50/X 56 LSD 212/DHE/Gy/Hyg/LAE/Meth

The Effect of Lysergic Acid Diethylamide on *Betta Splendens:* I.

WM. J. TURNER, M.D.
Central Islip, New York

●

Reprinted from *Diseases of the Nervous System,* Vol. XVII, No. 6, June 1956. p.193

Printed in U.S.A.

The Effect of Lysergic Acid Diethylamide on *Betta Splendens:* I.

WM. J. TURNER, M.D.
Central Islip, New York

In 1954 Abramson and Evans[1] reported that immersion of juvenile *Betta splendens* (Siamese fighting fish) for six hours in 100 cc. of water containing as little as 0.5 micrograms per cc. of LSD-25 resulted in a characteristic alteration of motility. Later they found that the monoethylamide of lysergic acid (LAE-32) had qualitatively the same effect.[2]

The remarkable properties of LSD-25 in the human being have so far been difficult to relate to phenomena in non-human species. Specific effects have been reported only in certain spiders by Witt[3] and in waltzing mice.[4] Our own fruitless search for specific effects covers observations made between 1952 and 1954 on bacteria, amoebae, paramoeciae, annelids, molluscs (both of fresh and salt water), crustaceans, a few insects and tadpole larvae. It can only be said that LSD-25 exerted no evident action on any of these organisms except at such high concentrations (over 10 micrograms per cc.) that the unique action of LSD-25 would be questionable. A single exception is the snail, *Ambularia cuprina* (Abramson and Jarvik[5]).

An understanding of the sites and mechanisms of action of LSD-25 is of the greatest importance for the light which might thereby be thrown on the schizophrenic psychoses. The very slight action of LSD-25 on the hundreds of species which have been subjected to it indicates a highly specific action; its effects on spiders, waltzing mice, *Betta splendens* and on man conceivably have nothing in common. Indeed, it might also be that none of these effects has anything to do with schizophrenia. We are nevertheless justified in searching to determine whether, by assuming relevance, we may learn something of value.

From Research Division, Central Islip State Hospital.

Materials and Methods:

We examined the effects of some 60 pharmacologic agents on 22 species of fish, using the effect of LSD-25[B] on juvenile *Betta splendens* as an investigative hub. The drugs were dissolved in water in concentration starting at twice the maximum adult human dose per 100 cc. and descending to concentrations which exhibited no effect on fish after six hours' exposure.

This report will deal only with observations made on fish treated in the manner described by Abramson and Evans. Usually the smaller fish were immersed in 100 cc. of solution, larger fish in a suitable volume (e.g., 500 cc.) as recommended by professional fish culturists. Water was artesian well or clear fresh pond water which was drawn at least 24 hours previously, so that there was no shock to fish on immersion.

Generally, in addition to untreated controls, no more than two drugs were studied concomitantly. Three or four fish were used in each test immersion. When a drug was found which might have an LSD-25 effect or which might be a blocking agent for LSD-25 it would be studied in a variety of ways: e.g., (1) simultaneous action of the drug and LSD-25, (2) preliminary intoxication with one drug followed by immersion in a solution combining both drugs.

Juvenile specimens of the following species were studied: *Betta splendens, Aequidens latifrons, Aequidens portalegrensis, Molleinisia latipinna, Brachydanio reria, Xiphophorous helleri, Trichogaster sumatrans,*

Trichogaster leeri, Platypoecilus maculatus, Tanichthys albomibes, Macropodus viridi auratus, Lebistes reticulatus, Pterophyllum scalare, Barbus titteya, Haplochromis multicolor, Pristella riddlei, Carassius auratus, Hemigrammus ocellifer, Corydoras leopardus, Cichlasoma meeki, Corydoras aeneas and *Barbus sumatranus.* Of many species adult specimens were also tested.

The *Betta splendens* tested varied in length from 8 to 20 mm. from nose to base of tail fin. Our observations included not only those on undisturbed fish but also on rheotaxis, on response to approach of foreign bodies and on feeding behavior.

Experimental Results:

In solutions containing 1.0 micrograms per cc. of LSD-25 none of the fish showed any marked accentuation of normal behavior and no evidence of behavior not characteristic of the species (e.g., no "schooling" in *C. leopardus,* but in *Pt. scalare*).

With regard to the effects of LSD-25 on *Betta splendens* we were able to confirm Abramson and Evans in every detail. The normal *Betta* was relatively inert, however, and if left undisturbed would at times be observed to "hang at the surface," or to curl its body and remain so for some moments, or to swim backwards or sideways. Against a dark background, too, dispersion of melanophores occurred. With respect to these phenomena, LSD-25 could only be said to have produced an exaggeration of normal behavior. The "barrel-rolling," however, had not been observed by us in normal fish at lower levels of LSD-25 (e.g., 1 microgram per cc.). Above 1 microgram per cc., fish would often be seen to lie on their sides, their bodies S-shaped, for minutes on end. Phenomena rarely if ever seen in normals which were prominent even at low levels were the "Cartesian diver" effect and the reduction in motility which Abramson and Evans called the trance-like state. This latter was partly a function of stimulation frequency and intensity. A normal fish would rapidly orient itself in swirling water and would swim with such speed as to remain almost fixed with respect to the wall of the vessel in which it swam. LSD-25 fish oriented somewhat less rapidly and were unable to maintain position as well as would the normal. A normal fish would avoid a pencil lead brought to within 2 mm. of its nose, although it would allow gentle touching of the tail fins. LSD-25 fish would avoid only after actual touching of the nose and would permit being pushed in a circle by pressure on tail, fin or body. Normal fish would rarely permit a wire loop to be passed over themselves from nose to tail, which would touch both their pectoral fins; LSD-25 fish readily permitted this. Avoidance, when it occurred, was by minimal action rather than the normal rapid darting away for a considerable distance. Normal fish unfed for 24 hours would go avidly after finely granulated food. LSD-25 fish seemed to remain unaware of the presence of food for some time (often for minutes), and then might be responsive only to a few pieces settling down. Once started, some fish continued to feed as long as particles were on the surface or were settling. Their aim for food, once they went for it, was good.

It was of particular interest to examine whether other psychoplegic and hallucinogenic drugs exerted an effect like LSD-25. Most of the species noted above were exposed to mescaline sulfate in concentrations up to 250 micrograms per cc. and to bufotenine[C] up to 100 micrograms per cc. A few were treated with bufotenine 500 micrograms per cc. during six-hour observation periods. No alteration of behavior was observed in any of the species under these conditions. Nor did there appear any enhancement or inhibition of LSD-25 effect.

With *Betta splendens* and occasionally with other species, examination of a number of sedatives was carried out, including paraldehyde, chloral hydrate, dormison,[R] a number of barbiturates, morphine, cocaine, chlorpromazine and reserpine.[C] While moderate doses exerted sedative effects on fish, other effects were variable (e.g., aggregation of chromophores by cocaine, dispersion by reserpine[6]), none of the above-mentioned sedatives exerted an LSD-25 effect, nor did they specifically enhance or block LSD-25 action.

Antihistamines and related compounds (pyribenzamine,[R] benadryl,[R] antistine,[R] trime-

ton,[R] bonamine[R] were also powerful sedatives on fish, but they did not enhance or block the LSD-25 effect.

A group of agents with autonomic stimulating and blocking properties had no LSD-25 effect and failed to block LSD-25 action. These included ephedrine, neosynephrine,[R] vasoxyl,[R] eserine, atropine, papaverine, artane,[R] serotonin, adrenaline and adrenochrome.

Hyoscine alone of all drugs studied clearly and greatly potentiated the action of LSD-25. In one experiment seven rows of seven columns each of quart Mason jars were set up. Into each was placed 500 cc. of water which had stood at least 48 hours and to each ten gallons of which had been added one quart of sea water. In each jar were placed three juvenile *Bettas*. Twelve hours later all fish were healthy and normally active. At the end of 12 hours, drugs were added. The hyoscine concentrations in the columns were 0.4, 0.1, 0.025, 0.00625, 0.00156, 0.0004 and 0.0 micrograms per cc.

At one hour the limit of definite effect with the two micrograms per cc. of hyoscine was 0.025 of LSD-25; with one microgram per cc. of hyoscine the limit of definite effect was 0.1 micrograms per cc. of LSD-25; with no hyoscine the limit of definite effect was 0.40 micrograms per cc. of LSD-25.

At two hours the LSD-25 effect was evident at 0.00156 micrograms per cc. of LSD-25 with four micrograms per cc. of hyoscine. It was evident in the two micrograms per cc. of hyoscine at 0.00625 micrograms per cc. of LSD-25. At 0.025 micrograms per cc. of LSD-25 the effect was already evident at 0.5 micrograms per cc. of hyoscine. In the absence of hyoscine 0.1 micrograms per cc. of LSD-25 was effective.

At two hours also an effect due to hyoscine alone occurred only at 16 and eight micrograms per cc. This consisted in a tendency to lie on the side quietly either at the bottom of the jar or at the top, sometimes with marked curling of the body. Stimulation would lead the fish to become very active for a brief period in contrast to the minimal avtivity of the fish intoxicated with LSD-25. In addition, the hyoscine reaction differed from LSD-25 in that there was no "Cartesian diver" effect, no backward swimming and no barrel-rolling. Further, undulation of the dorsal and tail fins was not stopped, although the undulatory frequency was slow. The combination of hyoscine and LSD-25 occasionally lead to periods of excitement and sometimes even to fighting in the lower hyoscine combinations.

Further observations were made at three and six hours. There was no marked increase in the manifestations of intoxication during this time. At six hours, indeed, there had been a recession of activity at the lowest level of LSD-25 concentration. The larger doses led to an extreme torpidity of the fish, which would cluster together, sometimes lying on their sides at the surface of the water. On transfer to fresh water the effect wore off slowly during a period of 12 to 24 hours.

With pilocarpine 150 micrograms per cc. an otherwise unobserved effect occurred with *Betta splendens*. About one half hour after immersion, the fish would stand on their heads at the bottom of the jar, their bodies at angles of 75° to 90° to the horizontal. Their pectoral fins hung limply downward and their backs were arched as in opisthotonos. The effect was of variable duration, often ending in one hour, but at times (as in 300 micrograms per cc.) persisting for 24 hours.

Betta splendens were first intoxicated with LSD-25 1.0 micrograms per cc., then pilocarpine was added to make the solution 150 micrograms per cc. with respect to pilocarpine. Within 10 to 15 minutes the LSD-25 effect had been modified and over a subsequent period of several hours the fish exhibited normal behavior. Now and then a fish might swim head down, but feeding behavior, response to swirling water and to the approach of foreign objects was normal. Fish previously intoxicated with 3.0 micrograms per cc. of LSD-25 transferred to pilocarpine 150 micrograms per cc. showed normal behavior within one-half hour. *Bettas* first subjected to hyoscine 4.0 micrograms per cc., then to hyoscine 4.0 micrograms oer cc., plus pilocarpine 150 micrograms per cc., failed to show the pilocarpine effect.

Lysergic acid monoethylamide (LAE-32)

had an LSD-25 action, but this was not shared by ergotamine,[R] dihydroergotamine,[R] methergine[R] or hydergine,[R] nor did these drugs block the action of LSD-25.

Of all the drugs studied, only pilocarpine specifically blocked LSD-25 effect. Hyoscine potentiated LSD-25 and blocked the effect of pilocarpine, but did not in itself alter behavior of the fish up to concentrations of four micrograms per cc.

Discussion

The observer who watches narcotized fish and compares them with LSD-25 fish, recognizes differences in body posture, fine fin movements and in response to stimuli. LSD-25 does not disconnect, as it were, the central from the peripheral nervous system. Fish intoxicated with LSD-25 resemble in many ways trout under tectal stimulation as described by Akert.[7] The impression of alteration, rather than of loss, of muscular tonus suggests a disturbance in the vestibular innervation, a disturbance arising out of alteration of signals, as from the optic tectum. Evarts[8] and Pennes[9] have presented evidence that LSD-25 decreases the height of the synaptic volley in the visual pathways of cats and monkeys. Marrazzi and Hart[10] have shown also that LSD-25 as well as bufotenine and mescaline decreases synaptic transmission in the cat. The thought, then, has been entertained by the writer that the action of LSD-25 on *Betta splendens* could be due to an action on the neurones of the visual pathways whereby the spatial and temporal organization of impulses, which code information to the tectum and thence to other parts of the central nervous system, suffers deformation. If this be indeed the site and the mechanism of action of LSD-25 on *Betta splendens,* might it not also be the site and mechanism of action productive of the psychic effects on man?

With this question in mind, the effect of hyoscine on LSD-25 with fish is especially interesting. It has long been known that tropanes (atropine, hyoscine and cocaine) are particularly potent agents for the production of psychosis with delusions as well as visual and auditory hallucinations.[11,12] It has also been known that synthetic drugs with hyoscine-like action which have been introduced in medicine for the treatment of paralysis agitans not infrequently induce psychosis. While the majority of these psychoses are delirious in nature, some of them seem indistinguishable from acute schizophrenic attacks.[13]

A review of the literature suggests that a synergistic action of hyoscine and other tropanes is much more effective in producing delirium, than is the pure drug alone. This seems to be the case also with LSD-25 and hyoscine—at least as far as our experience with fish is concerned. We can at present offer no theories to account for the synergistic action of hyoscine and LSD-25, nor for the absence of such effect with atropine. Similarly, we are unable to offer an explanation for the action of pilocarpine. Text books assert that pilocarpine acts directly on smooth muscle and on cells of the salivary glands.[14] While it is plausible that this relates to the difference between the action of pilocarpine and of physostigmine on the blocking of LSD-25 effect in *Betta splendens,* how pilocarpine could restore the coding of information, if this be its action, is beyond our conjecture.

Summary

The action of 60 pharmacologic agents has been examined on 22 species of fresh water fish. LSD-25 has a special action on *Beta splendens* which is not found in the other species. This action is partly shared by LAE-32 but not by serotonin, bufotenine, mescaline or adrenochrome. It is potentiated by hyoscine and counteracted by pilocarpine.

Acknowledgments

A. We are greatly indebted to Mr. Walton Scherer of Northport, N. Y., who made available to us large numbers of fish, tanks and space, often at considerable inconvenience. His knowledge of the behavior of fish has also been valuable.

B. Ampouls of LSD-25, LAE-32, dihydroergotamine, ergotamine and methergine were the gift of Sandoz Pharmaceuticals through Mr. Kenneth Ericson. LSD-25 as a dry powder was received from Dr. Harold Abramson.

C. We are indebted to Dr. Merrill E. Speeter of Upjohn Company for supplies of bufotenine and serotonin and to Dr. John C. Saunders of Ciba Pharmaceutical Products, Inc., for crystalline reserpine, regitine, privine, apresoline and priscoline.

The author is indebted to Mrs. Ann Carl and to Mrs. Grace Haas for their technical assistance.

REFERENCES

1. Abramson, H. A., and Evans, L. T.: Lysergic Acid Diethylamide (LSD-25): II. Psychobiological Effects on the Siamese Fighting Fish. *Science,* 120:990-991, Dec. 10, 1954.
2. Abramson, H. A.: Personal Communication.
3. DeWitt, Peter N.: Spider Webs and Drugs. *Scientific American,* 191:80-87, Dec. 1954.
4. Rothlin, E., and Cerlett, A.: Über einige pharmakologische Untersuchungen an Mauser mit congenitaler Drehsucht. *Helv. Physiol. Acta,* 10:319-327, 1952.
5. Abramson, H. A., and Jarvik, M. E.: LSD-25: IX. Effect on Snails. *J. Psychol.,* 40:337-40, 1955.
6. Turner, Wm. J. and Carl, Ann: The Effect of Reserpine on the Melanophores of Fish. *Science,* 121: 877-878, June 17, 1955.
7. Akert, K.: Die visuelle Greifreflex. *Helv. Physiol. Acta,* 7:112, 1949.
8. Evarts, E. V., Landau, Wm., Freygang, W., and Marshall, W. H.: Some Effects of LSD-25 and Bufotenine on Electrical Activity in the Cat's Visual System. *Am. J. Physiol.,* 182:594-598, 1955.
9. Pennes, Harry: Demonstration at LSD-Mescaline Round Table, A.P.A. meeting, Atlantic City, May 12, 1955.
10. Marrazzi, A. S., and Hart, E. R.: The Relation of Hallucinogens to Adrenergic Cerebral Neurohumors. *Science,* 121:365-367, 1955.
11. Lewin, Louis: Phantastica, Narcotic and Stimulating Drugs. Trans. from 2nd German edition by F. H. A. Wirth. *E. P. Dutton,* New York, 1931.
12. Henry, T. A.: The Plant Alkaloids. 4th Edition. 4th Edition. *Blakiston Co.,* Phila., 1949.
13. Pierik, Michael G.: Acute Hallucinosis secondary to Pagitane Hydrochloride Administration. *New Eng. J. Med.,* 251:1058, Dec. 23, 1954.
14. Burger, A.: Medicinal Chemistry, Vol. I, p. 373. *Interscience Publishers, Inc.,* New York, 1951.

Printed in U.S.A.