LSD: Its Effect Upon 5-Hydroxytryptamine in Embryonic Development of *Xenopus laevis*

The possibility that LSD is a causative agent in birth defect has been experimentally supported[1-3] although the evidence is not conclusive[4,5]. Reports of possible human birth defect are also available, but poorly documented[6,7]. Almost all of these findings have been based upon morphologic evidence of developmental error. To discover if LSD can induce biochemical abnormality in developing embryos, this laboratory has been investigating the 5-hydroxytryptamine (5-HT, serotonin) pathway in embryos of *Xenopus laevis*, the South African Clawed Toad. 5-HT was chosen for study because its interaction with LSD is well established[8-11]. Embryos of *Xenopus* were used because extensive measurements of 5-HT metabolism have already been made upon them[12-14].

Methods. These experiments were designed to measure 5-HT in embryos that had received an LSD dose which produced no observable morphological or behavioral error. A concentration of 0.06 mg/ml aquarium water with exposure for 6 h was finally chosen. Exposure to LSD for periods in excess of 6 h or at greater concentration than 0.06 mg/ml resulted in death, dwarfing, pigment loss and reduced swimming activity. Embryos were obtained and staged as described by NIEUWKOOP and FABER[15]. Embryos at neurula (Stage 19), hatching (Stage 35) and post-hatching (Stage 40) were exposed to the drug. After LSD exposure they were carried through multiple rinses of spring water and reared in aerated aquaria. When they had developed to premetamorphosis (Stage 48, about 5 days after Stage 40) they were assayed for 5-HT. 5-HT was measured by a modification of the ninhydrin method using an Aminco-Bowman spectrophotofluorometer[16,17]. Whole embryos were used in groups of 3 for each measurement and brains in groups of 10. Methods of brain dissection have been previously described[13,14].

Results. With an LSD dose of 0.06 mg/ml for 6 h whole embryo 5-HT levels were affected, although only at the neurula exposure time. The extent of altered 5-HT levels are outlined in the Table. Clearly these changes are not transitory toxic reactions since they are demonstrable 7 and 5 days following drug application. The contention that LSD is causing a developmental biochemical change in embryonic *Xenopus* is supported by these data.

Discussion. Because the sensitive periods for whole embryo and brain differ, it is concluded that whatever regulatory mechanisms are being affected by the drug must somehow differ in whole embryo and brain. This observation is not unwarranted since we know, that despite a similar biochemistry, the neuronal 5-HT system exists in isolation from the 5-HT scheme in the rest of the body[18,19]. In embryonic *Xenopus* it has been shown that patterns of development for the enzymes synthesizing and destroying 5-HT are quite different in brain and whole embryo[13,14]. These changes in developing *Xenopus* do not correlate with the data from adult mammalian brain, where alterations in 5-HT are more transient and believe due to increased vesicular retention[10,11] and reduced neuronal firing[20,21]. In the mammal though the biochemistry of 5-HT in developing brain differs in many ways from the adult brain[22] and thus, there is no reason

5-Hydroxytryptamine levels in Xenopus whole embryos and brains exposed to LSD for 6 h at a concentration of 0.06 mg/ml

Stage of exposure (0.06 mg/ml for 6 h)	5-HT levels as ng per embryo or brain, measured at stage 48, and comparison to controls (using p from Student-FISCHER t)
Whole embryos	
Unexposed controls	$N = 12$
	$\bar{X} = 17.04$
	$Se = 0.805$
Neurula	$N = 8$
	$\bar{X} = 13.91$
	$Se = 0.371$
	$P = 0.01$
Hatching	$N = 8$
	$\bar{X} = 14.49$
	$Se = 1.027$
	$P =$ n.s.
Post hatching	$N = 8$
	$\bar{X} = 16.81$
	$Se = 1.639$
	$P =$ n.s.
Brains	
Unexposed controls	$N = 17$
	$\bar{X} = 2.04$
	$Se = 0.308$
Neurula	$N = 8$
	$\bar{X} = 2.12$
	$Se = 0.479$
	$P =$ n.s.
Post hatching	$N = 8$
	$\bar{X} = 0.63$
	$Se = 0.355$
	$P = 0.001$

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to expect that experimental manipulation of the immature brain should yield results that mimic the adult response. The alteration of 5-HT ontogeny may be significant in LSD induced defects. There is evidence that abnormal 5-HT metabolism interferes with normal development and 5-HT induced birth defects of rat and mouse brain have been reported[23–25].

The obvious question of developmental abnormality in mammals, especially humans, resulting from LSD-induced disruption of embryonic 5-HT metabolism is a possibility, although not demonstrated in these experiments[26].

Zusammenfassung. Nach LSD-Einfluss auf Embryonen von *Xenopus laevis* zu verschiedenen Entwicklungsstadien wurden langandauernd veränderte 5-HT-Stufen im Gehirn und im ganzen Embryo gefunden. Die LSD-Empfindlichkeitsperioden waren für das Gehirn und den ganzen Embryo voneinander verschieden.

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Effekt des 6-Hydroxydopamin auf Hauthomotransplantation der Mäuse

Injektion von 6-Hydroxydopamin (6-HD) ruft bei neugeborenen Mäusen oder Ratten eine totale und definitive destruktion des peripheren Sympatikus hervor[1]. Da es schon bekannt ist, dass neben Immunosupression auch einige andere Faktoren eine Verlängerung des Hauthomotransplantates hervorrufen können, z.B. E-aminocapronsäure[2,3], Histaminliberatoren[3,4], NSD-1055[5], haben wir in diesen Experimenten versucht, bei Mäusen ohne peripheren Sympatikus eine Homotransplantation durchzuführen. Wir fragten uns, ob der Abfall von sympatischen Impulsen auf Gefässe eine verbesserte Durchblutung des Homotransplantates haben wird. Diese Frage war insofern berechtigt, da es schon bekannt ist, dass E-aminocapronsäure ähnlich wie Guanetidin die Freisetzung von Noradrenalin und Adrenalin bewirkt[6].

Zum Versuch wurden ein weisser Stamm Mäuse (Pasteur Institut, Novi Sad), nicht älter als drei Tage, und ein schwarzer DBA Stamm genommen. Die Tiere bekamen einmalig 50 γ/g i.p. 6-HD in 0,025% Askorbinsäure gelöst. Als die Tiere etwa nach 6–7 Wochen ca. 18 g erreichten, wurde die Homotransplantation mit unserer früheren Methode[4] durchgeführt. Im Versuch waren 12 weisse und 12 schwarze Mäuse. (Einige Tage nach der Operation gingen 3 weisse und 4 schwarze Mäuse ein.) Weisse Mäuse

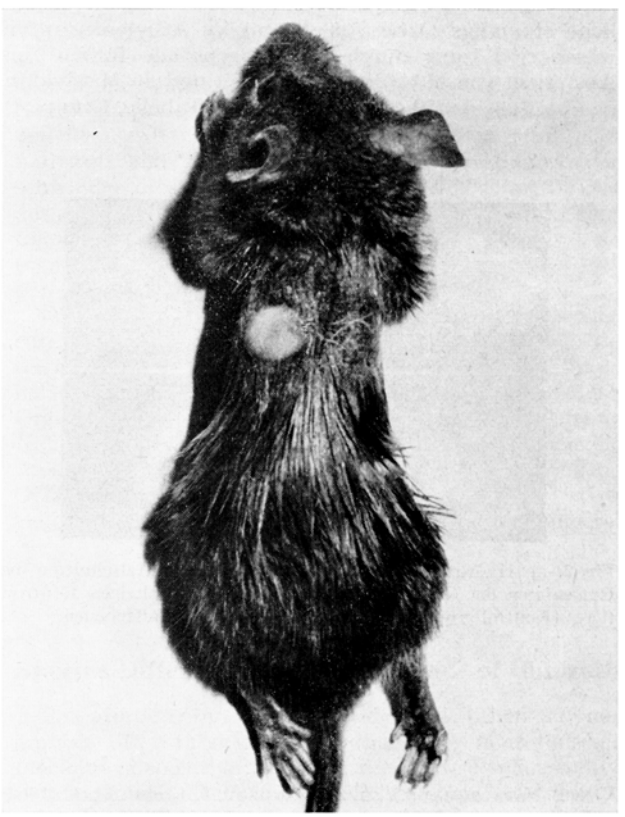

Fig. 1. Schwarze Maus, Homotransplantat links, Autotransplantat rechts. 25 Tage post op.

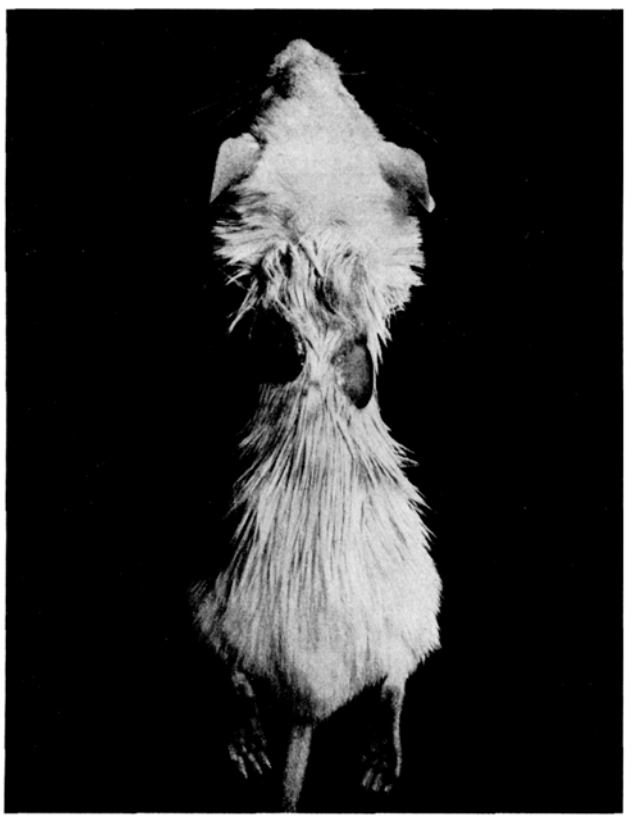

Fig. 2. Weisse Maus, Homotransplantat links, Autotransplantat rechts. 25 Tage post op.