J. Exp. Biol. (1962), 39, 557-566
With 1 text-figure
Printed in Great Britain

SIMULTANEOUS SHAPE DISCRIMINATION IN *OCTOPUS* AFTER REMOVAL OF THE VERTICAL LOBE

By W. R. A. MUNTZ*, N. S. SUTHERLAND* AND J. Z. YOUNG†
Zoological Station, Naples, Italy

(*Received 7 June* 1962)

In all previous investigations of the effects of vertical lobe removal on the discrimination of shape by *Octopus*, the animals were trained with the shapes presented successively, i.e. they were trained to attack one shape (the positive) when presented on its own, and not to attack another (the negative) when presented alone. Under these conditions, animals lacking the vertical lobes can learn to perform above chance with easy visual discriminations, but post-operative performance is much poorer than in unoperated controls whether animals are trained before or after the operation (refs. in Young, 1961). Animals are very severely impaired on a difficult discrimination, and may indeed give no evidence of learning. Some findings suggest that the poor performance is partly due to fluctuations in the tendency to attack that result from removal of the vertical lobes; thus operated animals tend to take any shape shown immediately after having been fed (whether it is positive or negative) and tend not to take any shape shown after receiving a shock.

However, when these operated animals are tested by giving trials without reward or punishment they sometimes respond differentially to the positive and negative shapes even though they had shown no signs of differential responses during training. Presumably this procedure stabilizes the level of attack so that differential responsiveness to the two shapes is no longer masked by fluctuations in this level. Another possible procedure for reducing the effects of fluctuations in the tendency to attack is to train animals with two shapes presented simultaneously. If the representations of the shapes are located outside of the vertical lobes any differential tendency to attack one shape rather than another should be revealed. When shapes are simultaneously presented there should still be a higher tendency to attack one than the other, irrespective of the general level of attack.

Initial attempts to train octopuses on simultaneous discrimination were not very successful (Boycott & Young, 1956; Sutherland & Muntz, 1959). It now appears that this was chiefly due to the animals not being punished for attacks on the negative figure, for subsequent work showed that simultaneous discrimination can readily be accomplished (at least on an easy discrimination) provided shocks were given for attacks on the negative shapes as well as rewards for attacks on the positive (Maldonado, unpubl.). Both shocks and rewards were therefore used in the following experiments.

Three experiments are reported, which show that animals without vertical lobes

* Present address: Institute of Experimental Psychology, Oxford.
† Present address: Department of Anatomy, University College London.

can discriminate moderately accurately between figures shown simultaneously even when the discrimination is a difficult one for normal animals.

GENERAL METHODS

Expt. 1 was performed before Expts. 2 and 3, and the simultaneous training procedure had been developed in many respects during the interval. Subjects in all cases were *Octopus vulgaris* Lamarck, obtained in the Bay of Naples and kept in opaque tanks 100 × 60 cm. with a depth of water of 40 cm. They were trained to discriminate between two shapes differing either in colour (white or black) or in form. The shapes were cut from ¼ in. thick Perspex and were presented on the end of transparent Perspex rods.

The stimulus object the animals were trained to attack will be referred to as the positive shape, and that which they were trained not to attack as the negative shape. Training was accomplished by rewarding attacks on the positive shape and punishing attacks on the negative. Rewards consisted of a small piece of sardine presented to the animal on the end of a wire; and punishments of an 8 V. a.c. shock delivered through a pair of electrodes attached to a probe.

Simultaneous training. In all the simultaneous training procedures the home of the octopus was placed in the centre of one of the shorter (60 cm.) walls of a rectangular tank. The shapes were inserted into the other end of the tank, and were divided from each other by a partition projecting into the tank towards the animal's home. The two shapes were presented at the same time; if the animal attacked the positive shape it received a reward. If it attacked the negative shape it received a shock. The side on which the positive and negative shapes were presented was varied in a random order.

Successive training. Successive training was carried out by the usual methods (see Young, 1961, for reference). Only one shape was shown at a time, and the positive and negative shapes were shown in a random order. If the positive shape was attacked within 20 sec. the animal was rewarded, and if the negative shape was attacked within 20 sec. the animal was given an electric shock. If there was no attack within 20 sec. the shape was withdrawn and the trial recorded as 'no attack'.

Operative procedure. The octopuses were anaesthetized with 3 % urethane and the vertical lobes were removed with fine scissors. At the end of the experiment the brains were fixed in formol, stained by Cajal's method, and sectioned serially at 25 μ. The proportion of the vertical lobe left intact was calculated by counting the number of sections in which any one of the five vertical lobules appeared. Examination of series of sections at 25 μ of normal animals of about the size used in the experiments shows that the vertical lobe tissue usually occurs in altogether about 400 sections. This has been assumed to give the amount of vertical lobe tissue originally present in each animal. In some animals the medial superior frontal lobe was removed.

EXPERIMENT 1

Methods

Subjects. The subjects were eleven octopuses. Seven of these animals had received positive training on a horizontal or vertical white rectangle as part of an experiment reported by Muntz (1962); none of them had received any shocks.

Stimulus objects. The stimulus objects were a black and a white 5 cm. square presented with one of the sides horizontal. In view of the marked preference shown by the animals for the white square (see below) the black square was made the positive shape in all cases, so that the animals were trained against this pre-existing preference.

Simultaneous training. The tank was arranged as described in General Methods. The shapes were placed in the water on the ends of transparent Perspex rods and moved up and down over a distance of about 3 cm. at a rate of about three times per second. A trial was only given if the animal was sitting centrally in its home in such a way that both shapes could be seen equally easily and at equal distances.

Training was by a modified correction procedure. If the animal attacked the negative shape it was given an 8 V. a.c. shock; 5 min, later the positive shape was shown alone and the animal was rewarded when it attacked. If the positive shape was attacked the animal was rewarded immediately. The animals were given eight trials per day in two sessions of four, one session being in the morning and one in the evening. Individual trials within each session were separated by intervals of about five minutes.

Initial preferences. The initial preference for the two shapes used was tested as described in Sutherland & Muntz (1959). They were presented on the two sides of the partition in a random order, no shocks were given and the animal was rewarded on every fourth trial irrespective of which shape was attacked.

Successive training. Successive training was as described in General Methods. Sixteen trials were given per day in two sessions of eight, individual trials being separated by 5 min. intervals.

Results

(1) *Initial preferences*

Six animals with their vertical lobes removed were given six trials each to determine which of the two shapes was preferred. One animal refused to attack for three trials; out of the remaining thirty-three trials the white square was attacked on twenty-eight occasions (= 85 %).

Three normal animals were similarly tested over twelve trials each, and a fourth animal over six trials. The white square was attacked on thirty-five occasions out of a possible forty-two (= 83·4 %). Thus both normal animals and animals without their vertical lobes showed a strong preference for the white square in the simultaneous situation. No tests were run on the successive situation. However, Young (1958) gives percentage attacks on black figures as 53 %, and on white figures as 66 %, in an experiment that used the successive situation and tested the initial preferences of octopuses to a series of figures. This confirms the preference for white shapes over black.

(2) *Simultaneous discrimination by normal and operated animals*

(*a*) *Initial learning.* The initial learning of four normal animals and three animals with their vertical lobes removed is shown in Fig. 1 A. The operated animals had 93, 85 and 80 % of the vertical lobe removed respectively. It can be seen that both groups learned readily, but that the unoperated animals learned consistently better. Over the last six training sessions the unoperated animals made an average of 91·6 % correct responses, while the operated animals scored 74·9 % correct responses. There was no overlap between the two groups, and the difference was significant ($t = 5 \cdot 84$, $n = 5$, $< 0 \cdot 01$).

(*b*) *Retention after vertical lobe removal.* On the evening of the sixth day the four normal animals were operated, an average of 92 % of the vertical lobe being removed. Training was resumed on the seventh day. The operation caused a significant drop in performance (t calculated between the scores on the sixth and seventh days was 5·41, $n = 3$, $P < 0 \cdot 05$), but the animals thereafter improved rapidly. Over days 9 to 12 they made an average of 77·5 % correct responses, which is nearly the same as the level of performance reached by the three no-vertical animals in initial training.

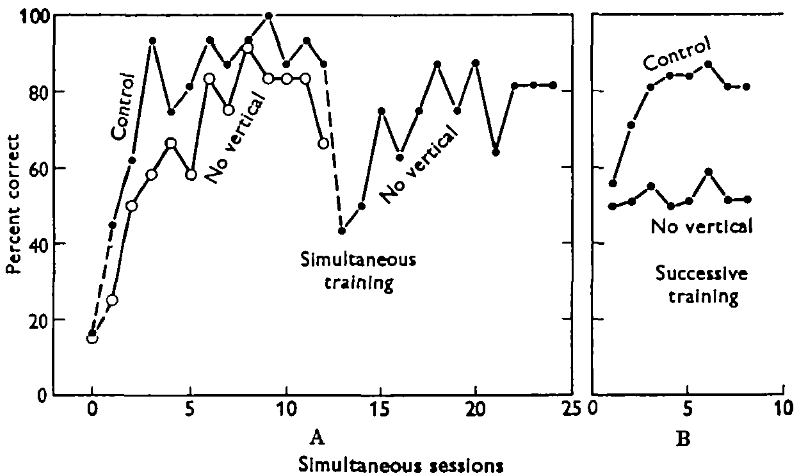

Fig. 1. Percentage correct responses by control and no-vertical animals when trained to attack a black but not a white square by first simultaneous and then successive presentation (Expt. 1).

(*c*) *Successive discrimination by normals and no-verticals.* The seven operated animals which had been trained in the simultaneous situation as described above were all subsequently trained on the same discrimination using the successive discrimination procedure. The average vertical lobe removal for all seven animals was 90 %. The results are shown in Fig. 1 B. In spite of the fact that the animals had all previously been discriminating at a fairly high level of accuracy on the same pair of shapes, it can be seen that they barely scored above chance in the successive situation. The average score over the 4 days of successive training was 53 %, which was not significantly different from chance ($t = 1 \cdot 43$, $n = 6$).

Four normal animals were also trained on the same discrimination by the successive method. These normal animals had had no previous experience of the shapes in the simultaneous situation, and were therefore at a disadvantage from this point of view as compared to the operated animals. In spite of this their superiority over the operated animals was very marked (Fig. 1 B), and by the second day they were performing at better than 80 % accuracy.

EXPERIMENT 2

Methods

Subjects. Subjects were eight *O. vulgaris.*

Stimulus objects. All subjects were initially trained to discriminate between a 5 × 5 cm. white square and a parallelogram of the same area. The training parallelogram (P 1) had acute angles of 45°. In each group two animals were trained with the square positive and two with the parallelogram positive. Four other parallelograms were subsequently used, making a series approximating nearer and nearer to a square. These had acute angles of 51°, 59°, 68°, and 79°, and will be referred to as P 2, P 3, P 4 and P 5.

Simultaneous training. The simultaneous training procedure used has been fully described in Sutherland, Mackintosh & Mackintosh (1963). It differed from the methods used in Expt. 1 in the following particulars: (*a*) The home of the animal was divided from the rest of the tank by a screen, in which there was a transparent sliding door. (*b*) The stimulus objects were not moved after they were placed in the water. (*c*) No correction procedure was used. The procedure followed was to place the stimulus objects in position and, after an interval of 5 sec., to raise the transparent door in the screen dividing the animal's home from the rest of the tank. If the animal did not make an attack within 30 sec. it was shown a piece of sardine in the centre of the doorway: as the animal advanced down the tank the sardine was withdrawn and an attack on one or other of the shapes usually ensued. The animals were given ten trials a day, in two blocks of five.

Successive training. Successive training was by the usual methods. Twenty trials were given a day, in two sessions of ten.

Results

(*a*) *Initial learning.* All eight animals were trained before operation as described in Sutherland *et al.* (1963). Four were trained in the simultaneous situation and four in the successive situation for about 1 month, at the end of which all animals were performing at an accuracy of over 90 % correct responses. The average level of correct response was 93 % (measured as the percentage of attacks on the positive stimulus object out of all attacks made). Operations were performed at this point.

(*b*) *Retention after vertical lobe removal.* Operations were performed in the morning, and 24 hr. after operation all eight animals began retraining, each with the procedure used for that animal throughout its original training.

All animals trained by the simultaneous method scored above chance throughout the initial period of retraining with the square and P 1 (days 1–4). The scores made and the number of trials given to each animal are shown in Table 1. One animal ceased attacking after five trials, two others after thirty, but all responded sufficiently often to show that the discriminatory habit had been retained. There was little evidence that any relearning was involved, the average score on the first day was 74 % and on the third 80 %. However, performance was less accurate than over the 10-day period immediately before operation, when each animal had averaged over 90 % correct responses.

Unfortunately three of the four animals ceased to attack the shapes within 5 days after the operation. It was found that neither would they attack crabs, and all died within a short time. The remaining animal was then given successively one day's retraining with each of P 2, P 3 and P 4, followed by 4 days' retraining with P 5. It scored 80 %, 70 % and 70 % correct with P 2, P 3 and P 4 but during the forty trials with P 5 scored practically at chance—53 % correct. Yet the same animal before it was operated had scored 75 % correct when trained for 4 days with the square and P 5. The difference between the proportions of attacks made on the positive figure before and after operation is significant ($\chi^2 = 4.5$, D.F. = 1, $P < 0.05$).

Table 1. *Animals trained before operation by simultaneous method*

(Number of correct responses in each ten trials (five for S 1). Expt. 2.)

Day	Parallelogram used	Animal and % removed			
		S 1 (85 %)	S 2 (85 %)	S 3 (90 %)	S 4 (87 %)
1	P 1	5/5	8	6	7
2	P 1	—	7	8	7
3	P 1	—	8	8	8
4	P 1	—	—	—	8
5	P 2	—	—	—	8
6	P 3	—	—	—	7
7	P 4	—	—	—	7
8	P 5	—	—	—	5
9	P 5	—	—	—	5
10	P 5	—	—	—	8
11	P 5	—	—	—	3
12	P 1	—	—	—	9
13	P 1	—	—	—	8
14	P 1	—	—	—	7
15	P 1	—	—	—	7

After the 4 days' retraining with the most difficult shapes this animal was given a further 4 days' retraining on the easy shapes; its performance over these days was much the same as over the first 4 days of retraining (78 % correct). In the experiments performed before operation it was found that the parallelogram was initially a more attractive shape than the square (Sutherland *et al.* 1963). Since the animal given the longest period of training was trained with the square as the positive shape, its consistently good performance on the easy discrimination must be the result of learning and not of a pre-existing preference.

When retrained by the successive method for 5 days after operation the group of animals originally trained by this method did not score significantly above chance. The individual scores were 35, 54, 65 and 60 %. The first two animals were trained with parallelogram positive, the second two with square positive.

On the sixth day of training the animals that had been given the successive procedure were retrained to take the stationary positive shape on its own with the partition and screen in place in the tank. By the end of the day all four were taking it within 20 sec. They were then retrained with the same shapes simultaneously presented. Two animals scored significantly above chance, making scores of 66 and 70 % over the 5 days of retraining (Table 2). The values of χ^2 for the difference from chance expectation are 5.1 and 8.0; the first is significant at the 0.05 level of confidence, the second at the 0·01 level: in both cases D.F. = 1. Since the scores of these two animals did not improve after the second day it seems likely that little relearning was involved and that previous training on a successive situation had carried over to a simultaneous situation (this occurs with normal animals, see Sutherland, 1960). Since the highest scoring animal was trained with square positive, the better-than-chance scores were due to learning and not to initial preferences.

The remaining two animals continued to score at chance level. They were the animals with the most extensive lesions, although the amounts of vertical lobe tissue remaining in the animals making the discrimination were only slightly greater (Table 2).

Table 2. *Animals trained before operation by successive presentation*

(Percentage correct responses. Expt. 2.)

Animal and % removed	S5 (95)	S6 (86)	S7 (96)	S8 (90)
100 trials successive (%)	65	35	54	60
100 trials simultaneous (%)	46	66	50	70

Table 3. *Animals trained before operation with* V *and* W *in simultaneous presentation*

(The figures show for each day the numbers of correct and incorrect choices for each animal. Below these are the percentage of correct choices for the group (Expt. 3).)

Octopus ...	S9	S10	S11	S12	S13	S14	S15	S16
Pre-op. Correct/incorrect	8–2	7–3	7–3	10–0	8–2	7–3	8–2	7–3
	80%				75%		75%	
	Vertical removed				Medial superior frontal removed		Dummy	
Amount removed ...	77%	88%	90%	95%	75%	75%	—	—
No reward Day 1	10–6	9–7	10–6	6–10	10–6	12–4	10–3	10–6
	55%				69%		69%	
Day 2	6–10	7–7	3–4	13–3	7–5	4–3	0–1	11–5
	55%				58%		65%	
Re-training	41–19	20–13	26–34	39–21	39–21	36–24	46–9	47–13
	59%				63%		81%	

EXPERIMENT 3

Methods

The subjects in this experiment were eight *O. vulgaris* trained by the simultaneous method as described in General Methods and in Expt. 2. The shapes used were a white W and a white V; the W was presented on its side. Details of these shapes are given in Sutherland (1959), where it was also shown that they constitute a moderately difficult discrimination for the octopus.

Results

(*a*) *Initial training.* Initial training was in every case done before operation, and is described in Sutherland *et al.* (1963). At the end of initial training all the animals were showing clear signs of discrimination (75–80 %, Table 3).

(*b*) *Retention after vertical lobe removal or removal of the medial superior frontal lobe.* At operation the vertical lobe was removed from four of the animals, and the medial superior frontal lobe from two of them. In two further animals the cranium was opened and the jelly round the brain removed without any lesion being made to the brain itself; these animals will be referred to as dummy-operated animals.

On the second day after operation all the animals were tested with the shapes used in training without either food or shocks being given. As can be seen from Table 3 all three groups showed signs of retention, but the animals with their vertical lobes removed performed at a lower level of accuracy than the other two groups.

These no-reward tests thus confirm that after removal of the vertical lobe animals show signs of retention though performing erratically. This was further shown when the animals were subsequently retrained with the same discrimination. Three days of retraining were given, with 20 trials a day. Three of the animals with vertical lobe lesions showed signs of correct performance, and the fourth none. The two animals with the medial superior frontal removed also improved to some extent. None of the animals with lesions showed anything like the improvement of the dummy-operated animals, which rapidly reached the pre-operation level of accuracy.

Discussion

The experiments that have been described show that simultaneous discriminations can be performed adequately by octopuses with their vertical lobes removed, even though the same discrimination is performed at a chance level in the successive situation. Although animals lacking the vertical lobe discriminate better in the simultaneous situation than in the successive situation, they still perform worse than normal animals also trained by the simultaneous method. This is true no matter what is the level of difficulty of the discrimination. Indeed, on a very difficult discrimination one operated animal failed to perform above chance although it had mastered the discrimination before operation and was able after operation to perform reasonably well on discriminations of moderate difficulty. It must be noted that in all the animals a small fraction of the vertical lobe was left intact. This is inevitable if the median superior frontal is to be left undamaged.

Several other experimental situations have been reported which also allow animals without their vertical lobes to perform at a reasonable level of accuracy on easy discriminations. Thus:

(1) Animals without their vertical lobes can learn not to attack crabs, and to distinguish a crab shown alone from a crab shown together with a white square, if the trials are spaced at short intervals. With trials at longer intervals little or no learning is apparent (Boycott & Young, 1956).

(2) Octopuses trained in a successive situation perform discriminations badly, or even at chance level, if they have vertical lobe lesions. However, if both rewards and

shocks are discontinued, quite accurate discrimination is often revealed (Young, 1958).

(3) Extinction experiments, in which a stimulus object is repeatedly presented without reward, have shown that extinction is specific to the stimulus object presented; this is the case with normal animals and with animals having vertical lobe lesions (Young, 1959).

(4) If an animal is trained to attack one shape a stimulus generalization gradient can be obtained by testing it with this shape and also a transfer shape, and giving rewards for neither. The stimulus generalization gradient thus obtained is the same for normal animals and for animals without their vertical lobes (Muntz, 1962).

One possible explanation of these results is that in the absence of the vertical lobe there are marked fluctuations in the level of attack following rewards or shocks, and there is also a tendency to attack more often than normal animals. On successive discriminations by no-vertical animals most mistakes are made by attacking the negative shape (see, for example, Young 1958) whereas normal animals typically make more mistakes by failing to attack the positive figure than by attacking the negative. Thus with trials at short intervals no-verticals can learn not to attack crabs because they receive many shocks, which both lowers and stabilises the level of attack (situation (1) above). Similarly, discontinuing both rewards and shocks would be expected to stabilize fluctuations in the level of attack (situation (2) above). In situations (3) and (4) only one response is required, and so fluctuations in the level of attack have comparatively little effect if a fairly large number of trials is considered. Finally, as is pointed out in the introduction, fluctuations in the general level of attack will have comparatively little effect in simultaneous discriminations since both shapes are presented together, and there should therefore be a higher tendency to attack the positive shape irrespective of the general level.

The experiments thus suggest that the memory representations lie, at any rate to a great extent, outside the vertical lobe, and that vertical lobe removal results in motivational difficulties. Such a statement, however, is certainly an over simplification; even in simultaneous discrimination no-vertical animals do not reach the level of performance of intact animals, and further work is required to find out why this should be so.

SUMMARY

1. Octopuses from which the vertical or median superior frontal lobes had been removed were able to discriminate between objects shown simultaneously, although they could not distinguish them when shown successively.

2. Discrimination by operated animals was however always less accurate than by controls.

3. A very difficult simultaneous discrimination could not be performed without the vertical lobes (although the same animal was able to make it before operation).

4. A discrimination learned by the simultaneous method before operation continues to appear (though less accurately) after vertical lobe removal.

5. The experiments therefore confirm previous evidence that the representations that ensure correct visual responses do not lie mainly in the vertical lobes, but elsewhere (probably in the optic lobes).

6. The function of the vertical lobes is considered to be to stabilize and perhaps lower the level of tendency to attack.

The work was done at the Zoological Station at Naples. We are most grateful to the Director, Dr P. Dohrn, and Staff for much assistance. We also wish to thank Mr G. Brownlee for his assistance with some of the experiments. We are grateful for much help received from the Nuffield Foundation. The research reported in this document has been sponsored in part by Air Force Office of Scientific Research, O.A.R. through the European Office, Aerospace Research, United States Air Force, and by the American Office of Naval Research (Contract Number N62558-2453.)

REFERENCES

BOYCOTT, B. B. & YOUNG, J. Z. (1956). Reactions to shape in *Octopus vulgaris* Lamarck. *Proc. Zool. Soc. Lond.* **126**, 491–547.

MUNTZ, W. R. A. (1962). Stimulus generalisation following monocular training in *Octopus*. *J. Comp. Physiol. Psychol.* **55**, 535–40.

SUTHERLAND, N. S. (1959). A test of a theory of shape discrimination in *Octopus vulgaris* Lamarck. *J. Comp. Physiol. Psychol.* **52**, 135–41.

SUTHERLAND, N. S. (1960). Visual discrimination of shape by *Octopus*: squares and rectangles. *J. Comp. Physiol. Psychol.* **53**, 95–103.

SUTHERLAND, N. S., MACKINTOSH, J. & MACKINTOSH, N. (1963). Simultaneous discrimination training of *Octopus*, and the transfer of a discrimination along a continuum. *J. Comp. Physiol. Psychol.* (in the Press).

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.

YOUNG, J. Z. (1958). Effect of removal of various amounts of the vertical lobes on visual discrimination by *Octopus*. *Proc. Roy. Soc.* B, **149**, 441–62.

YOUNG, J. Z. (1959). Extinction of unrewarded responses in *Octopus*. *Pubbl. Staz. zool. Napoli*, **31**, 225–47.

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