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Lysergic Acid Diethylamide Block of Lateral Geniculate Synapses and Relief by Repetitive Stimulation

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The mode of action of lysergic acid diethylamide (LSD) in blocking transmission through the lateral geniculate nucleus of the cat has been studied. The optic nerve and optic radiation fibers are unaffected by the drug, and the action is therefore on the synapse. LSD block is relieved to only a small extent by a 15-sec tetanus at about 500 per second to the optic nerve. A 10-min tetanus at the same frequency produces considerable relief of block which is usually not permanent. Since a 15-sec tetanus will relieve the depression produced by a previous 15-sec tetanus, its failure to relieve LSD block suggests that the output of transmitter during LSD block is normal, i.e., that the action of LSD is postsynaptic. It is inferred from this conclusion that output of transmitter after a 15-sec tetanus is increased above normal by not more than a few per cent; after a 10-min tetanus the increase is much greater and lasts for several minutes.

Introduction

Evarts, Landau, Freygang, and Marshall (11) and Bishop, Field, Hennessy, and Smith (4) have shown that lysergic acid diethylamide (LSD) blocks the synapse between optic nerve and optic radiation fibers in the dorsal nucleus of the lateral geniculate body of the cat. It was suggested tentatively (4) that LSD was acting postsynaptically rather like curare at the neuromuscular junction of striated muscle.

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The present paper attempts a further analysis of the mode of action of this drug on the geniculate synapses. The effects of repetitive stimulation on the normal lateral geniculate nucleus have been studied previously (2, 9, 10, 12). We have used the results of these investigations in studying the interaction between the effects of LSD and of tetanic stimulation of the optic nerve. It is concluded that LSD blocks synaptic transmission, most probably, by competitive antagonism to the normal transmitter.

Methods

The methods are described in greater detail in a preceding paper (2). Adult cats, both Dial-anesthetized and *cerveau isolé* preparations, were used. Lysergic acid diethylamide was injected into the carotid blood stream via a cannula in the lingual artery.[2] Stimulation of optic tract nerve endings was by means of a pair of insulated steel electrodes about 1 mm apart, placed in or just above the geniculate nucleus. The same electrodes were used for stimulating the optic radiation fibers. Records from the visual cortex were obtained using a silver-silver chloride agar wick electrode. In the experiments to be reported here, orthodromic stimulation of optic nerve fibers was confined to the fast-conducting group of fibers, designated t_1 (5). The testing interval was 5 sec throughout, except where otherwise indicated.

Results and Comments

Site of Action of LSD Block. LSD has no effect on the axons of either optic tract or radiation. This is illustrated in Fig. 1 which shows three pairs of records. The geniculate response to optic nerve stimulation is shown before (a), and after (b) 200 μg LSD. The response consists of three parts. Following the artifact (m) there is a small positive wave due to the arrival at the skull of impulses in the optic nerve. The following positive-negative wave (t_1) is the response of the fast-conducting group of optic tract fibers, and finally the negative wave (r_1) represents activity in the geniculate somata and radiation fibers (6). This r_1 wave becomes reduced to a synaptic potential (s_1) in b, but the tract response (t_1) is unaffected (4).[3]

A further indication that LSD was without effect on the tract response is provided by testing the excitability of the tract nerve endings. Synaptic

[2] For further details see reference (4).

[3] See also Fig. 2.

block might be obtained by depolarization or hyperpolarization of the nerve endings. Such changes should be revealed as an alteration in excitability of the fibers. The method of testing the excitability of the tract nerve endings is discussed in a preceding paper (2). Figure 1c and d, shows the response in the optic nerve (recorded in the same

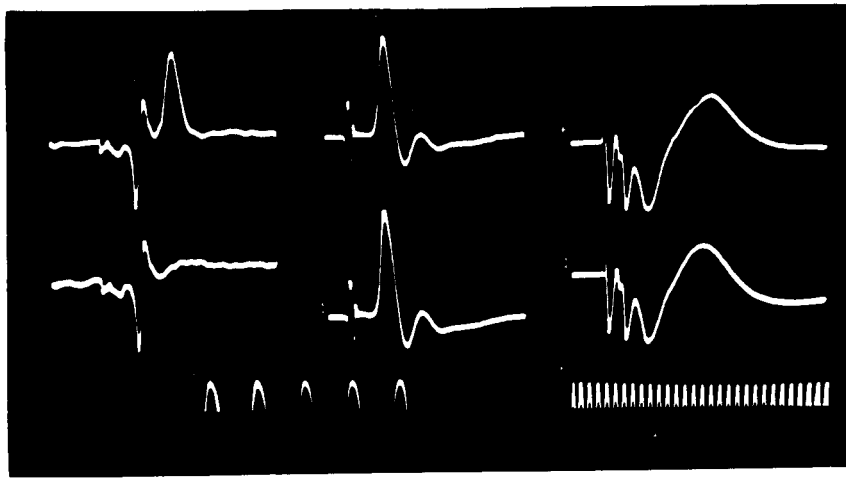


FIG. 1. Effect of LSD on optic tract, geniculate synapses, radiation axons, and visual cortex; a, c, f, before LSD; b, d, g, after LSD; a, b, response of geniculate nucleus to stimulation of contralateral optic nerve; c, d, response of optic nerve to stimulation of optic tract nerve endings; f, g, response of visual cortex to stimulation of radiation axons. a–d, same preparation, 200 μg LSD; f, g, another preparation in which the effect of LSD (250 μg) on geniculate nucleus was similar to that in a, b. Time calibration, e, applies to a–d; calibration, h, to f, g. m, stimulus artifact; t_1, response of fast-conducting optic nerve fibers; r_1, response of geniculate somata and radiation fibers; s_1, synaptic potential; $t_{1(a)}$, response of fast-conducting optic nerve fibers; $t_{2(a)}$, response of slow-conducting fibers, antidromically stimulated. Labeling of waveform in f is according to Chang and Kaada (7). Negativity is upwards in this and all other figures.

preparation and at about the same time as a and b, respectively) to stimulation of the contralateral tract nerve endings before and after the administration of the LSD. Stimulation is submaximal. Here the response consists of two negative waves ($t_{1(a)}$, $t_{2(a)}$), the responses of the two main groups of fibers (5). The optic nerve was crushed at the eyeball to make the responses monophasic, but in this experiment the crush was not quite complete and there is a small positive wave following each negative wave.

There is no change in the response after LSD indicating that there is no change in excitability of these fibers.

Records f and g are taken from another experiment and show the response of the visual cortex to stimulation of the radiation axons before (f), and after (g) 250 μg LSD producing an effect in the geniculate nucleus similar to that in a and b.[4] Wave 1 is absent from these responses. It is seen only on stimulation of the optic nerve and represents the action potentials in those fibers. Wave 2 is unaffected by the drug as are waves 3 and 4, although waves 5 and 6 are reduced in amplitude. The interpretation of waves 3 to 6 is not yet settled, but the main point here is that wave 2 which is the response of the radiation axons is unchanged by LSD.

The experiments indicate that LSD in the doses used has no action on transmission in the optic nerve nor in the main portion of the radiation axons. The results suggest that the action of LSD is on the synapse although the possibility of an action on the initial portions of radiation axons cannot be excluded.

Effect of 15-sec Tetanus on LSD Block. Figure 2A illustrates the effect of a 15-sec tetanus at about 520 per second on a geniculate block caused by a 200-μg dose of LSD given 1 min earlier. Figure 2B shows the response to LSD alone. In this experiment, there was no evidence that the tetanus in any way relieved the LSD block. The maximum post-tetanic r_1 response was about equal to that which would have been expected in the absence of a tetanus (Fig. 2B). Thereafter, the r_1 response declined again as it always does following a tetanus of this duration (2, 9, 10).

The effect of a 15-sec tetanus on an LSD block has been examined in seven experiments. The tetani were all at about 500 per second, of 15 to 20 sec duration, and were delivered between 1 and 5 min after the drug. In three experiments, the maximum post-tetanic r_1 response was not appreciably different from the level of recovery which would have been reached in the absence of the tetanus; in two experiments, the post-tetanic response was more than 10 per cent above this level; in the other two experiments it was more than 10 per cent below this level. The mean value for all experiments was 7 per cent above the normal recovery level.

In two of these experiments, an attempt was made to study the relationship between the degree of post-tetanic recovery and the normal curve of recovery from LSD block. One of these experiments is illustrated in

[4] The cortical responses are labeled according to Chang and Kaada's (7) nomenclature.

Figs. 3 and 4, the full details being given in the captions to the figures. A series of 15-sec tetani were delivered to the optic nerve at approximately 5-min intervals after an injection of 500 μg LSD, control recovery curves from a similar LSD block being obtained both before and after the series of tetani. The two control curves diverge from one another particularly

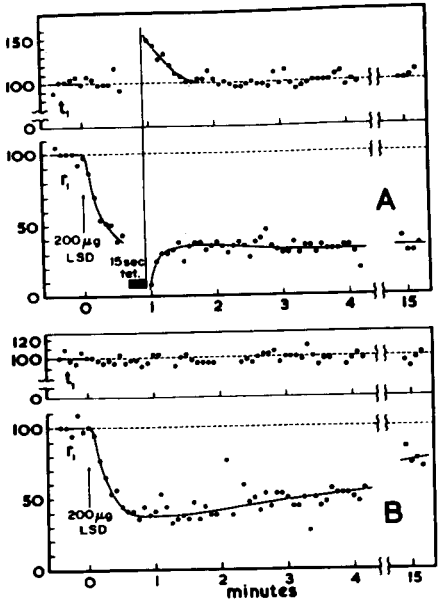


FIG. 2. Effect of 15-sec tetanus on response of geniculate nucleus depressed by LSD. In B, is graphed the response of the fast-conducting optic fibers (t_1) and postsynaptic cells and axons (r_1) before and following the close-arterial injection of 200 μg LSD. A is from the same preparation and is similar, but shows the effect of a 15-sec tetanus (at about 520/sec) given within 1 min of LSD. Ordinates, response as a percentage of normal response; abscissae, minutes.

between the fifth and fifteenth minute and it is, of course, impossible to be sure what the precise recovery curve after the second dose of LSD would have been in the absence of the tetani. However, after four of the seven tetani, the maximum post-tetanic response was no greater than either control curve and only the first and fourth produced a response appreciably above both control curves (117–129 and 107–113 per cent, respectively, of the control values). The degree of post-tetanic recovery during an LSD block is, therefore, very small. It is clear from these results that the block due to LSD must be different from that due to a

15-sec tetanus since the latter is relieved by a further 15-sec tetanus (2, 9, 10). The comparison was carried further by examining the responses during a tetanus.

Postsynaptic Response (r_1) during a Train of Stimuli. Figure 5 shows the geniculate responses during a train of 6 stimuli at about 360 per second.

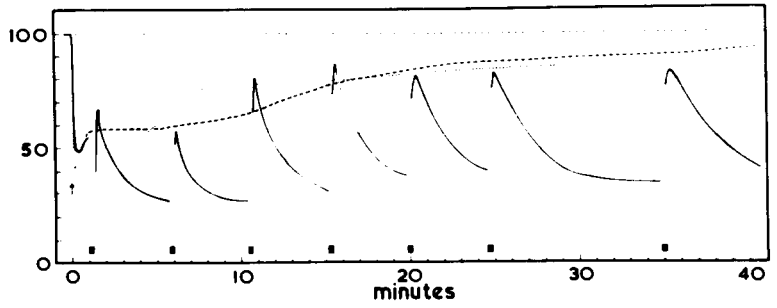


FIG. 3. Effect of a series of 15-sec tetani on the LSD depression of the postsynaptic geniculate response plotted as a percentage of the normal response. Dashed line: initial control intracarotid injection of 500 μg LSD before the tetani. Solid lines: effect of 15-sec tetani (465/sec) delivered at approximately 5-min intervals after the injection of a further 500 μg LSD. Dotted line: final control injection of 500 μg LSD after the depression produced by the series of tetani had been relieved by a 10-min tetanus (10). LSD injected at arrow. The solid bars near the time scale indicate the time of the tetani.

In a, the preparation was normal, and in b, the same preparation had received 50 μg LSD 1 min earlier; the first response in the train gives an indication of the degree of block. Succeeding r_1 responses increase to about the fourth, after which they decline progressively to remain very small throughout the rest of the tetanus. This effect has also been noted by Evarts and co-workers (11). A relief of block is also obtained during a short train of stimuli applied in a preparation blocked by a previous 15-sec tetanus (2). In this respect the depression produced by LSD is similar to that following a 15-sec tetanus.

Effect of 10-Min Tetanus on LSD Block. Figure 6 shows a series of graphs from the same preparation illustrating the effect of a 10-min tetanus on block due to LSD. The graphs A, B, C, D are in chronological sequence, the time of day being indicated by the vertical figures beneath each. After recovery from a small control dose of LSD (Fig. 6A), a second larger dose of LSD was given, followed by a tetanus from the first to the eleventh minute, after which the synapse was found to have recovered completely

(Fig. 6B). The recovery occurred in two stages, as is commonly found also after a 10-min tetanus in the normal preparation (2).

In five of the seven experiments in which the effect of a 10-min tetanus on LSD block was examined, the response was found to be fully recovered very soon after the end of the tetanus and subsequently exceeded the

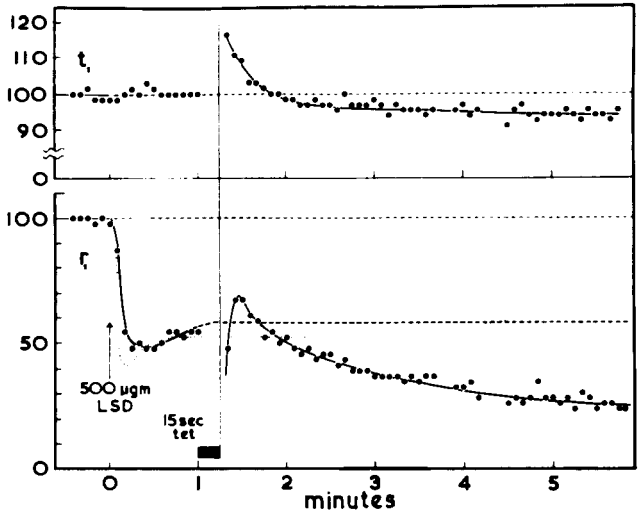


FIG. 4. Effect of 15-sec tetanus on LSD depression. Figure 4 shows the first few minutes of Fig. 3 on an expanded time scale using the same conventions for the three graphs, i.e., dashes, solid line, and dots. Upper graph shows response of t_1 fibers, lower graph that of the postsynaptic neurons (r_1). The plotted points and solid line graph the amplitudes of the t_1 and r_1 responses before and after the injection of 500 µg LSD, and then following a 15-sec tetanus delivered to the optic nerve 1 min after the LSD injection. The dashed and solid lines coincide in the first minute.

normal amplitude of the response. In the remaining two cases, the degree of recovery attained was greater than the corresponding point on the recovery curve following administration of the drug alone. On the average, the peak of the post-tetanic recovery curve was 24 per cent greater than the control curve at corresponding times. In the five cases in which the post-tetanic response was fully recovered, the percentage increase may have been limited by the available subliminal fringe.

In only two of the seven cases was the full recovery maintained. In the other cases, the response subsequently became depressed, the degree and duration of this depression varying considerably. In the experiment illus-

trated in Fig. 6B, the depression below the normal 100 per cent level commenced at 17 min, fell to 80 per cent of the normal value and had recovered to 97 per cent at 33 min. It seems probable that this depression was due to LSD remaining in the geniculate nucleus, since a delayed depression does not usually follow the 10-min tetanus in the normal preparation (2, 10).

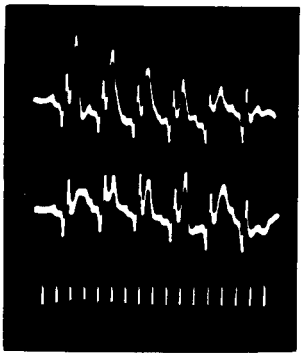


FIG. 5. Effect of train of 6 stimuli at about 360 per second on response of geniculate nucleus; a, normal; b, same preparation during LSD block (50 μg LSD, 1 min earlier).

The possibility that LSD might still be present in the geniculate nucleus after a 10-min tetanus, although the response was back to normal, raised the question whether the synapse might have become desensitized to the drug in some way. In order to test this possibility two experiments were performed in which a small dose of LSD was injected soon after the end of the tetanus. As shown in Fig. 6C, a small dose of LSD (40 μg) has its usual effect when given at a time when the response was nearly normal. The time course of the depression due to this dose is shown at greater length in Fig. 6D, so that it can be compared with the time course of depression due to the same dose at the start of the experiment (Fig. 6A). The degree and duration of the block was substantially the same. This observation, therefore, rules out the possibility of desensitization.

It appears that any LSD still present in the geniculate nucleus after the tetanus is free to combine with and dissociate from the receptor molecules. We are therefore led to the conclusion that the 10-min tetanus gives rise to a large post-tetanic increase in output of transmitter lasting 15 min or more.

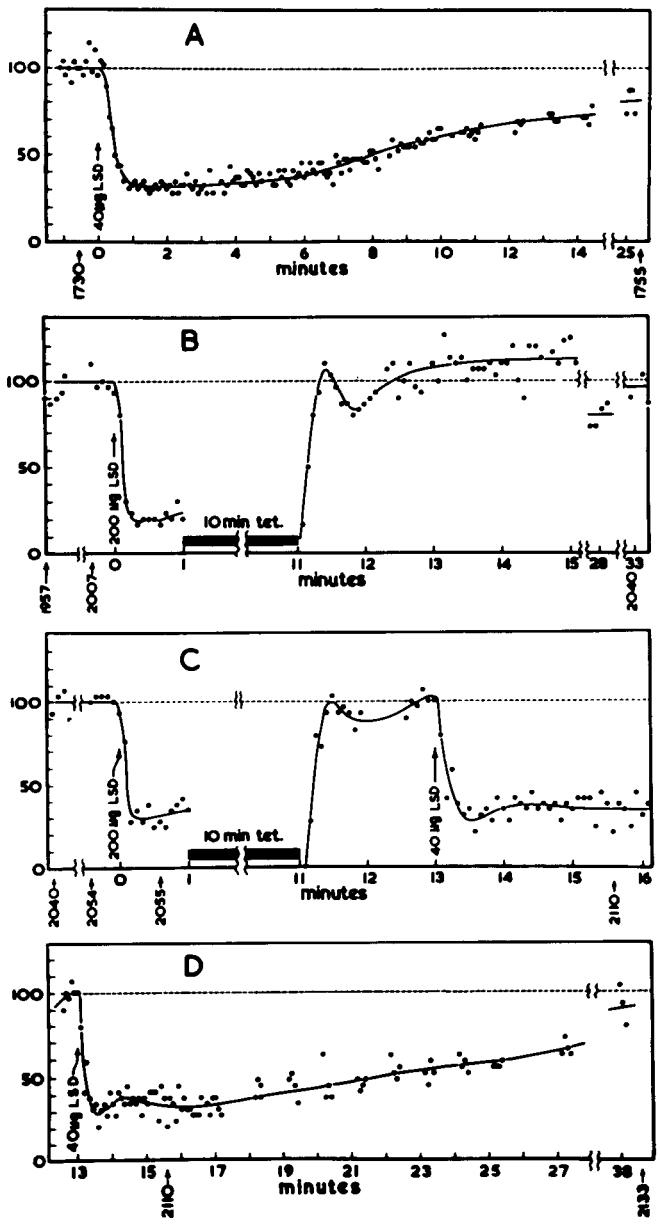
Discussion

Our finding that the 15-sec tetanus produces very little relief of LSD block is in disagreement with that of Evarts and Hughes (8, 9), who report post-tetanic potentiation up to 700 per cent that of the pretetanic level and a duration of potentiation lasting several minutes. Evarts and Hughes used a 20-sec tetanus, but in our experience this is not different in effect from a 15-sec tetanus. Nor is there any difference between *cerveau isolé* and barbiturate-anesthetized animals as far as this particular point is concerned. We are unable to explain the large quantitative difference between our results and those of Evarts and Hughes.

The experiments illustrated in Fig. 1 indicate that block of transmission through the geniculate nucleus by LSD is due to an action on the synapse or on the proximal portion of the postsynaptic fiber. However, it is unlikely that the electrical excitability of the geniculate cells is in any way affected since these cells will fire readily on the second, third, fourth, or fifth shock of a short train of stimuli (Fig. 5). The question then remains whether the immediate cause of the block is reduced output of transmitter or reduced sensitivity of the postsynaptic membrane.

The relief of block during a short train of stimuli (Fig. 5b) is probably not due to increased output of transmitter, since it has been observed (1, 6) that the synaptic potential usually declines progressively during a tetanus. It seems probable that this relief of block is due to a summation of synaptic potentials reaching threshold for firing on the second, third, fourth, or fifth stimulus in different cells. Thereafter, there is block due to refractoriness of cells and reduced amplitude of synaptic potential (1). These effects should be distinguished from those following the tetanus when there is no possibility of summation of synaptic potentials (because the test stimuli are now applied infrequently) nor of excitability changes in the cell (since the cell has been inactive during all but the first few stimuli of the tetanus).

FIG. 6. Effect of 10-min tetanus on response of geniculate nucleus depressed by LSD. The graphs are of amplitude of postsynaptic response and are in chronological sequence from top to bottom (A to D), the time being indicated beneath each graph at various intervals. Plotted points are as percentages of the mean level of response prior to administration of the drug (dashed lines). A shows the control response to 40 μg LSD. B shows the effect of a 10-min tetanus (600/sec) commenced 1 min after 200 μg LSD. C is similar to B but a further dose of 40 μg LSD was given 2 min after end of tetanus. The time course of effect of this dose is given on a longer time scale in D.


A
100
50
0
40mg LSD
0
2
4
6
8
10
12
14
25
minutes
1730
1755
B
100
50
0
200 mg LSD
10 min tet.
0
1
11
12
13
14
15
28
33
minutes
1957
2007
2040
C
100
50
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200 mg LSD
10 min tet.
40 mg LSD
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1
11
12
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14
15
16
minutes
2040
2054
2055
2110
D
100
50
0
40mg LSD
13
15
17
19
21
23
25
27
38
minutes
2110
2133

In the normal preparation, a 15-sec tetanus is followed by a phase of potentiation and then one of depression from which the synapse can be relieved by a further tetanus. It was suggested in a preceding paper (2) that the potentiation was due to an increase in output of transmitter and the depression to a reduction, and that a further 15-sec tetanus temporarily restored the output to normal values or greater. Hence the failure of the 15-sec tetanus to relieve the LSD block to any appreciable extent (Figs. 2, 3, 4) suggests that the output of transmitter prior to the tetanus (i.e., during the LSD block) was nearly normal. If so, it would not be surprising if the post-tetanic response showed very little increase. We therefore conclude that output of transmitter is normal during LSD block and therefore, that the blocking action of LSD is postsynaptic.

If this conclusion is accepted we can make some deductions concerning output of transmitter after either a 15-sec tetanus or a 10-min tetanus. In the normal preparation either tetanus produces a post-tetanic potentiation of about 10 per cent. The increase in the response is limited by the extent of the subliminal fringe which in the geniculate nucleus is not large (3, 13). In the LSD-blocked preparation, this limiting factor has been reduced by extension of the subliminal fringe and it is now seen that the post-tetanic effects of the two tetani are very different. In agreement with Evarts and Hughes (10) it has been found that in most cases a 10-min tetanus produced a complete relief of LSD block; the relief was permanent in two of our experiments. This relief of block does not appear to be due to insensitivity of the synapse to LSD, since a small dose of LSD during this time has its normal effect. The fact that a measure of depression usually returned after the relief of the block by the 10-min tetanus (absent in the normal preparation) indicates that some LSD remained at the synapse. It is difficult to say whether any of the LSD had actually been removed by the tetanus over and above the normal process of elimination. In this connection one might note that it is not necessary for the transmitter to displace the drug from the receptors; relief of block is possible provided a sufficient number of receptors remain unoccupied (14). It is concluded therefore that the output of transmitter after a 10-min tetanus is considerably increased. On the other hand a 15-sec tetanus produces very little post-tetanic increase in output.

The experiment illustrated in Figs. 3 and 4 and others in which more than one 15-sec tetanus have been applied during LSD block have shown that the post-tetanic response does not exceed the control recovery curve

by more than 10 to 20 per cent. In the different experiments the level of LSD block varied from 33 to 76 per cent, and in the experiment of Figs. 3 and 4, from 55 to 93 per cent. There was no relationship between the degree of post-tetanic relief of LSD block and the actual level of the LSD block at the time when the tetanus was applied. These facts again suggest that the output of transmitter following a 15-sec tetanus is only slightly above normal.

The average increase in r_1 response following a 10-min tetanus was 24 per cent, but in five of the seven experiments the post-tetanic response was more than 100 per cent, hence the value of 24 per cent is a minimal one. In one experiment, the post-tetanic response rose to 109 per cent at a time when the level of LSD block was estimated to be about 58 per cent, an increase of 88 per cent. Of course, it is not possible to say what this means in terms of amounts of transmitter.

The conclusion from these experiments is that the action of LSD is most easily explained as due to competitive inhibition of post-synaptic receptors; that after a 15-sec tetanus the output of transmitter from the optic nerve endings does not exceed the pretetanic level by more than a few per cent and for no longer than about 1 min (Fig. 4); that after a 10-min tetanus the output is considerably greater than the pre-tetanic level and this effect lasts for several minutes (Fig. 6B).

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