PROPRIOCEPTION AND VISUAL DISCRIMINATION OF ORIENTATION IN *OCTOPUS*

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INTRODUCTION

Octopuses can be trained to discriminate between figures seen that differ only in orientation. Thus a rectangle shown with its long axis vertical can readily be distinguished from an exactly similar rectangle shown horizontally (Boycott & Young, 1957; Sutherland, 1957). A wide variety of cues are potentially available to octopuses in the training situation used; the figures might, for example, be recognized as distinct because they are parallel or not parallel with the water surface, with the sides of the aquaria, or with the direction of illumination. Since the figures are moved up and down on the ends of rods inserted into the tank, they could also be distinguished as moving along or at right angles to their long axes (see Sutherland & Muntz, 1959) or as parallel or not parallel with the supporting rod. An alternative set of cues is potentially available from within the animal itself, in the form of proprioceptive information about the position of the animal in space, originating in stretch or pressure receptors and/or in the statocysts.

It will be shown below that octopuses cannot reliably discriminate between figures differing only in orientation after removal of the statocysts, and that correct performance in such discriminations depends upon correct orientation of the retina, and not upon central integration of visual and positional information.

MATERIAL AND TRAINING TECHNIQUE

Octopus vulgaris Lamarck. from the bay of Naples was used, experiments being made in July and August 1958 (EK series) and 1959 (FK series). The animals used were all very small compared with those used hitherto in octopus training experiments and weighed between 16 and 36 g. when killed after a period of training during which they grew appreciably in size.

Each individual was kept in a separate tank, either a glass accumulator jar (with black paper screening around it) or (more often, including the whole of the FK series) in a wooden aquarium measuring 40 × 20 × 15 cm. A jet of sea water provided circulation and aeration, and each animal was provided with two or three pieces of brick grouped at one end of the tank to form a 'home' in which it normally sat. Each tank had a lid.

Animals were required to learn to discriminate between the members of pairs of figures, cut out of card, presented successively and moved up and down at the end of the tank distant from the octopus's home. Animals were rewarded with a fragment of sardine or shrimp for attacking one (the 'positive') of the two figures, and were given a 3–5 V. a.c. electric shock when they attacked the other (the 'negative' figure). The experimental situation was thus as nearly as possible a copy of that originally devised by Boycott & Young (1955), which has been used in the majority of subsequent visual training experiments with larger octopuses (for a review of these see Young, 1960*a*). Training was at a rate of ten trials per day, 5+ and 5−, at intervals of not less than half an hour; the trials were systematized thus: + − + − + + − − + −, next day − + − + + − − + − + and so on.

OPERATIVE TECHNIQUE

The principal reason for using small octopuses in these experiments was that it is relatively easy to remove the statocysts from young animals. After anaesthesia in 3% urethane, an octopus of 20 g. or less is sufficiently transparent for the anterior vena cava to be seen pulsating beneath the skin. Avoiding this blood-vessel constitutes the only serious difficulty in removal of the statocysts from below. To remove each statocyst an incision is made in the skin and muscle beside the anterior vena cava to reveal the cartilage surrounding the brain. The white otolith of the statocyst can be seen clearly through the cartilage. (In very small specimens the skin and muscle are transparent too, and the otoliths can be seen before any incision is made.) The statocyst can be removed entire by picking out the endolymphatic sac with fine forceps through a hole made in the cartilage. The whole operation is very rapidly done, and if the initial incision is made parallel with the muscle fibres immediately underlying the skin, the wound heals quickly and completely. One week after operation the lesion is only detectable because the chromatophores in the skin overlying the wound tend to be more expanded than their immediate neighbours. The effect of the operation upon the behaviour of the animal is so striking and regular that a subsequent histological check upon the completeness of the operation was not considered necessary.

IMMEDIATE EFFECTS OF STATOCYST REMOVAL UPON MOVEMENT AND POSTURE

Boycott (1960) and Dijkgraaf (1960) have described the effects of unilateral and bilateral statocyst removal upon the movements and posture of *Octopus*. The animals used by them were all much larger than those used in the present series of experiments, but their behaviour after operation was sufficiently similar for there to be no point in redescribing it in full here. Briefly, removal of a single statocyst, left or right, produces no detectable changes in behaviour; but removal of both statocysts causes widespread abnormalities of movement and posture. Animals with both statocysts removed cannot swim straight and tend to spiral in an irregular manner. They are therefore unable to approach objects seen at a distance in the usual way by swimming up to them, aided by jets from the funnel. Pretrained animals tested after bilateral statocyst removal sometimes try to make such attacks, but rarely reach their objective. Octopuses with both statocysts removed can, however, crawl rapidly about their tanks, but again their progress, particularly when some of the arms are not in contact with the substratum, is erratic. The head tends to jerk from side to side, and the animal is liable to fall over its own arms if moving rapidly. Statocyst removal appears to have no effect upon muscular tone; the operated animals are in no way 'weak' and they climb out of their tanks if anything more frequently than unoperated animals. The main difficulty of training such animals is that though they react readily by starting towards objects seen, their approach path is often so erratic that the target fails to remain within the visual field; an animal that has started to come down the tank frequently stops, apparently unable to see the target until further movement of its own head brings this again into sight. Two changes from the usual visual training technique were made to allow for this difficulty. First, the duration of the individual trials was increased from 20 or 30 sec. to a full minute; this allows time for several 'false starts' and for a slow intermittent approach. Secondly (in the FK series of experiments only), the figures were shown relatively close to the animals instead of at the full length of the tank. In the last 10 days of the experiments with the FK series the figures were shown sufficiently closely for the animals to reach out and take them without alteration of bodily position, so that the position of the eye concerned did not alter appreciably in the course of the response.

EFFECT OF STATOCYST REMOVAL UPON THE ORIENTATION OF THE EYES

One of the most striking effects of bilateral destruction of the statocysts is upon the orientation of the eyeballs. In unoperated octopuses the slit-like pupil of the eye remains horizontal, or nearly so, however the animal is sitting in its tank (Fig. 1 *a–e*). After removal of one statocyst this remains true, even of the side of the animal from which the statocyst was removed. After bilateral operations all regularity of orientation is lost. The position of the pupil then depends upon the position of the head, and this in turn depends upon the position in which the animal happens to be sitting (Fig. 1*f*, *g*).

It will be shown below that figures differing only in orientation are distinguished by their positions relative to the retina, as indicated by the pupil, and that animals deprived of their statocysts after training make mistakes in such discriminations when their pupils are in an abnormal (i.e. vertical rather than horizontal) position.

EXPERIMENTAL RESULTS

Animals were trained to make either a black-white or an orientation discrimination. The black-white discrimination was between two circular figures each 12 mm. in diameter, the other was between 5 × 30 mm. rectangles shown with the long axis vertical or horizontal. All the animals were pretrained to attack the future positive of the two figures to be discriminated before the start of the discrimination experiments.

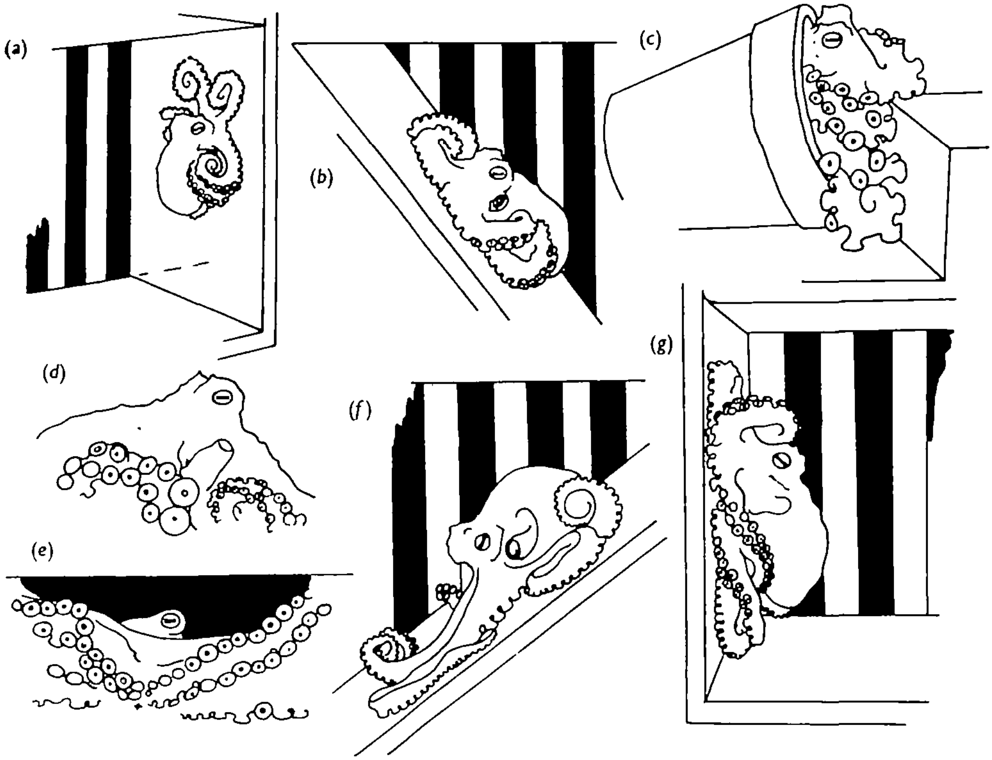

Fig. 1. Orientation of the eyes before and after bilateral statocyst removal. In unoperated animals the slit-like pupil normally remains horizontal or very nearly so (*a–e*), whatever the position of the octopus. After removal of both statocysts this ceases to be true, and the orientation of the retina, as indicated by the position of the pupil, thereafter depends upon the position in which the animal is sitting (*f–g*, octopus EKK). Pictures traced from projections of Kodachrome transparencies: *c*, *d* and *e* are of comparatively large (500 g.) octopuses, the rest of small (15–25 g.) animals in an aquarium set up in front of a vertically striped background; in *b* and *f* the aquarium, with the animals sitting on the bottom, has been tipped through 45°.

In the orientation discrimination experiments nine of the animals were trained with the vertical, and six with the horizontal rectangle as the positive figure; in the black-white discriminations four animals were trained with the white and three with the black circle as positive figure. Young (1958) has shown that untrained, though somewhat larger (500 g. ±), octopuses are slightly more likely to attack vertical than horizontal figures and white rather than black figures under the experimental conditions used here. Details of the direction of training in individual experiments are given in Tables 1 and 2, and in Fig. 3.

Three of the octopuses tested had both statocysts removed before training; the rest, nineteen in all, were pretrained to discriminate before operations in which one or both statocysts were removed. The duration of pretraining varied considerably, this being continued until the animal concerned was regularly making better than 75% correct responses; the octopus was then operated at the end of a day's set of ten trials, training being resumed next morning. Fig. 2 summarizes the results of experiments with pretrained animals. In it only the results of the last thirty preoperational and the first thirty or fifty postoperational training trials are given, although most of the animals were trained for much longer. Details of some individual performances are given in Fig. 3.

Table 1. *Details of attacks made after removal of both statocysts*

Figures to be discriminated		● (B) *v* ○ (W) Attacks on					▯ (V) *v* ▭ (H) Attacks on		
	Positive figure	+ve	−ve	Total trials		Positive figure	+ve	−ve	Total trials
Pretained animals									
FK1	B	62** (25)	18** (11)	140	FK7	V	12 (1)	17 (4)	180
FK3	W	26** (12)	6** (4)	100	FK8	V	47* (9)	21* (4)	180
FK6	W	61** (22)	16** (9)	130	FK9	V	3 (2)	8 (7)	80
EKR	B	13 (13)	5 (5)	50	FK10	V	42* (9)	21* (5)	180
EKS	B	18* (18)	5* (5)	50	EKF	H	12 (4)	9 (4)	90
					EKH	H	6 (5)	6 (5)	60
					EKI	H	16 (13)	8 (8)	70
					EKJ	H	1 (1)	1 (1)	50
					EKL	H	6 (6)	4 (4)	70
					EKM	H	5 (4)	9 (7)	70
Animals without preoperational training									
FK11	W	29**	7**	130	EK6	V	29	19	140
FK12	W	39**	9**	130					

** Difference significant at the 0·1 % level.
* Difference significant at the 1 % level.
Figures in parentheses show the number of attacks made in the first fifty trials after operation (as summarized in Fig. 2*b* and *c*).

It can be seen (Fig. 2) that removal of one statocyst did not affect the performance of octopuses in the orientation discrimination (8 animals). Removal of both statocysts, on the other hand, abolished the capacity to discriminate between the rectangles (10 animals), while not preventing discrimination of black and white circles (5 animals). The performance of three further octopuses from which the statocysts were removed *before* training confirms this result; the two trained on a black-white discrimination learned, while a third animal, trained using the rectangles, did not make significantly better than chance responses (Table 1).

The apparently simple result that bilateral statocyst removal abolishes the capacity to make orientation discriminations is seen to be more complicated when the performance of individual animals is considered. Individual records show that attacks upon the rectangles were *not* always made at random after operation; certain animals scored significantly better than might be expected on a chance basis, and nearly all showed a bias in favour of one or other of the test objects. The non-discriminatory responses of the group considered as a whole (Fig. 2*b*) are a consequence of summing the performances of individuals most of whom discriminated postoperationally to some extent, though not always in the direction learned during preoperational training; thus in the first fifty postoperational trials three out of ten animals tested actually took the negative object more often than the positive, and three took negative and positive equally often; only four out of the ten octopuses continued to discriminate in the direction learned during pretraining.

These results become explicable when the responses made are considered in relation to the postoperational habits of the individuals concerned. An octopus tends to return to a particular location on the side or bottom of its tank when not wandering about, and this determines the posture in which the individual is

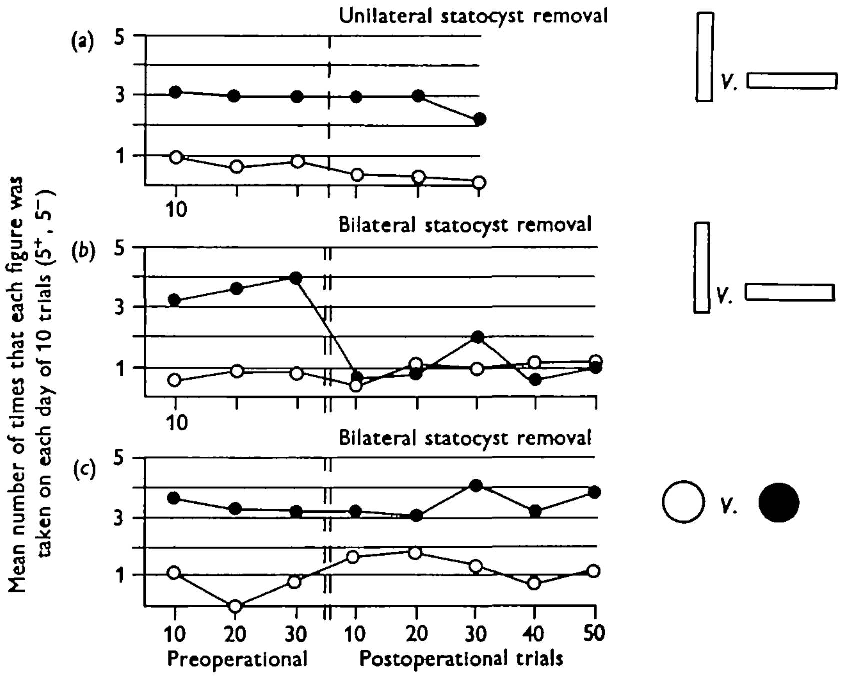

Fig. 2. The effect of statocyst removal on the performance of octopuses in visual discrimination experiments. In each plot ● shows the number of times that the positive figure was attacked and ○ the number of attacks on the negative. (*a*) A summary of the performance of eight animals from which one statocyst only was removed in the course of training; four of the animals had the left, and four the right statocyst removed; performance in the learned discrimination between horizontal and vertical rectangles was not affected by the operation. (*b*) A similar plot of the performance of ten octopuses which had both statocysts removed (four of these had the statocysts removed in two successive operations, their performance after removal of the first statocyst being included in plot *a*). Removal of both statocysts destroys the capacity to discriminate successfully between the rectangles. (*c*) A similar plot of the performance of five animals trained to discriminate between black and white disks. Removal of both statocysts does not prevent discrimination. These summaries are compounded from training experiments of variable length, and only the thirty trials immediately preceding operation and the first thirty or fifty postoperational trials are plotted. Details of some individual performances are given in Fig. 3.

usually found when at rest at the start of a training trial. The position that the operated animal takes up in its tank affects, among other things, the orientation of its retina, as indicated by the pupil (Fig. 1*f*, *g*). During the last 10 days of the 1959 (FK) series of experiments shown in Fig. 3 the position of the pupil was recorded together with the record of responses made. It was found that two of the operated octopuses (FK7 and FK9) tended to sit in positions such that their pupils remained vertical or nearly vertical (i.e. at right angles to the position held during preoperational training), while the other two (FK8 and FK10) generally sat with their pupils horizontal. FK7 and FK9, with pupils vertical, took the negative object more often than the positive and thus made more than random errors, while FK8

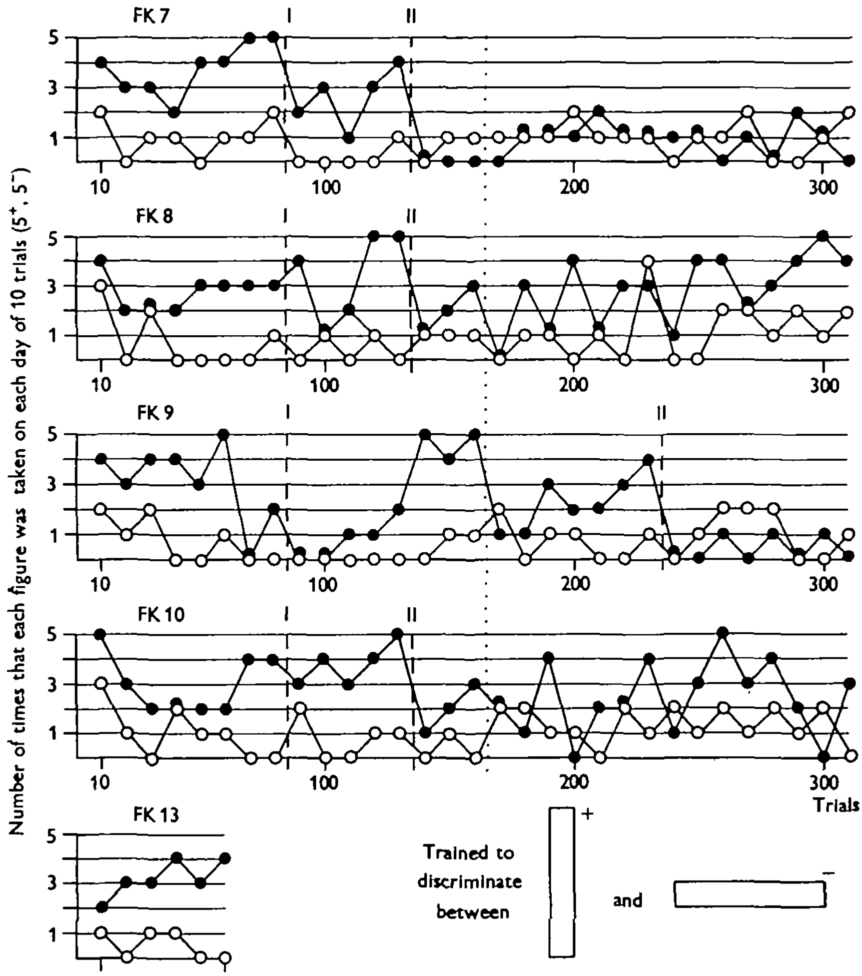

Fig. 3. Details of the performance of four octopuses from which both statocysts were removed in successive operations, and the performance of a fifth control animal. All were trained to discriminate between horizontal and vertical rectangles. The results are plotted as in fig. 2. Vertical broken lines marked I and II show when first one (LHS in the case of FK7 and FK8, RHS in FK9 and FK10) and then the second statocyst was removed. At all trials subsequent to the vertical dotted line and throughout the training of FK13 the horizontal rectangle was moved along its long axis (see text).

and FK10, with the pupils remaining predominantly horizontal, made considerably less; in preoperational training the performances of FK7 and FK9 were marginally better than those of FK8 and FK10 (Table 2). When the postoperational responses of these four octopuses are classified according to the position of the pupil at the

Table 2. *Details of postoperational attacks in training to discriminate between* ▯ *and* ▭ *(with the vertical rectangle as positive figure), considered in relation to the position of the eye used to direct the attack. Bilateral statocyst removals*

	Position of the pupil			
	—	|	/ \ or uncertain	
Correct responses/trials in which the eye was oriented as given above				
FK7	13/23	22/45	15/32	Total 49/100
FK8	37/48	13/23	21/29	61/100
FK9	11/23	16/42	8/15	35/80
FK10	42/54	9/20	13/26	64/100
Totals	103/148	59/130	57/102	—
Proportion of correct responses with the eye in the position above				
FK7	0·56	0·47	0·47	—
FK8	0·77	0·57	0·72	—
FK9	0·48	0·38	0·53	—
FK10	0·78	0·45	0·50	—
For all responses made by the four octopuses				
	0·70*	0·45	0·56	—

* Cf. 0·75 in preoperational training.
In preoperational training the proportions of correct responses made by these four animals were: 0·77, 0·74, 0·75, and 0·74 respectively.

time of the response, it is found that 70% (103) of the 148 responses made when the pupil was horizontal were correct; this is comparable with the performance of the animals in preoperational training, where 74–77% of the responses made were correct. In contrast to this, the responses made on occasions when the pupils were vertical were mainly incorrect, the animal tending to ignore the positive and attack the negative object; only 45% (59 out of 130) of the responses made on these occasions were correct (Table 2). It should be noticed that more than random errors were recorded, despite the fact that this analysis covers only the *last* 10 days of the training experiments shown in Fig. 3, and omits the first eighty postoperational trials in three out of the four experiments. During this period one of the initially perverse animals, FK7, was already relearning to make correct responses with the retina in the new, vertical, position; it was no longer attacking only the negative object (as at first, see Fig. 3) by the time the record of pupil position was begun.

Sutherland & Muntz (1959) have shown that direction of movement of rectangles is potentially a cue for discrimination of these figures in training experiments since untrained animals are more likely to attack a rectangle moving along its long axis than a rectangle moved at right angles to this when both are presented simultaneously. To find out whether this cue formed a basis for discrimination occurring after statocyst removal, the horizontal figure was moved along its long axis whenever presented in the course of the last 150 trials of the four experiments with FK animals described above, including throughout the whole of the period in which a record of pupil position was kept; performance did not appear to be altered in any way by this change in method of presentation (Fig. 3). A further unoperated animal (FK 13) was trained to discriminate between the rectangles at the same time; both rectangles were moved parallel to their long axes throughout this experiment, in which the animal learned as quickly as those trained in the more usual way, with both figures moved up and down (Fig. 3). The direction of movement seems to be irrelevant in these experiments, and cannot account for discrimination after statocyst removal.

DISCUSSION

After bilateral statocyst removal the eyes of *Octopus* no longer remain oriented with respect to gravity. The orientation of the retina, which normally remains constant with respect to gravity over a wide range of bodily positions (Fig. 1) becomes irregular, and the animal can no longer learn to discriminate between figures differing only in orientation.* Octopuses operated in this way can still, however, make black-white discriminations, and continue to distinguish between test figures and food, which is taken regularly even when the figures are not.

An analysis of postoperational responses by pretrained animals shows that the position of the retina relative to objects seen is a determining factor in the recognition of orientation; postoperationally attacks were made on the negative object (and not on the positive) when the eye used was at right angles to the position maintained during preoperational training (Table 2). This implies that postural information from pressure and stretch receptors and from the statocysts is not integrated centrally with the retinal input in the determination of orientation of figures seen. A simpler mechanism exists whereby the position of the retina relative to the external environment is kept constant as a result of the input from the statocysts; it remains constant even in blind animals (see Boycott (1960) and Dijkgraaf (1960)). Evidently the two eyes are 'locked' in some way, since removal of a single statocyst does not interfere with the orientation of either eye.

These results are interesting because of their relation to two hitherto separate lines of research upon the nervous organization of *Octopus*. They are interesting, first, because of their relation to the visual stimulus analysing mechanism. Sutherland (1957) has demonstrated that the performance of octopuses in visual discrimination experiments is consistent with the hypothesis that figures seen are classified centrally mainly on a basis of the ratio of their horizontal and vertical extents; octopuses can, for example, be taught to discriminate between horizontal and vertical rectangles, but not between the same rectangles shown at right angles, but obliquely. Sutherland (1957) further found that oblique rectangles are more readily distinguished from vertical than from horizontal rectangles, a finding that is explicable if the analysing mechanism at work sums more accurately in one direction than in the other *and* the orientation of the receptors is fixed relative to

* The slight improvement in postoperational performance by such initially perverse animals as FK 7 and FK 9 (Fig. 3) indicates, however, that learning to discriminate is potentially possible provided that the animal is always in the same position at the beginning of training trials and provided that figures are presented sufficiently close for the animal to take them without moving the head—i.e. under conditions in which the absence of the statocysts does not upset the constancy or orientation of the eyes relative to the figures.

horizontal-vertical in the external world. (If information about the orientation of the retina were fed into the analysing mechanism *after* computation of the apparent vertical and horizontal ratios there is no reason why $\setminus v$— should be any more difficult than $\setminus v\,|$).

Young (1960*b*) has recently shown an anatomical basis for the visual classification mechanism: the retinal rods are arranged in vertical and horizontal rows and are backed by predominantly vertical dendritic connexions within the optic lobes. It will be noticed that this arrangement is not only along the predicted axes, but is also asymmetric in the expected direction.

The eye-statocyst relationship is also interesting because it confirms what we already suspect about the role of proprioception in learning by cephalopods. Wells & Wells (1957) showed that octopuses can readily be trained to discriminate between objects differing in degree of surface irregularity (roughness) but not between objects differing only in the pattern or orientation of surface irregularities —they cannot be trained to make discriminations that depend upon taking into account the relative position of receptors on the arms. Moreover, attempts to teach octopuses to discriminate between objects differing in weight (Wells, 1961) have so far failed, which again implies that proprioceptive information (this time potentially available from 'stretch' receptors in the arms) is not available as a basis of learned adjustments in behaviour. The observation of Boycott (1960) that octopuses do not learn to use other proprioceptive information about the position of the arms and body in order to orientate themselves after statocyst removal has been repeated several times in the course of the present series of experiments, and would appear to confirm the over-all impression that proprioceptive information is not available for learning, although it clearly must contribute to many essentially reflex activities in movement and in the maintenance of posture (see, for example, Boycott & Young, 1950). On the evidence now available proprioceptive information from the statocysts would appear to be no exception to this general rule. This information is clearly used in reflex adjustments of the position of the eyes (see, for example, Dijkgraaf (1960) on the effects of rotation upon eye movements in blinded animals), but there is no need to suppose that it is available for central integration with other sensory inputs.

SUMMARY

1. Twenty-two small octopuses were trained to make visual discriminations before and after removal of the statocysts.

2. Removal of one statocyst (left or right) did not affect performance in a visual discrimination of rectangles shown horizontally and vertically (8 experiments), nor did it determine which eye was used to guide attacks upon things seen (a record of this was kept in four of the experiments).

3. Removal of both statocysts did not affect performance in a discrimination between black and white circles (7 animals) but did destroy the capacity to discriminate successfully between rectangles shown horizontally and vertically (11 animals).

4. After removal of both statocysts the orientation of the retina (as indicated by the slit-like pupils) is no longer constant over the normal wide range of bodily positions, but depends upon how the animal is sitting on the side or bottom of its tank.

5. An analysis of the postoperational responses of animals trained to make the orientation discrimination showed that nearly all of them were biased in favour of one or other of the test figures, not always that used as 'positive' figure during preoperational training. The postoperational scores of these trained animals depended mainly upon the positions in which they habitually sat in their tanks; ndividuals in positions such that the retina of the eye used to observe the test figures lay at right angles to the position held during pretraining tended to behave perversely.

6. These results indicate that orientation discrimination is dependent upon correct orientation of the retina, rather than upon central integration of proprioceptive and visual information. This is discussed in relation to a possible mechanism of visual discrimination proposed by Sutherland (1957), and in relation to what is already known about the role of proprioceptive information in learning.

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