Psychopharmacology (1997) 131 : 287–295

ORIGINAL INVESTIGATION

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Increased aggression after ethanol self-administration in male resident rats

Received: 17 September 1996/Final version: 9 December 1996

Abstract In order to study experimental alcohol intake that leads to heightened aggression, we established ethanol self-administration in aggressive rats. The focus was on low doses of self-administered ethanol and to assess their effects on aggressive behavior in resident rats, using a limited access paradigm followed by a 5-min confrontation with an intruder. In the first phase of the experiment, rats were established as "residents", and their consistent aggressive behavior in confrontations with an intruder was verified. In the second phase, these resident rats were trained to self-administer alcohol, using a sucrose-fading technique. In the third phase, alcohol self-administration was followed by intruder confrontations in order to study the effect of alcohol on aggression. Confrontations after ethanol consumption leading to low (5–20 mg/dl) and moderate (20–50 mg/dl) blood alcohol concentration (BAC) were compared to confrontations without alcohol, each animal serving as its own control. On average, the group showed no change in aggressive behavior after low or moderate ethanol intake. However, six out of 16 individuals significantly increased the number of attack bites and the duration of aggressive behavior by up to 90% after alcohol self-administration. When these rats were assigned post-hoc to an alcohol heightened aggression group, the group was characterized by a 40% increase in number of attack bites and a 90% increase in aggressive posture over control (BAC 0 mg/dl), whereas the alcohol non-heightened aggression group showed no significant changes. These results extend previous observations of increased aggression in a subpopulation of animals after experimenter-administered ethanol in mice, rats and monkeys to self-administered alcohol. Using this animal model, individuals showing enhanced or reduced aggression after oral alcohol self-administration can be characterized behaviorally, physiologically, and neurochemically.

Key words Aggression · Alcohol · Individual differences · Oral self-administration · Rat

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Introduction

Alcohol has been linked to many types of aggression and violence in humans in many cultures and historical periods (Bushman and Cooper 1990; Roizen 1993). Only recently the effects of alcohol on aggression, and specifically alcohol heightened aggression, have been studied in animal models. However, many studies have reported alcohol to decrease aggressive behavior, usually at high doses in many species, mainly due to the sedative, incapacitating, and intoxicating effects of alcohol (e.g. Krsiak and Borgesova 1973; Crowley et al. 1974; Lagerspetz and Ekqvist 1978; Smoothy and Berry 1983; Mos and Olivier 1988). In contrast, several studies have shown alcohol to increase aggression (e.g. Ellman et al. 1972; Chance et al. 1973; Miczek and Barry 1977; Pettijohn 1979; Blanchard et al. 1987a; Lister and Hilakivi 1988). Many of these latter studies focused on lower ethanol doses, the oral route of administration, and the time since administration when blood alcohol concentrations are in the ascending phase. Still, there are perplexing divergent outcomes in experimental studies of ethanol effects on aggressive behavior, when conditions are matched. Important progress was achieved when *individuals* became the focus instead of group averages. While the group as a whole may show no change, some individuals more than double their aggressive response after low doses of alcohol, whereas other individuals are equally or less aggressive. These individually differentiated alcohol effects on aggressive behavior were demonstrated for a

number of species, including squirrel monkeys, rats and mice (Miczek et al. 1992, 1993, 1994, 1997).

An initial task for the study of alcohol effects on aggression in animals is to select a specific type of aggression and a route of administration for alcohol. In this study, resident-intruder confrontations were used to engender aggressive behavior in laboratory rats, similar to aggressive behavior displayed by feral rats. The confrontations are repeatable and allow for detailed quantification of aggressive and motor behaviors, and comprise mostly "offensive" instead of "defensive" aggressive behavioral elements, which are differentially affected by alcohol (Blanchard et al. 1987b; Miczek et al. 1993). In addition, this type of aggression lends itself to controlled experimentation, in which duration of encounters and timing of aggression relative to alcohol administration can be manipulated, and discrete behavioral elements can be unambiguously monitored and quantified.

The second important factor in studying the effect of alcohol on aggression is the way in which alcohol is administered. Typically, the experimenter administers the alcohol, and this mode is the most practical for animal experiments but does not resemble alcohol intake in humans. Most studies on aggression have used alcohol administered as a bolus by the experimenter (see Bushman and Cooper 1990). A few studies have actually explored the effects of self-administered alcohol on dominance status and social interactions in group-housed animals (e.g. Ellison 1981; Blanchard et al. 1987c; Crowley and Andrews 1987). However, in large groups of animals it is difficult to monitor individual animals adequately. During alcohol self-administration in group-housed animals, more than one animal has access to the same drinking spout. Even if lick-sensors are used and continuous video registration of drinking behavior (Blanchard et al. 1987c), actual fluid intake or blood alcohol levels for individual animals cannot be monitored in real time. Similarly, it is difficult to ensure stable hierarchical structures in colonies with several males, because dominance status may change, and after initial interactions to establish dominance during group formation few aggressive encounters actually occur (Blanchard et al. 1984, 1987b).

In the first phase of the present experiment, rats were established as residents and tested for consistent aggressive behavior when confronted with an intruder. In the second phase, these resident rats were trained to self-administer alcohol, using a sucrose-fading technique adapted from Samson (1986). In the third phase, alcohol self-administration was followed by a confrontation with an intruder. In this way, both alcohol self-administration and the aggressive encounter are precisely specified in start and end time, and can then be monitored and quantified in detail for each individual rat. The objective was to establish whether certain individuals would show increased aggressive behavior after alcohol self-administration, as shown previously with experimenter-administered alcohol in monkeys, rats and mice. This is a prerequisite for a model which resembles the human situation more closely, and which may be used in future experiments to study neurobiological and physiological aspects of the individual differences in the alcohol-heightening effects on aggression.

Materials and methods

Subjects

Male Long-Evans rats (Charles River, Wilmington, Mass., USA), weighing 350–375 g at the start, were housed each with a female in a large stainless steel cage ($70 \times 45 \times 45$ cm) with sawdust bedding and a clear Lexane front panel. The cages were equipped with a wooden panel and a wooden structure to provide cover and gnawing material. The female rats' Fallopian tubes were ligated under ketamine (100 mg/kg) and xylazine (9 mg/kg) anesthesia, to prevent changes in behavior due to the presence of pups. Food and water were provided freely except during the daily period of ethanol self-administration (see below). The cages were kept in a temperature (20–21°C) and humidity (40–50%) controlled vivarium under a reversed light cycle (lights on between 2000 and 0800 hours). All experimental procedures were carried out in accordance with the NIH Guide for the care and use of laboratory animals.

Resident-intruder confrontations: stable baseline of aggression

Three weeks after being housed with a female, the male resident rats confronted a naive smaller male intruder rat (250–300 g) twice a week for 5 min, as described previously (Miczek 1979). In brief, the female rat was removed from the resident's cage for the duration of the confrontation. The confrontations were terminated 5 min following the first attack bite by the resident, after 20 bites, or after 5 min if no attack occurred. Typically, the resident displays a species-specific pattern of aggressive behavior, consisting of pursuits, threats and attacks. Latency to the first attack and total number of attack bites were monitored during at least five resident-intruder tests. Resident rats were selected that did not reach the maximum of 20 bites during confrontations, but still showed consistent attack behavior resulting in defeat of the intruder, as defined by the intruder showing a supine posture for at least 5 consecutive seconds and emitting 20–30 kHz ultrasonic vocalizations.

Ethanol self-administration

Sixteen resident rats were trained to drink a 10% ethanol solution using a sucrose-fading technique (Pfeffer and Samson 1986; Samson 1986). During this time, no further intruder tests were conducted. At first, a 10% sucrose (w/v) solution was presented for 6 days to induce drinking behavior. Thereafter, ethanol was mixed in with the sucrose in increasing concentrations (5, 7.5 and 10% ethanol v/v). After stabilizing intake of the 10% sucrose/10% ethanol solution, the sucrose was removed gradually (7.5, 5 and 2.5%) until a 10% ethanol solution without sucrose was presented. The fading procedure took 4–5 weeks, followed by a 10-day period to stabilize self-administration of the 10% ethanol solution (see bottom of Fig. 1A). Solutions were presented daily between 0800 and 1000 hours for 15 min. During the access period, the female was removed and the resident was confined to a smaller area of its home cage, using an inverted stainless steel hanging cage ($25 \times 20 \times 18$ cm). A plastic 50 ml centrifuge tube with a rubber stopper and curved ball-type sipper tube (Ancare Corporation, Bellmore, N.Y.,

USA) was mounted on the front of this smaller cage. Aliquots of 25 ml of solution were presented, and bottles were weighed before and after self-administration. Neither water nor food was available during this 15-min access period. Solutions were prepared fresh every day and kept refrigerated overnight to ensure a similar fluid temperature upon presentation. Food was removed overnight and presented after the access period, to prevent variation in alcohol absorption and blood alcohol concentration (BAC) due to a different stomach content (Goldstein 1983). Animals were weighed once a week for calculation of ethanol intake per bodyweight; the males gained weight over the course of the experiment to 500–600 g and the females to 400–450 g. Ethanol intake (in g/kg) was calculated using the following formula:

$$\text{Intake (g/kg)} = \text{Intake (ml)} \times \text{ETOH (\%)} \times \text{Density ETOH (0.794)/Bodyweight (g).}$$

Resident-intruder tests: effects of ethanol self-administration on aggressive behavior

After establishing stable ethanol self-administration (i.e. less than 10% daily variation in intake), intruder tests were conducted twice a week immediately following the 15-min ethanol access period. Maximally, four resident rats were tested between 0830 and 1100 hours, in random order. After 15 min ethanol access, in the absence of the female, motor behavior of the resident was recorded on videotape for 5 min, followed by the confrontation with a naive male intruder. As before, the confrontation lasted for 5 min after the first attack bite, regardless of the number of bites, or 5 min if no attack occurred. Behavioral responses were analyzed afterwards, using a customized computerized scoring program (Tufts data acquisition program; see Miczek 1982). The following non-social behavioral elements were recorded: walking, rearing, digging, self-grooming, inactive, lying, eating, drinking; the social behavioral elements recorded were: nasal contact, anogenital contact, allogrooming, attack bite, aggressive posture, sideways threat, chasing (see Miczek 1979). Duration of total aggressive behavior was calculated by combining the measures for aggressive posture, sideways threat, allogrooming and chasing. Behavioral data were grouped according to the blood alcohol concentration which was determined after each confrontation (see below), with each animal serving as its own control. Because of the scheduled daily ethanol self-administration sessions, "control" encounters were conducted between 0830 and 1100 hours but *before* ethanol self-administration, or more than 4 h afterwards (BAC 0 mg/dl). No solution was offered before "control" encounters, in order not to disrupt daily ethanol intake. In total, at least five control encounters and ten alcohol encounters were conducted for each individual, with at least 1 day in between encounters. The animals were fed after the intruder test or the self-administration session, whichever came last.

Blood alcohol concentration

Immediately following each intruder test, a 0.1 ml blood sample was taken from the orbital sinus under isoflurane inhalation anesthesia. Blood samples were stored in 0.9 ml trichloroacetic acid and analyzed for ethanol content using an NADH assay (Sigma, St Louis, Mo., USA) and UV detection at 340 nm wavelength (Spectronic 21, Bausch & Lomb, Rochester, N.Y., USA).

Data analysis and statistics

Frequency and duration of behavioral categories were analyzed for each animal using a repeated measures analysis of variance (ANOVA), with "days" as repeated variable. The behavioral data were grouped according to the concentration of blood alcohol that was measured immediately after the intruder confrontation: low alcohol (BAC 5–20 mg/dl), moderate alcohol (BAC 20–50 mg/dl), and control (BAC 0 mg/dl). Data were averaged across all animals for zero, low and moderate BAC and analyzed using an ANOVA. In addition, animals were assigned to an alcohol-heightened-aggression (AHA) group or an alcohol-non-heightened-aggression (ANA) group (see Results section). For comparison of the AHA and ANA groups the data were converted to percent of control, and analyzed using a one-way ANOVA with a Dunnett's post-hoc test.

Results

Baseline levels of aggression

Resident rats were included in the study that showed 5–15 bites during a 5-min confrontation with an intruder. In the experimental confrontations without alcohol (BAC 0 mg/dl) the average time spent in aggressive behavior (i.e. aggressive posture, sideways threat, allogrooming and chasing combined) was 88.6 ± 11.2 s. The average number of attack bites was 8.6 ± 1.5 (SEM). Although the levels of aggression during intruder tests held before the daily ethanol access period seemed slightly higher than during intruder tests held 4 h afterwards, these data were not significantly different.

Ethanol self-administration

During acquisition of ethanol self-administration, all resident rats readily consumed the 25 ml of a 10% sucrose solution presented. Adding ethanol to this solution decreased fluid intake, with most rats dropping their intake considerably when a 10% ethanol solution was presented (Fig. 1A). The average amount consumed on day 10 after acquisition was 4.5 ± 0.5 ml fluid, or 0.60 ± 0.09 g/kg ethanol. After acquisition of ethanol self-administration, ethanol intake yielded blood alcohol concentrations in a range from 5 to 50 mg/dl (Fig. 1B). Blood alcohol levels in excess of 50 mg/dl were rarely seen. No behavioral signs of intoxication, such as loss of coordination, were observed in any of the animals. For each animal, the relationship between ethanol intake, as calculated from fluid intake (in g/kg) and the actual blood alcohol concentration (in mg/dl), was established. Based on these data, confrontations were categorized as "low" (5–20 mg/dl) and "moderate" (20–50 mg/dl) "doses" of ethanol.

Effects of self-administered ethanol on motor behavior

Motor behavior (i.e. walking, rearing, grooming, digging) was monitored in the resident's home cage during a 5-min period preceding each intruder test. An increase in the duration of digging was observed

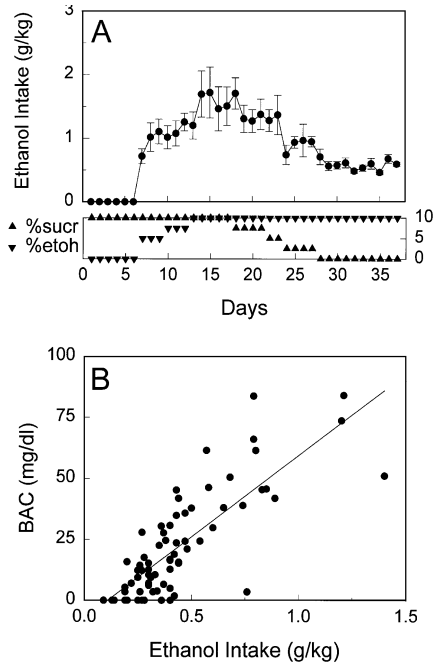

Fig. 1A, B Ethanol self-administration in resident rats. **A** Acquisition of self-administration using a sucrose-fading procedure. Ethanol intake in g/kg during a daily 15-min access period is calculated from intake of a sucrose/ethanol solution, presented in concentrations as shown in the *bottom part* of the graph ($n = 8$). **B** Blood alcohol concentration as a function of ethanol intake. Blood samples were taken immediately after a 5-min intruder confrontation which followed a 15-min ethanol self-administration session ($n = 4$)

at low BAC (11.8 ± 1.6 mg/dl) ($F_{2,3} = 7.07$, $P = 0.026$; Dunnett's *t*-test low versus 0 BAC $P < 0.05$). A decrease was observed in the frequency and duration of rearing after ethanol self-administration at moderate BAC (43.0 ± 6.8 mg/dl) (frequency: $F_{2,3} = 20.34$, $P = 0.002$; duration: $F_{2,3} = 14.7$, $P = 0.005$; Dunnett's *t*-test moderate versus 0 BAC $P < 0.05$ for frequency and duration). The increase in digging may represent an activating effect of low doses of alcohol, whereas the decrease in rearing may represent the onset of sedative effects of alcohol at higher doses. However, no significant changes were observed in walking or inactive behavior (Table 1). No evidence was found for intoxicating effects of alcohol, such as loss of coordination.

Effects of self-administered ethanol on aggressive behavior

Behavioral data were averaged for all resident rats ($n = 16$), in confrontations without alcohol (BAC 0 mg/dl), "low" alcohol (BAC 11.6 ± 1.4 mg/dl), and "moderate" alcohol intake (BAC 41.2 ± 3.0 mg/dl). Although there was a trend for an increase in some categories of aggressive behavior, no significant effects were found after drinking low or moderate amounts of alcohol, in comparison with confrontations without alcohol (Table 2).

Table 1 Effect of alcohol on locomotor behavior. Behavior was measured in the home cage of each resident rat 5 min prior to the intruder test. *Asterisks* indicate significant changes from BAC 0 mg/dl (repeated measures ANOVA, $P < 0.05$). Data are averages of raw data (± SEM) ($n = 4$)

BAC (mg/dl)	0	Low	Moderate
Frequency			
Rear	12.85 (± 1.65)	12.00 (± 2.11)	6.98 (± 2.41)*
Walking	31.40 (± 2.86)	34.90 (± 1.30)	32.10 (± 9.14)
Digging	1.98 (± 0.94)	2.87 (± 1.07)	1.24 (± 0.53)
Groom	2.67 (± 0.91)	0.72 (± 0.26)	0.96 (± 0.40)
Inactive	32.10 (± 1.75)	35.15 (± 1.02)	31.66 (± 8.22)
Eating	1.86 (± 0.65)	2.03 (± 0.69)	2.32 (± 1.16)
Drinking	0.85 (± 0.46)	1.59 (± 1.20)	1.06 (± 0.81)
Duration (s/5 min)			
Rear	43.27 (± 9.42)	33.52 (± 8.43)	19.76 (± 7.72)*
Walking	41.72 (± 3.53)	37.79 (± 3.92)	33.86 (± 11.56)
Digging	2.28 (± 1.25)	5.67 (± 1.9)*	1.79 (± 0.82)
Groom	18.24 (± 6.71)	5.07 (± 0.82)	6.23 (± 4.01)
Inactive	143.40 (± 9.17)	153.70 (± 20.97)	129.60 (± 29.26)
Eating	6.90 (± 0.75)	7.43 (± 3.50)	9.16 (± 4.35)
Drinking	5.51 (± 3.00)	7.59 (± 5.81)	5.91 (± 5.66)

However, there appeared to be marked individual differences in alcohol's effects on aggression. Some resident rats showed significant increases in frequency and/or duration of several categories of aggressive behavior, such as sideways threat, attack bites and aggressive posture. Figure 2 illustrates how resident rat R04 increased aggressive behavior after alcohol self-administration. Two behavioral elements are highlighted, frequency of attack bite and duration of sideways threat, with data from all confrontations presented rank-ordered according to blood alcohol concentration. It is apparent that the highest values of the frequency of attack bites and duration of sideways threat occur during confrontations after alcohol intake and not during control confrontations. In contrast, Fig. 3 illustrates a comparable data portrayal from a resident rat who did not show alcohol-heightened aggression.

Because of these clear individual differences in aggressive behavior after ethanol self-administration, the animals were divided post-hoc into two groups, an alcohol-heightened-aggression group (AHA) and an alcohol-non-heightened-aggression group (ANA), similar to a previous approach (Miczek et al. 1992). For each individual, the average frequency and/or duration of aggressive behavior during alcohol confrontations was compared to control confrontations. The criterion for inclusion in the AHA group was an increase of 40% or more in at least three aggressive behaviors (i.e. aggressive posture, sideways threat, attack bites, allogrooming, and chasing). Animals which failed this criterion were included in the ANA group. There were no apparent differences in ethanol intake between both

Table 2 Effect of alcohol on aggressive behavior. Behavior was measured in the home cage of each resident rat during a 5-min confrontation with a male intruder. No significant changes from BAC 0 mg/dl were observed (repeated measures ANOVA, $P < 0.05$). Data are averages of raw data (± SEM) for all animals ($n = 16$)

BAC (mg/dl)	0	Low	Moderate
Frequency			
Rear	4.36 (± 0.8)	4.44 (± 1.03)	4.58 (± 0.98)
Walking	37.64 (± 1.84)	45.89 (± 3.02)	41.91 (± 3.12)
Digging	1.93 (± 0.65)	2.19 (± 0.78)	2.33 (± 0.64)
Self-grooming	1.16 (± 0.26)	0.87 (± 0.19)	1.03 (± 0.16)
Nasal contact	17.65 (± 1.33)	18.90 (± 1.71)	17.32 (± 1.65)
Anogenital contact	4.20 (± 0.83)	4.79 (± 0.93)	4.47 (± 0.94)
Attack bite	8.63 (± 1.52)	8.34 (± 1.30)	9.47 (± 1.42)
Allogrooming	3.73 (± 0.61)	4.09 (± 0.71)	4.55 (± 0.80)
Aggressive posture	8.59 (± 1.56)	9.20 (± 1.34)	11.00 (± 2.61)
Sideways threat	15.31 (± 1.97)	18.28 (± 1.42)	19.05 (± 3.78)
Chasing	2.71 (± 0.58)	2.65 (± 0.55)	3.37 (± 0.71)
Duration (s/5 min)			
Rear	11.4 (± 3.01)	10.46 (± 3.00)	9.03 (± 2.44)
Walking	38.26 (± 2.98)	46.99 (± 5.27)	40.92 (± 3.64)
Digging	3.03 (± 1.07)	3.01 (± 1.19)	3.18 (± 0.90)
Self-grooming	2.81 (± 0.69)	2.16 (± 1.01)	3.92 (± 1.65)
Nasal contact	40.51 (± 4.26)	36.98 (± 5.07)	33.54 (± 4.66)
Anogenital contact	10.92 (± 2.28)	10.88 (± 2.39)	11.25 (± 2.23)
Allogrooming	7.81 (± 1.85)	7.55 (± 1.71)	9.06 (± 1.98)
Aggressive posture	32.88 (± 7.77)	33.14 (± 6.79)	38.09 (± 7.84)
Sideways threat	42.87 (± 4.66)	46.23 (± 4.35)	48.49 (± 6.70)
Chasing	3.06 (± 0.70)	2.73 (± 0.58)	3.53 (± 0.83)
Total aggression[a]	88.62 (± 11.22)	91.20 (± 8.24)	100.80 (± 14.52)
Attack latency	59.1 (± 10.0)	55.9 (± 9.2)	61.7 (± 12.3)

[a] Combined measure of allogrooming, aggressive posture, sideways threat, and chasing

groups [BAC AHA ($n = 6$): 30.3 ± 2.8 mg/dl versus ANA ($n = 10$) 27.5 ± 1.4 mg/dl].

As shown in Fig. 4, five out of six animals showed an increase in aggressive posture at moderate blood alcohol concentration, whereas three out of six showed an increase at low BAC. The duration of aggressive posture ($n = 6$) at moderate BAC is significantly higher compared to control confrontations (Fig. 4, bottom panel; $F_{2,5} = 7.39$, $P = 0.022$; Dunnett's t-test moderate versus 0 BAC $P < 0.05$). In the AHA group, five out of six rats showed a 30% or higher increase in total duration of aggressive behavior, and four out of six rats showed a similar increase in frequency of attack bites. In contrast, none of the animals in the ANA group showed an increase of more than 15% in those measures.

When data from the AHA ($n = 6$) and ANA ($n = 10$) groups were compared during control confrontations without alcohol, there were some significant behavioral differences. At BAC 0 mg/kg, AHA animals showed an increased duration of walking (AHA 46.7 s versus ANA 32.1 s; $F_{1,15} = 8.63$, $P = 0.015$), whereas ANA animals showed increased chasing (frequency: ANA 3.7 versus AHA 1.6; $F_{1,15} = 6.84$, $P = 0.026$; and duration: ANA 3.9 s versus AHA 1.7 s; $F_{1,15} = 5.40$, $P = 0.043$) and increased total time spent on aggressive behavior (ANA 110.3 s versus AHA 70.3 s; $F_{1,15} = 5.76$,

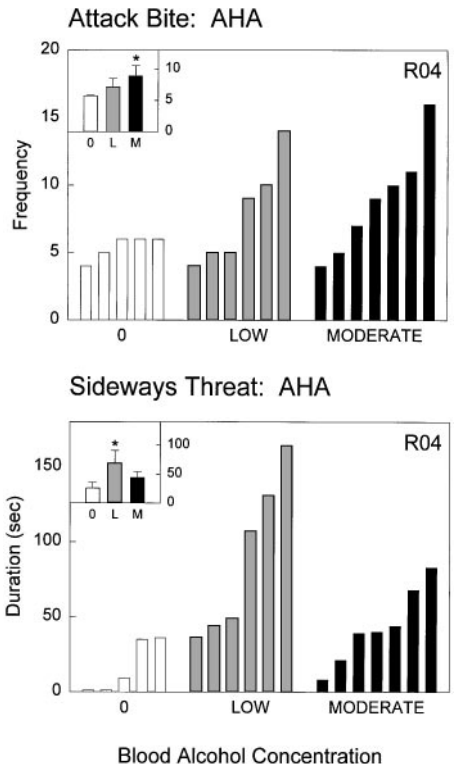

Fig. 2 Heightened aggression after ethanol self-administration. Aggressive behavior was measured during a 5-min intruder confrontation following a 15-min period of access to ethanol. All confrontations for one individual in the AHA group are shown, grouped by blood alcohol concentration (low BAC: 10.1 ± 1.8 mg/dl; moderate BAC: 49.6 ± 8.5 mg/dl) and rank-ordered. *Top panel:* frequency of attack bite. *Bottom panel:* duration of sideways threat. *Inserts:* averages ± SEM. *Asterisks* indicate a significant difference between alcohol and control confrontations (one-way ANOVA followed by Dunnett's test; $P < 0.05$). *AHA*, alcohol heightened aggression

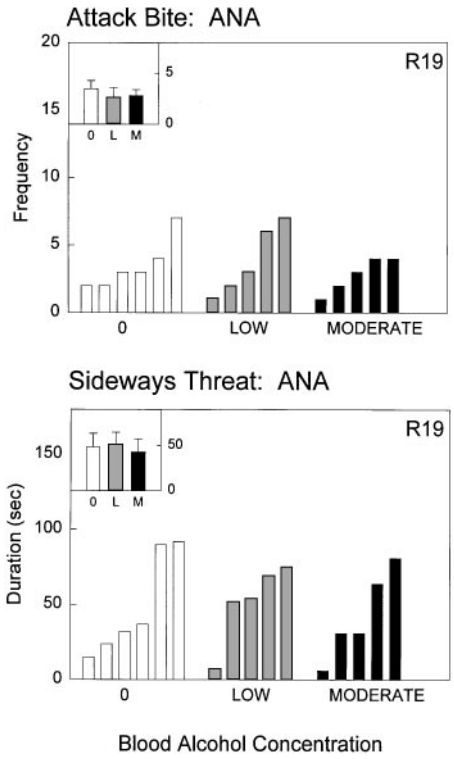

Fig. 3 Non-heightened aggression after ethanol self-administration. All confrontations for one individual in the ANA group are shown, grouped by blood alcohol concentration (low BAC: 5.6 ± 1.0 mg/dl; moderate BAC: 33.8 ± 9.0 mg/dl) and rank-ordered. *ANA*, alcohol non-heightened aggression. See also legend to Fig. 2

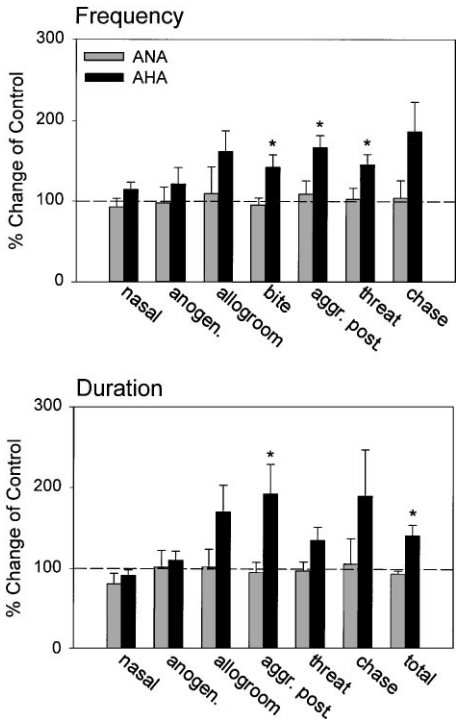

Fig. 5 Changes in social and aggressive behavior during a 5-min intruder confrontation following ethanol self-administration. Data are expressed as percentage change of control, i.e. confrontations without ethanol self-administration. Data for confrontations with low and moderate BAC have been combined for each individual and were averaged per group (AHA group: 30.3 ± 2.8 mg/dl; ANA group: 27.5 ± 1.4 mg/dl). *Top panel:* frequency. *Bottom panel:* duration. *Grey bars:* alcohol non-heightened aggression (ANA) group ($n = 10$). *Black bars:* alcohol heightened aggression (AHA) group ($n = 6$). *Asterisks* indicate a significant difference between ANA and AHA groups (one-way ANOVA, $P < 0.05$)

$P = 0.037$). In order to compare the effect of alcohol on aggression in both groups, all data were converted to percentage change of control.

The AHA group ($n = 6$) showed significant increases in aggressive behavior after ethanol self-administration compared to the ANA group ($n = 10$) (Fig. 5). Main effects were observed in the AHA group for the frequency and duration of aggressive posture (frequency: $F_{1,15} = 8.22$, $P = 0.017$; duration: $F_{1,15} = 7.39$, $P = 0.022$), frequency of sideways threat ($F_{1,15} = 6.09$, $P = 0.033$), frequency of attack bites ($F_{1,15} = 8.30$, $P = 0.016$), and total duration of aggressive behavior combined ($F_{1,15} = 14.6$, $P = 0.003$). Other aggressive behavior, such as allogrooming and chasing, showed a trend in the same direction. There were no differences between both groups in non-social behavior (walking, rearing, self-grooming) or in non-aggressive social behavior (nasal contact, anogenital contact) after ethanol consumption (data not shown).

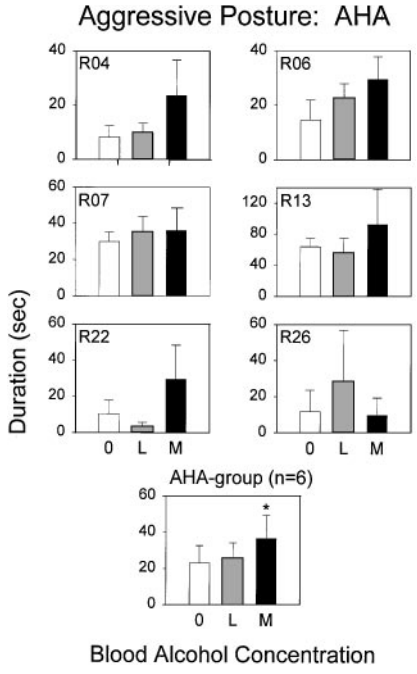

Fig. 4 Increase in duration of aggressive posture after ethanol self-administration. All individuals in the AHA group are shown (*top six panels*) as well as the group average (*bottom panel*). Data are averages ± SEM for control confrontations (*white bars*), confrontations at low BAC (*grey bars*, 5–20 mg/dl) and moderate BAC (*black bars*, 20–50 mg/dl). *Asterisks* indicate a significant difference between alcohol and control confrontations (one-way ANOVA followed by Dunnett's test; $P < 0.05$)

Discussion

Substantial increases in several elements of aggressive behavior were observed after a short period of alcohol self-administration in a subpopulation of Long-Evans rats. The increased aggression after alcohol did not characterize the group on average, but emerged only when the focus was on individual animals rather than the group. These results confirm previous studies in mice, rats and monkeys, which showed heightened aggression in certain individuals but not others after alcohol administered by the experimenter (Blanchard et al. 1987a; Miczek et al. 1992, 1993, 1994).

The presently implemented methodology successfully engendered self-administration of alcohol in resident rats, leading to BACs most commonly of 5–50 mg/dl. A clear correlation existed between the amount of fluid consumed and the actual blood alcohol concentration. This was true for most of the rats individually, although some tended to consume low volumes reaching low but not higher BACs. The intake was lower than observed in parallel studies with singly housed animals, in which the same method for acquisition of drinking was used (van Erp et al. 1994b). It is common to induce alcohol self-administration in individually housed rats, and typically BACs between 50 and 150 mg/dl are reached (e.g. Mello 1973; Grant and Samson 1985). A possible explanation for the lower intake of resident rats is the fact that these rats live with a female in an enriched environment, and are therefore less susceptible to the rewarding effects of alcohol than rats housed singly in stainless steel cages (Kulkosky et al. 1980). In addition, the rats in this study are substantially bigger (500–600 g bodyweight) than rats used in other studies (usually 200–400 g; e.g. Ellison 1981; Samson 1986), which may lead to differences in uptake, distribution and metabolism of the alcohol.

In the present study, food restriction was employed to prevent variation in BAC achieved due to a different stomach content. We cannot exclude that the ethanol self-administration may have partially been motivated by hunger, as the rats were fed after the ethanol access period. It should be stressed, however, that these animals were not food deprived, but were actually allowed to gain weight over the course of the experiment, from 350 to 600 g bodyweight.

The lower BACs in the resident rats suggest that no alcohol dependence developed (Mello 1973). However, these low doses were sufficient to induce significant changes in behavior and, most significantly, increased aggression in some animals. Interestingly, the intruder confrontation temporarily lowered the alcohol intake on the following day (from 3.6 ± 0.2 ml immediately before the confrontation to 3.0 ± 0.2 ml on the day after), and increased again on the day after that (to 3.3 ± 0.2 ml). This change indicates that the aggressive confrontation is a stressful event which leads to decreased self-administration similarly to other stressors, such as footshock (see Pohorecky 1990). A similar decrease in self-administration was observed in intruder rats after exposure to an aggressive rat (van Erp et al. 1994b).

A particular challenging problem for the current experimental procedure concerns the control conditions. In order to obtain control measures, intruder confrontations were scheduled before the daily ethanol access period or 4 h afterwards. The behavior in the confrontation before the ethanol access period could be affected by the fact that the resident is presented with an intruder instead of the daily ethanol solution, possibly leading to higher levels of aggression, adding to the variability in the control data. Another factor that may be important in a limited access paradigm is the occurrence of "mini-withdrawals" in between drinking sessions. This assumes that the rat is in withdrawal during the confrontation before the ethanol access period, which also might change the behavioral response during the intruder confrontation. However, the alcohol intake in these rats is too low to cause dependence (see above) and therefore argues against such a phenomenon.

An increase in aggression could occur in several ways, e.g. a decrease in latency to attack, an increase in frequency and duration of aggressive behavior, or a change in the pattern of aggression, such as a lengthening of aggressive "bursts" (Miczek et al. 1992). The animals in this study did not show a change in attack latency (Table 2), which would argue against disinhibition of aggression by alcohol. It was unclear whether there was a change in pattern of aggression, partly because there was considerable variation in fighting strategy between individuals, with some rats spending most of the time in sideways threat and others in aggressive posture. Another possibility for a change in aggressive behavior lies in the specific target for attack bites, i.e. on which body part the attack is aimed. Species-characteristically, male rats attack mostly the neck and back of the opponent, whereas attacks on the face and ventral surface are relatively rare (Blanchard and Blanchard 1977; Mos et al. 1984; Kruk et al. 1990). Both the submissive behavior displayed by the intruder and the target area of attack are postulated to be important adaptations for species survival, to prevent fatal injuries (Blanchard and Blanchard 1977). There are some indications that long-term alcohol consumption changes the attack pattern (Peterson and Pohorecky 1989). In this study, in some animals a change from biting in the neck region to biting at the face of the intruder was observed, a pattern that is specific for maternal aggression (Mos et al. 1990). Whether this is a genuine and long-lasting change in attack behavior due to alcohol requires further study.

Surprisingly, the selection of moderately aggressive rats before the start of the experiment did not prevent

the occurrence of a difference in baseline level of aggression throughout the experiment. In addition, individual differences in the response to ethanol were observed. Although this study only used moderately aggressive animals, several studies have addressed the possible rate-dependency of ethanol's effects on aggression. Both low and high aggressive rats and mice show increased aggression after ethanol (Miczek et al. 1992, 1993). Another study showed that moderately aggressive rats show an increase whereas highly aggressive rats do not (Blanchard et al. 1987a), possibly due to a ceiling effect which would prevent any further increase. In general, the aggression heightening effect of ethanol seems to be independent of the baseline level of aggression.

It has been suggested that alcohol may increase aggression through a disruption of communication between the attacker and the attacked (Miczek et al. 1992). In dominant squirrel monkeys, e.g. ethanol increases "aggressive" vocalizations (Weerts and Miczek 1996). In the current protocol, the intruder shows submissive behavior, i.e. a prolonged supine position, and emits 22 kHz ultrasonic vocalizations, which usually reduces the probability of further attacks. Alcohol may render the resident unresponsive to these signals of submission, leading to heightened aggression after alcohol administration. Lack of communication has also been suggested to be an important factor in increased aggressive behavior after alcohol drinking in humans (Virkkunen 1974). Unfortunately, little is known yet about the mechanism of action by which alcohol increases aggression in some individuals, but not others.

The present model of alcohol-heightened aggression in self-administering rats provides a model to study the differences in neurochemistry between those individuals that are prone to the aggression-enhancing effects of alcohol and those individuals that are not. Currently, studies in our laboratory are applying the technique of in vivo microdialysis to measure changes in catecholamine levels in nucleus accumbens and prefrontal cortex in alcohol drinking aggressive rats (van Erp et al. 1994a, Van Erp and Miczek 1995, 1996). Preliminary results suggest that dopamine levels in nucleus accumbens are increased during ethanol self-administration, confirming earlier observations (Wozniak et al. 1991; Yoshimoto et al. 1992), and during a subsequent intruder confrontation; no change was observed after confrontations without alcohol. In prefrontal cortex, serotonin levels decreased after the intruder confrontation. Whether individuals that are more aggressive after alcohol self-administration show different neurochemical responses under baseline conditions and/or under the influence of alcohol is under current investigation.

Acknowledgements We would like to thank Dr. Helena Barros for assistance in taking blood samples and BAC analysis, Dr. Hank Samson for advise on the sucrose fading technique, Dr. Joe DeBold for assistance with tube ligature surgery, and Ms. Carrie Rosengart for assistance with behavior scoring. This research was supported by USPHS grant R37AA05122.

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