VISUAL ACUITY IN *OCTOPUS PALLIDUS* AND *OCTOPUS AUSTRALIS*

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SUMMARY

Specimens of *Octopus pallidus* and *O. australis* were trained to discriminate vertical from horizontal rectangles, vertical from horizontal gratings, and vertical and horizontal gratings from uniform grey. In the discriminations that involved gratings a conditional simultaneous discrimination procedure was used, in which the two stimuli to be discriminated were presented stationary at the two ends of the tank, and a moving white disc was shown in front of each of them. Attacks on a disc were then rewarded or punished depending on the background against which it was shown. Animals rapidly reached performance levels of better than 80 % correct responses on all discriminations.

With one specimen of *O. pallidus* and three of *O. australis* when progressively finer gratings were used the discrimination broke down with stripe widths between 4·4′ and 9·7′, showing that for both species the minimum separable visual acuity is less than 9·7′.

The behaviour of the two species is very similar to that of *O. vulgaris*, except that they accept less food per day, so fewer trials could be given.

INTRODUCTION

While a great deal of information is available on the visual capabilities of the common European octopus, *Octopus vulgaris*, most of this concerns higher visual functions, such as the ability to discriminate shapes, the mechanisms by which such discriminations are learnt, and the function of the various parts of the central nervous system (see, for example, Wells, 1978; Messenger, 1981, for reviews). Comparatively few behavioural studies have been carried out on the animals' more basic visual capabilities, such as sensitivity and acuity. Thus in the case of sensitivity there appear to be no behavioural studies on *Octopus* at all, and in the case of visual acuity only two experiments have been reported, neither of which is conclusive. The first (Sutherland, 1963*a*) involved training the animals to discriminate between vertical and horizontal gratings presented in a successive discrimination situation, and yielded an estimate of 17′ or better for the minimum separable acuity. The second

study (Packard, 1969) used the optomotor response and gave a result between 27′ and 49′ for very small animals (less than 3 g), and of 27′ or better for slightly larger animals (12–22 g). These studies will be considered further in the Discussion.

The present experiments were done for two purposes: (1) to develop behavioural methods suitable for studying vision in Australian octopuses; and (2) to measure visual acuity in *Octopus* as an initial contribution to the study of basic visual mechanisms in these animals.

The behavioural methods used initially followed closely those developed by Boycott & Young (1956) for *O. vulgaris*, and were later modified to fit the needs of the current experiment. Although the final determination of visual acuity did not depend on these initial stages, some detail of them is given in order to compare the suitability of *O. pallidus* and *O. australis* with *O. vulgaris* as experimental animals.

MATERIALS AND METHODS

Subjects

The determinations of acuity were carried out on one specimen of *O. pallidus*, weighing 102 g at the start of the experiments, and three specimens of *O. australis* weighing 113, 109 and 59 g. They were caught in traps in Port Phillip Bay, and kept in individual black plastic tanks with transparent lids, measuring 80 cm × 20 cm and 40 cm in depth, on an open seawater circulation, in the Marine Science Laboratory, Queenscliff. At the start of the experiments the seawater temperature was 12°C. Heating was later introduced which raised the temperature of the water to 15 °C.

Pretraining and successive discrimination training

The first stages of the experiment followed in detail the procedures developed for *O. vulgaris*. A 'home', consisting of a plastic box or an earthenware flowerpot, was provided at one of the narrow ends of the tank, and the animals were trained to attack a vertical white rectangle 5·5 cm × 1·5 cm, cut out of 0·4 cm thick white Perspex, and presented at the other end of the tank on a transparent Perspex rod. The rectangle was moved vertically by hand, and attacks on it were rewarded by a small piece of fish or prawn presented on a wire probe. When the animals attacked this stimulus consistently a successive discrimination was set up by introducing the negative stimulus, a horizontal rectangle of the same dimensions. Attacks on the negative stimulus were punished by a 4·5 V d.c. shock delivered to the animal through a pair of electrodes attached to a probe.

Simultaneous discrimination training

In the next stage of the experiment a home, made from an inverted flowerpot with the bottom sawn off, was provided in the centre of the tank. The animals remained most of the time in these homes, with one eye pointing towards each of the narrow
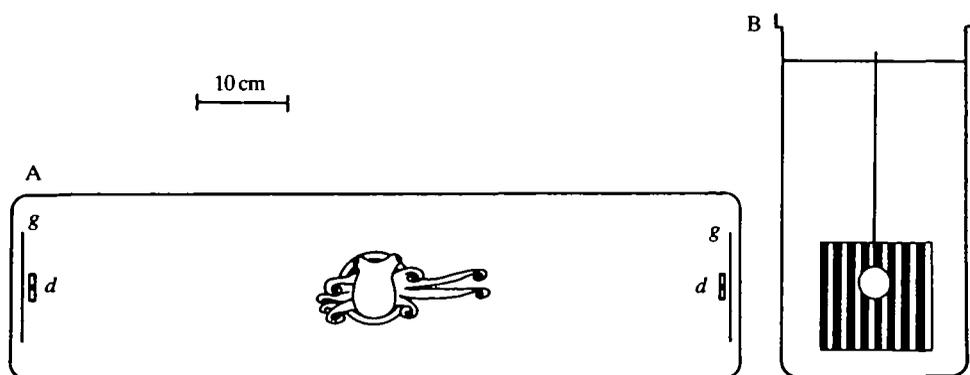
Fig. 1. Experimental set up for the simultaneous discrimination experiments. (A) Plan view, showing the octopus starting an attack from its home. *g*, gratings; *d*, white disc. (B) Elevation view of one of the ends of the tank, showing the stimulus display.

ends of the tank (Fig. 1). The animals were trained to discriminate between stationary gratings with vertical and horizontal stripes, inserted into the tank against the two narrow ends at the beginning of each trial (Fig. 1). The size of the gratings was 13·5 cm × 13·5 cm: they were produced photographically by enlarging or reducing commercially available gratings (Letraset) and printed on plastic paper which could be placed directly into the water without any added protection. Six gratings were used in all, with stripe widths of 7·5, 4·0, 3·5, 1·75, 1·1 and 0·5 mm. Since the distance of the gratings from the home was 390 mm, the individual bars subtended 66′, 35′, 15·4′, 9·7′ and 4·4′ at the home, respectively. The brightness of the white stripes, measured with a calibrated PIN silicon photodiode, varied between −0·4 and −0·9 log millilamberts in the different tanks. The light incident at the water surface in the tanks, measured with a Licor LI 185 A photometer fitted with a cosine collector, varied between −0·64 and 0·079 log μE m^{-2} s^{-1}: at a depth of 17 cm (roughly level with the stimuli) it was further attenuated by about 0·6 log units.

Since octopuses do not readily attack stationary stimuli, on each trial a white disc, 3·8 cm in diameter and mounted on a transparent Perspex rod, was moved vertically by hand in front of each of the gratings. The situation was thus a conditional simultaneous discrimination: the stimulus object being responded to (the white disc) was the same at each end of the tank but attacks were rewarded or punished, with food or electric shock as before, depending on the background (the grating) against which the disc was seen. Attacks to the end with the vertical grating were rewarded in all cases and the end at which this grating was presented was varied randomly between trials. After establishing the discrimination with coarse gratings, progressively finer gratings were used until the discrimination broke down. Both vertical and horizontal gratings were also tested against a grey background of intensity intermediate between the black and the grey stripes. Attacks to the grey end were punished, and to the end with the horizontal or vertical grating rewarded.

RESULTS

Suitability of O. australis *and* O. pallidus *for experimental study*

During the study eight octopuses were closely observed and studied for between 6 and 12 months, five *O. australis* and three *O. pallidus*. Eighteen other octopuses (eleven *O. australis* and seven *O. pallidus* with a mass range of 38–475 g) were kept for varying periods of up to 3 months. There was considerable variation between individuals in such things as readiness to attack and the percentage of time spent in their homes.

The readiness of different octopuses to attack the moving white target varied with their method of capture. Octopuses lifted in traps suffered little disturbance and would usually begin to feed during their first 24 h in the tanks. Animals collected by dredging or trawling, however, were usually damaged and not suitable for behavioural work.

The first batch of six animals was collected on 2 May 1986, and of these four were female. Between the 9th and 17th day after capture all the females laid eggs on the walls of their home. Since while brooding their eggs they refused to feed, they were of no value for learning experiments, and only male octopuses were therefore used.

It was also found that animals weighing more than 300 g were usually unsatisfactory experimental subjects because of their variable appetite and low activity level. Very small octopuses (less than about 60 g) could not be rewarded with food sufficiently often to achieve good learning, and these two groups were therefore also excluded from the experiments.

Successive discrimination

During pretraining octopuses learned to associate attacking a moving vertical rectangle with food. Medium-sized animals (100–200 g) that had not been recently fed usually came to attack the shape regularly for food reward within the first day of training. However, the number of rewards an animal would accept on any given day was both small and variable, and this determined the number of trials that could be given. Once the association of the positive stimulus with food was established, negative trials were introduced.

The rate of learning of two animals to perform a successive discrimination between a vertical and a horizontal rectangle is shown in Fig. 2. Because of the variable number of rewards that could be given, only trials on which an attack occurred were counted, and the figure shows the percentage of attacks made to the vertical rectangle out of the total number of attacks made. Both octopuses made between 8 and 15 attacks per day. Octopus number 2 (*O. pallidus*, mass 102 g) reached a level of 80 % correct responses on day 2, and by day 6 was scoring between 92 and 96 %. Octopus number 6 (*O. australis*, mass 100 g) refused to leave his home on days 2 and 5, but reached his final score of 90 % correct by day 6. Most errors (i.e. attacks on the horizontal bar) were made during the first three or four trials of the day when the octopuses were hungry and prone to attack any moving object. This bias is eliminated in the simultaneous discrimination situation.
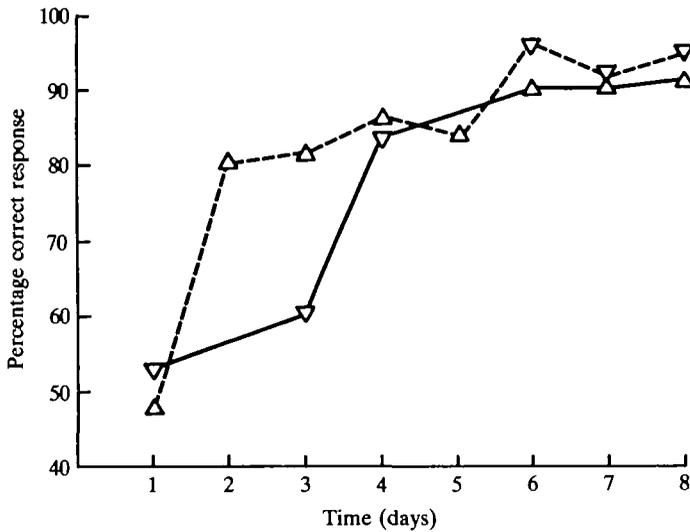
Fig. 2. The learning curves of two octopuses in a successive discrimination test. Upright triangles, *Octopus pallidus*; inverted triangles, *O. australis*.

Simultaneous discrimination

Visual acuity

Three octopuses, two of which (numbers 2 and 6) had been used previously in the successive discrimination, were trained to discriminate between vertical and horizontal gratings. Initially they were trained with the coarsest gratings, until their performance no longer improved. Progressively finer gratings were then used. The results are shown in Fig. 3A. As in the successive discrimination, the limited number of rewards the animals would accept meant that the number of trials varied from day to day. Table 1 shows the total number of trials given with each grating width (obtained in each case over several days).

The same three animals were then trained and tested on vertical gratings used in conjunction with the uniform grey stimulus. The results of these tests are shown in Fig. 3B and, as before, the number of trials given at each stripe width is given in Table 1. Finally, the animals were trained with horizontal gratings against uniform grey. A fourth animal was added at this stage of the experiments. The results are given in Fig. 3C and Table 1. Fig. 3D shows the results for the three discrimination conditions for all the animals pooled.

It is clear from the figures that *O. australis* and *O. pallidus* can discriminate between horizontal and vertical gratings, and can also discriminate these gratings from a uniform grey stimulus, provided that the visual angle subtended by the individual stripes was 9·7′ or greater. For both species the discrimination broke down between 4·4′ and 9·7′.

The data were also analysed by calculating the difference between the proportion of correct responses obtained under the various conditions, relative to the standard

Table 1. *Number of trials contributing to each of the data points shown in Figs 3, 4 and 5*

Animal	Discrimination	Grating stripe width (min)					
		4·4	9·7	15·4	30	35	66
2	Vertical *vs*	10	34	27	8	70	31
4	horizontal	9	21	24	27		23
6		10	24	10		10	14
2	Vertical *vs* grey	10	9	30	13		5
4		12	19	26	27		15
6		9	16	12	10		5
1	Horizontal *vs*	15	21	18	25		
2	grey	15	31	14	12		
4		10	21	17	19		
6		9	16	20	12		
	Total	109	212	198	153	80	93

Animal no. 2 was *Octopus pallidus*, animals nos 1, 4 and 6 were *O. australis*.

deviation of these differences, and comparing the result (z) to the significance points of the normal distribution. The difference between the 4·4' and 9·7' gratings, averaged over animals, was highly significant under all conditions (z = 3·23, 3·08 and 3·10 for the horizontal *vs* vertical, vertical *vs* grey, and horizontal *vs* grey discriminations, respectively). None of the other differences reached significance.

General aspects of behaviour

During each trial the octopus leaves its home to attack a target, and then returns to the home to eat the reward or to seek refuge, depending on whether the response was correct or incorrect. When the finest gratings (subtending an angle of 4·4' at the home) were presented, the octopuses were usually reluctant to attack either moving target. Such 'cautious' and 'hesitant' behaviour is typified by the octopus rising to sit on the upper rim of its home and then extending one or two arms alternately towards each end of the tank. Up to 3 min was allowed for an attack. Confident attacks could be re-induced by increasing the stripe width of the gratings.

It might be expected that, especially with the finer gratings, the animal would initially move towards one or other end of the tank more or less at random until the grating was close enough to be easily resolved, and then back-track to the other end of the tank if it had approached the negative stimulus. Such behaviour was in fact very infrequent, occurring on only 24 occasions over the whole course of the experiments, which is on less than 3 % of the trials given. As expected, back-tracking was most common with the 4·4' grating (16 occasions). When back-tracking occurred this did not necessarily correct an initially incorrect choice: with the 4·4' grating, for example, while an initially incorrect response was corrected on 10 of the occasions on which back-tracking occurred, on the other six occasions the animal initially moved
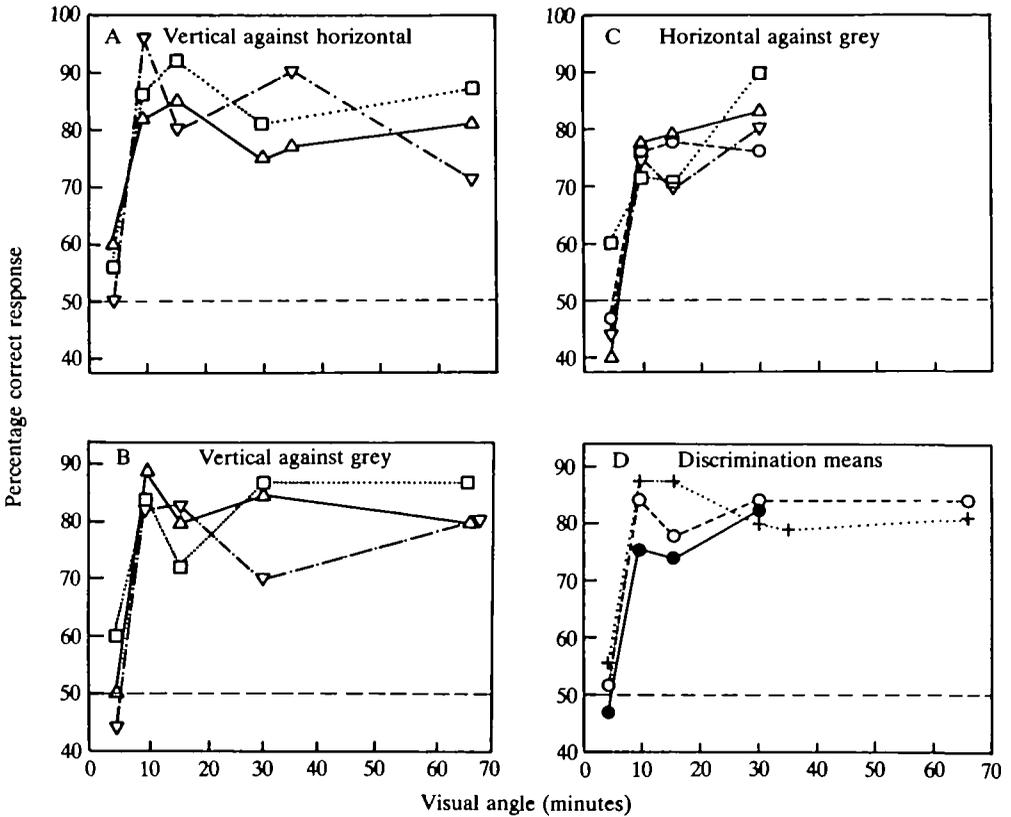
Fig. 3. Simultaneous discrimination tests. (A) Vertical against horizontal gratings; (B) vertical gratings against uniform grey; (C) horizontal gratings against uniform grey; (D) mean scores for the different discriminations. Symbols for A–C: circles, animal no. 1; upright triangles, animal no. 2; squares, animal no. 3; inverted triangles, animal no. 6. Animal no. 2 was *Octopus pallidus*, the other three animals *O. australis*. Symbols for D: crosses, horizontal/vertical; plain circles, vertical/grey; filled circles, horizontal/grey.

towards the correct stimulus, but eventually attacked the incorrect stimulus. Trials on which back-tracking occurred are not included in the results.

The results also show that even when gratings with wide stripe widths were used 100 % correct performance was not achieved, although it did on occasion occur within a given day. With the 66′ grating, for example, the average level of performance was only 82 % correct.

DISCUSSION

The results show that *O. australis* and *O. pallidus* are both suitable for behavioural work on vision. The experimental animals remained in excellent condition throughout the study, their weight increasing by between 60 % and 87 % during the 4 months from June to October. In most respects their behaviour was very similar to that

shown by *O. vulgaris*: 'cautious' behaviour such as that described here, and the failure to reach 100 % correct performance even on easy discriminations are, for example, also typical of *O. vulgaris*.

Compared to *O. vulgaris* the main difficulty was found to be the small number of rewards that they were prepared to accept. The number of trials that can be completed consequently varied markedly from day to day, and the accumulation of data was time consuming. In part, this may have been a result of the water temperature, which was low during the time of year that these experiments were carried out. In *O. vulgaris* behavioural data are difficult to obtain when the water temperature falls below about 18 °C.

The low frequency of back-tracking behaviour in the present experiments is surprising. By the time an animal approaches and completes an attack on a negative stimulus the grating must be resolvable, even with the finest gratings used. However, Maldonado (1964) has shown that in *O. vulgaris* once an attack has been launched it will be completed normally even if the lights in the experimental tank are turned off, showing that vision is not needed in the later stages of an attack. It was also clear that the capacity of moving stimuli, such as the white disc used in the current experiments, to elicit attacks increases the closer the stimulus is to the animal. It may be that as the animal approaches the disc during an incorrect response this positive effect becomes greater than the negative effect of the grating. The fact that even with the large gratings the animals only perform at just over 80 % correct also reflects the fact that attacks are often completed even when the stimulus is negative and easily resolvable by the animal.

The present results give an estimate of between 4·4' and 9·7' for the minimum separable acuity of octopuses. This is comparable to values obtained for many vertebrates, including fishes (see Muntz, 1974, for a review), and is considerably smaller than the previous estimates of Sutherland (1963a) and Packard (1969). It is, of course, the case that different species of octopus were used, but it seems unlikely that the differences, which are large, are solely due to this cause. In Sutherland's (1963a) experiment a successive discrimination situation was used, and such paradigms usually result in poorer discrimination than simultaneous discriminations (e.g. Mackintosh, 1974). Also, in order to measure visual acuity it is necessary to know the distance from the stimulus at which the discrimination is being made. In Sutherland's experiment this was assumed to be the distance from the negative stimulus at which the animals stopped on those trials where the attack was not completed, but this could obviously be a smaller distance than that at which the animals can in fact resolve the gratings. In Packard's (1969) study using the optomotor response there were difficulties associated with irregularities in the striped drums other than the stripes themselves, which had to be indirectly allowed for by subtracting control scores (obtained with plain drums or drums with horizontal stripes) from the experimental scores, and the validity of using such a procedure is unclear. Packard's study also used much smaller animals. Finally, any behavioural experiment can only give an upper limit for a visual capacity, since it is always possible that changing the experimental conditions will result in better performance.

The present study also used stationary gratings, which may be easier for the animals to resolve than moving gratings. Sutherland (1963b) has previously shown that octopuses can discriminate stationary stimuli. The basic problem in such a situation is that octopuses do not readily attack stationary objects. Sutherland overcame this by increasing the trial length, and by luring the animals out, using a moving neutral stimulus, on those positive trials where no attack occurred within 60 s: as the animal approached the neutral stimulus was removed so that the final attack was made on the stationary stimulus. In the conditional discrimination paradigm used in the present experiments the attacks were in all cases made to moving objects, and the problem does not arise.

The octopus pupil has the form of a horizontal rectangle, which could well affect the discrimination of horizontal and vertical gratings differently. For example, Heidermanns (1928) points out that, other things being equal, the horizontal shape of the pupil should result in chromatic aberration having a greater effect on the resolution of vertical contours compared with horizontal ones. However, diffraction will have the opposite effect. It is thus clear that the effect of the shape of the pupil could depend on pupil size, since chromatic aberration will be most severe when the pupil is large, and diffraction when the pupil is constricted. The optical character-istics of the lens could also be relevant. Sroczyński & Muntz (1985) have, for example, shown that the lens of the octopus *Eledone cirrhosa* is markedly astigmatic. Sutherland (1963a) found that his animals discriminated horizontal gratings of different periodicity from each other more readily than they did vertical ones, which may be related to these factors. In the present study there was no significant difference between the discrimination of horizontal and vertical gratings from grey.

Sroczyński & Muntz (1985) also measured the monochromatic resolution angles of lenses from the octopus *E. cirrhosa*, at a wavelength of 546·1 nm. Considerable variation between lenses was found, with resolution angles between about 1′ and 7′ being obtained when the lens was at full aperture, and angles on occasion down to the diffraction limit of 0·7′ when small apertures were used. However, even if the lenses of the animals used in the present study are similar, such levels of resolution would not be expected in the present situation since (1) the stimuli were illuminated with 'white' light, (2) cephalopod lenses show considerable chromatic aberration (Sroc-zyński & Muntz, 1985), and (3) cephalopods are apparently not capable of colour vision (Messenger, 1977; Flores, Igarashi & Mikami, 1978; Flores, 1983).

Finally, it is clear that the retinal mosaic of octopuses is much finer than necessary to achieve the visual acuity that is found experimentally (oversampling). Young (1960), for example, estimates the size of a retinal unit in *O. vulgaris* to be about 1·3′, and Packard (1969) estimates that the retina of this species has some 20 000 rhab-domes mm^{-2} irrespective of the size of the animal, which means that in an adult, with an eye diameter of perhaps 2 cm, each rhabdome would subtend about 1·2′. Unpublished histological studies of the retinas of *O. pallidus* and *O. australis* indicate that the dimensions of the rhabdomes of these species are comparable in size to those of *O. vulgaris*. Muntz & Raj (1984) found a similar oversampling of the image by the retina in another cephalopod, *Nautilus pompilius*. While uncommon

(Snyder, Bossomaier & Hughes, 1986), oversampling is not unknown in other animals. It occurs, for example, in the periphery of the human retina, and in the retinas of the ghost crab *Ocypoda ceratophthalma* (Doujak, 1986) and the freshwater fish *Acerina cernua* (Sroczyński, 1981). It has been suggested that oversampling may not be related to the animal's acuity at all, but rather be an adaptation to increase sensitivity. In cephalopods it is also possible that, since the retina develops from a simple invagination of skin, the retinal mosaic may be determined by the same factors that determine the size of the epithelial cells from which the retinal receptors arose.

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