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Relationship Between Initial Sensitivity to Ethanol and the High Alcohol Intake in Dependent Rats

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MARFAING-JALLAT, P AND J LE MAGNEN *Relationship between initial sensitivity to ethanol and the high alcohol intake in dependent rats* PHARMACOL BIOCHEM BEHAV **22**(1) 19–23, 1985 —The high spontaneous intake of ethanol, which can be induced in rats after a period of forced administration, may be used to study the altered state created in the C N.S by the chronic exposure to ethanol The relationship between the initial acute sensitivity to ethanol and this induced high oral intake has been examined in rats Initial sensitivity was determined in two groups of rats either by a test of motor impairment or by alcohol induced hypothermia After 15 days of daily IG administration of 10 g/kg, rats were submitted to the ethanol presentations which display the high voluntary intake. Two groups of controls were initially tested for their motor impairment or hypothermia respectively under ethanol and then treated for 15 days with saline injections. The results indicate a highly significant negative correlation between initial sensitivity and the level of dependence induced by a chronic treatment and manifested by a voluntary high intake. In control groups, the low intake of ethanol observed in the final test was not correlated to the initial sensitivity to ethanol as tested by hypothermia but weakly correlated to sensitivity measured by motor impairment The results are discussed in terms of mechanisms which determine the voluntary intake of ethanol in ethanol naive and dependent rats

Initial sensitivity Ethanol consumption Physical dependence Rat

VARIOUS acute responses to ethanol of naive rats and mice such as motor impairment, activity changes, hypothermia and sleep time have been used to measure initial sensitivity to ethanol. Individual and strain responses to these various tests are not identical and may be uncorrelated [1,19] For example, a correlation has been found in the responses of rats to a test of motor impairment and hypothermia, but these responses are not correlated with activity changes [18]. However, most of these tests show large individual and strain differences of responses which have been interpreted as differences of initial sensitivity Relationships have been studied between this initial response and other acute or chronic responses to ethanol and its effects ethanol intake [7, 11, 16, 17], acquired tolerance [5, 14, 20] and physical dependence [3, 15, 22] Some of these correlations have been shown to be dependent on the particular response used to measure either initial sensitivity or acute and chronic effects For instance, a chronic ethanol treatment in rats differently develops tolerance tested by sleep time or by hypothermia [19]

As far as physical dependence is concerned, a possible relationship between initial sensitivity and the state of the C.N.S induced by a chronic exposure to ethanol has been investigated only by using scores of signs of the withdrawal syndrome as an evaluation of the severity and degree of physical dependence. Goldstein [3] using this scoring of withdrawal signs has found that initially the least tolerant mice, determined by a longer sleeping time under ethanol, have the mildest signs of withdrawal after a chronic ethanol exposure

A new procedure has been recently developed in our laboratory [9] and in others [6] by which rats chronically treated with high doses of ethanol previously, are induced to take large quantities of ethanol when the treatment is discontinued This voluntary "ethanol induced" high intake is both considerably higher than the intake before treatment and higher than the water intake in alternate presentations or in a choice Reaching 10 g per kg and per day, i.e., the dose used in the previous forced intragastric administration, this intake may be considered as a manifestation of a "behavioral dependence" or self-maintained intoxication By parallel studies of IG and IV self-administrations [8,18] this "induced behavioral dependence" is demonstrated and assumed to result from a conditioned taste preference. Inasmuch as the level of this response might be related to the severity of this ethanol induced brain impairment, it could be used as a measure of this impairment, i.e , of physical dependence.

Using this measure of alcohol addiction in rats, we have undertaken a series of experiments to investigate the respective roles and interactions between various parameters of inducing the state of the C.N.S. which underlies the acquisition of dependence on ethanol. These parameters include

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initial sensitivity to ethanol, routes and periodicity of forced administrations, doses and duration of treatment. In the first series, relationships between initial sensitivity tested by both motor impairment and hypothermia, and the high oral intake of ethanol induced by 15 days of forced IG administrations have been examined.

METHOD

Subjects

Thirty-five male adult Wistar rats weighing 265±6 g were used. They were individually housed in cylindrical cages equipped for automatic and programmed administration of ethanol A 12 hour light/12 hour dark cycle (6 a.m.–6 p m) was maintained throughout the experiment. Standard laboratory chow (Pietrement) and water were available at all times except when otherwise indicated.

Assessment of Initial Sensitivity

Two measurements of the initial sensitivity were used the ethanol induced hypothermia and the drinking test

Rectal temperatures were determined according to the method described by Frankel *et al.* [2]. A digital thermometer was used (INTERSIL). The 2 mm diameter probe was inserted 4 cm into the rectum and was maintained during one minute. Baseline temperatures were measured at T=30 min after an IP administration of 3 ml of saline. The following day, the animals were injected with 2.5 g/kg of alcohol in 3 ml and temperatures were measured at T=15, 30, 60, 90, 120 minutes. The maximal hypothermia observed was taken as the measure of the sensitivity of the C.N.S. Hypothermia testing was conducted at 9 a.m. during the light cycle.

Drinking test was developed by Miceli and Le Magnen [10]. The time necessary for water-deprived rats, after administration of a challenge dose of alcohol to stand up and to reach the water bottle suspended inside the cage 22 cm above the floor, is taken as the measure of the sensitivity to ethanol. One day before the test, the rats were given an intraperitoneal injection of 4 ml of isotonic saline after 16 hours without water. Thirty minutes later, they were placed in the test apparatus. All rats showed a latency of drinking of less than 30 seconds. Then the rats were returned to their home cages where water was supplied until 6 p.m. at which time a new 16 hour water deprivation sequence was started. The following day, the testing procedure was carried out after an injection of 2 g/kg (4 ml) of ethanol. The latency of drinking was taken as the measure of initial sensitivity to ethanol.

Surgery

All rats were implanted with a chronic intragastric catheter under pentobarbital anesthesia. The catheter was fixed through a gastric fistula and tied firmly. Then, the distal part of the silastic catheter was passed under the skin and fixed to the skull by screws and dental cement. After surgery, the rats were placed in the Plexiglas cages equipped for chronic infusion.

Procedure

Six days after recovery from surgery, they were randomly assigned to one of four groups: Group I or hypothermia group (n=8), Group II or hypothermia control group (n=6), Group III or drinking test group (n=13), Group IV or drinking test control group (n=8)

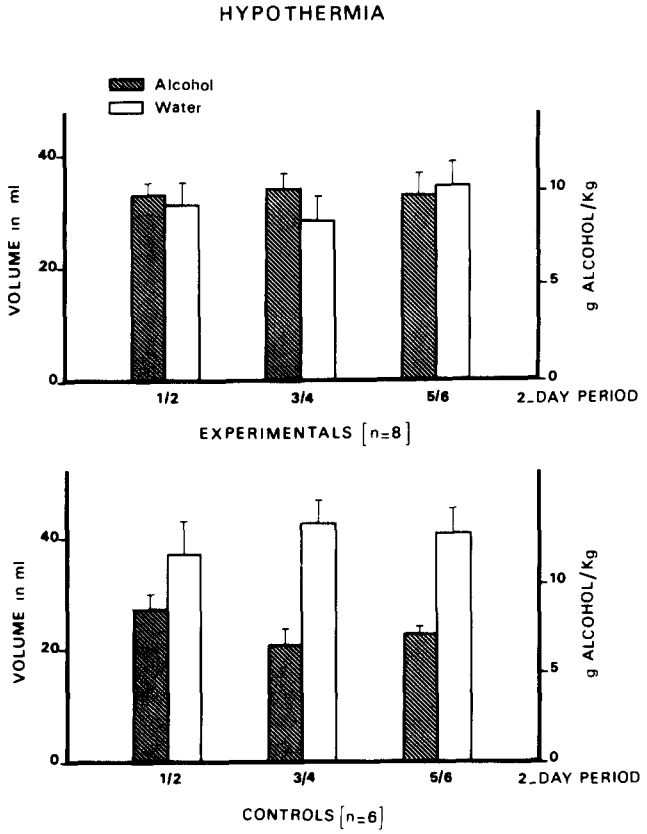

FIG 1 Twenty-four hr mean alcohol intake (in volumes and grammes of pure ethanol per Kg) versus 24 hr mean water intake in three successive couples of days in rats previously chronically treated by ethanol and untreated controls, both initially tested for their initial sensitivity by hypothermia under ethanol

The two experimental groups I and III were submitted to chronic ethanol treatment for 15 consecutive days. The rats received 10 g/kg/day through 5 IG administrations per day of 2 g/kg dose (3.36 ml) each, prepared from 95% ethanol diluted in physiological saline The administrations started at 2 a.m and the following administrations were at 6 30 a.m., 10 30 a.m , 3 p.m and 7:30 p m. A gap of 6½ hours during the dark cycle allowed the rats to drink and to eat without the disturbance due to the acute effects of ethanol. The two groups of controls (II and IV) were submitted to the same treatment but received saline administrations in place of ethanol

Twelve hours after the end of the treatment, all rats deprived of water for 24 hours were offered a 10% (v/v) ethyl alcohol solution for 1 day as the only source of fluid. Then, for 6 days, they were offered the alternate and successive presentation for 8 hour periods of ethanol solution or water. The 6 day period made it possible to assess the acquired preference for ethanol by comparing the 8 hour intake of alcohol and water according to the procedure previously described

Statistics

A Pearson's correlation was performed between measurements of initial sensitivity in relation to either preference

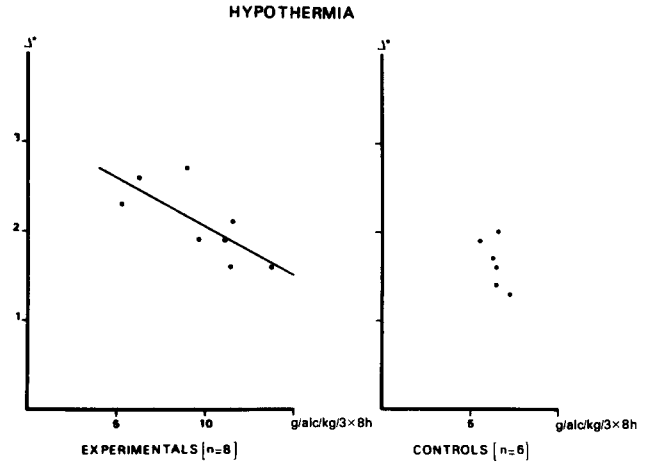

FIG 2 Correlation between the level of initial hypothermia under ethanol and the alcohol consumption of dependent rats during the last 2-day period of the alternate presentation

or the amount of alcohol consumed and was tested for significance by reference to tabled critical values for 2 tailed tests.

RESULTS

The results of the hypothermia groups (groups I and II) are shown in Fig 1. Ethanol treated rats took in average 9.84±0.44 g of alcohol per kg B.W. during the three 8 hour presentations of alcohol for 48 hr. This represents 52.5% of their total fluid intake. Control rats took in average 6.7±0.41 g of alcohol per kg B W during the same periods and 35.3% of their total fluid intake The difference between ethanol intake of treated and untreated rats is significant· $t=5.10$, $p<0.01$ (in g alc/kg), $t=4.55$, $p<0.01$ (in percentage)

Four out of eight previously treated rats have a mean level of alcohol consumption per 24 hours above the dose used in the preceding chronic treatment.

In the ethanol treated group, a negative and significant correlation is found between the level of hypothermia (2°±0.1) and the consumption of alcohol during the 2 last pairs of days of the alternate presentation, when expressed in percentage of alcohol consumed to total fluid intake (r=−0 746, $p<0.05$ and r=−0 893, $p<0.01$ respectively) Expressed in g of alcohol per kg, the same negative correlation is shown for the last pair of days only (r=−0.737, $p<0.05$) No significant correlation between the level of hypothermia and alcohol intake is exhibited by control rats (Fig. 2)

In the group initially submitted to the drinking test and chronically treated by ethanol, the average amount consumed was 11 72±0.63 g/kg during the three 8 hour presentations of alcohol for 48 hr. This represents 54.9% of their total fluid intake (Fig. 3). The average for the control rats was 5 25±0 29 g of alcohol per kg B.W. or 34.75±1.65% of their total fluid intake (Student $t=9.29$, $p<0$ 01 and $t=6.58$, $p<0.01$ respectively) A negative correlation between the latency of drinking and the amount of alcohol drunk per 24 hours during the last series of the alternate presentation can be seen in ethanol treated rats (r=−0 616, $p<0.05$ in g alc/kg and r=−0.629, $p<0.05$ in percentage) (Fig. 4). The correlation with the mean alcohol intake during the three 2 day periods expressed in percent of alcohol intake also is significant (r=−0 663, $p<0$ 05). Control rats show a

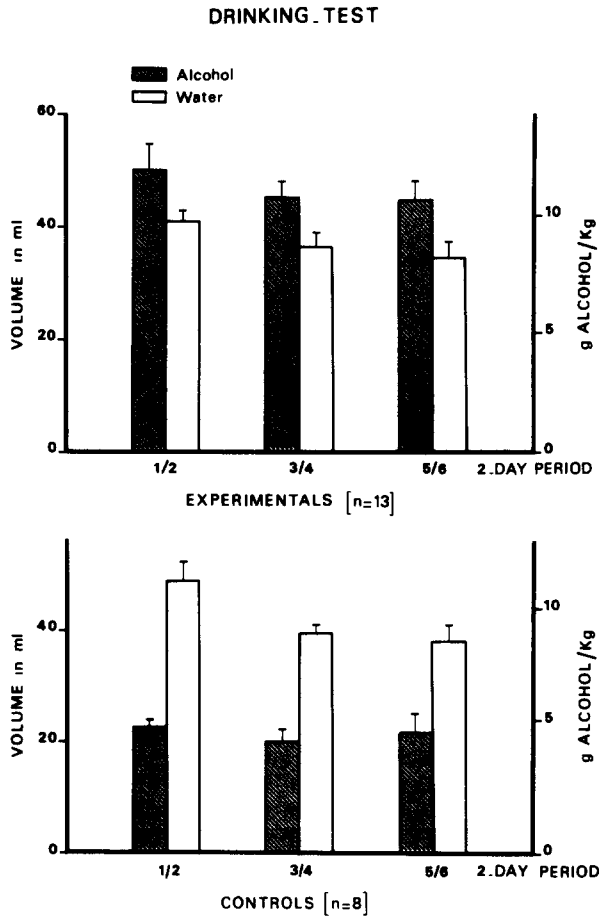

FIG. 3 Twenty-four hr mean alcohol intake (in volumes and grammes of pure ethanol per kg versus 24 hr mean water intake in three successive couples of days in previously chronically treated by ethanol and untreated controls, both initially tested for their initial sensitivity by "drinking test "

significant correlation between their low alcohol consumption during days 3 and 4 and their latency (199±8 min) in the drinking test (M=−0 765, $p<0.01$)

DISCUSSION

The limited correlation between motor impairment under ethanol measured by the drinking test and the initial voluntary intake just mentioned in ethanol naive rats is similar to correlations found between various tests of sensitivity and intake in rats and mice [7, 11, 16, 17]. When the observed ethanol intake in a choice does not exceed 1–2 g/kg per day, this interindividual relationship between initial sensitivity and intake is obviously only correlational and not causal. We have demonstrated that a single IP injection of at least 1.5 g/kg is required to induce aversion to a saccharin solution through the acute toxic effect of this dose acting as unconditioned stimulus [12]. In another study it has been shown that the level of aversion to this saccharine solution, induced by pairing oral intake with an IP injection of 1.5 g/kg ethanol, is highly correlated with the individual response to both the drinking and hypothermic tests [18]. Thus, only a short term oral intake of this dose of ethanol can determine the level of

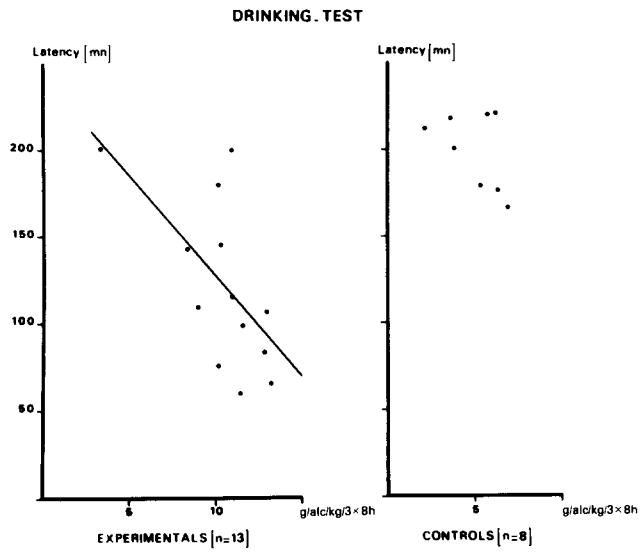

FIG 4 Correlation between the latency of drinking in the drinking test and the alcohol consumption of dependent rats during the last 2-day period of the alternate presentation

intake through post-ingestive effects and might therefore establish a causal relation between initial sensitivity and intake. Evidence exists that the initial low level of ethanol versus water intake results from an unlearned sensory aversion Consequently, its correlation with initial sensitivity is difficult to explain

The correlation between initial sensitivity and voluntary intake is absent in control rats tested by hypothermia A general negative correlation is found between both hypothermia and motor impairment under ethanol and the high level of intake induced by 15 days of forced administrations The less sensitive the rat, the higher its intake This result is in agreement with data from Sinden showing that rats becoming high IV self-injecting rats after a chronic treatment were initially the less sensitive rats [18]

The relation between initial sensitivity and the acquired tolerance by a chronic forced administration must be considered [14,19] It has been reported that a given chronic treatment enhances more the tolerance of initially less sensitive than that of the most sensitive rats Thus, in the present study, the least initially sensitive rats would be the most tolerant at the end of the treatment upon the withdrawal of ethanol

Is this high tolerance a cause of the subsequent high oral intake? In rats, a chronic ethanol treatment doubles the dose of injected ethanol just required to induce a conditioned taste aversion [8,12]. In such dependent rats, because this sensitivity is reduced by the acquired tolerance, a higher level of circulating ethanol is required apparently to limit and repress intake through the conditioned taste aversion process This allows the dependent rat to reach a new ceiling of oral intake This could explain the high correlation found in this study between the observed high level of intake and the initial sensitivity. However it is not explained why dependent rats enhance their intake of ethanol until this new and high level of its aversive effects. In other words it is not explained why ethanol is rewarding below this level instead of being aversive as it is in naive rats

The main characteristic of dependence on ethanol and on other addictive drugs is the relief by the acute action of the same drug of the state of C.N S created by the chronic action of the drug This provides the basis for a positive reinforcement or reward of oral intake or self-administration through a conditioned taste preference The recovery from illness has been demonstrated to be an unconditioned stimulus for conditioned taste preference [4, 13, 21] We assume that such a conditioned taste preference, though not directly demonstrated, is involved in inducing high intake in dependent rats

Only a further investigation of the exact nature of brain injuries caused by chronic intake or administration of ethanol would lead to a clearer understanding of our findings Meanwhile, since initial sensitivity is demonstrated to be a variable of the acquired dependence or alcohol addiction, it is important to pursue studies on other parameters of this acquisition in animals initially selected for their identical sensitivity to ethanol

REFERENCES

1 Crabbe, J C , D K Gray, E R Young, J S Janowsky and H Righter Initial sensitivity and tolerance to ethanol in mice correlations among open field activity, hypothermia and loss of righting reflex *Behav Neural Biol* **33:** 188–203, 1981
2 Frankel, D , J M Khanna, M Kalant and A E Le Blanc Effect of p-chlorophenylalanine on the acquisition of tolerance to the hypothermic effects of ethanol *Psychopharmacology* **57:** 239–242, 1978
3 Goldstein, D B and P Kakihana Alcohol withdrawal reactions in mouse strains selectively bred for long or short sleep times *Life Sci* **17:** 981–985, 1975
4 Green, K F and J Garcia Recuperation from illness flavor enhancement for rats *Science* **173:** 749–751, 1971
5 Grieve, S J , P J Griffiths and J M Littleton Genetic influences on the rate of development of ethanol tolerance and the ethanol physical withdrawal syndrome in mice *J Drug Alcohol Depend* **4:** 77–87, 1979
6 Le Bourhis, B , G Aufrere and D Choquart Study of alcohol dependence in the rat *Adv Exp Med Biol* **126:** 339–358, 1980
7 Lumeng, L , M B Waller, N J McBride and K Li Different sensitivities to ethanol in alcohol Preferring and non-preferring rats *Pharmacol Biochem Behav* **16:** 125–130, 1982
8 Marfaing-Jallat, P and J Le Magnen Ethanol-induced taste aversion in ethanol-dependent and normal rats *Behav Neural Biol* **26:** 106–114, 1979
9 Marfaing-Jallat, P and J Le Magnen Induction of high voluntary ethanol intake in dependent rats *Pharmacol Biochem Behav* **17:** 609–612, 1982
10 Miceli, D and J Le Magnen A simple drinking test for measuring the effects of ethanol on the central nervous system *Psychopharmacology (Berlin)* **66:** 257–261, 1979
11 Nikander, P and L Pekkanen An inborn alcohol tolerance in alcohol-preferring rats The lack of relationship between tolerance to ethanol and the brain microsomal (Na^+K^+) ATPase activity *Psychopharmacology (Berlin)* **51:** 219–223, 1977
12 Pacteau, C and J Le Magnen Ethanol taste aversions in naive and dependent rats Submitted for publication

13 Parker, L., A. Failor and C. Weidman. Conditioned preferences in the rat with a natural need state. a morphine withdrawal *J Comp Physiol Psychol* **82:** 294–300, 1973.
14. Riley, E. P. and E A Lockry. Effects of initial tolerance on acquired tolerance to alcohol in two selectively bred rat strains *Drug Alcohol Depend* **2:** 485–494, 1977
15 Sanders, B Withdrawal-like signs induced by a single administration of ethanol in mice that differ in ethanol sensitivity *Psychopharmacology (Berlin)* **68:** 109–113, 1980
16. Schneider, C W , S K. Evans, M. B Chenoweth and F L Beman. Ethanol preference and behavioral tolerance in mice biochemical and neurophysiological mechanisms *J Comp Physiol Psychol* **82:** 466–474, 1973
17 Schneider, C W , P Trzil and R. D'Andrea. Neural tolerance in high and low ethanol selecting mouse strains *Pharmacol Biochem Behav* **2:** 549–551, 1974
18 Sinden, J D *Les déterminants postabsorptifs des réponses de préférence et d'aversion pour l'ethanol chez le rat* Thése de 3ème cycle, 1982 Paris·
19. Tabakoff, B , R. F Ritzmann, T S. Rajer and R A. Dietrich Characterization of acute and chronic tolerance in mice selected for inherent differences in sensitivity to ethanol *Alcohol Clin Exp Rep* **3:** 198, 1979.
20 Tampier, L , M E Quintanilla and J. Mardones Genetic differences in tolerance to ethanol a study in UChA and UChB rats *Pharmacol Biochem Behav* **14:** 165–168, 1981
21. Trapold, M A and H L Sullivan. A withdrawal related reinforcing effect of alcohol *Bull Psychom Soc* **13:** 327–329, 1979.
22 Waller, M B , W J McBride, L Lumeng and T. K. Li Induction of dependence on ethanol by free-choice drinking in alcohol preferring rats *Pharmacol Biochem Behav* **16:** 501–507, 1982