

STUDIES IN REPTILIAN COLOUR RESPONSE

II. THE ROLE OF RETINAL AND DERMAL PHOTORECEPTORS IN THE PIGMENTARY ACTIVITY OF THE CHAMELEON

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IN a recent paper (Zoond and Eyre, 1934) the responses of blind and normal chameleons on different backgrounds and with strong and weak illumination were described. Starting with animals adapted to darkness (maximum pallor) colour indices, recorded at intervals of 2 min., were plotted. The time curves thus obtained demonstrated the following facts:

(a) In strong light on a black background the animals darken rapidly, and attain a maximum intensity after about 4 min.

(b) Blind animals under the same conditions behave in a similar way.

(c) In strong light, on a white background, intact chameleons darken perceptibly during the first 2 min. and then slowly become paler, but they never attain a pallor as extreme as in total darkness.

(d) Blind animals under the same conditions darken in much the same way as they do on a black background.

(e) In weak light, on a black background, intact chameleons darken quite as intensely as in strong light, but blind animals under these conditions remain pale.

(f) On a white background, in weak light, the intact animals become slowly *dark*, the blind animals remain relatively pale.

From these observations it was concluded that the pigmentary responses of the chameleon depend upon the stimulation of two separate receptor fields: the dermal and retinal photoreceptors. The dermal receptors have a higher threshold of stimulation than the retinal ones. Owing to the fact that in darkness the melano-phores are maintained in a state of tonic contraction, it was inferred that melano-phore expansion (*i.e.* darkening) is due to an inhibition of sympathetic impulses. Such inhibition occurs when the dermal photoreceptors are stimulated by light sufficiently intense to excite them, and when the retinal photoreceptors are exposed to an environment in which light falls from above upon a light absorbing background. Further, consideration of the time curves and their relation to one another suggested a possible interpretation of the response to white background in strong light. The theory was advanced that the stimulation of the retinal receptors by light from a light-scattering background gave rise to an inhibition of the inhibition due to the simultaneous direct stimulation of the dermal receptors.

The validity of such an explanation is open to direct experimental confirmation. It should be possible to show that the white background response cannot occur unless the skin and the eyes are simultaneously stimulated. Section of the optic nerves serves to isolate the activity of the dermal photoreceptors. In order to isolate the activity of the retinal receptors it is necessary to employ some method of excluding light from the skin, while at the same time leaving the eyes exposed to the requisite conditions of stimulation. This was achieved by Carlton (1903) with *Anolis carolinensis* by enclosing the animal in a dark box with its head projecting through a hole in the side. Actually Carlton did not envisage the distinction between the retinal and dermal receptors, his aim in performing these experiments was to discover "whether the illumination of one part of the body had any influence on the colour changes in those parts which were not illuminated." Accordingly, he carried out two series of determinations, the first on animals with the body inside the box and the head outside, and the second on animals with head inside and body outside. In the first series all the animals tested turned brown all over, in the second series contradictory results were obtained. Since Carlton was unaware of the importance of the nature of the background, his results shed little light on the present issue.

The procedure adopted in our experiments was to enclose the body of the chameleon up to the neck in a small sack made of broad ribbon. The sack had a longitudinal slit along the greater part of its length through which the animal was inserted. The head was made to project through a closely fitting hole, and the four limbs through four other little holes suitably spaced. When the animal had been inserted into the sack it was tied to a rectangular piece of cardboard by its feet, and then the longitudinal slit was closed above the animal's back by means of a puckering thread which could be instantaneously withdrawn by a single pull, thus permitting the observer to inspect the animal's body at any desired moment with practically no handling. It was necessary to tie the animals down because otherwise it was found impossible to prevent them from drawing their heads back into the sack. This procedure does not interfere with the normal colour responses, indeed the photographs reproduced in the previous paper (Zoond and Eyre, *loc. cit.*) were taken with the chameleons secured in this way.

For recording the black background response black ribbon was used, and the animals were tied to black cards. For the white background response the sacks were made of material which was black on the inside and white outside, and the animals were tied to white cards. Thus the chameleon's field of vision was occupied by black or white surfaces respectively. The efficiency of the sacks in excluding light from the skin was tested out on blind chameleons. In every case the characteristic pallor of the dark-adapted skin was maintained.

Ten animals were recorded in each experiment. After they had been sewn into the sacks and secured to the cards, they were left in a photographic dark room for twenty minutes to allow them to attain maximum pallor, and were then exposed to weak or strong illumination as in previous experiments. Their colour indices were recorded after 20 min. exposure.

Chameleons treated in this way displayed a curious tendency to go to sleep; in

every batch of ten animals there were several which had their eyes closed for part of the duration of exposure. It was not always possible to induce them to open their eyes permanently by prodding them. It was noticeable that animals which had been asleep tended to be paler when their colour index was recorded than those which had not. This tendency undoubtedly served to depress somewhat the mean values obtained. No satisfactory method of correcting this source of error has suggested itself, and we therefore deemed it safer to ignore it in making the determinations. Nevertheless it is necessary to bear this factor in mind when making comparisons between mean colour-index values.

In the data given below the mean values for a number of determinations are shown together with their standard deviations. The data refer to normal animals (retinal and dermal stimulation), animals with optic nerves cut (dermal stimulation only), and animals enclosed in sacks (retinal stimulation only). We have designated these three groups normal, dermal and retinal respectively. The means for the retinal groups are based on duplicate records of twenty animals. The means for the normal and dermal groups are quoted from the previous paper and are based on fifteen or twenty-five animals. All determinations were carried out at room temperatures ranging from 14 to 17° C.

(a) *The response to black background.*

Illumination	Normal	Dermal	Retinal
Daylight	4.68 ± 0.11	4.35 ± 0.15	3.74 ± 0.19
Lamplight	4.93 ± 0.04	2.05 ± 0.05	4.37 ± 0.12

The results show that melanophore expansion occurs when the field of vision is occupied by a light-absorbing surface and also when light of sufficient intensity falls upon the skin. The lamplight employed in these experiments was the same as was used in the previous investigation. It appears that this light is barely strong enough to stimulate the dermal receptors although it can evoke a strong retinal response to black background. Thus it follows that the response of normal animals to black background in daylight is a compound of dermal and retinal responses, whereas in weak light the response is purely retinal. It is certainly dangerous to speak of relative intensities of stimulation when one stimulus is light falling directly upon the skin, and the other is a complex visual field in which light falls from above upon a light-absorbing surface, but in so far as the two responses can be studied under identical conditions of illumination it is legitimate to conclude that the threshold of stimulation is lower for the retinal than for the dermal photoreceptors. The theory that the photic stimulation of the dermal receptors and exposure of the retinal receptors to a field in which light falls upon a light absorbing surface independently cause inhibition of the tonic innervation of the melanophores is confirmed.

There remains, however, the possibility that the differences between the five groups which show darkening are not insignificant. The mean difference between the normal and retinal groups divided by the standard deviation of the difference

gives the value $\frac{N-R}{\sigma_{n-r}} = 4.3$ both for the daylight and the lamplight determinations.

It cannot be assumed that these differences are entirely to be assigned to the depressing effect of the closing of the eyes during the group *R* determinations, although this factor probably accounts for a part of the difference. If these differences are regarded as significant, they are to be interpreted as showing that inhibition of the tonic innervation is not maximal in all circumstances.

(b) *The response to white background in daylight.*

Exposure	Normal	Dermal	Retinal
2 min.	3.49 ± 0.17	4.10 ± 0.21	1.85 ± 0.17
20 min.	2.43 ± 0.12	4.75 ± 0.11	1.50 ± 0.10

In view of the initial darkening shown by normal animals when exposed to daylight on a white background, it was considered advisable to obtain retinal group records after 2 min. exposure as well as after 20 min. The results for this group show that *when illumination of the skin is excluded, the white background response does not occur*. The melanophores remain throughout in the condition of extreme contraction, although the eyes received light from a light scattering surface. The mean for twenty dark-adapted animals recorded immediately on being taken from the dark room was 1.55 ± 0.10 . It is clear that after 20 min. exposure the retinal group animals had undergone no change. A very slight darkening appears to have occurred after 2 min., but its significance is doubtful. The significance quotient for the difference between the retinal group 2 min. mean and the mean for dark adapted normals is $\frac{R-N}{\sigma_{r-n}} = 1.5$.

The theory that the white background response is an inhibition of the inhibition resulting from the stimulation of the dermal photoreceptors receives striking confirmation from these results. The conditions are such that dermal stimulation alone produces melanophore expansion, retinal stimulation alone produces no effect, the two together result in an initial expansion followed by a partial contraction. These observations are only explicable on the assumption that the stimulation of the retinal receptors under these conditions does not directly affect the pigmentomotor apparatus, but exerts an influence upon another mechanism which is brought into play by the activity of the dermal receptors. This influence cannot be other than an inhibition, since it has been clearly demonstrated that melanophore expansion results from a release from tonic sympathetic control.

(c) *The response to white background in lamplight.*

Normal	Dermal	Retinal
3.98 ± 0.19	2.48 ± 0.16	3.11 ± 0.19

It was shown in the previous paper that when the light is sufficiently weak the white background response is reversed and the chameleons assume a dark shade. It was suggested that this response occurs over the range of intensity between the threshold for retinal and the threshold for dermal stimulation, though this is not a statement which can be made with emphasis before these thresholds have been photometrically determined. The lamplight employed in our experiments was not altogether without effect on the dermal receptors (dermal group). On a black background there was very slight darkening (2.0), but on a white background, which would reflect a greater amount of light, the darkening was more pronounced (2.5). Nevertheless the data show clearly that under these conditions the stimulation of the retinal receptors exerted a direct influence upon the pigmentomotor apparatus, and caused an expansion of the melanophores. The contrast between the retinal group values for white background in weak and in strong light leaves little doubt that the conditions of *retinal* stimulation in light of high and low intensity are physiologically fundamentally different. The value obtained for the retinal group, however, was considerably lower than for the normal group, and the difference is not negligible: $\frac{N-R}{\sigma_{n-r}} = 3.2$.

If the white background response in weak light is due entirely to retinal stimulation then it is not apparent why such a difference should exist. Provisionally it may be pointed out that the light employed was probably somewhat stronger than the optimum for the low intensity response. The distribution of the individual scores for the *retinal group* shows satisfactory evidence of two modal values, and in the records for the normal group there is also some indication of two modes. This suggests that the light intensity employed was on the borderline between the high intensity and the low intensity ranges, and that some of the animals in the experiment were giving the low intensity response, others the high intensity response. Such an assumption would serve to account for the two modes, and it would also explain the difference between the normal and retinal group means, but it can hardly be made with confidence until further determinations with light of lower intensity have been carried out.

SUMMARY.

1. A method has been devised for measuring the effect of retinal stimulation with the exclusion of light from the dermal photoreceptors.
2. Stimulation of the dermal photoreceptors and exposure of the retinal receptors to light falling on a black surface independently cause an inhibition of the pigmentomotor apparatus.
3. The response to white background in strong light depends upon the simultaneous stimulation of the retinal and dermal photoreceptors.
4. The stimulation of the retinal receptors causes an inhibition of the inhibition resulting from the direct stimulation of the dermal photoreceptors.
5. In weak light the white background response is reversed. This effect is due to retinal stimulation and is independent of the dermal receptors.

REFERENCES.

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