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DISUSE IN THE LATERAL GENICULATE NUCLEUS OF THE CAT

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SUMMARY

1. An attempt has been made to produce disuse in the lateral geniculate nucleus (LGN) of the cat, in the synapses between the optic nerve fibres and the principal cells of the nucleus. Evidence is produced that destruction of the visual receptor cells by iodoacetate or 1,5-di(*p*-aminophenoxy) pentane dihydrochloride effectively silences the optic nerve discharge and so achieves this result.

2. Disuse of the LGN synapses does not cause any decrease in synaptic efficiency. The LGN response to a single stimulus to the optic nerve was not appreciably altered, and the depression which normally follows single or repeated stimuli was much reduced or absent. This increased responsiveness was unaffected by prolonged tetanization of the optic nerve during the experiment.

3. Two possible explanations of the increased responsiveness are suggested: a post-synaptic 'decentralization'-hypersensitivity and an increased output of transmitter per impulse. The relevance of these results to theories of learning is discussed.

4. The LGN response of adult cats kept in complete darkness for periods of up to 966 days was not appreciably different from that in the normal cat and there was little or no increased responsiveness. This suggests that many retinal ganglion cells continue to discharge in total darkness for long periods. There is a possibility that disuse may develop after a long time in the dark.

INTRODUCTION

It is widely assumed that learning depends upon facilitation at specific synapses and that repeated use of a synapse brings about facilitation (see Eccles (1953, 1964) for references). Some support for this theory is provided by the demonstration of 'post-tetanic potentiation' at many synapses, although the duration of this effect, a few minutes at most, is clearly

inadequate to account for the relatively permanent changes which must accompany learning. It has also been argued by Eccles & McIntyre (1953) and by Eccles, Krnjević & Miledi (1959) that disuse of a synapse should produce depression of synaptic function. By disuse here is meant an absence of impulses in presynaptic nerve fibres. These workers obtained disuse of the Ia/motor neurone synapse in the cat by section either of the dorsal roots or of the muscle nerve. Unfortunately this method is complicated by effects resulting from the injury to the fibres concerned (Szentágothai & Rajkovits, 1955). It is therefore desirable to find a method which will produce disuse in a synapse without damage to either the presynaptic or the post-synaptic fibre.

This paper is concerned with an attempt to produce disuse in the dorsal lateral geniculate nucleus of the cat, in the synapses between the optic nerve fibres and the principal cells of the nucleus. It was hoped that direct injury to the neurones concerned would be avoided by the methods used: exclusion of light from the eyes and selective destruction of the receptor cells in the retina. It was known, of course, that in acute experiments the retinal ganglion cells showed a maintained discharge in complete darkness but it was thought that this might cease within a comparatively short time. There was also evidence, admittedly incomplete, that discharge of the retinal ganglion cells was dependent on functional receptor cells (Noell, 1953). We tested for the effects of disuse by electrically stimulating the optic nerve and recording from the lateral geniculate nucleus. The second method appears to produce disuse in the lateral geniculate nucleus whereas the first method does not or does so only after a long time. The results do not support the theory that disuse produces a decreased efficiency of the synapse. Preliminary reports have been published (Burke & Hayhow, 1960, 1962).

METHODS

Experiments were performed on eleven cats which were kept in total darkness ('dark cats') and on fourteen cats in which selective destruction of the visual receptor cells was attempted ('treated cats'). Experiments were also conducted on normal cats and some observations were obtained from normal cats which were the subject of previous work in this laboratory (Bishop, Burke & Hayhow, 1959). The dark and treated cats were all examined initially and judged to be in good health, to have normal light reflexes and to show normal visual behaviour (e.g. ability to follow visually a moving finger). Initially, all cats were at least 1 kg in weight and all were adult at the time of the experiment.

Procedure for dark cats. Originally it was hoped that it might be possible to keep one eye of the cat covered and allow the uncovered eye to serve as a control. An opaque plastic mask was made which covered one eye and the top and back of the head but left the other eye free and also allowed the cat to eat normally. The mask was held by two straps and was lined with foam rubber at pressure points and around the free eye. Only one cat (cat 477, see Table 1) was eventually used after this procedure. The method was abandoned because of numerous difficulties: the covered eye tended to become infected, the pressure points

developed sores, the mask was not well tolerated by the cat, and there was always the possibility that light might get in when the cat tried to dislodge it.

All the other dark cats were kept in total darkness for the periods indicated in Table 1. They had ample fresh uncooked minced meat to which was added a commercial preparation of vitamins A and D. There was also a plentiful supply of milk. All cats put on weight in the darkroom and all seemed in very good condition when removed with the exception of cat 603, which spent the longest time in the dark (966 days). This cat increased in weight from 1·9 kg to only 2·3 kg, there was a loss of elasticity in its skin and the skull was very thin and fragile. This cat was not eating well and did not drink its milk. At any given time there were never more than six cats, and usually fewer, in the darkroom. Each cat wore a light collar carrying a distinctively shaped metal tab so that the cat could be readily identified in the dark.

TABLE 1. Dark cats

Cat no.	Days in darkness	Histology of retina	Histology of LGN	Other remarks
477	56	—	—	Mask over right eye
532	99	—	—	2 sec dim light on day 37
608	145	—	—	—
609	189	—	—	—
611	230	—	—	Luminous bacteria on days 7, 17. 2–3 sec dim light on day 46
566	307	Apparently normal	Apparently normal	—
598	381	—	—	—
584	485	Apparently normal	Apparently normal	Luminous bacteria on day 471
586	520	Apparently normal	Apparently normal	Luminous bacteria on day 471
602	562	Apparently normal	Apparently normal	Luminous bacteria on days 106 and 526. 1 sec dim light on day 240
603	966	Apparently normal	Apparently normal	Luminous bacteria on days 471, 891

The darkroom consisted of a light-tight room 1·65 m square with two heavy refrigerator-type doors. The room was entered via a similar, slightly smaller room (the anteroom) which formed a light-tight trap. The cats could be placed in the anteroom and the second door of the darkroom opened to permit cleaning and replacement of food dishes. Air was circulated through the darkroom by an electric fan via light-tight ports. The doors were padlocked at all times and could only be opened by one or other of the authors. The darkroom and anteroom were rested for light-tightness before any cats were installed and also when all had been finally removed. Photographic plates were exposed and developed at the same time as unexposed plates. A more sensitive test was for one of us to remain in the darkroom or anteroom until dark-adapted and then search for light cracks. At the end of the experiments the darkroom was found to be still light-tight. In the anteroom there was a very small chink at the threshold of the door which had evidently developed due to the wear and tear on this region. No light was visible unless a strong bulb was placed on the other side of the closed door and then only if the gaze was in exactly the right direction. Normally the cats would be in the anteroom only for about 30 min each day. The chances of a cat getting photic stimulation in this way must be regarded as exceedingly remote.

On three occasions cats accidentally received 1–3 sec of very dim diffuse light because we had failed to locate them and put them in the anteroom before opening the outer door of the darkroom (see Table 1). The only other incidents which interrupted this regime of complete darkness were the occurrences of luminous bacteria in stale meat. This happened on two occasions when the darkroom had not been cleaned at the weekend. After the first occasion

TABLE 2. Treated cats

Cat no.	Treatment*	Days from start of treatment to experiment	Histology of retina†	Histology of LGN	Other remarks
607	M and B 968A 400 mg/kg orally on day 0 200 mg/kg on day 1 200 mg/kg on day 2	7	Grade II	A.N.‡	Weak light reflex. Behaviourally blind but responds to flickering light.
578	M and B 968A 200 mg/kg orally on day 0 100 mg/kg on day 1	10	Grade I	A.N.	Weak light reflex. Behaviourally blind.
605	M and B 968A 500 mg/kg orally on day 0	14	—	—	Moderate light reflex. Some recovery of vision.
460	IAA 10 mg/kg IV on day 0 12 mg/kg IV on day 1 12 mg/kg IV on day 7	23	Grade IV but no pigment cell invasion	A.N.	Weak light reflex.
461	IAA 24 mg/kg IV on day 0 12 mg/kg IV on day 1 12 mg/kg IV on day 5	28	A.N.	A.N.	Behaviourally blind 2 weeks before experiment.
601	M and B 968A 500 mg/kg orally on day 0	28	A.N.	A.N.	Good light reflex, can follow moving finger Retina ophthalmoscopically normal.
575	M and B 968A 500 mg/kg orally on day 0 500 mg/kg on day 6	30	Grade III	A.N.	Very weak light reflex. Behaviourally blind.

TABLE 2. (*cont.*)

Cat no.	Treatment*	Days from start of treatment to experiment	Histology of retina†	Histology of LGN	Other remarks
612	M and B 968A 50 mg/kg IV on day 0	43	Grade II	A.N.	Light reflex practically absent. Behaviourally completely blind.
614	M and B 968A 50 mg/kg IV on day 0	63	Grade II	A.N.	Weak light reflex. Behaviourally blind.
571	M and B 968A 500 mg/kg orally on day 0	220	Grade IV	A.N.	No light reflex. Behaviourally blind.
570	M and B 968A 500 mg/kg orally on day 0	228	Grade V	A.N.	No light reflex. Behaviourally blind. Pale optic disk.
592	M and B 968A 500 mg/kg orally on day 0	400	Grade V	A.N.	Weak light reflex. Pale optic disk.
597	M and B 968A 500 mg/kg orally on day 0 500 mg/kg on day 6 150 mg/kg on day 13 150 mg/kg on day 21	534	—	—	Weak light reflex. Pale optic disk.
600	M and B 968A 500 mg/kg orally on day 0	791	Grade V	A.N.	Very weak light reflex. Pale optic disk.

* IAA = sodium iodoacetate.

† The degree of retinal degeneration is expressed as one of 5 grades: I, 'arcade' formation (foci of proliferating cells of the pigment epithelium with distortion of the overlying retina); II, arcade formation plus mild damage to the outer segments of receptor cells; III, arcade formation plus marked damage to the outer segments; IV, severe loss of outer segments, mild reduction of outer nuclear layer, pigment cell invasion; V, loss of entire photoreceptor layer, pigment cell invasion.

‡ A.N. = Apparently normal.

the meat was subsequently covered with boiling water for a minute or so, a treatment that effectively destroys the bacteria. The second occasion was due to neglect of this practice when some fish was given instead of meat. The times of these incidents are shown in Table 1.

Meat with luminescent bacteria was examined with a photometer. The greatest luminance recorded was $0{\cdot}9 \times 10^{-4}$ cd/m^2 when a bright spot in the meat was placed about 0·5 cm from the head of the photometer probe. At 2 cm the luminance was $0{\cdot}18 \times 10^{-4}$ cd/m^2. In view of the very small amount of light which the cats may have received in these ways we do not feel that the results of the experiments have been jeopardized. In four cats there were no incidents of any kind (see Table 1).

The procedure for removing a cat from the darkroom for experiment was as follows. The cat was given an intraperitoneal injection of barbiturate in the darkroom. When the cat was fully anaesthetized a piece of flexible cardboard was inserted under the lids of each eye, the lids were held together with crocodile clips and the cat was removed from the darkroom. The preparation of the optic nerve for stimulation was done in as dim an illumination, and as quickly, as possible. In the later experiments (cats 602, 603, 608, 609, 611) an additional precaution was taken. As soon as the cat was removed from the darkroom we injected 0·5 ml. 2% lignocaine hydrochloride solution (Xylocaine, Astra) into each eyeball. We checked in a separate experiment on a normal cat that this procedure abolished the response in the lateral geniculate nucleus to a bright flash of light. The effect may depend partly on the local anaesthetic action of the lignocaine and partly on the increased intraocular pressure. The injection was repeated before exposing the cornea and making the final preparation of the optic nerve (see later).

Selective destruction of visual receptor cells. The treated cats were given one of two drugs. Originally we used sodium iodoacetate, which we gave by intravenous injection. Iodoacetate is believed to attack the visual receptor cells directly (Noell, 1951, 1952). Only two cats (cats 460, 461) were used after this treatment, which we abandoned because of the extreme toxicity of this substance. The remaining cats were given the compound 1,5-di(*p*-aminophenoxy)pentane dihydrochloride (M and B 968A), at first orally and later intravenously (see Table 2). This is much better tolerated by the cats, causes almost no side-effects and has almost no general toxicity in the doses we used. Some of the oral dose was lost because of vomiting. Because there was always some recovery of vision with both drugs, additional doses were given if necessary (see Table 2). For intravenous injection we used a 3% solution in sterile water injected over about 5 min. This substance causes a pigmentary degeneration of the retina which in turn leads to a degeneration of the receptor cells (Edge, Mason, Wien & Ashton, 1956; Ashton, 1957). The optic nerve shows no histological changes and only in the extreme stages of degeneration are there any signs of involvement of the inner layers of the retina, the changes being limited to a slight oedema and distortion (Ashton, 1957).

Histological controls. The observations of Ashton (1957) were confirmed in our experiments. The brain and retina were perfused with 10% formol saline. Sections of the retina were stained with haematoxylin and eosin and by the Nissl method; sections of the brain at the level of the lateral geniculate nucleus were stained by the Nissl method only. Table 2 summarizes the relevant information concerning the treatment and its effectiveness. For convenience of comparison with the electrophysiological results the retinal degeneration in each cat was graded (see legend to Table 2). In one cat given iodoacetate (cat 461) and in one cat given M and B 968A (cat 601) the retinas were apparently normal. Each of these cats had at one stage been behaviourally blind. Some degree of recovery was always noted in the M and B cats and cat 601 was found to have a good light reflex and good vision just before the experiment. Because of the histological and behavioural findings the results from these two cats have not been included in the mean values reported below. However, in the *t* tests performed on all the results it was checked that the addition of the results from these cats did not affect the degree of significance of any differences.

In comparison with the retinas of normal cats there was no obvious difference in the

appearance or density of the cells in the inner nuclear or ganglion cell layers in the treated cats, apart from the fact that they lay closer to the choroid and in the retinas in which there was most degeneration these layers were invaded by pigment cells. In these cats the cells of the lateral geniculate nucleus also appeared normal.

The retinas and brains of the cats which had been in the darkroom for the longest periods of time were also examined. The retina and the lateral geniculate nucleus in each of these cats were apparently normal. The phrase 'apparently normal' in this context means little more than that the cells were alive and healthy before the death of the cat. In the retinal cells of adult rabbits placed in darkness significant changes in morphology and chemical composition have been detected by the use of more sophisticated techniques (Brattgård, 1952; Bech, 1957; Einarson, 1957; De Robertis, 1958). Hence similar changes may have been present in our dark cats. No changes in LGN have been reported in adult animals kept in darkness.

Experimental procedure. In all experiments anaesthesia was induced with pentobarbitone sodium (Sagatal, May and Baker, 10–20 mg/kg I.P.) and maintained with allobarbitone (Dial, Ciba, 50 mg/kg I.P.). Body temperature was controlled within normal limits (37–39° C) by means of a heating blanket. The optic nerve was prepared for stimulation as follows: the eyeball was collapsed and tied over the rounded end of the silver anode electrode, the external eye muscles were ligatured and tied back and the optic nerve suspended free of orbital tissue. A silver cathode electrode was placed in contact with the optic nerve. The field response from the contralateral lateral geniculate nucleus was recorded by means of a stereotaxically directed steel micro-electrode inserted vertically through the intact cerebral cortex, an electrode in the neck muscles serving as a reference. The recording electrode was an electrolytically pointed steel beading needle which had been insulated with Bakelite varnish except at the tip. A similar electrode was used to record the massed unit discharge in the retina. The stimulating pulses were 0·05 msec in duration and were applied not more than once every 5 sec except where stated.

RESULTS

Response to single shock. It had been shown by Eccles & McIntyre (1953) that disuse of the Ia/motor neurone synapse resulted in a reduced motor neurone response and a proportionately higher and more prolonged post-tetanic potentiation. In our experiments it was clear that the LGN response was not appreciably reduced either in the dark cats or in the treated cats. In all experiments we confined our attention to the responses of the fast group of optic fibres (t_1) and the corresponding post-synaptic response (r_1) because of the greater accuracy of measuring these responses compared with those of the slower fibres (see Bishop, Jeremy & Lance (1953) for a discussion of fibre groups). The wave form and amplitude of the response varies according to the position of the recording electrode. In our experiments the electrode was normally directed at the centre of the LGN and inserted until an appreciable negative phase appeared on the t_1 response (see Fig. 3). There was no apparent difference in either the amplitude or the wave form of the response between the three groups of cats. Certainly there was no marked decrease or disappearance of the post-synaptic response as was observed by Eccles & McIntyre (1953) in the Ia/motor neurone synapse.

Threshold, latencies, refractory period. Measurements were made of the threshold, absolutely refractory period and latency of the t_1 response and of the synaptic delay and latency to peak of the r_1 response. Since there was no trend in the values in any group of cats the measurements have been averaged for each group and are shown in Table 3. The threshold

TABLE 3. Some parameters of function in optic nerve and LGN

Group	Threshold (V)	t_1 latency (msec)	Absolutely refractory period (msec)	Synaptic delay (msec)	Latency to r_1 peak (msec)
Normal	0·32 ± 0·019	0·72 ± 0·035	0·81*	0·38 ± 0·022	1·47 ± 0·041
	(0·21–0·43)	(0·60–0·90)	(0·70–0·92)	(0·25–0·47)	(1·33–1·68)
	10	8		8	8
Dark	0·28 ± 0·034	0·71 ± 0·027	0·71 ± 0·072	0·43 ± 0·027	1·50 ± 0·046
	(0·20–0·43)	(0·60–0·82)	(0·50–0·86)	(0·30–0·55)	(1·35–1·70)
	6	7	4	7	7
Treated	0·34 ± 0·041	0·83 ± 0·024	0·82 ± 0·061	0·37 ± 0·018	1·56 ± 0·037
	(0·15–0·51)	(0·65–0·94)	(0·63–0·95)	(0·26–0·49)	(1·35–1·74)
	9	11	4	11	11

Each set of results shows the mean ± S.E. of mean, followed by the range of the average values for each cat and the number of animals, except for the asterisked results which are taken from Bishop & Evans (1956) and which show the mean and the range of values. The only significant difference ($P < 0·05$) between groups is for t_1 latency; t_1 latency for treated cats is significantly longer ($P < 0·02$) than the value for normal cats.

voltage using a 0·05 msec pulse is a somewhat arbitrary value since it depends on the amount of fluid around the optic nerve, the distance apart of the electrodes and other factors, but in all experiments the nerve was prepared for stimulation in the same way and the same equipment was used for stimulation. The absolutely refractory period was obtained by determining the minimal interval at which a second response could be elicited by a second stimulus of maximal strength. The t_1 response consists of a positive/negative wave form followed by a negative r_1 wave (see Fig. 3). The latency of the t_1 response was taken as the time from the stimulus artifact to the positive peak of this response and the synaptic delay as the time from this peak to the commencement of the r_1 response, i.e. to the positive dip preceding the negative r_1 wave (cf. Brooks & Eccles, 1947).

The only significant difference between the three groups of cats was in respect of t_1 latency. There was no significant difference between the values for normal and dark cats but comparing the t_1 latency for treated cats with that for normal cats there was a probably significant difference ($P < 0·02$). This result implies a reduction in conduction velocity in the treated cats but, as mentioned above, there was no trend in the values, the increased latency being evident from the tenth day onwards. It is possible that in the 7-day treated cat the latency (0·65 msec) was entirely normal.

We have confirmed the observation of Ashton (1957) that M and B 968A causes a marked pallor of the optic disk and an attenuation of the retinal blood vessels. It is possible that a reduced blood supply might affect the metabolism of the retinal ganglion cells and optic nerve fibres and lead to this reduction in conduction velocity.

Absolutely refractory period was not measured in normal cats and comparison was made with the values obtained in a previous investigation in this laboratory (Bishop & Evans, 1956). The mean value for the treated cats (0·82 msec) was practically identical with that for normal cats (0·81 msec) and was not significantly different ($P > 0{\cdot}05$) from that of dark cats (0·71 msec).

In summary, the response to a single stimulus in the dark cats, even after 966 days in the dark, differed in none of the parameters mentioned from the response in a normal cat. In the treated cats the only difference was that the latency of the presynaptic response (t_1) was probably significantly longer than in normal cats. The absolutely refractory period of the optic nerve fibres was normal.

Post-tetanic responsiveness. The post-tetanic responsiveness of the LGN was examined to see whether results similar to those of Eccles & McIntyre (1953) on the Ia/motor neurone synapse could be obtained. The optic nerve was stimulated at about 500/sec for 15 sec. A test stimulus was applied via the same electrodes at 5 sec intervals before and after the tetanus. The tetanic stimulus strength was always supramaximal for the t_1 response. The test stimulus was sometimes supramaximal, sometimes about half-maximal. In a normal cat it has been shown that tetanic stimulation of this kind produces a small brief post-tetanic potentiation of the r_1 response followed by a deep depression of this response (post-tetanic delayed depression, PTDD) which may last for hours (Fig. 2*A*; see Hughes, Evarts & Marshall, 1956; Evarts & Hughes, 1957*a*, *b*; Bishop *et al.* 1959). The post-tetanic changes in the t_1 response are less dramatic. If the stimulus is submaximal the t_1 response shows a small post-tetanic depression; if the test stimulus is supramaximal there is a small potentiation lasting usually not more than 1 min (Bishop *et al.* 1959).

Figure 1 shows the results of such an experiment using a supramaximal test stimulus in a treated cat (M and B 968A given 220 days previously). Post-tetanically the t_1 response showed the usual potentiation. Contrary to the usual finding in a normal cat there was no PTDD in the treated cat. Figure 2 shows typical examples of the post-tetanic changes in r_1 responsiveness in a normal cat (*A*), a dark cat (*B*) and a treated cat (*C*). The PTDD in the normal and dark cats was similar, to about 50 % of the pre-tetanic level, whereas in the treated cat there was only about 5 % depression and recovery was complete in 20 min. When the peak values for t_1

supernormality, r_1 supernormality and r_1 subnormality (PTDD) were examined as a function of time in darkness or time after commencement of 'treatment' no trend in the values could be detected. Accordingly the results have been averaged for each group of cats. Table 4 shows the mean values, standard errors, range of values and number of cats for each group for each parameter of the recovery for both the responsiveness following a single shock (see later) and for post-tetanic responsiveness.

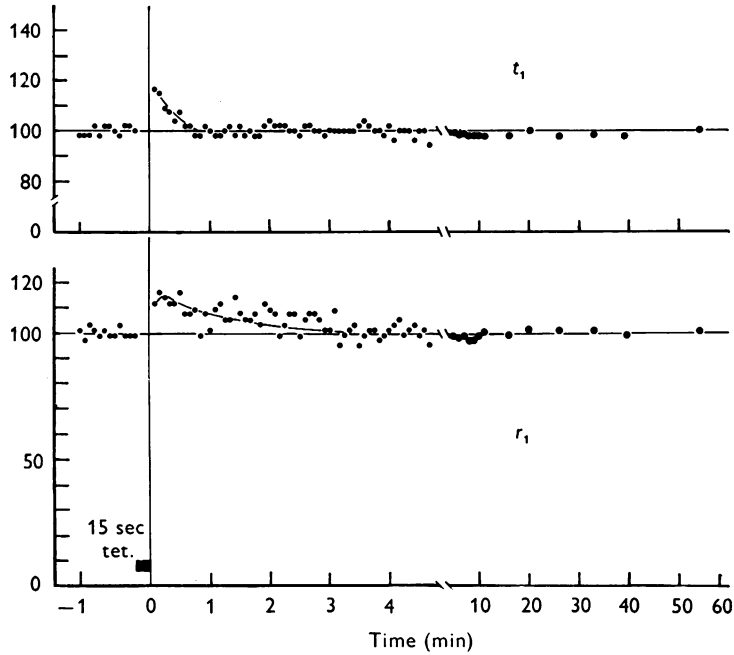

Fig. 1. Effect of 15-sec tetanus (480/sec) on presynaptic (t_1) and post-synaptic (r_1) responses in lateral geniculate nucleus of treated cat (cat 571). The amplitudes of the responses are expressed as percentages of the mean pre-tetanic level. The large dots are the means of four responses, the small dots are single responses. The vertical line marks the end of the tetanus. Supramaximal test stimulus.

Looking at post-tetanic recovery only, there is no significant difference ($P > 0{\cdot}05$) between the three groups of cats in the values for t_1 supernormality and r_1 supernormality, although the mean r_1 supernormality for treated cats is lower than that in the other groups and is very close to 100%. It was also apparent that there was no difference in the durations of the potentiations between the three groups. In the values for PTDD there is a highly significant difference ($P < 0{\cdot}001$) between treated cats and normal cats. In the treated cats the depression was to a mean value of 92·3% compared with 36·0% in normal cats. In the dark cats the depression was to 55·7% but this value was not significantly different

from that of normal cats. In the treated cats the reduced PTDD was apparent in the cat examined after the shortest interval (cat 607, 7 days, depression to 96 %).

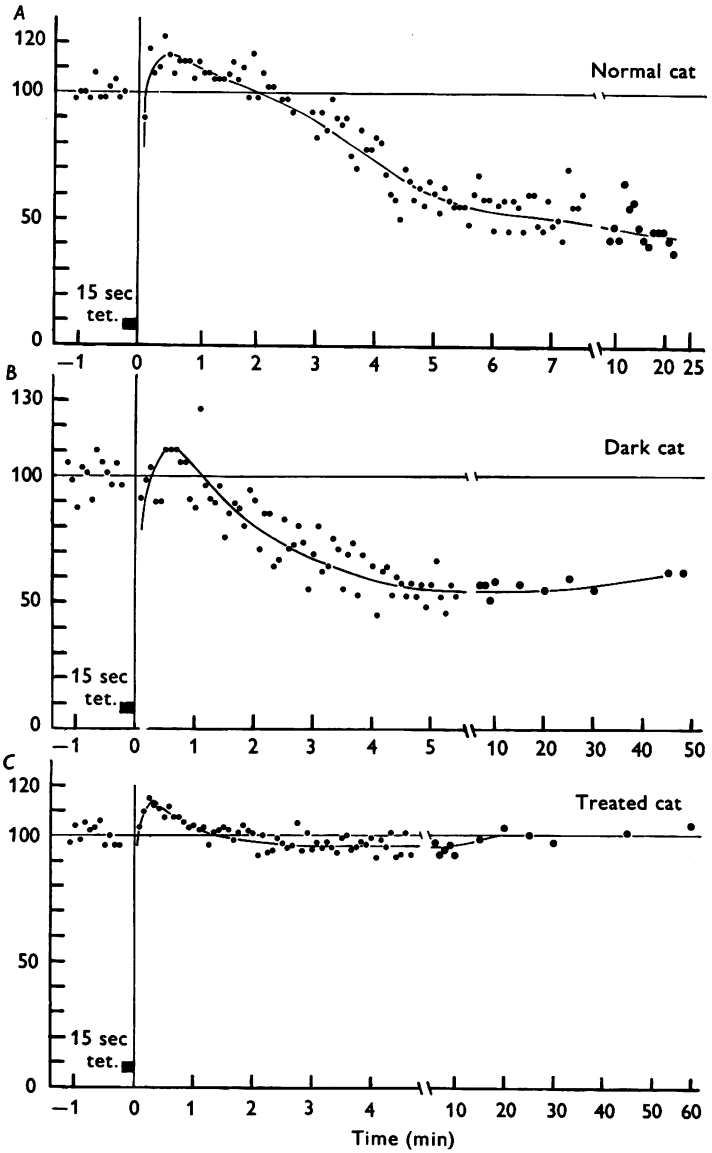

Fig. 2. Effect of 15-sec tetanus on post-synaptic (r_1) responses in LGN of normal, dark and treated cats. *A*. Normal cat, tetanus 611/sec. *B*. Dark cat (cat 602), tetanus 470/ sec. *C*. Treated cat (cat 607), tetanus 455/sec. The amplitudes of the responses are expressed as percentages of the mean pre-tetanic level. The large dots are the means of 4–6 responses, the small dots are single responses. The vertical line marks the end of the tetanus.

Summarizing these results, there was no difference in the amount of t_1 or r_1 potentiation, neither in amplitude nor in duration, between the three groups of cats. Only in the treated cats was the r_1 subnormality significantly less than in normal cats.

TABLE 4. Parameters of recovery after single or tetanic stimulation

	Two-shock recovery			Post-tetanic recovery		
Group	t_1 super-normality	r_1 super-normality	r_1 sub-normality	t_1 super-normality	r_1 super-normality	r_1 sub-normality (PTDD)
Normal	151* (132–180) —	113* (77–146) —	32* (20–44) —	117·1 ± 3·79 (100–142·5) 15	107·7 ± 6·88 (81–152) 8	36·0 ± 4·82 (21–62) 8
Dark	125·8 ± 6·47 (108–155) 6	121·5 ± 6·67 (100–149) 6	53·8 ± 4·96 (32–72) 8	116·1 ± 3·43 (100–130) 7	116·5 ± 2·99 (105–129) 8	55·7 ± 7·54 (23–84) 8
Treated	150·7 ± 7·54 (122–176) 7	108·2 ± 6·92 (82·5–140) 8	71·1 ± 5·52 (43–100) 8	119·3 ± 2·64 (109–131) 7	101·7 ± 3·26 (83–116) 10	92·3 ± 2·87 (69–100) 11

All values are expressed as a percentage of the control amplitude (the unconditioned test response in the two-shock recovery, the pre-tetanic test response in the post-tetanic recovery). All r_1 values (except the r_1 supernormality in post-tetanic recovery) are corrected for changes in t_1. Each set of results shows the mean ± S.E. of mean followed by the range of the average values for each cat and the number of animals, except for the asterisked results which are taken from Bishop & Davis (1960) and which show the mean and the range of values. There is a significant difference ($0{\cdot}025 < P < 0{\cdot}05$) between the t_1 supernormality (two-shock recovery) values for dark and treated cats. There is a highly significant difference ($P < 0{\cdot}001$) between the PTDD values for normal and treated cats. Further discussion in the text.

Responsiveness during repetitive stimulation. Since the post-tetanic recovery was so different in the treated cats the question was examined whether there was also a difference during the tetanic stimulation. Figure 3 shows the LGN response to a short train of stimuli in these cats. In this figure, in order to show each response clearly the rate is somewhat lower (about 300/sec) than in the tetanic stimulation experiments (about 500/sec). Each response consists of a diphasic (positive/negative) optic tract response (t_1) followed by a negative post-synaptic response (r_1). Figure 3*a* shows the responses in a normal cat, *b* in a dark cat, *c* and *d* in treated cats. In all four cats the t_1 response is maintained more-or-less unchanged in amplitude. In the normal cats the r_1 response is usually well maintained for three or four responses at this rate of stimulation and is then drastically reduced to a small response which is mainly a synaptic potential (Fig. 3*a*; see Bishop & McLeod, 1954). A similar effect was observed in the dark cats (Fig. 3*b*). In the treated cats the r_1 response is much better maintained, the example of Fig. 3*d* showing almost no decrease during seven responses. In all cats the response declined during about ten responses to a level

which was then maintained indefinitely, this level being appreciably higher in treated cats (Fig. 4*b*, *d*) than in normal (Fig. 4*a*, *c*) or dark cats.

Recovery following a single shock. Because the response in the treated cats was well maintained during repetitive stimulation it was natural to expect that there would also be less depression following a single shock. This is

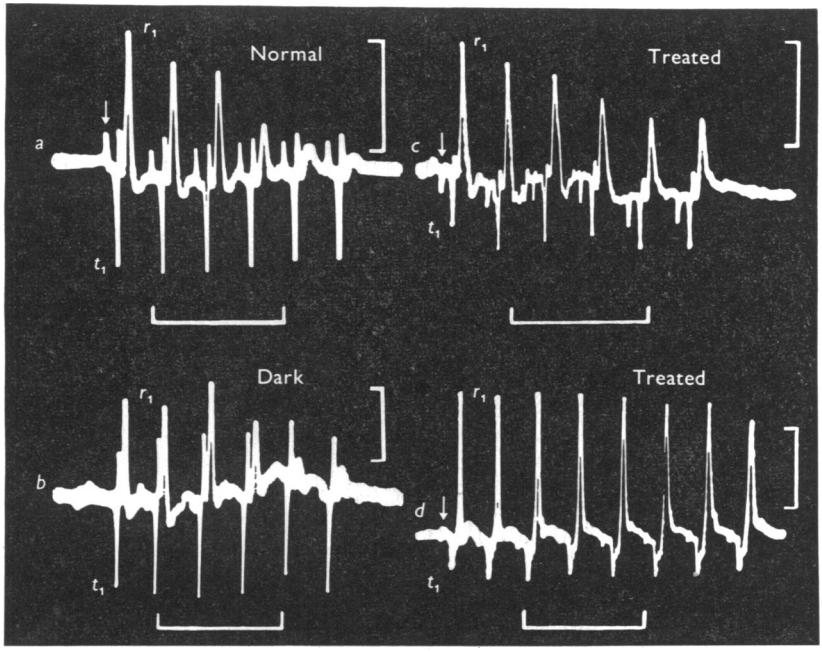

Fig. 3. LGN responses to a train of 6 or 8 stimuli at about 300/sec applied to optic nerve. Stimulus about twice threshold in each case. Response to a single stimulus consisted of a positive/negative deflexion (t_1) due to the large optic tract fibres, following by a negative wave (r_1) due to the LGN neurones. Arrows, stimulus artifacts. The t_1 response is only slightly affected during the train but the r_1 response is greatly depressed in (*a*) the normal cat and in (*b*) the dark cat (cat 566) but is much less depressed in the treated cats (*c*, cat 575; *d*, cat 570). Horizontal bars 10 msec, vertical bars 1 mV (*a*, *d*) 0·5 mV (*b*, *c*). Negativity upwards.

also a simpler situation to analyse and describe because during repetitive stimulation each response is presumably affected by the preceding stimuli and to a different degree by each stimulus. The recovery process in a normal cat after a single shock has been described in detail by Bishop & Davis (1960). They report a t_1 supernormality reaching a peak value of 151 % at an interval of 4·6 msec, the responsiveness returning to normal at 34 msec (mean values). There is also an r_1 supernormality to a peak value of 113 % at an interval of 4·7 msec, the supernormality ending at about 6·7 msec; there then follows a subnormality to a peak value of 32 % lasting about 2 sec (all mean values).

Typical recovery curves are shown in Fig. 5 (dark cat) and Fig. 6 (treated cat). Values for t_1 supernormality, r_1 supernormality and r_1 subnormality in dark and treated cats are summarized in Table 4, which also includes the values for normal cats given by Bishop & Davis (1960). The mean value for t_1 supernormality is identical in normal and treated cats (151 %) but the value in dark cats (126 %) is just significantly less ($P < 0{\cdot}05$) than

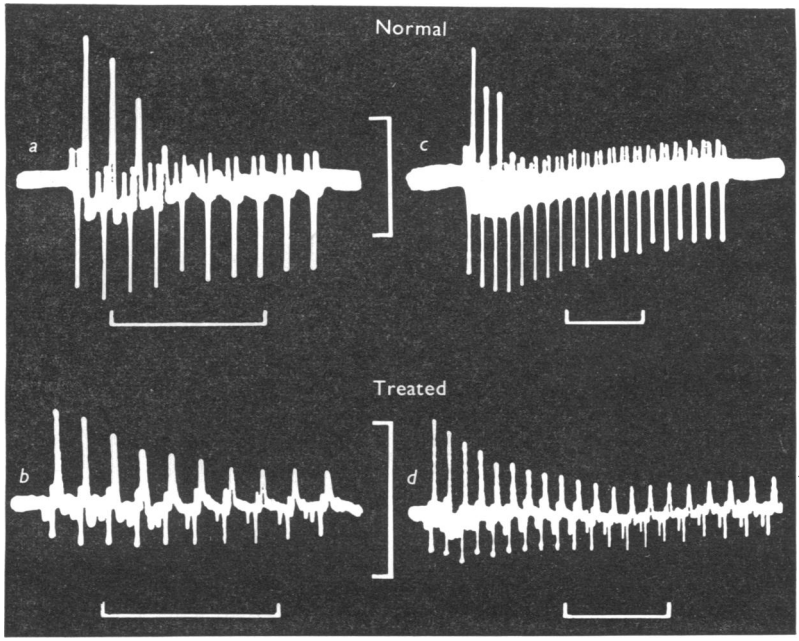

Fig. 4. LGN responses similar to those shown in Fig. 3 but with longer trains of stimuli. *a*, *c*, normal cat; *b*, *d*, treated cat (cat 575). No post-synaptic response is discernible in the normal cat after about 10 stimuli (*c*). In the treated cat the r_1 response is well maintained (*b*, *d*). Horizontal bars, 20 msec; vertical bars, 1 mV. Negativity upwards.

in treated cats and hence may be significantly less than in normal cats. The mean values for r_1 supernormality are not significantly different ($P > 0{\cdot}05$) between dark and treated cats (121·5 % and 108 % respectively), and since the mean value for normal cats (113 %) lies between these values and the range of values is similar we may conclude that no significant difference has been demonstrated between the three groups.

There is no significant difference ($P > 0{\cdot}05$) between the values for r_1 subnormality in dark and treated cats (53·8 % and 71·1 % respectively), but there would certainly be a significant difference between normal and treated cats because the range of values barely overlap (20–44 % and 43–100 % respectively). It is not possible to say whether the mean value

for dark cats (53·8 %) differs significantly from that of normal cats (32 %).

These results indicate a clear difference between normal and treated cats in respect of the r_1 subnormality. In agreement with the observations on repetitive stimulation they indicate that the r_1 response is much less depressed following a single response than is the case in the normal cat. The

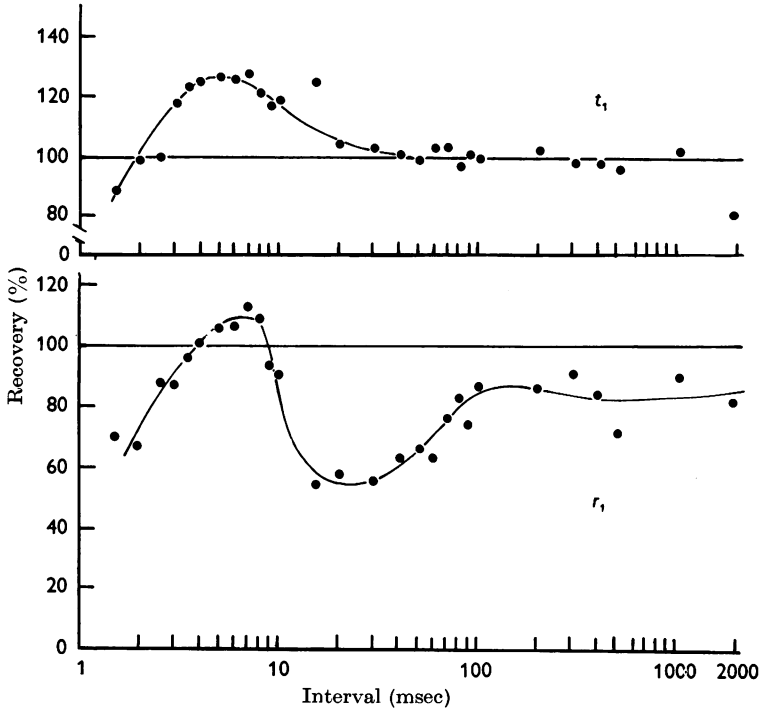

Fig. 5. Recovery of t_1 and r_1 responses following a single supramaximal shock to the optic nerve in a dark cat (cat 602). Test shock about half-maximal t_1. The responses are expressed as percentages of the control (unconditioned test) responses. Points are the means of four responses. Abscissa, interval between conditioning and test shock (logarithmic scale).

differences between normal and dark cats are smaller and may not be significant. It is however possible, at least as regards synaptic transmission, that a slow change does occur in the dark over a long period of time because the highest value for r_1 subnormality in dark cats was obtained in the cat which had been in the dark for the longest time (cat 603, 966 days, 72 %) and the lowest value for the cat which had been in the dark for only 145 days (cat 608, 32 %). However, as mentioned in Methods, cat 603 was stunted in growth and had abnormalities of skin and bone. There is a possibility that it was calcium-deficient and this may have affected the response.

Maintained activity in retinal ganglion cells. The results so far have indicated that in the treated cats there are significant changes in the quality of synaptic transmission in the LGN. Since the significant morphological change in the treated cats was destruction of the visual receptor cells we presumed that this led to a cessation of discharge in the optic

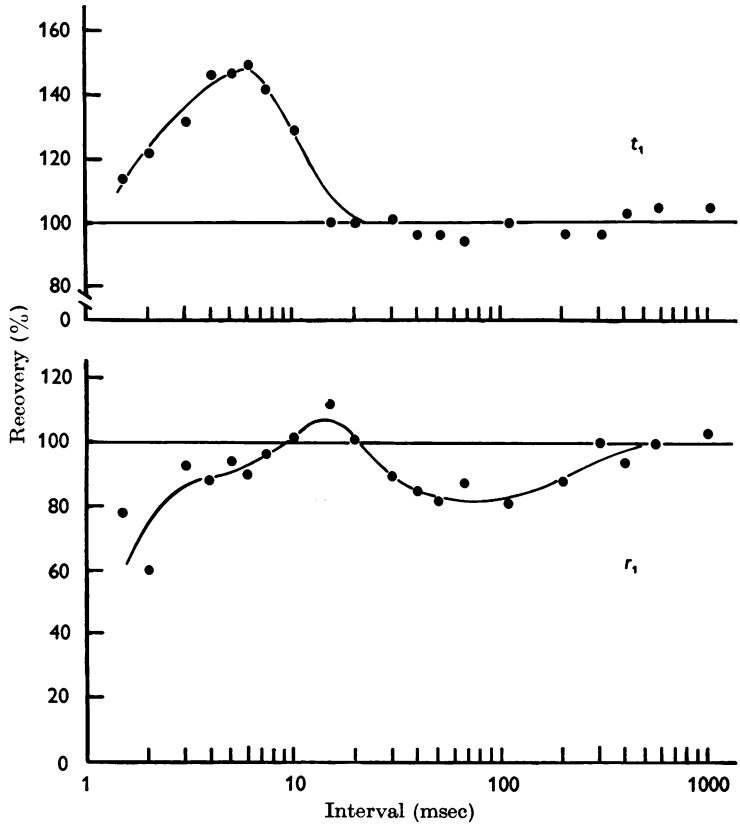

Fig. 6. As for Fig. 5 but in a treated cat (cat 597). The r_1 subnormality is less than in the normal or dark cats.

nerve and so to a disuse of the optic tract/LGN cell synapses. It had been shown in an acute preparation by Noell (1953) that injection of iodoacetate silenced the optic nerve discharge. It was necessary however to show that there was a similar cessation or diminution of discharge in our treated cats.

We recorded from the exposed retina in five normal cats and in two treated cats (M and B 968A given 2–3 weeks earlier). Four normal cats were used in preliminary experiments to determine the best type of electrode for recording a massed unit discharge. Eventually we selected a

varnished steel electrode with a fine exposed tip in preference to glass microcapillary electrodes or tungsten electrodes. However, there is no doubt that this type of recording is readily obtained with a wide variety of electrodes. Such a discharge was obtained in all four normal cats and gave us confidence that we would not miss such a discharge in a treated cat. In the first treated cat we were unable to record any retinal discharge either in darkness or in response to steady or modulated light. Since there was always the possibility that the particular combination of recording circumstances was unfavourable we did another experiment in which we recorded alternately from a normal cat and a treated cat using the same electrode. Several areas of the retina in each cat were probed and the electrode was transferred several times from one cat to the other. As may be seen from Fig. 7 the retina of the treated cat (*A*, *C*, *E*) was much 'quieter'

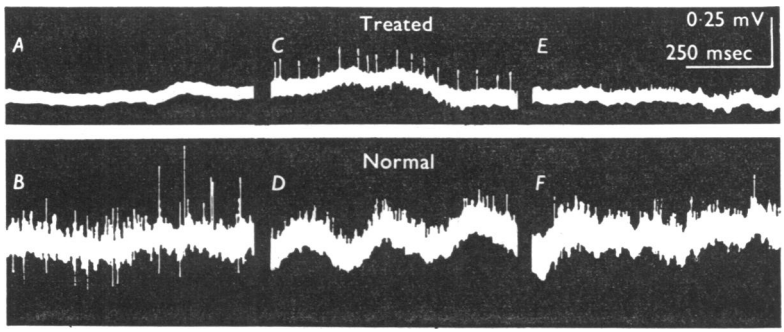

Fig. 7. Massed discharge of retinal ganglion cells in a treated cat (*A*, *C*, *E*, cat 605) and in a normal cat (*B*, *D*, *F*). *A*, *B*, in total darkness; *C*, *D*, in diffuse moderate illumination; *E*, *F*, in modulated light. Responses from three different retinal regions in each cat. Calibrations, 0·25 mV and 250 msec apply to all records. Spikes retouched.

than that of the normal cat (*B*, *D*, *F*). The fact that some discharge could be recorded from the retina of the treated cat was also confirmation that our recording was not at fault. In Fig. 7*A* no discharge at all was evident, in *E* a small discharge and in *C* a single unit, small by comparison with many units in a normal retina (Fig. 7*B*, *D*, *F*) but clearly distinguished against the very weak background discharge. This cat was used in an electrophysiological experiment (cat 605) but was not examined histologically. However, there had been a slight return of vision which is consistent with the weak retinal discharge we recorded.

We confirmed here an observation made by many previous workers (e.g. Kuffler, Fitzhugh & Barlow, 1957; Bornschein, 1958; Doty & Kimura, 1963; Rodieck & Smith, 1966; Rodieck, 1967) that there is normally a maintained discharge in retinal ganglion cells in the complete absence

of light. This is very relevant to our experiments on cats kept in darkness.

Maintained activity in LGN. In our treated cats we regularly observed an ongoing activity in the LGN. This was not surprising, however, because it has already been shown that many LGN cells discharge after destruction of both retinas (Bishop, Burke & Davis, 1962; Levick & Williams, 1964), suggesting that the cells are either spontaneously active or receive an input from some other source. The possibility should be considered, however, that spontaneous release of transmitter from the optic nerve endings might play some part in this maintained discharge, particularly if for some reason the cells develop a hypersensitivity.

The effect of prolonged tetanic stimulation of the optic nerve. The results described above suggested very strongly that a true disuse of the optic nerve/LGN synapses occurs in the treated cats and is responsible for the reduction in the amount of depression which normally follows activity. If the effects observed are due to disuse it ought to be possible to reverse these effects by repeated stimulation. In several treated cats we observed that stimulation for 10 min at 500/sec was without effect on the degree of subnormality following a 15-sec tetanus. In one cat the effect of a 15-sec tetanus was determined; there was no PTDD (cat 578). The optic nerve was then stimulated for 1 hr at about 500/sec and the optic nerve and LGN were then allowed to recover fully. One hour from the end of the tetanus a second 15-sec tetanus was given—again there was no PTDD.

These results suggest that if the disuse explanation is correct then the changes produced are not readily reversible; re-use of the synapses over a longer period of time may be necessary to restore them to normal. Incidentally this experiment indicates that the photic stimulation which the dark cats receive after removal from the darkroom before the optic nerve is ready for stimulation would be unlikely to reverse the effects of disuse, assuming that total darkness was effective in producing disuse.

Correlation of response with retinal degeneration in treated cats. Since the morphological effects of M and B 968A were not uniform in our treated cats we wondered if this irregularity could explain some of the variation in our results from these cats. The large doses of M and B 968A used in these experiments invariably produced a severe loss of vision by the following day. The pupils were widely dilated and the light reflex absent or very weak; the cat seemed unable to see and moved uncertainly. Over a period of 2 weeks there was usually some recovery and in one cat (cat 601) an apparently complete recovery. Table 2 summarizes our assessments of the histological appearance of the retina and the behaviour of the treated cats. The two sets of results are not in exact agreement but this may be because the histological ratings were all made together by direct compari-

son (although this was done 'blind') whereas the judgements of behaviour or light reflex were made at the time of the various experiments spread out over a number of years.

TABLE 5. Correlation of degree of retinal degeneration with electrical changes

Cat nos.	Grade of retinal degeneration	t_1 latency (msec)	Two-shock recovery: t_1 super-normality	Two-shock recovery: r_1 sub-normality	PTDD
600, 592, 570	V	0·76, 0·78, 0·94 (mean 0·83)	—, 173, —	—, 80, 100 (mean 90)	—, 97·5 100 (mean 99)
460, 571	IV	—, 0·81	—, 138	—, 72	85, 100 (mean 92)
575	III	0·87	—	—	96
607, 612, 614	II	0·65, 0·92, 0·86 (mean 0·80)	169, 176, 135 (mean 160)	68, 43, 70 (mean 60)	96, 69, 100 (mean 88)
578	I	0·85	—	—	100
461, 601	No degeneration	—, 0·89	—, 150	—, 35	73, 73 (mean 73)

The values are values for individual cats together with mean values for each group, expressed as in Tables 3 and 4. The only trend in the above results is in the two-shock r_1 subnormality in which the amount of subnormality lessens as the retinal degeneration increases. Further discussion in the text.

Table 5 lists the values of response parameters found to be significantly different from those of normal or dark cats. Only in respect of the amount of r_1 subnormality following a single shock is there any correlation with the degree of retinal degeneration, the subnormality decreasing as receptor cell destruction increases. For post-tetanic responsiveness there is no correlation, the cat with the least amount of retinal degeneration (cat 578, grade I) showing no PTDD. However, it is interesting that in the two cats in which recovery was more-or-less complete (cats 461, 601) the PTDD values were just outside the range obtained in normal cats (see Table 4). Both these cats had been behaviourally blind at one stage and so it is possible that they had not fully recovered from the effects of a period of temporary disuse.

DISCUSSION

Have we obtained 'disuse'? There is now good evidence that the discharge of retinal ganglion cells depends upon intact receptor cells. Noell (1953) has shown that iodoacetate, which is believed to act directly on the receptor cells, abolishes the optic nerve discharge in an acute experiment. We have shown here that M and B 968A produces a considerable reduction in the discharge in a chronic cat. Rodieck (1967) was likewise unable to find much activity in the retinal ganglion cells of a cat given M and B 968A 3 days previously. It could be argued of course that both these drugs may have also acted directly on the retinal ganglion cells but strong evidence

against this contention is provided by Rodieck (1967) who points out that in the cat given M and B 968A all the retinal ganglion cells which gave a maintained discharge were also affected by photic stimulation and vice versa.

As far as the present problem is concerned the important point is that administration of these drugs reduces or abolishes the retinal discharge and therefore we have succeeded in producing a 'disuse' of the optic nerve/LGN synapses in the treated cats. We would like to be able to say that the optic nerve fibres and LGN cells were not directly affected by these compounds. Strongly in favour of this view are the findings that both retinal ganglion cells and LGN cells retained their normal appearance under Nissl staining in all the treated cats examined (see Table 2), that is, from 7 to 791 days after administration of the drug, and the optic fibres were virtually unchanged in their physiological properties.

In the experiments of Eccles & McIntyre (1953) and Eccles *et al.* (1959) disuse of the Ia/motor neurone synapse was produced either by section of the dorsal roots extraganglionically or by section of the muscle nerve. The effects of disuse in this synapse were thus necessarily complicated by the injury caused either to the Ia fibres or to the motor neurone. Eccles *et al.* (1959) emphasized the desirability of producing disuse uncomplicated by the undesirable changes (such as shrinkage) which result from operative severance of the afferent fibres. The situation achieved in our treated cats seems to meet these conditions.

The effects of disuse. The experiments of Eccles and colleagues showed that disuse of the Ia/motor neurone synapse produced by the methods mentioned earlier had three major effects: (i) the motor neurone response to stimulation of the Ia fibres was much reduced or absent, (ii) post-tetanic potentiation reached a higher level on the operated side (results expressed relative to the pre-tetanic level), (iii) post-tetanic potentiation had a longer duration on the operated side. In contrast to these results, the effects of disuse at the optic nerve/LGN synapses was not depression of synaptic function but rather an increased excitability. There was no obvious change in the LGN response to a single shock to the optic nerve apart from a slightly longer latency of the presynaptic response. However, the depression which normally follows single or repetitive stimulation was reduced or absent. On the basis of this result and assuming that these two synapses will prove to be similar in this respect, we suggest that the reduced response in the motor neurone is due to injury to the afferent fibres.

Post-tetanic potentiation in the LGN of the treated cats was neither to a proportionately higher level nor more prolonged than in the normal cat. However, this fact must be considered in terms of the normal functioning of the optic nerve/LGN synapse. In this synapse the subliminal fringe is

small and post-tetanic potentiation is normally to only about 108% (Table 4). In the treated cats the potentiation was to 102% (Table 4) Although these two values are not significantly different a simple interpretation of the results is that the response in the treated cats has increased slightly and obliterated the subliminal fringe. In these circumstances no post-tetanic potentiation is possible nor, of course, can one speak of its duration. Our results are therefore not necessarily in disagreement with those of Eccles and colleagues as far as their second and third effects are concerned and it is possible that these are the true effects of disuse.

In both the optic nerve/LGN synapse and the Ia/motor neurone synapse post-tetanic potentiation is followed by a phase of depression, although this is much more marked and more prolonged in the former synapse. As a result of disuse of the synapse in each case there is reduction or abolition of this depression. In the optic nerve/LGN synapse the depression which follows a single response or which occurs during repetitive stimulation is likewise reduced as a result of disuse of the synapse. There are two possible explanations, not mutually exclusive, of these observations. The first is that they are examples of the well-known phenomenon of denervation- or 'decentralization'-hypersensitivity. Decentralization-hypersensitivity is presumably due to silencing (or resting) the neurones which are afferent to the synapse, and this hypersensitivity is not as marked as in denervation-hypersensitivity in which there is degeneration of the afferent fibres. This phenomenon has been described in detail and discussed fully by Cannon & Rosenblueth (1949) and Stavraky (1961).

A second explanation is that when a neurone is silenced it accumulates transmitter in its nerve endings and subsequent stimulation releases increased amounts of transmitter. Good evidence in support of this theory has been provided for the splenic nerves by Brown, Davies & Ferry (1961) and Brown, Dearnaley & Geffen (1966). It is also interesting that the increased noradrenaline output from the rested splenic nerves was only slightly reduced by periods of 'excess use' during the experiment (Brown *et al.* 1961). As in the LGN, this effect of disuse was not readily reversible.

It should be pointed out that the two kinds of depression in the LGN referred to above probably have different underlying mechanisms. Morlock, Pearlman & Marshall (1965) have presented evidence that PTDD is due to presynaptic changes. The subnormality which follows a single shock and which develops in the early part of repetitive stimulation is probably due to a recurrent inhibition mediated by collaterals of the principal cells (P cells) of the LGN and inhibitory interneurones (I cells) (Burke & Sefton, 1966 *a*, *b*, *c*). The reduction or absence of both kinds of

depression could be accounted for by either of the explanations mentioned above. Thus a post-synaptic hypersensitivity or an elevated general level of transmitter release could compensate for a decreased transmitter output during PTDD and for the post-synaptic hyperpolarization due to recurrent inhibition. Quite possibly both explanations apply.

It might be argued that hypersensitivity could also develop at the I cell/P cell synapse and that this would oppose the hypersensitivity at the optic nerve/P cell synapse. However, many P cells have a maintained activity which does not depend on the optic discharge (Bishop *et al.* 1962; Levick & Williams, 1964; Morlock *et al.* 1965) and it is not improbable that I cells may be activated from other parts of the brain. Thus there may be very little decrease in activity at the I cell/P cell synapse and hence no hypersensitivity.

It is interesting that the degree of r_1 subnormality in the two-shock experiment is dependent on the extent of retinal degeneration whereas this is not so for post-tetanic depression, at least within the range of degeneration produced in our experiments. This difference is not difficult to explain since the two mechanisms are different. It presumably reflects the more powerful (if less prolonged) inhibition due to the I cells compared with that occurring in PTDD.

Relevance to learning. Our results therefore give no support to theories of learning in which it is postulated that disuse of a synapse results in defective function (e.g. Eccles, 1953). Although the effect of disuse in synapses in the central nervous system is still not clear, there is overwhelming evidence that in peripheral junctions disuse increases rather than decreases synaptic efficiency (see Sharpless, 1964). But it may be asked whether our experiments, or those of Eccles and colleagues, are a fair test of the theory, even supposing that all synapses are fundamentally the same. It may be that it is only in the functional development of a synapse that plasticity of this nature can be demonstrated. In support of this statement is a considerable body of evidence showing that behavioural deficits in a visual task occur when animals are reared in darkness (e.g. Goodman, 1932; Riesen, 1947; Riesen, Kurke & Mellinger, 1953). In animals deprived of light in early life there are significant histological changes in retina and optic nerve (Chow, Riesen & Newell, 1957; Weiskrantz, 1958; Rasch, Swift, Riesen & Chow, 1961; Wendell-Smith, 1964), in the LGN (Wiesel & Hubel, 1963, 1965; Kupfer & Palmer, 1964) and in visual cortex (Gyllensten, 1959). Electrophysiological changes in cats deprived of light from birth were also reported by Baxter & Riesen (1961) in retina and by Wiesel & Hubel (1963, 1965) in LGN. On the other hand, Goodman (1932) could find no significant morphological changes in any part of the visual pathway in rabbits reared in total darkness. The dis-

crepancy between Goodman's results and those of others may be explainable in terms of species difference or the histological tests used. A recent review of this field is by Riesen (1966).

An alternative viewpoint is that learning can occur only in certain synapses which are appropriately connected and which perhaps require an appropriate morphological arrangement of some basic synaptic elements. It has been suggested, for example, that learning depends on reinforcement—that is, on positive feed-back—and the morphological basis for this could be facilitatory axo-axonal junctions (Burke, 1966). Neither the Ia/motor neurone synapse nor the optic nerve/LGN synapse may be 'learning synapses' in this sense. In any case it is desirable to continue testing the 'disuse' theory and to look for other synapses where disuse can be achieved in as 'pure' a form as possible.

The maintained discharge from the retina. No significant difference was found for any parameter of the response between normal and dark cats. There is a possibility, however, that such a difference might exist for the t_1 supernormality and r_1 subnormality in the two-shock recovery experiments. There is no ready explanation for the reduction of t_1 supernormality in the dark cats; for the time being we must reserve judgement on the significance of this effect. The r_1 subnormality is markedly affected by the value from one cat (cat 603, 72 %) which had been in the darkroom for 966 days but which had grown very little in that time and may have been calcium-deficient. Even if we exclude the results from this cat the mean value for r_1 subnormality so obtained (50·2 %) is still appreciably above the mean value for normal cats (32 %, Bishop & Davis, 1960). For PTDD also the mean value from the dark cats (55·7 %) is well above that of the normal cats (36·0 %, see Table 4) although because of the wide range of values the difference is not significant. The possibility must therefore be considered that some change occurs in the dark and perhaps becomes greater with time. Because the changes observed are considerably less than in the treated cats it is reasonable to infer that the LGN synapses never become 'disused' to the same extent and it follows from this that there must be a continuous discharge from at least some retinal ganglion cells in total darkness over long periods of time, even more than 2 years. It is well-known, of course, that retinal ganglion cells continue to discharge even after several hours of darkness (e.g. Kuffler *et al.* 1957; Bornschein, 1958; Doty & Kimura, 1963; Rodieck & Smith, 1966; Rodieck, 1967). There is now good reason to think that this discharge continues indefinitely and has significant trophic effects on the LGN cells.

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