LSD 428/(BOL)

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INTERACTION OF SEROTONIN AND LYSERGIC ACID DIETHYLAMIDE IN THE SIAMESE FIGHTING FISH

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Lysergic acid diethylamide (LSD) produces in human subjects emotional and perceptual changes which resemble those of schizophrenia (Stoll, 1947). The mode of action of this drug has been extensively investigated, for it is hoped that knowledge about LSD will provide clues to a possible biochemical error in schizophrenia (Woolley, 1955; Woolley and Shaw, 1954; Rinkel *et al.*, 1955; Hoagland *et al.*, 1955; Cerletti and Rothlin, 1955).

It is now widely believed that the central action of this drug is related to the function in the brain of 5-hydroxytryptamine (serotonin). The relationship, however, is complex for, centrally and peripherally, LSD produces effects both similar and opposite to those of serotonin. Apparently, LSD blocks serotonin at some central receptors and acts like serotonin at others. Experiments involving general effects on whole animals are necessary to evaluate these opposing actions.

LSD exerts complex behavioral effects on the Siamese fighting fish, *Betta splendens* (Abramson and Evans, 1954), and this test animal has been used in pharmacological problems involving LSD (Abramson *et al.*, 1957), modifications of LSD (Evans *et al.*, 1956), and tranquilizers (Walaszek and Abood, 1956). In this paper, effects on three aspects of *Betta* behavior under a controlled stress situation are described for LSD and serotonin, when given separately and together. These findings are compared with those obtained with chlorpromazine, pentamethylenetetrazol (Metrazol) and thiopental sodium (Pentothal sodium).

METHOD. A controlled stress situation is produced for *Betta* by partitioning off the air surface with a tight gauze "ceiling." *Betta* is unusual in having a highly vascularized accessary air sac or labyrinth, which operates like a lung (Emmes, 1953). Unable to renew the oxygen content of the labyrinth, fish become restless, somewhat hypoxic and finally show an increase in their specific gravity.

Drug effects on three aspects of the stress response were quantitatively determined. These were as follows: 1) activity, as recorded electrically, especially during the 8- to 12-minute interval after roof placement, 2) quiescent period, *i.e.* the interval after ceiling placement before fish first probed against this gauze partition (fish swam about during part of this time), and 3) duration of struggle, *i.e.* the interval after first probing the gauze ceiling before fish stopped moving for at least 30 seconds.

Two sets of apparatus were employed, one for electrical recording of fish activity and the other for visual determination of the quiescent period and duration of struggle. In each experiment, fish were paired on the basis of age, size and body shape, and only female juveniles, 3.5 to 5.0 cm. long, were used. One of each pair served as test animal, and the difference in its stress response from that of its control was determined.

Electrical recording of activity. The apparatus consisted essentially of an outer elliptical shell (10 by 15 cm.), an inner gauze enclosure (3 by 8 cm.) for the fish, and an electrode 4 cm. from either end of the enclosure. Muscle action potentials produced waves of altered charge along the fish epidermis and thus produced rapid changes in the electro-potential

field in front of and behind the fish. These electrical disturbances induced rapid fluctuations in the potential difference between the paired electrodes, and the latter were recorded by a Grass encephalogram. Activity, as estimated from the electromyograms, was the number of excursions per minute of specified amplitude, *i.e.* the height obtained with the instantaneous application of 10 to 40 microvolts.

Test and control media were carefully adjusted to the same pH (5.7) and the same low conductivity. The pH of the medium influenced fish activity. Ions tended to short out the electrical charges on the fish epidermis and thus to flatten the waves on the electromyograms. Records obtained with fish in buffered KCl and $MgSO_4$ solutions indicated that conductivity of solutions greatly affected estimates of activity but that ionic strength could be ignored.

In each experiment, fish were transferred to the inner gauze enclosures and left undisturbed for 30 minutes before the gauze ceiling was pushed down in place below the water surface. During this time, drugs, when present, took effect. Electromyograms were obtained for three 20-second periods, first during the 2- to 6-minute interval, then during the 8- to 12-minute interval, and finally during the 28- to 32-minute period after ceiling placement. For details of procedure and apparatus, see Trout (1957).

Visual observation. Rectangular lucite containers, 6 by 10 cm. and 6.3 cm. deep, were employed. Each was fitted with supports for a copper-rimmed gauze ceiling which rested 5.5 cm. above the bottom. During experiments, each fish was screened from view of the others. The observer sat away from the light, as quietly as possible, about four feet from the fish.

After transfer into 350 ml. of buffered medium, each fish was left undisturbed for at least 37 minutes. As soon after this time as it exchanged air at the surface and settled down again, the gauze ceiling was installed below the water surface. The quiescent period, *i.e.* the time after ceiling placement before the *Betta* first pushed against this gauze layer, was observed. The interval until the fish stopped moving for 30 seconds, *i.e.* the quiescent period, was also recorded.

Dosage levels. Preliminary experiments with serotonin, LSD, Metrazol, and chlorpromazine were performed with small numbers of fish at lower drug levels than are reported here. Dosages listed below were estimated to be roughly the lowest which would give statistically significant results with a reasonable number of trials. It is noted that LSD and serotonin (Fried and Antopol, 1957) and chlorpromazine (Trout, 1957) sometimes produce at high concentrations the opposite effects to those noted at lower levels.

Solutions for electrical recording of activity.

Test	Control
(1) 0.12 mgm./l. of LSD in 0.098 *mM* (sodium) succinate buffer, pH 5.7	0.100 *mM* (sodium) succinate buffer, pH 5.7
(2) 13 mgm./l. of serotonin (30 mgm./l. of serotonin creatinine sulfate titrated to pH 5.7 with NaOH)	0.11 *mM* succinate buffer + 0.074 *mM* creatinine
(3) Same as (2) + 0.08 mgm. l. of LSD	Same as (2) + 0.08 mgm. l. of LSD

Solutions for visual observations. All solutions contained 0.5 *mM* (sodium) succinate buffer, pH 5.7. Drugs were included as follows:

Test	Control
(1) 0.12 mgm. l. of LSD	None
(2) 17.4 mgm./l. of serotonin (40 mgm./l. of serotonin creatinine sulfate titrated to pH 5.7 with NaOH)	0.089 *mM* creatinine
(3) Same as (2) + 0.12 mgm. l. of LSD	Same as (2) + 0.12 mgm. l. LSD
(4) 100 mgm./l. of Metrazol	None
(5) 10 mgm./l. of chlorpromazine	None
(6) 10 mgm. l. of sodium thiopental	None

TABLE 1

Drug effects on activity level under stress*

Time† *min.*	Mean activity levels		Mean diff.	No. of expts. (fish pairs tested)	s.d.	s.e.	"t" value	Test of null hypothesis
	Test fish	Controls						
	LSD							
2–6	326	391	−65	8	159.6	56.5	1.15	Not significant
8–12	175	282	−107	8	91.6	32.4	3.30	$P < 0.01$
28–32	159	234	−75	12	115.6	33.4	2.25	$P < 0.05$
	Serotonin							
2–6	357	287	+70	14	235.0	62.8	1.11	Not significant
8–12	529	404	+125	14	202.4	54.1	2.31	$P < 0.05$
28–32	213	184	+29	14	178.1	47.6	0.61	Not significant
	LSD + Serotonin	LSD						
2–6	187	265	−78	11	255.7	77.1	1.01	Not significant
8–12	193	298	−105	11	156.3	47.1	2.23	$P < 0.05$
28–32	156	204	−48	11	156.7	47.3	1.01	Not significant

* Activity, as determined from electromyograms, is the number of excursions per minute of specified amplitude, *i.e.* the height obtained with the instantaneous application of 10 to 40 μV.

† "Time" is the period after ceiling installation during which myograms were recorded.

RESULTS. Table 1 shows that LSD and serotonin had opposite effects on the intensity of activity under the standard stress situation. Data show statistical significance for the 8- to 12-minute interval after ceiling placement, when fish had recovered from this minor disturbance, yet were not seriously fatigued. Serotonin alone increased the intensity of the stress response, but, when given with LSD, it potentiated the LSD lethargy.

Table 2 shows that LSD decreased the "quiescent period"; that is, LSD-treated fish probed the gauze ceiling sooner after its installation than did controls. Serotonin alone, at the dosage used, had little or no effect on the quiescent period; but, when combined with LSD, it inhibited the LSD effect. These findings cannot be interpreted in terms of central excitability. Metrazol prolonged the quiescent period, as did chlorpromazine; while sodium thiopental had no significant effect at a dosage (10 mgm./l.) which markedly shortened the duration of almost continuous movement.

Table 3 shows that LSD and serotonin both decreased the duration of struggle, *i.e.* the interval after first probing the gauze tent before fish stopped moving for at least 30 seconds. Serotonin also increased the LSD effect. Data in this table are handled in a somewhat unusual manner, chiefly for two reasons. First, control

TABLE 2

*Drug effects on the quiescent period**

Mean quiescent period *sec.*		Mean diff.	No. of expts. (fish pairs tested)	s.d.	s.e.	"t" value	Test of null hypothesis
Test fish	Controls						
LSD							
128	247	−119	11	134.6	40.6	2.93	$P < 0.02$
Serotonin							
190	172	+18	12	218.3	63.0	0.29	Not significant
LSD + Serotonin							
192	108	+84	12	116.5	33.6	2.50	$P < 0.05$
Chlorpromazine							
240	172	+68	10	77.2	24.4	2.77	$P < 0.05$
Metrazol							
427	214	+213	10	268.1	84.8	2.50	$P < 0.05$

* Quiescent period is the time after installation of the gauze ceiling before fish probed against it.

TABLE 3

*Drug effects on duration of struggle**

No. of fish pairs tested	Test fish			Controls			Mean value for $\log \frac{\text{time for test fish}}{\text{time for controls}}$	s.e.	"t" value	Test of null hypothesis
	Treatment	Median time *sec.**	Range *sec.*	Treatment	Median time *sec.*	Range *sec.*				
11	LSD	395	215–5,615	None	840	260–3,285	−0.310	0.129	2.40	$P < 0.05$
12	Serotonin	600	195–1,205	None	1,058	260–3,245	−0.268	0.093	2.87	$P < 0.02$
12	LSD and Serotonin	340	180–410	LSD	478	260–6,580	−0.447	0.151	2.96	$P < 0.02$

* Duration of struggle is the interval after first probing the gauze ceiling before fish stopped moving for at least 30 seconds.

fish demonstrated a widely varied duration of struggle. Secondly, distribution of values was markedly skew, with occasional fish "struggling" for several times the median duration. In evaluating statistical significance of group differences, each test fish was compared with its control; the mean, standard error, and "t" value were determined for:

$$\log \frac{\text{duration of struggle of test fish}}{\text{duration of struggle of control}}.$$

Discussion. LSD causes central effects similar to those of serotonin (Bogdanski *et al.*, 1956; Udenfriend *et al.*, 1956) but important qualitative differences in response have also been reported. Injection of LSD into the lateral cerebral ventricle of the unanesthetized cat produces sham rage, while the similar injection of serotonin causes lethargy and loss of muscular tone (Gaddum and Vogt,

1956). Cats thus treated with serotonin remain playful and responsive to petting (Feldberg and Sherwood, 1954).

Shore *et al.* (1955) demonstrated that LSD can block a serotonin effect in the brain, as well as peripherally. Serotonin produces a sedative effect in mice and prolongs the sleeping time after injection of a fixed dose of barbiturate. LSD, while having little effect itself on barbiturate sleeping time, inhibits the prolongation by serotonin as does brom-LSD (Cerletti and Rothlin, 1955).

In the last two or three years, the importance of similarities in LSD and serotonin effects have become increasingly recognized. High brain serotonin levels, produced by administering a serotonin precursor and a monoamine oxidase inhibitor, raise body temperature in the rabbit, as does LSD (Horita and Gogerty, 1957). LSD and serotonin inhibit transmission of nerve impulses at some central synapses (Marrazzi and Hart, 1955; Marrazzi, 1953; Evart *et al.*, 1955). Serotonin increases LSD depression of glycolysis and respiration of electrically stimulated brain slices (Lewis and McIlwain, 1954). Ginzel and Meyer-Gross (1956) suggest that LSD acts like serotonin in disturbing mood and perception in man, for these psychic changes can be blocked by pretreatment with brom-LSD.

Most of the findings of this paper are consistent with the above reports. Serotonin and LSD, when added separately, had opposite effects on the intensity of the stress response in fish and had the same effect in shortening its duration. But, when added with LSD, serotonin potentiated the LSD effect on intensity and increased its effect on duration.

One cannot, with certainty, explain the decreased "quiescent period" of LSD-treated fish. These fish did not appear to delay any longer than the controls in growing restless under the gauze ceiling, but they did show less hesitation in probing against it. It is possible that this effect indicates a perceptual or mood change. Behavior suggesting altered perception after LSD has been described for mice (Woolley, 1955) and guppies (Keller and Umbreit, 1956).

It was surprising to find that serotonin opposed the LSD-shortening of the quiescent period. Cerletti and Berde (1955) reported that serotonin antagonized LSD-induced expansion of certain fish chromatophores, but other inhibitions of an LSD effect by serotonin have not been reported.

SUMMARY

Siamese fighting fish (*Betta splendens*) were subjected to a controlled stress situation involving hypoxia. LSD depressed the intensity of activity under these conditions, whereas serotonin increased it. LSD and serotonin, however, had the same effect in reducing the duration of almost continuous activity. When the two drugs were added together, serotonin potentiated the effect of LSD on intensity and increased its effect on duration.

Evidence is also presented suggesting that LSD produced perceptual or mood changes in *Betta*.

REFERENCES

ABRAMSON, H. A., AND EVANS, L. T.: Science, **120**: 990, 1954.

ABRAMSON, H. A., SKLAROFSKY, B., BARON, M. O., AND GELFNER, H. H.: Science, **125**: 397, 1957.

BOGDANSKI, D. F., WEISSBACH, H., AND UDENFRIEND, S.: Fed. Proc., **15:** 402, 1956.
CERLETTI, A., AND BERDE, B.: Experimentia, **11:** 312, 1955.
CERLETTI, A., AND ROTHLIN, E.: Nature, London, **176:** 785, 1955.
EMMES, C. W.: Keeping and Breeding of Aquarium Fishes, p. 202, Academic Press Inc., New York, 1953.
EVANS, L. T., GERONIMUS, L. H., KORNETSKY, C., AND ABRAMSON, H. A.: Science, **123:** 26, 1956.
EVART, E. W., LANDAU, W., FREYGANG, W., AND WADE, W. H.: Am. J. Physiol., **182:** 594, 1955.
FELDBERG, W., AND SHERWOOD, S. L.: J. Physiol., **123:** 148, 1954.
FRIED, G. H., AND ANTOPOL, W.: Fed. Proc., **16:** 357, 1957.
GADDUM, J. H., AND VOGT, M.: Brit. J. Pharmacol., **11:** 175, 1956.
GINZEL, K. H., AND MEYER-GROSS, W.: Nature, London, **178:** 210, 1956.
HOAGLAND, H., RINKEL, M., AND HYDE, R. W.: Arch. Neurol. & Psychiat., **73:** 100, 1955.
HORITA, A., AND GOGERTY, J. H.: Fed. Proc., **16:** 308, 1957.
KELLER, D. L., AND UMBREIT, W. W.: Science, **124:** 407, 1956.
LEWIS, J. L., AND MCILWAIN, H.: Biochem. J., **57:** 680, 1954.
MARRAZZI, A. S.: Science, **118:** 367, 1953.
MARRAZZI, A. S., AND HART, E. R.: Science, **121:** 365, 1955.
RINKEL, M., HYDE, R. W., SOLOMON, H. C.: Dis. Nerv. Syst., **16:** 229, 1955.
SHORE, P. A., SILVER, S. L., AND BRODIE, B. B.: Experimentia, **11:** 272, 1955.
STOLL, W. A.: Schweiz. Arch. Neurol. u. Psychiat., **68:** 279, 1947.
TROUT, D. L.: Arch. Internat. Pharmacodyn., in press, 1957.
UDENFRIEND, S., BOGDANSKI, D. F., AND WEISSBACH, H.: Fed. Proc., **15:** 493, 1956.
WALASZEK, E. J., AND ABOOD, L. G.: Science, **124:** 440, 1956.
WOOLLEY, D. W.: Proc. Nat. Acad. Sc., **41:** 338, 1955.
WOOLLEY, D. W., AND SHAW, E.: Proc. Nat. Acad. Sci., **40:** 289, 1954.