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Response variability of ethanol-induced locomotor activation in mice *

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Abstract. Mice from a randomly bred strain were divided into two groups according to their locomotor responses to ethanol (0.8–3.0 g/kg): in two thirds of the tested animals ethanol increased locomotor activity (ethanol activated – EA), whereas in the remaining one third it did not (ethanol non-activated – ENA). Both groups did not differ in their locomotor activity after saline administration. Furthermore, EA and ENA mice presented a similar increase in locomotor activity after challenge with 1.0 and 2.0 mg/kg *d*-amphetamine. Chronic exposure to ethanol increased the ethanol-induced locomotor activation in both EA and ENA groups. The possibility that the lack of responsiveness of ENA mice to ethanol's acute activating effect could be due to a higher sensitivity to the depressant effect of ethanol is discussed.

Key words: Ethanol stimulation – Activity – Chronic ethanol – Amphetamine and ethanol – Ethanol

Data on the biphasic effect of ethanol, behavioral stimulation with low doses and depression with high ones, have accumulated during the last years. Different systems of central neurotransmitters have been suggested as mediating the ethanol-induced stimulation and depression (Erikson and Burnan 1971; Engel et al. 1974; Cott et al. 1976; Liljequist and Engel 1982; Mereu et al. 1984). It has been also reported that the biphasic effect is age dependent, with older mice showing an absence of locomotor activation following ethanol (Engel 1985). The issue of whether or not the phenomenon of tolerance, which is well known to develop to the depressant effect of ethanol, occurs in relation to ethanol's activating effect has been previously addressed. Studies with mice have shown a lack of tolerance development to the locomotor stimulating effect of ethanol (Masur and Boerngen 1980; Crabbe et al. 1982; Tabakoff and Kiianma 1982; Masur et al. 1986). In addition, relevant species and strain differences have been reported to occur regarding the responsivity to ethanol's excitatory effect (Randall et al. 1975; Frye and Breese 1981; Dudek and Abbott 1984; Masur et al. 1986).

The main purpose of the present study was to evaluate the intra-strain variability of the responsiveness to the stimulant effect of ethanol. First it was observed whether there were, within a randomly bred strain of mice, animals not showing locomotor activation following a wide dose range of ethanol. Having found it, the ethanol activated (EA) and the ethanol non-activated (ENA) groups were treated with *d*-amphetamine in order to study their locomotor response. Considering that central catecholamines have been reported as participating in ethanol's stimulant effect (Pohorecky 1977) and *d*-amphetamine acts by enhancing the release of catecholamines, the hypothesis that the ENA group reacts less to it has been raised.

Secondly, based on previous data showing that mice become more responsive to ethanol's stimulating effect after chronic exposure (Masur and Boerngen 1980; Masur et al. 1986), the EA and ENA groups were chronically treated with ethanol and had their locomotor activity measured after a challenge dose of ethanol.

Material and methods

Animals. Albino Swiss male mice from our own colony were used. They were housed 10–13 per cage and kept at a room temperature of $23 \pm 2°$ C on a 12-h light-dark cycle. At the beginning of the experiment they were 3 months old and weighed 37 ± 2 g (mean $\pm$ SD).

Apparatus. The locomotor activity was measured in cages containing three photocells to detect horizontal movements, and measuring 40 (length) × 25 (width) × 20 (height) cm.

Drugs. A 10% (w/v) ethanol/saline solution was administered intraperitoneally (IP) in a volume sufficient to achieve the desired dose. Saline was administered in equivalent volumes; *d*-amphetamine was dissolved in saline and injected IP in a dose of 1 or 2 mg/kg.

Experiment 1. Forty-eight mice were randomly assigned to the experimental group and 12 mice formed the control group. Once a week all animals were placed into the activity cages and the number of lightbeam interruptions was recorded over 30 min prior to the injections. This was done in order to minimize the enhancement of exploratory behavior known to occur when rodents are exposed to a new environment which could mask the increase of activity induced by ethanol. After injecting ethanol and saline, respectively, to the experimental and control groups, the mice

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were immediately replaced into the activity cage for an additional 30 min recording (test period).

The doses of ethanol administered IP were 0.8; 1.2; 1.6; 2.0; 2.4; 2.8; and 3.0 g/kg. The higher doses were alternated weekly with the smaller ones, e.g., the mice received in one session 0.8 g/kg and in the next one 3.0 g/kg. All animals were injected with the same dose in each session.

Mice were classified as ethanol activated (EA) when for at least one of the seven administered doses their locomotor activity exceeded the 0.99 confidence interval calculated from the mean locomotor activity displayed by the control group during the same test period. Mice not fulfilling this criterion were classified as ethanol non-activated (ENA). A week later the EA and ENA groups had their locomotor activity measured following the administration of saline in order to determine possible differences unrelated to ethanol.

Finally, the EA and ENA groups were injected IP with 1.0 and 2.0 mg/kg *d*-amphetamine and the control animals again with saline for the last two weekly sessions. Their locomotor activity was recorded during a 60-min interval.

The activity measures were carried out between 1:00 and 4:00 p.m.

Experiment 2. Thirty-five mice were randomly assigned to the experimental group, and ten mice to the control group. The experimental group was divided into EA and ENA sub-groups following the same procedure described for experiment 1.

The EA and ENA mice were daily injected with 2.0 g/kg ethanol IP, during a 30-day period. The locomotor activity was measured on days 15 and 30, using the same procedure and dose of ethanol. The control group was similarly treated and tested with saline.

Results

Experiment 1

Seven out of the 48 mice from the ethanol-treated group were lost during the experiment. From the remaining animals, approximately one third (13 out of 41) did not show an increase in locomotor activity within the dose range of ethanol used (0.8–3.0 g/kg), being classified as ENA. The remaining mice displayed increased locomotor activity following at least one dose of ethanol (EA group). Figure 1 shows the results obtained from two EA and two ENA representative mice. A large variability in the activating dose was found, as exemplified in the same figure. Thus while one animal showed activation following 1.6 and 2.0 g/kg the other one had increased locomotion only after 2.4 and 2.8 g/kg.

Figure 2 shows the percentage of mice stimulated by the different doses of ethanol used. It can be seen that the large majority of animals increased locomotion with one to two doses, while a small number were activated with a wider dose range.

Drug-free locomotor activity of EA and ENA groups showed no difference (Student *t* test, $P>0.05$). They were equally active during the first exposure to the activity cage (30 min before ethanol administration) as well as at the eighth exposure, when their locomotor activity was recorded 30 min before and 30 min after saline injection. The

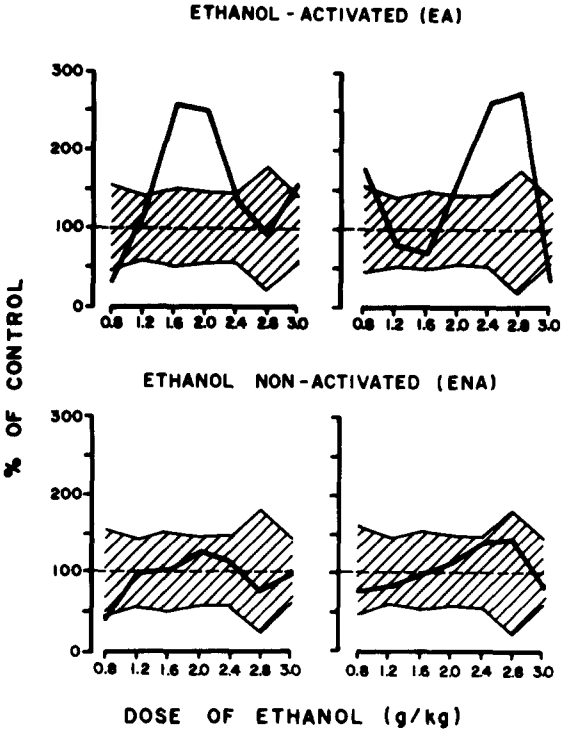

Fig. 1. Locomotor activity expressed as percentage of control of two mice from the ethanol activated (EA) group and two from the ethanol non-activated (ENA) group. Mean values of the control group (tested with saline) were considered as 100%. The *shadowed area* indicates 0.99 confidence interval

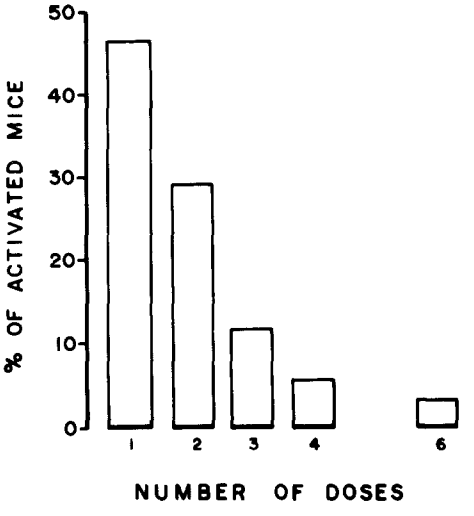

Fig. 2. Percentage of mice from the EA group ($n=28$) showing locomotor activation following one to six doses of ethanol. None of the animals was activated by the seven doses administered. Each animal received all doses

figures from the 60 min recording period were 569 ± 170 and 598 ± 120, respectively, for the ENA and EA.

Both groups (EA and ENA) showed a significant dose-dependent increase in locomotor activity following 1 and 2 mg/kg *d*-amphetamine (Fig. 3) compared to the control group (ANOVA followed by Ducan's new multiple range test: $P<0.05$). However, no difference was found between EA and ENA groups, indicating a similar responsiveness to amphetamine-induced locomotor activation.

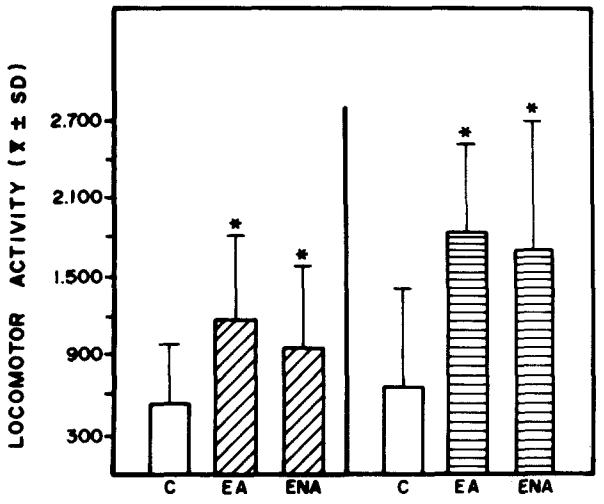

Fig. 3. Locomotor activity effects of 1 and 2 mg/kg *d*-amphetamine in ENA ($n=13$) and EA mice ($n=28$). Control animals ($n=12$) were tested with saline. $^*P<0.05$ when compared to controls. No difference between EA and ENA mice was found. □ Saline; ▨ Amphetamine (1 mg/kg); ▤ Amphetamine (2 mg/kg)

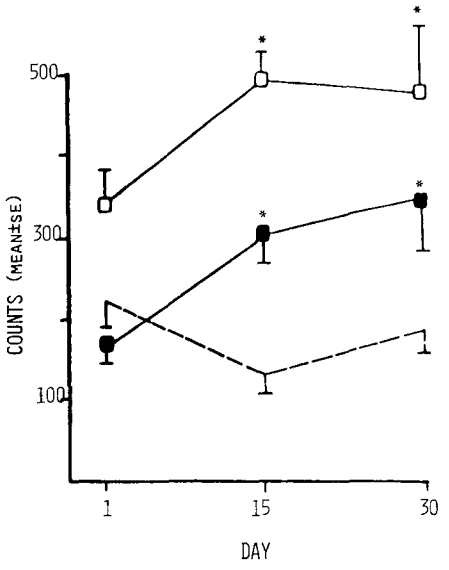

Fig. 4. Locomotor activity of EA (□—□; $n=23$) and ENA (■—■; $n=9$) mice after the 1st, 15th and 30th injection of 2.0 g/kg ethanol. The control group (----; $n=10$) was daily injected with and tested after saline. $^*P<0.05$ compared to control and to day 1. The EA significantly differed from ENA in the three tests

Experiment 2

Nearly one third of the mice (9 out of 32), as in experiment 1, failed to show locomotor activation following ethanol administration (three animals were lost during the long treatment period). Figure 4 shows the locomotor activity of EA and ENA groups injected at the beginning and on the 15th and 30th day of treatment with 2.0 g/kg ethanol. Ethanol initially did not increase the locomotor activity of the ENA group; however, after being treated with ethanol for 15 and 30 days, a stimulant effect was observed for this group. Furthermore, in the EA group the locomotor increase after chronic exposure to ethanol was higher when compared to the first ethanol administration. (ANOVA followed by Duncan's new multiple range test; $P<0.05$).

Discussion

The effect of ethanol on locomotor activity can be divided into two components – stimulation and depression – the latter being always achieved through dose increase.

As pointed out before (Reed 1985), human and experimental animals responses to ethanol are often reported as means, with variability being ignored. The present data show that mice belonging to the same strain displayed different sensitivities to the ethanol-induced locomotor activation. Thus, approximately one third of the animals did not show increased locomotor activity after acute administration of ethanol in doses ranging from 0.8–3.0 g/kg.

The question is, why do some animals apparently fail to present behavioral activation after ethanol? It has been shown that central catecholamines participate as mediators of the locomotor stimulant effect of ethanol. Thus, the inhibition of catecholamines synthesis by α-methyl-*p*-tyrosine (α-MT) antagonizes ethanol-induced locomotor activation, and doses of L-dopa without effect alone restore the locomotor stimulant action (Engel et al. 1974). The activation of central dopamine (DA) neurons was reported to occur by Mereu et al. (1984), who have showed that acute small doses of ethanol increased the firing rate of DA cells in the substantia nigra, pars compacta.

On the other hand, it has been suggested that the locomotor depressant effect of ethanol is mediated by the inhibitory neurotransmitter GABA (Cott et al. 1976; Liljequist and Engel 1982). Engel (1985) suggested that the biphasic effect of ethanol on the locomotor activity of mice could be explained based on the assumption that "small doses of ethanol produce a more marked effect on the catecholamine systems than on the GABA systems, masking the sedative effect of GABAergic activation and thus resulting in locomotor activation; after higher doses of ethanol the effects on the GABAergic mechanisms predominate, resulting in sedation and hypnosis".

The fact that EA and ENA mice were equally sensitive to the stimulant effect of amphetamine allows different interpretations. One possibility is that the catecholaminergic systems are affected differently by amphetamine and ethanol. However, data showing that old mice do not show ethanol's locomotor activation and are also less sensitive to amphetamine do not favour this hypothesis (Engel 1985). Another possibility could be that catecholaminergic responses of the EA and ENA mice to ethanol do not differ, the lack of ethanol-induced behavioral activation in the ENA mice being due to a hyper-responsiveness of the GABAergic system. Thus, the depressant component of ethanol could be counteracting the behavioral manifestation of the catecholaminergic activation in these mice. Data from experiment 2 showing that the initial absence of ethanol's stimulating effect in the ENA mice was reversed by chronic exposure to this drug could be taken to support this hypothesis. Further specific studies are required to help in clarifying this point.

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