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Social Structure and Ethanol Consumption in the Laboratory Rat

ROBERT J. BLANCHARD,[1] KEVIN HORI, PAUL TOM AND D. CAROLINE BLANCHARD

Department of Psychology and The Pacific Biomedical Research Center
Bekesy Laboratory, University of Hawaii at Manoa

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BLANCHARD, R. J., K. HORI, P. TOM AND D. C. BLANCHARD. *Social structure and ethanol consumption in the laboratory rat.* PHARMACOL BIOCHEM BEHAV **28**(4) 437–442, 1987.—When mixed-sex groups of rats in established colonies were given free access to 4% and then 8% ethanol solutions, relative ethanol consumption for individual subjects was consistent over the two solutions, with some subordinate males consuming much more ethanol than any of the dominant males. Overall, subordinate male consumption was significantly higher than that of dominants, suggesting that the social stress of subordination may be a factor in ethanol consumption. Offensive attack was reduced under 8% consumption conditions, compared to the pre-ethanol level. The significant negative correlation between initial offense level and the level of offense seen under 8% ethanol consumption agrees with previous work suggesting that more offensive males show a differential decline in offense with ethanol. Female colony members consumed significantly more ethanol than males, with some suggestion of increased drinking in response to social stresses. This pattern of results suggests that the colony situation may provide an important model for investigation of the relationship between social stress and ethanol consumption.

Alcohol Ethanol Stress Social stress Consumption Dominant Subordinate Rat

STRESS is consistently viewed as an important factor in voluntary human alcohol consumption [11,14] and knowledge of the affective and emotional changes produced by alcohol has long been considered crucial to understanding alcohol abuse [13]. Laboratory research on stress and ethanol consumption (reviewed in Cappell and Herman [5]) presents a mixed, but often positive relationship. Stressors such as foot shock, for example, tend to increase ethanol consumption in laboratory rats for a relatively brief period of time, but do not change consumption on a daily or longer basis, after shock is terminated [12]. In contrast, group-housing of rats, without additional stress factors, appears to selectively and consistently increase ethanol intake for some animals, while not increasing mean intake as compared to isolate controls [6].

The Ellison finding is particularly interesting in view of the variable, and sometimes very rapid, death rates for subordinate but not dominant, male rats in groups designed to provide important natural features of the social and physical environment [2]. While these early deaths do not appear to be the direct result of physical injury by the dominant male, they are correlated on a long-term basis with avoidance of this animal. If this situation produces social stress related to status within the dominance hierarchy, it should be possible to relate changes in voluntary ethanol consumption to pre-ethanol dominance status within the group as indexed by offensive and defensive behaviors to other group males.

METHOD

Subjects

The subjects were 30 adult female and 50 adult male *Rattus norvegicus* of the Long-Evans strain. All animals were 150–170 days old at the beginning of the study and had been housed singly in suspended Plexiglas cages from weaning.

Apparatus

Each colony was housed in a 1 meter square, 37 cm high Plexiglas enclosure, with a removable plywood frame top covered with hardware cloth. The floor of each enclosure was covered with sawdust, which was changed once a week. A 20×16×28 cm Plexiglas chamber with a wire grid floor was attached to one side of the enclosure. A cylindrical wire basket 8 cm in diameter was attached to the bottom of the chamber to discourage animals from sleeping in this chamber or establishing a defended territory there. The bottle containing ethanol solutions was attached to the wall of this chamber. The combination of a small chamber with central floor basket reduced crowding around the ethanol solution bottles, making it easier to identify the animals who were drinking. All colonies were maintained under a 12:12 light/dark cycle, and food and water—standard lab chow in a wall hopper, and two wall-mounted water bottles—were available at all times.

[1]Requests for reprints should be addressed to Robert J. Blanchard, Psychology Dept., 2430 Campus Road, Honolulu, HI 96822. This research was supported by NIAA Grant 060220.

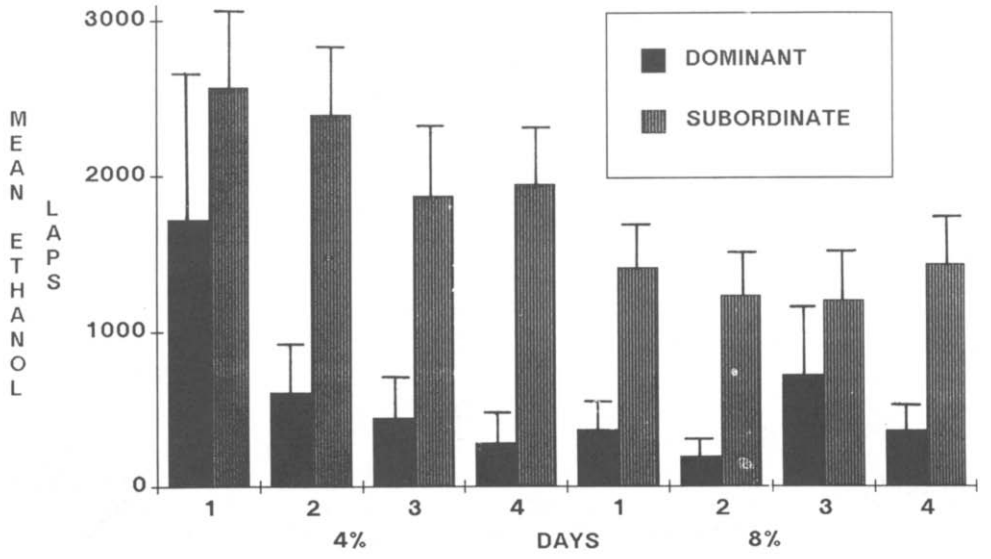

FIG. 1. Mean lap frequencies for dominant and subordinate males during 4 test days permitting voluntary consumption of 4% ethanol, and 4 test days with 8% ethanol.

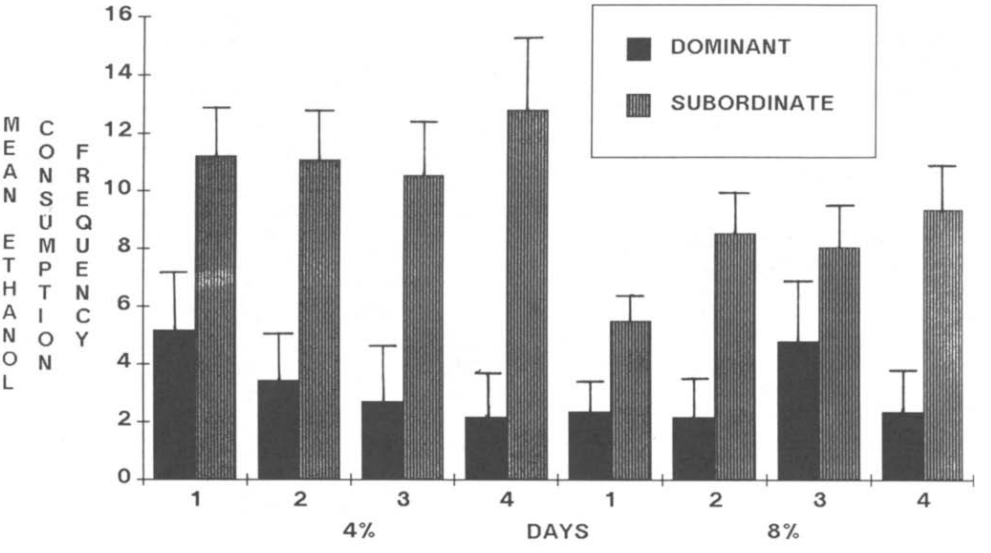

FIG. 2. Mean consumption (drinking bout) frequency for dominant and subordinate males during 4 test days permitting voluntary consumption of 4% ethanol and 4 test days with 8% ethanol.

Procedure

Social groups of animals were formed by placing five males and three females together in each colony enclosure. Group formation occurred 10 days prior to the beginning of videotaping in order to facilitate the formation of a stable dominance hierarchy prior to the introduction of ethanol.

Videorecordings of social interactions were made for two hours each day, on either days 10, 12, and 14 (for half the groups), or, days 11, 13, and 15 after colony formation. All recordings were started at 4:00 p.m., during the initial phase of lights out. Illumination was provided by three, 100 watt red lightbulbs.

Seven days after the last pre-ethanol social interaction recording (day 21 or 22 after colony formation), a 4% ethanol solution was placed in the small side chamber. This 4% solution was freely available to the subjects during a period of one week. All ethanol solutions were made up with 95% ethanol and distilled water and mixed to the appropriate concentrations. Ethanol was changed each day to assure freshness and to allow for any possible evaporation. Ethanol consumption was recorded through a 6 animal drinking monitor supplied by Columbus Instruments. This drinking system was hooked up to a microcomputer system, which recorded the number of laps of fluid. On average, 43 laps correspond to 1 ml of ethanol.

On the first, third, fifth, and seventh days of the seven day period of 4% ethanol availability, ethanol consumption was recorded for a 24 hour period. Analysis of ethanol consumption involved the frequency of laps per individual. This was accomplished through videotapes of the side chamber containing the nozzle of the ethanol bottle, to identify the drinker, in combination with the drinking monitor lap recordings for that bottle. On the day following the last consump-

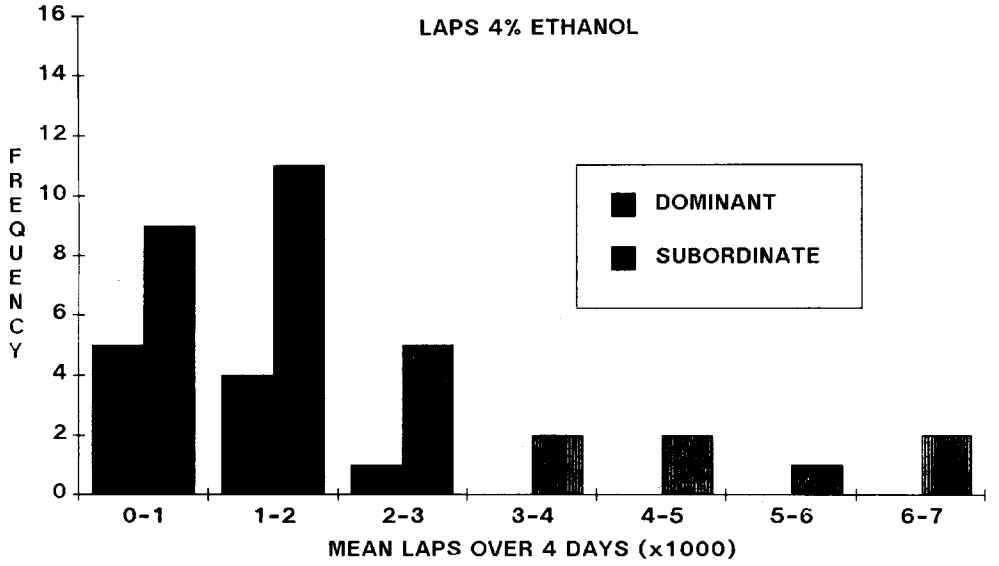

FIG. 3. Number of individual dominant or subordinate male rats with various total lap frequency scores during test periods with free access to 4% ethanol.

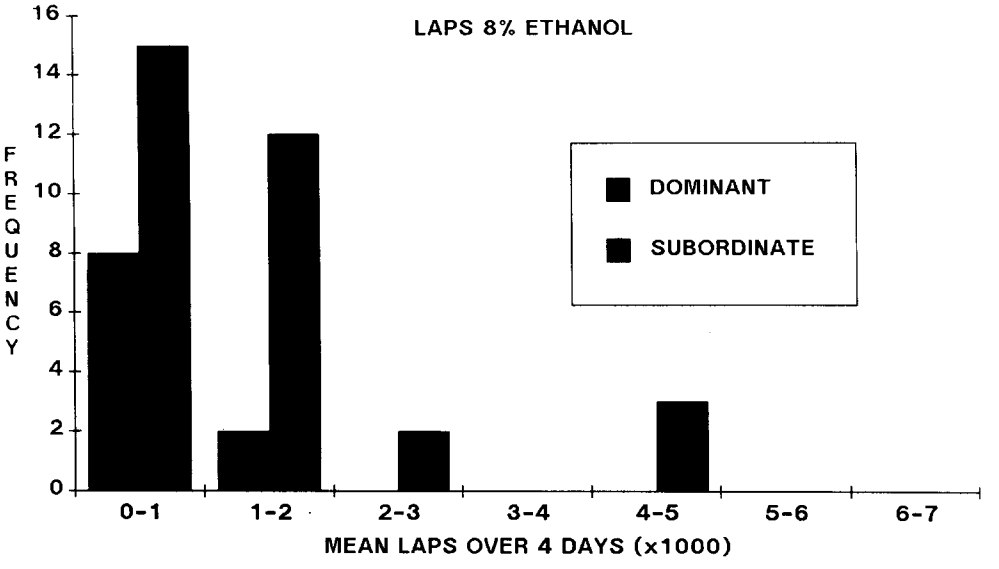

FIG. 4. Number of individual dominant or subordinate male rats with various total lap frequency scores during test periods with free access to 8% ethanol.

tion recording with 4% ethanol (day 29 or day 30) an 8% ethanol solution was offered for 7 more days, through day 35 or day 36. Consumption was then recorded on the first, third, fifth, and seventh day that the 8% solution was offered. Videorecordings of social interactions were also made for 2 hours beginning at lights out on the same days when ethanol consumption was recorded, during the 8% solution test days.

Videotape scoring and data analysis: All tapes of social interactions were later scored by an observer who did not know the ethanol status of the group, for frequencies and, where appropriate, durations of offensive behaviors; LA (lateral attack), OTO (on-top-of), and chase; total boxing (offensive and defensive box combined) and defensive behaviors; OTB (on-the-back), and flight; and bites. Measures taken during the pre-ethanol period were used to determine the dominant animal in each colony. This involved subtraction of the defense composite durations (OTB and flight) from the offense composite durations (OTO, LA, and chase) for all possible male dyadic encounters. For each colony a male was classified as dominant if his offense minus defense composite exceeded that of the other males by at least ten seconds. Two separate measures of ethanol consumption were analyzed; the frequency of drinking bouts, and lap frequency. Reliability checks indicated agreement between two trained observers of 0.92 or more with reference to specific behaviors, with perfect agreement with reference to the identity of animals, based on natural individual markings on these hooded rats. These markings had been photographed or drawn when the colonies were formed, and this documentation was used as a guide throughout the scoring of tapes.

RESULTS

One male met the criterion for dominance in each of the

TABLE 1

MEAN FREQUENCY (±SEM) OF ETHANOL CONSUMPTION FOR MALE AND FEMALE COLONY

	Members Across Days			
	Consumption Frequency		Lap Frequency	
	Males	Females	Males	Females
	Four Percent Ethanol Solution			
Day 1	9.74 ± 1.35	20.27 ± 2.70	2292.67 ± 400.11	4277.30 ± 684.80
Day 2	8.64 ± 1.28	18.93 ± 3.61	1873.59 ± 352.09	4099.03 ± 596.30
Day 3	8.02 ± 1.34	16.20 ± 2.09	1365.74 ± 283.68	3448.90 ± 544.39
Day 4	9.36 ± 1.80	19.23 ± 2.80	1344.29 ± 234.19	3383.00 ± 511.22
	Eight Percent Ethanol Solution			
Day 1	5.69 ± 1.08	13.43 ± 1.90	1150.83 ± 233.27	1719.23 ± 298.76
Day 2	6.69 ± 1.10	10.53 ± 1.53	1150.07 ± 252.17	1214.97 ± 236.18
Day 3	5.98 ± 0.91	9.50 ± 1.56	764.93 ± 171.24	775.50 ± 188.22
Day 4	7.95 ± 1.27	11.67 ± 1.33	1256.41 ± 249.96	1400.80 ± 232.26

ten colonies studied. For these 10 dominant males, mean offense minus defense duration scores were 208 seconds, as opposed to a mean of 9 seconds for subordinate males. With four freely interacting subordinates in each group, it might be expected that some subordinates would have substantial offense minus defense scores based on attacks toward other subordinate animals. However, there were very few substantial positive scores among the subordinates, in contrast to a wide range of positive scores for the dominant rats. This suggests that the presence of the dominant inhibits offense in interactions among the subordinates.

Figures 1 and 2 present ethanol consumption and lap frequency data for the dominant and subordinate males. A 2 (dominant/subordinate) × 2 (ethanol dose) × 4 (days) mixed design ANOVA of lap frequency indicated significant main effects of dominant/subordinate status, $F(1,39)=5.47$, $p<0.02$, and ethanol dose level, $F(1,39)=6.34$, $p<0.01$. Subordinate males made more laps than dominant animals, while lap frequency under the 8% ethanol condition was considerably lower than that of the 4% condition. A significant interaction of ethanol dose level × days was also found, $F(3,117)=3.57$, $p<0.01$. Day effects and other interactions were not statistically significant. However, over the first 4 days of measurement of ethanol consumption (4%), dominants' consumption as a proportion of subordinate consumption declined from about 94% (day 1) to 76% (day 2) to about 54% (days 3 and 4).

The same analysis run on male drinking bout frequency revealed a significant main effect of dominant/subordinate status, $F(1,40)=7.236$, $p<0.01$, and ethanol dose level, $F(1,40)=4.063$, $p<0.05$. No day effects or significant interactions were found. Again, the subordinates had a higher mean frequency of ethanol consumption (ethanol drinking bouts) than dominants.

Lap frequencies and consumption frequencies for males and females are presented in Table 1. These were analyzed using the same mixed design as for the dominant vs. subordinate analysis. The male category included both dominant and subordinate animals, but subordinates outnumbered dominants by 4 to 1 in each group, so the overall male figures are much more typical of the subordinates (which had higher lap frequencies) than of the dominant males. Nevertheless, the main effect of sex was highly reliable, $F(1,70)=11.366$, $p<0.001$, with the females showing much higher lap frequencies than the males. Ethanol dose level, $F(1,70)=69.180$, $p<0.001$, and repeated measures (days), $F(3,210)=5.577$, $p<0.001$, were also statistically significant, as was the sex × ethanol dose interaction, $F(1,70)=24.595$, $p<0.001$, and the ethanol dose × days interaction, $F(3,210)=2.804$, $p<0.04$. Subsequent ts indicated a significantly higher lap frequency for females versus males with the 4% ethanol solution, $t(70)=4.22$, $p<0.001$, but no reliable difference at the 8% dose level.

Analysis of consumption frequency indicated significant effects of sex, $F(1,70)=14.947$, $p<0.001$, ethanol dose, $F(1,70)=40.412$, $p<0.001$, and days, $F(3,210)=3.515$, $p<0.01$. As with the lap frequency data, these reliable differences reflect higher frequencies for females than males, higher frequencies of 4% as opposed to 8% consumption, and a tendency for consumption frequency to decline over days. A significant sex × ethanol interaction was also found, $F(1,70)=11.788$, $p<0.001$. Inspection of the data suggests that this interaction reflects a steeper decline in 4% ethanol consumption frequency over days in females than in males, but less of a decline over days for 8% consumption frequency, which was initially lower in both males and females than was 4% consumption.

Figures 3 and 4 illustrate the dramatic difference in the variability of laps taken of 4% or 8% ethanol, respectively, for the dominant rats as opposed to the subordinate males.

TABLE 2

MEAN LEVELS (±SEM) OF OFFENSE, DEFENSE, AND BOXING DIRECTED TOWARDS COLONY MALES UNDER NO ETHANOL VS. 8 PERCENT ETHANOL

		Dominant Males	Subordinate Males
Offensive Composite Frequency	no ethanol	16.33 ± 2.38	1.80 ± 0.23
	ethanol	7.53 ± 1.40*	2.04 ± 1.09†
Defensive Composite Frequency	no ethanol	0.16 ± 0.90	0.56 ± 0.09
	ethanol	0.72 ± 0.61	0.79 ± 0.37
Total Box Frequency	no ethanol	6.93 ± 1.17	6.08 ± 0.68
	ethanol	2.59 ± 0.77	3.39 ± 0.88†
Bite Frequency	no ethanol	0.52 ± 0.14	0.07 ± 0.05
	ethanol	0.44 ± 0.12	0.10 ± 0.04

*Significantly different from no ethanol condition ($p<0.03$).
†Significantly different from no ethanol condition ($p<0.05$).

No dominant rats consumed more than 3000 laps of 4% or 2000 laps of 8% ethanol over the four days of measurement, while a substantial proportion of the subordinate animals consumed up to 7000 laps of 4% or 5000 laps of 8% ethanol over the same period. Consumption of 5000 laps of 8% ethanol per day, incidentally, is roughly equivalent to a 2.0 g/kg dose of ethanol every 3 hours over the entire 4 day period of measurement.

The correlation between the number of laps under the 4%, and the 8% ethanol solutions for males was +.68, which was highly reliable. It is even more telling, perhaps, that 6 of the 7 subordinate rats making more laps than any dominant rat under 8% ethanol were also among the 9 animals making more laps than any dominant under 4% ethanol. Thus the subordinates showing extremely high ethanol lap scores were consistent across the two dose levels. It is also notable, however, that many subordinates' ethanol lap frequencies were consistently no higher than those of the dominant animals.

Female lap frequency scores were as variable as those of the subordinates, and again there was a reliable correlation between lap frequencies for the 4% and the 8% ethanol solutions (r=+.60). One interesting feature of the female scores was that females in only one colony showed an increase in lap frequency from the 4% to the 8% test days: This colony was one in which the dominant male and 2 subordinate males died after the 4% series was complete but before the end of the 8% series, a male mortality rate not paralleled in any other group.

Correlations were also obtained for male offense composite frequency prior to ethanol administration and during voluntary consumption of the 8% ethanol solution. A significant negative correlation was obtained, $r(43)=-0.35$, $p<0.05$. These offense composite frequencies, and also defense composite frequencies and boxing frequency, before and during ethanol consumption, are presented in Table 2. Because these frequencies were not normally distributed, Wilcoxon matched pairs analysis was used to evaluate changes from scores for the three days prior to ethanol administration versus scores recorded under 8 percent ethanol (days 36 or 37). A reliable decrease in offense composite frequencies (LA + OTO + chase) for dominant males was found when pre-ethanol measures were compared to measures taken during 8% ethanol administration (Wilcoxon $z=2.1915$, $p<0.03$) and the decrease for subordinates over the same measures was also reliable (Wilcoxon $z=2.13$, $p<0.03$). A significant decline was also observed for total boxing of subordinate males to other male colony members (Wilcoxon $z=1.89$, $p<0.05$). No significant differences were obtained for biting or defensive composites, nor were any significant differences obtained for any female offense or defense composites, which were very low both before and during the ethanol consumption periods.

DISCUSSION

The results of the present study are strikingly consistent with the suggestion [6] that those male rats showing greatly enhanced ethanol intake while or after living in a group situation might be different from light ethanol drinkers on the basis of position within the group dominance hierarchy. The present male subordinates showed a wide variation in ethanol consumption, with a number of subordinate males drinking far more ethanol than any of the dominant males of the groups. The apparent relative decline in consumption for dominant as opposed to subordinate males over the first 4 days of 4% availability suggests that some effect of ethanol consumption is less rewarding or more aversive to dominant males than to subordinates. This might, perhaps, represent differences in levels of anxiety for the two sets of males, or, it might reflect a deleterious effect on successful dominance-related behaviors for dominant males. Regardless of the specific mechanism, however, dominant males

here appeared to be learning not to consume ethanol over days, while subordinates did not. The reliable correlation obtained between pre-ethanol offense composites and subsequent (8%) ethanol consumption is also congruent with this suggestion. However, the magnitude of the obtained correlation ($r = -.35$) suggests that other important factors are also influencing ethanol intake.

The interpretation of this finding in terms of the stressful impact of low position within a dominance hierarchy is additionally somewhat complicated by the finding that the group females also showed a high and very variable intake pattern. However, recent measurements of ethanol intake patterns among male and female rats of a number of inbred strains, as well as the outbred N/Nih strain, suggest that the higher consumption of ethanol, and to some degree the higher variability in ethanol intake, may be typical of female as opposed to male rats [9]. In that study, females of the outbred strain were consuming ethanol at a rate approximately three times that of the males, in terms of g/kg body weight, even though all subjects were singly, rather than group, housed. Thus the very high female ethanol consumption data of the present study may simply reflect a sex difference regardless of housing condition and its related stresses, or, it may represent a stress-ethanol intake relationship that holds for female as well as male rats. In terms of the latter possibility, the unique increase in laps by females from the 4% to the 8% solution, for the only group in which the dominant male (plus two other males) died, may suggest that females, too, respond to stress within the group by drinking more. Certainly the finding that increasing ethanol consumption by male rats in mixed-sex groups leads to a higher proportion of attacks being directed to females of the group [4] would suggest that stress on female rats may increase with male ethanol consumption.

The results of the present study are also relevant to a number of issues concerned with the relationship of ethanol intake to aggression and defense within rat groups. First, these results suggest that the present groups displayed a clear dominance hierarchy which appeared shortly after group formation and continued over changing conditions of ethanol intake. This finding is in agreement with previous work indicating such stability [3]. The negative correlation between offense scores before, and during, 8% ethanol consumption is congruent with a finding that animals with higher pre-drinking offense scores tend to show disproportionate reductions following ethanol consumption [4]. That study additionally found that rats making low to intermediate offense scores initially, show an aggression increase at certain specific ethanol dose levels. While such an increase was not obtained for the present subordinate animals, the present study involved variable and self-selected drug doses which were unlikely to be consistently within the narrow, low-dose range where this ethanol-enhancement of aggression effect has been found.

Human research on the relationship of stress and ethanol consumption tends to suggest that it is social stress, specifically, which is most likely to be involved in precipitating high rates of problematic alcohol consumption: Marlatt [10] has reported that the situations most strongly associated with drinking relapses of discharged alcoholics include interpersonal conflict and social pressure. In a laboratory study, stress induced by the anticipation of shock failed to increase alcohol consumption in either social drinkers or heavy drinkers [7]. However, when anticipating important interpersonal evaluations, social drinkers did show increased alcohol consumption [8]. Both of these factors, that drinking increases with stress only in a subpopulation of subjects, and that social stress is highly effective in promoting this increase, appear to be well modelled in populations of rats living in natural group situations. Such situations thus appear to provide a promising situation for the study of the interaction of biological and environmental features impacting variations in ethanol consumption.

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