Visual Acuity of Essential Fatty Acid-Deficient Rats

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1. Rats were maintained on a diet deficient in fat and on a normal diet of rat cubes. 2. Rats were trained to discriminate between vertical and horizontal striations. The minimal stripe width that could be used for discrimination was determined. 3. In bright illumination (0.7 or 4.5 ft.lamberts) both deficient and normal rats had the same ability to discriminate between black and white stripes. 4. With an illuminance of 0.002 ft.lambert, supplemented rats could discriminate as efficiently as at 0.7 ft.lambert, but deficient animals were unable to discriminate at 0.002 ft.lambert. 5. Control rats had 14% of docosahexaenoic acid in their retinal fats but the deficient rats had only 1%. 6. Deficient animals had no vitamin A stores in the liver whereas the control animals had about 190 i.u./g. 7. The visual acuity of the rats used was about 45' of arc.

all-*cis*-Docosa-4,7,10,13,16,19-hexaenoic acid has been shown to be a major constituent of the fatty acids of ox retina (Bartley, van Heyningen, Notten & Renshaw, 1962; Hands & Bartley, 1962). To test whether loss of docosahexaenoic acid from the retina affected the visual acuity of the rat, retinal concentrations of this acid and the threshold of visual acuity under light- and dark-adaptation were measured in rats fed on a diet deficient in essential fatty acids and in rats normally fed.

100; inositol, 500; choline, 2000; biotin, 0.43; pyridoxine, 40; *p*-aminobenzoic acid, 250; calcium pantothenate, 240; vitamin A acetate, 75; tocopherol, 500; calciferol, 0.375; the last three were given in tributyrin (0.1 ml.) and the rest in 95% ethanol (0.1 ml.). For 10 weeks the two groups were fed for 24 hr./day, but subsequently the diet was offered for reduced periods, so that after 5 days the control animals had access to food for 1 hr./day and the deficient animals for 2 hr./day. Training the rats to jump then began at once.

EXPERIMENTAL

Animals. Wistar strain rats were used throughout. The animals were weaned at 3 weeks and kept for 2 weeks on a diet of rat cubes [a stabilized diet for laboratory animals, no. 41B, manufactured by Herbert C. Styles (Bewdley) Ltd.]. During the fourth and fifth weeks food was available to the animals at all times. At 5 weeks the rats were divided randomly into two groups. The controls continued to receive the diet of rat cubes and the deficient group was fed on a diet of the following composition: 20 parts (by wt.) of casein (Genatosan, low in vitamins, extracted twice with 50% ethanol, once with 90% ethanol and once with diethyl ether); 70 parts (by wt.) of sucrose; 5 parts (by wt.) of mineral salts. The salt mixture contained (parts by wt.): NaCl, 104; anhydrous MgSO₄, 166; NaH₂PO₄·2H₂O, 204; K₂HPO₄, 272; CaHPO₄·2H₂O, 324; ferric citrate pentahydrate, 70; calcium lactate pentahydrate, 780; KI, 0.1; CaSO₄·5H₂O, 2; MnCl₂·4H₂O, 5. The daily intake of vitamins (μg.) was: thiamine, 20; riboflavin, 40; nicotinamide,

Testing visual acuity

Apparatus. Animals were trained in a modified Lashley jumping stand (Munn, 1950). The subjects are placed individually on a Y-shaped jumping platform (*J* in Fig. 1) and have to jump to one or other of two landing platforms (*L*) each 5 cm. long and 9 cm. deep: a vertical partition (*P*) separates the two platforms. The landing platforms project from a vertical wall containing two 14 cm. square openings, one above each landing platform. Doors bearing the stimuli to be discriminated can be placed in each opening: rats are trained to select one stimulus and not the other. When the rat jumps to the correct stimulus (positive) it is allowed to push the door down, walk through the opening and eat for 20 sec. on a feeding platform (*F*) behind the wall. The other door is locked so that when a rat jumps to the wrong stimulus (negative) it cannot get through the opening to obtain food: instead it is left on the landing platform for 5 sec. and then removed from the apparatus. The apparatus was painted matt grey. The stimuli used were black, white and grey cards and patterns of black and white striations of varying breadths (2, 1, 0.5 and 0.25 cm.): the striations were

Fig. 1. Modified Lashley jumping stand. The apparatus is described in the text.

either horizontal or vertical. The stimuli were illuminated by a light placed inside the rear wall of the apparatus. During most of the experiments a fluorescent discharge tube was used, but in the experiment on dark adaptation a 15 w bulb was substituted. The illumination from the bulb could be reduced by the insertion of filters in front of it. The illumination measurements given below were made with an S.E.I. exposure photometer. Measurements below 0.01 ft.lambert were made with a cadmium sulphide photometer (Cooke, Trouton and Simms) that had been calibrated against the S.E.I. photometer at the upper end of its range.

Training. A week before training was due to begin, all animals were put on a feeding schedule: they were allowed food *ad lib.* for 2 hr./day. Deficient rats were given the deficient diet described above. This feeding schedule was maintained throughout the experiments: animals were fed on each day immediately their training ended and were therefore run under about 22 hr. hunger. Deficient rats sometimes lost weight suddenly under this regime and they were then given longer access to food: despite this several died in the course of the experiment. They tended to develop mouth sores that prevented them eating altogether.

All animals were first trained to jump to the landing platforms and run through the opening without doors being present to obtain food. At first the jumping platform was adjacent to the landing platforms, but it was moved back in stages until rats had to jump the gap. The final gap was

made 18 cm. for the normal rats and 15 cm. for the deficient animals. Since the rat's head protruded beyond the jumping platform just before jumping, and since deficient rats were much smaller than normal, this meant that all animals' eyes were about 20 cm. from the stimulus when the response of jumping was made. All animals received between 50 and 60 trials of this preliminary training.

During the remainder of training animals were run with both doors in place and the one bearing the negative stimulus was locked. A modified correction procedure was used. When an animal made a mistake it was removed from the apparatus, but was re-run later, without changing the position of the stimuli, until it jumped correctly. The position of the positive and negative stimuli was varied randomly from trial to trial according to Gellermann (1933) sequences. Ten trials a day were given, with an intertrial interval of about 5 min. Animals were given 20 sec. access to food when they made a correct jump and the food available for each animal was the same as was used in its home cage diet.

Analysis of the retinae

Rats were killed by stunning and decapitation, and the eyes were removed. Rat retinae were isolated by cooling the eyes in solid carbon dioxide and cutting them in half parallel to the cornea. After warming to room temperature, the aqueous humour was extracted with a Pasteur pipette and the retinae were removed with a camel-hair brush. Retinae from the same rat were combined and hydrolysed by heating at 60° under nitrogen for 1 hr. with aq. methanolic (1:1, v/v) 10% potassium hydroxide (1 ml.) and a crystal of quinol. The cooled mixture was diluted with water (5 ml.), extracted with chloroform (2 ml.) to remove unsaponifiable lipid and acidified with 50% (v/v) sulphuric acid, and the free fatty acids were extracted with chloroform (3 × 5 ml.). The extracts were washed with water (5 ml.), dried (over MgSO_4) and treated with an excess of ethereal diazomethane to give the methyl esters. Evaporation to dryness and dissolution of the esters in ether gave solutions that were applied by the method of Renshaw & Biran (1962) to a Pye Argon chromatograph (with Apiezon L as stationary phase).

RESULTS

Visual acuity of the Wistar rat

Preliminary experiment. In a preliminary experiment four normal rats and four deficient rats were trained to discriminate between horizontal stripes and a matt grey card equated for brightness. Successively narrower stripes were used until the discrimination broke down. The illumination was 4.5 ft.lamberts throughout. Since only one deficient rat survived to the end of the experiment, the results are only briefly summarized. With stripes of widths 2, 1 and 0.5 cm., the performance of the deficient rat was about the same as the mean performance of the normal rats. All animals mastered these discriminations to a level of 100% accuracy. When 0.25 cm. stripes were used, the accuracy of normal animals deteriorated sharply (63% correct) and they

failed to improve over 100 training trials. The deficient rat, however, made no mistakes at this stage. All five rats succeeded in discriminating between horizontal and vertical stripes of 0.5 cm. width to a criterion of ten out of ten successive jumps correct. No rat was able to discriminate between 0.25 cm. horizontal and vertical stripes: on this problem performance remained at chance level. It appears then that in good illumination the visual acuity of deficient rats may be as good as that of normals, and that stripes of 0.25 cm. width at a distance of 20 cm. are about at the limits of the resolving power of the eye of this strain of Wistar rats.

Main experiment. Four further deficient rats and five normal controls were used in this experiment: three other deficient animals died before they had learned the first problem, and their results are not included below.

All animals were first trained to jump to a white card and to avoid a black card: training continued until they reached a criterion of all ten jumps correct over a single day. The four surviving deficient rats learned the problem just as fast (average 4.8 days) as the five controls (average 5.4 days).

All rats were next trained to jump to horizontal stripes and not to vertical stripes. Rats were successively trained with stripes of different widths (2.0, 1.0, 0.5 and 0.25 cm.): each rat was required to reach a criterion of ten jumps all correct on one day on each stripe width before moving on to the next. The normal rats solved the first stage (2 cm. stripes) considerably faster than the deficient (the means were 2.4 and 8.7 days). Both deficient and controls then solved the next stage (1 cm. stripes) almost immediately (2.0 and 2.2 days). When 0.5 cm stripes were used, one deficient and two normal

animals jumped correctly on every trial on the first day. The remaining normal animals all reached criterion within 9 days. None of the three remaining deficient animals reached criterion (one was trained for 11 days, one for 9 and one for 5). However, only one deficient animal performed at chance on this problem: the other two scored over 70% correct, one from the outset of training, the other from the fourth day onwards. It is clear that both normal and deficient animals can discriminate 0.5 cm. stripes: the results suggest that the deficient animals have slightly more difficulty with stripes of this width than normal rats. Four normal animals and the one deficient animal that had solved the problem with 0.5 cm. stripes were trained for 10 days on the 0.25 cm. stripes. The mean score was 47.5% correct and no animal showed any tendency to score significantly above chance. The results confirm those of the preliminary experiment: under the conditions of illumination used, the minimum stripe width that can be resolved at a distance of 20 cm. lies between 0.5 and 0.25 cm. This corresponds to a visual angle of between 43' and 1°26'.

In the final stage of the experiment, tests were made of acuity under conditions of dark-adaptation. All rats were kept in near darkness for at least 1 hr. before being tested. Their cages were placed near the apparatus and were illuminated only by the light in the apparatus at the intensity to be used for that day's training. A summary of the results of this stage of the experiment is given in Table 1.

All animals were first retrained on the broadest stripes illuminated by a 15w bulb (luminance of stimuli 0.7 ft.lambert). Performance was almost perfect. On day 2 the 1 cm. stripes were used with the same illuminance. No mistakes were made by any animal. On day 3 the illumination was reduced

Table 1. *Ability of rats to discriminate between different visual stimuli at different intensities of illumination*

The experimental procedure is given in the text. The rats were required to make the choice between two alternative stimuli and the results are given as the number of times the choice was correctly made (expressed as a percentage). Chance performance is 50% correct; errorless performance is 100% correct. The methods of measuring luminance are given in the text. Deficient rats were maintained on the fatty acid-deficient diet described in the Experimental section.

Day	Normal rats			Deficient rats		
	Luminance (ft.lambert)	Stripe width (cm.)	Performance (% correct)	Luminance (ft.lambert)	Stripe width (cm.)	Performance (% correct)
1	0.7	2.0	98	0.7	2.0	95
2	0.7	1.0	100	0.7	1.0	100
3	0.002	1.0	98	0.002	1.0	40
4	0.002	0.5	98	0.002	2.0	53
5	0.002	0.25	60	0.008	2.0	80
6				0.008	2.0	80
7				0.7	2.0	100

to give a luminance of 0.002 ft.lambert and the 1 cm. stripes were again used. The performance of the deficient rats fell off dramatically: they averaged only 40% correct, whereas the normal animals continued to discriminate almost perfectly (98% correct). The probability of this difference being due to chance is less than 0.01 (by a binomial test).

On the following day the luminance was kept the same but the broadest stripes (2 cm.) were used with the deficient animals, and normal animals were tested with 0.5 cm. stripes. Once again the deficient rats performed at chance (52.5% correct) but the normal rats performed almost perfectly (98% correct).

The deficient rats were next tested on the broadest stripes in slightly brighter light (0.008 ft.lambert). Two animals were now able to discriminate with complete accuracy, but the other two continued to perform at chance over 20 trials. On the last day of the experiment deficient animals were retested with a luminance of 0.7 ft.lambert on the broadest horizontal stripes: all animals performed without a mistake. Thus the poor performance of the deficient rats over the period in which very low intensities of light were used was not due to any general debility of the animals, but was brought about solely by the reduction in the illumination.

On day 5, the normal rats were tested on 0.25 cm. stripes with a luminance of 0.008 ft.lambert and four performed at chance. One rat, however, made ten correct responses. This animal was retested the following day and again made no mistakes. When tested with the same stripes in brighter illumination (0.7 ft.lambert), it scored 75% correct over 20 trials. It seems that after prolonged training the 0.25 cm. stripes may be just within the resolving power of the eye at least for some animals. Further, it appears that, in the Wistar strain used, the visual acuity of normal rats is at least as good in very low illumination as in comparatively bright light.

Summary of results. The black-white problem was included to discover whether there was any general deficiency in the learning ability of deficient rats; this problem was learnt as fast by the deficient animals as by the normal controls. Moreover, when tested with stripes, deficient rats again reached approximately the same performance asymptotes as did normal rats, though at certain stages of the experiment they took longer to learn. The increased time to learn may have been due to differences in the level of hunger drive or to the greater difficulty deficient rats had in jumping owing to their physical weakness. The important result is that in bright illumination there is no significant difference in the visual acuity of deficient and normal rats as measured by the finest stripe width that can be resolved: although in the main experiment the deficient rats did slightly worse than normal rats with the 0.5 cm.

stripes, in the preliminary experiment the single deficient animal did better and even gave evidence of being able to discriminate 0.25 cm. stripes.

These results are in marked contrast with those obtained under conditions of dark-adaptation. Whereas the normal rats were unimpaired on 0.5 cm. stripes with a luminance of 0.002 ft.lambert, the deficient animals performed at chance level on the broadest stripes (2 cm.) under the same illumination, and two animals were unable to resolve these stripes with a luminance of 0.008 ft.lambert.

The results indicate that visual acuity for the strain of Wistar rats used is about 45' of arc: one animal after much training was clearly able to discriminate stripes subtending this visual angle, and the remainder of the normal animals were able to discriminate stripes of twice this visual angle with complete accuracy. The threshold found is slightly higher than Lashley's (1938) estimate of about 30' of arc: this difference is in the expected direction since Lashley used pigmented rats, whereas we used an albino strain. A further finding of some interest is that the normal rats' visual acuity was at least as good in dark-adaptation as in light-adaptation. One rat actually performed better with the minimum stripe width used with a luminance of 0.002 ft.lambert than with higher illuminances.

Vitamin A content of the livers

Analysis of the livers of the four control rats that had completed the visual tests showed them to contain 204, 198, 196 and 179 i.u. of vitamin A/g. The livers of the three essential fatty acid-deficient rats that had completed the visual tests contained less than 1 i.u./g., the fourth containing 5.9 i.u./g.

Fatty acid composition of the retinæ

The fatty acid composition of the retinæ of control rats was (mean of four animals, to the nearest 0.5%): methyl palmitate, 26.0; oleate and linoleate, 16.5; stearate, 30.0; arachidonate, 10.5; docosatetraenoate, 4.0; docosahexaenoate, 14.0%. That of essential fatty acid-deficient rats was (mean of four animals, to the nearest 0.5%): methyl palmitate, 41.0; oleate and linoleate, 19.0; stearate, 39.0; docosahexaenoate, 1.0%.

DISCUSSION

Docosahexaenoic acid, a major constituent of ox retinæ (Bartley *et al.* 1962), was shown to be present in normal rat retinæ but absent from the retinæ of essential fatty acid-deficient rats. This did not affect their visual acuity at high illuminations, as measured by their ability to resolve horizontal and vertical stripes. Under conditions of dark-adapta-

tion, however, the visual acuity of deficient rats was severely impaired: when a luminance of 0.002 ft.lambert was used, normal animals were readily able to resolve stripes subtending a visual angle of $1^{\circ}32'$, but deficient animals were wholly unable to resolve stripes with a visual angle of over 12° .

Examination of the livers of the essential fatty acid-deficient rats showed them to be deficient in vitamin A, although they were receiving adequate quantities (75 $\mu\text{g.}/\text{day}$; wt. of rat approx. 120 g.) in their diet. Lewis, Bodansky, Falk & McGuire (1942) found that in growing rats 2 i.u. of vitamin A daily was sufficient to ensure the maximal retinal content of vitamin, although growth was suboptimum. For optimum growth 25 i.u. of vitamin A was sufficient but little liver storage occurred. With 50 i.u. intake the blood values become maximal and substantial liver stores were laid down. The amount received by our rats (100 i.u. daily) should have been sufficient to supply all tissue needs with a substantial surplus for liver storage.

This vitamin A deficiency could explain the much reduced dark-adaptation of the essential-fatty acid-deficient rats (cf. Morgan, 1942). It could be due either to reduced absorption through the intestine, or to an increased rate of metabolism of the vitamin. Muelder & Kelly (1942) have shown that the inclusion of 10% of fat in the diet as cotton-seed oil facilitated the absorption of vitamin A, and Basu (1937) has reported that absorption of vitamin A from the intestine was dependent on a suitable fat; moreover, Ahmad & Drummond (1931) have shown that carotene is more completely absorbed when the diet contains 10% of fat than when the diet is fat-free. However, since Mahadevan & Ganguly (1961) suggest that the absorption of vitamin A through the gut is entirely as the palmitate ester and Enser & Bartley (1962) have shown that control and deficient rats show little difference in the intestinal content of palmitate, there is no clear reason to expect an impaired absorption.

An incidental finding of some interest was that, in the Wistar strain used, the visual acuity of normal rats appeared to be at least as good with very low illumination (0.002 ft.lambert) as it was under comparatively bright light (0.7 and 4.5 ft.lamberts). This may be connected with the absence of cones in the rat retina (though this is itself controversial; cf. Walls, 1934; Detweiler, 1943). There is no trace of duplex functioning of the rat retina in Morgan's (1942) curve of dark-adaptation obtained by measuring the absolute brightness threshold. We may therefore have measured acuity when it was already at asymptote even under low illumination.

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