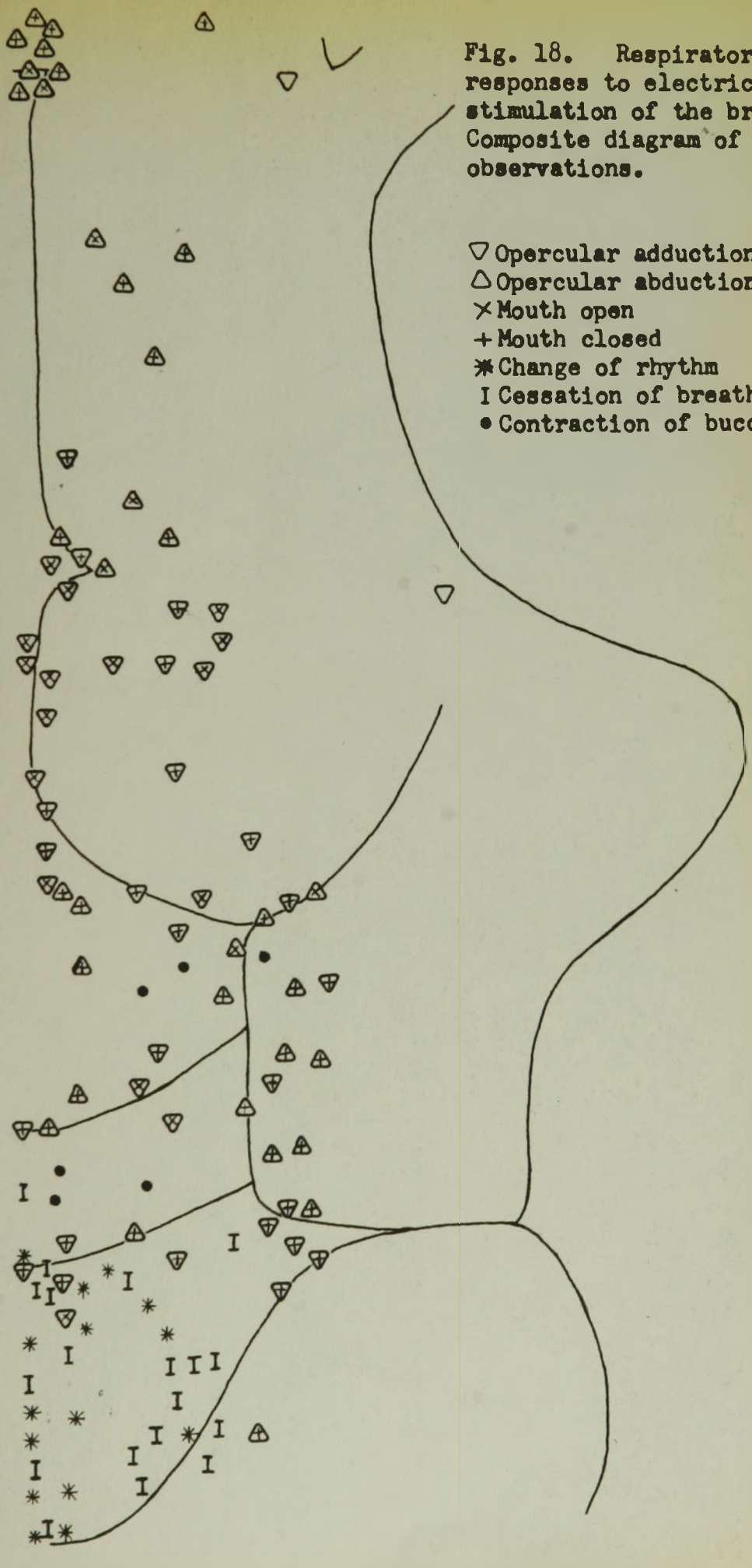


Fig. 18. Respiratory responses to electrical stimulation of the brain. Composite diagram of all observations.

▽ Opercular adduction
△ Opercular abduction
× Mouth open
+ Mouth closed
✳ Change of rhythm
I Cessation of breathing
• Contraction of buccal floor

plane for the sake of clarity. In actual fact, the responses obtained depended upon the depth of the stimulating electrode within the brain tissue. Only the predominating respiratory response is indicated on the diagrams: the details of the different responses will be given, where important, in the text. Exact localisations of these depths could only have been made by preparing cross sections of the brain at the site of electrode penetration. It was felt that such exactitude was not warrented by the preliminary survey being undertaken here.

Since no previous work on brain stimulation with respect to respiration in the fish has been reported in the literature, the most satisfactory parameters for the electrical stimulus were determined empirically. The stimulus used was 40v at 60/sec. throughout the experiments.

In all cases, the spinal cord was severed and the fish was allowed to recover from the anaesthetic before proceding with any experimentation.

There were four types of response to electrical stimulation.

a) i. When the fish was breathing normally, electrical stimulation produced no change in the rhythm whatsoever. (24 observations)

ii. When the fish was not breathing, stimulation produced no visible effect. (24 observations)

b) Inhibition of all breathing movements accompanied by a maintained contraction of some, or all, of the respiratory muscles. (79 observations)

c) Inhibition of all breathing movements leaving every part of the apparatus relaxed. (21 observations)

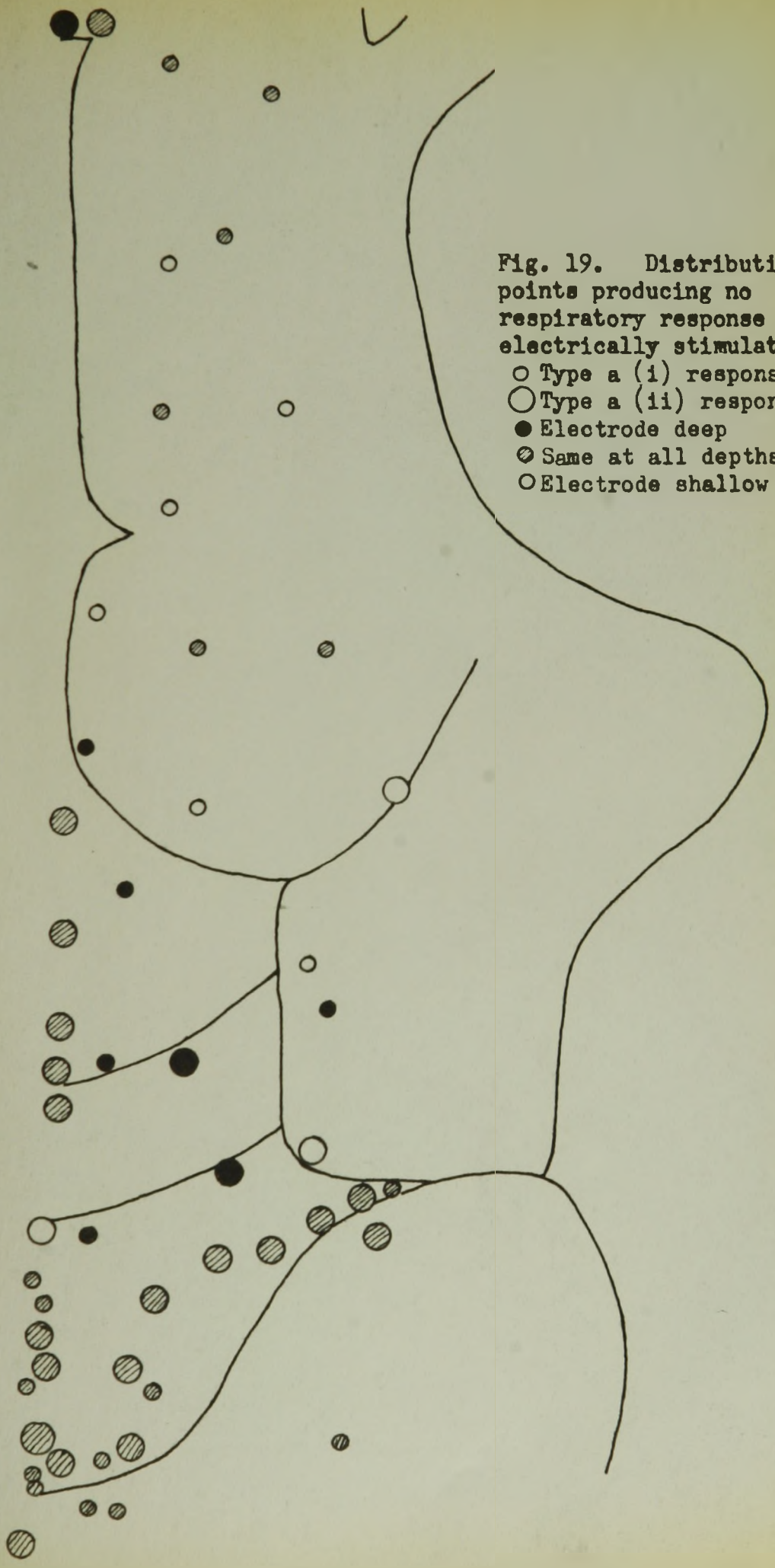


Fig. 19. Distribution of points producing no respiratory response when electrically stimulated.
○ Type a (i) response
◯ Type a (ii) response
● Electrode deep
⊘ Same at all depths
○ Electrode shallow

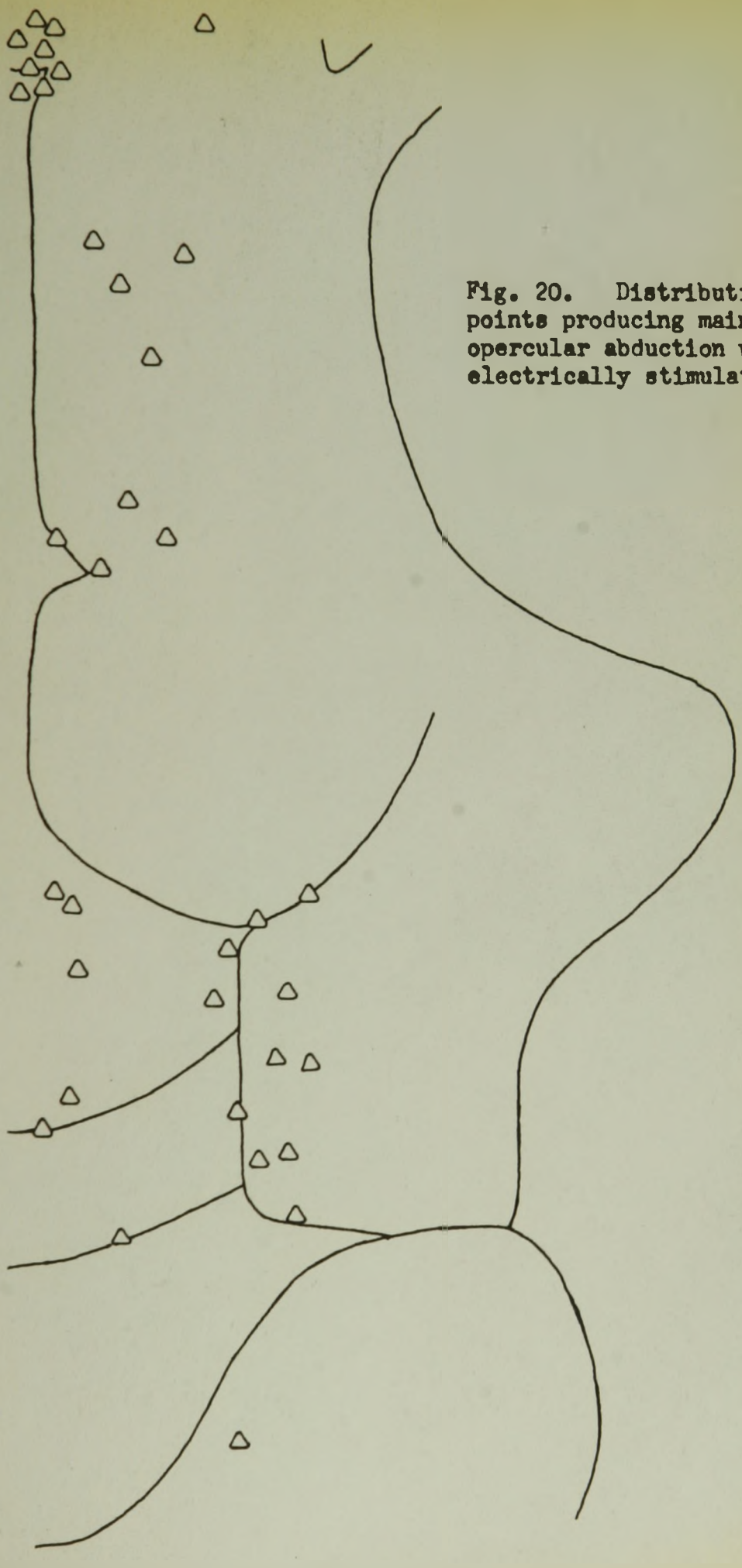


Fig. 20. Distribution of points producing maintained opercular abduction when electrically stimulated.

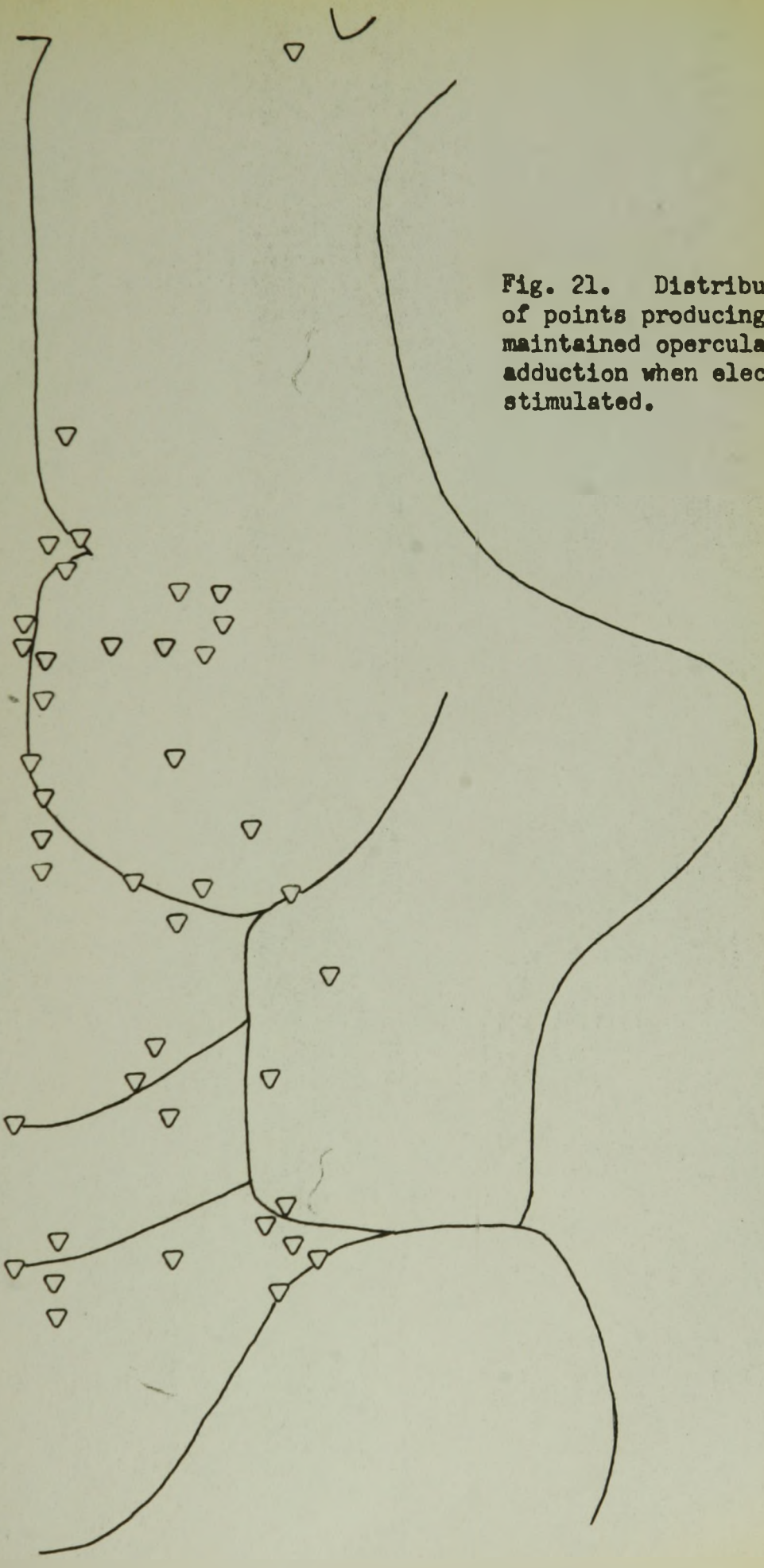


Fig. 21. Distribution of points producing maintained opercular adduction when electrically stimulated.

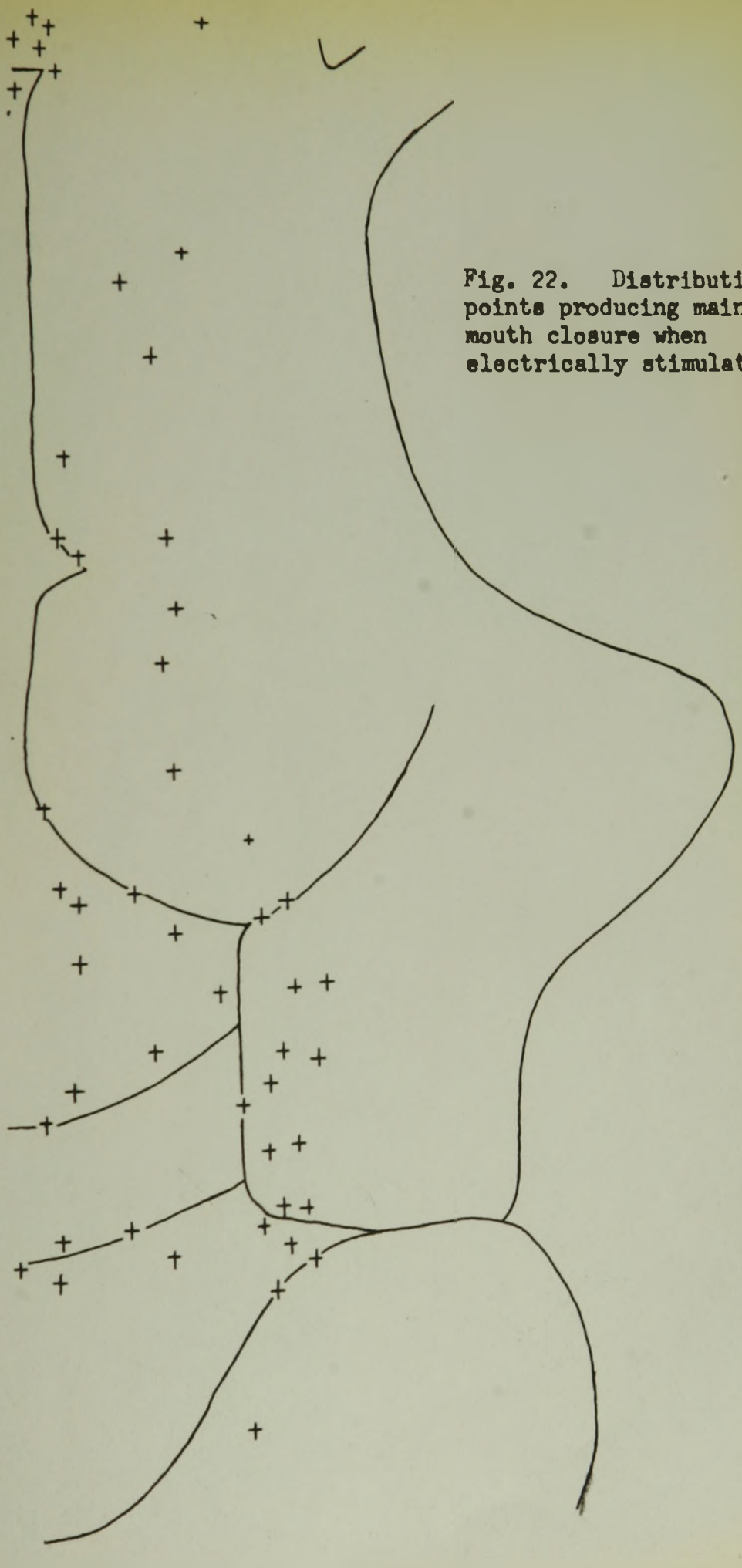


Fig. 22. Distribution of points producing maintained mouth closure when electrically stimulated.

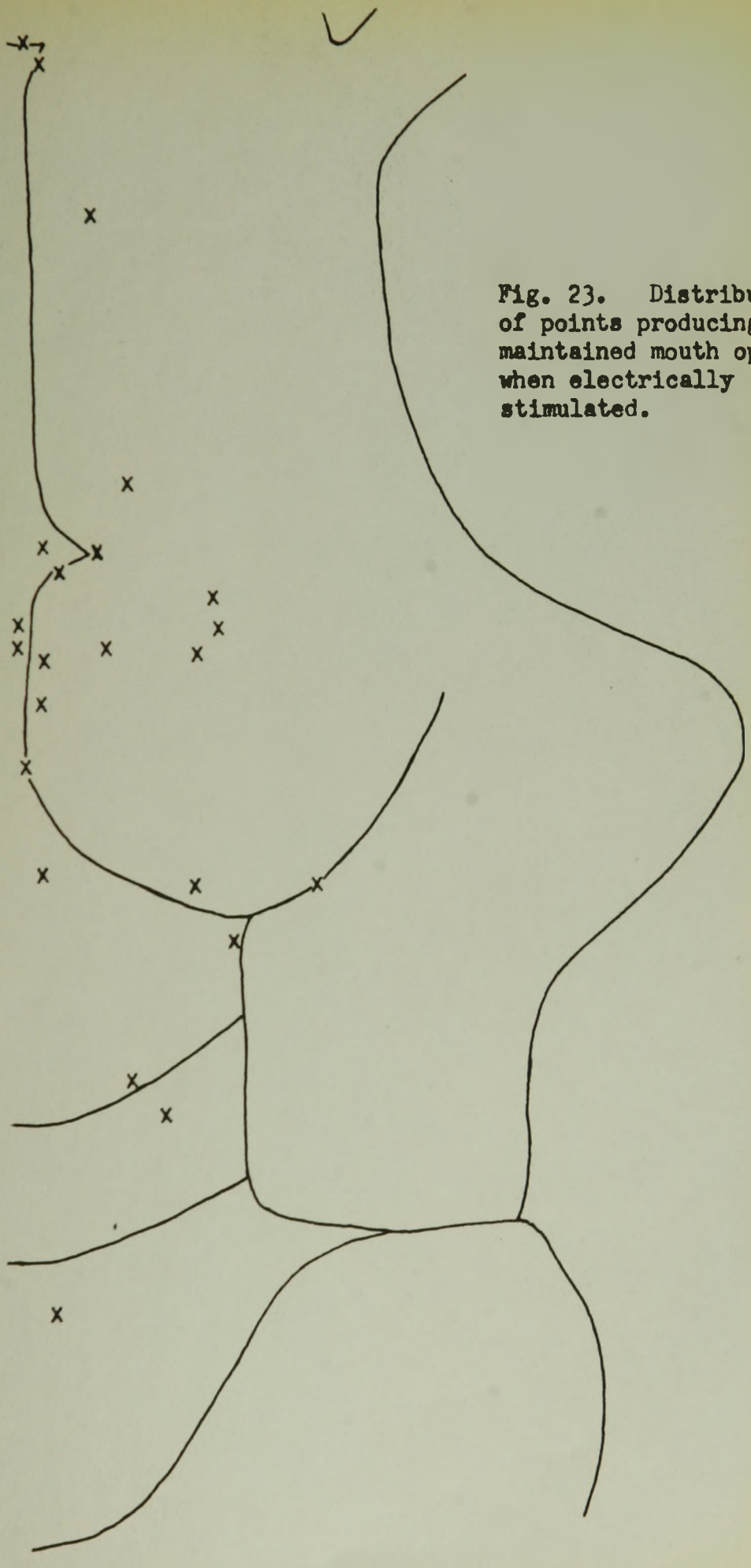


Fig. 23. Distribution of points producing maintained mouth opening when electrically stimulated.

d) Alteration of the respiratory rhythm either during or after the stimulus. (19 observations)

Type a) responses

Where there was no response of a respiratory nature, it may be inferred that the area is not directly connected with the respiratory system. These areas were most commonly found in the sensory parts of the medulla and in the optic lobes. However, as fig. 19 shows, the loci of ineffective stimulations show no clear anatomical grouping.

Type b) responses

These were the most common responses (figs. 20-23). Rhythmical movements cease altogether, but some muscle, or group of muscles, connected with the respiratory apparatus is held in a position of maximal contraction. This indicates that the tip of the stimulating electrode is situated either within a motor nucleus, or on a direct pathway leading to a motor nucleus.

Of the 79 points which gave type b) responses, 44 were stimulated at several depths. In the remaining 35, the electrode tip was only inserted into the superficial layers of the floors of the ventricles. In Table VII below, the depths of the loci stimulated have been grouped into two categories 'shallow' and 'deep'.

It can be seen from the diagrams and from Table VII that loci connected with a particular group of muscles, for example those opening the operculum, are scattered over a wide area. No grouping of these loci can be seen either in the horizontal or vertical planes.

As can be seen from the composite diagram, it is customary for both mouth and opercular muscles to be affected by the stimulation at

TABLE VII

RESPIRATORY RESPONSES OF TYPE b) PRODUCED BY STIMULATION OF THE BRAIN AT VARYING LEVELS

I Variation in response resulting from a change in depth of electrode tip

i) Type b) responses seen at all depths. Activation of different muscle groups determined by depth

No. of positions	Shallow	Deep
4	opercula abducted	opercula adducted
6	opercula adducted	opercula abducted
3	mouth closed	mouth open

ii) Type b) responses observed only at certain depths. No effect on respiration at other depths

No. of positions	Shallow	Deep
10	No effect	Type b) response
12	Type b) response	No effect

II No variation in response observed as depth of electrode tip altered

No. of positions	Shallow	Deep
7	Identical muscle responses	

one position. It is interesting to note that these responses do not always follow the combinations of muscle activity found during the normal breathing cycle. During most of the normal inspiratory phase, the mouth is open and the cheeks and opercula are abducted. During expiration the mouth closes and the cheeks and opercula adduct. However, as a result of electrical stimulation of the brain, opercular adduction may be accompanied by either an open or a closed mouth. It is therefore not possible to talk of an 'inspiratory response' or an 'expiratory response'.

Frequency of correlation of mouth and opercular movements in all observations

Normal combinations

i. Opercular adduction and mouth closure

30%

ii. Opercular abduction and mouth opening

8%

Abnormal combinations

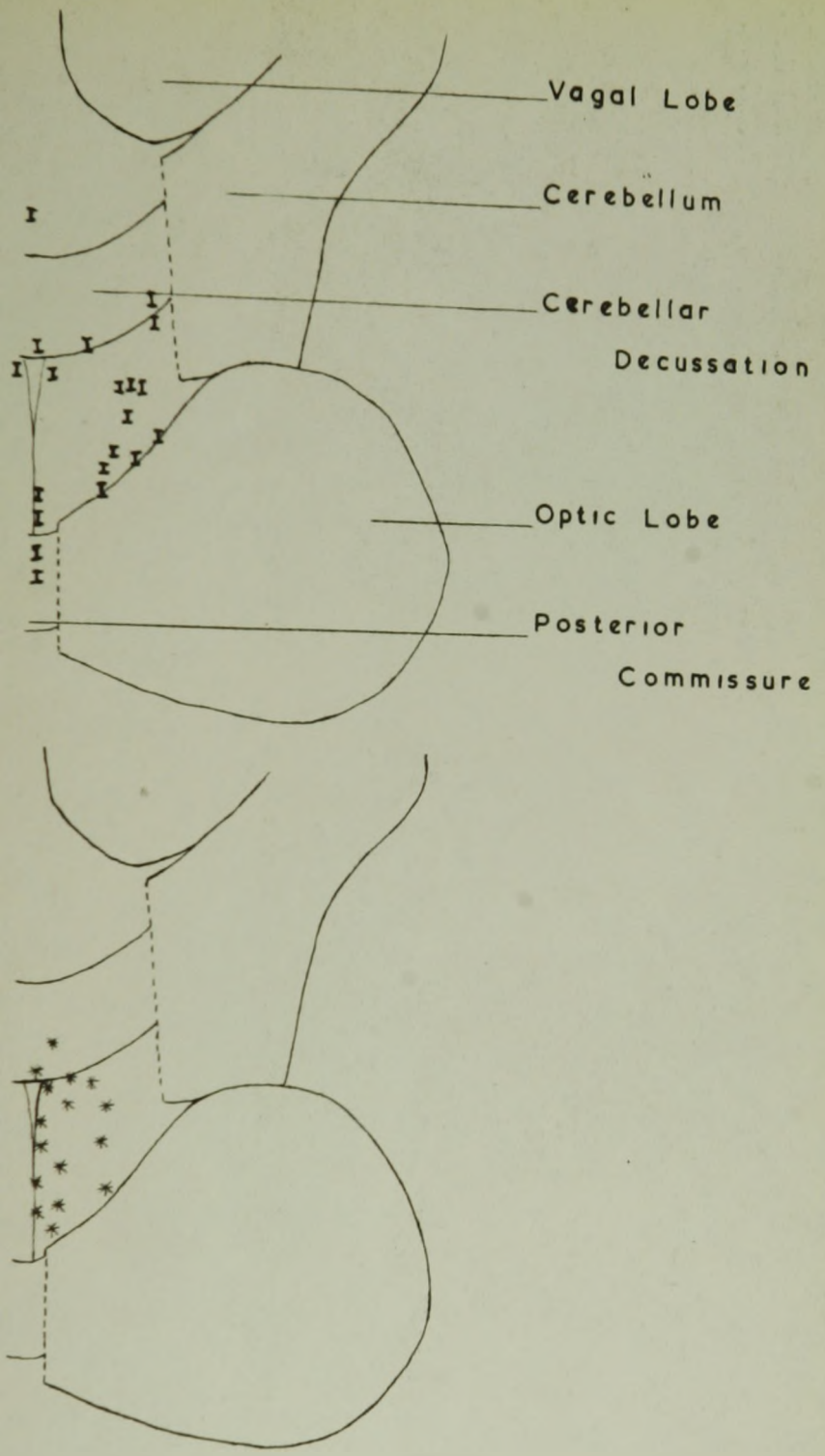
i. Opercular adduction and mouth opening

23%

ii. Opercular abduction and mouth closure

39%

A further complication arises from the fact that the responses of the two opercula may be different. For example, it was often found that the ipsilateral operculum would abduct and the contralateral one would adduct in response to stimulating a particular locus. The ipsilateral operculum showed a varying response with depth in these instances, but the contralateral one rarely altered.



Upper: Fig. 24. Distribution of points producing type c) responses when electrically stimulated.

Lower: Fig. 25. Distribution of points producing type d) responses when electrically stimulated.

Type c) responses

In contrast with the type b) responses, where cessation of breathing was always accompanied by some muscle contraction, those of type c) always involved complete relaxation of all parts of the respiratory apparatus. These loci are most noticeably grouped into a limited area of the brain. This area is bounded anteriorly by the posterior commissure and posteriorly by the cerebellar commissure, and lies fairly close to the midline (fig. 24). It extends from the surface of the floor of the ventricle to a deep level. At six points the needle penetrated the whole depth of the stem, and produced the same responses at all levels.

During the period of stimulation, the respiratory movements cease altogether and all parts of the apparatus adopt a relaxed position, but, when the electrical stimulation is turned off, normal movements begin again with unaltered frequency, even though the electrode is still in situ.

Type d) responses

This same area of the brain gives completely different responses if the fish is exhibiting an abnormal rhythm at the time of stimulation. Points giving type d) responses are shown in fig. 25. The abnormal rhythms are dealt with in a later section, and range from an irregular series of breaths separated by long pauses, to various types of periodic rhythms, which usually involve patterns of amplitude variation. Whenever the breathing rhythm was irregular at the time of stimulation, the rhythm became perfectly regular with respect to frequency and amplitude for the duration of the stimulus. At the end of the electrical

stimulation, the breathing returned to its former irregular rhythm. Occasionally, the change to a temporarily regular rhythm did not take place until immediately after the stimulation period.

As will have been noticed from fig. 19, if the fish was not breathing at the time of stimulation, electrical stimulation of this midbrain area just described produced no response at all. Those areas which gave type b) response would also elicit muscular contractions if electrically stimulated soon after the cessation of normal breathing.

Whether the inhibition produced by electrical stimulation was of type b) or c), it was sometimes possible for some part of the respiratory apparatus to escape from the inhibitory influence. The frequency of the movements of such a part was not always normal, but the activity had definite respiratory characteristics. This escape could come about spontaneously with absolutely constant stimulation, but it was also noticed when the electrical stimulus remained constant and the depth of the electrode tip was varied. The extent of this escape from inhibition varied a great deal. Sometimes one muscle alone would begin contracting rhythmically, for example the ipsilateral levator operculi, or all of them would escape, leaving only one group in a state of immobility, for example the adductor mandibulae. In some cases every part of the breathing apparatus escaped.

III THE OCCURRENCE OF ABNORMAL BREATHING RHYTHMS

It is a commonly observed fact that a fish will 'cough' if any foreign particles get entangled in the gill filaments. This is a cleaning reflex, involving sudden extreme dilation of all the respiratory

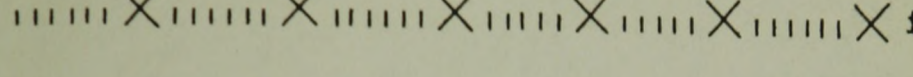
chambers, rapid filament movements and equally vigorous contraction of the apparatus. This produces a forceful current of water through the gills which flushes away the foreign matter. Bijtel (1947) has described this in more detail. It has also been noticed in the literature that some fish will cough frequently, even in perfectly clean water free of any particles which might irritate the gills. This is especially true in the case of the carp, and was observed many times in the course of these investigations.

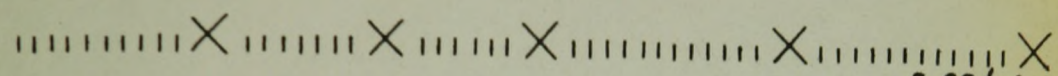
The conditions governing the appearance of a coughing rhythm, as with all other abnormal rhythms, are widespread and its occurrence is unpredictable. It is often associated with anaesthesia, whether the fish is in water or air. Between the coughs, respiration retains all its normal characteristics with respect to frequency and amplitude. The number of breaths between coughs varies, generally, between two and 25, but during any one period the number of breaths remains fairly constant. Particular rhythms have been observed to continue unchanged for periods as long as 50 minutes, or the frequency of the coughs may vary continuously. Normal breathing usually returns spontaneously.

Another rhythm which was frequently seen was one very similar to that described above, but all the normal eupnoic breaths in between the coughs were absent. During the pauses, the respiratory apparatus was immobile, then, towards the end of the pause, it would slowly begin to expand. This slow expansion culminated in a violent, maximal expansion of all the respiratory chambers, and the complete adduction of the filaments, characteristic of a cough. The length of the pause varied on an average from 5 - 100 secs. This rhythm was rarely seen in the normal,

Abnormal breathing rhythms

I Normal breathing with coughs at regular intervals

Carp 12/13  f 48/min

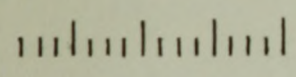
Bull. 32  f 22/min

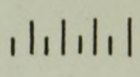
II Slow coughing rhythm. No eupnoic breaths

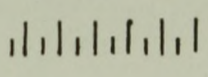
Carp 3/01 × 9 secs. × 9 × 9 ×

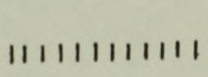
Carp 36 × 21 secs. × 17 × 6 ×
13 × 16 × 15 ×

III Pattern of fluctuating amplitude

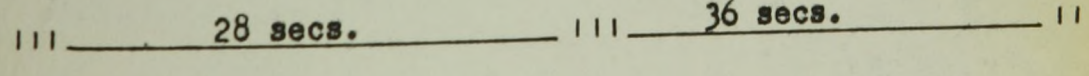
Carp 18/10 

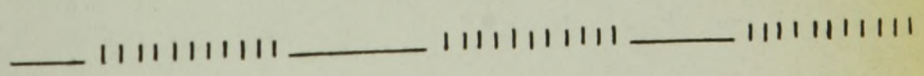
Carp 16/01 

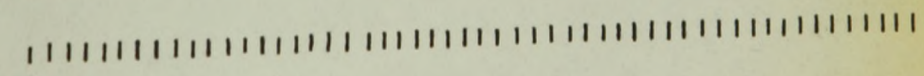
Carp 12/14 Filament movements 

Other parts 

IV Periodic breathing

Carp 19/01 
28 secs. 36 secs.

Rock Bass 28 R. Operc. 

L. Operc. 

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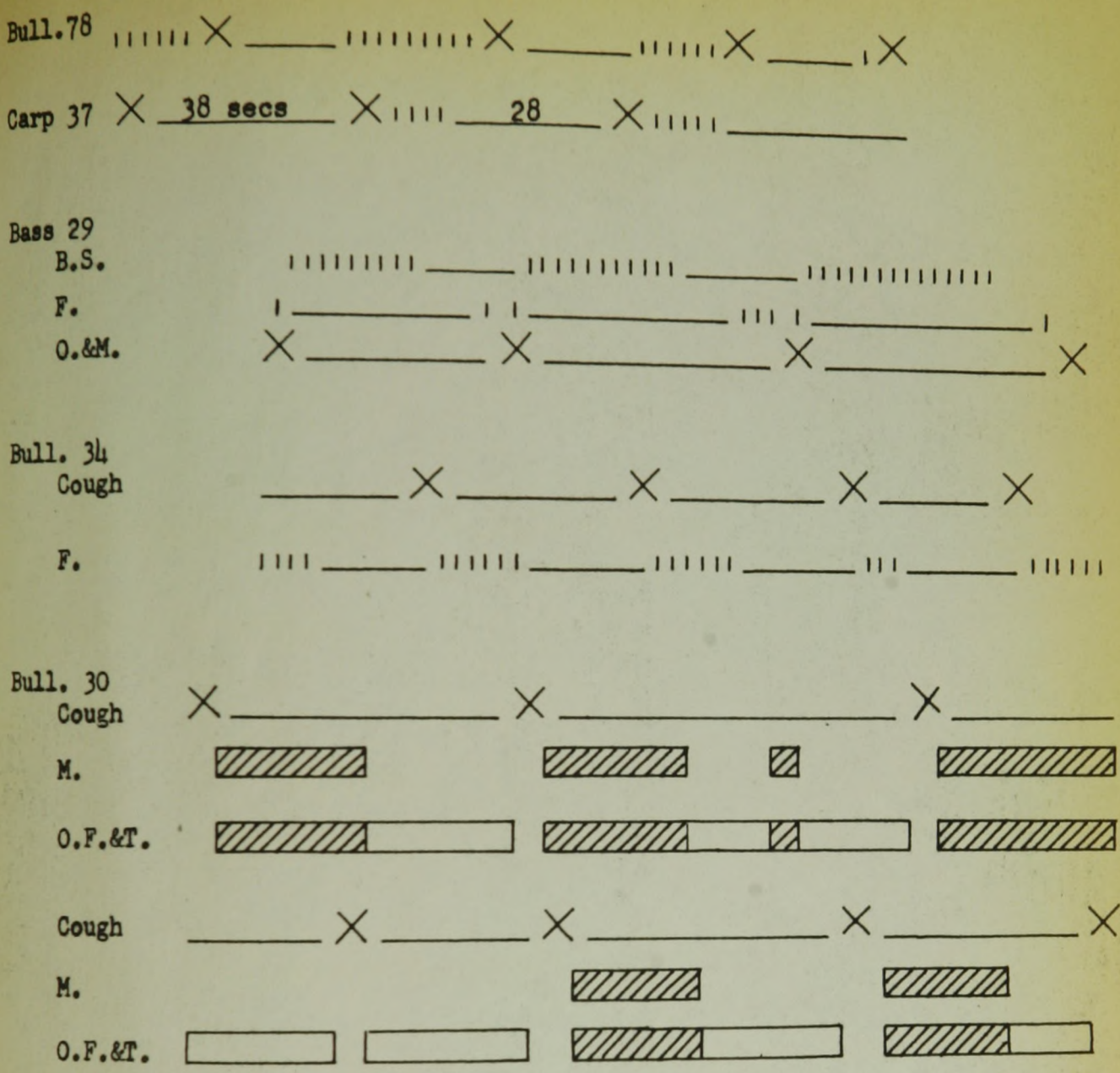


Fig. 26. Diagrams illustrating some of the abnormal breathing rhythms observed during the course of this investigation.

'conscious' fish in aerated water, but usually followed a period of apnoea, anaesthesia or water deprivation. This slow coughing rhythm was more commonly seen after damage had been inflicted upon the brain, especially upon the supra-medullary parts. On many occasions, the coughs did not involve all the parts of the apparatus. For example, the opercula would exhibit typical coughing movements while the mouth and filaments remained immobile. Sometimes, all parts would be involved for several minutes, then the activity would become limited to certain groups of muscles only. In most cases the rhythm was only temporary.

More complex rhythms involved fluctuating amplitudes and frequencies. When the fish was recovering from anaesthesia, in water or air, breathing was often seen to consist of a mixture of large and small breaths. The fluctuating amplitude was sometimes exhibited by all the moving parts, other times it was limited to one structure, for example the lower jaw or the filaments. Examples are given in fig. 26. Other rhythms observed showed a periodic grouping of the breaths, interspersed with periods of apnoea. The groups were often associated with coughs, or patterns of rhythmically fluctuating amplitudes. There were some extremely complicated patterns in this category (fig. 26). The coughs usually involved all the parts of the apparatus, but the breaths of the groups might be limited to a very few parts. On occasions, the operculum and mouth showed periodic rhythms, while the filaments and branchiostegal apparatus moved continuously in a normal breathing rhythm. During the groups of operculum and mouth movements, the frequency of the breathing changed considerably. These periodic rhythms sometimes

appeared spontaneously in the intact fish, but more often they were the result of brain damage. Again, the responses were so variable that they remain quite unpredictable.

D I S C U S S I O N

I THE FILAMENT MUSCULATURE

1. The origin of the spikes

There are four possible sources for the spike discharge found in the gill filaments: a) sensory nerve endings, b) motor nerve fibres passing to the filament muscles or through the gill arch to other muscles, c) motor end plates of the filament muscles and d) the filament muscle fibres themselves.

The curare and novocaine experiments eliminate the first possibility. If the spikes had originated from a sensory ending, they would have been depressed by novocaine and unaffected by curare. This was not found to be the case. They were eliminated by sectioning the nerves supplying the arch. If the impulses had been travelling centripetally, cutting the nerves centrally from the recording site should not have caused the spikes to disappear. Finally, the irregularity of the frequency and amplitude, coupled with the long duration of a spike is uncharacteristic of sensory activity.

The second possibility is unlikely because here again the characteristics of the bursts are most unlike typical nerve action potentials. Neither would these be inhibited by curare.

Curare is known to affect the motor end plate specifically. Since these spikes were suppressed by the application of curare, they originate

either from the motor end plate, or from the muscle fibres. However, the shape of the spikes does not have the characteristics of end plate potentials. These slow-rising, non-propagating potentials may be recorded only by intracellular electrodes introduced below the end plate itself, and the localisation of the electrodes would be much more critical than was found to be necessary in our experiments.

All evidence points to the action potentials of the muscles themselves as being the source of the spikes. The long duration and irregularities of the spikes are probably due to summation of the potentials from adjacent muscle units. They would be suppressed by curare because the fibres would be isolated from all centrifugal stimulation by the blocked end plates. The gradual decrease in amplitude shown occasionally with small doses of the drug, can be explained by the varying thresholds of the end plates to the blocking action of curare.

The only reference we have been able to find in the literature to spike potentials located in the gills is the work of Konishi (1957). He also ascribed these spikes to muscle activity, but makes no reference to the details of the filament muscles. Many of his records show two bursts for each breath, one negative and one positive. These he correlates with inspiration and expiration respectively. We have never been able to repeat this observation.

From our oscillographic work we can draw several conclusions. It has been established that the spike activity is of muscular origin. In Type I fish the activity is localised in the region of the adductor muscles; in Type II fish there are two sources, the adductor and abductor muscles. In all fish there is a burst for every breathing cycle, therefore

we can assume that these muscles are active during every breath. This theory is supported by the visual observations, as is discussed in the next section.

2. The role of the filament musculature

Rhythmical contractions of the filament muscles have been observed under the following very diverse conditions: a) during normal breathing, b) during shallow breathing in the absence of any water flow, c) in air, d) when all other parts of the apparatus had been paralysed and e) after loss of central coordination when the filaments exhibited a different rhythm from the rest of the apparatus. It is obvious therefore, that these muscles play a more active role than that attributed to them by Bijtel's hypothesis (1949). The only criterion she used for adductor muscle activity was overt parting of the tips of filaments on adjacent gill arches. Since Hofdijk-Enklaar (unpublished, quoted in Bijtel, 1949) failed to observe any such parting during normal breathing, Bijtel denied the adductor muscles any significance except during coughing movements.

However, our experiments have established that, not only is parting of the filaments exhibited under varied circumstances, but that, even when no actual parting is visible, adductor muscles maintain their rhythmical contractions throughout normal breathing. When all the adductor muscles contract synchronously, the whole gill assembly folds together, but no parting of the tips takes place (fig. 10). This results in the maintenance of the continuity of the gill 'curtain'. The active nature of this folding of the 'curtain' was demonstrated when the operculum was clamped away from the gills. There was no opercular

pressure on the gills, but the 'curtain' still folded down rhythmically towards the body wall. If one arch was isolated from the others, the contribution of the adductor muscles could be observed: the two hemibranchs rhythmically approached and parted from one another, a function which could not be ascribed to any other muscle.

This folding of the gill curtain is necessitated by the decrease in available space within the branchial chamber as the operculum adducts. If the filaments were passively attached to the arches, one could imagine a situation occurring analagous to that in a row of bowling pins set closely together. If the first one is pushed down, it will knock the next one over, which will in turn upset the third, fourth and fifth. In the case of the gills, the operculum would close down on the first hemibranch, which would be thrust against the underlying ones. The effect would be to occlude the first gill slits completely, so that no water at all would be able to pass over their lamellae. Only if the decrease in available volume is shared equally between all the arches could efficient ventilation be maintained at all phases of the respiratory cycle.

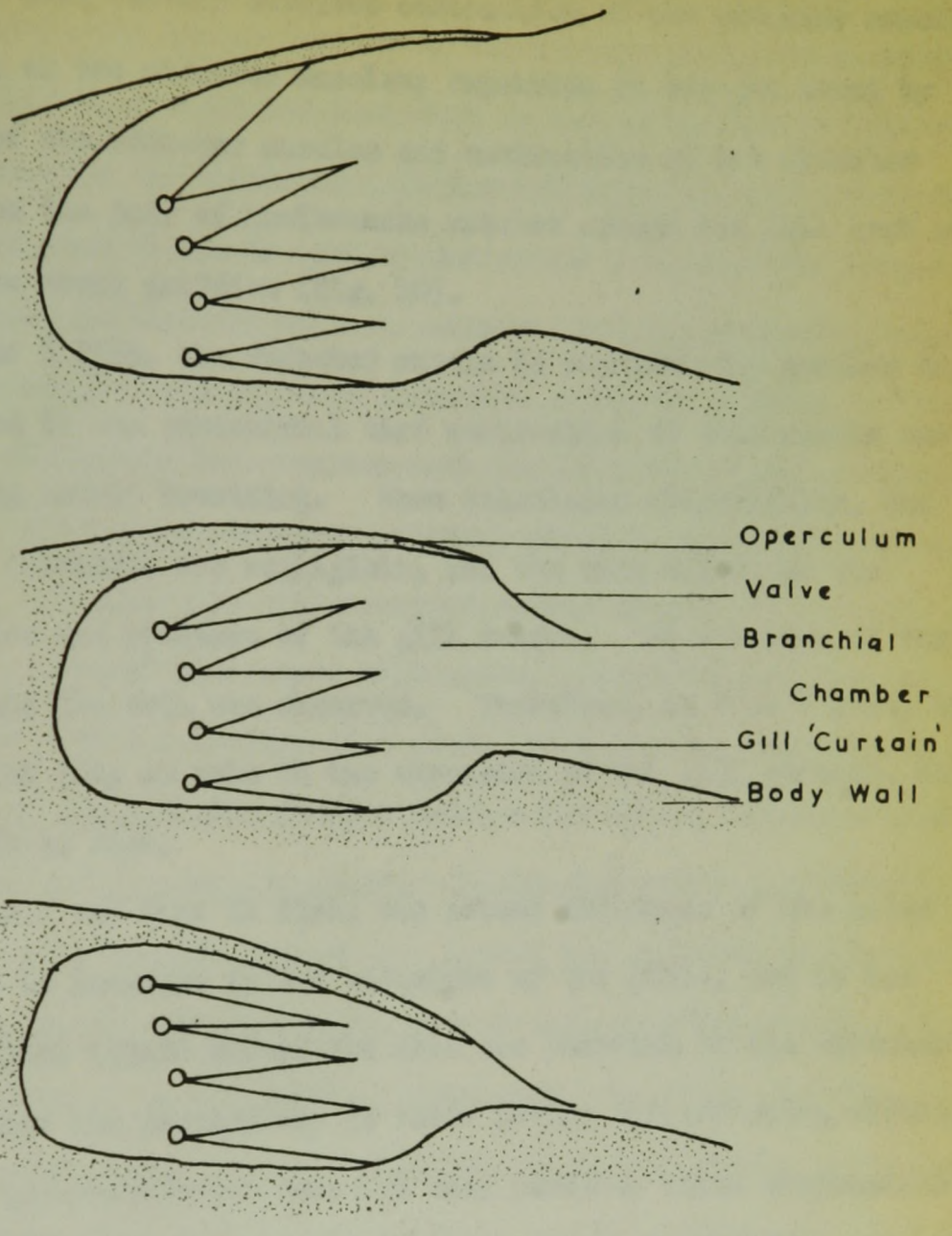
It seems most likely that this coordinated folding of the gill curtain is under direct control of the nervous system, and is effected by means of the adductor muscles. This would ensure that every arch contracted at the same time, and to the same extent, and the even flow of water would be undisturbed.

If adductor muscles are cut away or curarised, the filaments are seen to wave in an uncoordinated manner in the respiratory current. When an operculum is removed, the pressure differential across the gill curtain is grossly exaggerated. Under these conditions, the filaments without

muscles gape widely at the tips for long periods, and most of the respiratory current leaves through these openings. It seems very probable that, with the operculum in place, filaments in these injured areas would be unable to maintain their normal positions.

In Type II fish, not only were the adductor muscles observed contracting rhythmically during normal breathing, but also the abductor muscles were particularly active. They invariably contracted during opercular abduction. Electrical stimulation of the muscles themselves showed that they were not, as their name implies, the direct antagonists of the adductor muscles, because their contraction did not bring about an expansion of the 'V' between the two hemibranchs of an arch. In Type II fish, the two hemibranchs are joined for two thirds of their height by a 'diaphragm' (fig. 3). The adductor muscles are situated near the peripheral edge of this 'diaphragm', while the abductor muscles are inserted at the base of the hemibranch, connecting the oral filaments to the gill arch. This arrangement precludes any direct antagonism between the two sets of muscles. Electrical stimulation showed that the abductor muscles pulled upon the two united rows of filaments as a single unit, causing the filaments to pivot around the gill arch (fig.10).

This rotation, we feel, is an integral component of the expansion of the gill curtain during the opercular abduction, in Type II fish. As the available space in the branchial chamber increases, a coordinated mechanism is again necessary to prevent a gap occurring between the inner surface of the operculum and the tips of the oral hemibranch, or between the filaments and the body wall. Such gaps would disturb the even flow of the respiratory water across the gills. In Type II fish,



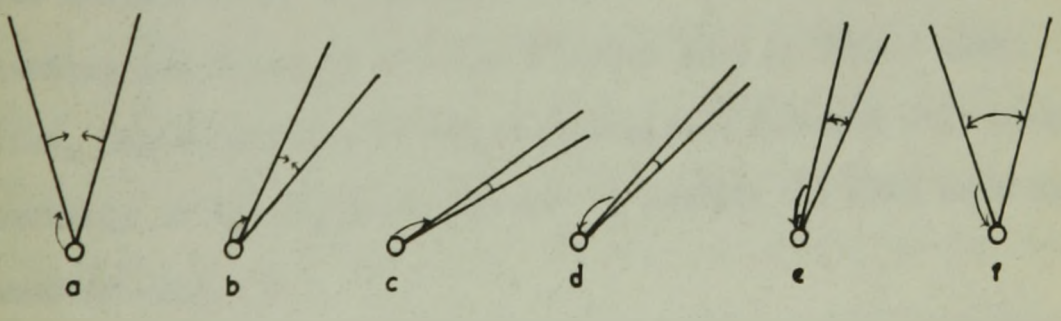


Fig. 10. A - C. Diagrams illustrating the coordinated contraction of the gill 'curtain' as the volume of the branchial chamber decreases at opercular adduction. a - f. Diagrams showing the cycle performed by one pair of filaments, showing the contractions of the adductor and abductor muscles.

folding of the gill curtain involves contraction of the adductor muscles and relaxation of the abductor muscles; expansion is brought about by a relaxation of the adductor muscles and contraction of the abductor muscles. Thus the pair of hemibranchs rotates around the gill arch and takes up a more erect position (fig. 10).

In Type I fish, the abductor muscle is considerably smaller than in Type II, and it was exceptional that contraction of this muscle was observed during normal breathing. When stimulated electrically, the pull upon the filaments was negligible, and the main effect of the abductor muscles was erection of the gill rakers. No rotation of the filaments around the arch was observed. Therefore, in Type I fish, the abductor muscles play no role in the expansion of the gill curtain, as they do in Type II fish.

In both Type I and Type II fish, the actual abduction of the pairs of hemibranchs is inherent in the structure of the gills, and is not muscular. Bijtel (1949) considered that the position of the abductor muscles precluded the possibility of their having any abductory effect. We agree with her observation that the only possible force responsible for abducting the hemibranchs, lies in the band of elastic fibres connecting the bases of the two filament rays in Type I fish. In Type II fish, the elasticity of the cartilaginous filament rays would resist the activity of the adductor muscles in bending the free ends towards one another (fig. 3).

The mechanisms for the control of the volume of water entering the branchial apparatus has been adequately dealt with in the literature (Van Dam, 1936; Henschel, 1939; Hughes, 1958, 1960).

By varying the amplitude of movements of the mouth parts and walls of the branchial chamber, the forces exerted by the suction and pressure pumps can be altered. No attempt has been made so far to explain the distribution of water over the lamellae. It has hitherto been assumed that, as the filament tips remain in contact during all phases of the breathing cycle, the whole respiratory current would be diverted laterally, passing through the spaces between adjacent filaments of one hemibranch. Thus, all the water would come into contact with the lamellae. Any parting of the tips would allow water to escape without passing over the lamellae, and would tend to reduce the efficiency of oxygen uptake.

Our experiments have shown that active parting of the filaments, due to adductor muscle activity, does occur under certain conditions. It is proposed that the adductor muscles exert a regulatory effect on the distribution of the respiratory water. If the hydrostatic pressure exerted on the gill curtain becomes too great, the delicate lamellae might become damaged, so the adductor muscles contract slightly, allowing a certain proportion of the respiratory current to escape through the parted tips. The slight loss in efficiency of oxygen uptake is sustained in order to protect the lamellae.

There is some evidence in support of the hypothesis in the literature. Hughes and Shelton (1958) measured the rate of flow of water across the gills, in relation to the mean water pressure in the respiratory cavities, and they found that the resistance to water flow exerted by the gills varied with the mean pressure. Saunders (private communication) observed that the percentage utilisation of oxygen in the respiratory water decreased as the minute volume increased. The increase

in minute volume occurred during hyperpnoea, caused by excess carbon dioxide in the medium, and during exercise. During forward swimming, the oxygen utilisation dropped from 50-60% to 30-50%.

Both the variable resistance observed by Hughes and Shelton and the drop in oxygen uptake seen by Saunders can be satisfactorily explained by our hypothesis. The adductor muscles provide a mechanism for the 'fine adjustment' of gill ventilation. If our assumption is correct, that one of the functions of the adductor muscles is a regulatory one, it must be expected that the contractions of these muscles will not be coordinated with the respiratory movements, but will be able to occur at any phase of the breathing cycle. This is particularly so since Henschel (1939) observed that the flow of water across the gills is continuous. The excessive water pressure on the gills might be produced by the inspiratory suction pump phase of breathing, or by the expiratory pressure pump phase. This expectation is borne out by our observations.

The problem of neural control of these muscles remains unsolved. A reflex mechanism seems the most likely. Our own histological work, and that of previous workers (Reiss, 1881; Droscher, 1882; Faussek, 1902), does not demonstrate any sense organs suitable for such a reflex. Satchell (1959) in his work on the skate, did find an increased centripetal discharge in the vagus when the pharynx was inflated with a rubber balloon. It is possible that such a system is operative in teleosts, in connection with this regulatory function of the adductor muscles. However, contractions of the adductor muscles occur during all phases of the breathing cycle, even when the fish is in air, when most normal stimuli would be absent. Some central mechanism, not necessarily dependent

upon water flow, must also control the activity of the filaments.

In many cases of recovery from deep anaesthesia and apnoea, the filament activity appeared exaggerated, and the parting of the tips too great to serve either of the functions proposed above. Sometimes, complex patterns of variation in the extent of parting were seen, which had no bearing on the activity of the rest of the respiratory apparatus. This behaviour during abnormal conditions indicates that the contractions of the filament muscles are not solely under reflex control, but that there is a central control system, which is particularly susceptible to interference by anaesthesia.

II THE NEUROGENESIS OF RESPIRATION

When discussing the respiratory mechanisms of the brain of teleosts, one factor must always be borne in mind: the final effector neurones activating the respiratory muscles lie within the rhombencephalon. They lie in extenuated nuclei within the medulla and in the brain stem below the cerebellum, primitively in one continuous special visceral column. The nuclei of V and VII have migrated away from this column and are found as discrete, subdivided nuclei in the metencephalon (Ariens Kappers, 1936). Any experimentation, therefore, performed on the respiratory centres, is likely to affect the adjacent motorneurones directly. It is extremely difficult to ascertain whether responses are derived from the respiratory mechanism or from these motorneurones. This factor is especially important when considering the source of the spike potentials recorded in the brain, and the responses to electrical stimulation. This situation is in direct contrast to that of higher

vertebrates, possessing respiratory apparatus innervated by spinal segmental nerves. Here the motorneurones are completely separated from the respiratory neurones.

In teleosts, it has always been assumed that the respiratory centre lies at the anterior end of the medulla and is completely autonomous with respect to the rest of the central nervous system (Woldring and Dirken, 1951; Hukuhara and Okada, 1956). It was thought that this area was capable of initiating and maintaining the respiratory rhythm, unaided by any extrinsic source of nerve activity from higher centres. The results of the present investigation do not support this hypothesis. Every aspect of our experimental approach to the central nervous system indicates that the ventral areas of the brain stem in the supra-medullary regions are of vital importance in the regulation of respiration.

However, the importance of a respiratory area within the medulla is not denied, and the first part of the discussion will be concerned with the medulla and its role in the production of the respiratory rhythm. The existence of supra-medullary modulators will then be considered in the second part.

1. The role played by the medulla in initiation and maintenance of respiration

When the medulla is isolated from all higher centres of the brain by transsection at the base of the cerebellum, most of the motor nuclei of V and VII are separated from the medulla. The exception may be that part of VII which has not migrated from its primitive position on the anterior end of the special visceral motor column (Ariens Kappers, 1936). Therefore, if the respiratory rhythm originates within the

medulla, after such a transsection, all those muscles innervated by fibres originating from nuclei anterior to the transsection should lose their rhythmicity. This was borne out by the results. In experiment 27 (Table III) rhythmical movements of the mouth were seen after transsection at level F; probably some fibres of VII had remained untouched by the section.

After all sections at level F, only groups of filaments were active; many muscles whose nerves were intact showed no contractions. This indicates that when the medulla is isolated from the rest of the brain, it is still capable of producing rhythmical movements, but the operation depresses the activity of the neurone system, and only a portion of the population is emitting respiratory stimuli.

Salmoiraghi and Burns (1960) working with the medulla of the cat, found that, after similar transsections, the proportion of neurones which could be found firing in the respiratory pattern decreased from 60% to 10%. This drop in the number of active respiratory neurones was correlated with a general depression of activity in the whole medulla. They proposed that the respiratory neurones are self-exciting, but rely on a high background level of general activity in adjacent neurones. A similar hypothesis might be put forward to explain our results, in which only a very few muscles seemed to be receiving any respiratory stimuli.

This independent activity of the medulla was retained not only when the medulla was isolated from higher centres, but also when it was separated from all centripetal inflow entering via IX and X. This was done either by sectioning the nerves themselves, or by transsecting

the brain at level E. Powers and Clark (1943) working on the trout, maintain that bilateral section of IX and X leads to a permanent cessation of respiratory movements. These results are not substantiated either in the present investigation, or in the work of previous authors (Hukuhara and Okada, 1956; Satchell, 1959; Shelton, 1959).

The nature of the centripetal inflow of these nerves was investigated in elasmobranchs by Satchell (1959). He found that bursts of action potentials could be elicited in afferent fibres of IX and X by distention of the pharynx. Such a reflex resembles the Hering-Breuer stretch reflex found in mammals. The significance of this mammalian reflex has caused much controversy in the literature. Some have postulated that the vagal afferent discharge is the driving force in producing rhythmical outflow from the respiratory centre (Pitts, Magoun & Ranson, 1939). More recently, the significance of the vagus as a 'pattern-initiator' has been relegated to a secondary role, of importance only under abnormal conditions (Breckenridge & Hoff, 1950; Kerr and Dunlop, 1954). Its importance under normal conditions is a tonic one, providing a facilitatory background for the respiratory neurones. The locus of its action has not been agreed upon. Wang, Ngai and Frumin (1957) say it could act directly on the medulla, but most agree that it has its effect indirectly through supra-medullary mechanisms. (Kerr,Dunlop,Best, & Mullner, 1954, and others). Salmoiraghi and Burns found that the respiratory spike activity of the cat medulla was not affected by sectioning the vagi.

It appears that the nerves IX and X have a function in the fish similar to that of the vagi in mammals. In order to understand the

significance of the vagal inflow in fish we must look at its central pathways. In teleosts the visceral sensory areas have become greatly enlarged forming the facial and vagal lobes. They have direct connection with the motor areas supplying the respiratory muscles. Dorsal root fibres enter the lobes and synapse with secondary neurones lying superficially. These send some axons directly to the motor nuclei which lie at the junction between the lobe and the rest of the medulla (Ariens Kappers, 1936). This is a much more direct connection than that between vagal receiving areas of the mammalian brain and the motor-neurones of the spinal cord. It is rather surprising therefore, that vagal afferent inflow does not have more direct effect upon the respiratory rhythm in fish. However, this great development of the visceral sensory area is mostly correlated with the importance of the gustatory sense. Teleosts, and especially siluomds, have numerous taste buds scattered all over the body, and the lobes are enlarged to accommodate their inflow. Gustatory stimuli are of more significance in feeding reflexes than in respiratory ones; the tactile and tension receptors would have greater importance. These non-gustatory stimuli enter descending secondary pathways, which run parallel with gustatory pathways to the caudal end of the IVth ventricle. Here most of the descending fibres enter the inferior gustatory nucleus (or lateral funicular nucleus as it is sometimes called) but some of them, carrying non-gustatory impulses, enter the nucleus commissurae infirmae, where all the correlation takes place between stimuli from the various sources. These descending fibres make up the post-vagal part of the vagal fasciculus solitarius. From this nucleus, fibres are sent directly to

motor nuclei or enter ascending tracts to the midbrain (Ariens Kappers, 1936).

In our experiments, the region of the nucleus commissurae infirmae has been seen to have some respiratory significance. Electrical stimulation of the midline at the end of the IVth ventricle, produced maintained contraction of muscles responsible for opening the operculum, and both opening and closing the mouth. Stimulation of the brain tissue with bipolar electrodes leads to very little spread of the electric current, and the motor nuclei of V and VII lie well outside any such area of spread, so we can only assume that the motorneurones were being stimulated by impulses originating from the nucleus commissurae infirmae. These experiments therefore give some indication of the central pathways followed by the vagal impulses, but there is at present no evidence to show their significance.

It is of interest to note that Ondina, Yamoto and Masland (1960) working with the cat, found a group of expiratory responses to electrical stimulation having loci situated in the nucleus solitarius and nucleus commissurae infirmae which, as in fish, receive descending fibres carrying vagal afferents. As the gustatory sense decreased in importance and lung breathing evolved, this part of the system, small in fish, hypertrophied to carry the Hering-Breuer inflow.

The sections made caudad to C did not affect breathing, neither did cautery of the region of the nucleus commissurae infirmae; therefore its activity is not essential for respiratory rhythmicity. Hyde reached similar conclusions in her work with elasmobranchs (1904).

Many parts of the medulla and midbrain respond to electrical

stimuli by producing muscular contractions in one or more muscles of the respiratory apparatus. When all these responses are analysed, a difference is immediately noticed between the behaviour of the fish and mammalian brains. In the literature on mammalian respiration, references are made to inspiratory and expiratory responses. Stimulation at one point induced all the parts of the apparatus to adopt an expiratory position, while stimulation at another point produced an overall inspiratory position (Baxter and Olzewski, 1955; Ngai and Wang, 1957). In fish, the responses to electrical stimulation cannot always be classified as inspiratory or expiratory. In 38% of our observations the whole apparatus did adopt either a typical inspiratory or expiratory position, but in 62% of the cases, the response was a mixed one. For example, the operculum might be in an inspiratory position while the mouth might be in an expiratory one. This may be related to the fact that during the normal breathing cycle, the sequence of events is very complex. (See the Introduction, part one.) In the mammalian cycle all parts expand, thus drawing air into the apparatus, and then all parts relax, forcing the air out again. In fish ventilation there is a double pumping system; the mouth and buccal cavity pump, and the operculum and branchial cavity pump. The opening and closing cycle of the operculum does not exactly coincide with the opening and closing cycle of the mouth. Mouth movements precede the opercular movements (Hughes and Shelton, 1957). Thus, at certain phases of the breathing cycle, one part of the apparatus may be undergoing the end of its inspiratory phase, while another part may be beginning its expiratory phase. This may explain the appearance

of the mixed responses to electrical stimulation.

It is, therefore, impossible to describe an 'inspiratory centre' or an 'expiratory centre' in the fish brain. In mammalian work, most agree that there are no discrete morphological centres in the respiratory system as Pitts, Magoun and Ransom (1939) and other earlier investigators had described, but that loci producing inspiratory or expiratory responses tend to be grouped into limited areas (Haber, Kohn et al, 1957; Ondina, Yamamoto and Masland, 1960). As was mentioned in the results, no grouping of the loci can be made at all, even with respect to the behaviour of one group of muscles. For example, loci which, when stimulated, give opercular adduction are scattered all over the medulla and midbrain.

During all the experiments on the brain, a most noticeable feature was the independent behaviour of the different muscles of the apparatus. For example, the opercula might inexplicably cease moving while activity continued unchanged in all the other parts. No permanent motor paralysis was involved, because, after a while, they would display normal activity once more. On other occasions, the respiratory rhythm was maintained by one group of muscles only, for example the lower jaw, and all the other parts stayed motionless. Again, no permanent paralysis was involved because all the motionless parts became active when the fish coughed. Another example of this independent behaviour occurred sometimes during electrical stimulation of the brain. When one muscle group was induced to contract, all the other parts of the apparatus ceased moving. Sometimes, although the stimulus remained constant, certain muscles could escape from this inhibition and regain

rhythmical activity.

In the literature on mammalian respiration, little mention is made of independent activity on the part of the separate components of the breathing apparatus. Two references only were found. Harris and Borison (1954) noticed that during pentobarbital anaesthesia, the diaphragm showed different responses from the abdominal apparatus when the brain was electrically stimulated. Hoff and Breckenridge (1954) mention that, occasionally, when most of the apparatus is exhibiting apneustic breathing, the facial musculature continues rhythmical movements of a normal frequency. No explanation is given for this phenomenon.

Turning to a group lower in the evolutionary scale than the teleosts, we see that Hyde (1904) found an even greater degree of independence in the elasmobranchs. By appropriate sections in the medulla, she was able to observe independent behaviour of the separate branchial pouches. Several different respiratory rhythms were manifested by the isolated parts.

Our experimental observations may best be explained by the following hypothesis. The respiratory rhythm is produced by a diffuse assemblage of neurones situated in the reticulatory system of the medulla. There is no major inspiratory or expiratory component of the population, but there are a number of 'networks' of neurones, each connected with a group of muscles. These networks are widely diffused, intermingled and interconnected. For example, neurones connected with the muscles which open the operculum form a network, which is 'sympatric' with other networks connected with the mouth, arches and other parts. Each network becomes active at a certain phase of the breathing cycle, and, as

at no phase are all the muscles relaxed, a portion of the respiratory neurones is active at all times. The network system can remain active in a completely isolated medulla and much of the neural population can be inactivated by damage to the medullary tissue without halting the activity of the remainder.

There is no indication at present as to the relationship between the reticulatory neurones and the motorneurones innervating the respiratory muscles. As mentioned at the beginning of this section, the two kinds of neurones lie close together and our experiments cannot readily distinguish between them.

Several theories have been proposed to explain how the neurones of the medullary respiratory system produce the rhythmical series of impulses. No work of this nature has so far been published regarding teleosts, and all the theories given are based on experiments on mammals. Many believe that the inspiratory neurones inherently emit a continual stream of impulses, which are periodically inhibited either by the expiratory neurones or by extrinsic sources, such as the vagal inflow or higher centres in the pons (Lumsden, 1924; Pitts et all, 1939; Wyss, 1954). Other hypotheses propose that the respiratory neurones themselves are capable of periodic activity due to inherent characteristics of the individual cells or to self re-exciting circuits, not unlike those of feedback mechanisms, within both the inspiratory and expiratory networks (Brodie and Borison, 1957; Burns and Salmoiraghi, 1960). Others have looked for pacemaker neurones which will dictate the rhythm to all the other neurones (Hoff and Breckenridge, 1949; Brodie and Borison, 1957).

In our experiments we could find no medullary locus which, when stimulated electrically or extirpated, produced any change in the frequency of the respiratory rhythm. The pacemaker theory therefore seems unlikely. We have also ascertained that the medulla can produce rhythmical respiratory movements in isolation from the rest of the brain and from the vagus. Hukuhara and Okada (1956) recorded spike potentials, grouped in bursts with a respiratory frequency, from the completely isolated medulla of a carp. Therefore, extrinsic sources of rhythmicity are not essential. It is difficult to ascertain whether the networks of neurones are inherently rhythmical or whether they rely upon reciprocal inhibition between the networks to produce the respiratory rhythm. Our results indicate that the networks do influence one another to a large extent. For example in one experiment (Bullhead 30, fig. 26) the mouth exhibited periods of inactivity lasting approximately a minute and a half. During these periods the other parts of the apparatus had a frequency of 50-60/min. In the intervening periods, when the mouth was active, all parts showed a frequency of 72-84/min. In this particular experiment, it therefore appears that the activity of the mouth network has enhanced the activity of the whole system. However, in addition, each network seems to have an inherent rhythmicity. During several experiments, the filaments were seen to be the only active parts; the adductor muscles were contracting rhythmically with a rapid rhythm of 80-100/min. As was seen in a previous section, the adductor muscles alone produce the movements of the hemibranchs in Type I fish. This indicates that only one network of neurones, that connected with the adductor muscles, was active and it was producing rhythmical outbursts

without inhibition from any other network.

We can, therefore, at this stage draw three conclusions: the respiratory rhythm is inherent in the network system, which does not rely on pacemakers; the different networks influence one another; the networks have a high degree of autonomy.

2. Supra-medullary modulators

Having discussed the behaviour of the medulla, consideration may now be given to the supra-medullary areas. The region found to be of respiratory significance was that part of the brain stem ventral to the cerebellum and the optic lobes. Our positions of reference were the anterior margin of the facial lobe, the cerebellar decussation (also known as the decussatio veli) and the posterior commissure. These approximate to levels F, I and L respectively in fig. . Our experiments showed that region F - I has different characteristics from I - L. The former region is metencephalic, and corresponds to the tegmental part of the pons of higher animals. The latter region contains the whole of the mesencephalic tegmentum and the isthmus area. Both regions contain extensions of the motor column and in particular the motor and sensory nuclei of V and VII. Many correlation centres and reticulatory nuclei are situated within these boundaries. Of special interest is the superior secondary gustatory nucleus, which receives tracts from the inferior gustatory nucleus, and possibly also from the nucleus commissurae infirmae. It lies in the isthmus region slightly anterior to the motor nucleus of V, to which it sends direct fibres. It also has connection with the reticulatory system. It is of importance in respiratory reflexes and serves to correlate gustatory and tactile information (Kapper, 1936).

When transsections were made at levels anterior to F, the fish showed many abnormalities in breathing and soon died. The cause of death cannot be ascribed to circulatory failure, because the bleeding caused by these transsections was no more extensive than at other levels. These abnormalities were not due either to inactivation of the motor nuclei in that area, because all parts of the apparatus were observed to be capable of moving. However, rhythmical breathing of normal characteristics was not seen unless the transsections were made at levels anterior to F (Table V). The medulla alone is capable of rhythmicity, as is the preparation consisting of the medulla plus the tegmentum up to level L, but when only the region F - J is in connection with the medulla, then all rhythmicity is inhibited.

Superficial cautery over this whole tegmental region had no effect, therefore significant neurones are situated deeper than one millimeter. When the anterior, mesencephalic part, I - L, was cauterised completely near the midline, there was no effect, but when the deep cautery was localised in the metencephalic area near the cerebellar decussation, the breathing became very abnormal. The type of breathing seen reminded one of the apneustic breathing seen in mammals, in that the inspiratory component, or abduction, was exaggerated. In mammals, this type of breathing is observed if the rostral part of the pons is removed leaving only the caudal part in connection with the medulla. An apneustic centre has been postulated for this region of the pons (Lumsden, 1924; Pitts, Magoun and Ransom, 1939; Wang, Ngai and Frumin, 1957). According to these authors, if the apneustic centre is allowed to act unchecked, by isolating it from the higher centres, it causes the inspiratory neurones

in the medulla to become overactive, thus causing the apneustic breathing. This higher control originates in another part of the pons located more rostrally. It is called the pneumotaxic centre. They say that the apneustic centre exerts a constant stimulating effect which must be inhibited during the expiratory phase of each cycle by a double feedback system involving the vagal stretch reflex and the pneumotaxic centre. Others believe that the medulla has inherent rhythmicity, and that the apneustic and pneumotaxic centres are parts of the general facilitatory and suppressor systems of the reticulatory network (Breckenridge and Hoff, 1950; Hoff and Breckenridge, 1952; Brodie and Borison, 1957). This is based on the observation that apneustic breathing is often correlated with general decerebrate rigidity. Normal breathing is only exhibited when the medulla receives a balanced output from these two systems. If there is any imbalance between the modulating influences impinging on the medullary centre, then the eupnoic rhythm is lost and breathing becomes apneustic or periodic (Hoff and Breckenridge, 1954).

Returning to the results of the transsection experiments on teleosts, it is seen that there is much evidence to suggest the presence of similar modulating influences in fish also. Removal of the anterior part of the tegmentum, I - L, seemed to create some imbalance leading to abnormal rhythms, and the behaviour after deep cautery of the posterior part, F - I, also suggested some imbalance. The kinds of abnormal rhythms seen in fish show great resemblance to those described in mammalian work.

The stimulation experiments also provide strong evidence for the hypothesis concerning supra-medullary modulating influences in fish.

Our transsection and cauterisation experiments have suggested that the region F - I is important as a modulator of respiratory activity. However, electrical stimulation of the area produced intense muscular contraction of some part of the respiratory apparatus accompanied by inhibition of all other activity. This suggests that motor nuclei or tracts were being stimulated directly. Our histological studies showed that the motorneurone column extends into this region. The importance of this area as a modulator cannot therefore be ascertained by this method.

Electrical stimulation of region I - L on the other hand, did not produce any muscular contraction, only a change in the breathing pattern. This response may be considered analagous to the response of the cerebellum to electrical stimulation. It has been established that the cerebellum is one of the most important areas supervising coordinated movements of the striated muscles. Yet, when it is stimulated electrically there is no direct muscular response observable. This is because the cerebellum influences the motor column indirectly through the red nucleus and other components of the reticulatory system. If, according to our hypothesis, there is a group of neurones in the tegmentum affecting the branchial muscles indirectly through the respiratory part of the reticulatory complex, stimulation of this group should produce no direct muscular response, only a change in pattern. This was borne out by our results.

The nature of the change induced in the breathing pattern depended upon the type of breathing being exhibited by the fish before stimulation. If the breathing was abnormal, stimulation of that part

of the tegmentum near the posterior commissure frequently transformed it into a regular one. According to the hypothesis of Hoff and Breckenridge (1954), this abnormal rhythm could have been due to an imbalance between the suppressor and facilitatory systems. It is possible that stimulation of this particular region in some way equilibrated the modulating influences, and allowed the normal rhythm to reappear for the duration of the stimulus. When the fish was breathing normally, electrical stimulation in this same region caused total inhibition. Further experimentation is needed to ascertain whether the same neurones are responsible for both types of response. The latter effect might be explained by assuming that the neurones stimulated were part of the suppressor system, and that their abnormally strong activity completely blocked all medullary activity.

It is of interest to note here that some authors (Baxter and Olszewski, 1955; Ngai and Wang, 1957) working with the cat, stimulated the pontine region and observed what they termed 'expiratory apnoea'; that is, a cessation of breathing in the expiratory position. Ngai and Wang (1957) mention this phenomenon specifically, and suggest that in the cat it might be a "mechanism for the inhibition of the rhythmical activity of the respiratory centre." In mammals, expiration consists largely of a relaxation of the respiratory apparatus, so it is possible that the results of this pontine stimulation in the cat are homologous with the responses we obtained from stimulating the region I - L.

On the basis of our experiments, we propose that the inherent medullary rhythm is modulated by two extrinsic, supra-medullary sources. One of these originates in the region between the facial lobe and the

cerebellar decussation, and the other lies more anteriorly, between the decussation and the posterior commissure. If those neurones lying in the posterior of the two regions are allowed to act unchecked upon the medulla, then an abnormal rhythm is produced. If both regions are intact, breathing is normal. Excess stimulation of the neurones lying in the anterior modulating region alters the respiratory rhythm radically. The neurones may have a suppressory function.

References have continually been made to mammalian work, partly because no such detailed work has hitherto been done on fish, but mostly because the author was struck by the resemblance between the mammalian and teleost systems of respiratory control. No such supra-medullary control areas have previously been suspected in fish. Their role in mammals is still not agreed upon. Some believe (Kerr, Dunlop, Best and Mullner, 1954; Wang, Ngai and Frumin, 1957) that the supra-medullary system is a recently evolved system which is superseding the primitive medullary system as the prime co-ordinator of rhythm. They suggest that the medullary system is vestigial in mammals and that it is only used under abnormal conditions. Our work shows that the supra-medullary system is a very ancient one. It may have changed as lung breathing evolved from gill breathing, but the basic origins of the mechanism were already present at the gill breathing stage. It would be of great interest to investigate some of the intermediate groups, and find out what the neural changes were that accompanied the structural changes in the breathing apparatus.

SUMMARY AND CONCLUSIONS

1. This thesis deals with the gill filament musculature and the neural mechanisms responsible for the respiratory rhythm.

2. Spike potentials having all the characteristics of muscle action potentials have been recorded from the gill filaments. The spikes are arranged in volleys; one volley occurring in every breathing cycle.

3. These electrical phenomena, coupled with visual observations, prove that the adductor muscles are active during normal breathing, contrary to the widely accepted theories in the literature.

4. In those fish where the two hemibranchs of one arch are bound together at the base (Type II fish in Bijtel's classification, 1943) the abductor muscles also are active during normal breathing. In those fish with unbound hemibranchs, (Type I fish)the abductor muscles are minute and rarely contract.

5. It is proposed that the adductor muscles play a two-fold role. The first is a positional one. The adductor muscles assist in the orderly folding of the gill 'curtain' as the volume of the branchial chamber decreases at opercular adduction, thereby ensuring efficient ventilation of all the lamellae. In Type II fish, the abductor muscles assist in the expansion of the gill 'curtain' at opercular abduction. In all fish, the natural elasticity of the connection between the filaments and the arch is important in the expansion of the 'curtain'.

The second function of the adductor muscles is a regulatory one. In times of increased gill ventilation, the adductor muscles perform extra contractions, causing a slight parting between tips of filaments of adjacent arches. Some of the excess water escapes through the tips thus relieving the pressure on the delicate lamellae which might otherwise be damaged. The adductor muscles act as the 'fine adjustment' in the regulation of water flow over the lamellae.

6. The medulla is able to maintain the respiratory rhythm after isolation from the remainder of the central nervous system.

7. It seems that the respiratory neurones are diffusely arranged in networks in the reticulatory system of the medulla, and that the muscles of the respiratory apparatus are associated each with their own network of respiratory neurones.

8. The different networks have a high degree of autonomy yet, at the same time, do influence one another.

9. There is no evidence for the existence of pacemaker neurones within the medulla.

10. In an area situated near the midline of the ventral brain stem, between the posterior commissure and the cerebellar decussation, is a group of neurones having an important modulating effect on the medullary respiratory system. Electrical stimulation of the 'modulator neurones', when the fish is breathing normally, induces a cessation of all movement, and the respiratory apparatus adopts a relaxed position. If the fish is breathing abnormally, electrical stimulation of the 'modulator neurones' transforms the rhythm to a normal one for the duration of the stimulus.

11. This modulator mechanism seems to bear resemblance to that found

in mammals, therefore the origin of the modulator mechanism in vertebrates is obviously much more ancient than had hitherto been suspected. The neural mechanisms controlling respiration probably originated at the gill breathing stage, and have not changed greatly during the transition to lung breathing.

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