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The Excitatory Component of Ethanol in Mice: A Chronic Study[1]

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MASUR, J. AND R. BOERNGEN. *The excitatory component of ethanol in mice: A chronic study.* PHARMAC. BIOCHEM. BEHAV. 13(6) 777–780, 1980.—The effects of chronic administration of initially depressant, ineffective and excitatory doses of ethanol on the locomotor activity of mice was studied. The results showed that (1) an excitatory effect of ethanol is observed after tolerance develops to its depressant action; (2) the effect induced by an initially excitatory dose of ethanol became more pronounced with chronic exposure to the drug; and (3) tolerance to the excitatory effect was not reached after 60 days of ethanol treatment.

Excitatory effect of ethanol Tolerance to ethanol Tolerance and excitatory effect of ethanol

IT was incidentally observed in our laboratory that a progressive hyperactivity in mice developed after continuous daily injection of an initially depressant dose of ethanol. This casual observation led us to a search in the literature where, surprisingly, data in this respect was lacking. Although tolerance to the effects of ethanol has been reported to occur in humans and in animals [4] the experiments performed are based in the depressant action of this drug, no attention being given to its excitatory component. This approach is manifested in the common conception of tolerance as "the tendency for regular drinkers to become more resistant to the intoxicant effect of alcohol, so that they can tolerate larger amounts on repeated exposures".

To the best of our knowledge, only in one paper was the hyperactivating action of ethanol considered in a tolerance study [3]. This absence of data is probably consequent to the fact that ethanol is primarily known as a depressant drug. However, it must be considered that the excitatory action is an important determinant factor for the use of alcohol, and as such should deserve more concern.

The present paper describes the results of several experiments designed to investigate the effects of chronic exposure to ethanol on the locomotor activity of mice. First, the acute depressant and excitatory doses of ethanol were determined in mice through a dose response study. This was followed by chronic experiments where (a) a possible emergence of the excitatory component of ethanol was investigated after tolerance developed to an initially depressant dose; (b) the possibility that an excitatory effect may emerge after long exposure to an initially ineffective dose of ethanol was also studied; and (c) the development of tolerance to an initially excitatory dose of ethanol was examined.

METHOD

Subjects

Naive albino Swiss male and female mice were used. They were maintained in wooden cages in groups of 20–30 in air conditioned laboratories at a temperature of 23±1°C. At the beginning of the experiment the mice were 2 months old.

Drug

Ethanol pro-analysis (Merck Laboratories). The concentration was 15% (weight/volume) in a 0.9% NaCl solution.

Apparatus

To measure spontaneous motor activity cages provided with 3 photocells and measuring 40×25×20 cm were used.

Statistical Analysis

One way analysis of variance (ANOVA) followed by Duncan's new multiple range test was employed. In the case when only two groups were compared, the Student *t* test was used. The level of significance considered was 0.05.

EXPERIMENT 1

The acute effect of ethanol on motor activity was studied using seven groups of 15 female mice each. Six doses of ethanol were tested ranging between 1.5 to 4.0 g/kg. The

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seventh group was injected with saline. Immediately after the intraperitoneal (IP) injection the animals were introduced in the experimental cages and the number of light beam interruptions were cumulatively recorded at 10 min intervals during 60 min. All sessions were performed in the afternoon.

EXPERIMENT 2: LONG TERM ADMINISTRATION OF INITIALLY DEPRESSANT, INEFFECTIVE OR EXCITATORY DOSES OF ETHANOL

Naive female mice, 60 days old at the beginning of the experiment received 30 daily IP injections of either 1.0, 2.5 or 3.5 g/kg. These doses in the acute experiment (Fig. 1) showed to have an ineffective, excitatory and depressant effect, respectively. For each dose, 15 mice were used. Immediately after the 1st, 15th and 30th injection, the motor activity of the animals was recorded according to the same procedure described for the acute experiment. For each dose of ethanol, two control groups with the same number of animals were used. In the first one ethanol was injected daily at the same dose as the experimental group to which it was assigned, but motor activity was recorded under saline (ethanol was given immediately after the 60 min test). The purpose of this group was to detect whether the chronic manipulations to which the experimental animals were exposed (daily injections, undernutrition due to alcohol, etc.) could *per se* alter the activity of the mice. Unfortunately, due to a laboratory accident the mice injected with 2.5 g/kg were lost, thus, these controls are only available for 1.0 and 3.5 g/kg of ethanol. However, in the experiment described below this failure was partially overcome. The second control groups, for each of the 3 doses of ethanol, were daily injected with saline and were tested also under saline at the same days (1st, 15th and 30th).

EXPERIMENT 3: PROLONGED ADMINISTRATION OF AN INITIALLY EXCITATORY DOSE OF ETHANOL

For 60 days, naive male mice were daily injected with 2.0 g/kg of ethanol, a dose which in a pilot experiment induced an excitatory effect. It is noteworthy that 2.0 g/kg was not able to induce excitation in female mice (see acute experiment).

Motor activity was recorded after the 1st, 30th, 45th and 60th day of ethanol treatment. This measure was performed during 60 min being the mice introduced into the experimental cage immediately after the injection. Two control groups were performed. In the first, the mice were injected daily with 2.0 g/kg of ethanol, but were tested under saline. The animals of the second control group were injected daily with saline and tested also under saline. Fifteen mice were used for each group.

RESULTS

EXPERIMENT 1

Figure 1 shows the effects of different doses of ethanol on the motor activity of mice. An excitatory action was observed with a dose of 2.5 g/kg, at the end of 60 min of observation, whereas depression occurred with 3.0, 3.5 and 4.0 g/kg. The doses of 1.5 and 2.0 did not alter the activity of the animals.

EXPERIMENT 2

Figure 2 (upper part) compares the motor activity of mice after the first injection with either saline or 1.0, 2.5 and 3.5

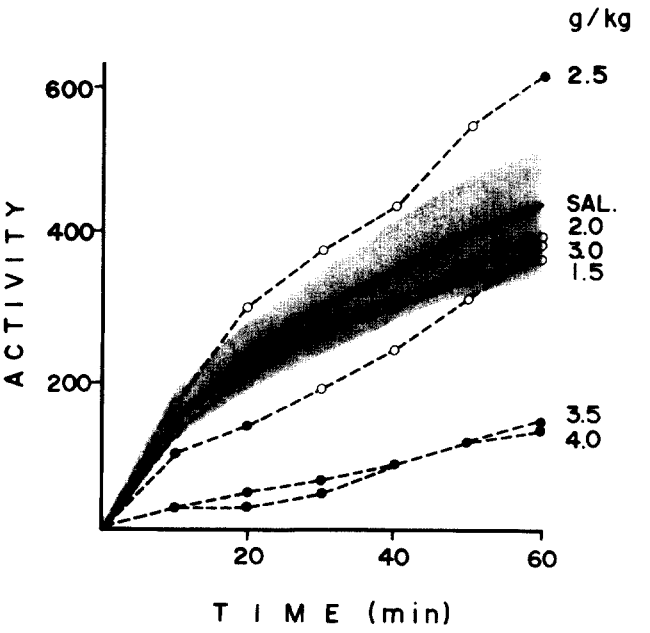

FIG. 1. Mean motor activity of female mice under different doses of ethanol and saline (SAL). The shadowed area represents two standard errors of the mean of the SAL group. Black dots indicate significant differences when compared to SAL (p at least 0.05).

g/kg of ethanol. As previously found in the acute experiment, the lowest dose was ineffective, whereas 2.5 and 3.5 g/kg induced excitation and depression, respectively. After fifteen days of treatment (middle part of Fig. 2), the dose of 1.0 g/kg remained without effect. The excitation induced by the dose of 2.5 g/kg of ethanol was increased several fold when compared with the first day of administration. Also the dose of 3.5 g/kg showed a marked difference when compared with the acute depressant effect. Besides the development of tolerance to the depressant effect, the animals presented a slight (although nonsignificant) excitation after 40 min of drug administration. The motor activity of the second control group, chronically treated with ethanol and tested under saline was similar to the control group chronically treated with and tested under saline, showing that chronic manipulations *per se* did not increase the activity of the mice.

After 30 days of treatment (lower part of Fig. 2), the 1.0 g/kg dose continued not to differ from the saline treated group. Also, the several fold increase in motor activity observed with 2.5 g/kg remained unchanged. The slight excitation disclosed after tolerance to the depressant effect of 3.5 g/kg (Day 15) appeared more clearly after 30 days of drug administration, reaching significant levels during the 50–60 min of observation. Concerning the control group chronically treated with ethanol and tested under saline, a significant depression was observed.

EXPERIMENT 3

The results are shown in Fig. 3. The initial excitation observed at Day 1 increased after the chronic administration. No tolerance developed within 60 days to the excitatory effects. The control group chronically treated with ethanol and tested under saline showed a decrease on motor activity which was significant at Days 30 and 45.

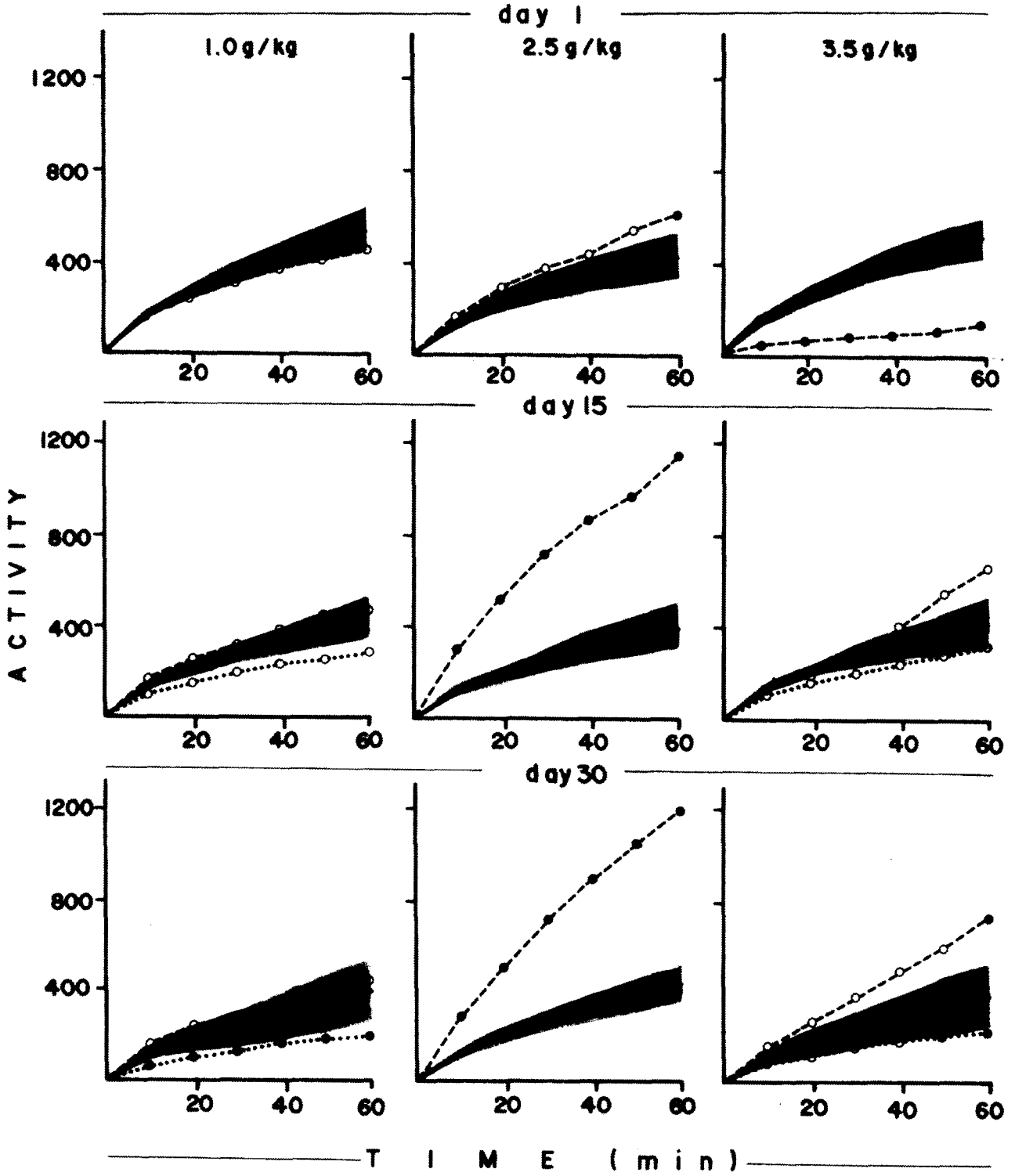

FIG. 2. Mean motor activity of female mice after the 1st, 15th and 30th injection of 1.0, 2.5 or 3.5 g/kg of ethanol (-----) compared to a control group (———) daily injected with saline and tested under saline. The other control group (.....) was daily injected with ethanol and tested under saline. At Day 1 both controls are represented together (———) as they were tested after a first injection of saline. The shadowed area indicates two standard errors of the mean, while black dots represent significant differences (p at least 0.05) when compared to the (———) group.

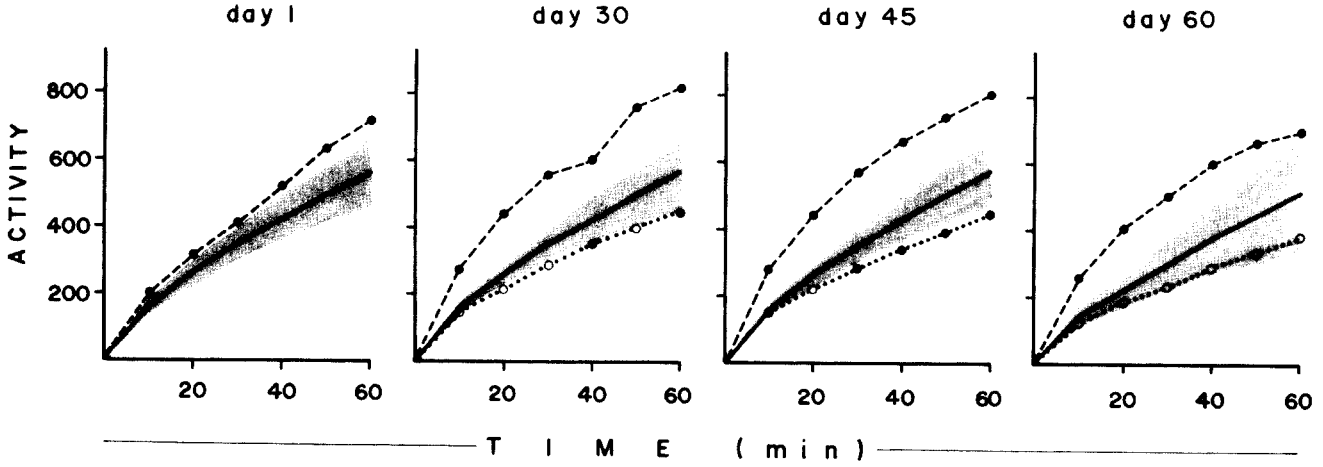

FIG. 3. Mean motor activity of male mice under chronic treatment of 2.0 g/kg of ethanol (-----) compared to a control group (———) daily injected with saline and tested under saline. The remaining of the figure can be read as in Fig. 2.

DISCUSSION

In a recent review on the action of ethanol, Pohorecky [6] commented on the need for detailed studies concerning the balance between the excitatory and depressant components of ethanol effects. The results here presented provide data on this subject. Thus, it was shown that (1) after the development of tolerance to an initially depressant dose of ethanol, an excitatory effect took place (Experiment 2) and that (2) the excitatory effect of a given dose of ethanol became more pronounced with daily administration of the drug (Experiments 2 and 3). The increase of excitation cannot be ascribed to an unspecific excitatory state resulting from long term manipulation or ethanol induced undernourishment for the animals when challenged with saline showed normal or even decreased locomotor activity. The increase of ambulation could be visualized as the outcome of a balance between the excitatory and inhibitory components of the ethanol effect. At the first exposures the depressant effect would either prevail or reduce excitatory responses. As tolerance develops to the depressant component, excitatory responses are uncovered. The development of tolerance only to the depressant effect has already been described for other drugs [1, 2, 5].

Another observation derived from our data is that tolerance to the excitatory component of ethanol is not easily achieved, as it was not reached even after 60 days of daily treatment. This is an interesting finding as it provides experimental evidence indicative of the possible relevance of the excitatory component of ethanol when chronically ingested.

Our data is in contrast with those described by Hunt and Overstreet [3], who reported in rats a parallel development of tolerance to the depressant and hyperactivating effects of ethanol. Among the factors that could account for the contrary findings is the different species studied, as we have unpublished data indicating that the excitatory action of ethanol is more evident in mice than in rats.

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