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| Title | Single Unit Analysis of the Olfactory Tract of the Crucian Carp (With 10 Text-figures) |
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| Citation | , 17(1), 208-223 |
| Issue Date | 1969-09 |
| Doc URL | http://hdl.handle.net/2115/27482 |
| Type | bulletin (article) |
| File Information | 17(1)_P208-223.pdf |

Instructions for use

# Single Unit Analysis of the Olfactory Tract
## of the Crucian Carp[1]

By

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*(With 10 Text-figures)*

Behavioral studies have shown that olfaction is well developed in many species of fishes and plays important roles in their lives, detection and choice of foods (Parker, 1910; Pipping, 1927), avoidance from enemies (von Frisch, 1941 a, b, Schutz, 1956), mating (Tavolga, 1956; Losey, 1969) and homing (Hasler and Wisby, 1951; Hasler, 1954).

The pioneering work on the electrophysiology of the fish olfactroy system was made by Adrian and Ludwig (1937). They showed that electrical response of the olfactrory tract to chemical stimulation of the olfactroy mucosa was a function of concentration. Recently it has been shown that the electrical activity of the fish olfactory bulb evoked by electrical or chemical stimulation of the olfactory nerves is influenced by the electrical stimulation of the olfactory tract of contralateral bulb or the other higher nervous structures (Döving and Gemne, 1966; Döving, 1966a; Hara and Gorbman, 1967; Hara, 1967). These result indicate that the olfactory tract of fish contains not only centripetal fibres but also the centrifugal fibres and also suggest that a complex mechanism of feedback control may be involved in the olfactory discrimination and memory in the fish. In order to elucidate the coding and controlling mechaism of olfactroy information at the level of the olfactory tract, it is necessary to know about the functional properties of individual fibre units within it. Although the several workers have studied on the nervous activity of olfactory tract of the teleost fish (Adrian and Ludwig, 1938; Boudreau, 1962; Döving, 1966b; Nanba et al., 1966), relatively little information & about them have been obtained because of the technical difficulty of recording electricl activities of the single fibres in the olfactory tract. In addition, they have never paid any attention to the effects of temperature change of the olfactory mucosa caused by application of the test solution or by the contact of any object at different temperature.

Recent electronmicroscopical observations by Westerman and Wilson (1968) have shown that the olfactory tract of the crucian carp (*Carassius carassius* L.) contains fine myelinated and unmyelinated fibres of about 73,000 (estimated number).

The present report will describe a qualitative analysis of functional properties of single fibres and their localizations in the olfactory tract determined, to make a basis for future study on the olfactory mechanism in the fish. During the present investigation it was found unexpectedly that the specially thermosensitive fibre units were included among the fibres of the olfactory tract. Mechanisms of thermoreception in the teleost fish will also be described.

**Material and Methods**: The crucian carps (*Carassius carassius grandoculis* Temminck et Schlegel) used in the experiments weighed 85–250 g and were 18–27 cm long. They were kept before use in a glass aquarium in which the tap water (12°C) was flowed continuously and were fed sufficiently with commercial compressed fish-meal foods.

*Preparation*: The fish was immobilized by the intraperitoneal injection of d-tubocurarine chloride (6 mg/kg fish weight). After the anterior part of the skull was opened, the fish was wrapped in a wet cloth and was fixed by a suitable holder under the dissection microscope. The connective tissue surrounding the olfactory tracts was removed. The exposed tract was divided into six fine (about $100 \mu$) strands (the medial tract was divided into four strands and the lateral one was divided into two strands). In order to investigate the localization of fibre units which were different functionally, these strands were named as $M_1$, $M_2$, $M_3$, $M_4$, $L_1$ and $L_2$ from the medial side of the tract, respectively.

*Recording and Stimulation*: For recording electrical activity of the olfactory tract, the anterior part of the brain pan was filled with paraffin oil. The strand was lifted up by a platinum wire recording electrode into the paraffin oil. As an indifferent electrode the silver plate was attached to the abdomen of the fish. Nerve activites were amplified through the AC amplifier (Nihonkoden Type AVB-1) and displayed on the cathode-ray oscilloscope (Nihonkoden Type VC-6). To record the unitary activity the further reduction of number of fibres in the strand was made on the electrode by means of a sharpened needle as the recording condition was monitored. Central connection, however, was cared to be maintained as far as possible.

The gills were flowed with oxygenic tap water led in through the mouth at a rate of flow of about 25 ml/min. And the blood vessels were left untouched as far as possible. In this manner the fish remained in good experimental condition for several hours. The physiological solution used has a following composition; 100 parts of M/6.5 NaCl, 2 parts of M/6.5 KCl and 2.1 parts of M/11 $CaCl_2$. This solution was buffered to pH 7.2 by $NaHCO_2$.

The chemical stimulation was applied to the olfactory mucosa by means of the three-ways stopcock connected with the glass reservoir for the flow of chemical solution and the lucite tube for the flow of distilled water. The nozzle (0.8 mm inside diameter) of the three-ways stopcock was inserted into the olfactory sac. The chemical solution (0.5–2.0 ml) was applied to the olfactory mucosa at the mean flow rate of 0.13 ml/sec by turning the three-ways stopcock to the reservoir of stimulus solution. During the interval of stimulation the olfactory mucosa was flowed with the distilled water continuously (0.3 ml/sec) by turning the stopcock. The 2 ml cylinder as the reservoir of stimulus solution was washed repeatedly with distilled water or exchanged for new one in every exchange of stimulus solution. As the stimulus substances, Sodium Chloride, n-Butyl Alcohol, Isoamyl Alcohol, n-Butyl Acetate and Isoamyl Acetate dissolved in distilled water were used. These substances were selected merely on trial and it is not clear whether these substances would be actually discriminated as different odours by the fish or not.

As we could preliminarily observe that the thermosensitive units did not respond to the mechanical stimulation, thermal stimulation of the olfactory mucosa was applied by the flow of distilled water at different temperatures. The temperature change of the surface of

mucosa was monitored by the thermister (Kokusaidenki Type NS. Time constant=0.39±
0.02 sec) attached to it and displayed simultaneously with the nerve activity on the oscillo-
scope. The experiments were carried out between February and September, 1968 under
room temperature (17.0–25.5°C).

## Results

### Chemosensitive units

The fibre units of this type did not respond to pure mechanical stimulation
(touching or pressing with the tip of a glass rod) and to thermal stimuli applied
to the olfactory mucosa. In the present experiment we could find only centripetal
ipsilateral chemosensitive fibre units. Most of the chemosensitive units fired
sponteneously but some of them showed a lower activity or no spontaneous
firing. Not all of the units did always respond to all of the chemical stimulants of
various concentrations (0.003125–0.5 M Sodium Chloride, 0.001–0.016 M n-Butyl
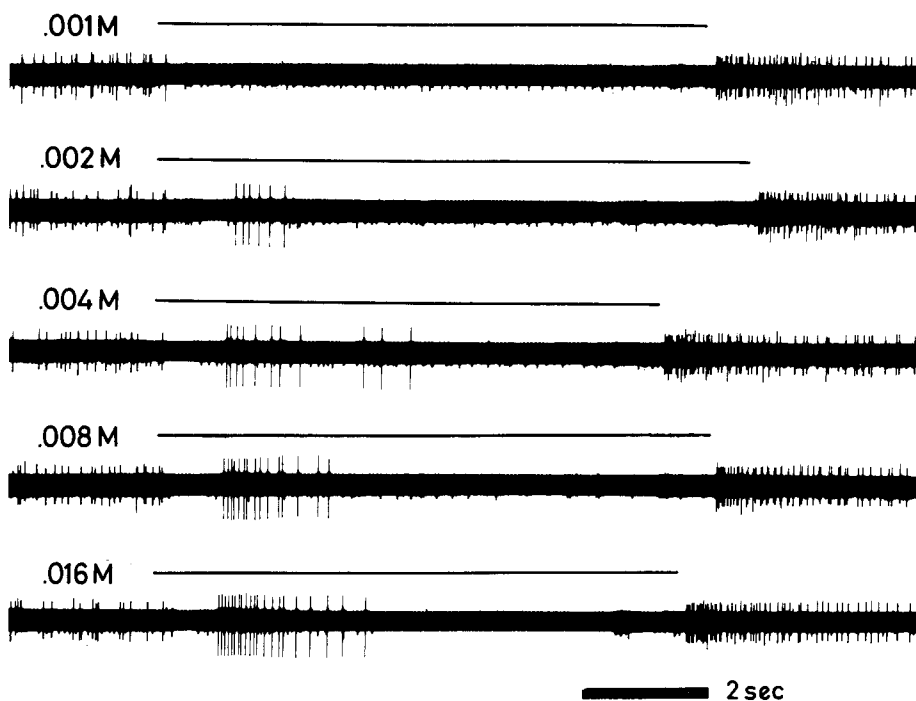Alcohol, 0.001125–0.018 M Isoamyl Alcohol, 0.001–0.016 M n-Butyl Acetate and



Fig. 1.   The response of the phasic unit to n-Butyl Acetate of various concentrations.
Here the small teperature change of the olfactory mucosa (from 18.3 to 17.9°C) was caused
by applicaton of stimulting solutions. The large spike unit is the phasic unit and the
small spike unit is the thermosensitive one (See in text). In this and the following figures
the horizontal bar above the record indicates the period of stimulation.

0.00875–0.014 M Isoamyl Acetate).

*Type of units*:   Although the response discharge patterns of units were varied with
the stimulus quality and concentration, generally speaking, the units could be
classified into two types; namely, "phasic" type and "tonic" type.

Figure 1. illustrates an example of the phasic unit resopnses to n-Butyl
Acetate.   This phasic unit showed no spontaneous firing.   When chemical
solution was flowed through the olfactory sac, the unit fired at the beginning of
the stimulation and the response soon ceased through the stimulation continued.
The impulse number was observed to increase with a increase of stimulus concentra-
tion.   This unit behaved as the same way to Isoamyl Acetate, but did not respond
to other stimulants;  Sodium Chloride, n-Butyl  Alcohol and  Isoamyl Alcohol,
regardless of the concentration.

On the other hand, the tonic units showed spontaneous firing and responded to
the stimuli with increase in frequency or with inhibition of the discharge.   Fig.
2a. illustrated the behaviour of such a unit to Isoamyl Acetate at various concent-
rations.   The increase in discharge frequency during stimulation was observed at
the concentration from 0.000875 to 0.00175 M.   But at the concentration of
0.0035 M, an inhibition of sponteneous discharges appeared after the simulation.
Furthermore,  a  marked  inhibition  period  was  observed  during  and  after  the
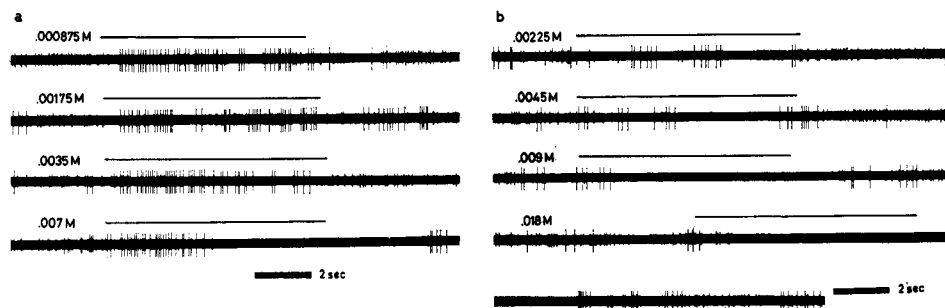


Fig. 2.   a:  The response of the tonic unit to Isoamyl Acetate  solutions of various
concentrations.   b:  The response of the same unit to Isoamyl Alcohol of various concent-
rations.   In these records, the olfactory mucosa was cooled from 21.75 to 21.0°C by appli-
cation of stimulating solutions.

stimulation and the discharge pattern became similar to that of the phasic units.
This unit behaved as the same way to n-Butyl Acetate.   The behaviour of the
same unit to Isoamyl Alcohol is shown in Figure 2b.   In this case the inhibition of
spontaneous  discharges  more  clearly  appeared.   The  similar  response  of  this
unit was observed in the case of n-Butyl Alcohol, but this unit did not response to
Sodium Chloride.

The discharge patterns of the tonic unit were actually much complexed, and
the inhibition of firing was observed after the stimulation  in  some units.   Figure

3a. shows the response of another tonic unit to n-Butyl Alcohol.  An increase in firing rate during stimulation was observed and the inhibition appeared after the stimulation.  The inhibition period became longer with increase of concentration and the total number of impulses during stimulation actually decreased with increases of concentration.  The response of this unit to Isoamyl Alcohol, Isoamyl Acetate and n-Butyl Acetate was similar to that to n-Butyl Alcohol.  But in the response to Sodium Chloride the inhibition period after stimulation was not observed (Fig. 3b).  This unit responded with increases in firing rate during stimulation, and the total number of impulses increases with the increase of concentration.  In this way the discharge patterns of tonic type unit seemed to be
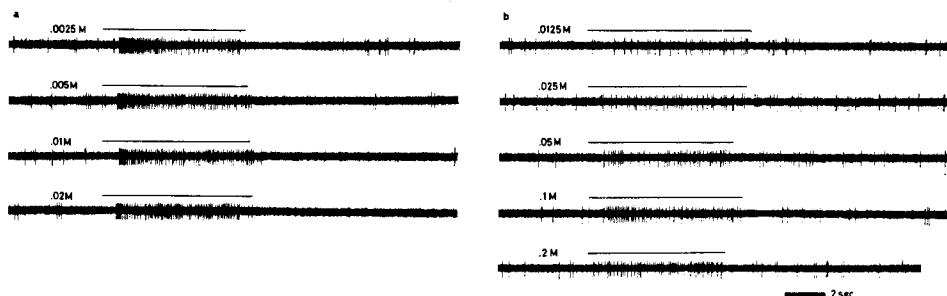


Fig. 3.  a: The response of another tonic unit to n-Butyl Alcohol of various concent-rations.  b: The response of the same unit to NaCl solutions of various concentrations. In these records, the olfactory mucosa was cooled from 22.25 to 21.8°C by application of stimulating solutions.

composed of excitation and inhibition components.  They were variable by the stimulus quality and concentration from unit to unit.

*Response characteristics of chemosensitive units to each chemical stimulant*:  Taking an extensive view of all the units, Sodium Chloride was observed to cause the tonic response and had no inhibitory effect in the concentration used (0.006125–0.5 M).  The esters (n- Butyl Acetate and Isoamyl Acetate) caused the phasic or tonic response, and the inhibitory effects by them sometimes were observed at higher concentration. The alcohols (n-Butyl Alcohol and Isoamyl Alcohol) caused the tonic response and rather inhibitory response comparing with the esters.

## *Thermosensitive units*

The units which responded specifically to thermal stimulation of the olfactory mucosa were found in the olfactory tract.  These units responded neither to pure mechanical stimulation nor to chemical stimulation of the olfactory mucosa.  Only the temperature change of the mucosa elicited the responses of the units.  Not only centripetal units but also centrifugal ones responding to thermal stimulation

of the contralateral olfactory mucosa were found.    The thermosensitive units could be classified into two types.    Namely, the one was the units excited by cooling and the  other  excited by warming the olfactory mucosa.    Such a differentiation of the  units  was  very  similar  to that  of the thermoreceptors previously reported in homoiotherms (Hensel et al., 1960)
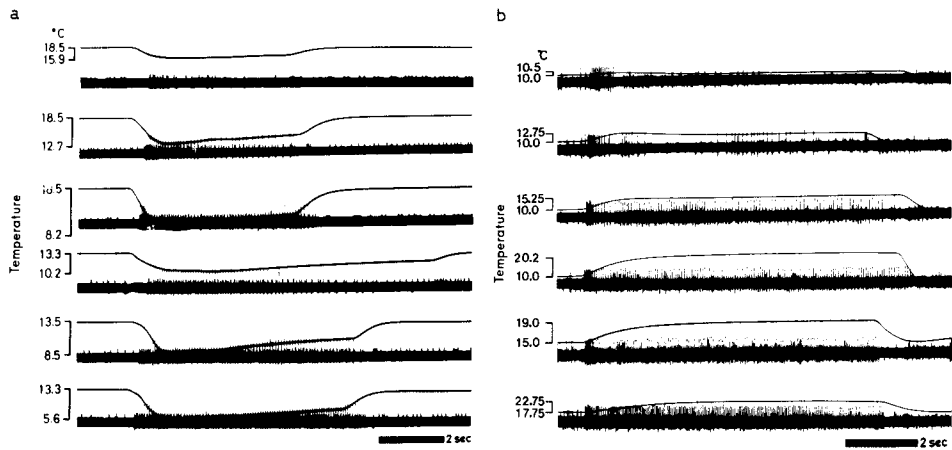


Fig. 4.    Impulse discharge of a cold unit (a) and a warm unit (b) when  the sudden temperature  change  was  applied  to  the  ipsilateral  olfactory  mucosa.
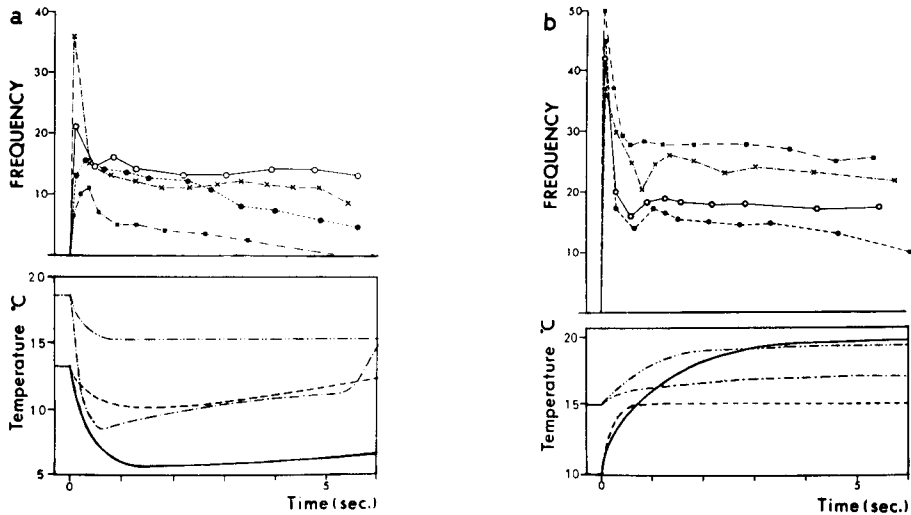


Fig. 5.    Frequency of impulses discharge in a cold unit (a) and a warm unit (b) when the temperature gradient was changed.    Each different line in the upper figure corresponds to the line in the lower figure.

### Ipsilateral thermosensitive units

*Cold units*: The units of this type responded to cooling the ipsilateral olfactory mucosa with an increase in discharge frequency and to heating it with a decrease or complete inhibition of the discharge. Figure 4a. illustrates the discharges of such a unit. In this record the olfactory mucosa was cooled from 13.5 or 18.5°C. Within those ranges of preadapting temperature the steady discharge was not observed (the further descriptions will be given later). When the olfactory mucosa was suddenly cooled, the discharge frequency increased transiently and reached a maximum and then declined gradually. Actually, the impulse discharge was elicited by cooling as much as a degree smaller than 0.1°C.

On sudden cooling, the discharge of the unit increased in frequency with an increase of the gradient of temperature change (Fig. 5a). The "dynamic" sensitivity (Hensel, Iggo and Witt, 1960) of this unit was 48.0 imp/sec/°C. This means that sudden cooling as much as 1°C would raise the discharge frequency by 48.0 imp/sec. The maximum discharge frequency of the units of this type was observed to be 65 imp/sec.

At constant temperatures the units exhibited steady discharges. Figure 6. shows graphically the steady discharge frequency of the another unit of this type at various constant temperatures between 5.5 and 17.7°C. The result was obtained after keeping the temperature constant for about 30 seconds. Within these ranges of temperature, the discharge frequency decreased with increase of temperature. The "static" sensitivity (Hensel, Iggo and Witt, 1960) of the unit was about -0.27 imp/sec/°C.

*Warm units*: The units of this type responded to heating of the ipsilateral olfactory mucosa with an increase in discharge frequency and to cooling of it with a decrease or complete inhibition of the discharge. Impulse discharge of the
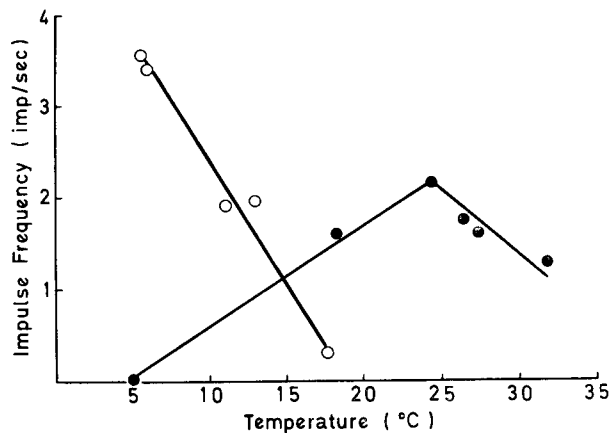


Fig. 6. Steady discharge frequency of a cold and a warm unit.

unit which responded to various temperatures (10.0, 15.0 and 17.5°C) is shown in
Fig. 4b. Within a certain preadapting temperature, steady discharges were
observed before warming. The time course of impulse discharge was also similar
to that of the units excited by cooling, and the discharge of the unit increased in
frequency with an increase of the gradient of temperature change (Fig. 5b). But
on sudden cooling such a "off" discharge reported in the "warm fibre" of the rat
tongue (Dodt and Zotterman, 1952) was not observed in the present study. An
increase in discharge frequency of the unit was actually observed by warming as
much smaller than 0.1°C, whereas the impulse discharge was also stopped by
cooling as much smaller than 0.1°C.

The dynamic sensitivity of this type of the units was 40.7 imp/sec/°C and
slightly lower than that of the units excited by cooling. The maximum discharge
frequency of these units was 55 imp/sec.

The steady discharge frequency of another unit of this type at various constant
temperatures between 5.0 and 32.0°C after keeping for about 30 seconds is shown
graphically in Figure 6. In this unit the maximum discharge frequency was
reached at temperature of 24.6°C. The static sensitivity of the unit was 0.11
imp/sec/°C in positive range and -0.19 imp/sec/°C in negative range, respectively.

### Contralateral thermosensitive units

The centrifugal thermosensitive units responding to thermal stimulation of
the contralateral olfactory mucosa could be classified also into two type in the
same way with ipsilateral thermosensitive units. The upper record of Figure 7.
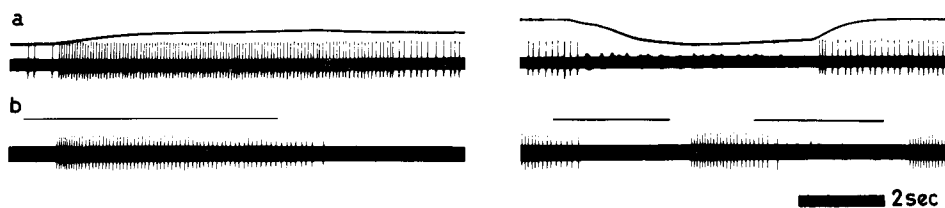


Fig. 7. Impulse discharges of the contralateral thermosensitive units during warming
and cooling of the contralateral olfactory mucosa. a: Warm unit. The olfactory mucosa
was warmed from 16.1 to 18.6°C and cooled from 20.6 to 16.1°C, respectively. b: Cold
unit. The olfactory mucosa was cooled from 17.5 to 11.0°C and warmed from 11.0 to
17.5°C, respectively.

shows the warm unit which responded to warming the contralateral olfactory
mucosa with an increase in discharge frequency and to cooling it with an inhibition
of the discharge. The lower one shows the other type of the units, namely the cold
unit which responded in the opposite way. Thermal stimulation of the ipsilateral
olfactory mucosa, of course, did not cause the responses of these units. The
discharge time course of these units was also similar to that of the ipsilateral
thermosensitive units and the sensitivity to temperature change of these units was
the same order as that of the ipsilateral ones. These results indicate that some

contralateral thermosensitive fibres of two different kinds may pass through the anterior commisure or the telencephalon and reach at the ipsilateral olfactory bulb.

<center>*Bimodal units:*</center>

Although the pure thermosensitive units did not respond to chemical stimuli unless they were accompanied by simultaneous thermal stimuli, the responses of the units of this type were strongly modified by chemical stimuli accompanied with thermal stimulation of the ipsilateral olfactory mucosa. The bimodal units could be classified into the 'warm unit' and the 'cold unit' as the same way to the thermosensitive units mentioned above. However, the 'warm unit' of this typs was excited by chemical stimuli even when the olfactory mucosa was actually cooled by application of stimulus solution. The 'cold unit' of this type was excited also by chemical stimuli, in contrast, even when the mucosa was warmed. And the bimodal units have also the specificities for the quality of chemical stimulus as the same way to the chemosensitive units.
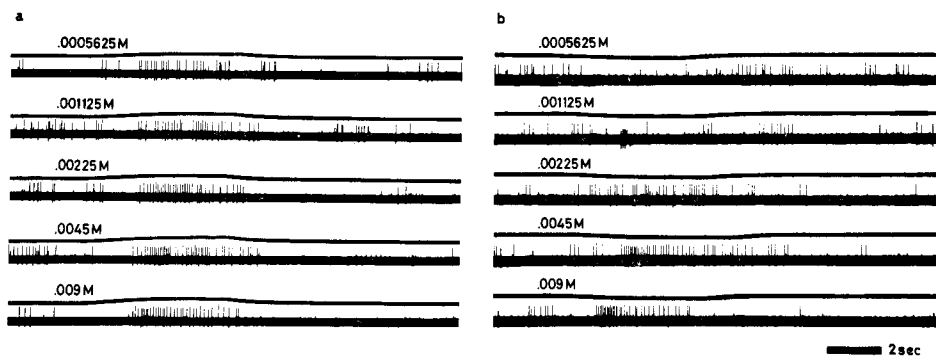


Fig. 8. The responses of the bimodal unit (warm unit) to Isoamyl Alcohol. a: The responses when the olfactory mucosa was warmed from 22.75 to 24.75°C by application of Isoamyl Alcohol solutions. b: The responses when the olfactory mucosa was cooled from 24.5 to 22.7°C by application of Isoamyl Alcohol solutions. The beam above each record indicates the temperature change of the mucosa caused by application of stimulus solutions.

Figure. 8a illustrates the responses of such a warm unit to Isoamyl Alcohol. In this the olfactory mucosa was warmed from 22.75 to 24.75°C by application of Isoamyl Alcohol solutions. The discharge pattern of this unit to Isoamyl Alcohol had the similar feature to that of the tonic chemosensitive unit. The discharge frequency increased with an increase in concentration. However, impulse number of the response actually decreased in higher concentration (0.009 M). Figure 8b shows the responses of the same unit when Isoamyl Alcohol solution was appled to the olfactory mucosa. In all these records the olfactory mucosa was cooled from 24.5 to 22.7°C by application of the stimulus solutions. In lower concentration range (0.0005625–0.001125 M) the discharge of this unit was inhibited. However, the response appeared with increasing concentration of stimulus substance. And

the discharge pattern of this unit became similar to that to stimulus solution warmed. This 'warm unit' responded specifically to Isoamyl Alcohol but did not respond to the other stimulants used, regardless the concentration of them.
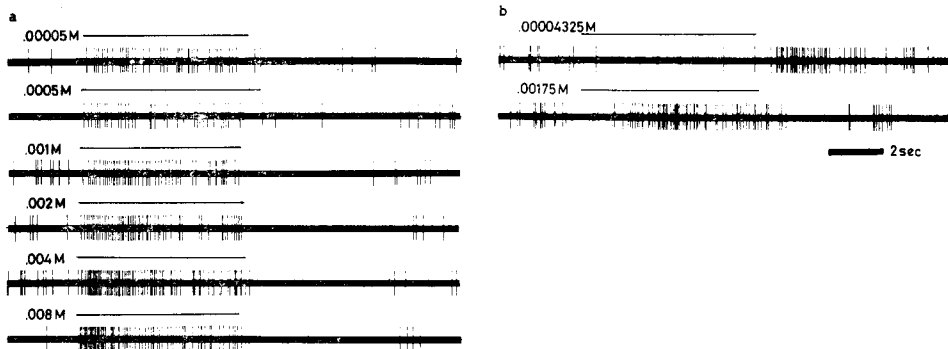


Fig. 9. The responses of the bimodal unit (cold unit) to n-Butyl Acetate and Isoamyl Acetate. a: The responses when the olfactoy mucosa was cooled from 20.8 to 20.75°C by application of n-Butyl Acetate solutions. b: The responses when the olfactory mucosa was warmed from 20.5 to 21.5°C by application of Isoamyl Acetate solutions.

On the other hand, Figure 9a illustrates the responses of the 'cold unit' of this type to n-Butyl Acetate. In this the olfactory mucosa was cooled from 20.8 to 20.75°C by application of the stimulus solutions. The magnitude of the response of the unit in the lower concentration range (0.00005–0.005 M) was somewhat equal. The discharge frequency in higher concentration range (0.001–0.008 M), however, increased with an increases in concentration of stimulus solution. In this way the effect of chemical stimuli appeared in higher concentration range. This unit behaved in the contrary ways to the 'warm unit' of this type for n-Butyl Acetate solution warmed (Fig. 9b). This unit rsponded also to Isoamyl Acetate but did not respond to Isoamyl Alcohol, n-Butyl Alcohol and Sodium Chloride, regardless the concentrations of stimulants.

## Mechanosensitive units

The centrifugal units responding to the extraolfactory stimulation were found. Figure 10a illustrates the units which excited to tactile stimulation of the body surface, particularly, by touching the tail skin of the fish. And application of Sodium Chloride, Indole or Skatol of high concentrations to the face skin was also efficient to excite the unit of this type.

On the other hand, the mechanosensitive units which were inhibited by tactile stimulation of the body surface were also found. The mechanical stimulation over entire body surface were efficient to inhibit the unitary activities of this type. The similar units were previously found by Döving and Gemne (1966) in the

burbot.  But in the present experiment a slight inhibition of the discharge of these units could be caused also by the flash stimulation to the fish eyes (Fig. 10).
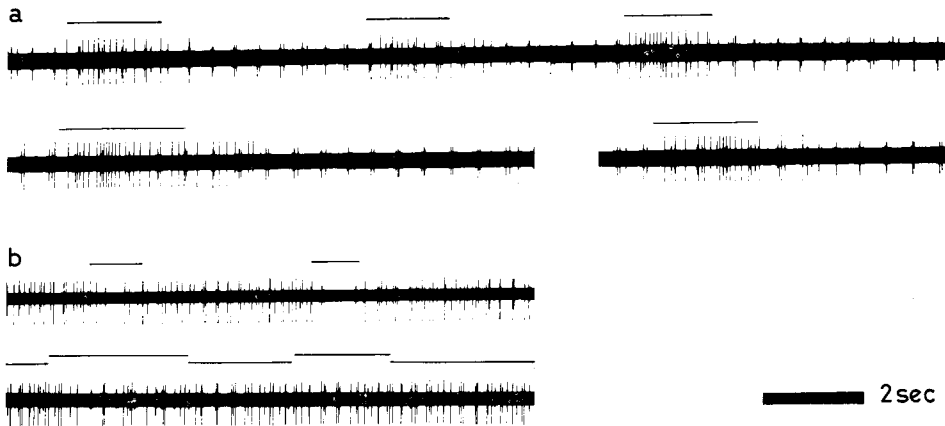
a

b



2 sec

Fig. 10.  Impulse discharges of mechanosensitive units.  a: The unit excited by tactile stimulation of the tail skin (the upper record) and by chemical stimulation of facial skin (the lower record).  b: The unit inhibited by tactile stimulation of the skin (the upper record) and by the flash stimulation to the eye (the lower record).

### Localization of units determined

Most of the chemosensitive units were obtained from $M_3$ and $M_4$ of the medial tract and some of them were obtained also from $L_1$ and $L_2$ of the lateral tract (20 units).  The ipsilateral thermosensitive units were obtained from $M_1$ and $M_2$ of the medial tract and $L_1$ and $L_2$ of the lateral tract (30 units).  The contralateral thermosensitive units were recorded from $M_3$ and $M_4$ of the medial tract (2 units).  The bimodal units localized only in the lateral portion of $L_1$ of the lateral tract (2 units).  The mechanosensitive units were obtained from $M_3$ and $M_4$ of the medial tract (12 units).  Judging from the recording condition and the spike height,  the diameter of chemosensitive fibres seemed to be smaller than those of the thermosensitive ones.

### Discussion

*Discharge patterns of the chemosensitive units*:  The results of the present work demonstrated that there were two main types in the chemosensitive units, namely, the 'phasic' and the 'tonic' type.  Although the discharge pattern was rather simple in the former units,  that of the latter was much complex and varied depending on the quality and concentration of chemical solution.  Furthermore, the chemosensitvie units did not always respond to all of the five chemical substances used in the present experiment.

According to the histological studies of the olfactory bulb of the teleost fish (Sheldon, 1912; Holmgren, 1920), the olfactory nerve fibres (the axons of the olfactory receptors) do not divide until they reach at olfactory glomeruli where they are in synaptic contact with dendrites of mitral cells and tufted cells. And each mitral cell has several main dendrites reaching out to branch in two or more glomeruli. Therefore, each mitral cell in fish olfactory bulb can accept the nervous information from several receptors in the mucosa, as well as from the tufted cells. Although the correspondence of the chemosensitive fibre unit in the tract with the bulber cell has not been determined histologically, if the chemosensitive fibre units in the present experiment are assumed to be the axons of the mitral cells in the bulb, and the olfactory receptors would have similar properties with those of the receptors in other animals, where the discharge of the receptor was either inhibited, accelerated and or unaffected by various chemical substances (Shibuya and Tucker, 1967; Gesteland et al., 1963), the complexity of the discharge patterns of the chemosensitive units may depend on the connecting ways of the mitral cell with various kinds of receptor cells.

Furthermore, we must be concerned in the higher nervous control on the discharge patterns of the chemosensitive units. Döving and Genme (1966) reported that the spontaneous discharge of the centrifugal unit in the olfactory tract of the burbot (*Lota lota* L.) was inhibited by tactile stimulation of the fish skin. In the present experiment with the crucian carp we found also this type of the centrifugal unit as the mechanosensitive fibre unit. The discharge of the unit was sometimes inhibited by flash stimulation to the fish eyes. In addition, the centrifugal units in which the discharges were accelerated by tactile stimulation of the skin or by application of chemical solution of high concentration to the facial skin were also found in the present experiment. As already pointed out by Döving and Gemne (1966) and Hara (1967), these facts suggest the discharge of the chemosensitive units is influenced excitatorily or inhibitorily by the other sense organs via higher nervous pathways.

*Thermoreception in teleost fish*: It has been shown that the ampullae of Lorenzini are the thermoreceptors in the elasmobranch fish (Sand, 1938; Hensel, 1955; Murray, 1962). On the other hand, some of the teleost fish have been shown to be able to percieve the temperature differences of 0.03 to 0.05°C (Bull, 1937) or of 0.05°C (Bardach and Bjorklund, 1957) by means of conditioning techniques. However, there was no evidence of existence of such a highly sensitive thermoreceptor in the teleost fish.

Recently, Späth (1967) reported that the sensory endings of the head and spinal nerves of *Leusisus* (*Cyprinidae*) do not register temperature stimuli unless these are accompanied by simultaneous mechanical stimulation and that they showed a reaction to temperature change which corresponds to that of the cold fibres of the homoiotherms. He explained that the thermal informations in teleost fish may be sent to the central nervous system not only by the thermo-specific receptors, but also by the multimodal receptors accompanied with mechanical

informations. Before discussing about the problems whether the 'unimodal' or
'multimodal' system is involved in the thermoreception of the teleost fish, we must
clear out whether the thermo-specific receptor exist or not in the olfactory mucosa
of the fish. In our experiment the thermosensitive units were shown to respond
to the temperature change of the olfactory mucosa smaller than 0.1°C and they
were differentiated to warm and cold units, which are coincided with the previous
behavioral observations. In addition to these facts, the existence of the bimodal
units in our experiment suggest that the thermo-specific receptor may localize in
the olfactory mucosa of the teleost fish. Because the existence of the bimodal
unit is thought to result from the integration in the olfactory bulb.

Recently, Wilson and Westerman (1967) studied on the ultrastructural ob-
servation of the olfactory epithelium of the crucian carp, *Carassius carassius* L.
And they identified the five distinct types of cells in the epithelium, namely, the
olfactory receptor cells, sustentacular cells, mucous cells, basal cells and foliaceous
cells. The existence of the foliaceous cell has not been previously described in
the olfactory mucosa of other animals. From its intricate structure they suggested
that the foliaceous cell may be a sensory receptor. We also agree with their
suggestion and it may be suggested that the foliaceous cell is a specific thermore-
ceptor in the teleost fish.

Furthermore, in the present experiment with the crucian carp, the contralateral
thermosensitive fibres were shown to exist in the tract. This fact suggests that
the inhibition or the acceleration control of the ipsilateral olfactory bulb by the
contralateral thermosensitive fibres may be associated with raising the accuracy
in detecting the temperature change. The mechanisms of this, however, is not
clear in the present experiment.

*Functional differentiation of the units*: Although the functional properties of all
finer fibres in the tract could not be always determined by the present method of
splitting the tract into fine strand, the present results showed that there are at
least four main functional types in the olfactory tract fibres: namely, the
chemosensitive, the thermosensitive, the bimodal and the mechanosensitive one.
Their localizations in the tract was somewhat definite. Especially in the thermo-
sensitive units, the warm unit were frequently recorded simultaneosly with the cold
units from the same position. Therefore, it seems that the fibres with the same
function gather and run along the olfactory tract. In addition to these fibres
defined functionally, we found more than 20 fibres which did not respond to any
stimuli used in the present experiment.

According to the electronmicroscopical observations by Westerman and
Wilson (1968), numerous fine (smaller than 0.1 μ in diameter) unmyelinated
fibres as well as myelinated fibres are packed in the lateral and medial tract of the
crucian carp. However, it is somewhat questionable whether all these fine
fibres have independently their functions, such as chemoreceptive or thermorecep-
tive very slow conduction velocity and the electrical shild between them may be
thought to be incomplete. Therefore, a large number of these fine unmyelinated

fibres may have any other function like neurosecretion (Jasinski *et al.*, 1966; Kandel, 1964).

## Summary

Nerve impulses of the single fibres were recorded from the olfactory tract of the crucian carp (*Carassius carassius grandoculis* T. et S.). The following functinally different fibre units were established by chemical and thermal stimulation of the olfactory mucosa and mechanical stimulation of the fish body.

1. The chemosensitive units responding to chemical stimuli were classified mainly into two types from the discharge patterns, namely the 'phasic' and the 'tonic' types. The phasic fibres discharged at the beginning of stimulation and the impulse number and discharge frequency increased with an increases of concentration of chemical solution. The tonic fibres showed the complex discharge patterns varying by the quality and concentration of chemical solutions, and the following discharge patterns were observed; and increase in discharge frequency during stimulation, and inhibition during stimulation and increase in discharge frequency during stimulation followed by an inhibition of discharges. Each unit did not always respond to chemical substances used (NaCl, Isoamyl Alcohol, Isoamyl Acetate, n-Butyl Alcohol and n-Butyl Acetate), regardless their concentrations.

2. The thermosensitive units responding specially to the temperature change of the olfactory mucosa were classified into two types of units; one was the warm unit, which was excited by warming and inhibited by cooling of the olfactory mucosa, and the other was the cold unit, which was excited by cooling and inhibited by warming of the olfactory mucosa. The discharge frequency of the thermosensitive units increased or decreased by temperature change of smaller than $0.1°C$ in the both units. And the thermosensitive units exhibited the steady discharge when the olfactory mucosa was kept at a constant temperature. In the thermosensitive units there were not only the centripetal ones responding to temperature change of the ipsilateral olfactory mucosa but also the centrifugal ones responding to temperature change of the contralateral olfactory mucosa.

3. The bimodal units responding to not only thermal stimuli but also chemical stimuli were classified into two types, namely the 'cold unit' and the 'warm unit'. The cold unit of this type responded to chemical stimuli even when the olfactory mucosa was warmed by application of chemical solution. The warm unit of this type behaved just in contrary ways to the cold unit.

4. The centrifugal mechanosensitive units of two types were found; namely, the unit which was inhibited by tactile stimulation of the fish skin and by flash stimulation of the eye and the unit which was excited by the tactile and the chemical stimulation of the fish skin.

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