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Ethanol and Cocaine Intake by Rats Selectively Bred for Oral Opioid Acceptance

KRISTIN R. CARLSON[1] AND LYCETT PEREZ

Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical Center, 55 Lake Ave. North, Worcester, MA 01655-0126

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CARLSON, K. R. AND L. PEREZ. *Ethanol and cocaine intake by rats selectively bred for oral opioid acceptance.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 309–313, 1997.—Lines which accept or reject the potent opioid etonitazene, and a randomly bred control line, were assessed for the specificity of selective breeding. Drug-naive subjects from generation 8 were offered a continuous choice between water and 10% ethanol for 20 days. There was no difference between the accepting and rejecting lines in preference for one fluid, or in amount of ethanol consumed. The same rats were then given a choice between water and increasing concentrations (0.08-0.64 mg/ml) of cocaine, 7 days at each concentration. There were no differences among the lines in preference for the drug, but the rejecting line drank more of the cocaine solution than the accepting line. Finally, these rats were subjected to the regimen used in choosing rats for selective breeding, 4 days of a water-etonitazene choice. In their preference for etonitazene the order of the lines was as expected: accepting > control > rejecting. In addition, the accepting line drank more of the etonitazene solution than the other two lines. These data suggest that selection has been rather specific and not for a generalized tendency to become intoxicated.

Opioid Selective breeding Cocaine Ethanol Etonitazene Drug abuse Rat

SELECTIVE breeding for the willingness to self-administer psychoactive drugs has been used with particular success in the field of alcohol abuse, where five pairs of rat lines which differ in drinking ethanol have been developed (6). The most thoroughly investigated are the Finnish AA (ALKO, alcohol) and ANA (ALKO, nonalcohol) lines (17) and the Indiana University P (preferring) and NP (non-preferring) lines (18). Both high-preference lines drink more ethanol than water, and both low-preference lines drink less, when 10% ethanol is presented as a choice with water; this is the criterion used for imposing selection pressure.

To our knowledge, only one program is developing lines of rats bred selectively for self-administration of a drug other than ethanol; we have recently described lines which ingest or avoid the potent opioid etonitazene (ETZ). Over seven generations an accepting and a rejecting line diverged bidirectionally from a randomly bred control line in free choice consumption of ETZ presented with concurrently available water (3). ETZ is a μ-selective opioid (19) which was used because it is some 1000-2000 times more potent than morphine in various behavioral tests (1,7,9,20,21,26); as a consequence, it can be diluted to the point where the taste is apparently not aversive to rats (2–5), and it is used frequently in oral self-administration experiments.

A question with implications for the genetics of drug abuse is whether self-administration is specific for ETZ, or whether these lines have been bred for a generalized tendency toward or away from becoming intoxicated. Preferences for drugs of various classes covary with some inbred strains; for example, oral ethanol, ETZ, morphine and cocaine serve as strong positive reinforcers for Lewis rats but none is a reinforcer for Fischer 344 rats (12,23–25). Similarly, C57BL/6J mice drink ethanol, morphine and cocaine, while DBA/2J mice will not (11,13). With respect to selectively bred lines, the AA line of rats prefers low concentrations of not only ethanol, but also ETZ and cocaine, over water, and it drinks more of them at all tested concentrations than does the ANA line (16). These data suggest that there may be a common genetic determinant to psychoactive drug consumption (12,14). To assess the generality of this hypothesis, we tested drug-naive rats of our accepting, control, and rejecting lines for oral self-administration of ethanol and cocaine.

[1]To whom requests for reprints should be addressed.

TABLE 1
SELECTIVELY BRED LINES

	Accepting		Control		Rejecting	
	Female	Male	Female	Male	Female	Male
Body weights (Mean ± SEM) at the beginning of the experiments						
Grams*†	230 ± 11	404 ± 23	289 ± 14	531 ± 17	269 ± 8	484 ± 26
Fluid consumed in ml/kg/day (Mean ± SEM) during the days it was the only fluid available						
Water†	114 ± 7	48 ± 2	90 ± 10	46 ± 3	117 ± 10	54 ± 2
Ethanol†	85 ± 5	24 ± 3	61 ± 4	24 ± 2	71 ± 7	44 ± 3
Cocaine†	127 ± 6	46 ± 2	115 ± 9	50 ± 3	137 ± 5	67 ± 4
ETZ‡	108 ± 16	62 ± 7	56 ± 4	50 ± 4	39 ± 5	62 ± 7

*Difference between the lines: $F(2, 33) = 14.4$, $p < 0.00001$; †Difference between the sexes: $F(1, 33) = 31.2$ to 216.2, $p < 0.00001$; ‡Difference between the lines: $F(2, 33) = 4.97$, $p < 0.02$; and $N = 12$–14 rats/line.

METHOD

Animals

Drug-naive rats of the generation 8 accepting ($N = 14$), control ($N = 12$), and rejecting ($N = 13$) lines were used; the number of subjects was determined by the number of fluid presentation devices, and both sexes were represented approximately equally in each line. Rats were 14 months old at the beginning of the study. The accepting and rejecting rats were chosen on the basis of being offspring of the generation 7 rats with the most extreme preferences appropriate to their line. Animals were housed individually in 30 × 34 × 16-cm-high Plexiglas cages with ad lib chow under a 12 L : 12 D cycle (lights on at 0700 h) at 22 ± 3°C. At the end wall of each cage was a fluid presentation device which eliminated the influence of any position preference when the rat was given a choice between water and a drug solution from side-by-side bottles with spouts (2,3). Each cage contained a 10 cm length of pine 2 × 4 which served as a suitable object for the stereotyped chewing which is characteristic of chronic intoxication with ETZ (5,26).

Drugs

Ethanol (200 proof, Pharmco) was prepared as a 10% v/v solution in tapwater, cocaine HCl (Sigma) as 0.08–0.64 mg/ml solutions in tapwater, and etonitazene base (NIDA, Rockville, MD) as a 2.5 μg/ml solution in tapwater.

Procedure

Rats were weighed daily, and the amount of water and drug solution drunk in each 24-h period was determined by weighing the bottles. Rats were taught to drink from the presentation device by giving them a day of water from one spout, followed by 2 days of water in both spouts, the latter day serving as a baseline water-water day. For 4 days only 10% ethanol was available from the presentation device, to insure that all rats experienced its effects. That concentration was then offered in a choice with water for 20 days, because several weeks' access is necessary for an accurate phenotypic measure of ethanol preference (17), and the 10% concentration gives the same results as a series of concentrations (8,17). In addition, 5 and 10% are the concentrations preferred by Wistar and AA rats (22), and a choice between 10% ethanol and water is used in all five ethanol selective breeding programs (6). A 2-wk washout period, in which water was supplied from a large bottle on the cage top, was given. After another water-water baseline day, 4 days of access to only 0.08 mg/ml cocaine was given. Increasing concentrations of cocaine were then paired with water, since we could find no information concerning preferred concentrations in a free access situation. Starting at 0.08 mg/ml, the concentration was doubled every 7 days up to 0.64 mg/ml. A 1-wk washout period was followed by a choice between ETZ and water, in order to determine whether these animals were representative of their lines. Our standard protocol to select rats for breeding was used: a water-water baseline day, 2 days with only 2.5 μg/ml ETZ available, and then 4 days of a choice between that concentration of ETZ and water. A longer choice period was not necessary, as preference behavior toward ETZ stabilizes within a few days (3).

Statistics

Three-way (line × sex × day) analyses of variance with repeated measures on the day variable (27) were used to test differences among the lines. Because there were significant line differences in body weight (see Results), the amounts consumed of drug solutions and water were expressed as ml/kg. For visual clarity, the ethanol and cocaine figures show the results in terms of blocks of days: for ethanol an arbitrary 5 days/block, and for cocaine 7 days/block (i.e., the number of days each concentration was used).

RESULTS

Table 1 shows that there were significant differences in body weight among the lines at the time of testing. Differences were not a function of age, since all the rats had been born within a week of each other and were over a year old. As would be expected, males were heavier than females, irrespective of line. These results were typical of previous (3) and subsequent generations (unpublished data).

Table 1 also shows that the lines did not differ, on a weight-adjusted basis, in consumption of water during the water-water baseline day, or in ethanol or cocaine intake when these were the only fluids available before the choice tests. Regardless of line, however, females drank significantly more per kg than males; there were no line-sex interactions, indicating that gender differences were in the same direction in all the lines. On the other hand, when ETZ alone was available for 2 days, the accepting rats drank more than the control rats and the

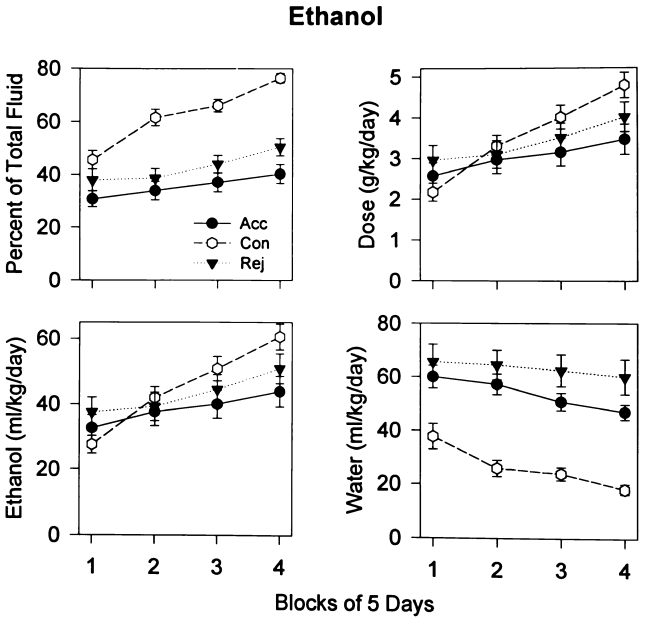

FIG. 1. Ethanol consumption over blocks of 5 days during the time the lines were given a choice between 10% ethanol and water. (Top Left) Ethanol intake as a percent of total fluid consumed. (Bottom Left) Ethanol intake in ml/kg/day. (Top Right) Dose of ethanol consumed in g/kg/day. (Bottom Right) Water intake in ml/kg/day. All values are Mean ± SEM. N = 12–14 rats/line.

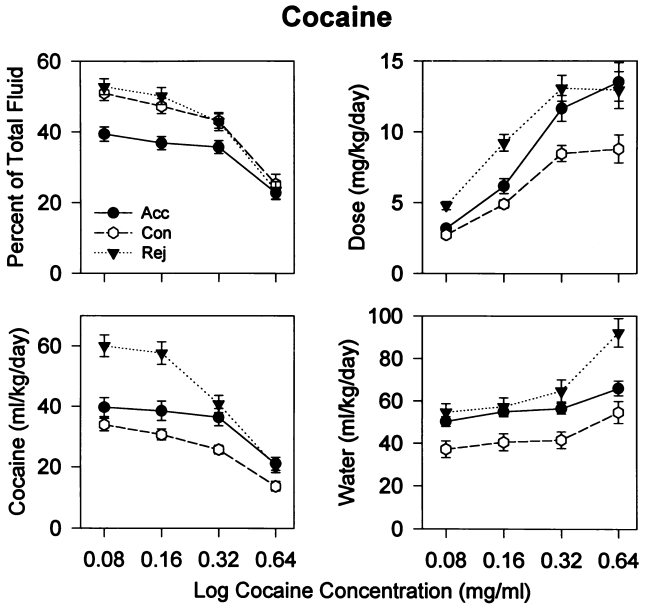

FIG. 2. Cocaine consumption as a function of the concentration of the cocaine solution, during the days the lines were given a choice between a cocaine solution and water. (Top Left) Cocaine intake as a percent of total fluid consumed. (Bottom Left) Cocaine intake in ml/kg/day. (Top Right) Dose of cocaine consumed in mg/kg/day. (Bottom Right) Water intake in ml/kg/day. All values are Mean ± SEM. N = 12–14 rats/line.

rejecting rats drank less, and there was no difference between males and females.

During the phase of a choice between ethanol and water (Fig. 1), the accepting and rejecting lines did not differ in any measure. As shown in the top left panel, the control line's preference for ethanol developed gradually after the first week, as is typical of unselected rats (17), whereas the selected lines intake as a percent of total fluid remained low and stable throughout the experiment. The control line's divergence from the selected lines resulted in a significant overall difference [$F(2, 33) = 5.04$, $p < 0.02$]. Because the amount of ethanol consumed did not differ among the lines (bottom left), neither did the doses received (top right). Water intake was significantly different overall [$F(2, 33) = 4.12$, $p < 0.05$], owing to the low intake of the control line (bottom right). Females were significantly different from males on 3 measures: they drank more ethanol [$F(1, 33) = 33.9$, $p < 0.00001$] and consequently received a higher dose [$F(1, 33) = 33.9$, $p < 0.00001$], and they drank more water as well [$F(1, 33) = 9.10$, $p < 0.005$]. In no measure, however, was there a line-sex interaction.

Figure 2 shows the data on cocaine intake during the choice phase; there was no line difference in preference (top left). The amount of cocaine ingested (bottom left), especially at the lower concentrations, and the resultant dose received (top right), were greater for the rejecting line, producing significant overall line differences [Amount: $F(2, 33) = 3.89$, $p < 0.05$; Dose: $F(2, 33) = 3.67$, $p < 0.05$]. Water consumption (bottom right) did not differ among the lines. As was the case with ethanol, females drank more cocaine solution [$F(1, 33) = 33.2$, $p < 0.00001$], consequently received a higher dose [$F(1, 33) = 21.5$, $p < 0.00001$], and also drank more water [$F(1, 33) = 30.1$, $p < 0.00001$]. Line-sex interactions were not significant.

Finally, in the choice phase between ETZ and water the lines ran true to genotype. In Figure 3 (top left) the expected pattern of accepting > control > rejecting was shown in ETZ intake as a percent of total fluid [$F(2, 33) = 3.39$, $p < 0.05$]. There were significant overall line differences in amount of ETZ consumed (bottom left; $F(2, 33) = 4.67$, $p < 0.02$] and consequently in dose received [top right; $F(2, 33) = 4.67$, $p < 0.02$], owing to the higher values attained by the accepting line. Water intake also differed among the lines [$F(2, 33) = 4.17$, $p < 0.05$]. There was only one gender difference: females drank significantly more water than males [$F(1, 33) = 59.6$, $p < 0.00001$]. Interestingly, in the 3 measures related to ETZ ingestion there was a significant line-sex interaction: accepting females had higher values than accepting males, but males had higher values than females of the other two lines, in ETZ intake as a percent of total fluid [$F(2, 33) = 6.17$, $p < 0.01$], ETZ consumption in ml/kg [$F(2, 33) = 6.01$, $p < 0.01$], and dose of ETZ received [$F(2, 33) = 6.01$, $p < 0.01$]. This finding stands in contrast to those with ethanol and cocaine, where there were no such interactions.

DISCUSSION

Selectivity of drug acceptance was shown in two parameters. When only a single fluid was available, the lines drank equivalent amounts of water, ethanol, and cocaine. However, acceptance of ETZ under the same conditions was significantly different among the lines, and in the direction for which they had been selectively bred. Second, when a choice between a drug solution and water was offered, there was no evidence that a preference for ethanol or cocaine tracked with preference for ETZ. The accepting and rejecting lines were no differ-

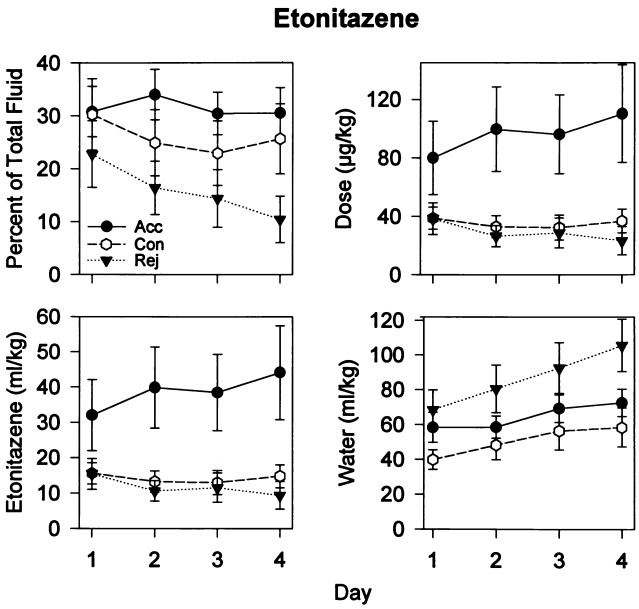

FIG. 3. Etonitazene (ETZ) consumption on each of the 4 days the lines were given a choice between 2.5 μg/ml etonitazene and water. (Top Left) ETZ intake as a percent of total fluid consumed. (Bottom Left) ETZ intake in ml/kg. (Top Right) Dose of ETZ consumed in μg/kg. (Bottom Right) Water intake in ml/kg. All values are Mean ± SEM. N = 12–14 rats/line.

ent in any measure related to ethanol consumption. Similarly, the lines showed equivalent consumption of cocaine as a percent of total fluid, and the rejecting line actually drank more cocaine solution than the accepting line on a ml/kg basis, exactly the opposite of what would be expected if there were a common genetic determinant. On a per body weight basis, females drank more ethanol and cocaine than males, but they also drank more water, and there were no line-sex interactions, indicating that the phenomenon was related to gender alone. In contrast, behavior toward ETZ solutions was characteristic of the selected lines, in the expected direction, and consistent with the results from prior (3) and subsequent generations. Further, as in our previous study (3), ETZ consumption by the accepting line produced clear stereotyped behavior indicative of intoxication (5,26), i.e., intense chewing on the block of wood in the cage.

These lines are unusual in the selectivity of their drug acceptance. The logical comparison is the five pairs of lines bred for ethanol preference; to our knowledge, only the AA line has been tested with drugs other than ethanol, and it prefers ETZ and cocaine over water (16), suggesting that selection in that ethanol preferring line has been for a factor common to several psychoactive drugs.

The ethanol doses consumed were low and comparable to those of Wistar rats (22), and were well below those of AA and P rats bred for ethanol preference (10,15,18,22), supporting the dissociation of ETZ and ethanol drinking in the present selected lines. Similarly, the cocaine doses attained were comparable to those of Wistar rats but less than those of AA rats drinking the same concentrations (16). It is apparent that as cocaine concentration increased, consumption by all the lines gradually fell off, but whether this was due to cocaine's interoceptive effects or its bitter taste is not known.

Although this must be tested directly in a future experiment, on several grounds we think it is unlikely that line differences in acceptance of ETZ are based on differing sensitivities to its weak bitter taste. In this choice paradigm rejection of ETZ solutions occurs gradually over days (2,3), whereas another bitter solution such as quinine becomes the non-preferred fluid in a matter of hours (2). The results with cocaine were inconsistent with the hypothesis, in that the rejecting line did not avoid the bitter taste of cocaine, in fact drank more than the other lines at the lower concentrations where a local anesthetic effect would not be a significant factor. Conversely, the accepting rats drank large amounts of ETZ but very little cocaine. In addition, the accepting and rejecting lines drank similar amounts of a fluid with a different strong taste, ethanol. The taste of the ETZ solution probably served as a cue for identifying which fluid contained the drug (4), but the available evidence does not suggest that the rejecting rats were more sensitive, or the accepting rats less so, to various tastes than rats of the control line.

Still to be determined is whether the lines also differ with respect to drinking other opioids. The technical problem of bitter taste is what led us to use ETZ in the first place, but other possible opioids are etorphine, which is about as potent as ETZ, and fentanyl, which is about 100 times more potent than morphine and can be diluted to levels which are acceptable to rats (2). Irrespective of those results, the specificity in drug acceptance already shown by the present lines makes them unique among selective breeding programs for drug self-administration.

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