OPTOMOTOR RESPONSES
AND NYSTAGMUS IN INTACT, BLINDED AND
STATOCYSTLESS CUTTLEFISH (*SEPIA OFFICINALIS* L.)

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INTRODUCTION

Cephalopods have well developed eyes and elaborate statocysts, with gravity receptors and angular acceleration receptors (Young, 1960). There are six or eight extra-ocular muscles (Glockauer, 1914) and the eye may move in the orbit as a result of information from either the eye itself or from the statocyst.

In *Octopus* eye movements, as a result of visual information, have been studied by Packard & Lucey (1964) and by Packard (1969), who found that nystagmus and a well-developed optomotor response were shown by animals in a revolving striped field. The influence of gravity on the position of the eye (and hence on posture, loco-motion and visual discrimination) was shown by Boycott (1960) and Wells (1960); and Dijkgraaf (1961) provided evidence that the cephalopod statocyst can detect angular acceleration: octopuses showed after-nystagmus at the cessation of rotation. This received physiological confirmation from the experiments of Maturana & Sperling (1963).

In *Sepia* visual influences on eye movement were described by Messenger (1968), who found, first, that the eyes moved in the yaw and pitch planes when prey was introduced into the visual field, and secondly, that they exhibited nystagmus in a revolving striped field. This effect has been quantitatively examined in the recent elegant study of Collewijn (1970). The influence of gravity (in keeping the eyes horizontal in uniform surroundings) was also noted by Messenger (1968); but Dijkgraaf (1963) had already demonstrated the activity of receptors sensitive to rotation in all three planes, although he was unable to blind his animals and confirm that this response was non-visual, as he inferred from the occurrence of after-nystagmus at the cessation of rotation.

Development of a new method of blinding as well as a technique for removing the statocysts prompted the following experiments, which confirm and extend Dijkgraaf's observations and establish that in *Sepia* visual nystagmus and optomotor responses are abolished by blinding; and that post-rotatory nystagmus is abolished by bilateral destruction of the statocysts.

MATERIALS AND METHODS

Subjects were adult cuttlefish, dorsal mantle length (DML) 80–165 mm, kept singly in white plastic tanks (100 × 50 × 50 cm) and fed on prawns, crabs or fish. The animals were observed free swimming in the large tanks and then, in a circular or rectangular

glass dish, in a nystagmus apparatus most kindly placed at my disposal by its designer, Mr A. Packard (Packard, 1969). Black and white stripes (5 cm width, subtending an angle of approximately 9° 30′) on a uniform background (a velvety, matt-white paper with only one join) were employed, and the apparatus permitted the background and/or the animal to be rotated in either sense.

Thirteen animals whose behaviour had been recorded in the nystagmus apparatus were subsequently operated upon, under 1 % urethane anaesthesia, to produce three examples of each of the three treatments: blinding, statocyst removal, and blinding with statocyst removal. 'Blinding' consisted of removing the lens, expelling the posterior chamber fluid and closing the orbit with a suture. This not only deprives the cuttlefish of keen vision; it renders it functionally blind (Messenger, 1968). Operations on the statocyst are best performed on an animal about 100 mm DML; the funnel is reflected posteriorly and the skin at its anterior base is opened to expose the large cephalic vein, which can be separated from the adjacent muscles with the back of a scalpel to reveal the cartilage floor of the statocysts, which lie very close to the midline, beneath the vein. (Dummy operations stopped at this point.) Each statocyst is opened separately with a pointed scalpel, the macula is removed and the interior is scraped with a small swab. Such a lesion to the statocyst, which was confirmed by *post mortem* dissection under a binocular microscope is referred to as 'statocyst removal'.

Since there was no variation between individuals undergoing the same treatment, and since the results were unequivocal, no further operations were made. Additional data, collected over several years, are referred to in the results (1 *a*, *b*) and in the discussion. Tests were made at various times after operation. The criterion for a valid test was subsequent survival for 24 h. Urethane anaesthesia, and exposure of the floor of the statocysts alone does not produce any alteration in the behaviour of cuttlefishes in this situation.

RESULTS

(1) *The behaviour after lesions*

(*a*) *Blind animals* (N = 5)

Blind *Sepia* are hyperactive. In well-lit tanks intact animals generally lie on the bottom, half buried in sand, but blind animals swim almost continuously, usually at the surface, with the most dorsal part of the mantle out of the water. They swim very slowly with the fins, often in wide circles; the sense of movement is reversible. Occasionally tight circling about the animal's yaw axis occurs and this, too, can be in either sense. Straight swimming is sometimes seen and always occurs if the animal bumps into the side of a tank; this also elicits rapid locomotion by 'jetting'. The colour of blind animals is in the range normal for cuttlefish in aquaria: it is generally uniformly mottled though once a striped pattern was seen. The most notable feature is the faint pair of 'eye-spots' (Holmes, 1940; Young, 1950; Boycott, 1958) nearly always present on the posterior mantle; these become intensely dark if the animal is touched.

(*b*) *Animals without statocysts* (N = 4)

Removal of the statocysts bilaterally is followed by extreme instability about all axes. Not only may the animal *oscillate* about one or more axis, especially the roll axis, but it may also *spin* (i.e. make several revolutions in the same sense) particularly

about the yaw axis (cf. *Octopus*, Messenger, 1967a). This may not be apparent at first sight because these preparations, like intact animals, usually sit at the bottom of the tank. But if a crab or prawn is introduced into their tank and they swim towards it they move slowly and jerkily, and an animal may spin several times on its own axis even in a distance of 60 cm. Such spinning is especially marked when the animal makes rapid escape movements by jetting, and involves a loss of control of the fins and funnel. Despite these locomotory deficiencies, such animals follow prey visually and can capture crabs; they show normal colour patterns.

As one would expect, cuttlefish that lack eyes and statocysts show the features produced by both these operations: they are unstable about all axes, do not respond to mechanical vibration, and swim continuously at the top of the tank.

(2) *Visual and motor responses in the test cylinder*

The results are unequivocal and are set out in Table 1. The following notes are for amplification only.

Table 1. *Results of testing in nystagmus apparatus*

Rotation of	Field	Intact	Blind	No statocysts	Blind, no statocysts
Field alone	Plain	No response*	No response	No response*	No response
	Striped	Eyes drift, head, body turn, swims† in same sense	No response	Eyes drift, head, body turns, swims† in same sense	No response
Animal alone	Plain	Passively turned and post-rotatory nystagmus		Passively turned, no post-rotatory nystagmus	
	Striped	Eyes drift, head, body turns, swims† in opposite sense	Passively turned, post-rotatory nystagmus	Eyes drift, head, body turns, swims† in opposite sense	Passively turned no post- rotatory nystagmus
Both together	Plain	Passively turned and post-rotatory nystagmus		Passively turned, no post-rotatory nystagmus	
	Striped	Passively turned; abbreviated or no post-rotatory nystagmus	Passively turned, post-rotatory nystagmus	Passively turned, no post-rotatory nystagmus	

* At speeds greater than 70°/s, below this irregular eyes movements—see text.
† 'Optomotor response'.

(a) *Intact animals* (N = 13)

When a striped cylinder is revolved about a stationary cuttlefish there are movements of the eyes, head and whole body, in that order. As a result of this, and within 6 or 7 s, the animal is swimming at the same speed and in the same sense as the cylinder. The nystagmic movements exhibit the usual slow phase ('drift') and fast phase ('flick'): the eyes drift in the same sense as the cylinder rotates, but flick in the opposite sense. These movements disappear by the time the animal is swimming.

When the animal is turned in a fixed cylinder all movements are in the opposite sense; thus clockwise revolution causes the eyes to drift anticlockwise. The head then turns anticlockwise and the animal starts swimming in that sense until its velocity is equal and opposite to that of the rotating dish.

When both background and animal are turned there are no visible responses; the animal is passively turned and there are no colour changes, which sometimes occur in both the first situations.

Although speed of rotation was not a parameter systematically varied in this study, responses to 5 cm stripes were obtained at speeds of 30°/s down to less than 5°/s (cf. Collewijn, 1970). When a 'uniform' background was moved about a still cuttlefish there was generally no optomotor response and head movements were extremely rare; eye movements, however, commonly occurred and only at speeds far beyond physiological significance (72°/s) were these completely absent (cf. Collewijn, 1970).

A consistent feature of revolution in a plain background is the conspicuous post-rotatory nystagmus. If both background and cuttlefish are turned, say anticlockwise, head and eyes turn anticlockwise when revolution abruptly stops. If the background is striped there may be very weak after-nystagmus, which is rapidly damped, or more usually there is none.

(b) Blind animals (N = 3)

The results are self-evident. As would be expected, removal of keen vision in no way impairs input from the statocyst, and after-responses, head movements, are regularly observed.

(c) Animals without statocysts (N = 3)

The striking feature of these animals is that post-rotatory responses are completely abolished, though eye movements relying on visual cues persist.

As described above, these preparations are unstable and do not move as readily as intact animals, so that the eye movements may lag and the eye and the animal stop pursuing the moving stripes. These preparations may show intense colour changes in this situation.

(d) Blinded animals without statocysts (N = 3)

These preparations responded to neither visual nor mechanical stimuli, though they were in excellent condition, as judged by the muscle tone. They flashed eye-spots and sometimes changed colour, emphasizing that chromatic behaviour can occur in the absence of visual input, as in *Octopus* (Packard & Sanders, 1969).

DISCUSSION
(1)   *The effects of lesions*

Most noteworthy is the hyperactivity after bilateral blinding. Denton & Gilpin-Brown (1961) have shown that there is a diurnal rhythm based on illumination: swim in the dark, settle in the light. The behaviour recorded here is in agreement with this and suggests that 'night' is continuous for this preparation from the moment of recovery. This is noteworthy—first because the retina could not have been directly injured during operation, although there must have been interference to the circulation; and secondly, whatever damage may have occurred unknowingly to the rhabdomes, none could have occurred to the parolfactory vesicles (POV) (Boycott & Young, 1956) which lie deep to optic lobe near the optic tract; this was confirmed

histologically in two animals. Nishioka *et al.* (1966) showed that these organs have the ultrastructure and biochemistry of photoreceptors and supposed that they mediated in a relatively long-term light-dependent activity. Messenger (1967*c*) found the POV were well developed in *Liocranchia reinhardti* and *Bathothauma lyromma*, which both live in deeper waters and have been fished over a depth-range of 0–500 and 0–3000 m respectively (open nets: Clarke, 1966). This again suggests a photoreceptor function separated from keen vision: perhaps vertical migration. These problems must remain unresolved for the present as removal of the POV alone is not possible; but it seems unlikely that in *Sepia* the POV play a role in regulating the light-dependent buoyancy changes.

It is emphasized that these preparations were in good physical condition; unlike moribund cuttlefish, which also float up to the top of the water, these fed and had good muscle tone; the skin was usually darkly mottled and papillate, and the tentacles were held retracted.

Although all animals showed some circling after blinding, this was not continuous or fixed in sense and was only rarely 'tight'; that is, it was never of the 'forced' kind known in *Octopus* (Messenger, 1967*b*), or indeed in *Sepia* (Boycott & Young, 1950), after asymmetrical lesions. Its significance is not clear.

The effect of unilaterally blinding cuttlefish has been seen many times ($N > 40$) because of a series of experiments designed to show how cuttlefish estimate the position of their prey in space (J. B. Messenger, in preparation). The behaviour differs little from that of an intact cuttlefish, except that visual stimuli on the blind side produce no response. There is a tendency to maintain an eye spot unilaterally on the mantle, on the side without the eye. This might be expected, since bilateral blinding produces semipermanent eye spots bilaterally (see above); but the significance of this is not clear. Much more interesting is that a unilaterally blinded male is susceptible to approaches from other males that may begin to copulate with it. If a male is blind, say, in the right eye, and is approached on the right by another male, it will not see the displayed fourth arm and will not, therefore, return the display that establishes it as a male. The approacher will then begin to copulate with it and a fight will ensue; if such a male is approached on the left side, however, it returns the display in the usual way and no copulation is attempted, emphasizing, incidentally, how entirely dependent on *visual* cues reproduction seems to be in *Sepia*.

Removal of the statocysts bilaterally is followed by marked locomotor instability as would be expected (cf. *Octopus*, Boycott, 1960; Wells, 1960). The effect of unilateral extirpation, which has little effect in *Octopus*, is not so certain, however. Four out of five preparations were unstable, showing spin and oscillation, and in one of these the thin median wall between the statocysts was found, at *post mortem* macroscopic examination, to be punctured; the statocysts are very close together in this genus and the effects of unilateral lesion can be properly assessed only after examination of serial sections of the brains of operated animals.

Of course any instability produced by a unilateral lesion would show up more in a swimming animal like a cuttlefish. Indeed Boycott (1960) describes a slight postural asymmetry in *Octopus* after unilateral statocyst removal that only shows up when it swims.

One animal was tested in the nystagmus apparatus; it showed clear post-rotatory

nystagmus, agreeing with Dijkgraaf's (1961) finding in *Octopus*; it also showed opto-kinetic nystagmus (as Collewijn, 1970, found) and optomotor responses in the expected sense. Visual tasks, such as pursuit of prey or sexually displaying to other cuttlefishes, survive operation to one or both statocysts.

(2) *Optokinetic nystagmus, after-nystagmus and the optomotor response*

These experiments provide further evidence (Packard & Lucey, 1964; Messenger, 1968; Collewijn, 1970) that in cephalopods there are coarse eye movements, which may be 'compensatory' or visually induced. It is not yet known whether cephalopods show fine saccades (Collewijn, 1970). Nystagmus, which may be interpreted as a device to stabilize the image on the retina, has been considered at length recently by Collewijn (1970) and our results add little to his. Our animals, however, were unrestricted so that optomotor responses, which can be seen as a further step towards a stable image, appeared. For the free-swimming animal there are in fact three feedback loops operating to reduce the error signal; if eye movements cannot reduce it, the head is moved and if this is inadequate the whole body moves. The first two loops of the system survive gross lesions to the statocysts, but the third may cease to operate because of locomotor dysfunction following the operation. It is interesting to recall that acute colour changes occur in this situation, the animal usually turning intensely dark. Such behaviour occurs at other times when *Sepia* is in a 'conflict' situation (cf. *Octopus*, Boycott & Young, 1958; Packard, 1963).

The results of revolving a 'plain' background agree with Collewijn's and suggest that the background used was anything but plain; even at quite fast speeds (30°/s) there were isolated eye movements. However, with unrestricted animals the optomotor responses were not elicited with any regularity by the 'plain' ground, in marked contrast to the consistent optomotor response to the movement of stripes. Further-more the background is evidently plain enough to permit after-nystagmus (see below).

Nystagmus and optomotor responses seem to be a fundamental feature of visuo-motor systems (e.g. Horridge, 1966). Their occurrence does not, of course, imply an area of specialized vision in the retina, although other evidence suggests there may indeed be one (Young, 1963; Messenger, 1968).

The effect of angular acceleration on the eyes first shown by Dijkgraaf (1963) is confirmed here, and post-rotatory turning of the head, at least, is shown to persist in blinded cuttlefish; the method of blinding prevents eye movements in such prepara-tions. But visual influences suppress the non-visual at the abrupt cessation of rotation. There is strong after-nystagmus in the absence of visual cues (blind animals, or plain background), but none when stripes are present. (There may be very brief after-nystagmus, never more than one 'flick'. Collewijn (1970), found no after-nystagmus electrically.)

(3) *Central control of eye movements*

The dual control of eye movements described here finds physiological and anatomical support from the work of Boycott (1961) on *Sepia* and Boycott (1960); Young (1970) on *Octopus*. The oculomotor muscles of each eye (and also the intrinsic muscles of the eye) are innervated from the ipsilateral lateral pedal lobe. Stimulation of this area is followed by ipsilateral or bilateral movements of the eyes (Boycott, 1961). Afferents

to the lateral pedal lobe include fibres from the ipsilateral optic lobe and the ipsilateral statocyst; and also the ipsilateral peduncle lobe, in *Octopus* (Messenger, 1967a).

The experiments reported here do not tell us how eye movements are affected by the contralateral statocyst and visual system, and though Collewijn has evidence that eye movements are conjugate in his experimental situation, films of attacking animals suggest that the eyes may sometimes act independently under normal conditions.

SUMMARY

1. Evidence is reviewed confirming that the eyes of *Sepia* move under the influence of gravity, angular acceleration and visual receptors.

2. Visual nystagmus and optomotor responses are described in free-swimming *Sepia*; these survive bilateral destruction of the statocysts, though they are abolished by blinding.

3. Post-rotatory nystagmus is shown by intact animals in a plain background; in bilaterally blinded animals there are post-rotatory movements of the head. These responses disappear if the statocysts are removed.

4. The post-rotatory nystagmus of intact animals is almost completely suppressed by visual cues.

5. Other effects of blinding and statocyst removal are described.

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