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# Behavioural and Electrical Responses of the Land Snail, *Ezohelix flexibilis* (Fulton), to Odours<sup>1)</sup>

By

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(With 5 Text-figures and 7 Tables)

It has been said that the land snail and the slug can perceive distant chemical stimuli and that their four tentacles play the dominant role in odour perception (Hanström, 1926; Schulz, 1938; Kittel, 1956). However, no electrophysiological study has ever been made on the olfactory response of these animals.

Odours applied to the olfactory epithelium of vertebrates (Ottoson, 1956, 1959; Shibuya, 1960; Shibuya and Takagi, 1963; Takagi, Shibuya, Higashino, and Arai, 1960) have been known to elicit slow negative or positive potential changes. The potentials of the frog olfactory epithelium have been attributed to the activity of the olfactory receptor cells (Takagi and Yajima, 1965).

In the present study, the behavioural response to odours was investigated statistically to obtain general information on olfaction in the snail and to clarify the role of the tentacles by carrying out experimental amputation on them. Secondly, as an approach to elucidation of the olfactory receptive mechanism in the snail, the electrical properties of the olfactory epithelium of the tentacle tip were studied comparatively with those of the vertebrates.

**Material and Method:** The snails, *Ezohelix flexibilis* (Fulton), the common species in Hokkaido, were used throughout the present experiments.

**Observation of behavioural response:** The test apparatus for observing the behavioural response to odours was built as shown in Fig. 1. The odourized air and deodourized air were passed into the glass box (63×11×11cm). The air for both streams was initially dried and deodourized by the silica gel and the activated charcoal and then humidified by the deodourized water. The air for the odourized stream was then passed through the water suspension of cut pieces of vegetables (cucumber, cabbage, lettuce and Japanese butter bur). The outlet tube of the testing glass box was connected with a suction pump to take out the odourized air filled in the box. The test snails were made hungry for 3-4 days before the experiments. Twenty or more individuals were placed at random on the middle part of the floor of the test box. The side from which the odourized air came in, was changed also at random to eliminate the learning effects from trial repetition. The tests were repeated at 30 minute intervals and each test took 15-20 minutes. The number of individuals that migrated to

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both sides of the test box was counted. Percentages of migrated animals were calculated. All data were analysed by paired comparison with arc sine conversion (Snedecor, 1956; Mayer and Thaggard, 1966). The *t*-test at 0.05 per cent level was used to determine the statistically significant difference.

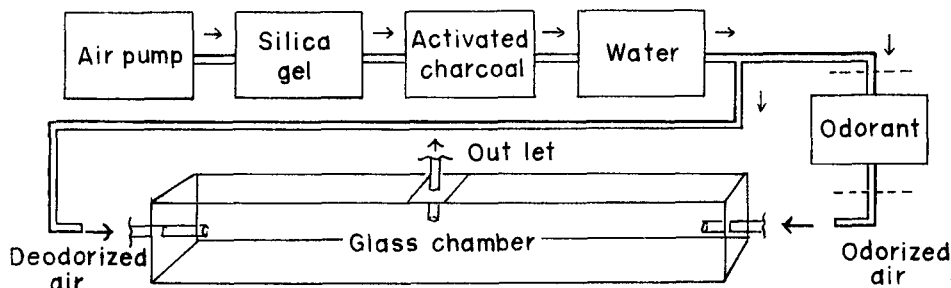


Fig. 1. Diagram of the experimental arrangement of the olfactory response. The odorant (between the two dotted lines) is replaced in every exchange of stimulant.

*Recording of slow potentials of the tentacular epithelium;* The tentacle tip was isolated and placed, with the receptive surface up, on filter paper, soaked in physiological saline, (Sato, Tamasige and Ozeki, 1960) over the Ag-AgCl indifferent electrode in a lucite chamber. The recording electrode, which was a salt bridge consisting of the physiological saline and gelatin in a glass pipette with a tip diameter of 100–150 $\mu$ , was attached to the receptive surface of the tentacle tip. The electrodes, filled with 3% gelatin-physiological saline, which were stored in the physiological saline for a week, were most suitable for electrical stability. Potentials of the epithelium were amplified by DC amplifier (Nihon Koden, Type ADH 1) and displayed on a cathode ray oscilloscope (Nihon Koden, VC 6).

Various odours were applied as follows: The deodorized air was obtained by passing it through the silica gel and the activated charcoal, and it was saturated with various chemicals. Saturated vapours were diluted to 1/2, 1/4, 1/8, and 1/10 by the cleaned and humidified air. The odourized air was put into the 5 cc syringe connected with the DC motor by which the volume and the flow rate of stimulant gas were controlled. Odours were applied to the whole surface of the tentacle tip from a distance of 2 cm by means of a glass tube with an inner diameter of 2 mm.

All experiments were carried out under room temperature (19–20°C) in autumn, 1966 and in spring, 1967.

## Results

### *Behavioural response to odours*

When a piece of filter paper, soaked in the odour substance, such as ethanol or amyl acetate, was advanced towards each tentacles, the animal was observed to pull its tentacle in quickly, although it did not pull in its tentacles when a piece of filter paper soaked with the pure distilled water was brought close to it. When the odours were applied to other parts of the body surface, the muscle of the part stimulated by the odours contracted slowly. And in fact all parts of the body surface responded more or less to chemical stimuli.

Table 1. Response of intact snails to odours of vegetables

		Arc sine $\sqrt{(\text{per cent})} \pm \text{S.E.} (\dagger)$ of snails moved to	Number of repetition
I.	Deodourized air	38.2 $\pm$ 2.2 (38.7)	10
	Deodourized air	38.7 $\pm$ 2.1 (39.3)	
II.	Cucumber-odourized air	45.4 $\pm$ 3.9 (51.2)*	10
	Deodourized air	22.8 $\pm$ 3.9 (18.0)	
III.	Japanese butter bur- odourized air	35.6 $\pm$ 3.9 (34.7)*	6
	Deodourized air	12.9 $\pm$ 6.9 (10.8)	
IV.	Cabbage-odourized air	42.5 $\pm$ 4.1 (46.2)*	7
	Deodourized air	26.1 $\pm$ 5.1 (22.6)	
V.	Lettuce-odourized air	42.6 $\pm$ 1.2 (47.4)*	9
	Deodourized air	20.2 $\pm$ 0.9 (14.5)	

\* Statistically significant difference at 0.05 level

† Actual percentages in parentheses

To determine what parts of the body play the dominant role in the olfactory perception of the snail, amputation experiments were undertaken using the test box. Data summarized in Table 1 show the response of an intact snail to the odours of vegetables. In the control where the deodourized air was taken in from both sides of the box, the migrated percentages to left and right sides were 38.7 and 39.3, respectively, and there was no significant difference. But the application of odours of vegetables caused the movement of snails toward them. In all the cases where stimulants were used, statistically significant differences between the responses to odourized and deodourized air were observed (Table 1. II, III, VI and V). These results show that snails after 3-4 days abstinence from foods are attracted by the odours of vegetables which are taken favourably as nutriments. And it is clear that this behaviour did not depend on the detection of the humidity difference or on visual perception but on the olfactory perception, because the applied air was sufficiently humidified and the vegetable matters were not given directly but the extracted odours from them were applied to the snails.

In the unilaterally amputated animals, where the large tentacle of one side was cut off, there was no statistically significant difference between the percentages of migrated animals to odourized and deodourized air. Odour-attraction did not appear clearly (Table 2. II, III and IV). The same results were also obtained from the bilaterally amputated animals in which both large tentacles were cut off (Table 3. II, III and IV).

But the amputation of one or both small tentacles did not deprive the snails of the odour-attraction behaviour (Table 4 and Table 5.).

Table 2. Response of snails, amputated one of large tentacles, to odours of vegetables.

		Arc sine $\sqrt{(\text{per cent})} \pm \text{S.E.} (\dagger)\ddagger$ of snails moved to	Number of repetition
I.	Deodourized air	29.8 $\pm$ 1.6 (25.0)	8
	Deodourized air	31.2 $\pm$ 2.7 (27.5)	
II.	Cucumber-odourized air	31.6 $\pm$ 1.2 (27.7)	10
	Deodourized air	29.7 $\pm$ 3.0 (25.8)	
III.	Cabbage-odourized air	28.0 $\pm$ 2.3 (23.0)	10
	Deodourized air	23.7 $\pm$ 3.4 (18.5)	
IV.	Lettuce-odourized air	23.9 $\pm$ 1.4 (16.7)	6
	Deodourized air	21.6 $\pm$ 2.4 (14.2)	

† Actual percentages in parentheses

‡ No significant difference in these tests

Table 3. Response of snails, amputated both large tentacles, to odours of vegetables

		Arc sine $\sqrt{(\text{per cent})} \pm \text{S.E.} (\dagger)\ddagger$ of snails moved to	Number of repetition
I.	Deodourized air	33.6 $\pm$ 2.0 (30.9)	6
	Deodourized air	35.9 $\pm$ 2.1 (34.5)	
II.	Cucumber-odourized air	32.2 $\pm$ 1.8 (28.6)	7
	Deodourized air	35.6 $\pm$ 1.9 (34.0)	
III.	Cabbage-odourized air	28.2 $\pm$ 1.5 (22.5)	6
	Deodourized air	30.1 $\pm$ 2.9 (25.8)	
IV.	Lettuce-odourized air	31.3 $\pm$ 2.2 (27.5)	7
	Deodourized air	32.1 $\pm$ 2.5 (28.9)	

† Actual percentages in parentheses

‡ No significant difference in these tests

Table 6 shows the response of the animals in which the tentacles were amputated entirely. There was no significant difference between the responses to odourized and deodourized air and the odour-attraction behaviour disappeared.

The above mentioned results in behavioural responses indicate that the four tentacles play a more dominant role than other parts of the body in the odour perception. And it is necessary for the animal not only to perceive the odours but also to know the direction of the odour source. These experimental results suggest important role of the tentacles in the orientation to the odour source. Further

descriptions on the odour orientation will be given in the discussion (p. 183).

Table 4. Response of snails, amputated one of small tentacles, to odours of vegetables

		Arc sine $\sqrt{(\text{per cent})} \pm \text{S. E.} (\dagger)$ of snails moved to	Number of repetition
I.	Deodourized air	33.4 $\pm$ 2.6 (33.5)	8
	Deodourized air	34.0 $\pm$ 2.6 (34.7)	
II.	Cucumber-odourized air	36.2 $\pm$ 2.0 (35.0)*	7
	Deodourized air	26.8 $\pm$ 3.2 (20.7)	
III.	Cabbage-odourized air	34.3 $\pm$ 1.7 (31.8)*	6
	Deodourized air	19.8 $\pm$ 5.7 (15.2)	
IV.	Japanese butter bur- odourized air	37.6 $\pm$ 3.5 (37.5)*	6
	Deodourized air	25.2 $\pm$ 1.2 (18.4)	

\* Statistically significant difference at 0.05 level

† Actual percentages in parentheses

Table 5. Response of snails, amputated both small tentacles, to odours of vegetables

		Arc sine $\sqrt{(\text{per cent})} \pm \text{S. E.} (\dagger)$ of snails moved to	Number of repetition
I.	Deodourized air	31.0 $\pm$ 3.7 (27.5)	6
	Deodourized air	29.3 $\pm$ 2.7 (24.0)	
II.	Japanese butter bur- odourized air	27.3 $\pm$ 2.7 (21.6)*	6
		16.6 $\pm$ 3.7 (10.0)	
III.	Cucumber-odourized air	25.5 $\pm$ 2.5 (19.1)*	6
	Deodourized air	14.7 $\pm$ 5.2 (10.0)	

\* Statistically significant difference at 0.05 level

† Actual percentages in parentheses

#### *Electrical response of olfactory epithelium*

1. *Slow potentials and various odours:* The surface of the tentacle tip was electrically negative ( $-4.1 \pm 1.2$  mV) with respect to the tentacle base and this negativity was confined to the yellow part of the tip. A potential change between the epithelial surface of the tentacle tip and the other point of its base could be produced by a puff of odourized air (the volume; 1 cc, the flow rate; 0.5 cc/sec), whereas there was little or no response to a puff of cleaned moist air. The slow monophasic potential had a faster rising phase and a slower exponential decline. The potential changes

caused by various odours could be divided into two kinds; one was negative and the other positive. Most odours caused the negative potential but odours such as methanol and ethanol caused the positive potential in the present experiment. The odours of vegetables which were taken by the snails as their favourite foods and ethylmercaptan which was contained a little in cabbage also caused the negative potential (Fig. 3, Fig. 4 and Table 7).

Table 6. Response of snails, amputated both small and large tentacles, to odours of vegetables

		Arc sine $\sqrt{(\text{per cent})} \pm \text{S.E. } (\dagger)\ddagger$ of snails moved to	Number of repetition
I.	Deodourized air	32.5 $\pm$ 1.7 (29.0)	9
	Deodourized air	31.2 $\pm$ 1.6 (27.1)	
II.	Cucumber-odourized air	34.7 $\pm$ 3.4 (33.1)	6
	Deodourized air	34.1 $\pm$ 1.8 (31.6)	
III.	Cabbage-odourized air	38.3 $\pm$ 3.3 (38.9)	6
	Deodourized air	34.1 $\pm$ 3.0 (32.0)	
IV.	Lettuce-odourized air	35.6 $\pm$ 3.1 (34.4)	7
	Deodourized air	35.8 $\pm$ 1.6 (34.4)	

† Actual percentages in parentheses

‡ No significant difference in these tests

Table 7. Slow potentials of olfactory epithelium caused by various odour substances

Odour Substances which cause a positive potential	
Methanol	
Ethanol	
Odour Substances which cause a negative potential	
n-Butanol	iso-Amyl acetate
n-Propanol	Methylmercaptan
Chloroform	Benzol
Ethyl ether	Toluol
Formaldehyde	Xylol
Acetone	Cucumber
Formic acid	Lettuce
Acetic acid	Japanese butter bur
Ethyl acetate	Cabbage
n-Butyl acetate	

It has been reported previously that saturated vapours of chloroform and ethyl ether cause the positive potential in the frog (Takagi, Shibuya, Higashino and Arai, 1960; Higashino and Takagi, 1964; Gesteland, 1964; Takagi, Wyse and Yajima, 1966) and in the newt (Shibuya and Takagi, 1962; Shibuya and Takagi, 1963). But in the present experiment on the snail tentacle, chloroform and ethyl ether did not cause the positive potential but only the negative potential.

2. *Responses and stimulus variables*: The relation between the response amplitude and stimulus concentration is shown in Fig. 2. An increase in the concentration led to an increase in the amplitude at the given volume and in the flow rate of both the negative and positive potential.

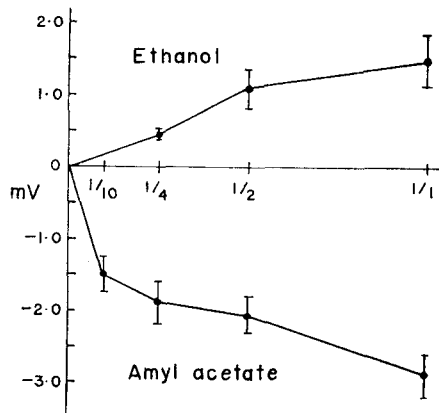


Fig. 2. The relationship between the potential amplitude and the concentration of stimulant. The volume and the flow rate of stimulant gas were fixed at 1 cc and 0.5 cc/sec, respectively. The widths of vertical lines through the points indicate the standard error.

And the same relationship between the amplitude of response and the flow rate of stimulating odourized air was observed at the given stimulus concentration and volume (Fig. 3).

When the duration of stimulation was prolonged, both the positive and negative potential were sustained until the end of stimulation (Fig. 4).

The above mentioned electrical properties of the olfactory epithelium of the snail with respect to stimulus variables, were very similar to those of the frog and the newt (Ottoson, 1956; Shibuya and Takagi, 1963), although there was not a distinct "off-response" in the snail.

3. *Decrease in potential amplitude by removal of the receptor layer*: The following experiments were undertaken to determine the origin of the negative and positive potentials. Initially, the potential amplitude caused by ethanol and amyl acetate respectively was measured. Then, the receptor cell layer (Schulz, 1938) was rem-

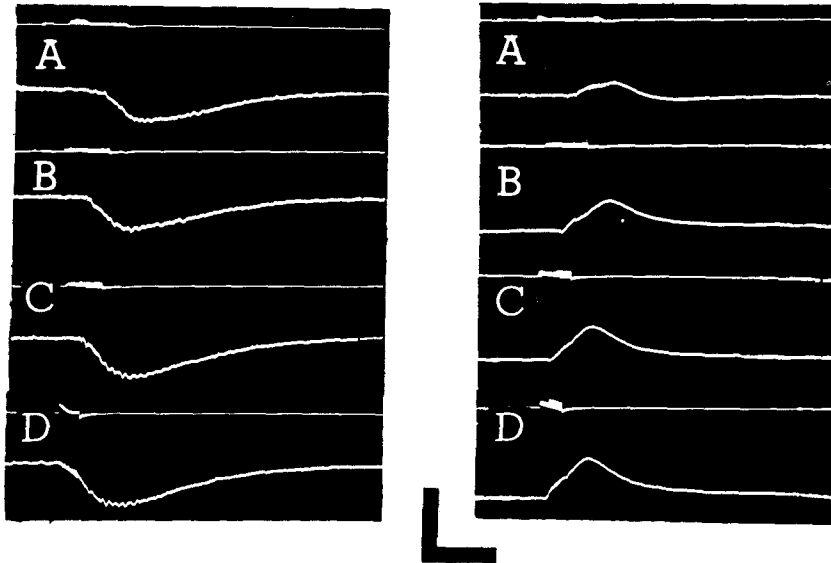


Fig. 3. The increase in potential amplitude with the increase in the flow rate of stimulant gas. The stimulants are 0.5 cc of 1/1 amyl acetate in the left row and the same volume of ethanol in the right row. A: 0.31, B: 0.42, C: 0.50, D: 0.89 cc/sec. The upper trace in each record indicates the stimulus duration. Calibration marks in this and all following figures indicate 5mV positive in the upward direction and 2 sec. in the right direction, respectively.

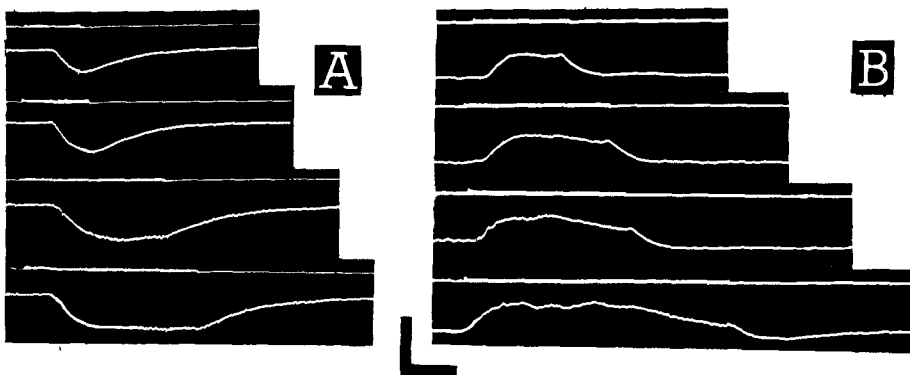


Fig. 4. The stimulus duration and the response. A: 1/1 amyl acetate at 0.5 cc/sec. B: 1/1 methanol at 0.25 cc/sec. The upper trace in each record indicates the stimulus duration.

oved mechanically by means of a pair of sharpened forceps under the dissecting microscope. After removal of the receptor layer, the measurement of the amplitude was made as before. Paraffin sections of the epithelium were made after recording the electrical response, to check whether the receptor layer was removed or not.



Fig. 5. Decrease in the potential amplitude. A: 1/1 amyl acetate. B: 1/1 ethanol. The upper trace and the lower one in A and B indicate the potential change before and after removal of the receptor layer, respectively. The volume and the flow rate of stimulant gas were fixed at 1 cc and 0.5 cc/sec, respectively. C: The microphotograph of the section of tentacular epithelium after removal of the receptor layer. Calibration mark indicates 100 $\mu$ .

Fig. 5, C shows the microphotograph of the tentacle tip after removal of the receptor layer. The supporting cell layer is not injured but the receptor layer is almost removed. The responses before removal of the receptor layer are shown in the upper records in Fig. 5, A and B. And the lower records in Fig. 5, A and B are the responses after removal of the receptor layer. The negative potential elicited by amyl acetate was reduced to  $18.5 \pm 6.3\%$  of the initial one in amplitude by removal of the receptor layer. On the other hand, the positive potential caused by ethanol was reduced to  $64.5 \pm 7.7\%$  of the initial one in amplitude. The removal of the receptor layer was more effective on the negative potential than on the positive one.

### Discussion

Schulz (1938) has examined the orientation of the snail, *Helix pomatia*, to the odour source. He observed that the snail both of whose tentacles on one side were amputated tended to creep in a curve towards the side on which the tentacles still remained. And when a large tentacle on one side and a small tentacle on the

opposite side were amputated, a fairly large number of animals appeared to move towards the side of the remaining large tentacle, while some animals crept to the side of the remaining small tentacle. From these observations he concluded that both the large and the small tentacles were concerned with the orientation to the odours. Furthermore, even when all of the tentacles were amputated, if the odourant was placed as close to the animals head as 2 cm, the animal could still orientate to the odor source.

An experiment is necessary in which odours are applied from a greater distance, since in Schulz's experiment the odour source was placed rather close to the animal (2-4 cm). The present experiment showed that the animals could orientate to the odour after the small tentacle amputation but that the orientation disappeared as a result of amputation of one of large tentacles. And the sensitivities of both large and small tentacles to the odours measured by the potential amplitude were of similar grade. These results indicate more clearly the dominant role of the large tentacle in orientation to the odours. This dominant role in the orientation seems to depend on the ability of free movement of the large tentacle in searching for the odour.

The present behavioural observations show that the snail can detect vegetables only by means of odour perception. Also in natural conditions, the snails seem to depend rather on odour perception than on visual perception in finding their foods, because their visual organ is rather simple compared with other animals and it is doubtful whether they can recognize the form and colours of the objects.

Communication among animals by means of odour perceptions have been known in various animals (cf. Wilson and Bossert, 1963) and especially, the mating reactions of some species of insects have been known to depend on odour perception (Schneider, 1963). In Addition to the food attraction behaviour of the snails, it is necessary to observe whether communications among snails depend on olfactory perception. In the present study it was briefly made as on the following lines.

The snails, hermaphrodite animals, when their genital organs mature, copulate frequently and exchange sperms. The matured individuals become more sticky on their body surface and the genital pore has a more marked appearance. In the laboratory these matured individuals were observed to move around actively, to swarm and to pair in the feeding case. This behaviour in mating suggests that the detection of the matured mate depends on odour perception and a specific odour substance may be involved in the mating of the snail. It may be said that odour perception is widely found and of great significance in the life of the snails.

Electrical responses of the snail olfactory epithelium with respect to stimulus variables had very similar natures as compared with those of the vertebrate olfactory epithelium. On the other hand, there were some differences, namely, chloroform and ethyl ether did not cause the positive potential, and the distinct "off-response" (Takagi and Shibuya, 1959; Takagi and Shibuya, 1960) was not observed in the snail epithelium. And the most interesting problem is the

origin of the slow potentials. Takagi and Yajima (1965) have investigated it in degenerating olfactory epithelium of the frog by dissecting the olfactory nerve. They observed that not only the positive potential but also the negative potential of the epithelium disappeared with the degeneration of the receptors. From their observations they concluded that the slow potential of the olfactory epithelium was originated in the receptors. Consequently, the slow potential was the receptor potential.

In the present experiment, a marked decrease in amplitude of the negative potential was caused by mechanical removal of the receptor layer, while the positive potential almost unchanged. These results suggest that the negative potential is the receptor potential in the snail. But it is not clear whether the origin of the positive potential in the snail lies in the residual receptors, which could not be completely removed, or in other tissues such as the supporting cells and gland cells in the epithelium of the tentacle tip.

### Summary

1. Behavioural and electrical responses of the land snail, *Ezohelix flexibilis* (Fulton), to odours were investigated.

2. The snails could perceive the odours of vegetables which were taken as their favourite foods and they approached the odour source. The odour-attraction behaviour of the snail disappeared when one of the large tentacles was amputated, whereas it remained when both the small tentacles were amputated but both large tentacles remained intact.

3. A puff of odourized air applied to the epithelium of tentacle tip elicited two kinds of potential changes. One was the slow negative potential and the other was the positive one. Most odours caused the negative potential but some odours, such as methanol and ethanol, caused the positive potential. Chloroform and ethyl ether did not cause the positive potential. The nature of potentials of the large tentacle was the same as those of the small tentacle.

4. An increase in the stimulus concentration led to an increase both in the positive potential amplitude and in the negative one. The same relation between the potential amplitude and the flow rate of stimulant gas was observed. When the duration of stimulation was prolonged, the negative potentials as well as the positive ones were sustained. There was no distinct "off-response".

5. A marked decrease in the amplitude of the negative potential was caused by mechanical removal of the receptor layer, although the positive potential almost unchanged.

6. Electrical responses of the olfactory epithelium of the snail were compared with those of other animals, and the origin of the slow potential was discussed.

7. The role of the large tentacle in odour orientation and communications by means of odour perception in the snail was discussed.

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