Pharmacology Biochemistry and Behavior
Volume 70, Issues 2-3, October-November 2001, Pages 387-396

doi:10.1016/S0091-3057(01)00621-9 Cite or Link Using DOI

Developmental alterations of ethanol sensitivity in selectively bred high and low alcohol sensitive rats

Laura J. Draski, Paula J. Bice and Richard A. Deitrich
Department of Pharmacology and Alcohol Research Center, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262, USA
Received 9 September 2000; revised 18 June 2001; accepted 11 July 2001 Available online 2 November 2001.

Abstract

Initial sensitivity and acute tolerance to ethanol have been implicated as risk factors in the development of alcoholism in humans. These behaviors were investigated in rats selectively bred for differences in hypnotic sensitivity following their first dose of ethanol in two different experiments. In Experiment 1, developmental profiles of the association between initial sensitivity and acute tolerance induced by a single exposure to ethanol were examined using male and female high, low, and control alcohol sensitive (HAS, LAS, and CAS) rats. Dose–response curves were constructed for duration of the loss of the righting reflex and for blood ethanol concentration (BEC) at the regain of the righting reflex. Animals were tested with a single ethanol dose ranging from 1.5 to 5.0 g/kg at either 15, 25, 40, 70, 120, or 180 days of age (DOA). For each group, acute tolerance to ethanol was estimated by the slope of the regression line using dose of ethanol and mean BEC at regain. In general, all rat lines showed an increase in hypnotic sensitivity to ethanol with age. To a large degree, the lower sensitivity observed in 15 and 25 DOA HAS and LAS rats was associated with an increase in the development of acute ethanol tolerance relative to older rats. Divergence of the LAS and CAS lines was evident by 25 DOA and remained stable with advancing age. However, HAS rats did not differ significantly from CAS rats until 40 DOA, after which the magnitude of the difference continued to increase with age. In Experiment 2, rats were treated with alcohol at 25, 70, or 180 DOA. Rats at 70 or 180 DOA required less ethanol to disrupt their motor coordination on a rotating dowel (rotarod). Blood ethanol levels were determined at the loss and subsequent regain of the ability to negotiate the rotarod. Total duration of inability to negotiate the rotarod also was recorded. HAS rats were less able to remain on a rotarod while under the influence of alcohol relative to LAS and CAS rats regardless of age. However, no evidence of acute tolerance was observed in this experiment and, in fact, there was evidence of reverse tolerance in that all animals had lower BEC values at regain of ability than they did at loss.

Author Keywords: Selective breeding; HAS; LAS; Ethanol sensitivity; Acute tolerance; Sleep time; Rotarod; Developmental; Ontogeny; Dose–response

Article Outline

1. Introduction

The utilization of selective breeding paradigms provides invaluable knowledge regarding genetic influences of alcohol-related phenotypes (see Deitrich, 1990 for review). Replicate lines of the high alcohol sensitive (HAS) and low alcohol sensitive (LAS) rats have been bred selectively for marked differences in CNS sensitivity to ethanol as measured by their hypnotic response to an acute dose of ethanol administered at ages greater than 60 postnatal days (Draski et al., 1992). Comparisons of blood ethanol concentration (BEC) when animals regained their righting reflex following ethanol hypnosis and examination of ethanol elimination rates indicate that the lines differ in neurosensitivity to ethanol. However, the neural mechanisms responsible for mediating the differential effects of ethanol observed in these selected lines remain unclear.

One method of uncovering the neural basis of a pharmacological phenomenon is to develop an ontological profile of the response. Age-related alterations in the drug-induced behavior then may be attributable to the maturation of specific neural systems. Such studies may be valuable in dissecting the timing of gene expression in younger vs. older animals in response to ethanol. By comparing the ontogeny of ethanol sensitivity in HAS and LAS rats, greater insight may be gained regarding the differential neural mechanisms governing high and low alcohol sensitivity.

Studies conducted in our laboratory utilizing the similarly selected long-sleep (LS) and short-sleep (SS) mouse lines suggest that differences in ethanol-induced sleep times are observable at the earliest age that this response could be ascertained (Keir and Deitrich, 1990). In general, mice younger than 30 days of age (DOA) are more sensitive to ethanol than adult mice. On the contrary, Silveri and Spear (1997) report a marked increase in sensitivity to ethanol during ontogeny in rats ranging from 16 to 96 DOA. Other studies examining the effects of aging on ethanol sensitivity in rats 90 DOA and older also demonstrated an increase in sensitivity with advancing age (York; York and Propp).

We investigated the development of ethanol sensitivity by constructing dose–response curves for ethanol-induced sleep time and BEC at awakening in male and female HAS, LAS, and control alcohol sensitive (CAS) rats at either 15, 25, 40, 70, 120, or 180 DOA. In addition, age-related differences in ethanol-induced, nonhypnotic impairment of motor coordination on a rotating rod (rotarod) were examined in a separate experiment at 25, 70, or 180 DOA.

2. Method

2.1. Experiment 1: ethanol-induced hypnosis

Approximately equal numbers of male and female HAS, LAS, and CAS rats were obtained from surplus litters produced by breeding pairs representing selected generations 16–18. Breeders were surveyed for new litters a minimum of once per day, with the day of birth recorded as DOA 0. Following weaning at 22–25 DOA, animals were group-housed in single-sexed, Plexiglas cages with laboratory rat chow and water provided ad libitum. Each subject was tested only once at 15, 25, 40, 70, 120, or 180 DOA. When multiple subjects from one litter were utilized, each rat of a given sex received a different dose of ethanol. The number of animals in each testing condition (age, dose, line, and sex) ranged from five to nine (7.1 average), with a total *N* of 1494. Ambient temperature in the testing room was 21±1 °C.

Rats utilized in the dose–response curves were administered an intraperitoneal injection of 15% w/v ethanol at one of the following 12 doses: 1.5, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.5, or 5.0 g/kg. Due to their extreme differences in sensitivity, we were unable to test animals of each line, sex, and age at all doses. Each group was given a minimum of four doses, with a difference of at least 1.5 g/kg ethanol between the lowest and highest dose. Since an ethanol dose of 3.0 g/kg was used in the initial phenotypic selection of the lines, this dose

typically was used as a starting point. Additional doses were used until the lowest whole or half gram per kilogram ethanol dose that still resulted in loss of the righting reflex had been identified. Since the slope of the dose–response curve was steeper for the 180 DOA rats, four 0.25-g/kg increment doses also were used.

Sleep time was defined as the interval from loss to regain of the righting reflex. Loss of the righting reflex was defined by the inability of the animal to right itself three times within 1 min after being placed on its back in a V-shaped Plexiglas trough. Immediately after the righting reflex was regained, a 40-μl blood sample was obtained from the retro-orbital sinus of each animal for determination of BEC. If an animal had not recovered within 10 h postinjection, a maximum sleep time score of 600 min was recorded and a BEC sample was collected at this time. Blood ethanol was determined enzymatically using alcohol dehydrogenase by a modification of the method described by Smolen and Smolen (1987).

2.2. Experiment 2: rotarod incoordination

Naive male HAS, LAS, and CAS rats were trained on a rotarod treadmill for rats for 3 days prior to testing at 25, 70, or 180 DOA. The rotarod dowel measured 7.5 in. in circumference and rotated at a fixed speed of 12 rpm. Animals were given a maximum of 15 attempts over the first two training days (maximum trials per DAY=10) to achieve the training criteria of four continuous minutes per day on the rotarod. On Day 3, all rats were required to stay on the rotarod for a continuous 4-min interval in a maximum of five attempts. Any rat not meeting these qualifications was not tested on Day 4. A total of 11 animals were tested in each condition.

On Day 4, rats received an intraperitoneal injection of ethanol and were immediately placed upon the moving rotarod. Rats, 70 and 180 DOA, were tested with an ethanol dose of 1.5 g/kg. However, a higher dose of ethanol (2.5 g/kg) was required to achieve similar measurable incoordination in the 25 DOA animals. Loss of coordination was defined as the animal falling off the rotarod three times within 10 s, while regain was identified as the ability to negotiate the moving rod for two consecutive minutes.

Immediately following loss of coordination, a 40-μl blood sample was obtained from the retro-orbital sinus of each animal and the BEC was determined as described above. Rats were placed in holding cages and checked approximately every 10 min for their ability to negotiate the rotarod. When coordination was regained, a second blood sample was taken immediately.

2.3. Statistical analyses

2.3.1. Experiment 1: hypnosis

Criterion for significance was set at $P<.05$ for all analyses. Since the reporting of BEC at regain of the righting reflex was critical to the interpretation of these studies, rats that did not lose the righting reflex were not included in the analysis. In addition, experimental groups were eliminated from the study when less than half of the group lost their righting reflex. Given that BEC may not accurately reflect brain ethanol concentration for the first 10 min postinjection, experimental groups were also eliminated from the study when the ethanol dose failed to produce a mean sleep time greater than 10 min. Because ethanol sensitivity varied greatly between the selected lines and the different aged animals, only one ethanol dose (3.5 g/kg) produced reliable sleep times in all groups. For these data, a three-factor analysis of variance (ANOVA) was conducted for the effects of selected line (three groups), age (six groups), and sex (two).

The contributions of acute tolerance to overall ethanol sensitivity were estimated by the linear relationships between ethanol dose and mean BEC at awakening for all groups. If acute tolerance was not evident, the mean BEC at regain would not differ as a function of ethanol dose and the slope of the relationship would be zero. If acute tolerance was a factor, BEC at regain would increase as the dose increased, and the slope of the line would be greater than zero. The linear regression correlation coefficients were used to determine if each slope was significantly different from zero. The selected lines were compared at each age using *t* tests for individual slopes.

Brain sensitivity was determined by examining the linear relationships between mean sleep time and BEC produced by a given ethanol dose. The *y*-intercepts extrapolated from these slopes estimate the minimum BEC necessary to produce ethanol-induced hypnosis. The selected lines were compared at each age by Student's *t* tests for intercepts corrected for multiple comparisons by the Bonferroni statistic for planned comparisons (Keppel, 1982).

To illustrate developmental differences in the emergence of high and low sensitivity to ethanol, the sleep time and BEC scores of individual male and female HAS and LAS rats were subtracted from the corresponding mean values for CAS animals. These data were analyzed by two-factor ANOVA with selected line (2) and age (6) as the main factors.

2.3.2. Experiment 2: rotarod

Since different ethanol doses were used, the time to loss of coordination on the rotarod and the total duration of incoordination were analyzed at each age by single-factor ANOVA with selected line as the main factor. BECs at time of loss and regain of rotarod coordination were analyzed by three-factor repeated-measures ANOVA, with selected line (3) and age (3) as the main factors and BEC as the repeated measure. Criterion for significance was set a $P<.05$ for all analyses. Post hoc comparisons were analyzed by Fisher's Protected Least Significant Difference (PLSD) tests to control for increases in family-wise Type 1 error (Keppel, 1982).

3. Results

3.1. Experiment 1: ethanol-induced hypnosis

Representative sleep time and BEC data from the dose–response analyses are presented in Table 1 and Table 2. Three-factor ANOVA of the 3.5-g/kg ethanol dose revealed significant main effects of selected line [$F(2,226)=164$], age [$F(5,226)=64.5$], and sex [$F(l,226)=35.6$], on sleep time. Corresponding significant effects were observed for BEC at regain for selected line [$F(2,225)=97.4$], age [$F(5,225)=14.2$], and sex [$F(l,225)=4.5$]. In general, HAS rats were the most sensitive to ethanol while LAS rats were the least, and males were more sensitive than females. All rats became more sensitive to the effects of ethanol as they became older. Significant interactions also were observed for the sleep time data. HAS rats became more sensitive to ethanol with age than CAS or LAS rats (Line×Age) [$F(10,226)=13.4$], while males also became more sensitive than females as they became older (Sex×Age) [$F(5,226)=7.2$]. In general, the greatest sex differences were observed in HAS rats (Line×Sex) [$F(2,226)=10.9$], and this difference became increasingly apparent with age (Line×Age×Sex) [$F(10,226)=3.6$]. While similar trends were observed for the BEC data, only the Line×Age interaction was significant [$F(10,225)=5.5$].

Table 1. Ethanol-induced sleep times

Mean ethanol-induced sleep times in minutes±S.E.M of male (M) and female (F) HAS, CAS, and LAS rats at 15, 25, 40, 70, 120, and 180 DOA. Data presented are a representative sample of the dose–response curve and do not include all doses measured. Minimum number of animals tested per GROUP=5.

Line	DOA	Ethanol dose (g/kg)									
		2.5		3.0		3.5		4.0		4.5	
		M	F	M	F	M	F	M	F	M	F
HAS	15	12±6	34±10	56±14	50±5	101±17	93±7	110±19	151±28	116±44	191±29
	25	2±2	1±1	20±6	29±7	44±9	74±16	116±20	102±12	150±19	154±22
	40	76±24	34±13	97±23	57±16	170±18	136±22	248±24	200±27		
	70	109±34	77±24	250±28	190±22	319±39	241±16	356±41	254±23		
	120	133±27	118±29	298±28	215±29	411±40	191±22	573±18	434±42		
	180	224±40	91±26	308±38	201±38	475±47	269±25				
CAS	15	21±9	29±5	42±4	48±9	76±10	80±7	129±21	145±22	189±26	210±16
	25	8±8	0±0	5±2	10±4	52±14	57±22	70±9	100±18	119±12	109±16
	40	9±6	2±1	49±15	32±8	63±13	46±4	100±9	94±8	143±14	177±29
	70	20±7	16±7	47±13	45±11	121±21	86±8	254±35	133±12	359±25	246±22
	120	21±5	18±4	91±10	52±6	177±24	134±17	318±30	176±22	468±50	260±26
	180	60±20	15±4	135±19	50±5	159±12	107±13	333±59	196±19		
LAS	15	4±4	0±0	42±8	14±8	64±12	54±8	86±9	75±4	96±12	117±20
	25			2±2	2±2	16±4	16±2	35±5	43±4	72±11	65±7
	40	0±0	1±1	16±11	16±3	26±5	25±4	61±7	63±5	84±8	107±10
	70	3±3	9±5	17±5	34±18	88±22	45±8	128±26	104±12	202±28	167±19
	120	16±5	9±2	44±5	17±4	67±9	88±20	195±25	169±12	386±47	241±17
	180	7±2	9±2	40±9	22±2	169±20	118±51	223±65	131±23	389±64	286±37

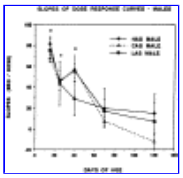
Table 2. Blood ethanol concentrations (mg/dl) at awakening

Mean BECs measured (mg/dl) at regain of the righting reflex±S.E.M. in male (M) and female (F) HAS, CAS, and LAS rats at 15, 25, 40, 70, 120, and 180 DOA. Data presented are a

representative sample of the dose–response curve and do not include all doses measured. Mean BECs are not reported for mean sleep times less than 15 min due to potential unreliability of data (marked “x”). Minimum number of animals tested per GROUP=5.

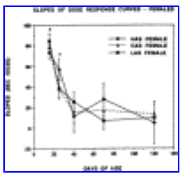
Line	DOA	Ethanol dose (g/kg)									
		2.5		3.0		3.5		4.0		4.5	
		M	F	M	F	M	F	M	F	M	F
HAS	15	x	259±6	292±16	312±9	348±11	356±11	375±8	376±18	419±10	411±17
	25	x	x	350±27	324±19	350±13	332±18	357±16	380±20	420±38	403±9
	40	274±27	312±19	322±14	346±17	318±10	321±18	323±13	339±23		
	70	245±27	260±23	217±15	215±18	257±14	257±10	268±18	286±18		
	120	266±9	269±22	224±20	254±19	276±14	328±17	243±10	263±19		
	180	205±23	269±19	227±19	231±21	275±21	302±20				
CAS	15	279±9	281±15	320±10	334±12	358±9	362±19	382±9	392±12	418±15	448±11
	25	x	x	x	x	364±17	384±21	390±9	377±15	409±5	408±14
	40	x	x	347±11	378±11	392±9	400±7	426±12	412±7	432±13	400±26
	70	312±10	302±12	332±11	329±14	330±15	353±5	333±18	339±9	330±12	339±14
	120	322±6	340±13	324±9	347±8	332±11	333±9	302±11	375±11	302±16	359±11
	180	288±16	330±11	318±14	337±12	339±18	351±12	362±17	385±16		
LAS	15	x	x	340±14	x	365±17	353±10	434±10	410±14	470±9	446±7
	25			x	x	406±12	402±9	429±10	430±19	467±14	458±15
	40	x	x	364±23	389±10	414±6	414±11	437±10	445±11	457±9	446±13
	70	x	x	347±8	369±14	368±12	387±14	389±13	386±8	400±13	403±17
	120	354±13	x	377±6	382±4	419±10	396±12	385±15	391±11	367±12	400±13
	180	x	x	354±16	365±11	339±22	389±14	403±23	391±11	388±27	402±19

Slopes derived from the linear regression of BEC vs. dose for rats between 15 and 120 DOA are plotted as a function of age in Fig. 1 and Fig. 2. Many of the 180 DOA rats slept longer than the maximum allowed sleep time interval of 600 min, and the BEC values taken at this time point were artificially inflated. Consequently, slopes were not calculated at this age. Significant positive slopes were observed in male and female rats at 15 and 25 DOA, and in males at 40 DOA. Slopes were not significantly different from zero at 70 or 120 DOA, suggesting that younger rats develop acute tolerance in response to increasing ethanol doses while older rats do not. No differences between selected lines were indicated by the *t* tests for independent slopes.



Display Full Size version of this image (7K)

Fig. 1. Contributions of acute tolerance to first-dose ethanol sensitivity over time in male rats. Each point represents the slope obtained by plotting mean BEC at awakening at a minimum of four ethanol doses. Slopes significantly greater than zero indicate the development of acute tolerance. (a) All slopes significantly different from zero at that age. No statistical differences were found between HAS, LAS, and CAS.

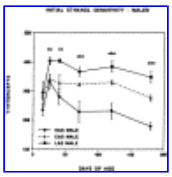


Display Full Size version of this image (7K)

Fig. 2. Contributions of acute tolerance to first-dose ethanol sensitivity over time in female rats. Each point represents the slope obtained by plotting mean BEC at awakening at a minimum of four ethanol doses. Slopes significantly greater than zero indicate the development of acute tolerance. (a) All slopes significantly different from zero at that age. No statistical differences were found between HAS, LAS, and CAS.

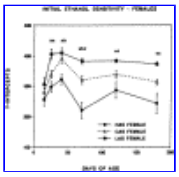
The extrapolated *y*-intercepts obtained by plotting the mean sleep time vs. the mean BEC at regain of the righting reflex are shown as a function of age in Fig. 3 and Fig. 4. These intercepts approximate the concentration of ethanol necessary to cause the animals to lose the righting reflex. Students *t* tests for differences in *y*-intercepts revealed no differences between the selected lines at 15 DOA. In general, LAS rats were differentiated from CAS and HAS rats starting at 25 DOA. On the contrary, HAS rats were not different from

controls until 40 DOA for females, and 70 DOA for males.



Display Full Size version of this image (6K)

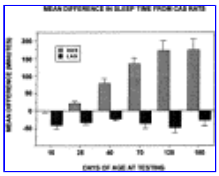
Fig. 3. Estimates of minimum BEC necessary to induce ethanol hypnosis in male rats across age. Each point represents the *y*-intercept extrapolated by plotting the mean BEC at awakening vs. the mean sleep time observed at each dose of ethanol. Selected lines were compared at each age by Student's *t* test for intercepts modified by the Bonferroni statistic for multiple comparisons. (a) HAS significantly different from LAS. (b) HAS significantly different from CAS. (c) LAS significantly different from CAS.



Display Full Size version of this image (6K)

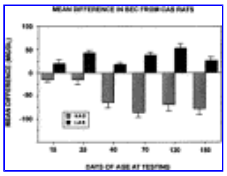
Fig. 4. Estimates of minimum BEC necessary to induce ethanol hypnosis in female rats across age. Each point represents the *y*-intercept extrapolated by plotting the mean BEC at awakening vs. the mean sleep time observed at each dose of ethanol. Selected lines were compared at each age by Student's *t* test for intercepts modified by the Bonferroni statistic for multiple comparisons. (a) HAS significantly different from LAS. (b) HAS significantly different from CAS. (c) LAS significantly different from CAS.

Similar developmental differences in the emergence of high and low sensitivity to ethanol were observed when individual HAS and LAS sleep time and BEC scores were standardized to the mean CAS values at each age (see Fig. 5 and Fig. 6). Significant main effects of selected line and age were observed for both sleep time [$F(1,84)=52.48$ and $F(5,84)=12.47$, respectively], and age [$F(1,60)=15.94$ and $F(5,60)=7.39$]. Significant interactions of selected line and age were observed for both sleep time [$F(5,84)=11.32$], and BEC [$F(5,60)=5.80$]. LAS rats differed from CAS rats at the earliest age tested, and the magnitude of this difference was not altered with age. On the other hand, HAS rats did not differ from CAS rats until 40 DOA, and the magnitude of this difference appears to increase with age.



Display Full Size version of this image (8K)

Fig. 5. Divergence of sleep time produced by 3.5 g/kg ethanol in HAS and LAS rats over age when compared to CAS rats.

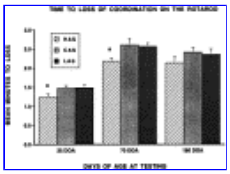


Display Full Size version of this image (7K)

Fig. 6. Divergence of BEC at awakening following 3.5 g/kg ethanol in HAS and LAS rats when compared to CAS rats.

3.2. Experiment 2: rotarod incoordination

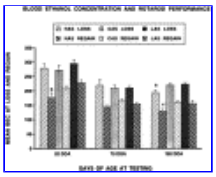
Following ethanol, HAS rats fell off the rotarod significantly sooner than CAS and LAS rats at 25 DOA [$F(2,30)=3.26$], and 70 DOA [$F(2,30)=3.75$], but not at 180 DOA (see Fig. 7).



Display Full Size version of this image (17K)

Fig. 7. Mean minutes to loss of the ability to negotiate a fixed-speed rotarod following ethanol administration to rats at 25, 70, or 180 DOA. (a) HAS significantly different from CAS and LAS.

On the contrary, BEC at loss was not different in the HAS rats at either 25 or 70 DOA, but was significantly lower than CAS and LAS at 180 DOA [$F(2,30)=4.58$]. BECs at regain also were lower in the HAS rats at 25 DOA [$F(2,30)=4.15$], and 180 DOA [$F(2,30)=3.17$], suggesting that the differences are in neurosensitivity and not metabolism (Fig. 8). Repeated-measures ANOVA revealed significant differences in BEC due to age [$F(2,90)=42.82$], as well as significant effects of line [$F(2,90)=3.99$].

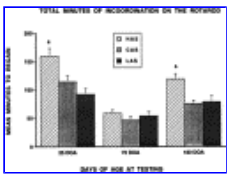


Display Full Size version of this image (17K)

Fig. 8. Mean total minutes of rotarod incoordination following ethanol administration in rats at 25, 70, or 180 DOA. (a) HAS significantly different from CAS and LAS.

With respect to age, 25 DOA rats of all lines lost and regained the ability to navigate the rotarod at higher BECs than the older rats, suggesting that sensitivity to the locomotor disrupting effects of ethanol increases with age in these rats. All groups regained their rotarod ability at a lower BEC than when they lost it [$F(l,90)=236.20$], indicating the development of sensitization or reverse tolerance (Fig. 8).

HAS rats also took significantly longer than CAS and LAS rats to regain the ability to negotiate the rotarod at 25 DOA [$F(2,30)=8.52$], and 180 DOA [$F(2,30)=7.18$], but not at 70 DOA (see Fig. 9).



Display Full Size version of this image (13K)

Fig. 9. Comparison of BEC at loss and regain of the ability to negotiate a fixed-speed rotarod following ethanol administration in rats at 25, 70, or 180 DOA. All groups regained coordination at significantly lower BECs than at loss. (a) HAS significantly different from CAS and LAS at loss. (b) HAS significantly different from LAS at regain. (c) HAS significantly different from CAS at regain.

4. Discussion

HAS rats were more sensitive to ethanol than CAS or LAS rats as young as 15 DOA, and age-related increases in ethanol-induced narcosis and incoordination were evident in all lines. Overall, immature HAS and LAS rats required much higher doses of ethanol to bring about loss of the righting reflex, and their corresponding BECs at regain of the righting reflex were much higher than older rats. The lower waking BECs observed in the older animals, particularly the HAS rats, suggests that age and line differences are attributable to either alterations in ethanol neurosensitivity, or to a slower acquisition of acute functional tolerance to ethanol (Tabakoff et al., 1980), or both. Similar developmental profiles were observed in Sprague–Dawley rats by Silveri and Spear (1997), who reported a marked increase in sensitivity to ethanol hypnosis during ontogeny. However, this pattern is contrary to that reported by Keir and Deitrich (1990) in LS and SS mice, where younger mice demonstrated a greater sensitivity to ethanol as evidenced by longer sleep times and lower waking BECs than older mice.

Fifteen-day-old HAS rats consistently slept longer than LAS rats at all common doses. However, the lines did not differ in first dose sensitivity to ethanol as estimated by the *y*-intercepts illustrated in Fig. 3 and Fig. 4. On the other hand, they did differ significantly in acute tolerance to ethanol as measured by the slopes obtained from the dose–response curves (Fig. 1 and Fig. 2). Thus, the initial divergence in HAS/LAS ethanol sensitivity appears to be due to differences in the development of acute ethanol tolerance. At 25 and 40 DOA, both measures were significant and the HAS/LAS differences in ethanol sensitivity appear to be due to a combination of acute tolerance and first dose sensitivity. After 40 DOA, relatively little dose-dependent acute tolerance was observed in either HAS or LAS rats. Consequently, the differences in these older animals are ascribed primarily to alterations in first dose sensitivity to ethanol. It should be noted that an accurate index of acute tolerance could not be determined for rats at 180 DOA. At the higher doses, many of the 180 DOA rats slept longer than the 600-min time limit placed on regain of the righting reflex. Since the index of acute tolerance used in this study is a reflection of the linear relationship between dose of ethanol and BEC at awakening, including the BECs obtained at the 600-min time point resulted in artificially inflated slopes. However, if data from the higher doses were excluded from these calculations, none of the slopes would differ significantly from zero at 180 DOA.

Similar developmental changes in expression of ethanol sensitivity have been reported in other studies. Even though their animals became less sensitive, instead of more sensitive, with age, Keir and Deitrich (1990) concluded that the differential sensitivity of the LS and SS mice prior to 18 DOA was due to differences in the development of acute tolerance. They also suggested that a combination of differences in first dose sensitivity and the acquisition of acute tolerance to ethanol contributed to the LS/SS differences later in development. Comparably, Silveri and Spear (1997) observed a developmental decline in acute tolerance that was associated with an increase in sensitivity to first-dose ethanol hypnosis. Little et al. (1996) also found that 20 DOA male Sprague–Dawley rats were less sensitive to the hypnotic effects of ethanol vs. 60 DOA rats. While evidence suggested that some tolerance developed in the 20 DOA rats, they did not determine if acute tolerance was responsible for the differences in sleep time.

Several factors may contribute to age-related alterations in ethanol sensitivity, including differences in ethanol-induced hypothermia, metabolism of ethanol, and volume of distribution of ethanol. Preweanling, juvenile, and young adult rats are more susceptible to ethanol-induced hypothermia due to their higher surface area to volume ratio. Themoregulatory control is attained gradually during the preweanling period (Adels and Leon, 1986), and mice as young as 10 DOA have demonstrated ethanol-induced hypothermia following doses of 3 g/kg ethanol (Hunt et al., 1991). However, higher susceptibility to ethanol-induced hypothermia would predict that the younger rats would be more sensitive to ethanol, rather than less sensitive as observed. In their developmental study, Silveri and Spear (1997) examined the effect of testing temperature on ethanol-induced sleep time in 16 DOA rat pups. In general, they found that the pups tested in a warmed (nest temperature) environment slept significantly longer, and had lower BECs at awakening than the pups tested at room temperature. While the authors did not compare body temperature at awakening between the groups, it is unlikely that the inability to thermoregulate contributed to the observed differences. Developmental alterations in the pharmacokinetic properties of ethanol have also been documented in rats (Kelly and Wanwimolruk). Alcohol dehydrogenase and aldehyde dehydrogenase, the main enzymes in ethanol metabolism, do not reach mature levels until approximately 20 DOA (Hollstedt and Rydberg, 1985). In one study, preweanling rats had slower rates of ethanol clearance than 60 DOA rats, and infant rats also were able to achieve higher maximum BECs than older animals (Kelly et al., 1987). Other studies in aged rats (9–12 months) confirmed that lower drug concentrations were obtained in neural tissues following ethanol administration relative to subadult (35 DOA) rats (Wanwimolruk and Levy, 1987). However, these factors would also render the young animals more sensitive to ethanol, rather than less sensitive as observed in this study.

Ontogenetic changes in total body and brain water content also may contribute to age-related differences in pharmacological sensitivity. The proportion of total body water to body mass is known to decrease with age in rodents and other animals (Abel and York, 1979). Due to this decline in the volume of distribution for ethanol with age, older rats have been reported to achieve higher BECs following ethanol administration than younger rats (York and Chan, 1993). However, when the ethanol doses were adjusted to produce the same blood ethanol level, 25-month-old rats still demonstrated greater ataxia and regained their righting reflex at lower BECs than the younger animals (York and Chan, 1993). These findings suggest that some but not all age-related increases in ethanol sensitivity may be due to systematic overdosing of older animals when administration of ethanol is based upon body mass. In the current study, we compared the BECs at the appearance of a specific target response, such as regain of the righting reflex. As in the York and Chan (1993) study, it appears that differences between the age groups resulted from changes in the neurosensitivity of the older rats. With few exceptions, older rats had lower BECs at the regain of the righting reflex and at loss and regain of rotarod coordination than younger rats. Greater water content in the brains of younger animals could also lead to diluted ethanol

concentrations. When brain ethanol levels at awakening were compared in rats ranging from 16 to 96 DOA, brain ethanol levels also became lower with age (Silveri and Spear, 1997). Thus, it is possible that the differences in the development of ethanol sensitivity and acute tolerance may be due to a combination of pharmacokinetic, body composition, and neurosensitivity changes with age.

Sex differences in hypnotic sensitivity to ethanol were evident with males having significantly greater sleep times and higher BECs at awakening than females. Moreover, these differences became more marked with age. These results are different from Silveri and Spear's (1997) where males also were more sensitive to ethanol-induced narcosis than females with age, but in the absence of any differences in BEC or brain ethanol concentration at awakening. Because the males and females in our study differed in BEC at awakening, the sex differences in hypnotic sensitivity cannot be explained by possible differences in the elimination rates of ethanol. Since the sex difference becomes greater as the animals get older, it is possible that ethanol sensitivity is correlated with increasing body mass. However, total ethanol load resulting from dosing based on body weight does not appear to fully account for the sex differences in this study. Unfortunately, little data regarding the mechanism of difference in ethanol sensitivity of male and female rats have been presented. However, these data eventually may prove valuable in this endeavor.

HAS and LAS rats differed in first-dose ethanol sensitivity at the earliest age tested. However, when the response of each selected line to a dose of 3.5 g/kg ethanol was compared to the nonselected control line, the developmental emergence of high and low alcohol sensitivity appears to differ. LAS rats were less sensitive than CAS rats at the onset of the developmental profile, and the relationship of their sensitivity relative to the CAS line does not change with age (see Fig. 5 and Fig. 6). On the contrary, the HAS rats do not appear to differ significantly in ethanol sensitivity from the CAS rats at the early ages of ontogeny, but become increasingly more sensitive to ethanol with age relative to the CAS rats. It should be emphasized that the genes responsible for controlling high and low sensitivity to ethanol may not be different alleles at the same loci. That is, some of the alleles responsible for high sensitivity to ethanol may occur at entirely different loci than the genes responsible for low sensitivity to ethanol. If the control line is thought of as displaying the normal profile of response to ethanol with age, it might be speculated that the genes responsible for controlling low sensitivity to ethanol in the LAS line are expressed prior to 15 DOA. On the other hand, the genes responsible for high sensitivity to ethanol in the HAS rats may not exert their action until sometime after 15 DOA.

One advantage in examining the developmental profile of drug response is that the behavioral functions may be correlated with the appearance and maturation of specific neuronal circuitry (Spear, 2000). Alcohol is known to enhance the inhibitory effects of gamma-aminobutyric acid (GABA) on chloride flux via ion channels coupled to the $GABA_A$–receptor complex (Harris and Harris). Furthermore, HAS rats have been shown to be more sensitive to the effects of ethanol on GABA-mediated chloride flux relative to LAS rats (Allan and Liu). Many multiple forms have been described for the $GABA_A$–receptor complex, with pharmacological expression being contingent upon the heterology of subunit composition (Levitan et al., 1988). The subunits appear to develop and be expressed at varying points of maturation. For example, mRNA levels of the alpha-1 subunit appear to increase with age, while the levels of alpha-2 subunits are initially high at birth and appear to decrease with age, apparently replaced by the alpha-1 subunit (Bovolin; Primus; Gambarana and Fritschy). Furthermore, the developmental expression of alpha-1 subunit mRNA in the cerebellum appears to be maximal at 21 DOA (Gambarana et al., 1991). The beta-2 and -3 subunits are present at all ages (Fritschy et al., 1995). Studies examining brain mRNA or cRNA expression in *Xenopus* oocytes suggest that GABA-mediated ethanol sensitivity requires the presence of the gamma-2_{long} subunit (Wafford and Wick). Bovolin et al. (1992) examined the ontological profile of gamma-2_{long} subunit mRNA in the maturing rat cerebellum and found that the mRNA content increased continuously with age after 7 DOA. The age-related changes in ethanol sensitivity observed in the rats may be related to the appearance and disappearance of these various GABA subunits.

Another neurotransmitter system implicated in the effects of ethanol is the NMDA–glutamate receptor system (Lovinger; Lovinger and Hoffman). This receptor system is known to undergo structural (Brady; Feldmeyer and Zhong) as well as functional (Horimoto and Nabekura) changes during development. In addition, Swartzwelder et al. (1995) found that NMDA-mediated synaptic activity in hippocampal slices from young animals is more sensitive to ethanol than in similar slices from adult animals. This is opposite of what would be expected from our results as well as those from Little et al. (1996). However, it would be consistent with the findings of Keir and Deitrich (1990) in SS and LS mice where it was found that 15-day-old mouse pups were much more sensitive to ethanol than older mice. This result has been challenged by Fang et al. (1997) who used a different strain of mice and different conditions. It is possible that the NMDA system is the controlling neurotransmitter system in neonatal SS and LS mice as well as in young rats, but the GABA system is the controlling neurotransmitter system in older mice and determines the sleep time response to ethanol. This would imply that the NMDA

system also is more susceptible to development of acute tolerance, since it is only in young animals that tolerance is seen to develop in these studies, those of Keir and Deitrich as well as in the studies of Little et al. (1996).

The experiments on development of tolerance as measured on the rotarod appear to tap a different set of genes that bear little relationship to the sleep time measure. Thus, the BEC at loss and regain of ability to run on the rotarod revealed almost no difference between the lines at 25, 70, or 180 DOA. A second experiment directly correlating acute functional tolerance by a slightly different procedure (Lundhal et al. in preparation), also revealed no correlation between these two measures. These results are similar to those obtained with selectively bred lines of mice, the SS and LS mice, as well as the high and low acute functional tolerance (HAFT and LAFT) mice. We have found that sleep time measures, acute functional tolerance as measured by a two-dose method (Erwin and Deitrich, 1996) and acute single dose tolerance, as measured by the procedure of Gill and Deitrich (1998), have no quantitative trait loci in common (Deitrich et al., 2000). It is clear from these studies that markedly different results can be obtained depending on the behavior tested.

In summary, this study is an attempt to dissect the contributions of genetics to the development and expression of sensitivity to ethanol as a function of age. The ontological profile of ethanol response was examined in selected rat lines demonstrating initial differential sensitivity to the hypnotic effects of ethanol. The selected lines differed in ethanol sensitivity from the earliest age tested, and the magnitude of this difference increased with age. Without exception, older rats of all lines were more sensitive to ethanol than younger rats. Developmental profiles of drug responses may provide important clues to determining mechanisms of neural action and involvement of specific genetic systems, such as the genes responsible for GABA response to ethanol.

Acknowledgements

This work was supported by grants AA05868, AA00093, and AA07464. The experiments reported here were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The authors wish to thank Tina Fay for her excellent technical assistance.

References

Abel, EL and York, JL, 1979. Age-related differences in the response to ethanol in the rat. *Physiol Psychol* **7**, pp. 391–395. View Record in Scopus | Cited By in Scopus (5)

Adels, LE and Leon, M, 1986. Thermal control of mother–young contact in Norway rats: factors mediating the chronic elevation of maternal temperature. *Physiol Behav* **36**, pp. 183–196. Abstract | View Record in Scopus | Cited By in Scopus (7)

Allan, AM, Mayes, GG and Draski, LJ, 1991. Gamma-aminobutyric acid-activated chloride channels in rats selectively bred for differential acute sensitivity to alcohol. *Alcohol: Clin Exp Res* **15**, pp. 212–218. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (18)

Bovolin, P, Santi, MR, Memo, M, Costa, E and Grayson, DR, 1992. Distinct developmental patterns of expression of rat alpha 1, alpha 5, gamma 2S, and gamma 2L gamma-aminobutyric acid A receptor subunit mRNAs in vivo and in vitro. *J Neurochem* **59**, pp. 62–72. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (57)

Brady, RJ, Gorter, JA, Monroe, MTM and Swann, JW, 1994. Developmental alterations in the sensitivity of hippocampal NMDA receptors of AP5. *Dev Brain Res* **83**, pp. 190–196. Abstract | View Record in Scopus | Cited By in Scopus (11)

Deitrich, RA, 1990. Genetics of alcoholism: how do we find the answers and what do we do then?. *Alcohol Alcohol* **25**, pp. 571–572. View Record in Scopus | Cited By in Scopus (2)

Deitrich, RA, Bludeau, P and Erwin, VG, 2000. Phenotypic and genotypic relationships between ethanol tolerance and sensitivity in mice selectively bred for initial sensitivity to ethanol (SS and LS) or development of acute tolerance (HAFT and LAFT). *Alcohol: Clin Exp Res* **24**, pp. 595–604. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (21)

Erwin, VG and Deitrich, RA, 1996. Genetic selection and characterization of mouse lines for acute functional tolerance to ethanol. *J Pharmacol Exptl Therap* **279**, pp. 1310–1317. View Record in Scopus | Cited By in Scopus (51)

Draski, LJ, Spuhler, KP, Erwin, VG, Baker, RC and Deitrich, RA, 1992. Selective breeding of rats differing in sensitivity to the effects of acute ethanol administration. *Alcohol: Clin Exp Res* **16**, pp. 48–54. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (42)

Fang, Z, Ionescu, P, Gong, D, Kendig, J, Harris, A and Eger, EI, 1997. Maturation decreases ethanol minimum alveolar anesthetic concentration in mice as previously demonstrated in rats: there is no species difference. *Anesth Analg* **85**, pp. 160–163. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (9)

Feldmeyer, D and Cull-Candy, S, 1996. Functional consequences of changes in NMDA receptor subunit expression during development. *J Neurocytol* **25**, pp. 857–867. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (49)

Fritschy, J-M, Paysan, J, Enna, A and Mohler, H, 1995. Switch in the expression of rat $GABA_A$-receptor subtypes during postnatal development: an immunohistochemical study. *J Neurosci* **14**, pp. 5302–5324.

Gambarana, C, Pittman, R and Siegel, RE, 1990. Developmental expression of the $GABA_A$ receptor alpha 1 subunit mRNA in the rat brain. *J Neurobiol* **21**, pp. 1169–1179. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (28)

Gambarana, C, Beattle, CE, Rodriguez, ZR and Siegel, RE, 1991. Region-specific expression of messenger RNAs encoding $GABA_A$receptor subunits in the developing rat brain. *Neuroscience* **45**, pp. 423–432. Abstract | View Record in Scopus | Cited By in Scopus (37)

Gill, K and Deitrich, RA, 1998. Acute tolerance to the atopic effects of ethanol in short-sleep (SS) and long-sleep (LS). *Mice Psychopharmacology* **136**, pp. 91–98. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (20)

Harris, RA, 1990. Distinct actions of alcohols, barbiturates and benzodiazepines on GABA-activated chloride channels. *Alcohol* **7**, pp. 273–275. Abstract | View Record in Scopus | Cited By in Scopus (16)

Harris, RA and Allan, AM, 1989. Alcohol intoxication: ion channels and genetics. *FASEB J* **3**, pp. 1689–1695.

Hoffman, PL, Rabe, CS, Grant, KA, Valverius, P, Hudspith, M and Tabakoff, B, 1990. Ethanol and the NMDA receptor. *Alcohol* **7**, pp. 229–231. Abstract | View Record in Scopus | Cited By in Scopus (47)

Hollstedt, C and Rydberg, U, 1985. Postnatal effects of alcohol on the developing rat. In: Rydberg, U, Alling, C and Engle, JA, Editors, 1985. *Alcohol and the developing brain*, Raven Press, New York, pp. 69–84.

Horimoto, N, Nabekura, J and Ogawa, T, 1996. Developmental changes in arachidonic acid potentiation of NMDA currents in cortical neurons. *NeuroReport* **7**, pp. 2463–2467. View Record in Scopus | Cited By in Scopus (6)

Hunt, PS, Spear, LP and Spear, NE, 1991. An ontogenetic comparison of ethanol-mediated taste aversion learning and ethanol-induced hypothermia in preweanling rats. *Behav Neurosci* **105**, pp. 971–983. Abstract | **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (22)

Keir, WJ and Deitrich, RA, 1990. Development of central nervous system sensitivity to ethanol and pentobarbital in short- and long-sleep mice. *J Pharmacol Exp Ther* **254**, pp. 831–835. View Record in Scopus | Cited By in Scopus (34)

Kelly, SJ, Bonthius, DJ and West, JR, 1987. Developmental changes in alcohol pharmacokinetics in rats. *Alcohol: Clin Exp Res* **11**, pp. 281–286. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (59)

Keppel, G, 1982. Design and analysis. In: *A researcher's handbook*, Prentice–Hall, Englewood Cliffs (NJ), p. 669.

Levitan, ES, Schofield, PR, Burt, DR, Rhee, LM, Wisden, W, Kohler, M, Fujita, N, Rodriguez, HF, Stephenson, A, Darlison, MG, Barnard, EA and Seeburg, PH, 1988. Structural and functional basis for $GABA_A$ receptor heterogeneity. *Nature* **335**, pp. 76–79. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (96)

Little, PJ, Kuhn, CM, Wilson, WA and Swartzwelder, HS, 1996. Differential effects of ethanol in adolescent and adult rats. *Alcohol: Clin Exp Res* **20**, pp. 1346–1351. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (70)

Liu, Y and Deitrich, RA, 1998. Role of GABA in the actions of ethanol in rats selectively bred for ethanol sensitivity. *Pharmacol, Biochem Behav* **60**, pp. 793–801. SummaryPlus | **Full Text + Links** | PDF (126 K) | View Record in Scopus | Cited By in Scopus (11)

Lovinger, DM, White, G and Weight, FF, 1989. Ethanol inhibition of neuronal glutamate receptor function. *Ann Med* **22**, pp. 247–252.

Lovinger, DM, White, G and Weight, FF, 1990. NMDA receptor-mediated synaptic ex citation selectively inhibited by ethanol in hippocampal slice from adult rat. *J Neurosci* **10**, pp. 1372–1379. View Record in Scopus | Cited By in Scopus (135)

Nabekura, J, Kawamoto, I, Horimoto, N and Ogawa, T, 1995. Developmental change of the potentiation of NMDA response by spermine. *NeuroReport* **6**, pp. 994–996. View Record in Scopus | Cited By in Scopus (3)

Primus, RJ, Jacobs, AA and Gallager, DW, 1992. Developmental profile of polyadenylated and non-polyadenylated $GABA_A$ receptor subunit mRNAs. *Brain Res Mol Brain Res* **14**, pp. 179–185. Abstract | View Record in Scopus | Cited By in Scopus (3)

Propp, EL, Erickson, CK, Past, ZM, Meton, CV and Omar, MA, 1990. Ethanol effects of blood ethanol parameters in four ages of Fischer rats. *Alcohol: Clin Exp Res* **14**, p. 330 Abstract.

Silveri, MM and Spear, LP, 1997. Decreased sensitivity to the hypnotic effect of ethanol early in ontogeny. *Alcohol: Clin Exp Res* **22**, pp. 670–676.

Smolen, A and Smolen, TN, 1987. 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* **7**, pp. 279–283. View Record in Scopus | Cited By in Scopus (11)

Spear, LP, 2000. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* **24**, pp. 417–463. SummaryPlus | **Full Text + Links** | PDF (414 K) | View Record in Scopus | Cited By in Scopus (479)

Swartzwelder, HS, Wilson, WA and Tayyeb, MI, 1995. Differential sensitivity of NMDA receptor-mediated synaptic potentials to ethanol in immature versus mature hippocampus. *Alcohol: Clin Exp Res* **19**, pp. 320–323. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (76)

Tabakoff, B, Ritzmann, RF, Raju, TS and Deitrich, RA, 1980. Characterization of acute and chronic tolerance in mice selected for inherent differences in sensitivity to ethanol. *Alcohol: Clin Exp Res* **4**, pp. 70–73. View Record in Scopus | Cited By in Scopus (17)

Wafford, KA, Burnett, DM, Leidenheimer, NJ, Burt, DR, Wang, JB, Kofuji, R, Dunwiddie, TV, Harris, RA and Sikela, JM, 1991. Ethanol sensitivity of the $GABA_A$ receptor expressed in Xenopus oocytes requires 8 amino acids contained in the gamma 2L subunit. *Neuron* **7**, pp. 27–33. Abstract | View Record in Scopus | Cited By in Scopus (166)

Wanwimolruk, S and Levy, G, 1987. Effect of age on the pharmacodynamics of phenobarbital and ethanol in rats. *J Pharm Sci* **76**, pp. 503–507. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (5)

Wick, MJ, Radcliffe, RA, Bowers, BJ, Mascia, MP, Lüscher, B, Harris, RA and Wehner, JM, 2000. Behavioural changes produced by transgenic overexpression of γ2L and γ2S subunits of the $GABA_A$ receptor. *Eur J Neurosci* **12**, pp. 2634–2638. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (21)

York, JL, 1983. Increased responsiveness to ethanol with advancing age in rats. *Pharmacol, Biochem Behav* **19**, pp. 687–691. View Record in Scopus | Cited By in Scopus (10)

York, JL and Chan, AW, 1993. Age-related differences in sensitivity to alcohol in the rat. *Alcohol: Clin Exp Res* **17**, pp. 864–869. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (15)

Zhong, J, Carrozza, DP, Williams, K, Pritchett, DB and Molinoff, PB, 1995. Expression of mRNAs encoding subunits of the NMDA receptor in developing rat brain. *J Neurochem* **64**, pp. 531–539. View Record in Scopus | Cited By in Scopus (157)

Corresponding author. Tel.: +1-303-315-7076; fax: +1-303-315-7097

Pharmacology Biochemistry and Behavior
Volume 70, Issues 2-3, October-November 2001, Pages 387-396