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THE LACK OF EFFECT OF LSD-25 UPON THE HISSING RESPONSE ELICITED BY HYPOTHALAMIC STIMULATION (1)

BY

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It has been observed that lysergic acid diethylamide (LSD), when given in high doses, produces "rage" in animals (5, 7, 12, 16).

In addition many workers have demonstrated (17, 19, 23, 28, 32, 34) that a variety of responses resembling those observed when an animal attacks or is attacked, and referred to by terms such as "affective defense" (19) and "emotional mimetic" (29), can be elicited by electrical stimulation of the hypothalamus of the unanesthetized intact cat. Additional studies indicate that various psychotropic drugs can alter the threshold for certain of the responses produced via hypothalamic stimulation (3, 6, 26, 33).

Since LSD-25 and hypothalamic stimulation both produce "emotional" behavior, it was decided to investigate the effect of LSD-25 on the threshold current necessary to produce such behavior via electrical stimulation of the hypothalamus. In this way we hoped to gain information indicating whether the emotional effects of LSD-25 can be ascribed to an action upon the hypothalamus.

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Materials and Methods

Cats, of either sex weighing between 2.5 and 3.5 kg, were implanted, using aseptic technique, with bipolar Formvar coated nichrome electrodes; the tips were spaced approximately 2 mm apart. The electrodes were aimed at the perifornical region (2) of the hypothalamus; the electrodes were wired to a Winchester socket, and the assembly embedded in dental acrylic reinforced by stainless steel screws driven into the skull. Antibiotics were given routinely for 5 days postoperatively. The animals were tested in a sound resistant cubicle containing a "one-way" mirror and a microphone. Current was delivered by a Grass S4G stimulator. A resistance of 94,000 ohms was placed in series with the output in order to convert the stimulator from a constant voltage source to approximate constant current output. The voltage drop across an additional 100 ohm series resistance was monitored on an oscilloscope and provided continuous observation of waveform and current amplitude. The stimulus was a 10 sec train of 60 pulse per sec, 2 msec pulse width square waves. The stimulator was set in the "biphasic" position.

A previous investigation in this laboratory demonstrated that of the variables observed upon hypothalamic stimulation—alerting or arousal, defense posture, hissing, growling, spitting, flight, attack, piloerection, mydriasis, defecation, urination, and salivation—hissing is the most useful. There is complete agreement between observers as to whether hissing occurs at a given current level and, therefore, thresholds can be reliably determined; we have not found this to be the case for the other responses. In this communication, hissing is the only dependent variable that will be reported in detail.

Each animal was given an initial test to determine the behavior that could be elicited and the approximate thresholds for the responses produced. Stimulation was begun at 100 μA and raised progressively in 40 μA steps until both hissing and attack directed at a toy mouse were elicited. If hissing was not produced when the stimulating current reached 1 mA, the animal was discarded. In this manner 12 cats were selected and divided, randomly, into 2 groups: an experimental group of 6 animals and a control group of the same size. In the formal testing procedure the animal was placed in the isolation cubicle and the electrodes connected to the stimulator; 10 min were allowed for adaptation to the test chamber. Stimulation was begun two steps (80 μA) below the threshold for any response observed during the initial test; the animal was stimulated for 10 sec at one minute intervals and the current raised one step (40 μA) at each 10 sec stimulation period. In this manner the minimal current producing hissing was determined. Stimulation was always continued at least two steps beyond the hissing threshold in an attempt to prevent the development of a conditioned response. If hypothalamic stimulation is aversive, then cessation of stimulation contingent upon the initiation of hissing could lead to the development of a conditioned operant response. We hoped to limit the experiment to unconditioned responses.

One threshold reading was obtained 15 min before injection, one 15 min after injection, and one every 30 min thereafter. The last determination was at 105 min post injection. All animals in the experimental group were dosed at 1.0, 2.0, and 3.0 mg/kg of LSD i.p. prepared in water for each experiment. At least one week was allowed to elapse between dose levels. The controls received an equivalent volume of physiological saline. At the conclusion of testing, the animals were perfused with saline followed by 10 per cent formalin, the head mounted in the stereotaxic instrument, and 1 cm coronal brain slabs containing the electrode tracts cut using a knife held in the stereotaxic carrier. These slabs were then frozen, sectioned, and the electrodes localized, using the photographic method of Guzman *et al.* (18).

Results

Control group. The data from the control group are shown in Table I. The absolute value for the initial pre-injection reading is shown and the change in threshold compared to this value given for the post-injection readings. Changes in threshold during a test occurred in both directions — above or below the initial threshold value. The greatest change in threshold observed in any animal was 80 μA. These results conform to those obtained in other studies performed in our laboratory in which it has been found that "spontaneous" variations in threshold of up to, but not greater than, 80 μA commonly occur.

Table I

Results obtained from the control group. The initial absolute threshold readings obtained 15 min before saline injection and the change in threshold at 15, 45, 75, and 105 min post injection are shown. It will be noted that no change exceeds 80 μA

Control

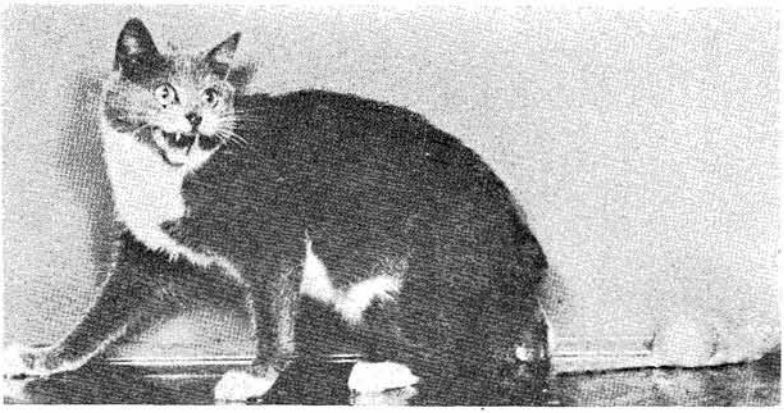
Animal No.	Threshold (μA)		Threshold Change (μA)			
	−15′	0	15′	45′	75′	105′
X-1	320	Inject saline i.p.	+40	0	+40	+40
X-2	360		0	0	+40	+40
X-3	320		0	0	0	0
X-4	320		0	0	0	0
X-5	560		0	+40	0	0
X-6	440		−40	−80	−80	−40

Experimental group. All 6 animals showed ataxia, mydriasis, and a peculiar, rigid "spread eagle" stance at all 3 dose levels. At 1 mg/kg five showed piloerection; none showed tachypnea. At 2.0 mg/kg five showed piloerection and two tachypnea. At 3.0 mg/kg six showed piloerection and four tachypnea. These signs appeared within 5 min after drug injection and were most pronounced between approximately 15 min and 1 hour post injection.

Occasionally the animals made passes in the air in front of them ("catching flies") and showed head movements from side to side that resembled those observed when an animal watches an object moving rapidly across the visual field. All the signs noted were somewhat diminished in intensity by the end of the 105 min test period, although this varied considerably from animal to animal. All signs had disappeared completely within 24 hours.

The most dramatic change induced by LSD was the development of extreme reactivity to auditory stimuli. This effect was tested by rapping on the side of the test chamber. After 1.0 mg/kg of LSD, 4 animals

A



B

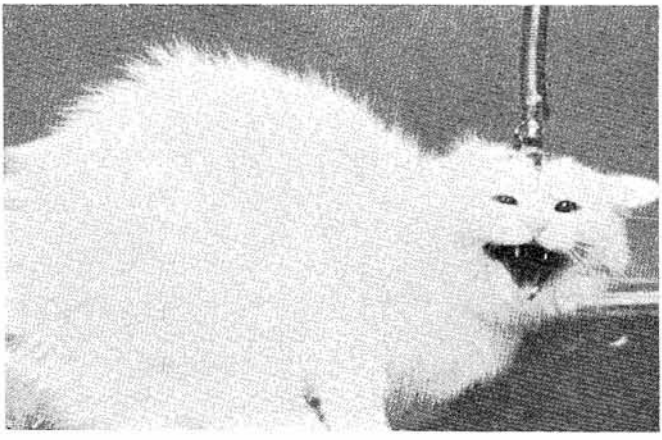


FIG. 1

A. Photograph of a stock animal (non-implanted) responding to a rap on the side of the test chamber. LSD 2 mg/kg i.p. was injected 30 min before this test. The hissing posture is displayed.

B. Implanted animal responding to electrical stimulation of the hypothalamus. Hissing posture, piloerection, and mydriasis are evident.

hissed when the chamber was rapped; at 2.0 and 3.0 mg/kg, all 6 animals hissed violently when stimulated in this manner (the saline controls and the experimental animals before LSD was administered never demonstrated this reaction). After 3.0 mg/kg, it was observed that minute sounds, which before treatment elicited no response, triggered a paroxysm of hissing. Fig. 1A shows a stock animal (non-implanted) which had received 2 mg/kg of LSD, i.p. one-half hour previously, responding to a rap on the side of the test chamber—hissing is evident. For comparison, Fig. 1B shows the response of an implanted animal (untreated) to hypothalamic stimulation. Again hissing is displayed. For these photographs the door of the test chamber was removed and replaced with a solid glass front.

Table II shows the results obtained from the 6 experimental animals;

TABLE II

Results obtained from the experimental group. The form is identical to that of Table I. Absolute thresholds and changes in threshold after 1.0, 2.0, and 3.0 mg/kg of LSD i.p. are given. No threshold change exceeds 80 μA

LSD-25

Animal No.	Threshold (μA)		Threshold Change (μA)			
	−15′	0	15′	45′	75′	105′
D-1	360		+80	−80	+80	+80
D-2	280		0	0	0	0
D-3	360	Inject	0	+40	0	+80
D-4	200	LSD-25	+80	+80	+40	+40
D-5	360	1.0 mg/kg	0	−40	0	+40
D-6	200	i.p.	0	0	0	0
D-1	360		+80	+80	+80	+80
D-2	200	Inject	+80	+80	+80	+80
D-3	400	LSD-25	0	−40	+40	+40
D-4	200	2.0 mg/kg	0	0	+40	+40
D-5	360	i.p.	0	−40	0	0
D-6	200		0	0	0	0
D-1	360		+80	+80	0	0
D-2	200	Inject	0	+80	+80	+80
D-3	400	LSD-25	0	−80	−80	−80
D-4	200	3.0 mg/kg	+40	+40	0	−40
D-5	320	i.p.	−40	−40	−40	+40
D-6	200		+40	+40	+40	+40

again the absolute threshold value before injection and the changes in threshold after injection are shown. It will be noted that no animal demonstrated a change in threshold exceeding 80 μA at any drug level. When the data were analyzed using Student's "t" test, no significant difference was found, at any drug level, between the 2 groups ($P > 0.05$).

Examination of the brain sections taken from the animals utilized in this experiment showed that electrode placements were confined to the region of the perifornical nucleus or, in a few cases, the ventromedial nucleus.

Discussion

Considerable evidence has accumulated indicating that LSD effects the afferent pathways in the brain at the level of the retina (4, 31) and lateral geniculate body (7, 11, 13) in the visual system; and peripheral to, or at the level of input to, the brain stem reticular formation in the case of the transmission of auditory information (8).

Other studies have indicated that LSD produces abnormal electrical activity in the hippocampus (30), and in the entorhinal cortex, rostral midbrain reticular formation and the thalamic nucleus ventralis anterior (1). At the cortical level it has also been reported that LSD alters the transcallosal evoked response (27).

According to the workers in Zurich (14, 19, 20, 21, 23, 24) who have studied the system in great detail, "emotional" responses can be elicited from a complex beginning with the dorsal portion of the nucleus basalis and adjacent parts of the nucleus centralis and nucleus medialis of the amygdala, i.e., fields from which several components of the stria terminalis arise, through the stria terminalis to the bed nucleus of the stria, and thence into the preoptic area and caudally throughout the hypothalamus and continuing into the periaqueductal midbrain.

In a more recent study Fernandez de Molina and Hunsperger (15) extended their previous investigation, based upon electrical stimulation alone, by combining stimulation with lesions of the system discussed. They found that the growling and growling-hissing responses elicited by stimulation of the amygdala were abolished by ipsilateral coagulation of the "hissing zone" situated in the central gray of the midbrain, or by ipsilateral coagulation of the hypothalamic "hissing zone" (rostral portion of the hypothalamic perifornical region). These workers found, further, that hissing elicited from the hypothalamus was unaltered after bilateral coagulation of the amygdala but that destruction of the stria

abolished the response elicited from the ipsilateral amygdala while at the same time not altering the response from the ipsilateral hypothalamus.

Although this picture has been disputed in some of its details by other workers (9, 22, 35), the evidence clearly indicates the involvement of the structures outlined by FERNANDEZ DE MOLINA and HUNSPERGER (15) in the mediation of agonistic behavior, but there is some question concerning the precise inter-relationship and connections between the various structures within the system. It does appear to be well supported that an intact midbrain hissing zone is necessary for the production of hissing via either peripheral or hypothalamic stimulation and that the hypothalamic hissing zone projects to the midbrain hissing zone.

Our results suggest that LSD does not significantly affect the excitability of the system mediating hissing that is activated by stimulation of the perifornical region of the hypothalamus. This proposition, in turn, suggests that the ability of LSD to produce hissing in response to previously innocuous stimuli is due to an effect upon structures anterior—in a physiological sense—to the perifornical hypothalamus.

In a study related to our work URSIN (36) found that LSD did not alter the threshold for elicitation of the "attention response" produced via stimulation of the "amygdaloid nuclear complex". We observed, upon hypothalamic stimulation, a response very similar to the attention response Ursin produced by amygdaloid stimulation, when our stimulating current was 40 to 80 μA below that producing hissing. It is possible that Ursin was stimulating in the amygdaloid region mediating agonistic behavior and that he would have produced such responses if higher stimulating voltages had been employed. If so, Ursin's evidence can be interpreted as showing that LSD also does not affect the system of Fernandez de Molina and Hunsperger at the amygdaloid level. Ursin, however, utilized much lower dose levels of drug than those employed in our investigation (5–200 μg/kg versus 1–3 mg/kg).

Previous research has shown (8, 25) that LSD dramatically lowers the threshold for "arousal" (from sleep), in response to auditory stimuli, whether behavioral or EEG parameters are measured; the threshold for arousal, however, produced by direct stimulation of the reticular formation was unaltered by this drug.

BRADLEY and KEY (8) pointed out that it was unlikely that this effect was due to an action on the direct sensory projection system, since LSD does not alter the evoked response from the auditory cortex produced by click stimuli; and, they suggested that this action was produced by a "sensitization" of the reticular formation to collateral input from sensory pathways. These workers also noted that chlorpromazine,

which is an LSD antagonist, tends to block arousal to peripheral stimuli, but that it also does not affect the arousal threshold measured by direct stimulation of the reticular formation. We have previously noted that LSD induces abnormal electrical activity in the reticular formation.

It may be suggested that at low doses LSD increases the excitability of the sensory collateral—reticular formation synapses and that at very high doses, such as those employed in the present study, this effect becomes so pronounced that the intense excitation produced spreads to the structures mediating agonistic behavior outlined by Fernandes de Molina and Hunsperger. We cannot, at present, delineate what pathways may be involved in the transmission of this excitation.

It must be pointed out that certain assumptions are involved in the preceding discussion : 1. that the excitability of the neurons mediating hissing located in the hypothalamus is accurately assessed by our threshold measuring technique; 2. that the alteration of the excitability of the neuronal mechanisms involved in this response, efferent to the hypothalamus, (for instance the midbrain "hissing zone") would be reflected by changes in the threshold current required to produce hissing via stimulation of the hypothalamus. In other words, if LSD increased the excitability of the lower centers, less intense afferent bombardment of these centers would be required to elicit hissing.

Furthermore, in extrapolating the results of this investigation to the effect of LSD upon the human brain, not only the species difference, but also the very large doses, compared to those employed in the clinic, utilized in this study must be taken into consideration.

Recognizing these factors, the results presented here support the hypothesis that the behavioral effects of LSD are not produced by action upon the hypothalamus or related mesencephalic loci.

Summary

Injection of lysergic acid diethylamide (LSD) at doses of 1.0, 2.0, and 3.0 mg/kg i.p. failed to alter the threshold for the hissing response elicited via stimulation of the perifornical region of the hypothalamus to a greater extent than that produced by physiological saline.

It was concluded that this evidence is consistent with the hypothesis that the behavioral effects of LSD are not produced by action on the hypothalamus or the intimately related "hissing zone" in the periaqueductal mesencephalon.

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