Avian vision

Diane Thomson

Abstract

This thesis is in two parts.

The first part is a literature review of the avian retina. The review deals with issues in the literature concerning each type or layer of cells in the retina, as well as problems involving synaptical interactions in the retina. The chapter on oil droplets discusses problems in photopigments and avian colour vision as well. Centrifugal fibres and their possible functions are discussed. The avian pecten and hypotheses about its function are dealt with. Finally the functional and evolutionary significance of the avian retina with regard to the avian visual system as a whole is discussed.

The second part concerns experiments on colour preference in the pigeon, with regard to the effects of dark adaptation and intensity of the stimulus. Subjects were rewarded for pecking either a blue or a red key presented simultaneously in various combinations of intensities and under three conditions of light or dark adaptation. It was found that the intensity of the blue stimulus affected the number of pecks to blue in a monotonically increasing function. There was also an interaction between intensity of the blue stimulus and the effects of dark adaptation. The results are discussed in light of motivational factors and rod-cone interactions.

AVIAN VISION

PART I: THE AVIAN RETINA

PART II: MOTIVATION AND VISUAL MECHANISMS:

COLOUR PREFERENCE IN THE PIGEON, THE EFFECTS OF DARK ADAPTATION AND THE INTENSITY OF THE STIMULUS

DIANE THOMSON

M.SC. THESIS

UNIVERSITY OF DURHAM

APRIL 9, 1972

Preface

This thesis is presented in two parts. The first is a review of the literature on the avian retina and the second concerns experimental work done on the effects of dark adaptation and stimulus intensity on colour preference in the pigeon. The two sections represent samples of approaches to the study of avian vision, the first primarily physiological and the second behavioural in nature. They are by no means exclusive, either mutually or of any other approach to the study of animal behaviour. The present attempt is, rather, to bring in as many disciplines as possible to aid in uncovering the mysteries of avian vision, but always with the bias of a background in psychology.

There are a great many people to whom I am indebted.

The great fund of knowledge of my supervisor, Dr. J.D. Delius has been a constant source of inspiration. I owe many ideas to Dr. D. Parker. Mrs. K. Bennetto's criticism was invaluable. And the technical assistance of Mr. D. Harper was vital.

I would also like to thank Dr. B.J. Frost for his encouragement and patience, Dr. D. Mewhort for his advice and programs in statistics and Miss E. Cole and Mrs. E. Thomson for their typing.

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PART ONE: THE AVIAN RETINA

Chapter I

Introduction

The retina, a light-sensitive extension of the brain is a layer of photoreceptors and their connections to the brain and to each other which lies at the back of the eye behind the vitreous, the lens and the pupil. In pigeons the retina is twice as thick as that of man, but the pigeon brain possesses no real cortex. Visual information is of prime importance to the pigeon, as it is to humans, but it is processed at the level of the retina to a greater extent in birds than it is in mammals. (Maturana, 1962).

The features of the avian eye contained in, and adjacent to the retina which can be observed by ophthalmoscopy are a) concavity or general chorio-retinal surface of the posterior half of the eye, b) the pecten, c) the optic nerve entrance, d) the areas of acute vision, or the maculae, e) the fundal blood vessels, and, f) opaque nerve fibres (Wood, 1917). In the pigeon retina the fovea centralis is slightly ventral to a line dividing the eye in half, two millimetres above the pecten which extends about one millimetre temporal and dorsal to the fovea. The area lateralis or "red field" (so-called because of its red appearance lent by the concentration of red oil droplets in the cones in the area) is another specialized area located in and almost

entirely constituting the dorsal posterior quadrant of the retina. It is not sure whether or not this area contains a true fovea, a smaller centre of higher photoreceptor concentration (Galifret, 1968) than in the rest of the retina. A view of the pigeon retina with cornea lens and vitreous removed from the eye is shown schematically in Fig. 1. (All figures referred to in Part One are found in Appendix A).

The layers of the avian retina as shown in Fig. 2 are neatly stratified. The outermost layer, the pigment epithelium and choroid, is a heavily pigmented layer both relatively and absolutely thicker in the avian retina than in the human retina (Walls, 1942), (perhaps because the bird retina depends more on the choroid for its nutrition), with ragged expansions of each cell near its nucleus, containing granules and needles of pigment, extending as far as the inner segments of the visual cells. In birds, the pigment called fuscin (Walls, 1942) of the epithelial cells changes position according to the intensity of illumination. With stronger illumination the pigment in the cell expansions moves closer to the external limiting membrane reducing the lateral diffusion of light while in dim light the pigment moves into the cell bodies, exposing the photoreceptors to more light than when the pigment was in the cell expansions. It is the movement of the pigment itself and not a change in the shape of the cells which is involved in this pigment

migration (Nishida, 1963). The scleral ends of the visual cells meet with the choroid in what Polyak (1955) defines as the second or basillary layer of the retina. This layer contains the outer and inner segments of the rods and cones as well as the intermediate zone of each, all three of which appear to play a part in photoreception which will be discussed more fully in Chapter 3. Of the visual cells the rod is often the more elongated, more sensitive to lower intensities of light, than is the shorter cone whose sensitivity is greater in daytime illumination. In birds the cones contain coloured oil droplets located between the inner and outer segments of the cone which are possibly responsible for colour vision (as discussed in Chapters III and IV). Between the photosensitive segments and the nuclei of the receptors lies the outer limiting membrane which is produced by the joining of the top ends of the radial fibres of Müller and which keeps the rods and cones in place. In the outer nuclear layer consisting of the closely packed bodies of receptor cells which contain the nuclei of the rods and cones, the nuclei of the cones are usually in a more outward location than those of the rods. The fibres of the rods and cones extend through the outer plexiform layer to meet the horizontal cells and the bipolar cells which continue to a layer of Müller cell nuclei where they meet the amacrines, all in the inner nuclear layer.

The amacrine fibres pass thence through the inner plexiform layer to meet the brain cell-like ganglion cells whose fibres extend into the optic nerve through the inner limiting membrane, part of the retinal supporting or neuroglial tissue bounding the retina at the vitreous.

Horizontal, bipolar, amacrine and ganglion cells possess connections across the retina in their own layer as well as to others (Polyak, 1955). All three nuclear layers and the inner plexiform layer are thickened in the avian retina, thereby making the entire retina thicker than in man (Walls, 1942). Most avian retinae are one and a half to two times as thick as in man. The thickness of the avian retina as compared with that of the human retina is .16 mm in the avian retina as compared with .09 mm in the human retina at the ora serrata, .24 mm in the avian compared with .10 mm in the human retina at the bottom of the fovea and .31 mm in the avian compared with .14 mm in the human retina between the fovea and the ora serrata (Chard and Gundlach, 1938). In the bird, bipolars, amacrines and horizontal cells all outnumber those in man (Oehme, 1964). A clue to the functional significance of the thickened retina in the bird may lie in the complexity of response given by single cells in the various layers to various visual stimuli (Maturana, 1962), the equivalents of which take place higher in the nervous system of mammals. These single cell responses will

be discussed more fully in Chapter VII.

There are several types of maculae and fovea found in birds. The fundi of birds can be divided into six different types as Wood (1914) divided them according to the type of macular region they possess. The first is an amacular fundus which appears upon ophthalmological examination to possess no macula or fovea at all as for example does the fundus of the California Valley Quail. (The six types of fundi are illustrated in Fig. 3.) Secondly the nasal monomacular fundus possesses a single macular region containing a smaller fovea in the nasal region of the fundus as does the Stellar Jay, and is found in the majority of birds. The temporal monomacular fundus, the third type, which is found mostly in owls has a macula with a smaller fovea in the temporal region of the fundus. Fourthly, the bimacular fundus with a deeply marked principal nasal region and a subsidiary temporal area as in the Belted Kingfisher can be divided into two types. In eyes of regular shape the nasal macula is well developed with a deep and sharply defined fovea as in Alcedo, Sterna and Terchyimeta. In eyes of irregular shape the temporal fovea is deeper with a better marked macular retion, as exhibited especially in Hawks. The fifth kind of fundus Wood labels the infulamacular fundus because of the band-like central area associated with a well defined fovea or macula as in the Greater Yellow Legs. The

infula-bimacular fundus, the sixth type exhibits two macular regions joined to or associated with the band-like area as in the Common Flamingo. This last type of fundus can also be divided further into two different types of fundi. The Sparrow Hawk, for example has a well defined nasal macula with a deep fovea situated near the centre of the retina and a shallow temporal macula joined by a short ribbon-like area not extending beyond either macula, while in the Tern the band encloses a nasal macula and there is another above and apart from these other areas.

In most of these types of retinae the central or more nasal macula possesses a well defined, steep fovea, while the temporal macula is less well defined, more shallow and flat and sometimes without a fovea. The temporal area is used for binocular vision, while the steeper nasal area is used for monocular vision. The shape of the depression in the fovea or macula (steep or shallow) is important functionally. It can be assumed that the shallow foveal area is useful for binocular, short range vision while the steep foveal area is useful for monocular long range vision since first of all the location in the retina of these two types of areas dictates their use; that is, the shallow area is located in the temporal region of the retina where it "looks" straight ahead, at the same place as the temporal area of the other eye does while the steep area is usually

in the centre of the retina, located to receive stimulation from the side of the bird's head, and not possibly from the same place as the analagous area in the other eye. The shallow area is better suited to binocular vision also because light does not have to be refracted through other layers of the retina to reach the photoreceptors as it does in the steep area (see Fig. 4) and therefore the image received by the photoreceptors in a shallow area is more clearly defined than it is in a steep area. However the refraction is somewhat compensated for in the steep fovea by the distribution of the photoreceptors in a ridge around the deepest part of the foveal depression.

The habits of an avian species are dependant upon its visual apparatus and are therefore dependant upon the type and number of maculae it possesses. Pumphrey (1948) noticed that an area centralis was absent in many grain feeding birds, which would use short-range binocular vision for feeding. The area dorsalis of the pigeon, called the red field because of the high concentration of red oil droplets in the cones in the area is useful for the pigeon's grain feeding habits while its central area is useful for more distant vision while flying. Hawks also possess two maculae, but the central one is better developed and steeper as Wood pointed out in his classifications of fundi, enabling them to detect small objects on the ground while flying at a

great height. Sea birds typically possess a ribbon-like area in their retina which seems suited to fixation of the horizon and to a preferential increase in sensitivity to vertical movements of objects in relation to the horizon as Lockie (1952) suggested. In general, then the shape of the macula or fovea is a fairly obvious indicator of an avian species' mode of life. The round, steep central fovea is useful for spotting small far-away objects, the round shallow temporal area for seeing small objects at close range and the horizontal area for seeing things on the horizon.

The visual cells of the pigeon seem to be distributed in such a way as to take full advantage of foveal refraction. Although the density of photoreceptors in the pigeon has never actually been determined densities in the outer nuclear layer average around 85,000 cells per square millimeter, approaching 92,000 close to the fovea and probably in the area dorsalis (Galifret, 1968). Visual cell counts however may be higher than this in the fovea since the cells other than visual are pulled away from the fovea and stand in a thickening of their layer around it (as can be seen in Fig. 3) Galifret (1966) quotes a count of 400,000 cells per square millimeter in the optic axis in the pigeon. The fundus of Motacilla, another diurnal bird contains 120,000 cones per square millimeter (Hamilton, 1961).

The ratio of cones to rods is higher in diurnal birds

than in nocturnal. The buzzard (diurnal) parafovea is two thirds cones and the kestrel (also diurnal) is four fifths cones (Oehme, 1964). The ratio in pigeons is not known.

In birds as in other species, the summation of the rods in the bipolars is greater than that of the cones (Pumphrey, 1948). In the rod system as defined by Polyak (1955) rods are related synaptically to all diffuse varieties of centripetal bipolars but not to midget bipolars or to cones. Only some brush and flat-top bipolars are part of the rod system. The arrangement of the receptor and conductor units in the rod system is topical or spatial with a fair preservation of topographical relations present in the bacillary rod layer, but because of the large dendritic expansions of diffuse bipolars, reciprocal overlapping and summation in the bipolars the physiological units of the rod system are larger and the grain is less fine than in the cone system. The rod system is inhibited by the cones, except in dim illumination, below the cones' threshold.

In the cone system as defined by Polyak (1955) the cones are related synaptically to all varieties of centripetal bipolars, but principally to midget bipolars. In the complete cone system found in the peripheral retina there is summation of the cones in the bipolars but still a finer grain than in the rod system, while the monosynaptical cone system, found in the fovea possesses "direct lines" to the

brain with one cone related to one midget bipolar and thus has a finer grain still than the complete cone system. In terms of receptive fields then, cells in the rod system, specifically here, bipolar and ganglion cells in the rod system should have larger receptive fields than do cells in the cone system at least partly because of the greater summation in the rod system. Sizes of receptive fields in the cone system or the rod system have never been shown to differ, because the cells which determine the receptive field of another higher cell have never definitely been determined. However, Gouras (1965) found ganglion cells in the primate retina which showed a purkinje shift, demonstrating that both rods and cones were affecting the response of the same ganglion cell. It may be, then, that there are not separate pathways to the brain for rods and for cones. At least in primates it has been demonstrated that there is overlap in rod and cone systems. This does not exclude the possibility that some photoreceptors may possess their own "private line" to higher centres. However, in view of the amount of processing known to occur in the avian retina, the possibility of little or no overlap between rod-bipolar-ganglion cell and cone-bipolar-ganglion cell systems seems highly unlikely.

Receptive fields in higher cells of the retina, such as ganglion cells are not, however, simply determined by vertical interactions. Amacrine and horizontal cells also

play a major part in determining their responses. The interactions taking place higher in the retina will be discussed at some length in Chapter VIII.

The avian retina appears, as does the mammalian retina, to grow primarily from the inside out, that is centrifugally from the brain. In the developing retina of the chick (Coulombre, 1955) the first critical period occurs on the fourth to the fifth day marked by active cell proliferation. At this time the onset of increase in retinal area and thickness, the appearance of axons from the ganglion cells and the formation of the inner limiting membrane by the Müller fibres occur, accompanied by a marked decrease in mitotic activity and the appearance of melanin granules in the pigment epithelium. The second period on the eighth to the tenth day sees the appearance of the inner plexiform layer with a second rise in apyrase activity, a cessation of mitotis in the pigment epithelium and its confinement to the margins of the neural retina. The processes of the ganglion and amacrine cells arborize horizontally in the inner plexiform layer, ganglion cells begin to be reorganized into a layer one cell in thickness and the inner segments of the rods and cones begin to appear. During the third period at fifteen days the oil droplets of the cones begin to show colour and increase in diameter at the same time as astaxanthin, a carotenoid present in the oil droplets, is first

detectable. There is a third peak in apyrase activity. The accumulation of astaxanthin is correlated with an increase in the diameter of the red oil droplets as the outer segments of the rods and cones can be seen. The processes of the cells of the pigment epithelium appear and mitosis ceases in the neural retina. The area, however, continues to increase because of a decrease in cellular strata, the separation of carotenoids in the cones, the separation of the pigment epithelial cells and increases in cell size. Between the thirtieth and forty-fifth day of life the pigment epithelium is still developing in the chick retina, its endoplasmic reticulum changing from vesicular to network-like (Nishida, 1964).

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Chapter II

The Pecten

The avian pecten, looking somewhat like a radiator, composed mainly of blood vessels, projects into the vitreous of the eye in the ventral half, from the head of the optic nerve with which its base roughly coincides. Two types of avian pecten are shown in Fig. 5. Its framework is composed of neuroglial cells of optic cup origin. It is pigmented most heavily in the part that projects into the vitreous. It is not an extension of the choroid whose function is also supposed to be retinal nutrition, as has been argued in the past (Thompson, 1929), but has its own separate vascular supply (Wingstrand and Munk, 1965).

There are two types of avian pecten, the first belonging to paleognathous birds (primitive and flightless) which has a central vertical panel buttressed on either side and end by lateral vanes and the other to neognathous birds which is like an undulant pleated fin. There are many variations of the avian pecten. For example, the kiwi pecten is really a conus papillaris, like that of reptiles, without vanes or pleats. But rather than being primitive it may be degenerate since the kiwi's eye, too small for its socket appears to be degenerate (Walls, 1942). The pecten varies from species to species with respect to the basal area,

freedom from the nerve head, number of folds, closeness of approach to the ventral ciliary body and to the ventral periphery of the lens. Its location is always such that the long axis of the base of the structure is always directed along the former course of the embryonic fissure of the optic cup. It conforms to the fissure as does the head of the optic nerve since it develops from the head of the nerve.

The function of the pecten has been a much debated topic. Of the several possibilities two seem the most likely; that of the nutrition of the retinal visual cells and that of movement detection. Menner (1938) noticed that the size of the pecten seemed related to the eating habits of the different species of birds, it being smallest in nocturnal birds and increasing in size through the seminivorous to insectivorous to diurnal predators who most need detection of movement. The pecten, he said, as others have (Crozier and Wolf, 1943; Pumphrey, 1948) casts fingerlike shadows of its pleats on the retina, each shadow creating a blind spot. The repeated on-off effects of these shadows give movement "greater saliency in consciousness" as Menner put it. Crozier and Wolf (1943) projected a pecten-like shadow onto a human retina and found that the flicker acuity for small dark times was enhanced. They hypothesized a changing integration of rod and cone effects as the light time fraction was altered. Walls (1942) argued that the size of the pecten is not great

enough to cast a shadow on the retina, but the previously stated evidence would seem to indicate otherwise.

One could determine whether a bird is using its pecten as a visual aid in some way by observing the head movements of the bird. If the bird moves its head into such a position that the shadow of the pecten is across the area of the retina which is receiving visual stimulation then one could reasonably assume the shadow of the pecten is in some way altering the bird's perception of the stimulus. It may be found, for example, that a moving stimulus would evoke a head position in which the shadow of the pecten is used whereas a non-moving stimulus would not. On the other hand, if the bird were using the shadow of the pecten in order to mask out the too-bright image of the sun one would expect a very bright spot to elicit a head movement such that the pecten would be brought into a position which masks the spot of light.

It would be possible to measure head movements, as suggested to the author by Dr. B.J. Frost by projecting a spot of light from a fixed position on the bird's head onto a translucent screen which would be photographed during a long exposure time. The brightest spots on the photograph would indicate the most frequent positions of the bird's head.

The pecten, however, may have more than one function. The degree of its development is apparently correlated with the dominance of photopic vision and with the use of monocular vision. Walls (1942) has noted that the size of the pecten and the number of its pleats reflect the metabolism of the cones, that is, it is largest in diurnal active birds and smallest in nocturnal sluggish birds. According to Walls' argument if the choroid of a particular eye cannot supply a cone-rich visual cell population in an extensive retina we may expect some additional nutritive device, advantageously situated to supply the inner reaches of the retinal tissue. The ciliary processes are too far anterior to the main mass of the retina and their secretion passes too largely and too directly to the anterior chamber and is too promptly drained from there. The pecten is the logical answer to the necessity of a supplementary nutritive device.

The size of the pecten in different birds has been determined by Kijikawa (1923), Franz (1911) and others (Wood, 1917) but one needs to know the number of folds as well as the size, the total blood vessel area, the blood capacity and rate of flow, the area and volume of the retina and the rate of oxygen and glucose consumption in order to obtain ratios for inter-species comparisons. In the majority of birds the length of the base is one half the horizontal diameter of the eye and the number of folds is usually 14-17.

(Garrulus glandarius has 30). Most of the ground feeding birds, gallinaceous and perching birds feed upon small objects and have a high capacity for resolution and accomodation in proportion to the size of their eyes. Greater ranges of accomodation are found in the largest eyed birds. Predaceous birds, like hawks and eagles have fewer and coarser folds in their pectens. Owls and swifts accomodate little and have a lower number of folds. Rabl (1900) found that the number of pecten folds correlates with the relative size of the ring-wolst which is involved in accomodation. It would then appear that the pecten plays a part in the sauropsidian accomodation mechanism, but Walls argues that the correlation is a result of the retinal metabolic rate since accomodation goes with diurnality, high visual acuity and activity as does the pecten size and number of folds. In the nocturnal birds the base of the pecten is less than half the eyeball diameter. Birds which are relatively inactive or nocturnal such as the cassowary, Apteryx, owls, European goatsucker, Cereopsis and owl parrots have pectens with relatively few folds or none, while active day birds such as the ostrich, rhea and parrot have a greater number of folds in their pecten (Walls, 1942). Wagner in 1837 had noticed this correlation of pecten size and number of pleats with diurnality even before the duplicity theory had entered the scene.

Cutting off the blood supply to the pecten results

in almost complete anoxia in the corpus vitreum and in the inner retinal layers (Wingstrand and Munk, 1965). Degeneration, especially in the ganglion cells and the optic nerve, then also occurs in the retina. The anoxia and degeneration would seem to provide evidence for an oxygen supplying role of the pecten to the inner layers of the retina. Wingstrand and Munk (1965) also observed that oxygen pressure was greater near the pecten than near the retina allowing diffusion of oxygen through the vitreous in amounts large enough to be functionally significant. The pecten, then, appears to replace intra-retinal blood vessels like those found in the mammalian retina, as an oxygen supply for retinal cells.

There is little evidence for other early theories of the pecten's function. It is not likely to prevent monocular diplopia during binocular vision by suppressing the binocular field, nor to act as a dark mirror for a too bright image or as a proprioceptive sense organ for regulation of accomodation or a heat radiator or to smooth out intra-ocular blood pulsations. These fascinating propositions are dealt with at more length by Walls (1942) and by Mann (1924).

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Chapter III

Visual Cells

The most recent complete description of avian visual cells has been made of the chicken's receptors using phase contrast microscopy by Meyer and Cooper (1968). The visual cell layer in the chicken retina constitutes one third of the retinal thickness. In this layer three types of cells were found: single rods, single cones and double cones as shown in Figure 6. Undoubtedly many of the cones in the chicken retina are double, as Rabinovitch and his co-experimenters (1954) thought that all the cones in the chick retina were double. All three types of visual cells are present in other avian species. (Walls, 1942).

The rods have a uniformly thick outer segment, theirs being thicker than the outer segment of the cones (Morris and Shorey, 1967), the distal extremities of which are embedded in the cytoplasm of the pigment epithelium. The inner and outer segments are approximately the same length with their junction in the middle of the cell layer. In the inner segment of the rod is the hyperboloid of Krause (1894) an elongated conical-shaped dark body proximal to the ellipsoid. It is smooth in texture, surrounded by the cytoplasm of the inner segment and contains glycogen. No oil droplets have been observed in the rods.

The single cones are the shortest receptors of the chicken retina. Morris and Shorey (1967) found two types of these in the chick retina but Morris (1970) later found a third type of single cone. One type of single cone has a dark oil droplet and the mitochondria in the ellipsoid are dense with cristae. The second type of single cone has a lighter oil droplet and fewer cristae. The third type has an oil droplet still less dense than either of the other two. In all three types their conical outer segments take up one third of the entire length of the receptors. The inner segment has a single circular oil droplet which occupies almost the entire thickness of the distal end of the receptor but is surrounded by cytoplasm. The ellipsoid, granular with mitochondria, occupies the distal third of the inner segment. In a very few cells the continuation of the ellipsoid to a rectangular body is separated by the oil droplet which usually is distal to both portions of the ellipsoid.

The double cones having two unequal but independant components with separate nuclei, have a tall thin chief cone and a broad accessory cone in close proximity to each other along most of their inner segments. The outer segment of the accessory cone is very slender and reaches almost to the pigment epithelium while its inner segment is very broad with an ellipsoid, a paraboloid and a myoid. Within the distal apex of the ellipsoid Meyer and Cooper (1968) found

a small oil droplet in close proximity to the oil droplet of the chief component. Under electron microscopy (Morris and Shorey, 1967), however this structure appeared to be a small granular vesicle rather than an oil droplet.

The chief component of the double cone is the tallest of the cones. Its outer segment is like that of the single cone but it is one quarter of the length of the cell. The inner segment has a prominent circular oil droplet as well as the usual ellipsoid, myoid and paraboloid. It is difficult to observe double cones unless they have been sectioned in the same plane as that in which they lie side by side. That plane is subject to variation from pair to pair of cones. Their functional significance is not entirely known, although it is thought that the pedicles from the two cells may terminate in different levels of the outer plexiform layer. Also the two inner segments may be associated for metabolic economy or in order to bring the outer segments closer together for greater acuity. These ideas are discussed in Kalberer and Pedler's article on alligator receptors (1963).

Flattened saccules have been observed in the outer segment of the receptors of several avian (Cohen, 1962; Wada, 1965; Morris, 1970) and other non-avian (Kalberer and Pedler, 1963; Laties and Liebman, 1970) species. These saccules are continuous with the cell membrane along the outer segment length in cones but only at the base in rods in pigeons

(Cohen, 1962). They appear to be groups of double lamellae 100 Å thick and 50-70 Å apart arranged in parallel rows perpendicular to the cell wall (Wada, 1965). In amphibians it has been guessed that cone saccules are open to extra cellular space whereas rod saccules are not since a chlortrizinyl dye, Procion Yellow, which does not cross nerve cell membrane, after being injected into extracellular space also appeared inside the cone outer segments under election microscopy (Laties and Liebman, 1970).

Young (1971) noticed that saccule formation continues throughout life in rods but not in cones and that the outer segments of developing rods in amphibia are cone shaped. He therefore formulated an hypothesis to account for the distinction between rods and cones based on saccule formation characteristics of each. While the replacement of the first small saccules by larger ones continues in rods until the saccule diameter becomes stabilized thereby producing a cylindrical outer segment, in cones saccule formation is arrested at the stage where small older saccules have been displaced by larger ones just enough to form a conical outer segment. (See Fig. 7.)

Some cones in pigeons have been found to contain three independant columns of saccules in their outer segments (Cohen, 1962). These varieties could not be correlated with single or double cone varieties based on inner segment

characteristics. The three separate columns of saccules within one cell may contribute to the sensitivity of the eye allowing three separate functions in one cell. To test the possibility of separate functions in the three columns one would have to take separate electrical recordings from each column or determine chemical differences between the columns. The methodological problems involved in either approach would be enormous, and needless to say their discussion in detail is beyond the scope of this thesis.

Most avian retinae contain both rods and cones. In nocturnal species the two types of receptors are not well differentiated on the basis of characteristics observable through light microscopy while in diurnal birds they are, with differing foot pieces (Walls, 1942). The number of double cones is higher in diurnal birds and ratio of cones to rods is higher in diurnal birds than in nocturnal. Generally, however, even the fovea of most birds contains at least some rods. The ratio of rods to cones was guessed to be higher in the pigeon fovea than in the human fovea (Waelchli, 1883) perhaps indicating a retina adapted for both daytime and some night-time vision.

Receptor density has been determined for a few species of birds. In the fovea of the house sparrow (Passer donesticus) there are 400,000 cones per square millimeter, while the hawk (Buteo buteo) has 1,000,000 cones per square millimeter in

the fovea (Rochon-Duvigneaud, 1943). Receptor densities in the pigeon may be guessed at through counts of cell bodies in the outer nuclear layer by Galifret (1968). The number of cells near the fovea is about 92,000 per square millimeter and an overall average is about 85,000 per square millimeter. However, the pulling away of the receptor nuclei near the foveal depression makes extrapolation from one layer to another difficult and the outer segments are probably far more densely packed than the cell bodies of the receptors in the central fovea. Galifret (1966) had earlier quoted a count of 400,000 cones per square millimeter in the pigeon fovea. The starling (Oehme, 1961) has 480,000 receptors per square millimeter in its fovea with ten times as many cones as rods while it has 260,000 receptors per square millimeter in the periphery with 7.5 times as many cones as rods. Owls (Oehme, 1961) have from 425,000 to 300,000 receptors per square millimeter in the fovea and from 200,000 to 35,000 receptors per square millimeter in the periphery with 1.3 times as many cones as rods in the fovea and the same number of cones as rods in the periphery in the Asioflamen. For other species of owls Oehme (1961) found from .7 to .5 times as many cones as rods in the fovea and from .6 to .4 times as many cones as rods in the periphery.

The overall receptor density is a good indication of a species' powers of resolution, or acuity. The hawk, for

example, commonly noted for its great visual acuity has vast numbers of receptors in its fovea as noted above. On the other hand, the proportions of types of receptors in the retina indicate whether a species is diurnal or nocturnal. The nocturnal owls have a smaller proportion of cones in all areas of their retinae than does the diurnal starling as can be seen from figures provided by Oehme (1961) above.

A visible pattern of four double cones in a square with a single cone in the centre has been found in the avian retina (Engstrom, 1958). A similar pattern is found in fish, sometimes with odd single cones among the four single with one double-pattern. Electrophysiological work on teleosts (Svaetichin, 1956) suggests that the centre cone is generally light sensitive with the two double cones opposite representing the same complementary colour pair. This type of cone pattern is present in the Great Tit (Engstrom, 1958) while in fish the pattern groups may overlap. There are variations in other avian species as well.

Morris (1970) found by statistical analysis that the types of receptors in the chick retina were evenly spaced in the receptor mosaic except for one type of single cone which tended to occur in pairs. The types of receptor were arranged in a pattern which was in the form of a hexagonal lattice, (see Figure 8). The pattern in the mosaic was regarded as the outcome of an evenly spaced distribution of the types of

receptor which may provide an interpretation for patterns found in the distribution of receptors in other species as well. Whether the functional significance of these patterns is anything more than even distribution, making the retina functionally uniform within a given area, has yet to be determined.

In the duplex avian retina it is almost certain that the visual pigment contained in the rods is universally rhodopsin. In the pigeon rhodopsin was extracted from the retina first by Hess in 1923. Scotopic spectral sensitivity curves of the pigeon, found behaviourally (Blough, 1957) and electrophysiologically (Donner, 1953; Graham et al, 1935; and Ikeda, 1965) and in the chicken (Armington and Thiede, 1956: Armington and Crampton, 1958) agree with the spectral absorption curve for rhodopsin.

Iodopsin, first discovered by Wald in 1937, appears to play a part in cone vision in the chicken retina. The photopic spectral sensitivity curve of the chicken determined electrophysiologically (Armington and Thiede, 1956) agrees with the absorption spectrum of iodopsin. A behaviourally determined photopic threshold curve for the crow (Orlov, 1961) also agrees with the absorption curve of iodopsin. In the pigeon, however, agreement has been found between photopic spectral sensitivity and what Bridges (1962) calls visual pigment 544 because of its extinction spectrum maximum.

There is also evidence in Bridges' results for the influence of rhodopsin in pigeon cone vision. He found that 85% of the pigment extracted from the cone-dominated pigeon retina was rhodopsin. Rhodopsin has also been extracted from the pure cone retina of the squirrel (Dartnall, 1960), indicating again the possibility of rhodopsin as a cone pigment.

In several avian species cone pigments other than iodopsin have been found or there has been a failure to find iodopsin (Dartnall, 1969; Arden and Tansley, 1955; Dowling, 1964; Sillman, 1969). The precise chemical nature of various cone pigments has not been determined but work in this area has been attempted. For example, noting the existence in nature of two kinds of rod substance which are sometimes found combined in one retina Wald, Brown and Smith (1953) analagously combined chicken "photopsin" (that which is presumed to be the cone pigment; in this case iodopsin) with $retinene_2$ to form a substance that they call cyanopsin. It was later found that the photopic sensitivity is shifted towards the longer wavelengths, that is towards the maximum absorption expected from cyanopsin, in provitamin A_2 fed chicks (Auerbach, Rowe and Budowski, 1966) indicating the possibility of its existence in nature since presumably feeding the chicks provitamin A_2 produces $retinene_2$ in their cones. A pigment with the absorption maximum expected from cyanopsin was later found in the tadpole retina (Morton and Pitt, 1969).

Sillman (1969) extracted pigments from the retinae of several species of birds, fourteen of which had one pigment with an absorption maximum between 500 and 506 nm. From five species he extracted two pigments. The predominant pigment in these species had an absorption maximum near 502 nm. while the second pigment's absorption maximum was found to be between 480 and 490 nm. All of these pigments have absorption maxima close to that of rhodopsin and therefore presumably are similar to rhodopsin. In none of the species he examined did he find iodopsin or anything resembling it or any other cone pigment. His results are definitely in conflict with those mentioned earlier who found cone pigments. It is obvious that further analysis, chemical or otherwise, is necessary of visual pigments. If it were found to be true that there are no cone pigments in the avian retina one might consider the possibility that oil droplets are the primary vehicles of photoreception in the cones.

It is important to question not only the work done on the chemistry of the photoreceptors to date but also that on their morphology. Electron microscopy and phase contrast microscopy although a far cry from the earlier light microscopical work, are not unquestionably lucid. Much of the evidence they provide still needs interpretation which is subject to the individual training and bias of the observer. This sort of work already done is magnificent in comparison

with what was done before, but it must be verified by other observers before we can accept it with maximum confidence.

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Chapter IV

Oil Droplets

Oil droplets, a feature of the sauropsidian retina including the avian retina, were discovered over 120 years ago, perhaps by Hannover (1840) or perhaps by Valentin who is credited with their discovery by Walls and Judd (1933). Electron microscopical evidence shows oil droplets in the avian retina within the distal apex of the inner segment of cones in close association with the mitochondria which constitute the bulk of the ellipsoid (Cohen, 1963; Craig et. al., 1963; Maysuska, 1961; Okuda, 1962; Takayama, 1961; Villegas, 1960; Yasazuma, et. al., 1958). They may arise during phylogeny from the bulging of the primitive inner segment (Ueno, 1961). There exist red, orange, yellow, yellowish-green and colourless oil droplets in various distributions in various avian retinae.

Oil droplets consist of unsaturated lipids (Sidman and Wislockie, 1954; O'Rahilly and Meyer, 1963) and the coloured ones also contain carotenoid pigments (Waelchli, 1881; Kuhne, 1878). Droplets lacking lipid properties have been found in the chicken (Kolmer, 1936) and the kestrel (Frances, 1955). Of the carotenoids only the existence of astaxanthin in the oil droplets has been fully substantiated (Meyer et. al., 1945; Strother and Wolken, 1960; Studnitz

et al., 1943; Wald, 1948). Waelchli's transmission spectra, represented an early attempt to demonstrate the chemical composition of the various carotenoids found in the oil droplets. Waelchli (1883) found at least three different pigments but his results were questioned by Walls and Judd (1933) because the tests were not carried out on in vivo material. Wald and Zussman (1397) crystallized three pigments from the chicken retina. The yellowish-green pigment may have been gallaxanthin. Other investigators (Loevenich et al., 1943; Capranica, 1877; Francis, 1955; Fox, 1953; Kolmer, 1936; Karrer and Jucker, 1950; Goodwin, 1954; Kuhne and Ayres, 1878; Kuhn et. al., 1939; Kuhne, 1879; O'Rahilly and Meyer, 1963; Schultze, 1867; Sidman and Wislockie, 1954; Studnitz et. al., 1943; Strother and Wolken, 1960; Waelchli, 1883; Wald and Zussman, 1937, 1938; Wald, 1948; Wallenfells and Bielig, 1941; Walls and Judd, 1933; Waelchli, 1881) have also tried to determine the character of the yellowish-green pigment, as well as the other coloured pigments. Bridges (1963) obtained three chromatographically separated carotenoid fractions from the pigeon retina, the pink zone representing the astaxanthin, the upper yellow zone either the sarcinene or sarcinoxanthia and an impure yellow zone.

Detailed analysis of the problems involved in the chemical analysis of oil droplets, although beyond the scope of this thesis, is important in the study of the effects oil

droplets have on avian visual behaviour. Meyer, Cooper and Gernez (1965) give a brief, but fairly complete discussion of findings related to the chemical nature of avian retinal oil droplets.

It is questionable whether all oil droplets derive from different weakly coloured precursors or from the same colourless oil droplets. Several investigators (Witkovski, 1964; Konishi, 1965; Walls and Judd, 1933) have claimed that oil droplets are colourless which first appear in the chick and the Japanese quail embryo. Hahn (1916), however, claimed to have found pale green droplets before the colourless ones appeared. Meyer and his coworkers (1965) had difficulty identifying the first droplets appearing in the chick retina as colourless or pale green. Nevertheless it is evident that pale droplets precede the development of the more strongly coloured ones.

In the chicken retina all cones contain oil droplets, the colour of which may depend on the cone type (Meyer et. al., 1965). Red droplets were found in the single cones, yellow and greenish-yellow in the chief and accessory parts respectively of the double cones. Because the different types of cones are different lengths, their oil droplets lie at various depths. Thus it is possible that oil droplets of the same colour lie at the same depth within a given area of the retina in the chicken. For each colour of an oil

droplet in an area there is a different depth. This depth distribution of colours is not necessarily the same over the whole retina, as the length of one type of cone may differ from area to area. Waelchli (1883) claimed to have found variations in depth distributions from area to area in the rooster retina.

Consistencies in depth distributions, although certainly a possibility have not been found in other species. Indeed, it is difficult enough to determine whether certain types of cones contain oil droplets at all, without determining their colour. Oil droplets have been observed in the single cones and the chief component of the double cones in the pigeon retina (Cohen, 1963) and probably also exist in the accessory component of the double cone which is difficult to observe as mentioned in the previous chapter.

Nevertheless it is possible to conclude at this point that all pigeon and chicken and probably all diurnal avian cones contain coloured oil droplets. Colourless oil droplets have not been observed in the chicken retina (Blasser, 1927; Dobrowolsky, 1871; Hoffman, 1877; Krause, 1894) or in the pigeon and turkey retina (Strother, 1963) or several other diurnal avian species (Rochon-Duvigneaud, 1943). It is commonly accepted that strongly coloured oil droplets are characteristic of diurnal birds. More information on the distribution of oil droplets within a particular species is

in order and will probably come about through more refined microscopical and staining techniques.

Tiny "microdroplets" have been found in the cones of the pigeon (Pedler and Boyle, 1969). These are numerous small red bodies in the inner segments of some photoreceptors similar in fine structure to the principal oil droplets (determined electron microscopically). They have been found only in cells with a large red oil droplet and not in the receptors containing granular bodies. They are generally found in the "red field" of the pigeon retina which could act as a fine grain red filter in this retinal area, with droplets influencing the light reaching more than one receptor. The microdroplets could conceivably add to the over-all filtering effect of the droplets as Pedler and Boyle (1969) suggested. The possibility that microdroplets only affect the light entering the particular receptor which contains them will not be ruled out until the refractive index of the droplets as compared with that of the surrounding tissue has been determined.

It is now apparent that the coloured oil droplets contribute to, if they do not account entirely for, hue discrimination in birds. Schultze (1866) had early pointed out their possible role in colour vision. Any light entering the receptors containing oil droplets must first pass through the oil droplets. Since they filter any light passing

through them (in fact Meyer et. al. (1965) have stated that oil droplets absorb 80% of the light that enters them) their filtering effect is probably of functional significance. Microspectrophotometry has confirmed the filtering function of oil droplets (Strother, 1963) through the shapes of their absorption curves and their location relative to the iodopsin as shown in Figure 9. The problem, then, is to determine in what way oil droplets affect the light entering and passing through them, and whether or not their influence is combined with the influence of cone pigments or a cone pigment.

Most researchers have assumed that there is at least one cone pigment in the avian retina. Sillman (1969), however, failed to find any cone pigments whatsoever in the retinae of several species of birds. If there were no cone pigments in birds the oil droplets could account entirely for the photochemical reactions of the cones and therefore probably most of the receptors' colour vision mechanism. But there is also the possibility that the cones contain rhodopsin or some other presumably scotopic pigment. Bridges (1962) presented some evidence for the influence of rhodopsin in pigeon cone vision (see Chapter III).

However, a great amount of evidence exists (as will be cited below) for the existence of avian cone pigments. The issue seems to be whether or not there is more than one pigment interacting with the various coloured oil droplets

in the cones within one retina. Walls and Judd (1933) postulated a multiplex retina containing cones with oil droplets which were independent elements. They considered oil droplets to be modifiers of colour perception, filters permitting the development of physiologically multiplex photopic apparatus capable of adaptation to varying conditions of illumination. This view of oil droplet function could be called the multiple pigment hypothesis since it assumes different types of pigments in different types of cones with different colours of oil droplets. Donner (1958) also came out in support of the multiple pigment hypothesis. He denied the existence of a single cone pigment with oil droplets as filters-system because he did not find agreement between the absorption spectra of the oil droplets and a one pigment system. He postulated colour 'modulators' the curves of which are shown in Figure 10 based on microelectrode recordings from units in the pigeon retina, probably fibres in the optic nerve and ganglion cells. But as King-Smith (1969) pointed out, his evidence is dependent on the assumption that the 'modulator' type sensitivity curves he observed from the retinal ganglion cells could be equated to cone sensitivity curves and the evidence (Dartnall, 1953) suggests that this equation cannot be made.

King-Smith (1969) found evidence for a one-pigment theory in her absorption spectra of the pigeon's oil droplets

shown in Figure 11. She noticed that above 620 nm transmission is nearly constant for all colours of oil droplets. When this fact is considered in light of Hamilton and Coleman's (1933) behavioural finding that there is sudden deterioration of hue discrimination in pigeons with wavelengths above 620 nm, it leads to the conclusion that overall cone sensitivities for the different cone types should be equal at each wavelength and thus there should be only one cone pigment in pigeons.

It is possible that some avian species have more than one cone pigment while others have a single pigment in all the cones. For it is true that different species produce different spectral sensitivity curves (evidence cited above). However, these differences may be due only to having particular colours of oil droplets different from other species. But even if one could assume that there is only one pigment in all the cones in a particular species, it is not at present known whether all species are likely to possess the same cone pigment or not. We know that pigments and oil droplets are interacting in the avian cones to produce colour vision, but it is this interaction which makes the analysis of the mechanisms of avian colour vision difficult.

However it would be possible to add sensitivity curves of the oil droplets derived from their spectral absorption curves, to the sensitivity curves found in the

ganglion cells and subtract both of these from cone sensitivity curves based on recordings from the cones' inner segments to obtain a hypothetical spectral sensitivity curve of a cone pigment. This could then be compared to the absorption spectra of pigments which have previously been extracted from avian retinae. The main difficulty here is in obtaining reliable recordings from the inner segments of cones. As yet none have been reported.

Meyer, Stuckey and Hudson (1971) have recently been able to raise quails with carotenoid-free ("colourless") retinal oil droplets by dietary exclusion of carotenoids and work on their spectral sensitivities is now proceeding. Behavioural and physiological studies on these "colourless" quails may very well yield conclusive evidence on the function of oil droplets and cone pigments.

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Chapter V

Bipolar Cells

Moving on through the inner nuclear layer which in the avian retina is slanted outward because of the slanting of the bipolars and the horizontal radial fibres of Muller, we come to the bipolar cells so named because of their bipolar appearance. Their bodies are closely packed in the inner nuclear layer and only rarely displaced into another layer, especially rarely in the beautifully layered avian retina. From each cell body two vertical processes project and divide further into smaller branches. Polyak (1955) defined basically two types of bipolar cells, common or polysynaptic rod and cone bipolars and monosynaptic bipolars which are related only to the cones in his retinal system. In the barn swallow (Polyak, 1955) and probably in other avian species as well, the bipolars appear to be anatomically separated into groups of one type.

Mop bipolars are of the common or polysynaptic type each being related to a group of rods and cones. There is a reciprocal overlapping of the dendritic mops of the adjoining mop bipolars so that one group of rods and cones which is related to one mop bipolar is also partially related to others. There is also an overlapping of mop bipolars with other types of bipolars and horizontal cells.

The axons of the mop bipolars descend to the lowest part of the inner plexiform layer. Apparently there is no selection of synaptical relations of mop bipolars with specific types of ganglion cells.

It would appear then that brush and flat-top bipolars are only connected to the cones and again do not select which type of ganglion cell they will connect with.

Midget bipolars are the only true monosynaptic cone bipolars. They do not contact ganglion cell bodies but synapse with them in the inner plexiform layer. Usually one midget bipolar connects with one midget ganglion cell but has polysynaptic relations with the other varieties of ganglion cells.

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Chapter VI

Association Cells

The association cells of the retina are found in the inner nuclear layer apparently serving to distribute impulses or alter them in some way as they come from the rods and cones and other cells of the retina. Polyak's (1955) monumental work on vertebrate vision still serves us well in looking at association cells in the avian retina. They are of three types; the horizontals, the centrifugal bipolars and the amacrines. The horizontals are in the one or two uppermost rows of the inner nuclear layer and their dendrites spread in all directions. They are larger and possess more branches when in the periphery of the retina. They are activated only by cones but stimulate both rods and cones. The centrifugal bipolar bodies are found in the lower zones of the inner nuclear layer and have a larger nucleus than the centripetal bipolars. They connect with the photoreceptors, ganglion cells and centripetal bipolars, probably transmitting influences from the ganglion cells and possibly the centripetal bipolars to the rods and cones, perhaps inhibiting the photoreceptors. The amacrines typically possess no scleral process and are very small in the fovea. Their expansions are horizontal and can be seen as streaks across the inner plexiform layer in the avian retina.

The thickening of the inner plexiform layer in the

avian retina may be due to an increase in synaptical connections in this layer, especially of the cells which are primarily horizontal in extent. Pedler (1970) has argued that the pigeon retina is primarily horizontally organized upon finding many more horizontal processes under the electron microscope than had hitherto been observed. While its primary organization may not be horizontal it is increasingly evident that far more processing goes on at the retinal level in birds than in man as we shall see from results of recordings from ganglion cells. The horizontal streaks may also indicate binding by supporting tissue in synaptical fields. Yet in man synaptical fields possess particularly less neuroglia. If there is, in fact, more neuroglial support of synaptical structures in the avian retina than in the human retina it may evidence different types of synaptical organization in the two different types of retina, the one more rigid than the other. Let us go to the ganglion cells for more evidence.

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Chapter VII

Ganglion Cells

The ganglion cells are numerous in the avian retina (Polyak, 1955). Electron microscopy has revealed the presence of large numbers of unmyelinated and small myelinated fibres not resolvable with the light microscope (Bingelli and Paule, 1968) doubling previous estimates of the number of fibres in the optic nerve and the ganglion cell layer of the pigeon. Counts of the cells in the ganglion layer exceeded those of the fibres, perhaps showing the displacement of neuroglial amacrine cells into this layer as previously described by Cajal (1889).

Ganglion cells can be either polysynaptic or oligo-or-monosynaptic (Dowling, 1970). There is one axon per cell which leaves the eyeball and continues into the optic nerve and into the brain as part of the optic tract. The axon is not myelinated in the eyeball but becomes so once it is outside. There are several identifiable types of ganglion cells (Polyak, 1955). The umbrella or parasol ganglion cells synapse with many bipolars and their arborizations spread far, especially in the peripheral retina. The shrub ganglion connects with several types of bipolars and seems to have a spatial summating role. Small diffuse ganglion cells synapse with a few bipolars of all varieties. Garland ganglion cells offer dendrites to brush, flat-top and midget bipolars,

connecting with the bodies of mop bipolars. They produce summation from large areas. Giant ganglion cells are found peripherally, while in the central area and fovea midget ganglion cells have individual synapses with midget bipolars in two separate tiers in the inner plexiform layer and several synapses with other types of bipolars, both giving and taking stimulation to and from the other cells.

Taking unit responses in the optic nerve of the pigeon, Maturana (1962, Maturana and Frenk, 1963) found several types of units. Verticality detectors responded as long as the vertical edge was in the receptive field but did not respond to edges 20^{o}-30^{o} from the vertical or to a change in the diffuse lighting or to small objects or spots of light. The receptive field here was a fraction of a degree in diameter. Horizontality detectors respond only to a horizontal edge moving in the receptive field or to a tip or edge in the surround if the edge extends into the surround but the response disappears at a deviation of 20^{o}-30^{o}. General edge detectors of two kinds were found, one with a large receptive field 2^{o}-3^{o} in diameter and another with a smaller receptive field $\frac{1}{2}^{o}$ in diameter. The cells with large fields detect small objects moving within the field while those with smaller fields respond to background changes as well. Both of these types of units respond with one or two spikes to on, off or both of diffuse light, with six to ten spikes to an edge moving

across the field and respond to a spot on or off and most strongly to a spot moving across the field. The receptive fields are uniformly on or off. Directional movement detectors respond maximally to an edge moving in a particular direction, with three or four bursts of three or four spikes each. The magnitude diminishes with deviation from the optimal direction. There is no assymmetry in these fields to explain the directional sensitivity. Convex edge detectors respond strongly to a convex moving edge and not to a straight edge. They do not require as in the frog (Muntz, 1962) a particular direction of contrast and they do not respond to diffuse illumination changes but may respond weakly to the on and off of a spot in the centre of edges. Luminosity detectors give a maintained response to illumination.

The ganglion cells of the pigeon then, resemble in complexity and specificity of operations they perform, the cortical cells of the cat, as described by Hubel and Weisel (1959). The response of the cat ganglion cells is not specific to the specific configurations of the stimulus. Maturana thus differentiates two types of visual systems; that of amphibians, reptiles and birds which is deterministic with the fundamental characteristics of the stimulus discerned at the level of the retina and that of mammals which is indeterministic where the fundamental elements of the stimulus are transferred to the cortex as a combination of the output

of the whole neighbourhood of ganglion cells. The deterministic system is more economical, requires fewer cells but is more rigid and restricted in the amount of information that is transferred to the brain. The indeterministic system requires more cells in the brain but can do more complex operations on the fundamental visual information. It is essentially more plastic and has greater possibilities for further development. In general it is not just the summation of afferent impulses which discharge a specific cell but a particular spatial and temporal configuration of afferent impulses which constitute its adequate stimulus. The interplay of excitatory and inhibitory processes is necessary to form the proper stimulus configuration that excites a cell if the adequate visual pattern is present. The story of excitatory and inhibitory interaction is told in the synaptical organization. Thus we will go on to that topic in the next chapter.

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Chapter VIII

Synaptical Organization of the Retina

The two plexiform layers, the outer, between the receptors and the bipolars and the inner, between the bipolars and the ganglion cells are the main layers of the retina containing synapses. In birds these two layers take up a greater proportion of the retinal thickness than they do in mammals. This could very well be because there are more synapses in the avian than in the mammalian retina. We have just seen in the previous chapter that the complexity of the response contingencies of retinal ganglion cells in the pigeon to visual stimuli is comparable to that of those in the mammalian cortex. It is therefore reasonable to assume that more processing is occuring at the retinal level in birds than in mammals. And it is therefore not surprising to find larger synaptical (plexiform) layers and probably more synapses.

Let us examine the characteristics of synapses in general so that we can use this information as it applies to birds. There appear to be two types of synapses (Dowling, 1970). The ribbon synapse is characterized by a dense ribbon or bar in the presynaptic cytoplasm surrounded by an array of vesicles. It has multiple postsynaptic elements, increased membrane density postsynaptically and a synaptic cleft wider

than non-synaptic extracellular spaces and is found in the receptor terminals and in the bipolars in the inner plexiform layer. The second is the conventional synapse characterized by a dense aggregation of vesicles clustered close to the presynaptic membrane and only one post synaptic element. This is found in the horizontal and amacrine cells.

The bipolar dendrites in the outer plexiform layer and the ganglion dendrites in the inner plexiform layer have never been observed to make presynaptic contact. Thus of the three types of neurons with processes in each synaptic layer only the processes of two make synapses. In the outer plexiform layer ribbon synapses are made only by receptors and conventional synapses only by horizontal cells while in the inner plexiform layer ribbon synapses are made only by the bipolar cells and conventional synapses mainly by amacrine cells. It is therefore possible to identify a process under electron microscopy by the nature of its synaptical contacts. Synaptical pathways were thus determined by Dowling (1970).

In the outer plexiform layer the majority of synapses are the ribbon synapses of the receptors. Here the unusual invaginated synaptic complexes in the receptor terminals could allow for the interactions between the horizontal and bipolar cell processes. For example, the horizontal processes could regulate the transmitter flow from the receptor to the bipolar cell dentrite. The horizontal processes can be both

pre- and post-synaptic and thus appear well suited to mediate lateral and reciprocal interactions between adjacent neurons. This versatility is found in most horizontal and amacrine processes. In the outer plexiform layer then, processes from the bipolars and horizontals are post-synaptic at the superficial contacts on the receptor. The horizontals contact bipolar dendrites or the dendrites of other horizontals and interact with the bipolars at the receptor terminals as described above, so that the bipolars are affected by the nearer receptors and also far ones via the far-reaching horizontals.

In the inner plexiform layer there are more synapses of greater variety than in the outer plexiform layer. Species differences in this layer are more apparent. The bipolars connect with the amacrine processes and the ganglion dendrites in several types of synaptic arrangements. The dyad, a type of synapse which has two post synaptic elements, can consist of a ganglion cell dendrite and an amacrine process, two amacrine processes, or, rarely, two ganglion dendrites. This type of synapse, although it exists in the avian retina is not particularly predominant there. We shall see why in a moment. There are many conventional synapses of amacrine cells on the bipolar terminals, as well as on the ganglion dendrites and other amacrine processes. Dowling noted two particularly unusual types of synapses at the bipolar

terminals. At many such dyads the amacrine cell synapses back onto the bipolar terminal forming a reciprocal synapse. At others the amacrine synapses onto another amacrine which in turn synapses onto another type of cell or in some cases another amacrine in a serial synapse suggestive of the local interaction.

In retinae with more complex receptive fields, such as that of birds, the dyad pairings consist mostly of two amacrine cell processes. There are abundant amacrine synapses per unit area and there are many serial synapses. The ratio of amacrine/bipolar synapses is generally directly proportional to the complexity of the receptive field organization of the retina. The more complex the organization, the fewer direct bipolar to ganglion cell contacts there are. The amacrines thus appear to mediate complex retinal interactions and may play a part in the vertical receptor-bipolar-amacrine-ganglion chain. More evidence for the role amacrines play in retinal interaction lies in the type of responses obtained from the various retinal cells. These were taken by Dowling (1970) in the mudpuppy retina which contains both simple and complex receptive fields.

Receptors, horizontals and bipolars all show a slow response probably because they need propqgate impulses only for a short a distance. They can also have hyperpolarizing potentials which here signal excitation although they are

usually inhibitory. The receptive fields of receptors are small and are not affected by surrounding annular stimulation. Those of the horizontal cells are larger and are affected both by a small spot and annular stimulation. Bipolars have two types of receptive fields. One hyperpolarizes and the other depolarizes to stimulation by a spot while both responses are reduced by annular stimulation in an antagonistic centre-surround receptive field organization.

Amacrines respond transiently to static retinal illumination regardless of the configuration with both a slow potential and usually two spikes.

Ganglion cells show two types of responses. One resembles that of the amacrines but has a number of spikes which seems to be related to the amount of depolarization of the cell. The other resembles that of the bipolars with a sustained slow potential and many spikes in response to central illumination and is inhibited by surround illumination.

The various types of receptive fields are produced by various types of synaptic interaction. A summary diagram of vertebrate synaptical organization in the retina is shown in Figure 12. The horizontal cells summate from a wide area of receptors which respond autonomously to stimulation. The latencies of the horizontals match those of the bipolars since both are activated by the receptors at the same synapses. The bipolar cells are directly activated by the receptors and

antagonized by the surrounding horizontals which have a lateral extent greater than that of the bipolars thus producing the antagonistic centre-surround receptive fields of the bipolars. The amacrines could turn off the bipolars locally through the reciprocal synapse arrangements and in the retinae containing more complex receptive fields could produce the second type of response described for the ganglion cells, that is one which resembles that of the bipolars. In simply organized retinae the ganglion cells receive stimulation directly from the bipolars and act more like amacrine cells, perhaps displacing more complex responses to the central nervous system where they can interact more closely with other neural systems. In a complex retina such as the avian retina the serial synapses of the amacrines may mediate directional selectivity by inhibiting cells in a series so that a spot moving in one direction evokes a vigorous response in a ganglion cell, but moving the other way evokes none. The transient responses of the amacrines may also indicate other types of moving convex edges found by Maturana (1962) in the pigeon. The functional organization of the retina is only the beginning of the story of the visual system, but it must be understood before higher order interactions can be fully understood.

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Chapter IX

Centrifugal Fibres to the Retina

Evidence of efferent retinal fibres is available for cephalopods, insects and birds. There is no conclusive evidence for them in amphibians, reptiles and mammals. But by far the strongest and most evidence is available for efferent retinal fibres in birds, and most of the avian work on them has been done on pigeons.

The centrifugal fibres to the retina in the pigeon originate in the isthmo-optic nucleus and travel to the contralateral retina (Cowan and Powell, 1963) where they form several kinds of terminals in the inner nuclear layer. Cajal (1911) described pericellular nest terminals of efferent retinal fibres, the same as the "convergent" type of Maturana and Frenk (1965). A few endings penetrate deeply into the inner nuclear layer to an unspecified termination (Ogden, 1968). The "divergent" terminals (Maturana and Frenk, 1965) contact several types of cells at the junction of the inner nuclear and plexiform layers. There is also a single terminal on the axon hillock of each "displaced ganglion cell" (Maturana and Frenk, 1965).

The anatomical evidence for efferent retinal fibres is inconclusive since it depends on the assumption that prograde degeneration is faster than retrograde degeneration

and this is not necessarily the case. Ogden (1968), however, gives a very complete review of the literature on anatomical evidence for centrifugal fibres in several species.

Physiological evidence is somewhat more rewarding here. Scholes and Roberts (1964) observed an increase in the amplitude of the ERG of the chick following intravenous injection of nembutal. The increase did not occur after section of the optic nerve. They thus concluded that the amplitude increase was the result of CNS effects on the retina. Ogden (1968) however, pointed out that section of the optic nerve would inevitably also result in damage to the pecten. The absence of the effect after surgery, then, could as likely have been caused by lack of blood supply to the retina, and therefore also less effect of the drug.

Ogden (1968) provided evidence for the role of retinal efferent fibres in the pigeon through surgical interruption of the optic tract, procain blocking of the retina and stimulation of the lateral tectum. Both optic tract section and procainization of the retina produced a marked increase in oscillatory activity. This result was unlikely to be from ganglion cell membrane degeneration. Ogden reckoned it to be a release from control of an oscillatory mechanism, similar to one previously postulated for neurons in the thalamus (Anderson and Sears, 1964; Anderson and Rudjord, 1964). Stimulation of the tectum preceding

light flashes reduced oscillations recorded from ganglion cells as compared to oscillations recorded after light flashes alone. So tectal stimulation also showed evidence of efferent control of an oscillatory mechanism. The efferent terminals are located so as to alter amacrine output (Dowling and Cowan, 1966), so Ogden thought that the activity of the amacrines could be controlling the oscillatory mechanism.

Holden (1966) suggested another function of retinal efferent fibres. He thought that in the pigeon a single channel limit in the brain might produce suppression of signals from one eye while the other predominates. This suppression might be effected through the centrifugal pathway to the retina.

Holden (1968a) identified output cells of the isthmo-optic nucleus by anti-dromic activation. He then used the collision technique to demonstrate conclusively one feature of their response. When they generate action potentials, conduction takes place centrifugally from the cell soma towards the terminals in the retina. Holden (1968b) then found that the majority of the output cells in the isthmo-optic nucleus could be fired synaptically from the lateral tectum, with early firing representing monosynaptic activation and later firing more complex routes, while one tenth of the output cells here could be fired synaptically from the retina at long and short latencies. The firing of the output cells

activated via the retina could be blocked by former antidromic invasion, but the antidromic invasion itself could in turn be blocked by a conditioning stimulus to the retina and to the lateral tectum. He demonstrated that excitatory synapses acting upon output cells are activated from the tecto-isthmo-optic tract while inhibitory synapes can be activated both from the optic nerve head and from the lateral tectum. Let us look at retinal responses to centrifugal effects for more evidence of their function.

Miles (1970) found that stimulation of the efferent tract to the retina in chicks always increased the responses of retinal units. The centrifugal effects were most readily seen when normal visual responses had been reduced by habituation or surround inhibition. He found two different effects resulting from efferent stimulation. In cells where the surround-inhibited responses were restored through efferent stimulation the effect of stimulating was to emphasize the centre response. The efferent input effect was found to be the uncovering of a visual centre response when the entire field was illuminated rather than the introducing of a novel response to surround illumination (tested with small spots). Miles hypothesizes two separate mechanisms which could mediate the efferent effects. The first is selective facilitation of the central excitatory system and the second is suppression of the surrounding inhibitory

system (disinhibition). In view of Holden's (1968) evidence for both excitatory and inhibitory centrifugal effects on the retina it is possible that both mechanisms could be operating in the retina.

In cells where responses were restored by efferent stimulation after they had habituated there was an increase in firing to centre illumination, but none to overall illumination. In this case the centrifugal effects probably facilitate excitatory systems dominating the field centre. These cells exhibited an increase in the apparent extent of the field centre as revealed by annular stimulation of the field. It may be that excitatory and inhibitory systems overlap at receptive field centre and surround boundaries and thus the disturbance in their balance caused by centrifugal stimulation (in this case probably excitatory) results in an enlargement of field centre size.

The centrifugal system in birds involving projections of retina upon tectum and nucleus isthmo-opticus, tectum upon nucleus isthmo-opticus and nucleus isthmo-opticus back onto the retina (Figure 13) apparently plays a part in processing of visual information in the retina. Its role may be both excitatory and inhibitory in nature and is certainly not simple. The main effect of the system is as Miles (1970) pointed out to make retinal cells respond to more stimuli, and more readily to stimuli, which super-

ficially appears to be a disinhibitory function. The mechanisms of the disinhibition could, however be excitatory as well as inhibitory. What we need to know are more specific stimulus and response attributes connected with centrifugal effects on the retina, both at the level of the retina and of the nucleus isthmo-opticus. For example, responses of isthmo-optic output cells could be recorded to visual stimuli as Holden (1968b) suggested. Responses of these output neurons to stimulation of motor nerves in the neck, wings and legs could also be studied to determine whether or not centrifugal effects on the retina are the result of motor activity.

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Chapter X

Conclusion

The Functional and Evolutionary Significance of the Avian Retina

It should be remembered that the evolution of the vertebrate eye is not the slow step-by-step progress which characterizes the evolution of the invertebrate eye.from a single photosensitive cell to a multi-celled eye with lens, cornea and the other trappings of the most sophisticated invertebrate eye. Instead, each vertebrate species has developed its own type of eye which has strong similarities to the eyes of other vertebrates but which is adapted to its own special environment. All the eyes of vertebrates have a three-layered retina and a pigmentary epithelium. But highly specialized developments are found in three groups - teleostean fishes, Sauropsida (lizards and birds) and primates. All three have a fovea, for greater acuity, good accommodative mechanisms, good colour vision, binocular viewing and the visually dominated neopalium which replaces the olfactary archipallium. Of these three groups birds have relatively the largest and absolutely the most specialized eyes, the most efficient focusing apparatus, a pecten, the most complex macular arrangement and the highest visual acuity. In particular, the avian retina is remarkable for its beautiful layering, its thickness in general and in

particular the thickening of the inner plexiform layer, the great density of receptors and the existence of coloured oil droplets in the cones. Let us keep in mind then, that the avian retina, although limited in its efficaciousness by the complexity and efficiency of the central visual system to which it projects, is a very sophisticated perceptual machine which has developed in response to the particular environment of the bird, quite separately from the retinae of other vertebrates, adapted to their own purposes.

Consider the avian pecten. It is suited to the needs of birds since it provides a nutritive device other than the choroid to the visual cells without taking away from acuity as would retinal blood vessels. And it is visual acuity upon which birds depend for self defense, or finding prey while flying at great distances from the land. If the pecten is also a device for motion detection, as has been hypothesized (Menner, 1938) then its existence in the avian eye is doubly adaptive for a species needing long range vision in an open sky, where movement of apparently tiny objects is barely discernable to the human eye.

The visual cells of the avian retina are also adapted to the life style of the species in which they reside. Most birds possess both rods and cones, useful for day and night-time vision. The nocturnal owls possess a greater concentration of rods and some diurnal birds, such as the

kiwi, almost all cones. The density of avian visual cells is great reaching a maximum in the hawks, long noted for their acuity. Again, as previously illustrated by the existence of the avian pecten, birds have a great need for acuity in their life-style.

Visual pigments of birds represent a problem perhaps best considered in conjunction with oil droplets, both pigments and droplets contributing to colour vision, and in particular spectral sensitivity. It has not, at this point been determined whether avian cones contain only one kind of pigment or several pigments, or whether all avian species possess the same cone pigment or pigments. However, it is known that the colours of oil doplets differ from species to species. We are safe in assuming, then, that different avian species will exhibit different spectral sensitivity curves when determined behaviourally.

The spectral sensitivity of a species is important in its habits, perhaps mainly in its feeding habits. Hue discrimination, a behavioural phenomenon not entirely unrelated to spectral sensititivy is important in picking out food from non-food. A perhaps more complex problem - colour preference - borrowing factors both from spectral sensitivity and hue-discriminatory powers, relates motivational and discernably physiological factors in a bird's mode of life. It is known that different species of birds exhibit preferences

for different colours (see Part II). For example, ducklings, coots, and young geese seem to exhibit a preference for green, while fruit and seed eating birds, chickens, gulls and pigeons exhibit red and blue preferences. And the preference changes with changes in motivation (hunger and thirst) and dark adaptation (see Part II). The avian visual system must then, possess mechanisms sensitive to changes in illumination and other environmental conditions which enable the bird to react adaptively to these changes. We know that dark adaptation produces changes in visual pigments adaptive for changing conditions of illumination. It is also known that sex, maturity, castration, light and spermatogenic activity affect the proportion of coloured oil droplets in the avian retina (Pezard, 1957). It is not too great a leap of faith to say that motivational states in general, affect the retina, specifically here colour vision mechanisms and perhaps therefore, colour preference. What we have then, is not just a suitably adapted retina but a retina adaptable to changes.

The organization of the avian retina throughout its layers may be, as Polyak (1955) said, in two systems, the rod system and the cone system, or it may not be as later evidence of a purkinje shift in one ganglion cell (Gouras, 1965) indicated. This is an issue of significance in all vertebrate species. What we are mainly concerned with in

just the avian retina is how much processing occurs there, and what it produces in the way of input into the central nervous system. Obviously study of the synaptical interaction is important, but only when it tells us what the outcome of that interaction is.

In the avian retina, the large amount of processing accomplished there means that the retina's input to the brain is more restrictingly coded than is that of, say the human retina. At the optic nerve the relevant features of the visual stimulus have already been extracted from the basic input at the visual cells, so that the brain is restricted to dealing with only the information that the retina has "let through" to it. Thus the avian visual system is a "deterministic" one as Maturana (1962) described it (see Chapter VII) as opposed to the "indeterministic" visual system of mammals.

However, Karten (1969) points out the lack of knowledge about projections not only in the avian but also in the mammalian visual system necessary to draw homologies between the two systems. He does suggest that the thalamofugal system of birds seems readily comparable to that of mammals.

The centrifugal fibres to the avian retina are another indication of its complexity. In mammals correlations of inputs from different senses almost entirely take place in

the cortex. In birds the dorsal area of the midbrain assumes immense importance as a correlating centre for sensory, gratavistic and photostatic impulses (Duke-Elder, 1958). And the midbrain also influences the retina, through the centrifugal fibres from the isthmo-optic nucleus, so that some of the correlating is being done at even the retinal level. The retina, then, plays its own part in the evolution of the vertebrate brain, even although the eye as a whole does not show the same sort of progression. The reflex mechanism in the lower levels of the visual system becomes gradually subordinated to the controlling and integrating influence of a cerebral cortex. Birds are found half-way in vertebrate visual system evolution, where much of the processing takes place at the level of the midbrain (see Figure 14) which also has intimate and reciprocal connections with the retina.

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APPENDIX A

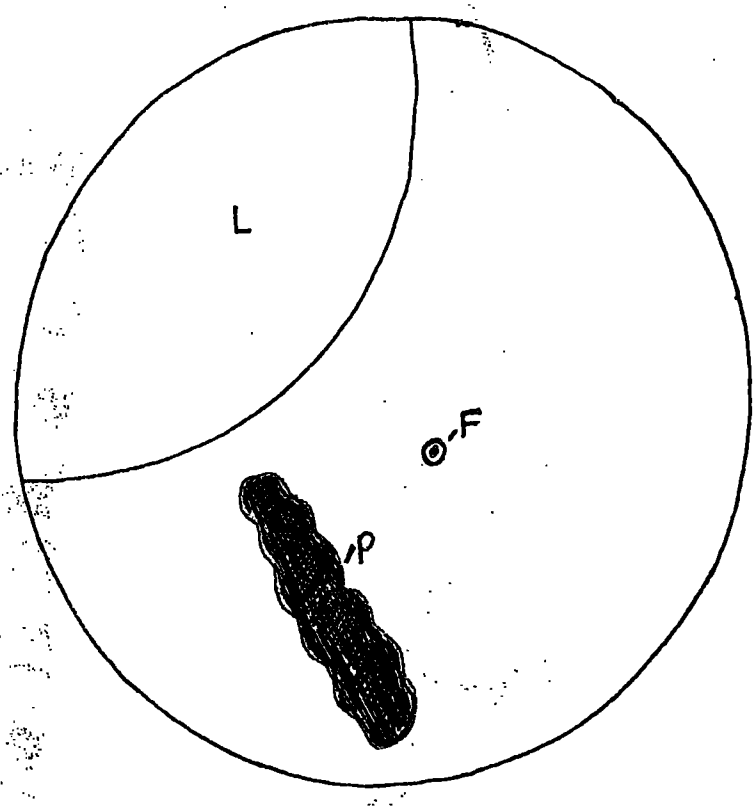


Figure 1. An ophthalmolscopic view of the pigeon's fundus showing the fovea (F), pecten (P) and area laterals (L) or "red field". After Galifret, 1966.

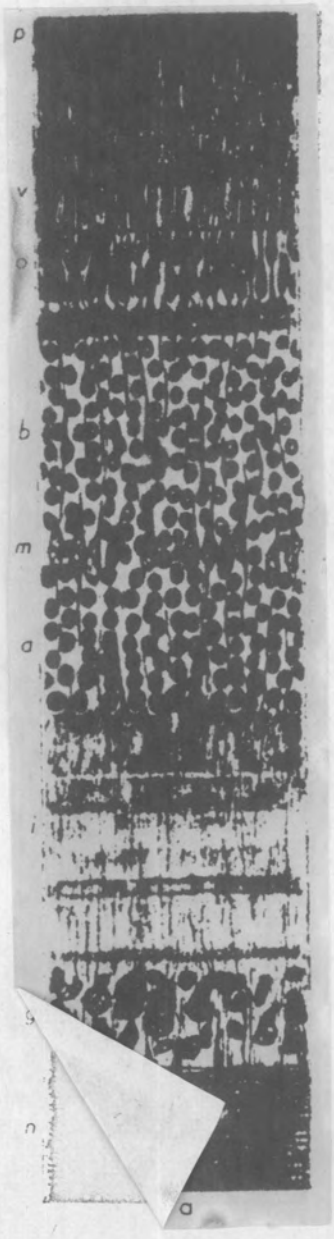


Figure 2. Layers of the avian retina. p, pigment epithelium; v, visual cells; o, outer nuclear layer; b, bipolar cells; m, muller-fibre nuclei; a, amacrine cells; i, inner plexiform layer; g, ganglion cells; n, nerve fibres. (Walls, 1942)

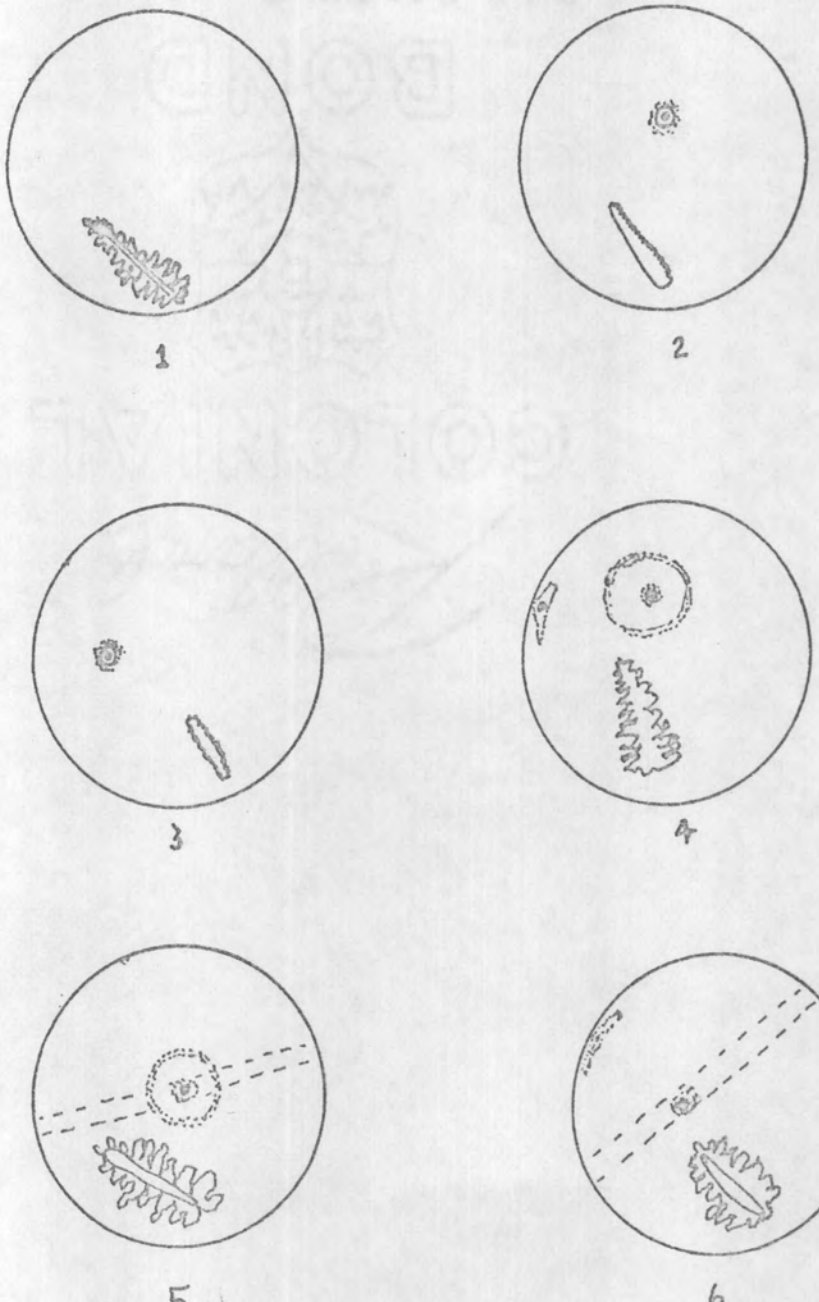


Figure 3. Various types of avian fundi as observed through the ophthalmoscope. 1. Amacular fundus - Californis Valley Quail 2. Nasal monomacular fundus - Stellar Jay 3. Temporal monomacular fundus - Owl. 4. Bimacular fundus - Belted Kingfisher 5. Infulamacular fundus - Greater Yellow-Legs. 6. Infulabimacular fundus - Common Flamingo. (After Wood, 1917)

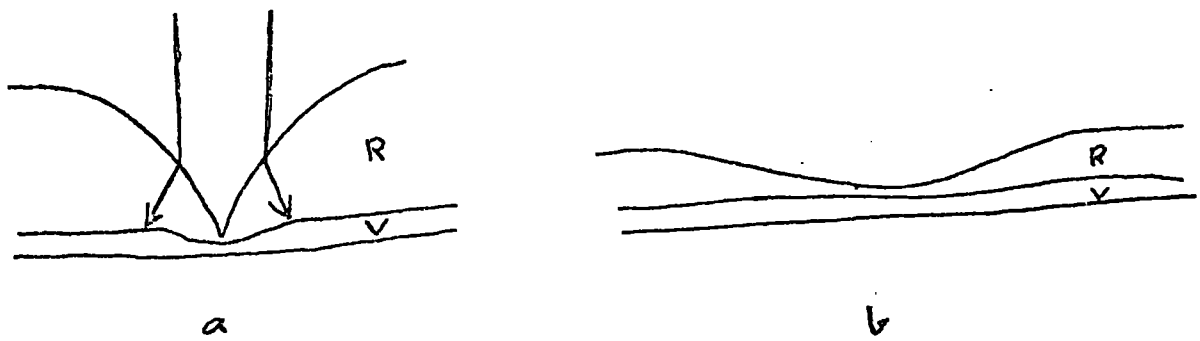


Figure 4. Two types of fovea shown in cross section of retina. Arrows in a represent light being refracted through retinal layers (R) before impinging on receptors (V).

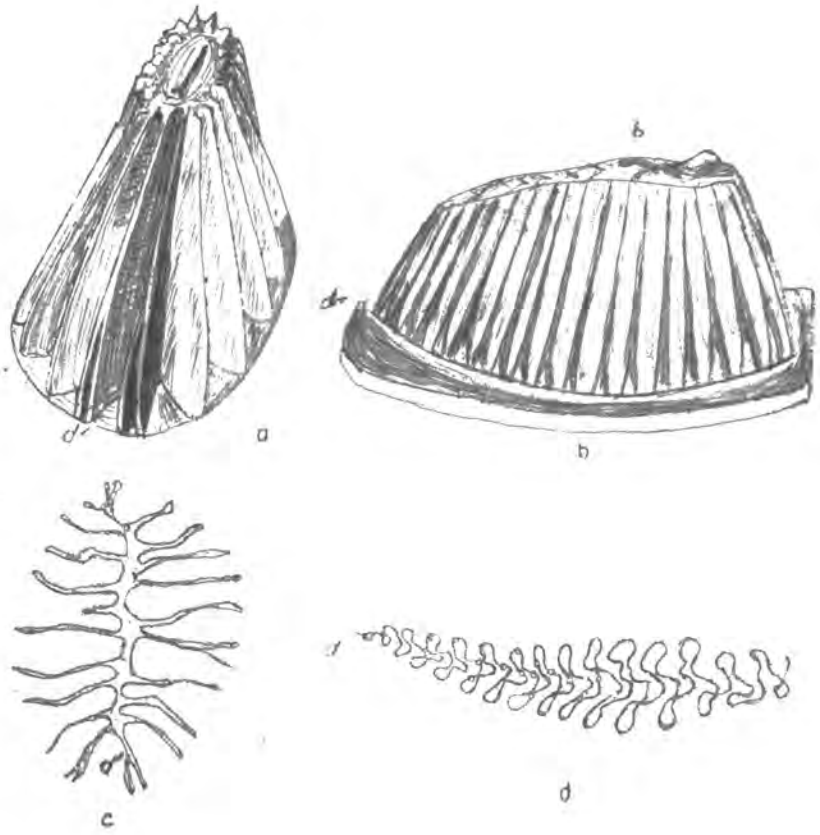


Figure 5. Avian pectens. a, pecten and optic disc of ostrich. b, portion of eyeball wall bearing pecten of domestic fowl, exemplifying type present in most birds. c, section of a near and parallel to its base. d, section parallel to base of red-tailed hawk showing pleated structure characteristic of the common type of pecten shown in b.
(Walls, 1942)

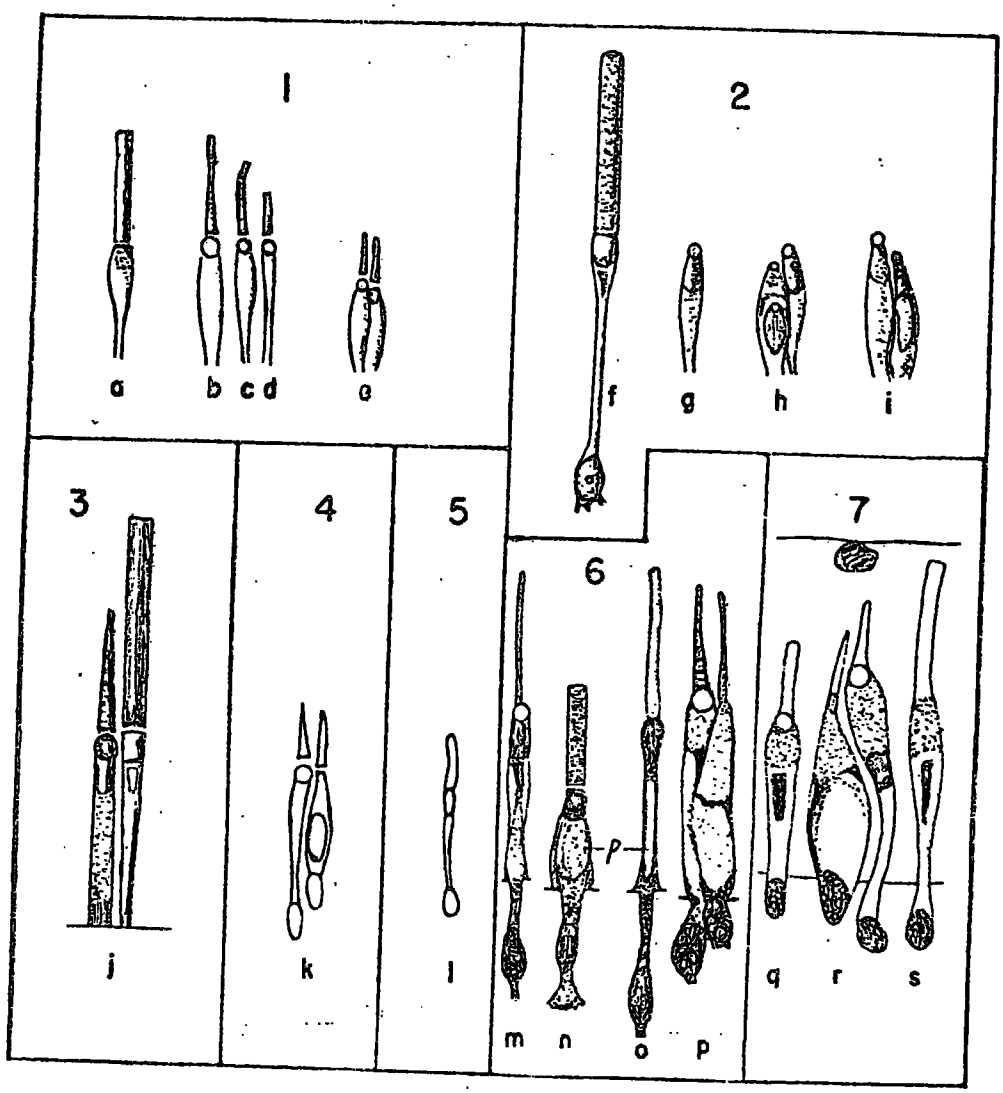


Figure 6. Avian visual cells as depicted by various investigators: (1) Schultze (1867): a, rod; b,c,d, single cones; e, double cone. (2) Hoffman (1877): f, rod; g, single cone; h and i, double cones. (3) Schultze (1872): j, single cone. (4 and 5) Detwiler (1943): k, cones; l, rod. (6) Walls (1942): m, single peripheral cone; n, peripheral rod; o, fundal rod; p, peripheral double cone. Structures labeled p are paraboloid. (7) Meyer and Cooper (1968): q, single cone; r, double cone; s, single cone. Coloured circles in receptors represent coloured oil droplets as indicated by investigators. Note in q, r and s that yellow oil droplets occupy a distal row and red oil droplets form a proximal layer. The yellowish-green droplets are intermediate in position.

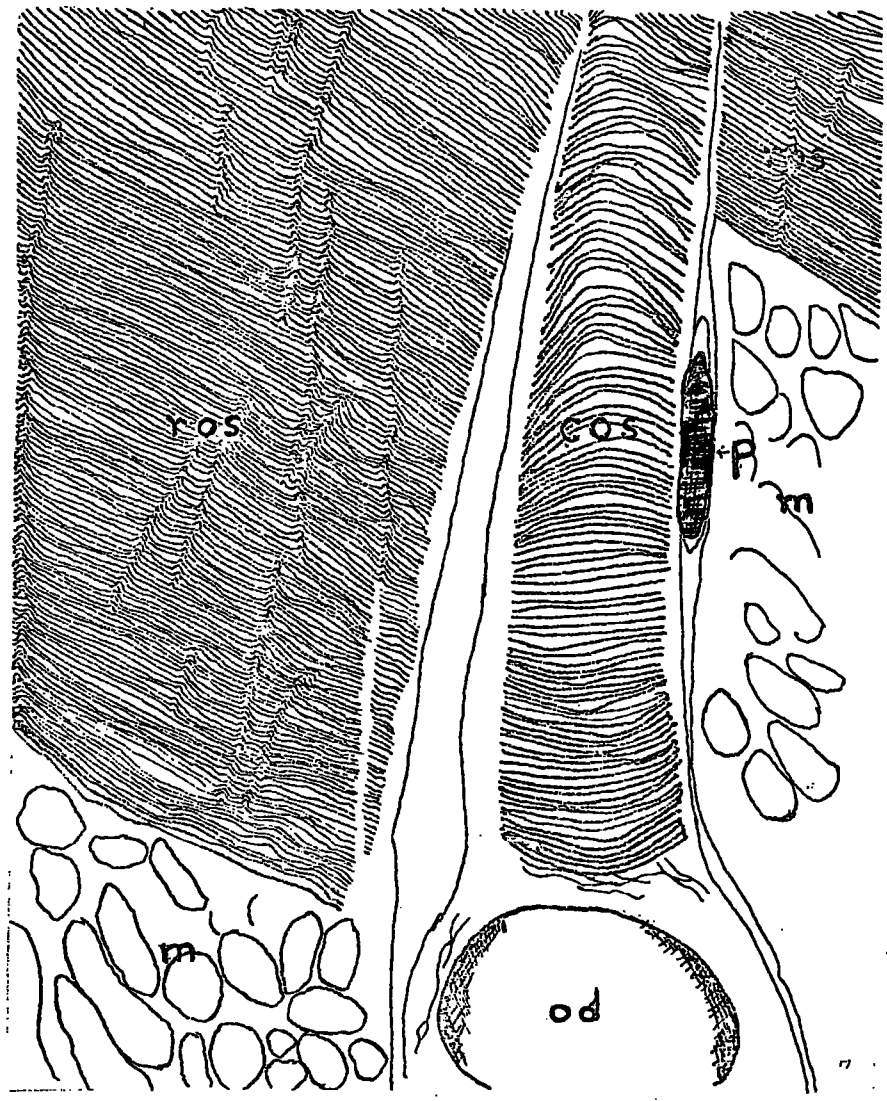


Figure 7. Visual cells showing columns of saccules in rod (ros) and cone (cos) outer segments. od, Oil droplet; m, mitochondria; p, pigment granules in a cytoplasmic extension of the pigment epithelium. Drawing after Young, (1970).

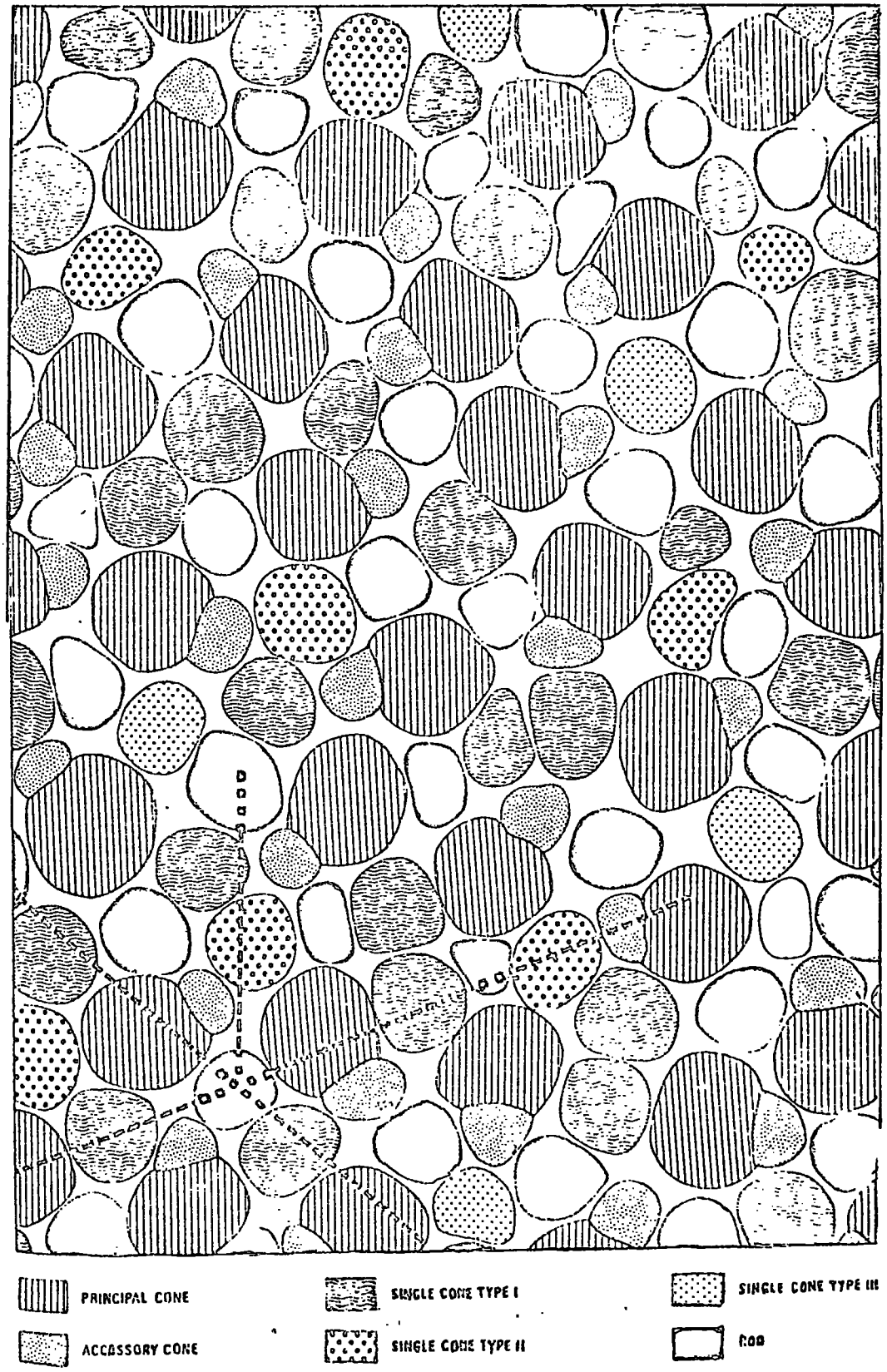


Figure 8. Receptor mosaic in the peripheral retina. Dotted lines show three primary rows. (Morris, 1970).

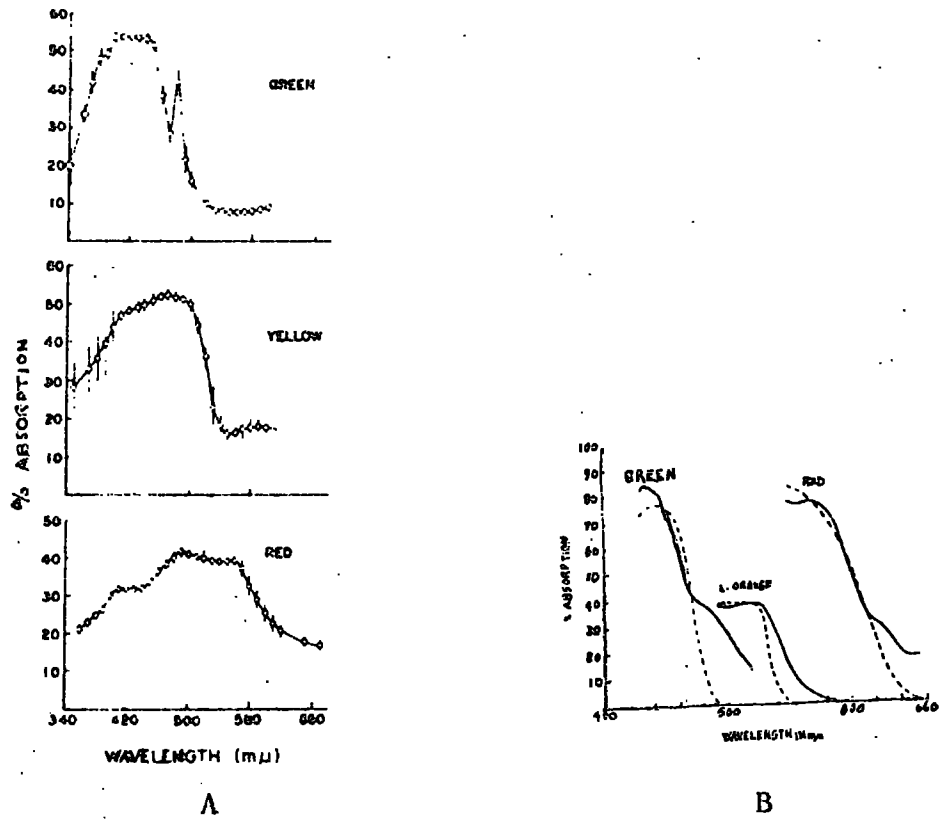


Figure 9. Absorption spectra of oil droplets in chicken (A) and pigeon (B) (----,calculated; _____, observed). Strother and Wolken (1960) and Strother (1963).

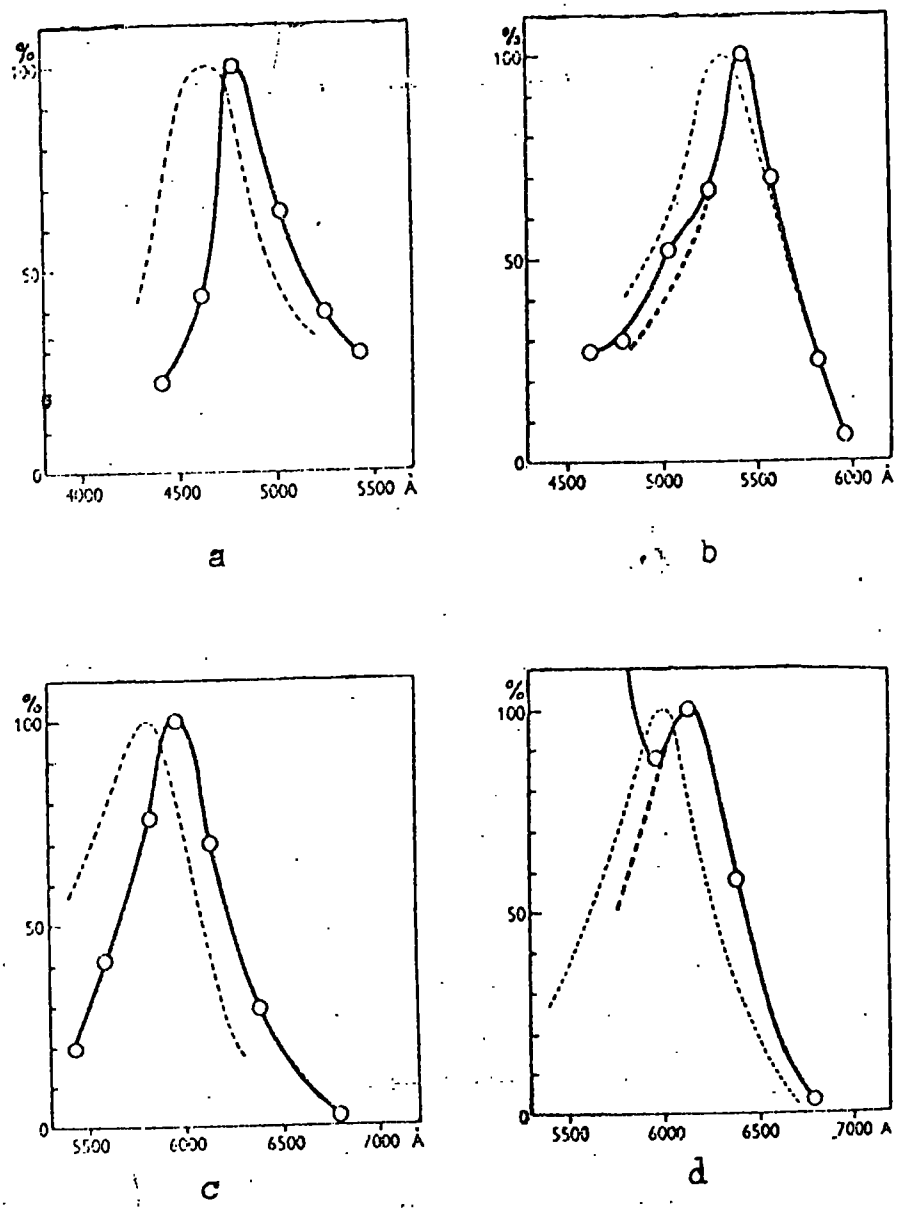


Figure 10. Donner's (1953) modulator curves (o-o) compared with modulator curves of frog(---) (Granit, 1941). a, blue modulator; b, green modulator; c, yellow modulator; d, secondary hump at 6130 A.

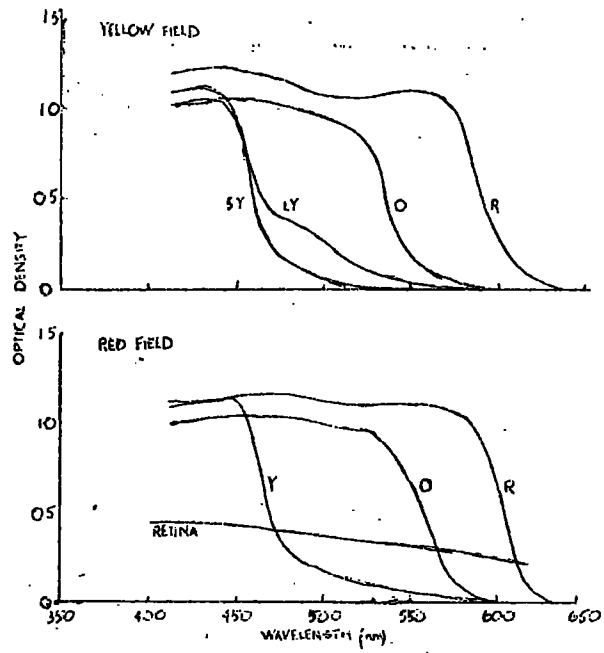


Figure 11. Absorption spectra of red (R), orange (O), yellow (Y) and small yellow (SY) oil droplets in the yellow field and red fields of the pigeon retina. (King-Smith, 1969).

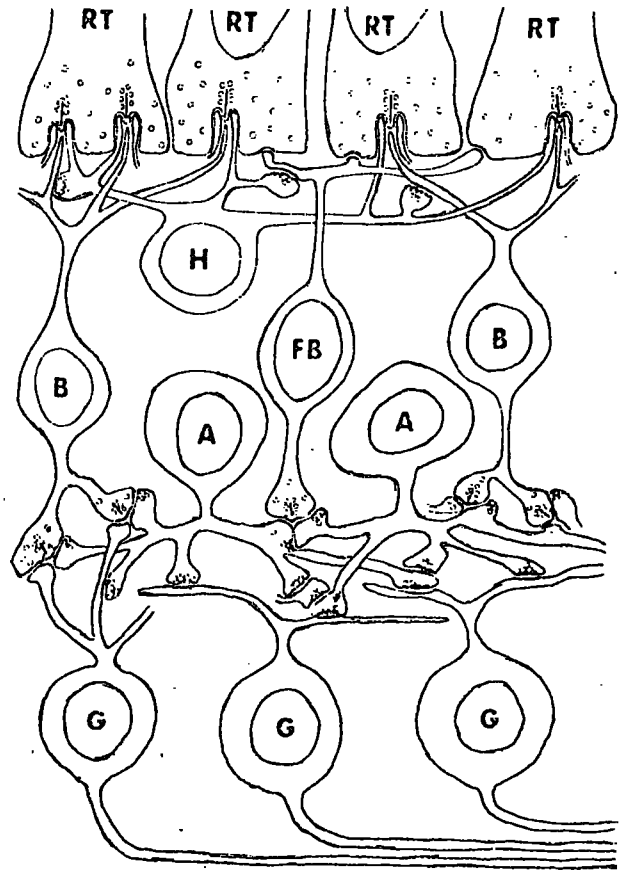


Figure 12. Summary diagram of the arrangements of synaptic contacts found in vertebrate retinas. In the outer plexiform layer, processes from bipolar (B) and horizontal (H) cells penetrate into invaginations in the receptor terminals (RT) and terminate near the synaptic ribbons of the receptor. The processes of flat bipolar cells (FB) make superficial contacts on the bases of some receptor terminals. Horizontal cells make conventional synaptic contacts onto bipolar dendrites and other horizontal cell processes (not shown). Since horizontal cells usually extend further laterally in the outer plexiform layer than do bipolar dendrites, distant receptors can presumably influence bipolar cells via the horizontal cells. In the inner plexiform layer, two basic synaptic pathways are suggested. Bipolar terminals may contact one ganglion cell dendrite and one amacrine process at ribbon synapses (left side of diagram) or two amacrine cell (A) processes (right side of diagram). When the latter arrangement predominates in a retina, numerous conventional synapses between amacrine processes (serial synapses) are observed, and the ganglion cells (G) are contacted mainly by amacrine processes (right side of diagram). Amacrine processes in all retinas make synapses of the conventional type back onto bipolar terminals (reciprocal synapses).

(Dowling, 1970)

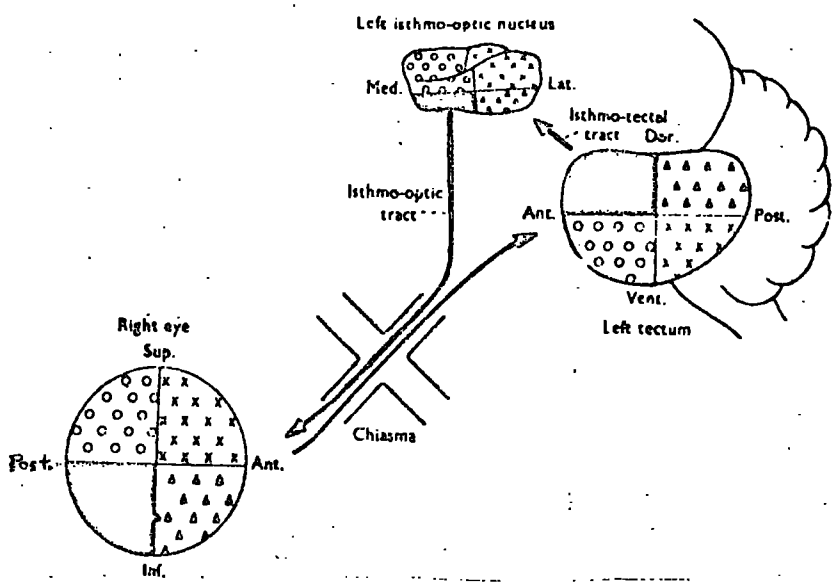
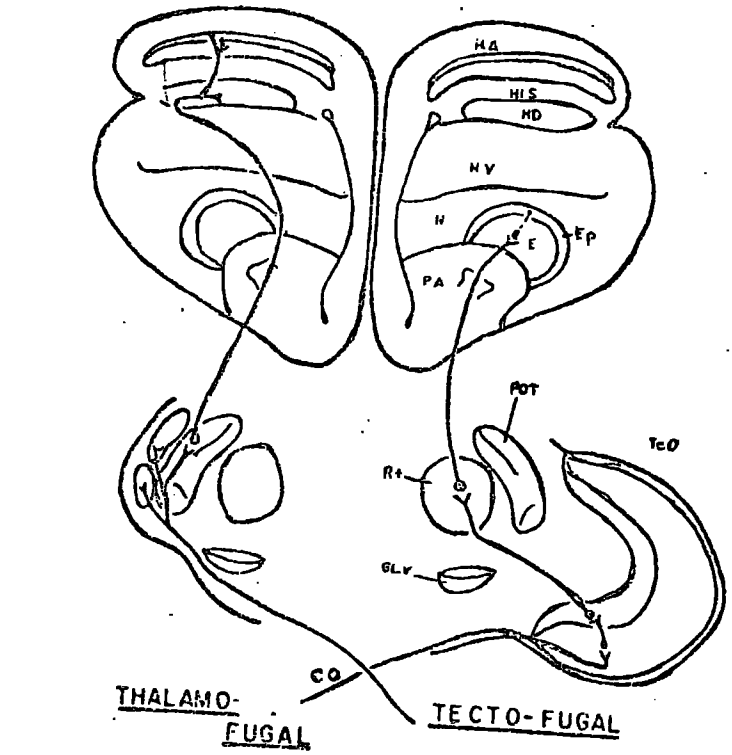


Figure 13. Diagrammatic representation of the retinotopic organization of the projection, in the pigeon, of retina upon tectum, tectum upon nucleus isthmo-opticus (isthmo-tectal tract) and nucleus isthmo-opticus back onto the retina (isthmo-optic tract). (McGill, Powell and Cowan, 1966)



CO	=	Chiasma opticum
E	=	Ectostriatum
Ep	=	Perlectostriatal region
GLv	=	Nucleus geniculatus lateralis, pars ventralis
HA	=	Hyperstriatum accessorium
HD	=	Hyperstriatum dorsale
HIS	=	Hyperstriatum intercalatus suprema
HV	=	Hyperstriatum ventrale
N	=	Neostriatum
PA	=	Paleostriatum augmentatum
POT	=	Principal optic nucleus of the thalamus
Rt	=	Nucleus rotundus thalami
TeO	=	Tectum opticum

Figure 14. Diagrammatic summary of some major ascending visual pathways in birds. Karten, 1969.

PART TWO: MOTIVATION AND VISUAL MECHANISMS: COLOUR PREFERENCE IN THE PIGEON; THE EFFECTS OF DARK ADAPTATION AND INTENSITY OF THE STIMULUS

The development of pecking preferences in young birds has been studied in various attempts to get at motivational mechanisms. In particular, studies of colour preference present interesting problems of visually and motivationally controlled behaviour. While ducklings, coots and young geese seem to exhibit a preference for green (Oppenheim, 1968; Davies, 1961; Kear, 1964; Kear 1966) fruit and seed eating birds, chickens, gulls and pigeons exhibit red and blue preferences (Turcek, 1963; Hailman, 1967, 1968; Delius, 1968; Delius and Thompson, 1970; Thompson, 1970). The adaptive significance of colour preferences has for the most part been attributed to species specific eating habits, that is, birds peck at the colour of the food which they normally eat.

However, a red and blue preference exhibited by gull chicks was interpreted by Hailman (1968) as a green avoidance, a variation on the usual adaptive significance theme. Rather than approaching colours of food, chicks avoid colours of non-food. Plotting retinal receptive fields Hailman (1964)

showed red and blue-sensitive centres with surrounds sensitive to green and yellow. Colour preference does not change with age in chicks while shape preference does, suggesting not only that colour preference may be of earlier significance adaptively but also that physiological correlates of colour preference develop earlier, prior to hatching.

What would seem to be the most obvious physiological correlate of colour preference is the oil droplet distribution in the bird's retina. Oil droplets lie between the inner and outer segments of the cone and seem to act as colour filters. They can be red, orange, yellow, yellowish-green or colourless, the distribution of each colour being different in different species. The frontally binocular fields of the pigeon retina have a preponderance of red oil droplets, acting as a fine grain red filter over the area (Pedler and Boyle, 1969), and probably influence spectral sensitivity in that area of the retina. Colour preference data, however does not strictly correspond to spectral sensitivity (Thompson, 1971). We must therefore look further afield for mechanisms governing colour preference.

Motivational states have been shown to affect colour preference. Delius (1968) found that hungry pigeons pecked at yellow-green and green stimuli, while thirsty pigeons pecked more at stimuli towards the blue-green and blue end of the spectrum. The shift in preference, here due to a

change in motivational state may have been mediated by a physiological change resembling dark adaptation producing a preference toward the blue end of the spectrum. For, as Delius (1968) pointed out, it has been suggested that central factors are capable of modulating adaptation. Ethanol ingestion in man affects flicker fusion in a manner similar to the onset of scotopic vision (Granger and Ikeda, 1968). Also receptive field organization of monkey retinal units is changed by frontal lobe stimulation in a manner analagous to dark adaptation (Spinelli and Pibram, 1966).

If the motivationally induced preference for blue is indeed mediated by a physiological change resembling dark adaptation, then dark adaptation itself could produce a shift in colour preference towards blue. In the present experiment pigeons were tested for a preference between red and blue under three conditions of dark adaptation.

It is also known that the intensity of the stimulus affects preference within a single hue in a monotonic function for red and blue in light-adapted herring gull chicks (Delius and Thompson, 1970). The present experiment also varies the intensity of the red and blue stimuli within each level of dark adaptation in order to determine the effects of dark adaptation on the interaction between hue and intensity.

A pilot study was first conducted to determine effects of intensity of hue of the stimulus and dark adaptation level.

In this study intensity of the hue was varied in four steps and dark adaptation was varied in three steps, being, no dark adaptation, fifteen minutes of dark adaptation and half an hour of dark adaptation. It was discovered later, however, that complete dark adaptation in some birds is not accomplished for at least an hour after being placed in the dark (Blough, 1957). The experiment therefore was repeated, with subjects undergoing two hours of dark adaptation before being tested.

In the preliminary study a significant preference for blue was found ($t = 9.941$; $p < .001$) but there were no effects of dark adaptation or of intensity of the stimulus, nor were there any interactions. Raw data and analysis of variance details are given in Appendix B. Three of the birds used in the preliminary study were the same as those used in the second experiment.

Method

Four pigeons of various breeds and sources were deprived to and maintained at 80% of their original body weight. They were given water ad lib throughout the experiment. Subjects were trained to peck at either of two keys (they were rewarded for pecking either key) illuminated from behind by two incandescent bulbs shining through heat filters, interference filters and ND filters. The optical apparatus was enclosed in a box so that no stray light was allowed to enter the experimental room from outside the skinner box. Subjects were rewarded for each single peck, but had to cross a platform to a hopper containing food, by which action the keys were reilluminated and the apparatus reset to raise the hopper upon a key being pecked. This was so that only the first peck after the previous reward was rewarded. The hopper was illuminated and lifted to a feeding position for three seconds after a key had been pecked.

The skinner box was metal on three sides painted medium grey inside, and had a perspex lid with three fluorescent tubes one, two, or three of which could be turned on at a time to illuminate the inside of the box. There was never any illumination other than these fluorescent tubes inside the box, except for the keys or the hopper light.

The interference filters which transmitted approxi-

mately equal intensities, were red (639 nm) and blue (422 nm). The red filter was always used with a neutral density filter of 1.0 log unit in order to equate the red and blue for brightness for pigeons (Blough, 1957). The brightness of the keys with just coloured filters and no neutral density filters was 1.4 log ft. lamberts. Neutral density filters besides the one of 1.0 log unit always used with the red filter, of .8, 1.6, 2.4, 3.2 and 4.0 log units were used with the interference filters to vary the intensity of the keys in six steps, the sixth being no extra ND filter. Each subject was presented with all thirty-six possible combinations of intensities of red and blue keys twenty times, ten trials with the blue on the right and ten trials with the blue on the left. These were repeated under three levels of illumination, the first with all three fluorescent tubes turned on, the second with one and the third with none. With the first illumination condition the birds were completely light-adapted, with the second they had been dark adapted for half an hour and with the third they had been dark adapted for two hours. Subjects usually ran eighty trials per day under one adaptation and illumination condition, except in the third adaptation condition where they were only allowed to remain in the box for ten minutes after being dark adapted so that they would not become light adapted while being tested.

Within each adaptation condition the order of presentation of the stimuli was randomized. The same stimuli were presented for ten trials in a row, then the sides reversed for another ten trials.

The number of pecks to the blue key in ten trials, the side the blue key was on, and the time of day was recorded for the three conditions of illumination and adaptation, and the combinations of intensities of the red and blue keys for each trial.

Results

Raw data for all birds used in the experiment are shown in Table I. (All tables and figures pertaining to this experiment are found in Appendix C). It was found that two of these birds (2 and 4) exhibited marked side preferences, and so their data were not considered in the analysis. Data for just the two birds retained for analysis are shown in Table II, where the number of pecks to left or right are now shown, but combined to show only the number of pecks to blue.

A three-way analysis of variance, within subjects design, was carried out on the data for the two birds. A source table is shown in Table III. The only significant effects were of the intensity of the blue stimulus by itself and an interaction between the intensity of the blue stimulus and the level of dark adaptation of the subjects. The means for each intensity of the blue stimulus are shown in Table IV and the means for each intensity of the blue stimulus under each level of adaptation are shown in Table IV and graphically in Figure 1.

A trend analysis was carried out on the characteristics of the effect of the intensity of the blue stimulus (B) and its interactions with the effects of dark adaptation level (A) and of the intensity of the red stimulus (R). A source table for this analysis is shown in Table V. The significant effects

were of B linear times A and of B cubic times A times R. The B cubic effect approached significance ($p = 0.0664$). When the F ratio for the B linear effect is obtained by employing the pooled error term (see Table III) it reaches significance ($F = 39.3949$, $p < .025$).

Discussion

It is possible to conclude from these results that the effect of intensity of the stimulus on pecking preferences is dependent on the spectral composition of the stimulus. That effect also interacts with the effect of the level of dark adaptation. The failure to find any effect of the intensity of the red is in agreement with the findings of Delius and Thompson (1970) in gulls. The blue-intensity effect is perhaps surprisingly, analogous to their findings with white stimuli, that is, a decreasing monotonic function of decreasing intensity of the stimulus. One might have expected the intensity of the blue effect to be more similar to their intensity of green effect which was a U shaped function. In terms of spectral composition blue is perhaps closer to green than it is to white.

The lack of the effect of dark adaptation by itself may have been because of the paucity of subjects used in the analysis. Looking at the data for the two birds with side preferences (see Table I, birds 2 and 4) one can see that their side preferences were disturbed in the dark adapted condition (Level 1). It seems evident that pecking preferences do change with dark adaptation. What is needed is more data from more subjects on the effect of dark adaptation by itself on colour preference. The effect observed in this

experiment although not significant was in the direction of an inverted U function, with the greatest preference for blue shown at the middle level of dark adaptation. (Means for Levels 1, 2 and 3 were 14.7361, 14.9306 and 14.0893 respectively). If the increased preference for blue in the middle adaptation condition could be reliably demonstrated it might partially be explained by the disturbance of preferences generally under the condition of dark adaptation, that is, by the lack of increased preference under complete dark adaptation.

There was however, a significant interaction between the effects of intensity of the blue and the level of dark adaptation. It appears that intensity of blue has less effect in a dark adapted condition than it does in more light adapted conditions. In light-adapted and partially dark adapted conditions blue is more preferred when it is more intense and the relationship is linear as the trend analysis showed. Under dark adaptation, however the intensity of the blue has little effect, perhaps again illustrating a lack of preference in scotopic conditions. For not only are lower intensities of blue more preferred under scotopic conditions but also higher intensities of blue are less preferred. However, the mean number of pecks to blue under the dark adapted condition is not significantly different from the other two levels of adaptation. This suggests that

the same preference for blue is exhibited, but intensity is no longer a significant determinant of pecking preferences.

It may be that the level of dark adaptation was only effective for the two lowest intensities of blue stimulus (see Fig. 1). Certainly the differences between adaptation conditions (or at least between L1, and L2 and L3 combined) are greater at lower intensities of blue. If the effect is only real at lower intensities of blue, then the relatively increased preference for blue is in keeping with Delius' (1968) hypothesis that dark adaptation may be analogous to changes in motivational states. He found a shift to blue under hunger, from green-blue under thirst. A preference shift to blue has also been demonstrated (Curtius, 1954) when chromatic stimuli are presented against a dark background (perhaps producing the same conditions as in the dark adapted condition here) rather than against a light background.

In terms of rod-cone interaction the hypothesis put forward by Delius and Thompson (1970) states that the blue modulator system is subject to rod inhibition in the photopic state and the red is not, since rods are not sensitive to red, and therefore the blue preference is released under dark adaptation. It may be that what is released under dark adaptation is sensitivity to blue, a retinal phenomenon.

This is evidenced in the fact that the "darker" blues are preferred only in scotopic as opposed to photopic conditions. But even if brightnesses under scotopic conditions were compared to equivalent brightnesses under photopic conditions, as equated by Blough's (1957) sensitivity curves for pigeons, the range presented is not enough for adequate comparison at all levels of intensity. The trend evident in the data (B linear times A) would seem to indicate that comparing data points for the L2 and L3 conditions to those in the L1 condition shifted to the right, would not make any significant difference in the analysis. It is therefore not possible to account for the results simply in terms of changed spectral sensitivity under scotopic conditions.

The combined effects of intensity and dark adaptation on the preference for blue is a fairly complex interaction and perhaps an effect quite different from either that of intensity by itself or of dark adaptation by itself. What we should seek to know before a more complete interpretation of the interaction is undertaken is whether there is any effect of dark adaptation by itself using only lower intensities of blue on blue preference, or whether it is, as would appear from the data here, entirely dependent on the intensity of the stimulus.

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APPENDIX B

	BLUE	1				2				3				4			
	RED	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
A1	S1	7	5	5	9	8	10	4	8	7	9	9	10	1	1	3	5
	S2	0	1	0	0	0	0	0	3	1	1	1	6	1	1	3	5
	S3	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	S4	5	5	5	8	5	7	5	8	3	9	6	7	5	8	6	7
A2	S1	4	10	10	10	8	7	8	10	8	5	10	10	6	2	10	6
	S2	8	5	9	6	6	8	8	9	5	5	10	7	5	8	5	5
	S3	10	8	10	10	10	10	6	10	10	8	5	10	10	8	9	7
	S4	5	5	5	10	10	5	10	9	6	10	5	10	9	9	10	4
A3	S1	5	10	10	1	7	6	6	10	2	8	7	10	5	5	7	9
	S2	5	5	7	5	6	5	5	5	5	7	5	5	6	5	5	10
	S3	9	10	9	8	10	7	10	10	9	5	4	6	5	5	6	9
	S4	5	6	5	8	9	10	5	10	6	6	5	7	2	7	6	5

TABLE i: Data for four birds in pilot study. Number of pecks to blue out of ten for three levels of dark adaptation (A), four intensities of blue and four intensities of red.

Source	SS	df	MS	F
R	510419861503.9	3	170139910144	1.844
B	201249849344	3	67083280383.9	.461
RB	568332976127	9	63148105728	1.040
S	1171663093760	3	390554320896	
RS	812916801535	9	90324974495.9	
BS	1308748677120	9	145416519680	
RBS	1639988592640	27	60740317183.9	
A	1249059536896	2	624529768448	1.858
RA	470519578624	6	78419918848	.749
BA	278436380672	6	46406062079	.337
RBA	1081971048448	18	60109500416	.656
SA	2016762920960	6	336127131647	
RSA	1883633614848	18	104646311936	
BSA	2479061204992	18	137725607936	
RBSA	4947098730495.9	54	91612905472	
TOTAL	20619852775424	191		

Table ii: Source table for analysis of variance carried out on data from four birds used in pilot study. R, intensity of red stimulus; B, intensity of blue stimulus; A, level of dark adaptation.

APPENDIX C

Bird 1

	Blue	1		2		3		4		5		6	
	Side	L	R	L	R	L	R	L	R	L	R	L	R
	Red												
	1	8	0	6	9	5	8	0	10	0	2	0	6
	2	0	10	0	10	1	3	0	10	0	9	10	6
Level 1	3	0	0	3	10	0	10	2	9	2	8	0	5
	4	10	10	9	10	0	10	3	9	0	10	0	8
	5	10	2	4	10	3	10	4	10	2	10	0	9
	6	0	8	0	8	6	10	2	10	0	10	2	10
	1	3	10	6	10	1	10	10	0	0	2	0	0
	2	8	10	0	10	7	10	8	9	1	0	10	10
Level 2	3	0	8	10	10	10	9	6	10	10	0	2	1
	4	0	10	10	10	10	10	10	9	0	10	0	10
	5	0	10	4	10	3	10	10	8	2	9	0	10
	6	3	10	10	10	10	10	1	10	7	7	6	2
	1	0	10	3	10	0	10	9	3	0	7	0	2
	2	9	10	10	10	9	10	6	10	2	0	0	2
Level 3	3	10	9	8	10	10	9	0	10	0	9	0	2
	4	10	10	10	9	2	10	10	10	8	4	0	2
	5	9	10	10	10	5	10	1	9	5	4	6	3
	6	5	10	1	10	10	10	9	10	1	8	4	5

(cont'd.)

Bird 2

	Blue	1		2		3		4		5		6	
	Side	L	R	L	R	L	R	L	R	L	R	L	R
	Red												
	1	0	4	8	3	0	10	0	10	5	10	0	6
	2	1	2	5	5	0	8	2	4	1	4	0	9
Level 1	3	0	6	1	8	0	10	1	2	3	10	0	10
	4	8	1	0	10	10	0	10	4	0	10	6	10
	5	1	6	1	0	8	10	8	2	10	9	10	0
	6	5	7	7	0	3	5	7	8	0	8	6	5
	1	0	10	0	10	0	10	7	1	0	10	0	8
	2	0	10	0	10	0	6	10	1	0	10	0	10
Level 2	3	0	10	0	10	0	10	0	10	0	10	0	10
	4	0	10	0	10	1	10	9	1	0	10	0	10
	5	10	1	0	10	0	10	0	10	0	10	0	10
	6	0	0	0	10	0	10	0	10	0	10	0	10
	1	0	10	0	10	0	10	0	10	0	10	0	10
	2	7	6	0	10	0	10	0	10	8	0	0	4
Level 3	3	8	7	0	10	0	10	0	10	2	6	0	10
	4	0	10	3	10	0	0	9	4	0	9	0	10
	5	2	10	0	10	0	10	0	10	0	10	0	10
	6	0	10	2	10	10	3	0	10	7	6	0	10

(cont'd.)

Bird 3

	Blue	1		2		3		4		5		6	
	Side	L	R	L	R	L	R	L	R	L	R	L	R
	Red												
	1	10	10	10	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	9	10	10	10
Level 1	3	10	10	10	10	10	10	10	10	10	10	10	8
	4	10	10	10	8	8	10	10	5	10	8	10	10
	5	9	5	10	10	10	10	10	10	10	10	10	4
	6	9	10	10	10	10	10	9	4	10	10	9	8
	1	10	10	9	0	10	10	10	6	10	1	9	1
	2	10	10	10	10	10	9	9	9	8	7	8	8
Level 2	3	10	6	10	9	10	10	10	3	9	5	9	4
	4	10	9	10	10	10	9	8	10	9	1	8	3
	5	10	9	10	7	10	10	10	4	9	6	9	0
	6	9	10	10	10	9	9	10	6	9	5	10	9
	1	10	10	10	10	10	10	10	10	10	10	10	9
	2	6	10	10	10	10	10	10	10	0	9	4	9
Level 3	3	7	10	10	10	10	10	10	7	6	2	10	9
	4	10	10	10	10	10	10	4	9	9	9	10	8
	5	10	0	10	10	10	5	10	9	10	8	10	8
	6	10	10	10	10	3	10	10	6	6	4	10	8

(cont'd.)

Bird 4

	Blue	1		2		3		4		5		6	
	Side	L	R	L	R	L	R	L	R	L	R	L	R
	Red												
Level 1	1	10	0	9	0	6	8	9	3	5	0	5	10
	2	10	0	10	0	10	0	10	0	4	0	8	0
	3	8	0	10	0	9	1	6	0	10	2	5	3
	4	10	1	10	1	5	3	10	0	10	0	8	3
	5	9	7	10	2	10	0	3	3	10	0	9	0
	6	10	4	10	6	10	4	9	3	7	5	10	0
Level 2	1	10	0	10	0	6	2	9	0	10	0	10	0
	2	10	0	10	0	10	0	10	1	9	0	10	0
	3	10	0	10	0	8	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0	10	0	10	0
	5	10	0	10	0	9	1	8	2	10	0	9	0
	6	10	0	10	0	10	0	10	0	10	0	10	0
Level 3	1	10	0	10	0	10	0	10	0	9	0	10	0
	2	10	0	10	0	10	0	10	0	0	5	10	0
	3	10	5	10	0	10	0	10	0	10	1	10	0
	4	10	0	10	0	9	10	10	0	10	0	10	0
	5	10	0	10	0	10	0	10	0	10	0	10	0
	6	10	0	10	1	10	1	10	0	10	0	10	0

Table I: Raw data for all four birds used in the experiment. Number of pecks to blue out of ten for two sides (L, left; R, right) six intensities of the blue stimulus, six intensities of the red stimulus and three levels of dark adaptation.

	Blue	1		2		3		4		5		6	
	S	1	2	1	2	1	2	1	2	1	2	1	2
	Red												
	1	18	20	15	20	13	20	10	20	2	20	6	20
	2	10	20	10	20	4	20	10	20	9	19	14	20
Level 1	3	9	20	13	20	10	20	11	20	10	20	5	18
	4	20	20	19	18	10	18	12	15	10	18	8	20
	5	12	14	14	20	13	20	14	20	12	20	9	18
	6	18	19	10	20	16	20	12	13	10	20	12	17
	1	13	20	16	19	11	20	10	16	2	11	0	10
	2	18	20	17	20	17	19	17	18	1	15	20	16
Level 2	3	18	16	20	19	19	20	16	13	10	14	3	13
	4	10	19	20	20	20	19	19	18	10	10	10	11
	5	20	19	14	17	13	20	18	14	11	15	10	9
	6	13	19	20	20	20	18	11	16	14	14	8	19
	1	10	20	13	19	10	20	12	18	7	15	2	15
	2	19	20	20	17	19	20	16	12	2	16	2	19
Level 3	3	19	20	18	20	19	15	10	11	9	8	6	9
	4	20	20	19	20	12	14	20	13	12	10	2	15
	5	19	18	20	19	15	15	10	11	9	8	9	10
	6	15	20	11	19	20	17	19	11	9	8	9	10

Table II: Number of pecks to blue stimulus out of 20 for two birds over six levels of each intensity of blue and red stimuli and three levels of dark adaptation.

Source	SS	d.f.	MS	F	P
Total	6008.500	215	27.947		
Within	4932.926	214	23.051		
A	28.361	2	14.181	0.0985	0.9098
error	287.787	2	143.894		
R	157.056	5	31.411	0.6109	0.6999
error	257.093	5	51.419		
B	1638.111	5	327.622	9.5651	0.0150
error	171.259	5	34.252		
AR	151.083	10	15.108	1.5980	0.2354
error	94.546	10	9.455		
AB	293.028	10	29.303	3.6008	0.0279
error	81.380	10	8.138		
RB	306.333	25	12.253	1.1056	0.4018
error	277.074	25	11.083		
ARB	650.528	50	13.011	1.2063	0.2546
error	539.287	50	10.786		

Table III: Source table for analysis of variance on data for two birds. A, level of adaptation; R, intensity of red stimulus; B, intensity of blue stimulus.

	Intensity of blue stimulus					
Level	1	2	3	4	5	6
1	15.9167	16.5833	15.3333	14.7500	11.9167	13.9167
2	16.2500	18.5000	18.0000	15.5000	10.5833	10.7500
3	18.3333	17.9167	16.3333	13.5833	9.4167	8.9167
$\underline{m}$	16.8333	17.6667	16.5555	14.6111	10.6389	11.1984

Table IV: Mean number of pecks to blue out of 20 for each intensity of blue and for each level of dark adaptation. Intensity, 1--6, brightest to darkest. Adaptation; level 1, 2 hours dark adaptation; level 2, $\frac{1}{2}$ hour dark adaptation; level 3, no dark adaptation; $\underline{m}$, mean number of pecks to blue out of 20 for each intensity of blue.

Source	SS	df	MS	F	P
B lin	1349.340	1	1349.340	28.0778	0.1244
error	48.057	1	48.057		
B lin A	22.406	2	111.203	19.8281	0.0468
error	11.217	2	5.608		
B lin R	56.508	5	11.302	1.0537	0.4779
error	58.629	5	10.726		
B lin AR	137.661	10	13.766	0.6025	0.7819
error	228.469	10	22.847		
B quad	70.583	1	70.583	0.8890	0.5181
error	79.398	1	79.398		
B quad A	58.609	2	29.305	1.3924	0.4179
error	42.092	2	21.046		
B quad R	70.980	5	14.196	0.7622	0.6140
error	93.126	5	18.625		
B quad AR	152.220	10	15.222	1.332	0.2254
error	93.206	10	9.321		
B cub	165.632	1	165.632	92.0178	0.0664
error	1.800	1	1.800		
B cub A	8.435	2	4.218	0.3378	0.7473
error	24.973	2	12.487		
B cub R	78.920	5	15.784	2.3634	0.1833
error	33.393	5	6.679		
B cub AR	125.952	10	12.595	2.8730	0.0557
error	43.840	10	4.384		

Table V: Source table for analysis of trends of B (intensity of Blue) effects and their interactions. B lin, B linear; B quad, B quadratic; B cub, B cubic; A, level of adaptation; R, intensity of red stimulus.

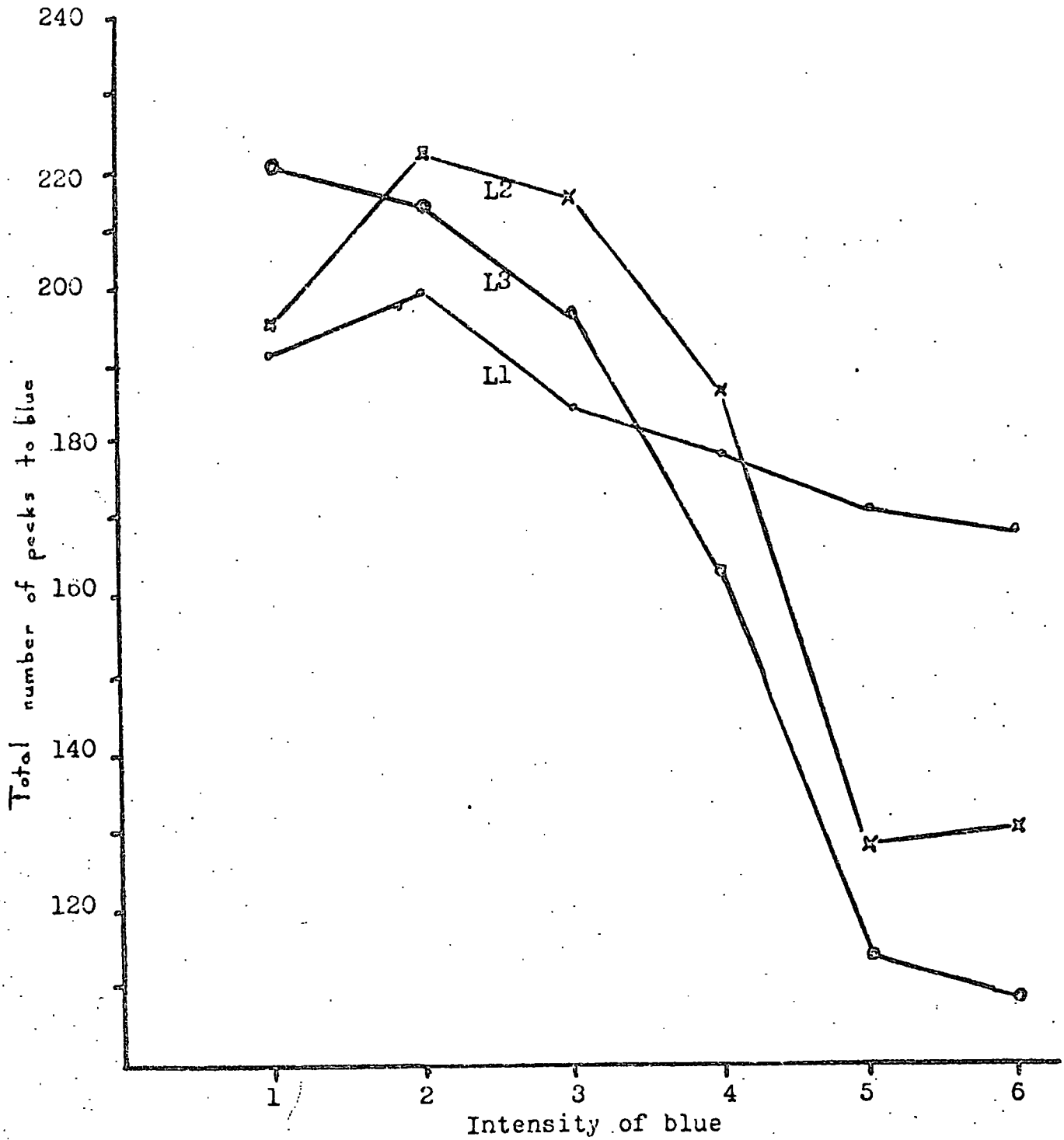

Figure 1: Total number of pecks to blue out of 240 over all intensities of red, for each intensity of blue (1-6, brightest to darkest) and for each level of dark adaptation. L1, 2 hours; L2, ½ hour; L3, no dark adaptation.

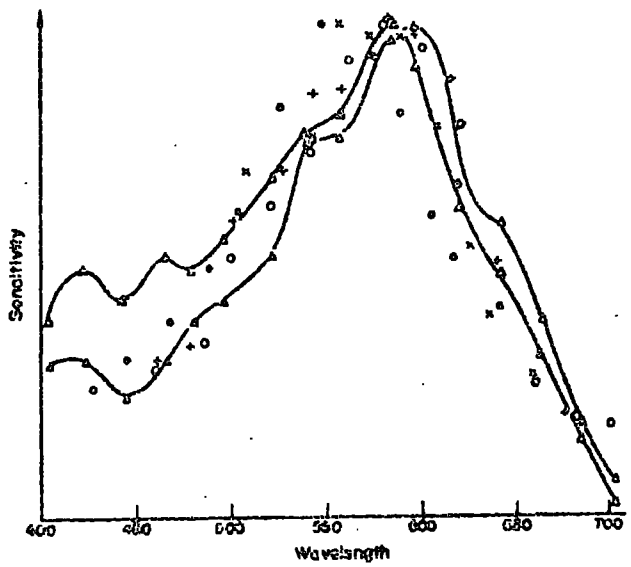

Figure 2. Electroretinographic spectral sensitivity of gulls (ΔΔΔ, juveniles; ΔΔΔ, adults, Thompson, 1970); electrophysiological sensitivity of pigeons (ooo, Granit, 1942; +++, Donner, 1953; •••, Ikeda, 1965) and chicken (×××, Armington and Thiede, 1956). (Thompson, 1970)