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Effect of Ethanol on Aggression and Timidity in Mice

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Abstract. The effects of ethanol (0.4, 0.8, 1.6, and 2.4 g/kg p.o.) on behavior of aggressive, timid, and sociable male mice treated with the drug on paired interactions with non-aggressive males given water were investigated. Under control interactions, aggressive mice attacked their partners, timid mice showed defensive-escape activities though their partners were completely non-aggressive, and sociable mice intensively investigated their partners. A low dose of ethanol (0.4 g/kg) increased while higher doses (0.8 to 2.4 g/kg) reduced aggressive activities in aggressive mice. Ethanol (0.8 g/kg) also evoked aggressive behavior in non-aggressive timid mice but no dose of ethanol stimulated aggression in non-aggressive sociable mice. Ethanol altered timid defensive-escape activities only in the highest dose of 2.4 g/kg: this dose increased defences and escapes in aggressive males while it reduced defensive upright postures in timid mice. However, 2.4 g/kg of ethanol reduced also another upright movement (exploratory rearing) in timid mice. Sociable activities were not increased by any dose of ethanol tested. By contrast, 0.4 g/kg of ethanol reduced sniffing and following partners in sociable mice. Thus, ethanol exhibited relatively strong aggression-stimulating effects in aversively disposed subjects while the drug was not able to supress timid defensive escape behavior and to stimulate active non-aggressive contacts between strange male mice.

Key words: Ethanol – Aggression – Timidity – Fear – Sociability.

There is still limited knowledge on the effects of ethanol on social behavior in animals. Investigation has been focused mainly on ethanol effects on behavior in aggressive animals. Some studies reported reduced whereas others found increased intraspecies aggression after ethanol in various species (Tamimie, 1968; Chance et al., 1973; Kamback, 1973; Kršiak and Borgesová, 1973).

Studies testing the effects of a wide dose range of ethanol brought evidence that low doses of ethanol stimulate while higher ones inhibit spontaneous intermale aggressive behavior in fishes (Peeke et al., 1973) and in rats (Miczek and Barry, in press). The facilitation of on-going aggression does not seem to indicate an extraordinary aggression-stimulating potency as many drugs from various categories of psychotropic drugs have been shown to increase aggressive behavior in aggressive animals (Kršiak, 1974; Miczek and Barry, 1976). A question arises whether and under what conditions, ethanol can evoke aggressive behavior in non-aggressive animals.

There is a paucity of studies providing opportunity for dose response-data of ethanol on non-aggressive social behavior such as flight or social investigation in rodents. A medium and a higher dose of ethanol (1.2 and 3.0 g/kg, respectively) reduced defensive postures and sociable activities (sniffing and following partners) in rats (Kršiak and Borgesová, 1973). A non-ataxic dose of ethanol increased or unchanged flight and reduced social and sexual investigation in pairs of male mice (Chance et al., 1973; Cutler et al., 1975). The latter studies did not discriminate between passive and active flight. Mice can show defensive-escape activities not only as passive responses coerced by aggressive partners (passive flight) but also actively on interaction with completely non-aggressive partners (active flight termed timidity, Kršiak, 1975). The active flight of timid mice, which is selectively inhibited by benzodiazepines, seems to be a good measure for prediction of anxiety-reducing activity of drugs (Kršiak, 1975).

Ethanol effects on social behavior have been mostly tested on interactions of pairs of animals. In

some studies, where ethanol was administered to both interacting animals (Chance et al., 1973; Cutler et al., 1975), it is difficult to discriminate direct effects produced by administration of ethanol itself from those exerted by interaction with ethanol-treated partners. Ethanol-treated mice increased active flight in their untreated partners (Borgesová et al., 1971) and this effect was potentiated when both interacting mice were given ethanol (Kršiak and Borgesová, 1972). Behavioral changes induced by interaction with drugged partners have been found also in other experiments (e.g., McDonald and Heimstra, 1965; Silverman, 1966a; Kršiak and Steinberg, 1969; Cairns and Scholz, 1973; Kršiak and Borgesová, 1973; Miczek, 1974). Therefore, to discriminate pharmacologic effects from those induced by interaction with drugged partners ('interactional' effects, Kršiak and Borgesová, 1972), it is advisable to treat only one member of each pair or to use appropriate controls (Kršiak, 1974).

In the present study, effects of four doses of ethanol ranging from 0.4 to 2.4 g/kg on social behavior of aggressive as well as non-aggressive male mice were investigated. Interactions of pairs composed of a singly housed male and a non-aggressive group-housed male were used. Under this arrangement, some of the singly housed mice attacked their partners (aggressive mice) while some other isolates showed defensive-escape activities though their partners were completely non-aggressive (timid mice). The rest of the isolates intensively investigated their partners (sociable mice). To avoid confounding pharmacological and interactional effects, ethanol was always given only to one member of each pair – to the isolate. By recording several acts and postures involving similar type of movement but occurring in another behavioral context in the same animals, it was possible to assess selectivity of ethanol effects. Though the general aim of the present study was to obtain dose-response data on ethanol effects on social behavior in different types of mice, it was of particular interest to find whether ethanol is able to evoke aggressive behavior in non-aggressive mice and/or to reduce selectively the active flight (timidity).

METHOD

Subjects and Procedure

Male albino random-bred mice weighing 20–22 g at the beginning of the experimental housing were used. They were housed singly in self-cleaning cages or in groups of 10. The cages used for the individual housing were made of solid metal walls 13 cm high with wire-mesh floors (8 × 16 cm) which were placed on trays with wood shavings. Except on experimental days, the isolates were not handled throughout the isolation period. The mice kept in groups were housed in standard plastic cages 25 cm high with solid bottoms (22 × 38 cm) covered with wood shavings. All mice were housed in a natural day-and-night cycle under temperatures ranging from 22 to 24°C. Food and water were available permanently ad lib.

The mice were observed in transparent cages (20 × 30 × 20 cm) with wood shavings on the floor and open tops. The observation were performed in a quiet experimental room from 8:30 a.m. to 4:00 p.m. under moderate artificial dispersed lighting.

Social interactions were started after 3–6 weeks of isolation always involving one singly housed and one group-housed mouse in the observational cages. The isolates were allowed 30 min adaption in the observational cages before the group-housed partners were introduced; interactions ended after 4 min. Altogether three interactions were repeated 1 week apart with 89 pairs of singly vs. group-housed mice. Each isolate was paired with the same group-housed partner throughout the experiment. Ethanol (0.4, 0.8, 1.6, and 2.4 g/kg) was given as a 20% wt/vol solution in distilled water p.o. 30 min before the interaction to singly housed mice. Each isolate was treated once weekly and each received successively two doses of ethanol or water in a randomized order according to a Latin-square design (each mouse served as its own control). Thus, each dose was tested in 44–45 isolates. Group-housed mice were always given only water.

Measures

The incidence of the following behavioral acts and postures similar to those described by Grant and Mackintosh (1963) was recorded by a keyboard counters system: Sociable activities: Social sniff – sniffing the partner's head, flanks, genital or tail. Climb – the mouse places its forepaws on the partner's back, mostly in the shoulder region, and usually sniffs this area at the same time (Grant and Mackintosh called this "attempt mount"). Follow – following the partner by quietly walking. Timid activities: Alert postures – a sudden interruption of all movements with eyes and ears being directed toward the other mouse (attend). Escape – a rapid running or jumping away from the opponent (retreat and flee). Defence – the mouse responds to the partner's social behavior by raising the forepaws, hunching the back, or by rearing up on the hind legs with the head going up and forelegs extended (defensive or submissive upright posture). Aggressive activities: Attack – a fierce lunging at the partner from various sides often associated with biting. Aggressive unrest – walking around the partner (walk around, mince), or on its own axis (circle) walking to and from the partner (to-fro) and chasing the partner. Tail rattle – rapid vibrations of the tail were classified as an ambivalent activity reflecting both aggressive and flight tendency. Locomotion (non-social activities): Walk across cage – any walking which is apparently not related to the partner. Rear – the mouse stands only on his legs and usually sniffs air or walls at the same time.

The interobserver reliability of the recorded items was satisfactory, as determined by two observers recording independently the behavior of 18 mice in interactions lasting 200 s. The r_S values ranged from 0.7 to 0.8. The observer did not know which kind of treatment was given to the tested animals.

Data Analysis

The isolates were classified in three groups according to their behavior in the control interaction: aggressive isolates (exhibiting attacks), timid isolates (exhibiting escapes or defensive postures but no attacks), and sociable isolates (not exhibiting attacks, escapes,

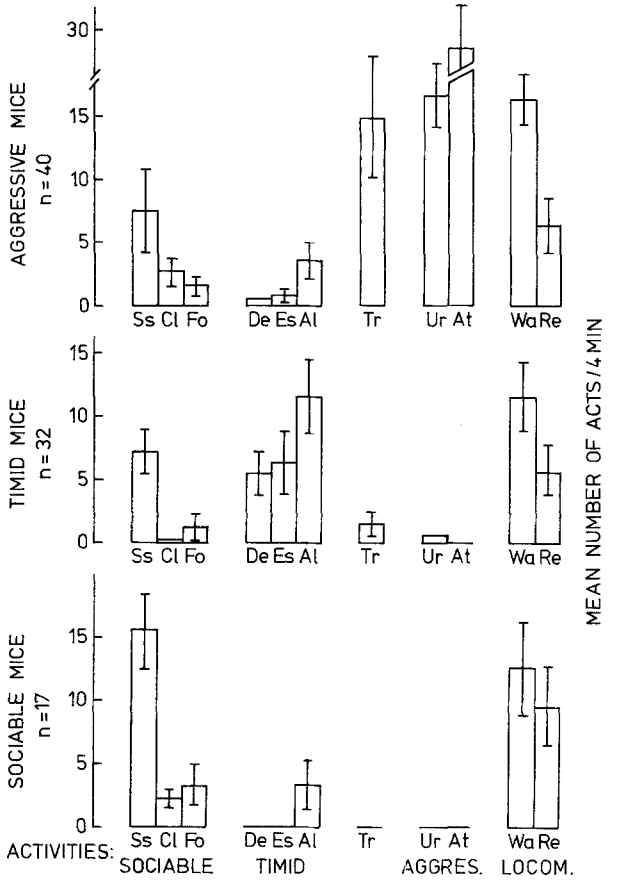

Fig. 1. Behavior of aggressive, timid, and sociable singly housed male mice in the control paired interaction with non-aggressive male mice. Code for abbreviations: *Ss* social sniffing, *Cl* climbing over partner, *Fo* following partner, *De* defensive postures, *Es* escape, *Al* alert posture, *Tr* tail rattling, *Ur* aggressive unrest, *At* attack, *Wa* walking across cage, *Re* rearing. Limits of confidence of means for $P = 0.05$ are given

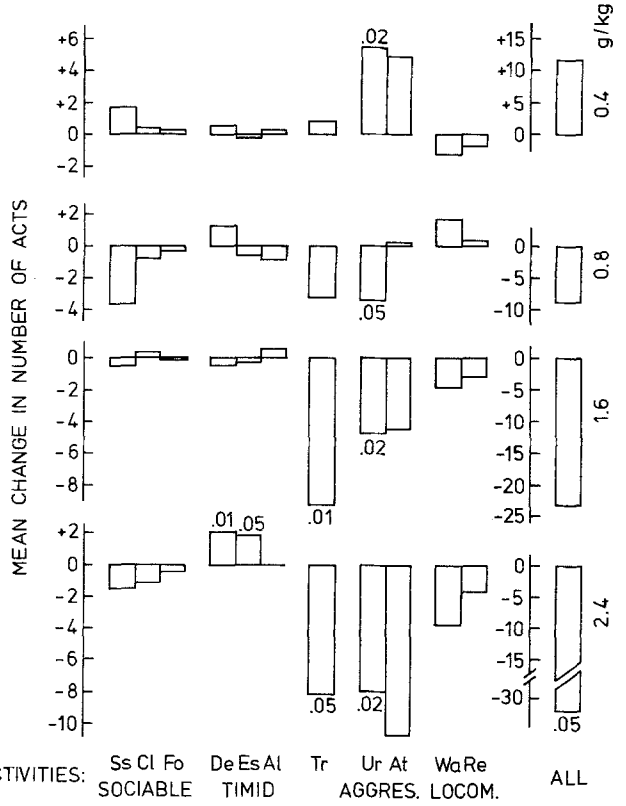

Fig. 2. Behavior of aggressive singly housed male mice given 0.4, 0.8, 1.6, and 2.4 g/kg of ethanol in paired interactions with non-aggressive male mice. The ordinate scale shows the number of acts during 4 min expressed as the mean difference from activity in the control interaction (depicted in Fig. 1)

or defensive postures). Since the individual type of behavior remained comparably stable upon repeating interactions under the conditions of the present experiment (Kršiak, 1975), the differences between the control and experimental values were examined by the two-tailed Wilcoxon matched-pairs signed-ranks test (Siegel, 1956) separately for each category of isolates.

RESULTS

Behavior in the Control Interaction

Forty singly housed males attacked their partners in the control interaction (Fig. 1). The aggressive isolates showed also a higher amount of aggressive unrest, tail rattling, and of locomotion. The second category of isolates ($n = 32$, Fig. 1) did not show attacks but in contrast exhibited a greater number of defensive postures, escapes, and alert postures even though their partners were completely nonaggressive. These isolates are therefore called the "timid" isolates (Kršiak, 1975). The rest of the isolates ($n = 17$, Fig. 1), which did not show attacks or defensive postures and escapes, exhibited a greater amount of social sniffing. They are called the "sociable" isolates.

Group-housed mice did not attack the isolates, neither did they show aggressive unrest, tail rattling, or alert postures. The activity of group-housed mice was largely composed of locomotion (walking across cage and rearing) while their active social behavior was limited to a smaller amount of approaching and sniffing the isolates. Defensive postures and escapes occurred in the group-housed mice only when interacting with aggressive isolates as passive responses to aggressive behavior of their partners.

Effects of Ethanol

Aggressive activities were stimulated by lower doses and inhibited by higher doses of ethanol. A dose of

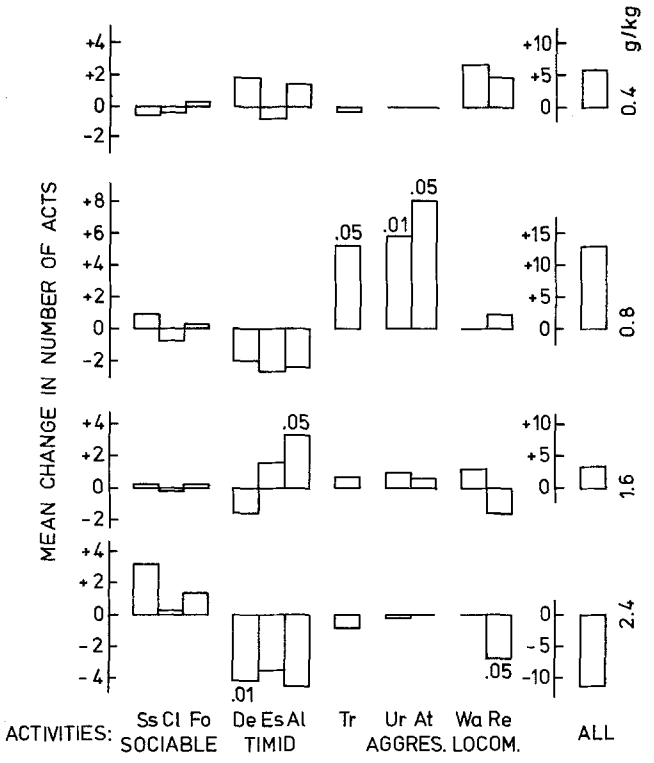

Fig. 3. Behaviour of timid singly housed male mice given 0.4, 0.8, 1.6, and 2.4 g/kg of ethanol in paired interactions with non-aggressive male mice. The ordinate scale shows the number of acts during 4 min expressed as the mean difference from activity in the control interaction (depicted in Fig. 1)

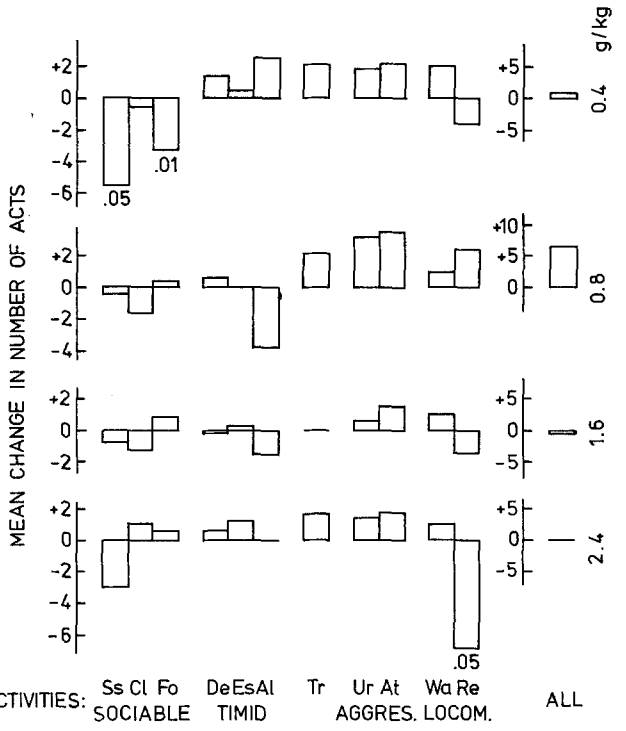

Fig. 4. Behavior of sociable singly housed male mice given 0.4, 0.8, 1.6, and 2.4 g/kg of ethanol in paired interactions with non-aggressive male mice. The ordinate scale shows the number of acts during 4 min expressed as the mean difference from activity in the control interaction (depicted in Fig. 1)

0.4 g/kg of ethanol increased the number of aggressive unrests in aggressive mice and 0.8 g/kg of ethanol stimulated significantly tail rattling, aggressive unrest and attacks in timid isolates (Figs. 2 and 3). On the other hand, 0.8, 1.6, and 2.4 g/kg of ethanol reduced aggressive unrest and tail rattling in aggressive mice (Fig. 2). However, the number of attacks was not decreased significantly even after 2.4 g/kg of ethanol in aggressive mice.

Timid activities were affected only by higher doses of ethanol. The number of alert postures in timid mice (Fig. 3) and that of defensive postures and escapes in aggressive mice (Fig. 2) was increased significantly after 1.6 and 2.4 g/kg of ethanol respectively. On the other hand, 2.4 g/kg of ethanol reduced incidence of defensive postures in timid mice (Fig. 3). However, this dose of ethanol also reduced another upright movement – rearing.

Sociable activities were not increased significantly by any dose of ethanol tested. A decrease of sniffing and following partners after 0.4 g/kg of ethanol in sociable isolates (Fig. 4) was the only significant change in sociable activities found.

Ethanol did not change significantly locomotion of isolates, with the exception of rearing, which was reduced after 2.4 g/kg of ethanol in timid and sociable mice (Figs. 3 and 4).

Lower doses of ethanol tended to increase while the higher ones to decrease the total number of all activities combined. However, this was never due to a uniform change of all activities in one direction.

DISCUSSION

It has been shown that low doses of ethanol increase while high doses of the drug decrease spontaneous intermale aggressive behavior in the convict cichlid *Cichlasoma nigrofasciatum* (Peeke et al., 1973) and in the rat (Miczek and Barry, in press). Present results indicate that ethanol exhibits a biphasic effect on aggressive behavior also in the mouse: a low dose of 0.4 g/kg stimulated while higher doses (0.8, 1.6, and 2.4 g/kg) reduced aggressive activities in aggressive mice. However, ethanol has not been reported to evoke aggressive behavior in non-aggressive animals. In the present study, ethanol stimulated aggressive behavior not only in aggressive mice but also in non-aggressive mice that exhibited timid defensive-escape activity on interaction with non-aggressive partners.

Of the other psychotropic drugs tested in the same model in a wider dose range, barbitone has stimulated some aggressive activities in timid mice while chlordiazepoxide, diazepam, chlorpromazine, imipramine, *d*-amphetamine, and LSD evoked little or no aggression in timid isolates (Kršiak, 1975). Accordingly, ethanol appears to be a relatively potent aggression-stimulating drug in male mice. However, it should be stressed that ethanol has not evoked aggression in non-aggressive sociable mice. Thus, ethanol stimulated aggressive behavior only in males which responded by aversive reaction (aggressive or timid) to a strange partner.

Also, other animal studies indicate that ethanol does not always evoke direct aggressive behavior between males even when tested in a wide dose range. Crowley et al. (1974) and Cressman and Cadell (1971) have not observed stimulation of aggressive dominant behavior when ethanol was given to male monkeys in a stable group whose members were familiar to each other, while ethanol given to singly housed male monkeys (Chamove and Harlow, 1970; Kamback, 1973) increased self-aggression and various aggressive responses. Nevertheless, ethanol still evoked some aggression-like displays, described as playful fighting, in the former two studies (Crowley et al., 1974; Cressman and Cadell, 1971). It is interesting to note that Crowley et al. (1974) have not observed playful aggression-like displays in their monkeys after various doses of pentobarbitone, methamphetamine, or morphine. This suggests that further investigation of aggression-evoking potency of ethanol should be focused also on aggression-like displays which lost aggressive function, and on ritualized and redirected derivatives of aggressive displays. It seems important to know whether ethanol facilitates efficiently not only direct aggressive behavior but also ritualized and redirected derivatives of aggressive displays which are supposed to have a bond-forming function in a number of animal species (Lorenz, 1966). The evidence for this effect on analogous behavior in man, such as laughter (Lorenz, 1966), may be relevant for better understanding of some causes and mechanisms of group drinking in man.

Ethanol significantly altered timid defensive-escape activities only in the highest dose tested (2.4 g/kg): this dose stimulated occurrence of defences and escapes in aggressive males while, by contrast, it reduced defensive upright postures in timid mice. As 2.4 g/kg of ethanol also reduced exploratory rearing in timid mice, it cannot be excluded that the lowered number of defences was due to a lowered capability to raise the front part of the body. On the other hand, chlordiazepoxide and diazepam inhibited defensive postures, escapes and alert postures of timid mice in relatively low doses which did not reduce concomitant behavioral activities (Kršiak, 1975). Other drugs tested in the same experimental situation exhibited a less selective timidity-reducing effect (barbitone, chlorpromazine, and imipramine) or rather increased (*d*-amphetamine and LSD) the isolation-induced timidity in mice (Kršiak, 1975). The lack of timidity-reducing effect of ethanol in singly housed mice seems to be in agreement with negative results of a major part of extensive literature on the experimental study of the tension-reducing effects of ethanol in animals and man reviewed by Cappell and Herman (1972). Ethanol has also a weak effect on passive defensive and submissive postures showed by subordinate rats on interactions with dominant partners (Kršiak and Borgesová, 1973; Miczek and Barry, in press).

Sociable activities were not increased by any dose of ethanol tested. By contrast, 0.4 g/kg of ethanol reduced sniffing and following partners in sociable isolates. These results corroborate and extend findings of previous studies which used a narrower dose range of ethanol and found reduced or unchanged amount of social and sexual investigation, gregariousness or contact behavior between male mice or rats (Chance et al., 1973; Cutler et al., 1975; Kršiak and Borgesová, 1973; Cappell and Latané, 1969; Tikal and Benešová, 1972). Thus, ethanol tested in relatively wide dose range and in diverse experimental conditions has not stimulated contact non-aggressive behavior between male mice or rats. Also in this respect ethanol seems to differ from benzodiazepines and barbiturates which stimulate sociable activities in male rodents. Diazepam, chlordiazepoxid, and barbitone increased sociability in timid and aggressive singly housed male mice (Kršiak, 1975 and in preparation). Amylobarbitone increased social investigation in rats (Silverman, 1966b) and chlordiazepoxide stimulated social sniffing in golden hamsters (Poole, 1973).

In summary, effects of ethanol on social behavior between strange male mice can be characterized by marked aggression-stimulating effects in aversively disposed subjects and a lack of timidity-reducing and sociability-stimulating activity. The profile of activity of ethanol on social behavior of male mice seems to differ from that of some other central depressants, particularly that from benzodiazepine anxiolytics.

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