THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 151, No. 3

AN ELECTROGRAPHIC STUDY OF *d*-LYSERGIC ACID DIETHYLAMIDE AND NINE CONGENERS

ARLENE K. SCHWEIGERDT,[1] ANN H. STEWART AND HAROLD E. HIMWICH

Thudichum Psychiatric Research Laboratory, Galesburg State Research Hospital, Galesburg, Illinois

Accepted for publication September 24, 1965

ABSTRACT

SCHWEIGERDT, ARLENE K., ANN H. STEWART AND HAROLD E. HIMWICH: An electrographic study of *d*-lysergic acid diethylamide and nine congeners. J. Pharmacol. **151**: 353–359, 1966. Ninety-six rabbits were used for an electrographic analysis of *d*-lysergic acid diethylamide (LSD) and 9 congeners. Experiments performed on the groups of intact animals showed that sustained drug-induced EEG arousal patterns were obtained with LSD, *d*-lysergic acid monoethylamide (LAE), *d*-lysergic acid morpholide (LSM), *d*-1-methyl-lysergic acid diethylamide (MLD) and *d*-lysergic acid dimethylamide (DAM), but not with 1-methyl-3-lysergic acid butanolamide (UML), *d*-isolysergic acid diethylamide (*d*-iso-LSD), *l*-lysergic acid diethylamide (*l*-LSD) and 2-brom-*d*-lysergic acid diethylamide (BOL). All drugs which produced EEG activation were psychotomimetic, but the reverse did not necessarily hold true. One hallucinogen, *d*-lysergic acid amide, failed to elicit EEG arousal. In order to ascertain a site of EEG alerting, a series of transections consisting of sections above the midbrain, below the midbrain and at the level of the first cervical vertebra were made. Cervical transection neither abolished the evoked activation when LSD was given previous to the section nor inhibited the appearance of EEG arousal in rabbits transected prior to the administration of the drug. However, the arousal reaction was abolished by transections caudal to the midbrain, indicating an effective locus for LSD between these planes. Even though LSM, MLD and DAM evoked EEG alerting in animals with intact brain, the drug-induced arousal could be neither initiated nor maintained following cervical transection. In contrast, LAE elicited activation following transections posterior to the midbrain whereas sections above the midbrain abolished the drug-induced pattern. These results indicate effective loci for EEG alerting of the hallucinogens in the lower brainstem region. In general, our results show that those psychotomimetic compounds containing the N-diethyl configuration exhibited loci of action in the lower brainstem. Furthermore, it has been suggested that the indole group and the N-diethyl or N-dimethyl configuration are important in producing changes in the site of EEG activation as well as in psychotomimetic behavior.

That LSD and its congeners can affect behavior is well documented (Hoch *et al.*, 1953; Abramson *et al.*, 1955; Von Felsinger, 1956; Hirsch *et al.*, 1956; Isbell *et al.*, 1956; Solms, 1956). Exact analyses of the effects of these substances in the brain are, however, few in number. Woolley and Shaw (1954) have suggested that LSD affects behavior by competitive inhibition with serotonin and normal brain constituents. Welsh (1954) has shown that LSD inhibits transmission mediated by serotonin in the accelerator nerve of the heart in the clam, *Venus merceneria*, and Gaddum (1953) has found LSD to be a potent antagonist of serotonin on smooth muscle *in vitro*. As further means of analysis, studies on the electrical activity of the brain have been made on LSD homologs. Bishop *et al.* (1958) reported that BOL, a nonhallucinogenic congener, depresses synaptic transmission in the lateral geniculate body. Evarts *et al.* (1955), studying the effects of LSD on the visual system of the cat, demonstrated that low doses of LSD caused a decrease in amplitude of the geniculate postsynaptic response, although transmission

Received for publication December 31, 1964.
[1] Present address: Louisiana State University, Baton Rouge, Louisiana.

within the retina and the cortical level was little affected. In addition, administration of LSD in low concentrations facilitates evoked auditory and visual primary responses in the unanesthetized, immobilized cat (Purpura, 1956).

Bradley (1958) found that in unrestrained, conscious animals, LSD elicited low-amplitude fast activity in the EEG. On the other hand, LSD had no effect on either *encephale* or *cerveau isolé* cat preparations. It was suggested that the alerting effect of LSD on EEG may be exerted directly by the action of the drug on receptors related to the collaterals entering the reticular formation. In addition to the direct effect on the reticular level, afferent stimuli were necessary to provoke alerting. Previous investigations from our laboratory (Rinaldi and Himwich, 1955; Himwich *et al.*, 1959) have revealed that low doses of LSD (0.003–0.015 μmol/kg) caused a decrease in amplitude and an increase in the frequency of the EEG, whereas slightly higher amounts (0.030 μmol/kg) of LSD elicited continuous alert patterns. The present study is concerned with an electrographic analysis of LSD and 9 homologs (fig. 1) in an effort to elucidate the actions of these compounds on the spontaneous electrical activity of the rabbit brain and to determine sites of action in producing EEG alerting.

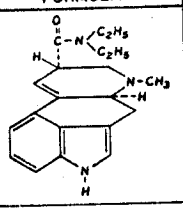
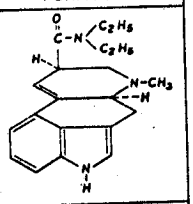
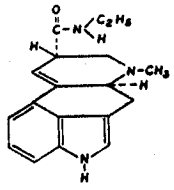
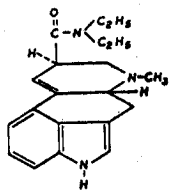
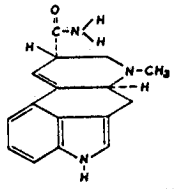
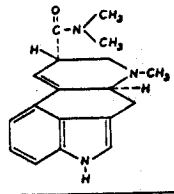
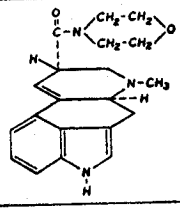
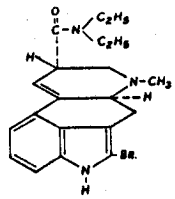
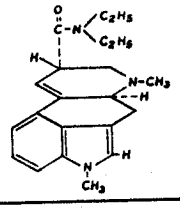
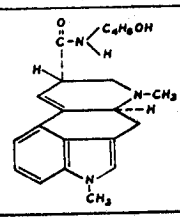
METHOD. A total of 96 albino rabbits ranging in weight from 2.5 to 3.0 kg were used. Animals were tracheotomized under ether and local Pontocaine anesthesia, curarized and artificially respired at a rate of 34 strokes/min with a volume of 150 ml/stroke. Brain wave recordings were made on an 8-channel Grass Model III D electroencephalograph (EEG). Monocoaxial electrodes were implanted according to the maps of Sawyer *et al.* (1954) in the anterior cortex (motor), anterior 3, lateral 4, vertical plus 9; amygdala, anterior 2, lateral 5.5, vertical plus 5; caudate nucleus, anterior 3, lateral 4, vertical plus 4.5; posterior cortex (limbic), posterior 4, lateral 4, vertical plus 8.5; hippocampus, posterior 4, lateral 4, vertical plus 5.5; thalamus (VPL), posterior 4, lateral 4, vertical minus 1; and reticular substance, posterior 10, lateral 2, vertical minus 9. For purposes of convenience, these will be referred to as EEG, even though they were direct tissue recordings from cortical and subcortical sites. Histologic examinations were undertaken and the placements of electrodes were verified. For the brain and spinal cord transections, the procedures were identical with those employed by Brodey *et al.* (1963) and Schweigerdt and Himwich (1964) (fig. 2). Following each experiment, the animals were sacrificed and the brains removed to ascertain if the transection was complete and in the prescribed plane. Ten compounds were investigated: LSD; LAE; *d*-lysergic acid (LA); LSM; MLD; *d*-iso-LSD; *l*-LSD; DAM; BOL; and UML. All drugs were dissolved in distilled water and injected into the femoral vein *via* a polyethylene cannula. Pilot studies carried out on the animals with unoperated brains were initially conducted in order to determine the minimal dosage of each of the 10 drugs that evoked EEG arousal. These predetermined dosages were then used subsequently in all experimental procedures. Total dosages used in the production of alerting were as follows: LSD (0.062 μmol/kg); LAE (0.169 μmol/kg); LSM (0.148 μmol/kg); MLD (0.296–0.592 μmol/kg); and DAM (0.169 μmol/kg).

In all experiments, blood pressure readings were monitored by a polyethylene cannula inserted into the femoral artery and connected to a mercury manometer. Animals with tracings that did not show normal maintenance of blood pressure (80–120 mm Hg) after transection were not included in the study.

RESULTS. A normal resting pattern consisted of high amplitude slow waves interrupted by relatively rapid spindles (14/sec). Afferent stimulation produced by hand clapping or by pinching the footpad changed the resting pattern markedly. All leads showed a sharp reduction in amplitude and an increase in frequency in addition to the elimination of spindles (alert pattern). Under these conditions, the control alert pattern lasted for 10 to 20 sec.

Experiments performed on groups of 5 intact animals showed that sustained drug-induced arousal patterns were obtained with LSD, LAE, LSM, MLD and DAM. EEG arousal patterns for LAE and MLD did not appear until approximately 30 min after the injection. Once established, the alert pattern for each of the 5 drugs held for 30 min or longer. Increase in the dosages of LA, *d*-iso-LSD, *l*-LSD or UML failed to elicit EEG alerting. In 6 of 10 animals, 0.248 μmol/kg to 0.496 μmol/kg of BOL also failed to produce EEG alerting, although arousal patterns were maintained in the other 4, but for only approximately 15 min.

d-Lysergic acid monoethylamide. Experiments

COMPOUNDS ABBR.	STRUCTURAL FORMULA	COMPOUNDS ABBR.	STRUCTURAL FORMULA
D-LYSERGIC ACID DIETHYLAMIDE * LSD		D-ISO-LYSERGIC ACID DIETHYLAMIDE D-ISO-LSD	
D-LYSERGIC ACID MONOETHYLAMIDE * LAE		L-LYSERGIC ACID DIETHYLAMIDE L-LSD	
D-LYSERGIC ACID * LA		D-LYSERGIC ACID DIMETHYLAMIDE * DAM	
D-LYSERGIC ACID MORPHOLIDE * LSM		2-BROM-D-LYSERGIC ACID DIETHYLAMIDE BOL	
D-1-METHYL-LYSERGIC ACID DIETHYLAMIDE * MLD		1-METHYL-D-LYSERGIC ACID BUTANOLAMIDE UML	

* HAVE HALLUCINOGENIC ACTIVITY.

8 D AS-2

FIG. 1. Chemical structures of compounds investigated.

performed on 5 intact animals showed EEG arousal patterns after 0.169 μmol/kg LAE. EEG of 4 animals transected at the precollicular prepontine plane (transection 1) and given LAE did not disclose an arousal pattern. Similarly, in 4 rabbits transected after the drug-induced arousal was established, the section abolished this pattern. In animals transected caudad to the midbrain (transection 3) and injected with LAE, an alerting pattern was obtained within the same time interval as that for intact animals (fig. 3).

d-Lysergic acid diethylamide. In 5 intact animals, EEG activation was seen immediately following injection of 0.062 μmol/kg LSD. Animals transected caudad to the midbrain (transection 3) either before or after LSD administration did not exhibit the drug-induced arousal. A total of 10 animals was transected at the level of the first cervical vertebra (transection 4) in experiments with LSD. Cervical transection neither abolished the activation when LSD was given previous to the section nor inhibited the appearance of EEG arousal in animals transected prior to the administration of the drug (fig. 4).

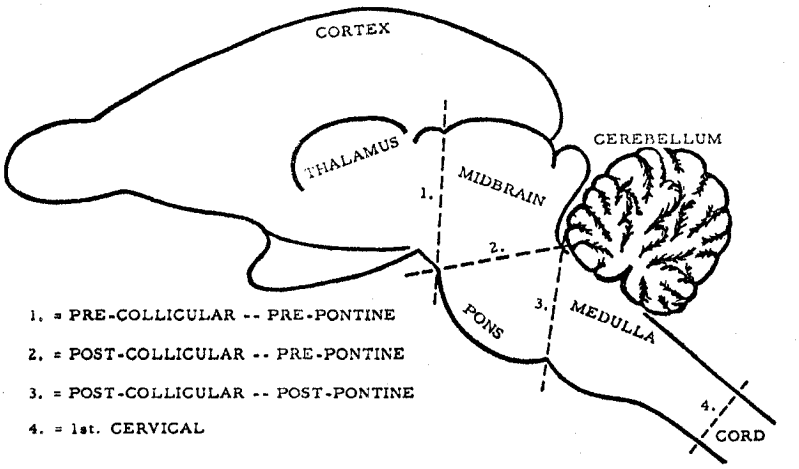


FIG. 2. Schematic representation of the rabbit brain showing planes of transections. In our experiments transections 1, 3 and 4 were employed.

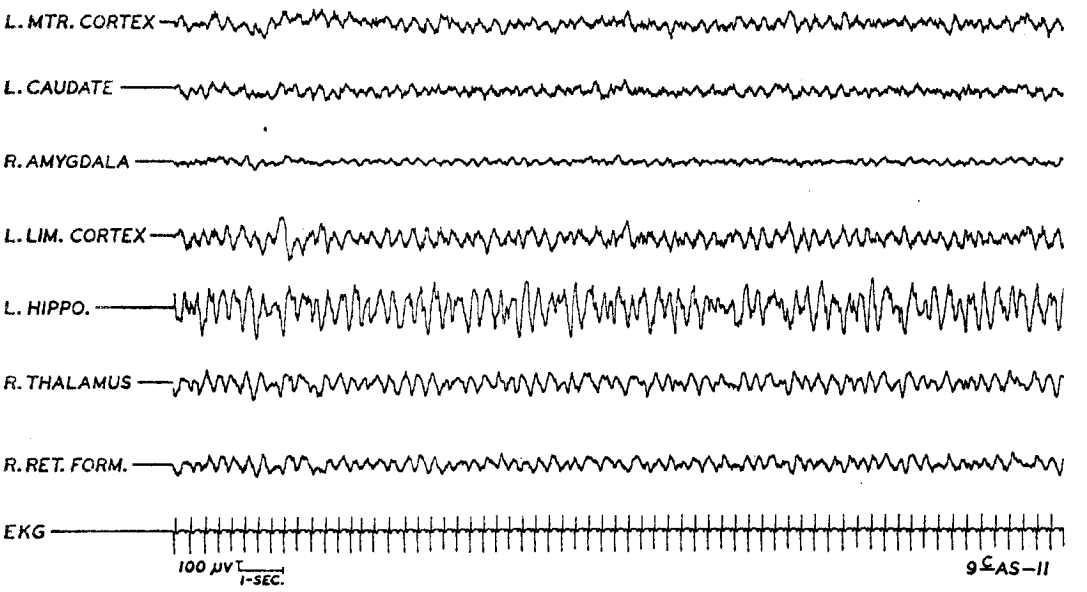


FIG. 3. Continued maintenance of EEG arousal pattern evoked by 50 μg/kg i.v. LAE.

d-Lysergic acid morpholide, d-lysergic acid dimethylamide and d-methyl-lysergic acid diethylamide. In experiments performed on 3 groups of 5 intact animals, EEG activation patterns were obtained with LSM, DAM and MLD. The total quantities of each agent needed to produce sustained arousal were: LSM, 0.148 μmol/kg; DAM, 0.169 μmol/kg; and MLD, 0.296 to 0.592 μmol/kg. Cervical transections performed on 3 groups of 4 rabbits each with LSM, DAM or MLD did not show EEG arousal when the drugs were administered after the transection. Likewise, in animals transected after the arousal reaction was established, the drug-induced alerting was eliminated by that cut. Because these animals transected at the cervical level did not exhibit arousal even though adequate blood pressures were maintained, it was felt that additional sections at the postcollicular, postpontine level were not necessary. As noted in table 1, the results for LAE, LSD, LSM, DAM and MLD

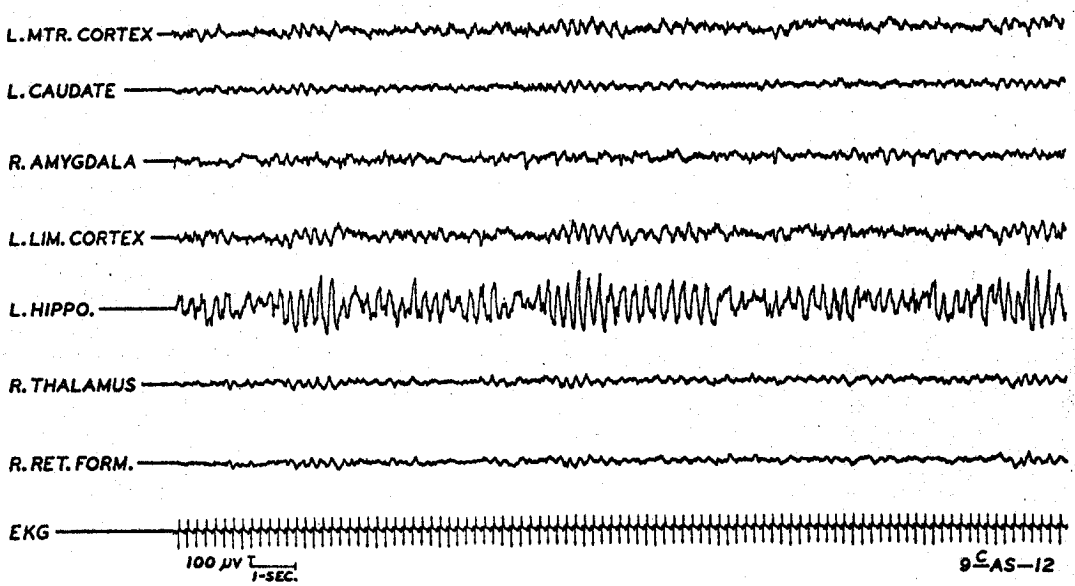


FIG. 4. Continued maintenance of 20 μg/kg i.v. LSD.

TABLE 1

Effects of brain and spinal cord transection on drug-induced arousal

Drug	No. of Animals	Drug Administration	Site of Transection	Effect	Necessary Level for EEG Arousal
LSD	4	Pretransection	Postcollicular, postpontine	Alerting stopped	Between midbrain and first cervical vertebra and the cephalad input to reticular formation
	4	Posttransection		Alerting stopped	
	5	Pretransection	First cervical vertebra	Alerting continued	
	5	Posttransection		Alerting continued	
LAE	4	Pretransection	Precollicular, prepontine	Alerting stopped	Midbrain region
	4	Posttransection		Alerting stopped	
	4	Pretransection	Postcollicular, postpontine	Alerting continued	
	4	Posttransection		Alerting continued	
LSM	2	Pretransection	First cervical vertebra	Alerting stopped	Lower brain stem region and spinal afferent input
	2	Posttransection		Alerting stopped	
DAM	2	Pretransection	First cervical vertebra	Alerting stopped	Lower brain stem region and spinal afferent input
	2	Posttransection		Alerting stopped	
MLD	2	Pretransection	First cervical vertebra	Alerting stopped	Lower brain stem region and spinal afferent input
	2	Posttransection		Alerting stopped	

were the same whether the drugs were administered before or after transection. Changes in blood pressure were studied for each of the drugs. LSD induced an initial elevation in systemic blood pressure (30–40 mm Hg) following injection, whereas the remaining compounds induced only slight increases (5–10 mm Hg) following administration.

DISCUSSION. Our results show that, of the 10 compounds studied, only LSD, LAE, MLD and DAM evoked sustained alert EEG patterns in rabbits with intact brain. In addition, our findings in transected animals reveal that LAE, the monoethyl *d*-lysergic acid amide derivative, acts effectively at the midbrain level. The inability of LSD, the diethyl derivative, to evoke

EEG alerting in animals transected at the postcollicular, postpontine level suggests that this drug possesses a locus of action in the lower brainstem region. In contrast, LSM, MLD and DAM did not exhibit EEG alerting in *encephale isolé* preparations. These latter findings are in agreement with those reported by Bradley (1958), namely, that in the cat LSD alerting could be neither initiated nor maintained after cervical transection. Bradley suggests that the action of this hallucinogen (LSD) in cats may be to sensitize receptors at the level of the reticular formation, so that they become more sensitive to afferent impulses arriving *via* collaterals and especially those coming centripetally in the spinal cord. This explanation would apply to the failure of LSM, MLD and DAM to invoke EEG arousal in spinal animals since cervical transection would abolish much of the afferent background necessary for the so-called spontaneous EEG arousal reaction even in the absence of direct stimulation on the part of the experimenter. On the other hand, in the rabbit, unlike the cat, LSD did evoke alerting in cervical-sectioned animals. It would seem that LSD is more stimulating to the receptors at the reticular level than are LSM, MLD or DAM in the rabbit. In any event, it is likely that the tonic reticular inputs from supraspinal regions were adequate to elicit EEG arousal with LSD in the rabbit transected at the cervical level. However, with LSM, MLD and DAM, not only were supraspinal tonic impulses necessary, but also those of the spinal cord as well. Our data on cervical transected animals also suggest that the dissimilarity between rabbits and cats may be due to a species difference, because the results with LSD agree with previous findings of psychotomimetic indoles obtained in rabbits. These studies with psilocin and MP-809 (Brodey *et al.*, 1963) and bufotenin and 5-HTP (Schweigerdt and Himwich, 1964) disclosed that psychotomimetic indole agents possessing the N-dimethyl side chain configuration exhibit loci of action for EEG arousal posterior to the midbrain, whereas homologs without the N-dimethyl configuration were effective at the midbrain level. Our data on animals injected with LSD, the diethyl congener, makes it possible to extend this hypothesis from the N-dimethyl configuration to the N-diethyl configuration. Further support for this conclusion that these configurations are effective at the lower brainstem level is exhibited by the fact that LAE, with a single ethyl group, affected EEG alerting in animals transected caudad to the midbrain and, therefore, had a higher site of action at the midbrain level.

It is noteworthy that of the drugs studied, those which evoked sustained EEG arousal (LSD, LAE, LSM, MLD and DAM) are clinically hallucinogenic (Solms, 1956; Isbell, 1959; Abramson *et al.*, 1955; Gogerty and Dille, 1957; Isbell *et al.*, 1956). With the exception of LAE, these hallucinogens had an effective locus of action for EEG alerting at the lower brainstem region. It is true that one hallucinogen, LA, without an N-substituted ethyl group, failed to elicit EEG activation. LA, however, possesses relatively weak psychotomimetic activities (Solms, 1956). Thus the diethyl or monoethyl configuration may be a necessary condition for evoking drug-induced arousal patterns though at different levels. Substitution of a dimethyl group on the amino nitrogen (DAM) or the enclosure of the two ethyl groups into a morpholine ring (LSM) does not change the ability of these substances to produce EEG alerting. With the addition of a methyl group on the indole ring (MLD) or the substitution of one ethyl radical with a hydrogen, as in LAE, a period of latency is seen before the drug-induced arousal. This delay suggests either that these drugs do not pass the blood-brain barrier readily, or that some active intermediate is formed in the intervening period. With the exception of BOL, the nonhallucinogenic compounds, *d*-iso-LSD, *l*-LSD and UML, failed to elicit EEG alerting. We found it necessary, however, to administer BOL in total dosages which approached toxic levels to obtain even brief periods of activation in some animals. Our results reveal the importance of the indole group as well as the essential role of the N-diethyl configuration. It appears that both parts of the molecule of the hallucinogen are necessary for EEG alerting at the lower brainstem region. Thus it becomes apparent that strategic differences in chemical structure induce changes in drug action and may influence behavior as well.

ACKNOWLEDGMENTS. The authors wish to thank Drs. Rudi Bircher and Leonard Achor of Sandoz Pharmaceuticals for the supply of drugs

in the study. We also wish to thank Mrs. Kathryn Bost for careful and patient technical assistance.

REFERENCES

ABRAMSON, H. A., JARVIK, M. E., KAUFMAN, M. R., KORNETSKY, C., LEVINE, A. AND WAGNER, M.: LSD-25 I: Physiological and perceptual response. J. Psychol. **39**: 3-60, 1955.

BISHOP, P. O., FIELD, G., HENNESSEY, B. L. AND SMITH, J. R.: Action of *d*-lysergic acid diethylamide on lateral geniculate synapses. J. Neurophysiol. **21**: 529-549, 1958.

BRADLEY, P. B.: The central action of certain drugs in relation to the reticular formation of the brain. *In* Reticular Formation of the Brain, ed. by H. H. Jasper, L. D. Proctor, R. S. Knighton, W. C. Noshay and R. T. Costello, pp. 123-149, Little, Brown and Company, Boston, 1958.

BRODEY, J. F., STEINER, W. G. and HIMWICH, H. E.: An electrographic study of psilocin and 4-methyl α-methyl tryptamine (MP-809). J. Pharmacol. **140**: 8-18, 1963.

EVARTS, E. V., LANDAU, W., FREYGANG, W., JR. AND MARSHALL, W. H.: Some effects of lysergic acid diethylamide and bufotenin on electrical activity in the cat's visual system. Amer. J. Physiol. **182**: 594-598, 1955.

GADDUM, J. H.: Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. J. Physiol. **121**: 15P, 1953.

GOGERTY, J. H. AND DILLE, J. M.: Pharmacology of *d*-lysergic acid morpholide (LLM). J. Pharmacol. **120**: 340-348, 1957.

HIMWICH, H. E., VAN METER, W. AND OWENS, H. F.: An EEG analysis of psychotomimetic drugs. *In* Neuropsychopharmacology, ed. by P. B. Bradley, pp. 329-333, American Elsevier Publishing Company, Inc., New York, 1959.

HIRSCH, M. W., JARVIK, M. E. AND ABRAMSON, H. A.: Lysergic acid diethylamide (LSD-25). XVIII. Effects of LSD-25 and six related drugs upon handwriting. J. Psychol. **41**: 11-22, 1956.

HOCH, P. H., PENNES, H. H. AND CATTELL, J. P.: Psychoses produced by administration of drugs. *In* Metabolic and Toxic Diseases of the Nervous System, ed. by H. H. Merritt and C. C. Hare, vol. 32, pp. 287-296, The Williams & Wilkins Co., Baltimore, 1953.

ISBELL, H.: Comparison of the reactions induced by psilocybin and LSD-25 in man. Psychopharmacologia **1**: 29-38, 1959.

ISBELL, H., BELLEVILLE, R. E., FRASER, H. F., WIKLER, A. AND LOGAN, C. R.: Studies on lysergic acid diethylamide (LSD-25). I. Effects in former morphine addicts and development of tolerance during chronic intoxication. Arch. Neurol. Psychiat., Chicago **76**: 468-478, 1956.

PURPURA, D. P.: Electrophysiological analysis of psychotogenic drug action. I. Effect of LSD on specific afferent systems in the cat. Arch. Neurol. Psychiat., Chicago **75**: 122-131, 1956.

RINALDI, F. AND HIMWICH, H. E.: The cerebral electrographic changes induced by LSD and mescaline are corrected by Frenquel. J. nerv. ment. Dis. **122**: 424-432, 1955.

SAWYER, C. H., EVERETT, J. W. AND GREEN, J. D.: The rabbit diencephalon in stereotaxic coordinates. J. comp. Neurol. **101**: 801-824, 1954.

SCHWEIGERDT, A. K. AND HIMWICH, H. E.: An electrographic analysis of bufotenin and 5-HTP. J. Pharmacol. **144**: 253-259, 1964.

SOLMS, H.: Relationships between chemical structures and psychoses with the use of psychotoxic substances. J. clin. exp. Psychopathol. Quart. Rev. Psychiat. Neurol. **17**: 429-433, 1956.

VON FELSINGER, J. M., LASAGNA, L. AND BEECHER, H. K.: The response of normal man to lysergic acid derivatives (di and monoethylamides). J. clin. exp. Psychopathol. Quart. Rev. Psychiat. Neurol. **17**: 414-428, 1956.

WELSH, J. H.: Hydroxytryptamine: A neurohormone in the invertebrates. Fed. Proc. **13**: 162-163, 1954.

WOOLLEY, D. W. AND SHAW, E.: A biochemical and pharmacological suggestion about certain mental disorders. Proc. nat. Acad. Sci., Wash. **40**: 228-231, 1954.

ZSIGMOND, E. K., FOLDES, F. F. AND FOLDES, V. M.: The *in vitro* inhibitory effect of LSD, its congeners and 5-hydroxytryptamine on human cholinesterases. J. Neurochem. **8**: 72-80, 1961.