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THE CHEMICAL SENSES IN FEEDING AND SOCIAL BEHAVIOR OF THE CATFISH, *ICTALURUS NATALIS*

by

Jelle Atema

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in
The University of Michigan
1969

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ABSTRACT

THE CHEMICAL SENSES IN FEEDING AND SOCIAL BEHAVIOR OF THE CATFISH, ICTALURUS NATALIS

by

Jelle Atema

Chairman: John E. Bardach

A. A first report is given on the behavior of experimentally "tasteless" fishes. Lesions in the brain selectively eliminated either one or the other of the two taste systems of the catfish. Changes in feeding behavior were recorded on motion pictures.

Facial lobe ablation caused serious impairment of two steps in the feeding behavior chain. 1) Localization of food was gravely affected and did not return to normal levels of performance even after several months, although gradual improvement could be observed. 2) The food-pick-up reflex was absent for several weeks, improved with time and finally returned to an almost normal level of performance. 3) Alert and ingest (the first and last step in the chain) were not affected.

It is suggested that 1) The remainder of sensory taste neurons after facial lobe ablation could still be sufficient to alert the fish to the presence of food; but that a full set of functional taste receptors is required to provide the directional clues in localization. Spatial projection of the body in the facial lobe is suggested. 2) Reinforcement of tactile input to the mandibular motor neurons in the presence of remaining taste may finally result in the reappearance of the pick-up reflex.

Vagal lobe ablation exclusively blocked the last step (ingest) of the feeding behavior chain. Tactile reinforcement and the presence of remaining taste may result in the reappearance of the reflex after several weeks.

B. Individual catfishes were subjected to a series of operations that eliminated gradually the olfactory structures and the forebrain. The effects of the ablations were measured in changes in behavior as expressed in frequencies of behavior units. Agonistic social behavior was emphasized. Macroscopical and histological microscopical inspections of the brains were performed.

In the first stage a series of 10 experimental animals were each observed for one hour in their normal social interactions with a group of 3 resident animals, after which their olfactory epithelium was destroyed, thus eliminating the olfactory receptor cells. In the second stage, one week later, the fishes were observed in their interactions with the same resident fishes, after which their olfactory tracts were severed, thus disconnecting the olfactory bulb from the telencephalon. In the third stage, one week later, the fishes were again observed. After this observation both telencephalic hemispheres were removed. In the fourth and last stage, one week later, the fishes were observed once more and killed for histological inspection of their brains. Some fishes were observed over longer periods of time. Two fishes were added to the series; these underwent 3 successive sham operations. Thereafter their forebrains were removed and their behavior observed in an additional fifth stage. Two of the experimental animals were kept for several months after forebrain removal. Some other forebrainless fishes were observed for up to 18 months.

Elimination of the olfactory receptor cells (cauterization of the olfactory epithelium) resulted in an "aggression peak" in social interactions following the operation. Additional severing of the olfactory tracts did not appreciably alter this "noseless" behavior, except that the latter operation had an effect on the general condition of the animals. Changes in general behavior after forebrain ablation are in accord with many reports in the literature. They center around lack of recognition ability, unpredictability, "laziness" and "emotional polarization."

The forebrain ablation effects are discussed in relation with comparable ablations in mammals and remarkable similarities are pointed out.

Normal behavior is restored after several months without regeneration of the forebrain.

C. The effects of chemosensory and forebrain eliminations were tested for their specificity by subjecting taste deficient animals to social interactions and smell deficient and forebrain ablated animals to feeding tests.

Blocking the olfactory input and even removal of the telencephalon had no effect upon feeding. Taste deficient animals, however, showed

minor changes in social behavior. These changes increased in effect when the olfactory input was blocked in addition to one gustatory input.

It was concluded that feeding is relatively simple reflex behavior, steered in the catfish by two distinct taste systems and by the tactile system. Social behavior, in contrast, requires the fully intact brain in the presence of all sensory inputs, even those that may not be used specifically to gain information about the social situation. The forebrain contains the integration centers and it balances social behavior.

DEDICATION

To the creatures of our world:
that they may benefit from
our efforts.

ACKNOWLEDGEMENTS

Of the many people involved in the shaping of thoughts and experiments that form the basis of this work, my friend and the chairman of my doctoral committee, John Bardach, must be mentioned first. His confidence and support have been of invaluable importance. His approach to the many facets of life goes beyond biological limits and will continue to serve as a source of inspiration.

I want to thank the members of my committee for their scientific criticisms and many valuable discussions that helped greatly in molding this work into its present form. Specifically I want to thank you, Doc Leonard, for the improvement of my cultural climate and you, Doctor Von Bee, for the many enjoyable pilot studies. I am grateful to you, Roger, for taking, on short notice, the place of Dr. James Olds. And I want to thank Dr. Olds for many stimulating discussions during the earlier phases of this work. He is to a large extent responsible for the similarities between my bullhead and his rats.

To you, John Todd, who taught me the arts of ethology as well as the blessings of contemporary English, and who served as a continuous source of anarchistic ideas, I am especially indebted for your loyal partnership. The unorthodox atmosphere in and out of the laboratory was further enhanced by your presence, William O. McLarney. Besides this and many other important non-specific effects I am grateful to you, Bill, for the specific and substantial improvements that you have made in the language of this work and its author's.

Dex, you will forever be remembered by me and the family for serving as grandpa and doughnut man. In the latter function your contribution to this work can be measured by the pound.

U, Professor Dijkgraaf, en jou, Ad Kalmijn, wil ik hier noemen om officieel te getuigen van mijn waardering en bewondering voor uw wetenschappelijke werk en denken, dat mij zo voortreffelijk heeft voorbereid op de taak die mij hier te wachten stond.

My work and life would have been far less enjoyable without the support, criticisms, help and presence of Dr. Ora Safriel, Carol Ferreira, Trudy Villars, Ilyse Rosenthal, Claudia Rouster and Mary MacDowell.

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CHAPTER I

INTRODUCTION

The Chemical Senses in Aquatic Life

Human sensory impressions are subjective. Our knowledge of anatomy and physiology and our common environmental influences and language permit, however, fairly satisfactory comparisons between individuals. Evaluation of the sensory impressions of terrestrial animals is made difficult by the anatomical and physiological differences between the animals and ourselves. In the aquatic environment this difficulty is compounded, for we are dealing not only with different anatomy and physiology but with an unknown milieu. In terrestrial life the visual, acoustical and to some extend the chemical stimulus fields with their sharp images, and airborne sounds and odors are familiar to us. The underwater world, then, poses many special problems. The same stimulus classes are still present, but the physical properties of water as a medium cause shifts in their biological importance.

Water is "thicker" than air. The visual field is limited from about 60 meters in the clearest tropical waters to practically zero in murky pools and in the sea below 200 meter depth there is no other light than bioluminescence. Often the images are blurred. Yet the acoustical properties of water permit a fast and clear conduction of sounds (far-field) and the near-field effects of vibrations have much more impact in water than in air. The "chemical landscape" is more stable because the currents are slower in this thick medium. Electrical fields, which are absent from our terrestrial stimulus milieu, are used to an as yet unknown extend for underwater communication and orientation.

It is therefore not surprising to find among aquatic organisms that receptors for acoustico-mechanical and chemical stimuli are generally well developed. Although many sharks have good eyes, they are attracted by smell and mechanical vibrations from long distances; vision is utilized only at close range (Eibl-Eibesfeldt and Hass, 1959). The pike (Esox), a

chemically insensitive and highly visual predator, still uses near-field vibrations to guide its strike direction towards the prey; and can rely entirely on these vibrations if surgically blinded (Wunder, 1927).

Chemical and mechanical receptors have probably had the longest evolution, because chemical and mechanical stimuli are likely the ones to which the first forms of life in the ocean were sensitive. In the earliest stages of life, the chemical senses may have been relatively undifferentiated. Later, as some Lower Devonian fossils indicate, an arthropod inhabited the seas showing structures very similar to some of modern Limulus, which latter was found very responsive to clam extracts and several pure amino acids (Barber, 1956). It is thus a permissible inference that a true chemoreceptor organ was in use some 300 million years ago.

It is possible that the chemical senses, because of their long evolution, developed and maintained a special psychological function among an organism's sensory equipment. For instance one of the chemical senses, smell, classically situated in the first body segment of an organism, will lead an animal to the source of odor, which can mean food for the survival of the individual and sex for the survival of the species.

Olfaction is still closely associated with areas of positive reinforcement in the brain. Taste acts as a negative reinforcer, a controlling agent over food intake; even when present as only a general chemical sensitivity, it warns against dangerous situations: Salt and acid perception register changes in salinity and pH, bitter usually indicates poisonous material and sweet is connected with "good," edible, nutritive substances. The four basic taste modalities are found in all vertebrates and invertebrates as far as they have been studied and seem to be universal.

In fact, they may have developed associative significance very early in phylogeny. Salinity and pH measurement would provide a means for staying in an optimal milieu; all dangerous material is lumped in the bitter class, and all nutritious substances in the sweet class. The association of bitter with danger and sweet with satisfaction has evolved in our emotional reactions to tastes. We can find this exemplified in all languages. Our subjective feelings about the four taste modalities may have ancient roots.

The concept of positive and negative reinforcers is particularly interesting to discuss when we deal with an animal whose life depends on

its chemical senses, the reason why catfish was chosen here as experimental animal. But before we turn to our catfish experiments, the place of the forebrain in relation to the olfactory system must be mentioned, as well as the taste-smell relationship.

Through the evolution of the forebrain and therefore through mechanisms primitively under olfactory dominance, higher vertebrates became less subject to the specific enforcement of stereotyped behavior (Herrick, 1924). Originally the forebrain was exclusively olfactory, but invasion into this area by various systems of non-olfactory fibers initiated tissue differentiation within the pallial field, resulting in more effective adjustment to the external milieu (Herrick, 1933). This development accompanied the vertebrate invasion of the terrestrial habitat with its abrupt milieu changes that call for such an adjustment.

The specific behavioral role of olfaction is all but clear; Herrick's careful wording and Pribram and Kruger's definition (1954) of three "rhinencephalic" systems in mammals indicate only an indirect connection of the olfactory bulb with the brain areas most likely involved in emotional and social behavior. However, a relation of olfaction and behavioral plasticity was recently suggested for fish and higher vertebrates (Atema, et al., 1969).

Other sensory systems, taste among them, have in the course of evolution sent their projection fibers into telencephalic and diencephalic areas. The primary taste centers of fishes are located in the dorsal wall of the medulla oblongata between the root of the nervus vagus and the cerebellum from where they project through tertiary fibers into the hypothalamus, where relation to the olfactory system is suggested (Ariëns Kappers, et al., 1936). The hypothalamus, today's most extensively investigated and most complicated chordate brain area, contains "feeding centers" in the rat (Morgane, 1961). It is generally closely associated with the telencephalon on the one hand and the hormonal system, via the hypophysis, on the other. (Fiedler, 1967).

The role of smell and taste, their complementary function and interaction, their differences and the possibility of overlapping function have been areas of curious interest in sensory physiology. They have

important implications for our understanding of aquatic life. Yet it is not too long ago that one thought that fishes had no sense of smell!

Earlier Studies on the Biological Function of the Chemical Senses of the Catfish

Experiments on the chemical senses of the catfishes have been conducted for several years by Bardach and co-workers, resulting in a large body of data dealing with peripheral taste perception of the yellow bullhead, Ictalurus natalis. It was the study of their behavior, however, that became of paramount importance; more specifically the role of the senses in their behavior. A chance observation and our surprise discovery that in this species olfaction is not required for feeding, but may be required for individual recognition (Todd, et al., 1967) inspired this study.

Evidence for chemical communication

Numerous observations and ethological analyses of behavior in groups of blind and normal bullheads suggested that the chemical characteristics of the fishes rather than, for instance, visual characteristics were crucial to the elicited behavioral responses. When water was introduced from other fish tanks it could elicit clear agonistic or escape responses which were dependent on the relative social status of the fishes. It could easily be seen from the observed response times that visual and mechanical factors played a subordinate role.

It was most clearly demonstrated when we had a chance to observe over a long period of time the responses of two small bullheads that had involuntarily spent a few hours in the aquarium (territory) of their full-sized neighbor who nearly killed them. When brought back to their own aquarium, they subsequently established territories, each with a clay tile as its center, into which the other fish was not allowed. When we introduced 50 cc of water from the neighbor's tank (through the air-driven filter to prevent conveyance of nonchemical information), their response was startling: after about one minute, the time required to distribute the foreign water throughout the tank, the fish stiffened,

erected their fins, darted left and right over the bottom and then huddled together in one shelter. There they remained, side by side for fifteen minutes. At that time the one who was the regular owner started to push and display and finally expelled the other who went back to his own tile.

Testing about every other week, this response was obtained up to three months after the incident, but the response was never observed with water from the tanks of other fish. Other apparently severe stresses, however, such as a door slamming in the laboratory, provoked the same "cooperative" behavior. This behavior has since been observed frequently. Besides showing the existence of good memory, the water switching experiment appears as the clearest evidence for chemical communication.

"Since bullheads do not normally live in aquaria," (Todd, 1968) I should mention here the scant evidence for chemical communication in the natural habitat which suggests that we do not deal with laboratory artifacts. I had a chance to study bullhead behavior during the young-guarding period (last weeks of June) in Lake-on-the-Mountain, (Picton, Ontario, Canada) a clear spring-fed lake, with a depth approximately 6-10 feet for the most part. Because of its shallow depth, it was ideal for skin-diving and long observations.

There were several bullhead pairs in the area guarding their young. After a few minuties the fish became used to the presence of a large body at the surface and exhibited what appeared to be their normal activities of chasing predators of the young. These predators, mainly the pumpkinseed sunfish, Lepomis gibbosus, could slowly approach from behind the watching parent and stay there for some time; but then suddenly the bullhead would turn around, attack and chase.

This situation was simulated under aquarium conditions. One guarding female parent was caught together with ca. 1,000 fry and put in a 1000 liter aquarium where she resumed her guarding behavior. Her activity slowly declined over the next few days, which was also found to be the case in the lake at the same time. When presented with a pumpkinseed, however, she became more active. The significance to our argument is, that the predator was safe if he did not move, unless the cruising bullhead approached, apparently by chance, an area of ca. 10 cm. around him.

She then started a fast search causing the pumpkinseed to flee and thus directing her attack. From the chain of events it is most likely that a chemical signal triggered the bullhead's search for a possible predator and that water movement acting upon the lateral line directed the attack. Vision cannot be ruled out in this phase since movement detection in retinal programming is probably phylogenetically older and more universally present than form recognition. However, in comparable but intraspecific situations we observed a similar chain of reactions in blinded bullheads. In general, vision is not strongly selected for in these fish, where blind and half-blind populations exist in nature (Weisel and McLaury, 1964).

In one case where intraspecific chemical recognition most likely occurred in the natural environment, the male returned from a wild chase of several fry predator and attacked the guarding female at full speed to within a few centimeters, then suddenly stopped and slowly approached here; the female had not moved during all this.

For completeness' sake, it must be mentioned that we have never been able to register sound from a bullhead under any circumstances, even during fighting. On the other hand the lateral line, especially in the head region, is very well developed. Free neuromasts occur, as do pit organs.

Evidence for olfactory communication

Our suspicion of the social role of olfactory sense was aroused when three bullheads with previously destroyed olfactory epithelium were put together in a large tank after they had served in another set of experiments. After a short while they began acting aggressively towards each other; the intensity of aggression increased rapidly and soon two seized each other by the mouth and remained locked pulling and pushing for the longest mouth fight we had ever observed: 24 minutes. Normally a few seconds were decisive. The third fish's behavior was even stranger. Since there was no mouth available, he swam nervously around the wrestlers and finally grabbed both of them over their heads near the mouth and hung on for some ten minutes and later another few minutes. He contributed his share to the orgy of pushes and pulls.

In the following days the three fish became severely damaged by the incessant violent attacks. Fins and tails were bloody and ragged and

the bodies showed many toothmarks. Needless to say, this never occurs in normal groups. In subsequent weeks, the aggression diminished; some peck order became established and the wounds healed. At this stage a close examination of the nose showed that several olfactory lamellae had regenerated. No further histological or physiological tests were done to check whether or not smell was again functional, as suggested by behavior. Therefore, it was tentatively concluded that it is the sense of smell that has a major function in social relations.

When we then demonstrated that bullheads are able to discriminate between tank water of individual bullheads and that this discrimination disappears when their olfactory epithelium is destroyed (Todd, et al., 1967) it seemed clear that the sense of smell is used for individual recognition, which in turn is the basis for social behavior.

Olfactory Functions in Fishes

It has often been stated (for instance Herrick, 1924) that the olfactory system has two different functions: a specific olfactory function and a non-specific arousal function. Some authors claim that smell even in a typical macrosmat as the dogfish has an arousal function only while specific information is gathered through other senses (Kleerekoper, 1967). This is probably the case in many instances and in many species but it seems rather unlikely for example, that this would be the sole use of a shark's enormous nose. Tester (1963b) gives ample evidence for specific and directional olfactory responses in sharks. In many vertebrates smell alone is known to release specific behavior patterns and to orient behavioral responses. We can think of hunting dogs, rabbit colonies (Mykytowycz, 1968), and prairie dog towns (King, 1964).

In fishes, olfaction plays a role in the search for food and the avoidance of predators in homing and in social behavior. In several of these categories smell acts as an alerting signal only. The subsequent directed responses are guided by other senses. But in some categories, the fish is able to perform a complete oriented response to an olfactory stimulus. Localization of food by smell has been demonstrated for many species of fish by a number of authors, among these Bateson, 1890; von Uexkull, 1895; Baglioni, 1910; Parker, 1910 (b), 1911, 1914; Sheldon, 1911; Copeland, 1912; Parker and Sheldon, 1913; von Hess, 1913; Green,

1925; Pipping, 1927 (a, b); Wunder, 1927; Bardach, et al., 1959; Teichmann and Teichmann, 1959; and Tester, 1963a. A predator's odor elicited escape responses in some species (Göz, 1941; Brett and MacKinnon, 1954; George, 1960). The well-known "Schreckstoff" reactions were first described by von Frisch (1938). Other authors have supplemented this material (Heintz, 1954 a, b,; Schutz, 1956; Verheijen, 1956; Pfeiffer, 1960, 1966). Experiments have shown that a home area can be smelled by some fish: Lepomis megalotis megalotis (Gunning, 1963, 1965) and Salmo clarkii (Miller, 1954); and Hasler (1966) reviews the literature of the famous salmon homing phenomenon. Few experiments have dealt with olfaction in social behavior (Myrberg, 1961; Kuenzer, 1962; Kühme, 1963; Todd, et al., 1967; Todd, 1968). The latter two demonstrated that Göz's top performer, Phoxinus "34", was not an exception among smell-dependent fishes and that individual recognition can be mediated via the nose. It would not be surprising if in the near future several more important olfactory functions were discovered and described.

Olfaction and the "Rhinencephalon"

The above studies have all considered a function of the sense of smell per se in fish behavior. A next and closely related question is to what extent the olfactory related neural centers exert their influence upon these same behavior patterns. Hence, the forebrain,* so often referred to as "rhinencephalon," became of special interest to us. If indeed olfaction is crucial in such complicated responses as seen in social behavior, then we must expect the detailed evaluations to take place in some higher order "olfactory" centers. For this task these centers must be in close relation with other sensory centers, directly and/or indirectly through other higher order correlation areas. The vertebrate brain literature and especially those papers dealing with the phylogenetic development of the forebrain contains plenty of evidence for the role of olfaction in such correlation centers. Earlier reference to Herrick (1924) stated boldly that the neural substrate for behavioral plasticity is found in primitively olfactory areas. "Higher", "sophisticated" behavior based on

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*The term forebrain is used in accordance with the literature. More correct would be to use the term telencephalon.

individual experience might well be correlated with olfactory dominance in the brain (Atema et al., 1969). The "rhinencephalic" brain areas in higher vertebrates (homologous to those of fishes) make up an important part of the limbic system, or the visceral brain, which is said to be the site of emotion (Papez, 1937). In mammals there is a clear relationship between parts of this complicated system and social behavior. For a review of the extensive and controversial literature on the "rhinencephalon" see Pribram and Kruger (1954).

Evidence from many sides thus indicates that olfactory related correlation centers have a major function in the complicated performances of social and emotional behavior. Initiating and regulating neural centers for such behavior must be situated somewhere along the olfactory system or in the forebrain.

Experiments on the function of the chemical senses in the catfish, an animal that shows an exaggerated development of both smell and taste systems, and a remarkably large brain among teleost fishes, are described below. They will delineate the interplay between these systems and their specific role in behavior.

CHAPTER II

EXPERIMENTS ON TASTE AND FEEDING BEHAVIOR

Peripheral Taste Structures

Like all catfishes, the yellow bullhead, Ictalurus natalis (Ictaluridae, Siluriformes = catfishes) has no scales. Concomitantly the order has developed an abundance of taste buds all over the skin. Figure II, 1, shows the distribution and average density of external taste buds as we counted these on the bullhead. Individual fishes show peculiarities in distribution as well as in densities. Figure 1 was generalized from five adult animals of about 25 cm length. Over the whole body surface approximately five taste buds per mm^2 can be counted. This number is a little higher for the dorsal and a little lower for the ventral side. There are usually none at the rims of the fins. On the caudal fin taste buds are ordered in rows next to the fin rays. The flat sides of the spine of the pectoral fins and dorsal fin show higher concentrations; in one fish we observed a very high concentration (15 taste buds/mm^2) on a fleshy structure extending beyond the dorsal spine, which gave it almost the appearance of an additional barbel. The areas that are regularly covered by skin flaps or appendages, such as the skin area that becomes obscured when the maxillary barbels fold backwards and the area that is covered by the opercular flaps, have no taste buds at all. The concentration increases to 7/mm^2 toward the rostral end of the fish in front of the dorsal fin. The increase is more pronounced in the dorsal area than at the ventral surface. In the nasal area and on the lips, taste buds are even more abundant (10/mm^2). Highest concentrations are found on the barbels.

Catfishes owe their name to the possession of barbels, the so-called whiskers, that actually serve as a set of external tongues with a combined gustatory-tactile function. The maxillary barbels (Figure II,2) have taste buds in densities of more than 25/mm^2 at the rostral surface,

15-25/mm^2 at the caudal surface, 10-15/mm^2 at the flattened dorsal surface and 10/mm^2 at the ventral surface.

The total number of taste buds on the eight barbels is estimated at 20,000, on the lips at 3,000 and on the rest of the body skin at 155,000. The total number of external taste buds would thus be over 175,000.

The taste bud distribution inside the mouth is schematically represented in Figure II, 3, and is in the order of 3-5/mm^2. High concentrations are found on small mounds and ridges in the median plane both on the roof and the bottom of the mouth. On the gill arches taste buds are found on the surface that is turned toward the inside of the pharyngeal cavity. They are concentrated on mounds, in rows along the spines and in small tufts. In certain areas densities up to 50/mm^2 may occur (Figure II, 4).

The total number of taste buds on the gill arches of a 25 cm long bullhead is estimated at 12,500, and the number inside the mouth at 8,000. The number of internal taste buds would amount to over 20,000. An average adult bullhead thus has over 200,000 taste buds.

Each taste bud contains a cluster of taste cells, supporting cells and nerve fibers. Several excellent papers give detailed descriptions of the microscopic and sub-microscopic structure of the taste bud in various animals; for fish, one is referred to a recent review on taste by Bardach and Atema (1969).

Central Taste Structures

The innervating nerve fibers (Figure II, 5) from each taste bud form a "stalk" under the bud. The "stalks" join together and collect in a branch of the facial nerve when the taste bud is on the body, in the vagal nerve when it is a pharyngeal taste bud. Free nerve endings extend up to the outer epidermal layers between and around the taste buds. These small nerve fibers join the taste fibers on their course to the brain. Large motor nerves of the cranial musculature take the same route and are packed in the same perineural sheet of connective tissue as these sensory bundles. Together they make the components of the massive nerve trunks that are the most obvious feature of the bullhead brain, the facial-trigeminal complex (Figure II, 5 and 6).

All the nerves of the taste system are joined by tactile-sensory and partly by motor components from the periphery to the brain. Surgical operation on nerves of any one component without affecting the others is thus made impossible. However, shortly after where the nerve trunks enter the brain the different components are separated and each modality collects in a separate center. At such primary centers, these sensory systems become accessible to selective surgical techniques. The dorsal parts of the facial and vagal lobes are the primary gustatory nuclei (Figure II, 7).

The gustatory VII nerve can be seen in its totality where it runs under the acoustic tubercle of the cerrebellum and comes up into the dorsolateral wall of the facial lobe (Figure II, 8). One part of the nerve terminates in the medial area and another part in the lateral area. There is a third tract in a more ventral position that is connected via the reticular formation with the homolateral motor nuclei of cranial nerves V, VII, IX, X and with the fasciculus longitudinalis medialis, which serves oculomotor and trunk musculature nerves (Herrick, 1905). From the large dorso-lateral area of the lobe a massive tract of medullated fibers runs in a ventral direction turning caudad when it reaches the spinal V tract. Its major part leads to the inferior secondary gustatory nucleus, situated near the commissura infirma at the caudal end of the medulla. This nucleus receives besides the descending secondary gustatory tract from the facial lobe, fibers from the vagal lobe, and is further related to spinal V tract, funicular nuclei and commissura infima. The remaining part of the descending gustatory tract runs into the spinal cord. From the smaller mesial area of the facial lobe a very distinctive ascending secondary gustatory tract can be seen going to the superior secondary gustatory nucleus under the cerebellum near the valvula. This tract also contains some fibers of cranial nerves IX and X. A minor part crosses over in the dorsal commissure of that nucleus.

A tertiary gustatory tract connects the secondary nucleus with the hypothalamus. From a lateral lobe of the hypothalamus the tractus lobo-bulbaris runs to the medulla and spinal cord mingling with the above tertiary fibers in the hypothalamic area.

In the bullhead there is no true distinction between glossopharyngeal (IX) and vagal (X) nerves and for our purposes we will refer to the complex as Xth or vagal nerve. Its gustatory component serves pharyngeal taste buds and can be seen rising into the vagal lobe along its lateral wall. A superficial root runs into the dorsal nucleus, which shows macroscopically as the vagal lobe (Figure II, 7). The nucleus is connected through ascending and descending secondary tracts with the superior and inferior gustatory nuclei respectively. The lateral area of the vagal lobe has direct connections with motorneurons in the ventral horns of the medulla and spinal cord, and also contributes to the ventral commissure of that area. A deep root of the vagal nerve goes to the nucleus intermedius X, a caudal extension of the similar nucleus intermedius VII (Herrick, 1905).

Summarizing the histological data, we see that both gustatory VII and X terminate in sensory areas in the dorsal wall of the medulla where primary nuclei have so much expanded that large facial and vagal lobes bulge out in a manner characteristic of the brain of many species of catfish. The primary centers have not only direct connections with homolateral motor nuclei (mostly from the vagal lobe), but they also send strong fiber tracts to a number of correlation centers (mostly from the facial lobe). The inferior secondary nucleus receives tracts from both gustatory nuclei, from tractile areas (spinal V tract) and from the contralateral inferior nucleus. It projects into the spinal cord. The superior secondary nucleus also receives tracts from both gustatory nuclei (but mainly from the facial lobe) and has a strong contralateral connection. It projects into the hypothalamus, which receives fibers from almost all brain areas. (Schnitzlein, 1964).

The sensory nuclei of the vagal lobe have their main motor connections with motor nuclei in the homo and contra lateral vagal area. The facial lobe sends strong tracts into the somatic motor centers of the medulla and spinal cord; it also relates to other sensory systems through higher correlation areas in the diencephalon.

Normal Feeding Behavior

It is well known that catfishes upon touching their skin with a piece of meat respond with goal-directed movements and pick up the food.

When searching over the bottom with their mandibular and maxillary barbels, they invariably stop and dig in the sand when a piece of food is present. Merely tactile responses can be elicited but not repeatedly over a long time. However, when meat juice is added to the tactile stimulus, the fish again responds with great enthusiasm (Herrick, 1905).

Taste as a true distance receptor was demonstrated by Bardach et al. (1967) for the bullhead. Blinded fish with destroyed olfactory epithelium become alerted to the presence of food and localize it accurately showing normal search patterns. All our experiments on bullheads without a sense of smell confirmed that there is no observable difference in feeding patterns between them and normal animals. Even forebrain removal does not affect the feeding per se.

From the above-mentioned data, it was assumed that bullheads are alerted and guided to the food by their facial taste system. Once it is in their mouth and in contact with internal taste buds, the vagal taste system presumably provides for a final judgement over ingestion. We have observed on occasions how pieces of food were picked up, kept in the mouth for a few seconds, spat out, were immediately picked up and spat out again, and so on for more than five times. It seemed to us that the food was judged attractive by external taste and repugnant by internal taste; an oscillation of reflexes resulted. This process usually ended with indigestion.

Normal food search and ingestion can be described in the behavioral units of Table II, 1, that form a temporal sequence, sometimes with alternatives. The unit symbols are given in parentheses.

TABLE II, 1

(a) Alert: the fish interrupts whatever he was doing and responds with a sudden stiffening of his body, the fins erect; quick lateral jerks can also be seen.

(s) Search: directed cruise, sometimes nervous and jerky, sometimes smooth in an almost straight line, usually with frequent loops.

(h) Hit: (not a behavioral unit) the moment he touches the food; when it is on the bottom the tail moves up and the lips touch the bottom in one quick movement.

(d) Dig: headstand on bottom with lateral head movements (see Figure II, 9).

TABLE II, 1 (Continued)

(pu) Pick-up: an object is taken in the mouth (by suction).

(cw) Chew: mandibular movements, presumably testing the object for tactile and gustatory qualities.

(sp) Spit: the object is rejected.

(i) Ingest: the object is passed through the pharynx into the stomach.

(reg) Regurgitate: the object is returned from the stomach.

The normal feeding chain in unit symbols would be: a-s-h-pu-i. The other four units in the table are elaborations or alternatives. We can now postulate that "alarm" is elicited by smell (according to most authors) and/or external taste, that "search" is guided by external taste, that "hit" and "pick-up" are triggered by tactile stimuli in addition to external gustatory stimuli, and that "ingest" follows upon approval from internal taste perhaps aided by tactile information.

In 200-liter aquaria normal, undisturbed bullheads can be seen to respond to the introduction of a piece of food (liver or dry pellets in our experiments) within a few seconds to a few minutes depending on the relative positions of fish and food and the water currents between them. The fish will then cruise nervously through the water in a seemingly random manner. This cruising is due to the small size of the tank, where concentration differences are ill-defined and scents whirl around rather randomly. Within a few minutes the fish will have hit the food, picked it up and ingested it in one quick sequence. If more pieces of food are introduced, the fish will only stop ingesting when his stomach is actually bloated.

Ablation of Central Taste Structures

Deprivation of one or another sensory modality in animals has contributed much to our knowledge of some specific functions of the receptor systems. However, this experimental procedure was never employed in the case of the gustatory and tactile senses for the anatomical reasons described in the beginning of this chapter. Confirmation of how the senses interacted in feeding was therefore lacking. In the following section, I

will describe the procedure of selective "degustation" in the bullhead, a method that could be employed equally well in all genera of catfishes whose brains I have seen. The method seems generally appropriate to many other similar problems in sensory physiology.

Our experimental animals were trapped locally in Winan's Lake, a little north of Ann Arbor, Michigan, in the fall of 1967 and 1968. They were kept in large groups, where territoriality was minimal, on a 16-8 hour day-night schedule. They were fed trout developer dry food pellets, supplemented with fresh beef liver. Bullheads selected as experimental animals were kept in individual 75 liter aquaria for several weeks prior to operation and observed in their normal behavior.

Experimental fish were anaesthetized in a 0.5% solution of tricaine methane sulfonate and kept moist during the operation, which usually took an hour. A rectangular piece of skin (10 X 6 mm) was removed in the dorso-medial line at the very caudal end of the skull (Figure II, 10 and 11). The exposed musculature (a small part of musculus adductor mandibulae) was detached from the bone crest and a piece of skull of the same size as the removed skin was bared. A dental drill and round burr were employed to carve a slit (8 X 3 mm) in the bone carefully avoiding the main dorsal blood sinus that is attached to the cranium at this level. This was accomplished by not drilling all the way through the bone. The piece of cranium was then removed leaving the sinus intact (Figure II, 12).

If too much of the skull is removed laterally, the posterior vertical semicircular canal of the labrinth will be damaged (Figure II, 11 and 13). The facial lobes can be seen through lose connective tissue with oil drops that covers the brain in most fishes. This loose tissue was removed and saved. The membranous roof of the fourth ventricle, the choroid plexus, was also removed, thus exposing the facial lobes, located just in front of the blood sinus. Then the caudal part of the cerebellum can be seen (Figure II, 14). For the removal of the entire dorsal part of the facial lobe suction was applied by mouth aspiration through a small piece of polyethylene tubing. Fiber tracts in the lobe can be seen and saved and good control is possible in this way. The whole operation was done under a dissecting microscope with 7-30 X magnification. The hole was filled with the original loose liquid tissue and sealed with vaseline.

The vagal lobes were removed in a similar way. This operation is complicated by the fact that the position of the forementioned blood sinus is exactly dorsal to the vagal lobes, but with careful manipulation, damage can be avoided. The sinus can be slightly moved and the ablation can be done under complete visual control.

Three facial and two vagal lobe ablations were performed. The third facial lobe ablated fish died a few days after operation possibly because the ablation extended more ventrally into the tract area; the spinal V tract was severed unilaterally. The first facial lobe ablated fish suffered from unilateral labyrinth damage caused by the removal of a piece of skull. Central readjustment was achieved in three weeks, which corresponds to data in the classical literature on labyrinth damage (Von Bechterew, 1883; Magnus, 1924).

The pertinent data on facial and vagal lobe ablated fish are collected in Table II, 2, where the results of routine histological inspection of the brain are also shown. The brains were cut in 50 μ transverse sections and stained with cresyl violet for cell nuclei. The histological procedures were similar to those employed to assess forebrain ablation damage (Chapter III). The fish were tested for feeding and for social behavior and sacrificed after 2 to 135 days.

Motion pictures (Kodachrome II, Super 8 mm) were taken with a Bauer C2B camera to analyze details of motor patterns in the feeding behavior chain and their changes after brain lesions.

Results of Ablations

Histological inspection of the ablated areas shows that no regeneration took place and that the major part of the sensory areas of the facial (respectively vagal) lobes was removed (Figure II, 15 and Table II, 2).

Bullheads with ablated sensory areas of the facial lobe respond to the presence of food and they exhibit a clear search pattern. It was possible to observe alarm (a) and search (s) behavior when food was introduced as soon as one day after the operation (as in the case of subject TL_2 (Table II, 3). In one more day, the fish was able to localize food pieces albeit with great difficulty. It was not clear if the food objects

were recognized as such even when touched with the barbels. Yet the fish spent considerably more time over and around the food than elsewhere in the aquarium. Localization improved with time. Careful tactile investigation of food objects was observed.

In daily food presentation tests gradual improvement of localization was observed and after a week even the motor patterns of chew and ingest appeared in the presence of food, which the fish was still unable to pick up. Hits became more frequent and more accurately aimed, but the appropriate response of opening the mouth and sucking the food in did not follow. It seemed as if during this period the tactile centers with the help of still intact chemical senses became trained to distinguish the different food objects from the gravel substrate and to recognize them as food. The combined sensory information thus available was, however, not sufficient to trigger the appropriate motor neurons, that are programmed for the "pu" pattern. Instead an antagonistic motor response was frequently observed - at the moment of hitting the food the closed jaws were clamped together even more strongly.

Under all circumstances it was clear that the motor centers and muscles proper were not affected, because several behavior units that require the same muscles were recorded regularly; for example, yawn, gulp, and mouth display (for general behavior units see Todd, 1968).

During this period the operated fish approached the food area slowly and repeatedly. The food was pushed with the lips over the substrate for distances up to 25 cm. Then renewed, deliberately slow approaches and hits were made. Even the most tempting bait, a fresh, wiggling worm, could not trigger the "pu" reflex.

TL_1 was first successful in "pu" 17 days after the facial lobes were ablated; TL_2 took 28 days (Table II, 3). It is possible that the stress from several experimental interactions with other fish, in which TL_2 was badly bitten, had a negative influence on his "learning" period. TL_1 was not exposed to other fish until four weeks after he had resumed feeding. These interactions will be discussed in Chapter IV.

After the food was taken in the mouth, normal ingestion followed immediately. This proves that there is no facial control over this part of the feeding behavior. Feeding improved daily. The first successful "pu", of which we have also a film record, was still very problematic.

The food was dropped a few times and the coordination of mouth opening and gulping was very poor, so that the food only slowly entered in the mouth. It was only after another week (for TL_2; 11 days for TL_1) that some fluency in the interplay of the motor patters was observed. The whole feeding chain (a-s-h-pu-i) could then be performed in a nearly normal manner. The only differences were a slower and more deliberate "h" and "pu" when compared to the easy feeding virtuosity -- as it now seemed -- of the normal bullhead.

The function of the sense of smell in the feeding of taste deficient fish was tested by additionally destroying the olfactory epithelium (Chapter III for method). For TL_1 this resulted in one day on which he did not feed and appeared not in optimal condition; on the second day, however, normal feeding behavior was restored.

TL_2 had his first day of easy feeding after facial lobe ablation on the day following additional nose operation. From this it can be concluded without restriction that the presence of a nose is without effect on feeding behavior. It can further be concluded that the effects of facial lobe ablation are strictly limited to the localization (hit) and pick-up phases of the feeding behavior chain.

Both vagal lobe ablated fish were alert and responsive the day after the operation. Even feeding seem unaffected at first. Changes in feeding behavior became apparent only after several pieces of food were introduced. The alert, search, hit and pick-up behavior appeared completely normal. However, after the fish had picked up the third piece of food, it became obvious that he still had the first two in his mouth. After the fifth he began spitting them out and immediately picking them up again often before they had reached bottom. This behavior much resembled the oscillation between two different reflexes sometimes observed in the feeding chain in normal bullheads and described earlier in this chapter. In this case, however, it ended more than one hour later, when the fish finally sank to the bottom near the rejected pieces of food.

Even after slow motion analysis of the movie frames it was not possible to detect any significant differences in the feeding behavior until the "ingest" unit. Timing and coordination were perfectly normal within the units as well as in their sequence.

The condition of TL_4 appeared good for seven days after the operation. He died on the tenth day without an obvious cause. In the other fish (TL_5) ingestion was restored after 3-5 weeks.

It was concluded that vagal lobe ablation effects are strictly limited to the last phase of the feeding behavior chain: swallowing the food after it is in the mouth. The impression is that the fish, once the food is in his mouth, does not recognize it anymore as such, does not know what to do with it, and finally spits it out; whereupon his unaffected facial taste system outside the mouth does recognize it as food and triggers pick-up again.

Additional operation effects (Table II, 4)

Even when the labyrinth itself was not touched, balance problems appeared after facial lobe ablation. As late as two weeks after the operation, TL_2 could be observed flipping over in headstand while digging for food.

Another swimming problem was observed mainly in the vagal lobe ablated animals; they were too heavy and, like sharks, had to swim in order to stay off the bottom. This caused faster than normal cruising in a nose-up position, sometimes with the snout above the water surface enhancing the shark-like appearance. When they stopped cruising, they sank to the bottom in a resting position, (see unit list of Chapter III).

Most of the time both facial and vagal lobe ablated fish kept their mouths firmly locked. Opercular movement had to make up for the resulting inefficient flow of water over the gills. The fish could be seen regularly gulping and spitting air (behavior units Todd, 1968).

Experiments on Tactile Feeding

In order to test the tactile contribution to the pick-up reflex, tasteless dummy pellets were presented. The dummies were made from a variety of materials: rubber, polyethylene, glass and ceramic. They were of different texture and color, but similar in size and shape to the food pellets. The whole collection together with a few food pellets was presented to normal and deolfacted fish and to the ones without facial or vagal taste.

Fish from all four categories responded in exactly the same way: Dummies were only taken in the mouth when they had been in contact with food material or with a human hand. (Bullheads are positively attracted to the chemicals of human skin; among the attractants is L-serine, personal observation.) Counts indicated that no dummy was ever ingested; they were rejected after a few chew movements and even sorted out from the food pellets which were ingested.

Normal and operated fishes alike, characteristically trailed their barbels over the gravel in their search for food and regularly came in contact with the dummies but failed to pick them up. The experiment proves once more that tactile information alone is not sufficient to trigger the pick-up reflex.

Discussion

It should be clear that the described ablations do not affect the motor centers themselves, because all the operated fishes could be seen opening their mouths in gulp, yawn, display, etc.

Both facial and vagal sensory inputs are directly connected with the motor centers in the ventral area of the respective lobe (beginning of this chapter). These motor centers, among them the mandibular, also receive fibertracts from other sensory areas (tactile). The change in neural "tone" caused by the absence of either facial or vagal lobe stimulation could distort the balance of sensory influences in the motor centers that control the mandibular musculature. This may account for the strange mouth behavior as well as for the swimming problems. The vagal nerve innervates the swimbladder bilaterally and regulates its volume (Fänge, 1953). Sensory areas of the facial and vagal lobes are both connected with the vagal motor centers. Air gulping and spitting are possibly related to swimbladder problems.

Facial taste ablation in general affects the fishes more seriously than vagal taste ablations. One reason for this could be simply that more neural tissue is removed in facial lobe ablation. The facial nerve supplies over 175,000 taste buds, whereas the vagal nerve serves only 20,000. The drop in general sensory input is thus considerably greater in facial ablation. Furthermore, the facial system has more ascending connections,

thus relating to higher integration centers in the diencephalon. Hypothalamic connections particularly open the possibility of connections with the hormonal system (Fiedler, 1967).

The hypothalamus of rats, cats and monkeys contains areas that are involved in regulating food and water intake (Anand and Brobeck, 1951; Anand, et al., 1955; Albert and Storlien, 1969). Even though there may be precursors of the mammalian "feeding centers" in the catfish hypothalmus, these would be influenced only indirectly by ablation of the primary taste centers in the medulla. It is suggested that lesions in the mammalian lateral hypothalamic areas cause a reduced motivation for feeding (Morgane, 1961). Lack of motivation is certainly not the case in the present study where the fish search for food for hours, but are simply unable to localize it. Rather than looking for a neural substrate of feeding behavior in the (remote) diencephalic area a neural circuit in the medullar centers can be seen as solely in charge of a successful feeding process.

Senses which may contribute to feeding in the catfish are olfaction, facial and vagal taste and (trigeminal) touch. Elimination of smell proved that it does not play a limiting role in the feeding behavior of the bullhead. Facial or vagal tastes alone are also ruled out, since the fish would not be able to feed at all after either facial or vagal lobe ablation when regeneration is ruled out. After a few weeks, however, the fish began to feed again. Trigeminal stimulation alone is not sufficient either, since the fishes did not feed on touch after taste ablation. Also tasteless dummy pellets were not considered by either normal or taste ablated fish.

There remains the combined influences of taste (facial and vagal) and touch (trigeminal). Information of both systems is apparently required to designate a piece of material as worthwhile picking-up and, once in the mouth, as edible. It takes several weeks for the fish to adjust to a new balance of sensory inputs of taste and touch after selective elimination of a taste system.

The alerting response of the bullhead to the presence of food, which surprisingly is not an olfactory function,could be explained by stating that both facial and vagal taste can do so. After earlier experiments in which the bullhead was able to localize the food on taste alone (Bardach, et al., 1967), it is no surprise to find that facial

stimulation is capable of alerting the fish. Also the ascending facial connections in the brain make it plausible that such a function exists. It is, however, rather unexpected to find that after facial lobe ablation and without a nose the fish is still alerted to the chemical presence of food. This information could be mediated through vagal taste. However only few ascending fibers from the vagal lobe are known. The common chemical sense or the nervus terminalis could be suspected, but this is at best unlikely.

One other possibility can be offered. With the present ablation procedure it will never be possible to remove all and exclusively the gustatory sensory nuclei. Many cells could remain and many of those may retain functional connections. The remaining cells could still provide sufficient information to alert the fish. Localization, however, requires a more complete set of neural connections with the peripheral taste cells. It would not be surprising to find a spatial projection of the peripheral skin in the facial lobes of catfishes, in much the same way as we find the retina projected in the optic tectum of teleost fishes (Schwassmann and Kruger, 1965). Future experiments with additional facial and vagal lobe ablation and vagal lobe electrical stimulation in the absence of food may largely solve the question.

In the case of vagal lobe ablations it is fully expected that the fish is still alerted by the chemical presence of food through its intact facial system. This system acts as a distance receptor in catfishes, and has the somatic neural connections in medulla and spinal cord.

The problem of how the feeding behavior was restored after facial and after vagal lobe ablation cannot be solved from data available at present. Shifts in the balance of sensory inputs that together trigger a behavioral response seem likely. After facial ablations we observed frequent barbel touch and pushing of food objects. This may have facilitated tactile neural connections. Thus there remained a qualitative notion of the presence of food through unaffected taste. After vagal ablations frequent chew movements were noticed and these would provide tactile facilitation in the same way. The "balance-shift" theory thus explains how the pick-up reflex slowly reappears. It also takes into account that accurate localization is not possible again (in 135 days).

CHAPTER III

EXPERIMENTS ON SMELL, FOREBRAIN AND SOCIAL BEHAVIOR

Peripheral Smell Structures

It is assumed that in many fishes, smell plays at least an alerting role in feeding, but in the bullhead facial and vagal nerve innervated taste systems alone are entirely sufficient to enable the fish to feed, as demonstrated in Chapter II. It was therefore at first surprising not to find any obvious role of the olfactory system in this fish, especially in view of its peripheral and central olfactory structures, which are among the best developed ones found in teleost fishes.

Under and in front of the nasal barbel lies the nasal cavity or olfactory sac. It is connected through separate in-and out-flow openings with the external milieu, a condition indicative of good "smellers" (Pipping, 1927[a]). Opening the roof of the nasal sac of an average adult fish exposes 46 densely packed page-like lamellae (Figure III, 5). They provide an estimated surface area of 250 mm^2 in one sac with a volume of 30 mm^3. Of this surface about 2/5 or 1 cm^2 is olfactory epithelium; the remaining area is covered by ciliar epithelium (Holl, 1965). With an average receptor cell density of 250,000/mm^2 the total number of olfactory receptor cells in both olfactory organs approximates 50 million.

Numerous small nerve fibers leave the peripheral olfactory organ and collect in a short nerve, consisting of a lateral and a medial part. The two different parts come from the medial and the lateral row of lamellae (Figure III, 6). After a few millimeters the fibers enter the frontal surface of the olfactory bulb where they characteristically spread out over its surface. The olfactory bulb is located immediately below the olfactory sac.

The core of the olfactory bulb contains the cell bodies of the mitral cells, whose axons in turn make up the major part of the olfactory tracts on their course to the forebrain (see also Figure II, 12). The olfactory nerves are in contact with dendrites of the mitral cells. The

olfactory bulb can be considered as the primary sensory area for smell, in the same way as the facial and vagal lobes are primary taste centers. In their relation with other brain areas, however, there are considerable differences (see sections on anatomy).

The olfactory tract of fish is divided into a lateral and medial bundle, each of which can be subdivided into lateral and medial parts (Sheldon, 1912, Döving and Gemne, 1965). The tracts come from different areas of the olfactory bulb and establish connections with different parts of the telencephalon (Figures III, 7 and 8). The lateral olfactory tract sends fibers into the ventromedial area of the amygdaloid complex and into the piriform area. The medial olfactory tract divides into two strong tracts that enter the precommissural basal area (the olfactory tubercle of higher vertebrates) and the septal area (Droogleever Fortuyn, 1961). Functional differences between lateral and medial olfactory tracts are not known but may well be significant in the light of their different connections at both ends. Anatomy thus suggests that smell plays an important role in the bullhead.

Anatomy of the Teleost Forebrain

The following description is based on papers by Droogleever Fortyn (1961), Schnitzlein (1964), and Fiedler (1967); on serial sections of bullhead brains; and on low magnification microscopical preparations of bullhead brains.

Most secondary olfactory centers, the tuberculum, the septal area, and part of the amygdala are located in the caudo-ventral area of the telencephalon. The amygdaloid complex can be seen in the caudo-lateral part of the telencephalon and it is replaced frontally by the piriform area that forms the entire lateral and rostro-lateral part of the brain. Its borders are clearly marked by the sulcus ypsiloniformis, (Figure II, 7). The piriform area can also be regarded as a secondary olfactory center.

The main olfactory connections in the bullhead telencephalon are with the olfactory tubercle, located in the basal area medial from the fissura endorhinalis, and with the septal area, located dorso-medial from the tubercle in the area of the anterior commissure (Figure III, 8 and 9). The hippocampal formation of the dorso-medial wall of the telencephalon possibly

receives some olfactory fibers from the medial olfactory tract. The hippocampal commissure forms the dorsal part of the anterior commissure (Figure III, 10).

Septal area and tuberculum send a strong fiber tract, the medial olfacto-hypothalamic tract, to the hypothalamus as part of the medial forebrain bundle. Other connections are made within the telencephalon; the tuberculum sends many fiber bundles into the corpus striatum and some into the piriform area; the bilateral septal areas communicate via the anterior commissure.

The amygdaloid complex is an important intermediary station between olfactory centers and general telencephalic and diencephalic areas. Its dorso-lateral nucleus is in close relation with hippocampal and piriform regions and its fibers contribute significantly to the ventral part of the anterior commissure. This tract is homologous with the fornix of higher vertebrates (Schnitzlein, 1964). The ventromedial amygdala, the main receiving area for the lateral olfactory tract, relates mostly to the piriform area of the telencephalon and via a strong, medullated fiber tract, the stria terminalis, to preoptic and hypothalamic centers.

In the dorsal area between hippocampus and piriform lobe a rather small area, the general pallium or paleopallium, stands out. It is considered the precursor of all cortical development and it is the area that develops most in vertebrate phylogeny. This area, which is not clearly delineated in catfish, is related through small neurons to all neighboring areas: piriform lobe, hippocampal formation and striatum. The latter consists of a mass of undifferentiated cells in the central area of the telecephalon. Striatum and paleopallium are connected with the dorsal thalamus, its entopeduncular nucleus (n. prerotundus) and corpus glomerulosum (n. rotundus) by means of the dorsal portion of the lateral forebrain bundle. The ventral portion of the lateral forebrain bundle connects striatum and paleopallium with the tuber posterior hypothalami.

The tuber posterior contributes to the medial longitudinal fasciculus with fibers that come in relation with motor centers in medulla and spinal cord. An ascending tract in the same fasciculus carries sensory information, possibly including taste information from the medulla to the hypothalamus.

In addition to the stria terminalis from the amygdaloid complex and portions of the lateral forebrain bundle, the hypothalamus receives a significant contribution from the whole medial forebrain bundle. The hypothalamus is thus related to the tuberculum olfactorium, supracommissural septal area and hippocampus.

The main fiber tracts in the telencephalic peduncle, the stalk that connects the forebrain with the rest of the brain, are medial and lateral forebrain bundle, stria terminalis and tractus teniae. The latter is a small tract between nucleus teniae and habenula. The nucleus teniae is situated in the ventromedial amygdala. When the telencephalon is completely removed (see Figure III, 11) all these tracts are severed and the hypothalamus, as well as the ventral and dorsal thalamus and epithalamus (habenula) are stripped of some of their most prominent neuronal connections. In the peduncular area we find two more nuclear groups, the area preoptica parvocellularis and caudoventrally, the area preoptica magnocellularis. Parts of the latter area are likely to remain intact when complete forebrain ablation is done.

The hypothalamus is in direct relation with the hypophysis which in turn controls the total hormonal balance of a vertebrate.

Literature on Teleost Forebrain Ablations

The forebrain of fishes has been a focal point for physiological, behavioral and anatomical investigations. Its accessibility may at first have been the main reason for this emphasis, but the realization that it was this area from which phylogenetic brain development had taken place (Herrick, 1924 and 1933) kept the forebrain in the center of attention. Ablations of this part of the brain have been practiced for several decades and an extensive literature has accumulated. It should be mentioned that many of the older studies were undertaken with different motives in mind, often merely to block the sense of smell (Strieck, 1924; Neurath, 1949 for instance). Although these studies offer some interesting observations also from our point of view, they will not be discussed here.

When Janzen (1933) removed forebrains in goldfish he noted that opercular and eye movements became significantly more regular after ablation. This suggests that the effects of non-olfactory sensory stimuli on the

patterns of opercular and eye movements, normally effective and reliable as demonstrated in sensory threshold studies (Dijkgraaf and Kalmijn, 1963), are blocked. A similar explanation fits the fact that forebrain ablated fish "follow" better in an optokinetic drum (Janzen, 1933): a visual reflex dominates behavior and is less affected by other sensory information. Forebrain removal is without effect upon learning of certain visual tasks (Scharrer, 1938; Nolte, 1932; Bernstein, 1961a), but can affect others in an instrumental avoidance response (Hainsworth, et al., 1967).

The intensity of schooling behavior, often regarded to be predominantly visually triggered (Shaw, 1961), decreases after forebrain removal (Hosch, 1936; Noble, 1936; Berwein, 1941, e.a.). According to Hemmings (1966) schooling in the roach, Rutilus, has a strong olfactory basis, which would account for decreased schooling after forebrain ablation. Details of reactions necessary for schooling may still very well be visually guided. Berwein (1941) also found that a school of forebrainless minnows took much longer to reject a strange minnow than a school of normal controls. In general terms this could mean that the evaluation of a complex pattern (such as clan recognition might require) becomes more difficult without a forebrain. But again it could also indicate that clan recognition is achieved mostly under olfactory influence (Todd, et al., 1967).

Changes in social behavior after forebrain ablation have been studied and the general observation is that all behavior patterns remain intact, but that responsiveness to other fish clearly diminishes (Hale, 1956b, Aronson, 1957). For instance, Hale (1956b) in the sunfish (Lepomis) and Fiedler (1967) in the wrasse (Crenilabrus) counted a considerably lower number of aggressive displays; but once aggression was elicited in the ablated fish, it was as violent as in normal controls (Hale). Carefully placed lesions in different forebrain areas of male stickleback (Gasterosteus) caused quantitative changes in specific behavior patterns, such as zig-zig dance, fanning and leading the partner, indicating that the balance between fighting, sexual and guarding behavior was disturbed by malfunctioning of the forebrain (Segaar and Nieuwenhuys, 1963).

Hale (1956a) further found that forebrain ablated sunfish take much longer to pass a gate into a feeding compartment than normal fish, corresponding to the effects observed in Tilapia (Aronson and Herberman, 1960).

They found that forebrain ablated Tilapia respond much later and with a much greater variability in the latency to an instrumental conditioning situation, in this case the pushing of an introduced target. Removal of the olfactory bulbs had no effect, however.

We must add here that goldfish are less hesitant in a strange environment when their forebrains are removed (Janzen, 1933). Apparently they do not recognize the environment as being strange.

This lack of recognition can be considered one of the general effects of forebrain removal; comprised in it is social malfunctioning. Considering the observations in the conditioning experiments, where the simpler tasks are performed normally and the more complicated ones suffer considerable decrements, we could summarize in a general way by saying that the forebrain organizes complicated sensory input patterns into flexible adaptive behavior (output).

Comparison with Mammalian Studies

Today we recognize many parts in the fish forebrain which have their counterparts in the highest mammalian brains: Amygdaloid complex, hippocampus, septal areas, piriform and pallial area, striatum and several fibertracts such as lateral and medial forebrain bundle, fornix, hippocampal and anterior commissure. As is well known, several of these structures have at some time or other been grouped in the limbic system.

Mammalian, and more specifically, primate work has focused on the limbic system for its supposed function in emotional and social behavior. This was most clearly formulated by Papez (1937), who wrote: ". . . that the hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus and their interconnections constitute a harmonious mechanism which may elaborate the functions of central emotion, as well as participate in emotional expression. . . The structures described here are usually represented as dealing with some phase of the olfactory function."

However, later experiments that were more specifically aimed at disclosing the role played by limbic structures in emotional and social behavior, failed to support the idea as a whole. From these experiments a more detailed classification of the limbic brain areas has emerged (Pribram and Kruger, 1954) which supports our present concept of the brain's phylogeny. In primates we can distinguish three systems that are

anatomically and functionally superimposed upon one another. The first system is directly connected with the olfactory bulb and consists of the olfactory tubercle, diagonal band area, prepiriform cortex, and corticomedical nuclei of the amygdala. The second system has direct connections with the first but not with the olfactory bulb. It consists of subcallosal and frontotemporal juxtallocortex, septal nuclei and basolateral nuclei of the amygdala. The third system has direct connections with the second but not with either the olfactory bulb or the second system. It is made up on Ammon's formation, entorhinal, retrosplenial and cingulate juxtallocortex. Papez suggested the third (most exterior, and phylogentically newest) band to be the site for emotion. However, there is much evidence that the second (older) system is involved rather than the third. The entire isocortical mantle in cats can be removed (Bard and Mountcastle, 1948) without producing the emotional changes ("rage reactions") that occur when the amygdaloid complex belonging to the second system is damaged specifically or additionally. In another series of experiments, however, (Schreiner, et al., 1952) the same ablations resulted in diminution of aggressive behavior, which is in accord with temporal lobe ablation experiments in monkeys (Klüver and Bucy, 1939). Whatever the specific effects are, they all can be grouped together under "emotional" changes. Most interesting is that Herrick's (1924, 1933) neurological theories on the evolution and phylogeny of the forebrain in vertebrates can be confirmed by physiological data from several vertebrate classes.

Forebrain ablations in fishes have been conducted to determine forebrain function usually by means of visual discrimination tests (Aronson, Bernstein, Hosch, and others). Comparison of these tests with mammalian studies is practically impossible because of the extensive differences in exactly those areas that are involved in discrimination: namely the higher cortical centers of the "third system". The area of best comparison is in the older structures of the "second system", that are related to emotional and social behavior. Ablation of these areas in fish and in primates bring about similar changes in such behavior. This would indicate that the brain areas involved are homologous and analogous and that they have developed in an extremely conservative manner throughout the vertebrate evolution. It is on these changes that the present study focuses.

Methods

Experimental and surgical procedures

Neither our own previous experiments on bullhead catfishes nor the voluminous literature on teleost forebrains indicate what role smell plays in social behavior. And it is not clear to what extent we observe actions of the telencephalic and diencephalic correlation centers that are related to many sensory systems, including olfaction.

For this reason we designed a new experiment: a series of ablations along the olfactory pathway, each previous step of ablation acting as a control for the next in the ethological analysis of social behavior. We chose agonistic behavior, since we had ample experience with such situations.

Our experimental animals were selected from the groups of bullheads as already described in Chapter II.

A group of three bullheads was given at least two weeks to establish a stable community in a 1500 liter aquarium with a gravel substrate and several tile shelters (Figure III, 13). Three fishes of clearly different sizes were selected in order to facilitate easy recognition of α, β and γ fish and to prevent dominance shifts such as we have observed sometimes between equal sized fish. Short daily observations informed us about their relationship, which usually stabilized in one to two weeks. One more week was given before the first introduction of an experimental animal into the resident community. In the first observation hour the fish are not operated upon.

The experimental animals had been alone in a 75 liter aquarium for at least two weeks, usually longer. After these preliminary measures the experimental animal was carefully taken from his home tank and introduced into the large community tank. Detailed notes were taken on the behavior of all four animals for one hour. One observer kept a running comment in the behavioral units as previously used (Todd, 1968); sometimes two observers were required. Selected units appear in Table III, 2. After the hour was over, the experimental animal was immediately removed and anesthetised in 0.5% methane tricaine sulfonate solution. The nasal sacs were opened and the olfactory lamellae completely removed by cauterization

under visual control (30X magnification). Recovery from anesthesia was facilitated by running tapwater over the gills. The fish was brought back to its home tank when swimming and breathing reflexes reappeared. The whole operation took about 10 minutes. Minor bleeding sometimes occurred; infections were rarely seen. One week was allowed for recovery as stress products in trout urine returned to almost normal levels about one week after operation (MacKim, 1966). Feeding and other behavior was observed during this week. The animals usually fed immediately after the operation. Sham operations performed here involved opening of the olfactory sac, but no cautery.

Exactly one week after surgery the noseless fish was again introduced into the same community tank. The behavior of the four animals was carefully recorded again for one hour after which the experimental animal was removed and anesthetized for a further operation, much like the medulla ablations of Chapter II.

A portion of the occipito-frontal skin was removed, the muscles were severed from the bony crest on the skull and a square piece of bone was removed exposing the frontal part of the forebrain and the caudal end of the olfactory tracts, (Figure II, 11). The bone resection was accomplished by making a slit as described before (Chapter II), avoiding the blood vessels that are attached to the roof of the cranium. The piece of bone was lifted slowly to avoid rupture of the underlying blood vessels. The almost liquid gel that fills the cranial cavity was partly removed and saved giving a clear view of the olfactory and optic tracts. The olfactory tracts were completely severed, but the optic tracts were never touched. Sometimes the blood vessels that lay immediately ventral to the tracts were damaged, which caused bleeding. No difference in recovery over the next week was ever observed, however. The exposed cavity was filled with the liquid gel and sealed with vaseline. The fish was revived and returned to its tank. Again one week was allowed for recovery. Feeding usually started after two days. Sham operations at this stage included removal of the bony window but not the cutting of the olfactory tracts.

When the week was over the third hour-long observation was made after which the entire forebrain was removed. This last operation was relatively easy as trepanation of the skull had already been accomplished.

After the vaseline seal was lifted, the nuclei were easily removed by suction (Chapter II). The operation ended when the fiber tracts running in the telencephalic peduncle were exposed. In a few cases a small part of the ventro-lateral area remained intact (see section on Histology). Bleeding was always minor. The wound was refilled with vaseline and the fish brought back to its tank. Feeding during the third recovery week sometimes started as early as 16 hours after the operation and never later than the second day.

The last hour-long observation for operated fish was a week after the above operation. In most cases the experimental animals were then anesthetized, weighed, measured and sexed (since bullheads have no definite external sex characteristics); they were preserved in formalin. Some, however, were kept to be reintroduced at a later stage (see section on Later Observations). The brains of all fishes were collected and preserved in formalin. For histological inspection 50 μ transverse serial sections were made using a freezing microtome and starting at the frontal stump of the ablated brain. Cresyl violet staining was used for cell nuclei. Some communities were used for several experiments, others were used only once. All fishes were weighed, measured, and sexed. The sham operation here consisted of anesthetizing the fish, exposing the brain and resealing the cranium.

One week later the thus far only sham operated fish were forebrain ablated.

Recording of results

Behavior can be described in a sequence of more or less distinct behavior units. Changes in behavior will become manifest in these units, in their frequency of occurrence and in their sequence. Frequency of unit occurrence or unit score was chosen to measure behavior. Unit scores can be calculated for small time intervals to demonstrate behavior changes during one observation session. However, in this case, where we were primarily interested in the effects of the successive operations upon social behavior and, more specifically, agonistic behavior, the unit scores for the whole hour of observation will be treated first. This enables direct comparison of these scores by fish in different states of surgical treatment.

Todd (1968) described 75 behavioral units observed in bullheads. These units need not be treated individually, but may be subdivided or lumped so as to facilitate analysis of specific facets of behavior. Since the research reported here concerns itself chiefly with the gross operation-induced effects on agonistic behavior, our discussion will be clarified if these units are gathered into four groups on the basis of aggressive content:

Group A, aggressive behavior, included all those units aimed at driving away another fish.

Group B, curious behavior, involves the attempted acquisition of information about another fish. Curious behavior may terminate in Group A or Group C behavior.

Group C comprises escape behavior, i.e., behavior in which the subject attempts to avoid or escape from another fish.

The fourth group, neutral behavior, will be designated Group O and will be discussed later; by definition it does not involve interaction of the subject with another fish.

Table III, 1, represents the complete series of experimental fish. It contains relevant information on the operations and histological examination. Sham operated fish are represented by Roman numerals.

The running commentaries on behavior taken during observation hours were rewritten including only 22 selected, mainly agonistic, units. Table III, 2, shows the units, their symbols and definition. Ten additional symbols were used to designate individual fishes and tile shelters.

The data were parsed in two-minute intervals, thus arbitrarily splitting up the original commentary. This made it possible to compute the unit scores in each interval without losing the, sometimes important, time pattern.

The data were also grouped in classes of behavior units and plotted against the thirty time intervals. (Appendix Figures 1 and 2). These classes are ranked according to their aggressive contents and are numbered from 6 (most aggressive units) to -2 (least aggressive units). This classification is represented in Table III, 3.

The frequency of behavior units scored by the experimental fish was multiplied by the class number and then added algebraically in each time interval. Classes 2, 1 and 0 were not included. The histograms of

Appendix Figure 2 represent these numbers as they were calculated for the four observation hours and for all experimental fish.

The behavior units were also converted into numbers and plotted on graphs. But the time pattern is lost in doing so. From the graphical representations such as Appendix Figure 1 the score of units in each behavior unit class was computed for each observation hour. The results are presented in Table III, 4, and plotted in Figure III, 1, a and b. In Figure III, 1, a the scores are grouped according to observation hours. Thus the differences in behavior of individual fish before and after each operation are shown. Figure III, 1, b shows the changes that occur in each class of behavior units following the several operations, again listed for individual fish.

Even though individual differences are important, general effects of the operations can be shown when the resulting scores in each behavior unit class are averaged, separating operated and sham-operated fish. The average scores for each unit class and for each introduction are represented in Table III, 5 and in Figure III, 2, a and b. Scores of sham operated fish appear hatched, others in black. In Figure III, 2, a, the scores are grouped according to observation hours (= state of operation) and in Figure III, 2,b according to classes of behavior units.

Further condensation is obtained by grouping comparable classes of behavior units together in unit class groups. Unit classes 6, 5, 4 and 3 constitute the "aggressive" group (A); classes 2 and 1, the "curious" group (B) and classes -1 and -2, the "escape" group (C). For the aggressive group the average scores (Table III, 5) of unit class 6 were multiplied by three, the scores of classes 5 and 4 by two and class 3 by one. Adding the results gives the numbers of Table III, 6 (A). The numbers are again plotted in a histogram (Figure III, 3), with the algebraic weighting (above) serving particularly to distinguish between aggressive and curious behavior. Operated and sham-operated fish are represented as before.

The "curious" behavior group (B) and the "escape" behavior group (C) are formed from the added scores of their unit classes (Table III, 6, B and C).

Figure III, 1,b and Table III, 4, demonstrate in which observation hour, i.e., at which state of neural impairment, the different fishes scored highest in each unit class. These peak scores are presented in Table III,

7. The sham-operated fish are in parentheses. Observation hour no. 5 shows only formerly sham operated fish after they have been forebrain ablated. Fifth introductions of two other fish are discussed separately (see section Later Observations). The peak scores of Table III, 7 were added and represented in Figure III, 4. For the first observation hour (normal fish) we can add the peak scores of sham operated fishes, since neither have undergone surgery on first introduction. Number of peak scores in fifth observation hours (forebrain ablated, former shams) can be added to the number of peak scores in fourth observation hours (forebrain ablated fish) for the same reason, providing a clear comparison between normal and forebrain ablated behavior. When comparing the number of peak scores in all four observation hours the shams (hatched) have to be separated from the other fish (solid black).

Results

Histology

Histological examination of the brains of operated animals was done to establish the place and the amount of lesion. The results of macroscopical and microscopical inspection of the brains of the experimental animals are summarized in Table III, 1. Forebrain ablation was deemed complete in 9 cases. In these cases the caudoventral preoptic area was present up to a level slightly caudad from the middle of the optic chiasma.

In four cases parts of one hemisphere remained. Macroscopical evidence for a remaining piece of telencephalon can be seen in the photograph of Figure III, 14. In 4 fishes (no. 0, 3, 9 and I) such a unilateral piece of caudal telencephalon was found. It contains the olfactory tubercle, and parts of the septal areas and amygdala, besides the complete preoptic area.

In two cases (fish no. 7 and 8) degeneration was observed in the epithalamus and dorsal thalamus area. The histological techniques that we used were only designed to localize the place of ablation. It is therefore possible that most degenerated fiber tracts and nuclear areas, for which special stains are required, were not traced.

It is interesting to note that regeneration did not occur; in the case of no. 0 not even in 17 months. The small caudal telencephalic area that was found was similar to the areas found in nos. 3, 9, and I, which lived without a forebrain for 26 days, 16 days and 16 weeks respectively. It must be assumed that these were incomplete ablations from the beginning.

The olfactory sacs were examined under low power magnification for regeneration of lamellae, but they were generally found empty. Fishes no. 1 and 2 had a few small irregular lamellae at the rostral end of the olfactory sac. Fish no. 9 had several lamellae in the rostral end. The three fish lived 50 weeks, 25 days, and 18 weeks respectively after the olfactory lamellae were destroyed. Since the olfactory tracts were severed one week after removal of the lamellae and since the forebrains were removed two weeks later, it must be assumed that functional regeneration did not take place. The observed remmants of lamellae could have been left

from an incomplete destruction. It is also possible that non-nervous tissue regenerated.

Fishes no. 0, I and II never had their lamellae destroyed, but only their forebrains removed. In the case of no. 0 degeneration was not apparent macroscopically, even after 17 months. However, only histological examination could indicate if olfactory receptor cells were indeed present. This was not attempted.

Changes in Behavior

A general operation effect appeared at the first and second stages of surgery. The sham operation, simulating ablation of the olfactory bulb, produced a skittish fish, as evidenced by the greatly increased frequency of escape units and corresponding decrease in aggressive units in Figure III, 3 a, Column 3 as compared to Column 2. Individual differences between operated animals were not pronounced at this stage, nor is there any neurological basis for expecting differences. Such difference as can be observed consisted of a slight decrease in frequency of aggression and an equally slight increase in escape behavior. This was precisely the trend observed in sham operated animals, and may thus be ascribed to operation. The most pronounced effect of sensory deprivation is shown by comparison of Column 4 with the preceding Columns. It can be seen that forebrain ablated animals were more aggressive than either normal or olfactorily ablated ("noseless" and "tract-cut") fish, an increase from about 30 to 50 (170%) in aggressive scores. In contrast the only change in the sham operated animals at this stage was an across the board decrease in number of behavior units that occur in one observation period. The aggressive scores decreased from about 10 to 0.5 (5%). Decrease in activity is a good indication of habituation. The final stage in the experiment was to subject the sham operated animals to forebrain ablation. As can be seen from Column 5, this caused a change in behavior identical to that observed in other forebrain ablated fish. Aggressive scores reach about 40 after a "normal" level of about 10 (400% increase).

A more detailed analysis of behavior is obtained by plotting the behavior unit classes that make up the groups A, B, C and O. Figure III, 2, a and b show which of the classes are mainly involved in the changes

that appeared in the groups of behavior units, as described above. Behavior within the aggressive group (classes 6-3) was rather stable and not significantly affected by the first or the second operation. The "curious behavior" group was made up of approach and follow units. The two olfactory operations did not affect the approach units (class 2), but caused a decrease in the frequency of follow units (class 1). "Escape behavior" (classes -1 and -2) was similar in the distribution of its unit components and scores increased after each operation.

Especially interesting here are the units of the 0-group that were not represented before. The frequency of the unit 0-explore, an active "curious" behavior unit, but by definition not social and hence not included in unit class 2 or 1, followed the general trend of a decrease over consecutive observation hours, as demonstrated by the "curious" group. The 0-RP ("resting position") unit, indicating general activity, increased in frequency over the consecutive hours and peaked dramatically in the forebrain ablated animals, observation hours 4 and 5. The frequency of the 0-strep ("stress pump") units followed the trend of the escape behavior group: increasing over the first three hours and decreasing after that. These selected non-social units thus emphasize trends that were shown in agonistic social units.

For an overview of the changes in behavior units of individual animals one is referred to Figures III, 1, a and b. Trends exhibited by individual animals underscore the described results.

Graphs of individual animal scores, at two minute intervals (Appendix Figure 2), show a phenomenon that could not be extracted from the presentation thus far. Eight out of ten operated animals showed an aggression peak in the first 20 minutes of the second observation hour (behavior after nose cauterization). In 7 out of 10 the pattern of unit scores during the third observation hour (tract-cut) resembled the pattern of the second hour, but at a lower over-all aggression level. Another phenomenon, which we will call "emotional polarization," appears in these figures: forebrainless behavior was characterized in many individual cases by scattered short aggressive outbursts, alternated by sudden "RP" and fleeing bouts of high intensities.

Another way in which the polarization expresses itself is in the distribution pattern of behavior. Forebrainless fish as well as normal fish

each had a typical distribution pattern of behavior units during the observation hours. This pattern is most clearly demonstrated in Figure III, 4, where peak scores of individual animals are considered. Normal animals showed higher unit frequencies in low aggressive and escape classes. This pattern was reversed in forebrain ablated animals most of which scored frequency peaks in classes of higher aggressivity, 5 and 6. Even the relation between classes -1 and -2 was reversed. The sham operated animals showed these phenomena very convincingly when their forebrain was removed (observation hour 5).

The peak score Figure III, 4 also demonstrates that general activity was decreased by removal of the forebrain. This tendency is expressed in an increase in the frequency of the 0-RP (resting) unit.

Later observations on forebrain ablated animals

Fish no. 9 was reintroduced to his community (No. 5) 10 weeks after forebrain ablation. His behavior was highly aggressive and indiscriminate. His unit scores are included in Table III, 4, fish no. 9, observation hour no. 5. This fish was aggressive from his first (normal) introduction and was more aggressive on each succeeding occasion. During his fourth (first forebrainless) observation hour some flee and avoid units appeared and it was mainly the resident α fish who started aggressive encounters. After the fifth introduction, however, no. 9 attacked all resident animals indiscriminately with damage-inflicting behavior units (25 scores in unit class 5, the highest we ever recorded). It is also the only time that an experimental animal had mouth fights with both α and β resident fish.

Fish no. 10 was reintroduced to his community (No. 6) 14 weeks after forebrain ablation. His behavior, although it included a brief mouth fight, was characterized by continuous and high intensity flees, with resting positions and stress pump in between. The scores (Table III, 4) in classes 0-RP, 0-Strep, and -2 (flee) are the highest we recorded. The fish had been rather typical in his behavior throughout the four observations. He had a mouth fight with the α fish in the fourth observation hour that lasted over half an hour.

Both of these observations show impressively the unbalance and unpredictability of ablated behavior: One fish, having shown aggressive tendencies in the past, goes into an indiscriminate rage, the other fish flees in panic whenever a resident approaches.

No. 9 was again introduced 17 weeks after forebrain ablation. He was immediately (seconds after introduction) attacked by the ∝ resident and they had a short mouth fight. After this fight fish no. 9 avoided and fled from the ∝ resident, who stayed aggressive. Panic and high intensity flees were not observed, however.

The first two forebrain ablated fish, no. 0 and no. 1 were observed in interactions with similar sized large fish. No. 1 was observed 10 1/2 months (46 weeks) after forebrain ablation, and no. 0 after 16 1/2 months (72 weeks). Both fish were introduced once to each of two resident fish. The residents had been alone for several months in the experimental 200 liter aquaria. In the four interactions the behavior was indistinguishable from normal. It appeared balanced as well as flexible. With one resident both experimental fish tended to be dominant, but very rarely exhibted behavior units from the 5-class. The experimental fish were both dominated to some extent by the other resident when introduced in his tank. Flee, however, was always relatively calm, bordering on avoid.

No. 0 showed the clearest example of balanced behavior. He was dominant over one resident but never used any damage inflicting units; typical were "ap, MD, Th"; a few "Q" and one "N" were seen in the beginning. However the other resident fish seemed dominant over no. 0 who showed both "ap" and "av" behavior at first. Later he fled more frequently but after ten minutes he shifted back to "ap", followed by "MD, Th", which resulted in two short mouth fights. Afterwards his behavior was still "MD, Th", but now alternated with periods of "av".

Although personality differences between experimental as well as resident fish play an important role, it seems justified to generalize that the forebrain ablated animals went through a period of 4-5 months of unbalanced agonistic social behavior. However, after this period (10 months) behavior became indistinguishable from normal as determined by direct observations on their feeding behavior and responses to general environmental stimuli. "Laziness", so characteristic for this ablation, gradually disappeared in the course of many months. The question of how a more normal behavioral balance was regained without the forebrain is left open, since histology showed no regeneration even after almost 1 1/2 years.

General behavior observations

In addition to the described experiments most aspects of the behavior of these and several other forebrainless bullheads were studied. It was found in general that in the first postoperational month bullheads show an obvious lack of initiative. Resting positions are frequent and mild stimuli of all sorts fail to elicit responses; even eye movements or changes in breathing rate are hard to provoke. When stimulated sufficiently through any one sensory input a normal response pattern will follow, but frequently with an unusual intensity and duration.

For example: a bullhead without forebrain will not respond for a long time to the mild threat of a slowly approaching, mouth-displaying tank mate. But when the approach becomes faster and the displaying mouth bigger, he may suddenly go either into a panic flight or into a high intensity aggressive display (MD, Th, Q), not rarely ending in bites and mouth-fights of unusual length (25 minutes as opposed to a few seconds in most encounters of untreated fish).

Another example is the behavior during feeding. When a group of normal bullheads is fed, rank orders and territories break up and food search and feeding get priority. When a forebrain ablated bullhead is among such a group, he may start out searching and feeding. But soon the fast milling about of other fish will elicit aggressive behavior and he will be seen spending his time chasing the other fish, which usually do not pay any attention to these attacks as long as the feeding and searching behavior lasts. Afterwards, territories, rank order and aggressive displays among normal fish are reinstated.

Frequently normal fish show hesitance and "frustration" in dealing with a forebrain ablated animal. These observations may serve as examples of "balanced" behavior. Normal behavior is dependent on the interplay with the expected (learned) responses of an opponent (behavior feedback). When these responses are strange to him, he holds back and often stops action completely. After a while he can be seen to "try out" the other fish.

Similar behavioral "hold back" is observed when one fish starts window pushing (WP), a behavior unit that was tentatively explained as escape behavior occurring in small aquaria (150 liters). A window pushing

fish swims "in trance", often violently, along the tank walls and is less responsive to his environment much like a forebrain ablated animal. Other fish hesitate to attack a fish in such a state. They go into their ambivalent approach-avoid behavior, which appears well balanced and appropriate (adaptive) to the strange situation.

Discussion

The results of our ablation studies on the role of smell and the forebrain in the social behavior of a catfish will be discussed from the following two vantage points. First a remarkable similarity was found in certain aspects of fish and mammalian forebrain functions and ablation effects. These similarities are relevant in considerations of the evolution of the forebrain. Secondly smell seems to be important in the performance of learned tasks, such as occur in conditioning experiments and in catfish social behavior, when individual recognition is required.

The bullhead's obvious behavior changes after forebrain removal appear as a lack of ability to evaluate complicated patterns of multisensory stimulation. The fish is no longer able to evaluate the total picture correctly. All sensory perception -- except olfactory -- is still present and each sense is able to elicit normal behavior patterns. (For example, operated fish feed in a normal way when alone.) But when several sensory inputs are present, a proper evaluation and response cannot be made; one response type will completely dominate the others so that the animal overreacts (or "underreacts") to the situation. In other words, it seems as if each input is correctly connected with its specific motor output, but that superimposed influences of some higher correlation center are distorted. Such a center could be one that receives information from several sensory systems via higher order neurons and maintains a delicately balanced influence over the motor centers.

More than one of these centers may exist in parallel as positive and negative reinforcers, such as for example, the hypothalamic hunger and satiation centers in mammals (Morgane, 1961). It is my belief that after forebrain ablation many of those centers are still in operation but that another superimposed modulator, which in turn is responsible for the balance between the correlation centers, is affected or removed. The basis for

this interpretation is that the behavioral units are not changed and also that sequences of units often appear unaltered. The literature on teleost forebrain ablations provides further evidence for this assumption as summarized earlier.

A most striking similarity of opinion is expressed by Gloor (1958), who describes the mammalian limbic system ("counterpart" of the fish forebrain) as a modulator of integrated behavior patterns that are represented and elaborated at hypothalamic or reticular levels. It is not an organiser of behavior patterns but a modulator playing upon them through facilitation and inhibition.

As neural substrate for this organization of behavior Herrick (1948) proposed activation of lower centers via the olfacto-habenular tracts (inhibition) or via the olfacto-thalamic and hypothalamic tracts (facilitation). Although Herrick was dealing here with the amphibian forebrain, similar concepts have been suggested for fishes (Aronson, 1948, Hale, 1956a). Theories from mammalian work have regarded the hippocampus as inhibitory gating center, or noise filter and the amygdala as a signal amplifier (Douglas and Pribram, 1966; Douglas, 1967). It seems highly suggestive from many vertebrate accounts that the same overall principles of forebrain function pertain throughout the vertebrate subphylum.

This is expressed not only in a comparison of forebrain functions, but also in the results of its removal in fishes and mammals. Indeed, if we consider the vast differences in development between the fish forebrain and its mammalian counterparts, the similarities in ablation effects upon behavior are most remarkable. Telencephalon ablations in fish correspond in a gross way to temporal lobe ablations in mammals. (The temporal lobe includes hippocampus and amygdala; structures that developed from dorsomedial and caudo-lateral areas in the fish telencephalon.) The famous Klüver and Bucy (1939) experiments on the effects of temporal lobe ablations on the behavior of rhesus monkeys showed that after lobectomy the monkevs became stereotyped and responded in a compulsive way to all visual stimuli (hypermetamorphosis.) They also indicated no recognition and detection of the meaning of objects on a visual basis, although vision itself was not impaired ("psychic blindness"). Their emotional behavior was changed in such a way that they showed no fear of animate or inanimate objects, even those that normally were frightening.

Iawata and Ando (1968) repeated these experiments in an intraspecific context. They found inhibited emotional behavior in general and lack of normal fear and caution especially. However, two lobectomized monkeys, brought together seven months after the operation, fought so viciously that they had to be separated after 30 minutes. Such intense fighting never occurs in normal social relationships. The authors describe this as being due to a lack of rank relationship (social agnosia).

Recently a report came out describing the effects of uncus and amygdala lesions on social behavior of rhesus monkeys in the field: "Operated subjects showed social indifference, failed to display appropriate aggressive and submissive gestures, were expelled from their social group and eventually died." (Dicks, et al., 1969). The authors further described that two of the younger subjects with amygdala lesions only, survived and rejoined their social group, indicating that here, as in our fish, recovery is possible.

These observations are remarkably similar to our own studies on forebrain ablations in bullheads. We found stereotyped aggression, no territoriality, no exploratory behavior, no individual recognition, altered social feeding behavior and general "laziness." Two overall effects that stand out in the bullhead are lack of initiative (see also Janzen, 1933 and Hosch, 1936) and decreased recognition ability (see also Hosch, 1936 and Berwein, 1941). The latter must be important in territoriality and social feeding behavior. Aggression and individual recognition are closely interdependent in bullheads. Lack of initiative, a phenomenon that may be different from the recognition complex, is shown in the general laziness and the lack of exploratory behavior. Decreased recognition ability is apparent for animate as well as for inanimate bodies. The fact that in some studies (Klüver and Bucy, 1939) a strong attachment to visual stimuli was observed, could be due to the tests given, which were mainly of a visual nature. They are not necessarily indicative of a strong connection between the temporal lobe and the optic system. Evidence from several sides thus indicates that the forebrain of fishes and comparable brain areas of mammals are closely involved in the regulation of emotional and social behavior.

This behavior also seems to be affected by a blocked olfactory channel, particularly when recognition is involved because bullheads can turn extremely aggressive after their olfactory input has been removed.

When normal animals are compared to those subjected to either of the two types of olfactory ablation (Figure III, 3,b, Columns 1, 2, and 3, of each group), it can be seen that there is an increase in escape behavior, a decrease in curious behavior and no apparent change in frequency of aggressive behavior. However, considering the likelihood of habituation brought on by repeated introduction of the test fish to the same communities (note the decrease to 5% of the original aggressity level in sham operated animals), the net result of olfactory ablation could well be a tendency toward aggressive behavior. The aggressivity peak shown by many fishes after losing their sense of smell is another expression of the same phenomenon.

This effect can be explained in two different ways. The lack of olfactory input could change the normal function of the forebrain and thus indirectly influence the organization of social behavior (non-specific olfactory function). The other possibility is that aggressivity is caused by the lack of recognition of individual fish and of their social status, which in turn is caused directly by the absence of its main information channel: smell (specific olfactory function). Evidence for a specific olfactory function in social behavior comes from a conditioning experiment with bullheads in which discrimination between members of the same species disappeared with the removal of smell input through nose ablation (Todd, et al., 1967). The forebrain still functioned normally, but other sensory information was no longer integrated with the, evidently important, olfactory information. The overall behavior of this species indicates that not only smell but other senses as well are involved in social interactions: lateral line and tactile stimulation appear important in their mutual thrusts and quiver and also in interspecific communication in the responses to fry predators. The forebrain most likely provides for an important neural substratum where multi-sensory feedback and integration takes place. Removal of the forebrain consequently has far reaching consequences in all behavior where this integration is important. Social behavior undoubtedly is among such behavior as will be further clarified in the next chapter, which discusses roles of the chemical senses in catfish behavior in general. The well developed nose of the bullhead may have a specific function in intra and interspecific recognition and is thus of survival value to the individual and the species in their relation with other aquatic inhabitants.

CHAPTER IV

RELATIONS OF SMELL AND TASTE

The previous two chapters have dealt separately with the sense of taste in feeding behavior and the sense of smell in social behavior. In this chapter relations -- differences and similarities -- between the two chemical senses will be discussed, for which some additional experimental data will be presented.

The value of neural ablation techniques increases when the resulting changes in behavior can be ascribed unambiguously to the neurosurgical interference, i.e., if they are specific for the particular ablation. Specificity tests can rely on behavioral, histological and electrophysiological observations, all requiring that the broadest range of possible effects be investigated.

For this reason olfactory and forebrain ablated animals were tested for changes in general and in feeding behavior in addition to the social effects described in Chapter III; vice versa facial and vagal lobe ablated animals were tested for effects on general and social behavior in addition to the changes in feeding behavior as presented in Chapter II. Cross examination of this sort shows not only to what extent ablation of one brain area influences different categories of behavior, but it also shows how ablations of different brain areas affect one category of behavior. It therefore provides a valuable test for ablation specificity. Supporting evidence obtained by different techniques of brain research, moreover, add significantly to the value of results obtained by ablation only. Thus our results obtained by ablation of gustatory brain centers are backed by evidence from other studies that employed histological, physiological and behavioral measuring techniques as described in Chapter II. That taste centers govern feeding reflexes very specifically was also demonstrated by the results of cross examination tests on smell and forebrain ablated animals. The results are summarized below.

The experimental animals treated in Chapter III were tested for feeding responses at all stages of surgery. The tests were conducted like those for the taste deficient animals of Chapter II.

In our aquaria and with the food employed (liver, dry pellets, worms) we have never been able to observe that elimination of the sense of smell clearly changes feeding behavior. In the case of the already taste deficient fish TL_2 (facial lobes ablated) feeding was even resumed on the day following additional smell elimination. Forebrainless animals are more difficult to arouse in general. It is not surprising therefore to find that they also require a higher chemical stimulation to start searching for food. They may become more easily disinterested in their search in some cases. Yet their search behavior indicated that feeding, even after forebrain ablation, must be considered normal, albeit slower sometimes.

Thus feeding behavior is governed by a brain activity restricted largely to the area of the medulla and one for which far less multisensory integration is required than for social behavior. It seems further that smell input is not necessary in any part of the feeding behavior chain. In this respect bullheads, and probably catfishes (Siluriformes) in general, may occupy an exceptional position among fishes. The abundance of taste buds all over their bodies and the functional differences between the facial and vagal taste system may have reduced the importance of the nose as a distance receptor in feeding, a role which was assumed by the facial gustatory sense in catfishes. A role of smell in the feeding behavior of many fishes was reported by many authors referred to in Chapter I. However, taste in these fishes was never eliminated as it has been in our experiments and interesting results can be expected from continued research in this area.

The complementary cross examination experiment was to test taste deficient bullheads in social interactions according to the methods of Chapter III. The experimental animals of Chapter II were therefore observed in a total of 25 such interactions with various other, normal, bullheads. Five of these interactions were reintroductions to the same normal fish after the experimental animal had its olfactory epithelium destroyed in addition to facial lobe ablation. The results are summarized below.

Facial lobe ablated TL_1 appeared essentially normal in his repeated interactions. When the interaction took place in his own aquarium, his aggression was milder than expected; and in another aquarium his behavior was slightly bolder. In other cases it appeared insecure, not persistent in either attack or flee. Tactile investigation of the other fishes was typical for this ablation and to this degree never seen in normal animals. It reminded one of food inspecting behavior with repeated pushes. After additional elimination of the sense of smell, we observed an increase in this "tactile behavior". In social feeding the thus operated fish was attracted more to the fast moving normal fish than to the food, in a way similarly known from forebrain ablated animals.

TL_2, after facial lobe ablation, followed this pattern in general, but was more inclined to fleeing. On all second introductions to the same normal fish fleeing was almost instantaneous. After additional elimination of smell, the flee tendency increased even more.

Both vagal lobe ablated animals, TL_4 and TL_5, behaved normally. TL_5, in his own aquarium (and territory), attacked an introduced normal fish, lost the mouth fight and subsequently fled from his opponent. When introduced into a strange aquarium, he also fled. TL_5 displayed very aggressive behavior towards an introduced fish. He alternated with sudden rests on the bottom. This phenomenon was already described in Chapter II where the general behavior after vagal lobe ablation is discussed.

It was thus demonstrated that bullheads with ablated facial or vagal gustatory lobes suffer minor deficiencies in their social agonistic behavior. Since tactile and gustatory inputs are closely connected and balanced at the medullar level, it may not be surprising to find increased "tactile behavior" after gustatory damage, even in the investigation of species mates. At the same time, this may be an indication that normal taste is required for undisturbed social behavior. Additional smell elimination caused a further increase in disturbance.

Well balanced social behavior then seems to require a full set of unimpaired sensory inputs. These are probably integrated at telencephalic and thalamic levels. When one taste system and smell are seriously affected or eliminated, we even observed an effect known from actual forebrain removal: visual stimuli become of greater and sometimes overriding importance

as seen in social feeding behavior. Taste deficiencies have effects upon social behavior but it does not necessarily follow that taste is used in interactions with species mates.

It is likely that the ablations, besides specific effects upon directly related braincenters, have farther reaching non-specific effects upon the performance of even remote brain areas. Fiedler (1967) has followed ablation effects with histological techniques and found extensive trans-neuronal degeneration a few weeks after forebrain removal. The brain must be seen as an entity, despite the fact that in fishes several centers seem to operate as rather independent sub-units. The independence is much greater in the medullar region than elsewhere, as demonstrated by the localized effects of facial and vagal lobe ablations, and also by the fact that forebrain ablation hardly affected feeding. The brain functioning as an entity is most prominent in the forebrain -- and thalamic area -- where deficits in sensory inputs are relayed and therefore become apparent in forebrain regulated behavior, such as the complex social behavior of the bullhead.

SUMMARY

A. A first report is given on the behavior of experimentally "tasteless" fishes. Lesions in the brain selectively eliminated either one or the other of the two taste systems of the catfish. Changes in feeding behavior were recorded on motion pictures.

Facial lobe ablation caused serious impairment of two steps in the feeding behavior chain. 1) Localization of food was gravely affected and did not return to normal levels of performance even after several months, although gradual improvement could be observed. 2) The food pick-up reflex was absent for several weeks, improved with time and finally returned to an almost normal level of performance. 3) Alert and ingest (the first and last step in the chain) were not affected.

It is suggested that 1) The remainder of sensory taste neurons after facial lobe ablation could still be sufficient to alert the fish to the presence of food; but that a full set of functional taste receptors is required to provide the directional clues in localization. Spatial projection of the body in the facial lobe is suggested. 2) Reinforcement of tactile input to the mandibular motor neurons in the presence of remaining taste may finally result in the reappearance of the pick-up reflex.

Vagal lobe ablation exclusively blocked the last step (ingest) of the feeding behavior chain. Tactile reinforcement and the presence of remaining taste may result in the reappearance of the reflex after several weeks.

B. Individual catfishes were subjected to a series of operations that eliminated gradually the olfactory structures and the forebrain. The effects of the ablations were measured in changes in behavior as expressed in frequencies of behavior units. Agonistic social behavior was emphasized. Macroscopical and histological microscopical inspections of the brains were performed.

In the first stage a series of 10 experimental animals were each observed for one hour in their normal social interactions with a

group of 3 resident animals, after which their olfactory epithelium was destroyed, thus eliminating the olfactory receptor cells. In the second stage, one week later, the fishes were observed in their interactions with the same resident fishes, after which their olfactory tracts were severed, thus disconnecting the olfactory bulb from the telencephalon. In the third stage, one week later, the fishes were again observed. After this observation both telencephalic hemispheres were removed. In the fourth and last stage, one week later, the fishes were observed once more and killed for histological inspection of their brains. Some fishes were observed over longer periods of time. Two fishes were added to the series; these underwent 3 successive sham operations. Thereafter their forebrains were removed and their behavior observed in an additional fifth stage. Two of the experimental animals were kept for several months after forebrain removal. Some other forebrainless fishes were observed for up to 18 months.

Elimination of the olfactory receptor cells (cauterization of the olfactory epithelium) resulted in an "aggression peak" in social interactions following the operation. Additional severing of the olfactory tracts did not appreciably alter this "noseless" behavior, except that the latter operation had an effect on the general condition of the animals. Changes in general behavior after forebrain ablation are in accord with many reports in the literature. They center around lack of recognition ability, unpredictability, "laziness" and "emotional polarization."

The forebrain ablation effects are discussed in relation with comparable ablations in mammals and remarkable similarities are pointed out.

Normal behavior is restored after several months without regeneration of the forebrain.

C. The effects of chemosensory and forebrain eliminations were tested for their specificity by subjecting taste deficient animals to social interactions and smell deficient and forebrain ablated animals to feeding tests.

Blocking the olfactory input and even removal of the telencephalon had no effect upon feeding. Taste deficient animals, however, showed

minor changes in social behavior. These changes increased in effect when the olfactory input was blocked in addition to one gustatory input.

It was concluded that feeding is relatively simple reflex behavior, steered in the catfish by two distinct taste systems and by the tactile system. Social behavior, in contrast, requires the fully intact brain in the presence of all sensory inputs, even those that may not be used specifically to gain information about the social situation. The forebrain contains the integration centers and it balances social behavior.

TABLE II, 2

NAME	SEX	WEIGHT(g) / LENGTH(mm)	DATE OF OPERATION	DATE KILLED	HISTOLOGICAL POST MORTEM INSPECTION OF BRAIN	GENERAL REMARKS
TL_1	0	250/262	6 I 69	8 III 69	tracts under facial lobes left intact, slight unilateral vagal damage, slight cerebellar damage, total facial lobe ablated	bleeding from sinus. after operation hangs to right side (posterior semicircular canal damaged and ramus recurrens cut), 3 week recovery. 10% of frontal lamellae regenerated.
TL_2	0	111/218	18 II 69	3 VII 69	clean facial lobe ablation	bloodless clean ablation, no lamellae regenerated
TL_3	0	240/285	19 III 69	21 III 69	overall dorsal medulla damage	right side ramus recurrens cut, left side spinal V tract cut, bleeding, normal nose
TL_4	0	175/238	19 III 69	29 III 69	vagal damage, facial lobes intact	bleeding, normal nose
TL_5	0	151/234	29 III 69	3 VII 69	clean vagal lobe ablation, facial lobes intact	bloodless, normal nose

TABLE II, 3

FIRST REAPPEARANCE OF UNITS AFTER
FACIAL OR VAGAL LOBE ABLATION

Facial (FL) and vagal (VL) lobe ablated animals

Days after Operation

	TL_1(FL)	TL_2(FL)	TL_4(VL)	TL_5(VL)
Food response (a)	7	1	1	1
Food search (s)	7	1	1	1
Food localize (h)	7?	2	1	1
Appearance motor patterns chew (cw)	-	9	-	-
Appearance motor patterns ingest (i)	-	9	-	-
Food pick up (pu)	17	28	1	1
Food spit (sp)	-	-	1	1
Food ingest (i)	17	28	-	?
Easy feeding (a, s, h, pu, i)	28	*35	-	±35
Nose cauterization day	50	34	-	-
Feeding afterwards	52	35	-	-

*After nose ablation

TABLE II, 4

	TL_1(FL)	TL_2(FL)	TL_3(FL)	TL_4(VL)	TL_5(VL)
Mouth opening difficulties	+	+	+	+	+
Opercular difficulties	-	-	-	+	(-)
Balance difficulties	+	(+)	+	-	-
Shark-like swimming	-	+	0	+	+
Gulp-spit air	-	(+)	-	+	+

TABLE III, 1

LIST OF EXPERIMENTAL FISH

INCLUDING POST MORTEM OBSERVATIONS

Fish No.	Sex	Weight(g) / Total Length(mm)	Date of First Introduction	Interaction with Community No.	Date Killed
0		300/290	---	---	2 VII 69
1		200/260	21 VII 68	1	3 VII 69
2		160/240	27 IX 68	1	22 X 68
3		155/240	4 X 68	2	13 XI 68
4		185/255	4 XI 68	2	25 XI 68
5		130/230	5 XII 68	2	3 I 69
6		185/255	1 XI 68	3	25 XI 68
7		175/245	1 XII 68	3	7 I 69
8		135/230	5 II 69	4	27 II 69
9		170/235	21 II 69	5	1 VII 69

TABLE III, 1 (Continued)

No.	Brain Post Mortem	General
0	1 mm of left caudal hemisphere present; right pre-optic area intact. (See Figure III, 14).	olfactory lamellae normal, no degeneration apparent macroscopically.
1	complete ablation, foamy tissue fills braincase, especially in forebrain area.	minor irregular lamellae found in rostral olfactory sac; slight infection after second operation.
2	complete ablation, one side of optic tectum slightly damaged.	small frontal part of olfactory lamellae intact; antibiotics treatment 2 days after second operation; did not feed prior to 4th observation hour.
3	2.5 mm of left caudal hemisphere present, with only slight dorsal damage; right side only pre-optic area present.	difficulty feeding 2 days prior to 3rd observation hour; did not feed prior to 4th observation hour.
4	complete ablation, degenerated pre-optic area present.	
5	complete ablation.	
6	complete ablation	
7	complete ablation; epithalamus (and dorsal thalamus) slightly damaged and degenerated.	
8	complete ablation, right pre-optic area present; on left long ventral strip of non-nervous tissue present; epithalamus degenerated.	
9	0.5 mm of left caudal hemisphere present (pre-optic area)	partial regeneration of lamellae at rostral end on both sides.

(cont.)

TABLE III, 1 (Continued)

Fish No.	Sex	Weight (g) / Total Length (mm)	Date of First Introduction	Interaction with Community No.	Date Killed
10		$\frac{175}{232}$	10 III 69	6	2 VII 69
I		$\frac{180}{240}$	6 I 69	2	5 II 69
II		$\frac{160}{240}$	2 I 69	3	3 II 69

TABLE III, 1 (Continued)

No.	Brain Post Mortem	General
10	complete ablation, ventral pre-optic area intact	
I	1.5 mm of right caudal hemisphere present, with slight dorso-medial damage, left side pre-optic area present	olfactory lamellae normal
II	complete ablation, pre-optic area largely intact	olfactory lamellae normal

TABLE III, 2

DEFINITION OF BEHAVIOR UNITS* AND THEIR SYMBOLS

1. (A) attack: an unspecific unit, including ap, ch, MD, N, Bi in any combination.
2. (ap) approach: cr directed toward another fish.
3. (av) avoid: turn and cr away from another fish; intermediate between lv and fl.
4. (Bi) bite: similar to N, but mouth wider open, longer contact with the other fish.
5. (C) collide: occurs when the swimming paths of two fish accidentally intercept.
6. (ch) chase: usually fast and persistant fw of another fish accompanied by aggressive units (MD). Figure III, 17.
7. (cr) cruise: neutral swimming.
8. (E) enter: going into a shelter.
9. (exp) explore: repeatedly cr around an area or pot with barbels in contact with objects; "curious" behavior. Figure III, 17.
10. (fl) flee: fast moving away from another fish.
11. (fw) follow: slowly going after a fish without aggressive displays; "curious" behavior.
12. (lv) leave: moving away from a pot, place or fish.
13. (MD) mouth display: opened mouth to various degrees, directed toward a fish. Figures III, 15 and 17.
14. (MF) mouth fight: two fish grab each other by the jaw and push-pull. Figure III, 16.
15. (N) nip: short mouth contact with part of another fish, see Bi.
16. (p) push: lateral or frontal (attempt to) displacement of another fish.

*Adapted from Todd, 1968.

(cont.)

TABLE III, 2 (Continued)

17. (Q) quiver: lateral head and body vibration.

18. (R) roll: tilt about longitudinal axis, occurs as a defensive lateral display and as preceding Th. Figure III, 15.

19. (RP) resting position: parts of ventral side of fish in contact with substrate without moving.

20. (strep) stress pump: high intensity breathing.

21. (t) touch: (frontal) contact with another fish.

22. (Th) thrust: lateral head and body swings aimed at the opponent, sometimes at significant objects, such as pots. Figure III, 15.

23. (Θ) no response: used when after an ap or display the other fish does not react in any obvious way.

P 1, 2, 3, 4: symbols for the pots and other shelters

α, β and γ : symbols for the fish in the community according to dominance.

TABLE III, 3

CLASSIFICATION OF BEHAVIOR UNITS

Behavior units are ordered in classes according to their aggressive contents, and are weighted accordingly (+6 through -2). The classes are lumped together in groups of units.

GROUP	CLASS	UNIT SYMBOLS
"aggressive"	+6	MF
	+5	A, Bi, ch, N, Q
	+4	R, Th
	+3	MD
"curious"	+2	ap, p
	+1	fw, t
"non-social"	0	C, cr, E, exp, lv, RP, strep
"escape"	-1	av
	-2	fl

TABLE III, 4

UNIT FREQUENCY IN 11 BEHAVIOR CLASSES FOR THE 5 OBSERVATION HOURS, LISTED FOR INDIVIDUAL FISH

Unit Fish No.	Classes: Ob. Hr. No.	6	5 aggr	4	3	2 ap	1 fw	0 exp	0 RP	0 strep	-1 av	-2 flee
1	1	1	2	7	5	2	1	2	0		4	31
	2	1	0	20	8	11	0	1	2		0	24
	3	0	0	4	0	1	0	1	11		0	55
	4	41	8	28	11	2	0	0	0		1	5
2	1	0	0	7	0	0	0	1	1	1	0	23
	2	0	0	0	0	1	2	0	4	6	19	10
	3	0	0	0	0	15	0	9	9	10	12	3
	4	0	0	0	0	2	0	1	20	3	1	1
3	1	2	1	7	10	7	1	4	0	1	3	2
	2	0	0	0	0	10	2	3	0	0	6	4
	3	0	0	9	3	13	2	0	1	5	7	2
	4	0	9	18	9	7	2	0	19	10	0	0
4	1	0	19	37	31	4	5	6	0	1	0	0
	2	0	14	49	45	8	3	6	0	0	13	4
	3	0	16	32	45	4	1	6	0	0	11	21
	4	0	4	24	5	12	2	4	0	0	11	16
5	1	1	6	19	21	12	0	11	3	0	7	4
	2	0	4	12	10	8	0	3	15	3	14	24
	3	0	11	15	23	6	0	2	9	1	14	11
	4	1	0	8	2	3	0	1	23	10	0	22
6	1	0	0	5	2	18	7	12	5	0	0	0
	2	0	0	8	17	17	2	4	7	4	0	0
	3	0	0	1	0	11	1	3	9	6	18	17
	4	0	7	7	14	4	0	0	19	1	2	8
7	1	0	0	41	19	15	3	4	0	1	9	0
	2	0	3	12	15	18	1	0	5	1	0	0
	3	0	4	16	15	17	3	0	15	8	0	0
	4	0	0	1	0	0	0	0	30	2	1	0
8	1	0	0	1	0	35	27	7	5	1	2	0
	2	0	0	6	3	23	8	3	2	0	1	0
	3	0	0	2	0	22	5	6	8	0	3	0
	4	1	9	20	16	13	1	0	12	0	10	1

(cont.)

TABLE III, 4 (Continued)

		6	5	4	3	2	1	0	0	0	-1	-2
			aggr			ap	fw	exp	RP	strep	av	flee
9	1	0	1	4	6	12	3	4	1	0	0	0
	2	0	6	13	24	18	2	7	5	0	0	0
	3	0	9	20	26	29	2	2	12	1	3	4
	4	0	5	7	7	24	0	0	31	1	1	11
	*5	5	25	12	15	40	11	2	0	13	0	1
10	1	0	3	10	18	12	5	1	28	1	4	38
	2	5	2	4	3	2	0	1	5	0	4	41
	3	0	0	1	0	1	0	0	41	3	1	37
	4	0	2	5	6	1	0	0	14	4	4	6
	*5	1	0	5	5	5	1	0	37	28	24	61
I	1	0	1	10	4	17	1	3	12	0	1	0
	2	2	0	6	9	17	1	2	5	0	2	1
	3	0	0	1	0	13	0	2	2	0	14	34
	4	0	0	0	0	10	0	1	17	0	6	19
	5	2	12	22	28	3	1	0	23	0	0	11
II	1	0	0	1	2	14	1	6	17	3	0	0
	2	0	0	0	0	2	0	2	20	6	1	0
	3	0	0	0	0	8	0	2	25	3	2	0
	4	0	0	0	0	6	0	1	23	2	0	0
	5	0	2	0	1	0	0	0	30	0	0	0

*Later observation

TABLE III, 5

AVERAGE UNIT FREQUENCY IN 11 BEHAVIOR UNIT CLASSES FOR THE 5 OBSERVATION HOURS

Observation Hours	Behavoir Unit Class Nos.										
	6	5	4	3	2	1	0(Exp)	0(RP)	0(Strep)	-1	-2
1	*.2 0	3.2 .5	13.8 5.5	11.1 3	11.7 15.5	5.2 1	5.2 4.5	4.3 14.5	.67 1.5	2.9 .5	9.8 0
2	.7 1	2.9 0	12.4 3	11.5 4.5	11.6 9.5	2 .5	2.8 2	4.3 12.5	1.56 3	5.4 1.5	10.7 .5
3	0 0	4 0	10 .5	11.2 0	11.9 10.5	1.4 0	2.1 2.5	11.5 13.5	3.8 1.5	6.9 8	15 17
4	8.3 0	4.4 0	11.8 0	6 0	7.7 8	.5 0	.6 1	16.8 20	3.44 1	3.1 3	7 9.5
5	1	7	11	14.5	1.5	.5	0	26.5	0	0	5.5

*Operated fish at left, sham operated fish at right of each column

TABLE III, 6

AVERAGE UNIT FREQUENCY IN 3 BEHAVIOR GROUPS FOR THE 5 OBSERVATION HOURS

Observation hours	Behavior Groups A "Aggressive" Behavior		B "Curious" Behavior		C "Escape" Behavior	
1	*32.2	9.5	16.9	16.5	12.7	.5
2	32.5	10.5	13.6	10	16.1	2
3	28.2	.5	13.3	10.5	22.7	25
4	52.5	0	7.3	8	9	12.5
5		42.5		2		5.5

*Operated fish at left, sham operated fish at right of each column

TABLE III, 7

PEAK SCORES OF INDIVIDUAL FISH FOR THE 5 OBSERVATION HOURS

The individual fish are designated with their code number. In parentheses the fish that scored equally high unit frequency peaks in more than one observation hour. Arabic numerals: operated fish; Roman numerals: sham operated fish (in column 5: forebrain ablated)

Behavior Unit Classes: Observation Hours:	1	2	3	4	5
6	3 (5)	10 (I)	---	1 (5) 8	(I)
5	4 10	---	5 7 9	1 3 6 8	I II
4	2 5 7 10 II	4 6	9	1 3 8	I
3	3 7 10 II	(4) 6	(4) 5 9	1 8	I
2	5 6 8 10 (I) II	1 7 (I)	2 3 9	4	---
1	1 4 6 (7) 8 9 10 (I) II	2 (3) (I)	(3) (7)	(3)	I
0 (exp)	1 3 (4) 5 6 7 8 (10) I II	(4) 9 (10)	2 (4)	---	---
0 (RP)	---	---	1 10	2 3 5 6 7 8 9	I II
0 (Strep)	4 8	II	2 6 7 (9)	3 5 (9) 10	---
-1	1 7 (10)	2 4 (5) (10)	3 (5) 6 9 I II	8 (10)	---
-2	2	3 5 10	1 4 6 I	8 9	---

Figure II, 1

Distribution of taste buds on the skin of an adult catfish, Ictalurus natalis.

Scale of Grid = 1 cm. Each dot represents 100 taste buds. (Number of dots per square = number of taste buds per mm^2.) Solid black = more than 15 tastebuds per mm^2.

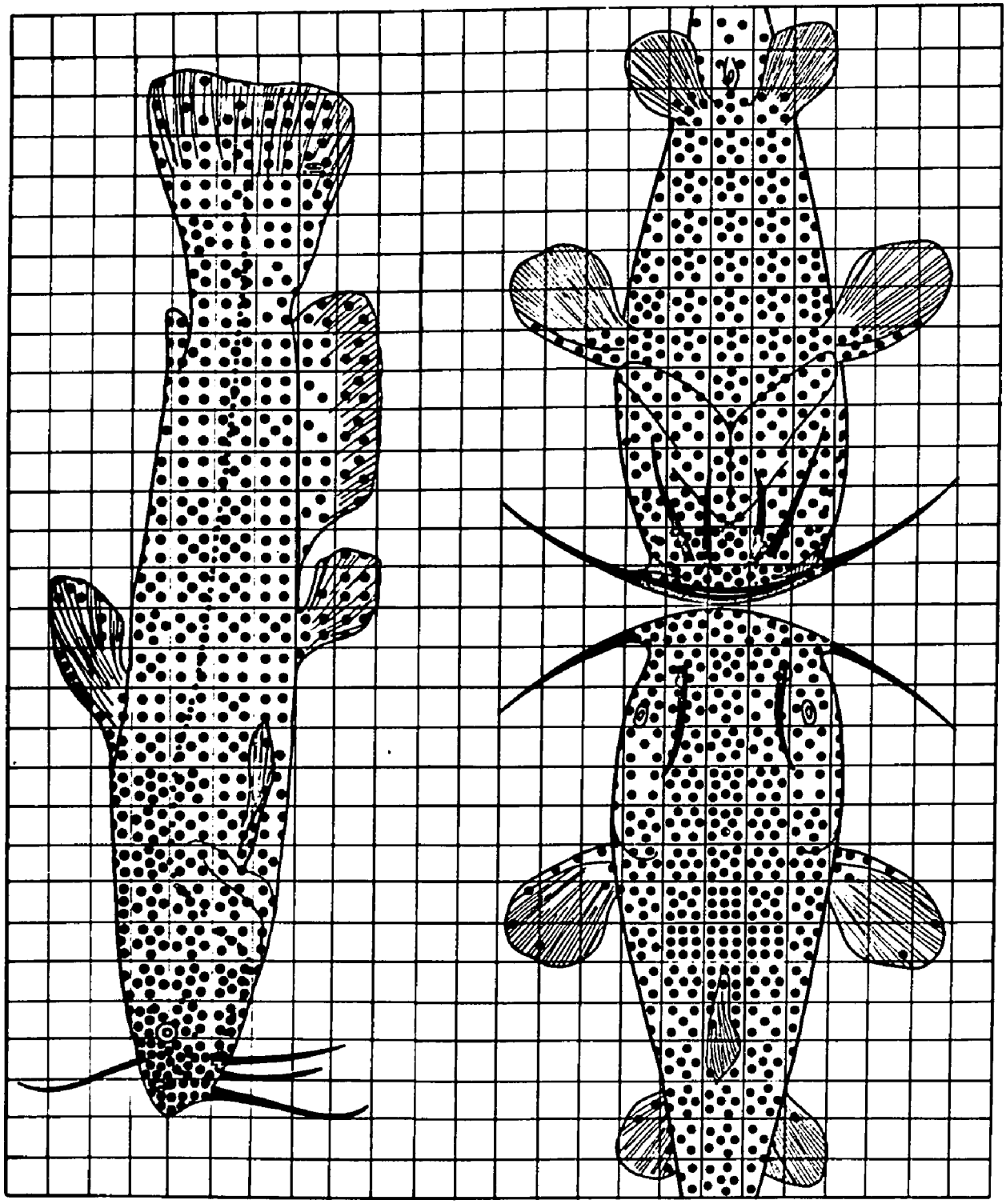


Figure II, 2

Distribution of tastebuds on head, lip and maxillary barbel.

A. 1:1 representation of taste buds (linear enlargement 5X)

B. enlargement of parts of the maxillary barbel (10X)

C. photograph of the distal end of the maxillary barbel (15X)

1. maxillary barbel; 2. inflow naris; 3. outflow naris; 4. nasal barbel; 5. pore of lateral line canal; 6. supporting cartilage; 7. branch of cranial nerves V + VII; 8. tastebud; 9. mucus with protruding taste hairs; 10. eye.

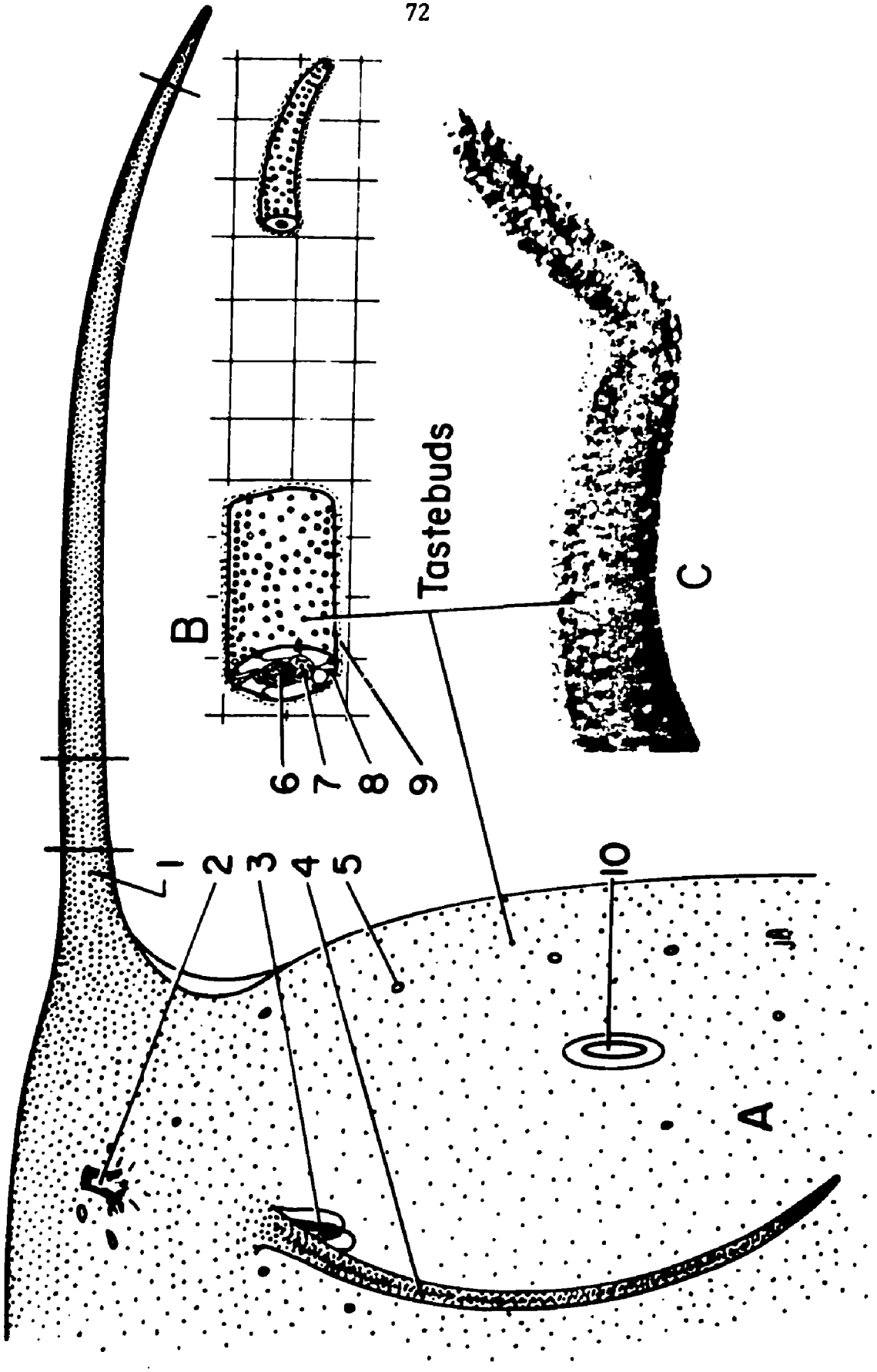

Tastebuds
B
C
A
1
2
3
4
5
6
7
8
9
10

Figure II, 3

Distribution of taste buds in the mouth and pharynx. Numbers indicate taste buds per mm^2 for that area. High concentrations on ridges are shown by arrows and attached numbers.

1, 2, 3, 4: gill arches corresponding to Figure II, 4.

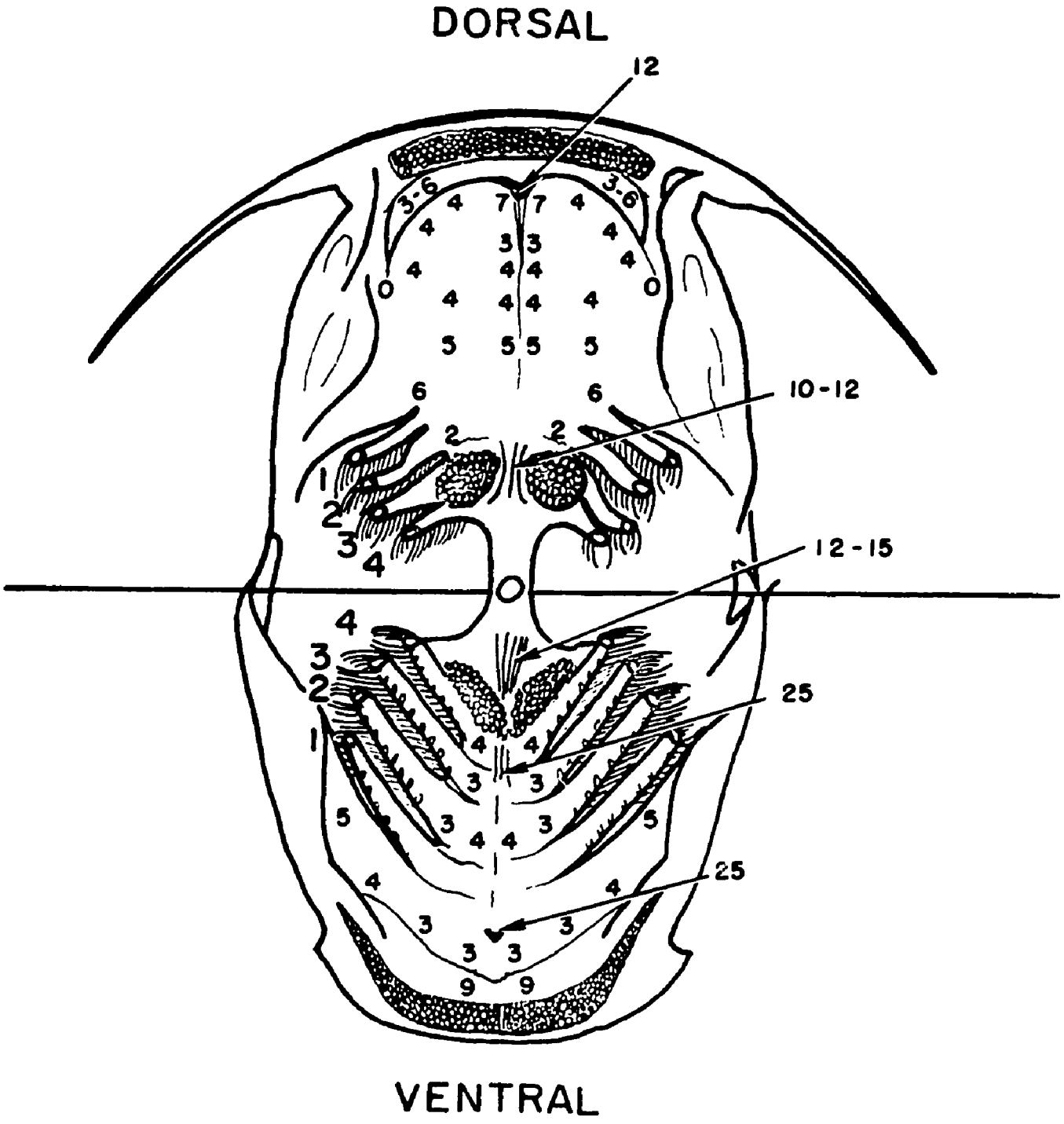

DORSAL
12
10-12
12-15
25
25
VENTRAL

Figure II, 4

Distribution of taste buds on the gill arches. Numbers indicate densities per mm^2. Frontal view of the arches at left, caudal at right. Dorsal views show the surface facing the inside of the pharynx.

1, 2, 3, 4: gill arches corresponding to Figure II, 3.

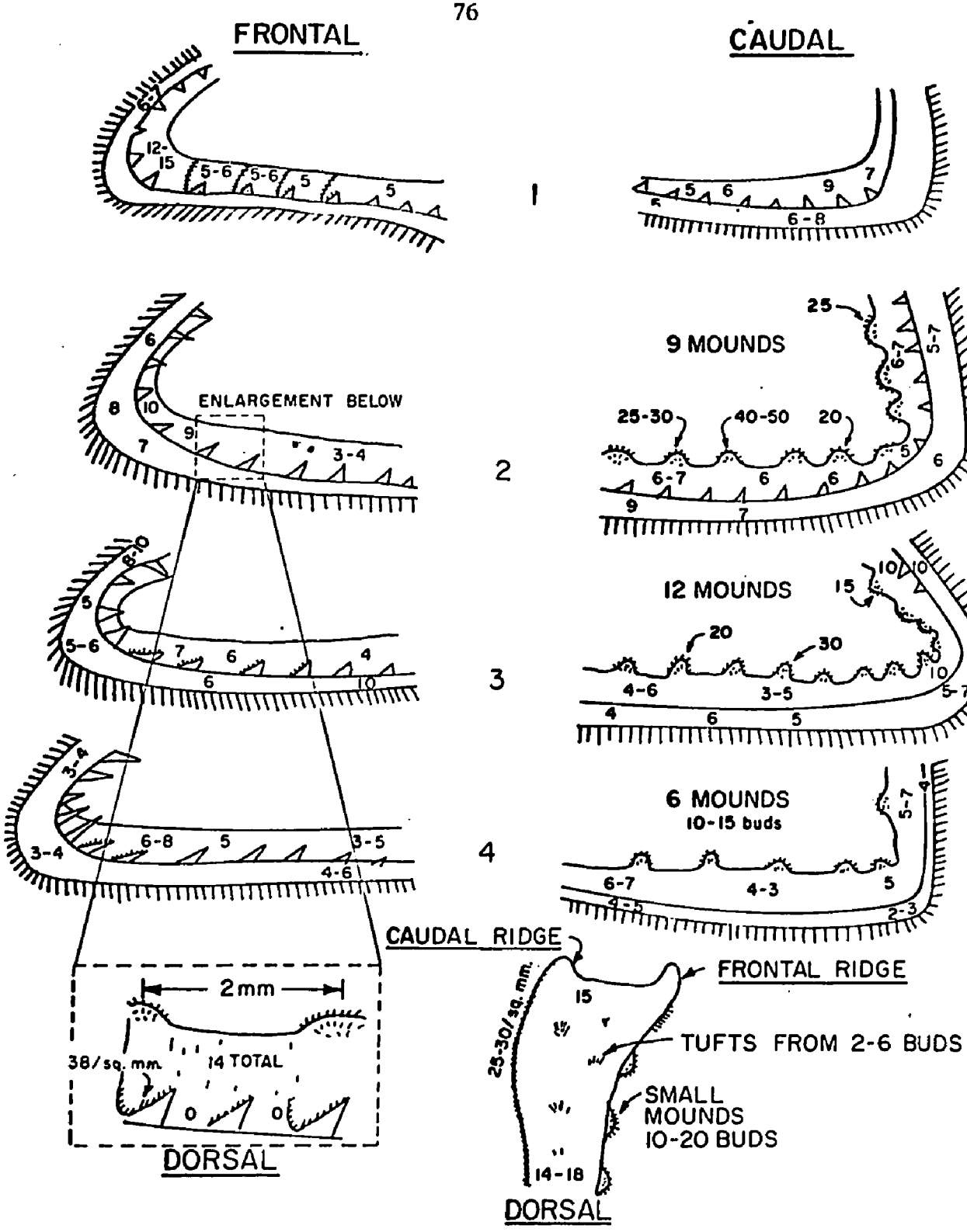

FRONTAL
CAUDAL
1
2
3
4
ENLARGEMENT BELOW
9 MOUNDS
12 MOUNDS
6 MOUNDS
10-15 buds
25-30
40-50
20
2mm
38/sq. mm.
14 TOTAL
DORSAL
CAUDAL RIDGE
FRONTAL RIDGE
TUFTS FROM 2-6 BUDS
SMALL
MOUNDS
10-20 BUDS
25-30/sq. mm.
14-18
DORSAL

Figure II, 5

A. Innervation of taste buds in the skin; 5 buds are shown; the nerves (V and VII) form a stalk under the bud and join together on their way to the brain.

B. Innervation by branches of the facial nerve (VII) of taste buds in the head region. 1. ramus recurrens; 2. ramus supra ophthalmicus superficialis; 3. ramus maxillaris; 4. ramus mandibularis; VIII. nervus acousticus; X. nervus vagus.

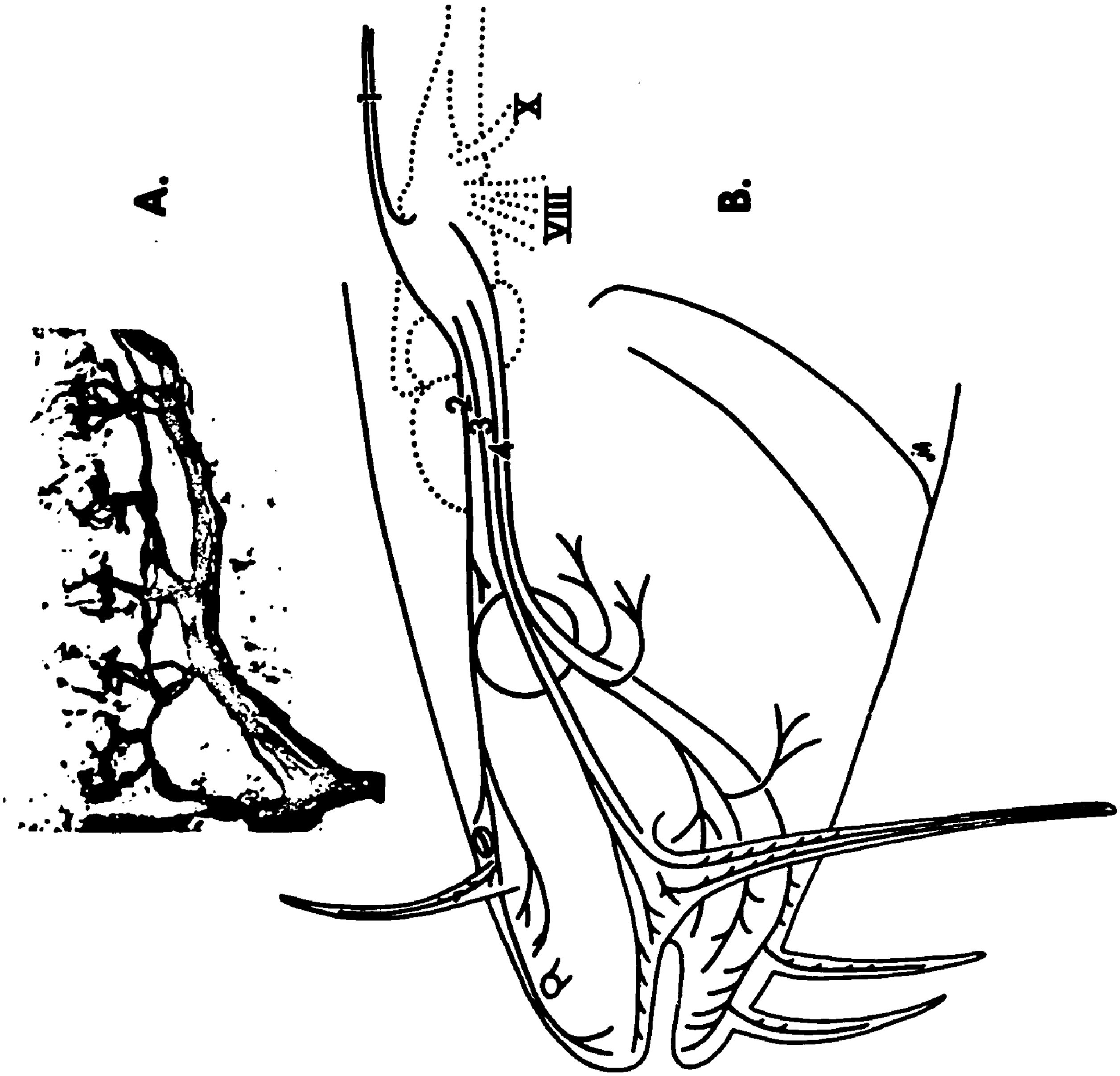

A.
B.
X
VIII
2
3
4



Figure II, 6

Lateral view of the brain (left side) showing the massive nerve trunks of the Vth and VIIth cranial nerve. The ramus recurrens VII arches back over the facial lobes. VIII, nervus acousticus.

sc. spinal cord; c. cerebellum; H. hypothalamus



Figure II, 7

Dorsal view of the brain.

1. olfactory tracts; 2. optic nerve; 3. telencephalon; 4. optic tectum; 5. cerebellum; 6. facial lobe; 7. vagal lobe; 8. funicular nuclei; 9. spinal cord; 10. labyrinth (anterior semicircular canal); 11. ramus recurrens VII; 12. vagal nerve X.

a. amygdala; p. piriform area; y. sulcus ypsiloni-formis

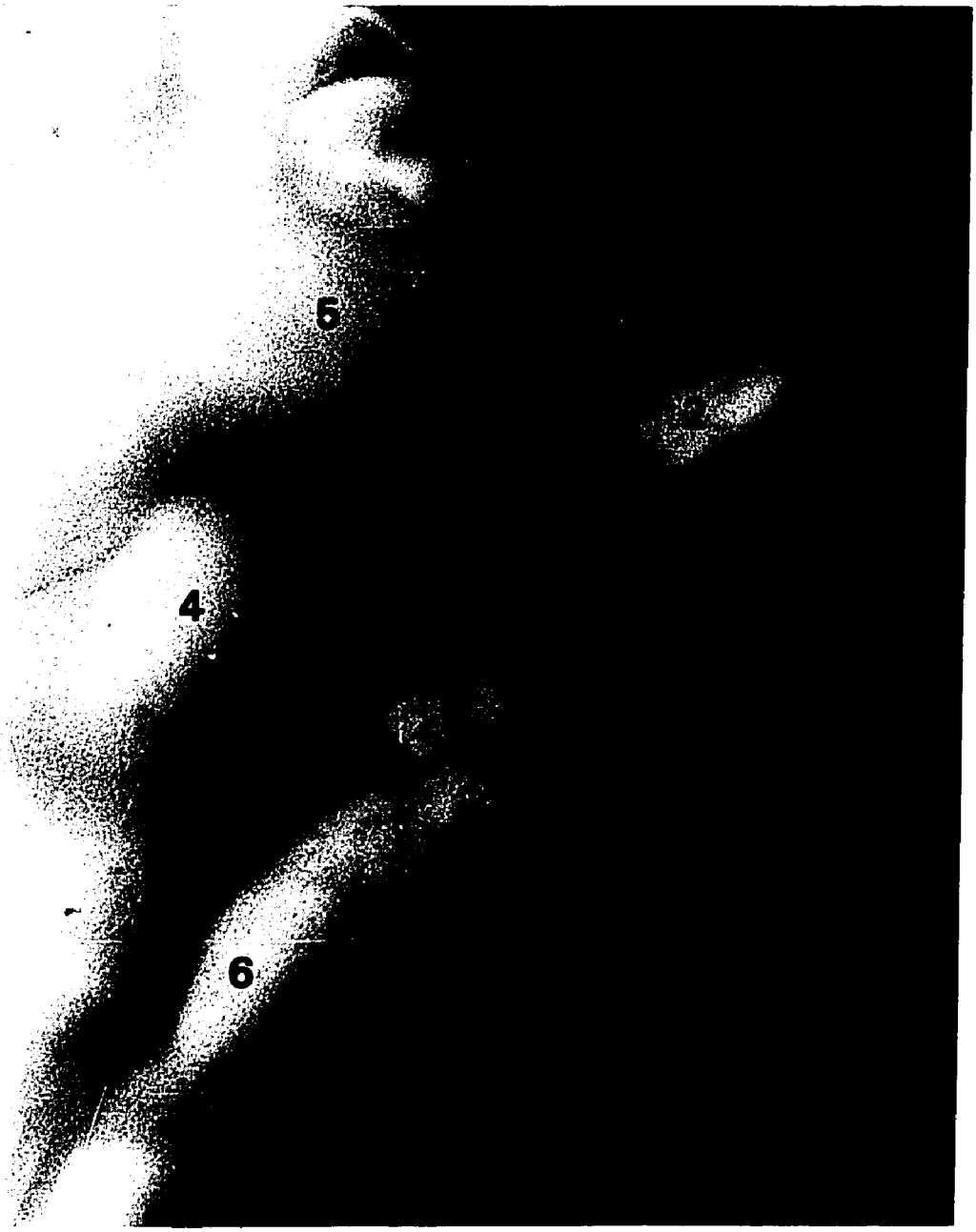
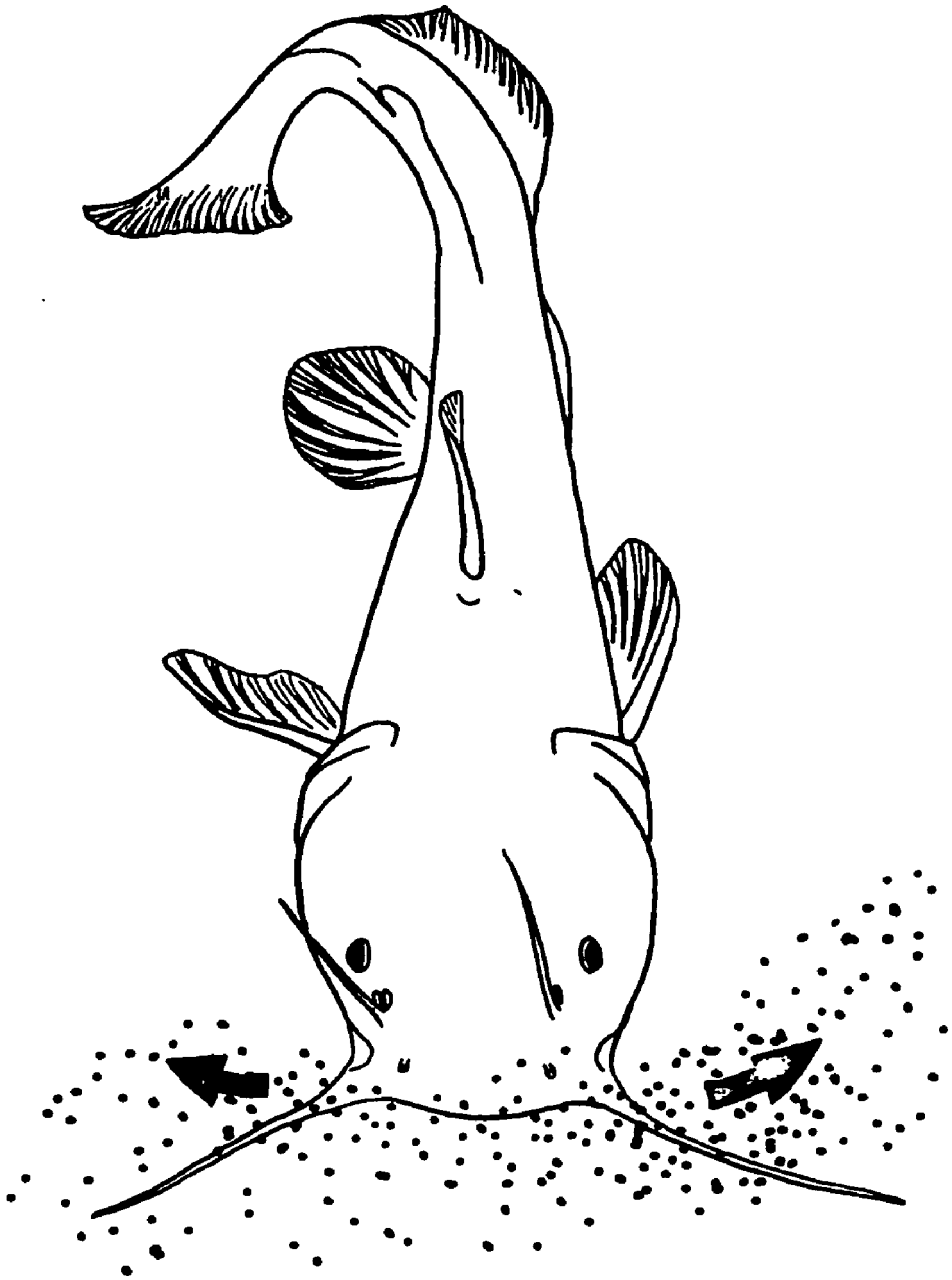


Figure II, 8

Gustatory component of the facial nerve (VII) entering the facial lobe. The gustatory component of the mixed nerves V + VII can be seen separating from the trunk just inside the brain. Part of the cerebellum is removed to show this area. The dorsal area of the right facial lobe is also removed to show how the gustatory nerve fibers curve under the cerebellum and up into the facial lobe. Left side of brain intact. (30X)

1. gustatory VII; 2. mixed trunk of cranial nerves V and VII; 3. remaining part of right facial lobe; 4. left facial lobe; 5. left side of cerebellum; 6. vagal lobes.



DIG AND HEADSTAND

Figure II, 10

Relation of brain, nose, eyes, labyrinth and some cranial nerves. Natural size.

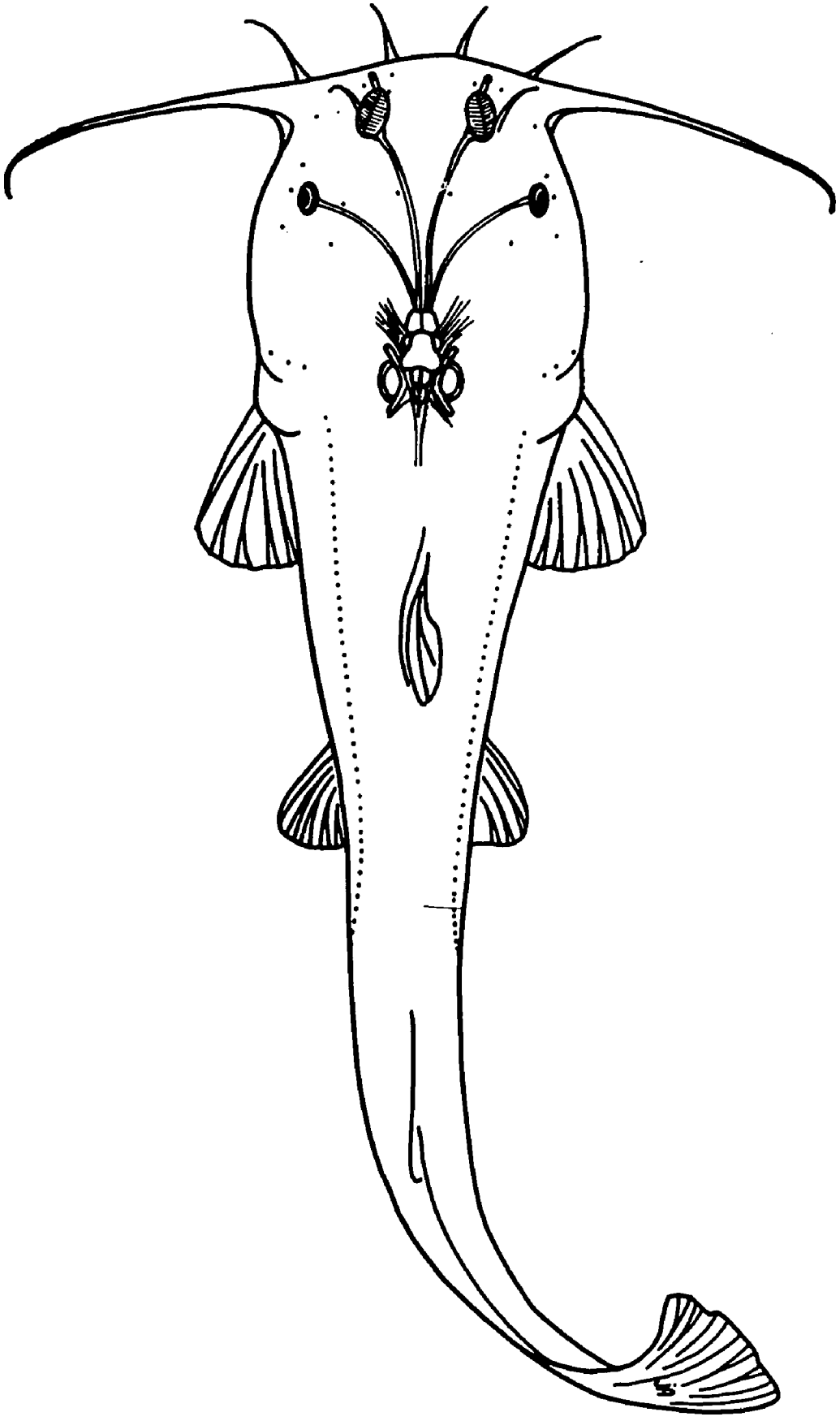


Figure II, 11

Brain in situ and operation windows

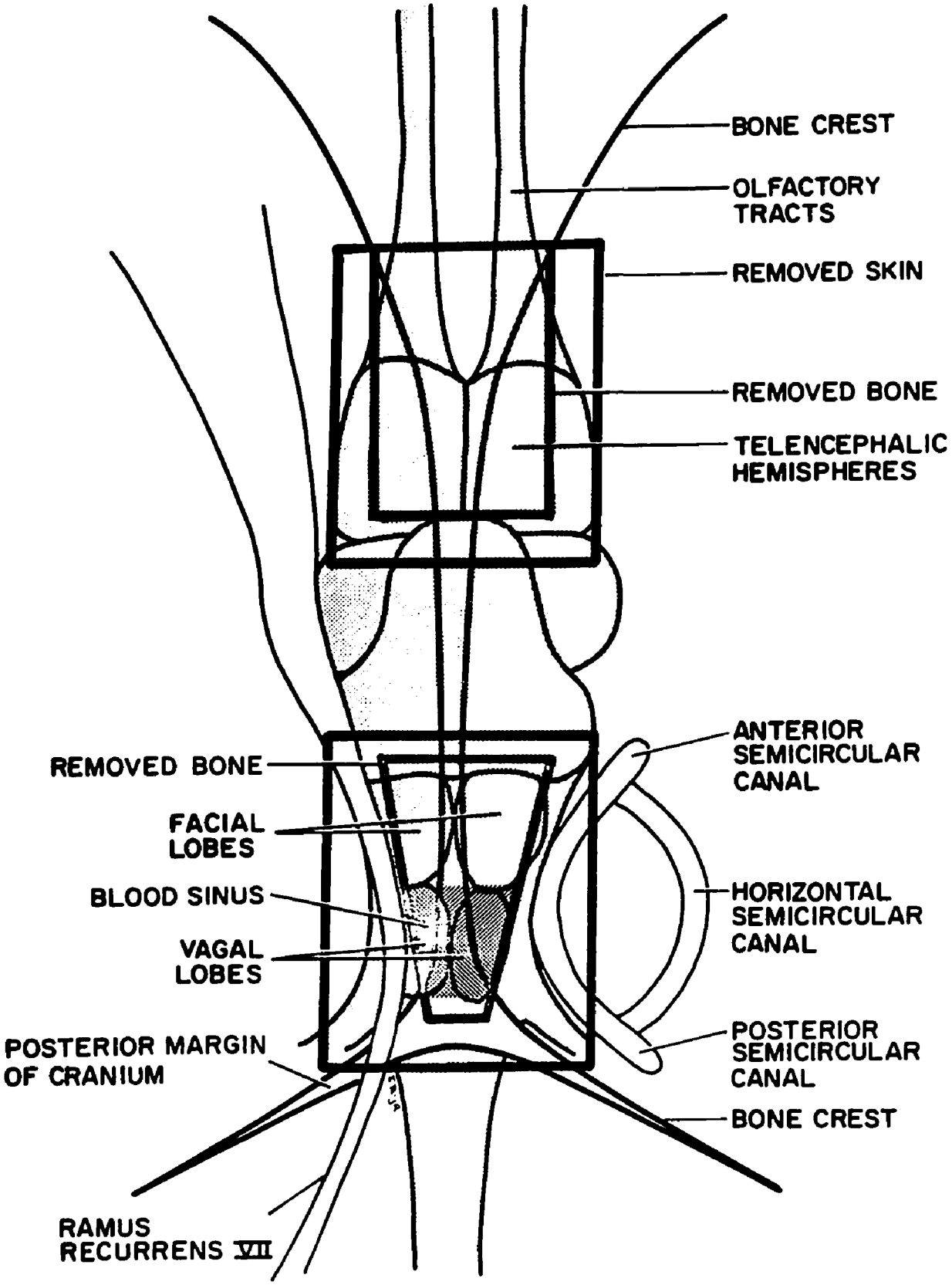

BONE CREST
OLFACTORY TRACTS
REMOVED SKIN
REMOVED BONE
TELENCEPHALIC HEMISPHERES
REMOVED BONE
FACIAL LOBES
BLOOD SINUS
VAGAL LOBES
POSTERIOR MARGIN OF CRANIUM
RAMUS RECURRENS VII
ANTERIOR SEMICIRCULAR CANAL
HORIZONTAL SEMICIRCULAR CANAL
POSTERIOR SEMICIRCULAR CANAL
BONE CREST



Figure II, 12

Operation window for facial lobe ablation. Note blood sinus (B); FL. facial lobes. (See also Figure II, 11).



Figure II, 13

Relation of brain, labyrinth and ramus recurrens VII.
A. dorsal view; B. lateral view

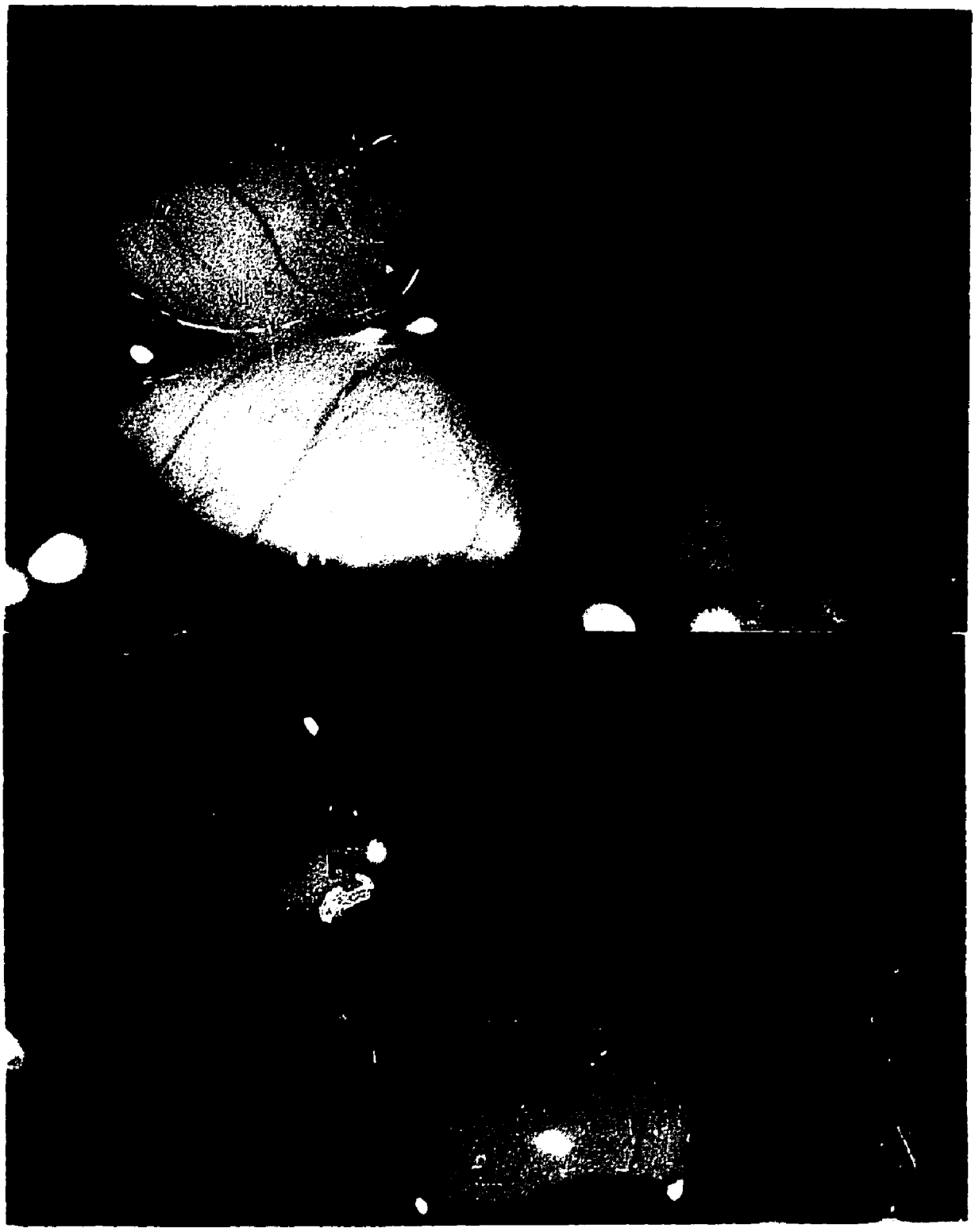


Figure II, 14

Facial lobes exposed before and after ablation.
A. normal; B. one lobe ablated

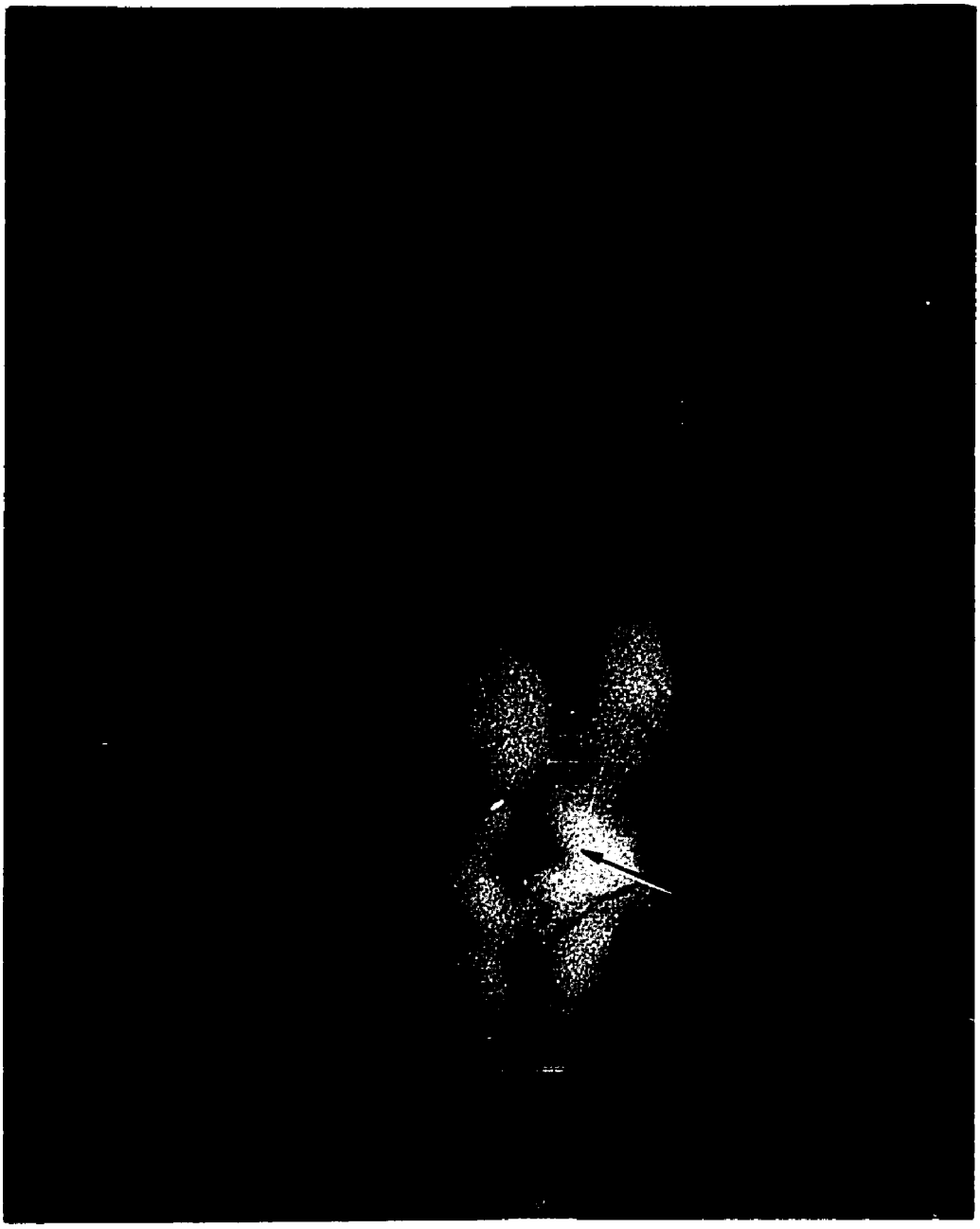


Figure II, 15

Dorsal view cf ablated vagal lobes, post mortem

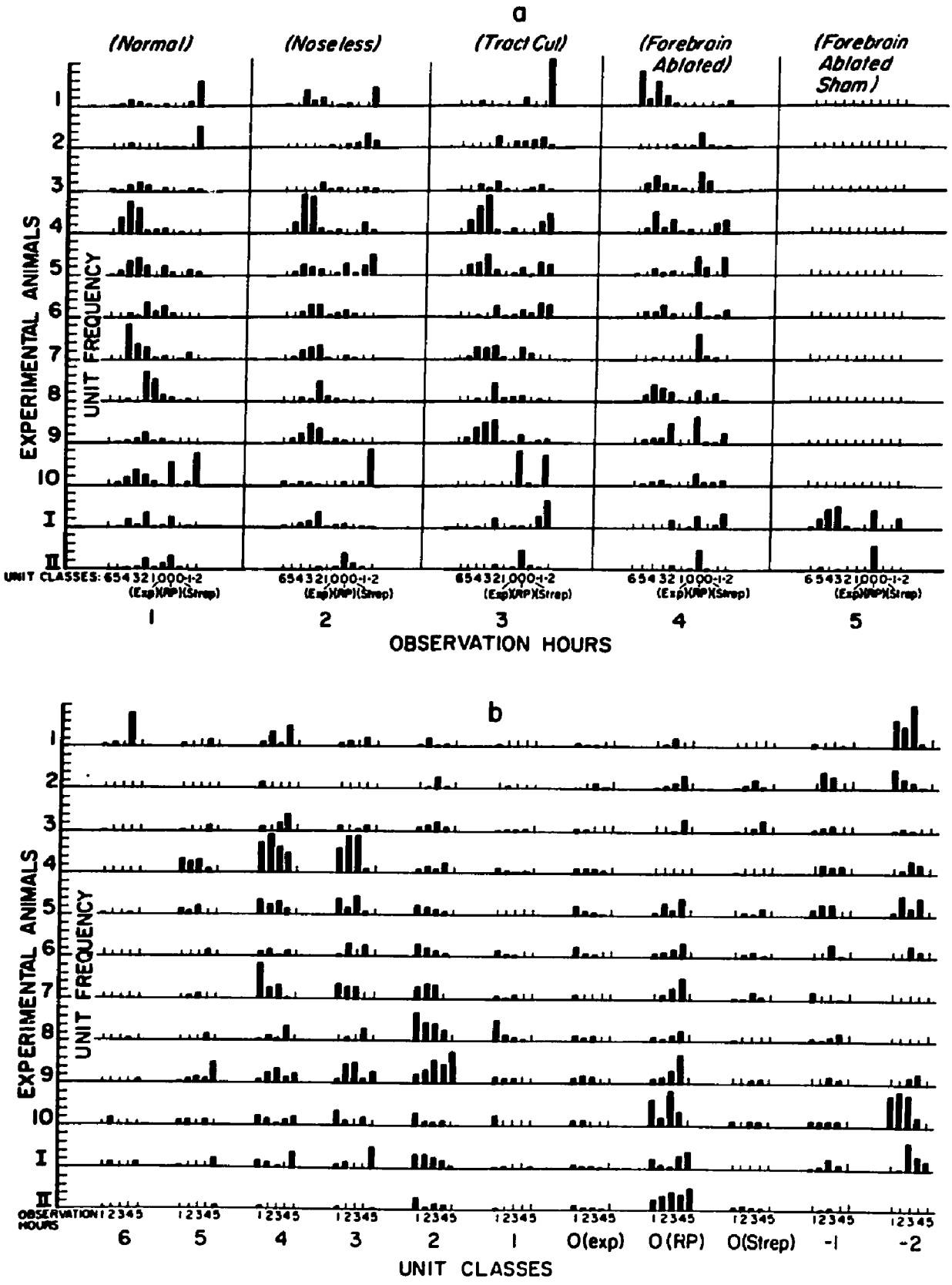


Figure III, 1 Unit Frequency of Individual Animals

a) In Each Unit Class, Grouped for the 5 Observation Hours

b) In Each Observation Hour, Grouped for the 11 Unit Classes

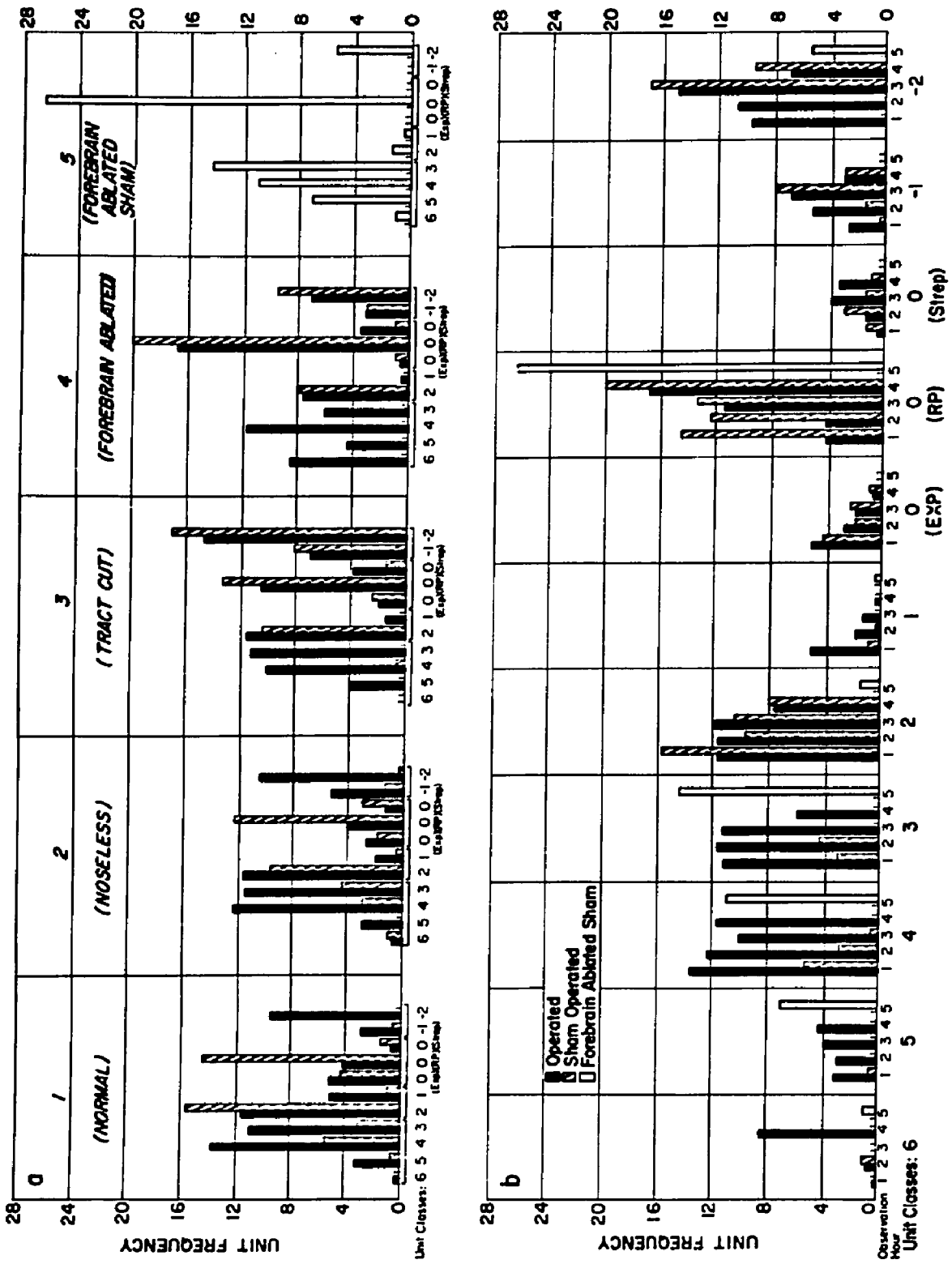


Figure III, 2 Average Unit Frequency in Behavior Classes (Operated vs. Sham Operated)
a) In Each Unit Class, Grouped for the 5 Observation Hours
b) In Each Observation Hour, Grouped for the 11 Unit Classes

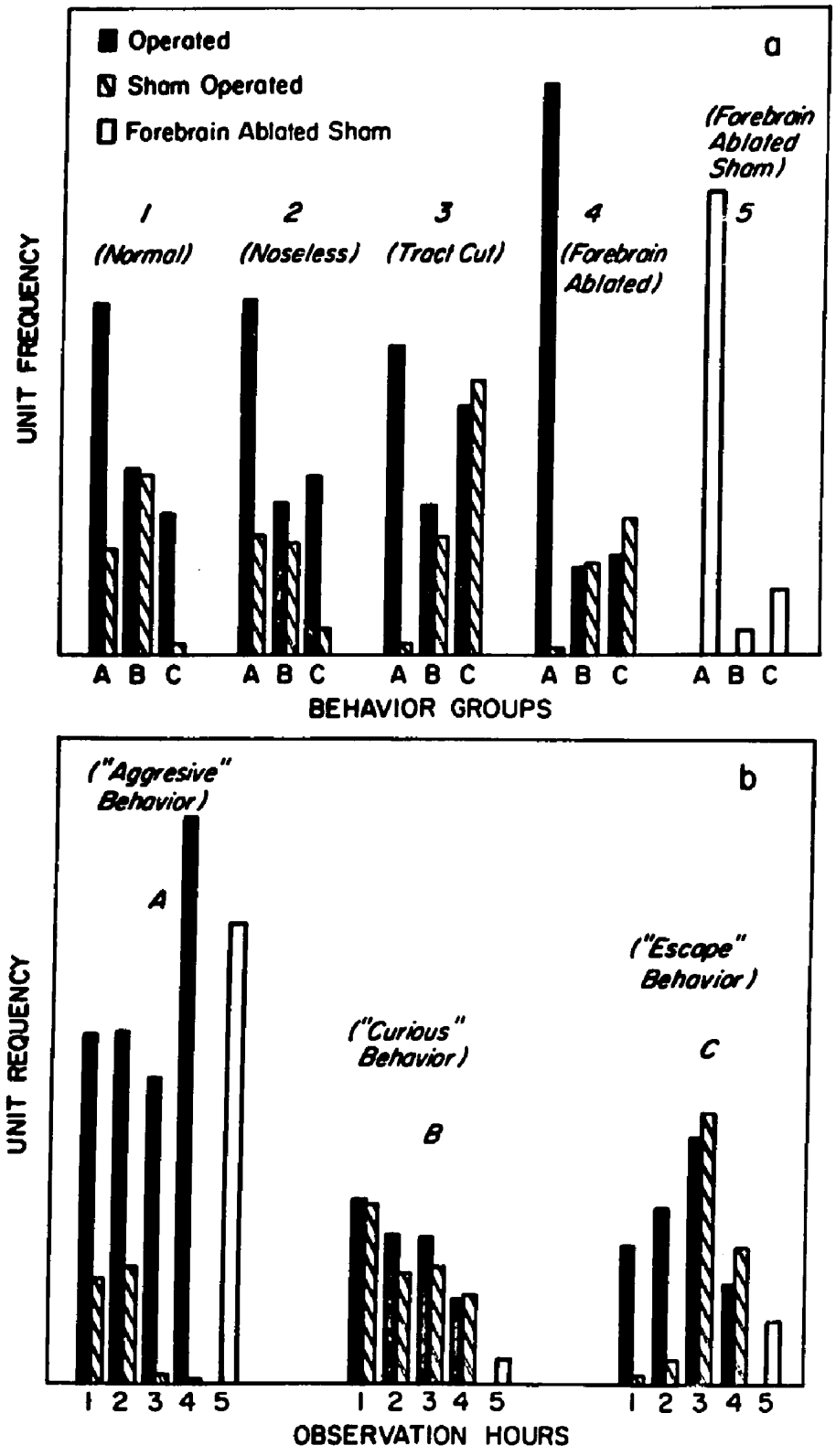


Figure III, 3 Average Unit Frequency in Behavior Groups (Operated vs. Sham Operated)
a) For the 5 Operation Hours
b) For the 3 Behavior Groups

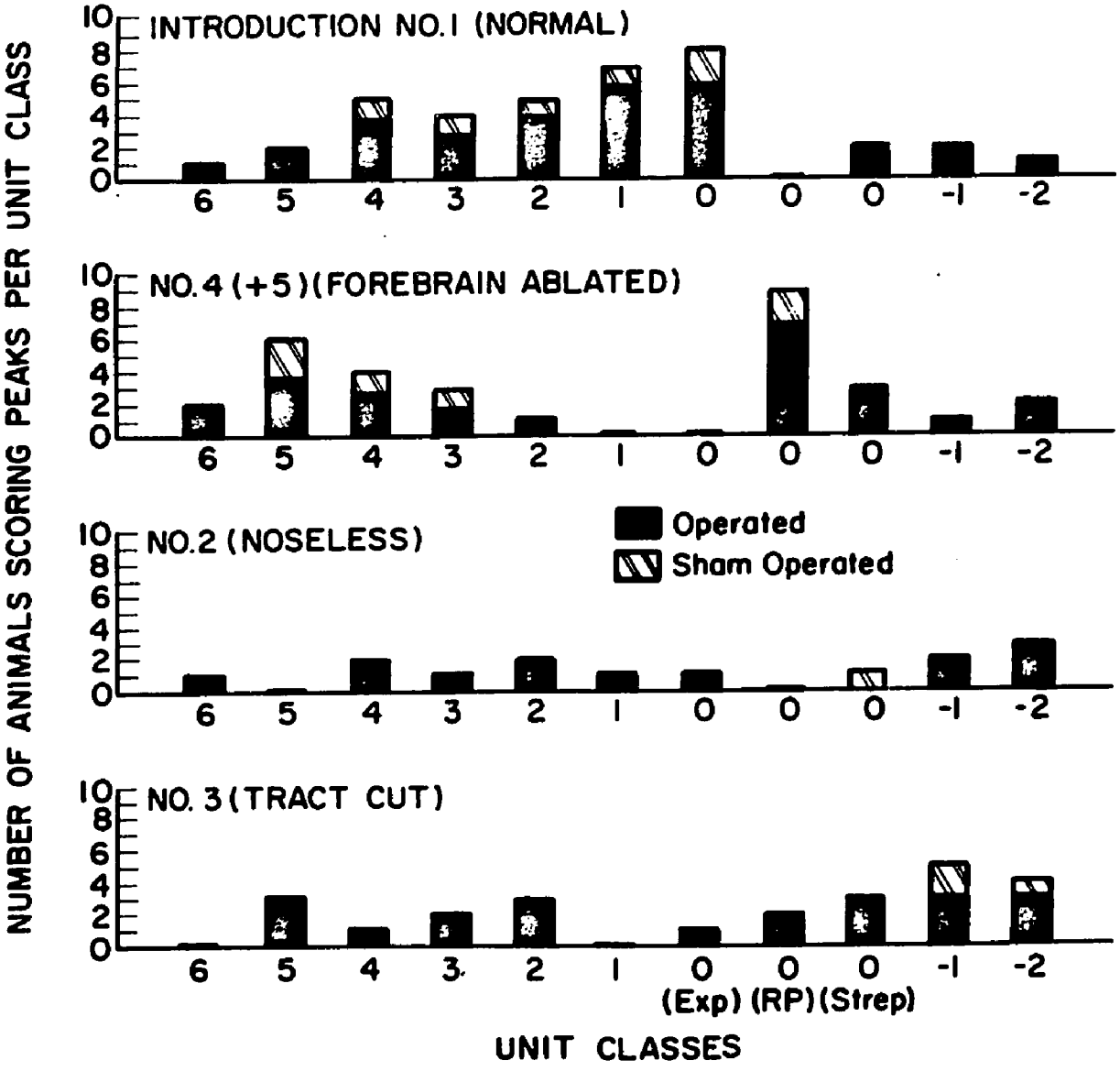


Figure III, 4 Peak Scores

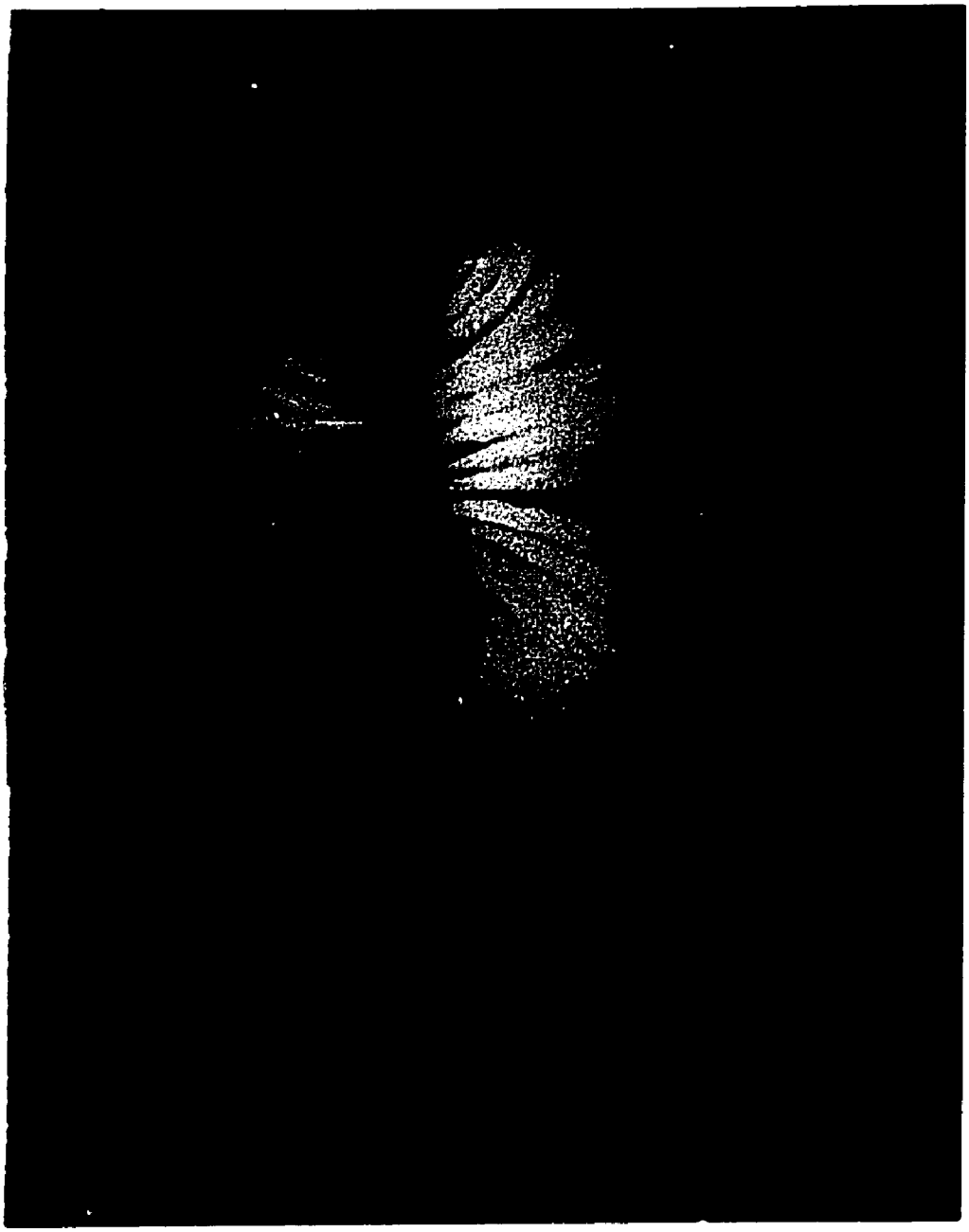


Figure III, 5

Dorsal view of a teleost olfactory "rosette". Olfactory lamellae are arranged around the central raphe. Olfactory epithelium is restricted to the lower 1/3 of the lamellae. The rest is covered with ciliar epithelium.

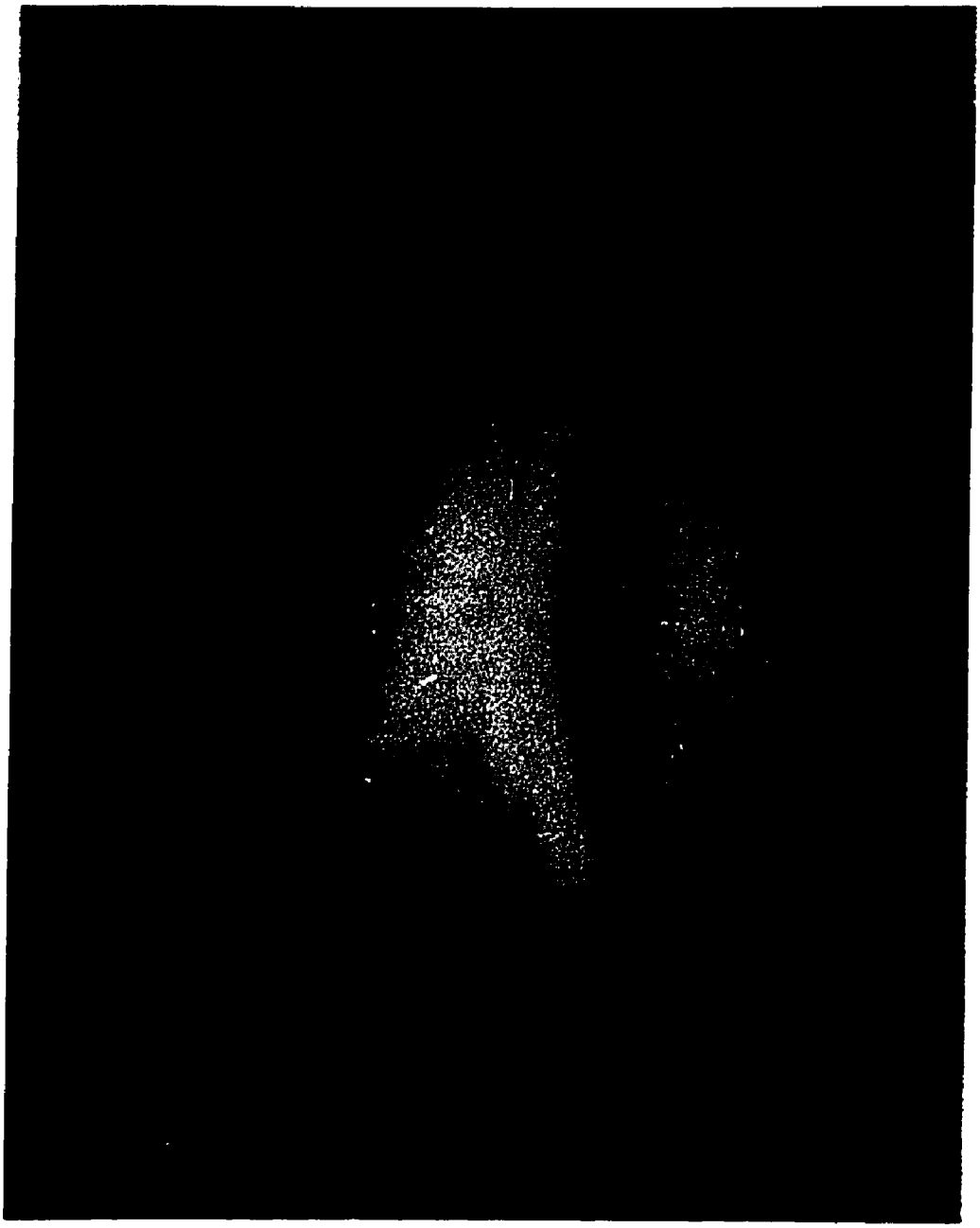


Figure III, 6

Olfactory nerve innervating epithelium. Ventral view of olfactory "rosette". The nerve is clearly divided in a medial and a lateral part.



Figure III, 7

Dorso medial and frontal view of left olfactory tracts entering left telencephalic hemisphere.

L. lateral olfactory tract; M. medial olfactory tract; p. piriform area; hc. hippocampal lobe; T. tuberculum olfactorium; AC. anterior commissure; S. septal areas.

Note ascending fibers in tubercular area (arrow).

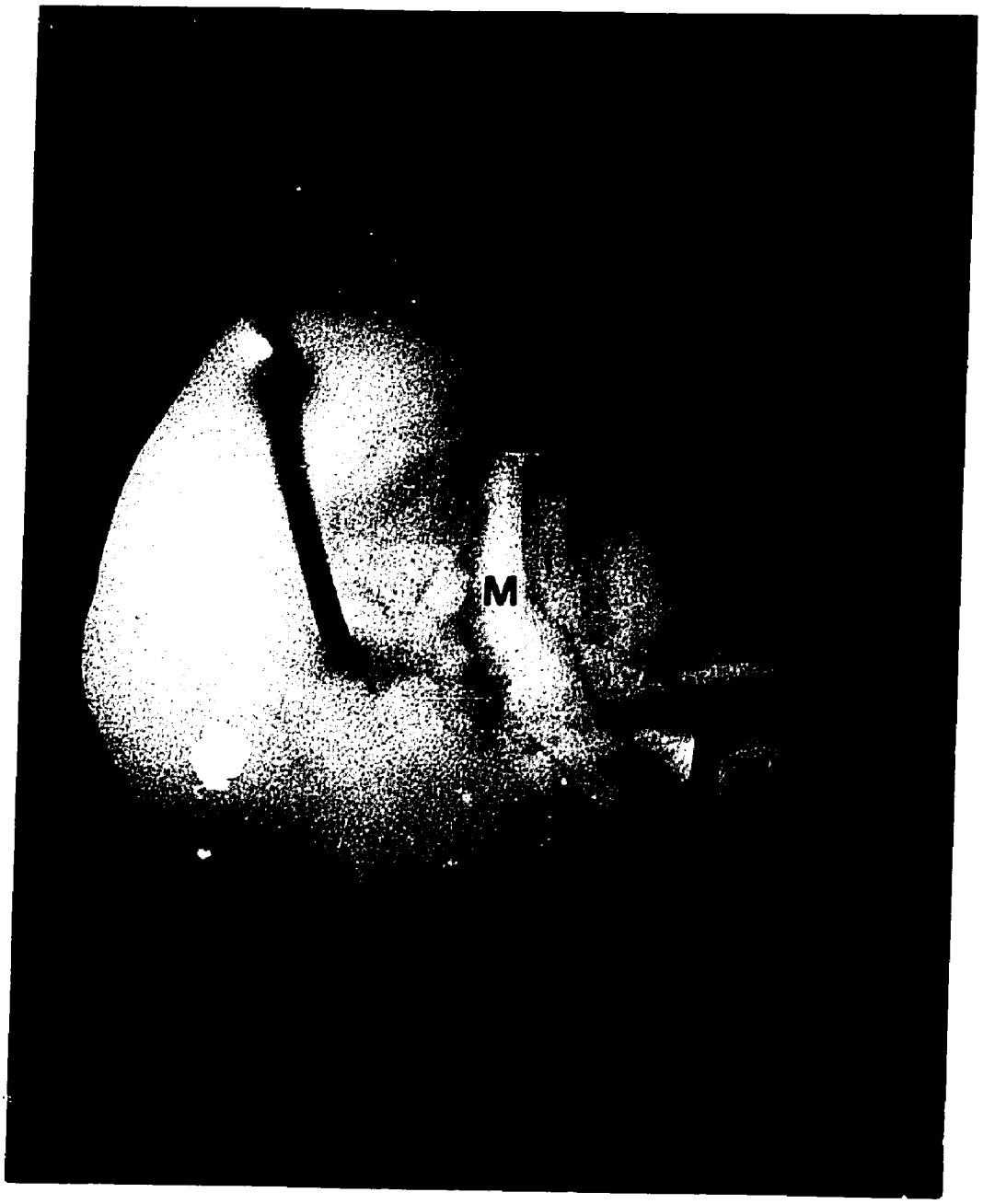


Figure III, 8

Dorso medial view of one hemisphere showing relation of olfactory tracts and forebrain areas. Hippocampal and septal areas and part of striatum and amygdala are removed.

Medial olfactory tract (M) ends in precommissural septal area. Lateral olfactory tract (L) curves over anterior commissure (AC) into amygdaloid complex (a). Note large single fibers in anterior commissure.

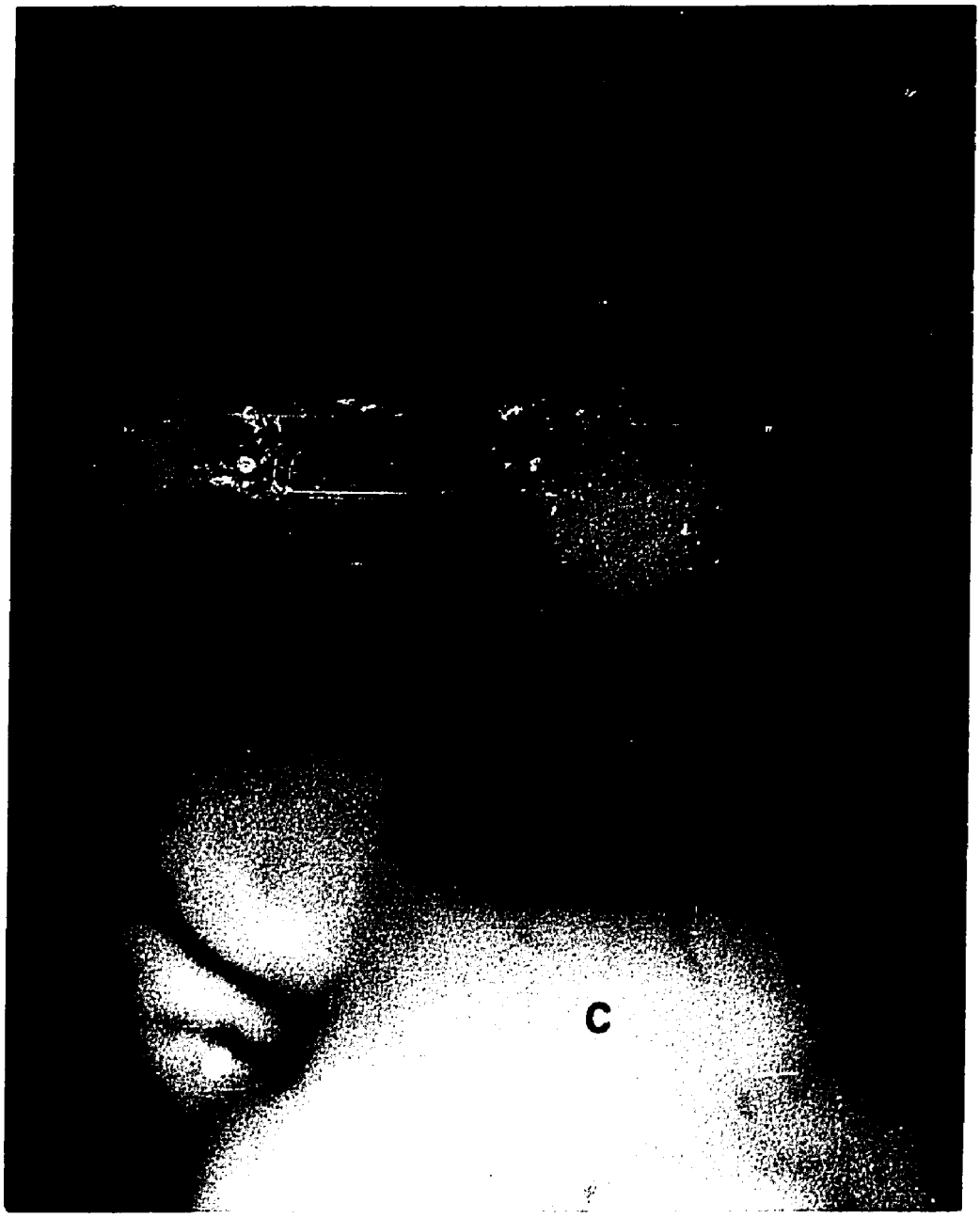


Figure III, 9

Dorso-medial view of one hemisphere showing fibers from tuberculum and septal areas ascending into dorsal area of forebrain.
AC. anterior commissure.
Hippocampal formation and part of septum and amygdala are removed.

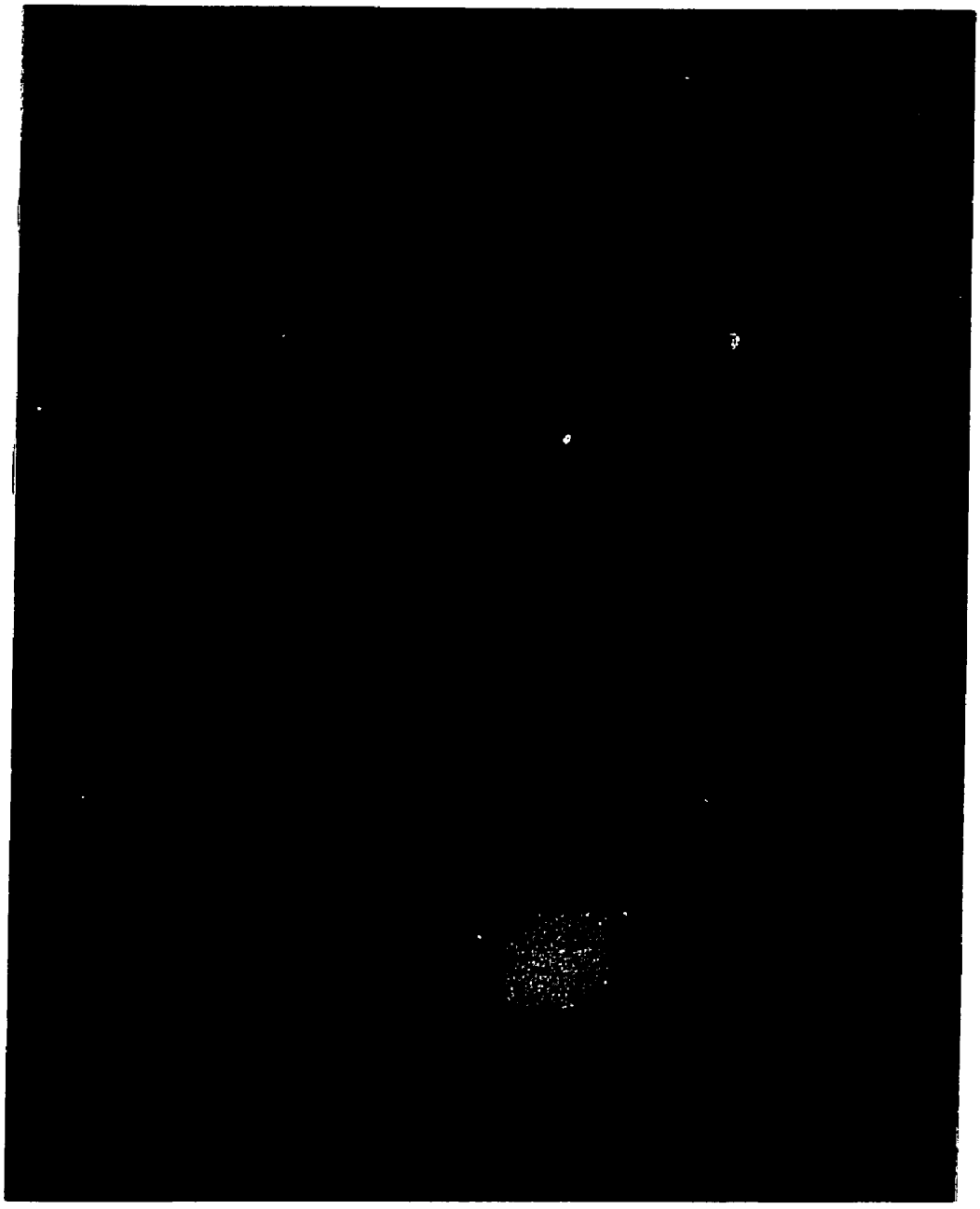


Figure III, 10

Dorsal view of hemispheres and anterior commissure (AC). Rostral lobe of cerebellum (C) removed to expose habenular nuclei (h). y. sulcus ypsiliformis; a. amygdaloid complex; p. piriform area; t. optic tectum

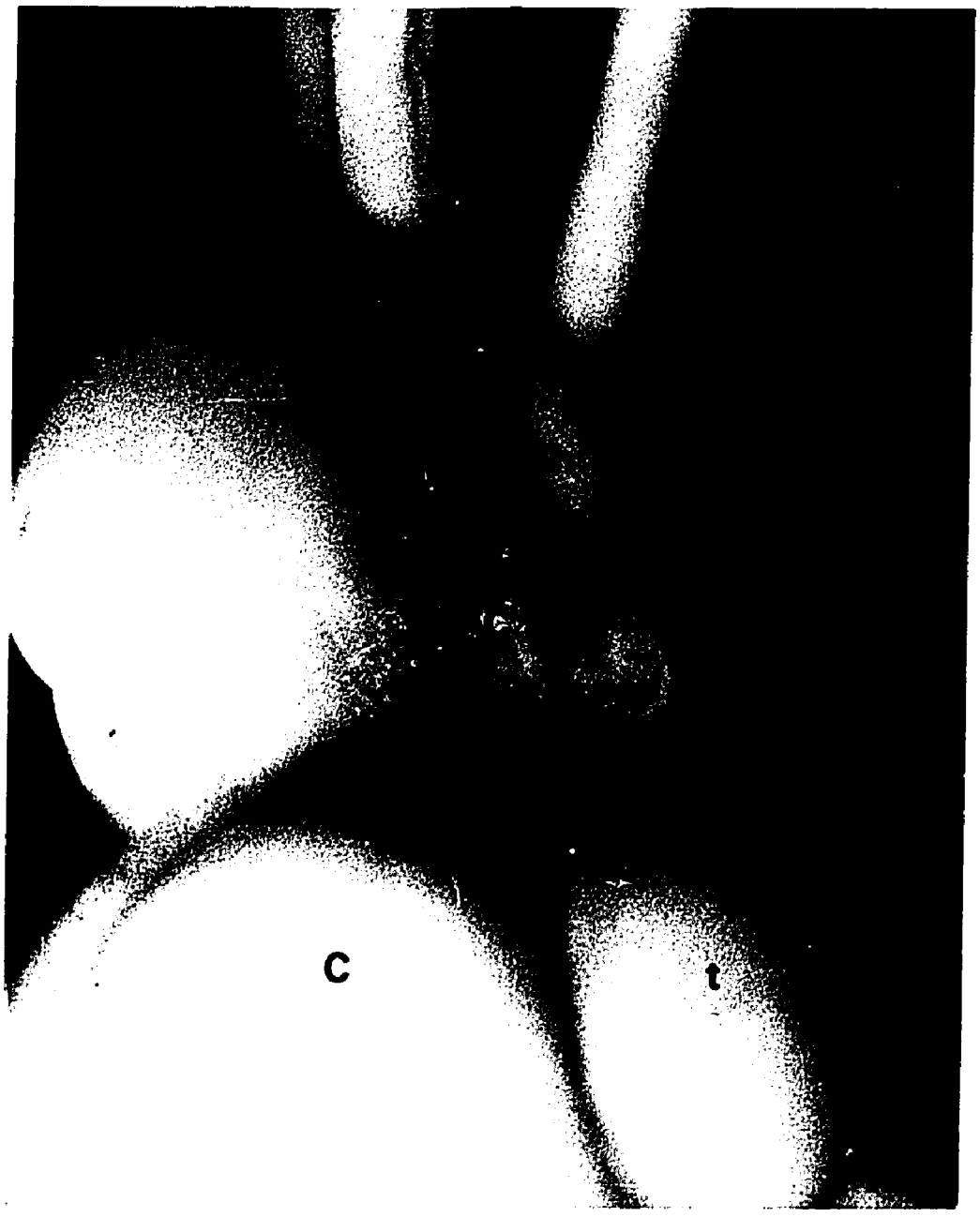


Figure III, 11

Dorso lateral view of left hemisphere and ablated right hemisphere. Ch. optic chiasma; AC. anterior commissure; ped. telencephalic penduncle; I. inferior lobe (rostral end); t. optic tectum; C. cerebellum



Figure III, 12

Ventral view of the brain, showing hypothalamic inferior lobes and hypophysis. Ch. optic chiasma; tel. telencephalic hemispheres; se. sulcus endorhindalis; V + VII. facial-trigeminal complex; VIII. acoustic nerve; X. vagal nerve; sp. spinal cord

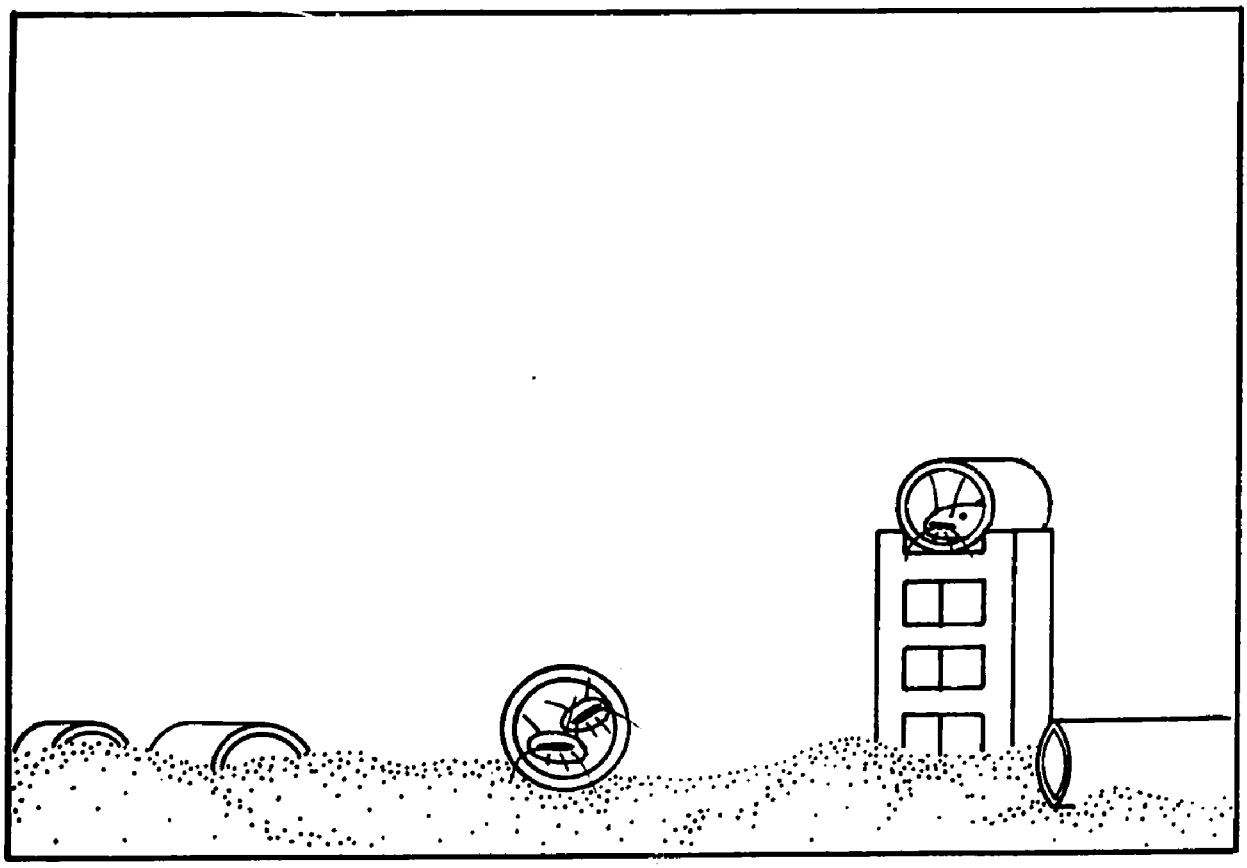


Figure III, 13

Resident fish in 1200 liter community aquarium

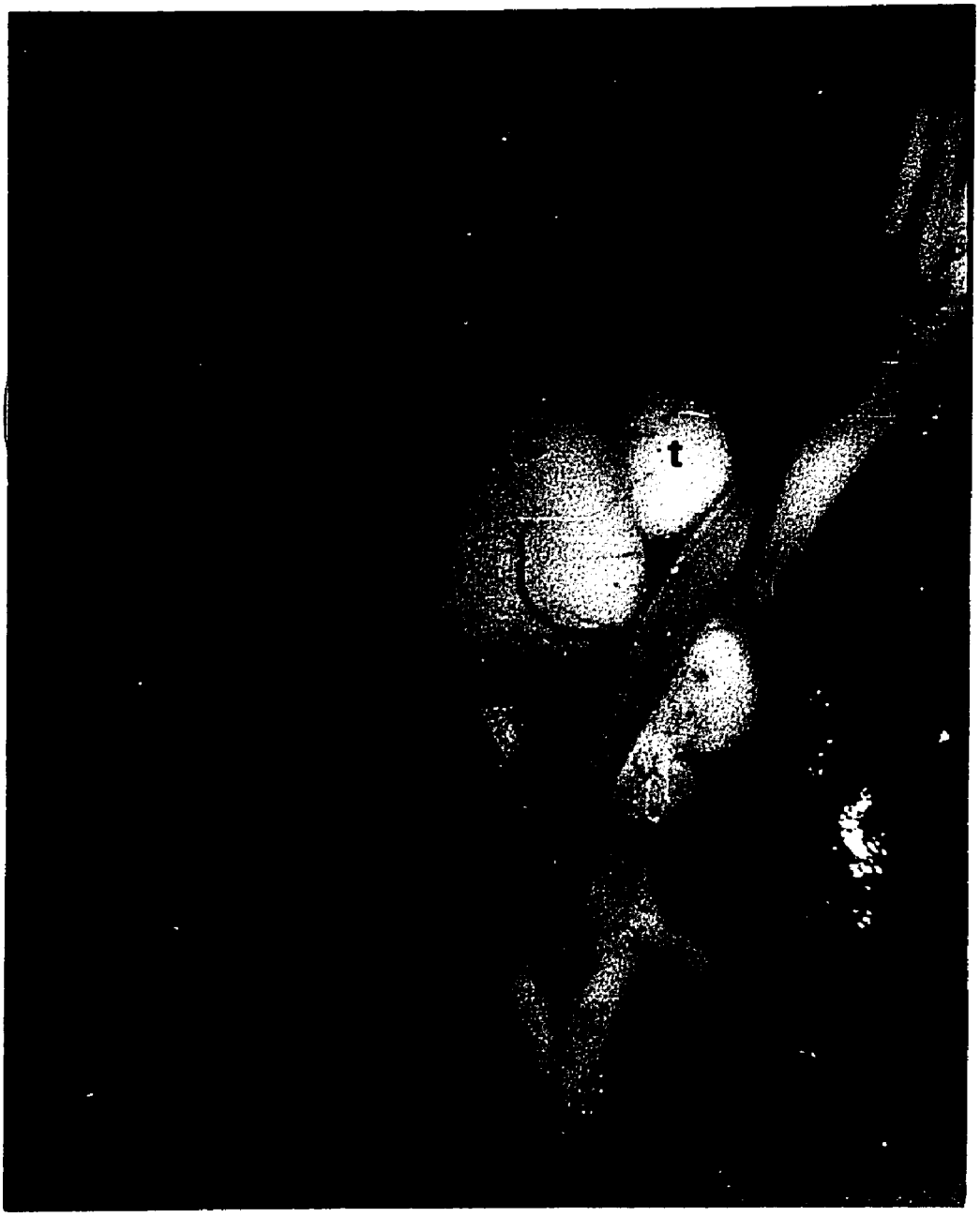
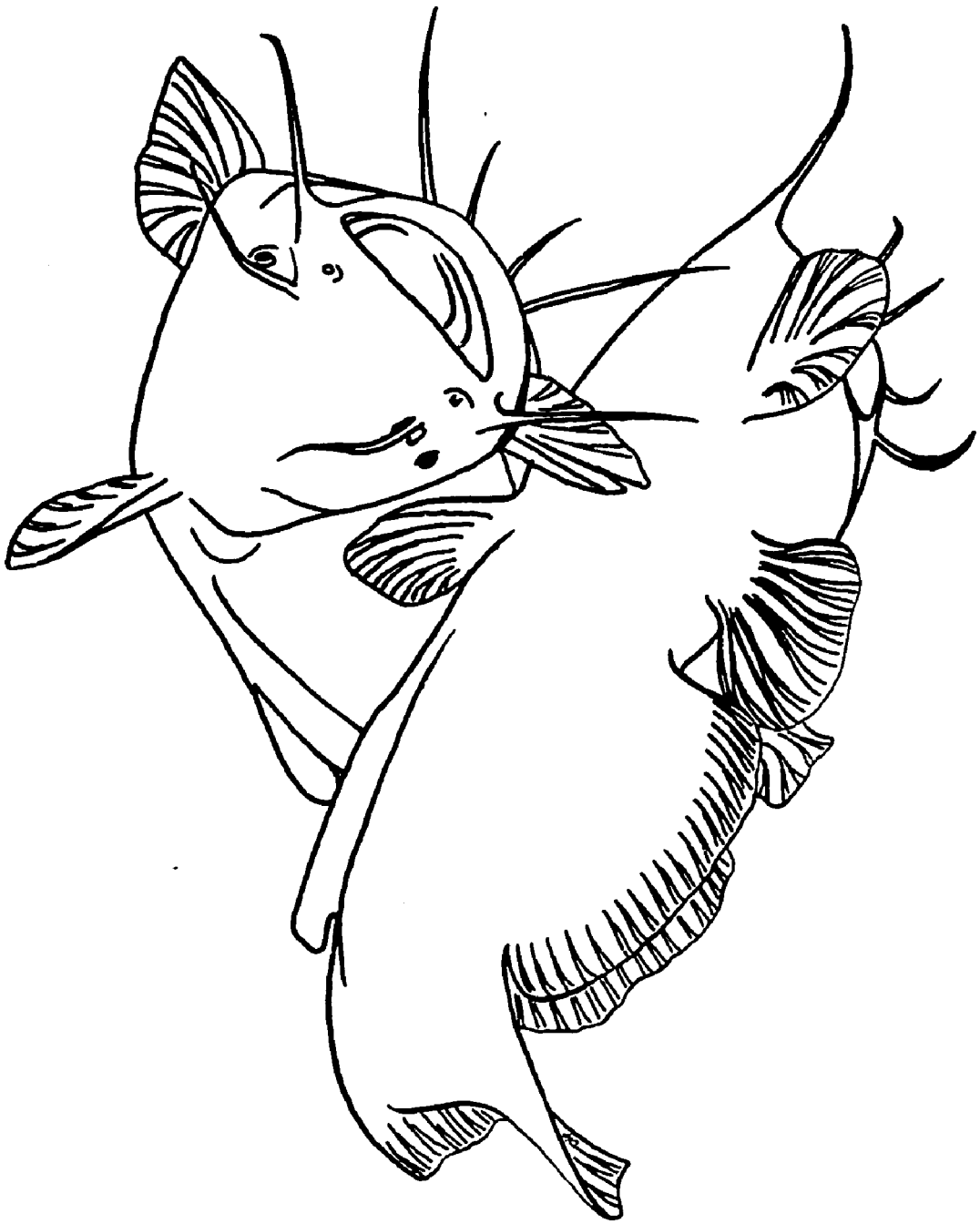
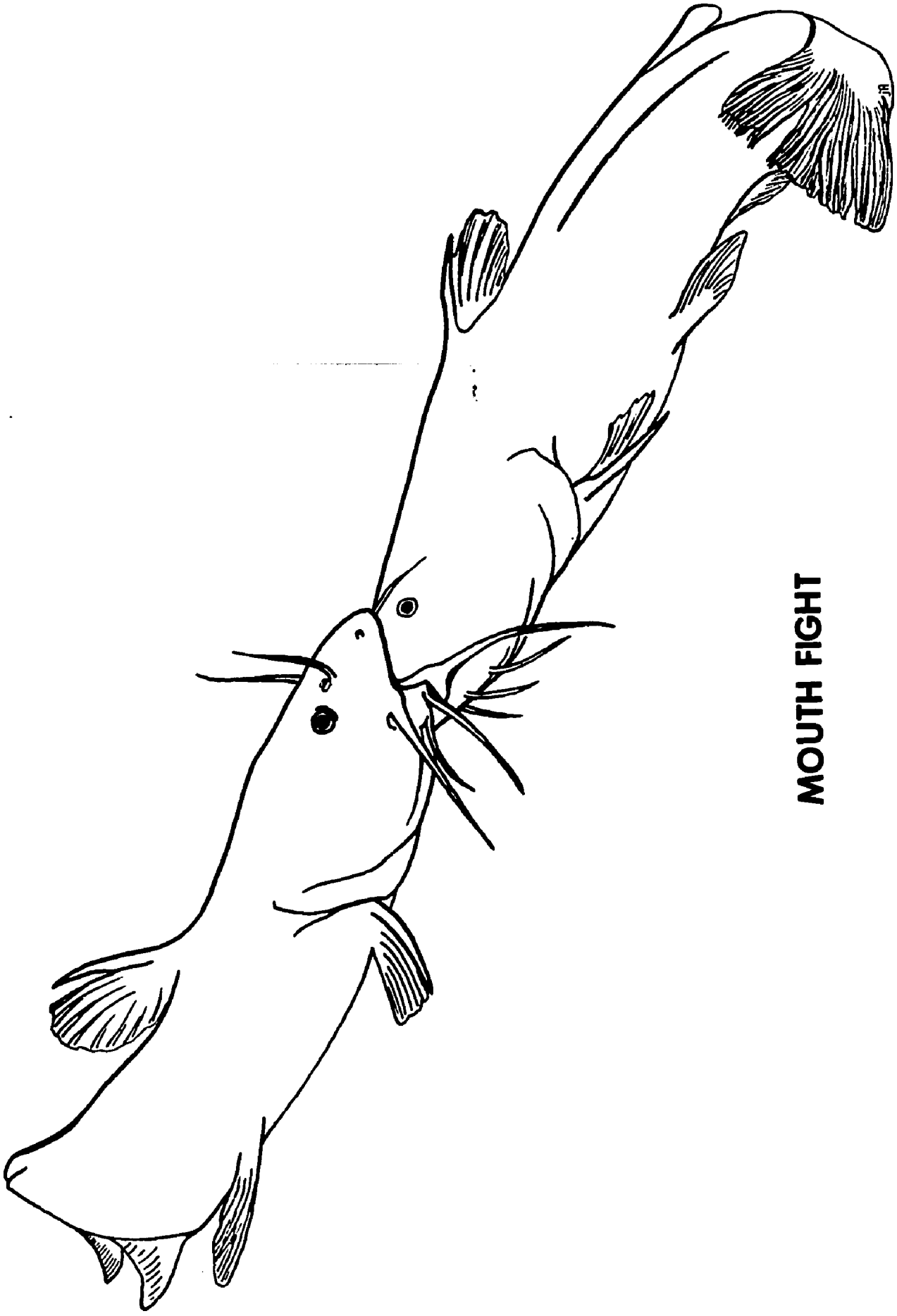


Figure III, 14

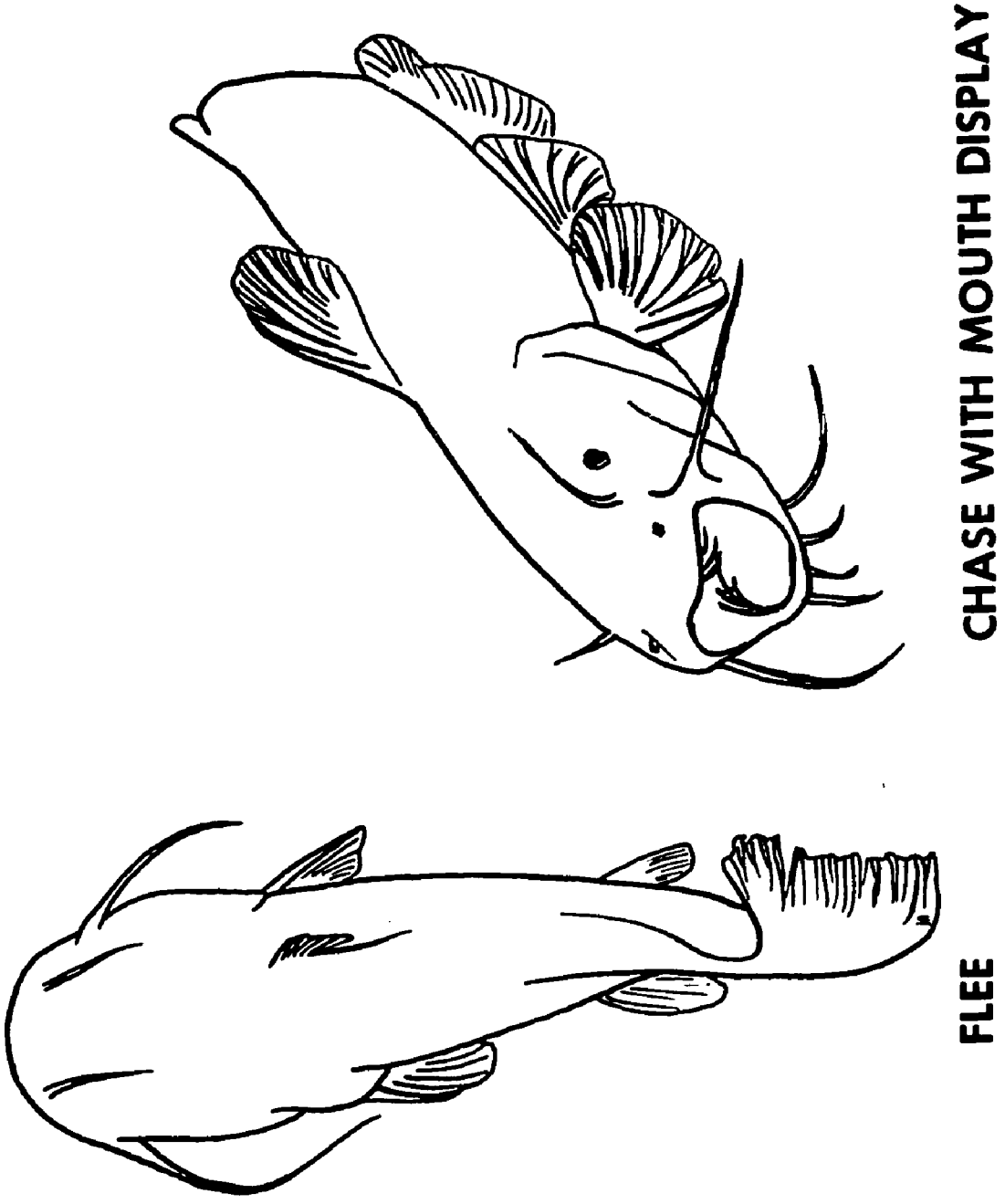
Dorsal view of the brain of forebrain ablated fish No. 0, showing remaining piece of left hemisphere (arrow). Ch. optic chiasma; C. cerebellum; t. optic tectum.



MOUTH DISPLAY AND THRUST



MOUTH FIGHT


CHASE WITH MOUTH DISPLAY
FLEE

APPENDIX FIGURES 1

Unit frequency in 11 behavior classes for the 4 observation hours, presented for experimental and resident animals individually.

Horizontal axis: time in minutes

vertical axis: behavior unit classes

X - experimental animal

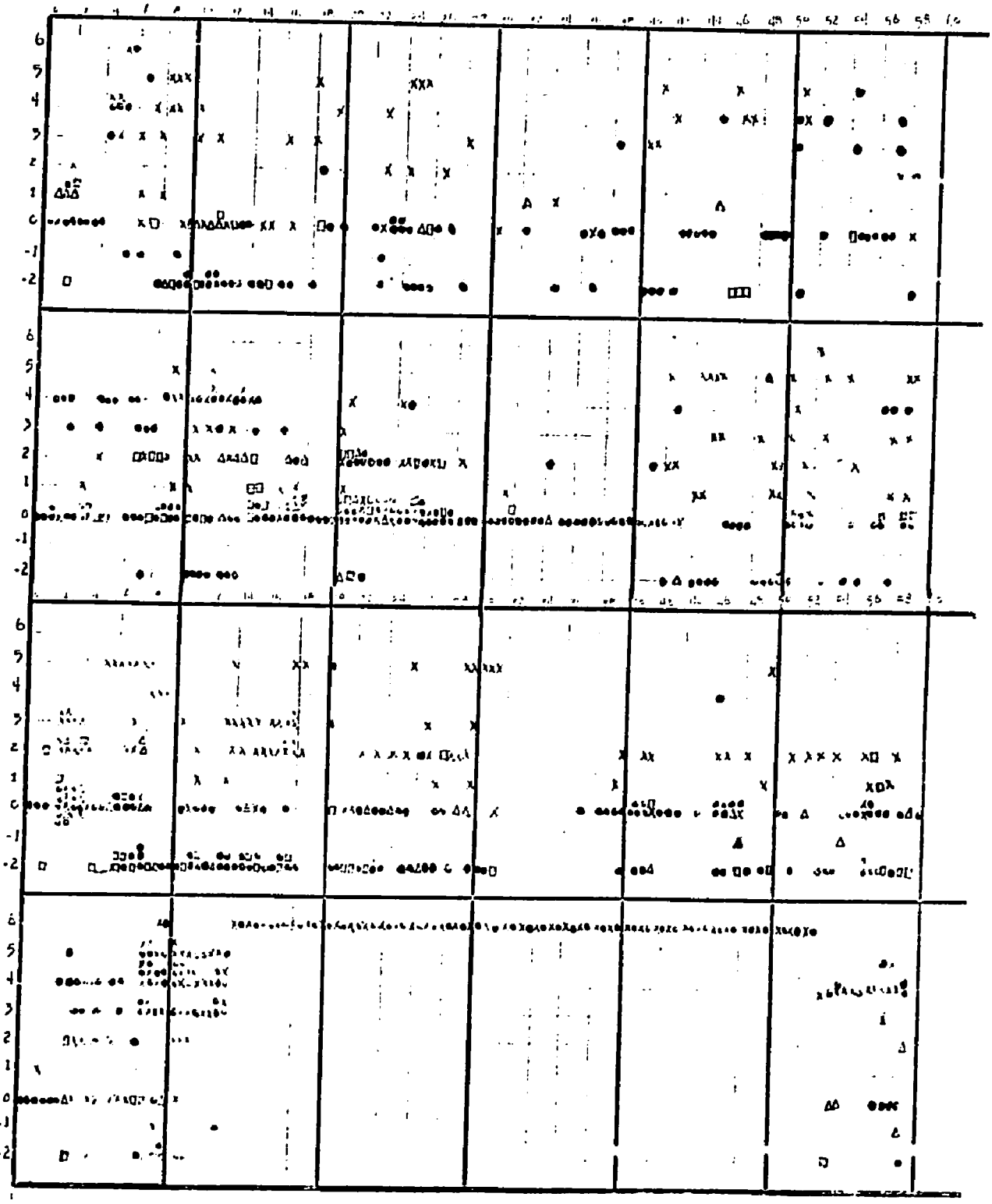
● - α resident animal

□ - β resident animal

△ - γ resident animal

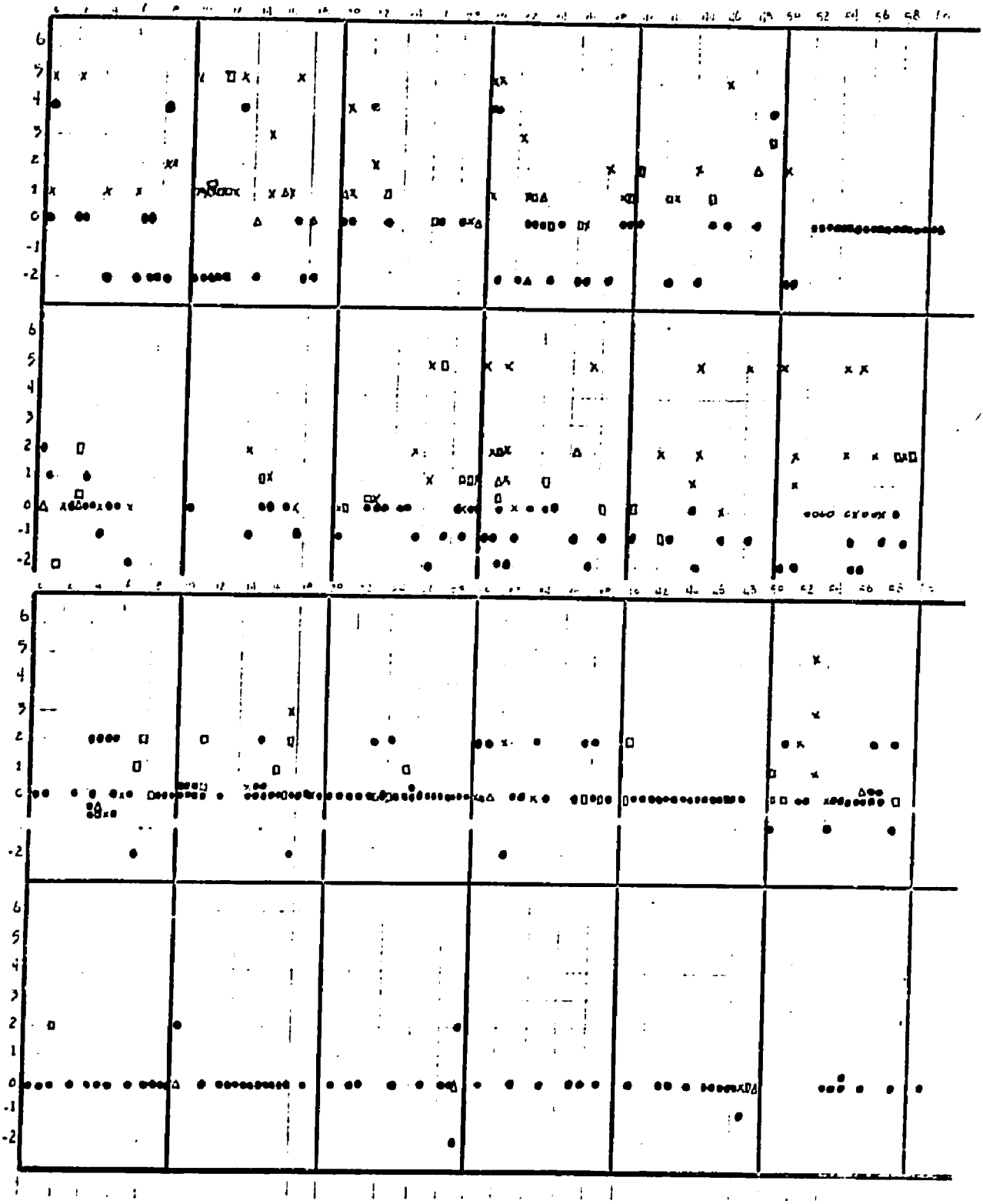
EXPERIMENTAL ANIMAL NO. 1

COMMUNITY NO. 1



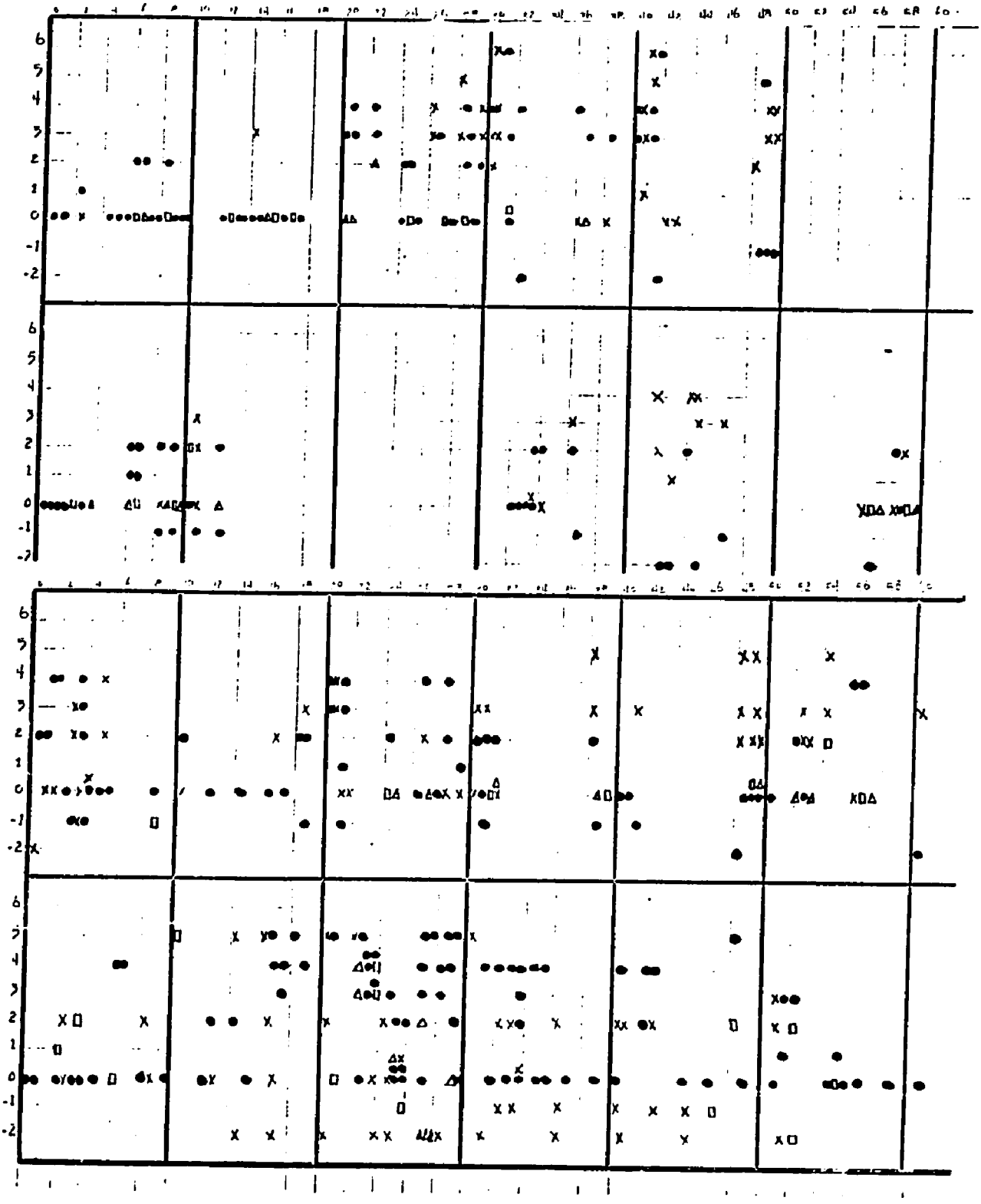
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COMMUNITY NO. 3



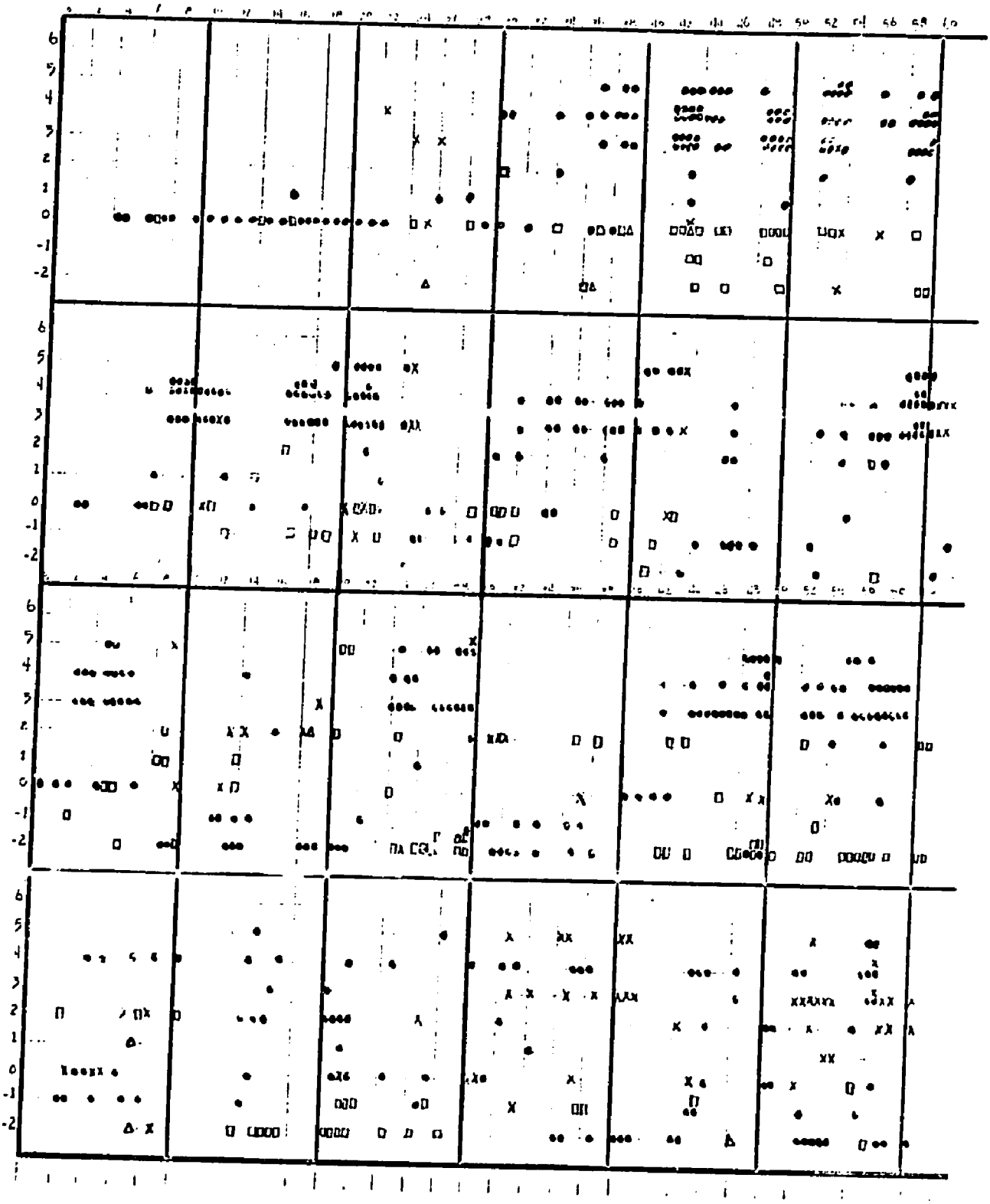
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COMMUNITY NO. 2



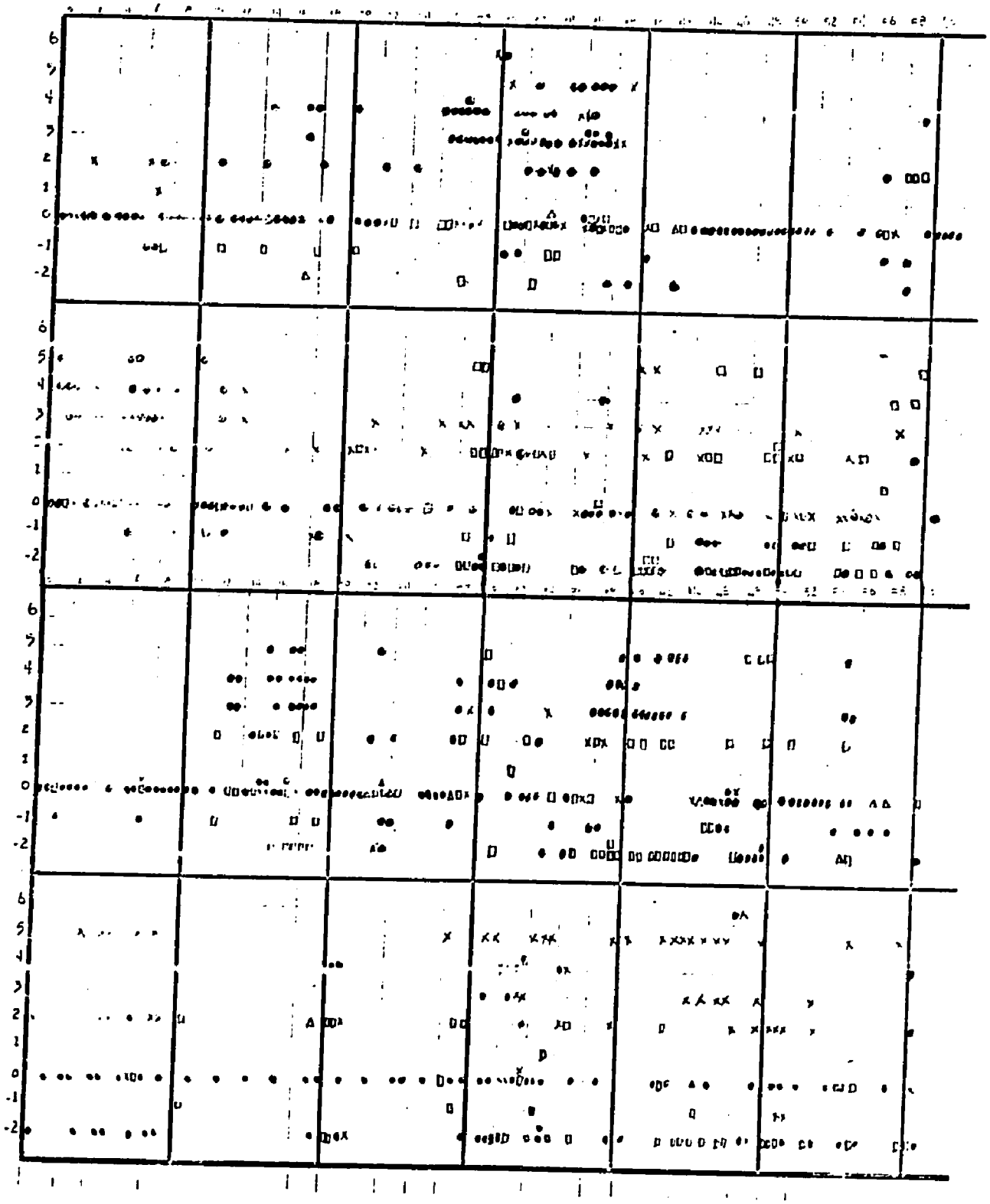
EXPERIMENTAL ANIMAL NO. 4

COMMUNITY NO. 2



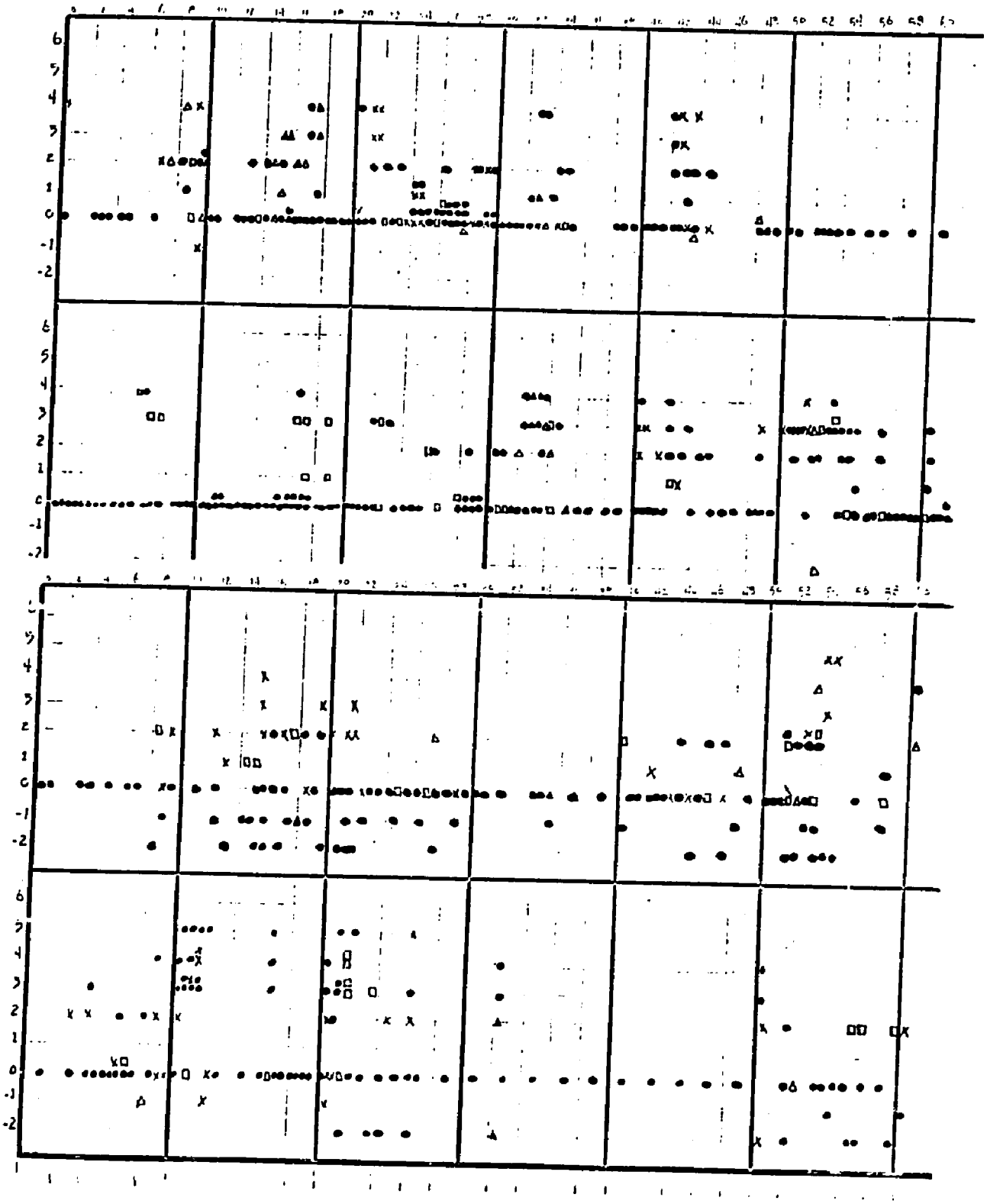
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COMMUNITY NO. 2



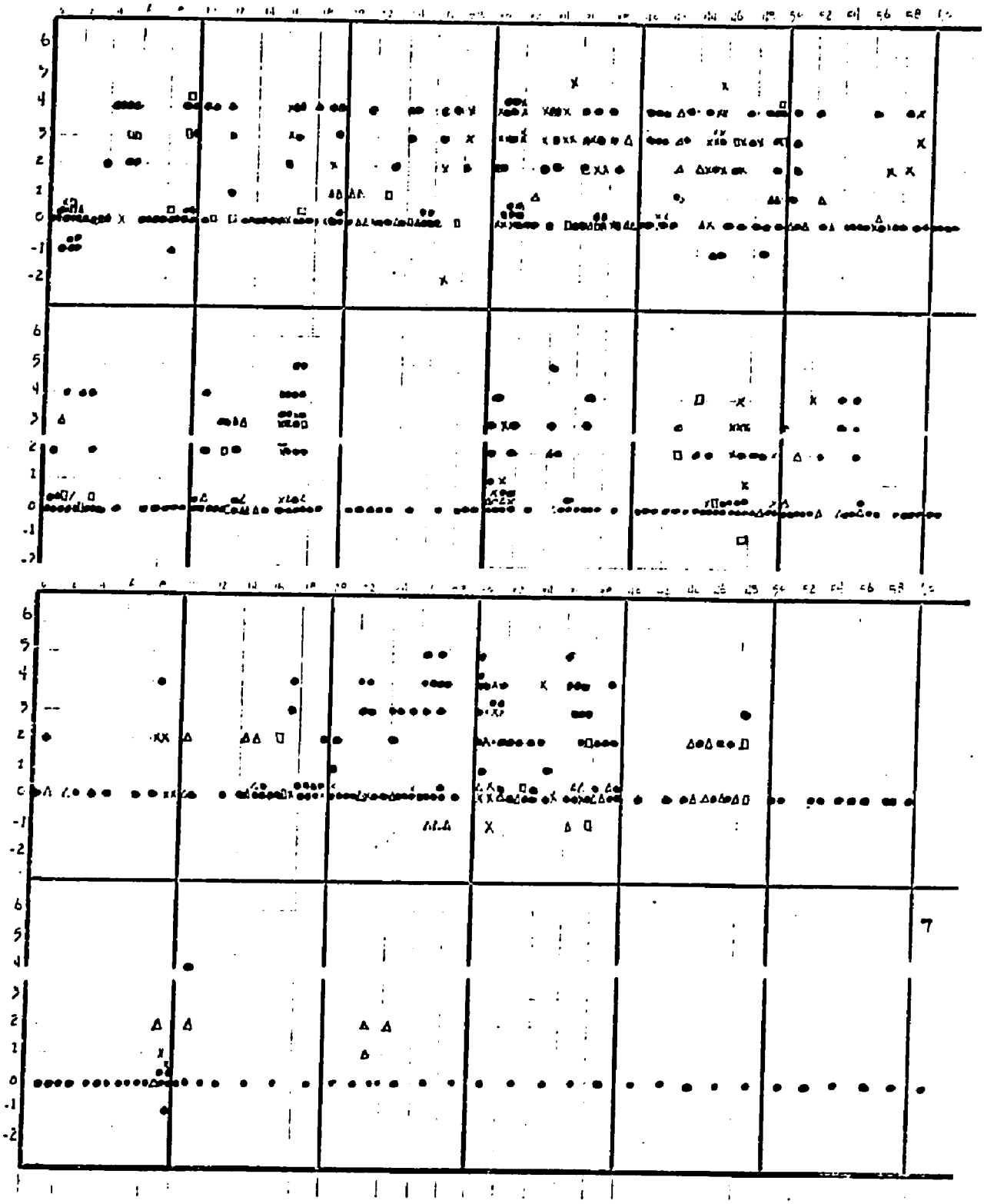
EXPERIMENTAL ANIMAL NO. 6

COMMONITY NO. 3



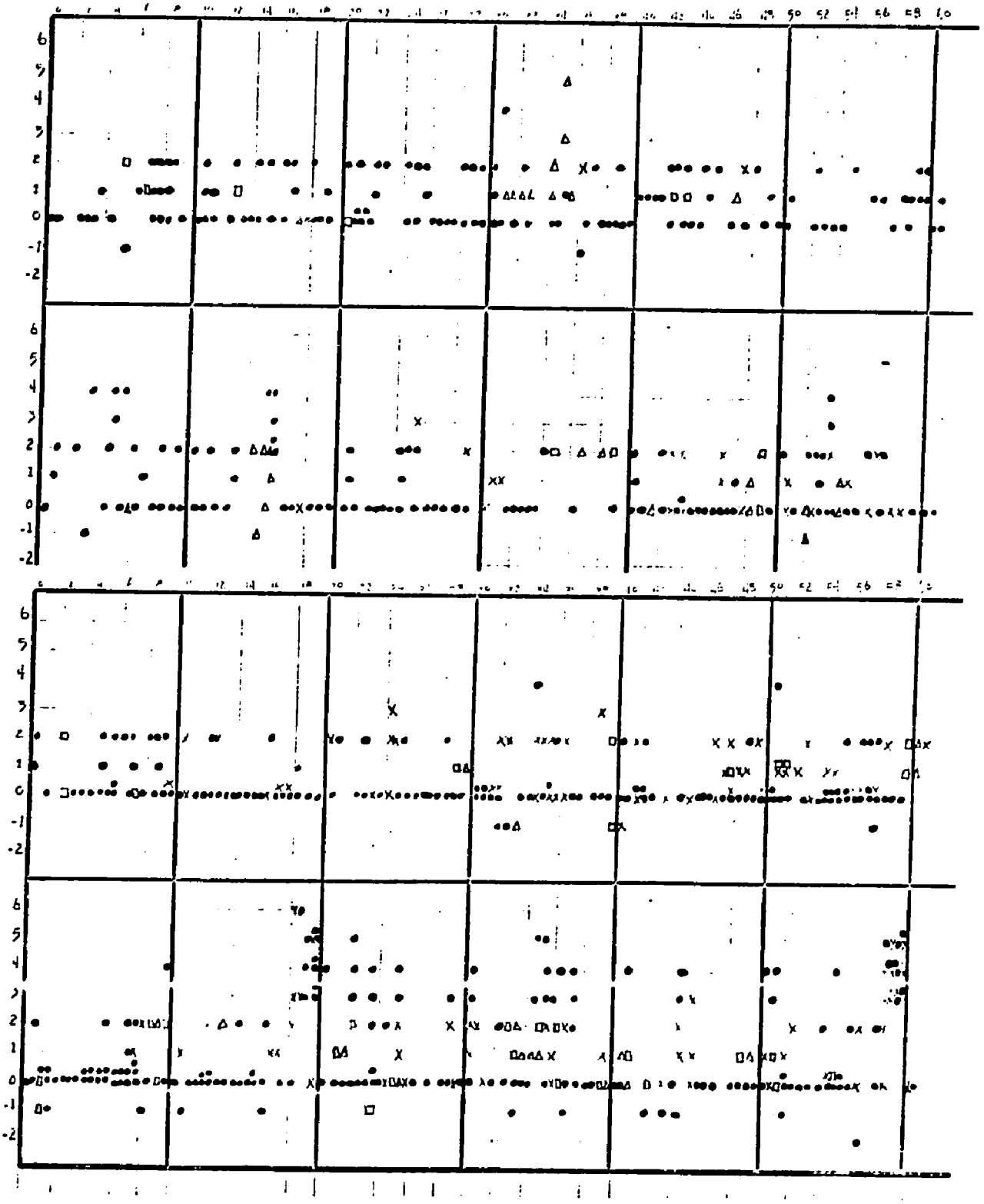
EXPERIMENTAL ANIMAL NO. 7

COMMUNITY NO. 3



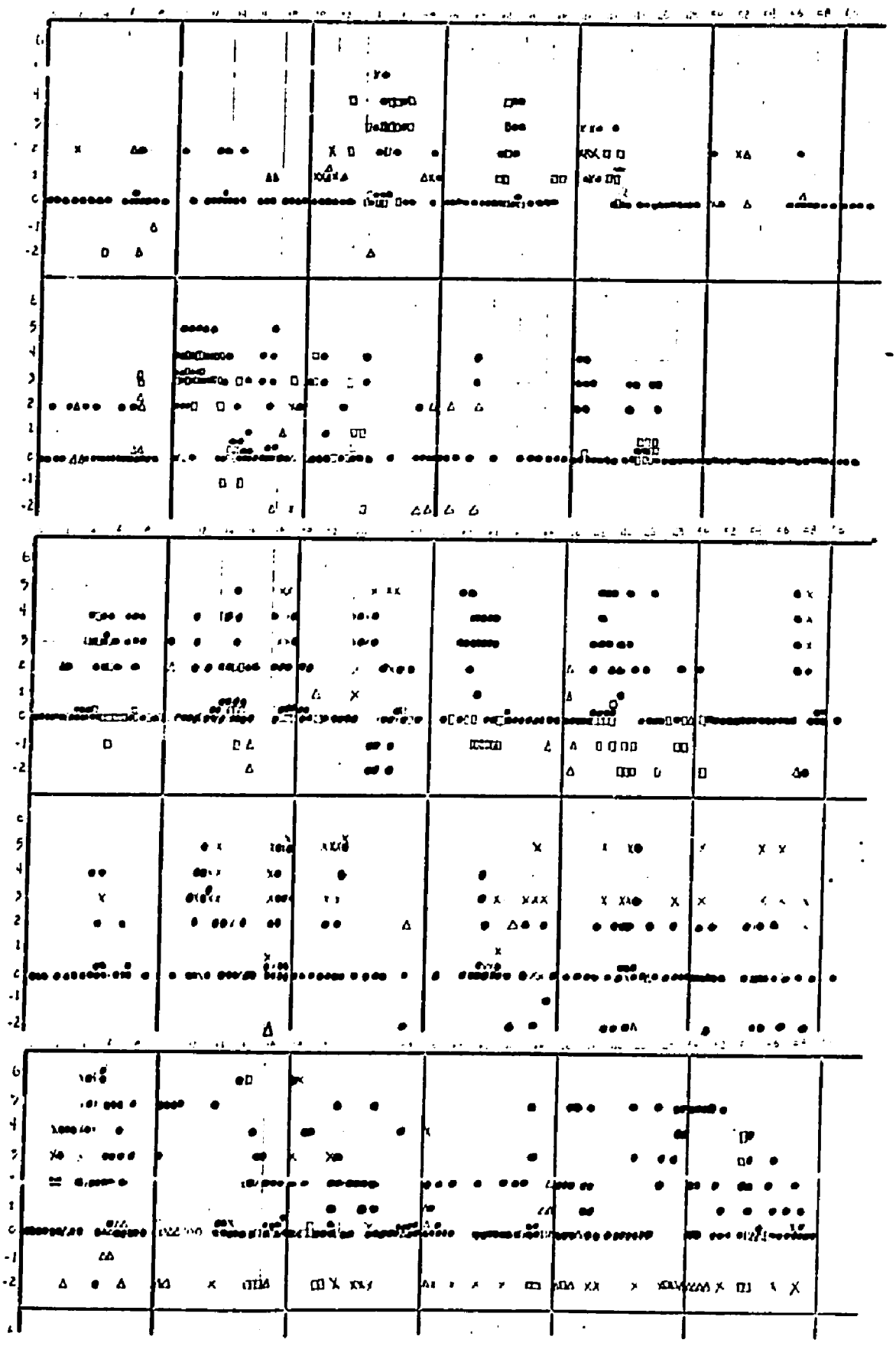
EXPERIMENTAL ANIMAL NO. 8

COMMUNITY NO. 4



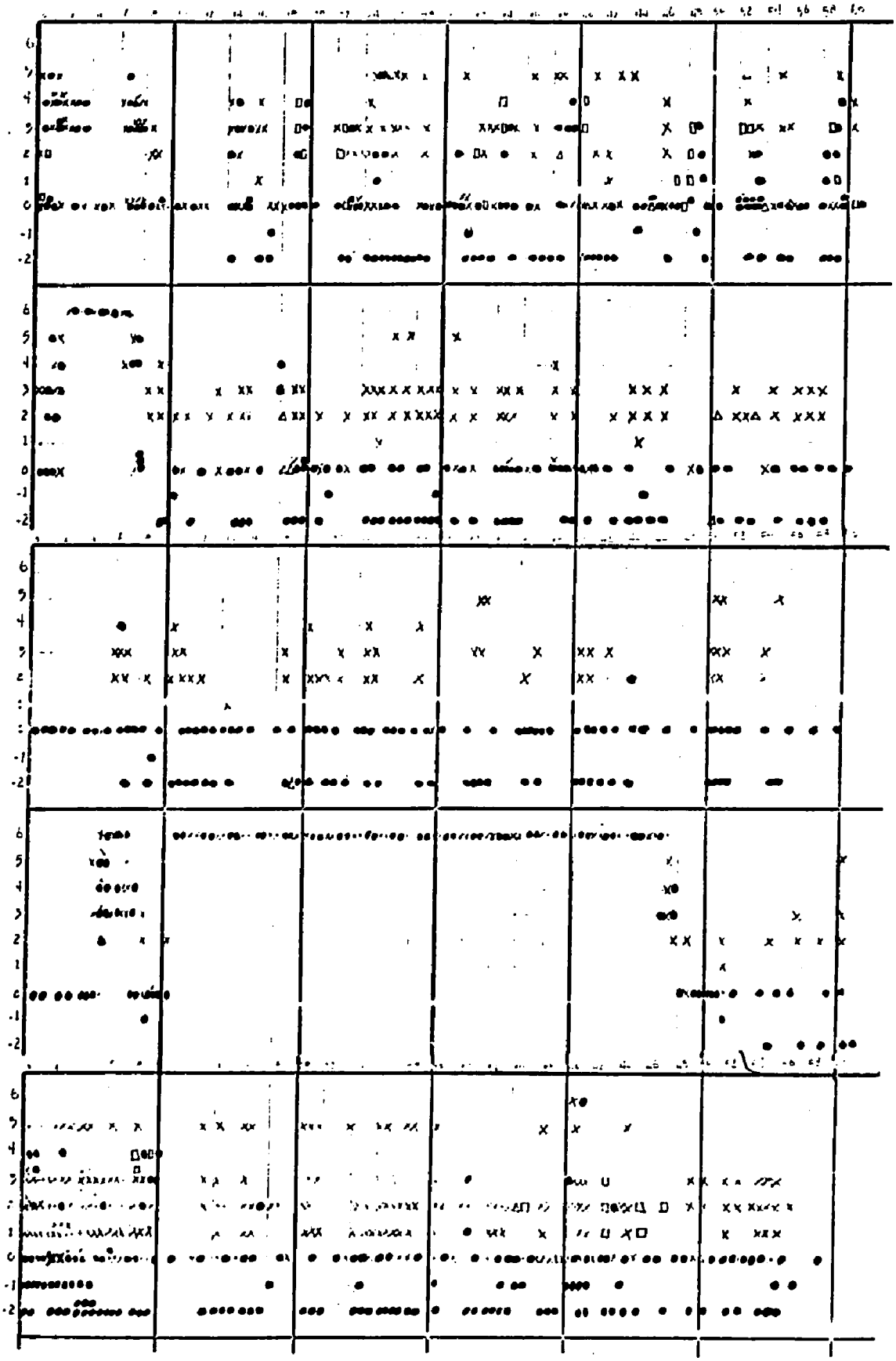
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COMMUNITY NO. 5



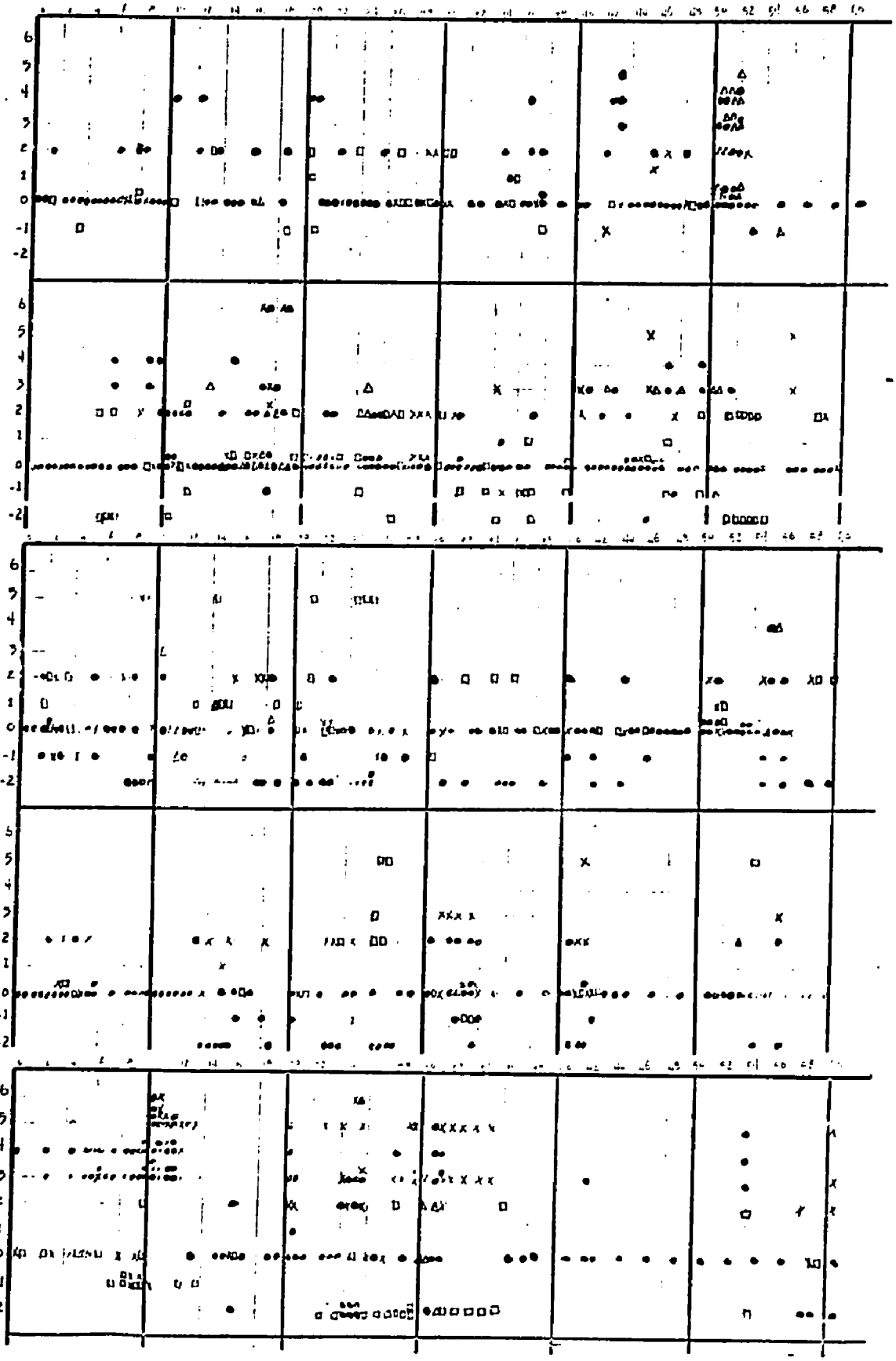
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COMMUNITY NO. 6



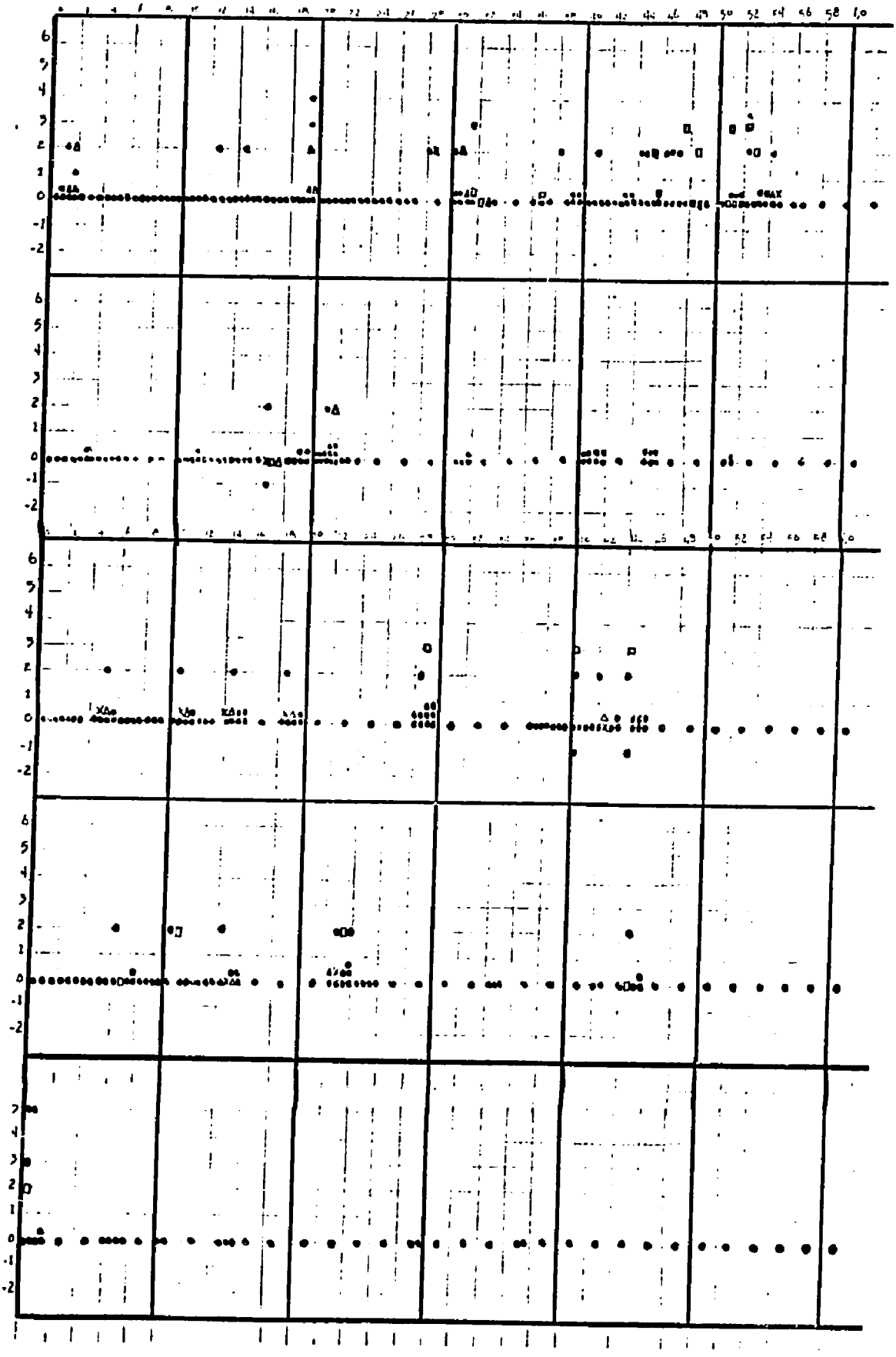
EXPERIMENTAL ANIMAL NO. I

COMMUNITY NO. 2



EXPERIMENTAL ANIMAL NO. II

COMMUNITY NO. 5

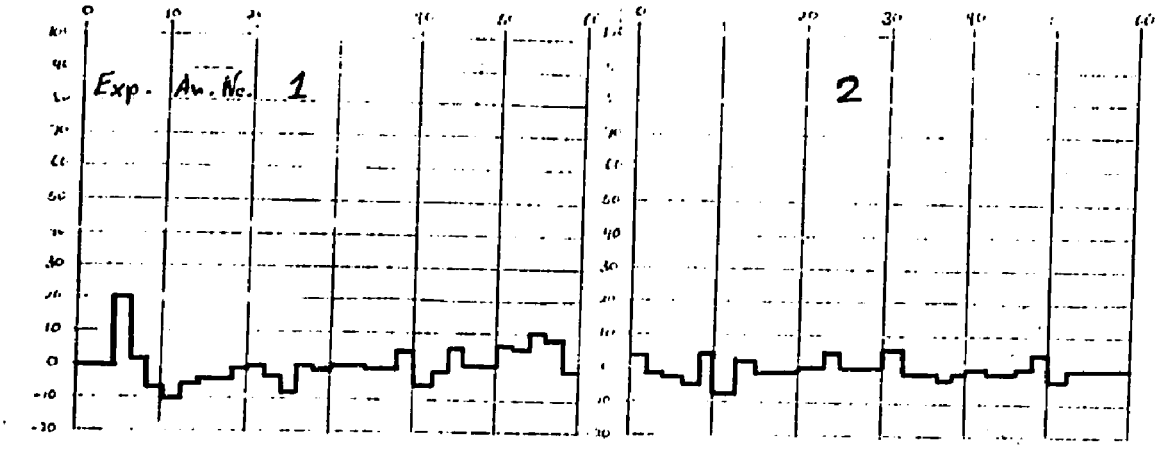


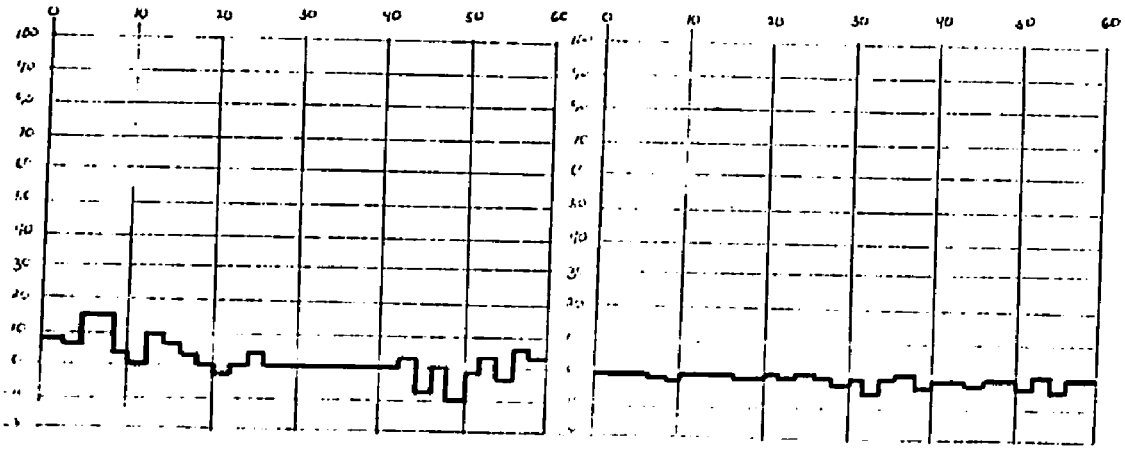
APPENDIX FIGURES 2

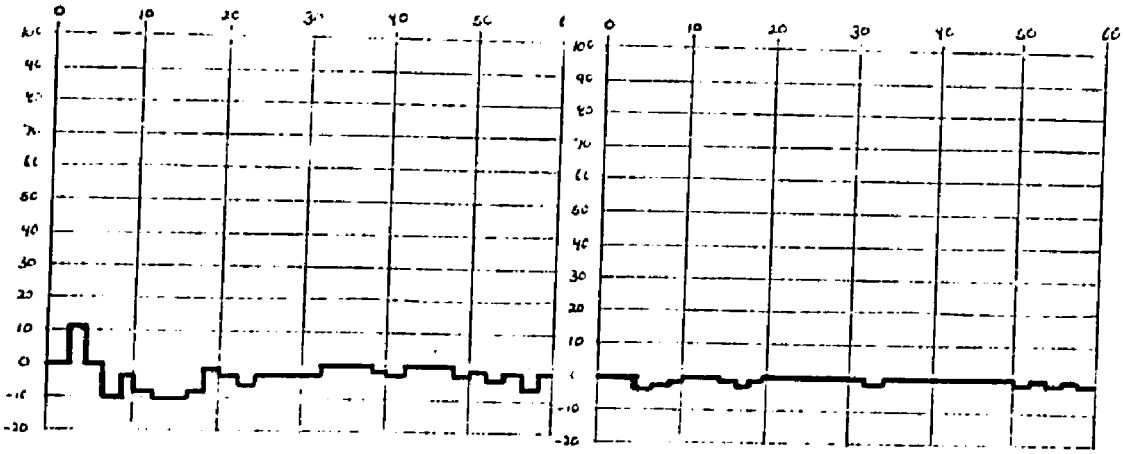
Pattern of aggressivity during each observation hour, presented for experimental animals only. Each column represents one experimental animal in the four observation hours.

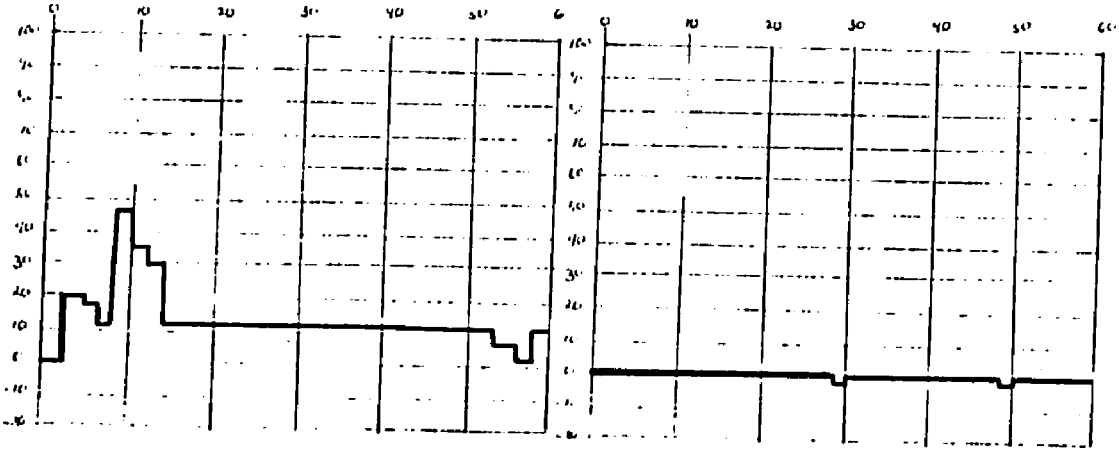
horizontal axis: time in minutes

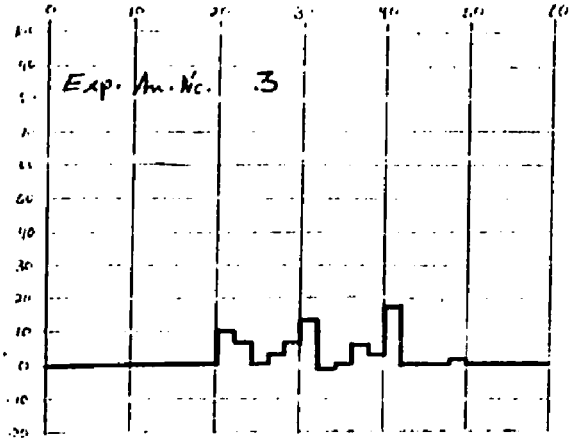
vertical axis: aggressivity in arbitrary units (see text)

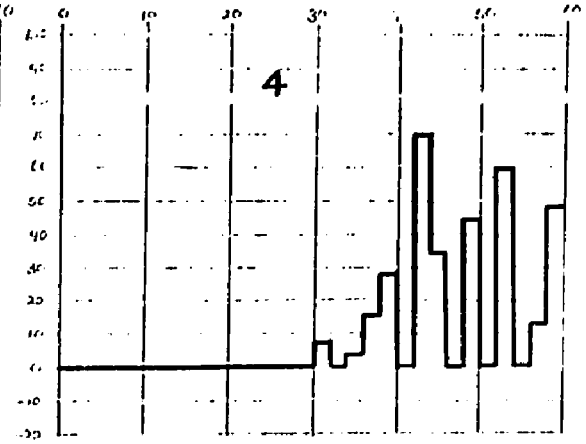

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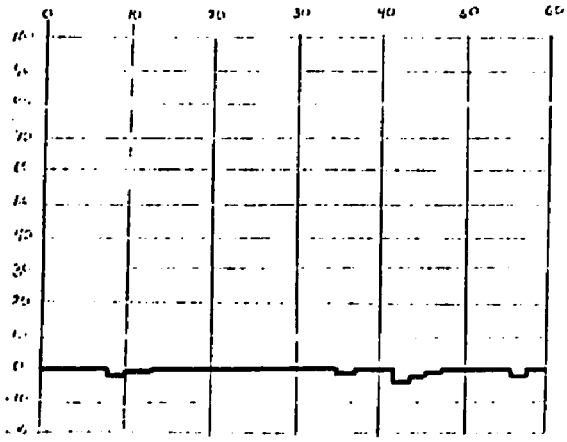


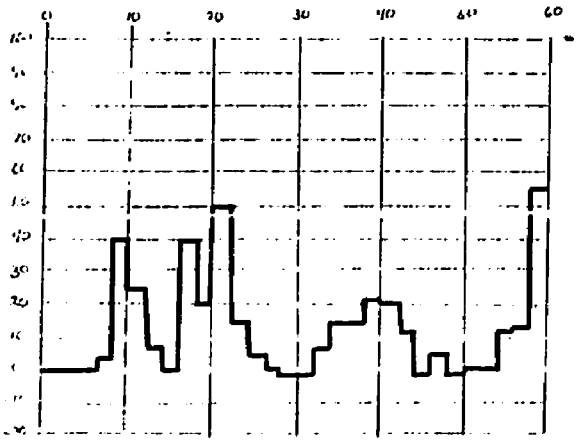


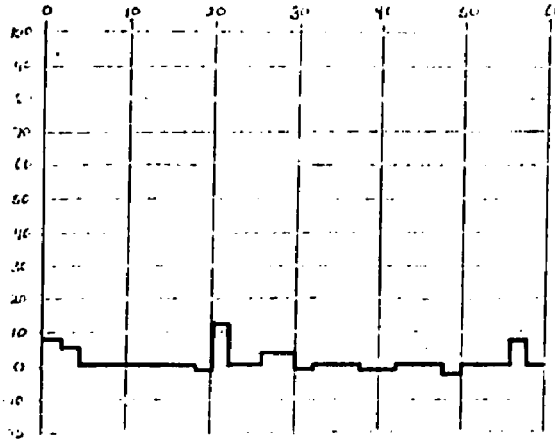


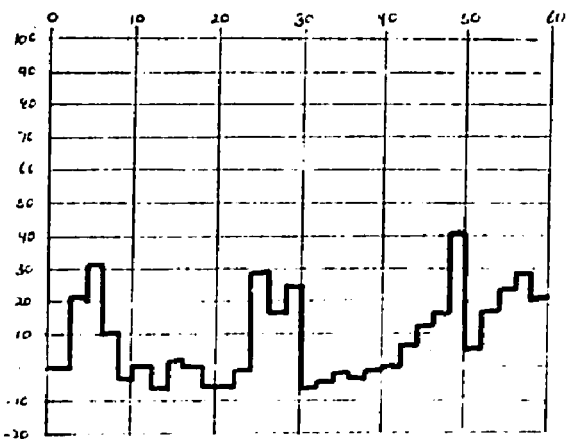

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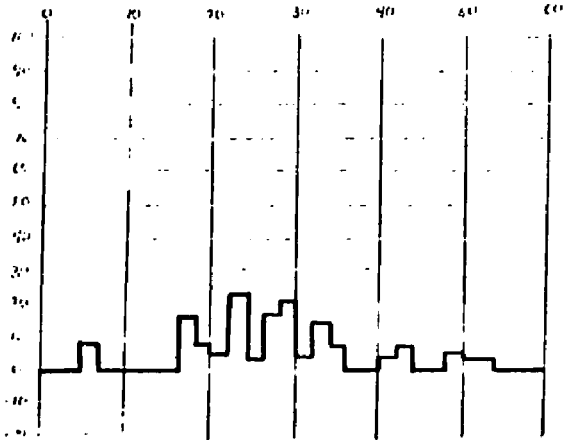

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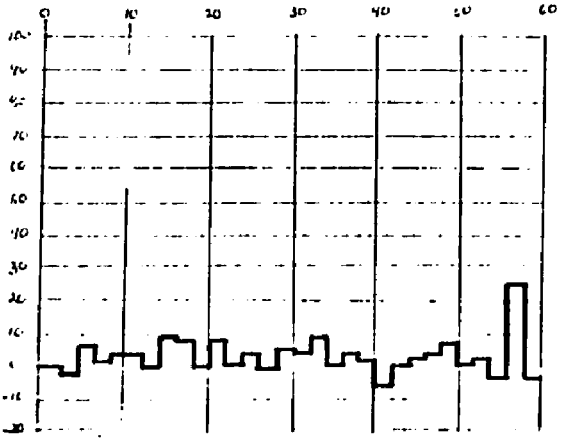


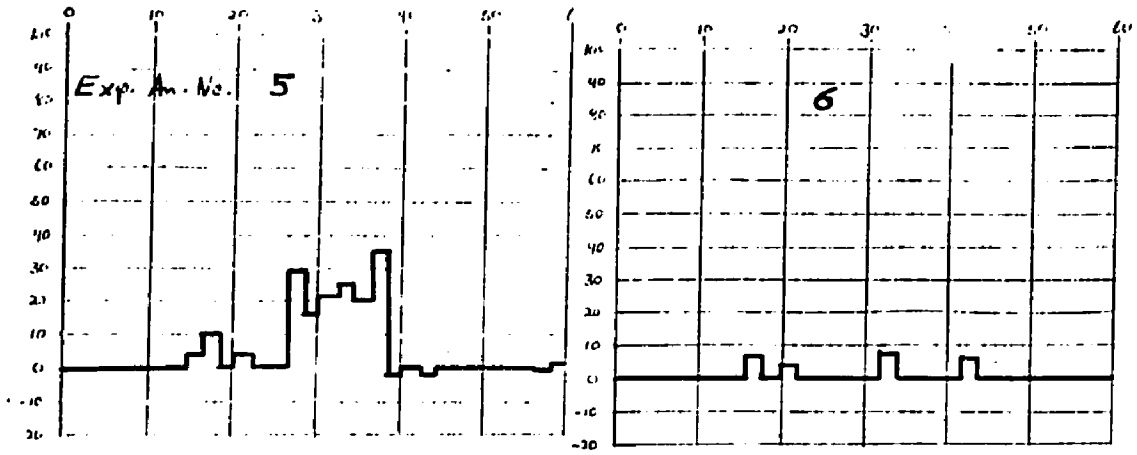


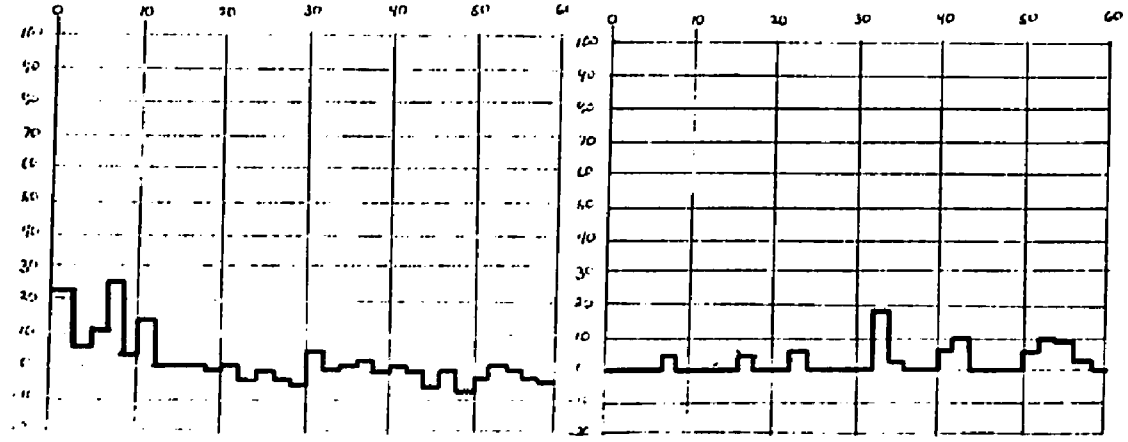


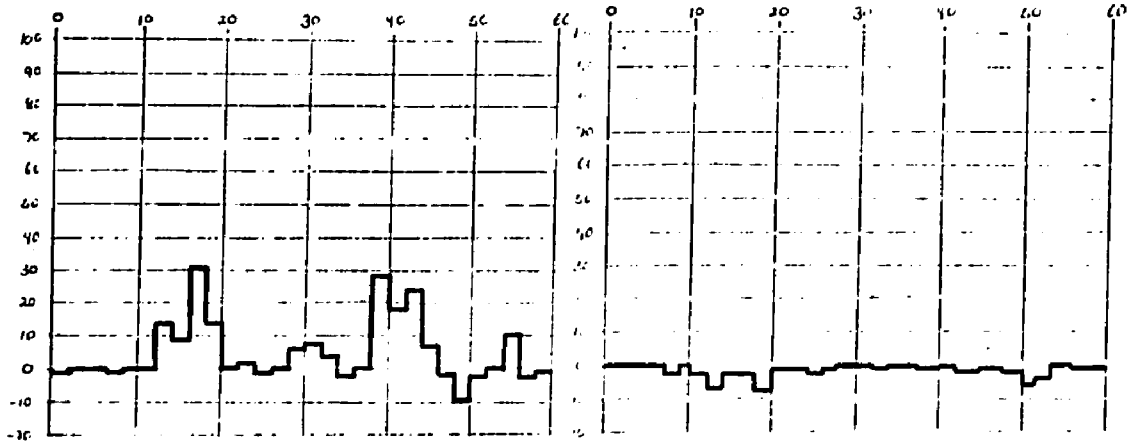


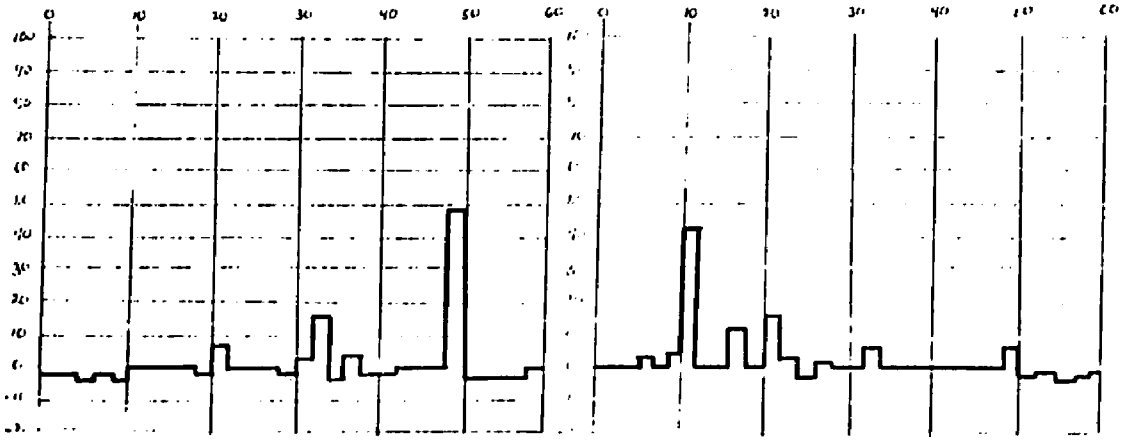


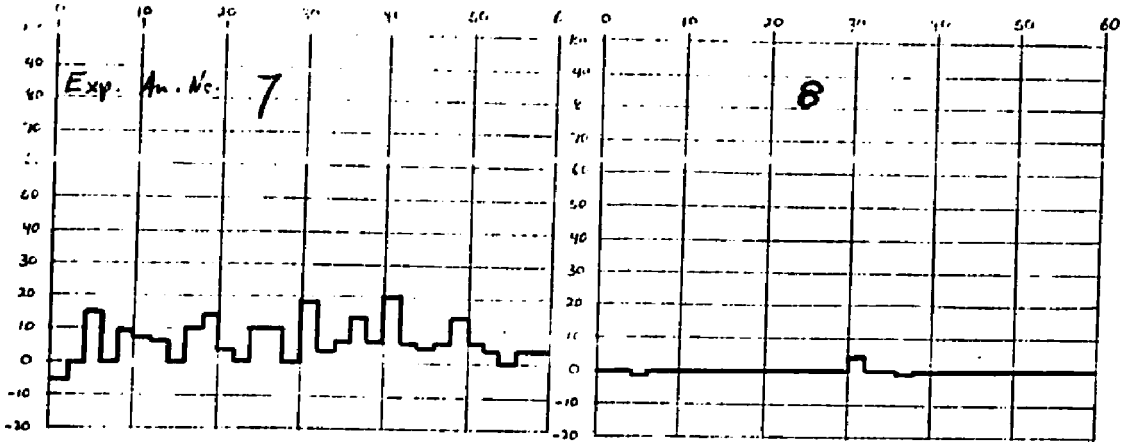


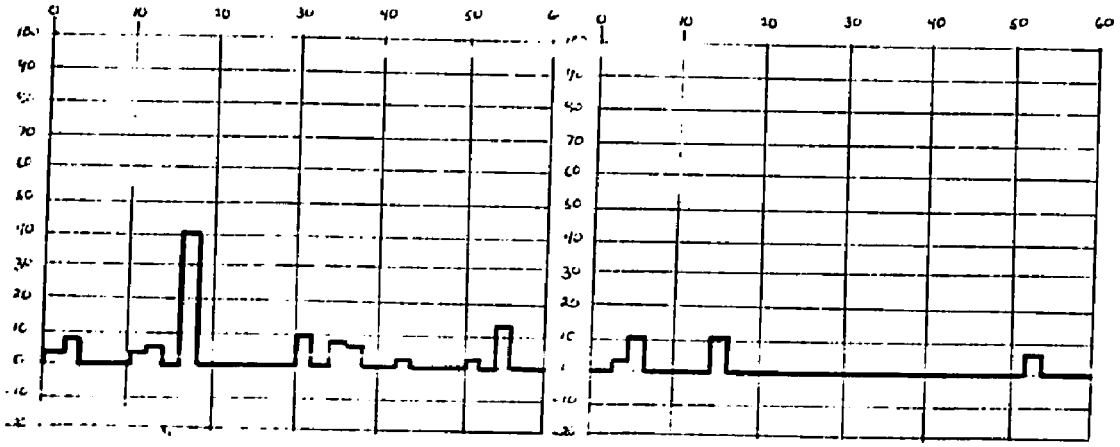

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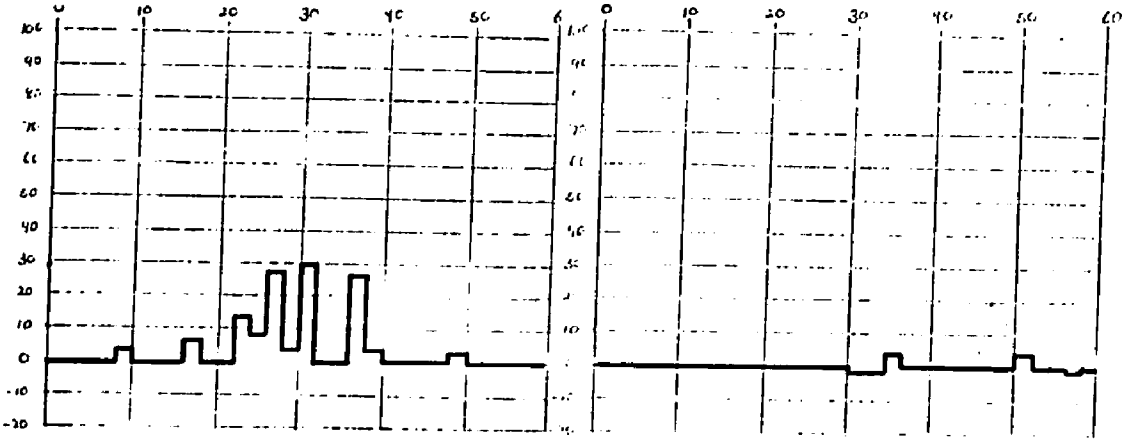


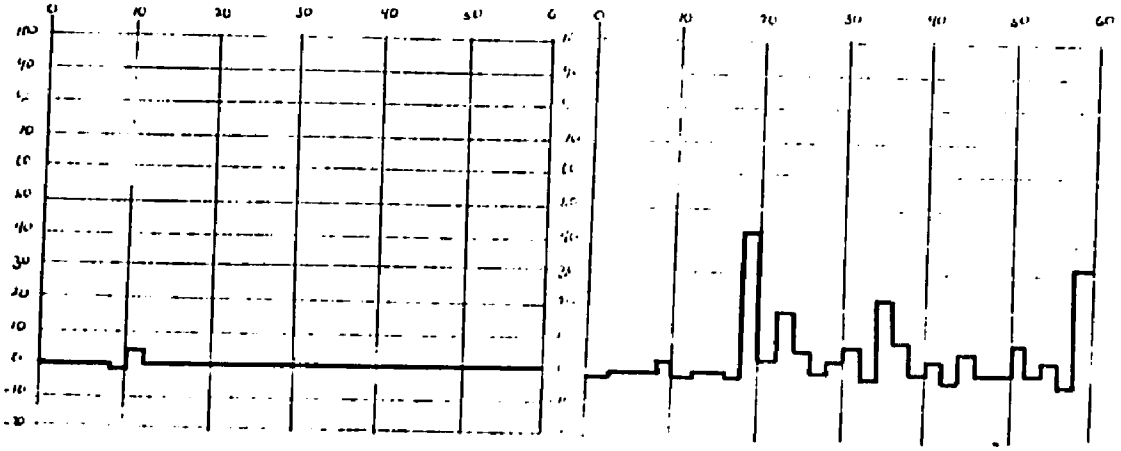


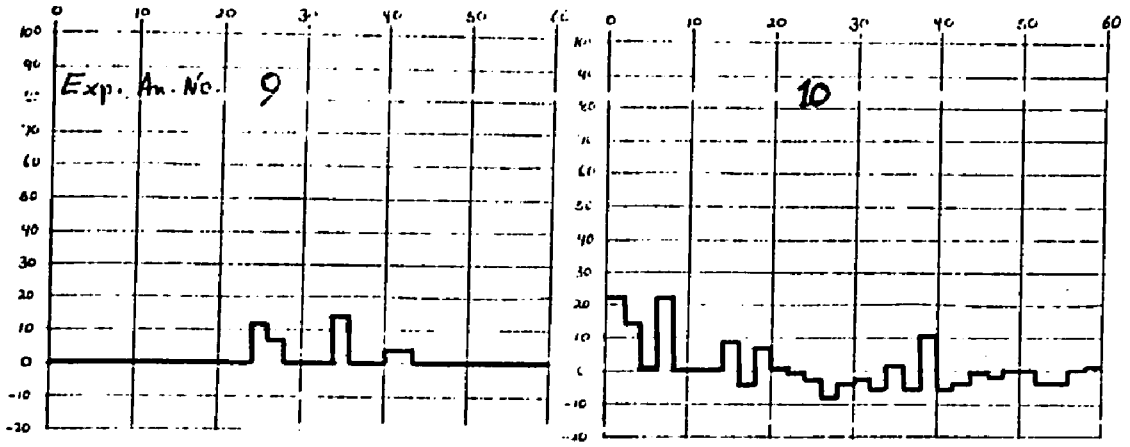


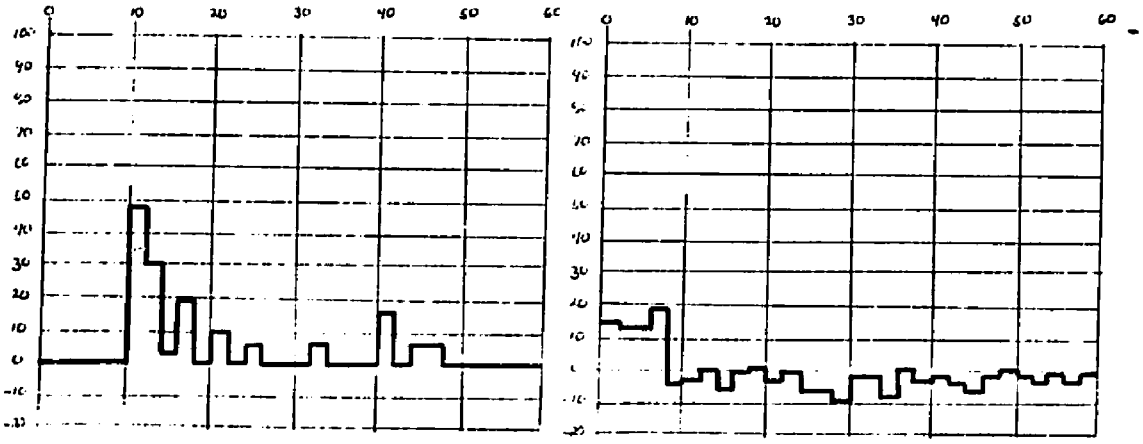

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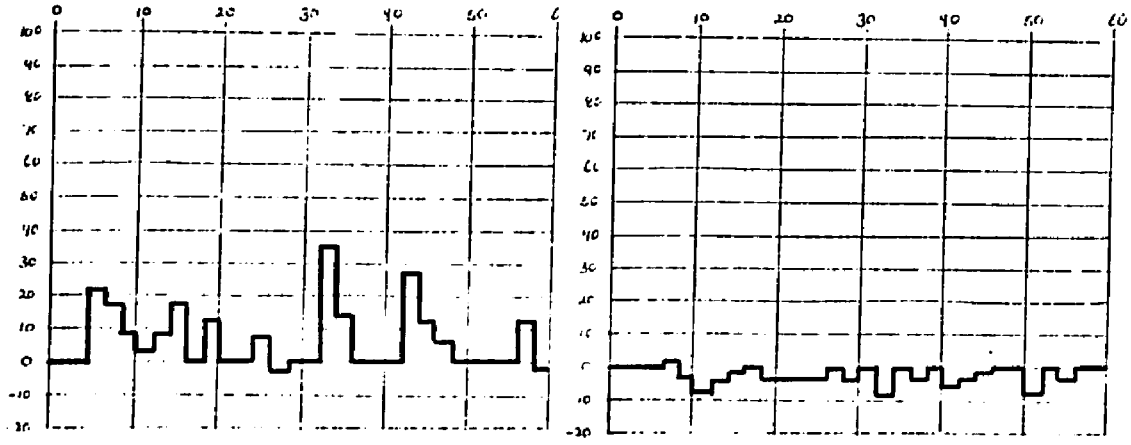


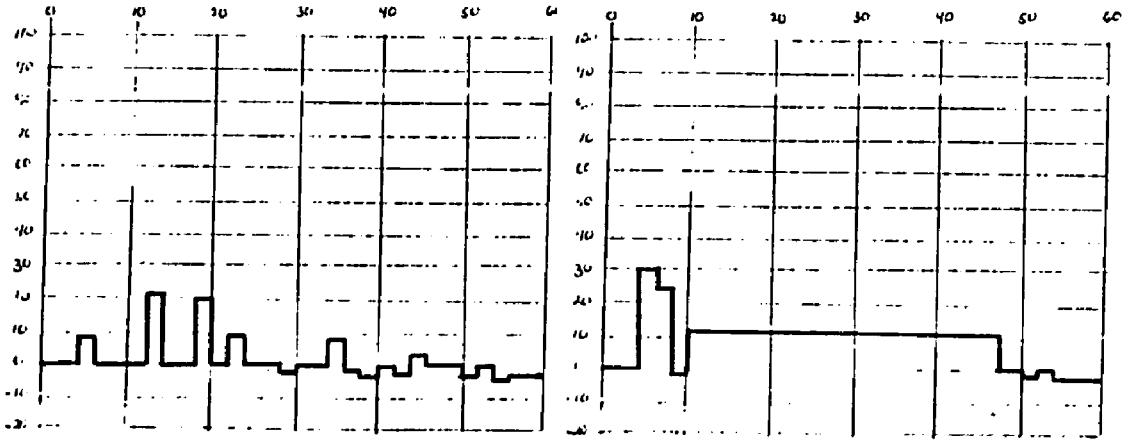


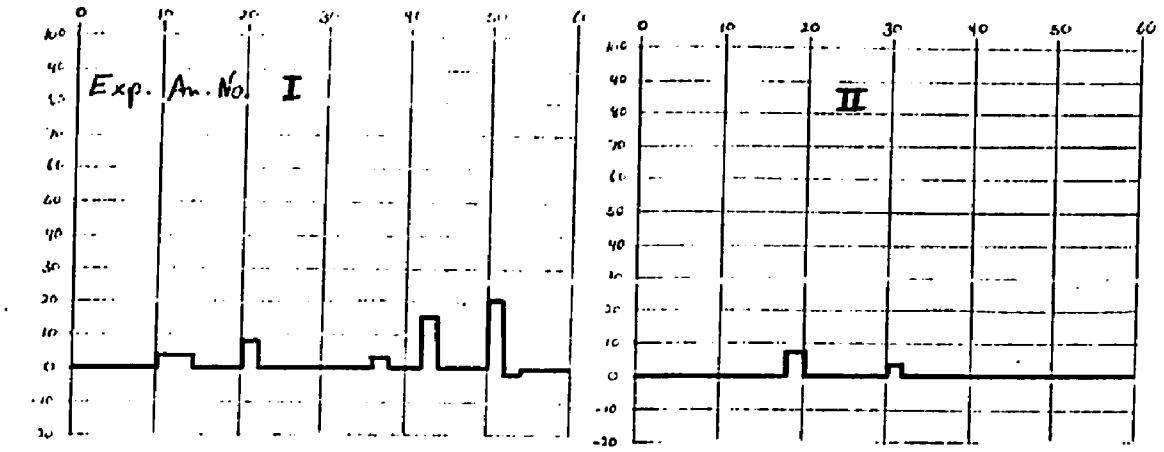


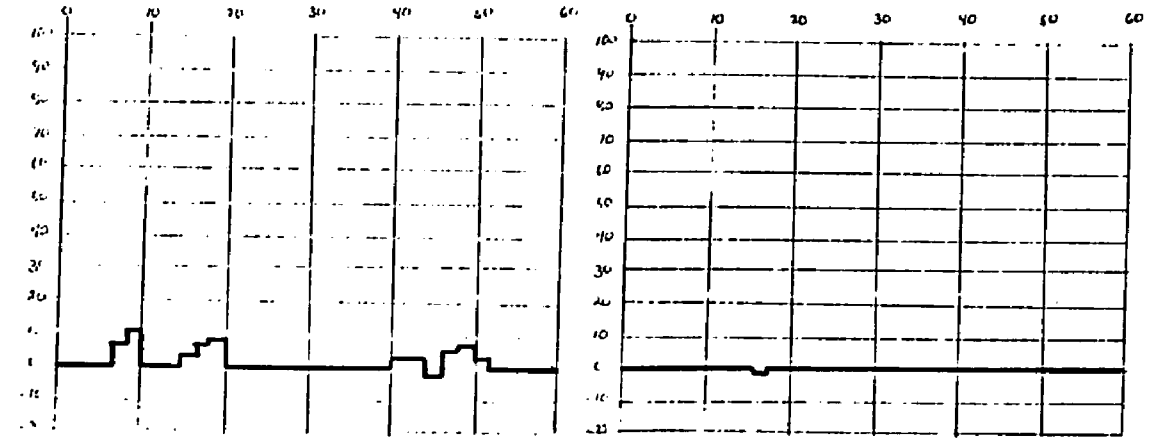

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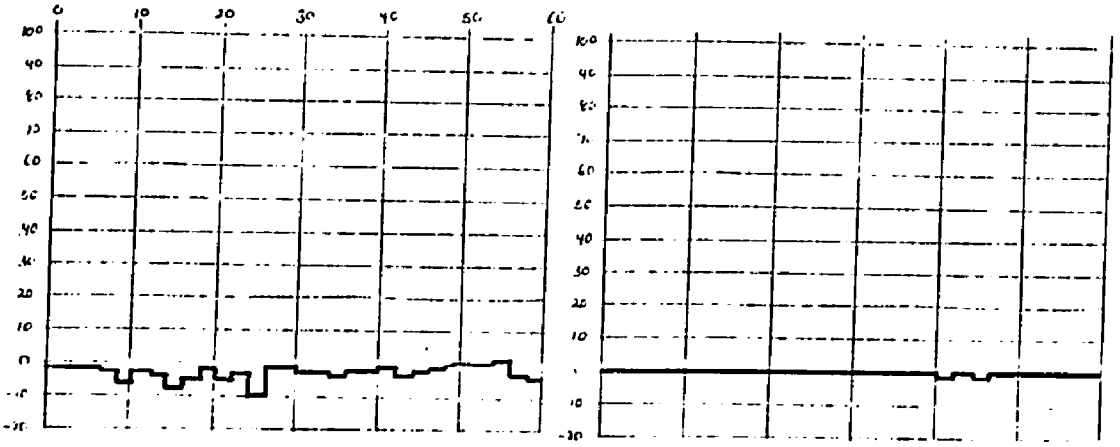


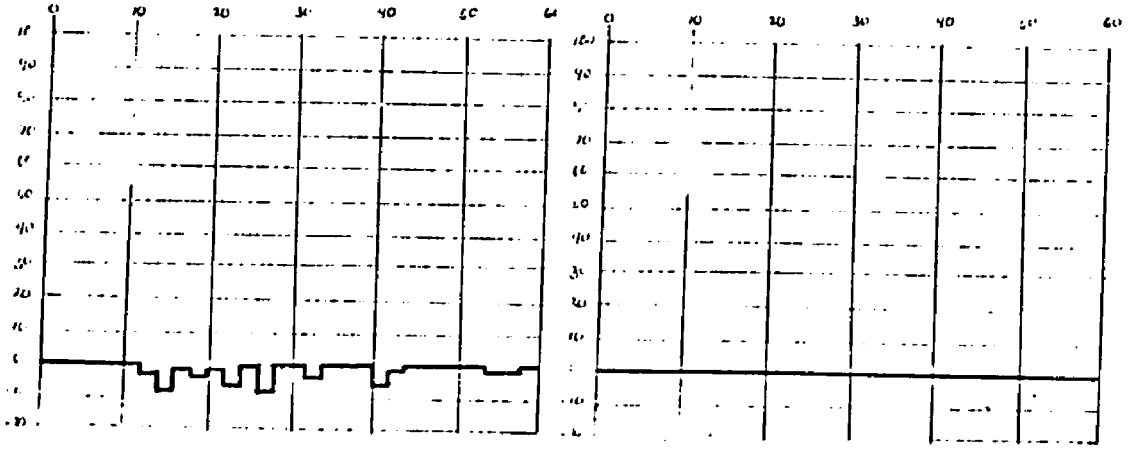





Exp. An. No. I
II







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