Research Article

Different Lines of Rats Selectively-Bred for High Alcohol-Drinking Demonstrate Disparate Preferences for Nicotine Self-administration

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Abstract *Background.* Alcohol and nicotine are commonly co-abused. The search for a common core of neural, behavioral, and genetic factors underlying addiction has been the goal of addiction research. *Purpose.* Genetic predisposition to high alcohol intake has been studied in rats by selectively breeding rats that have high preference for alcohol. The current experiments were conducted to determine if the level of intravenous nicotine administration for the various lines of alcohol-preferring rats differs from that for nonalcohol-preferring controls. *Study design.* Adult alcohol-naïve selectively-bred alcohol-preferring male rats from four lines (P, AA, HAD-1, sP) and their control nonalcohol-preferring rats (NP, ANA, LAD-1, sNP) were trained and given access to self-administer nicotine (0.03 mg/kg/infusion). *Results.* The results show that the P rats self-administered significantly more nicotine than NP rats. In contrast, there were no significant differences in nicotine self-administration between the sP and sNP or the AA and ANA rats. Unexpectedly, high alcohol-drinking HAD-1 rats self-administered significantly less nicotine than low alcohol-drinking LAD-1 rats. *Conclusion.* This suggests that some genetic factors that underlie high-alcohol intake have more general effects in promoting high nicotine intake tendencies, while other genetic factors are more specific to only heavy drinking.

Keywords addiction; alcoholism; alcohol-preferring rats; nicotine self-administration; genetics

1. Introduction

Genetic factors appear to play a major role in both excessive drinking and tobacco smoking. Furthermore, epidemiological data, clinical observations, and animal research support the notion of common neuronal pathways for both nicotine and alcohol addiction [1,2,3,4,5,6]. Alcohol and nicotine exert their reinforcing properties through common neural substrates: enhancing dopamine release in the nucleus accumbens, and modulating the opioid system [7,8,9,10,11]. It is possible that a shared genetic determinant accounts for the coabuse of these two drugs [12]. Nicotine may be more rewarding or less aversive to rats that have been selectively bred for alcohol preference. Another possibility is that selectively-bred

alcohol-preferring rats, similar to a subpopulation of human alcoholics, substitute nicotine in the absence of alcohol or they use it to enhance the effect of alcohol. However, it is not clear if all selectively-bred alcohol-preferring rats such as P, HAD-1, AA, and sP rats will behave in the same manner when have access to nicotine. Although all of these lines demonstrate high alcohol preference compared to their control nonalcohol-preferring lines, they have different neurochemical profiles due to unique genetic backgrounds [13]. For instance, dopamine neurons in the posterior ventral tegmental area (VTA) of P rats have been shown to fire a greater percentage of action potentials in bursts, and a greater number of bursts, compared with posterior VTA dopamine (DA) neurons in NP rats. However, there were no differences in VTA DA neuronal activity between both replicate lines of HAD and LAD rats [14].

The four different alcohol-preferring and nonpreferring pairs of selectively bred rat lines come from different sources. The first strain of alcohol-preferring rats (P rats) and their counterpart, alcohol-nonpreferring rats (NP rats), were developed by Drs. T. K. Li and Lawrence Lumeng at the Walter Reed Army Hospital by selective breeding from a closed colony of Wistar stock in 1977. These lines were later transferred to their current home at the Indiana School of Medicine in Indianapolis, Indiana [13]. The second strain of rats used was developed to reduce the level of inbreeding. The high alcohol-drinking rats (HAD) rats) and their counterpart, low alcohol-drinking rats (LAD-rats), were developed by utilizing a within-family selection and rotational breeding on an N/NIH heterogeneous stock composed of eight inbred lines [15] from Indiana School of Medicine [16]. The third line is the alcohol-preferring Alko Alcohol (AA) and alcohol avoiding Alko nonalcohol (ANA) rats, which were developed by selective

breeding from a closed colony of Wistar rats in Helsinki in 1968 [17]. In the 1980s, these lines were mated with the F1 cross of the Brown-Norway and Lewis lines, followed by selective breeding [18]. The fourth strain is comprised of the Sardinian alcohol-preferring (sP) and alcohol nonpreferring (sNP) rats. The line was developed from a Wistar foundation at the University of Cagliari, Italy [19]. All of these alcohol-preferring lines meet the established criteria for an animal model of human alcoholism with some differences in their drinking patterns [13]. These lines, similar to human alcoholics, respond variously to disparate "anticraving" drugs [13].

Coabuse of nicotine and alcohol may be explained, in part, by alcohol's functional interaction with the neuronal nicotinic system [20]; similarities in biologic factors underlying both nicotine and alcohol addictions may also explain coabuse of the two drugs [11,12,21]. The main goal of this project was to compare IV nicotine self-administration between different lines of selectively bred alcohol-preferring rats with respect to their controls. To determine inherent reward-motivated behavior of the strains, performance on an operant behavior task for food delivery (an intrinsic reward) was compared between food-restricted rats from each line prior to nicotine self-administration. Selective breeding for high-alcohol intake and preference is hypothesized to be associated with an innate neurocircuitry that predisposes these lines of rats to nicotine self-administration. The extent of this association may be dependent on the genetic background of each line. In support of this, alcohol-preferring lines of rats with different genetic backgrounds are expected to self-administer nicotine at different rates during the studies. Concordance and discordance of alcohol and nicotine self-administration among the four pairs of lines can serve as the basis for a better understanding of possible shared genetics of alcoholism and nicotine addiction.

2. Materials and methods

2.1. Animals and housing

Adult male selectively-bred alcohol-preferring rats (P, sP, AA, and HAD-1) and their alcohol nonpreferring counterparts (NP, sNP, ANA, and LAD-1) were obtained from colonies maintained at the University of Indiana School of Medicine, Indianapolis, IN, USA. Animals were kept in a colony room under a relatively constant room temperature of $22 \pm 1\,°C$ and on a 12:12 reverse light-dark cycle (7:00 AM to 7:00 PM dark). All animals had ad lib access to water, and were fed 5001 Rodent Chow (Lab Diet, Brentwood, MO, USA) once daily throughout the study to maintain approximately 85% ad lib weight. Food amounts were adjusted from 8 g to 16 g per day as they grew, to provide a lean healthy growth curve. Animals were fed 20–30 min after completing nicotine self-administration

session. Average weights for the separate lines of rats at the beginning of the experiments were as follows: P rats $= 271 \pm 7$, NP rats $= 253 \pm 8$, AA rats $= 242 \pm 4$, ANA rats $= 278 \pm 6$, sP rats $= 365 \pm 8$, sNP rats $= 333 \pm 6$, HAD-1 rats $= 190 \pm 10$, and LAD-1 rats $= 245 \pm 15$. All procedures used in this study were approved by the Duke University Animal Care and Use Committee.

2.2. Experimental design and procedures

Intravenous nicotine self-administration in alcohol-preferring rats

The aim of these experiments was to determine if the baseline levels of intravenous nicotine self-administration in alcohol-preferring P, sP, AA, and HAD-1 rats were significantly different from their control counterparts (NP, sNP, ANA, and LAD-1 rats). Before beginning IV nicotine self-administration trials, all rats were trained to receive a food reinforcement for lever-pressing behavior using dual lever test chambers (Med Associates, Vermont, USA). Rats were initially hand-trained in the operant conditioning chambers, followed by three daily pellet sessions that followed an FR-1 schedule of reinforcement. The chambers were randomized, such that half the animals were reinforced for left lever responses, and the other half for right lever responses. Following pellet training, each rat had a catheter surgically implanted into the jugular vein, which was then fastened to a port (Instech-Solomon, Plymouth Meeting, PA, USA) enabling the rat to receive nicotine infusions. A mixture of ketamine (60 mg/kg) and domitor (15 mg/kg), injected IP, was used for anesthesia [22]. Once the catheter was connected to the port, the port was sutured subcutaneously on its back for easy access to the tether delivery line. The catheters were flushed daily with a 0.3 mL solution containing 100 units/mL heparinized saline (Baxter Health Corporation, Deerfield, IL, USA) and 8 mg/mL Gentamicin (American Pharmaceutical Partners, Shaumburg, IL, USA) to prevent possible infection. Following recovery from the surgery, rats began nicotine self-administration trials in the operant dual-lever chambers. Each chamber was equipped with two levers; a house light, located at top center of the chamber; a tone generator, for creating white noise; a cue light above each lever; and a stainless steel tether, to cover and protect the drug delivery line. Each port was connected to a High Speed Micro-Liter Syringe Pump (MED-Associates, Georgia, VT, USA) with polyethylene tubing for drug delivery. During each session, the rats wore covance infusion harnesses (Instech-Solomon, Plymouth Meeting, PA, USA) connected to the stainless steel tethers. Following catheterization surgery, rats began daily sessions of nicotine reinforcement, totaling 10 over a period of two consecutive weeks (i.e., five sessions/week). Each nicotine self-administration session lasted for 45 min. During the

sessions, a lever press on the active side resulted in an immediate 50 μL infusion of nicotine (0.03 mg/kg nicotine base) in less than 1 s and the activation of the feedback tone for 0.5 s. Lever presses on the inactive side were only recorded. Each infusion was immediately followed by a one-minute timeout, during which the cue light above the active lever went out and any lever presses were recorded, but not reinforced [22, 23, 24]. The number of infusions/session and the amount of nicotine infused (mg/kg/session) were determined. The experimental events and all data collection were controlled by a Windows-based computer programmed with MED-PC software.

2.3. Preparation of drugs

Solutions of nicotine bitartrate were prepared biweekly in sterilized isotonic saline and kept refrigerated in the dark between sessions. The pH of the nicotine solution was adjusted to the 7.0–7.3 range using NaOH. The solution was then passed through a 0.22 μm Nalgene filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. The doses of nicotine were calculated as a function of the nicotine base weight. Nicotine bitartrate was purchased from Sigma Aldrich, Saint Louis, MO, USA.

2.4. Data analysis

The nicotine self-administration (number of nicotine infusions/session) data was assessed for significance using a two-way analysis of variance. Genetic line was used as the between subjects factor, and session as the repeated measure. The number of nicotine infusions per session was the dependent measure. Significant interactions were followed up by tests of the simple main effects. The correlation (R^2) between the initial three-day food pellet intake and the number of nicotine infusions was calculated for each rat within each line and was also compared across lines. An alpha level of $P < .05$ (two-tailed) was used as a cutoff for statistical significance.

3. Results

3.1. P and NP rats

The overall ANOVA revealed a significant main effect of line [$F(1, 30) = 4.60$, $P < .05$]. The main effect of session and the line x session interaction were not significant. The P rats self-administration rates averaged 11.66 ± 1.33 infusions/session, while the NP rats averaged 7.12 ± 1.07 infusions/session over the 10 nicotine sessions (Figure 1(a)). The P rats also self-administered a significantly [$F(1, 30) = 8.48$, $P < .01$] greater number of food pellets during three-day training sessions (P rats $= 242.0 \pm 9.8$ and NP rats $= 196.2 \pm 8.7$ pellets/session). The correlation between the number of food pellets delivered and the number of nicotine infusions was significant in P rats, $R^2 = 0.12$, ($P < .05$), Table 1.

3.2. sP and sNP rats

When sP rats were compared with the sNP control rats, there were no significant differences in average nicotine self-administration rates between the sP and sNP rats over 10 sessions (Figure 1(b)). The sP rats averaged 9.63 ± 1.71 nicotine infusions per trial over the 10 session span, while the sNP rats averaged 9.89 ± 1.14 infusions ($P = .90$). There was a significant ($P < .001$) difference in the number of food pellets delivered to the sP rats (232.2 ± 10.3 pellets/session) compared to the number delivered to the alcohol nonpreferring sNP rats (167.8 ± 7.5 pellets/session). However, as demonstrated in Table 1, the correlation between the number of food pellets delivered and the number of nicotine infusions received was not significant $R^2 = 0.13$, ($P = .12$).

3.3. AA and ANA rats

Using the same procedure, we compared another selectively-bred alcohol-preferring rat line (AA rats) with its alcohol nonpreferring counterpart (ANA rats). Our results show that AA rats self-administered slightly more nicotine, but the difference did not reach the level of significance [$F(1, 20) = 3.92$, $P = .062$] than their counterpart ANA rats over the 10 trials of nicotine self-administration. The average numbers of nicotine infusions/session were 5.71 ± 0.95 and 3.62 ± 0.55 for AA and ANA rats, respectively (Figure 1(c)). ANA rats earned significantly more [$F(1, 20) = 9.76$, $P < .01$] food pellets on average during the three-day training sessions (AA $= 103.1 \pm 4.8$, ANA $= 124.2 \pm 4.7$ pellets/session). However, as shown in Table 1, the correlation between the average number of food pellets/session and the average number of nicotine infusions/session was not significant, $R^2 = 0.04$, ($P = .40$).

3.4. HAD-1 and LAD-1 rats

The high alcohol-drinking (HAD-1) rats self-administered significantly ($P < .01$) less nicotine than low alcohol-drinking (LAD-1) rats averaged over 10 sessions, with LAD-1 rats averaging 3.9 ± 0.5 infusions/session and the HAD-1 rats averaging 2.3 ± 0.4 infusions/session (Figure 1(d)). Food motivated responding was not significantly affected by line in this study (HAD-1 $= 70 \pm 3.7$; LAD-1 $= 85.7 \pm 5.4$ food pellets/session). Consequently, the correlation between the number of food pellets delivered and the number of nicotine infusions was not significant, $R^2 = 0.11$, ($P = .06$).

Overall, Table 1 illustrates that there was only a statistically significant correlation between the number of nicotine infusions and the number of food pellets consumed within the pair lines of drinking and nondrinking rats for P rats. However, there was a significant correlation between the number of food pellets earned and the number
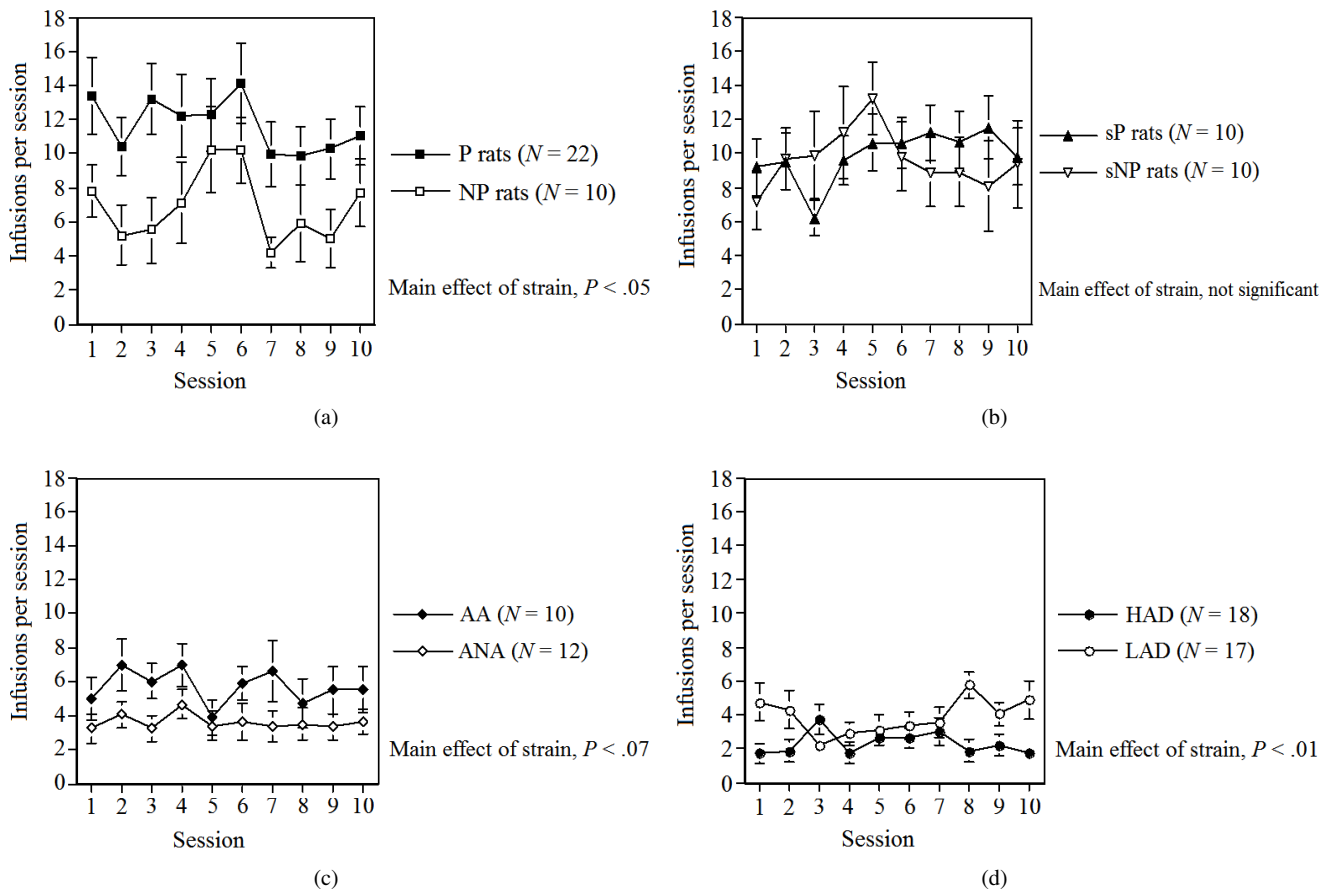
Figure 1: (a) IV self-administration of nicotine (0.03 mg/kg/infusion) in alcohol-preferring (P) and alcohol-nonpreferring (NP) rats. $N = 22$ P and $N = 10$ NP rats. (Graph adapted from Rezvani et al. [25]). (b) IV self-administration of nicotine (0.03 mg/kg/infusion) in alcohol-preferring (sP) and alcohol-nonpreferring (sNP) rats. $N = 10$ for each line. (c) IV self-administration of nicotine (0.03 mg/kg/infusion) in alcohol-preferring (AA) and alcohol-nonpreferring (ANA) rats. $N = 10$ AA and $N = 12$ ANA rats. (d) IV self-administration of nicotine (0.03 mg/kg/infusion) in alcohol-preferring (HAD-1) and alcohol-nonpreferring (LAD-1) rats. $N = 17$ for HAD-1 and $N = 18$ for LAD-1. Data represent mean infusions $\pm$SEM.

of nicotine infusions received across the pairs of lines ($R^2 = 0.44$, $P < .0005$).

3.5. Comparison of nicotine self-administration between lines

In addition to the analyses of nicotine self-administration rates within for each pair of lines, we conducted an analysis comparing rates between the lines (Figure 2). There was a significant main effect of line [$F(3, 101) = 20.35$, $P < .0005$]. There was also a significant line x high-low preferring line [$F(3, 101) = 3.51$, $P < .025$]. Comparisons between individual alcohol-preferring lines showed that AA rats had significantly fewer nicotine infusions than P rats ($P < .0005$) and sP rats ($P < .05$). HAD-1 rats had significantly fewer nicotine infusions than P rats ($P < .0005$), sP rats ($P < .0005$), and AA rats ($P < .05$). Among the alcohol nonpreferring rats, ANA rats had significantly

fewer nicotine infusions than NP rats ($P < .05$) and sNP rats ($P < .0005$). LAD-1 rats had significantly fewer nicotine infusions than NP rats ($P < .05$) and sNP rats ($P < .0005$).

3.6. Comparison of body weight between lines

A comparison of the body weights of the alcohol-preferring lines at the initiation of the experiment showed that sP rats had significantly ($P < .002$) greater body weights (365 ± 8 g) than all other alcohol-preferring lines and HAD-1 rats had the lowest body weight (190 ± 10 g). This pattern persisted throughout the experiment. Comparing the body weights of alcohol-preferring rats with their corresponding alcohol nonpreferring rats showed that ANA and LAD-1 had significantly ($P < .002$ and $P < .02$, resp.) greater body weights than their alcohol-drinking counterparts, AA and HAD-1 rats. However, alcohol-preferring sP rats showed significantly ($P < .005$) greater body weights than alcohol nonpreferring sNP rats.

Table 1: Number of nicotine infusions and number of food pellets and the correlation between these two variables in four lines of alcohol-preferring rats and their corresponding control alcohol nonpreferring rats. The P values indicate that there is no statistically significant correlation between food intake and nicotine self-administration within these lines except in P rats. The data represent means $\pm$SEM.

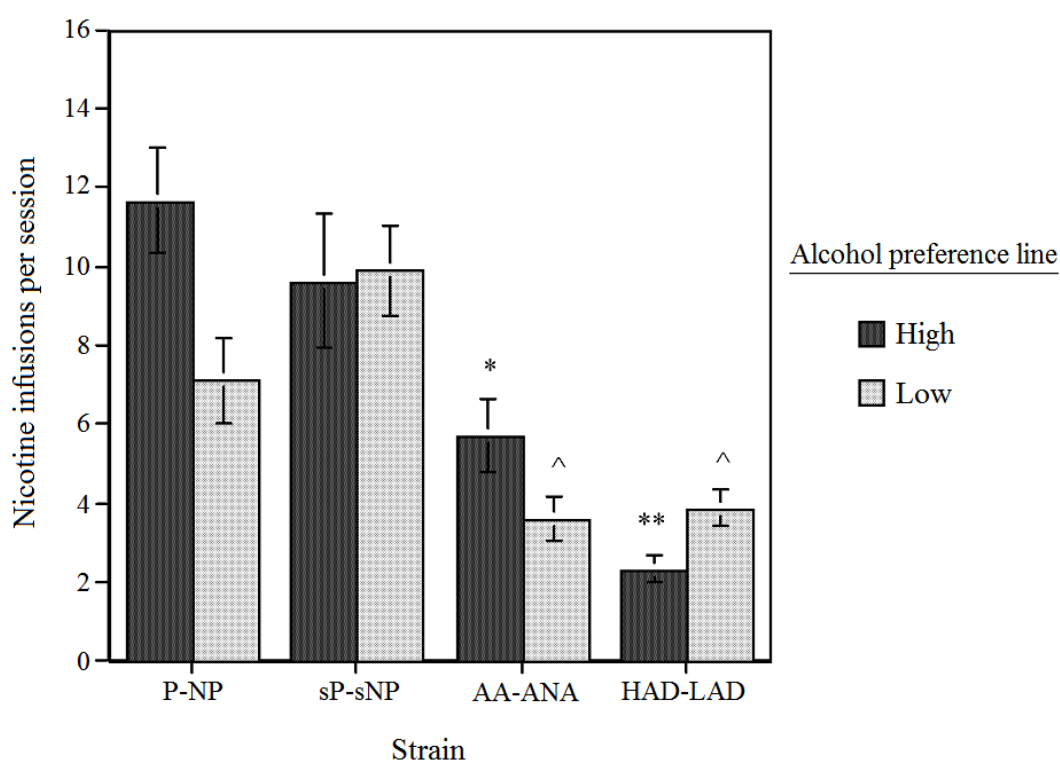| Rat lines	Number of nicotine infusions	Number of food pellets	R^2	P value
P	11.66 ± 1.33	242 ± 9.8	0.12	.05
NP	7.12 ± 1.07	196.2 ± 8.7		
sP	9.63 ± 1.71	232.2 ± 10.3	0.13	.12
sNP	9.89 ± 1.14	167.8 ± 7.5		
AA	5.71 ± 0.95	103.1 ± 4.8	0.04	.40
ANA	3.62 ± 0.55	124.2 ± 4.7		
HAD-1	2.3 ± 0.4	70 ± 3.7	0.11	.06
LAD-1	3.9 ± 0.5	85.7 ± 5.4		

Figure 2: Comparison of mean nicotine infusions per session over sessions 1–10 for high and low alcohol-preferring rats of each line. Data represent mean infusions $\pm$SEM. $P < .0005$ versus P rats, and $P < .05$ versus sP rats, $P < .005$ versus P and sP rats and $P < .05$ versus AA rats, $P < .05$ versus NP and $P < .0005$ versus sNP rats.

4. Discussion

Our findings indicated that the number of intravenous nicotine infusions differ across lines of alcohol-preferring and nonpreferring rats with unique neurochemistries. Compared with alcohol nonpreferring NP rats, alcohol-preferring P rats infused significantly more nicotine/session than NP rats. The sP and sNP rats demonstrated no divergence in levels of IV nicotine self-administration while the AA rats seem to lie between the other two lines with regard to nicotine self-administration. Their nicotine self-administration levels are nearly significantly higher than ANA rats. Contrary to other lines, the alcohol nonpreferring LAD-1 line infused significantly more nicotine than its alcohol-preferring counterpart (HAD-1). There were also significantly different levels of nicotine self-administration among the alcohol-preferring lines, with the P rats demonstrating the highest and the HAD-1 rats exhibiting the lowest amount of nicotine intake.

Analysis of food pellet intake during the three-day training period for food-restricted animals showed that alcohol-preferring P rats consumed significantly more food pellets than their control NP rats. The correlation between food

intake and the number of nicotine infusions was statistically significant in P rats. Alcohol-preferring sP rats also earned more food pellets than their nonalcohol-preferring controls sNP rats. The food acquisition for LAD-1 rats was not statistically significantly different from the food intake for HAD-1 rats. The correlation between the number of food pellets consumed and the number of nicotine infusions was not statistically significant in LAD-1 rats. This data collectively suggests that the consummatory behavior in several lines of rats does not play a major role in nicotine intake. However, the fact that the P rats consumed significantly more pellets than NP rats, and that there was a significant positive correlation between numbers of pellet delivered and the number of nicotine infusions, may suggest a role of consummatory behavior in alcohol preference and nicotine self-administration in this particular line of alcohol-preferring rat. It is noteworthy that among alcohol-preferring rats, P rats seem to be more reward-driven, as they self-administer more nicotine [25] and cocaine than NP and Wistar rats [26].

During the past three decades, several lines of alcohol-preferring rats have been developed through bidirectional selective breeding from a variety of outbred/heterogeneous stocks of rats. These lines include the Chilean lines [27], Finnish ALKO alcohol accepting (AA) rats [28], alcohol-preferring (P) rats [16, 29], Sardinian alcohol-preferring (sP) rats [30], and high alcohol-drinking (HAD-1) rats [16]. A major advantage of selective breeding is that the phenotype of interest (i.e., high alcohol intake) is driven by genetic factors without environmental influences. Since these lines have been derived from distinctive heterogeneous foundation stocks, they have unique genetic backgrounds. Consequently, the strains have disparate neurocircuitry, different patterns of drinking, and possibly contrasting susceptibility to nicotine self-administration.

Both alcohol and nicotine exert their reinforcing properties, in part, by enhancing dopamine release in the nucleus accumbens [8, 11]. For example, compared with alcohol nonpreferring NP rats, alcohol-naïve alcohol-preferring P rats are highly susceptible to nicotine self-administration. Alcohol-naïve P rats self-administer nicotine at a rate 3 times greater than NP rats [31]. Our results have confirmed that difference, albeit with less significance. Thus, a shared genetic determinant may account for the coabuse of these two drugs. However, our data shows that other alcohol-preferring lines (such as HAD-1, AA, and sP rats) do not behave in the same manner as alcohol-preferring P rats when given access to nicotine. Although all of these selectively-bred alcohol-preferring lines drink significant amounts of alcohol, they have unique neurochemical profiles owing to their separate genetic backgrounds [32, 33]. They also respond variably to existing anticraving drugs [13]. Based on their different genetic makeups, we speculated that their propensity to nicotine intake would vary.

Compared with alcohol nonpreferring NP rats, the alcohol-preferring P rats have been shown to have lower levels of both serotonin and its metabolite (5-hydroxy-indole-acetic-acid (5-HIAA)) in limbic and cortical regions, as well as lower levels of dopamine and its metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)) in the accumbens and anterior striatum [34, 35, 36]. Previous studies have also demonstrated that P rats have reduced the number of dopamine D2 receptors in the accumbens and the VTA [32, 37], and fewer 5-HT2 receptors in certain brain regions compared with NP rats [37]. An innate lower level of basal neuropeptide Y (NPY) and corticotropin-releasing factor (CRF) in the amygdala, the hippocampus, and the frontal cortex of the P rats has also been reported [38, 39]. Compared with their counterpart, P rats have been shown to have higher densities of μ opioid receptors in both the accumbens shell and the core [33]. These innate disparities in neurochemistry may partially explain higher nicotine and alcohol intake in P rats compared with NP rats.

The Sardinian alcohol-preferring (sP) and nonpreferring (sNP) rats, which were developed from Wistar rats, also vary neurochemically and behaviorally. Alcohol-preferring sP rats are more sensitive to the motor-impairing and sedative/hypnotic effects of alcohol than their nonalcohol-preferring sNP rats [40]. sP rats also show more anxiety-like behavior than sNP rats [30, 41]. Neurochemically, acute administration of alcohol increases extracellular levels of serotonin in the frontal cortex of sP rats, but not sNP rats [23]. Similar to P rats, sP rats have an innate lower densities of D2 receptors in subregions of the mesolimbic DA system compared to its nonalcohol preferring counterparts [32, 42]. In addition, sP rats also demonstrated a contrasting profile of opioid receptors in various regions of the brain; sP rats have lower densities of opioid receptors in the accumbens shell as well as in caudate putamen than sNP rats [43]. Compared with sNP rats, higher levels of CRF in the central nucleus of the amygdala in sP rats have been reported [24]. Differences in 5-HT, DA, opioid systems as well as the hypothalamic-pituitary-adrenal axis and possibly other neurochemical systems in the brain may underlie the behavioral differences reported in sP versus sNP rats. Since the number of nicotine infusions was not significantly distinct in these lines, the neurochemistry deviations may not play a role in nicotine intake in these lines, suggesting that factors contributing to alcohol intake may be independent of those contributing to nicotine intake.

Similar to other alcohol-preferring rats, AA rats demonstrate heterogeneous neurochemical profiles compared with their control ANA rats. The neurochemical profile of AA rats, in terms of levels of serotonin and dopamine metabolites, is dissimilar to both their controls and other

alcohol-preferring rats. As opposed to the P and HAD rats, AA rats have higher levels of serotonin [44] and dopamine metabolites (such as DPOAC and HVA) in certain regions of the brain in comparison to ANA rats [45]. AA rats also depart from ANA rats in their profiles of μ opioid receptors [46], N-Methyl-D-aspartic acid (NMDA) receptors [47], and NPY receptors within the amygdala and hippocampus [48]. Abnormal- or low-neuronal NPY activity has been shown to promote high alcohol-drinking by modulating the NPY Y1 and Y2 receptors [49]. Similar to sP and sNP rats, the contributing factors for alcohol intake may also be independent of the contributing factors for nicotine intake in these lines of rats.

Similar to P versus NP rats, HAD rats have disparate profiles for dopamine, serotonin, NPY, and GABA, in relation to LAD rats. Furthermore, HAD rats have lower levels of serotonin and 5-HIAA in cortical and limbic regions than their nonpreferring counterparts [34]. The same authors demonstrated that HAD rats also have lower levels of dopamine and its metabolites in the anterior striatum and accumbens. Higher densities of GABAergic terminals in the accumbens can also be found in HAD rats, relative to LAD rats. Likewise, reduced levels of NPY in the central amygdala are found in HAD rats [50].

The current set of studies, concerning several lines of selectively-bred high-alcohol consuming rats, demonstrates a complicated interaction between genes which underlie high alcohol-drinking and nicotine self-administration. The comparisons of the four lines studied revealed various relations: high consumption of both alcohol and nicotine in the P rats, trends toward consumption of both in the NP-line, no relationship between alcohol and nicotine consumption in the sP and AA lines, and divergent consumption of alcohol and nicotine in the HAD-1 line. Because of the similarities in neurochemical profile and alcohol intake of P and HAD-1 rats, and differences in levels of nicotine self-administration the genetic basis for nicotine and alcohol intake in these two lines are likely separate. Existing information on their neurochemical profiles, however, is currently unable to explain the individual mechanisms.

Numbers of nicotine infusions did not correspond with the body weight. P rats infused significantly more nicotine than alcohol nonpreferring NP rats, but there were no compelling variations between their body weights throughout the experiment. While there was a convincing difference between the body weights of AA and ANA rats, there was no significant difference in the number of their nicotine infusions. sP rats had a significantly greater body weight than sNP rats, but their nicotine intake were not importantly unique from each other. Alcohol nonpreferring LAD-1 rats, which infused considerably more nicotine than its counterpart alcohol-preferring HAD-1 rats, displayed significantly greater body weights than HAD-1 rats. The differences in body weight data is unable to explain the magnitude of nicotine infusion in these lines, suggesting that nicotine intake is independent of body weight and probably under a separate genetic control.

Analysis of gene expression in the VTA of these four pairs of rat lines selectively bred for high or low alcohol intake showed 1,295 individually named genes that were significantly different between the high alcohol and low alcohol drinking lines (for more genetic analysis see [51]). Although our results provide preliminary data indicating the possibility of a common genetic explanation for coabuse of nicotine and alcohol, especially in P rats, more comprehensive studies are needed to determine the specific genetic factors contributing to coabuse of these two addictive drugs. Additional studies, determining the profile of neuronal nicotinic receptors in these lines and the receptors' roles in both alcohol and nicotine intake, would help to reveal further genetic explanations for coabuse.

5. Conclusions

Overall, our results show that there are a variety of genetic bases underlying high alcohol and nicotine self-administration. This genetic diversity indicates that specialized treatments may more successfully treat alcoholism and tobacco addiction. The high rate of nicotine self-administration in the P-rat, combined with its high alcohol intake, suggests that preclinical studies in this rat model may reveal compounds, such as sazetidine-A (an $\alpha4\beta2$ nicotinic receptor desensitizer) that are effective for both conditions [25] and may lead to clinical studies of such compounds for the treatment of comorbidity of alcohol and nicotine addiction.

Conflict of interest The authors declare that they have no conflict of interest.

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