Pharmacology Biochemistry & Behavior, Vol. 20, pp. 349–353, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Heightened Aggressive Behavior by Animals Interacting With Alcohol-Treated Conspecifics: Studies With Mice, Rats and Squirrel Monkeys

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Received 6 April 1983

MICZEK, K. A., J. T. WINSLOW AND J. F. DEBOLD. *Heightened aggressive behavior by animals interacting with alcohol-treated conspecifics: Studies with mice, rats and squirrel monkeys.* PHARMACOL BIOCHEM BEHAV **20**(3) 349–353, 1984.—Drug-free mice, rats and squirrel monkeys showed more aggression toward alcohol-treated conspecifics than under control conditions. Quantitative ethological analysis was used to assess the dose-dependent effects of ethyl alcohol on a range of aggressive, submissive, defensive, escape responses as well as on non-agonistic behaviors such as associative responses, grooming, and locomotor activities. Two experimental situations were studied: resident-intruder confrontations in mice and rats, and interactions between members of established groups of squirrel monkeys. After PO administration of ethyl alcohol to intruder mice and rats, the non-drugged resident mice and rats attacked, threatened, and pursued intruders at higher frequencies during 5-min encounters. Similarly, subordinate squirrel monkeys who were members of three established groups, when given alcohol, were grasped, displaced, and displayed to more frequently by non-drugged group members than after water control injections during the first 40 min after injection. This change in aggressive behavior by non-drugged animals was related to the alcohol dose given to the intruder or subordinate animal; near-ataxic alcohol doses (3.0 g/kg in mice, 1.7 g/kg in rats, 1.0 g/kg in squirrel monkeys) altered the behavior of animals whose prevalent pattern is defensive and submissive so that they were the subject of most frequent and intense aggression.

Alcohol Ethanol Aggression Agonistic behavior Defensive responses Indirect drug effects

RESEARCH on the link between alcohol and aggression has focused chiefly on the aggressor. Systematic laboratory studies have examined alcohol effects on a range of aggressive and defensive behavior patterns in a variety of situations and species ranging from fish to primates (for reviews see [17,21]). We have confirmed the biphasic effect of alcohol on aggression by demonstrating that low acute doses of ethanol may enhance attack and threat behavior of mice and rats confronting intruders [7, 18, 23].

We now focus on a large and consistent increase in aggressive behavior by drug-free animals who interact with an animal that is given alcohol. These substantial effects on aggression may be brought about by alcohol-induced changes in the opponent's or partner's behavior. A detailed analysis of the interactive pattern of fighting may reveal potential sources for this important alcohol effect. When all combatants are subjected to a drug treatment, the indirect drug effects remain undetected [21,24]. Mice or rats may attack other members of their species more often when the opponents have received *d*-amphetamine, chlordiazepoxide, delta-9-tetrahydrocannabinol, phencyclidine, LSD or alcohol [2, 13, 14, 15, 16, 18, 29, 31]. This is not true of all drugs, however, for example, chlorpromazine treatment rendered mice less likely subjects of attacks by drug-free opponents [3].

In order to examine the species generality of the indirect or social alcohol effects, we studied resident mice and rats confronting alcohol-treated intruders, and we investigated the agonistic interactions of squirrel monkeys toward an alcohol-treated group member.

METHOD

Animals

Male and female adult Swiss-Webster mice (COBS CFW (SW) BR, Charles-River Breeding Laboratories, MA) were housed in polycarbonate cages (48 cm long, 27 cm wide, 20 cm high). The mice were housed either in male-female pairs or in unisexual groups of ten according to experimental assignment. Adult male and female Long-Evans rats (Charles-River Breeding Labs.) were housed in pairs in wooden cages, with a clear Plexiglas front (47 cm wide, 77.5 cm long, 49 cm high). Cage floors were lined with pine shavings. Access to Purina Rodent Chow and water was unrestricted. Separate vivaria for mice and rats were kept at constant temperature (22±1°C), humidity (30–40%), and light cycle (12 hr on/12 hr off). Additional male rats were housed singly in standard hanging stainless steel wire cages.

Twelve adult male and female squirrel monkeys (*Saimiri sciureus*) belonged to three separate groups, described in detail previously [19]. Each group was housed in a large room (2.4 m long, 2.2 m wide, 2.4 m high). The composition of each group was similar to each other and comprised nine to ten animals, including adults, juveniles and infants of both sexes. The monkeys were of Peruvian and Columbian origin. Temperature (22–26°C) and light (12 hr on/12 hr off) followed a daily cycle, and humidity was kept constant at 45–55%. The rooms could be viewed through large one-way vision windows (103 cm wide, 119 cm high).

Ethanol Preparation and Administration

Using 100% ethanol (U.S. Industrial Chemicals Co.) and distilled water, separate solutions for each dosage were prepared in concentrations ranging from 1% to 17% w/v. In mice, the following ethanol doses were administered in a volume of 1 ml/100 g body weight: 0.3, 1.0, 1.7 g/kg; the 3.0 g/kg dose was prepared in a 15% concentration and administered in a 2 ml/100 g volume. In rats, the ethanol doses included 0.1, 0.3, 0.6, 1.0 and 1.7 g/kg which were given in volumes of 1 ml/100 g body weight. In monkeys, the following doses were given: 0.1, 0.3, 0.6, and 1.0 g/kg; the injection volume was 3 ml/kg. The route of administration was PO using a stainless steel gavage in all three species.

Behavioral Testing and Measurements

The behavioral tests consisted of confrontations between residents and intruders following the protocols by Miczek and O'Donnell [22] for mice and Miczek [15] for rats. Group-housed male mice, termed "intruders," were introduced individually into the cage of a stimulus resident male. The female and pups were removed from the cage for the duration of the intruder test. All male mice, housed in male-female pairs, reliably attacked, threatened, and pursued intruders as shown previously [22,23]. Intruder mice reacted to the attacks by the resident stimulus animals with a pattern of defensive, escape and defeat responses [25]. Single-housed male rats were placed as "intruders" into the cages of resident male-female pairs. Confirming earlier experiences with these test situations, after repeated tests resident males showed the pattern of attack, threat, and pursuit toward intruder males who responded with defensive, submissive and escape reactions (e.g., [6,15]). As previously, resident-intruder tests were scheduled in the latter portion of the light phase of the photocycle for mice, and in the dark phase for rats.

Resident-intruder tests were conducted for 5 min in mice and for 10 min in rats, beginning with the stimulus male's first attack bite. Two experienced observers, one focusing on the resident situmuls animal and the other on the intruder, analyzed the videotaped tests. The observers' reliability was established before the start of the alcohol experiments by viewing and analyzing videotapes, until less than 10% variation was obtained. Each observer depressed one of 16 possible keys on a portable console when a defined behavioral item occurred and released the key when it stopped. The consoles were interfaced directly with a PDP 11/23 laboratory computer (Digital Equipment Corporation, Maynard, MA). The catalogue of behavioral items included escapes, defensive upright posture, walking, rearing, grooming for the alcohol-treated intruder mice, and attack bites by the resident stimulus animal. Similarly, for intruder rats, escapes, defensive upright posture, submissive supine posture, crouching as well as walking and rearing were recorded for intruder rats; in addition, sideways threats, attack bites, aggressive posture, pursuits, nipping bites, ano-genital contacts and locomotion were monitored in resident rats.

The protocol for experiments in the squirrel monkeys followed a previously developed format [19]. Interactions in the groups of monkeys were observed and recorded using the focal animal technique [1]. Five min after the alcohol administration, an observer who was unfamiliar with the treatment, was seated in front of the one-way vision window and operated a MORE microprocessor device for data collection (Observational Systems, Seattle, Washington). The observer focused on the alcohol-treated monkey and recorded continuously for two hours, in 20-min segments, the social and solitary behavior of this animal using an exhaustive behavioral catalogue. The list of behavioral items, based on the definitions by Hopf *et al.* [10], included social behaviors such as touching, huddling, inspecting the partner, aggressive elements such as displacing, grasping, restraining, and displaying directed toward another group member in the form of genital displays and chin thrusts. Instances of successful and attempted food thefts were recorded. In addition, all occurrences of social and aggressive behavior that were directed toward the focal animal were recorded; these included being touched, being inspected, being joined in a huddle, being displaced or yielding, being grasped, being restrained, being displayed to, and having food stolen from. Further elements of social significance, but without a specific target animal, included vocalizations and olfactory marking such as urine washing, anogenital rubbing, back rolling, rubbing the chest, back, or muzzle, and sneezing. These elements occur most often in dominant squirrel monkeys [9]. All incidences of feeding, drinking, foraging, locomotor activity, stationary alert posture, and sitting posture with curled tail were measured. The salient elements of aggressive, submissive and solitary behavior in squirrel monkeys are illustrated in Miczek and Gold [20].

Experimental Design

Three to five baseline tests were conducted in order to adapt the animals to the oral injection procedure, and to establish stable levels of aggressive behavior. Thereafter, intruder mice and rats, and subordinate monkeys underwent a schedule of five to six experimental treatments, including vehicle control. In mice and rats, experimental treatments consisted of administration of acute alcohol doses 15 min before being placed into the cage of the stimulus animal. In the monkey experiments, each designated animal was removed from the group, administered with alcohol, returned to the group, and observations of group interactions began 5 min after alcohol administration. The sequence of alcohol doses which included the water vehicle was systematically varied. Each experimental treatment was scheduled once a week for the mice and twice a week for rats and monkeys.

Statistical Evaluation

Frequency and duration of behavioral elements of mice and rats were analyzed with a one-way fixed factor analysis of variance. The data are based on the 5- or 10-min test periods after the first attack bite. Monkeys were assigned to categories of dominant or subordinate based on the amount of aggressive and submissive behavior they engaged in. The resulting groups concurred with previous observations [19,20]. In this report we focus on the data from the subordinate group. The data of 20-min segments were analyzed with a two-way fixed factor analysis of variance. Dunnett's t-test comparisons were calculated when significant F values were obtained. A p level of <0.05 for two-tailed distributions was accepted as statistically significant.

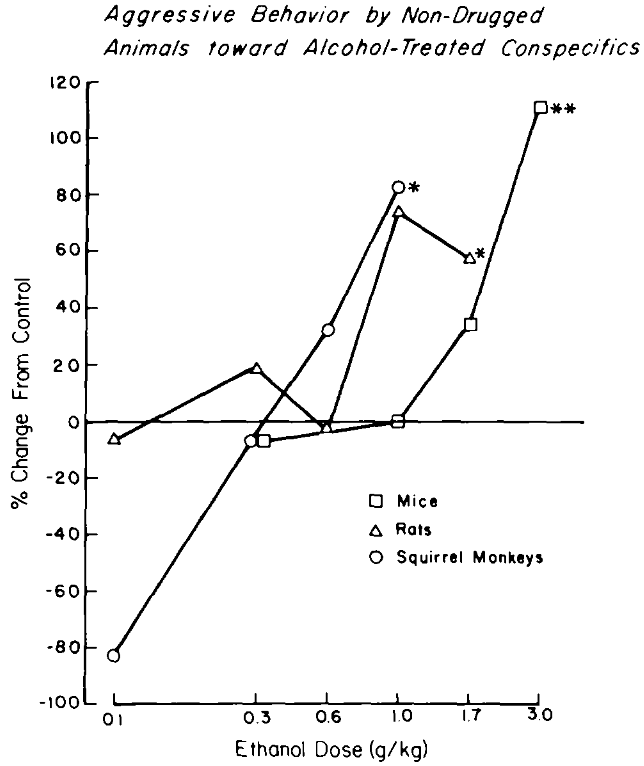

FIG. 1. Changes in aggressive behavior by non-drugged mice (□), rats (△), and squirrel monkeys (○) as a function of the alcohol dose that is administered to the animal toward whom aggression is directed. The alcohol effects are expressed as percent changes from the control level. During control tests, the average frequency of attack was 25.3±3.3 (Mean±S.E.M.) for resident mice, and 15.2±2.0 for resident rats; the average frequency of aggressive elements by non-treated monkeys was 1.8±0.5. Statistically reliable changes are indicated by asterisks (*$p<0.05$, **$p<0.01$).

RESULTS

During water vehicle control treatments resident mice and rats attacked intruders promptly and reliably; the mean frequency of attack bites was 25.3 (±3.3 S.E.M.) for mice, and 15.2 (±2.0 S.E.M.) for rats; the mean latency to the first attack bite was 5.9 (±0.9 S.E.M.) sec for mice, and 31.6 (±5.9 S.E.M.) sec for rats.

Drug-free mice, rats, and squirrel monkeys significantly altered their behavior toward alcohol-treated animals. Specifically, stimulus resident mice attacked, threatened and pursued intruder mice with increasing frequency, when the latter received higher doses of alcohol. After receiving the 3 g/kg alcohol dose, intruder mice were attacked twice as often as in the water vehicle control condition. Similarly, stimulus resident rats attacked alcohol-treated intruder rats with higher frequencies, when the latter received higher alcohol doses. The measures for attack bites and aggressive postures in undrugged stimulus rats were nearly twice as high after the intruders had been administered with 1.7 g/kg alcohol than after water vehicle. Figure 1 portrays these effects as percent change from control by summing the elements of attack, threat and pursuit into a summary value of "aggressive behavior."

In squirrel monkeys, alcohol produced a similar pattern of effects. Alcohol treatment caused subordinate monkeys to be grasped, to be displayed to, and to be displaced at significantly elevated frequencies during the first 40 min of observation. This change is portrayed in summary values in Fig. 1.

TABLE 1

EFFECTS OF ALCOHOL ON LOCOMOTOR ACTIVITY (MEAN DURATION IN SECONDS ± S.E.M.)

Alcohol (g/kg)	Mice (9)	Rats (9)	Squirrel Monkeys (5)
0	13.21 ± 4.27	27.10 ± 9.99	201.1 ± 74.80
0.1	—	15.30 ± 5.03	254.4 ± 94.44
0.3	8.69 ± 1.53	10.20 ± 3.10	200.3 ± 98.44
0.6	—	15.60 ± 5.74	113.8 ± 32.00
1.0	9.51 ± 3.30	22.70 ± 4.10	80.2 ± 38.12
1.7	9.93 ± 2.48	18.60 ± 5.01	—
3.0	11.86 ± 4.39	—	—

Numbers in parentheses indicate number in study.

Analysis of the escape behavior by alcohol-treated mice revealed that the intruders responded appropriately to the increased attacks by the stimulus animals. More than 50% of the intruder's escapes follow within one second of the resident stimulus animal's attacks under control conditions, and even at the highest alcohol doses this synchrony between the intruder's escapes and the resident's attacks was preserved. However, at the highest alcohol dose (3.0 g/kg), intruder animals spent significantly less time in the defensive upright posture, and also their rearing activity outside of the fighting episodes declined. Concurrently measured locomotor activity in the form of walking across the cage and grooming remained unimpaired (Table 1).

At the highest alcohol doses (1.0, 1.7 g/kg) intruder rats engaged significantly longer in the submissive supine posture ($p<0.05$), and less in the defensive upright posture ($p<0.05$). Locomotor behavior remained unaltered by the currently studied alcohol doses (Table 1), but the 1.7 g/kg dose impaired rearing activity. Similarly, during the first 45 min after alcohol administration subordinate monkeys yielded more often to other group members ($p<0.05$). Alcohol doses up to 1 g/kg did not alter their feeding, drinking, foraging, vocalizing, olfactory marking, nor their total locomotor activity (Table 1), although signs of ataxia started to appear at the highest dose. At the 1 g/kg alcohol dose, the monkeys showed significantly more frequent and prolonged bouts of inactive responses such as sitting and huddling that were accompanied by touching and inspecting the partner. One hour after alcohol administration the pattern of motor activities had returned to control levels.

DISCUSSION

Alcohol consistently altered the behavior of animals whose prevalent response pattern is submissive and defensive so that they were the subject of heightened aggression. This effect was dose-dependent, occurred in all three species, and in two different situations, resident-intruder confrontations and group interactions. Drug-free mice and rats attacked, threatened, and pursued alcohol-treated intruders more often, and subordinate squirrel monkeys, when given alcohol, were the recipients of more frequent aggressive responses from their drug-free group members. The present findings are consistent with earlier reports on similar indirect aggression-heightening alcohol effects in mice [31] and rats [18]. However, this effect is not always seen, in spite of detailed measurements of the non-drugged animals' behavior [2, 5, 12].

In view of the many drug effects on aggression that occur only in a specific situation and species (for reviews see [17,21]), the present observations may have wider generality. Attack behavior towards an intruder, and aggressive behavior in established social groups represent behavior patterns with different intensity and rate, and may have separate biological functions; yet, alcohol reliably enhanced the aggression-provoking cues in all three species.

Most research focuses on alcohol effects in subjects who engage in aggressive behavior or are prone to do so (for reviews see [21,24]). The most intriguing finding is that low acute alcohol doses can enhance aggressive behavior in fish, mice, rats, dogs, and monkeys under circumscribed conditions (e.g., [4, 11, 18, 23, 26, 27, 30, 31]).

In intruder mice and rats, higher alcohol doses reduced the display of defensive postures in reaction to increased attacks. This decline in defensive postures has been interpreted to indicate less anxiety in intruder animals [31]. Yet, the concurrent impairment of rearing outside of the fighting context favors the suggestion that at the higher alcohol doses animals are unable to raise the front part of their body to perform defensive postures [11].

What are the critical behavioral and physical features that are changed by alcohol and that provoke more frequent aggression? Dixon [8] studied social behavior in non-drugged mice, interacting with intruders that drank diazepam for one or 14 days; he pointed to drug-induced changes in the olfactory properties of mouse urine as a source for increased aggression. Indeed, alterations in hormone-dependent pheromone secretions profoundly alter aggressive behavior in rodents and also primates [28]. Considering the relatively short time course of alcohol effects in intruder or subordinate animals, a hormonal or pheromonal mechanism for provoking aggression appears less likely. By contrast, behavioral changes in alcohol-treated animals may represent inappropriate social signals in situations of conflict. Although undetected by the human observer, species members readily recognize alcohol-induced subtle changes in the social behavior of the intruder or group member, and they adjust their behavior accordingly.

Our observation of a rise in aggressive behavior due to interaction with an alcohol-treated animal seems relevant to the human situation. High levels of aggression may be seen *towards* individuals whose behavior is altered by alcohol.

ACKNOWLEDGEMENTS

The present research was supported by U.S.P.H.S. research grant AA-01522. We thank R. Russo for expert technical assistance. We also acknowledge the assistance of J. Baler, S. Maggied, P. Meehan, M. Nuccitelli.

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