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Schedule Induced Ethanol Polydipsia in Psychogenetically Selected Lines of Rats[1]

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MARTIN, J. R. AND K. BAETTIG. *Schedule induced ethanol polydipsia in psychogenetically selected lines of rats.* PHARMAC. BIOCHEM. BEHAV. 14(6) 857–862, 1981.—Schedule induced ethanol polydipsia was established in 28 male and female rats of two psychogenetically selected lines bred for extremes in active avoidance performance. These rats were maintained at 80% normal body weight and given 36 consecutive daily 45-min sessions with 3% (w/w) ethanol available. During the acquisition phase, food pellets were delivered intermittently on a FT-1 min schedule. Baseline tests preceded and followed the acquisition phase and involved presentation of 45 food pellets together at the start of a test. Roman High Avoidance (RHA/Verh) rats exhibited greater ethanol intake than Roman Low Avoidance (RLA/Verh) rats and female rats drank more ethanol (adjusted for body weight differences) than male rats during baseline and acquisition phases. Furthermore, baseline ethanol intake increased significantly from the initial block to the final block of sessions following acquisition testing, but remained significantly lower than ethanol intake during the final block of acquisition tests. In a second experiment, naive female rats of the two psychogenetic lines were given baseline tests or sessions with food intermittently delivered on a FT-2 min schedule following a period of free feeding or a 20-hr fast. During each 1-hr session 3% ethanol was continuously available. In the initial phase of acquisition, fasted RHA/Verh rats drank more ethanol than RLA/Verh rats. In the second phase, undeprived rats of these two lines did not differ in ethanol intake. In a final acquisition phase, fasted RHA/Verh and RLA/Verh rats did not differ significantly in ethanol consumption. Under both deprivation regimens, baseline ethanol intake increased from the initial to the final baseline test. The ethanol consumption of fasted rats in the final acquisition test was significantly greater than that in the final baseline test. Thus, although pronounced and relatively enduring differences in the schedule induced ethanol intake of these two rat lines were observed under the condition of chronically reduced body weight, this strain difference was relatively weak and observed only in the initial phase of acquisition when 20-hr fasted rats were tested.

Schedule induced polydipsia Psychogenetic selection Sex and strain differences Ethanol Body weight level Roman High-and Low Avoidance rats

A NUMBER of animal models for human alcoholism have been developed in recent years to facilitate the investigation of the etiology, prevention and treatment of human addiction to ethanol. One approach to developing a rat model of alcoholism has relied on the use of selective breeding procedures to obtain heuristic genotypes [3, 7, 9]. Strains of rats bred for bidirectional extremes in behavior other than alcohol consumption have sometimes fortuitously exhibited differential ethanol self-selection. The Maudsley strains that were selectively bred for extreme defecation scores in an open field test of emotional reactivity and the Roman strains that were bred for superior or inferior shuttle box active avoidance performance have received the most attention in this regard. Roman High Avoidance rats have generally been reported to consume more ethanol than Roman Low Avoidance rats; in contrast, the results obtained in different studies with the Maudsley strains have not been entirely consistent [1, 2, 10, 11].

Research concerning ethanol consumption by psychogenetically selected strains of rats has focused on ethanol drinking in the home cage situation. However, excessive ethanol intake has also been produced in the usual commercially available strains by training in a schedule induced polydipsia paradigm [4, 6, 8]. This experimental procedure involves intermittent food delivery to fasted rats given free access to an ethanol solution. When rats were given six 1-hr sessions per day with scheduled food delivery, the rats drank excessive quantities of ethanol and exhibited some evidence of subsequent physical dependence [5]. The present study investigated the acquisition of such schedule induced ethanol polydipsia in Roman High Avoidance (RHA/Verh) and Roman Low Avoidance (RLA/Verh) rats of both sexes.

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These bidirectionally selected lines of rats were produced by a continuing psychogenetic breeding program using a breeding nucleus provided by P. L. Broadhurst (University of Birmingham, England). On the basis of previously published experiments demonstrating greater ethanol consumption by mature RHA rats than RLA rats, it was expected that the former psychogenetic line would also exhibit greater schedule induced ethanol polydipsia than the latter rat line. In addition, the present investigation was designed to evaluate the effect of the sex of the subjects and the use of a regimen of acute food deprivation or free feeding on such schedule induced behavior in these psychogenetic rat lines.

EXPERIMENT 1

The initial experiment evaluated the development and maintenance of schedule induced ethanol polydipsia in RHA/Verh and RLA/Verh rats of both sexes chronically maintained at 80% of free-feeding body weight. Baseline tests, with all food pellets provided together at the start of a session, preceded and followed the 30-day period of schedule induced polydipsia testing to determine the importance of the scheduled food delivery in maintaining excessive ethanol drinking.

METHOD

Animals

The animals were 7 male RHA/Verh, 7 female RHA/Verh, 7 male RLA/Verh, and 7 female RLA/Verh naive rats obtained from the Institute colony. The rats were housed in individual Macrolon cages with continuous access to tap water. The rats were 3–4 months old at the start of the experiment and were maintained at 80% of their free-feeding body weight level throughout the experiment with daily food rations (Nafag laboratory pellets, no. 890). The mean (±SEM) free-feeding body weights of these four groups were: male RHA/Verh rats (358±9 g), female RHA/Verh rats (241±9 g), male RLA/Verh rats (362±9 g), and female RLA/Verh rats (229±4 g). The fluorescent lights in the animal quarters were set for a 12:12 hr light-dark cycle with light onset at 20:00 hr.

Apparatus

Testing was done in two ventilated operant conditioning chambers that were mounted in sound attenuating cubicles and equipped with house lights. At one end of the experimental chamber a pellet receptacle was attached to the wall and rested on the floor adjacent to an opening through which the spout of a bottle protruded. With each operation of the pellet dispenser, a 45-mg Noyes pellet was delivered into the food cup. The drinking spout and the wire floor served as the two electrodes of a drinkometer circuit activated by a lick. The behavior of a subject could be remotely monitored via a video camera. Programming and data recording equipment was located in adjacent rooms.

Procedure

Following the gradual reduction of body weight to 80% of the normal body weight level over a 2-week period, the rats began testing. Behavioral testing consisted of daily 45-min sessions beginning 1 hr after light offset on 36 successive days. Each rat was tested at about the same time each day. During the initial 3 baseline tests (days 1–3), 45 Noyes pellets were placed in the food cup before the start of the session. Throughout these and all subsequent sessions, rats had continuous access to 3% (w/w) ethanol. Intake was determined by weighing the bottles before and after each test. In addition, cumulative recorders provided a permanent record of the pattern of drinking. The bottles were kept full in order to minimize spillage and all values were corrected for spillage due to handling. The pellet dispenser was activated once each minute during the baseline phase, but the pellets were not delivered into the food cup. During the 30-day acquisition phase (days 4–33), pellets were delivered to the food cup according to a FT-1 min schedule (one Noyes pellet delivered automatically each minute regardless of the behavior of the subject). During the final 3 days of this experiment (days 34–36), baseline testing was again done. After the completion of each daily session throughout this experiment, the rats were weighed and given sufficient food in the home cage to maintain 80% of the free-feeding body weight level.

RESULTS AND DISCUSSION

Figure 1 shows the mean ethanol intake (calculated as g 100% ethanol per kg body weight) of RHA/Verh and RLA/Verh rats of both sexes during baseline and acquisition phases of the experiment. Overall statistical analysis of these data across the 30-day acquisition phase (averaged in 3-day blocks) was done with a 2×2×10 ANOVA repeated measures design. The factors were rat line, sex, and repeated testing. The RHA/Verh rats consumed more ethanol than the RLA/Verh rats, $F(1,24)=24.4$, $p<0.01$, and female rats consumed more ethanol than male rats, $F(1,24)=18.0$, $p<0.01$. The analysis of the effect of repeated testing was corrected for absence of homogeneity of variance-covariance [12] and yielded a significant main effect, $F(1,24)=15.1$, $p<0.01$. There were no significant interactions between these factors. A separate 2×2×2 ANOVA repeated measures design was used to analyze the ethanol intake data collected during the 3-day block of baseline testing carried out prior to the acquisition phase of the experiment and that carried out at its conclusion. The factors were rat line, sex, and repeated testing. The RHA/Verh rats drank more ethanol than the RLA/Verh rats, $F(1,24)=6.3$, $p<0.05$, the female rats drank more ethanol than the male rats, $F(1,24)=13.3$, $p<0.01$, and baseline intake was higher at the conclusion of the experiment than at its start, $F(1,24)=37.4$, $p<0.01$. Furthermore, there was a significant interaction between the factors sex and repeated testing, $F(1,24)=5.8$, $p<0.05$. It was also of interest to determine whether schedule induced polydipsia was produced by the experimental procedure, so a comparison of ethanol intake during the final 3-day block of the acquisition phase and that during the 3-day block of post-acquisition baseline testing was done with a 2×2×2 ANOVA repeated measures design. The factors evaluated were rat line, sex and repeated testing. The RHA/Verh rats consumed more ethanol than the RLA/Verh rats, $F(1,24)=11.5$, $p<0.01$, the female rats drank more than the male rats, $F(1,24)=20.3$, $p<0.01$, and ethanol intake was greater during the final block of acquisition testing than during the following block of baseline tests, $F(1,24)=63.5$, $p<0.01$. There were no significant interactions between these factors.

These results demonstrated that RHA/Verh rats consistently consumed more ethanol than the RLA/Verh rats during baseline tests and during testing when food was delivered according to a FT-1 min schedule. Similarly, female rats

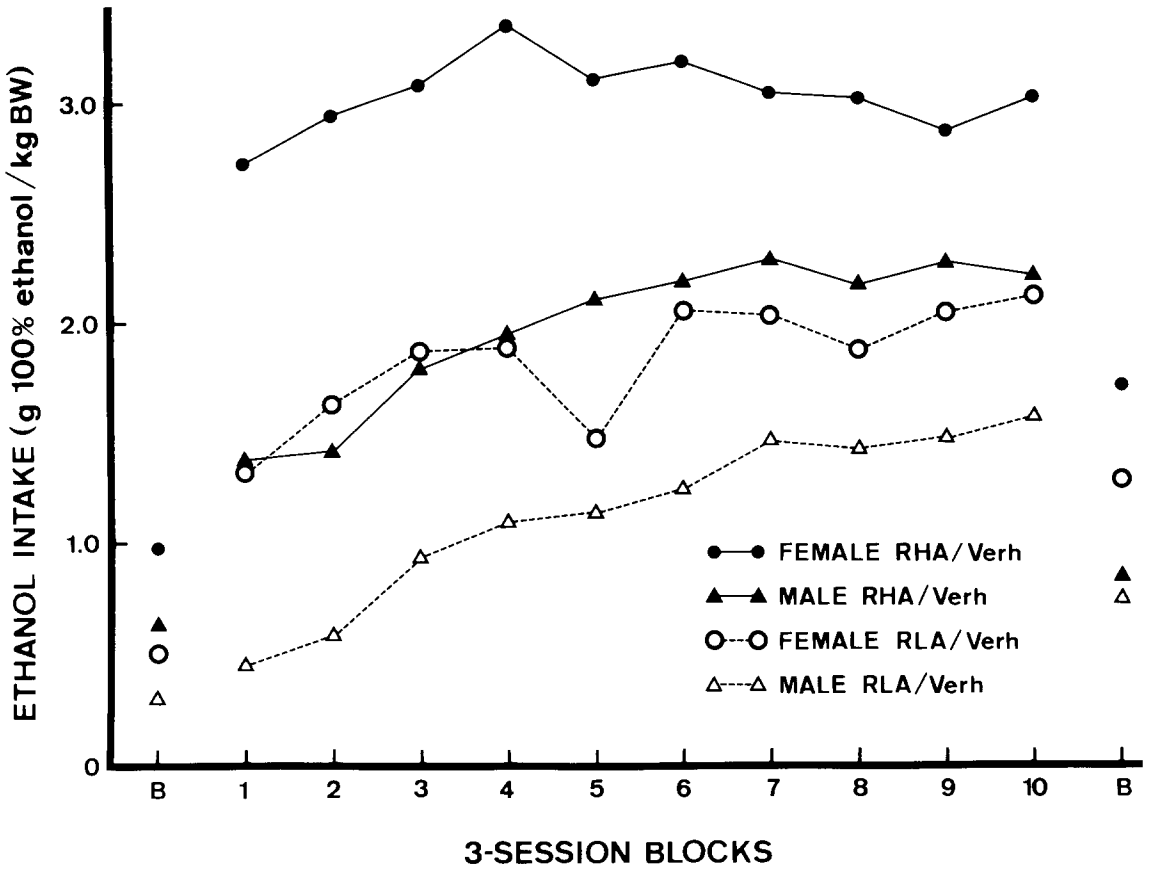

FIG. 1. Mean ethanol intake (g 100% ethanol per kg body weight) by RHA/Verh and RLA/Verh rats of both sexes during baseline tests (B) and sessions with intermittent food delivery on a FT-1 min schedule. Rats were maintained at 80% of their free-feeding body weight level throughout the experiment.

consumed more ethanol than male rats under these experimental conditions. It should be noted, however, that ethanol intake was corrected for differences in body weight prior to analysis. Baseline intake was significantly higher following the 30-day acquisition phase than before this phase. Thus, in evaluating whether schedule induced behavior was produced in this experiment, the intake during the final block of acquisition tests was compared with that of the final block of baseline tests, rather than the initial block of baseline tests. The ethanol intake during scheduled food presentation was significantly greater than during the baseline testing, providing evidence that schedule induced ethanol polydipsia had been produced. The cumulative records of drinking showed that drinking bouts immediately followed pellet delivery, although this pattern was less consistent late in the test session when long pauses between pellet-related bouts sometimes occurred.

EXPERIMENT 2

The results of Experiment 1 demonstrated that schedule induced ethanol polydipsia was greater in female rats than in male rats, with female RHA/Verh rats consuming particularly large quantities of 3% ethanol during the initial portion of acquisition testing. The present experiment was designed to investigate schedule induced drinking of 3% ethanol by RHA/Verh and RLA/Verh female rats following either a 20-hr fast or after a period of ad lib access to food. Occurrence of schedule induced ethanol polydipsia under the latter condition, presumably in the absence of caloric deprivation, would be particularly important in arguing that such a rat model provides a valid reflection of human alcoholism.

Animals and Apparatus

The subjects were 8 female RHA/Verh and 8 female RLA/Verh naive rats obtained from the Institute colony. These rats were approximately 3 months old at the start of the experiment. All rats were individually housed beginning 2 weeks prior to behavioral testing, but had continuous access to food and water prior to the start of the experiment. The mean (±SEM) free-feeding body weight of the RHA/Verh rats was 237±4 g and that of the RLA/Verh rats was 223±6 g. Other details concerning the animals and equipment were the same as in Experiment 1.

Procedure

The rats received 24 successive daily 1-hr sessions during the nocturnal portion of the light-dark cycle. Each rat was tested at approximately the same time each day. On Day 1, the free-feeding rats received a baseline test. Prior to the start of this session, 30 Noyes pellets were placed together in

the food cup and, although the pellet dispenser was activated every 2 min, no pellets were delivered to the pellet receptacle. A 3% (w/w) ethanol solution was available during this and all subsequent sessions in this experiment. During the initial 3 hr after this first baseline test, the rats had free access to food in the home cage but were then food deprived for the 20-hr interval prior to the next session. On Day 2, the fasted rats received a second baseline test and were then given 3 hr access to food in the home cage. During the subsequent 8 tests (days 3–10), 20-hr fasted rats received Noyes pellets on a FT-2 min schedule for 1-hr sessions. The subjects were then given ad lib access to food for the following 5 day period (days 11–15) during which daily schedule induced polydipsia testing sessions were given. An acute deprivation regimen was then reinstituted for a subsequent 5 sessions (days 16–20) and the rats were 20-hr fasted when tested with scheduled food presentation during this phase. The subjects were also 20-hr fasted prior to 3 more baseline sessions (days 21–23) and then received a final baseline test after a 23-hr period of free feeding (day 24). Intake of 3% ethanol was determined by weighing the bottle before and after each session and correcting this value for spillage due to handling.

RESULTS AND DISCUSSION

Figure 2 shows the mean ethanol intake (calculated as g 100% ethanol per kg body weight) of female RHA/Verh and RLA/Verh rats during baseline tests and sessions with intermittent food delivery on a FT-2 min schedule. The daily body weight data from these two rat lines were combined (the body weight trends for these groups were very similar) and are presented at the bottom of Fig. 2. Separate statistical analysis was done on data from the initial period of intermittent food delivery to 20-hr fasted rats (days 3–10), the second period of intermittent food delivery to undeprived rats (days 11–15), and a final period of intermittent food delivery to 20-hr fasted rats (days 16–20). The factors in each ANOVA were rat line and repeated testing. The analysis of the effects of repeated testing in each of these experimental phases was corrected for the absence of homogeneity of variance-covariance [12]. Fasted RHA/Verh rats receiving scheduled food delivery in the initial phase drank more ethanol than RLA/Verh rats, $F(1,14)=9.4$, $p<0.01$, and the effect of repeated testing during this phase was significant, $F(1,14)=18.1$, $p<0.01$. There were no significant main effects or interaction during the second phase indicating that there was no difference in ethanol intake between undeprived RHA/Verh and RLA/Verh rats during sessions with intermittent food delivery. There was also no main effect of rat line on ethanol consumption during the third experimental phase when food was delivered intermittently to 20-hr fasted rats, however, the effect of repeated testing was significant, $F(1,14)=10.4$, $p<0.01$.

Ethanol intake during baseline tests before and those after testing under the condition of intermittent food delivery were compared with a nondirectional Wilcoxon matched-pairs signed-ranks test. Fasted RHA/Verh rats ($p<0.02$) and fasted RLA/Verh rats ($p<0.05$) drank more during the final baseline test (day 23) than during the initial baseline test (day 2). Similarly, undeprived RHA/Verh rats ($p<0.01$) and undeprived RLA/Verh rats ($p<0.02$) drank more during the final baseline test (day 24) than during the initial baseline test (day 1) under this feeding regimen. It was of further interest to determine whether schedule induced polydipsia was produced under conditions of prior acute fasting or ad lib food access in the present experiment, so additional Wilcoxon tests were done. Fasted RHA/Verh rats ($p<0.01$) and fasted RLA/Verh rats ($p<0.01$) consumed more ethanol on the final day of scheduled food delivery (day 20) than on the final day of baseline testing under this same deprivation regimen (day 23). In contrast, undeprived RHA/Verh and RLA/Verh rats exhibited no significant differences between intake on the final day of scheduled food delivery (day 15) and on the final day of baseline testing under this ad lib feeding condition (day 24).

The results of this second experiment indicate that during initial exposure to 3% ethanol during intermittent delivery of food pellets on a FT-2 min schedule, fasted female RHA/Verh rats consumed more ethanol than fasted female RLA/Verh rats. However, during subsequent testing of undeprived rats and a later a repetition of the earlier test phase with fasted rats, there were no differences in ethanol intake by these two rat lines. There was a significant increase in ethanol intake from the initial to the final baseline test under conditions of both deprivation and ad lib feeding prior to testing. The final baseline ethanol intake of 20-hr fasted rats was significantly less than intake in the final test with intermittent food delivery, thus, demonstrating the occurrence of schedule induced polydipsia under this particular deprivation regimen.

GENERAL DISCUSSION

The present results extend earlier demonstrations of strain differences in ethanol consumption by Roman High Avoidance and Roman Low Avoidance rats to schedule induced ethanol polydipsia. Thus, at maturity, RHA rats generally exhibit greater ethanol preference, consume more ethanol in longterm home cage tests, and exhibit more pronounced schedule induced ethanol drinking, with the strain difference most pronounced for 3–10% ethanol solutions ([1, 2, 11] and present experiments).

The first experiment investigated the acquisition of schedule induced ethanol polydipsia in RHA/Verh and RLA/Verh rats of both sexes that were maintained at 80% of their free-feeding body weight level. The RHA/Verh rats consumed more 3% ethanol in baseline sessions and throughout the acquisition phase than did RLA/Verh rats. Furthermore, the ethanol intake (all ethanol data were calculated as g 100% ethanol per kg body weight) of female rats consistently exceeded that of male rats during baseline tests and sessions when food delivery was intermittent. Ethanol intake increased from the initial block to the final block of baseline tests, but even then remained significantly lower than during the final block of tests during the acquisition phase. The results of this experiment clearly demonstrate the potentially important influence of genotype on schedule induced ethanol polydipsia. Furthermore, following the correction of intake data for differences in body weight, the female rats consumed significantly more ethanol than the males. Even when absolute intake of ethanol is considered, female rats consistently consumed more ethanol than like-strain males for the initial 12 days of the acquisition phase. Thereafter, the absolute intake of males exceeded that of like-strain females for the remainder of the acquisition phase. The females reached the approximate peak for absolute intake during the initial 12 days of acquisition tests and thereafter intake remained relatively constant. In contrast, male rats reached their approximate peak in absolute intake only after 3 weeks of acquisition testing and then intake re-

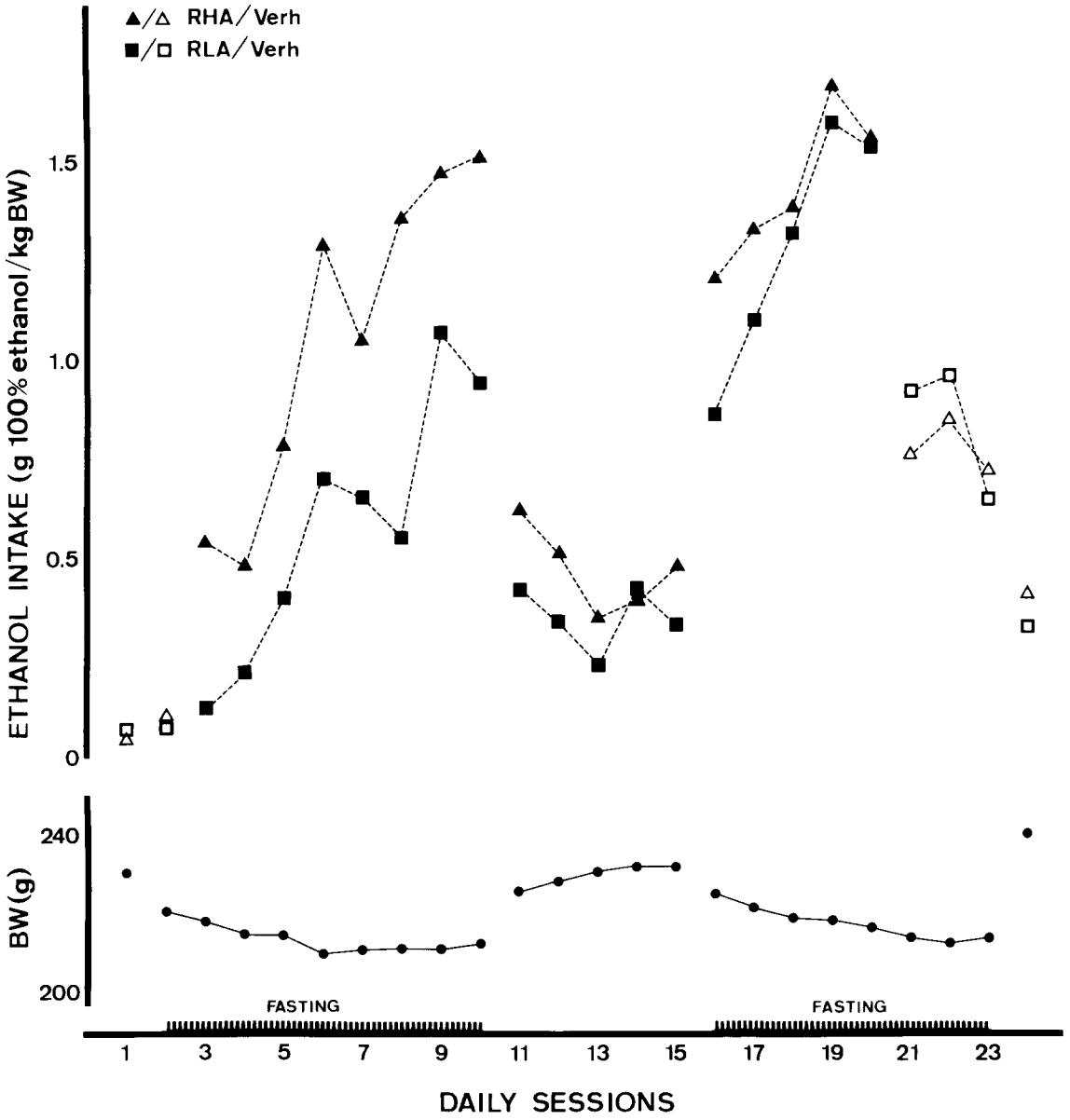

FIG. 2. Mean ethanol intake (g 100% ethanol per kg body weight) of female RHA/Verh and RLA/Verh rats during baseline tests (open symbols) and sessions with intermittent food delivery on a FT-2 min schedule (solid squares and triangles). Rats were tested following an interval of free feeding or after a 20-hr fast (indicated by striped band). The mean daily body weight of both groups combined is shown at the bottom of the figure (solid circles).

mained relatively stable for the remainder of the acquisition phase.

In the second experiment naive female RHA/Verh and RLA/Verh rats were tested in a schedule induced polydipsia paradigm following free feeding or a 20-hr fast. Female RHA/Verh rats drank significantly more 3% ethanol during the initial 8 daily acquisition sessions given under the fasting regimen than did female RLA/Verh rats. However, there was no significant strain difference during subsequent sessions when the subjects were undeprived or in a later phase of scheduled food delivery to 20-hr fasted rats. The reason for the absence of a clear strain difference in this latter phase is not obvious, but may reflect a ceiling effect resulting from an initially weak schedule effect and repeated testing. The loss of body weight on the fasting regimen used in the present experiment was relatively small and cannot be easily related to the magnitude of the schedule induced drinking. Thus, schedule induced ethanol drinking occurred in the two psychogenetic rat lines even when body weight was nearly normal. However, under the present testing conditions only an initial strain difference in schedule induced ethanol intake was observed. As was previously seen, ethanol intake under

the fasting and the free-feeding regimens increased significantly from the initial to the final baseline sessions. The basis for this increase may reflect any or all of several factors including increased ethanol preference, conditioned drinking in the test situation, and the development of ethanol tolerance or dependence. In any case, the present baseline data suggest that investigations of schedule induced ethanol polydipsia should not depend solely on initial baseline sessions when characterizing the magnitude of the schedule induced drinking effect.

The use of a schedule induced polydipsia procedure to maximize 3% ethanol drinking in RHA/Verh and RLA/Verh rats resulted in a high, consistent level of ethanol ingestion (mean ethanol intake calculated as g 100% ethanol per kg body weight over the final 15 days of the acquisition phase of Experiment 1) by female RHA/ Verh rats (3.0 g/kg/45 min session), and relatively lower intake by male RHA/Verh rats (2.2 g/kg/45 min session), female RLA/Verh rats (2.0 g/kg/45 min session), and male RLA/Verh rats (1.4 g/kg/45 min session). In comparison, previous investigations concerning the consumption of ethanol concentrations ranging up to 10% in the home cage by mature RHA rats have generally reported maximum 24-hr intake of about 3–11 g/kg body weight [1, 2, 10, 11]. The strain difference in ethanol consumption observed between RHA/Verh and RLA/Verh rats in the present study appear to be most pronounced and stable when body weight was maintained at about 80% of the free-feeding level and ethanol consumption was considerably less consistent when rats received a series of test sessions, each following a 20-hr fast. The present investigation provides further support for the relation between psychogenetic selection for avoidance conditionability and high ethanol consumption. Furthermore, the present experiments demonstrated the value of the schedule induced polydipsia procedure to increase the already high level of ethanol intake reported for RHA rats [1, 2, 10, 11]. Use of RHA rats may prove to be particularly advantageous in experiments requiring not only high chronic ethanol consumption, but also rapid acquisition of the schedule induced ethanol polydipsia phenomenon. In future research, the addiction liability of these two psychogenetic rat lines to ethanol, as well as to other abused drugs, merits additional investigation.

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