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NO EFFECT OF LSD UPON 5-HYDROXYTRYPTAMINE LEVEL IN MOUSE EYE

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ABSTRACT

1. LSD has been tested in mice to determine whether it causes a change in 5-hydroxytryptamine concentration in eyes, similar to the effect described in brain tissues.
2. No such effect was seen.

AN antagonistic physiological relationship between lysergic acid diethylamide (LSD) and 5-hydroxytryptamine (5-HT) has been known for some time (Gaddum, 1953), and considerable interest has centred upon the relation of these two substances in the brain (Smythies, 1970). Variations in brain 5-HT following LSD administration have been described, and this interaction represents one of the best-described actions of the drug (Giarman and Freedman, 1965). Another region of 5-HT localization in intimate association with the brain is the lateral eye, an early embryonic outgrowth of the forebrain. We have administered LSD to adult mice and assayed their eyes for 5-HT. Our results indicate that the 5-HT elevation characteristic of brain tissue is not demonstrable in the eyes.

MATERIALS AND METHODS

Carworth CFW albino mice (*Mus musculus*) 4·5–5 months old were used. They were given LSD by intraperitoneal injection. Two separate dose levels of 50 μg. LSD per kg. body-weight and 200 μg. per kg. body-weight were used. Sham-injected animals were used as controls. The mice were killed by cervical dislocation 20 and 60 minutes after LSD injection and 40 minutes after sham injection. The eyes were removed, weighed, and homogenized immediately in 1 ml. 0·1 *M* HCl with 0·5 per cent ascorbic acid. The homogenates were transferred to stoppered tubes and extracted for 5-HT. The extraction was carried out according to Quay (1963) and the determinations were made fluorimetrically. Tubes with homogenates received 0·1 ml. 1 per cent EDTA solution, 200 mg. NaCl, and 3 ml. diethyl ether. They were stoppered, shaken, and centrifuged. The ether phase was aspirated and the tubes then received 1 ml. *p*H 10 borate buffer and another 3 ml. diethyl ether. They were again stoppered, shaken, and centrifuged and the ether was aspirated. Each tube then received 3 ml. *n*-butanol and was once more stoppered, shaken, and centrifuged. The butanol phase of each tube was then transferred to another set of larger tubes containing 0·5 ml. 0·1 *M* HCl, 0·5 per cent ascorbic acid, and 8 ml. heptane. These larger tubes were then stoppered, shaken, and centrifuged. Most of the heptane/butanol phase was aspirated and the tubes were stored in the cold overnight. From the final acid phase of each tube, 0·25 ml. was placed in a microcuvette. Concentrated HCl (0·075 ml.) was added and mixed in, and each sample was read in an Aminco-Bowman Spectrophotofluorometer at 540–550 nm. max. with activation at 295 nm. Standards of known concentration and blanks were carried through the whole procedure and read with the samples.

RESULTS

As indicated in *Table I* there is no significant change in the μg. 5-HT per g. eye tissue, either 20 or 60 minutes following LSD administration. Although not so expressed in *Table I*, we also made comparisons of amounts of 5-HT per eye pair, but found no difference.

DISCUSSION

Among the characteristics usually used to describe the psychoactive substance LSD is an antagonism to 5-HT (Smythies, 1970). In the brain LSD causes an elevation of 5-HT while the 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), falls (Rosecrans,

Lovell, and Freedman, 1967). Since no changes are found in the enzymes involved in 5-HT synthesis and destruction, it is usually assumed that 5-HT is being isolated for a period from the usual enzymatic events (Giarman and Freedman, 1965). The isolation is considered to be due to increased vesicular retention (Diaz, Ngai, and Costa, 1968) and this, in turn, is ascribed to decreased firing of the 5-HT-containing neurons (Foote, Sheard, and Aghajanian, 1969).

Table I.—CONTENT OF 5-HT IN MOUSE EYE-PAIR AS μg. 5-HT per g. FRESH TISSUE

EXPERIMENT	DOSE OF 50 μg. LSD (per kg. body-weight)	DOSE OF 200 μg. LSD (per kg. body-weight)
Control		
N	8	8
$\bar{X}$	0·327	0·339
S.E.	0·025	0·065
20 minutes		
N	8	8
$\bar{X}$	0·240	0·256
S.E.	0·037	0·050
P	N.S.	N.S.
60 minutes		
N	8	8
$\bar{X}$	0·267	0·354
S.E.	0·056	0·061
P	N.S.	N.S.

$\bar{X}$ = Mean; S.E. = standard error of the mean; P = Probability from Student-Fisher t-test; N.S. = not significant.

5-HT has been found in the vertebrate eye. It has been described in the eyes of a broad spectrum of vertebrate types by Welsh (1964) and a number of mammals by Levine (1962). Enzymes associated with 5-HT metabolism have been identified in the eyes of a wide variety of vertebrates, including humans (Eranko and Merenmies, 1961; Eranko, Niemi, and Merenmies, 1961; Kojima, Iida, Majimi, and Okada, 1961; Shanthaveerappa and Bourne, 1964). Embryonic localization of 5-HT in chick eye (Hoff, 1971) and 5-HT-associated enzymes in toad eye (Baker and Quay, 1969) indicate the early existence of this system.

The elevations of 5-HT reported in the brains of rats exposed to LSD were less than 20 per cent above control values (Smythies, 1970). In our eye measurements the variation within any group is great enough to perhaps obscure a change of that magnitude. Comparison of other mammalian eye measurements with various brain measurements indicates that eye levels are more variable than brain levels (Erspamer, 1966). We ourselves have found that 5-HT concentrations in mouse brain regions are far more constant than in eyes, even among siblings (Hoff and Baker, 1971).

While it has not been possible to demonstrate any LSD-induced change in eye 5-HT, we cannot rule out such a possibility. However, if normal variation in eye samples is large enough to obscure a drug effect, then perhaps any such effect is of minimal physiological significance.

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