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Blood Ethanol and Free-Choice Ethanol Intake in Rats and Their Progeny

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KULKOSKY, P. J. *Blood ethanol and free-choice ethanol intake in rats and their progeny.* PHARMAC. BIOCHEM. BEHAV. 13(3) 449–452, 1980.—Outbred male and female Wistar rats demonstrated large and consistent interindividual variations in nocturnal blood ethanol level (BEL) during ad lib access to food, water and an ethanol solution. Mean individual BEL ranged from 0–109 mg/dl, grand mean=25.1 mg/dl (5.5 mM). BELs of all rats correlated with free-choice ethanol intakes, but females showed significantly greater ethanol intake (female and male means=10.5 and 8.2 g/kg/24 hr) that was unaccompanied by significantly greater BEL (female and male means=30.6 and 19.6 mg/dl). Across three linearly derived generations, progeny of rats matched for high BELs displayed significantly higher BELs (mean=32.8 mg/dl) than offspring of rats matched for low BELs (mean=25.1 mg/dl), but there was no significant difference across generations in ethanol intake (respective means=8.4 and 8.1 g/kg/24 hr). In the third derived generation, there were significant differences between high and low BEL progeny in both BEL (respective means=35 and 15 mg/dl) and ethanol intake (respective means=8.2 and 6.3 g/kg/24 hr).

Ethanol Blood ethanol Free-choice ethanol intake Male and female rats
Animal models of alcoholism Selective breeding

ANIMALS become physically dependent on alcohol and consequently exhibit alcohol withdrawal syndrome only when high blood ethanol levels are continuously maintained [27]. This suggests a measurable trait, voluntary blood ethanol maintenance, that might be selectively bred to develop a free-choice animal model of alcoholism. A valid small animal replica of human intoxication, alcohol dependence and withdrawal is yet unattained in a free-selection design with nondeprived, physiologically unmanipulated subjects [19].

Previous studies of drinking behavior [5, 6, 20, 28] and responses to administered ethanol [21,25] show that large individual differences in ethanol-related traits can be identified and selectively bred. However, no strain of rodent has been discovered or developed to maintain blood ethanol voluntarily and consistently at levels high enough to induce alcohol dependence and withdrawal (e.g., [7]). Unforced and continuously high blood ethanol, and thus the much-sought freely self-addicting strain has not yet been found because animals that exhibited high ethanol consumption also possessed sufficiently high ethanol metabolism to prevent blood ethanol accumulation [4,26], or there is a lack of correlation between studied traits of behavioral response to administered ethanol and ethanol consumption and metabolism [24]. Typically, the apparently or relatively high ethanol intakes exhibited by studied strains or species do not approach their ethanol eliminative capacities (e.g., [9]), and thus blood ethanol levels remain low. For example, the high alcohol dehydrogenase activity of the golden hamster allows for prodigious ethanol intake without continuously elevated blood ethanol, liver abnormality, or withdrawal syndrome [14,18].

Formation of conditioned taste aversion to ethanol restrains ethanol consumption from repeatedly exceeding metabolic capacity and thus prevents continuously high blood ethanol [1, 3, 17, 22, 23]. However, the ability to form conditioned taste aversion has been selectively bred successfully [13], so it appears possible to breed a strain that does not effectively avert to ethanol and thus sustains high blood ethanol.

The present study identifies large individual differences among rats in blood ethanol attained during free-choice ethanol intake. Extremes of this trait are then selected for breeding matches to explore the transmissibility of the trait of voluntary blood ethanol level to progeny. A previously described added-congener technique [15] was employed to maximize free-choice ethanol intake and blood ethanol. This method results in maintenance of blood ethanol in a few outbred rats at levels shown to produce measurable behavioral impairment [8], although randomly-selected rats as a group do not sustain intoxication or exhibit obvious withdrawal signs [15]. Blood samples were taken at midnight because previous determinations ([15], Kulkosky, unpublished observations) had established that mean blood ethanol level was at peak then, relative to levels determined at various intervals across the day-night cycle.

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METHOD

Animals

Animals were 20 male and 20 female experimentally naive Charles River Wistar descended rats (outbred, Crl:COBS (WI)BR). The rats were approximately 5 weeks of age when individually housed in wire mesh stainless steel cages [16] at an ambient temperature of 20°C and 12:12 L:D lighting cycle (8 a.m.–8 p.m. light), and had ad lib access to NIH Rat and Mouse Ration (Ralston Purina Checkers) in stainless steel hoppers and deionized water throughout the experiment.

Apparatus

Two hundred fifty and 100 ml calibrated drinking tubes (Wahmann) fitted with valveless stainless steel spouts were used to measure fluid consumption to the nearest 1.0 ml. Spectrophotometric determination of blood ethanol content was performed at 340 nm on a Zeiss M4 QIII spectrophotometer with yeast alcohol dehydrogenase (Boehringer Mannheim) and NAD^+ (Sigma Grade III).

Procedure

Each rat was individually housed and given ad lib access to chow and deionized water for an initial period of 26 days. Animals then received an ad lib 2-bottle choice of water and a previously described [14, 15, 16, 18] 3% glucose+0.125% sodium saccharin+1% NaCl solution for a period of 13 days. Ethanol (from U.S.P. 95%) was then gradually added to this solution from 0.5 to 2.5% w/v, increasing 0.5% per day. The solution then remained at 2.5% ethanol for a total of 10 days. Tail blood samples were taken from all rats in a previously described manner [29] at midnight on the fifth, eighth, and tenth days of this period and assayed for ethanol content in a previously described manner [2]. Male and female rats exhibiting consistently high blood ethanol levels were mated (3 pairs), as were rats with consistently low blood ethanol levels (2 pairs). The offspring of these matings (N=19 "high", N=24 "low") were weaned at about 21 days of age and underwent a regimen of fluid availability and blood sampling as described above. Two further generations were derived linearly; the third generation (N=18 "high", N=8 "low") was derived from the subsequent matings of the offspring of the initial group (2 pairs each for "high" and "low"), and the fourth generation (N=21 "high", N=10 "low") was produced from matings of the third generation (2 pairs each for "high" and "low"). These third and fourth generations were also tested as above. Outbreeding was practiced until the fourth generation, when some sib-sib matings were necessitated by a small number of surviving animals.

Data were analyzed with 2-way analysis of variance, 2-sample t-tests (2-tailed), and regression correlation analysis, with $p<0.05$ as significant.

RESULTS

Mean (± standard error, SEM) blood ethanol level (BEL, in mg/dl) for male and female rats of the initial generation at the three blood samplings are presented in Table 1. BELs of male and female rats did not differ significantly, $F(1,114)=3.87$, $p>0.05$; the effect of sampling day was significant, $F(2,114)=3.11$, $p<0.05$, but the interaction was not. Individual BELs ranged 0–140 mg/dl across samplings (range of mean BEL=0–109 mg/dl), and showed significant

TABLE 1

MEAN (± STANDARD ERROR) BLOOD ETHANOL LEVELS (IN mg/dl) OF MALE AND FEMALE RATS OF THE INITIAL GENERATION AT THE THREE SAMPLING DAYS

	Sample 1	Sample 2	Sample 3	Mean
Males (N=20)	28.7 ± 7.9	11.7 ± 3.3	18.5 ± 6.6	19.6 ± 5.2
Females (N=20)	34.9 ± 8.1	19.3 ± 4.6	37.8 ± 8.8	30.6 ± 6.1
Grand mean (N=40)	31.8 ± 5.6	15.5 ± 2.9	28.1 ± 5.7	25.1 ± 4.1

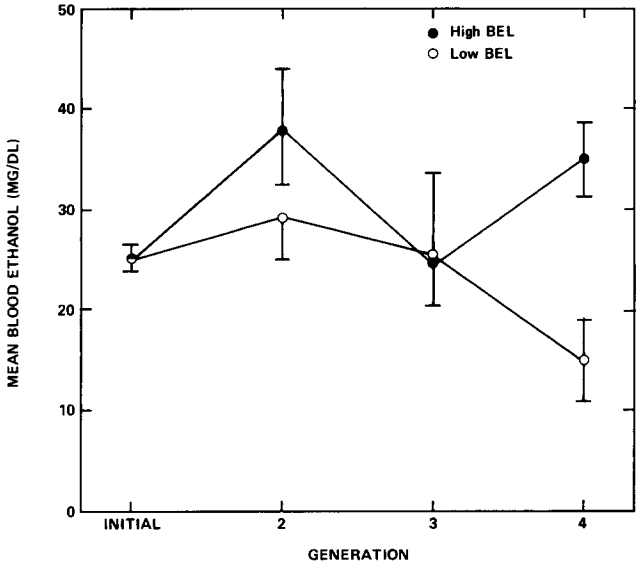

FIG. 1. Mean ± standard error blood ethanol level (in mg/dl) of the entire initial generation and the offspring of rats mated for high blood ethanol level (High BEL, solid dots) and the offspring of rats mated for low blood ethanol level (Low BEL, open dots), across three linearly derived generations.

sample-to-sample correlations, i.e., Samples 1–2, 1–3, and 2–3, $rs(38)=0.46$, 0.69, and 0.57, respectively, $ps<0.05$. Ethanol intakes of male and female rats averaged 8.2 and 10.5 g/kg/24 hr, respectively, and differed significantly, $F(1,114)=14.9$, $p<0.05$. Individual mean BEL was significantly correlated with individual mean ethanol intake $r(38)=0.72$, $p<0.05$. This correlation of blood ethanol with ethanol intake was also significant on each sampling day. Individual ethanol intakes obtained at the three samplings were also significantly correlated, i.e., Samples 1–2, 1–3, and 2–3, $rs(38)=0.65$, 0.58, and 0.67, respectively, $ps<0.05$.

Mean (± SEM) BEL of the entire initial generation and the subsequent three generations of offspring derived from matings of male and female rats with high mean BEL (grand mean ± SEM=57.8 ± 3.8 mg/dl) or low mean BEL (grand mean ± SEM=5.2 ± 4.5 mg/dl) is shown in Fig. 1. Across derived generations, offspring from matings of rats that had exhibited high BELs showed significantly higher BELs (mean ± SEM=32.8 ± 2.6 mg/dl, N=58) than offspring of rats that had exhibited low BELs (mean ± SEM=25.1 ± 3.0

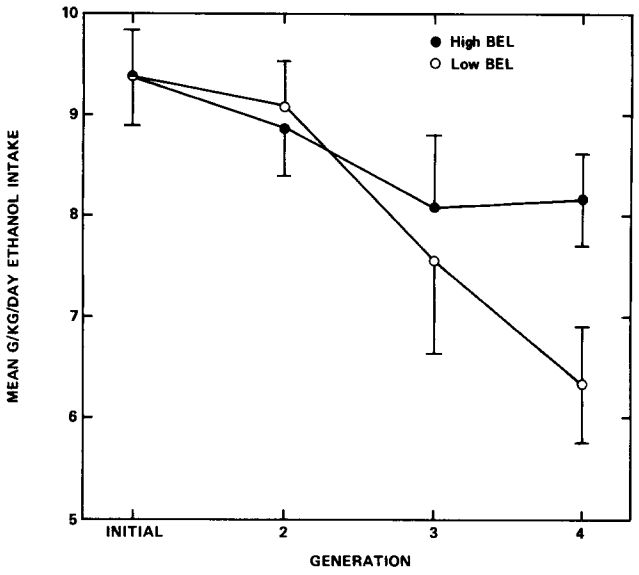

FIG. 2. Mean ± standard error grams per kilogram of body weight per day intakes of absolute ethanol of the entire initial generation and the offspring of rats mated for high blood ethanol (High BEL, solid dots) and the offspring of rats mated for low blood ethanol level (Low BEL, open dots), across three linearly derived generations.

mg/dl, N=42), $F(1,94)=4.72$, $p<0.05$. The effect of offspring generation, $F(2,94)=2.13$, $p>0.05$, and the interaction were not significant. Only the fourth generation showed a significant difference between high and low BEL groups by t-test, $t(29)=3.28$, $p<0.05$.

Mean ± SEM g/kg/day ethanol intake of the entire initial generation and the three generations derived linearly from sires and dams matched for high or low mean BELs is shown in Fig. 2. Across derived generations, ethanol intakes of offspring of high BEL sires and dams did not differ significantly from intakes of offspring of low BEL sires and dams, $F(1,94)=1.94$, $p>0.05$. The effect of offspring generation was significant, $F(2,94)=4.99$, $p<0.05$, but the interaction was not. Only the high and low BEL rats of the fourth generation showed a significant difference in ethanol intake by t-test, $t(29)=2.33$, $p<0.05$.

DISCUSSION

Data of the initial generation show outbred rats exhibit large and stable interindividual variations in voluntary maintenance of nocturnal blood ethanol levels. Similar stable large intragroup variations in blood ethanol maintenance have been reported at a greater range of levels for rats orally self-administering ethanol during schedule-induced ethanol consumption [12]. Free-choice ethanol intakes and blood ethanol levels are typically correlated in the present group, but variations in drinking pattern and/or blood ethanol elimination prevent a closer correspondence of ethanol intakes and blood levels.

The initial generation shows a significant difference between the sexes in ethanol intake that is unaccompanied by a consistent, significant sex difference in blood ethanol level. Greater ethanol intake in female rats accompanied by faster blood ethanol elimination has been previously reported [4,9]. Analogous cases of greater ethanol metabolism that allows for higher ethanol intake without greatly higher blood ethanol levels are the comparatively high ethanol intake and preference of the golden hamster, which is accompanied by comparatively high alcohol dehydrogenase activity [14,18], and the variation in inbred mouse strain ethanol preference that is paralleled by strain variations in ethanol metabolism [10, 11, 26].

Results from the procedure of selective matings of rats exhibiting either high or low blood ethanol levels indicate that significant differences in voluntary blood ethanol maintenance can be transmitted to progeny, although this process is slow and the transmissibility of this trait to offspring is very weak. Repeated matings of animals demonstrating significant tenfold differences in blood ethanol finally resulted in a significant twofold difference in this trait only in the fourth generation. However, the high variability and slowness in transmission of this blood ethanol trait to progeny is in accord with results from selective breeding of other alcohol-related traits [5, 6, 20, 21, 25, 28].

Results of the present experiment do not show rapid production of a strain that voluntarily maintains blood ethanol consistently at intoxicated and physical-dependence- and withdrawal-inducing levels. Nevertheless, when selectively breeding for an animal model of alcoholism, it appears more in accord with parsimony to select for the biological trait yet identified as most directly and necessarily associated with intoxication, physical dependence, and withdrawal syndrome. The approach outlined here has an advantage over selection for ethanol consumption alone because it avoids inadvertent coterminous selection for high ethanol metabolism in parallel with high ethanol intake. The present method also obviates the problem of lack of correlation between traits of response to administered ethanol and voluntary maintenance of blood ethanol. The presently-developed high blood ethanol strain displays a blood ethanol range within that attained by humans during "social" drinking, rather than chronic alcoholism.

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