Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 415–419, 1999

0091-3057/99/$–see front matter

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PII S0091-3057(99)00053-2

Differences Between Alcohol-Preferring and Alcohol-Nonpreferring Rats in Ethanol Generalization

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MCMILLAN, D. E., M. LI AND D. J. SHIDE. *Differences between alcohol-preferring and alcohol-nonpreferring rats in ethanol generalization.* PHARMACOL BIOCHEM BEHAV **64**(2) 415–419, 1999.—Alcohol-preferring (P rats) and alcohol-nonpreferring rats (NP rats) were trained to discriminate intraperitoneal injections of 0.5 g/kg ethanol, or subcutaneous injections of 0.6 mg/kg nicotine from saline. P rats learned the ethanol discrimination more rapidly and made a higher percentage (88%) of their responses on the ethanol lever after ethanol and a lower percentage (7%) after saline than NP rats (78 and 15%, respectively). In substitution tests, increasing doses of ethanol produced increases in the percentage of responses on the ethanol lever with similar ED_{50}s (0.43 and 0.44 g/kg) in P and NP rats. P rats trained to discriminate ethanol from saline made more responses on the ethanol lever after nicotine (80%) and *d*-amphetamine (63%) than NP rats (33 and 40%). The ethanol stimulus did not generalize to morphine in either P or NP rats. NP rats trained to discriminate ethanol from saline responded more on the ethanol lever after bupropion (77%) than P rats (49%). In rats trained to discriminate nicotine from saline, the nicotine discriminative stimulus did not generalize to ethanol in either P or NP rats, suggesting that the genetic difference in the stimulus generalization of ethanol was not symmetrical. © 1999 Elsevier Science Inc.

Ethanol Drug discrimination Genetic differences Stimulus Generalization Rats

THERE is evidence that genetic factors play an important role in the development of alcoholism (2). One approach to the study of hereditary factors in alcoholism is to use animal models. Genetic selection has developed several rat lines that differ widely in oral ethanol intake, such as the alcohol-preferring line (P rats) and the alcohol-nonpreferring line (NP rats) (1). When presented with a free choice between 10% (v/v) ethanol and water, P rats consume large amounts of ethanol, while NP rats do not.

Drug-discrimination procedures are widely used to study the stimulus properties of abused drugs. It has been suggested that interoceptive stimulus state produced by drugs is related to, although not identical to, the "subjective effects" produced by the drugs (7). The discriminative stimuli produced by abused drugs presumably contribute to their reinforcing effects, and consequently, to their abuse liability. Recently, Gordon et al. (3) trained P and NP rats to discriminate the presence or absence of ethanol. P rats trained to discriminate the presence or absence of ethanol generalized the ethanol discriminative stimulus to nicotine. NP rats did not. These data suggested a genetic difference in ethanol discrimination in P and NP rats. Our experiments expanded on this observation.

METHOD

Subjects

P and NP rats were obtained from the University of Indiana when the rats were about 90 days old. They were housed in a light- and temperature-controlled colony room when not being tested. Access to food was restricted to maintain the rats at about 85% of their free-feeding weights. In the ethanol discrimination experiments, 8 P and 6 NP rats were studied. In the nicotine discrimination experiments, 6 P and 6 NP rats were studied.

Apparatus

Experiments were conducted in Gerbrands test chambers enclosed in sound-attenuating enclosures. Each chamber contained two Gerbrands levers mounted above and lateral to the food cup into which 97 mg food pellets could be delivered.

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The test chamber was lighted by a houselight, which came on at the beginning of training and test sessions.

Procedure

Rats were trained by successive approximations to press both levers to obtain food pellets. Once responding was established on both levers under a fixed-ratio 10 response (FR 10) schedule, discrimination training began. Before each session rats were injected intraperitoneally with either physiologic saline solution, or 0.5 g/kg ethanol diluted with physiologic saline solution to a concentration of 10 g/100 ml. Responses were reinforced under the FR 10 schedule on one lever (counter balanced across rats) if 0.5 g/kg ethanol had been administered before the session, and on the other if saline had been administered before the session. Injections were given 10 min before the session, and animals were placed into the darkened chamber until the onset of the houselight signaled the beginning of the training session. Training sessions were conducted 5 days a week. Another group of 6 P and 6 NP rats was trained to discriminate 0.6 mg/kg (−)nicotine hydrogen tartrate (doses as the salt) from physiologic saline using similar training procedures. The pH of the nicotine solution was adjusted to 7 with dilute NaOH. Injections were given subcutaneously, 15 min before the session.

After responding stabilized, substitution tests were conducted with other doses of ethanol, nicotine, or other drugs administered before the session instead of the training drug. Rats were tested until one food pellet had been received during these test sessions. The food pellet was delivered when the FR 10 requirement had been completed on either of the two levers. Test sessions were conducted on Tuesdays and Fridays, with training sessions continuing on other weekdays.

RESULTS

Figure 1 shows the establishment of the ethanol discrimination in P and NP rats. Within 10 training sessions after both ethanol and saline administration, the P rats were showing clear evidence that ethanol was being discriminated from saline. After about 60 training sessions with ethanol and saline, discrimination performance appeared to have reached asymptote in the P rats, with more than 90% of the responses occurring on the appropriate lever after both ethanol and saline administration.

The NP rats were slower to learn the discrimination. About 40 sessions under each training condition were required for NP rats to perform at the level seen after 10 sessions under each training condition with P rats. When performance became asymptotic, NP rats responded on the appropriate lever about 80% of the time after both ethanol and saline administration. Variability was also somewhat higher in NP rats than in P rats (error bars in Fig. 1).

Figure 2 summarizes the data from substitution tests in P and NP rats trained to discriminate ethanol from saline (top frames), or nicotine from saline (bottom frames). At the 0.2 g/kg dose of ethanol, both P and NP rats trained to discriminate ethanol from saline responded more often on the saline lever than on the ethanol lever (top frames). With increasing ethanol doses, increased responding occurred on the ethanol lever for both groups of rats. The ethanol dose–response curves were very similar for both groups of rats. The ED_{50} for ethanol was 0.43 g/kg in P rats and 0.44 g/kg in NP rats.

When nicotine was administered to P rats, increasing doses of nicotine produced increased responding on the ethanol lever. The two highest doses of nicotine resulted in approximately 80% of the responses occurring on the ethanol lever. This generalization of the training dose of ethanol to nicotine did not occur in NP rats, where most of the points on the dose–effect curve for nicotine were within one standard error of the saline training mean.

When substitution tests were conducted with *d*-amphetamine in P rats, all doses produced more responding on the ethanol lever than after saline, and after the two highest doses of *d*-amphetamine almost 70% of the responses were on the ethanol lever. In contrast, after *d*-amphetamine administration to NP rats, the percentage of responses on the ethanol lever never rose above 40%, and most of the points on the dose–response curve were within one standard error of the mean for saline training sessions.

When substitution tests were conducted with morphine, low doses produced responding confined largely to the saline key in both groups. Higher doses of morphine produced 40 to 50% of the responses on the ethanol key with little difference between P and NP rats. When substitution tests were conducted with bupropion in P rats, higher doses produced in-

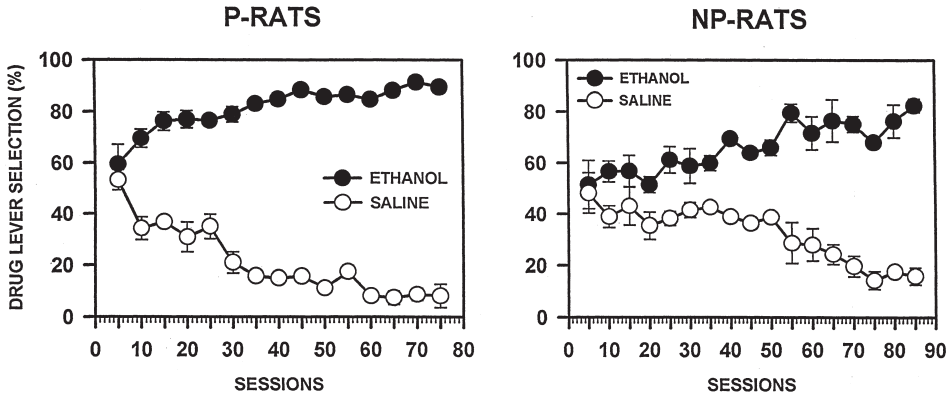

FIG. 1. Acquisition of 0.5 g/kg ethanol discrimination in P (left frame) and NP (right frame) rats. Each point represents a mean of five training sessions. Brackets represent ± one standard error. When no brackets are shown, the standard error was less than the diameter of the point representing the mean.

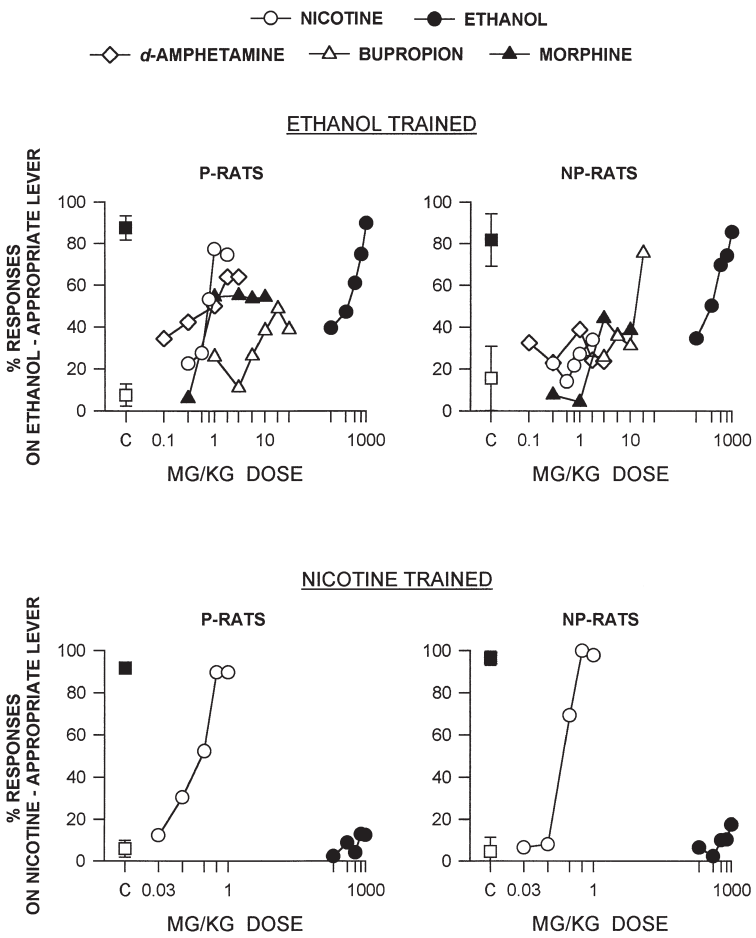

FIG. 2. Dose–response curves for generalization of the ethanol discriminative stimulus (top frames), or the nicotine discriminative stimulus (bottom frames) in P (first column) and NP (second column) rats. Each point represents a mean of six to eight rats. Squares at C represent mean performance during training sessions after ethanol or nicotine (filled squares) and after saline (unfilled squares). Brackets at C show ± one standard error.

creased responding on the ethanol lever, but the percentage of responses on the ethanol lever never exceeded 50%. In contrast, in NP rats the highest dose of bupropion produced almost 80% responding on the ethanol lever, which was equivalent to that seen during ethanol training sessions.

Figure 3 shows the acquisition of the discrimination between 0.6 mg/kg nicotine and saline in the other groups of P and NP rats. As with the acquisition of the ethanol discrimination, both P and NP rats showed evidence of learning the nicotine discrimination within 10 training sessions. P rats reached asymptotic performance more rapidly (about 20 sessions) than NP rats (more than 30 sessions). Although the final performance of both groups of rats was similar, NP rats responded less often on the nicotine lever than did the P rats. Thus, the large differences between P and NP rats in acquisition of the ethanol discrimination were not observed for the acquisition of the nicotine discrimination.

The bottom frames of Fig. 2 show the dose–response curves for substitution tests with ethanol and nicotine in the group of rats trained to discriminate 0.6 mg/kg nicotine from saline. Both P and NP rats responded on the appropriate lever more than 90% of the time after both saline and the training dose of nicotine. Increasing doses of nicotine produced increased responding on the nicotine lever in both P and NP rats, and the dose–response curves were very similar for the two groups. The nicotine discriminative stimulus did not generalize to ethanol in either group. Thus, the generalization between ethanol and nicotine in P rats was from ethanol to nicotine, but not from nicotine to ethanol.

Because the ethanol discriminative stimulus generalized to nicotine in P rats but the nicotine stimulus did not generalize to ethanol, it was of interest to study interactions between ethanol and nicotine in P rats. In P rats trained to discriminate nicotine from saline, doses of 0.6 and 0.8 g/kg ethanol decreased the percentage of responses on the nicotine lever after all doses of nicotine (Fig. 4). Thus, although the ethanol stimulus generalizes to nicotine in P rats trained to discriminate 0.5 g/kg ethanol from saline, ethanol appears to block the nicotine stimulus in P rats trained to discriminate 0.6 mg/kg nicotine from saline.

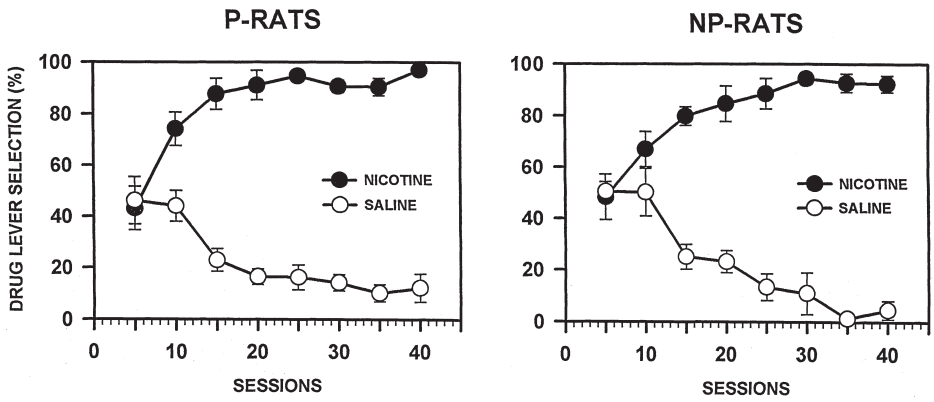

FIG. 3 Acquisition of 0.6 mg/kg nicotine discrimination in P (left frame) and NP (right frame) rats. Each point represents a mean of five training sessions. Brackets represent ± one standard error. When no brackets are shown, the standard error was less than the diameter of the point representing the mean.

DISCUSSION

These experiments replicate the original observation by Gordon et al. (3) that in P rats, but not in NP rats, the ethanol discriminative stimulus generalizes to nicotine. The experiments add to those of Gordon et al. (3) by showing that the discrimination is not symmetrical, that is, there is generalization from ethanol to nicotine, but not from nicotine to ethanol. Furthermore, doses of 0.6 and 0.8 g/kg ethanol appear to block the nicotine discriminative stimulus in P rats trained to discriminate 0.6 mg/kg nicotine from saline.

It is not clear if the difference between P and NP rats in the generalization of ethanol to nicotine represents a qualitative or quantitative difference. Because the ethanol dose–response curves were very similar in P and NP rats trained to discriminate ethanol from saline, it seems unlikely that the difference in the generalization from ethanol to nicotine in P and NP rats is only quantitative.

Figure 1 showed that asymptotic stimulus control by ethanol was somewhat weaker in NP rats than in P rats, and this difference in stimulus control at the time of testing might provide a basis for the differential generalization from ethanol to nicotine. However, one would expect that weaker control by the discriminative stimulus in NP rats would result in broader generalization of the ethanol discriminative stimulus, which is the opposite effect from that observed. Nevertheless, it is possible that better stimulus control might have been established in NP rats with a higher training dose of ethanol, and that this would have resulted in generalization of the ethanol discriminative stimulus to nicotine in NP rats.

Another argument that these genetic differences between P and NP rats are qualitative is that differences in the generalization of the ethanol discriminative stimulus are observed across other drug classes. For example, there was also a greater degree of stimulus generalization from ethanol to

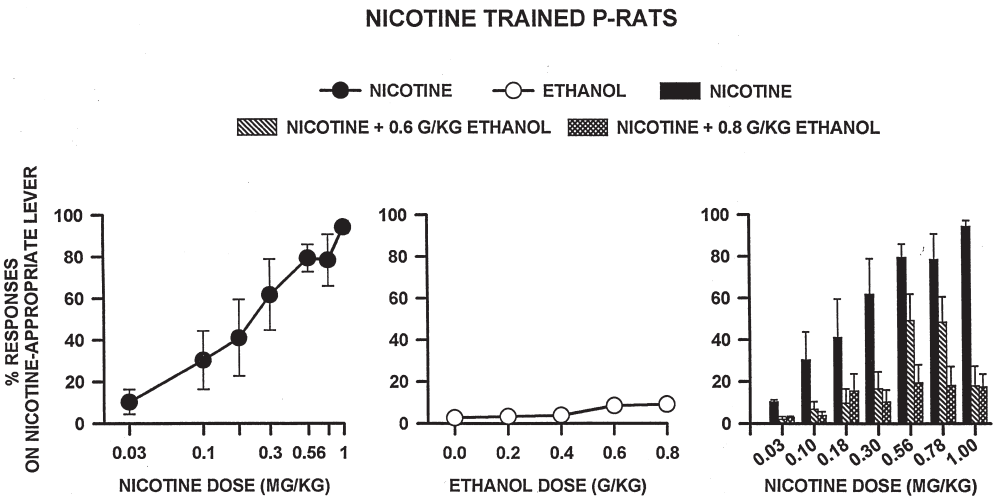

FIG. 4. Interaction between nicotine and ethanol in P rats trained to discriminate 0.6 mg/kg nicotine from saline. The left frame shows the nicotine dose–response curve and the middle frame shows the ethanol dose–response curve. The right frame shows the nicotine dose–response curve in the presence of saline (black bars), 0.6 g/kg ethanol (crosshatched bars), and 0.8 g/kg ethanol (dotted bars). Brackets represent ± one standard error.

d-amphetamine in P rats than in NP rats, although even in the P rats responding on the ethanol lever did not reach the levels seen after administration of the training dose of ethanol.

Another possible explanation for the generalization from ethanol to nicotine and *d*-amphetamine in P but not in NP rats is that the generalization gradients from ethanol to all drugs is broader in P rats than NP rats. This explanation does not fit the data. First, there was little difference in the generalization curves for morphine in the two strains. More strikingly, the ethanol stimulus generalized to bupropion in NP rats, not in P rats. This finding is the opposite to that with nicotine where the generalization occurred in the P rats and not the NP rats. Thus, P rats trained to discriminate ethanol from saline show a greater degree of ethanol stimulus generalization to some drugs than NP rats, but this relationship is reversed for at least one other drug.

It is difficult to explain why the discriminative stimuli produced by ethanol generalize to nicotine, but the discriminative stimulus effects of nicotine do not generalize to ethanol. It is possible that the stimulus properties of the two drugs overlap in P rats, but are not identical. Animals trained to discriminate ethanol from saline may attend to the part of the stimulus complex produced by ethanol that overlaps with the stimulus complex produced by nicotine, but animals trained to discriminate nicotine from saline attend to a part of the nicotine stimulus complex that is not shared by ethanol.

Ethanol interacts with multiple receptors (4), and the complex receptor interactions of ethanol may explain why the ethanol discriminative stimulus generalizes to 5-HT agonists, $GABA_A$ modulators, NMDA antagonists, and benzodiazepine agonists (5,8), although usually not nicotine. Previous studies have suggested that whether or not the ethanol discriminative stimulus generalizes to some of these drugs depends on the training dose of ethanol (5). Although the training dose of ethanol used in the present studies was very low (0.5 g/kg) compared to that used in most studies, the route of administration used in the present experiments was intraperitoneal, a dose that other investigators have not been able to establish as a discriminative stimulus with oral administration (5). Neither the interaction of different training doses with different receptor subtypes nor differences in ethanol absorption would explain why the ethanol stimulus generalizes to nicotine in P rats, but not in NP rats, unless P and NP rats differ in receptor populations or the pharmacokinetics of ethanol.

The differences between P and NP rats in the generalization of the ethanol stimulus to nicotine suggest that either the ethanol stimulus or the nicotine stimulus is different in P and NP rats. It seems likely that this genetic difference relates to ethanol rather than nicotine. First, we have not observed any clear differences between nicotine discrimination in P and NP rats. The nicotine discrimination is learned rapidly in P and NP rats, and their asymptotic performance is very similar. Furthermore, the nicotine stimulus does not generalize to ethanol in either P or NP rats. In contrast, the ethanol discrimination is learned more rapidly and to a higher level in P rats than NP rats, and there is generalization from ethanol to nicotine in P but not in NP rats. This argues that we are looking at a genetic difference in the stimuli produced by ethanol in P and NP rats, not a genetic difference in the stimuli produced by nicotine. In this context, it is interesting to note that there is a recent report of a genetic correlation between smoking and perceived ethanol intoxication in women (6).

ACKNOWLEDGEMENTS

This work was supported in part by NIDA Grant DA 02251.

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