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Genetic Differences in Ethanol Drinking of the Rat Following Injection of 6-OHDA, 5,6-DHT or 5,7-DHT Into the Cerebral Ventricles[1]

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MELCHIOR, C. L. AND R. D. MYERS. *Genetic differences in ethanol drinking of the rat following injection of 6-OHDA, 5,6-DHT or 5,7-DHT into the cerebral ventricles.* PHARMAC. BIOCHEM. BEHAV. 5(1) 63–72, 1976. – The preference characteristics for ethanol of four different strains of rats were determined in a two-choice situation by offering water and ethanol in a concentration which was increased from 3 to 30% over a 12-day test sequence. Using stereotaxic procedures, 50 μg 5,6-dihydroxytryptamine (5,6-DHT), 200 μg 6-hydroxydopamine (6-OHDA) or 100 μg 5,7-dihydroxytryptamine (5,7-DHT) were then injected acutely into the lateral cerebral ventricle in a 20 μl volume. Rats of the Sprague-Dawley strain increased their ethanol preference following the lesioning of the serotonergic system by 5,6-DHT, whereas similar destruction of catecholaminergic neurons by 6-OHDA markedly suppressed ethanol intake; Long-Evans rats displayed a similar trend in ethanol drinking patterns. However, animals of the Holtzman strain manifested the increased drinking after 5,6-DHT, but showed no suppression of drinking following 6-OHDA. The preference of rats of the Wistar strain was unaffected by 5,6-DHT but attenuated by 6-OHDA. 5,7-DHT had little or no effect on ethanol consumption in any of these strains. These findings thus suggest that genetic factors are an important determinant in an animal's response to a drug that affects 5-HT or NE systems in the brain, particularly when ethanol selection is investigated.

Ethanol drinking Genetic characteristics 5,7-Dihydroxytryptamine Strain differences
5,6-Dihydroxytryptamine Serotonin Ethanol preference 6-Hydroxydopamine Catecholamines

RECENTLY we reported that the reduction of cerebral serotonin by a direct injection of 5,6-dihydroxytryptamine (5,6-DHT) can enhance the rat's selection of different solutions of ethyl alcohol [25]. Conversely, the reduction of cerebral catecholamines by 6-hydroxydopamine (6-OHDA), administered in the same manner, exerts an opposite effect and reduces the intake of ethanol. Although the cerebral site(s) at which the two neurotoxins act to mediate these changes is unknown, these findings confirm the idea [22] that a functional imbalance in the activity of the monoamines in the CNS, or some aspect of their metabolism, serves to alter the pattern of ethanol preference.

In view of several inconsistent findings with respect to the characteristics of ethanol drinking following the administration of other substances [27], the possibility exists that a genetic factor influences the metabolic response to ethanol during treatment with a drug [18,29]. Recently, Deitrich and his colleagues [12, 13, 14] have demonstrated that changes in the hepatic enzyme system associated with phenobarbital treatment does depend on the strain of rat. Further, Myers and Melchior [26] have shown that L-tryptophan administered in the rat's diet can alter its subsequent intake of ethanol, but that the magnitude of this alteration depends entirely upon the particular strain tested.

Therefore, the present study was undertaken to determine whether a genetic factor could also influence the animal's response to a neurotoxin injected intracerebrally. In these experiments, rats of four strains were matched according to their pattern of selection of 12 concentrations of ethanol. Then an acute injection into the lateral cerebral ventricle of either 6-OHDA or 5,6-DHT was made. In three strains, the effect of 5,7-dihydroxytryptamine (5,7-DHT) given similarly was likewise examined.

METHOD

Male rats of the Wistar, Holtzman, Long-Evans and

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Sprague-Dawley strains, of five months of age or older, were kept in individual cages and maintained on a 12 hr light-dark cycle at a temperature which ranged from 21 to 23°C. Powdered or pelleted Wayne Lab Blox was available ad lib. throughout the experiment. Both food intake and the body weight of each rat were measured every 48 hr.

Ethanol Preference

Each animal's preference-aversion curve for ethanol and water was determined by means of the three-bottle, random rotation testing procedure of Myers and Holman [24]. In this method, the animal's cage is fitted with three 100 ml Kimax drinking tubes: one contains water; another a volume/volume solution of ethanol, prepared with deionized water and 95% ethanol, which was increased in concentration on each of 12 successive days from 3 to 30%; and a third dummy tube that was empty. By rotating the three bottles on each day of the 12-day test sequence according to a predetermined random schedule, the development of a position habit is prevented. Fluid intakes were recorded and the tubes containing water and the freshly prepared solution of ethanol were changed at the same time on every day.

When the first 12-day ethanol-water test sequence was completed, animals within each strain were matched according to their average intake of ethanol in terms of g/kg per day. Subsequently, the rats were divided into four equal groups having similar drinking patterns.

Intraventricular Injection

Each rat was anesthetized with ether, and a craniotomy hole was drilled in the calvarium above either the right or left lateral ventricle. The stereotaxic coordinates employed for the intraventricular injection were in the DeGroot orientation at: AP, 4.8; Lat, 2.5; and Hor, 3.0. After the tip of an injection needle was lowered to the appropriate depth, the patency of the ventricle was verified as 20 μl of the given drug or control solution were permitted to flow in by gravity over an interval of 15 to 30 sec. The specific drugs given were as follows: (1) 200 μg of 6-OHDA; (2) 50 μg of 5,6-DHT; or (3) 100 μg of 5,7-DHT. These doses, expressed as the amount of free base, are known to destroy a substantial number of particular monoaminergic neurons after an intraventricular injection [5, 6, 7, 8, 9, 10, 16, 33, 34]. The vehicle which also served as the control solution consisted of an artificial CSF for the rat comprised of 5-ions, Na^+, Ca^{++} K^+, Mg^{++} and Cl^-, [21] to which 0.1 mg/ml ascorbic acid was added.

Testing Procedure

After ten days had elapsed following the injection, each animal was retested for its ethanol preference on the 12-day sequence, referred to hereafter as Post 1. At an interval of 60 days following the injection, the same preference test sequence was repeated, referred to hereafter as Post 2. To test for the differences in the ethanol intakes of the groups of each strain in terms of g/kg, analyses of variance and the Dunnet test [36] were carried out. Alterations in the proportion of ethanol selected over water were examined statistically [31] with the Wilcoxon matched pairs signed-ranks test.

Six additional animals of the Long-Evans strain were given intraperitoneal injections of 50 mg/kg of pargyline and 25 mg/kg of desmethylimipramine (DMI) followed 30 min later by an intraventricular injection of 200 μg of 6-OHDA. This procedure lesions catecholamine containing neurons but purportedly destroys a larger percentage of dopamine (DA) than norepinephrine (NE) containing neurons [32]. Since only two of the animals survived this procedure, no other animals of any of the other strains were subjected to it.

Biochemical Validation of Neurotoxin Injection

In order to validate the intraventricular injections themselves in terms of an estimate of the efficacy of the neurotoxins given in the lateral cerebral ventricle, three groups of Sprague-Dawley rats were injected intraventricularly with 200 μg of 6-OHDA (N = 5), 5,6-DHT (N = 5) or the 5-ion control solution (N = 5). The animals were sacrificed 8 to 10 days later and spectrophotofluorometric assays were performed to determine the content of norepinephrine or dopamine as well as serotonin (5-HT) in the striatum, hypothalamus, and mesencephalon.

Each rat was decapitated, the overlying calvarium turned out by rongeurs and the brain extracted immediately by spatula onto an iced petri dish. The boundaries of the scalpel cuts used to delineate the hypothalamic and mesencephalic samples were the same as in the method employed by Myers *et al.* [28]. The striatal pieces were dissected away from the portion of the brain designated as anterior in this same method. Then each bilateral sample of tissue was transferred at once to an individual test tube containing 3.3 ml of iced acidified n-butanol and weighed. The entire procedure usually required less than 400 sec.

After homogenization of the tissue, 3.0 ml of the supernatant n-butanol layer were shaken with a mixture of 4.5 ml heptane and 1.0 ml water and then centrifuged. One ml of the lower aqueous phase was added to sodium acetate and alumina to separate the indoleamines and catecholamines. Following shaking and centrifugation, 2 ml of the supernatant was placed on a Dowex 50 column previously buffered to pH 6.5. 5-HT was eluted from the column with 0.3 ml of 0.1 N sodium hydroxide. The eluate was collected into 0.5 ml sodium acetate buffer, pH 4.0. The fluorescence due to serotonin was measured on an Aminco Bowman spectrophotofluorometer with settings of 285/340 nm.

Following a procedure [19] modified after Chang [11], the catecholamines were eluted from the sedimented alumina by shaking with 1.1 ml of 0.5 M sodium phosphate buffer, pH 6.0. After centrifugation, a 1.0 ml aliquot was removed and oxidized with 0.05 ml of 0.1 N iodine reagent. Precisely 2 min later, 0.1 ml of alkaline sulfite solution was added. Following another 2 min period, 0.1 ml 5 N acetic acid was added. The solution was then heated for 2 min, cooled and the fluorescence due to NE measured at 380/470 nm. The fluorescence due to DA was read at 320/380 nm after a 4 min period of heating.

The differential action of the intraventricular injections of 5,6-DHT and 6-OHDA on the content of serotonin, norepinephrine and dopamine is presented in Table 1. In essence, the data do provide a validation of the injection procedure, since 6-OHDA caused the greatest depletion in catecholamine content particularly in the hypothalamus and mesencephalon. Similarly, 5,6-DHT injected intraventricularly caused the most intense effect on the serotonin content in each region examined. These findings confirm those obtained by other investigators [5, 6, 7, 8. 9, 10, 16, 33, 34] but no extrapolation to strain of rat is intended.

TABLE 1

THE LEVEL OF NOREPINEPHRINE, DOPAMINE, AND SEROTONIN IN $\mu g/g \pm$ SEM IN THE STRIATUM, HYPOTHALAMUS,AND MESENCEPHALON OF RATS INJECTED WITH 6-OHDA, 5,6-DHT OR A CONTROL SOLUTION CONTAINING 5-IONS

Monoamines	Injection	Striatum	Hypothalamus	Mesencephalon
Serotonin	6-OHDA	1.85 ± .65	1.25 ± .28	1.36 ± .45
	5,6-DHT	.49 ± .16	.43 ± .10	.50 ± .08
	Control	1.04 ± .22	.52 ± .13	.61 ± .18
Norepinephrine	6-OHDA	.56 ± .07	.33 ± .05	.23 ± .04
	5,6-DHT	.99 ± .21	.74 ± .10	.37 ± .06
	Control	.60 ± .15	1.04 ± .17	.71 ± .25
Dopamine	6-OHDA	2.07 ± .30	.04 ± .00	.24 ± .14
	5,6-DHT	4.91 ± .62	.29 ± .07	.13 ± .10
	Control	5.64 ± .88	.31 ± .09	.29 ± .07

RESULTS

An overall analysis of the fluid intakes showed that the critical factor which influences the magnitude of the effect of a neurotoxin in altering ethanol drinking is the genetic characteristics of the rat. Therefore, the results are presented here according to the strain tested.

Basic differences in ethanol preference in terms of g/kg intakes were found for each strain. The results of the three ethanol test sequences for the four control groups are illustrated in Fig. 1. In every case, the well-known acclimation effect [35] is evident in that the selection of ethanol increased during the second (Post 1) test sequence begun 10 days after the acute injection of the control 5-ion solution. However, the ethanol intakes declined 60 days later during the third test sequence (Post 2) in each group of animals except those of the Sprague-Dawley strain.

Absolute Intakes of Ethanol

As described previously [25], 5,6-DHT injected intraventricularly causes the Sprague-Dawley rat to increase its consumption of ethanol. Conversely, 6-OHDA given similarly suppresses the overall intake of this fluid. As shown in Figs. 2 and 3, this altered pattern of ethanol ingestion persists for two months after the acute injection of the two neurotoxins into the cerebral ventricles ($p<0.01$).

Although Fig. 2 shows that the animals of the Long-Evans strain increased their intake of ethanol following intraventricular 5,6-DHT ($p<0.01$, Dunnett test),their g/kg intake returned essentially to the pre-5,6-DHT level during the final sequence. Furthermore, the amount of ethanol ingested by the Long-Evans rats following the injection of 5,6-DHT did not differ statistically from that consumed by the control animals of the same strain, which were injected with the 5-ion solution, during either the first or second post-injection sequence. Likewise, 6-OHDA did not alter the g/kg intake of ethanol in the Long-Evans animals (Fig. 3), in that neither a significant increase nor decrease in ethanol selection occurred.

The drinking pattern of the rats of the Holtzman strain was affected differentially by the neurotoxins. An enhanced preference for ethanol occurred after the intraventricular injection of 5,6-DHT. Figure 2 illustrates the significant increase from the pre-injection sequence in g/kg

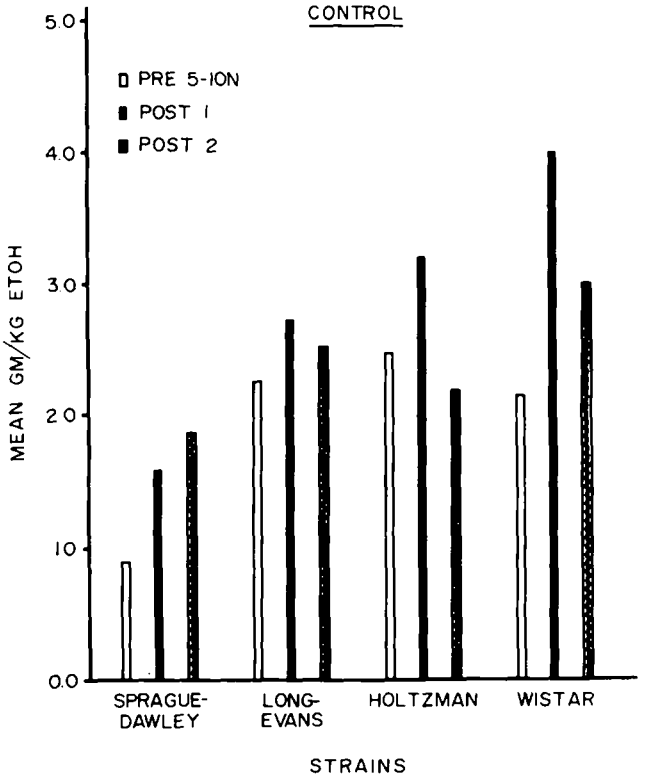

FIG. 1. Mean g of ethanol per kg body weight consumed by the control rats injected with 5-ion solution. The 12-day ethanol test sequences were carried out: (1) just before surgery (Pre-5-ion); (2) beginning 10 days after the acute injection (Post 1) and (3) 60 days after this injection (Post 2).

ethanol for the six animals in this group, $F(2,33) = 8.286$, $p<0.01$ during both of the post-injection sequences ($p<0.01$, Dunnet test). Conversely, the six Holtzman animals given intraventricular 6-OHDA did exhibit the typical acclimation-rise in ethanol intake after the first injection ($p<0.05$, Dunnett test) but during the final test sequence (Post 2), the amount consumed tended toward the pre-6-OHDA level.

As illustrated in Figs. 2 and 3, the rats of the Wistar strain increased their intake of ethanol significantly over

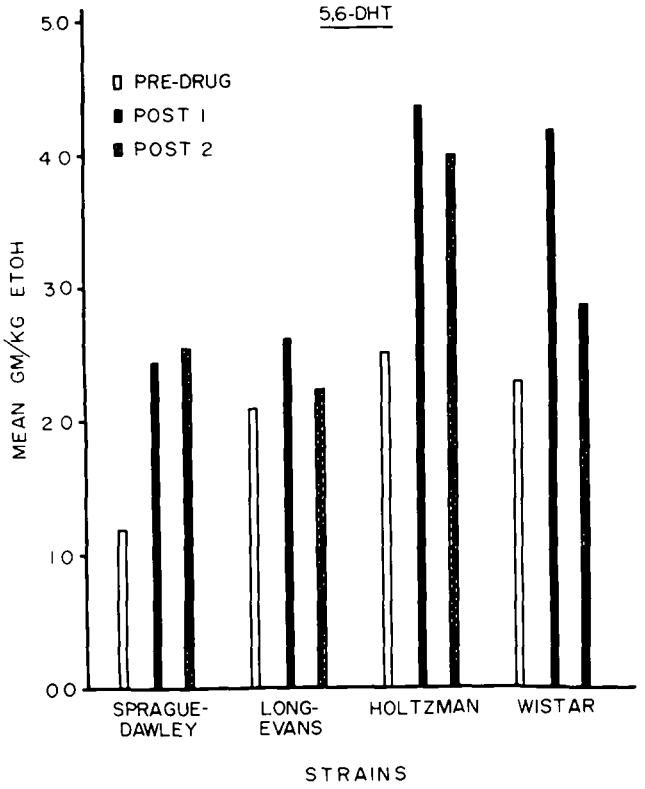

FIG. 2. Mean g of ethanol per kg body weight consumed by the 5,6-DHT injected rats of each strain during 12-day ethanol sequences carried out: (1) just before surgery (Pre-Drug); (2) beginning 10 days after the acute injection of 5,6-DHT (Post 1) and (3) 60 days after this injection (Post 2).

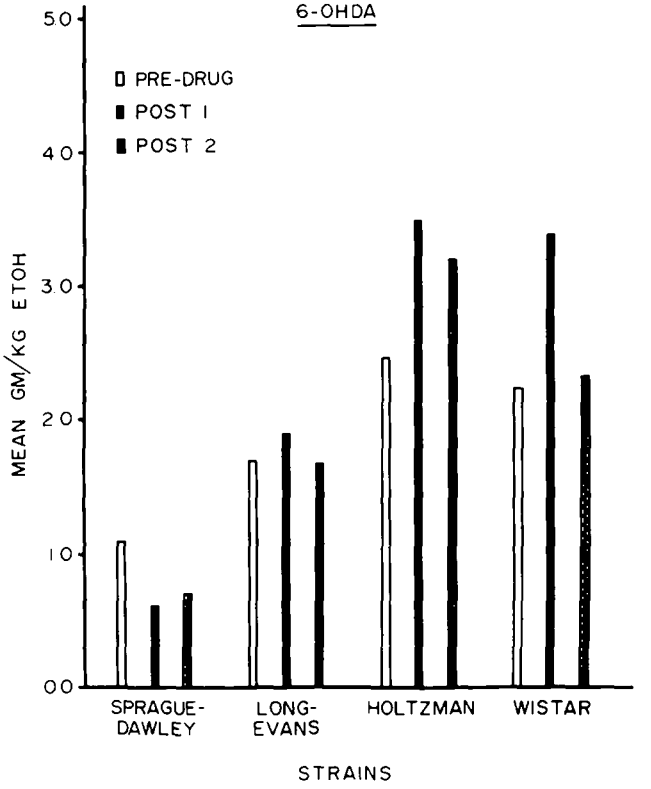

FIG. 3. Mean g of ethanol per kg of body weight consumed by the 6-OHDA injected rats during 12-day ethanol sequences carried out: (1) just before surgery (Pre-Drug); (2) beginning 10 days after the acute injection of 6-OHDA (Post 1) and (3) 60 days after this injection (Post 2).

their own control level during both of the post-injection sequences ($p<0.01$, Dunnett test) except for the animals in the 6-OHDA group (Fig. 3). Their ethanol intake was significantly less than that of the control group during both post-injection test sequences ($p<0.01$, Dunnett test); in addition, the g/kg intake was virtually at the pre-6-OHDA level during the last test sequence (Post 2).

Pattern of Ethanol Concentration Selected

The preference-aversion functions for ethanol were calculated in terms of the proportion of ethanol to water consumed at each of the 12 concentrations of ethanol offered during the first test sequence (Post 1) following the intraventricular injection of 5,6-DHT, 6-OHDA or the 5-ion control solution. Generally, the analysis of this second sequence, at which time an effect of either neurotoxin was ordinarily observed, revealed a concordance with the g/kg intakes of each strain. Figure 4 depicts the enhanced selection of ethanol produced in the Sprague-Dawley strain by the intraventricular injection of 5,6-DHT. On the other hand, the injection of 6-OHDA resulted in a preference-aversion curve that never intersected with that of either the 5-ion or 5,6-DHT injected groups. The mean proportions (Fig. 4, right) calculated for this first post 12-day sequence demonstrate the sharp differences between the three groups.

The rats of the Long-Evans strain exhibited the same sort of trend, in that 5,6-DHT evoked only a weak, and not significant, enhancement of ethanol drinking in the 3 to 30% range of concentrations. A slight but significantly lower ($p<0.01$, Wilcoxon test) intake of ethanol was produced by 6-OHDA; as shown in Fig. 5, the proportion of ethanol consumed by the 6-OHDA group never reached that of either the 5-ion or 5,6-DHT animals. Again, the mean proportions (Fig. 5, right) computed over the 12-day sequence confirm this finding.

Of special interest are the results of the Holtzman and Wistar strains. As shown in Fig. 6, a reversal in the action of the neurotoxin is evident. 6-OHDA given to rats of the Holtzman strain evoked a change in proportional intake that was greater than that of the 5-ion injected group. In fact, the proportion of ethanol consumed was significantly greater for both the 5,6-DHT and 6-OHDA animals than the 5-ion injected group ($p<0.01$, Wilcoxon test). The rats of the Wistar strain exhibited no change whatsoever in ethanol preference as a result of the intraventricular injections of 5,6-DHT or 6-OHDA (See Fig. 7), although the g/kg intake increased due to a loss in body weight (see Fig. 15).

Action of 5,7-DHT Across Strains

In terms of the mean g/kg intake, the intraventricular injection of 5,7-DHT generally had very little effect on the ethanol selection of the Sprague-Dawley and Wistar strains. However, the six animals of the Holtzman strain, as presented in Fig. 8, did increase their ethanol consumption $F(2,33) = 9.961$, $p<0.01$ but only during the Post 1 test sequence ten days after the neurotoxin had been given ($p<0.01$, Dunnett test). During the second post-test interval,

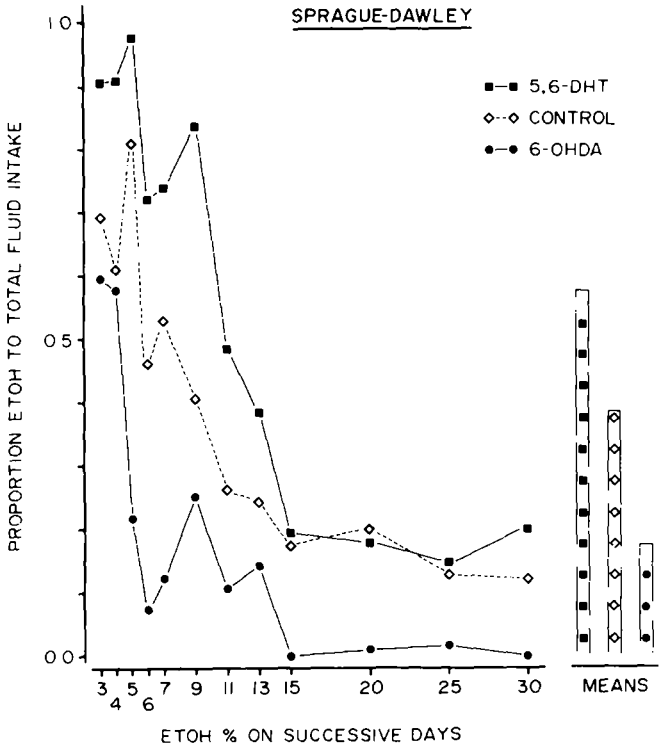

FIG. 4. Average proportion of ethanol to total fluid intake for each percent concentration offered on successive days to rats of the Sprague-Dawley strain. These values were obtained during the first preference sequence 10 days after the acute injection (Post 1) of 5,6-DHT, 6-OHDA or the 5-ion control solution. The mean of each proportion is shown at the right.

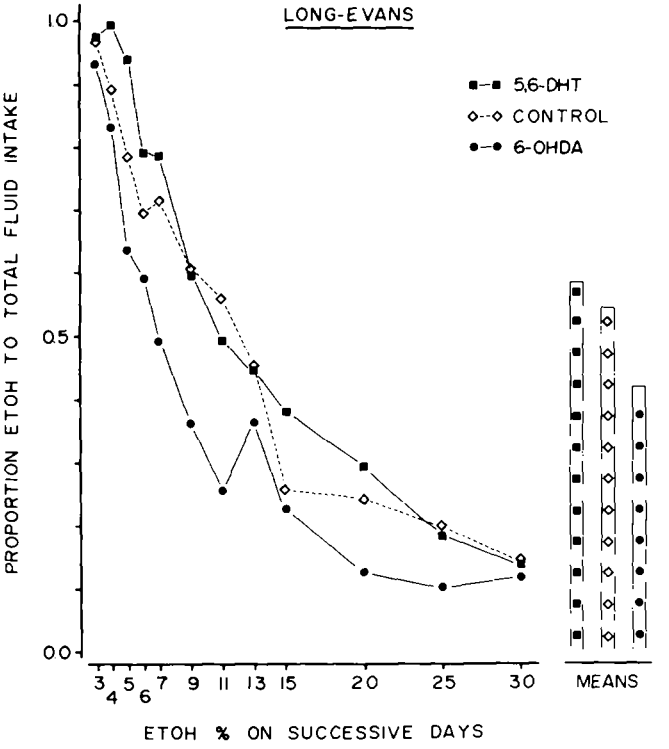

FIG. 5. Average proportion of ethanol to total fluid intake for each percent concentration offered on successive days to rats of the Long-Evans strain. These values were obtained during the first preference sequence 10 days after the acute injection (Post 1) of 5,6-DHT, 6-OHDA or the 5-ion control solution. The mean of each proportion is shown at the right.

60 days after the injections, the amount of ethanol taken by the Holtzman rats returned to that seen in the control group of Holtzman animals. 5,7-DHT enhanced only slightly the ingestion of ethanol in the Sprague-Dawley strain across the 12 concentrations.

In terms of the proportion of ethanol to total fluid intake, the Holtzman rats also exhibited a higher preference during both Post 1 and Post 2 test sequences ($p<0.01$, Wilcoxon test). Similarly, the animals of the Wistar strain also increased their ethanol consumption during each of the two post-injection sequences over the pre-injection test level, as shown in Fig. 9, but this increase was not statistically different from that of the pre-5,7-DHT sequences.

Pargyline-DMI Pretreatment of 6-OHDA Rats

Only two of the six animals of the Long-Evans strain survived the pretreatment with pargyline and desmethylimipramine prior to the acute intraventricular injection of 6-OHDA. Although no consistent alteration in ethanol drinking behavior could be ascertained, the pathological *sequelae* even in these two animals prevents any extrapolation of the action of this drug combination on ethanol selection.

Food Intake and Body Weight

During both of the post-injection ethanol test sequences, the intake of food of the Sprague-Dawley strain of rats remained stable. As shown in Fig. 10, no change in food intake occurred as a result of the injections of the two neurotoxins or the 5-ion control solution. However, during the 12-day preference sequence, 60 days following the intraventricular injections (Post 2), the 5,6-DHT and 6-OHDA injected rats exhibited a slight increase in body weight over the 5-ion control and 5,7-DHT treated animals. These differences are illustrated in Fig. 11.

Although the food intakes of the animals of the Long-Evans strain fluctuated initially, Fig. 12 shows that the magnitude of food consumed was relatively stable during the three 12-day ethanol test sequences. Similarly, the 5,6-DHT, control and 6-OHDA groups, as illustrated in Fig. 13, exhibited a progressive increase in body weight indicative of the typical growth pattern.

The feeding pattern of the rats of the Wistar strain was essentially unaffected by the intraventricular injections. However, as shown in Fig. 14, feeding was suppressed during the initial phases of the second ethanol test sequence after the injection had been given (Post 2). It is possible that this strain of animals required time to become accustomed to the powdered food diet in contrast to the pelletized food on which they were maintained during the interval between the second and third test sequences. Again, the body weight of the groups had declined, as presented in Fig. 15, by the first 12-day sequence after the injections were given, but the magnitude of loss was relatively the same for each group.

In rats of the Holtzman strain, the intake of food increased during the first post-injection sequence with the 5,7-DHT animals showing the greatest enhancement of food

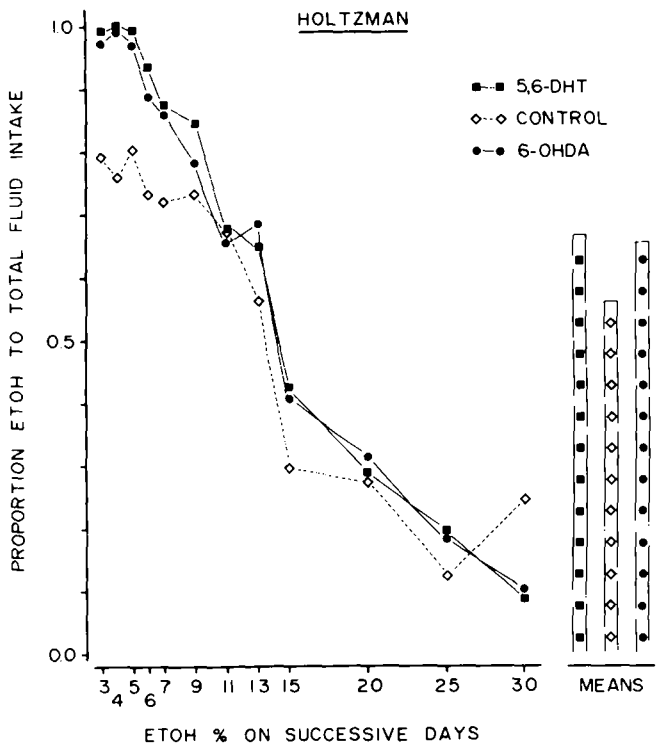

FIG. 6. Average proportion of ethanol to total fluid intake for each percent concentration offered on successive days to rats of the Holtzman strain. These values were obtained during the first preference sequence 10 days after the acute injection (Post 1) of 5,6-DHT, 6-OHDA or the 5-ion control solution. The mean of each proportion is shown at the right.

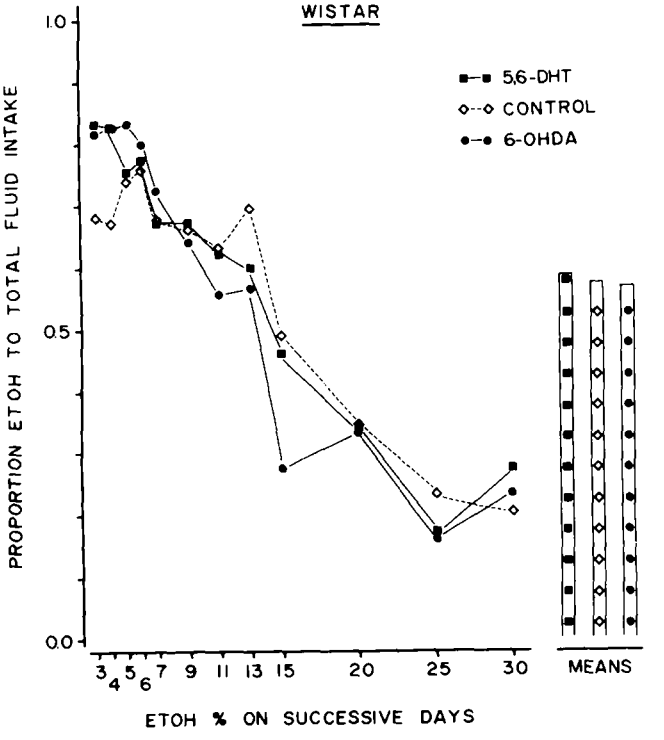

FIG. 7. Average proportion of ethanol to total fluid intake for each percent concentration offered on successive days to rats of the Wistar strain. These values were obtained during the first preference sequence 10 days after the acute injection (Post 1) of 5,6-DHT, 6-OHDA, or the 5-ion control solution. The mean of each proportion is shown at the right.

intake. Although the relative amount of food consumed declined to the pre-drug level during the Post 2 preference sequence, Fig. 16 shows that the 5,7-DHT-treated rats maintained a relatively elevated food intake. Of some interest is the converse effect on body weight which is portrayed in Fig. 17. The weight of the Holtzman strain of rats was most severely affected by the acute injection of 5,7-DHT, and this deficit persisted even 60 days after the drug had been given. Furthermore, those animals of the Holtzman strain which had been given 5,6-DHT and 6-OHDA exhibited a greater immediate weight loss than the control group as reflected in the Post 1 preference sequence. However, 60 days after the injection, these two groups had regained this weight and surpassed the level of the control group.

During the 10-day period following surgery, there was generally an overall decline in the body weight of the rats produced by the intraventricular injections. Table 2 presents the changes in body weight which occurred between the day on which the first ethanol preference sequence began following the intraventricular injection. It is clear that the magnitude of the change in body weight, whether gain or loss, was dependent upon the strain of animal tested. For example, in the animals of the Long-Evans strain, 5,6-DHT caused a relatively minor loss in body weight whereas 6-OHDA and the 5-ion solution did not interfere with the rat's weight.

DISCUSSION

The present results show that the pharmacological response to a neurotoxin is influenced greatly by the genetic attributes of the animal to which the compound has been administered. Because of the general agreement in the literature that 6-OHDA and 5,6-DHT given intraventricularly reduces substantially the content of cerebral catechol- and indoleamines respectively, the role of these endogenous substances in ethanol selection does not seem to be uniform in every strain of rat. In other words, if one accepts that the neurotoxin exerts a potent lesioning and amine-depleting effect, the resultant level of amine and the subsequent magnitude of change may be a more crucial factor to the ethanol drinking pattern of one strain than in another. Our observation with the animals of the Sprague-Dawley strain bears this out in terms of the marked contrast in the effects of intraventricular 6-OHDA and 5,6-DHT observed with the Wistar and Long-Evans animals.

These findings are not surprising in view of several earlier lines of investigation. First, there is a remarkable genetic variation in the synthesis and abundance of the biogenic amines in the brain of the rat and in receptor sensitivity to these compounds [30]. Moreover, if the same strain of rat is raised and maintained under different environmental or dietary conditions, distinct variations are seen in the levels of 5-HT and NE in the whole brain. This variation is manifest also in the catecholamine values of the cerebral hemisphere, diencephalon and midbrain as well as in the values of 5-HT in the midbrain and medulla [20]. Further, when the monoamine oxidase inhibitor, pargyline, is administered systemically, the consequent change in the content of 5-HT and norepinephrine in several areas of the

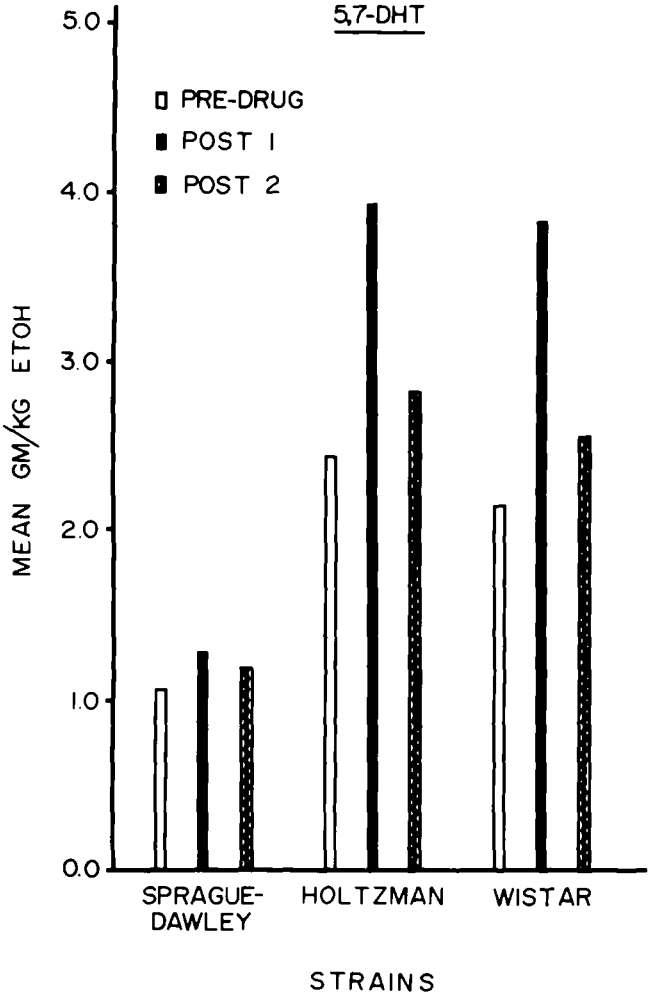

FIG. 8. Mean g of ethanol per kg body weight consumed by the 5,7-DHT injected rats. The 12-day ethanol sequences were carried out: (1) just before surgery (Pre-Drug); (2) beginning 10 days after the acute injection of 5,7-DHT (Post 1) and (3) 60 days after this injection (Post 2).

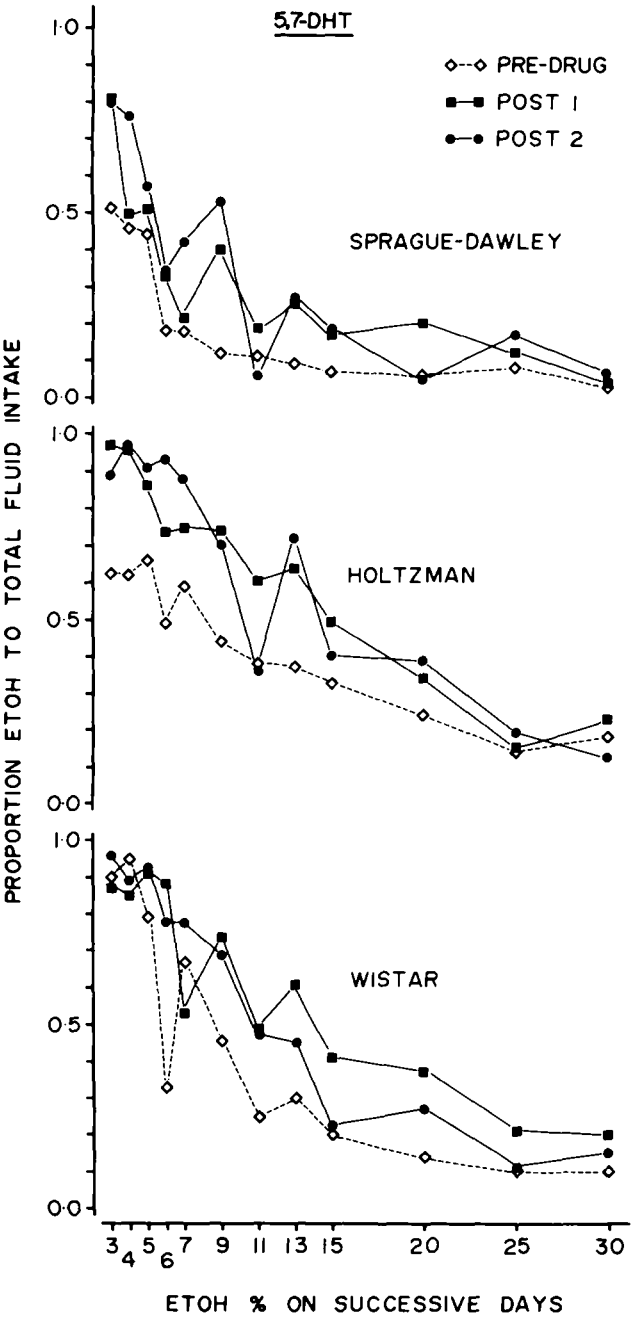

FIG. 9. Average proportion of ethanol to total fluid intake for each percent concentration of ethanol offered on successive days to rats of the Sprague-Dawley, Holtzman and Wistar strains: (1) just before surgery (Pre-Drug); (2) beginning 10 days after injection of 5,7-DHT (Post 1) and (3) 60 days after the injection (Post 2).

brain depends upon the rearing conditions [20]. As reviewed by Barchas *et al.* [4], the differences in enzyme levels and biosynthetic pathways for both the serotonergic and catecholaminergic systems have now been described for different strains of animals.

Second, the characteristics in ethanol preference among inbred strains of mice have been well-documented over the years (see review of Rodgers, [29]). On the basis of experimental evidence showing that upon waking from an anesthetic dose of ethanol, C57BL mice which have a high preference for ethanol, possess a lower ethanol level in the brain and in blood and a higher blood level of acetaldehyde than DBA mice, which have a low preference for ethanol. Lin [17] proposes that there is a difference in brain sensitivity to ethanol across strains. Recently, Goldstein and Kakihana [15] found that mice of the C57BL strain, which exhibit a high preference for ethanol, are more resistant to the convulsive effects of reserpine than mice of other strains which have a low preference for ethanol. Also, mice of the latter strains are more susceptible to convulsions during withdrawal from ethanol than mice of the higher preference C57BL strain [15].

Third, the genetic characteristics of ethanol preference correlate somewhat with the level of monoamines in the brain. In examining strains of rat bred selectively to prefer or reject ethanol, Ahtee and Eriksson [1,2] find that those animals which prefer ethanol over water possess a higher content of 5-HT in the brain. In rats of the same strain, those that select ethanol exhibit a higher concentration of dopamine in the brain, whereas norepinephrine is not significantly different from that seen in the low preference

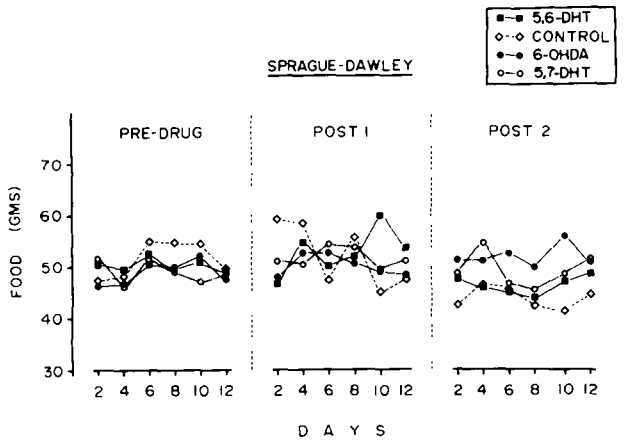

FIG. 10. Mean food intakes for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Sprague-Dawley strain.

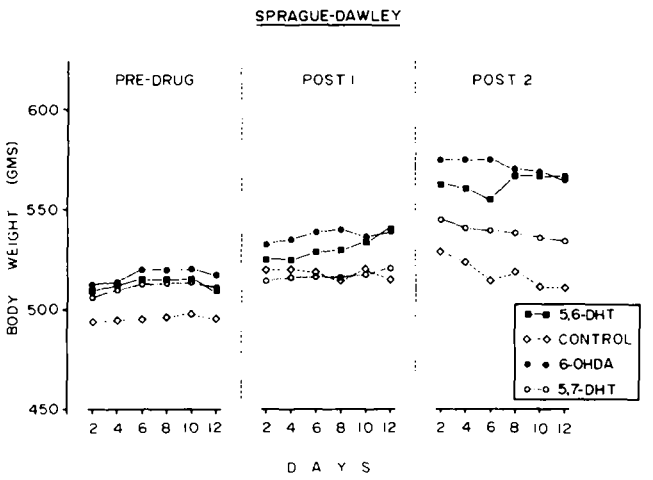

FIG. 11. Mean body weights for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Sprague-Dawley strain.

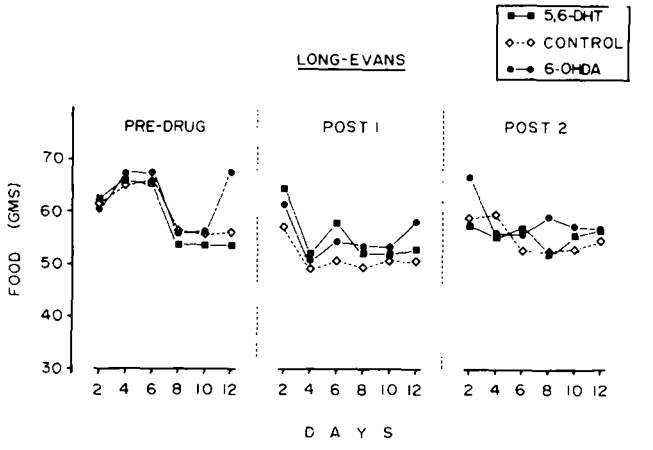

FIG. 12. Mean food intakes for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Long-Evans strain.

TABLE 2

AVERAGE LOSS (-) OR GAIN (+) IN BODY WEIGHT OF FOUR STRAINS OF RAT 10 DAYS FOLLOWING THE ACUTE INJECTION OF 5,6-DHT, 5,7-DHT, 6-OHDA OR 5-ION SOLUTION INTO THE CEREBRAL VENTRICLE

Strain	Injection	Body Weight Day of Surgery	Start of Post 1	Change in g Body Weight
Sprague-Dawley	5,6-DHT	545.6	525.8	−19.8
	Control	534.6	520.4	−14.2
	6-OHDA	557.0	533.2	−23.8
	5,7-DHT	553.0	514.2	−38.8
Long-Evans	5,6-DHT	541.4	532.4	− 9.0
	Control	492.0	508.2	+16.2
	6-OHDA	497.2	515.0	+17.8
Wistar	5,6-DHT	571.7	526.5	−45.2
	Control	540.7	525.7	− 15.0
	6-OHDA	596.2	560.8	−35.4
	5,7-DHT	615.8	587.4	−28.4
Holtzman	5,6-DHT	509.0	465.3	−43.7
	Control	504.5	502.5	− 2.0
	6-OHDA	495.3	456.0	−39.3
	5,7-DHT	479.2	432.7	−46.5

strain [3]. Interestingly, when the amount of tryptophan is artificially increased in the diet of the rat, ethanol drinking is enhanced mainly in rats of the Royal Victoria strain; this amino acid fails to alter the drinking pattern of rats of the Sprague-Dawley strain. Thus, one strain can be affected by the central but not peripheral administration of an amine-altering compound; another strain of rat may be affected by the peripheral but not central administration of the compound. Diametrically opposing effects of these substances may occur in still other strains [27].

These findings could account for some of the apparent discrepancies that are evident in the literature on pCPA and ethanol drinking. For example, in 11 of 12 investigations in which the action of pCPA on ethanol drinking has been

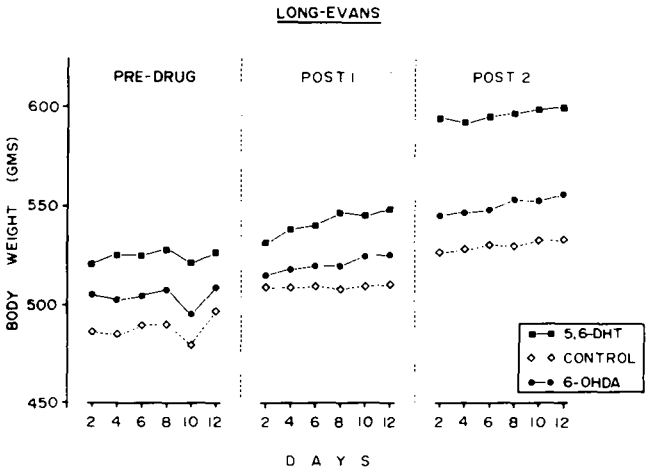

FIG. 13. Mean body weights for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Long-Evans strain.

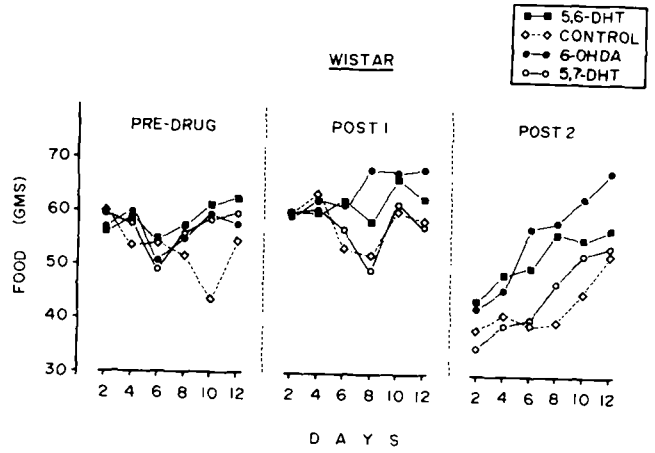

FIG. 14. Mean food intakes for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Wistar strain.

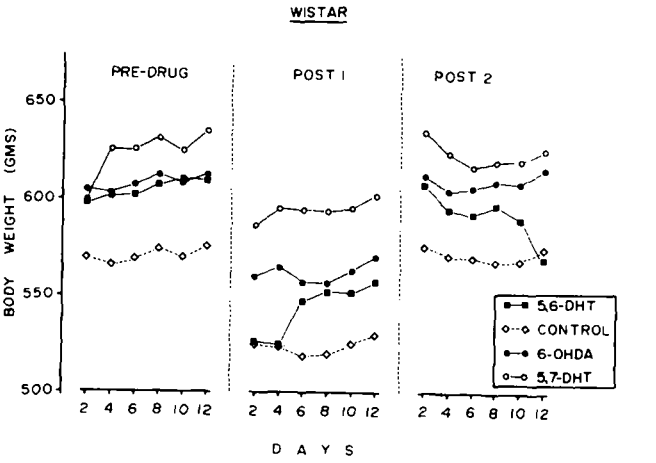

FIG. 15. Mean body weights for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Wistar strain.

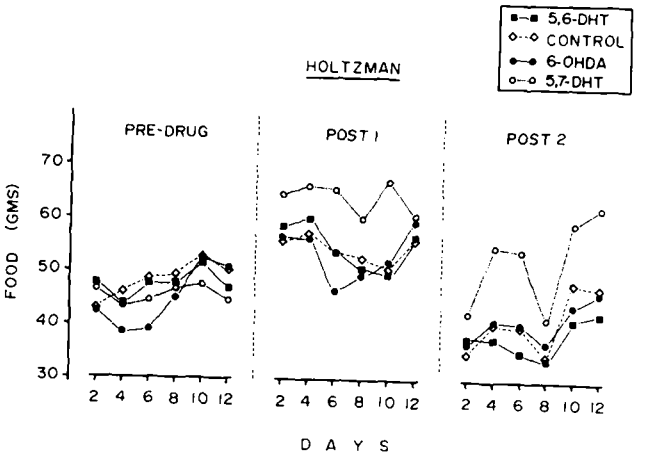

FIG. 16. Mean food intakes for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Holtzman strain.

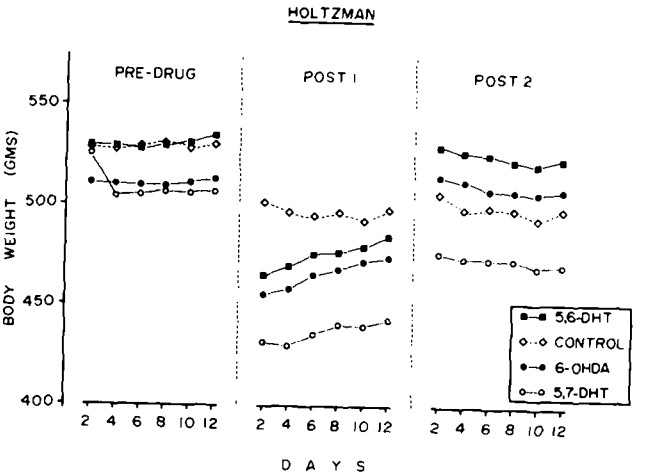

FIG. 17. Mean body weights for successive 2-day periods during each 12-day ethanol preference test obtained for the 5,6-DHT, 5-ion control, 6-OHDA and 5,7-DHT injected animals of the Holtzman strain.

examined, pCPA causes a diminution in ethanol intake either during or after its administration, or under both circumstances [27]. In relation to the present experiments, pCPA alters the drinking pattern most sharply in rats of the Long-Evans and Wistar strains, whereas those of the Sprague-Dawley and Holtzman strains show little or no response to pCPA insofar as their ethanol drinking until after the drug is terminated.

It is likewise important to note that changes in body weight and/or food intake following the intraventricular administration of 6-OHDA and 5,6-DHT may also be differentiated on the basis of strain. For example, in the present study, the Holtzman animals were most severely affected by the injection of 5,6-DHT. Similarly, the injection of 5,6-DHT directly into the hypothalamus of the Sprague-Dawley rat causes a profound albeit transient impairment in food intake [23]. In view of these findings, it appears that assays of circumscribed areas of CNS tissue should ultimately be undertaken in every strain of rat following an injection of a neurotoxin into the brain. In fact, it is possible that the magnitude of a neurotoxin's effect is determined to some degree by a genetic characteristic of neuronal tissue, which is as yet unknown.

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