INFLUENCE OF PREVIOUS PREFERENCES ON THE MEMORY OF *OCTOPUS VULGARIS* AFTER REMOVAL OF THE VERTICAL LOBE

By J. Z. YOUNG

Department of Anatomy, University College London and Stazione Zoologica, Naples

INTRODUCTION

In spite of much investigation, the part that the vertical lobe plays in the memory system of *Octopus* remains obscure in many respects. The capacity to change behaviour in the light of experience is certainly impaired after its removal, but is not entirely abolished (Boycott & Young, 1955, 1958; Young, 1960a, b). Investigation of the residual memory gives insight into the whole learning system. Learning to attack a given figure, or learning not to attack it, are both slow after the operation, but is the effect symmetrical in these two directions? Animals without the vertical lobes may show paradoxical behaviour in a discrimination situation, attacking the figure that is associated with shocks more often than that rewarded with food (Boycott & Young, 1957). The present study investigates whether this effect is symmetrical for training in the two directions with figures one of which is originally more likely to be attacked than the other. Further, investigation is also made of the question of the duration of records in the memory after removal of the vertical lobes.

METHODS

Octopuses (200–500 g) were kept singly in opaque tanks 100 × 60 cm. with re-circulating sea water to a depth of 40 cm. At one end a 'home' of bricks was formed, stimuli were presented at the opposite end and the time to attack was recorded with a stop-watch. If no attack was made within 20 sec. the trial was ended.

Removal of the vertical lobe was performed under urethane anaesthesia (3% in sea water). The operations were planned to avoid damage to any other lobes. This involved leaving small amounts of vertical lobe tissue intact at the front end, in order to avoid damage to the median superior frontal and anterior subvertical lobes. At the end of the experiment the brains were fixed in 10% formalin in sea water, stained with Cajal's method and sectioned. The maximum amount of the lobe remaining intact was 25% and the mean 13% for all the animals used.

The animals were all treated alike initially. When brought from the sea they were isolated and left without food for 3 days. They were then tested with a crab attached to a thread. The crab was withdrawn if the octopus attacked and before it could be seized. These tests were given in three sessions, each of eight trials with 10 min. intervals, and they gave a measure of each animal's tendency to attack. After removal

of the vertical lobe twenty-four further tests were given in the same way. There was some reduction in tendency to attack (see Nixon & Young, 1965).

After completion of tests with a crab the animals were divided into two groups, one group with a slightly higher rate of attack than the other. The seven animals of the less active group (A) were trained to attack a vertical rectangle and the six of the other group (B) to attack a horizontal one. The figures were cut from white Perspex (10 × 2 cm.), attached to transparent Perspex rods and presented with up-and-down movement in the vertical plane three times per second. If the octopus attacked the figure then it was given 0·5 g. of fish (sardine) on the end of a wire. If no attack was elicited the shape and wire were inserted close to the animal's home and then moved away to induce the octopus to follow and attack the object. Food was given to each octopus after every trial, within the home if the animal had not attacked. Trials were given at exactly 5 min. intervals in sets of eight, morning and evening.

After six sets of these 'pre-training' trials the octopuses that had been trained to attack the vertical rectangle were then also trained not to attack a horizontal one and vice versa. In negative training if the animal attacked the shape it received a 10 V a.c. shock, from a pair of electrodes placed on one of the arms. Eight sessions were given, and each consisted of sixteen tests, morning or evening. Each set began with two trials with the positive figure followed by two with the negative and continued in the same order at 5 min. intervals.

The octopuses were then tested for their capacity to learn not to attack crabs, shown at various intervals. For each attack the octopus received a 12 V. a.c. shock. Groups A and B were divided again into two further groups. These subgroups were given trials at either 4 or 8 min. intervals, as shown below.

At this time a group of six normal octopuses were shown crabs in the same way. These were unoperated animals that had previously been used for a different visual training experiment that had terminated 5 days previously, since when they had received no food. They had therefore undergone a longer period without food than the animals without vertical lobes and presumably their hunger parameter was greater. No further food was given to any of the animals until the end of the experiment.

RESULTS

Differences associated with previous preference

Before any operative procedures were carried out crabs were shown and were attacked at 86 % of trials by group A and 93 % by group B. After operation the attacks were 62 and 77 % respectively. The attacks during pretraining with the rectangles were 91 % for the vertical (group A), but only 72 % for the horizontal one (group B). This confirms preference for a vertical rectangle in *Octopus* (Young, 1958; Sutherland & Muntz, 1959).

A further wide difference between the groups appeared immediately discrimination training was begun (Fig. 1). In the group where the vertical rectangle was positive it was attacked much more often than the horizontal from the first session onwards. The difference in attacks was 25 % at the first two sessions and increased to a maximum of 58 % in the fourth session and remained at about 50 % thereafter. There was

therefore some evidence of effective learning, retained between sessions, but no continuous systematic change.

When the horizontal rectangle was positive (group B) 'perverse' behaviour was very marked. The vertical rectangle was attacked more than the horizontal throughout the first four sessions. In the last four, however, the difference decreased (though
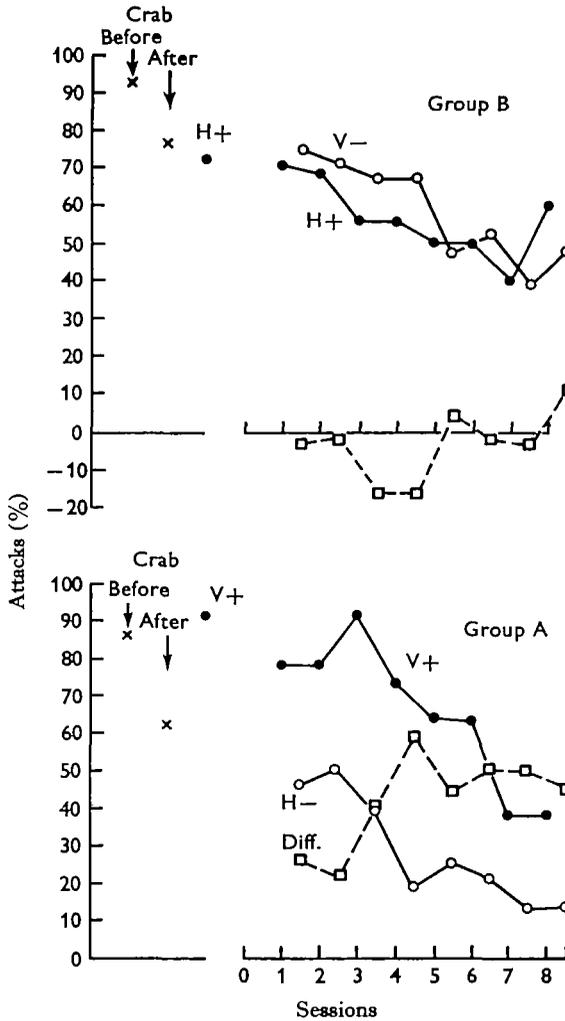

Fig. 1. The first two points (crosses) for each group show the percentage of attacks at crabs before and after operation for removal of the vertical lobes. The third point (filled circle) shows the proportion of attacks at the positive (vertical or horizontal) rectangle. The remaining points show the percentage attacks in each session at the positive (filled circles) and negative (open circles) figures. The squares show the differences between the percentage attacks at the positive (x) and the negative (y) figures in each session expressed as a ratio of all attacks, $(x-y)/(x+y)$.

not steadily) and was reversed, so that at the last session the difference was 11 % in favour of the positive figure. Here too, therefore, there is slight evidence of learning.

It is important to investigate how much learning goes on within each session and

is then forgotten before the next. Fig. 2 shows the attacks for the trials of all the sessions added together. Thus the first pair of points shows all attacks made at the positive (or negative) figures at the first and second pairs of trials of all the eight training sessions. The second pair of points show the third and fourth pairs of trials and so on for all of the sessions. It must be remembered that the first two trials of each session were positive, the second two negative and so on. The figure thus represents the results of the whole of this part of the experiment as a single 'composite session'.
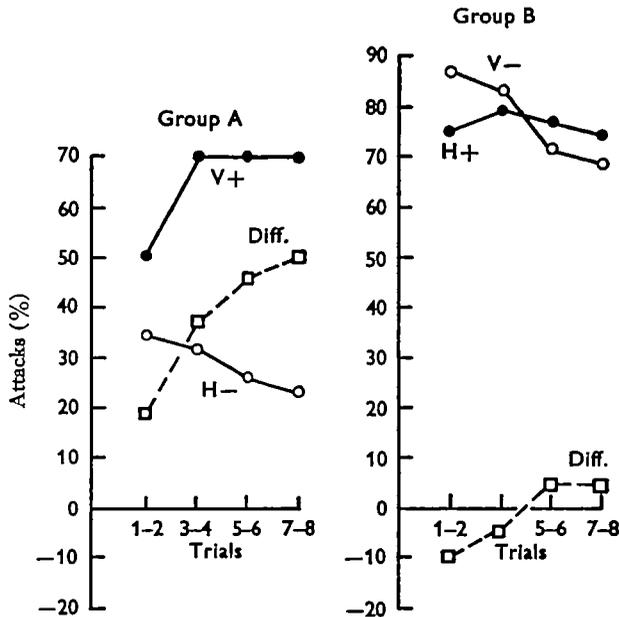

Fig. 2. Learning during sessions. Figures for pairs of trials added together from all sessions. The first filled circle represents the percentage of attacks at the positive figure during the first two positive trials of all sessions added together. The second filled circle is for trials 3 and 4, etc. Open circles represent the percentage of attacks at the negative figure. The squares show the difference between the positive (x) and negative (y) figures expressed as ratio of all attacks, $(x-y)/(x+y)$. The difference increases throughout the 'composite session' for both directions of training.

With the vertical rectangle as positive there was a striking improvement within the sessions (Fig. 2). The difference between the attacks at the two figures rises steadily from 19% at the first trials to 51% at the last. When the horizontal rectangle was positive there was a change in the same direction, but much less marked, from -9% (perverse) to $+5\%$, within the composite session.

Since there is this learning within sessions a good measure of the more enduring learning is provided by the responses at trials 1 and 2 of each session (Fig. 3). With the vertical rectangle as positive the excess of attacks at that figure was only 4% at the first pair of trials of the first session but increased steadily so that at the first pair of trials of the last session 66% of all attacks were made at the positive figure.

No such change appeared when the horizontal rectangle was positive. The responses at the first trials were 'perverse' at all sessions except the second and there was no systematic change in the degree of difference.

A marked feature of the discrimination training was a steady decline in the tendency to attack either rectangle (Fig. 1). Thus attacks at the horizontal positive figure declined from 71 % of trials in the first session to 40 % in the seventh (though they recovered to 60 % in the eighth); attacks at the vertical shape declined in parallel. When the vertical rectangle was positive attacks declined from 78 % in the first two sessions to 38 % in the last two.
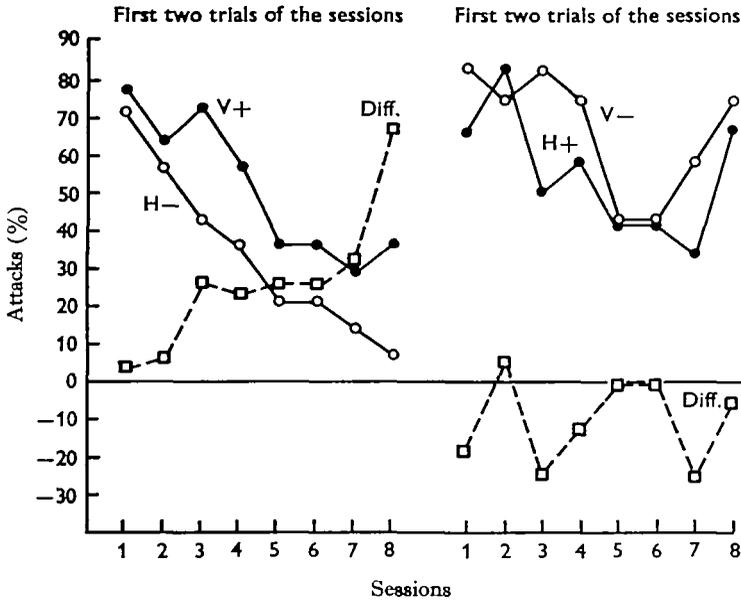

Fig. 3. Learning between sessions. The percentage of attacks in the first two trials of each session on the positive (filled circles) and negative (open circles) figures. The difference between the two figures expressed as a ratio of all attacks (squares), $(x-y)/(x+y)$.

These decreases do not necessarily indicate any particularly important feature of the learning process and may be simply a result of overfeeding with fish. 0·5 g. is a large fish reward and the total daily intake of 8 g. may be too large to maintain the animal at an optimum level of attacking. Towards the end of the training some of the animals refused to accept the fish, although all attacked crabs in the next stage of the experiment.

Training not to attack crabs

After the discrimination training seven of the animals—four from group A and three from group B—were used to investigate the effects on the tendency to attack crabs of shocks delivered at various intervals. The four animals from group A were first given fourteen trials at 4 min. intervals. All had stopped attacking after eight trials, but two attacked again on the twelfth and thirteenth (Fig. 4). After fourteen trials the intervals were increased to 8 min. and there were no attacks in the next five trials. Tests were then resumed 4 hr. later at 8 min. intervals. All the animals attacked again and received shocks, but the attacks decreased faster than before and at the fourth and fifth trials none were made, although later all the animals came out from time to time. In this session, during fourteen trials at 8 min. intervals, there

were 45 % of attacks against 39 % for fourteen trials at 4 min. intervals in the previous session. Tests were then continued at 16 and later 32 min. intervals, with further attacks as the individual memories no longer suppressed the tendency to attack.
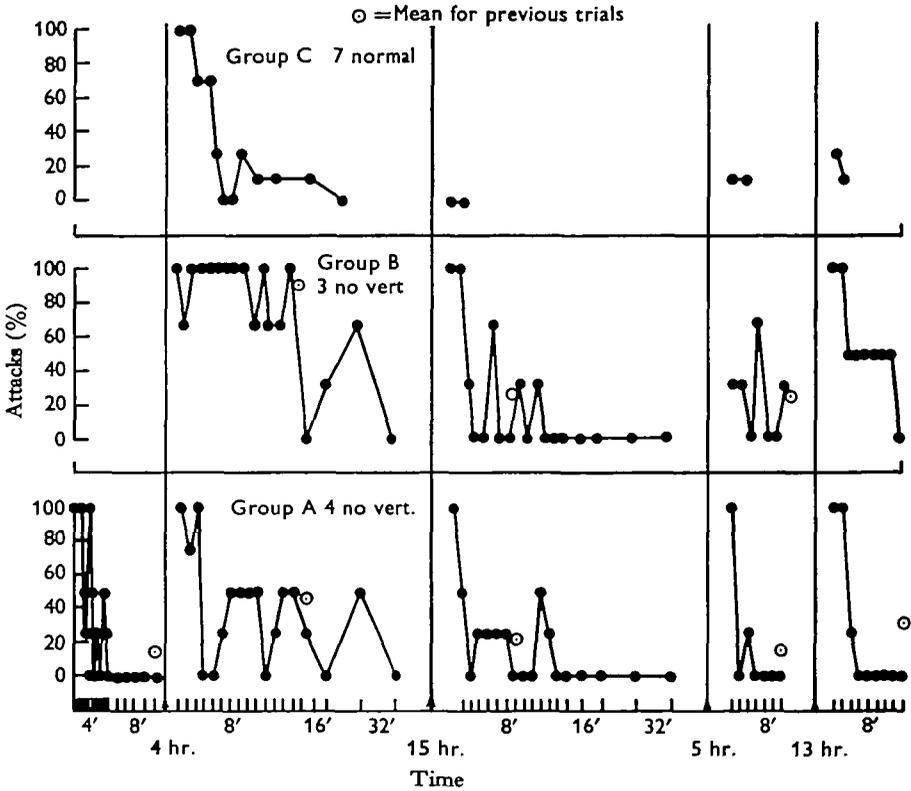

Fig. 4. Learning not to attack a crab. The vertical lobe had been removed in the animals in groups A and B; group C were normal animals. The tests were made at 4 min. intervals in group A, and then at greater intervals. Groups B and C were tested at 8 min. intervals and subsequently at longer intervals.

The full significance of these tests at 4 and then 8 min. intervals appears by comparison with tests given at 8 min. intervals to the second group of three animals (from group B). These were given at the same time as the second set of tests to group A, but without any tests 4 hr. previously. Group B attacked at 90% of the fourteen trials at 8 min. intervals (Fig. 4). Two of the animals attacked at every trial. Trials were then continued at intervals of 16 and later 32 min. Curiously enough there was a decline in attacks at these longer intervals.

A third group (C) of seven normal animals were tested simultaneously with groups A and B at 8 min. intervals. Attacks fell off much more steeply than in either of the series with operated animals. By the sixth trial none of the seven animals attacked (Fig. 4). The experiment was only continued for eight trials at 8 min., as there were only two attacks in the last three of these. Trials at intervals of 16 and 32 min. showed only very few further attacks by these normal animals. It is clear therefore: (1) that setting up of the inhibition is much poorer when trials were all given at 8 min.

intervals than when given initially at 4 and then 8 min. intervals; (2) that 4 hr. after trials at 4 min. intervals there is considerable retention of the influence that restrains attacks; (3) that in normal animals the restraint is imposed much more rapidly than in those without vertical lobes, even with trials at 4 min. intervals.

The experiments were continued the next day (15 hr. later) with a further series of tests at 8 min. intervals. Groups A and B now attacked again but showed a similar pattern of rapid decline of attacks, so that no attacks were made after four and three trials by groups A and B respectively. The percentage of attacks in fourteen trials was much lower than before, 26 % for A and 23 % for B. The control animals of group C were also shown crabs but none of them attacked, a striking contrast with the operated animals.

Five hours later at further tests some of the operated animals failed to attack and all rapidly ceased to do so; there were 18 % attacks by A and 24 % by B. Two of the control animals made single attacks. The experiments of this day therefore extend the second of the above conclusions to show that, after trials at 8 min. intervals, effects persist in animals without vertical lobes for at least 15 hr. A further conclusion can be added: (4) That the retention is much less effective in these operated animals than in normal animals.

On the next and final day of the experiment all the operated animals attacked, and the fall in attacks was rather less rapid than on the previous day, 32 % and 65 % attacks (in eight trials) by groups A and B respectively. Two of the control animals attacked. These results thus confirm the fourth of the above conclusions. It seems that with trials twice daily at 8 min. intervals the memory of the operated animals cannot completely prevent attacks for overnight periods of 15 hr., though it may do so for 5 hr.

DISCUSSION

The paradoxical 'preference' for the figure from which shocks are received has previously been reported after training in which the less preferred figure was 'positive' (Boycott & Young, 1957). It is now clear that this is the only direction in which the phenomenon appears. It is not therefore a result of some subtle effect of removing the influence of the vertical lobe but is a further manifestation of the preferential tendency to attack one configuration rather than another. This is, however, a most interesting and unexplained phenomenon, which may prove to be an integral feature of the classifying systems that are involved in these learning mechanisms. Such preferences have certainly been reported for *Octopus* in many visual situations (Sutherland & Muntz, 1959). There are also tactile preferences, but interestingly enough these only appear clearly in animals without vertical lobes (Wells & Young, 1965). In order to understand the significance of these preferences more information would be needed about the classifying mechanisms that are involved. In the visual system these have been supposed to include the elongated dendritic fields of cells of the optic lobe (Young, 1960c, 1961, 1964). There are nearly twice as many branches with a vertical direction as in any other, and there are more horizontal than oblique branches (Young, 1962). In the interpretations that have been proposed classifying cells for responding either to vertical or to horizontal contours have been postulated, because training can take place in either direction, in spite of the

preference. It may be that the asymmetry is simply the result of unequal numbers of classifiers, though the biological significance of the initial inequality remains to be found. Probably it is connected with the feeding habits and in particular with the movements of crabs. Crabs unfortunately are geometrically complicated figures and their relevant visual features have not been analysed.

The very slow growth of long-lasting effects in the memory after vertical lobe removal may be due to one or more of the following: (1) failure of multiplication of food or pain signals, (2) failure of their distribution to sufficient memory units at each trial, or (3) failure to maintain the 'addresses' of these units, between the appearance of the figure and arrival of food or shock. The fact that improvement of performance within the sessions is marked shows that short (or 'immediate') memory does not entirely depend upon the vertical lobe circuit. This might logically be held to show that long-term memory is a function mainly of the vertical lobe, short-term memory of the optic lobes. This appears not only from such experiments as the present ones but also from ones using a simultaneous discrimination (Muntz, Sutherland & Young, 1962).

The interpretation that has been adopted is that the representation is held within the optic lobes and that the vertical lobe circuit is needed both to establish it there and for its effective use to control behaviour (Young, 1964). This double function must be postulated to be consistent with the facts: (1) that the vertical lobe is necessary for some manifestations of enduring memory but not for all of them; and (2) that after its removal previously established representations are less effective and yet traces of them can be detected.

The experiments with crabs provide further evidence that the vertical lobe serves to increase the duration of the effects of shock. In animals without this lobe shocks are effective in preventing attacks at 4 min. but not at 8 min. intervals. Nevertheless, the effect of shocks at 8 min. intervals can be seen many hours later. There is at present no clear explanation for this paradox that the effects of shocks are seen not soon after they have been given but later (Wells, 1959).

Retention over all periods is much less effective in these operated animals than in normal octopuses (Fig. 4). A simple general explanation is that the lesion produces a defect in the short memory and this leads to defective recording in the long memory. This interpretation cannot however be regarded as proven as against the formally more direct explanation that the vertical lobe is itself the seat of a major part of the longer-lasting memory store. Such difficulties in interpreting even unambiguous experimental results emphasize the complexity of the neural memory mechanism.

SUMMARY

1. Octopuses from which the vertical lobe had been removed attacked a vertical rectangle more readily than a horizontal one when both shapes were moved vertically.

2. During discrimination training the results differed markedly according to which figure was rewarded with food.

3. When a vertical rectangle was positive the excess of attacks at this figure increased both within and between sessions, the animals showing considerable ability to learn.

4. When a horizontal rectangle was the positive figure the responses to it were at first 'paradoxical', the negative shape being attacked more often. There was then improvement within each session, but little if any between the sessions.

5. Octopuses without the vertical lobe quickly ceased to attack crabs shown at 4 min. intervals when shocks were given for attacks, and the effect could still be detected 4 hr. later.

6. When crabs were shown at 8 min. intervals, and shocks given for attacks, there was no decline in attacks over fourteen trials. Fifteen hours later, however, some residual signs of the shocks were evident when trials were resumed at 8 min. intervals. Although all of the animals at first attacked they soon stopped doing so. After a further 15 hr. all attacked again but soon ceased to do so after receiving shocks.

7. Normal animals, by contrast, all ceased to attack the crabs shown at 8 min. intervals after receiving four or five shocks. The memory is much more retentive in normal animals, so that 2 days after receiving shocks the octopuses seldom attacked the crabs.

REFERENCES

BOYCOTT, B. B. & YOUNG, J. Z. (1955). A memory system in *Octopus vulgaris* Lamarck. *Proc. Roy. Soc.* B, **143**, 449–80.

BOYCOTT, B. B. & YOUNG, J. Z. (1957). Effects of interference with the vertical lobe on visual discriminations in *Octopus vulgaris* Lamarck. *Proc. Roy. Soc.* B, **146**, 439–59.

BOYCOTT, B. B. & YOUNG, J. Z. (1958). Reversal of learned responses in *Octopus vulgaris* Lamarck. *Anim. Behav.* 6, 45–52.

MUNTZ, W. R. A., SUTHERLAND, N. S. & YOUNG, J. Z. (1962). Simultaneous shape discrimination in *Octopus* after removal of the vertical lobe. *J. Exp. Biol.* 39, 557–66.

NIXON, M. & YOUNG, J. Z. (1965). In preparation.

SUTHERLAND, N. S. & MUNTZ, W. R. A. (1959). Simultaneous discrimination training and preferred directions of motion in visual discrimination of shape in *Octopus vulgaris* Lamarck. *Pubbl. Staz. zool. Napoli*, **31**, 109–26.

WELLS, M. J. (1959). Functional evidence for neurone fields representing the individual arms within the central nervous system of *Octopus*. *J. Exp. Biol.* 36, 501–11.

WELLS, M. J. & YOUNG, J. Z. (1965). In preparation.

YOUNG, J. Z. (1958). Responses of untrained octopuses to various figures and the effect of removal of the vertical lobe. *Proc. Roy. Soc.* B, **149**, 463–83.

YOUNG, J. Z. (1960a). Unit processes in the formation of representations in the memory of *Octopus*. *Proc. Roy. Soc.* B, **153**, 1–17.

YOUNG, J. Z. (1960b). The failures of discrimination learning following the removal of the vertical lobes in *Octopus*. *Proc. Roy. Soc.* B, **153**, 18–46.

YOUNG, J. Z. (1960c). The visual system of *Octopus*. (1) Regularities in the retina and optic lobes of *Octopus* in relation to form discrimination. *Nature, Lond.*, **186**, 836–9.

YOUNG, J. Z. (1961). Learning and discrimination in the octopus. *Biol. Rev.* 36, 32–96.

YOUNG, J. Z. (1962). The optic lobes of *Octopus vulgaris*. *Phil. Trans.* B, **245**, 19–58.

YOUNG, J. Z. (1964). *A Model of the Brain*. Oxford: Clarendon Press.