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# The Effects of Chronic Administration of Ethanol on Startle Thresholds in Rats

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*Abstract.* The thresholds for startle responses to electric shock were measured in adult male Wistar strain rats given ethanol daily in doses rising from 3 to 7 g/kg over a 30-day period, and in controls receiving equicaloric doses of sucrose. Tests made 23, 36, or 47 h after ethanol (i.e., during partial or complete ethanol withdrawal) gave threshold values significantly lower than those obtained with sucrose-treated controls. The difference became greater after longer ethanol treatment and larger doses. However, when threshold measurements were made under the acute influence of ethanol in the experimental group, the mean values were virtually equal to those of the sucrose controls. This normalization, by ethanol, of a disturbance produced by absence of ethanol in a chronically treated animal is indicative of physical dependence. Following termination of ethanol treatment there was a gradual return of startle thresholds almost to control values over a relatively short period, indicating that the changes underlying the hyperexcitability are readily reversible.

*Key-Words:* Ethanol — Tolerance — Dependence — Withdrawal Reaction — Startle Threshold — Rat.

The chronic ingestion of large amounts of ethanol is well known to give rise to increased tolerance and to physical dependence which is manifested as withdrawal symptoms ranging in severity from "shakes" and insomnia to delirium tremens. According to one hypothesis (Collier, 1965), increased tolerance and physical dependence could represent two aspects of the same cellular response to chronic exposure to a drug. Pharmacological investigation of the mechanism of these changes, and of the relationship between them, would be helped substantially by the development of sensitive and reliable methods for measuring tolerance and physical dependence in laboratory animals. One method for measuring minor degrees of ethanol intoxication was described recently (Gibbins *et al.*, 1968), and has been employed successfully to demonstrate the time characteristics of acquisition and loss of tolerance, during and after chronic administration of ethanol to rats (LeBlanc *et al.*, 1969). The present paper describes a method for following the course of development of physical dependence on ethanol.

It has been reported that ethanol, administered by gavage in doses of 1—4 g/kg, produces a significant elevation of the threshold stimulus for electroshock seizures in both rats (Allan and Swinyard, 1949) and mice (McQuarrie and Fingl, 1958). In the latter study it was noted that the elevation of threshold was followed by a decline to subnormal values for a variable period of time following termination of the alcohol intoxication. The duration and intensity of this reactive hyperexcitability were found to be proportional to the intensity and length of the preceding period of intoxication, ranging from a few hours after a single dose of ethanol, to several days following a two week program of administration of 2 g/kg every 8 h. McQuarrie and Fingl suggested that the post-withdrawal hyperexcitability was an easily measurable manifestation of physical dependence.

However, electroshock seizures are complex responses which are difficult to correlate with spontaneous withdrawal phenomena. Spontaneous convulsions have been observed in severe withdrawal reactions in man (Victor, 1966), dogs (Essig and Lam, 1968), monkeys (Ellis and Pick, 1970) and mice (Freund, 1969), but not in rats. Moreover, ethanol was shown to have different effects upon different parameters of electroshock seizures (McQuarrie and Fingl, 1958). Therefore it seemed desirable to explore another measure of hyperexcitability, more closely related to normal sensory pathways and motor responses, which might provide an indication of lesser degrees of withdrawal reaction. The "flinch jump" procedure (Kimble, 1955; Evans, 1961, 1962; Hoffman *et al.*, 1964) was selected for this purpose. Essentially, this consists of measurement of the threshold intensities of electric shock, delivered through a grid on which the animal is standing, required to make it flinch (i.e., to lift one paw) or to jump off the grid. Comparison of the thresholds for the animals before, during, and after chronic ethanol treatment was found to provide a useful index of the degree of alcohol dependence produced.

## Methods

The apparatus consists of 4 Lucite test chambers, each about 20 cm long, 13 cm wide, and 25 cm high, with a grid floor through which brief shocks of various intensities can be delivered at variable time intervals. The grid is composed of 16 stainless steel rods, 3.2 mm in diameter set 1.25 cm apart. The back and both ends of each compartment are painted black. The grid is electrified (alternate bars connected, shock not scrambled) by a step-up transformer with a center-tapped secondary through a small autotransformer from an A.C. power source. The secondary voltage rating of the transformer is 240-0-240, and the output 42 volt amps. A double-pole 4-position switch serves the dual function of changing the output voltage connections from a given value to double that value, and

switching a voltmeter to suit the connections. The shock voltage is delivered to each of the individual grid floors through a 450,000 ohm resistor (non-inductive) so that each animal has his own limiting resistor. Assuming that the animal contributes an additional 50,000 ohms, the total resistance in each circuit is 500,000 ohms. This provides a convenient basis for determining the intensity of the shock from the voltage registered on the voltmeter. A tape-programmer and interval timer are used to control the presentation of shock stimuli. A signal light is activated each time the equipment delivers a stimulus, even when the voltage of the latter is set at zero.

Thresholds were determined by the method of constant stimuli (Guilford, 1954; Hoffman *et al.*, 1964). Each animal was given a 2 min period of adaptation in the compartment after which 6 different shock intensities (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mA) were presented 10 times each in a pre-determined random order. The duration of each shock was 0.5 sec and the inter-shock interval varied randomly about a mean of 12 sec. At the end of each block of 12 trials the animal was removed from the compartment and the grid floor wiped clean.

The subjects' responses to the shock stimuli were classified as "flinch", "jump", or "no response". "Flinch" was recorded when the animal made an abrupt startle-like movement without removing more than one paw from the grid. "Jump" was recorded if two or more paws were removed from the grid in response to the shock stimulus (Kimble, 1955; Evans, 1961). Throughout the experiment the animals were tested by the same three experimenters. One set the shock intensities while each of the other two observed and recorded the responses of two animals at a time, as dictated by the signal lights. The latter two observers did not know which animals had received which treatment. In a preliminary experiment in which the same two observers independently classified the responses of the same pairs of 10 animals during 8 test sessions of 120 trials each, the inter-observer reliability coefficients were all found to exceed $r = 0.93$. These results agree very well with those reported by Evans (1961) and support his conclusion that the visually detected response patterns are satisfactorily discriminable.

Twenty-four naive, male Wistar strain rats (initial weights 288 to 416 g) were randomly assigned to 2 groups of equal size. For the first 6 days of the experiment both groups were intubated with a 50% (w/v) solution of sucrose in tap water, in an amount calorically equivalent to a 3 g/kg dose of ethanol, and tested on days 2, 4 and 6, 23 h after intubation. Thereafter, the experimental animals were given ethanol (30% w/v in tap water) in regularly increasing doses and the controls equal volumes of the equicaloric solution of sucrose. Beginning on day 6 with 3 g/kg the ethanol dose was increased by 1 g/kg every 6 days until a level of

7 g/kg was reached on day 31. This dose was maintained until day 37, after which both ethanol and sucrose treatments were discontinued.

Threshold determinations were made every second day during the chronic treatment period and daily during the withdrawal period. With the exceptions noted below, the animals were tested on the second and fourth days of a given dose level, 23 h after intubation, at which time previous experience (Hawkins *et al.*, 1966; Khanna *et al.*, 1967) had indicated that there would be little or no residual alcohol in the blood. On the sixth day at each dose level the test was done 30—35 min after intubation, at the probable time of peak blood level. This time was chosen because earlier work (Kalant and Czaja, 1962) had shown that doses of as much as 4 g/kg by gavage produced their maximum intoxicating effect in 30—60 min. The tests on days 32 and 37 were given 36 h after intubation, and the test on day 34 at 47 h after. The reason is explained in the section on "Results". Blood samples (0.05 ml from the tip of the tail) were taken immediately after the test sessions on days 12, 18, 24 and 30, and immediately before testing on day 28, and later analysed for alcohol concentration by the deproteinization and gas-chromatographic methods described by LeBlanc (1968).

## Results

The mean values for the startle thresholds of the two groups were quite stable and not importantly different during the first six days of the experiment. Fig.1 shows that beginning on day 8 and continuing throughout the chronic treatment period the trend lines diverge conspicuously, the ethanol group showing lower mean thresholds than the control groups. The overall mean flinch thresholds for the ethanol and sucrose groups during the chronic treatment period (but excluding the values on days 12, 18, 24 and 30) were 0.21 mA and 0.24 mA respectively; the jump thresholds were 0.34 mA and 0.36 mA respectively. During the withdrawal period (from day 38 on) there is a gradual increase of both flinch and jump thresholds in the ethanol group almost to control values.

Tables 1 and 2 summarize the analysis of the threshold data. It can be seen that drugs, doses, and drugs × doses were all significant for both measures, although to a lesser degree for the flinch than for the jump thresholds. This means that the flinch and jump thresholds of the ethanol animals were lower than those of the controls; that the thresholds varied with the amount of sucrose or ethanol administered, and that the effects of change in dose were different for the two treatments.

In addition, days, and days × drugs are significant for jump threshold. This means that thresholds on the second test day at a given dose level were different from those on the first, and that the two treatments differ

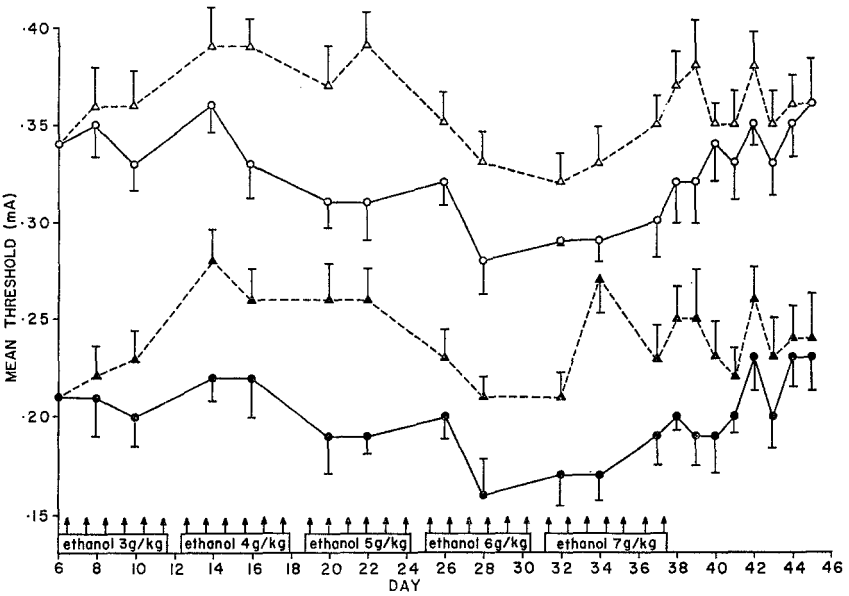


Fig. 1. Effects of chronic administration of ethanol, and of its withdrawal, on jump and flinch thresholds in rats: △ jump threshold, sucrose group; ○ jump threshold, ethanol group; ▲ flinch threshold, sucrose group; • flinch threshold, ethanol group. Each point is the mean of 12 animals; S.E. indicated by vertical bars. The boxes indicate days on which specified doses of ethanol or equicaloric sucrose were given. Arrows indicate times of intubation, either 30 min before (days 12, 18, 24 and 30) or immediately after the threshold tests. Threshold measurements made on days 12, 18, 24 and 30 are not represented (see Figs. 2 and 3)

Table 1. *Analysis of variance of flinch response*

| Source | *dF* | Mean square | *F* ratio | *P* |
|---|---|---|---|---|
| Drugs | 1 | 0.17658 | 11.23 | $< 0.005$ |
| Subjects (drugs) | 22 | 0.01573 | | |
| Doses | 4 | 0.02477 | 29.14 | $< 0.001$ |
| Drugs × doses | 4 | 0.00456 | 5.36 | $< 0.001$ |
| Subjects × doses (drugs) | 88 | | | |
| Days | 1 | 0.00187 | 3.74 | N.S. |
| Drugs × days | 1 | 0.00077 | 1.54 | N.S. |
| Subjects × days (drugs) | 22 | | | |
| Doses × days | 4 | 0.00234 | 2.41 | N.S. |
| Drugs × doses × days | 4 | 0.00040 | 0.41 | N.S. |
| Subjects × doses × days (drugs) | 88 | 0.00097 | | |

Table 2. *Analysis of variance of jump response*

| Source | *dF* | Mean square | *F* ratio | *P* |
|---|---|---|---|---|
| Drugs | 1 | 0.16068 | 8.68 | $< 0.01$ |
| Subjects (drugs) | 22 | 0.01851 | | |
| Doses | 4 | 0.02987 | 23.90 | $< 0.001$ |
| Drugs × doses | 4 | 0.00370 | 2.96 | $< 0.025$ |
| Subjects × doses (drugs) | 88 | 0.00125 | | |
| Days | 1 | 0.00392 | 5.76 | $< 0.05$ |
| Drugs × days | 1 | 0.00345 | 5.07 | $< 0.05$ |
| Subjects × days (drugs) | 22 | 0.00068 | | |
| Doses × days | 4 | 0.00321 | 3.91 | N.S. |
| Drugs × doses × days | 4 | 0.00006 | 0.07 | N.S. |
| Subjects × doses × days (drugs) | 88 | 0.00082 | | |

in this respect. Specifically, in the ethanol group the threshold tends to be lower on the second day than on the first, while the reverse is true in the sucrose group.

To illustrate further the differences between groups, the ratios of alcohol:control group mean threshold values were calculated for each test day. The results are shown in Figs. 2 and 3. A fairly steady decline in threshold values for the ethanol group relative to the controls is seen between days 6 and 22. Following cessation of alcohol treatment there was a return of the relative thresholds for both flinch and jump toward the control values, which was almost complete by day 45.

On days 12, 18, 24 and 30, when the experimental animals were tested under the acute influence of ethanol, the mean threshold values were virtually equal to those of the control group. The mean blood alcohol levels of the experimental group immediately after testing on days 12, 18, 24, and 30 were 104, 144, 175 and 200 mg/100 ml respectively.

On day 26 an unexpected rise in relative thresholds for both flinch and jump was noted in the alcohol group. Blood samples taken immediately before the test on day 28 revealed that 5 of the alcohol-treated animals had blood ethanol levels ranging between 50 and 150 mg/100 ml, indicating that the preceding daily dose had not been completely metabolised in all cases. The results on days 26 and 28 therefore cannot be considered true measures of maximal post-alcohol hyperexcitability. The same consideration presumably applied to the tests on days 32—38, especially since the dose had been increased further. To test this interpretation, no alcohol was given on day 33, so that the test on day 34 was made 47 h rather than 23 h after the last preceding dose of ethanol.

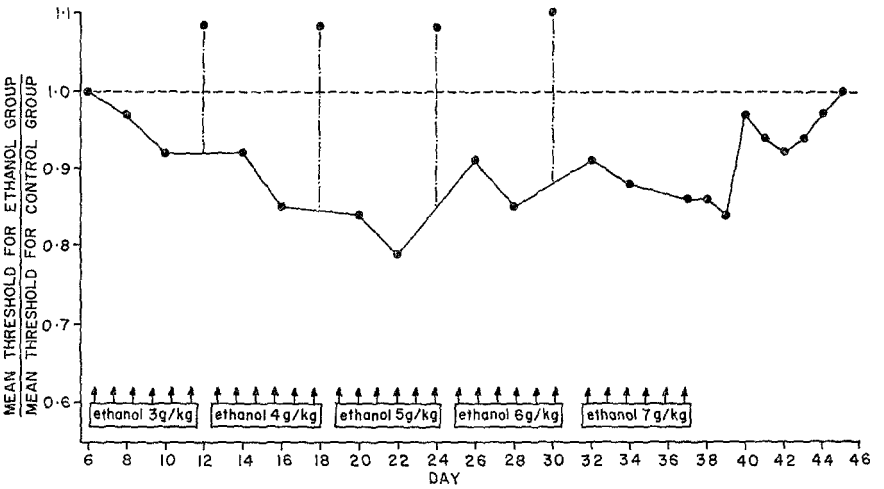


Fig. 2. Effects of chronic administration of ethanol and of its withdrawal on threshold ratio for flinch responses. Each point represents the mean threshold for the ethanol group divided by the mean threshold for the control group. The boxes indicate the days upon which the specified ethanol doses were given. The arrows indicate whether the animals were intubated 30 min before (days 12, 18, 24 and 30 marked by vertical broken lines), or immediately after the threshold tests

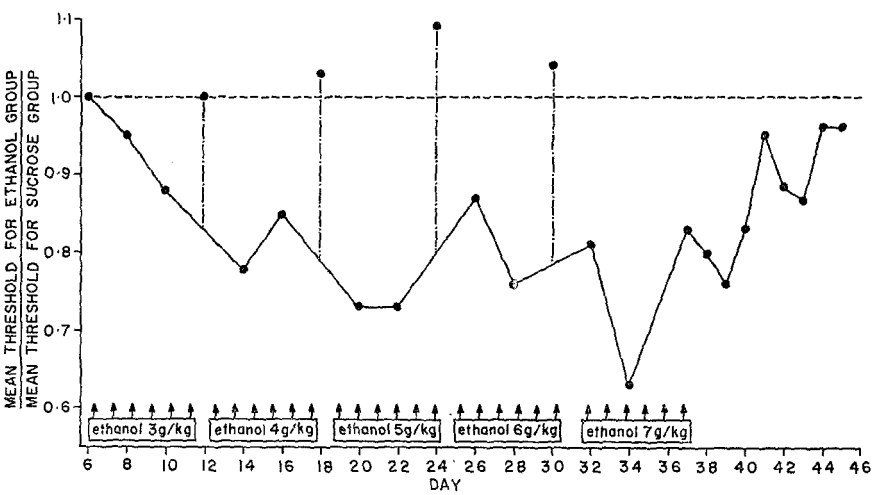


Fig. 3. Effects of chronic administration of ethanol and of its withdrawal on threshold ratio for jump responses. Each point represents the mean threshold for the ethanol group divided by the mean threshold for the control group. The boxes indicate the days upon which the specified ethanol doses were given. The arrows indicate whether the animals were intubated 30 min before (days 12, 18, 24 and 30 shown by vertical broken lines), or immediately after the threshold tests

There appeared to be a substantial decline in the relative threshold for flinch but not for jump. Despite continued high dosage, there did not appear to be any further lowering of threshold on subsequent tests.

Following cessation of ethanol treatment, from day 38 on there was a gradual return of both thresholds almost to control values over an 8-day period.

## Discussion

Measurements of stimulus thresholds for flinch and jump responses in the present study were made under several different conditions with respect to ethanol treatment and to environmental factors. The mean values for both responses by the control group fluctuated during the later part of the experiment, apparently because of variation in measured atmospheric humidity. However, since the analysis of the actual test values (Tables 1 and 2) showed significant differences between the two treatment groups for comparisons on the same day, it is permissible to use the inter-group threshold ratio (alcohol : controls) as an illustrative simplification.

It is evident from Figs. 2 and 3 that the flinch and jump thresholds changed in parallel in response to treatment. Despite some views to the contrary, the two responses are generally considered to represent merely different degrees of the same startle reflex (Landis and Hunt, 1939; Hoffman *et al.*, 1964). Therefore their parallel course reinforces the conclusions drawn from the experiment. As treatment was continued the ethanol animals became progressively more irritable and difficult to handle, although gross tremor or convulsions were not observed. There was a clearly evident fall in threshold ratio for both responses from the beginning of the alcohol treatment period. This is consistent with the finding that alcohol withdrawal signs, including tremor and convulsions, could be produced after as little as 5 days of alcohol treatment in mice (Freund, 1969) and 10—18 days in monkeys (Ellis and Pick, 1970). The decrease was greater with increasing alcohol dosage from day 6 through to day 22, with the sole exception of the flinch threshold on day 16.

The rise in ratio on days 26 and 28 reflected an artifact of experimental procedure. The preceding dosage increment had evidently raised the daily dose to more than the animals could metabolize in 23 h. The finding indicates that observations of alcohol withdrawal phenomena made at a single fixed time after administration of the last preceding dose may be misleading, and that it is probably desirable in such cases to verify by blood analysis that the subjects are actually in a state of withdrawal. The ideal procedure for comparative studies would be to use a fixed time after return of the blood alcohol level to zero. Since no blood ethanol measurements were made from day 28 on, it is impossible to

know whether the absence of continued decrease in thresholds during the last 9 days of the treatment period was due to residual ethanol or to the attainment of maximum degree of physical dependence. It is interesting to note that in previous work (LeBlanc *et al.*, 1969) the same type of dosage schedule resulted in the production of maximum attainable tolerance to ethanol in 19—21 days, and further dose increments did not increase it.

On days 12, 18, 24 and 30 the threshold measurements were made during the period 30—35 min after the preceding dose of ethanol. Under these conditions, after doses of 3, 4, 5 and 6 g/kg the thresholds in the alcohol-treated animals, which were markedly reduced when measured 23 h post-ethanol, returned to approximately the same levels as in the controls. This is by definition an evidence of physical dependence, inasmuch as it is a correction or normalization by ethanol of a disturbance produced by absence of ethanol in a chronically treated animal. Further, the present method provides a means of quantifying the degree of dependence at earlier stages than the fully developed picture described by other investigators. The changes underlying the hyperexcitability are readily reversible. The time of return to normal in the present work is of the same order as that required for normalization of the electroconvulsive seizure threshold in animals which had received ethanol for two weeks (McQuarrie and Fingl, 1958), and for reversal of tolerance to ethanol (LeBlanc *et al.*, 1969) and of cross-tolerance to amobarbital in ethanol-treated rats (Ratcliffe, 1969).

The present work shows that chronic treatment with increasing doses of ethanol gives rise to an increasing degree of post-alcohol hyperexcitability, as demonstrated by reduction of the threshold for response to stimulation via normal sensory pathways. This schedule of administration was chosen specifically because it had been used in earlier studies of the development of tolerance (LeBlanc *et al.*, 1969) and because it causes minimal mortality among the experimental animals. Unfortunately it contains two simultaneous variables, i.e. the duration of treatment and the dose level. The relative effects of these two must be examined separately in future work. The present findings do not permit a conclusion about the maximal degree of dependence which can be produced, but they are compatible with the hypothesis that tolerance to, and physical dependence on, ethanol are intimately related processes, or two manifestations of the same process.

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