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Oil Droplet Distribution and Colour Discrimination in the Pigeon

THE retina of the pigeon has at least four kinds of cones, which can be distinguished by the oil droplets which they contain. The colours of three of these are in the visible part of the spectrum; the fourth appears colourless or slightly green[1,2]. These oil droplets are found in the paraboloid of the cones and can act as sharp cut-off filters that absorb practically all visible light below 600 (red), 550 (orange), 480 (yellow) and 430 (greenish) nm respectively. The four kinds of cones are not uniformly distributed across the retina and appear in different proportions in the four retinal zones (Table 1). Different pigeons have different proportions in the different quadrants, but their relative distribution is similar to the one shown here.

Table 1 Percentage of Oil Droplets in the Different Retinal Quadrants of One Fresh Dark Adapted Pigeon Retina

	Oil droplets			
Retinal quadrant	Red	Orange	Yellow	Greenish
Posterior dorsal	20	47	19	14
Dorsal	16	9	59	16
Posterior ventral	21	9	51	19
Anterior ventral	19	2	76	11

The data were obtained by counting about 500 droplets in different fields of each quadrant.

At present only one kind of cone photopigment has been identified in the bird retina[3]. But irrespective of further possible photopigments, the oil droplets must influence the colour space of the bird. It is therefore significant for the understanding of colour coding whether or not colour "naming" is independent of where the image falls on the retina in an animal possessing a non-uniform retina.

We therefore studied the capacity of pigeons to learn colour discriminations with one part of the retina, and then transfer this learning to other parts of it. A bird like the pigeon is particularly suited for this purpose because of its wide visual field and the relative immobility of its eyes which, although presenting bursts of high frequency tremors, have practically no wide angle movements[4]. As a result of this, a position of the image on the retina can be reasonably determined by controlling the position of the head. Rather than immobilizing the bird's head, a behavioural technique was devised so as to ensure that the stimulus image should fall on a relatively well defined retinal area for each pigeon, according to its particular pecking habits.

The experimental set-up was as follows: the pigeon was placed in an adapted Skinner box painted with non-reflecting black, which had twenty-four round windows which could be illuminated with coloured lights. The size and distance of these windows were such that they subtended the same visual angle (about 2°) to the pigeon's eye while it was pecking (behavioural "fixation"). The angular positions of these windows with respect to the animal's head during fixation are expressed in spherical polar coordinates, as adapted from Bishop *et al.*[5], where the polar axis is the vertical line which passes through the centre of the head, with azimuth (first number in parenthesis) positive to the right and negative to the left of the sagittal plane, and elevation (second number in parenthesis) positive above and negative below the horizontal plane. They are given to the next degree. Four windows were in the frontal panel (±60°, ±35°). The remaining twenty were arranged, ten in each lateral wall, half in the horizontal upper row and the other half in the horizontal lower row; their coordinates were (±100°, ±36°); (±109°, ±35°); (±131°, ±30°); (±144°, ±24°); and (±152°, ±19°).

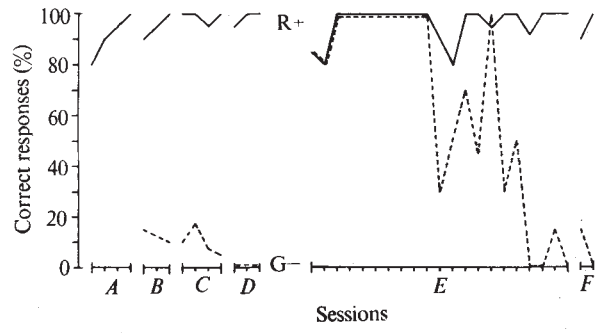

Fig. 1 Colour discrimination as % correct responses in pigeons Madame and Lorenzo for red-positive (——), and green-negative (- - -) stimuli. Session parameters are: *A*, Red-positive, frontal; *B*, discrimination red-positive-green-negative, frontal; *C*, generalization red-positive-green-negative, posterior; *D*, generalization to illumination of entire box, red-positive-green-negative; *E*, discrimination red-positive-green-negative, posterior; *F*, generalization, red-positive-green-negative, frontal. Each point on the graph represents about 15 min training.

The light sources were standard 6 W, 110 V bulbs. Their spectral composition was not analysed, but the bulbs were all of the same type and used for less than 50 h, and the voltage stability was adequately controlled. Slides with Wratten filters (92, 22, 99, 98 with transmittance maxima of 620, 570, 530 and 435 respectively) were mounted in front of each opening. A white diffusing paper eliminated vision of the lamp filament. The colour slides were interchangeable in order to avoid space cues. The intensity of the light source was varied at random by using Wratten neutral filters (0.10, 0.40 and 0.90 absorbance).

In the middle of the frontal panel, two transluscent response keys were mounted one above the other. Variable pecks on the upper key (K1) caused a coloured stimulus to appear, one at a time. The duration was controlled by an electric timer connected to a standard Grason-Stadler programming relay rack. At the beginning of the training the stimulus was on for 15 s so that the animal's response could be shaped into the task. Then, gradually, the stimulus was shortened to a flash of about 200 ms as measured by a photoelectric cell. When the discrimination proper started, only short flashes were used.

Pecks on the lower key (K2) contingent on the lightening of the adequate (positive) colour were rewarded with grains of corn. The training technique was such that the bird had to peck continuously, the upper key for "calling colours" and the lower one for food. Four birds were trained initially to coloured stimuli in the frontal panel (azimuth ±60° and then tested in the lateral (azimuths ±100° to ±131°) and more posterior portion of the panel (azimuth ±152°). The other two were trained to discriminate colours appearing in the most posterior field, which required about 4 h, as compared with 30 min for the

first group. In both cases, however, generalization to other areas was immediate and perfect (90–100%). A systematic observation was that the pigeons never turned their heads during the short flashes posterior test presentations, but rather gave appropriate response (peck K2) immediately. This further assured us that behavioural fixation was occurring. All trials were run on extinction (unreinforced). In the case of the pigeon called Madame a final test was done, in which on pecking K1, the entire box became illuminated red, green, blue and white from a general coloured light source. The bird responded perfectly by pecking K2 only when everything turned red. Training conditions for each bird are summarized in Table 2. Fig. 1 illustrates the percentage of correct responses for one bird, which can be considered as typical.

Table 2 Original Discrimination Training and Generalization Tests for each Pigeon

Pigeons	Stimuli Positive	Stimuli Negative	Training	Areas Generalization
Madame	Red	Green	Frontal	Posterior and general illumination
Blanca	Green	Blue	Frontal	Posterior and lateral
Molly	Orange	Green	Frontal	Posterior
Suzy	Green	Red	Frontal	Posterior and lateral
Lorenzo	Red	Green	Posterior	Frontal
Cresta	Orange	Blue	Posterior	Frontal and lateral

These results show that the pigeon which had learned to recognize certain colours when its image fell on a retinal area of a particular receptor composition had no difficulty to treat it in the same way when the test image was made to fall in another retinal area of different receptor composition.

The following can be concluded. (1) Pigeons can make colour discriminations in every portion of their visual field, independent of the size and relative brightness of the stimulus. (2) A colour discrimination learned in one part of the visual field can be immediately generalized to another part of the visual field.

In synthesis, although cones are found in different proportions in different parts of the retina, the mechanism of colour recognition is independent of these proportions; a colour discrimination learned with the image falling on one part of the retina can also be done when the image falls on other parts of the retina. Thus, whichever operational constancy makes the pigeon treat as equivalent these different situations, it is certainly independent of the actual proportions of the different kinds of cones which can be excited at any part of the retina.

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Function of DNA Polymerase III in DNA Replication

RECENTLY an *in vitro* system for DNA replication has been described. This system could be divided into two fractions (A and B) both of which are necessary for proper DNA replication[1]. Fraction A, the "soluble" fraction, contains those proteins which do not tightly bind to membranes or native DNA. Fraction B, the "insoluble" fraction, consists of DNA and membranous structures and proteins which are bound to either of them. It was shown that the soluble fraction contains at least one component which is needed at about *in vivo* concentration[1]. Studies of one such component are described in the following.

We have isolated *E. coli* mutants which are temperature sensitive with respect to *in vivo* DNA replication (unpublished work). With some of these mutants the *in vitro* replication is also temperature sensitive. The mutants fall into two groups: mutants which contain a temperature sensitive soluble fraction (A) and mutants which are defective in the insoluble fraction (B). This was determined by testing whether lysates of mutants could be complemented by wild type fraction A. Further complementation tests showed that four mutants defective in the soluble fraction (1026, 1040, 1061, 1126) are unable to complement each other; they belong to one complementation group. All of them map at the *dnaE* locus (J. A. Wechsler, personal communication).

Table 1 Properties of Purified Polymerase III

Test	Polymerase III assayed in	KCl concentration (M)	Incorporation stimulated (pmol. TMP/10 min)
1	Low concentration (test tube)	0.2	<0.1
		0	2.0
2	High concentration (on 'Cellophane')	0.2	13
		0	12
3	Complementation test (with *dnaE* lysate)	0.2	9

5 μl. of an enzyme preparation containing 1 mg protein/ml. was used for all tests. The buffer contained Tris-HCl 20 mM, $MgCl_2$ 5 mM, EDTA 0.1 mM, ATP 1 mM, all four dXTPs 20 μM at *p*H 7.8. Exonuclease III treated calf thymus DNA (3 μg) served as template in tests 1 and 2. Assay 1 was carried out in 0.1 ml. Assays 2 and 3 were performed corresponding to the method described earlier[1].

The *dnaE* gene product has been purified by standard biochemical purification techniques. The complementation test served as assay for its activity. The complementing activity which specifically stimulates DNA replication in *dnaE* mutant lysates at non-permissive temperature is a labile protein of molecular weight about 150,000 as judged by gel filtration on agarose. After about 1,000-fold purification it is free of polymerase II, endonuclease, and ATPase activity. Recently Gefter *et al.*[2] showed that the third and smallest DNA polymerase activity detected in *E. coli*[3] is temperature sensitive when isolated from the complementable strains *dna* 1026 and *dna* 1040. This implies that the *dnaE* gene product is polymerase III. It is confirmed by our finding that wild type *dnaE* gene product contains a DNA polymerase activity with properties similar to polymerase III and certainly different from polymerases I and II.

Polymerase III activity has been reported to be maximal at low ionic strength (≤0.05 M KCl)[3]. In assay conditions at physiological salt concentration, the activity is about 20-fold lower than the maximal value. This property seems at first sight puzzling unless one assumes that polymerase III acts *in vivo* in a nonionic environment; for example, in the bacterial membrane.