Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 84 (2006) 524–534
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Rapid ethanol tolerance mediated by adaptations in acute tolerance in inbred mouse strains

Richard A. Radcliffe [a,b,*], Kirsten L. Floyd [a], Michael J. Lee [b]

[a] *Department of Pharmaceutical Sciences, University of Colorado at Denver and Health Sciences Center, 4200 E. Ninth Ave., Campus Box C-238, Denver, CO 80262, United States*
[b] *Institute for Behavioral Genetics, University of Colorado, 447 UCB, Boulder, CO 80309, United States*

Received 24 April 2006; received in revised form 27 June 2006; accepted 30 June 2006
Available online 8 August 2006

Abstract

It has been postulated that decreased acute sensitivity to ethanol is an important genetically-mediated risk factor for the development of alcoholism. Previous work in mice and rats has indicated that ethanol sensitivity can be reduced in a genotype-dependent manner by a single dose of ethanol 24 h prior to testing, so-called 'rapid' tolerance. The current studies were undertaken to determine if the observed rapid tolerance was mediated by alterations in initial sensitivity or acute functional tolerance (AFT), the two primary components of acute sensitivity. Separate groups of C57BL/6, DBA/2, ILS, and ISS inbred mouse strains were administered a single pretreatment dose of saline or ethanol (5 g/kg). The original and modified versions of the loss of righting reflex test, ethanol-induced hypothermia, and ataxia on a stationary dowel rod were tested 24 h later. Dependent on the test and strain, varying degrees of rapid tolerance were observed; a pronounced sensitization was detected in one case. There was a concomitant increase in the rate and/or magnitude of AFT with little change in initial sensitivity suggesting that rapid tolerance was mediated primarily by alterations in AFT. This conclusion may have implications for the contribution of acute sensitivity to human alcoholism.

Keywords: C57BL/6; DBA/2; Genetics; Ethanol; Inbred mice; Tolerance; Level of response; Hypothermia; Ataxia; Righting reflex

1. Introduction

Human studies have confirmed an important contribution of genetics to the risk for alcoholism (alcohol abuse and dependence; American Psychiatric Association, 1994), yet progress in gene identification has been limited (Dick and Foroud, 2003; Enoch and Goldman, 2001; Glazier et al., 2002; Schuckit et al., 2004b). This is primarily because alcoholism is a *complex trait*, specifically meaning that trait variance derives from multiple genes, diverse environmental influences, gene–environment interactions, and differing combinations of psychiatric or other risk factors (Almasy, 2003; Li, 2000; McGue, 1994; Schuckit, 2002). All of these issues complicate the localization of individual alcoholism genes, each of which generally contributes only a small portion of the variance. Human and model organism geneticists have had somewhat more success through analysis of endophenotypes – simpler traits that contribute to the overall phenotype – rather than examining alcoholism directly. One such endophenotype has been referred to as "low level of response" with which individuals who have been found to be innately less sensitive to an acute alcohol challenge as measured with a variety of different tasks are at increased risk for developing alcoholism (Schuckit, 1998, 2002; see also: Conrod et al., 2001; King et al., 2002; Morzorati et al., 2002; Newlin and Thomson, 1999). Variation in acute alcohol sensitivity is almost certainly not the sole genetic determinant of alcoholism risk; rather it is probably an important interacting genetic factor in the context of other physiological, psychological, or environmental risk factors. Thus, the study of the genetic and molecular basis of alcohol sensitivity is a rational strategy in the pursuit of a basic understanding of alcoholism and for the development of new or novel therapies for its treatment.

Measurements of acute responses to alcohol, themselves complex traits, can be obscured by an individual's innate level

* Corresponding author. Department of Pharmaceutical Sciences, University of Colorado at Denver and Health Sciences Center, 4200 E. Ninth Ave., Campus Box C-238, Denver, CO 80262, United States. Tel.: +1 303 315 1597; fax: +1 303 315 6281.
E-mail address: Richard.Radcliffe@UCHSC.edu (R.A. Radcliffe).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.06.018

of sensitivity and by potentially opposing neuroadaptive processes that occur on both the ascending and descending limbs of alcohol distribution (Newlin and Thomson, 1990). In the context of acute responses, *initial sensitivity* can be considered to be the blood ethanol concentration (BEC) at the time at which a specific behavioral or other endpoint is achieved and ideally measured on the ascending limb of distribution. This can be conceptualized as the response at a given blood ethanol concentration in the absolute absence of acute adaptation of any kind. But because many responses are experimentally difficult to accurately evaluate immediately after alcohol administration, the measure of initial sensitivity is often confounded by acute functional tolerance (AFT; Mellanby, 1919; Newlin and Thomson, 1990). AFT is an acute pharmacodynamic adaptation that counteracts the cellular disturbance created by the presence of alcohol. For behaviors or other neuronal responses that do show AFT, true initial sensitivity is difficult to determine, at least within the limits of experimental error, because neurons start adapting virtually immediately after they come into contact with alcohol; this would represent the early stages of AFT (Goldstein, 1989; Palmer et al., 1985; Radlow, 1994). Thus, 'sensitivity' for most alcohol responses is typically comprised of the combined effects of true initial sensitivity and AFT, with the contributions of each dependent on the time course of the response and AFT kinetics, and modified by genetic and environmental factors. Note that it is recognized that acute sensitization is also possible; indeed, acute sensitization was observed in the current study. However, most studies of acute adaptive processes with regard to alcohol have reported AFT and thus the literature generally has focused on AFT as the predominant neuroadaptive effect.

AFT tends to dissipate fairly soon after alcohol has been cleared (Erwin et al., 2000), but more enduring forms of tolerance are just beginning to emerge during that first exposure to alcohol. *Rapid* tolerance is evident up to at least 24 h after a single administration of alcohol while *chronic* tolerance is associated with continuous or multiple dosings over an extended period lasting from days to years (Crabbe et al., 1979; Kalant et al., 1971; Khanna et al., 1996; Le et al., 1979; Melchior and Tabakoff, 1984). Rapid tolerance may represent the initial stages of chronic tolerance, but the relation between rapid or chronic tolerance and AFT has not been completely resolved (Kalant et al., 1971; Khanna et al., 1991; Tabakoff et al., 1982; Wu et al., 2001).

Genotype-dependent effects have been reported for all three types of tolerance for a variety of responses (e.g., Erwin and Deitrich, 1996; Erwin et al., 1992; Radcliffe et al., 2005; Rustay and Crabbe, 2004) and recently a robust rapid tolerance was observed for duration of the loss of righting reflex (LORR) in the Inbred Long Sleep (ILS), but with no effect in the Inbred Short Sleep (ISS) mouse strains; in fact, the ISS showed signs of sensitization 24 h after an initial exposure to alcohol (Radcliffe et al., 2005). However, the LORR test used in that experiment was the same procedure as that used for the selective breeding of the LS and SS (McClearn and Kakihana, 1981), and not well suited for the determination of initial sensitivity for onset of LORR or AFT; i.e., the difference between BEC at onset and BEC at recovery of LORR. Ponomarev and Crabbe (2002) recently developed a modified procedure for the determination of LORR with which it is possible to obtain a more accurate blood alcohol sample at the loss of the righting reflex thus providing a method to better estimate both initial sensitivity and AFT. The intent of the studies presented in this report was to take advantage of this modified version of the LORR test to examine the effects of rapid tolerance on initial sensitivity and AFT following acute alcohol administration. Experiments were conducted using the ILS and ISS, and also the C57BL/6 and DBA/2, mouse strains that have been widely used in alcohol research (Belknap and Atkins, 2001; Crabbe et al., 1994a; Crawley et al., 1997; Phillips et al., 1996). Experiments were performed using the original and modified methods of testing for duration of LORR 24 h following an alcohol pretreatment. In addition, a lower-dose measure of alcohol sensitivity, ataxia on a stationary dowel rod (Gallaher et al., 1982), was examined for the effect of rapid tolerance on AFT in the four strains.

2. Methods

2.1. Animals

C57BL/6 (B6), DBA/2 (D2), ILS, and ISS mice were obtained from the Institute for Behavioral Genetics, University of Colorado (Boulder, CO). All experiments were conducted on male animals ranging from 70 to 90 days of age at the time of testing. From the time they were weaned, mice were always group housed, usually 5 to a cage, sometimes 4. Animals were maintained in a constant temperature (22–23 °C), humidity (20–24%), and light (12L/12D) environment. All experiments were conducted at the University of Colorado at Denver and Health Sciences Center (UCDHSC). The procedures described in this report have been established to ensure the absolute highest level of humane care and use of the animals according to the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and have been reviewed and approved by the UCDHSC IACUC.

2.2. Rapid tolerance

Rapid tolerance is defined as occurring within 24 h of a single alcohol administration, but at some point after the dose has completely cleared (Crabbe et al., 1979). Animals were administered a single intraperitoneal (i.p.) injection of saline vehicle or alcohol (ethanol; 16% w/v in saline) on the first day; this is referred to as the pretreatment dose. For any given experiment, 2 or 3 mice in a cage of 5 were pretreated with saline; the remaining 2 or 3 animals were pretreated with alcohol. Cage-mates were from at least two separate litters. The pretreatment dose of 5 g/kg was selected because it was found to be not overtly toxic and it elicited a robust rapid tolerance compared to other doses (Radcliffe et al., 2005). Tolerance was then assessed 24 h following the pretreatment dose with the use of the behavioral tasks described below. Rapid tolerance was defined as a decrease in the measure of alcohol sensitivity in the

alcohol-pretreated group in comparison to the saline-pretreated group. Mice were tested only one time; i.e., independent groups of mice were used for each of the behavioral tasks. The animals were not behaviorally tested on day 1. They were simply administered the pretreatment dose and placed back into their home cage with their original cage-mates. The pretreatment procedure was conducted in this way to minimize associative learning that might contribute to tolerance, although it was probably not possible to completely eliminate associative learning.

2.3. Classic loss of righting reflex test

The 'classic' duration of the loss of righting reflex (LORR) and the blood ethanol concentration at regain of righting reflex (BECRR) were determined as previously described (Radcliffe et al., 2005). Animals were placed on their back in a V-shaped trough following i.p. alcohol administration and the time at which they could no longer right themselves was recorded. LORR was the elapsed time from this point until the time at which they could right themselves at least 3 times within a 1 min span. BECRR was defined as the mean BEC from two retro-orbital blood samples drawn at this time. The LORR test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS). Different test doses were used among the strains in an attempt to deliver equipotent duration of LORR doses (see below for discussion of the rationale for this approach). A second experiment was conducted in which a retro-orbital blood sample was taken after the loss of the righting reflex (BECLR; initial sensitivity) as well as at regain. An increase in BECRR from BECLR was interpreted as development of AFT which is quantitatively expressed as the difference

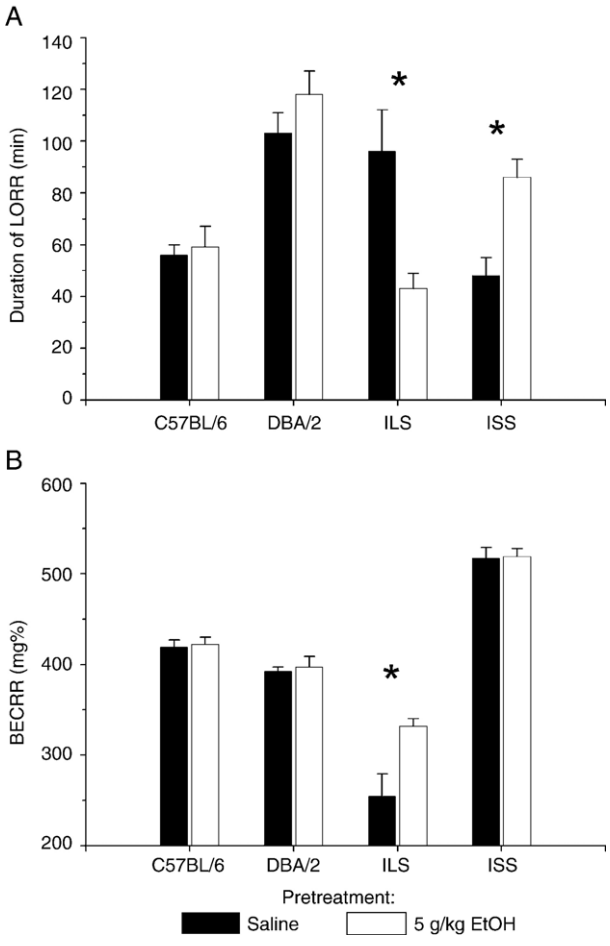

Fig. 1. The classic LORR test with one blood-draw in B6, D2, ILS, and ISS mice 24 h following an acute alcohol pretreatment. Mice were administered 5 g/kg on day 1 and then tested for duration of LORR (A) and BECRR (B) on day 2. Day 2 test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS). Asterisks indicate significant within-strain effect of pretreatment (*$p<0.01$); $n=8$–10 per group.

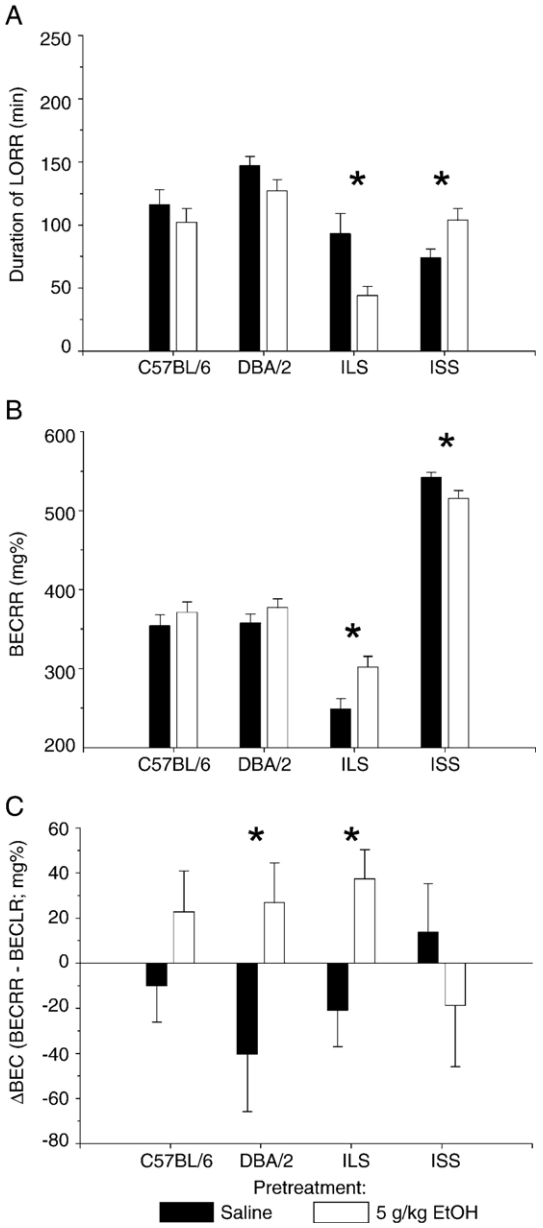

Fig. 2. The classic LORR test with two blood-draws in B6, D2, ILS, and ISS mice 24 h following an acute alcohol pretreatment. Mice were administered 5 g/kg on day 1 and then tested for duration of LORR (A), BECRR (B), and ΔBEC (C) on day 2 using the classic method, but with two blood-draws (BECLR and BECRR). Day 2 test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS). Asterisks indicate significant within-strain effect of pretreatment (*$p<0.01$); $n=10$–11 per group.

Table 1
One sample *t* statistic for test of ΔBEC vs. zero in the two blood-draw classic LORR test

Strain	Pretreatment	*t*
C57BL/6	Saline	−0.65
	5 g/kg EtOH	1.24
DBA/2	Saline	−1.58
	5 g/kg EtOH	1.52
ILS	Saline	−1.30
	5 g/kg EtOH	2.88 *
ISS	Saline	0.63
	5 g/kg EtOH	−0.69

* Significantly different than zero, $p<0.05$.

between BECRR and BECLR (ΔBEC). BEC values were determined by spectrophotometry with the use of a reliable enzyme assay (Lundquist, 1959). Values for LORR duration are expressed in minutes; BECRR and BECLR are expressed as mg alcohol per dl blood (mg%). Body temperatures were measured during the course of LORR testing (second experiment only) with the use of a rectal probe inserted approximately 1 cm into the rectum. Temperatures were obtained just prior to the administration of alcohol (time 0) and at 30, 60, 90, and 120 min after alcohol administration.

2.4. Modified loss of righting reflex test

A 'modified' version of the classic LORR test was recently introduced to better estimate parameters of AFT (Ponomarev and Crabbe, 2002). Immediately after alcohol injection, the mouse was placed in a small, closed Plexiglas cylinder that was rotated 90° every 2–3 s. Test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS). Different test doses were used among the strains in an attempt to deliver equipotent LORR doses (see below for discussion of the rationale for this approach). Loss of righting was defined as the time at which the mouse remained supine for at least 5 s; a retro-orbital blood sample was drawn at this point for determination of BEC at the loss of righting reflex (BECLR; initial sensitivity). With this procedure, determination of loss of righting can be accomplished much more quickly than with the use of the V-shaped trough and therefore a much more accurate measure of initial sensitivity was obtained (Ponomarev and Crabbe, 2002). The animals were tested for recovery of LORR every 3–6 min

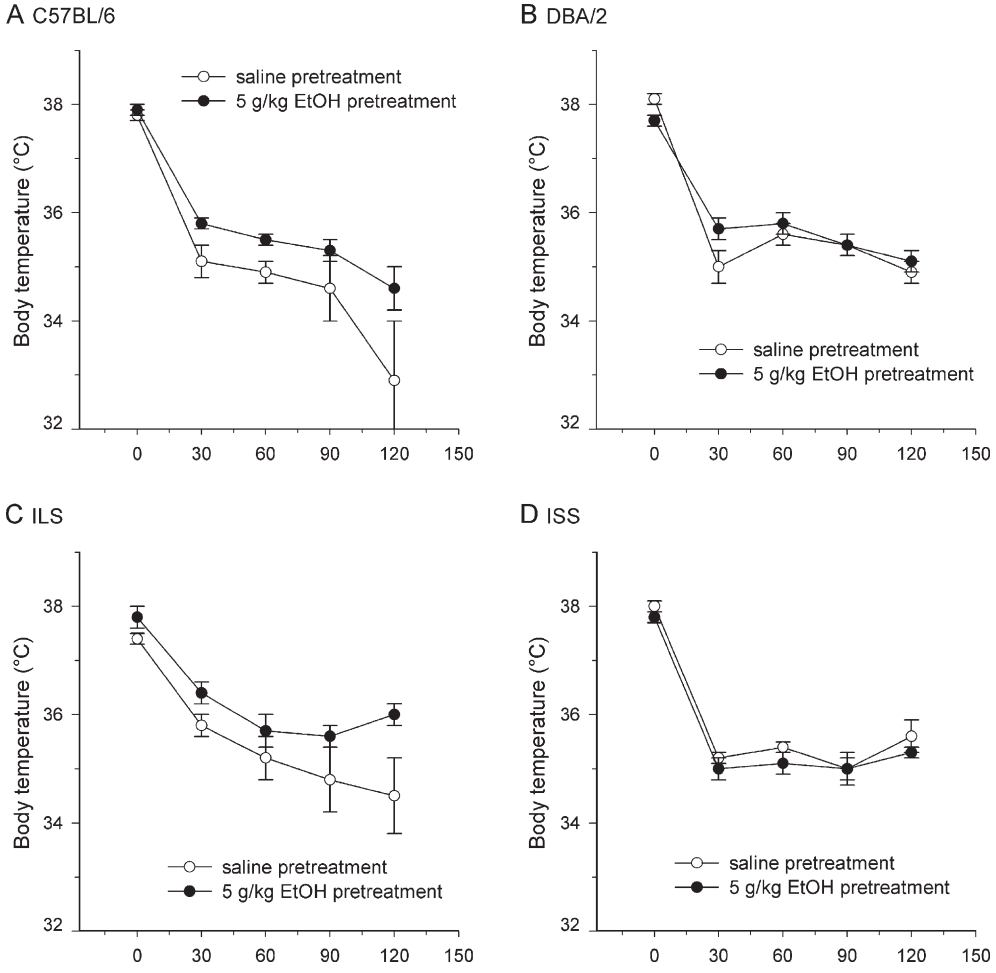

Fig. 3. Alcohol-induced hypothermia in B6 (A), D2 (B), ILS (C), and ISS (D) mice 24 h following an acute alcohol pretreatment. Mice were administered 5 g/kg on day 1 and rectal temperatures were measured during LORR testing on day 2 (classic method with two blood-draws; mice are the same as those used for experiment shown in Fig. 2). Day 2 test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS); $n=10–13$ per group. Within-strain two-way ANOVA (time-by-pretreatment): significant main effect of time in all 4 strains ($p<0.001$); significant main effect of pretreatment in B6 only ($p<0.05$); significant interaction effect in D2 only ($p<0.05$).

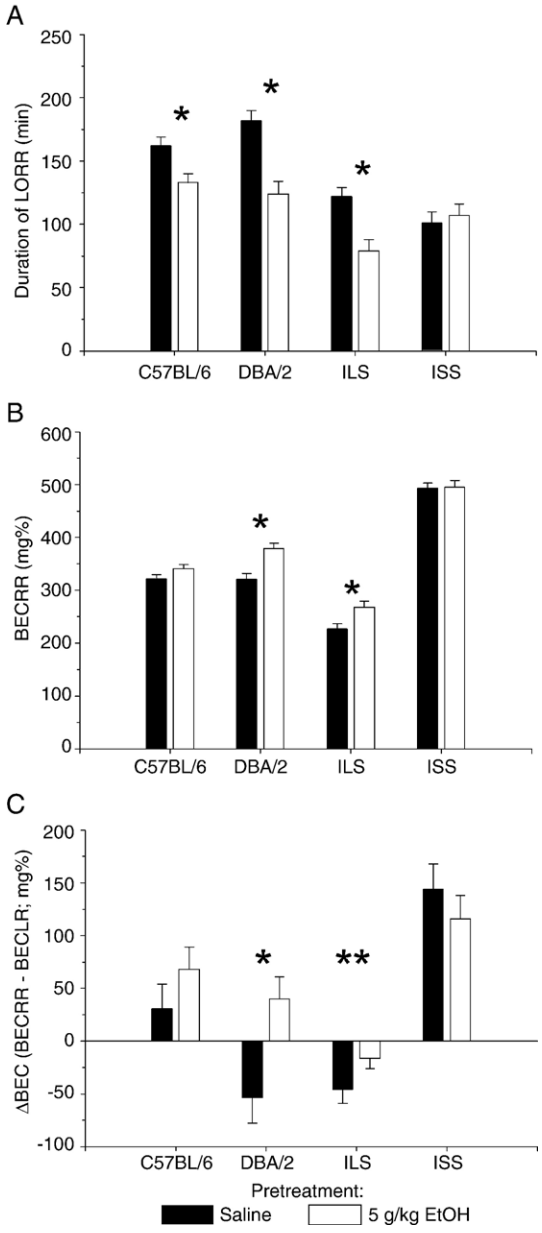

Fig. 4. The modified LORR test in B6, D2, ILS, and ISS mice 24 h following an acute alcohol pretreatment. Mice were administered 5 g/kg on day 1 and then tested for duration of LORR (A), BECRR (B), and ΔBEC (C) on day 2 using the modified method with two blood-draws (BECLR and BECRR). Day 2 test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS). Asterisks indicate significant within-strain effect of pretreatment (*$p<0.01$; **$p<0.05$); $n=10$–13 per group.

thereafter. A second blood sample was drawn (BECRR) when the animal was able to right itself within a 5 s period after being placed in a supine position or could not be placed on their backs after 8 successive 90° turns of the cylinder. Duration of LORR was defined as the elapsed time between the loss and regain of the righting reflex. An increase in BECRR from BECLR was interpreted as development of AFT which is quantitatively expressed as the difference between BECRR and BECLR (ΔBEC). BEC values were determined by spectrophotometry with the use of a reliable enzyme assay (Lundquist, 1959). Body temperatures were measured during the course of LORR testing as described above.

2.5. Acute functional tolerance on the stationary dowel rod

AFT on the stationary dowel rod was tested as previously described (Radcliffe et al., 1998). Animals were first administered 2 g/kg, i.p. (12% w/v). Within 2–4 min, the mouse typically was unable to remain on a horizontal wooden dowel rod (1.3 cm diameter) elevated 0.6 m above a floor covered with mouse bedding. The mouse was tested approximately every 5–10 min until it regained the ability to balance on the dowel for at least 1 min. At this time a retro-orbital blood sample was drawn for the determination of the blood ethanol concentration (BEC1). A second dose was administered and the procedure was repeated (1.75 g/kg, i.p., 12% w/v). When the mouse again recovered, a second blood sample was drawn (BEC2). AFT was defined as the quantitative difference between BEC2 and BEC1 (ΔBEC). BEC values were determined by spectrophotometry with the use of a reliable enzyme assay (Lundquist, 1959).

2.6. Data analysis

The mouse strains used for this study differ considerably in their LORR response, especially the ILS and ISS. An attempt was made to deliver equipotent doses (for duration of LORR) across the strains. The reason for using equipotent doses was to create a situation in which the strains had an equal opportunity to show AFT since AFT is a time-dependent process (Radlow, 1994). As can be seen in Results, this approach did not work as well as was desired. For this reason, only within-genotype analyses were conducted for LORR and BECRR. The same doses were used across all strains for the stationary dowel rod test of ataxia; therefore, primary analyses were conducted across strains. The specific statistical tests used for each of the experiments are described in Results and/or in the figure legends, and the number of animals used is shown in the figure legends. Values shown in figures and tables represent mean±S.E.M.

3. Results

3.1. Experiment 1: classic LORR test, single blood draw

Fig. 1 shows the effects of an alcohol vs. saline pretreatment on the classic LORR test. Notice that compared to the ISS, the ILS show a nearly two-fold longer duration of LORR and BECRR is almost half after a saline pretreatment despite

Table 2
One sample t statistic for test of ΔBEC vs. zero in the modified LORR test

Strain	Pretreatment	t
C57BL/6	Saline	1.32
	5 g/kg EtOH	3.32**
DBA/2	Saline	−2.24*
	5 g/kg EtOH	1.91
ILS	Saline	−3.48**
	5 g/kg EtOH	−1.94
ISS	Saline	5.47**
	5 g/kg EtOH	5.24**

* Significantly different than zero, $p<0.05$.
** Significantly different than zero, $p<0.01$.

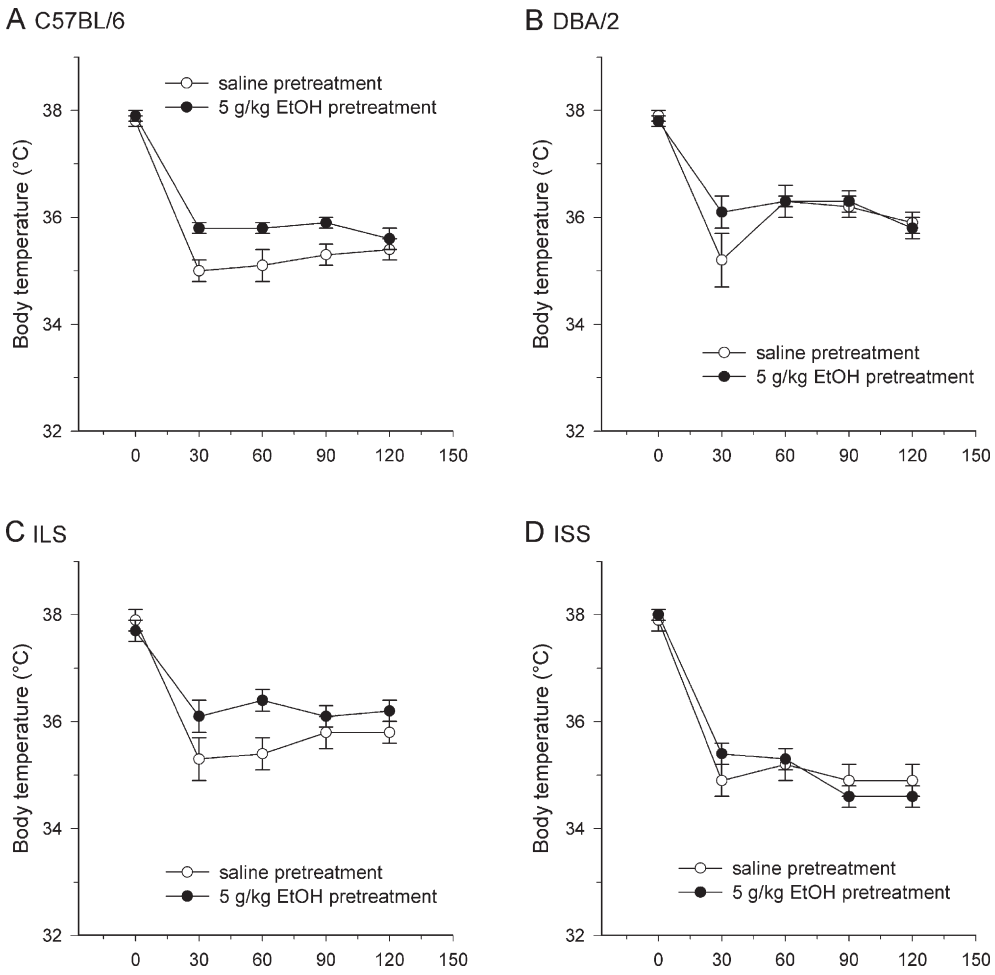

Fig. 5. Alcohol-induced hypothermia in B6 (A), D2 (B), ILS (C), and ISS (D) mice 24 h following an acute alcohol pretreatment. Mice were administered 5 g/kg on day 1 and rectal temperatures were measured during LORR testing on day 2 (modified method; mice are the same as those used for experiment shown in Fig. 4). Day 2 test doses were 4.1 g/kg (B6 and D2), 2.8 g/kg (ILS), and 5.2 g/kg (ISS); $n=10–13$ per group. Within-strain two-way ANOVA (time-by-pretreatment): significant main effects of time in all 4 strains ($p<0.001$); significant main effect of pretreatment in B6 only ($P<0.05$); interaction effect in ILS only ($p<0.05$).

receiving approximately half the test dose. This is a long-standing, oft repeated observation, but still remarkable. In the ILS, alcohol pretreatment caused a significant decrease in duration of LORR; i.e., tolerance. The opposite was observed in the ISS: this strain became sensitized. As expected, a concomitant increase in BECRR was observed in the ILS, indicative of pharmacodynamic rather than pharmacokinetic tolerance. BECRR in the ISS, however, was unaffected. Alcohol pretreatment had no effect on duration of LORR or BECRR in either the B6 or D2 strain.

3.2. Experiment 2: classic LORR test, two blood draws

Duration of LORR, BECRR, and ΔBEC results obtained using the classic test with two blood-draws are shown in Fig. 2. The second blood-draw was taken at the loss of the righting reflex (BECLR) and is an estimate of initial sensitivity. Alcohol pretreatment caused a significant decrease in LORR and a concomitant increase in BECRR in the ILS, similar to the classic test with one blood-draw. The ISS showed a significant increase in duration of LORR, also similar to the single blood-draw classic test. In this case, however, there was also a significant decrease in BECRR for the ISS. The alcohol pretreatment caused a decrease in the duration of LORR with a concomitant increase in BECRR in the B6 and D2, but none of the effects were significant.

Each of the ΔBEC values (Fig. 2C) was first evaluated to determine if it was significantly different from zero. After saline pretreatment, the B6, D2, and ILS showed negative ΔBEC values after saline pretreatment that were not significantly different from zero (Table 1). These three strains all had positive ΔBEC scores after alcohol pretreatment, indicative of the acquisition of AFT (i.e., BECRR greater than BECLR). This value was significantly different from zero only in the ILS. ΔBEC was not significantly different from zero after either saline or alcohol in the ISS.

The ΔBEC scores were next tested for a significant effect of alcohol pretreatment within each strain. Alcohol pretreatment caused a significant increase in ΔBEC over saline values for the D2 and ILS strains (Fig. 2C). A trend in that direction was observed in the B6. ΔBEC was non-significantly decreased following alcohol pretreatment in the ISS.

Fig. 3 shows alcohol-induced hypothermia measured during the two blood-draw classic LORR test. Two-way ANOVA was conducted (within-strain) to determine effects of pretreatment

Table 3
Latency to onset of LORR and BEC at the loss of the righting reflex (BECLR) in the classic and modified LORR tests

Strain	Pretreatment	Classic test		Modified test	
		Latency to onset (s)	BECLR (mg%)	Latency to onset (s)	BECLR (mg%)
C57BL/6	Saline	62±2 *	365±15 *	48±3	290±22
	5 g/kg EtOH	68±3	348±20	45±4	271±19
DBA/2	Saline	62±4 *	397±31	48±3	375±24
	5 g/kg EtOH	62±3	350±24	55±3	339±16
ILS	Saline	69±6	269±13	69±9	281±9
	5 g/kg EtOH	88±13	264±18	74±4	284±11
ISS	Saline	78±3 *	529±22 *	58±8	364±24
	5 g/kg EtOH	84±4	534±19	59±5	379±19

* Significant main effect of testing method, within-strain ($p<0.01$).

and time. Hypothermia was attenuated after alcohol pretreatment in the B6, D2 (at 30 min only), and ILS with a significant main effect of pretreatment in the B6 ($p<0.05$) and a significant interaction in the D2 ($p<0.05$); the pretreatment effect approached significance in the ILS ($p=0.10$). There was no effect of alcohol pretreatment in the ISS. A significant main effect of time was detected in all strains resulting primarily from the large difference between rectal temperature at time zero and all subsequent time points.

3.3. Experiment 3: modified LORR test

Fig. 4 illustrates the results of alcohol pretreatment using the modified LORR test. As with the classic test (Figs. 1 and 2), alcohol pretreatment caused a significant decrease in LORR duration in the ILS; however, there was no effect in the ISS (Fig. 4A). Similar to the two blood-draw classic test, both the B6 and D2 were found to have a decreased duration of LORR 24 h following alcohol pretreatment and now the difference was significant. BECRR was a reflection of LORR duration in each of the strains: increased in B6, D2, and ILS, with no change in ISS (Fig. 4B). The BECRR difference in the B6 was not statistically significant.

Table 4
BEC1 and BEC2 for the stationary dowel test of ataxia

Strain	Pretreatment	BEC1 (first regain of balance; mg%)	BEC2 (second regain of balance; mg%) *
C57BL/6	Saline	198±6	260±13 **
	5 g/kg EtOH	209±5	265±15 **
DBA/2	Saline	204±9	282±11 **
	5 g/kg EtOH	220±6	293±12 **
ILS	Saline	186±12	198±19
	5 g/kg EtOH	204±12	254±9 **, †
ISS	Saline	205±11	220±15
	5 g/kg EtOH	194±6	225±6 **

* Significant strain effect ($p<0.001$) and pretreatment effect ($p<0.05$) for BEC2; no significant interaction (strain-by-pretreatment two-way ANOVA).
** Significant difference between BEC1 and BEC2, $p<0.01$ (within strain and treatment group).
† Significant difference between saline and alcohol pretreatment for BEC2, $p<0.05$ (within strain).

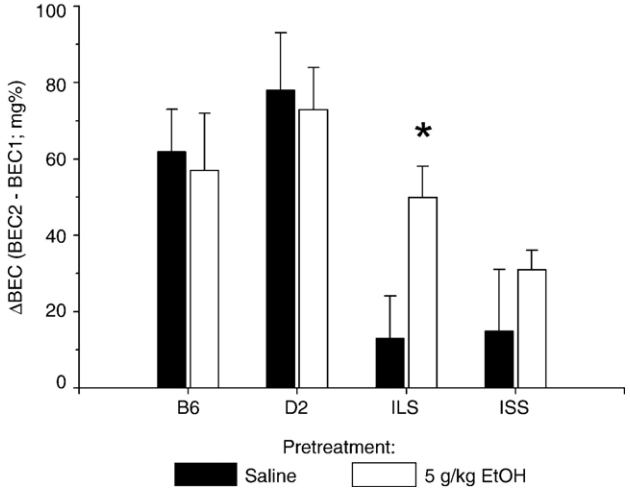

Fig. 6. ΔBEC using the stationary dowel test of ataxia in B6, D2, ILS, and ISS mice 24 h following an acute alcohol pretreatment. Mice were administered 5 g/kg on day 1 and then tested for their ability to balance on a stationary dowel rod on day 2 as described under Methods (day 2 test doses were the same for all strains). Asterisks indicate significant within-strain effect of pretreatment (*$p<0.05$); $n=7$–10 per group.

Fig. 4C shows ΔBEC for the four strains 24 h following saline or alcohol pretreatment. These values were tested to determine if they were different from zero; the results of this analysis are shown in Table 2. Saline-pretreated ISS showed positive ΔBEC scores that were significantly different from zero, indicating development of AFT. The same trend was observed in B6. In contrast, the saline-pretreated D2 and ILS showed a significant negative ΔBEC (acute sensitization). Among the alcohol-pretreated animals, ΔBEC was significantly greater than zero in the B6 and ISS with no significant effect in the D2 or ILS.

The alcohol pretreatment caused ΔBEC to become significantly less negative in the D2 and ILS (Fig. 4C). This effect was so pronounced in the D2 strain that rather than acute sensitization, the D2 showed AFT following alcohol pretreatment. Alcohol pretreatment caused ΔBEC to be increased over saline in the B6 strain, but this was not significant. ΔBEC in the ISS decreased slightly and non-significantly as a result of alcohol pretreatment.

Rectal temperatures were measured during the modified LORR test, shown in Fig. 5. Two-way ANOVA was conducted (within-strain) to determine effects of pretreatment and time. In general, the results were very similar to hypothermia measured during the classic LORR test (Fig. 3). There was a main effect of time for all strains, due primarily to the change from time zero which was significantly different from all subsequent time points. The alcohol pretreatment attenuated alcohol-induced hypothermia during the day 2 LORR test in the B6, D2 (at only 30 min), and ILS. This main effect was significant only in the

B6 while approaching significance in the ILS ($p=0.07$). There also was a significant interaction effect only in the ILS. Alcohol pretreatment had no effect on subsequent alcohol-induced hypothermia in the ISS.

Table 3 shows a comparison of the latency to onset of LORR and BECLR in the four strains following a pretreatment of saline or alcohol using the two different tests (data derived from the same mice used for the classic test shown in Fig. 2 and for the modified test shown in Fig. 4). Two-way ANOVA was conducted within-strain to determine effects of testing method and of pretreatment. In comparison to the modified test, both latency to onset and BECLR with the classic test were overall higher in the B6, D2, and ISS strains. The effect was significant in all three strains for latency; BECLR was significant for only the B6 and ISS. Latency to onset and BECLR were unaffected by test method in the ILS. The alcohol pretreatment had no effect on latency or BECLR as indicated by the absence of a significant main effect of pretreatment or of an interaction effect in any of the strains.

3.4. Experiment 4: stationary dowel rod test

Mean BEC1 and BEC2 values after saline or alcohol pretreatment for the stationary-dowel test of alcohol-induced ataxia are shown in Table 4. Two-way ANOVA (strain-by-pretreatment) indicated no significant effect of strain, pretreatment, or interaction effects for BEC1. However, there was a significant strain effect and pretreatment effect for BEC2. *Post hoc* analysis revealed that there was a pretreatment effect of alcohol on BEC2 in only the ILS strain. Assessment of BEC1 compared to BEC2 (within strain and pretreatment group; one-way ANOVA) showed that in all cases except for the ILS and ISS following saline pretreatment, the BEC2 scores were greater than BEC1, indicative of the acquisition of AFT. These results are reflected in the ΔBEC scores shown in Fig. 6 for which two-way ANOVA indicated significant main effects of strain and pretreatment with the pretreatment effect significant in only the ILS.

4. Discussion

Following intraperitoneal alcohol administration in the mouse, blood and brain alcohol concentrations rise rapidly to peak within 10 min or less (Smolen and Smolen, 1989). Because of this rapid rate of distribution and because determination of LORR using the classic method takes a relatively long period of time, initial sensitivity (BECLR) tends to be overestimated and, as a consequence, AFT will be underestimated. The modified procedure of Ponomarev and Crabbe (2002) was designed to reduce the amount of time it takes to determine LORR with the goal of obtaining an improved estimate of initial sensitivity. Indeed, with the exception of the ILS, latency to LORR onset and BECLR were generally higher using the classic compared to the modified procedure suggesting that the modified method was able to more accurately estimate initial LORR sensitivity. Thus, the modified procedure gave an opportunity to investigate the mechanistic basis of rapid tolerance; i.e., was the previously observed rapid tolerance due to a decrease in initial sensitivity, an increase in AFT, or some combination of the two?

Consistent with previous studies, a high-dose alcohol pretreatment prior to the single blood-draw classic LORR test caused the duration of LORR response to become sensitized in the ISS with no effect on BECRR, while the ILS showed rapid tolerance to both duration of LORR and BECRR (Radcliffe et al., 2005); no significant effect of alcohol pretreatment was found in the B6 or D2 strains using this procedure. The two blood-draw classic method yielded similar results, but with some notable differences. The ILS response was the same regardless of whether one or two blood-draws were taken. However, with two blood-draws, the B6 and D2 started to appear as though they had acquired rapid tolerance for both BECRR and duration of LORR, although the effect was not significant. The ISS showed the most dramatic difference in that now BECRR, as well as duration of LORR, was significantly sensitized. The reason for the discrepancies is not known. The second blood-draw was taken as the animals were just losing consciousness and it is possible that the fairly stressful retro-orbital procedure activated pathways that influenced alcohol sensitivity.

As noted, the B6, D2, and ILS all showed a decrease in duration of LORR as a result of the alcohol pretreatment in the two blood-draw classic test, although it was significant in only the ILS. The same was true using the modified test, but in this case it was significant for all three strains. The duration of LORR results from both tests were reflected in the BECRR scores which were concomitantly increased. The BECRR difference was not significant in the B6 strain probably because of its smaller effect size. It also is possible that the alcohol pretreatment altered alcohol metabolism in the B6. In any case, the results suggest that the B6, D2, and ILS generally developed rapid tolerance for high-dose sensitivity, whereas the ISS did not. Only the ILS showed rapid tolerance for a lower-dose test, the stationary dowel rod, as evidenced by the increase in BEC at regain of balance following the second alcohol dose (BEC2). Overall, the results point to task- and genotype-dependence for the development of rapid tolerance. There also may be dose-dependent effects (for both the pretreatment and test doses); however, dose–response functions were not directly examined in this study (see Radcliffe et al., 2005).

Measures of initial sensitivity (BECLR for the LORR tests and BEC1 for the stationary dowel test) were obtained to determine if a change in this parameter was responsible for the rapid tolerance or, in the case of the ISS, sensitization. For the ILS, BECLR was almost identical for the saline compared to the alcohol pretreated animals regardless of which LORR test was used. The B6 and D2 actually showed a modest non-significant decrease in BECLR while the ISS was slightly increased, also non-significantly, the opposite of what was occurring with BECRR in these strains. The first measure of sensitivity for the stationary dowel rod test (BEC1) was slightly increased by an alcohol pretreatment in the B6, D2, and ILS, and slightly decreased in the ISS, but this was non-significant in all cases. Overall, these results indicate that initial, or at least early sensitivity was minimally altered by the alcohol pretreatment.

Alcohol pretreatment caused ΔBEC to be increased in the B6, D2, and ILS for both the classic and modified LORR tests; significance was attained in both experiments for only the D2 and ILS. An increase in stationary dowel rod ΔBEC also was observed in the ILS, the ILS being the only strain that showed a significant effect of any kind with that test. In contrast, ΔBEC non-significantly decreased in the ISS with the classic LORR test, consistent with the rapid sensitization that was observed. Together, the initial sensitivity and ΔBEC results suggest that rapid tolerance (or sensitization) was mediated primarily if not exclusively by an adaptation in AFT. This idea is consistent with the results of an experiment conducted by Wu et al. (2001) in which it was found that an increase in the rate and/or magnitude of AFT was more important towards the development of chronic tolerance than a decrease in initial sensitivity. The similarity of the results from the two studies also lends support to the hypothesis that rapid tolerance is an early manifestation of chronic tolerance (Khanna et al., 1991).

The primary reason for using the modified LORR method is because it is possible to obtain artifactual negative ΔBEC scores (sensitization) using the classic method since that procedure is prone to overestimation of BECLR, as discussed above. However, significant sensitization after saline pretreatment was observed in the D2 and ILS using the modified method. This result is not consistent with the genetic studies of Ponomarev and Crabbe (2004) who used the modified method and found a negative ΔBEC in only 1 of 20 inbred mouse strains, an effect that was not statistically significant. The discrepancy could be the result of the different doses used, at least concerning the D2: 4.1 g/kg in the current study vs. 3.0 g/kg in Ponomarev and Crabbe (2004). The rate of distribution would have been more rapid with the higher dose and therefore BECLR would have been more prone to overestimation relative to the lower dose. This then could have contributed to the negative ΔBEC. Concerning the ILS, the doses used in the two studies were similar (2.8 g/kg vs. 3.0 g/kg) and therefore the observed sensitization may have been a real effect with the caveat that BECLR determined using the modified method, as with the classic method, almost assuredly overestimates true initial sensitivity due to the inability to obtain BECLR at the exact instant of loss of function coupled to the possibility that some amount of AFT had already developed.

Also noteworthy is that Ponomarev and Crabbe (2004) reported that the D2 developed more AFT than the B6, contrary to the current results. Although the modified procedure is well described (Ponomarev and Crabbe, 2002, 2004), it is possible that there was enough variation in the way in which the method was conducted to account for at least a portion of the difference. Another more likely possibility is, again, the different doses used. If the B6 developed AFT more slowly than the D2, but ultimately possessed the capacity for a higher magnitude of AFT, then it is possible that the D2 achieved its full magnitude of AFT at the lower dose while the B6 required a higher dose, and thus a longer duration of LORR, to achieve its maximum AFT. This would be an interesting genetic dissociation between rate of development and magnitude of AFT. Clearly more experimentation is required to examine this hypothesis.

Previous reports have suggested that the LORR difference between the ILS and ISS was due purely to variance in initial sensitivity (Tabakoff et al., 1980; Tabakoff and Ritzmann, 1979; Smolen and Smolen, 1987) or to variance in a combination of initial sensitivity and AFT (Keir and Deitrich, 1990). The current study is more consistent with the latter possibility. AFT, represented as the difference between BECLR and BECRR, was not observed in either the ILS or ISS by other investigators (Tabakoff et al., 1980; Tabakoff and Ritzmann, 1979) nor was it observed in the current study using the classic method (after saline pretreatment). However, use of the modified procedure indicated a very robust AFT in the ISS following either saline or alcohol pretreatment while the ILS actually showed acute sensitization, not AFT. Although initial sensitivity (BECLR) was much greater in the ISS with either the classic or modified procedure, a direct comparison was not possible since different test doses were used in the strains. While potential differences in initial sensitivity cannot be ruled out, the current results are consistent with a large portion of the ILS/ISS difference in LORR being due to differential acquisition of AFT, either in rate or magnitude.

The ISS LORR sensitization with no effect on BECRR after alcohol pretreatment (classic procedure, one blood-draw) suggests a pharmacokinetic rather than pharmacodynamic effect and it was previously speculated that a concomitant hypothermia sensitization was responsible (Radcliffe et al., 2005). This idea is supported by the results of the modified test in which LORR sensitization was not observed in the ISS and there was not a pretreatment effect on alcohol-induced hypothermia either. Being in a small enclosed space may have prevented some loss of body temperature thus contributing to the apparent absence of hypothermia sensitization. However, hypothermia sensitization also did not occur with the two blood-draw classic procedure and yet a substantial LORR sensitization was still observed. Moreover, BECRR was also sensitized in that experiment. The results suggest a dissociation between hypothermia and LORR, at least regarding the rapid tolerance paradigm, and are consistent with experiments in which significant genetic correlations were not observed between LORR and hypothermia in standard inbred and recombinant inbred mouse strains (Crabbe et al., 1994b; Erwin and Jones, 1993).

Rapid tolerance to the hypothermic effects of alcohol has been observed in inbred mouse strains for over 25 years, (Crabbe et al., 1979). It was thus a surprise that in our previous study hypothermia in the ISS was found to be sensitized following an alcohol pretreatment and that no rapid tolerance was observed in the ILS (Radcliffe et al., 2005). However, the current results indicate that rapid hypothermia tolerance was generally observed in the B6 and ILS with a modest effect in the D2 and no effect in the ISS. Procedural differences perhaps can account for the discrepancy between the experiments (including measuring body temperature over a longer time frame in the present study). It is notable that the ISS is an outlier for both hypothermia and measurements related to the LORR response. All three of the other strains showed rapid tolerance for hypothermia, duration of LORR, and BECRR in one or more of the current experiments. The ISS, however, showed either no effect or sensitization for duration of LORR and BECRR, and no hypothermia effect. The

ISS also had a substantially higher test dose than the other strains, although inspection of Figs. 3 and 5 reveal that hypothermia (after saline pretreatment) is similar among all of the strains, despite the different test doses. Nonetheless, it is possible that a floor effect was achieved with the ISS such that lower test doses might reveal an effect of the alcohol pretreatment on hypothermia. Prior studies have indicated that test dose was not an important factor with regard to rapid tolerance for duration of LORR and BECRR. Equivalent, rather than equipotent doses elicited the same basic pattern for these responses: no effect of alcohol pretreatment in the ISS, and rapid tolerance in the ILS (Radcliffe et al., 2005). Body temperature was not measured in that experiment and so it remains to be determined if there is a test dose effect for the hypothermia response.

In the context of the human condition, what is the contribution of genetics and of prior alcohol experience to variance in acute alcohol sensitivity? Given the caveat that only a limited number of strains was assessed, the current results suggest that AFT is more genetically sensitive to prior alcohol exposure than initial sensitivity. Tolerance has been shown to be a poor predictor of chronic alcoholism, but this was in subjects who had already met diagnostic criteria for alcohol abuse or dependence (de Bruijn et al., 2005). The rate or magnitude of rapid or chronic tolerance development and its interaction with acute sensitivity might be more important during the *initiation* of heavy drinking behavior and perhaps not especially relevant for *maintaining* it once it has been well established (Schuckit et al., 2004a). An inherently low acute sensitivity and/or a very quick development of rapid or chronic tolerance may simply permit an individual to drink more early in their drinking career thereby accelerating the neuronal changes that trigger the progression from casual to pathologic drinking behavior. Such effects could also promote social interactions with others who are already heavy drinkers thus reinforcing that this is an acceptable if not desirable behavior (Schuckit et al., 2004a). Another possibility is that other traits that are pleiotropic to acute sensitivity may be the root cause of the association with alcoholism. For example, studies have shown a relationship between AFT and contextual learning and also between alcohol sensitization and contextual learning, suggesting a common mechanistic basis for alcohol-induced plasticity and learning and memory (Radcliffe et al., 1998; Quadros et al., 2003). This is supported by a postulated overlap in the molecular mechanisms of synaptic plasticity and alcohol tolerance, including AFT (Chandler et al., 1998). Addictive processes have an important learning component (Everitt and Robbins, 2005; Hyman, 2005) and it is possible that low sensitivity (manifested through AFT and modulated through exposure to alcohol) contributes to alcoholism through pleiotropy with learning-related phenomena.

The main finding of this investigation is that rapid tolerance, and possibly sensitization, is mediated primarily by alterations in AFT: a decrease (increase for sensitization) in alcohol sensitivity as determined through the measurement of endpoints occurring on the descending limb of the alcohol distribution curve is a result of increased (decreased for sensitization) acquisition of AFT. Ongoing studies are being conducted to continue the examination of the genetic relationship between initial sensitivity, AFT, and rapid tolerance, and ultimately will address the question of how these pharmacological parameters influence the "low level of response" as it relates to human alcoholism.

Acknowledgements

The authors would like to thank Dr. Igor Ponomarev for helpful discussion and also Dr. George Koob and the reviewers of this manuscript for their valuable insight and suggestions. This work was supported by NIH grant AA13177.

References

Almasy L. Quantitative risk factors as indices of alcoholism susceptibility. Ann Med 2003;35:337–43.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th Ed. Washington, D.C.: American Psychiatric Association; 1994.

Belknap JK, Atkins AL. The replicability of QTLs for murine alcohol preference drinking behavior across eight independent studies. Mamm Genome 2001;12:893–9.

Chandler LJ, Harris RA, Crews FT. Ethanol tolerance and synaptic plasticity. Trends Pharmacol Sci 1998;19:491–5.

Conrod PJ, Peterson JB, Pihl RO. Reliability validity of alcohol-induced heart rate increase as a measure of sensitivity to the stimulant properties of alcohol. Psychopharmacology 2001;157:20–30.

Crabbe JC, Rigter H, Uijlen J, Strijbos C. Rapid development of tolerance to the hypothermic effect of ethanol in mice. J Pharmacol Exp Ther 1979;208:128–33.

Crabbe JC, Belknap JK, Mitchell SR, Crawshaw LI. Quantitative trait loci mapping of genes that influence the sensitivity and tolerance to ethanol-induced hypothermia in BXD recombinant inbred mice. J Pharmacol Exp Ther 1994a;269:184–92.

Crabbe JC, Gallaher ES, Phillips TJ, Belknap JK. Genetic determinants of sensitivity to ethanol in inbred mice. Behav Neurosci 1994b;108:186–95.

Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, et al. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology 1997;132:107–24.

de Bruijn C, van den Brink W, de Graaf R, Vollebergh WA. Alcohol abuse and dependence criteria as predictors of a chronic course of alcohol use disorders in the general population. Alcohol Alcohol 2005;40:441–6.

Dick DM, Foroud T. Candidate genes for alcohol dependence: a review of genetic evidence from human studies. Alcohol Clin Exp Res 2003;27:868–79.

Enoch MA, Goldman D. The genetics of alcoholism and alcohol abuse. Curr Psychiatry Rep 2001;3:144–51.

Erwin VG, Deitrich RA. Genetic selection and characterization of mouse lines for acute functional tolerance to ethanol. J Pharmacol Exp Ther 1996;279:1310–7.

Erwin VG, Jones BC. Genetic correlations among ethanol-related behaviors and neurotensin receptors in long sleep (LS)×short sleep (SS) recombinant inbred strains of mice. Behav Genet 1993;23:191–6.

Erwin VG, Radcliffe RA, Jones BC. Chronic ethanol consumption produces genotype-dependent tolerance to ethanol in LS/Ibg and SS/Ibg mice. Pharmacol Biochem Behav 1992;41:275–81.

Erwin VG, Gehle VM, Deitrich RA. Selectively bred lines of mice show response and drug specificity for genetic regulation of acute functional tolerance to ethanol and pentobarbital. J Pharmacol Exp Ther 2000;293:188–95.

Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 2005;8:1481–9.

Gallaher EJ, Parsons LM, Goldstein DB. The rapid onset of tolerance to ataxic effects of ethanol in mice. Psychopharmacology 1982;78:67–70.

Glazier AM, Nadeau JH, Aitman TJ. Finding genes that underlie complex traits. Science 2002;298:2345–9.

Goldstein DB. Animal models developed by selective breeding: some questions raised and a few answered. In: Kiianmaa K, Tabakoff B, Saito T, editors. Genetic aspects of alcoholism. Helsinki: Finnish Foundation for Alcohol Studies; 1989. p. 229–38.

Hyman SE. Addiction: a disease of learning and memory. Am J Psychiatry 2005;162(8):1414–22.
Kalant H, LeBlanc AE, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 1971;23:135–91.
Keir WJ, Deitrich RA. Development of central nervous system sensitivity to ethanol and pentobarbital in short- and long-sleep mice. J Pharmacol Exp Ther 1990;254:831–5.
Khanna JM, Chau A, Shah G. Characterization of the phenomenon of rapid tolerance to ethanol. Alcohol 1996;13:621–8.
Khanna JM, Kalant H, Shah G, Weiner J. Rapid tolerance as an index of chronic tolerance. Pharmacol Biochem Behav 1991;38:427–32.
King AC, Houle T, de Wit H, Holdstock L, Schuster A. Biphasic alcohol response differs in heavy versus light drinkers. Alcohol Clin Exp Res 2002;26:827–35.
Le AD, Poulos CX, Cappell H. Conditioned tolerance to the hypothermic effect of ethyl alcohol. Science 1979;206:1109–10.
Li TK. Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. J Stud Alcohol 2000;61:5-12.
Lundquist F. The determination of ethyl alcohol in blood and tissue. Methods Biochem Anal 1959;7:217–51.
McClearn GE, Kakihana R. Selective breeding for ethanol sensitivity: short-sleep and long-sleep mice. In: McClearn GE, Deitrich RA, Erwin VG, editors. Development of animal models as pharmacogenetic tools. Washington, D.C.: U.S. Government printing; 1981. p. 147–59.
McGue M. Genes, environment, and the etiology of alcoholism. In: Zucker R, Boyd G, Howard J, editors. The development of alcohol problems: exploring the biopsychosocial matrix of risk. Washington, D.C.: U.S. Government printing Office; 1994. p. 1-40.
Melchior CL, Tabakoff B. A conditioning model of alcohol tolerance. Recent Dev Alcohol 1984;2:5-16.
Mellanby E. Alcohol: its absorption into and disappearance from the blood under different conditions. Med Res Comm Spec Rep (Lond) 1919;31:1-48.
Morzorati SL, Ramchandani VA, Flury L, Li TK, O'Connor S. Self-reported subjective perception of intoxication reflects family history of alcoholism when breath alcohol levels are constant. Alcohol Clin Exp Res 2002;26:1299–306.
Newlin DB, Thomson JB. Alcohol challenge with sons of alcoholics: a critical review and analysis. Psychol Bull 1990;108:383–402.
Newlin DB, Thomson JB. Chronic tolerance sensitization to alcohol in sons of alcoholics: II. Replication reanalysis. Exp Clin Psychopharmacol 1999;7:234–43.
Palmer MR, Basile AS, Proctor WR, Baker RC, Dunwiddie TV. Ethanol tolerance of cerebellar purkinje neurons from selectively outbred mouse lines: in vivo and in vitro electrophysiological investigations. Alcohol Clin Exp Res 1985;9:291–6.
Phillips TJ, Lessov CN, Harland RD, Mitchell SR. Evaluation of potential genetic associations between ethanol tolerance and sensitization in BXD/Ty recombinant inbred mice. J Pharmacol Exp Ther 1996;277:613–23.
Ponomarev I, Crabbe JC. A novel method to assess initial sensitivity and acute functional tolerance to hypnotic effects of ethanol. J Pharmacol Exp Ther 2002;302:257–63.
Ponomarev I, Crabbe JC. Characterization of acute functional tolerance to the hypnotic effects of ethanol in mice. Alcohol Clin Exp Res 2004;28:991–7.
Quadros IMH, Souza-Formigoni MLO, Fornari RV, Nobrega JN, Oliveira MGM. Is behavioral sensitization to ethanol associated with contextual conditioning in mice? Behav Pharmacol 2003;14:129–36.
Radcliffe RA, Erwin VG, Wehner JM. Acute functional tolerance to ethanol and fear conditioning are genetically correlated in mice. Alcohol Clin Exp Res 1998;22(8):1673–9.
Radcliffe RA, Floyd KL, Drahnak JA, Deitrich RA. Genetic dissociation between ethanol sensitivity and rapid tolerance in mouse and rat strains selectively bred for differential ethanol sensitivity. Alcohol Clin Exp Res 2005;29:1580–9.
Radlow R. A quantitative theory of acute tolerance to alcohol. Psychopharmacology 1994;114:1–8.
Rustay NR, Crabbe JC. Genetic analysis of rapid tolerance to ethanol's incoordinating effects in mice: inbred strains and artificial selection. Behav Genet 2004;34:441–51.
Schuckit MA. Biological, psychological, and environmental predictors of the alcoholism risk: a longitudinal study. J Stud Alcohol 1998;59:485–94.
Schuckit MA. Vulnerability factors for alcoholism. In: Davis K, editor. Neuropsychopharmacology: the fifth generation of progress. Baltimore: Lippincott Williams & Wilkins; 2002. p. 1399–411.
Schuckit MA, Smith TL, Anderson KG, Brown SA. Testing the level of response to alcohol: social information processing model of alcoholism risk—a 20-year prospective study. Alcohol Clin Exp Res 2004a;28:1881–9.
Schuckit MA, Smith TL, Kalmijn J. The search for genes contributing to the low level of response to alcohol: patterns of findings across studies. Alcohol Clin Exp Res 2004b;28:1449–58.
Smolen A, Smolen TN. Demonstration of a threshold concentration for ethanol at the time of regaining the righting response in long-sleep and short-sleep mice. Alcohol Drug Res 1987;7:279–83.
Smolen TN, Smolen A. Blood and brain ethanol concentrations during absorption and distribution in long-sleep and short-sleep mice. Alcohol 1989;6:33–8.
Tabakoff B, Ritzmann RF. Acute tolerance in inbred and selected lines of mice. Drug Alcohol Dep 1979;4:87–90.
Tabakoff B, Ritzmann RF, Raju TS, Deitrich RA. Characterization of acute and chronic tolerance in mice selected for inherent differences in sensitivity to ethanol. Alcohol Clin Exp Res 1980;4:70–3.
Tabakoff B, Melchoir CL, Hoffman PL. Commentary on ethanol tolerance. Alcohol Clin Exp Res 1982;6:252–9.
Wu PH, Tabakoff B, Szabo G, Hoffman PL. Chronic ethanol exposure results in increased acute functional tolerance in selected lines of HAFT and LAFT mice. Psychopharmacology 2001;155:405–12.