

## NEONATAL TREATMENT WITH 5,7-DIHYDROXYTRYPTAMINE INDUCES DECREASE IN ALCOHOL DRINKING IN ADULT ANIMALS

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It has long been suggested that serotonin (5-HT) neurotransmitter system activity is associated with ethanol (ETOH) intake and dependence. The authors studied the effects of neonatal 5,7-dihydroxytryptamine (5,7-DHT) lesions on voluntary alcohol drinking in adult Wistar rats. At 3 days after birth animals were pretreated with desipramine (DMI) and then given a bilateral injection of 5,7-DHT into lateral ventricles. Afterwards, the rats were kept under standard laboratory conditions until at least 2 months of age following which they were tested. 5,7-DHT induced a marked and permanent decrease in brain 5-HT content, measured in the prefrontal cortex, hippocampus and striatum, but did not modify noradrenaline content in these structures. Lesioned animals, both males and females displayed lower preference for ETOH than sham-lesioned animals. Total fluid intake was significantly higher in 5,7-DHT-lesioned than sham-lesioned rats. A significant decrease in body weight was observed in 5,7-DHT-treated rats. This effect was not caused by a significant change in food intake. Both groups showed high preference for a 0.1% saccharin. In conclusion, the present results demonstrated that neonatal treatment with 5,7-DHT evoked long-lasting neurochemical changes and reduction of ETOH intake in adult rats. Neonatally 5,7-DHT-treated rats may be considered as a suitable model in further research on the relationship between the function of central 5-HT system and alcohol intake and dependence.

**Key words:** 5,7-DHT neonatal lesion, alcohol drinking, rats, serotonin

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*Abbreviations: 5,7-DHT – 5,7-dihydroxytryptamine, DMI – desipramine, ETOH – ethanol, 5-HT – serotonin, icv – intracerebroventricular, NE – noradrenaline*

## INTRODUCTION

Brain serotonin (5-HT) is believed to regulate development of alcohol tolerance and dependence [17, 25]. In some studies, 5-HT depletion resulted in increased alcohol consumption in animals and humans [10, 12, 20]. Compounds which affect serotonergic neurotransmission, such as the selective 5-HT reuptake inhibitors, have been reported to decrease ethanol (ETOH) intake in animal models of alcoholism [5, 6]. Low central 5-HT turnover, less frequent social interactions and excessive alcohol consumption in primates are similar to the overall pattern of antisocial functioning, excessive aggression and impaired impulse control exhibited by human alcoholics and is consistent with type II alcoholism [4, 9, 11]. A principal neurobiological feature of type II alcoholism is a CNS 5-HT deficit as measured by low 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the cerebrospinal fluid [4].

While many reports have described the effects of 5,7-dihydroxytryptamine (5,7-DHT) treatment in adult laboratory animals on their behavior and receptor mechanisms, relatively little information exists about the subsequent behavioral alterations induced when developing rats are treated with this neurotoxin.

There are conflicting reports regarding the effect of 5,7-DHT on serotonergic neurons in developing neonatal rats. Evidently, both the age of the rats and the route of 5,7-DHT administration are crucial factors underlying the vulnerability of brain serotonergic neurons to the neurotoxin. According to the literature, it looks that intracisternal administration of 5,7-DHT in 3-day-old rats leads to a long lasting loss of serotonergic terminals and cell bodies (i.e. cell death) throughout the brain (80–98% of 5-HT neurons) [22, 27, 28]. This approach seems to be imperative for experiments where the permanent destruction of virtually entire serotonergic system is desired. The present study was performed to investigate the effects of treating newborn rats with 5,7-DHT on their subsequent (during adulthood) alcohol voluntary intake.

## MATERIALS and METHODS

### Chemicals

5,7-Dihydroxytryptamine creatinine sulfate was obtained from RBI (Natick, MA 01760, USA). Desipramine hydrochloride (DMI) was purchased from SIGMA/RBI (St. Louis, MO 63178, USA). Saccharin sodium salt was obtained from Aldrich (Gillingham, UK).

### Animals and 5,7-DHT administration

Timed pregnant Wistar rats were obtained from a licenced breeder (HZL, Warszawa, Poland) and singly housed in clear plastic cages containing wood chip bedding material. The ages of newborn rats were determined by checking for births every day at 08:00 a.m. and 16:00 p.m. Each litter contained 8–10 pups. At 3 days after birth (the day of birth being postnatal day 0), pups were injected initially with DMI (desipramine hydrochloride, 20 mg/kg *ip*) followed 60 min later by an intracisternal injection of 70 µg 5,7-DHT (5,7-dihydroxytryptamine creatinine sulfate, dissolved in 0.1% saline solution of ascorbic acid, bilaterally in the volume of 5 µl). Solvents were delivered with a flow rate of 1 µl/12 s. Neonates were individually removed from the litter and were placed on a flat surface under a bright light. In this manner, the sagittal and transverse sinuses overlying the cranium, as well as bregma and lambda, could be seen through the transparent intact dermis. The needle having a polyethylene sleeve up to 2 mm from the tip was positioned 1.5 mm anterior to lambda and 2 mm lateral to the sagittal plane [1, 18]. After the needle was lowered into two lateral ventricles, and 5,7-DHT solution or vehicle was injected, the needle was left in place for about 30 s. Control sham-lesioned rats received the DMI injection and vehiculum instead of 5,7-DHT. Surgical anesthesia was induced by ether. Dams were maintained with the litters until 28 days of age when they were transferred (along with their foster litter mates) to group cages and housed divided by sex (3–4 animals/cage). Lesioned and control pups were weighed once a week. The rats were housed at 22 ± 1°C, under a 12-h alternating light-dark cycle (light on at 7:00) and 60% relative humidity. They were allowed free access to food (Bacutil, Poland) and tap water until at least 2 months of age following which they were tested.

Treatment of the rats in the present study was in full accordance with the respective Polish and European regulations and was approved by the local Ethics Committee.

### Long term free-choice ethanol drinking

Voluntary ETOH consumption procedure was conducted in the respective home cages. The rats were singly housed in standard macrolon cages with sawdust bedding changed twice weekly. The alcohol solutions were prepared from 95% stock ETOH and tap water. The subjects were assigned randomly to one of two experimental groups, i.e. to an ETOH-drinking group ( $n = 6$  for males and  $n = 6$  for females) or to a water-drinking group ( $n = 6$  for males and  $n = 6$  for females). The animals in the ETOH-treated group were exposed (for 22 days) to increasing concentrations of alcohol (2–8%, v/v) and tap water in a two-bottle choice situation [14, 15]. All solutions were presented in the drinking bottles with graduated drinking tubes which were rotated daily to prevent position preference. Fluid intake was measured daily at 10:00 a.m. and body weight was recorded once a week throughout the study.

### Saccharin drinking test

During the 48-h saccharin drinking test, the animals were given the choice between a saccharin solution (0.1% w/v) and tap water. The position of bottles was changed after 24 h to prevent position preference. For each fluid, an intake was measured, averaged across 2 days of the experiment and corrected for body weight of the subject (ml/kg/24 h).

### Food intake

During initial 3 days of ETOH drinking, the animal's food was weighed and 24 h later, the food was weighed again. The amount of food the rats had consumed during the test period was measured. All the pellets spilled into the cage during this time were included into the analysis. This procedure was performed for consecutive days and the mean amount of food eaten for 24 h was calculated.

### Water intake

The amount of water in the drinking bottle of each rat was weighed and 24 h later the water was weighed again and the amount of water the rat had drunk during the test period was measured. This

procedure was performed for 3 consecutive days before the ETOH drinking experiment had started. The mean volume of water consumed for 24 h was calculated.

### Neurochemical analysis

All biochemical procedures were in accordance with our previous studies [26]. The 4 months old rats were killed by decapitation using a guillotine and their brains were quickly removed, placed on a cooled plate, dissected and immediately frozen at  $-80^{\circ}\text{C}$  until the time of neurochemical analysis. Prefrontal cortex, hippocampus and striatum were taken for the analysis. Frozen tissue sections were homogenized in 15 volumes of ice-cold 0.05 M perchloric acid with an internal standard added. Homogenates were centrifuged at  $15\,000 \times g$  and filtered through 0.22-  $\mu\text{m}$  membranes (Millipore, Milford, MA). Contents of 5-HT, 5-HIAA and noradrenaline (NE) were measured using a liquid chromatography with electrochemical detection HPLC system (Shimadzu, Japan). The HPLC system consisted of a Shimadzu LC-9A pump, with a programmable flow rate, equipped with a 20- l injection loop (Rheodyne, CA). Separation of monoamines and their metabolites was carried out on a Nucleosil 7C-1B column  $250 \times 4$  mm (Macherey-Nagel, Germany) thermostated at  $32^{\circ}\text{C}$  in a Shimadzu CTO-6A column oven. An electrochemical detector (Shimadzu L-ECD-6A) was set at + 0.8 V potential versus calomel reference electrode. The mobile phase was citric acid (7.5 g/l),  $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$  (5 g/l), EDTA  $\text{Na}_2 \times 2\text{H}_2\text{O}$  (10 mg/l), octanesulphonic acid (180 mg/l), and methanol (12.5% v/v). The flow was programmed from 1.0 to 1.2 ml/min over 18 min for each analytical run. The mobile phase was continuously degassed with helium. Integration of the chromatograms was performed with a Shimadzu C-R4AX Chromatopac-computing integrator. Dihydroxybenzylamine (DHBA) was used as an internal standard.

### Statistical analysis

All statistical analyses were performed using Statistica<sup>®</sup> software package. A three-way analysis of variance for repeated measures, followed by a multiple comparisons with the Newman-Keuls test was used to determine statistically significant differences when comparing ETOH drinking, alcohol preference and total fluid intake (lesion  $\times$  sex  $\times$  concentration). A two-way analysis of variance,

followed by a multiple comparisons with the Newman-Keuls test was performed to determine statistically significant differences when comparing levels of 5-HT and NE among the groups (lesion  $\times$  sex). Food, water and saccharin intakes were also compared using a two-way analysis of variance and the Newman-Keuls test when appropriate. Data were shown as means  $\pm$  SEM;  $p < 0.05$  was considered as statistically significant. Daily fluid consumption data (ml) for water, alcohol and the saccharin solution were converted into: intake of the solution (g/kg for ETOH, water and total fluid intake, and ml/kg for saccharin solutions), and preference for the alcohol and saccharin expressed as a percentage of total daily fluid consumed (%). For each solution, the mean consumption was used for statistical analysis. Daily food intake was presented as a mean from 3 consecutive days (g/kg).

## RESULTS

Table 1 presents the effect of early postnatal 5,7-DHT and DMI treatment on the concentrations of 5-HT and NE in the hippocampus, striatum and prefrontal cortex of adult 4-month-old rats. The 5-HT contents were significantly reduced in all structures tested, in male as well as female rats. A significant lesion effect was observed in the prefrontal cortex  $F(1, 20) = 176.6$ ,  $p < 0.001$ , hippocampus  $F(1, 20) = 105.7$ ,  $p < 0.001$  and striatum  $F(1, 20) = 104.2$ ,  $p < 0.001$ . Further statistical analysis (post hoc Newman Keul's test) indicated that 5-HT level in animals subjected to 5,7-DHT differed significantly from sham-lesioned animals, in male and female groups ( $p < 0.01$ ). On contrary, the *icv* injection of 5,7-DHT did not induce a noradrenergic depletion. Two way ANOVA did not reveal any significant main effect of lesion [prefrontal cortex  $F(1, 20) = 2.0$ , ns; hippocampus  $F(1, 20) = 0.5$ , ns; striatum  $F(1, 20) = 2.2$ , ns],

sex [prefrontal cortex  $F(1, 20) = 1.3$ , ns; hippocampus  $F(1, 20) = 4.9$ ,  $p < 0.05$ , ns post hoc; striatum  $F(1, 20) = 0.4$ , ns] or lesion  $\times$  sex interaction [prefrontal cortex  $F(1, 20) = 1.5$ , ns; hippocampus  $F(1, 20) = 0.9$ , ns; striatum  $F(1, 20) = 2.2$ , ns]. Figures 1, 2 and 3 present the ETOH intake, total fluid intake and ETOH preference. Analysis of the ETOH drinking data in a three-way ANOVA revealed a significant main effects of lesion:  $F(1, 20) = 5.7$ ,  $p < 0.05$ , sex:  $F(1, 20) = 5.1$ ,  $p < 0.05$ , ETOH concentration:  $F(6, 120) = 34.8$ ,  $p < 0.001$ , lesion  $\times$  concentration interaction:  $F(6, 120) = 5.1$ ,  $p < 0.01$  and

Table 1. Monoamine concentrations (ng/g of tissue) in 5,7-DHT- and sham-lesioned male rats. Means  $\pm$  SEM of 6 separate samples per group are presented. \*\*  $p < 0.01$  vs respective controls, n.s. – not significant

Brain structure/ monoamine and group	Serotonin		Noradrenaline	
	Sham operated	5,7-DHT lesioned	Sham operated	5,7-DHT lesioned
MALE RATS				
Prefrontal cortex	249 $\pm$ 21	43 $\pm$ 13**	179 $\pm$ 4	181 $\pm$ 10 n.s.
Hippocampus	190 $\pm$ 24	36 $\pm$ 9**	227 $\pm$ 19	199 $\pm$ 24 n.s.
Striatum	260 $\pm$ 13	73 $\pm$ 37**	126 $\pm$ 5	106 $\pm$ 16 n.s.
FEMALE RATS				
Prefrontal cortex	236 $\pm$ 13	48 $\pm$ 12**	150 $\pm$ 14	182 $\pm$ 17 n.s.
Hippocampus	166 $\pm$ 11	24 $\pm$ 5**	249 $\pm$ 3	253 $\pm$ 15 n.s.
Striatum	228 $\pm$ 18	23 $\pm$ 10**	116 $\pm$ 10	102 $\pm$ 13 n.s.

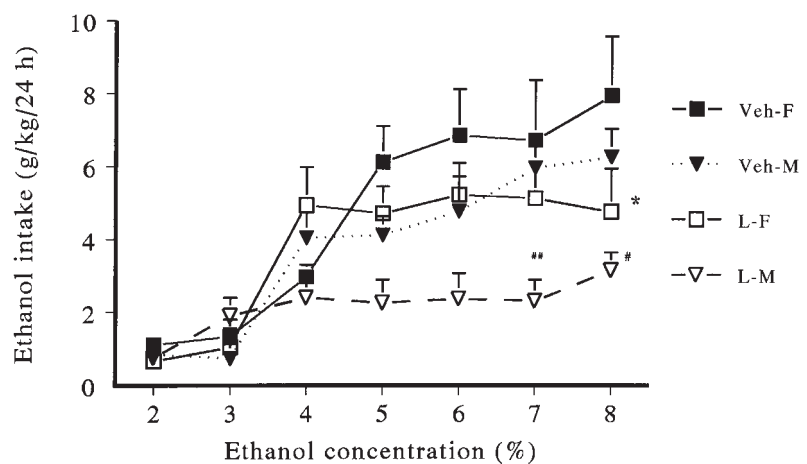


Fig. 1. Effects of a neonatal 5,7-DHT treatment on subsequent voluntary ethanol consumption in rats in the two bottle choice test. Each point represents the mean daily absolute alcohol intake in g/kg. Means ( $\pm$  SEM) from 6 rats are given. Veh-F and Veh-M denote vehicle-treated female and male rats; L-F and L-M denote 5,7-DHT-lesioned female and male rats. \*  $p < 0.05$  vs respective Veh-F, #  $p < 0.05$ , ##  $p < 0.01$  vs respective Veh-M

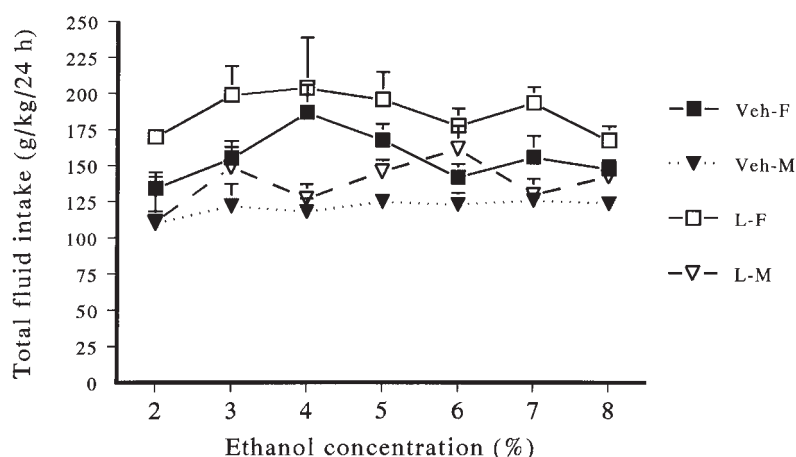


Fig. 2. Effects of a neonatal 5,7-DHT treatment on subsequent total fluid intake in rats in the two bottle choice test. Each point represents the mean daily fluid intake in g/kg. Means ( $\pm$  SEM) from 6 rats are given. For other explanations see Fig. 1

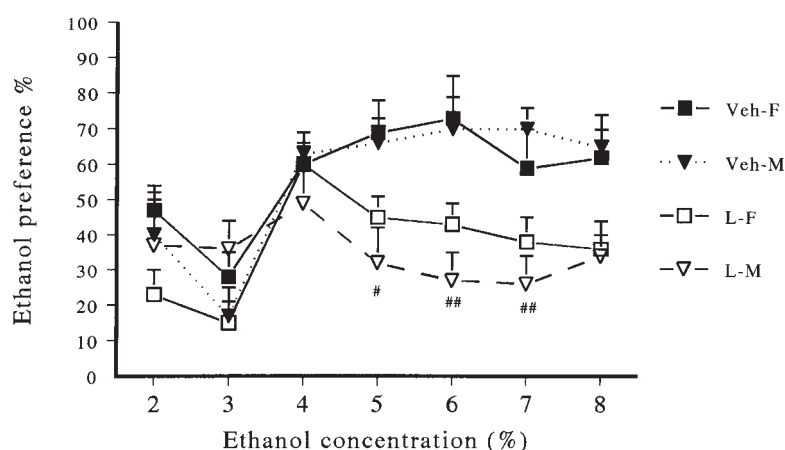


Fig. 3. Effects of a neonatal 5,7-DHT treatment on subsequent ethanol preference in rats in the two bottle choice test. Each point represents the mean daily preference in %. Means ( $\pm$  SEM) from 6 rats are given. For other explanations see Fig. 1

sex  $\times$  concentration interaction:  $F(6, 120) = 2.6, p < 0.05$ . Further statistical analysis indicated that sham female rats consumed significantly more 8% ETOH than lesioned females ( $p < 0.05$ ). Sham male rats consumed significantly more 7% and 8% ETOH than lesioned males ( $p < 0.01$  and  $p < 0.05$ , respectively). Lesioned females drank significantly more 4% ETOH than 3% ( $p < 0.01$ ), sham females drank significantly more 5% ETOH than 4% ( $p < 0.05$ ) and sham males drank significantly more 4% ETOH than 3% ( $p < 0.05$ ) (Fig. 1).

Analysis of the total fluid intake data in a three-way ANOVA revealed a significant main effect of lesion:  $F(1, 20) = 7.0, p < 0.05$ , sex:  $F(1, 20) = 20.8, p < 0.001$  and ETOH concentration:  $F(6, 120) = 2.5, p < 0.05$ . Further statistical analysis showed no significant difference between sham and lesioned females as well as between sham and lesioned males (Fig. 2).

Analysis of the alcohol preference data in a three-way ANOVA revealed a significant main effect of lesion:  $F(1, 20) = 12.7, p < 0.01$ , ETOH concentration:  $F(6, 120) = 13.1, p < 0.001$ , lesion  $\times$  concentration interaction:  $F(6, 120) = 5.2, p < 0.001$  and lesion  $\times$  sex  $\times$  concentration interaction:  $F(6, 120) = 2.4, p < 0.05$ . Further statistical analysis indicated that lesioned male rats pre-

Table 2. Water and saccharin drinking, food consumption and weight in 5,7-DHT- and sham-lesioned male rats. Means  $\pm$  SEM of 6 animals per group are presented. \*\*  $p < 0.01$  vs respective controls, n.s. – not significant

Parameter	Sham-operated males	5,7-DHT-lesioned males	Sham-operated females	5,7-DHT-lesioned females
Water drinking (ml/kg/24 h)	69.5 $\pm$ 3.1	65.2 $\pm$ 6.0	74.5 $\pm$ 3.6 n.s.	63.3 $\pm$ 7.1 n.s.
Saccharin drinking (ml/kg/24 h)	140.0 $\pm$ 19.7	142.2 $\pm$ 18.9	149.7 $\pm$ 6.8 n.s.	125.8 $\pm$ 11.1 n.s.
Saccharin preference (%)	79.0 $\pm$ 3.4	80.3 $\pm$ 2.7	82.2 $\pm$ 1.7 n.s.	79.0 $\pm$ 3.9 n.s.
Food consumption (g/kg/24 h)	52.1 $\pm$ 1.3	48.8 $\pm$ 1.1	49.1 $\pm$ 1.9 n.s.	50.1 $\pm$ 1.5 n.s.
Body weight (g)	516.0 $\pm$ 11.0	455 $\pm$ 13**	333.0 $\pm$ 5.0 n.s.	307.0 $\pm$ 6.0 n.s.

ferred significantly less 5%, 6% and 7% ETOH than sham male rats ( $p < 0.01$ ,  $p < 0.05$  and  $p < 0.05$ , respectively) (Fig. 3).

All animals drank large quantities of the saccharin solution during the continuous access phase (Tab. 2). Analysis of the data with two-way ANOVA did not reveal any significant main effect. No significant interaction was seen as regards lesion  $\times$  sex. This indicates that lesions had no effect on the magnitude of saccharin drinking and preference.

With respect to water and food intake, two-way ANOVA did not show any significant main effect and no significant interaction as regards lesion  $\times$  sex (Tab. 2). Comparison of body weights indicated a significant main effect of lesion:  $F(1, 20) = 21.4$ ,  $p < 0.01$  and sex:  $F(1, 20) = 309.4$ ,  $p < 0.01$  (Tab. 2). A post hoc Newman-Keuls test showed that lesioned male rats were significantly smaller than sham lesioned male rats ( $p < 0.01$ ). Similar trend was observed for female rats ( $p = 0.062$ ).

## DISCUSSION

The present experiments were performed on the premise that investigation of voluntary ETOH intake in adult rats, neonatally treated with 5,7-DHT, might help to better understand the neurobiological aspects of ETOH dependence. It was intended to provide a comprehensive model for the further analysis of behavioral changes associated with ETOH dependence.

As shown in neurochemical analysis, neonatal 5,7-DHT treatment was associated with a marked reduction of striatal, hippocampal and cortical content of 5-HT. This indicates that the neurotoxic effect was stable in course of time, thus suggesting that no regeneration of serotonergic neurons took place. Pretreatment with DMI effectively protected catecholaminergic neurons from toxic effects of 5,7-DHT, as evidenced by the lack of NE depletion. These biochemical results are in concert with findings of other authors, reporting the permanent loss of up to 90% of brain 5-HT content after the intracisternal administration of 5,7-DHT [2, 3, 16, 19, 22, 27].

In contrast to the results obtained in adult rats treated with 5,7-DHT [12, 17], early postnatal neurotoxin-induced lesions resulted in a decrease in ETOH consumption and preference. Thus, sham-lesioned female rats consumed significantly more 8% ETOH than lesioned females. Sham-lesioned

male rats consumed significantly more 7% and 8% ETOH and displayed higher preference for 5%, 6% and 7% ETOH than lesioned males. Male and female rats displayed differential responses to increasing concentrations of ETOH; that is, it has been shown that males are more vulnerable to ETOH than females. The mechanisms responsible for this phenomenon remain to be explained. In Cloninger's original formulation of type II alcoholism, he proposed that this early-onset alcohol abuse with impaired social functioning and excessive aggression is prevalent among men [11]. Many of the characteristics associated with type II alcohol dependence have been identified with 5-HT dysfunction, for example with abnormalities in 5-HT transmission [11].

The present results suggest that early postnatal 5-HT depletion decreases predisposition for alcohol consumption. Our finding is contradictory to the papers demonstrating that animals with reduced central 5-HT neurotransmission enhance their consumption of ETOH [9, 11, 12, 17]. However, all the studies reporting increased ETOH intake in 5-HT-lesioned rats were performed after administration of 5,7-DHT in their adult life. In the present study 5,7-DHT lesion was conducted during a critical period of postnatal development, where traumatic life events seem to evoke persistent changes, which occur even in the adulthood [see e.g. 18]. Such event, like intracisternal administration of 5,7-DHT is imperative for experiments where the permanent destruction of virtually entire serotonergic system is desired, but may also have profound effects on brain development. Such event, may also induce adaptative changes in other systems in the brain. Our result is also in opposition to the finding published by Kiianmaa and Attila [13], where newborn rats pretreated with DMI were treated with 5,7-DHT subcutaneously. This treatment resulted in a 53% decrease in 5-HT concentrations in the cerebral cortex accompanied with its 60% increase in the pons medulla when determined in adult rats. Furthermore, the 5-HT content in the midbrain remained unchanged. Thus, it seems that the degree of destruction of the 5-HT nerve terminals was not sufficient to disrupt the interaction between 5-HT system and ETOH intake. In the same paper, no difference was found between the voluntary 10% ETOH intake of the 5,7-DHT-treated and control animals when measured at the age of 3 months. One may suppose that this negative result was due

to the moderate and insufficient lesion produced by *sc* administration of 5,7-DHT. More extensive damage to the central 5-HT neurons caused by *icv* injections of neurotoxin was apparently seen in our animals.

The results of the present study indicate that the weight of the control sham-lesioned male rats was significantly higher than that of the rats in the 5,7-DHT-treated groups, when measured at the end of the experiment. The same trend was observed in the female rats. On the other hand, the daily food intake in control rats and in the 5,7-DHT-treated rats was similar during the initial 3 days of ETOH drinking. Our result is in line with the study of Breese et al. [2] showing that the treatment with 5,7-DHT at 3 days of age produced a weight deficit during the course of development, which persisted in rats at 50 days of age. Similar weight loss was observed after 5-HT depletion with para-chlorophenylalanine [23]. Interestingly, all these findings do not support the view that brain 5-HT depletion results in hyperphagia and increased body weight [24].

Taste factors may play an important role in the development of consumatory patterns. There are several lines of evidence suggesting a relationship between the intake of and preferences for flavored solutions and ETOH [8, 21], thus indicating that intake of sweetened solutions may be a suitable predictor for subsequent ETOH intake. It was recently shown in our laboratory that parameters of saccharin drinking behavior were highly correlated with the initial acceptance of low ETOH concentrations (2–6%) in Wistar rats. However, this relationship disappeared during further weeks of higher concentrations ETOH presentation [14]. The hypothesis of a general association between the intake of ETOH and sweetened solutions was not supported by the findings of the present experiment, where all groups of rats presented similar high preference for 0.1% saccharin solution. The results of the recent study with three different strains of rats have also indicated that saccharin consumption does not predict long-term alcohol self-administration [7].

On the basis of the present preliminary study we were able to observe long-lasting biochemical changes in consequence of neonatally administered 5,7-DHT. We believe that this model may be useful in further research on the relationship between the central 5-HT system and alcohol drinking and dependence.

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