



The alcohol deprivation effect model for studying relapse behavior: A comparison between rats and mice

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ABSTRACT

Understanding the psychological mechanisms and underlying neurobiology of relapse behavior is essential for improving the treatment of addiction. Because the neurobiology of relapse behavior cannot be well studied in patients, we must rely on appropriate animal models. The alcohol deprivation effect (ADE) is a phenomenon in laboratory animals that models a relapse-like drinking situation, providing excellent face and predictive validity. In rodents, relapse-like behavior is largely influenced by the genetic make-up of an animal. It is not clear which other factors are responsible for variability of this behavior, but there seems to be no correlation between levels of baseline alcohol intake and the occurrence, duration, and robustness of the ADE. Rats that undergo long-term alcohol drinking for several months with repeated deprivation phases develop a compulsive drinking behavior during a relapse situation, characterized by insensitivity to taste adulteration with quinine, a loss of circadian drinking patterns during relapse-like drinking, and a shift toward drinking highly concentrated alcohol solutions to rapidly increase blood alcohol concentrations and achieve intoxication. Some mouse strains also exhibit an ADE, but this is usually of shorter duration than in rats. However, compulsive drinking in mice during a relapse situation has yet to be demonstrated. We extend our review section with original data showing that during long-term alcohol consumption, mice show a decline in alcohol intake, and the ADE fades with repeated deprivation phases. Furthermore, anti-relapse compounds that produce reliable effects on the ADE in rats produce paradoxical effects in mice. We conclude that the rat provides a better model system to study alcohol relapse and putative anti-relapse compounds.

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Introduction

An alcohol-addicted brain is characterized by neurobiological alterations on all system levels, encompassing changes in gene expression regulation, molecular alterations, and synaptic and cellular changes, eventually resulting in long-lasting alterations in neuronal network activity. This happens on a small scale, i.e., in local neurodynamic changes, but alterations also occur in global neurotransmission and in the functional coupling of various brain regions (Noori, Spanagel, & Hansson, 2012; Spanagel et al., 2013). These system level alterations may persist after detoxification. The behavioral effects of these brain changes are exhibited in a relapse situation when an individual is exposed to alcohol-related stimuli or stress (Noori, Helsinki, & Spanagel, 2014; Spanagel, Noori, & Heilig, 2014). Thus, even after successful detoxification and abstinence treatment, an alcohol-dependent individual remains at risk for relapse. Therefore, it is of fundamental importance to understand the psychological underpinnings and neurobiology of relapse

behavior. The psychological concept that aims to understand a relapse situation builds on the negative reinforcement theory. Thus, the avoidance of an aversive state during withdrawal – which is characterized by depressed mood and elevated anxiety – triggers relapse to alcohol use (Koob, 2013). However, in most patients, these symptoms abate over 3–6 weeks of abstinence, while relapse risk persists long beyond this period. However, more subtle changes, such as increased behavioral sensitivity to stress, support the clinical relevance of negative emotionality for protracted abstinence and relapse (Heilig, Egli, Crabbe, & Becker, 2010; Spanagel, Noori, et al., 2014). Other psychological concepts posit a residual deficit state in the reinforcement/reward system, or sensitization of this system to alcohol-related cues, or both, which drive relapse behavior even after protracted abstinence (Robinson & Berridge, 2003). Furthermore, drug craving may incubate over time, leading to an enhanced relapse risk several months after detoxification (Pickens et al., 2011). Understanding the psychological mechanisms and underlying neurobiology of relapse behavior is essential for improving the treatment situation. Because the neurobiology of relapse behavior cannot be well studied in patients, we must therefore rely on appropriate animal models. The alcohol

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deprivation effect (ADE) is a phenomenon in laboratory animals that models a relapse-like drinking situation.

Modeling relapse behavior

The current psychiatric diagnostic classification system DSM-5 is based upon clinical observation and symptom reports by patients and is by nature built on anthropomorphic terms. As diagnoses are made according to DSM-5, logic dictates that animal research must adhere to DSM-5.¹ Therefore, our animal models should be based on DSM-5 criteria. Is this possible? Modeling the entire spectrum of a complex human mental disorder such as addiction in animals is not possible due to its complexity. However, we can translate anthropomorphic terminology into objective and behaviorally measurable parameters and thus model at least some key criteria of the disorder. With regard to relapse behavior, this is a straightforward endeavor, as a relapse is defined as the recurrence of a past condition, namely excessive and uncontrolled drinking after a phase of abstinence. The alcohol deprivation model provides excellent face validity to relapse behavior seen in alcoholics.

In animals given voluntary access to alcohol for a certain period of time and then deprived for several days/weeks/months, re-presentation of alcohol leads to a robust but temporary increase in alcohol intake over baseline drinking, relapse-like drinking referred to as the ADE (Sinclair & Senter, 1967). The ADE can be achieved under both operant (Hölter, Landgraf, Zieglänsberger, & Spanagel, 1997) and home-cage free-choice drinking conditions (Sinclair & Senter, 1967). Concurrent access to more than one alcohol concentration (e.g. 5%, 10%, and 20% v/v ethanol solutions vs. water) does influence the magnitude and duration of the ADE (Rodd-Henricks et al., 2001; Spanagel & Hölter, 1999). The magnitude of the ADE also depends on the duration of access to alcohol and on the length of abstinence. It has been demonstrated that only long-lasting alcohol consumption, for at least 6–8 weeks, will lead to a reliable ADE (Wolffgramm & Heyne, 1995), and that at least 2 days of withdrawal are needed to increase alcohol consumption by more than 50% (Sinclair, Walker, & Jordan, 1973). The ADE is not a universal phenomenon for all animal species. For instance, a negative ADE has been observed in hamsters (Sinclair & Sheaff, 1973). An increase in alcohol consumption following the period of forced abstinence has so far only been observed in rats (Sinclair & Senter, 1967), mice (Salimov & Salimova, 1993), and monkeys (Sinclair, 1971). However, in rats and mice, the occurrence and magnitude, as well as the duration, of the ADE are strongly dependent on the genetic background and thus a huge variability among strains is observed (Rosenwasser, Fixaris, Crabbe, Brooks, & Ascheid, 2013; Vengeliene et al., 2003) (Fig. 1).

ADE in rats

In rats, the ADE is a very robust phenomenon but not all animals show relapse-like drinking behavior. Whether a rat will have an ADE or not mainly depends on the genetic background of the

¹ The authors are aware of the National Institute of Mental Health (NIMH)-driven Research Domain Criteria project (RDoC) (Cuthbert & Insel, 2013) that aims to define basic dimensions of brain and behavioral functioning to be studied across multiple units of system analysis. The intent of this initiative is to translate progress in basic neurobiological and behavioral research to an improved integrative understanding of psychopathology and to the identification of new ways of classifying psychopathology for mental disorders. Although we applaud this paradigmatic shift which has the potential to revolutionize the entire field of psychiatry in the future, we argue that it does not improve the current situation, as the DSM-5 was just released in May 2013 and will rule our psychiatric diagnoses for at least the next 10–15 years. As a consequence, we further argue that basic and preclinical researchers should gear their animal models toward DSM-5.

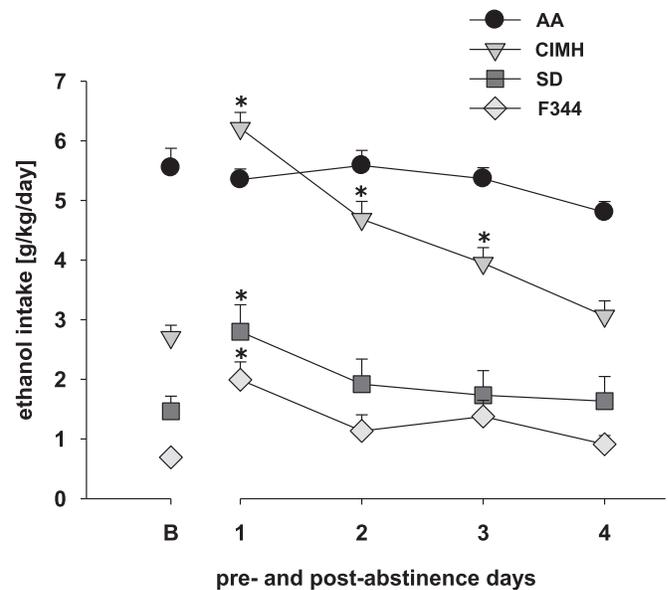


Fig. 1. Total ethanol intake (g/kg/day) by male rats from different strains/lines before and after an alcohol deprivation period of 2 weeks: alcohol-preferring rats (AA, National Public Health Institute, Helsinki, Finland, $n = 15$), Wistar (our own breeding colony at the CIMH, $n = 34$), Sprague-Dawley (SD, our own breeding colony at the CIMH, $n = 14$) (see also Vengeliene et al., 2005), Fisher (F344, our own breeding colony at the CIMH, $n = 8$). All animals were tested in a 4-bottle free-choice paradigm and had *ad libitum* access to tap water and to 5%, 10%, and 20% ethanol solutions (v/v) as well. The 2-week deprivation period was introduced after 8 weeks of continuous alcohol availability. The measurement of the last week of ethanol intake is given as baseline drinking – “B”. Data are presented as mean \pm SEM. * Indicates significant differences from baseline drinking.

animal. Hence, Wistar rats from our own breeding colony at the Central Institute of Mental Health (CIMH) in Mannheim increase their alcohol intake and preference approximately 2-fold–3-fold during the first re-exposure day, and the ADE then declines to previous basal levels within approximately 4 days (Fig. 1). Such an ADE is a very robust phenomenon that can even be described as a mathematical function (Sinclair & Senter, 1967), and a threshold criterion has been introduced to clearly define the onset of an ADE (Villarín Pildain, Vengeliene, & Matthäus, 2013). Sprague-Dawley rats usually have lower alcohol intake than Wistar rats, and the ADE is restricted to a single post-abstinence day (Vengeliene, Vollmayr, Henn, & Spanagel, 2005) (Fig. 1). Fisher rats generally do not consume pharmacologically significant amounts of alcohol when given free access to water and alcohol solutions. Nonetheless, a 2-month access to alcohol followed by 2 weeks of abstinence can lead to a small increase in alcohol consumption during the first 24 h of re-exposure (Fig. 1). Certainly, there are individual variations within every animal group which are caused by the genetic heterogeneity of outbred animals. Data on gender differences in the expression of an ADE are limited, but female rats from our Wistar colony as well as female Sprague-Dawley rats exhibit an ADE (Füllgrabe, Vengeliene, & Spanagel, 2007; Vengeliene et al., 2005).

The ADE phenomenon has also been extensively studied in various alcohol-preferring rat lines including the sP, HAD, and AA lines. These lines do not show an ADE after a single deprivation period (Agabio et al., 2000²; Rodd-Henricks, McKinzie, Murphy, et al., 2000; Sinclair & Tiihonen, 1988; Vengeliene et al., 2003) (Fig. 1). However, after repeated deprivation phases, a pronounced

² In this study, ADE was found to be limited to the first hour of renewed intake in sP rats, which was not dependent on the deprivation duration (1–180 days).

Table 1
Summary of ADE studies in mice.

Genetic strain	Pre-deprivation			Abstinence period	ADE		Reference
	Ethanol %	Exposure	Time		Onset	Duration	
C57BL6/J	10	18h daily	4d	4d	=	0	Khisti et al. (2006)
	13 (baseline 10–7d)	18h daily	7d	4d	=	0	Camp et al. (2011)
	15 (baseline 6w)	Weekly	1d	6d	=	0	Melendez et al. (2006)
	15 (baseline 6w)	Weekly	1d	2w	=	0	
	7.5, 15, 30 (baseline 6w)	Weekly	1d	2w	=	0	
C57BL6/N	15	Continuous	6w	2w	–	5d	
	10	18h daily	4d and 14d	4d	+	4d	Khisti et al. (2006)
DBA/2J	3 or 10	Continuous	12d	4d	=	0	Tomie et al. (2013)
CBA × C57BL/6F1	30; sweetened	Continuous	8w	2, 3, or 4d	+ ^a	1.5h	Salimov and Salimova (1993)
	30; sweetened	Continuous	16w	2w	+	1.5h	Salimov, Salimova, Shvets, and Maisky (2000)
129X1/SvJ × C57BL/6N	16	Continuous	14w	2w	+	2d	Cowen, Schumann, Yagi, and Spanagel (2003)
	16	Continuous	24d	2w	+	1–2d	Cowen, Schroff, Gass, Sprengel, and Spanagel (2003)
C57BL/10SnY (AKM&A(4R))	16	Continuous	14w	3w	+	1d	Sanchis-Segura et al. (2006)
	30; sweetened	Continuous	15w	3d	+	1.5h	Salimov, Salimova, Ratkin, Shvets, and Maisky (1995)
129SvEvBrd/C57/B L6-Tyrc-Brd	16	Continuous	18d	2m	+	3d	Zghoul et al. (2007)
WSC-1 (8-way cross of various inbred strains)	6	Continuous	8w	2w	+	1d	Tambour, Brown, and Crabbe (2008)
HS/lbg (WSP/WSR)	10; sweetened	Continuous	1m	2w	=	0	Ford et al. (2011)
129 × 1/SvJ × CD1	8	Continuous	3m	2w	=	0	Molander et al. (2012)
129S2/Sv × C57BL/6J × (C57BL/6 × SJL)F2	8	Continuous	3m	2w	=	0	

^a In 50% of the mice, an ADE was present. d, w, and h indicate days, weeks, and hours, respectively. +, =, – indicate occurrence of an ADE, no ADE, and negative ADE, respectively.

ADE is seen in HAD rats (Rodd-Henricks, McKinzie, Murphy, et al., 2000) but not in AA rats (unpublished data) and sP rats (Serra et al., 2003). Alcohol-preferring P rats exhibit a robust ADE after only a single deprivation phase by increasing their alcohol consumption approximately 2-fold on the first re-exposure day (McKinzie et al., 1998), and the ADE is prolonged in P rats after repeated deprivations (Rodd-Henricks, McKinzie, Shaikh, et al., 2000). Hence, it appears that genetic susceptibility to high alcohol consumption and other associated behavioral traits (Roman et al., 2012) are not necessarily related to the manifestation of an ADE.

In summary, relapse-like behavior in rats is largely affected by the genetic make-up of an animal. Furthermore, data from alcohol-preferring rat lines and from our huge data base on individual drinking patterns in Wistar rats from our CIMH colony show that levels of baseline alcohol intake do not correlate with the robustness of the ADE, suggesting that baseline alcohol drinking behavior and relapse-like drinking behavior are controlled, at least in part, via different brain systems (Vengeliene, Bilbao, Molander, & Spanagel, 2008).

ADE in mice

Unlike in the rat, the mouse ADE model has not extensively been studied. This is surprising, if one considers that the ADE phenomenon had already been described in mice in 1993 (Salimov & Salimova, 1993), and that transgenic mice provide an excellent model system for identifying genetic and molecular factors involved in disease processes.

Thus, although transgenic mouse models provided crucial information for the understanding of the genetics and neurobiological pathways mediating many alcohol-related phenotypes (Bilbao, 2013a; Crabbe, Phillips, Harris, Arends, & Koob, 2006; Stacey et al., 2012), the genetic and molecular factors underlying the ADE are less studied. One potential reason for this lack of knowledge may be

that, since the first description of an ADE in mice 20 years ago, the very few studies on the onset and duration of an ADE in mice have produced an inconsistent outcome due to the wide range of procedural variability used (Table 1). In particular, in the 15 studies reporting ADE in mice, ethanol concentrations vary from 3 to 30%, continuous exposure or non-continuous exposure varies from 1 day to 16 weeks, and abstinence periods range from 2 days to 2 months. Regardless of those procedural differences, in most cases it is the strain used that is critical for the onset and duration of an ADE in mice. Perhaps the most valuable data come from the C57BL/6 substrains. Both the C57BL/6J and C57BL/6N mice are the most commonly studied and widely used inbred strains, and they provide the opportunity to test sophisticated time-specific and site-specific genetic models, which are often based on these genetic backgrounds (Bilbao, 2013a). C57BL/6J mice have been used to study alcohol-related phenotypes since it was discovered that this strain has a high alcohol-preference phenotype (McClearn & Rodgers, 1959). However, this strain does not show an ADE after a first deprivation period, a result confirmed by four different laboratories using different ranges of alcohol concentrations, time exposure, and deprivation periods (Camp et al., 2011; Khisti, Wolstenholme, Shelton, & Miles, 2006; Melendez, Middaugh, & Kalivas, 2006; Spanagel lab, unpublished data). It has been proposed that the high basal intake of these mice (approximately 10 g/kg/day) may explain the lack of ADE, due to a ceiling effect, similar to what has been reported in high alcohol-preferring rat lines. However, after repeated cycles of drinking and deprivation periods, a robust ADE can also ensue in C57BL/6J mice, but this very much depends on the length of initial deprivation (Melendez et al., 2006). In contrast to the J line, the generation of an initial ADE in the C57BL/6N sub-strain seems to depend more on the procedure used. Thus, while an 18-h daily exposure to a 10% alcohol solution during a period of 4 or 14 days leads to an increase in alcohol intake during the post-abstinence days following 4 days of deprivation (Khisti et al., 2006), a continuous exposure to alcohol, using the same

deprivation period, does not trigger an ADE in these mice (Tomie, Azogu, & Yu, 2013). Our lab has also characterized the ADE in this sub-strain, and has found that a continuous, long-term voluntary access to different concentrations of alcohol solutions (for at least 2 months), followed by a 2-week deprivation phase, leads to a robust ADE that can, however, only be observed during the first post-deprivation day (please see original investigation below).

In summary, relapse-like drinking is more inconsistent in mice as compared to rats. In several reports, many mice failed to exhibit an ADE and it appears that both procedural (duration and conditions of initial alcohol exposure and deprivation period) and genetic (strain) variables contribute to the occurrence of an ADE. In general, if there is an onset of an ADE in mice the duration is usually not longer than one day, which is different from the duration of an ADE observed in most rat experiments. Given the huge number of mouse strains used in basic and preclinical research, a future challenge is to use standardized protocols in an attempt to identify genotypic differences of transgenic mouse models that would potentially improve our understanding of the genetic factors underlying relapse behavior.

Long-term alcohol consumption with repeated deprivation phases in male Wistar rats – a model of compulsive drinking in a relapse situation

This animal model is designed to demonstrate compulsive drinking during a relapse situation – for an extensive discussion about compulsive behavior in rodents see the review article by Hopf and Lesscher in this issue. The main principles of this rat model are described in detail by Spanagel and Höltter (1999). In this model, Wistar rats from our own breeding colony at the CIMH are used, since they are the most likely to show an ADE, which is relatively long-lasting. Alcohol, unlike cocaine or heroin, is a weak reinforcer and as such requires long-term exposure to induce compulsiveness during a relapse situation. Such conditions might be difficult to achieve with relatively short instrumental training procedures. Therefore, voluntary long-term oral alcohol consumption is a prerequisite for development of compulsive drinking, i.e., in our experimental procedure rats must have free access to alcohol for at least 8 months (Spanagel, Höltter, Allingham, Landgraf, & Ziegängsberger, 1996). In addition, this access should be interrupted repeatedly with forced abstinence phases. At the end of this procedure, compulsive drinking during an ADE can be measured. For this purpose two procedures are used – a taste adulteration test

with quinine and monitoring of the circadian drinking rhythmicity. In the taste adulteration test the taste of alcohol solutions is altered with bitter quinine. Alternatively, animals could be offered a highly palatable sucrose solution instead of water (Spanagel et al., 1996; Wolffgramm & Heyne, 1995; see also Hopf & Lesscher, in this issue). An animal is expected to naturally choose a more palatable (or less aversive) fluid as a drinking source. Those animals that exhibit an ADE despite alcohol taste adulteration with quinine or competitive choice of a highly palatable fluid are classified as compulsive animals in this experimental setting. It was demonstrated that taste adulteration reduced post-abstinence drinking in Wistar rats after a short-term alcohol experience, suggesting at least partial control of the behavior at this stage. However, long-term chronic alcohol consumption repeatedly interrupted with deprivation phases was shown to lead to an animal's complete loss of control over drinking behavior, as the taste adulteration procedure no longer modified the ADE (Spanagel et al., 1996). This behavior resembles continuation of drug use despite clear evidence of overtly harmful consequences and neglect of alternative interests. Another sign of compulsive alcohol drinking is an alteration of the normal circadian drinking pattern (Spanagel, 2009). Circadian disturbances have been reported in human addicts (Danel & Touitou, 2004). In long-term drinking rats that were repeatedly deprived of alcohol for several weeks, re-exposure leads to increased drinking frequency and loss of diurnal drinking rhythmicity during the first post-abstinence days. These changes occur with consumption of more highly concentrated alcohol solutions, which presumably are chosen because the rats are trying to rapidly increase their blood alcohol concentrations (Vengeliene, Noori, & Spanagel, 2013). To sum up, our model of compulsive drinking during a relapse situation demonstrates the loss of predictability of the drinking behavior of an animal, measured as the failure to naturally choose a more palatable drinking fluid as well as loss of a typical circadian drinking pattern.

Original investigation on long-term alcohol consumption with repeated deprivation phases in C57BL/6N mice

Introduction

In the following, we will present our attempt to establish a model of compulsive drinking behavior during a relapse situation in C57BL/6N mice. Establishing such a model in mice has a potential value in behavioral neuroscience and reverse genetics (Spanagel,

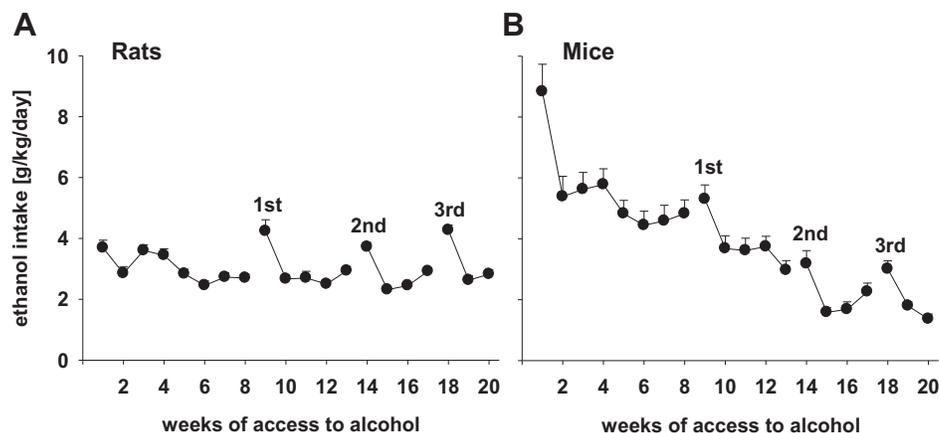


Fig. 2. An overview of a 20-week drinking course shown as ethanol intake (in g of total pure ethanol consumed per day per kg of body weight, g/kg/day) in (A) male Wistar rats (our own breeding colony at the CIMH) and (B) male C57BL/6N mice (Charles River, Sulzfeld, Germany). The figure shows the first 6 months of voluntary alcohol drinking, which included three 2-week deprivation phases (1st–3rd). Ethanol intake was calculated as the daily average across one week. Data are presented as means \pm S.E.M.

2013), because of the availability of a huge number of genetically modified mouse lines that could be tested in this model, thus contributing to our understanding of the mechanisms involved in the transition from controlled to compulsive alcohol use.

In order to establish the model of long-term alcohol consumption with repeated deprivation phases in mice, male C57BL/6N mice were subjected to the same procedures as mice in a long-term alcohol consumption model established for Wistar rats (see previous section). Hence, mice were given concurrent access to water and different alcohol solutions for one year, which was interrupted with repeated deprivation phases. For direct comparison with our rat model, we ran a rat group in parallel. The choice of C57BL/6N mice was based on our earlier studies, showing that these mice voluntarily consume considerable amounts of alcohol and exhibit a robust ADE.

Methods

Subjects

Thirty-four 2-month-old male Wistar rats and twenty-nine 2-month-old male C57BL/6N mice (Charles River, Sulzfeld, Germany) were used for the long-term alcohol consumption with repeated deprivation-phases procedure. All animals were housed individually in standard rat/mouse cages (Ehret, Emmendingen, Germany) under a 12-h artificial light–dark cycle (lights on at 7:00 AM). Room temperature and humidity were kept constant (temperature: 22 ± 1 °C; relative humidity: $55 \pm 5\%$). Standard laboratory rodent chow (Ssniff, Soest, Germany) and tap water were provided *ad libitum* throughout the experimental period. All experimental procedures were approved by the Committee on Animal Care and Use (Regierungspräsidium Karlsruhe), and carried out in accordance with the local Animal Welfare Act and the European Communities Council Directive of 24 November, 1986 (86/609/EEC).

Long-term alcohol consumption with repeated deprivation phases

After 2 weeks of habituation to the animal room, rats were given *ad libitum* access to tap water and to 5%, 10%, and 20% ethanol solutions (v/v) as well. C57BL/6N mice received *ad libitum* access to tap water and to 6% and 16% ethanol solutions (v/v).³ Alcohol drinking solutions were prepared from 96% ethanol (Sigma-Aldrich, Taufkirchen, Germany) and then diluted with tap water. Spillage and evaporation were minimized by the use of custom bottle caps. The positions of bottles were changed weekly to avoid location preferences.

The first 2-week deprivation period was introduced after 8 weeks of continuous alcohol access. After this deprivation period, all animals were given access to alcohol again and 8 more deprivation periods were introduced in a random manner, i.e., the durations of subsequent drinking and deprivation phases were irregular, i.e., approximately 2–5 weeks in order to prevent adaptive behavioral mechanisms. Total baseline ethanol intake (in grams of total pure ethanol consumed per day per kg of body weight, g/kg/day) was calculated as the daily average across the 7 measuring days. To determine the occurrence of an ADE, total ethanol before and after an abstinence phase was measured daily for 1 week. The long-term voluntary alcohol drinking procedure, including all deprivation phases, lasted a total of 1 year.

³ We decided to use a 3-bottle free-choice paradigm because of space limitations of a mouse standard cage to introduce 4 bottles. The concentrations – 6 and 16% ethanol solutions (v/v) – were used because we observed in multiple experimental settings that more highly concentrated ethanol solutions can yield a decrease in alcohol consumption in mice.

Pharmacological studies

Please note that the data presented in Fig. 4A, showing the effect of acamprosate, lamotrigine, GYKI52468, and Org25935 on ethanol intake during the ADE in Wistar rats was taken from our previously published studies (Sanchis-Segura et al., 2006; Spanagel, Vengeliene, et al., 2014; Vengeliene, Heidbreder, & Spanagel, 2007; Vengeliene, Leonardi-Essmann, Sommer, Marston, & Spanagel, 2010).

The pharmacological studies in mice followed the exact same treatment schedule as in the above-mentioned studies in rats. In particular, the pharmacological studies in mice were introduced at the end of the 6th and 7th alcohol deprivation periods. In order to study the effects of drug treatment, mice were divided into groups ($n = 9–10$) in such a way that the mean baseline total alcohol intake was approximately the same in each group. Baseline drinking was monitored daily for 1 week. After the last day of baseline measurement, the alcohol bottles were removed from the cages, leaving the animals with free access to food and water for 10–14 days. Thereafter, all animals were subjected to a total of five IP (intraperitoneal) injections (starting at 7:00 PM with 12-h intervals) of either vehicle, 200 mg/kg of acamprosate, 5 mg/kg of lamotrigine, 10 mg/kg of GYKI52468, or 6 mg/kg of Org25935 (for dosing see Lidö, Marston, Ericson, & Söderpalm, 2012; Sanchis-Segura et al., 2006; Vengeliene et al., 2007, 2010). Acamprosate (Lipha, France) was dissolved in isotonic saline; lamotrigine (generously provided by GSK, Verona, Italy), GYKI52468 (Tocris Cookson Inc., Bristol, UK), and Org25935 (Schering-Plough Research Institute, Newhouse, UK) were dissolved in a small amount of polyethylene glycol 400 (PEG 400, Sigma-Aldrich, Taufkirchen, Germany) and then diluted with water (*aqua ad iniectabilia*, Braun, Melsungen AG, Germany). All solutions were freshly prepared and injected as a volume of 10 mL/kg intraperitoneally (IP). Control experiments were performed following administration of the respective vehicle. The alcohol bottles were reintroduced after the second injection of either compound and the occurrence of an ADE was determined. Total ethanol (g/kg of body weight/day) and water intake (mL/kg of body weight/day) were measured daily for the subsequent week. Each mouse's body weight was recorded 24 h before the first injection and 12 h after the last injection.

Statistics

Data derived from ADE drinking were analyzed using a one-way analysis of variance (ANOVA) with repeated measures (factor – day). A one-way ANOVA was used for the analysis of pharmacological studies (factor – treatment group). Whenever significant differences were found, *post hoc* Student Newman–Keuls tests were performed. The chosen level of significance was $p < 0.05$.

Results and discussion

During this study, we discovered that there are several differences in the behavior between Wistar rats and C57BL/6N mice. One obvious difference was a clear drop in baseline alcohol intake over the time-course of the experiment in C57BL/6N mice – this is an observation we made in our laboratory multiple times and, although not systematically examined, usually voluntary alcohol drinking starts to decline after approximately 3 months of voluntary home cage drinking. After the initial acquisition period, lasting for the first few alcohol-exposure weeks, rats maintained relatively stable baseline alcohol consumption levels (Fig. 2A). In contrast, ethanol consumption by mice continued dropping after these drinking acquisition weeks (Fig. 2B). Within approximately 15 weeks of alcohol access the average intake was so low that the majority of the mice most likely did not consume enough alcohol to reach pharmacologically significant blood alcohol levels. The level

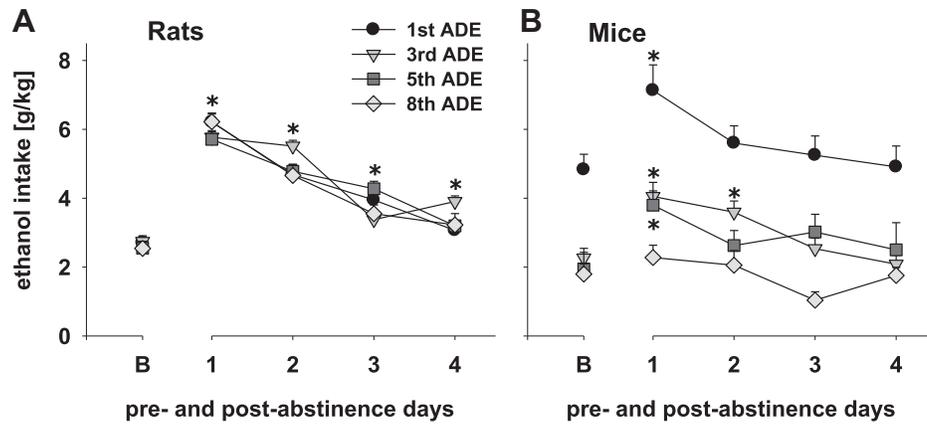


Fig. 3. Total ethanol intake (in g/kg of body weight per day) before and after an alcohol-deprivation period of 2 weeks in (A) male Wistar rats (our own breeding colony at the CIMH) and (B) male C57BL/6N mice (Charles River, Sulzfeld, Germany). The figure shows the first (1st ADE), the third (3rd ADE), the fifth (5th ADE) and the eighth (8th ADE) abstinence cycle. The measurement of the last week of ethanol intake is given as pre-abstinence baseline drinking – “B”. The entire alcohol drinking procedure, including all 8 deprivation phases, lasted one year. Data are presented as means \pm S.E.M. * Indicates significant differences to baseline drinking, $p < 0.05$.

of baseline ethanol intake remained as low as 2 g/kg per day for the remainder of the experiment. This could be caused by either changes in pharmacokinetics of alcohol with age or a reduced role of reward-dependent mechanisms in aged mice, as demonstrated by Wang, Liu, Harvey-White, Zimmer, and Kunos (2003) (see also Bilbao, 2013b).

Another difference between Wistar rats and C57BL/6N mice was a very short duration of the ADE. In mice the ADE lasted for only 1 day, while in rats increased post-abstinence drinking lasted for 3–4 days. One further feature which discriminated relapse-like drinking in mice from rats was its obvious lack of stability. In Wistar rats, post-abstinence drinking did not change over the time-course of the experiment (Fig. 3A); however, in mice this behavior completely disappeared at around the 8th deprivation phase (Fig. 3B).

Our relapse model in rats was validated pharmacologically using acamprosate (Spanagel et al., 1996; Spanagel, Vengeliene, et al., 2014), naltrexone (Hölter & Spanagel, 1999), and nalmefene (Vengeliene, not published). These three abstinence-promoting drugs are used in the treatment of alcohol-dependent patients (Litten et al., 2012; Mann, Bladström, Torup, Gual, & van den Brink, 2013; Spanagel & Kiefer, 2008). This demonstrated an excellent

predictive validity of our model for the human condition. Thereafter, this model has become widely used in examining the efficacy of putative pharmacological agents for preventing alcohol relapse (e.g., Sanchis-Segura et al., 2006; Spanagel & Vengeliene, 2013; Vengeliene et al., 2007, 2010). In order to pharmacologically validate the relapse model in mice, we used several compounds known to reduce post-abstinence drinking in humans and rats: acamprosate, lamotrigine, GYKI52468, and Org25935. As was mentioned above, acamprosate is approved for clinical use as a relapse-preventing drug, and it is often used as a reference compound in rats (e.g., Spanagel, Vengeliene, et al., 2014; Vengeliene et al., 2010). The anti-convulsant drug lamotrigine is used to treat alcohol-dependent patients with either comorbid bipolar disorder or schizophrenia (Kalyoncu et al., 2005; Rubio, López-Muñoz, & Alamo, 2006). This drug not only improves the mood of patients but also decreases alcohol craving and consumption. Lamotrigine was also extremely effective in reducing the ADE in our rat model (Vengeliene et al., 2007). Both the AMPA receptor antagonist GYKI52468 and the glycine transporter GlyT1 blocker Org25935 are not used in humans but were very potent in reducing relapse-like drinking in rats (Sanchis-Segura et al., 2006; Vengeliene et al.,

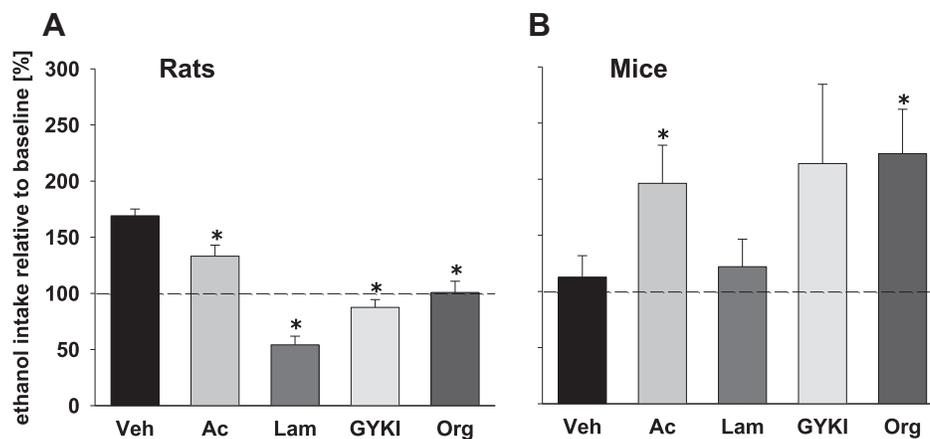


Fig. 4. Total ethanol intake after an alcohol deprivation period of 2 weeks shown as an average intake of the first four post-abstinence drinking days in (A) male Wistar rats (our own breeding colony at the CIMH) and (B) male C57BL/6N mice (Charles River, Sulzfeld, Germany). The percentage of each rat's total daily ethanol intake during post-abstinence drinking days was calculated with respect to baseline drinking (dashed line). All rats were injected with either vehicle (Veh) or one of the four drugs: 200 mg/kg of acamprosate (Ac), 5 mg/kg of lamotrigine (Lam), 10 mg/kg of GYKI52468 (GYKI), or 6 mg/kg of Org25935 (Org). Each animal received a total of five IP injections of one of the compounds. The alcohol bottles were reintroduced after the second injection. Data are presented as means \pm S.E.M. * Indicates significant differences from the control vehicle group, $p < 0.05$.

2010) (Fig. 4A). All drugs were administered repeatedly during the onset of relapse.⁴ To our surprise, the study demonstrated that all four compounds either tended to increase or significantly increased the amount of alcohol consumed during relapse-like drinking in mice as compared to the vehicle-treated animals (Fig. 4B). An explanation for this opposite reaction to drug treatment in mice is presently lacking but certainly needs independent replication. In summary, long-term alcohol consumption with repeated deprivation phases in C57BL/6N mice does not lead to an addiction-like behavior over time. Rather, these mice show reduced alcohol intake, until reaching only pharmacologically insignificant levels of blood alcohol concentration within approximately 3 months. Furthermore, by the end of the experimental year, relapse-like drinking in mice was no longer present. Finally, the model could not be validated pharmacologically, since neither acamprosate nor lamotrigine could reduce post-abstinence drinking.

Conclusions

The ADE is considered to be an animal model of relapse because of its excellent face validity. In animals, relapse-like behavior is largely affected by the genetic make-up of an animal; some animals will show more pronounced relapse-like behavior than others will. It is not clear which other factors are responsible for the variability of this behavior, but there seems to be no correlation between levels of baseline alcohol intake and the onset, duration, and robustness of the ADE. With repeated deprivation phases, some rat strains might develop a compulsive drinking behavior during a relapse situation, characterized by insensitivity to taste adulteration with quinine, loss of circadian drinking patterns during an ADE, and a shift toward consuming highly concentrated alcohol solutions to rapidly build up blood alcohol concentrations and produce intoxication. Thus, over time, chronically drinking Wistar rats from our own breeding colony lost control over alcohol consumption. Some mouse strains also exhibit an ADE but this is usually of shorter duration than in rats. However, compulsive drinking in male C57BL/6N mice during a relapse situation could not be demonstrated. During long-term alcohol consumption, C57BL/6N mice showed a decline in alcohol intake, and with repeated deprivation phases, the ADE faded away. This is an important observation, which is very similar to studies on cocaine addiction in mice. Although in rats, typical criteria of cocaine addiction could be demonstrated after long-term intravenous self-administration by several laboratories (Cannella et al., 2013; Chen et al., 2013; Deroche-Gamonet, Belin, & Piazza, 2004; Vanderschuren & Everitt, 2004), it has so far not been possible to establish, despite major efforts, a similar mouse model. Given that anti-relapse compounds produce reliable effects in rats but not in mice, one has to conclude that the rat is probably a better model system to study alcohol relapse and addictive behavior. Certainly, other mouse strains should be tested using this and other animal models/paradigms to confirm this statement.

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⁴ We always use either a sub-chronic or chronic treatment schedule (i.e., by applying the drug systemically via osmotic mini-pumps (Hölter, Danysz, & Spanagel, 1996, 2000)) to provide better translation of our pharmacological interventions in rats to the clinical treatment situation and to test for tolerance phenomena.

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