DIFFERENTIAL EFFECT OF LSD UPON HABITUATING AND EXTINGUISHING EVOKED RESPONSES

GUY C. SHEATZ, PH.D. * AND DONALD F. BOGDANSKI, PH.D. *

ABSTRACT

LSD, in small doses (10 μg/kg) which affect behavior only in the absence of an active environment and which only minimally alter the spontaneous EEG, draws a clear distinction between habituating and extinguishing evoked auditory responses by preferentially facilitating the latter. It also establishes a condition favorable to prolongation or oscillation of the response. Response components may be visible as long as 5 sec. following the click. LSD potentiated responses do not follow the usual fast extinction curve, but they are easily reduced by distraction and by small doses of nembutal.

Terminology itself implies a fundamental difference between the states of habituation and extinction, even though these states are indistinguishable by the criterion of behavioral indifference to a given stimulus. It is thought that the process of conditioning establishes irreversible changes in the CNS which cannot be completely erased by a repeated unreinforced stimulus. Such differences are well documented by many behavioral tests, but the underlying central nervous mechanisms have so far eluded direct analysis. In the course of a series of experiments on the effects of drugs upon the evoked responses, it became apparent that LSD was able to reveal a different "set" of the CNS in the habituated and extinguished state by exhibiting a differential effect upon EEG responses to clicks. The experiments described in the following account were designed to subject this observation to further test.

METHOD

In 8 monkeys and 7 cats, bipolar, enameled, .01 inch thick (# 316) stainless steel electrodes were implanted in a total of 42 brain loci, following a technique previously described by Sheatz. The monkeys were partially restrained in chairs placed in a semi-soundproofed, sightproofed, electrically shielded cage, and studied 1-15 months. Electrode locations were verified post mortem by histologic techniques. Brain responses evoked by approximately 80 db clicks were simultaneously recorded on a Grass 3D electroencephalograph and camera-oscilloscope. Responses were analyzed formally by superposition of enlarged EEG traces and by film. Statistical analsis was made on amplitude measurements from all active brain areas. Near the end of these experiments, a computer, built in our shop, after the design of Cox and Evarts, [6] became available for visual presentation of the average response.

Recordings were taken in several or all of the four psychological states related to a stimulus; novel, habituated, conditioned, extinguished. The clicks were separated by 10-30 sec. and were presented continuously or in series of 6-11 separated by intervals of 1-5 minutes. Habituation or extinction were achieved by presentation of the clicks for periods of hours or months. Conditioning was obtained by a puff of air delivered to the subject's face by blowing into a 10-foot tube at the sound of the click. The state of conditioning or extinction was gauged by features of the EEG response to the clicks.

* Department of Neurophysiology, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D. C. Dr. Bogdanski now at NIH, National Heart Institute, Sec. Chem. Pharm., Bethesda 14, Md.

Received for publication: April 27, 1964.

LSD, 4-20 μg. per kg., was administered in the vein or lung at strategic times in the four psychologic states to study the interaction of state and drug. A 10 μg. dose was soon taken as the standard and used in the majority of the experiments.

Behavioral:

Within seconds after the I.V. administration of 4-20 μg/kg LSD to monkeys, behavioral changes appeared which led in a few minutes to one of several behavioral configurations, which then persisted for one-half to several hours. (Licking of the lips and chewing of the tongue was sometimes transitory and sometimes persistent). (1) In conditions most favorable for recording of the EEG, random movement soon ceased and the animal became immobile, staring with fixed eyes for many minutes without change. (2) Some subjects in these conditions would gradually lose muscular tonus and sink down in the chair, suddenly arouse, straighten up, and look briefly about. (3) One of the more aggressive males struck out into space with one arm, every 30-50 sec. (4) More rarely, a monkey that was habitually restless in a recording session would become more so under LSD. All animals gave less behavioral attention to random noises.

In all cases monkeys assumed an essentially normal manner when approached. They made the customary gestures to discourage closer contact or pushed away proffered hands. They accepted food and ate voraciously. There was very little evidence for reported increases of viciousness or aggression.

A dichotomy appears between behavioral apathy of the isolated monkey and the activation pattern of the EEG. The latter is not, however, entirely typical of arousal, since sleep waves may occasionally appear.

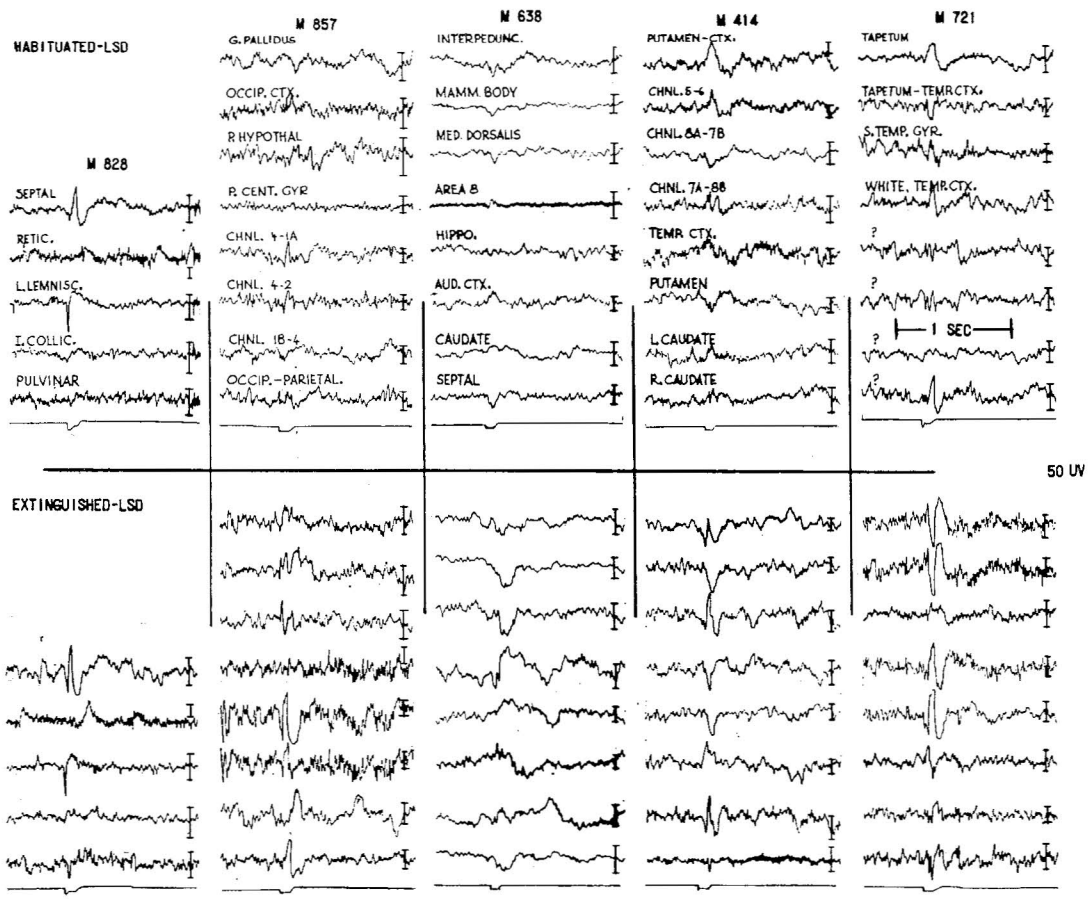

Fig. 1. *EEG Traces. Contrasting effects of LSD in Habituation and Extinction.*
The 5 monkeys are identified by an 'M' and a number at the head of the vertical columns. Habituation and long-term extinction (one to several months, M #721 and M #828), are displayed above the horizontal dividing line and short-term extinction below. The notch in the bottom line indicates the onset of an 80 db (approx.) click in an approximately 30 msec (microphone measurement) envelope. The electrode placements as abbreviated in this figure are more fully indicated in Table I.

The effect on the EEG is more obviously a reduction in amplitude of spontaneous activity than an increase in frequency. The general action of LSD on the EEG is opposite in direction to that which would a priori be correlated with an increased responsivity to clicks, but the reduction in random movement seems favorable under most experimental conditions for an increase in evoked response.

LSD Action in Habituation and Extinction:

These two states, while entirely similar behaviorally, electroencephalographically, and in the shape

TABLE I

MATHEMATICAL COMPARISON OF THE EFFECTS OF LSD IN HABITUATION VERSUS EXTINCTION

	Channel	Area	N	X uV	N	X uV
M	4	Septal area	176	51.7	118	65.0
8	5	Lat. Retic., near Coch. Nuc.	"	26.9	"	100.2
2	6	Nuc. Lat. Lemniscus	"	100.1	"	93.5
8	7	Brach. Inf. Collic.		0		0
	8	Pulvinar, Near M. Genic.	"	43.3	"	52.0
	1	Glob. Pallidus	42	26.2	30	46.7
M	2	Occip. Ctx., White	"	29.8	"	59.5
8	3	Post Hypothal.	"	20.8	"	94.5
5	4	Post Cent. Gyrus	"	28.8	"	55.5
7	5	Channels 4 to 1A	"	64.0	"	109.0
	6	" 4 to 2	"	69.0	"	117.5
	7	" 1B to 4	"	49.0	"	92.5
	8	" 2 to Parietal Ctx.	"	61.5	"	79.6
	1	Interpeduncular NuC.	60	19.5	95	39.6
M	2	Mammillary Body	"	15.7	"	50.1
6	3	Medialis Dorsalis	"	17.5	"	51.0
3	4	Ctx. Area 8	"	32.8	"	71.0
8	5	Hippocampus	"	24.9	"	87.1
	6	Auditory Cortex	"	36.1	"	77.6
	7	Caudate Nucleus	"	16.4	"	69.5
	8	Septal Area	"	39.8	"	40.1
	1	Parietal to Putamen	139	126.6	95	132.0
M	2	Channels 5 to 6	"	93.1	"	110.0
4	3	" 8A to 7B	"	41.4	"	75.0
1	4	" 7A to 8B	"	40.0	"	57.5
4	5	Temp. Ctx.	"	93.7	"	98.1
	6	Putamen	"	61.7	"	92.0
	7	L. Caudate Nuc.	"	43.7	"	65.0
	8	R. Caudate Nuc.	"	48.4	"	30.2
	1	Tapetum	240	34	120	164
M	2	Tapetum to S. Temp. Gyrus	"	43	"	120
7	3	S. Temp. Gyrus	"	49	"	34
2	4	White, S. Temp. Gyrus	"	49	"	96
1	5	?	"	39	"	109
	6	?	"	41	"	49
	7	?	"	20	"	72
	8	?	"	63	"	108

Data were compiled from peak to peak measurements of each evoked response from 10 minutes to 1-½ hours subsequent to LSD injection. The monkeys are identified as in Fig. 1, and the brain areas apply to Fig. 1, numbering channels 1 to 8 from top to bottom of the respective EEG traces. Implant areas not verified histologically are followed by a '?' mark, and rely on Horsley-Clarke target coordinates. In the vertical column head, 'N' refers to the number of responses measured and 'X bar' to the average response in microvolts.

and size of the evoked response, are quite different as judged by the action of LSD upon the evoked response. The chief differences are illustrated in Figure 1 and Table I. In 27 out of 36 brain areas the evoked response is more conspicuous in the extinguished than in the habituated state. The increase consists both in amplitude and complexity of form changes. Certain subcortical structures exhibit such increase in a ratio of 3 or 4 to 1: pontine, medullary, lower reticular substance, posterior hypothalamus, mammillary body, hippocampus, one of three caudate areas recorded from, and the tapetum; while in cortical areas, including the auditory, more modest differences are noted The one primary afferent pathway represented, the nucleus of the lateral lemniscus, is clearly not much influenced: In this structure, evoked responses are of lesser amplitude in the extinguished than in the habituated state, and may actually be nearly as small as in extinguished animals not treated with LSD. The lower average response in the LSD-habituated state reflects the greater variability as well as lesser amplitude of the evoked responses.

A fundamentally different click presentation schedule may be used under LSD. In the normal conditioned animal, presentation of more than 6-12 clicks in sequence, even if separated by 10-30 seconds, leads rapidly to response attenuation. Under LSD, however, continuous presentation does not lead to diminution. While evoked responses continue unabated, the animal may be in a completely immobile "LSD trance," and not display the sightest trace of the startles which might ordinarily accompany such large responses. If the animal's attention is now briefly pre-empted visually, these large responses are reduced to very low levels. Thus, electrically, as well as behaviorally, the animal can reverse the effects of this dose of LSD.

It must be pointed out that 2 of the monkeys (M721 and M828), in the top section of Figure 1 and Table I, are actually in a state of 'long term' extinction rather than habituation. One of them, M721, had never been well conditioned, having had only 3 pairings of clicks and unconditional stimulus. Both had been under continuous day-night extinction for 1-2 months prior to this series of experiments. No effort was made to quantitate the difference between habituation and long term extinction, but it is clear that they are essentially together in a different category than short term extinction.

The foregoing data were compiled from peak to peak measurement of each evoked response. Reliable insight into the initial fast components as well as the general morphology of the response is afforded by computer analysis (fig. 2) of recordings obtained in a monkey who had not been studied in habituation. It is seen that changes in form may be more striking (experiment repeated on 4 different occasions) than change in over-all amplitude. Reference is made to the short latency positive and negative components.

It has been pointed out that it is easy to reverse both the behavioral and EEG effects of this dose of LSD. To test whether the same effect could be obtained pharmacologically, a small dose (3 mg per kg) of nembutal was injected at the height of the

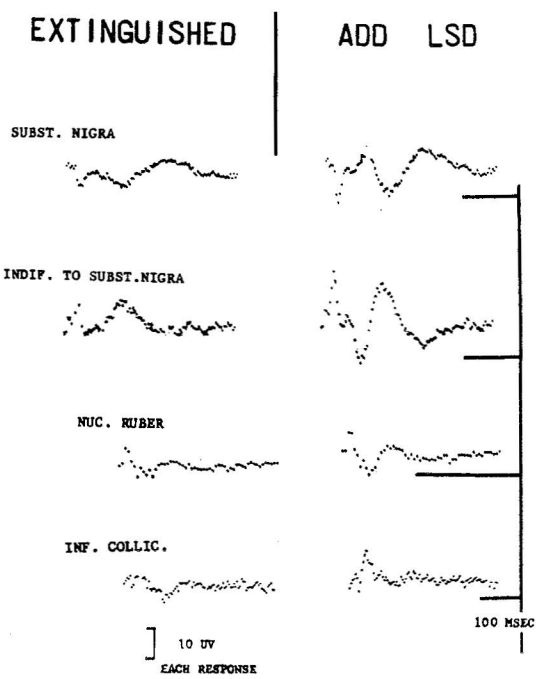

Fig. 2. *Computer Integrated Average Responses.* 50 auditory responses summed in each trace. Each dot in the trace represents 3.2 msec. 75 db clicks were presented at 10 sec. intervals. LSD records taken 20-50 minutes after 15 μg per kg. injection.

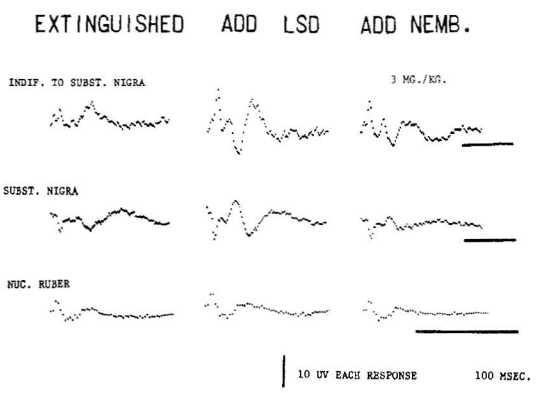

Fig. 3. *Reversal of LSD Effect by Small Doses of Nembutal.* 50 auditory responses summed in each trace. The LSD records were obtained 15 min. after administration of the drug, and the nembutal records, 10 min. after it's administration.

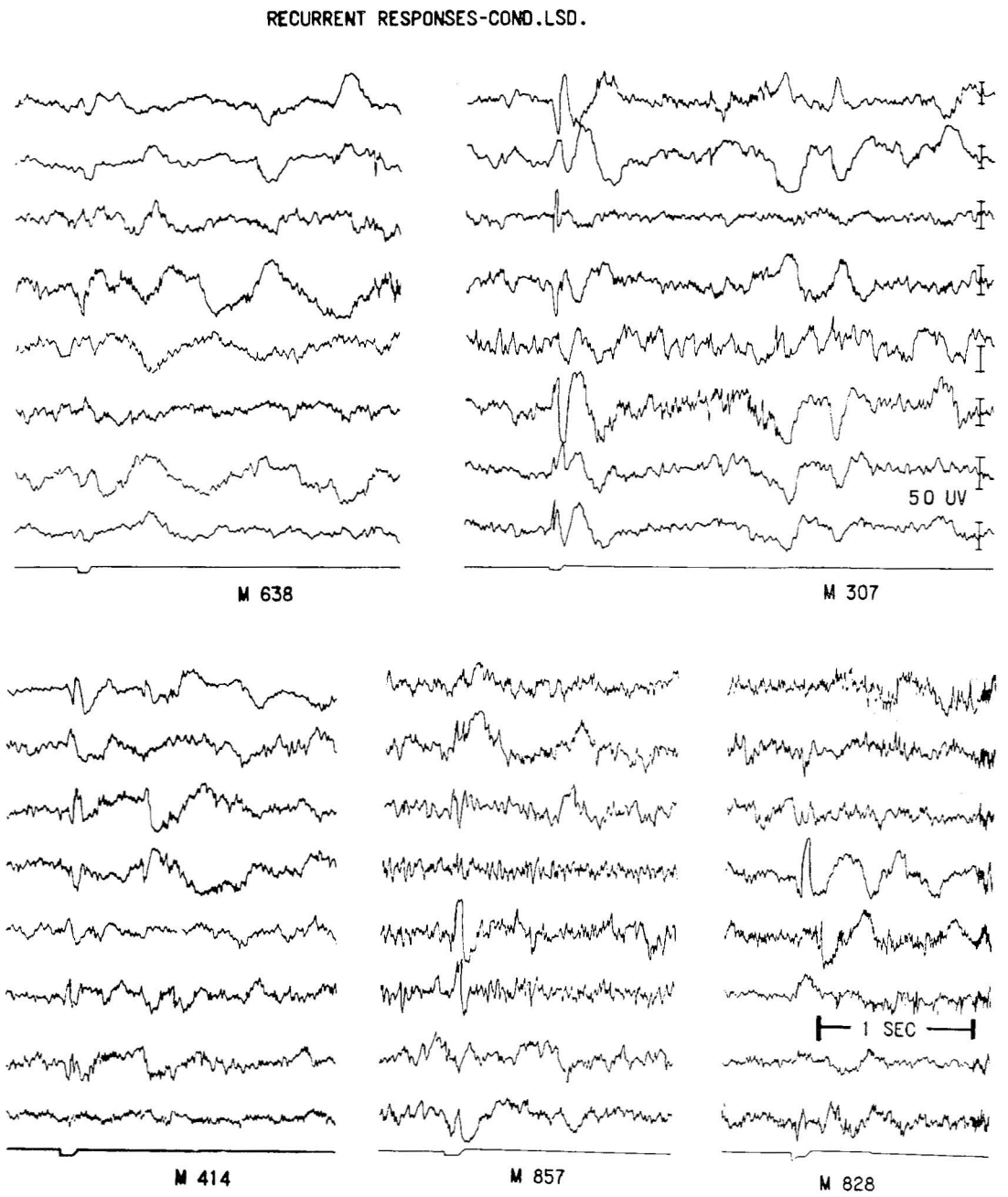

Fig. 4. *Fully Conditioned under LSD, Recurrent Responses.*

For all monkeys except M 307, the brain areas, time scale and amplitude scale are the same as designated in Fig. 1 and Table I. For M 307 the verified brain areas are (top to bottom), 1 Pedestal (ground) to central grey of midbrain, 2 amygdala to hypothalamus, 3 central grey to tegmentum, 4 tegmentum to central grey (by different combinations of the bipolar pairs than in 3), 5 basal nucleus of amygdala, 6 lateral hypothalamus, 7 tegmentum bordering pretectal area, 8 dorsal central grey of midbrain. All large waves in these EEG traces are 'recurring' response components.

LSD effect. It is seen (fig.3) that the LSD potentiation was removed without reducing the response below its control values.

Reinforcement under LSD:

These experiments were designed for maximal contrast between habituation and extinction; therefore, since LSD has a rather short circulatory half-life, reinforcement was not routinely performed under the high points of the drug curve. However, the following observations may be reported: Variously in different animals, a few reinforcements would increase, reduce or leave unaltered the amplitude and form of the maximal conditioned response. Fortunately, the first animal used, M307, was of the first category, and the response was so dramatic as to warrant a thorough study of the drug effect. Reductions of the evoked response were less commonly observed, and appeared to occur only in animals who became overactive in conditioning.

Responses brought to their fullest delineation by reinforcement under LSD are peculiarly liable to temporal extension. This extension may take the form of rhythmical repetition of the last slow wave component of the response or the reappearance of a response pattern seconds after the first has been terminated (Fig. 4). They are comparable in curve area to the original response. When such phenomena appear, they are present in 10-50% of the responses during maximal conditioning. Their variability is marked with respect to repetition rate, onset, duration, size and configuration. They may or may not be accompanied by, or loosely associated with, a modified startle response.

EPICRISIS

If it is assumed that the functional properties of afferent systems are little if at all affected by conditioning processes, the phenomena described above would seem attributable to a preferential effect of LSD upon central mechanisms of higher integration. Interesting though this conjecture may be, it is hard to substantiate in the absence of accurate knowledge regarding place and mode of action of LSD. Physiological demonstration of its action in the CNS, other than on primary afferent systems has not been easy. Purpura [16] has concluded that a major effect of LSD is to inhibit axodendritic and to facilitate axosomatic synapses regardless of their location in the brain. In his experiments, recruitment, evoked from the centromedian thalamic nucleus was decreased in excitability by small doses. Pupura's conclusion is compatible with a recent notion [13] that LSD "acts on thalamo-cortical areas where functional impairment produces schizophrenia." Elkes et al. [7] found that doses of LSD 5 to 10 times those used in the present experiments abolish the spiking phase of barbiturate anesthesia, and Pupura proposes in view of this finding, that the effect of LSD on the EEG is actually one of depression rather than activation of cortical axodendritic activity. Psychologists often attribute LSD symptomatology to a depression of higher inhibitory functions which helps in "releasing unconscious material," [17] or in "permitting re-examination of significant experiences from the past, which sometimes were relived with frightening realism." [5] While the above observations seem to fit into the theory of an inhibitory "release" effect of LSD on the CNS, they seem incompatible with the great facilitation of evoked responses observed in the present experiments. The majority of our recording sites were outside the afferent auditory system proper, and it thus appears likely that the eliciting impulses were mediated by multineuronal paths similar to those related to the reticular formation, and believed to be prim targets for neurotropic drugs.

We find the potentiating action of LSD on the extinguishing responses abolished by very small doses of nembutal (2-3 mg./kg.) which would only so affect multineuronal paths by an inhibitory action. [10] But, if LSD indeed potentiates evoked responses in the psychological state of extinction by some mechanism of "release," then nembutal would have to be reducing it by facilitation. In our experiments the process of extinction seemed weakened by LSD, for continuous presentation of clicks during the 1-2 hour experimental session may not result in diminution of evoked responses in subjects known to exhibit under normal conditions

a gradual decrease of response amplitude. It is important to note that the large responses in question do not show the stability which characterizes those recorded from lower neural structures. They are, for example, instantly blocked by such simple expedients as holding a mirror in front of the monkey's face. When the distraction is removed, the responses soon return to full size.

Further indications of an involvement of higher nervous structures are found in the way in which stimuli and symptomatology are modified by environmental and social settings. [8] Threatening non-rewarding circumstances lead to an increase in severity of the symptoms of LSD intoxication. [4] It is easy to see, in this light, how the extinguishing stimulus, which only a short time before caused drastic reactions throughout the learning brain, might be peculiarly susceptible to the effect of LSD.

In contrast, the lesser facilitation occuring in the habituating response could be simply explained in terms of known effects of LSD on afferent structures. Purpura [15] found evoked responses in auditory and visual areas facilitated by doses of LSD similar to those used in our experiments. He found, as did Evarts and Marshall, [9] in the lateral geniculate body, that high doses adversely affect impulse transmission through the medial geniculate body. LSD action in the optic system can be traced as far peripherally as the retina. [1] Auditory hyperacusis was noted by many of Stoll's subjects in the absence of auditory hallucinations. [19] The reaction time in the conditioned avoidance test is shortened as well as the withdrawal time in analgesic tests, presumably by "sensitization of the CNS for afferent impulses." [20] However, in our experiments the potentiated habituating response showed itself to be quite variable, and this is not consistent with a purely afferent effect. The efferent effect could, however, be modulated by the more central "wave-like" syndrome of LSD described by Stoll. [19]

The above consideration of the afferent auditory system as a relatively simple direct pathway must be qualified by recent findings. The fully conditioned evoked response persists at the cortical level in spite of complete section of the brachium of the inferior colliculus, and the presumable, but hitherto undefined, parallel corticopetal pathway is peculiarly sensitive to drugs. [11]

Under conditions of LSD influence, the evoked response may undergo early, late, and "recurrent" modifications. The early accentuation is consistent with the above references to a facilitating effect on afferent impulse transmission. The late and recurrent changes find accord with the experiments of Killam and Killam, [14] in which EEG seizures in the hippocampus evoked by stimulation of the precomissural fornix were found to be prolonged by LSD. Also, intermittent photic stimulation has enhanced, or brought out LSD symptoms, [3] and caused irradiation of evoked responses. [12] Ordinarily, a monkey that is sufficiently apprehensive to startle to a conditioned stimulus will do so following a brief reaction time, but a subject in LSD trance may display a modified startle as late as one or more second after the stimulus. In our experiments, the recurrent response could not often be correlated with any behavioral characteristic.

In a preliminary account of these experiments, [2] attention was addressed to the mechanism of action of LSD. The findings were, that neither adrenergic (d-amphetamine) nor cholinergic (physostigmine) effects were remotely similar to those of LSD. However, the serotonin precursor, 5 hydroxytryptophan (5-HTP) was found to have effects similar in character to those of LSD, and of nearly equal magnitude. It appears conceivable to us that LSD is in some way related to serotonin as regards its mechanism of action. The apparent 1000 to 1 advantage of LSD over 5-HTP, on a dosage basis, presumably reflects the losses which the latter substance undergoes in

conversion to serotonin and finding its active site in the brain.

Although critical comparison is impossible, our preliminary work with cats suggests a species difference with regard to our experimental method between cats and monkeys. LSD facilitation was so poor in most of the cats carried through the preconditional phase, that further work was confined to the monkey. Only in two of the cats was potentiation of the habituating response clear enough to be noticeable upon casual EEG inspection. Other workers have been more successful,[15] and have observed facilitation of auditory stimuli in our dose range. Most physiological work has employed a much higher dosage than that used in the present study.

While these experiments have demonstrated a different reactivity of the habituating and extinguishing CNS to LSD, it must be pointed out that as extinction continues indefinitely (one or more months in our experiments), the differences dwindle and approach an asymptote that would be very difficult to determine by the present method. This would argue for a lesser neurophysiological difference between the habituated and extinguished state than is usually believed to exist. However, this may be attributable in our experiments to the potential weakness of the air puff in comparison with the more widely used electric shock as an unconditioned stimulus.

REFERENCES

1. Apter, J. T. and Pfeiffer, C. C.: *Am. J. Opthal.,* 42:206-211, 1956.
2. Bogdanski, D. F., Galambos, R., and Sheatz, G. C.: *Am. Soc. Expth. Pharm. & Therap.,* Miami, 1959.
3. Bradley, P. B., Elkes, C. and Elkes, J.: *J. Physiol.,* 21:50-51, 1953.
4. Boston Psychopathic Hospital; Six Staff Members: *Experimental Psychosis. Sci. Amer.,* 192, 6:34-39, 1955.
5. Busch, A. K. and Johnson, W. C.: *Dis. Nerv. Syst.,* 11:241-243, 1950.
6. Cox, R. R. and Evarts, E. W.: *EEG Clin. Neurophysiol.,* 13:478-480, 1961.
7. Elkes, J., Elkes, C. and Bradley, P. B.: *J. Ment. Sci.,* 100:125-128, 1954.
8. Elkes, J.: *Neuropharmacolgoy, Trans. Third Conf. New York.* Joshua Macy, Jr. Foundation, 1957.
9. Evarts, E. W. and Marshall, W. H.: *Trans. Amer. Neurol. Assoc.,* pp. 58-60, 1956.
10. French, J. D., Verzeano, M. and Magoun, H. W.: *A.M.A. Arch. Neurol. Psych.,* 69:509, 1953.
11. Galambos, R., Myers, R. E. and Sheatz, G. C.: *Am. J. Physiol.,* 200:23-29, 1961.
12. Gastaut, H., Feffer, S. and Costells, C.: *Confin. Neurol.,* Basel, 13:102-120, 1953.
13. Hoff, H. and Arnold, O. H.: *Proceedings of the 1st Internat. Congress of Neuro-psychopharmacology.,* Rome, Sept., 1958.
14. Killam, K. F. and Killam, E. K.: *J. Pharmacol.,* 116:35, 1956.
15. Purpura, D. P.: *Transactions of the Amer. Neurological Assoc.,* 80th Meeting, 60-67, 1955.
16. Purpura, D. P.: *Arch. Neurol. Psychiat.,* Chicago, 75:132-143, 1956.
17. Sandison, R. A.: *Bull. Org. Mond. Santé,* 21:495, 1959.
18. Sheatz, G. C.: *Electrical stimulation of the brain.* Univ. Texas Press. 1961.
19. Stoll, W.: *Schweiz. Arch. Neurol. Psychiat.,* 60: 1-45, 1947.
20. Taeschler, M. and Cerletti, A.: *Munchen. Med. Wchnschs.,* 102:1000, 1960.