SOME EXPERIMENTS ON THE CHEMOTACTILE SENSE OF OCTOPUSES

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INTRODUCTION

The results reported here were obtained in a series of experiments carried out in Naples during the summers of 1963 and 1964. The experiments followed Wells's (1963) demonstration that octopuses could be trained to make chemotactile discriminations; their object was to produce data that could be compared with the findings from analogous experiments with insects (Dethier, 1963) and mammals (Beidler, 1962; Pfaffmann, 1959).

In the event, the octopus-training experiments proved far more tedious and time-consuming than had been anticipated, largely because of the difficulties of avoiding contamination and the development of potentially discriminable physical differences in the spongy objects used as carriers for the test solutions. It is clear that the technique developed is a possible but uneconomic means of obtaining information about this subject and at the present time it is not intended to proceed farther with the matter in this way. The results obtained, however, are interesting as far as they go and are reported here even though incomplete because they show that at least some of the properties of Octopus chemoreceptors are the same as those of chemoreceptors in mammals and insects.

MATERIAL

_Octopus vulgaris_ Lamarck from the Bay of Naples was used. The animals were blinded by cutting the optic nerves (Wells & Wells, 1956), and then given crabs and pieces of fish several times daily until their feeding responses to objects touched against the arms were both rapid and regular. Training generally began about a week after blinding. The animals had no pretraining experience of the test objects.

Since some of the experiments took a month or more to complete, all of the animals were killed and examined at the end of training. Blinding is commonly followed within a matter of weeks by a considerable increase in the size of the optic glands and gonads and such animals may eventually cease to feed (Wells & Wells, 1959). None of the results reported here are from octopuses with visibly enlarged gonads, and those from animals that ceased to feed regularly have been discarded.

TRAINING TECHNIQUE

The octopuses were kept in individual ‘Eternite’ tanks 100 x 50 x 30 cm. deep. Generally they sat on the sides of their tanks, close to the water surface, with the arms outstretched along the walls. They were trained by means of rewards (fish) and

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punishments (8 V. a.c. shocks) to discriminate between cylindrical test objects made of Perspex covered with a layer of polyethylene sponge or absorbent paper about 5 mm. thick. Training techniques have been described in full elsewhere (Wells & Wells, 1957). At each training trial an object, suspended on a nylon line, was removed from a 2 l. jar of the test solution and presented to the octopus by touching it gently against the back of one of the animal's outstretched arms. The octopus would then twist its arm and grasp the object with the suckers. It was scored as having taken an object when it passed it under the interbrachial web towards the mouth, and as having rejected the object if it thrust it away or simply let go and dropped it after a period of examination with the suckers.

Trials were at 5 min. intervals in groups of twenty, arranged thus: $+ - + - + + - - + - + - + - + - + - + - + - (i.e. 10+, 10-)$. There were two such groups of trials per day, 6 hr. apart. Animals generally began by taking all the objects.

The experimental method has a theoretical defect. Octopuses are known to detect small differences in the texture of things that they touch (Wells & Wells, 1957) and it is, strictly speaking, impossible to prepare identical sponge-covered objects. This was particularly a problem in the case of the paper-covered cylinders. The material used (sold as washing-up cloths in Naples) is tough, but softens with continual immersion, so that with use it becomes progressively smoother to the touch.

There is one obvious solution to this problem; throw the objects away and introduce a new pair every few trials. But this is hardly feasible in experiments that may continue for hundreds of trials, if only because of the time taken to prepare new objects. In practice it was more convenient to try to eliminate the possibility of the animals learning to recognize the texture of individual test objects by careful matching and by cycling the objects so that an octopus was unlikely to handle any particular cylinder in more than half a dozen successive positive or negative trials.

At any one time a number of objects were in circulation and these were treated as follows:

1. At the end of each day (forty trials) the test solutions were thrown away, and the objects were squeezed out by hand and washed in tap-water overnight.

2. Before starting tests next day the objects were transferred to solutions of different strength or withdrawn, to be replaced by fresh objects from a pool kept soft in tap-water. Where possible an object positive on one day became negative on the next. Care had to be taken so that no object was transferred to a solution much more dilute than that occupied on the day before since this could lead to contamination despite the overnight washing.

3. Whenever the number of available objects permitted, two or three cylinders were used for each solution, so that even within a series of forty trials an octopus did not always receive the same object as carrier for any particular solution.

4. Cylinders were discarded as soon as they became noticeably eroded and new objects were soaked at least overnight before use.

With these precautions one might think it justified to assert that mechanotactile recognition cannot be responsible for the 'chemotactile' discrimination results. Unfortunately, checks on the responses of individual animals not infrequently appeared to show preferences for one out of two or three alternative cylinders used to present the same test solution. The numbers are too small to be statistically signi-
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significant, because of the daily turnover of the objects, so the phenomenon itself is uncertain. But it is worrying because it leads to the suspicion that the octopuses were perhaps sometimes making choices on a basis of what were, in effect, erroneous hypotheses about the nature of the relevant difference between the test objects. A choice of, say, the smoother of two spongy objects could well sometimes give runs of better-than-chance responses, extending from day to day if by chance the textures of the positive and negative cylinders happened always to differ in this way. There would be no corresponding runs of incorrect responses, because the animals do not persist with hypotheses that turn out to be incorrect.

This is a serious theoretical difficulty, but perhaps not very important in practice. The fact that it was always possible to arrive at concentration differences or dilutions that the animals could not distinguish implies that even if textural differences do contribute to the performance of the animals under these conditions, they are not detected consistently enough to determine statistically significant discriminatory scores.

Throughout this account molarities refer to the amount of substances added to normal Mediterranean sea water; the molarity of substances already present is not taken into account. pH was read using glass electrodes and a Beckmann 'Zeromatic' meter.

RESULTS

(1) The discrimination of differences in concentration

Octopuses can distinguish solutions of hydrochloric acid, quinine and sugar in sea water from plain sea water; they can also distinguish diluted sea water from the full-strength solution (Wells, 1963). But no attempt was made in these preliminary studies to discover whether octopuses can be trained to respond differently to different concentrations of the same dissolved substance.

To investigate this, solutions of potassium chloride were used. Potassium chloride was chosen because (1) it is not liable to be adsorbed on surfaces. Thus the concentration presented to the octopus is never greater than the concentration of the solution in which the test objects are kept between trials. (2) Detection of small quantities of KCl added to sea water is almost certainly due to K\(^+\) ions alone, Cl\(^-\) already being present in such great concentration as to make the detection of additional chloride ions most unlikely. One knows what the animal is detecting if it discriminates at all. And (3) because predators in general seem to be relatively sensitive to KCl.

It proved easy to train octopuses to distinguish between sea water and sea water with M/10 KCl added. This constitutes a tenfold increase in the sea water KCl, which is otherwise approximately M/100 (Sverdrup, Johnson & Fleming 1942), and most animals learned to detect the difference between the two solutions within the first forty trials. Four of the octopuses had further training, during which the KCl solutions were progressively diluted until the animals were discriminating between plain sea water and sea water with M/20 (L6, L41), M/50 (L22) or M/100 (L45) KCl added.

Experiments were then made to determine the smallest difference in concentration that the animals were able to detect over the range M/10–M/100 added KCl. In each case a second, initially very dilute, KCl solution was introduced in place of the plain sea water, and training continued until the animal made five errors or less in a group
Fig. 1. For legend see foot of opposite page.
of twenty training trials. The strength of the weaker solution was then increased and training continued until the animal again made five errors or less in a group of twenty trials. Training with solutions of increasing strength was continued in this way until the animal failed to learn to discriminate between the two test solutions, the 'just noticeable difference' being taken as the difference between the last two solutions successfully distinguished by the octopus. Summaries of six such experiments are given in Fig. 1. Each plot shows the number of errors made per group of twenty trials and the places between groups of trials when the concentration of one of the test solutions was changed. In the case of L21, for example, the octopus was first trained to accept a porous object dipped in plain sea water and to reject a similar object from a jar containing sea water with M/10 KCl added. The first point plotted in Fig. 1 shows the score (one error in twenty trials) made in the last of a number of groups of training trials carried out under these conditions. The sea water, the positive stimulus in this case, was then discarded and replaced by sea water containing a very little (M/5 x 10⁴) added KCl. This did not produce an appreciable decline in performance (the octopus made three errors in the next twenty trials) so the solution was changed again, to M/10⁴. This process continued, with a slight progressive increase in the proportion of errors made, until discrimination failed at M/12.5 vs. M/10 KCl added to the sea water; over the next 100 trials L21 made 39 errors, a score that is not significantly different from chance at the 1% level.

The rest of Fig. 1 records a series of essentially similar experiments. From these it appears that effective discrimination breaks down at M/10 vs. M/12.5-13 (L21, L22), M/20 vs. M/26-27 (L6, L41), M/50 vs. M/64 (L22) and M/100 vs. M/180 (L45—an inconclusive experiment since with further training discrimination of M/150-160 was achieved). It will be noticed that over this range, the just noticeable difference is a constant fraction—about one third—of the molarity concerned. This is interesting because it approximates to the values obtained in analogous experiments with mammals and insects (Dethier & Rhoades, 1954; Pfaffmann, 1959). The mechanisms that cephalopods have evolved for contact chemoreception would appear to be subject to at least some of the same limitations as those found in the other groups.

(2) Thresholds

Again using KCl, several attempts were made to determine the minimum concentration that the animals are able to detect. As before, the animals were first trained on a comparatively strong (M/10) KCl solution, which was replaced by a more dilute one as soon as the octopus made five errors or less within a group of twenty trials. The results of experiments with six animals are summarized in Fig. 2. In the case of L20, the score for each group of twenty trials is plotted, as in Fig. 1. From this it can be seen that with each successive dilution of the KCl solution errors at
first rose and then fell. In the other Fig. 2 plots, where the scale along the abscissa has been reduced so that each point summarizes 100, not 20, trials the rise and fall with each succeeding dilution is not generally apparent.

It would appear from these experiments that the addition of KCl to sea water is detected down to additions of the order of M/10⁶ (this adds about 0.1% to the existing KCl in Mediterranean sea water). Beyond that, performance becomes more irregular and is generally not significant at the 1% level over runs of 100 trials. It will be noticed, however, that the scores made at M/10⁶ were always below the 50% error level, and the performance of the five animals tested at M/10⁶ considered as a whole (910 errors in 2280 trials) leaves little doubt that the animals do in fact continue to discriminate, though at a low level of accuracy, well beyond M/10⁵. Indeed the results with two of the animals suggest that even M/10¹⁰ may be within the possible range. With these two (L4 and L7, Fig. 2) a 200-trial period in which M/10¹⁰ KCl was presented in place of sea water was preceded and followed by 100-trial runs with

![Fig. 2. Summarizing a series of attempts to find the absolute threshold for KCl detection.](image)
M/10⁶ solution against sea water. In both cases performance was worse over the period during which traces of KCl were added to the sea water to be distinguished from the M/10⁶ solution. Thus the score with M/10⁶ vs. s.w. (sea water) was 171 errors in 400 trials (χ² = 8.4, P < 0.01), while M/10⁶ vs. M/10¹⁰ yielded 204 errors in 400 trials.

This sort of result, taken together with the progressive decline in performance with increasing KCl dilution, suggests that one is really dealing with chemotactile discrimination rather than discrimination arising from the detection of small differences in test object texture, a problem already discussed in the section on training technique above.

![Graphs showing acid-discrimination experiments](image)

Fig. 3. Scores in acid-discrimination experiments; errors per twenty trials. In these experiments the negative solution was first sea water and then a solution of dilute acid in sea water. The strength of this solution was increased by ten times after the octopus made three errors or less in a group of twenty trials. Changes in the molarity of the negative solution are given along the abscissa in each case. **T** = transfer experiments (see Fig. 4) in which unrewarded tests with other molarities of the same acids were included among normal training trials; the scores given here in these cases refer to the training trials only.

(3) **Discrimination of acidity**

To mammals and insects acetic acid appears to taste stronger than mineral acids at the same pH. This observation has been used to support the view that acid discrimination is based on the adsorption of H⁺ ions on the surface of chemoreceptors (Beidler, 1961). In 1963 some attempts were made to discover whether octopuses too can discriminate acetic from other acids at the same pH.

Training the animals to distinguish between acids of approximately M/100 and sea water presented no difficulties. All save one of the animals made 75% or more correct responses in the second or third group of twenty training trials. Acetic, hydrochloric and sulphuric acids were used, the strengths of the initial solutions...
being respectively 0.064, 0.005 and 0.0022M. These concentrations were chosen as likely to be approximately equally stimulating since they evoke an equal electrical response from the taste buds of rats (Beidler, 1961). In sea water, acids of these strengths have pH of 3.3 (acetic), 2.7 (HCl) and 3.1 (H2SO4).

Five animals were trained. Each octopus was taught to take objects dipped in one of the acid solutions and to reject objects immersed in sea water alone until it made three errors or less in a group of twenty trials. A second solution, of a different acid, was then introduced in place of the unacidified sea water. Initially the second solution was 100 times as dilute as the first. As in the KCl experiments, this was increased to ten times and finally to the strength that would have been the equivalent (to rats) of the acid used as positive stimulus had the solutions been made up in distilled water. In most instances performance was disturbed by increases in the strength of the second solution (Fig. 3) but further training restored discrimination until the octopuses were reliably distinguishing between acid solutions of the standard 'equivalent' strengths.

In two cases (K22, H2SO4 positive vs. HCl negative, and K25, CH6COOH positive vs. H2SO4 negative) a further 40–60 trials were carried out in which the solutions were carefully matched for pH, by diluting one of them. Again, this did not appear to prevent the octopuses from separating the objects (Fig. 3).

A series of transfer tests was then carried out. In the course of these, acid solutions stronger or weaker than the solutions used in the final stages of training were presented in tests interspersed among normal trials, during which training on the original solutions was continued. The order of presentation was arranged so that in the course of two groups of twenty presentations each animal had the stronger and weaker test solutions of each acid five times as well as twenty normal training trials. No rewards or punishments were given at test presentations.

Most of the results are consistent with the view that acids are classed on a basis of their pH, and that acetic acid tastes more acidic than one would expect on this basis alone. Fig. 4 summarizes the results of transfer tests. These show that once they have been trained to accept acetic acid at pH 3.3 (0.064M in s.w.) octopuses will almost always accept the same acid at a lower pH and reject it at a higher.

Transfer tests with mineral acids used as negative stimuli in training show rejection of solutions at higher pH than the training pH—this is hardly surprising since the animals had already been specifically trained to reject acids in this range during the course of their training. At lower pH than the training pH, however, the same acids were taken, which implies (1) that the animals are not primarily concerned with any qualitative difference between the substances and (2) that a mineral acid of low pH is either inherently very attractive or mistaken for the somewhat less acid acetic. Since there was no indication of a preference for the more acid of two solutions in the early trials with naïve animals the 'mistake' type of explanation would appear to be the more probable. The fewer tests following training to discriminate between HCl and H2SO4 seem to confirm this view in that they clearly show some confusion between these acids at the same pH—the 'negative' acid solution was always taken in transfer tests when it happened to coincide with, or exceed, the pH of the positive used in training (Fig. 4).
Fig. 4. The results of transfer tests. After training to discriminate between a pair of acid solutions (Fig. 3), tests with the same acids at different molarities were included among normal training trials. In each series of experiments there were forty presentations of the test objects. Twenty of these were normal training trials and the rest were tests with stronger and weaker solutions of the acids used in training (ten tests with each, five with the stronger solution and five with the weaker). The plots show how many times each solution was taken in each series of forty presentations. Scores in the training trials are included here, but since they were twice as numerous as the tests the figures have been halved so as to bring them to the same ordinate scale. ●, Positive acid; ○, negative acid.
DISCUSSION

These results add to the overall picture of the range of stimuli to which octopuses can be trained to respond and somewhat increase our knowledge of the sensitivity of some of the mechanisms concerned. They indicate that contact chemoreception in molluscs is subject to the same limitations as in the better-known systems of mammals and insects. In all three groups discrimination of differences in salt concentration breaks down at a concentration ratio of about 1:3, and acid discrimination appears to be based on pH, with a bias due to the degree of dissociation of the acids concerned, so that weaker acids taste stronger than would be expected from their pH alone.

Graziadei (1964) has recently described the structure of receptors in the suckers of Octopus, which are almost certainly chemo sensitive. They are very numerous (of the order of $10^4$ for each sucker) and alone of the several types of receptor found in the suckers (see Graziadei, 1962; Wells, 1964) penetrate to the surface. Structurally these 'chemoreceptors' resemble the olfactory cells of vertebrates, with cilia and structures derived from cilia at their external ends. Individual cells have from one to five of these structures, perhaps indicating more than one type of contact chemoreceptor (Graziadei, 1964).

Arguments about the relative merits of absorption and adsorption as explanations of the mechanism of chemoreceptor stimulation are unaltered by the cephalopod information. As a method of investigating chemoreception, training experiments with Octopus have no advantages over the more elegant techniques possible with insects and mammals (Dethier, 1963; Hodgson & Roeder, 1956; Beidler, 1961) and at the present time it is not intended to continue with the matter in this way. The relevant sensory nerves are too small and inaccessible for current electrophysiological techniques.

SUMMARY

1. A method of training octopuses to make chemotactile discriminations is described.
2. The animals can readily be taught to distinguish between porous objects soaked in sea water and similar objects soaked in sea water with M/10 KCl added.
3. Using pairs of objects soaked in solutions with different amounts of KCl added, minimum distinguishable concentrations were determined over the range M/10–M/100. Effective discrimination broke down when the ratio of the two test concentrations was of the order of 1:3. This approximates to the values found in chemical discrimination experiments with mammals and insects.
4. Attempts were made to determine the minimum detectable concentration of added KCl by progressive dilution of the M/10 KCl solution used in the initial training. Additions to sea water down to M/10^6 were distinguished and there was some indication that octopuses can, in fact, detect added KCl at much smaller concentrations; the addition of M/10^10 KCl produced a significant decline in the ability of animals under training to discriminate between M/10^6 KCl and sea water.
5. Experiments on acid discrimination indicated that acids were distinguished on a basis of their pH, with acetic tasting noticeably more acid than HCl or H_2SO_4.
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at the same pH. Again, the results closely parallel those that have been obtained with mammals and insects.

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REFERENCES


