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Low alcohol preference among the "high alcohol preference" C57/BL10 mice; factors affecting such preference

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Abstract

The effects of age, ethanol concentration and minor stress on the variation in alcohol preference of C57 strain mice were determined. In two bottle choice tests, an older population of mice contained slightly more low-preference mice than a younger population. A wide range of ethanol preference was consistently seen in young mice for 8% and 6% ethanol, but the previously reported biphasic pattern of distribution was revealed only with 8% ethanol. Very few animals showed high preference for concentrations of 10% or 12% ethanol. Moving low alcohol preference mice to a new location (but not repeated cage changing or ultrasonic noise) significantly increased the alcohol preference. Exploratory locomotor activity did not correlate with the subsequent alcohol consumption. Blood and brain alcohol concentrations showed that the differences in alcohol preference were not due to differences in metabolism of ethanol. The C57 strain mice with low preference for alcohol provides a valuable model for the study of the effects of minor stress on alcohol consumption. © 2002 Published by Elsevier Science Inc.

Keywords: Alcohol; C57 strain; Locomotor activity; Alcohol preference

1. Introduction

The C57 strain of mouse, originally bred by C.C. Little in 1921, has been widely used for many years, as an alcohol-preferring strain (McLearn and Rodgers, 1959; Phillips and Crabbe, 1991; Belknap et al., 1993). Animals of the strain, C57BL/10 (line ScSn) were originally bred in Bristol University Medical School for approximately 12 years, then the breeding line was moved to Durham University in 1995. Although the C57 strain is widely known and used as a strain with a high voluntary consumption of alcohol, we previously found that when mice from our breeding line were placed in a free-choice situation with a bottle of dilute (8% vol/vol) alcohol and a bottle of tap water, a considerable percentage had a low preference for alcohol, consuming the majority of their fluid in the form of water (Little et al., 1999). A biphasic distribution was seen in young animals in a free choice, two-bottle, preference test. The large majority of animals had either low alcohol preference (ratio of 8% alcohol to total fluid of 0.35 or less) or a high preference (ratio 8% alcohol to total fluid of 0.7 or more) very few falling in the intermediate range. In the present study, the alcohol preference of these mice was investigated in further detail and the factors affecting their ethanol consumption were examined.

The previous study on this line of mice (Little et al., 1999) demonstrated that the alcohol preference showed no correlation with gender and that selective breeding from the "in-house" stock did not demonstrate evidence of a simple genetic link. Mice with low alcohol preference were also found among animals bought from an outside breeder, so the variation in alcohol consumption was not confined to the line bred in our laboratories. Our earlier work also showed that repeated daily intraperitoneal injections of saline significantly increased the alcohol preference of mice characterised as "low preference" (Little et al., 1999). The present study further characterises the factors affecting such preference, including the effects of environmental disturbance.

The experiments first characterised the effects of age and of alcohol concentration on the alcohol consumption and preference. Secondly, we carried out studies on the effects of a different form of mild stress on alcohol consumption, to

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provide further information concerning the factors that might increase the alcohol preference of the "low preference" mice. These studies concentrated on environmental disturbances that would normally be encountered in laboratory animals. Thirdly, we examined whether or not there was any correlation between the locomotor activity of the mice and their alcohol preference that would provide a predictive test for determining the alcohol preference of individual animals in the two-bottle choice test. Fourthly, the blood and brain alcohol concentrations were measured in the high- and low -preferring mice after voluntary alcohol drinking and after acute injections of alcohol, in order to determine whether or not the differences in alcohol consumption were due to differences in metabolism or distribution of alcohol.

2. Methods

The animals in these investigations originated from the Bristol Medical School animal facility. When the study started, these C57BL/10 mice had been bred in this establishment for 12 years, with no introduction of new stock during that time. Such breeding continued when the research group moved to Durham University, where breeding continued under conditions as similar as possible to those in Bristol. The bimodal distribution of alcohol preference in young mice, discovered in Bristol, was also found in animals subsequently bred in Durham.

All mice were housed at 21 ± 1 °C, with $55 \pm 10\%$ relative humidity, and a 12-h light/dark cycle, with the light phase between 08:00 and 20:00 h, and free access to tap water and laboratory rodent chow (CRM) at all times. All animals were bred "in-house." The conditions of housing of the breeding pairs were kept consistent among the pairs. The mean litter size was seven, and the pups were weaned at 19 to 21 days, at which point they were transferred to new cages in single-sex groups of 10 per cage. In these, mice from different litters were mixed and this housing was kept consistent between this time and the alcohol screening or other tests. Unless otherwise stated, the tests were carried out on mice aged 6 to 10 weeks. As our initial studies had shown that there was no influence of gender on the alcohol preference (Little et al., 1999), both male and female mice were used in the studies.

2.1. Locations of tests

For all studies, the screening for alcohol preference was carried out in a laboratory adjacent to that in which the animals were bred. The experiments on environmental disturbance were carried out either in laboratories on different floors of the Medical School, as described below in Section 4, or in a laboratory adjacent to that in which the mice were bred. All studies were conducted under current British Home Office regulations.

2.2. Ethanol preference measurements

All tests of preference were made on mice in single housing (cage size $30 \times 14 \times 14$ cm. with solid floor). They were placed in the single cages 1 week before the beginning of either alcohol preference testing or the locomotor activity measurements in order to accustom them to the change in housing.

In the alcohol preference measurements, two fluid bottles were made available to the animals, for the whole of every 24-h period. For all the studies, one bottle contained tap water and the other, alcohol diluted with tap water. The alcohol concentration used was 8% vol/vol, with the exception of the experiment to investigate the effects of different alcohol concentrations on the alcohol preference (see below). In all cases, the positions of the bottles in the different cages were randomised with respect to which side of the cages they were placed. In all experiments, the ratio of alcohol to water consumed and the total fluid consumption was calculated.

Following the first demonstration of the existence of individuals in the C57 strain mouse with low preference for alcohol (Little et al., 1999), a screening procedure was established in the laboratory. This procedure was used prior to the studies carried out on the effects of environmental disturbance, in order to identify mice with low alcohol preference.

Two bottles, one containing 8% alcohol (vol/vol) and one containing tap water, were continuously available. Measurements of fluid intake were made, three times per week (Monday, Wednesday and Friday, at 09:00–10:00 h, and the amount drunk from each bottle used to calculate the ratio between 8% ethanol and water preference. The mean ratios for the last week of measurements were used to allocate mice to the preference categories. The cages were not cleaned during the last week of the screening procedure, in case this altered the ethanol consumption. Mice with a ratio of 0.7 and above for the consumption of 8% alcohol over water were classed as "high" drinkers, and those showing a ratio of 0.35 and below were classed as "low" drinkers. The "intermediate" preference animals, were those individuals that were not in the above classifications of low or high drinkers.

2.3. Effects of age on alcohol consumption and preference

In order to examine the effect of age on the distribution of ethanol preference, the standard screening procedure was carried out on one group of 96 mice, 7 to 9 weeks of age, (25 to 34 g) and one group of 96 mice, 30 to 52 weeks of age (35 to 45 g).

2.4. Effects of alcohol concentration on alcohol consumption and preference

The original choice of 8% ethanol for the preference testing was based on earlier work (Taberner et al., 1983),

which showed that the maximal difference between consumption of ethanol and water was seen with 8% ethanol. In order to compare the preference distributions for a range of concentrations of ethanol, an experiment was carried out on our C57 strain mice in which the screening procedure was carried out using the different concentrations of ethanol. Four groups of mice, ($n = 48$ per group) were offered a two-bottle choice between water and either 6% or 8%, or 10% or 12% ethanol. Different groups of mice were used for the study of each concentration of alcohol and in every group the mice were naive to alcohol at the beginning of the experiment. Measurements of fluid consumption were carried out three times per week for 3 weeks, as described above for the alcohol-preference screening.

2.5. The effects of environmental disturbance

In the first experiment, the alcohol-preference screening procedure was carried out in the environment in which the animals were weaned, as described above. A group of mice was screened, for alcohol preference, as above, then 34 animals with low preference were moved from the animal facility, down seven floors in an elevator, to a new laboratory on the ground floor. Another 34 mice with low preference were left in the room in which they were housed after weaning. Both rooms had the same light cycle times. Another week of alcohol choice measurements was made on each group of 34; then both groups were left undisturbed, in each environment, for 2 months, with no access to alcohol during this period. Another screening procedure was carried out at the end of this 2-month period.

2.6. The effects of ultrasonic noise

As the experiment above showed an effect of environmental disturbance on alcohol preference, a further experiment was carried out to examine the effects of one components of the change in location namely ultrasonic noise, as such noise would have been experienced during the elevator trip. Comparison was made with the ethanol preference in a group of mice taken to a different room, for the same amount of time but without the exposure to ultrasonic noise.

After the standard screening procedure, a group of low-preference mice ($n = 12$), were moved to another room, exposed to a 5-min period of ultrasonic noise from an ultrasonicator (Quay dental B22/100), then returned to their original laboratory. Ethanol-preference measurements were then made daily, in this room, for 7 days and comparison made with the preference of a second group of animals ($n = 12$) that remained in their original location throughout the test period. Both groups were then retested for fluid preference for 1 week after a period of 2 months without access to alcohol.

2.7. The effects of repeated cage changing

Mice ($n = 10$), classed as low alcohol preference animals after the standard screening procedure, were maintained in individual cages and subjected to a daily change of cage, into similar-sized cages containing fresh sawdust. This was carried out every day, for 5 days, with a free choice available between alcohol and water during this time. Comparison was made with a second group of $n = 10$ low-preference mice that were left undisturbed in their individual home cages. The alcohol preference was measured in both groups daily for 7 days, starting the day before the first cage change, and retested for fluid preference for 1 week after a period of 2 months without access to alcohol.

2.8. Locomotor activity measurement

Mice that had not been previously exposed to alcohol were placed individually in the locomotor activity boxes for 30 min for locomotor measurements. The next day the alcohol-preference screening procedure was commenced. Because the measurement of locomotor activity involved exposure to a novel environment, which might affect the subsequent ethanol consumption, a control group was screened for ethanol preference, in parallel, but without prior exposure to the activity meters. Each group of mice contained 46 individuals.

Spontaneous locomotor activity was measured using Opta-Varimex-Mini activity meters operated by the interruption of 15 infrared beams, between 10 a.m. and 4 p.m. (during the light phase). A clear perspex cage ($50 \times 32 \times 15$ cm), containing a small amount of sawdust was placed between a metal frame containing the infrared emitters and sensors placed 1 in. apart. The activity measurements were divided into static, mobile and rearing counts. Mobile activity was measured by the consecutive breaking of beams. Static activity was measured by counts of beam breaks in a nonsequential fashion, e.g., grooming and digging in the sawdust. Rearing activity was counted when the upper layer of beams was broken.

2.9. Brain alcohol concentrations

Trunk blood samples and whole brains were taken from separate groups of mice at the end of the 3-week screening procedure, in groups of low drinkers, intermediate drinkers and high drinkers, as defined above ($n = 6$ per group). These samples were taken at midnight because the alcohol consumption of the mice would have been greater during the dark phase.

Secondly, in order to determine whether or not there was any difference in the metabolism of alcohol between the high- and low-preferring mice, brain and blood samples were taken 15 min after the intraperitoneal injection of 1.5 g/kg alcohol.

For measurement of the alcohol concentrations, whole brains were removed, weighed and frozen at −70 °C in an isopentane/acetone ice trap and stored at −25 °C until use. For the assay, the brains were thawed then homogenised in 1 ml 1.2 M H_2SO_4, neutralised with 0.4 ml 5 M KOH. Pyrophosphate buffer (10 mM sodium pryrophosphate, 20 mM semicarbazide and 1 mM glycine), pH 8.8, was added to make a final volume of 2 ml, and the mixtures were vortexed and centrifuged at 2500 × g for 5 min at 25 °C. The supernatants were decanted and 100μl of the supernatant added to 40 μl sodium tungstate and 40 μl of 0.6 M H_2SO_4. The mixtures were then centrifuged at 3000 × g and 25 °C for 20 min.

Assays were performed in triplicate, using 5 μl alcohol dehydrogenase (Sigma) at 3600 units/ml, 10 μl of 0.5 M nicotinamide adenine dinucleotide and 1 ml phosphate buffer per cuvette. The reaction was started by the addition of 50 μl of substrate and extinction at 340 nm measured 30 min later. The mean of the triplicate recordings for each sample was calculated and the corresponding ethanol concentration obtained from the equation for a standard curve.

2.10. Statistical analysis

Fisher's exact probability test was used to compare the numbers of mice out of a group classed as low or high drinkers and chi-squared analysis for results containing three categories of alcohol preference. The ethanol preference and consumption, and the ethanol levels, were compared by one-way analysis of variance where there was more than one treatment group and by Student's *t* test for comparison between two treatment groups. Correlation comparisons were made by calculation of Pearson coefficient.

3. Results

3.1. Effects of age

The distribution of ethanol preference with age is illustrated in Fig. 1. The biphasic distribution reported previously (Little et al., 1999) was clearly seen in the mice aged 7 to 9 weeks. In the older animals, however, the distribution was not biphasic, but there was still a large proportion of animals with a low preference for ethanol. There were no differences in total fluid consumption between the two groups and no correlation between the ethanol preference and the body weight, as illustrated in Fig. 1c (Pearson coefficient .11, $P > .1$).

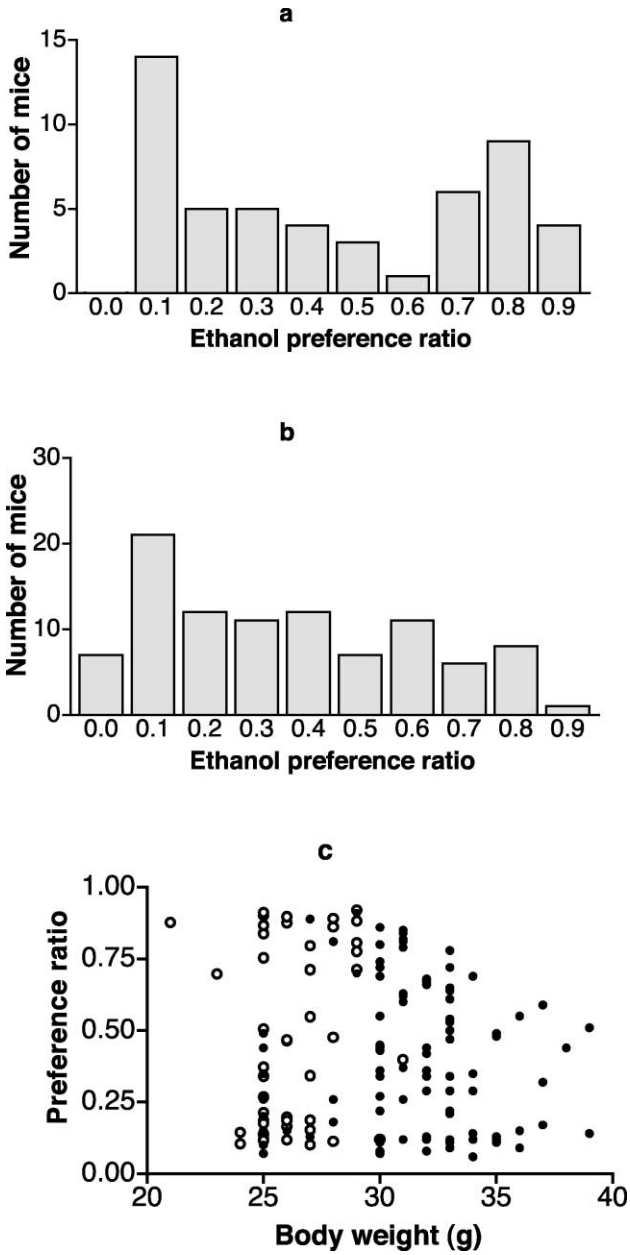

Fig. 1. The distribution of preference for ethanol in two different age groups of mice. The graphs show the numbers with each ratio of ethanol/water consumption (rounded up to the nearest higher single decimal place), during the last week of the 3-week screening period. (a) The distribution of preferences in a group of 96 mice, aged 7 to 9 weeks and weighing between 25 and 34 g. (b) The distribution of preferences in a group of 96 mice, 30 to 52 weeks of age, weighing between 35 and 45 g. (c) Correlation between body weight and ethanol preference for mice aged 7 to 9 weeks (○) and those aged 30 to 52 weeks (●). There was no significant correlation between the preference and the body weight for either group of mice.

3.2. Effects of alcohol concentration on alcohol consumption and preference

When different concentrations of ethanol were offered in the two-bottle choice, mice were found to demonstrate low, intermediate and high preference for each of the ethanol concentrations, but the distributions of preference differed for the different concentrations (Table 1). Chi-squared analysis overall showed significant differences between the distribution of preference at the different concentrations ($\chi^2 = 44.91$; $P < .0001$). For the 6% concentration there was

Table 1
Ethanol preference for the last week of the 3 weeks' measurements, when different concentrations of ethanol were offered, each in a separate two-bottle choice test. Values are the numbers of mice within each ethanol-preference category and the ethanol consumption in grams per kilogram per day (g/kg/day), mean ± S.E.M., for each preference group, for each concentration (conc.) of ethanol

Ethanol conc. (%)	Low preference	Low preference (g/kg/24 h)	Medium preference	Medium preference (g/kg/24 h)	High preference	High preference (g/kg/24 h)
6	12	2.8 ± 0.4	18	12.1 ± 1.4	18	12.7 ± 2.1
8	23	3.1 ± 0.5	5	11.0 ± 1.3	19	16.6 ± 1.3
10	14	5.7 ± 1.0	30	14.9 ± 1.4	4	14.5 ± 2.5
12	16	7.2 ± 1.0	29	17.9 ± 1.4	3	16.6 ± 4.2

little difference in the number of mice in each category and the distribution was not significantly different from that which would have been expected if there had been no difference between the categories ($P > .1$). For 8%, 10% and 12% ethanol concentrations, however, the distribution were significantly different from that expected if there had been no differences between the categories ($P < .05$ for 8% and $P < .01$ for 10% and 12% ethanol). The distribution for 8% ethanol appeared biphasic, as seen in all previous groups of this line of mice tested with 8% alcohol, as there were considerably larger numbers in the low and high preference categories than in the intermediate. For the 10% and 12% concentrations, the preference distributions were very similar, with very few showing high preference and large numbers in the intermediate category.

The amount of ethanol consumed, in grams per kilogram every 24 h, is also given in Table 1. Analysis of variance gave $P < .0001$ for differences between ethanol consumption for the separate categories. The amount of ethanol consumed by the low-preference mice in each group was significantly lower than that consumed by the animals that fell into the higher preference categories. For the intermediate and high-preference category mice, the amount of ethanol consumed was not significantly different. The total fluid consumption did not show significant differences between the groups (data not shown).

3.3. Effects of environmental disturbance

The subsequent effects on alcohol preference of transporting mice from the animal facility on the seventh floor of the Medical School to the ground floor laboratory are illustrated in Table 2. The proportion of high-preference mice increased significantly ($P < .005$) in the mice that were moved prior to the first screening, compared with those that remained in the original laboratory, when preference was measured after 2 months in the ground floor laboratory. No changes were seen in total fluid consumption of either low- or high-preference mice after the 2-month period.

3.4. The effects of ultrasonic noise

Exposing the mice to a short period of ultrasound did not significantly alter the alcohol preference or alcohol consumption when compared with the results from the parallel group of mice that did not undergo the experience (data not shown).

3.5. The effects of repeated cage changing

Repeated cage changing did not alter the preference of low-preference mice, when compared with the results from the parallel group of mice that did not undergo the experience (data not shown).

3.6. Locomotor activity

There were no significant correlations between the alcohol preference and any of the measures of locomotor activity prior to the preference testing ($r > .1$). Fig. 2 illustrates the mobile locomotor activity (Fig. 2a, Pearson coefficient .12, $P > .1$) and rearing counts (Fig. 2b, Pearson coefficient .12, $P > .1$) between 0 and 15 min after the animals were placed in the locomotor activity cages. A similar pattern was seen for the static activity measurements (data not shown). The results were also analysed for 15 to 30 min after the animals were placed in the cages, in case changes were seen at that time when the overall activity was considerably reduced, but no significant correlations were seen during this time interval (data not shown).

There was no significant difference ($P > .5$) between the alcohol preference of the mice that were placed in the activity meters compared to those of mice that

Table 2
Effect of change of environment on ethanol preference in low-preference mice. Values are the numbers of mice out of the two groups of 34 that showed preference ratios for ethanol/water of 0.35 or less. Values are from the last week of 3 weeks of screening, using the means of the measurements for that week

	First screening	After move	2 Months later
Original environment	34	34	10 (29%)
New environment	34	34	3* (9%)

* $P < .005$ compared with those that remained in original environment.

were screened without exposure to the activity meters (Fig. 2c).

3.7. Blood and brain concentrations of alcohol

The blood and brain concentrations of alcohol in mice with low, intermediate and high preference for 8% alcohol are illustrated in Fig. 3a. The concentrations correlated with the amount of alcohol consumed by the different categories of alcohol preference. Fig. 3b illustrates the concentrations of alcohol in blood and brain 15 min after intraperitoneal administration of 1.5 g/kg alcohol to mice with the three categories of preference for 8% alcohol.

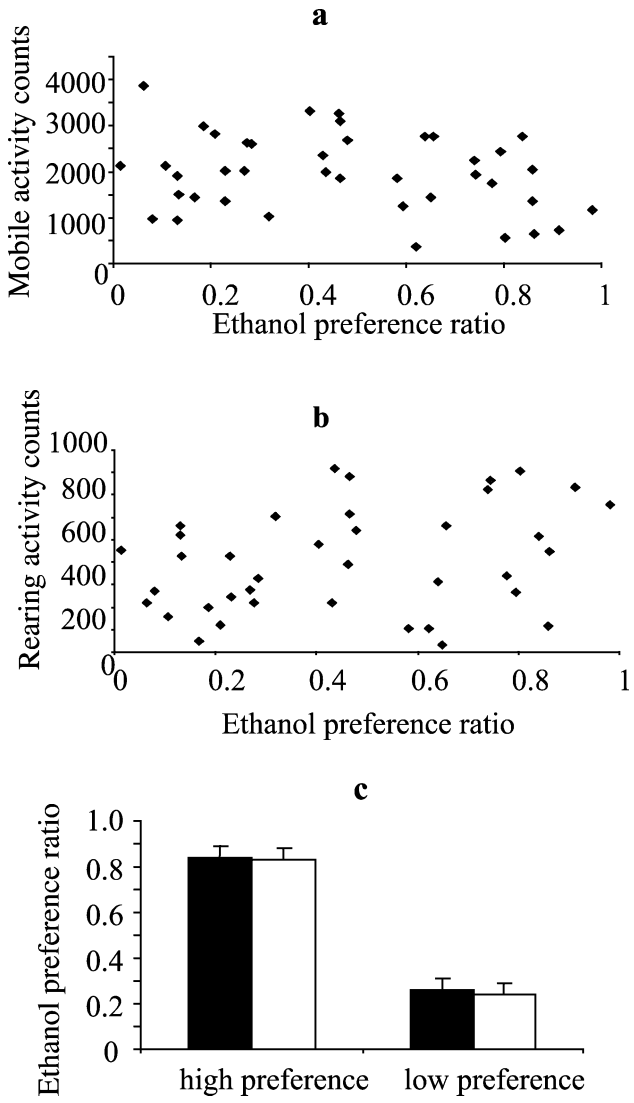

Fig. 2. (a) Correlation between measurements of mobile locomotor activity and subsequent ethanol preference. (b) Correlation between measurements of rearing and subsequent ethanol preference. (c) The numbers of mice with high alcohol preference (ratio 8% alcohol to total fluid of 0.7 or above) and low alcohol preference (ratio 8% alcohol to total fluid of 0.35 or below), measured before (dark columns) and after (open columns) the measurements of locomotor activity.

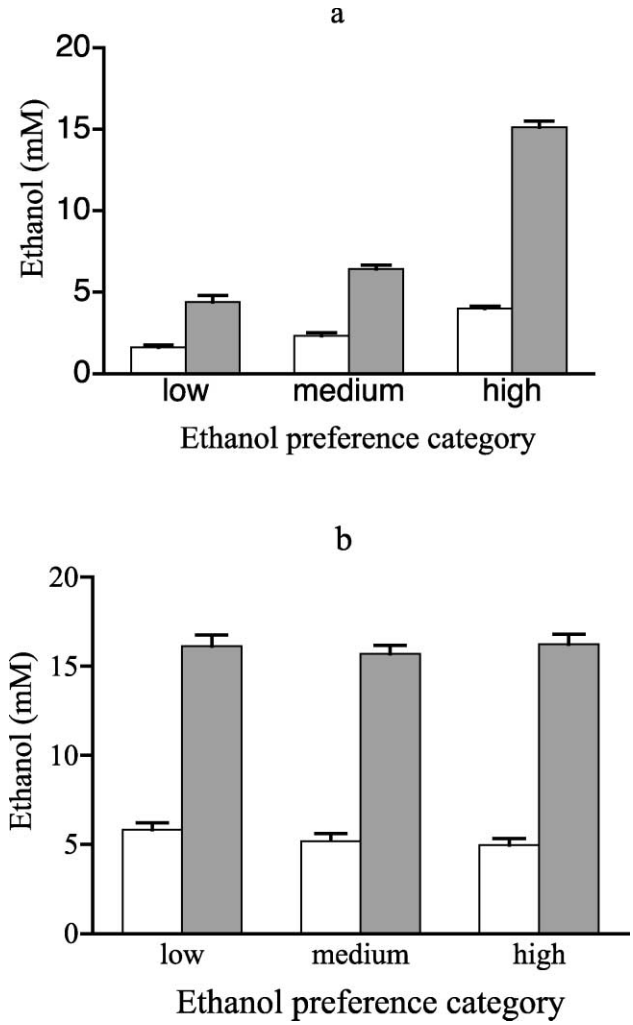

Fig. 3. Brain and blood concentrations of alcohol. (a) Alcohol concentrations in brains of mice sampled at 00:00 h at the end of the last week of 3 weeks of alcohol-preference screening. (b) Alcohol concentrations in brain (open columns) and blood (shaded columns) of mice given 1.5 g/kg alcohol, 15 min previously, by the intraperitoneal route. There were no significant differences between the preference categories in the latter set of measurements.

There was no significant difference between the groups ($P > .1$).

4. Discussion

In all the alcohol-preference screening the results clearly confirmed our original demonstration (Little et al., 1999) that a proportion of mice of the C57/BL10 strain, bred in-house, have a low, rather than a high, preference for alcohol. In our first studies, a concentration of 8% (vol/vol) ethanol was used because it had been established in earlier work (Taberner et al., 1983) that this concentration demonstrated the clearest changes in preference. The experiment in the present studies on the preference for different concentrations of ethanol showed that the patterns of ethanol preference

differed for the four alcohol concentrations studied (6%, 8%, 10% and 12% vol/vol).

For the 8% ethanol concentrations, there was a linear relationship between the preference and the amount of ethanol (in grams per kilogram) that was consumed. This was not apparent for the other ethanol concentrations, although the low-preferring mice consumed less ethanol in every group. It was notable that the small number that fell into the "high-preferring" classification for the 10% and 12% ethanol did not actually consume more ethanol than those that fell into the intermediate group, suggesting an upper limit to the amount that is consumed voluntarily. The amount of ethanol consumed by the intermediate and high preferring groups is within the range that, in our previous work, caused a clear withdrawal syndrome when taken in for a minimum of 6 weeks (Morton et al., 1992). This is a large amount of alcohol even for high-preferring strains of rodents; high-preferring rat strains are normally reported to consume 6–9 g/kg/day (Colombo, 1996; Samson et al., 1998; Files et al., 1998). The low-preferring mice can drink less than 1 g/kg/day, an intake similar to other strains of low or alcohol-avoiding rats (Samson et al., 1998; Files et al., 1998).

In our previous study we demonstrated that daily injections of saline significantly increased the preference of low-preference mice, while moving cages of animals once along a corridor then either back to the original laboratory or to a new room did not alter the preference distribution when this was measured over 3 weeks after the disturbance (Little et al., 1999). The present study extended these experiments and demonstrated that moving the mice down seven floors, via a lift, increased the preference of low-preference mice. This effect, however, was slow in onset, and was seen 2 months after the move, while in the previous study the effects of cage moving on ethanol preference was examined only up to 3 weeks after the manoeuvre. The lack of change in the alcohol preference following exposure to ultrasound showed that this component of the experience was unlikely to have contributed to the change in alcohol preference, although it should be noted that the ultrasound frequency distribution may not have been identical to that produced by the elevator. Repeated cage changing in the present experiments had no significant effects, either immediate or delayed, on the ethanol preference.

The lack of correlation between the measurements of locomotor activity and the subsequently measured alcohol preference suggests that the alcohol consumption is not related to exploratory activity. Previous work has shown that operant self-administration of amphetamine by rats can be correlated with their exploration of novel environments (Piazza et al., 1989, 1990).

The concentration measurements showed, firstly, that the differences in alcohol consumption by mice demonstrated different alcohol-preference levels is reflected in the blood and brain concentrations of alcohol. Secondly, the differences in preference for alcohol are unlikely to be due to differences in alcohol metabolism, as there were no differences in blood or brain concentrations of alcohol in mice with difference alcohol-preference levels after intraperitoneal injection of a specific dose of alcohol.

The effect of changing location in increasing alcohol consumption demonstrated in the present studies may be an important, previously unrecognised, factor affecting behaviour in laboratory mice. Such changes may have been missed in previous work, as the effect was slow in onset. In addition, animals bought from outside sources undergo the stress of transport before they reach laboratories. It is possible that while the animals used in many laboratories show high alcohol preference, they may not have begun life with such preference. An important aspect is the nature of the change in the alcohol consumption, whether, for example, it involves alterations in the rewarding, or aversive, effects of alcohol. Further studies on this model are in progress to investigate these aspects.

Acknowledgments

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