EFFECT OF LYSERGIC ACID DIETHYLAMIDE [LSD-25] AND OTHER DRUGS ON TROPICAL FISH

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The tropical fish is a small, easily handled organism and presents a number of total behavior parameters on which drug effects may be tested. In 1954, Abramson and Evans (2) first described the vegetative, motor and behavioral responses of Siamese Fighting Fish (Betta splendens) to Lysergic Acid Diethylamide (LSD-25). This response, known hereafter as the "LSD-25 reaction," consists of coming to the surface and hanging in a vertical, nose-up-tail-down position, backward swimming accomplished mainly by pectoral fin movements, "Cartesian diver" effect (vertical sinking to the bottom and rising to the top), "barrel roll" effect, and a marked decrease in spontaneous activity. Also there develops a typical body kink and a darkening of the body color.

No other species has been reported to show exactly the same response to LSD-25 as Siamese Fighting Fish, although Guppies (Lebistes reticulatus) have been reported to show a repeatable response of another kind (9, 15).

Many drugs, including sedatives, antihistaminics, autonomic stimulating and blocking drugs, ergot drugs, and respiratory enzyme poisons have been studied in tropical fish (1, 5-8, 12-14, 16). Of these others, only intraperitoneal injection of 25μg of psilocybin[2] has been reported to evoke a completely typical LSD-25 reaction in Siamese Fighting Fish (1). Several respiratory enzyme inhibitors such as sodium azide and potassium cyanide have reportedly produced reactions very similar to the LSD-25 reaction (4, 17). Hyoscine has been reported to potentiate the LSD-25 reaction and pilocarpine has been reported to return the behavior to normal (15). Brain extract also has been reported to block the LSD-25 reaction in Siamese Fighting Fish and serotonin to have no effect in some studies (3), and a definite effect in others (14).

In Guppies, tryptamine has been reported to potentiate the LSD-25 reaction and reserpine to return the behavior to normal (9). The present study was designed to investigate: 1) the effect of LSD-25 on species of tropical fish other than Siamese Fighting Fish, 2) the effect of temperature on the drug reactions in tropical fish, 3) the effect of simultaneous treatment of tropical fish with LSD-25 and reserpine, LSD-25 and norepinephrine, LSD-25 and serotonin, and 4) the effect of sodium azide and potassium cyanide on tropical fish.

METHODS

We screened thirty species of tropical fish (eight fish for each species) for reaction to LSD-25, using concentrations of 4μg/ml and 1μg/ml. Specific gravity, feeding routine and water hardness were constant throughout all the experiments. The fish were placed in 300 ml of drug solution at 22°C(72°F), pH = 7.8, and observed for one hour. Specimens of the following species were screened for reaction to LSD-25: Siamese Fighting Fish (Betta splendens), Cupanus Dayi (Macropodus cupanus dayi), Pearl Gourami (Trichogaster leeri), Paradise Fish (Macropodus opercularis), Dwarf Gourami (Colisa lalia), 3-Spot Gourami (Trichogaster trichopterus), White Cloud (Tanichthys albonubes), Red Minor (Hyphessobrycon callistus), Angel Fish (Pterophyllum scalare),

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[2] Phosphoric acid ester of 4-hydroxy-dimethyltryptamine.

Guppy (Lebistes reticulatus), Mexican Swordtail Fish (Xiphophorus helleri), Zebra Fish (Brachydanio rerio), Neon Tetra (Hyphessobrycon innesi), Malabar Killy (Apolcheilus lineatus), Rosy Barb (Barbus conchonius), South American Cat-Fish (Corydoras aeneus), Window Cleaner (Plecostomus plecostomus), Whiptail Cat-Fish (Loricaria parva), Net-like Cat-Fish (Corydoras reticulatus), Talking Cat-Fish (Acanthodoras spinosissimus), Fire-Mouth (Cichlasoma meeki), Rummy-nosed Tetra (Hemigrammus rhodostomus), Black Tetra (Gymnocorymbus ternetzi), Moonfish (Xiphophorus maculatus), Rough Black Killy (Pachypanchax playfairi), Kuli Loach (Acanthophthalmus khuli), Lemon Tetra (Hyphessobrycon pulchirpinnis), Aphyosemion calliurum,[3] Bumble Bee Goby (Brachygobius xanthozonus), and Aphyosemium wakeri.[3]

Those species showing a consistent and repeatable reaction in LSD-25 were similarly screened with sodium azide (10μg/ml) and potassium cyanide (0.5μg/ml).

Because of reports in the literature of discrepancies in behavior at different temperatures (5), a study was set up to demonstrate the effects of various drugs and drug combinations at different temperatures on tropical fish. Angel Fish, White Cloud, Red Minor, Pearl Gourami and Siamese Fighting Fish were chosen as test fish because of their easily demonstrable reaction to LSD-25. Ten fish of each species were taken from the general tanks and placed in groups of five in beakers containing 300 cc of control water of the same temperature, hardness, specific gravity and pH, and the beakers were placed into a water bath of the desired temperature and allowed to come to equilibrium. The fish were then put into either control beakers with no drugs or into beakers with drugs or drug combinations already at the same desired temperature and with identical water. They were observed every 15 minutes for 90 minutes. We then removed them from the drug and placed them back into the original beakers, still at the same temperature, and observed them again every 15 minutes for 90 minutes. The drugs and drug combinations used were LSD-25 (1μg/ml), LSD-25 (1μg/ml) + serotonin (1 mg/ml), LSD-25 (1μg/ml) + norepinephrine (50μg/ml), LSD-25 (1μg/ml) + reserpine (10μg/ml), and sodium azide (10μg/ml).[4] The temperatures used were 35°C(95°F), 30°C(86°F), 25°C(77°F), 20°C (68°F) and 15°C(59°F). Neither reserpine, serotonin, or norepinephrine alone have any notable effect on tropical fish behavior.

[3] This species of tropical fish has no common name.

RESULTS

Of the 30 species screened, eleven were found to have repeatable reactions in LSD-25. Both Cupanus Dayi and Paradise Fish showed reactions similar to Siamese Fighting Fish. They stayed at the surface, demonstrated the body kink and showed some back ward swimming motions. They differed, however, from Siamese Fighting Fish in that they had periods of extreme excitability consisting of attempts to jump out of the solution and rapid swimming at the surface. These periods, each lasting one to three minutes, were seen during the first forty-five minutes of their one-hour exposure to 1μg/ml LSD-25. Siamese Fighting Fish also have a similar excitement stage, which is seen early in the exposure LSD-25, and lasts ten to twenty minutes. Cupanus Dayi and Paradise Fish generally remain more active than Siamese Fighting Fish and do not show barrel roll, Cartesian diver, or nose-up-tail-down behavior.

3-Spot Gourami, Dwarf Gourami, and Pearl Gourami all come to the surface with the nose-up-tail-down position at an angle of about 30°–40° and show decreased activity. These five above-mentioned species

[4] LSD-25 and serotonin were kindly supplied by Sandoz Pharmaceuticals. Reserpine supplied by Ciba Pharmaceuticals. L-Arternol Bitartrate supplied by Winthrop Laboratories.

TABLE 1

Total Number of Reactions Seen in Each Species for Each Drug at Each Temperature During 90 Minutes of Exposure to the Drugs

Drug	LSD-25					LSD-25 + Serotonin					LSD-25 + Norepinephrine					LSD-25 + Reserpine					Sodium Azide					Control				
Temperature	35°	30°	25°	20°	15°	35°	30°	25°	20°	15°	35°	30°	25°	20°	15°	35°	30°	25°	20°	15°	35°	30°	25°	20°	15°	35°	30°	25°	20°	15°
Siamese Fighting Fish	53	60	52	34	13	60	55	52	31	14	60	60	50	23	21	53	60	58	51	12	28	31	40	35	30	10	12	2	22	12
Red Minor	50	50	39	36	38	57	55	38	40	40	58	44	44	43	33	‡	48	52	58	46	22	4	4	5	1	0	4	0	0	0
White Cloud	60	60	60	46	52	60	60	55	49	43	60	60	60	52	55	30*	58	60	60	60	17	14	11	15	1	6	32	0	0	0
Angel Fish	46	60	57	31	21	60	59	57	38	7	60	60	59	11	16	36†	60	60	43	12	16	11	10	0	0	0	4	0	0	0
Pearl Gourami	60	57	52	30	37	60	32	58	24	48	60	59	32	14	21	60	54	47	30	15*	21	13	2	4*	8*	0	2	0	0	0*

* Observation period 45 minutes because of toxicity in the fish.
† Observation period 60 minutes because of toxicity in the fish.
‡ All fish toxic in 5 minutes and removed from drug.

and Siamese Fighting Fish are of the family Anabantidae, which is characterized by the presence of an auxiliary breathing apparatus in addition to the ordinary gills.

White Cloud, Red Minor and Lemon Tetra react to LSD-25 by coming to the surface, increased backward swimming and decreased activity. They swim horizontally within the top centimeter of water.

Angel Fish come to the surface, tend to cluster together in tight groups with little activity and exhibit swimming in backward circles.

Guppies exhibited only part of the reaction described by Keller and Umbright (9) in 4μg/ml of LSD-25, and almost no reaction in 1μg/ml LSD-25. They showed increased surface and backward swimming.

The remaining 19 species of fish occasionally showed some abnormal behavior, but no consistently repeatable patterns. None of the eleven species reacting to LSD-25 and screened with sodium azide showed much reaction to sodium azide except Siamese Fighting Fish, which will be discussed later. Contrary to reports in the previous literature, potassium cyanide appeared to be much more toxic to tropical fish than sodium azide (4, 7). All fish showed some toxic reaction in 0.5 μg/ml potassium cyanide.

In the temperature experiments, the single reaction of remaining at the surface was used as a representative of the LSD-25 reaction in all fish. (A separate recording of body kink and nose-up-tail-down position was made for Siamese Fighting Fish.)

The total number of surface swimming responses observed in the 90 minutes in the drug was recorded for each species in each drug at each temperature.[5] The data (see Table 1) were analyzed by the Sign Test and Friedman 2-Way Analysis methods for the differences between reactions to the drugs or drug combinations, reactions at various temperatures, and differences between species (Table 2).

There were no significant differences in the reactions to LSD-25, LSD-25 + reserpine, LSD-25 + serotonin, and LSD-25 + norepinephrine within any one species. The LSD-25 + reserpine combination, however, was somewhat toxic in less than 90 minutes at 35°C(95°F) to all fish except Pearl Gourami. Reserpine alone at this temperature was found to be most toxic to Red Minor, and also was toxic to White Cloud and Angel Fish. This toxicity was not seen with the other drugs. There were significantly more responses to the LSD-25 (and LSD-25 + other drug combinations) than to sodium azide in all species. Sodium azide

[5] Since there were 10 fish of each species, each observed 6 times in 90 minutes, if all the fish reacted within the first 15 minutes and maintained the reaction, there would be 60 reactions observed.

TABLE 2
Statistical Analysis of Data

Factors Compared	Type of Test Used	p
A. In Each Species:		
1. LSD-25 *vs.* LSD-25 + serotonin *vs.* LSD-25 + reserpine *vs.* LSD-25 + norepinephrine	Friedman 2-Way Analysis	>0.80
2. Average of (LSD-25, LSD-25 + Drug Combinations) *vs.* sodium azide	Sign Test	0.03
3. Sodium azide *vs.* Control	Sign Test	0.03
B. In Each Drug:		
1. Pearl Gourami *vs.* Angel Fish *vs.* Red Minor *vs.* Siamese Fighting Fish	Friedman 2-Way Analysis	>0.50
2. Average of above 4 species *vs.* White Cloud	Sign Test	0.03
3. 35°C *vs.* 30°C *vs.* 25°C	Friedman 2-Way Analysis	0.60
4. Average (35°C, 30°C, 25°C) vs. 20°C for LSD-25, LSD-25 + serotonin, LDS-25 + reserpine, and LSD-25 + norepinephrine	Sign Test	0.03
C. At Each Temperature:		
1. Siamese Fighting Fish *vs.* other species in sodium azide	Sign Test	0.03

did, however, elicit significantly more responses than the controls.

There were no differences between Pearl Gourami, Angel Fish, Red Minor, and Siamese Fighting Fish in their reactions to LSD-25 and LSD-25 with the drugs in combination. White Cloud showed significantly more reactions to the LSD-25 (and LSD-25 + drug combinations) than any other species. Siamese Fighting Fish showed significantly more response to sodium azide than did the other species but this was still significantly less than to LSD-25 (or LSD-25 + drug combinations).

Analysis of the temperature factor showed that there were no significant differences at 35°C(95°F), 30°C(86°F) and 25°C(77°F), in the total number of responses recorded for each drug. Because of toxicity, however, fewer responses were recorded for LSD-25 + reserpine at 35°C(95°F) than for LSD-25 and LSD-25 and other drug combinations. There were significantly less responses at 20°C(68°F) than at the higher temperatures for LSD-25 by itself, with serotonin, and with norepinephrine, but the LSD-25 + reserpine combination retained its high level of effectiveness. Pearl Gourami were very sensitive to the 15°C(59°F) temperature, which was toxic even to the controls. The maximum total responses were seen at 35°C (95°F) for all drugs except LSD-25 and LSD-25 + reserpine. For these two drugs maximum responses were seen at 30°C (86°F).

DISCUSSION

The total LSD-25 behavior pattern as described for Siamese Fighting Fish seems to be specific for this fish. Other species in the same family show somewhat similar reactions but no species of fish shows all the behavior changes. The common behavior change shown by all the species was that of swimming at the surface and an increase in backward swimming. Cano (5) found that Guppies in LSD-25 had an increase in the pressure in the swim bladder. He suggests this as an explanation of the surface swimming phenomenon seen in tropical fish in LSD-25. We did not investigate this point. A reaction of the air bladder was "indicated" by Loeb (10) in a study of the surfacing effect of LSD-25 on a variety of species, although he has not demonstrated this.

It has further been suggested that the LSD-25 reaction could be explained on the basis of anoxia (4, 17) because of reported similarities in the response of Siamese Fighting Fish to potassium cyanide, sodium azide

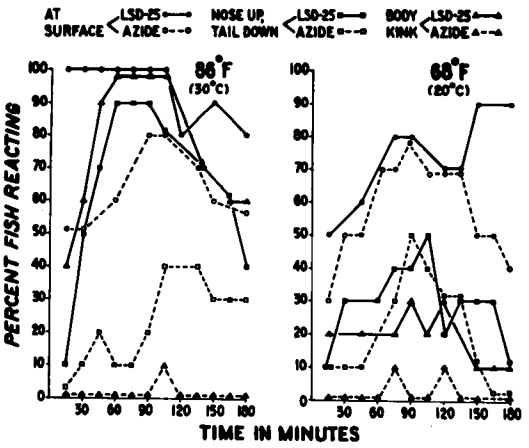

FIG. 1. Siamese Fighting Fish: LSD-25 and sodium azide.

and LSD-25. In our study, in a concentration of sodium azide ten times that of LSD-25, all fish at 30°C(86°F) showed some increase in surface swimming as compared to the controls, but much less than in LSD-25. However, at lower temperatures the LSD-25 effect was reduced.

In sodium azide, the nose-up-tail-down behavior of Siamese Fighting Fish is not affected by lowering of the temperature, whereas in LSD-25, the nose-up-tail-down behavior decreases significantly with the lowering of temperatures (Figure 1).

None of the other associated behavior changes seen in LSD-25 were consistently present in sodium azide. Backward swimming was not increased in any species and Siamese Fighting Fish specifically showed no body kink, barrel rolling or Cartesian diver behavior.

Since none of the ten species besides Siamese Fighting Fish showing consistently repeatable reactions to LSD-25 showed similar reactions to sodium azide or potassium cyanide, and Siamese Fighting Fish reactions only partially overlap and then only in a limited temperature range, it must be concluded that the behavioral changes seen with LSD-25 cannot be accounted for by assuming that LSD-25 acts mainly like a respiratory enzyme poison such as sodium azide or potassium cyanide.

Since serotonin failed to modify the LSD-25 reaction when the fish were exposed to a solution of 1μg/ml of LSD + 1 mg/ml of serotonin, eight fish each of species Angel Fish, White Cloud and Siamese Fighting Fish were analyzed for serotonin by the method of Shore and Olin (11). Four control fish of each species were analyzed before any drug exposure and four fish of each species were analyzed after a one hour exposure to 1 mg/ml of serotonin. The average amount of serotonin in the control fish was 0.127μg/gm of fish. For the test fish the average amount of serotonin was 0.627μg/gm at 35°C(95°F) and 0.673μg/gm in fish at 20°C(68°F). Serotonin therefore entered the fish. Lack of effect may have been due to an inability of serotonin to cross the blood-brain barrier which has been demonstrated in higher animals or to a lack of interaction of serotonin and LSD-25.

Reserpine also did not modify the LSD-25 reaction when the fish were exposed to LSD-25 (1μg/ml) and reserpine (10μg/ml) simultaneously. Five Siamese Fighting Fish were therefore injected intraperitoneally with 125μg of reserpine (0.05 cc of injectable Serpasil). The injection was made just anterior to the anal opening with a 27 gauge needle and a 1 cc syringe. No change in behavior was seen. It is therefore unlikely that the inability of reserpine to modify the LSD-25 reaction was due to non-absorption of reserpine.

SUMMARY

Thirty species of tropical fish were screened for LSD-25 reactions. Eleven were found to have repeatable behavior changes but none showed all the behavior changes seen in Siamese Fighting Fish. A common characteristic shown by all reactors was that of surface swimming.

Since none of the ten species besides Siamese Fighting Fish showing consistently repeatable reactions to LSD-25 showed

similar reactions to sodium azide or potassium cyanide, and Siamese Fighting Fish reactions only partially overlap and then only in a limited temperature range, it must be concluded that the behavioral changes seen with LSD-25 cannot be accounted for by assuming that LSD-25 acts mainly like a respiratory enzyme poison such as sodium azide or potassium cyanide.

Reserpine (10μg/ml) was found to be toxic for three species at high temperatures. Neither serotonin (1mg/ml), norepinephrine (50μg/ml) or reserpine (10μg/ml) either blocked or potentiated the reaction of LSD-25 (1μg/ml) on any of these fish at any temperature.

The number of reactions generally declined with temperature from 35°C(95°F) to 15°C(59°F) except for sodium azide, where temperature did not have a profound effect. The optimal temperature in our experiments appeared to be 30°C(86°F) because of the toxicity seen with some drugs at higher temperatures. Biochemical analysis showed that serotonin did enter the fish, and intraperitoneal injection of reserpine produced no changes.

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