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Selective Breeding for Oral Opioid Acceptance or Rejection in Rats

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CARLSON, K. R., C. M. SAULNIER-DYER AND M. S. MOOLTEN. *Selective breeding for oral opioid acceptance or rejection in rats.* PHARMACOL BIOCHEM BEHAV **53**(4) 871–876, 1996.—Lines of rats were selectively bred to diverge bidirectionally from a randomly bred control line in the propensity to self-administer an opioid orally. These lines seek or avoid the high-potency opioid etonitazene in a situation in which it is presented continuously as a choice with water. Over seven generations, preferences were measured and selection pressure imposed to develop the accepting and rejecting lines. These animals represent the only contemporary selective breeding program for opioid preference or self-administration, and hold the promise of being a useful resource in the drug-abuse field.

Opioid Opiate Selective breeding Etonitazene Drug abuse Genetics Rat

THE WIDE VARIATION in the propensity of humans to self-administer many drugs may be partly caused by genetic factors. The paradigm example of a genetic component to drug abuse is alcoholism, as has been demonstrated in numerous family, twin, and adoption studies [reviewed in (39)]. It has also been suggested that human opioid abuse might be at least partially genetically determined (4,35).

Selective breeding has been used to develop many lines of rats and mice which differ with respect to their responses to abused drugs or in their propensity to ingest drugs [for reviews, see (16,28)]. The latter effort has met with particular success in the field of alcohol abuse, where five pairs of rat lines differing in their willingness to drink ethanol have been developed. The most thoroughly investigated are the Finnish AA (ALKO, alcohol) and ANA (ALKO, nonalcohol) lines (42) and the Indiana University P (preferring) and NP (nonpreferring) lines (29).

Only two prior attempts have been made to breed selectively for opioid preference. In the first (32), Sprague–Dawley rats were made dependent on morphine by injection, and then were subjected to a regimen of varying fluid availability which induced morphine drinking. After a morphine–water choice trial, morphine was withdrawn, the rats were ranked for severity of the abstinence syndrome, and the most susceptible and most resistant were identified. These two groups were selectively bred and were found to diverge significantly over the three generations tested in the percentage of morphine drunk in the choice trial. These data suggest that opioid intake can be brought under genetic control, even when selection is based on the severity of withdrawal symptoms rather than on the ingestive behavior itself. More recently, Sprague–Dawley rats which drank large amounts of morphine-adulterated liquid diet were used to selectively breed a high-preference line (36). Over two selected generations preference increased, but in generations 3–8, preference for morphine was not transmitted to offspring and there was a high mortality rate among newborn pups.

Many drugs, including opioids, serve as reinforcers when ingested by rodents and other species, and oral drug self-administration is a practical and accepted animal model of drug abuse in humans [reviewed in (30)]. Its use has been limited in opioid research, however, by the bitterness of opioids in solution; disguising the taste is often necessary to induce significant consumption [e.g., (27)], with possible confounding effects. This problem can be overcome by employing very dilute solutions of the high-potency opioid etonitazene (ETZ). This synthetic opioid is selective for the μ receptor (31) and qualitatively equivalent to morphine, but approximately

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In abstracts reporting parts of this work (8–10), the lines were named preference, random, and aversion. The names have been changed to accepting, control, and rejecting, respectively, to reflect more accurately the animals' behavior.

1000–2000 times more potent in the ability to serve as a positive reinforcer (37,44), in producing physical dependence (47) and such behaviors as catalepsy and analgesia (1,18,37), and in identification as an opioid in a drug discrimination task (21,40). At behaviorally active concentrations, the taste of ETZ does not appear to be aversive to rats, and the drug is used frequently in oral self-administration paradigms (7, 11,12).

In preference paradigms, the consumption of the drug is compared with that of concurrently available water. The usual procedure is to present two bottles side by side in the home cage. However, rats have strong position preferences and will often drink from the same bottle regardless of its contents (12,13,20). Thus, daily alternation of the side of the drug bottle can measure merely the strength of the rat's position preference, rather than preference for the drug. We used a device developed in this laboratory, which presents both drug and vehicle at the same position in relation to the rat, thus permitting an accurate measure of an animal's preference for one or the other (7).

We report here the successful establishment of opioid-accepting and -rejecting lines of rats, and compare them to each other and to a randomly bred control line.

METHODS

Animals

At all times, the rats were housed under a 12 L : 12 D cycle (lights on at 0700 h) at 22 ± 3°C with ad lib chow. When not under experimentation, they were kept in same-sex groups of 3–4 in 30 × 34 × 16-cm-high Plexiglas cages with ad lib water, and when females were with a litter they were housed in large maternity cages. Pups were weaned from their mothers at 21–28 days old. While being tested for drug preference, each rat was housed individually as described below.

Drug

Etonitazene base (NIDA, Rockville, MD) was prepared as a 2.5-μg/ml solution in tapwater.

Selective Breeding

The foundation stock was 30 males and 30 females of the maximally heterogeneous N : NIH strain (24), representing the genetic diversity necessary to begin such a project (14,28). These rats were tested for ETZ preference (see next section), and those with the highest consumption of ETZ as a percentage of total fluid were mated to begin the accepting line; those with the lowest percentage began the rejecting line; and from the remainder the control line parents were chosen at random. This resulted in three to five pairs to begin each line. In each generation all rats were tested for preference, and those with the most extreme preferences appropriate to their line were chosen, generally >40% for the accepting line and <15% for the rejecting line. Individual selection, in which animals were chosen solely on the basis of their individual scores, was used, rather than within-family selection. Breeders in the control line were always picked at random, without regard for their preference, while avoiding sibling pairs. The number of mating pairs per line was gradually increased to 10 by generation 4, and has been maintained at this number in subsequent generations.

Preference Testing

The rats were housed continuously and individually in 30 × 34 × 16-cm-high Plexiglas cages with ad lib food. At the end wall of each cage was a switching device (7) which eliminated the influence of the rats' position preferences when they were given a choice between drug and water, by presenting both solutions at what the rat apparently viewed as the same location. Two 50-ml centrifuge tubes with leakproof spouts were mounted side by side behind a shutter with a small round hole through it. The shutter was driven back and forth by a motor, placing the hole alternately every two min in front of one of the spouts. The rat had access to the hole and the spout behind it through a large rectangular opening in its cage wall. When both spouts contained water, the vast majority of rats drank equally from both, but any rat exhibiting a preference under these conditions was excluded from further experimentation. Each cage contained a 10-cm length of pine (2 × 4); while rats were drinking ETZ they developed intense stereotyped chewing on the wood, a characteristic of chronic intoxication with ETZ (12,47).

On the 1st day, rats had access to one spout containing water behind a stationary shutter. They were then given 2 days to learn to drink water from both spouts with the shutter moving; the latter one also represented a water–water baseline day. Beginning with generation 2, for 4 days they then had available only one spout which contained 2.5 μg/ml ETZ with the shutter stationary, to ensure that all rats had experienced the effects of this drug (otherwise, one could argue that the rejecting line rats did not choose ETZ because they had never tried it). Finally, they were given a choice between 2.5 μg/ml ETZ and tapwater for 7 days with the shutter moving. ETZ and water consumption were measured daily by weighing the centrifuge tubes, and the rats were weighed daily. Testing was done when they were 3–5 mo old, equalizing as much as possible the number of rats from each line in each batch of 40 rats (the number was determined by the number of switching devices). Beginning with generation 7, the number of days in the ETZ-only phase was reduced to two, because rats drank the same amount of ETZ during the last 2 days as they had during the first 2 days. Similarly, the number of choice days was reduced to 4, because acceptance or rejection was well established by then, and there was little additional information to be gained from the last 3 days (see Results).

Statistics

For each generation, differences among the lines in the amount of ETZ consumed during the ETZ-only phase, and in ETZ consumed as a percentage of total fluid or as a percentage of baseline intake during the choice phase, were analyzed by analysis of variance with repeated measures on the day variable (48).

RESULTS

Rats of all lines remained healthy and fertile. Although accepting males weighed less than those of the other two lines in generation 4 and later, the females weighed the same and there were no line differences in litter size in any generation (average about seven pups). Thus, by generation 4 and thereafter, when it was possible to have 10 breeding pairs per line, the number of rats in each line tested for preference averaged 67.9 (range 57–84).

During the ETZ-only phase of testing, there were no differences between the accepting and rejecting lines in the amount of ETZ consumed until generation 6, when the accepting line drank significantly more ETZ solution when it was the sole source of fluid than did the other two lines (29.5 vs. 19.0

and 19.1 ml; $p < 0.00001$). This difference was also found in generation 7 (28.4 vs. 21.9 and 20.3 ml; $p < 0.00001$).

Figure 1 shows the divergence of the selected lines over generations in preference for ETZ, both as a percentage of the total fluid intake (top) and as a percentage of baseline water intake from that spout (bottom). In all but generation 1, the difference among the three lines was statistically significant ($F = 11.1$–87.7, $p < 0.00001$). In generations 2–7, the control line continued to have preference scores between the other two lines, and both the accepting and rejecting lines differed significantly from those unselected rats.

Detailed data from generation 7 are shown in Fig. 2. On day 0, the baseline water-water choice day, there were no differences among the groups in the amount of water consumed from the spout which would subsequently deliver ETZ (top panel). In the ETZ-water choice phase, however, the lines were significantly different in the consumption of ETZ as a percent of total fluid (top panel; $p < 0.00001$) and of baseline (bottom panel; $p < 0.00001$). As in previous generations, avoidance of ETZ was established in a few days in the rejecting line, and in general, the behavior of all the lines either remained the same or stabilized within the 4 choice days.

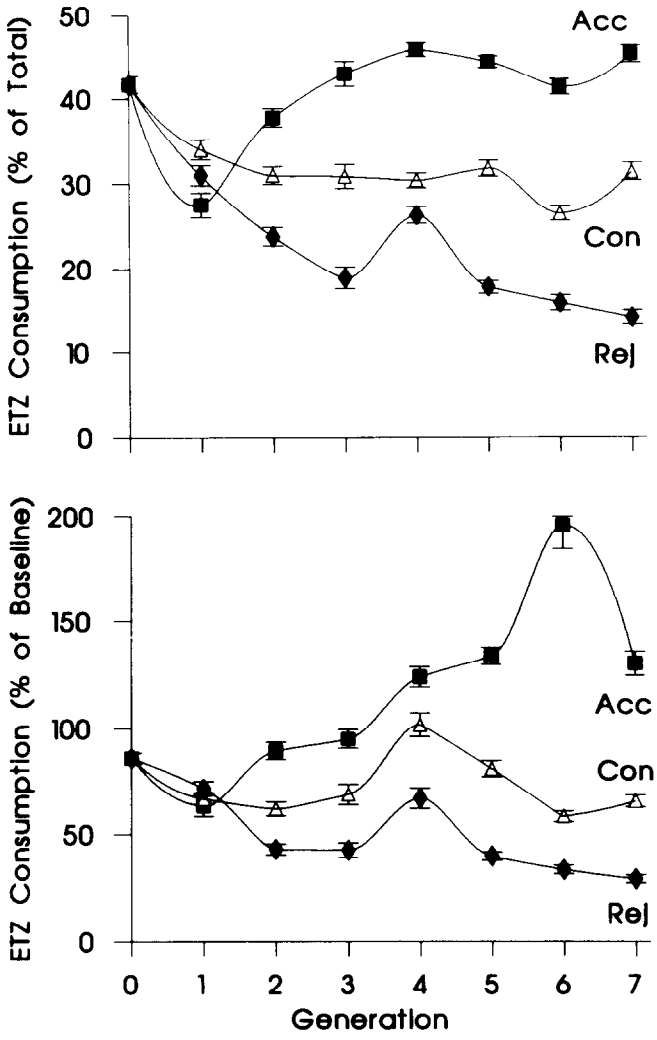

FIG. 1. (Top) ETZ consumption as a percentage of total fluid intake (mean ± SEM) during the ETZ-water choice phase for the accepting (Acc), control (Con), and rejecting (Rej) lines in each generation. (Bottom) ETZ consumption as a percentage of baseline intake from that spout (mean ± SEM).

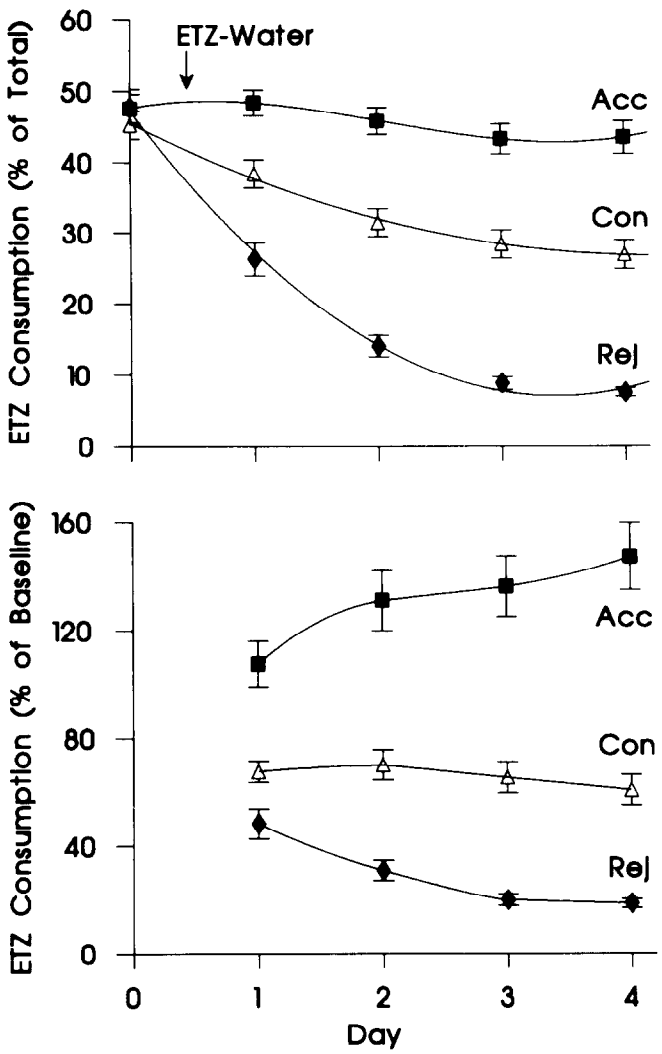

FIG. 2. (Top) ETZ consumption as a percentage of total fluid intake (mean ± SEM) for generation 7 rats of the accepting (Acc), control (Con), and rejecting (Rej) lines. The day 0 data represent the percentage drunk in a water-water choice from the spout which would subsequently deliver ETZ. Arrow: an ETZ-water choice was imposed during days 1–4. (Bottom) ETZ consumption as a percentage of the baseline intake from that spout during day 0 (mean ± SEM).

If one considers ETZ consumption as a percentage of baseline water intake from that spout, then the rejecting line exhibits a strong aversion and the accepting line a true preference for the drug (Fig. 2, bottom). However, a comparison of ETZ and water in terms of ETZ consumption as a percentage of total fluid shows that the rejecting line avoided ETZ in preference for water, but that the accepting line did not yet drink ETZ as >50% of total fluid (Fig. 2, top). The explanation lies in the absolute amounts of fluid consumed, as shown in Fig. 3 (top). For every generation except the first, the accepting line drank significantly more total fluid and sig-

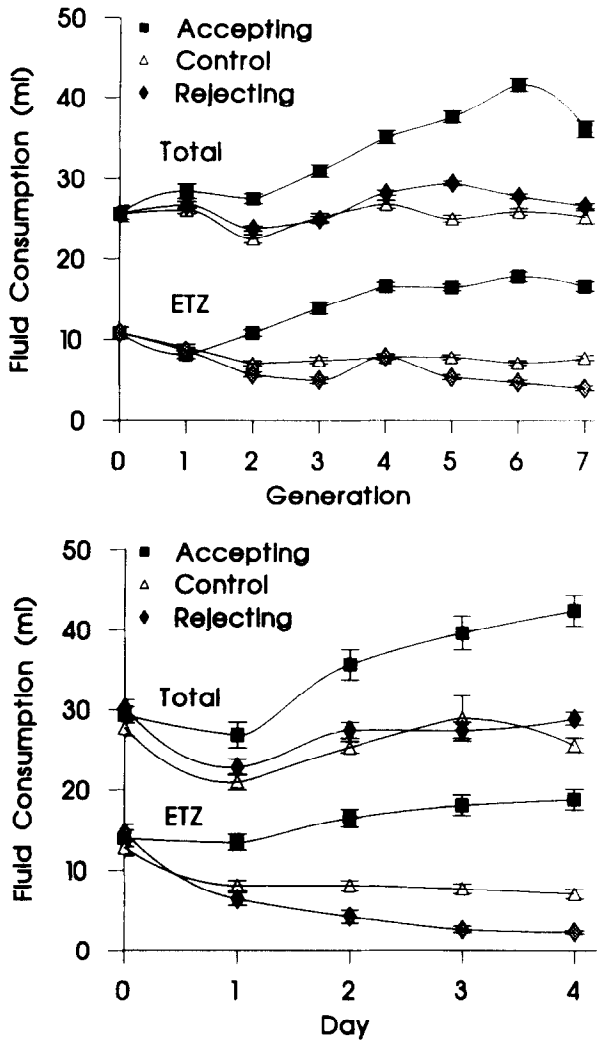

FIG. 3. (Top) Fluid consumption (mean ± SEM) during the ETZ-water choice phase for the three lines in each generation. The upper set of data lines represents total fluid intake (ETZ + water) and the lower set ETZ intake. (Bottom) Fluid consumption (mean ± SEM) during the 4 days of the ETZ-water choice phase for generation 7 rats of the three lines. The upper set of data lines represents total fluid intake (ETZ + water) and the lower set ETZ intake.

nificantly more ETZ than the other two lines (p = 0.01-0.00001), thus keeping the percentage of ETZ to total at about 50%.

The fluid consumption of generation 7 is shown in Fig. 3 (bottom). There were no differences among the lines on day 0, either in total fluid intake or in water intake from the spout which would later contain ETZ. Total and ETZ intake by the accepting line rose gradually on choice days 2-4, whereas the control and rejecting lines had fairly constant total fluid intake. The rejecting line's decreased consumption of ETZ was compensated for by increased water intake, keeping total fluid consumption at the same constant level as that of the control line.

The possibility that the accepting line's increased ETZ intake was caused by polydipsia was not supported by the total fluid intake on day 0. With the exception of generations 3-5, when the control line drank slightly less than the others, there were no differences in fluid consumption (data not shown). Most important, there was never any difference between the accepting and rejecting lines, although their intakes were very different when a choice between ETZ and water was offered.

In no generation was there a difference between the sexes of any line in ETZ preference, i.e., the amount of ETZ consumed in relation to water, or in the absolute amount of ETZ drunk, during the ETZ-water choice phase. However, females of the accepting line took in a significantly higher dose of ETZ than males; because their body weights were lower, the dose they received was higher, even though they drank the same volume of ETZ as the males. For example, in generation 7 the accepting females consumed 253 ± 12 μg/kg per day vs. the males' 140 ± 7 μg/kg per day ($p < 0.00001$). Because the other lines drank relatively little ETZ (Fig. 3), there were no sex differences in dose for the rejecting animals and only small, insignificant ones for the control animals.

DISCUSSION

This study demonstrates bidirectional selective breeding for opioid acceptance. It is clear that the lines are diverging, although the course of each line is not straight. This was expected, as the response to selection pressure is often uneven, with one line changing more than the line selected to go in the opposite direction (17,33,34) and with variations in progress over generations (15,17). Thus, it is necessary to maintain a control line to determine whether both lines are diverging from a randomly bred population (5,16,28), as are these selectively bred lines.

The two previous attempts to breed selectively for opioid preference began with a Sprague-Dawley foundation stock. One project was abandoned after three generations (32), and the other was unsuccessful in establishing a high-preference line and also encountered high infant mortality (36). The present program has not experienced the latter difficulties, most likely because the foundation stock was maximally heterogeneous and because the duration of exposure to the opioid during selection tests was far shorter. In addition, the rats remained fertile, probably because the necessary 10 pairs were bred per line (14,17).

Although maintaining independent replicate lines (two accepting, two rejecting, and two control lines) is considered theoretically desirable (14), in practice that proved impossible. There were not enough rats with strong preferences in the foundation stock to establish replicate lines, and even if there had been, the expense of maintaining them would have been prohibitive. This appears to be the situation with most modern selective breeding programs related to drugs of abuse; only two of nine such programs using rats have established replicate lines (16,38). Further, the use of replicate lines in eliminating spurious correlations has been challenged (41).

Although this possibility has not yet been tested directly, selection does not appear to be for polydipsia in the accepting line. These rats were not polydipsic when only water was available on day 0, and they only gradually increased their total fluid consumption when ETZ was presented with water. In addition, elevated intake of ETZ during the ETZ-only phase was not observed until generation 6, whereas increased fluid intake during the choice days began with generation 2. If polydipsia were responsible, it seems logical that it would be present during both phases.

It might be argued that the rejecting line is more sensitive to the weak bitter taste of ETZ, and as a consequence avoids

drinking the drug. Two pieces of evidence are inconsistent with this position. In a short preliminary study, drug-naive generation 4 rats were presented with a choice between water and increasing concentrations of quinine; at each concentration, the rejecting line demonstrated an aversion equivalent to those of the other lines. Second, as shown in Fig. 2, both the accepting and rejecting lines showed gradual changes in ETZ consumption over the first 3 choice days. Using this presentation device, preferences based on taste are established in a few hours, whereas opioid preferences develop over days (7). Thus, our working hypothesis is that acceptance and rejection are based on postingestional effects, presumably in the CNS. A similar conclusion has been reached in most studies using ethanol-preferring and -avoiding selectively bred lines (3, 19,46).

We have only preliminary data concerning the specificity of selection. Generation 2 breeders (with high levels of ETZ preference or aversion) were offered a choice between water and increasing concentrations of ethanol; the lines showed similar preferences at all concentrations. However, these data were obtained very early in the selection process and represented only 1 day per concentration. If later confirmed and extended to other drugs such as cocaine, those observations would sharply differentiate these animals from most inbred strains and selectively bred lines. Preferences for different drug classes often covary; for example, ethanol, ETZ, morphine, and cocaine by the oral route serve as strong positive reinforcers for Lewis rats, but none is a reinforcer for Fischer 344 rats (22,43-45). With respect to selectively bred lines, in one report of breeding for differential morphine ingestion the line of rats which drank the most morphine also drank more ethanol than the other line (32). In addition, AA rats preferred low concentrations of ETZ and cocaine solutions over water, and they drank more of them at all tested concentrations than did ANA rats (26), suggesting that there may be a common genetic determinant to psychoactive drug consumption (22, 23). It has also been shown that μ-opioid antagonists decrease ethanol drinking in AA rats (25), suggesting an ethanol-opioid relationship in this line.

The importance of pharmacokinetic factors in strain differences has been demonstrated recently with Lewis and Fischer 344 rats (6). If differences in the metabolism of ETZ have been developed by selective breeding, they could be responsible for the observed differences in acceptance of the drug. This possibility remains to be addressed in future work.

Although drugs of many classes which are abused by humans are also self-administered by animals, genetically determined differences in preference or self-administration have been studied only with respect to ethanol, cocaine, and ETZ (23). Regarding selective breeding, the vast majority of programs are for responses to acute drug administration (16); within the opiate field, it is remarkable that there is only one such program, for the analgesic response to levorphanol (2). To our knowledge, there are currently only two selective breeding programs for preferences other than for ethanol: one for cocaine (38) and the present for ETZ. Thus, this colony represents a unique resource which should prove useful in studying the genetic basis of opioid preferences.

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