THE ELECTRICAL RESPONSES OF THE OLFACTORY EPITHELIUM OF SOME FISHES

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The electrophysiological studies on the olfactory nervous system have been very few compared with those on the other sensory organs. It is well known that the fish can well smell out odorous substances in water. By observing the behaviour of fishes, Parker (1910, 1911) made it clear that the catfish and the killifish are able to smell odorous materials in water, and provided evidence that gustatory and olfactory perceptions are not the same in these fish. Olmsted (1918) observed that the catfish could not respond to olfactory stimulation when the olfactory organ was eliminated, and that blinded fishes found foods as readily as normal fishes did. Adrian and Ludwig (1938) recorded the discharges produced by olfactory stimulation in the olfactory nerve of the catfish, carp and tench. The discharges increased rapidly to maximum after a latency of 0.5 to 5 sec., and then decreased very slowly. When a decoction of aniseed was used as stimulus, discharges appeared not only when it was given, but also when it was washed out with water (‘off-effect’). It was suspected that this ‘off-effect’ might be due to mechanical stimulation of flowing water. Gasser (1956) studied the structure of the olfactory nerve fiber of the pike by means of an electron-microscope, and recorded the action potential of the nerve produced by electrical stimulation. The electrical activity of the olfactory epithelium, however, has not been studied in fish, though it has in the dog (Hosoya and Yoshida, 1937 a, b) and in the frog (Ottoson, 1956, 1958; Takagi and Shibuya, 1959, 1960 a). Takagi and Shibuya (1959, 1960 a, b, c) recently found that there are three types of responses, that is, the on-, the on-off- and the off-responses in the olfactory epithelium of the frog and the toad.

Thus, studies on the olfactory nervous system of fishes have been limited only to the activity of the olfactory nerve. In order to clarify the function of the olfactory nervous system, it seems necessary to study the activity of the olfactory epithelium first, and then that of the higher olfactory pathway. It also seems important to compare these activities in the fish with those in the terrestrial animals. This paper is concerned with the responses of the olfactory epithelium of some fishes. The results are compared with the olfactory responses in the frog and the toad.

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METHODS

Materials. The carp (*Cyprinus carpio*), catfish (*Parasilurus asotus*), *Channa argus*, eel (*Anguilla japonica*), mudfish (*Misgurnus anguillicaudatus*) in Pisces and lamprey (*Entosphenus japonicus*) in Cyclostomata were used.

As described by Adrian and Ludwig (1938) and Allison (1953), the olfactory sac of these fishes is covered with dorsal skin and bones, and a pair of the olfactory sacs is situated in front of both eyes. The sac has two apertures, an anterior and a posterior nostrils. While swimming, water flows along the olfactory organ from the anterior nostrils to the posterior ones. The olfactory organ consists of a number of thin plates which project into the cavity of the olfactory sac. It is therefore presumed that the olfactory organ has a large

![Diagram of olfactory organ](image)

**Fig. 1.** Dorsal view of the olfactory organ. *A*, *Channa argus*; *B*, catfish. an, anterior nostril; pn, posterior nostril; p, a plate of the organ; uj, upper jaw.
receptive surface. Olfactory organs have different shapes in different fishes. That of *Channa* consists of 15 to 18 pinkish plates which stand side by side (fig. 1 A). The edge of each plate is black tinged with gold. The olfactory organ of the catfish is horseshoe-shaped and consists of from 88 to 90 small triangular blackish plates (fig. 1 B). The organ of the eel is elliptical in shape, has a colour between gray and black and consists of a number of very small plates. The number of plates is much the same in the catfish. The plates in the olfactory organ of the carp and the mudfish are generally small and poorly developed compared with those of the above fishes. The colour of these plates is white with many black dots. The lamprey has only a single olfactory sac, and the plates are different in size. The thickness of the olfactory epithelium covering a plate is about 40 \( \mu \) in *Channa*, about 25 \( \mu \) in the eel and about 65 \( \mu \) in the lamprey. The surface of the plates is covered with numberless olfactory hairs (the length of hairs was 12 to 15 \( \mu \) in *Channa*). The olfactory cells were found to be situated among the sustentacular cells, as was stated by Dogiel (1886). The diameters of the olfactory and sustentacular cells in *Channa* were less than 1.5 \( \mu \) and about 3 \( \mu \) respectively.

**Preparation.** After a fish was firmly fixed on a metallic stand, it was put in the middle of a container. Water was poured into the container up to the height of the gills. Then, under ether narcosis, the olfactory epithelium and nerve were exposed by removing the dorsal skin and bones. The eyeballs and the surrounding muscles were removed to avoid their occasional spontaneous movements, and then the fish was decapitated. This head preparation could be used for as long as several hours in winter. Some experiments were performed in situ.

**Stimulating and recording apparatus.** A glass pipette macro-electrode with tip diameter of about 100 \( \mu \) and filled with fish-Ringer's solution was used to record the potentials of the olfactory epithelium. A glass pipette micro-electrode filled with 3 M KCl was also used to record the potentials. It was inserted near the macro electrode situated on the epithelium. For recording of the resting potential of the olfactory epithelium, micro-electrodes with very sharp tip were used. For recording of the action potential of the epithelium, a micro-electrode with a very sharp tip but with a low resistance (20–30 megohms) was selected. The olfactory nerve discharges were recorded by lifting the nerve with a hook of 0.2 mm. thick silver wire.

As odorous substances, the dried pupa of the silk-worm (which has been used as foods for cultured fishes) was used. After pulverized, 10 grams of pupa was put into distilled water of 100 ml., and was boiled for about two minutes. Then, the solution was filtered, and the filtrate was used as stimulant. Besides this fluid, butyric acid (10^-1-10^-1%) and decayed water were used as stimulants. A stimulating apparatus which consists of double glass tubes with slender tips and change cocks was devised. With this apparatus, a stimulant was applied onto the epithelium through the inner tube, and after stimulation the epithelium was washed with distilled water through the outer tube. In some cases, by mean of 5 ml. syringes 1 or 2 ml. of a stimulant were applied onto the olfactory
epithelium through a lucite tube of 0.7 mm. inside diameter.

RC coupled amplifiers with a time constant of 1.5 sec. were used. Potentials were recorded with a four-element ink-writing recorder. The slow potentials of the olfactory epithelium were sometimes recorded in the olfactory nerve, because the potential was found to propagate electrotonically to the nerve. Thus, as mentioned later, the intervention of diffusion potential could be avoided. Since the slow potential of the epithelium was sometimes larger than the olfactory nerve discharge, an amplifier of a short time constant (0.01 sec.) was used to record only the nerve discharges. The fish-Ringer's solution has a following composition: 100 parts of $1/7.5 \text{ m}\ NaCl$, 2 parts of $1/7.5 \text{ m}\ KCl$, 2.1 parts of $1/11 \text{ m}\ CaCl_2$.

RESULTS

The resting potentials of olfactory epithelium

When the tip of a micro-electrode pierced the mucous membrane, potential changes of 4 to 18 mV suddenly appeared. The averages of the resting potentials of the olfactory epithelium were 12.4 mV in the eel, 8.7 mV in the mudfish, 8.6 mV in the catfish, 7.7 mV in the lamprey, 7.6 mV in the *Channa* and 7.6 mV in the carp respectively.

When the micro-electrode was advanced further into the epithelium, the potential changes were observed frequently and at random. The electrode was always tried to advance vertically to the epithelial surface, but it was not easy due to the random distribution of the olfactory plates. It might frequently occur that a micro-electrode was advanced diagonally to the surface of the epithelium. Consequently, the small potential changes observed may originate in the impalement of neighbouring cells by micro-electrode. It was therefore not possible to determine the origin of the potential changes except the initial one. It seems that the initial resting potential indicates the potential across the external limiting membrane. The resting potential of the olfactory epithelium of the toad was found to be 9.5 mV on the average (Takagi and Shibuya, 1960 b). The resting potentials found in fishes well coincide with the ones in the toad. Moreover, the magnitudes of these potentials are comparable to that of the resting potential of the toad's retina (Yoshida, 1953).

Action potentials of the olfactory epithelium

The on-response. When an odorous fluid was applied into the olfactory sac, a slow action potential with a steep rise and a gradual exponential decline was recorded by macro-electrodes (fig. 2). The magnitudes of the slow potentials were various between 300 $\mu$V and 3 mV according to the kinds of stimulating fluids. The shapes of the on-response were different in different kinds of fishes. The slow potentials in the *Channa* and the carp are shown in fig. 2 a, b. The duration of the slow potential was increased in proportion to the stimulating period. The slow potential in the lamprey had a slightly steeper decline than in the carp (fig. 2 c). The slow potential in the catfish generally had a short duration of 0.5 to 0.7 sec. (fig. 2 d). While the slow potentials in the above fishes had exponential decline, the slow potential in
the eel had a steep rise and a steep decline. It always had a duration of about 0.4 sec. in spite of the lengths of stimulation (fig. 2e). In correspondence with these slow potentials, olfactory nerve discharges appeared. The relation between the slow potential and the olfactory nerve discharges seems interesting. The details of the research will be published in the following paper (Shibuya and Takagi, unpublished). Adrian and Ludwig (1938) found that the application of water after stimulation produced olfactory nerve discharges ('off-effect'). Though distilled water was applied onto the olfactory epithelium after stimulation, the off-effect was not observed in the Channa.

In the present experiment, the tips of electrodes were in contact with the olfactory epithelium. It is therefore doubted whether the observed slow potential is an artifact due to the diffusion potential which may occur in the tip of the electrodes when stimulated. Taking advantage of the electrotonic spread of the epithelial potential to the olfactory nerve, the slow potential was often recorded in the olfactory nerve. When compared, the slow potential recorded in the epithelium was found to be identical with the one recorded in the nerve (fig. 3). It was made clear that diffusion potential, if any, does not considerably modify the slow potential which originates in the activity of the olfactory epithelium.

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**FIG. 2.** The slow potentials (on-response) of the olfactory epithelium of fishes recorded by a macro-electrode. *a*, *Channa argus*; *b*, carp; *c*, lamprey; *d*, catfish; *e*, eel. Stimulant: pupa fluid. The horizontal lines below each record show olfactory stimulation.

**FIG. 3.** The slow potential in the olfactory epithelium of *Channa* (top) and the electrotonic potential recorded simultaneously in the olfactory nerve (bottom). Stimulant: pupa fluid. The horizontal line below record shows olfactory stimulation.
The on-off-responses. When the application of a stimulating fluid was stopped, the slow potential sometimes appeared again in the olfactory epithelium of fish. Thus, the on-off- and the off-responses were observed in fishes.

The relation between the magnitudes of the on- and the off-responses was usually not constant. Sometimes, the off-response gradually increased its magnitude during repetitive stimulation. The on- and the off-responses were usually electro-negative (fig. 4a). But, only in the Channa, the off-responses were found to be electro-positive (fig. 4b). It was clear that this electropositive potential is an off-response, because the olfactory nerve discharges appeared coincidently.

The responses recorded by micro-electrode

The response recorded by macro-electrode is the sum of the potentials of many cells in the olfactory epithelium. In order to record the potentials of as a small number of the cells as possible, a micro-electrode was used. It was inserted as close as possible to a macro-electrode. Thus, potentials recorded by both electrodes were compared. The on-response recorded by micro-electrode had a magnitude of 2 to 5 mV. Various types of responses were observed in the epithelium of the carp (fig. 5). While a simple on-response was recorded
by macro-electrode, on- and off-responses were recorded by micro-electrode. The on-response was larger than the off-response in fig. 5a, both responses were of similar magnitudes in fig. 5b, and the on-response was much smaller than the off-response in fig. 5c. Similar slow potential could be recorded by micro-electrode inserted into the olfactory nerve. The off-response recorded by micro-electrode was also electro-positive in the Channa (fig. 6a). Besides, a pure off-response was rarely found in the Channa by this technique (fig. 6b).

![Image of Fig. 6](image)

**Fig. 6.** The electrotonic potential recorded in the olfactory nerve of Channa by a micro-electrode. a, the on-off-response; b, the off-response. The off-response was electro-positive in Channa. Stimulant: pupa fluid. The horizontal lines below each record show olfactory stimulation.

**Spontaneous discharges of the olfactory epithelium**

By micro-electrode, spontaneous spike discharges were sometimes recorded in the olfactory epithelium of the Channa, catfish and carp. It was found in the carp that the frequency of the spontaneous discharges increased in response to olfactory stimulation (from 2/sec. to 8/sec. in fig. 7), but that it decreased gradually after stimulation (fig. 7). This change occurred on the falling phase of the slow potential. It is supposed that these spontaneous discharges act as background activity, by which complicated olfactory sensation could be produced.

![Image of Fig. 7](image)

**Fig. 7.** Spontaneous spike discharges in the olfactory epithelium of carp. Top, the on-response recorded by macro-electrode; Bottom, the on-response with spontaneous spike discharges recorded by micro-electrode. Stimulant: pupa fluid. The horizontal line below record shows olfactory stimulation.
DISCUSSION

It was found that the on-responses produced by olfactory stimulation in the olfactory epithelium of fishes had different shapes and durations in different kinds of fishes. The shapes of the on-responses in the Channa, carp and lamprey resemble well those of the on-responses in the frog and the toad (Ottoson, 1956; Takagi and Shibuya, 1959). But the response in the catfish had a steep rise and a steep decline, and especially in the eel, it had a steepest rise and fall, and a shortest duration among the six kinds of fishes studied. The mechanisms which produce these different shapes and durations have been studied but is still to be clarified yet.

Hosoya and Yoshida (1937a, b) found that the slow potentials of the olfactory epithelium produced by olfactory stimulation are larger in the epithelium with darker colour. Onagawa (1957a, b) showed in the rabbit that the olfactory epithelium with dark colour was very sensitive to odours. The olfactory epithelium is pink in the Channa, it is white with black dots in the carp, and it is between gray and black in the catfish and the eel. In the present experiment, it was found in fish that the olfactory epithelium with darker colour had a slow potential of shorter duration. Indeed, a slow potential of the shortest duration was found in the eel whose olfactory epithelium has darkest colour among the six kinds of fish. It is well known that the eel has very keen olfactory sensitivity to foods. It is therefore presumed that there is a close relation between a short duration of the slow potential and the keenness of olfactory sensitivity.

Three types of responses, the on-, the on-off- and the off-responses were recorded in the olfactory epithelium of the frog and the toad (Takagi and Shibuya, 1959, 1960a, b, c). It was presumed in these amphibians that three response types may originate in the three types of olfactory cells reported by Dogiel (1886) (Takagi and Shibuya, 1960b). Three types of responses were also observed in the olfactory epithelium of fish. Coincidently, three types of olfactory cells are known in the olfactory epithelium of fish, as well (Dogiel, 1886; Jagadowski, 1901). Consequently, it is presumed that the three types of responses in fish may originate in the three types of the olfactory cells.

SUMMARY

1. The electrical responses to olfactory stimulation were studied by a macro- and a micro-electrode in the olfactory epithelium of six kinds of fishes, the Channa argus, eel, catfish, carp, mudfish and lamprey.
2. The averaged resting potentials of the olfactory epithelium of these fishes were found to be between 7.6 and 12.4 mV.
3. The shapes of the slow potentials produced in the olfactory epithelium by olfactory stimulation were different in different fishes. The potential in the Channa, carp and lamprey showed a steep rise and an exponential slow decline. The potential in the catfish and eel had a far steeper rise and decline and it had a short and constant duration in spite of the durations of stimulation.
4. Three types of responses, the on-, the on-off- and the off-responses were
found in the olfactory epithelium of these fishes. The relative magnitudes of the on- and the off-responses were various and not consistent during repetitive stimulation.

5. By means of a micro-electrode spontaneous spike discharges were sometimes recorded in the olfactory epithelium. They increased in number in response to olfactory stimulation.

6. The colour of the olfactory epithelium, the duration of the slow potential and the keenness of olfaction were compared. It was presumed that the short duration of the slow potential is closely related with the olfactory sensitivity.

7. The olfactory responses of fish were compared with those of the frog and the toad. The origin of the three types of responses was considered.

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