

RESEARCH ARTICLE

Strain-specific responses of inbred mice to ethanol following food shortage

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Abstract

Specific inbred mouse strains such as C57BL/6J and DBA/2J show differences in consumption of and reaction on drugs of abuse. For example, C57BL/6J mice voluntarily consume greater amounts of ethanol than DBA/2J mice. Recently, it could be shown that a short environmental experience—12 days of food shortage followed by a recovery period—has a strong impact on strain-specific reactions to amphetamine. The purpose of the present study was to examine whether food shortage experience has an effect on ethanol responses. The effect of a period of 12 days food restriction which resulted in a weight loss of 20% body weight and which was followed by a complete recovery period was studied on ethanol self-administration and ethanol-induced locomotor activity in C57BL/6Ico and DBA/2Ico inbred mouse strains. The experience of food shortage led to a higher ethanol intake and preference in C57BL/6Ico mice compared to control animals without food shortage experience. In contrast DBA/2Ico showed no difference in ethanol intake or preference following this experience. The effect of ethanol onto locomotor activity of both mice strains was affected only in the case of DBA/2Ico mice, where food shortage experience resulted in a significantly higher ethanol-induced locomotor activity. The present data show that in inbred mouse strains environmental experiences can have a strong impact onto the effects of ethanol. In conclusion, in the field of preclinical alcohol research gene × environment interactions in specific inbred mouse strains can contribute strongly to the outcome of studies and more specifically food shortage can profoundly affect the outcome of alcohol studies in mice.

Introduction

In the field of drug abuse research specific inbred strains of mice such as C57BL/6J and DBA/2J are used widely to identify possible mechanisms of drug addiction. The base of these investigations are consistent behavioural differences between these strains,¹ which can help to identify genes responsible for different drug-induced responses.^{2–6} Clear differences in the amount of

drugs with an addictive potential consumed voluntarily can be found between the inbred strains C57/BL6J and DBA/2J. In a two-bottle free-choice paradigm C57/BL6J mice show a higher voluntary intake than DBA/2 mice of nicotine,^{7,8} morphine and amphetamine,^{9,10} as well as of ethanol.^{11–13} Also measurements of rewarding properties of ethanol in place-conditioning tests or testing of motor-stimulating

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effects of ethanol show significant differences between C57BL/6J and DBA/2J.^{14–17} Against the background of these findings, Cabib *et al.*¹⁸ showed that strain differences in behaviour appear to be dependent not only on genetic factors but also on environmental experiences. Thus different behavioural responses of C57BL/6J and DBA/2J mice to an application of amphetamine are reversed or abolished after recovery from a short period of food shortage.¹⁸

In the case of amphetamine these results demonstrated a direct interaction between the environment and the genome of the tested animals. However, it is not yet known if these interactions can be found in general for all psychotropic substances. To elucidate this question further we examined the influence of a short food shortage experience on ethanol consumption and ethanol-induced locomotor activity in the two inbred mouse strains C57BL/6Ico and DBA/2Ico.

Methods

Animals

In order to follow exactly the protocol of Cabib *et al.*,¹⁸ male C57BL/6Ico and DBA/2Ico purchased from Charles-River (Charles-River, Sulzfeld, Germany) were used for the experiments. The history of these substrains have not been documented so far in the literature despite the fact that numerous studies have been performed with these substrains. In 1981 Iffa-Credo (L'Arbresle, France) had obtained several founder lines of C57BL/6J (approx. generation 140) and DBA/2J from Jackson Laboratories. After 23 years of further breeding, these lines should now be considered as two new substrains and should be designated as C57BL/6Ico and DBA/2Ico. At 8 weeks of age all mice were housed singly in standard breeding cages (Macrolon Type III) with food (Sniff rodent food pellets, Soest, Germany) and water *ad libitum*. Mice were kept in a 12-hour dark/light cycle (lights on between 6.00 a.m. and 6.00 p.m.; light intensity 180 Lux) with constant temperature and humidity (temperature: $22 \pm 1^\circ\text{C}$; humidity $55 \pm 5\%$) in rooms of the animal facility of the Central Institute of Mental Health, Mannheim, Germany.

The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body and carried out following the German Law on the Protection of Animals.

Food shortage

Two weeks after habituation to the home cage available food was reduced progressively to reach a 20% weight loss of the animals. After reaching this weight loss the food regimen was held to maintain this loss for 12 days. Meanwhile a control group of animals received food *ad libitum*. To check body weight and animals' well-being, animals were examined and weighed daily at 10.00 a.m. After this food shortage period food was again available *ad libitum*. Ethanol drinking and locomotor activity experiments started 2 days after animals reached their initial body weight before the food shortage period.

Drugs

Ethanol drinking solutions were made up from 96% ethanol (Merck, Darmstadt, Germany) diluted in tap water to reach a 10% (v/v) solution. For ethanol injections in the locomotor activity experiments solutions of ethanol (96%, Merck, Darmstadt, Germany) were prepared in 0.9% saline (Fresenius Kabi, Bad Homburg, Germany) to reach a dose of 1 g ethanol per kg body weight.

Ethanol self-administration

After the food shortage period mice were given free access to a 10% (v/v) ethanol solution and tap water in two separate drinking bottles positioned on the top of the home cage. The location of the bottles was changed in an irregular manner to avoid side preferences and the consumed amount of ethanol solution and tap water, respectively was measured by weighing the bottles. Spillage and evaporation were minimized by the use of self-made glass cannulas in combination with a small plastic bottle (Techniplast, Milano, Italy). Under these conditions ethanol concentration in a given solution remained constant for at least 1 week, when measured with an alcoholometer (GECO, Gering, Germany). The amount of ethanol consumption in g ethanol per kg body weight and day as well as the ethanol preference, i.e. the percentage of consumed ethanol solution of the total fluid intake, was determined weekly up to day 35.

Locomotor activity

These experiments were performed in the same room where mice were kept during all experiments. All activity experiments were performed

during the second half of the light phase. Locomotor activity was measured in activity boxes (True Scan System, Coulbourn, USA). The number of interrupted photocells as a measure of locomotor activity was recorded in 2-minute intervals. Locomotor activity was assessed by quantifying the total covered distance in 1 hour. At the experimental day, animals were first habituated to the boxes for 11 hour. After another hour in their home cages animals received intraperitoneally either the ethanol solution (1 g/kg body weight) or vehicle. Immediately after injection locomotor activity (i.e. total distance in cm) was monitored.

Data analysis

All data were expressed as mean \pm SEM. Statistical analyses were performed with ANOVA. *Post-hoc* analyses were done with the Newman–Keuls test where appropriate. All analyses were performed with Graph Pad Prism 3.0 (GraphPad, San Diego, USA). Statistical significance was accepted if a *p* value less than 0.05 was obtained.

Results

Food shortage

Within 4 days progressive reduction of food resulted in a reduction of body weight of both C57BL/6Ico as well as the DBA/2Ico onto 80% of the weight at the beginning of the food shortage period. An application of up to \sim 2 g of food pellets/day/mouse held the body weight of food-deprived mice (C57BL/6Ico-FD, DBA/2Ico-FD; $n = 24$, respectively) 20% below the weight of the control groups (C57BL/6Ico, DBA/2Ico; $n = 24$, respectively; Fig. 1). After a period of 12 days, mice of the FD groups again received food *ad libitum*, which led to a fast recovery within 1–2 days to the ‘normal’ body weight of the control groups.

Ethanol consumption

According to published data^{11–13} ethanol consumption in g ethanol/kg body weight/day as well as ethanol preference of C57BL/6Ico was significantly higher compared to DBA/2Ico ($p < 0.05$). However, it is important to note that the C57BL/6Ico substrain has a lower intake and preference as well as a different drinking pattern over time, as reported for C57BL/6J mice.^{11–13}

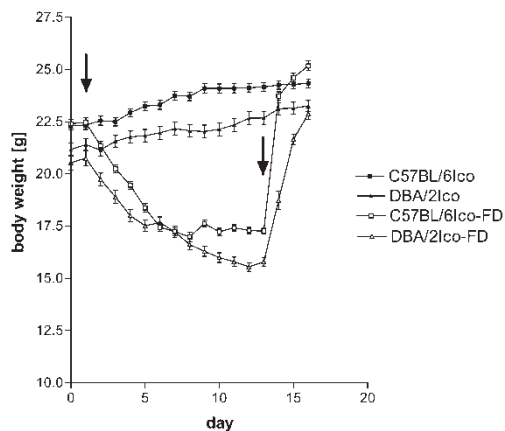


Figure 1. Effect of a period of food shortage (FD) and *ad libitum* feeding on the time-course of body weight in C57BL/6Ico and DBA/2Ico ($n = 24$ per group). Left arrow: begin of food shortage, right arrow: end of food shortage. Data presented as mean \pm SEM of the body weight.

The food shortage period led to a significant difference in the voluntarily consumed amount of ethanol in g/kg body weight in tested C57BL/6Ico ($p < 0.05$) but not in DBA/2Ico mice (Fig. 2). Ethanol preference was also significantly higher in C57BL/6Ico after food shortage experience compared to the control group ($p < 0.05$), whereas DBA/2Ico mice showed no significant difference in ethanol preference with or without food shortage (Fig. 2).

Locomotor activity

Locomotor activity—defined as the total covered distance in 1 hour—was not influenced in C57BL/6Ico after ethanol injection (1 g/kg body weight), whereas in DBA/2Ico ethanol led to a decrease of the locomotor activity. Food shortage had no effect on saline or ethanol-induced locomotor activity of C57BL/6Ico (Fig. 3). In contrast DBA/2Ico showed significant difference in ethanol-induced locomotion after food shortage: ethanol injected in mice with food shortage experience led to a significantly higher activity compared to the controls without food shortage (strain \times dose interaction: $F = 25,02$, $p < 0.001$) (Fig. 3).

Discussion

Our results demonstrate that the experience of food shortage can change the quality of responses

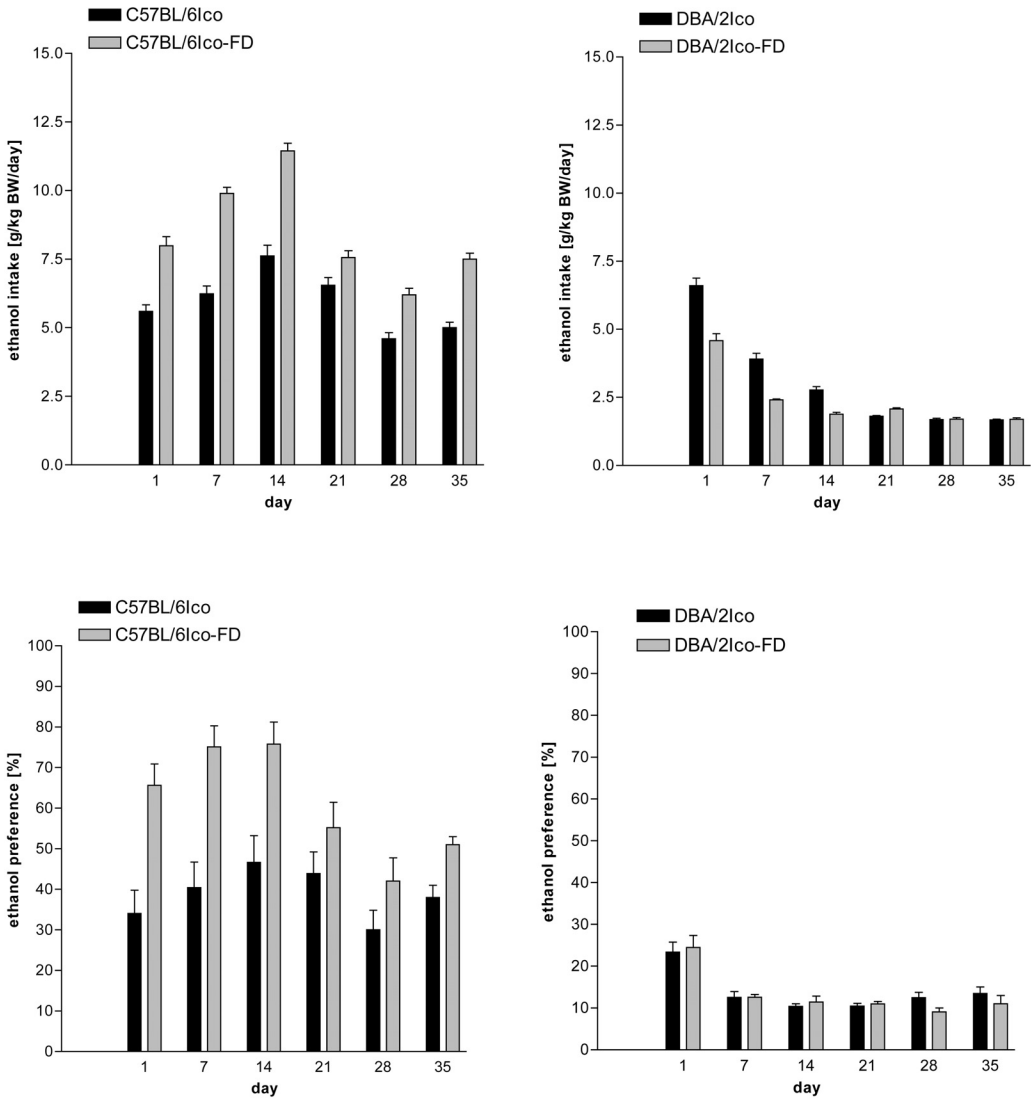


Figure 2. Ethanol consumption (g/kg body weight/day) and ethanol preference of the control groups (C57BL/6Ico and DBA/2Ico) and groups with a experience of food shortage (C57BL/6Ico-FD and DBA/2Ico-FD). All data of n = 12 per group are presented as the mean \pm SEM of the ethanol intake or preference, respectively.

of inbred mouse strains to ethanol. Thus, after a recovery period, inbred mice which underwent food shortage over a period of 12 days exhibited changes in the intake and preference of self-administered ethanol and in the motor-stimulating effects of ethanol. Interestingly, our experiments revealed different food shortage-related changes in tested mouse strains: food shortage resulted in a higher ethanol intake and preference in C57BL/6Ico, whereas DBA/2Ico showed no

change in basal ethanol drinking scores. On the other hand, only in DBA/2Ico mice which had experienced food shortage could changes in ethanol-induced locomotor activity be observed. The induction of strain-specific behavioural differences by an environmental experience is in accordance with the findings of Cabib *et al.*,¹⁸ who found that a similar food shortage schedule abolished the motor-stimulant effect of amphetamine in C57BL/6Ico but not in DBA/2Ico mice.

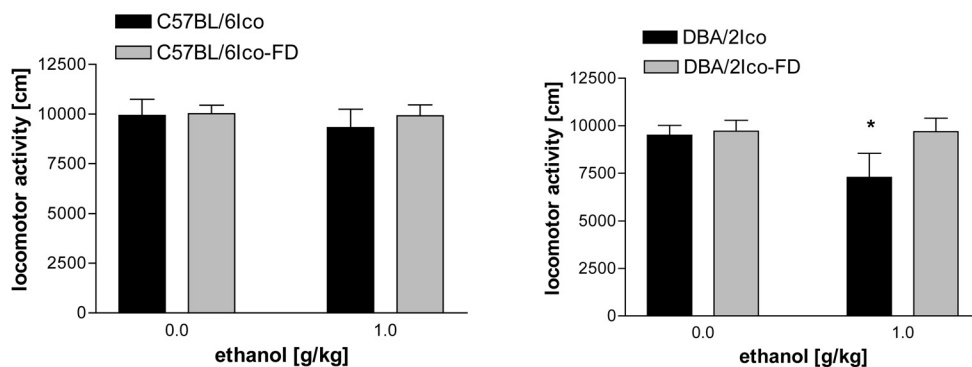


Figure 3. Effect of food shortage (FD) on ethanol-induced locomotion in C57BL/6Ico and DBA/2Ico. All data ($n = 12$ per group) are the mean \pm SEM of the total measured distance in 1 hour after saline or ethanol injection, respectively.

Furthermore, a reversal of the amphetamine-induced place preference of DBA/2Ico mice from aversion to a preference was shown under these conditions.¹⁸

A strong impact of environmental factors on drug-related behaviour in mice has also been demonstrated by Crabbe *et al.*¹⁹ who showed that even under highly standardized experimental conditions, the magnitude of behavioural responses to drugs of abuse of several inbred mice strains depends on the specific testing laboratory where the experiments were conducted. An exception to this finding concerns measures obtained in ethanol self-administration experiments. In the Crabbe *et al.*¹⁹ experiments basal ethanol drinking scores were closely comparable across different laboratories, suggesting robust phenotypes of C57BL/6J and DBA/2J mice with respect to basal ethanol drinking scores. In the same vein, in mice lacking the neuronal nitric oxide gene we have demonstrated recently that basal ethanol drinking scores did not differ when the ethanol self-administration experiments were repeated several times in the same way at three different locations (Munich, Magdeburg and Mannheim). All drinking experiments yielded similar results; the knock-out mice had about a sixfold higher ethanol intake than the wild-type animals.²⁰ Thus, in contrast to other behavioural measures used in drug abuse research, such as tolerance and sensitization, which show often large variability, measures obtained in ethanol self-administration experiments with inbred mice strains and knock-out mice should show only little variation, even when conducted in different laboratories and different settings.

However, one should be aware of the fact that other environmental factors can have a significant impact on ethanol drinking scores. In the present study we could show that food shortage can have long-lasting consequences on ethanol drinking scores (up to 6 weeks or possibly longer). Although food shortage should not occur under standardized conditions there is always a likelihood, for example during transport from the supplier or over weekends and holidays, that mice undergo a period of food shortage. It has to be emphasized that such an event can have a delayed effect on ethanol intake as observed recently in mutant mice lacking the corticotrophin-releasing hormone receptor 1 (CRHR1). Following a short period of stress—and food shortage can be also considered as a stressful event—CRHR1 mutants did not exhibit changes in ethanol drinking scores; however, 3 weeks after the stress period the voluntary ethanol intake in CRHR1 was markedly increased,²¹ demonstrating that a gene–environment effect can have a delayed onset.

Another important environmental factor besides food shortage, concerning the response to drugs of abuse, is the home cage environment. In a series of experiments Sachser *et al.*^{22,23} showed the high preference of mice to live under conditions of an enriched environment. Mice work hard—in terms of lever pressing—to reach a cage enriched with only two insets: a plastic box with several openings and a wooden scaffolding. These small changes in home cage conditions can profoundly affect responses to drugs of abuse. Recent results suggest that exposure to an enriched environment is effective in reducing

amphetamine self-administration rates,²⁴ whereas it increases ethanol drinking scores.²⁵ We have observed further that the cage size (standard cage size types II and III) can change basal ethanol drinking scores in inbred mouse strains (unpublished observations). Therefore, it would be useful for authors to state the cage size used in their drinking studies.

How could the impact of the described environmental changes on drug effects be explained? It is known that the described environmental changes can result in the release of stress hormones, in particular of corticosterone,^{26,27} and differences in the release of corticosterone in C57BL/6J and DBA/2J mouse strains have been described under basal as well as under stress conditions.^{28,29} Corticosterone can directly affect mesolimbic dopaminergic activity,³⁰ which in turn modulates alcohol reinforcement processes.³¹ For example, food shortage increases the dopaminergic function of mesolimbic neurones and leads thereby to changes in drug self-administration behaviour.^{30,32} Therefore, the behavioural changes in the response to ethanol observed following food shortage in C57BL/6Ico and DBA/2Ico mice, respectively, might be a result of different activities of dopaminergic neurons. In fact, C57BL/6J and DBA/2J mice display different dopamine receptor function^{33,34} and dopamine content and turnover of mesolimbic neurones^{35,36} under basal conditions as well as under stress conditions.

In summary, the present study shows that food shortage in inbred mouse strains can have long-lasting effects on ethanol drinking scores and on acute responses to injected ethanol. Although it is clear that a specific phenotype results from a gene–environment interaction the present study shows that environmental experience can have a profound impact on the assessment of ethanol's action in mice.

Acknowledgements

This work was supported by grant no. BMBF FKZ 01GS0117 (Identification and functional analysis of target genes involved in alcohol addiction) to R.S., grant no. BMBF FKZ EB 01011300/6 (Individual adapted pharmacotherapy) to R.S. and by the National Health and Medical Research Council, Australia to M.S.C.

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