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Lack of Increased Intermale Fighting Behavior in Mice After Low Ethanol Doses

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PÄIVÄRINTA, P. *Lack of increased intermale fighting behavior in mice after low ethanol doses.* PHARMACOL BIOCHEM BEHAV **42**(1) 35-39, 1992.—The effect of ethanol on intermale fighting behavior, measured mainly as the total fighting time, was studied using Swiss-Webster mice in 5-min encounters in a neutral arena (i.e., not the home cage). Ethanol treatment compared to control treatment had no statistically significant effect on fighting behavior when given to both equal-sized members of a pair of males socially isolated for a) 5 or 10 days at a dose of 0.4 g/kg IP; b) 4 weeks at 0.8 g/kg IP; and c) 38 weeks at 0.4 g/kg IP. Moreover, no significant effect was found when ethanol was given only to the expected dominant member of a pair, that is, to: a) a male isolated for 48 weeks confronting a younger and smaller group-housed male at 0.4 g/kg PO; and b) a male that had been pair housed with a female conspecific for 5 weeks confronting a group-housed male of equal age and weight at 0.4 g/kg IP. The results suggest that under these conditions ethanol does not lead to increased fighting behavior in Swiss-Webster male mice.

Fighting behavior Aggressive behavior Social isolation Ethanol Swiss-Webster mice

MANY studies have linked ethanol consumption with a variety of violent acts (2). Attempting to determine the relationship between the pharmacological effects of ethanol and aggression in humans is, however, extremely difficult and conflicting results have been obtained (2). Consequently, animal models have been used, with the bulk of the experiments being conducted on mice. The most common model of aggression in this species is the intermale aggression observed under conditions inducing social conflict. In this model, a male mouse is usually single housed (socially isolated) or, less frequently, pair housed with a female mouse for a period of days to months to induce aggressivity and then confronted with another male mouse. The results from studies investigating ethanol effects on intermale aggressive behavior have, however, been contradictory. Sometimes increased aggression has been seen (1,5,7), but more often ethanol has been found to have no effect or to reduce aggressive behavior (1,3). The use of varying experimental settings and approaches to quantify aggressive behavior make it hard to evaluate which factors are critical in the emergence of the claimed aggression-heightening effect of ethanol.

The present report summarizes our attempts to find out a specific situation where ethanol would enhance murine intermale aggressivity, with the final goal being the development of a reliable animal model of ethanol-induced aggressive behavior for pharmacological studies. A variety of conditions were selected for testing on the basis of earlier findings suggesting that these would be the most likely conditions to produce ethanol-induced increases in aggression. The total fighting time of an encountering mouse pair is a good indicator of the general level of aggressivity and can be reliably measured with a static charge sensitive bed (SCSB) system (12); thus, it was the main measure of aggressivity used in the present studies.

About the only universal factor in studies that have found increases in aggression after ethanol treatment has been the dose of ethanol, which in each case has been in the range of 0.3-1.0 g/kg. It also seems that studies using outbred albino mice have yielded most of the positive findings. Consequently, doses in this range were given in this study to outbred albino Swiss-Webster mice. A number of studies suggest that ethanol stimulates murine aggressive behavior only in a neutral arena, that is, not the home cage (1). This has been suggested to reflect the fact that ethanol can lead to behavioral disinhibition; in a novel place, aggressive behavior is otherwise suppressed but can be released by ethanol (10). Consequently, all experiments in the present study were conducted in a neutral cage. It has also been suggested that the effect of ethanol may depend upon the baseline level of aggressiveness, increasing low rates of aggression, and suppressing high rates (10). To induce various degrees of aggression, isolation periods of varying length were used (14): short term (5 and 10 days), intermediate (4 weeks), and long term (38 and 45 weeks). In addition, one experiment was carried out using pair housing (5 weeks) with a female instead of single housing. This procedure is known to be about as effective in inducing aggressive behavior as single housing (6) and has been associated with positive ethanol-aggression results (10,15).

Four of the six experiments conducted used an experimental setting where two isolates of equal size confronted each other, both being treated similarly. This design has been reported to yield positive results (5,7) and is also very suitable for SCSB recording of fighting behavior since it is not necessary to know which mouse of a pair might be the initiator of

TABLE 1
EXPERIMENTAL DESIGNS USED

Encountering Pair	Isolation time	Ethanol Administration			Treated Animal	Age (weeks)	Experiment number/ Results in Table
		Dose	Route	Before Test			
Isolate vs. isolate	5 days	0.4 g/kg	IP	20 min	Both	13 vs. 13	5/6
Isolate vs. isolate	10 days	0.4 g/kg	IP	20 min	Both	15 vs. 15	6/6
Isolate vs. isolate	4 weeks	0.8 g/kg	IP	30 min	Both	16 vs. 16	1/2
Pair housed vs. group housed	5 weeks of pair housing	0.4 g/kg	IP	20 min	Pair housed	18 vs. 18	4/5
Isolate vs. isolate	38 weeks	0.4 g/kg	IP	30 min	Both	64 vs. 64	3/4
Isolate vs. smaller group housed	45 weeks	0.4 g/kg	PO	30 min	Isolate	55–57 vs. 21–24	2/3

All fight-pairs consisted of male Swiss-Webster mice and encounters (5 min) took place in a neutral cage.

fighting or otherwise more aggressive. Some rat studies suggest, however, that for the proaggressive effect of ethanol to become evident the opponent must be subordinate to the isolated rat of the pair (8,9) and only the isolate must be administered ethanol. We observed in pilot experiments that after a few weeks of isolation or pair housing with a female mice developed a clear dominance over equal-sized or smaller group-housed mice. This led to fighting that was started and supported by the dominant mouse (no-treatment situation). Consequently, in two of the present experiments group-housed mice were used as opponents; in the other of these experiments, the opponent was also younger and smaller to make the dominant-subordinate situation even more evident. It is likely that SCSB recordings in these two experiments reflect the aggression level of the dominant animal.

Some of the experiments used pretesting without ethanol to establish two groups matched for aggressiveness. Although pretesting decreases the amount of fighting in later test encounters, the literature suggests that it does not interfere with production of proaggressive effects by ethanol since several of the studies reporting positive results have employed pretesting (13).

Ethanol was administered IP in all but one experiment, where it was given PO. IP administration is the general method, but the PO route has sometimes been associated with proaggressive effects (1). The experiments were done in dim light during the dark period, the natural wake period of mice.

METHOD

Quantification of Fighting Behavior

The equipment used to measure fighting behavior were an SCSB system and a video unit described in detail previously (12). In short, the SCSB system is a body-movement-sensitive electrical mattress interfaced to a microcomputer, capable of reliably quantifying fighting behavior as the total fighting time within a given time period, which in this study was 5 min. Fighting produces distinctively strong signals on the SCSB as compared to locomotor or other activity (except jumping) that after being digitized are easily picked up by the computer; locomotor activity can be measured simultaneously. Jumps add to the total fighting time, but usually these are of minor significance due to the short duration of an individual jump. Also, isolated Swiss-Webster mice show generally little jumping activity (12).

Subjects

Swiss-Webster mice (Charles River, Kingston, NY) were used. Animals arrived at 6 weeks of age and were group housed in Macrolon size III cages, 8–10 mice per cage. They were allowed to adapt for at least 3 weeks to the reversed 12L : 12D cycle with lights on at 2330. A portion of the male mice were then individually housed for 5 days to 45 weeks or pair housed with a female mouse for 5 weeks in Macrolon size II cages. The animal room had a temperature of 22–26°C and a relative humidity of 40–60%. Food (Ewos, R3, Södertälje, Sweden) and water were available ad lib except during testing.

Behavioral Experiments

Six experiments were conducted. Aggression was measured from videotaped records in the first and with the SCSB system in the other five. Experimental designs are shown in Table 1. For each encounter (lasting 5 min), a clean cage (Macrolon size II) was put on an SCSB mattress and a styrofoam lid was placed on top of the cage after putting a confronting mouse pair into the cage. Experiments were conducted during the dark period at 1300–1700 in the same room where animals were kept. The only light was that from the red 25-W lamp normally on during the dark period and a 15-W lamp at the injection table 3 m away from the arena.

Experiment 1. Male mice isolated for 4 weeks (n = 34, weight ± SD = 39.2 ± 2.9 g, age 16 weeks) were divided into encounter pairs matched for body weight. The pairs were then tested once for baseline fighting behavior with no treatment. The pairs were distributed into two treatment groups, ethanol (n = 9 pairs) and control (n = 8 pairs). Two days later, both mice of a pair in the ethanol group received ethanol 0.8 g/kg IP (0.1 ml/10 g body weight) 30 min before the encounters; animals in the control group received a similar treatment with saline. The experiment was videorecorded and reviewed on slow motion for measurement of total fighting time, number of fights, latency to first attack, number of bites, number of attacks, number of chases, and number of tail-rattles. Successive fighting episodes occurring 5 s or more apart were counted as separate fights when determining the number of fights. Attack, bite, chase, and tail-rattle were defined as described by Grant and Mackintosh (4). The experimental design was partly adopted from Lister and Hilakivi (7).

Experiment 2. Male mice isolated for 45 weeks (n = 16, weight 48.9 ± 5.7 g, age 55–57 weeks) were put into encounter pairs with younger and smaller group-housed male mice as opponents (n = 16, weight 39.8 ± 2.2 g, age 21–24 weeks). The pairs were distributed into two groups (both n = 8 pairs). The isolates of one group received ethanol 0.4 g/kg PO (in 8% tapwater solution) 30 min before the first encounter, no treatment before the second encounter 1 week later, and 1 week later, PO water before the third encounter. The isolates of Group 2 received the same treatments but in reversed order (water → no treatment → ethanol). Group-housed opponents always remained nontreated. The experiment was SCSB recorded for total fighting time.

Experiment 3. Male mice isolated for 38 weeks (n = 28, weight 41.9 ± 3.7 g, age 64 weeks) were divided into 14 encounter pairs matched for body weight and had three pretest encounters without treatment (3 days between all confrontations). Measures from the third pretest were used to distribute the pairs into two groups showing equal amounts of fighting. Subsequently, 30 min prior to the first treatment encounter half the pairs received ethanol 0.4 g/kg IP (0.1 ml/10 g body weight) and half the pairs an equal volume of saline, both subjects of a pair receiving the same treatment. On the second treatment encounter, the treatment order was reversed, that is, the pairs that previously received ethanol were now given saline and vice versa. Total fighting time was SCSB recorded. This experiment was treated as a repeated-measures design where each subject (= mouse pair) received two treatments, ethanol and saline.

Experiment 4. Male mice (n = 26, weight 37.7 ± 2.6 g, age 18 weeks) were pair housed with female mice of the same age for 5 weeks. The male of each pair then encountered a group-housed male opponent of equal weight and age initially three times without treatment and once with treatment, with 3 days between encounters. On the basis of the total fighting time of the third pretest encounter, the pairs were distributed into two similarly fighting groups, ethanol (n = 13 pairs) and control (n = 13 pairs). The ethanol group received ethanol 0.4 g/kg IP (0.1 ml/10 g body weight) and the control group an equal volume of saline 20 min before the encounters. Only pair-housed mice received treatments. Total fighting time was SCSB recorded. The pair-housing technique was adopted from Miczek and O'Donnel (10), and Yoshimura and Ogawa (15).

Experiment 5. Male mice isolated for 5 days (n = 68, weight = 31.3 ± 2.2 g, age 13 weeks) were divided into encounter pairs matched for body weight and the pairs distributed into two treatment groups matched for pair weight, ethanol (n = 17 pairs, weight of pairs = 62.6 ± 4.5 g) and saline (n = 17 pairs, weight of pairs = 62.4 ± 4.4 g). A pair consisted of mice unfamiliar to each other, that is, they were taken for isolation from different group cages. Both members of a pair received the same treatment, ethanol 0.4 g/kg IP (0.1 ml/10 g body weight) or saline 20 min before encounters. The experiment was SCSB recorded for total fighting time. On the basis of visual observations, if a pair showed no fighting but did exhibit jumping it received a total fighting time value of 0.

Experiment 6. This experiment was identical in design to Experiment 5 except that mice were isolated for 10 days (n = 68, weight = 33.2 ± 2.2 g, age 15 weeks; ethanol group, n = 17 pairs, weight of pairs = 66.5 ± 4.5 g; saline group, n = 17 pairs, weight of pairs = 66.3 ± 4.4 g).

Statistics

The measures of fighting behavior after ethanol and control treatments were analyzed with a two-way analysis of variance (ANOVA) with repeated measures in the first four experiments and a one-way ANOVA in Experiments 5 and 6. In addition, regression analysis was used in the first experiment to find possible significant correlations within the aggression measures.

RESULTS

Ethanol did not significantly increase aggression in any of the experiments. The F value representing the effect of ethanol relative to saline on the seven measures of aggression in Experiment 1 (Table 2) ranged from $F(1, 15) = 0.0003$, $p > 0.98$ to $F(1, 15) = 0.884$, $p > 0.36$. The corresponding value in Experiment 2 (Table 3) for total fighting time was $F(1, 14) = 0.70$, $p > 0.41$; for Experiment 3 (Table 4), $F(1, 12) = 2.4$, $p > 0.14$; for Experiment 4 (Table 5), $F(1, 24) = 0.57$, $p > 0.45$; for Experiment 5 (Table 6), $F(1, 32) = 0.39$, $p > 0.53$; for Experiment 6 (also Table 6), $F(1, 32) = 0.57$, $p > 0.45$.

Significant correlations were found in Experiment 1 between different measures of aggression. The number of bites correlated with the total fighting time in the four situations (two groups × two encounters) with r values 0.95–0.98 ($p < 0.0002$). The number of fights and the number of attacks correlated with r values 0.78–0.84 ($p < 0.021$).

TABLE 2

MEASURES OF AGGRESSIVE BEHAVIOR OF 4-WEEK ISOLATED MICE AS PAIRS (EXPERIMENT 1)

Measure of Aggressive Behavior Group	First Encounter (No Treatment)	Second Encounter (Ethanol 0.8 g/kg IP or Saline)
Total fighting time (seconds)		
Control group	12.8 ± 8.6	9.8 ± 6.0
Ethanol group	16.8 ± 9.4	10.8 ± 5.3
Latency to first attack (seconds)		
Control group	34.2 ± 44.5	51.2 ± 92.8
Ethanol group	57.2 ± 82.5	75.6 ± 82.7
Number of bites		
Control group	39.8 ± 27.6	39.3 ± 29.4
Ethanol group	54.0 ± 33.4	36.6 ± 23.8
Number of attacks		
Control group	13.4 ± 8.8	11.3 ± 7.1
Ethanol group	18.0 ± 8.2	17.1 ± 8.8
Number of fightings		
Control group	5.1 ± 2.0	4.6 ± 3.1
Ethanol group	6.0 ± 2.7	6.9 ± 4.9
Number of tail-rattles		
Control group	11.5 ± 4.9	12.1 ± 10.9
Ethanol group	11.2 ± 6.2	11.8 ± 8.4
Number of chasings		
Control group	1.0 ± 1.8	1.5 ± 2.0
Ethanol group	1.6 ± 2.1	2.2 ± 2.0

Both mice in a pair were treated. Control group, n = eight pairs; ethanol group, n = nine pairs. All pairs exhibited fighting behavior at both encounters. Means ± SD are indicated.

TABLE 3

TOTAL FIGHTING TIMES OF MOUSE PAIRS CONSISTING OF LONG-TERM (45 WEEKS) ISOLATES AND YOUNGER/SMALLER GROUP-HOUSED MICE AS OPPONENTS (EXPERIMENT 2)

Group Treatment	Total Fighting Time (seconds)	Pairs Fighting (%)
Group 1 (n = eight pairs)		
Encounter (ethanol 0.4 g/kg PO)	4.6 ± 5.6	75
Encounter (no treatment)	2.2 ± 2.3	75
Encounter (water PO)	1.9 ± 2.6	62.5
Group 2 (n = eight pairs)		
Encounter (water PO)	6.4 ± 7.5	100
Encounter (no treatment)	3.4 ± 3.6	87.5
Encounter (ethanol 0.4 g/kg PO)	0.8 ± 1.0	75

Opponents received no treatment. Means ± SD are indicated.

TABLE 4

TOTAL FIGHTING TIMES OF 14 PAIRS OF LONG-TERM ISOLATED MICE (38 WEEKS, EXPERIMENT 3)

Treatment	Total Fighting Time (seconds)	Pairs Fighting (%)
No treatment		
1	17.6 ± 13.6	100
2	12.8 ± 14.9	78.6
3	9.8 ± 12.5	92.9
Ethanol 0.4 g/kg IP	5.7 ± 7.3	92.6
Saline	8.7 ± 10.5	85.7

Both mice in a pair received the same treatment. Means ± SD are indicated.

TABLE 5

TOTAL FIGHTING TIMES OF MICE PAIRS CONSISTING OF A PAIR-HOUSED MALE (5 WEEKS) AND GROUP-HOUSED OPPONENT (EXPERIMENT 4)

Group Treatment	Total Fighting Time (seconds)	Pairs Fighting (%)
Ethanol group (n = 13 pairs)		
No treatment		
1	11.5 ± 7.6	100
2	7.4 ± 7.3	100
3	2.9 ± 2.2	100
Ethanol 0.4 g/kg IP	4.1 ± 3.5	92.3
Control group (n = 13 pairs)		
No treatment		
1	11.9 ± 11.8	100
2	8.5 ± 5.8	100
3	3.3 ± 3.4	92.3
Saline	3.3 ± 3.1	92.3

Means ± SD are indicated.

TABLE 6

TOTAL FIGHTING TIMES OF PAIRS OF SHORT-TERM ISOLATED MICE (ISOLATED FOR 5 OR 10 DAYS, EXPERIMENTS 5 AND 6)

Duration of Isolation Treatment Group	Total Fighting Time (seconds)	Pairs Fighting (%)
5-day isolation (n = 17 pairs/group)		
Ethanol 0.4 g/kg IP	0.1 ± 0.6	5.9
Saline	0.3 ± 0.5	23.5
10-day isolation (n = 17 pairs/group)		
Ethanol 0.4 g/kg IP	0.7 ± 1.8	11.8
Saline	0.3 ± 0.8	11.8

Both mice in a pair received the same treatment. Means ± SD are indicated.

DISCUSSION

Low ethanol doses did not significantly increase or decrease intermale fighting behavior in any of the experimental situations. The results are in agreement with the majority of findings suggesting that ethanol has no significant effect on intermale aggressiveness in mice (1,3).

The first experiment was videorecorded to obtain more detailed information of the possible aggression-enhancing effects of ethanol that might not be apparent from recording only the total fighting time with the SCSB system, as was done in later experiments. Nevertheless, there was no evidence that would suggest even a tendency of increased aggressive behavior after ethanol treatment. Within the seven measures of aggressive behavior, total fighting time correlated highly significantly with number of bites, reflecting the possibility that these two measures describe a similar aspect in the repertoire of aggressive behavior. The number of fights correlated quite well with the number of attacks, but neither correlated significantly with fighting or bites, suggesting that these former two measures describe another aspect of aggressive behavior that is not related to total fighting time or number of bites. This experiment was similar to one by Lister and Hilakivi (7) that obtained ethanol-induced increases in aggression in that both isolates of a pair were given 0.8 g/kg ethanol IP 30 min before the encounter in a neutral arena. In a later study, Lister and Hilakivi suggested the isolation period must be short, only about 5 days, to produce clear ethanol effects (5). Our results from experiments 5 and 6 do not, however, support this hypothesis with Swiss-Webster mice. Neither with 5 nor 10 days of isolation did ethanol have a significant effect on fighting. In looking at the results of Lister and Hilakivi (7), it seems that the NIH-Swiss mice are sensitive to isolation housing: After 10 days of isolation, 92% of the NIH-Swiss mice had turned aggressive, whereas only 12% of our Swiss-Webster mice showed fighting behavior after the same isolation period. It is possible that strain differences in sensitivity to isolation housing between Swiss-Webster and NIH-Swiss mice account for the reported difference in ethanol effects, but it is harder to explain why we observed no proaggressive effect with any of the isolation periods tested. If a certain baseline aggressiveness is needed for ethanol to show its proaggressive effect, it would have been expected to emerge somewhere within the range of isolation durations, 5 days to 45 weeks, used in the present experiments. Another difference is that Lister

and Hilakivi (7) used a combined aggression score, whereas total fighting time was used in the comparable experiments here.

In the second experiment, a procedure Maier and Pohorecky (8) used with rats was employed. They concluded that if there is no clear dominance hierarchy between the animals in the confrontation the effects of ethanol on aggressive behavior are variable (8). Using juvenile males as stimulus animals to ensure that the isolated adult rat was clearly the dominant animal in the test situation, they found an increased aggression score after treating the isolated rat with ethanol (8,9). In Experiment 2 here, however, ethanol did not make the isolated mouse more aggressive when a clearly subordinate (younger and smaller) mouse was used as an opponent. This could be due to species differences in this situation.

The two earlier studies that used pair housing with females yielded positive results (10,15). The experimental design used by Miczek and O'Donnel (10) was similar to that in Experiment 4 in that both used pair-housed vs. group-housed Swiss-Webster mice encountering in a neutral arena. The main differences were that, although both studies used several encounters per pair, Miczek and O'Donnel (10) used several different ethanol doses and changed the opponent for every encounter. These authors also used PO administration and testing during the light period of the light–dark cycle, whereas we used IP and dark period. It cannot be ruled out that ethanol some way interferes with the habituation to the fight partner.

Comparison between the experiments here show a clear effect of isolation on the total fighting time. First encounter fighting scores are bigger the longer the isolation period is if both mice of a pair are isolates (Experiments 1, 3, and 6). When the opponent is an equal-sized group-housed mouse, total fighting time is shorter than if the opponent is an isolate (Experiment 4); if the opponent is considerably younger and smaller than the isolate, fighting time is even shorter (Experiment 2). Total fighting also decreases progressively after the first encounter. The reason for this phenomenon may be habituation to the fight partner and/or to the test arena.

We have previously shown that after long-term isolation Swiss-Webster mice show strong stimulation of locomotor activity (recording done with one mouse per cage) after a low-dose ethanol treatment (11). The present results suggest that locomotor stimulation is not related to stimulation of fighting activity.

In conclusion, the present experiments failed to identify a situation in which small doses of ethanol increase intermale murine fighting behavior. Perhaps such experimental situations do exist, but the published reports in the field did not provide an adequate guide for discovering them.

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