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# P and NP Rats Respond Differently to the Discriminative Stimulus Effects of Nicotine

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GORDON, T. L., S. M. MEEHAN AND M. D. SCHECHTER. *P and NP rats respond differently to the discriminative stimulus effects of nicotine.* PHARMACOL BIOCHEM BEHAV 45(2) 305-308, 1993.—Rats selectively bred for ethanol preference, that is, alcohol-preferring (P) and -nonpreferring (NP) rats, were trained to discriminate the interoceptive stimuli produced by IP-administered 1,000 mg/kg ethanol (10% v/v) in a two-lever, food-motivated, operant task. Once criterion performance was attained, dose-response experiments indicated an $ED_{50}$ value for P rats = 354.1 mg/kg, whereas NP rats generated an $ED_{50}$ value of 495.1 mg/kg, not significantly different from each other. In contrast to these similar sensitivities to ethanol, administration of doses of nicotine (0.4-1.2 mg/kg, SC) indicated that P rats were significantly more sensitive to the ethanol-like effects of nicotine then were NP rats. The results provide additional evidence for a possible hereditary cooccurrence of alcohol and nicotine sensitivity.

Drug discrimination Ethanol P/NP rats Nicotine

THE established notion that there is a genetic predisposition to alcoholism (6) has lead to the development of a number of selectively bred strains of mice and rats that differ in their response to ethanol. Mice have been selectively bred for differences in ethanol's hypnotic effect, that is, long-sleep (LS) and short-sleep (SS) mice; differences in resistance to ethanol withdrawal seizures, that is, withdrawal seizure prone (WSP) and withdrawal seizure resistant (WSR) mice; as well as differences in the locomotor stimulatory effects of ethanol, that is, FAST and SLOW mice (25). A number of selectively bred rat lines have also been developed based upon differential sensitivity to ethanol effects. High- and low-alcohol-sensitive rats (HAS/LAS) are characterized by differential sleep time in response to administration of large doses (3-3.6 g/kg) of ethanol (29). In addition, several lines have been bred for differential volitional intake of ethanol. These include high- and low-alcohol-drinking (HAD/LAD) rats (16), alcohol-accepting and -non-accepting rats (AA/ANA) (10), and alcohol-preferring and -nonpreferring rats (P/NP) (18). All of these lines have provided useful models for the study of the physiological basis of ethanol intake and preference responses to acute ethanol administration.

The drug discrimination paradigm requires an animal to make one response (typically to press one of two levers) after administration of a drug and another response (i.e., press the second lever) following the vehicle, and it has been useful in delineating differential behavioral responsiveness to ethanol in both heterogeneously bred (1,26) and selectively bred rat lines. Thus, ANA rats have been shown to learn to discriminate 1,000 mg/kg ethanol faster than AA rats and to maintain superior discriminative performance throughout experimentation (34). HAS/LAS rats have also shown differential sensitivity to the discriminative effects of ethanol, with HAS animals having a slightly greater, but transient, selectivity to the discriminative effects of 600 mg/kg ethanol (14). In contrast, HAD/LAD rats trained to discriminate 500 mg/kg ethanol from saline showed no differences in their discriminative performance (15). To date, no evidence has been presented to suggest differences in ethanol discrimination in the P/NP line of rats. Therefore, one aim of the present study was to train P and NP animals to discriminate 1,000 mg/kg ethanol (10% v/v) from its vehicle in an effort to assess ethanol sensitivity as measured by discriminative performance during acquisition and then dose-response testing with lower ethanol doses.

Human behavior genetic analyses have reported a genetic component in human tobacco use (4) and recent investigations focused on a possible common genetic link between smoking and alcohol use (13). In light of the inherent difficulties in studying human genetic variability, genotypic variance in nicotine sensitivity has been clearly demonstrated in both mice and rats (9,19). Examination of ethanol-sensitive mouse lines have found correlations between ethanol and nicotine sensitivity on a number of behavioral and physiological measures (8). While relatively few studies have examined nicotine sensitivity in rats selectively bred for ethanol preference (9), recent work from this laboratory (12) indicated that nicotine produced differential effects on locomotor activity in the P/NP line with depression of activity seen only in NP animals. Based upon

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this finding, the second aim of the present study was to investigate whether P and NP rats trained to discriminate ethanol would generalize this discriminative performance to nicotine.

## METHOD

### *Subjects*

Six male P and six male NP rats received from Indiana University School of Medicine were individually housed in suspended metal wire cages. These were housed in a vivarium facility kept at an ambient temperature of 20–22°C on a 12 L : 12 D cycle with lights on at 0600 h. Although water was available ad lib, restricted access to commercial rat chow after each session allowed maintenance of rat weights at 85 ± 5% of their free-feeding weights. This procedure facilitated motivation of operant performance for food reward.

### *Drug Discrimination Training*

The experimental environment consisted of 12 standard rodent operant test chambers (Lafayette Instrument Corp., Lafayette, IN). Each chamber was equipped with two operant levers and a food receptacle at an equal distance between the levers. Solid-state equipment (LVB Corp., Lehigh Valley, PA), used to control and record the sessions, was located in an adjacent room.

The procedure used to train rats to discriminate between ethanol and its vehicle has been described in detail elsewhere (26). In brief, daily discrimination training started after initial shaping to lever-press on both levers on a food-reinforced fixed-ratio schedule of 10 (FR 10). Ten minutes prior to placement into the test chamber, rats were injected IP with either 1,000 mg/kg ethanol (10% v/v) in distilled water or an equal volume of distilled deionized water (vehicle). Depending upon whether the rat was administered ethanol or vehicle, it obtained reinforcement by pressing either the ethanol lever or the vehicle lever, respectively. After every 10th press on the appropriate lever, a 45-mg Noyes pellet was delivered through the food receptacle. Responses on the incorrect lever were recorded but produced no programmed consequence.

To randomize for possible position preference, lever assignments were ethanol: left and vehicle: right for half the rats and ethanol: right and vehicle: left for the other half. These assignments remained constant throughout the experimentation. The number of responses made on both levers before 10 responses were made on the correct lever was recorded. This number reflects the accuracy of rats' lever selection. Each rat was trained/tested once each weekday for a daily session of 15-min duration. Ethanol (E) or vehicle (V) injections were given according to a daily 2-week repeating sequence: E-V-V-E-E and V-E-E-V-V. The training criterion was reached when animals selected (pressed 10 times first) the lever appropriate to that day's treatment on 8 of 10 consecutive training sessions.

Once all rats attained the training criterion, training sessions of 15-min duration with alternating administrations of 1,000 mg/kg ethanol or its vehicle were continued on every other day. This procedure endeavored to ensure and maintain behavioral discrimination to the trained drug dose. It was intended that if a rat was observed to make more than two incorrect lever selections in 10 consecutive maintenance sessions the data on that rat's performance would be deleted from the results. This, however, did not occur. On days interspersed between maintenance sessions, rats were administered either 750, 500, or 250 mg/kg ethanol and, 10 min later, placed into the experimental chamber and allowed to lever press until 10 responses were made on either of the two levers. When 10 responses had accumulated on either lever, the animal was immediately removed from the experimental chamber, without receiving reinforcement, to preclude training at an ethanol dose different from the dose to which animals were trained. The first lever pressed 10 times was designated as the "selected" lever. Each ethanol dose was administered in a random order on two occasions with each test session preceded by one vehicle and one ethanol maintenance session. In this way, animals' experience on test days was counterbalanced with respect to any possible after-effects that may have been produced by the previous day's training condition.

### *Generalization Tests with Nicotine*

Nicotine bitartrate was prepared fresh daily in distilled deionized water for SC injection. The dose of nicotine was calculated as salt and prepared to yield a volume of 1.0 ml vehicle/kg body weight. Nicotine bitartrate (with salt constituting 65% of calculated dose) was administered in doses of 0.4, 0.8, and 1.2 mg/kg 10 min prior to testing on two occasions at each dose, once following saline maintenance and once following an ethanol maintenance session. Rats were immediately removed, without receiving reinforcement, upon accumulating 10 responses on either lever.

### *Measurement and Statistics*

The percentage of rats "selecting" the lever appropriate for the training drug was the quantal measure of discrimination. Quantal data are presented as percent correct first choice responses on the ethanol-correct lever and were subjected to the Litchfield-Wilcoxon procedure (17), which employs probits vs. log-dose measurements. A computer-generated analysis (31) yielded a separate $ED_{50}$ value (with 95% confidence limits) for P and NP rats. In addition to the quantal measurement, the total number of lever presses on both levers made before 10 lever presses accumulated on either lever constitutes the quantitative measurement. This measurement is derived by dividing the number of responses on the ethanol-appropriate lever by the total number of responses made on both levers prior to fulfillment of the selection criterion (FR 10). The quantitative data have the advantage of allowing the determination of statistically significant differences between drug treatments by application of *t*-test analysis (30).

## RESULTS

The results of discrimination testing after doses of ethanol lower than the training dose (A), as well as three doses of nicotine (B), appear in Table 1. Decreasing doses of ethanol generally produced decreased discriminative performance both in quantal and quantitative measurements for P and NP rats. Analysis of the dose–response curves yielded an $ED_{50}$ value of 354.1 mg/kg for P rats and 495.1 mg/kg for NP rats. As the 95% confidence limits of these two $ED_{50}$ values overlap, they are not significantly different from each other.

Administration of nicotine at doses of 0.4–1.2 mg/kg produced a dose-responsive increase in ethanol lever selection by P rats as the dose increased, whereas NP rats exclusively selected the saline-appropriate lever at all nicotine doses tested. Testing of higher doses of nicotine was precluded by the appearance of behavioral disruption (fewer than 75% of subjects responding within 10 min of placement in the apparatus) in both P and NP rats at 1.6 mg/kg. Analysis of the quantitative

TABLE 1

DOSE-RESPONSE RELATIONSHIP AFTER (A) ETHANOL OR (B) NICOTINE ADMINISTRATION IN P AND NP RATS

| | P Rats ($n$ = 6) | | NP Rats ($n$ = 6) | |
|---|---|---|---|---|
| Dose Drug (mg/kg) | Quantal | Quantitative (SD) | Quantal | Quantitative (SD) |
| (A) | | | | |
| 1,000 | 100.0 | 90.1 (1.7) | 95.8 | 82.7 (8.5) |
| 750 | 100.0 | 93.8 (2.1) | 83.3 | 73.0 (2.3) |
| 500 | 58.3 | 58.9 (6.4) | 41.7 | 45.9 (32.7) |
| 250 | 33.3 | 43.4 (9.2) | 8.3 | 22.8 (5.5) |
| 0 (saline) | 0.0 | 17.3 (6.4) | 5.6 | 21.6 (1.4) |
| $ED_{50}$ | 354.1 mg/kg | | 495.1 mg/kg | |
| (95% CL) | (245.9–509.9) | | (382.3–641.2) | |
| (B) | | | | |
| 1.2 | 58.3 | 51.1 (2.6)* | 0.0 | 23.1 (1.4) |
| 0.8 | 33.3 | 36.3 (4.8)* | 0.0 | 14.0 (6.9) |
| 0.4 | 16.7 | 25.8 (11.3) | 0.0 | 17.3 (9.6) |
| Maintenance sessions | | | | |
| Ethanol | 100.0 | 88.8 (11.6) | 94.0 | 79.0 (10.5) |
| Saline | 0.0 | 4.7 (2.6) | 0.0 | 9.3 (6.2) |

*$p < 0.01$.

measurement, by application of Student's $t$-test, indicated a significant ($p < 0.01$) difference between P and NP rats at both 0.8 mg/kg ($t$ = 3.74) and 1.2 mg/kg ($t$ = 13.60) nicotine.

## DISCUSSION

The drug discrimination paradigm was used to evaluate possible differences between rats selectively bred for differential ethanol drinking preference. Results indicate that P rats do not significantly differ from NP rats in sensitivity to decreasing doses of ethanol when each group of animals is trained to discriminate 1,000 mg/kg ethanol (Table 1), although P rats were somewhat, but not significantly, more sensitive as indicated by a lower $ED_{50}$ value. These results are similar to those shown to occur in HAD/LAD rats, which do not differ in their sensitivity to ethanol discriminative stimuli (15). Although the previous study employed a lower dose relative to the present study, that is, 500 mg/kg ethanol (10% w/v), the possibility exists that the necessity for repeated administrations of these (moderate) doses of ethanol as required in the discriminative training procedure obscures any possible differences that may reflect inherited alcohol sensitivity. However, this paradigm does allow for examination of neurotransmitter systems that may be involved in differential ethanol preference (28). In addition, another behavioral measurement, locomotor activity, has been shown to differ after ethanol administration in both HAD/LAD (15) and P/NP (27) rats.

In contrast to the similar discriminative effects after ethanol doses in P and NP animals, the results of nicotine administration suggests that P rats are more sensitive to nicotine's ethanol-like interoceptive stimuli than are NP rats. Although the highest ethanol lever selection of 58.3% occurred at a dose of 1.2 mg/kg nicotine in P rats, a result that may be considered to indicate only partial generalization, there was a significantly increased quantitative measurement in P animals when compared to NP animals, which, indeed, never chose the ethanol-appropriate lever with any dose of nicotine administered.

A number of studies have shown that mice bred for differential responses to ethanol also show differential sensitivity to the effects of nicotine (8,9). For example, nicotine administered to LS mice produced a greater decrease in nicotine-induced seizure susceptibility than it did in SS mice, suggesting that the two selectively bred lines of mice might differ in their sensitivity to nicotine and that common genes may regulate the sensitivity to both ethanol and nicotine (7). Relatively few studies have shown differences in nicotine sensitivity in rats bred for ethanol sensitivity (9). However, P/NP animals have been shown to be differentially sensitive to nicotine-induced spontaneous motor activity (12) and the results of the present study again point to divergent effects of nicotine in the two lines.

Using in vivo microdialysis to allow for the assay of dopamine (DA) content of the nucleus accumbens, various investigators reported that both nicotine (2,3,22) and ethanol (32, 33,35) increase extracellular dopamine. In addition, both nicotine and ethanol have been shown to modulate 5-hydroxytryptamine (5-HT) and GABA neurotransmission (5,11,24,35). Neurochemical studies of naive P/NP rats have shown differences in serotonergic (5-HT), GABAergic (GABA), and dopaminergic (DA) systems in the CNS (21,23). Further, deficits in 5-HT concentrations in P animals are thought to account for their alcohol-seeking behavior (20,23). Thus, actions at any of these neurotransmitter systems in P/NP rats may be responsible for the observed nicotine generalization to the ethanol cue.

Finally, these data add to the growing body of evidence from animal studies and human behavior genetic analyses that suggest a potential for hereditary cooccurrence of alcohol and nicotine sensitivity.

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