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Differential vulnerability to motor deficits in second replicate HAS and LAS rats following neonatal alcohol exposure

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Abstract

Children exposed prenatally to alcohol suffer from a variety of behavioral alterations. However, variation exists in the pattern and severity of these alcohol-related neurodevelopmental disorders. We examined the influence of alcohol sensitivity in the etiology of fetal alcohol effects by studying rat lines selectively bred for extremes in alcohol-induced sleep time: high-alcohol-sensitive (HAS) and low-alcohol-sensitive (LAS) rats. Using subjects from the first replicate, we previously reported that HAS rats exposed to alcohol during development were more vulnerable to ethanol-induced hyperactivity and motor deficits compared to LAS rats. To determine if these effects were, in fact, related to the trait for which these subjects were selected, the present study examined the consequences of developmental alcohol exposure in second replicate HAS and LAS rats. Second replicate HAS and LAS rats, as well as Sprague–Dawley rats, were exposed to 6.0 g/kg/day ethanol on Postnatal Days (PD) 4–9, a period of brain development equivalent to the third trimester, via an artificial rearing procedure. Artificially and normally reared controls were included. Activity was measured on PD 18–21 and parallel bar motor coordination on PD 30–32. Ethanol exposure produced hyperactivity in all genetic groups, and there were no differences among HAS and LAS rats. In contrast, consistent with findings from the first replicate, ethanol-exposed HAS rats were more impaired on the motor coordination task compared with LAS rats. These data suggest that genetically mediated responses to alcohol may relate to behavioral vulnerability to motor deficits following developmental alcohol exposure. They also provide evidence that genetic factors play a role in fetal alcohol effects and suggest that phenotypic markers may indicate individuals at high risk for some fetal alcohol effects.

Keywords: Genetic; Fetal alcohol; Teratogen; Activity

1. Introduction

Prenatal alcohol exposure can produce central nervous system (CNS) damage and consequent behavioral alterations (West et al., 1994; Mattson and Riley, 1998; Roebuck et al., 1998; National Institute on Alcohol Abuse and Alcoholism, 2000). However, there is great heterogeneity in the behavioral outcome of children exposed to alcohol during gestation (Abel, 1995; Abel and Hannigan, 1995; Abel and Sokol, 1986; Ernhart et al., 1987; Burd and Martsolf, 1989; Bookstein et al., 2002). Many factors may contribute to this variation, including alcohol dose, pattern of consumption, timing of exposure, nutritional status, polydrug use, and prenatal care. Genetic factors can also contribute to this variation. For example, animal studies have demonstrated that genotype influences mortality, growth reductions, physical malformations, craniofacial anomalies, brain damage, and behavioral alterations induced by developmental alcohol exposure (Chernoff, 1977; Giknis et al., 1980; Goodlett et al., 1989; Gilliam and Kotch, 1990; Boehm et al., 1997; Cavieres and Smith, 2000; Debelak and Smith, 2000; Su et al., 2001).

As part of our continuing investigation into the relationship between genetically mediated responses to alcohol and vulnerability to alcohol's teratogenic effects, we recently reported differential vulnerability to alcohol's teratogenicity in rat lines bred for extremes in sensitivity to alcohol's initial hypnotic effects, the high-alcohol-sensitive (HAS) and low-alcohol-sensitive (LAS) rats (Thomas et al., 1998b; 2000). The HAS and LAS lines were selectively bred for extremes in alcohol-induced sleep time

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(Draski et al., 1992). In other words, HAS rats require a longer period of time to recover from alcohol's acute hypnotic effects compared with LAS rats. Alcohol exposure during the third trimester brain growth spurt, a period of brain development that occurs during the early neonatal period in the rat, produced more severe hyperactivity (Thomas et al., 1998b) and motor coordination deficits (Thomas et al., 2000) in HAS rats, compared with LAS rats. These findings suggest that differences in CNS sensitivity to alcohol's hypnotic effects may be related to vulnerability to fetal alcohol effects.

One of the advantages of the HAS and LAS lines is that two parallel selections were conducted, producing a second replicate of HAS and LAS lines. If similar results are found in both replicates, it provides stronger evidence that the differential vulnerability to alcohol's teratogenic effects is, indeed, related to the trait for which these lines were selected and not due to spurious cosegregation of traits. The present study examines the vulnerability of second replicate HAS and LAS lines to alterations in activity level and motor coordination following ethanol treatment during the third trimester equivalent. In addition, we tested Sprague–Dawley (S–D) rats to compare results with a commonly used rat strain.

2. Methods

2.1. Subjects

Second replicate HAS and LAS dams and studs were provided by Dr. Richard Dietrich from the University of Colorado Health Science Center, whereas S–D breeders were purchased from Charles Rivers. Animals were bred at the San Diego State University Animal Care Facility. Within each genetic group, a male and a female were housed overnight and the presence of a seminal plug the following morning designated Gestational Day 0. Pregnant dams were then singly housed with food and water ad libitum. On the day following birth, litters were culled to 10 pups, with five males and five females when possible.

Within each line, subjects were randomly assigned to one of three treatment groups: ethanol-exposed (EtOH), gastrostomy control (GC), or normally reared suckle control (SC). Thus, this study used a $2 \times 3 \times 3$ [Sex (male, female) × Genotype (HAS, LAS, S–D) × Treatment (EtOH, GC, SC)] design. Each group contained 7–16 subjects, for a total of 211 subjects. The number of subjects for each group is shown in Table 1. All procedures used in this study are in compliance with the NIH Guide for Care and Use of Laboratory Animals.

2.2. Artificial rearing

To control for nutritional status, alcohol administration was accomplished via an artificial rearing procedure. On postnatal day (PD) 4, subjects were randomly assigned within litter to one of the three treatment groups (EtOH, GC, SC). Subjects in the EtOH and GC treatment groups were gastrostomized and artificially reared using the "pup-in-a-cup" method (Diaz and Samson, 1980). Subjects in the SC group were fostered to a lactating S–D dam along with nonexperimental subjects, maintaining the litter size at 10.

Table 1
Subject number and mean (±S.E.M.) body weights (g) for each treatment group.

Genotype	Treatment	*n*	Body weight	
			PD 18	PD 30
HAS	EtOH	M (7), F (9)	29.0 ± 0.6	74.5 ± 1.9
	GC	M (12), F (11)	29.6 ± 0.6	76.1 ± 1.2
	SC	M (16), F (14)	41.7 ± 0.8 **	92.0 ± 2.0 **
LAS	EtOH	M (10), F (13)	30.0 ± 0.6	76.7 ± 1.4
	GC	M (12), F (8)	29.3 ± 0.8	75.3 ± 1.8
	SC	M (15), F (14)	37.5 ± 1.0 **	88.4 ± 1.9 **
S–D	EtOH	M (10), F (8)	32.5 ± 0.7	90.9 ± 3.6
	GC	M (12), F (9)	33.3 ± 0.9	90.7 ± 3.4
	SC	M (15), F (16)	43.4 ± 0.8 **	112.0 ± 3.6 **

Artificially reared groups (EtOH and GC) lagged in growth compared to the SC groups within each genetic group, an effect that was still present at the time of behavioral testing (PD 18 and PD 30).

** Significantly different from EtOH and GC within genetic group.

Subjects undergoing gastrostomy surgery were anaesthesized with a halothane/oxygen (50/50%) mix. A gastrostomy tube was surgically implanted and held in place with press-fit washers (see Thomas et al., 2000 for more details). Following surgery, pups were placed individually in cups filled with wood chips and artificial fur. Wood chips from the mother dam's cage were also added to provide familiar odor cues. Each cup floated in a water-filled tank that maintained the temperature inside the cup at 35 °C. Each pup's gastrostomy tube was attached to a syringe containing a nutritionally balanced milk diet (West et al., 1984). Every 2 h, a timer-controlled infusion pump (Model 980566; Harvard Apparatus) delivered the milk diet into the gastrostomy tubes for a 20-min delivery period. Pups were weighed each morning and the mean body weight (in grams) was calculated. The daily volume of milk diet (in milliliters) was calculated as 33% of the mean body weight for pups maintained on each artificial rearing apparatus. Pups were bathed twice a day and their anal–genital areas were stimulated to facilitate excretion. Double-distilled water was injected into the gastrostomy tubes twice each day to keep the tubes patent.

From PD 4 to PD 9, ethanol (6.8% vol/vol) was added to the diets of EtOH subjects during the first four consecutive feedings each day, for a total dose of 6.0 g/kg/day. Feedings began between 0800 and 1100 h in the morning. During ethanol feedings, isocaloric maltose–dextrin was added to the diets of GC subjects. Milk diet only was delivered during the remaining eight feedings each day. Subjects were maintained in the artificial rearing environment and fed milk only on PD 10 and PD 11 to allow the pups to undergo any

withdrawal before being fostered back to a lactating dam. On PD 11, India ink was injected in the subjects' paws for later identification and, on PD 12, subjects were fostered back to a Sprague–Dawley dam along with the SC pups. The pups remained with the lactating dam until PD 21, at which time they were weaned. Litters remained group-housed until separated by sex on PD 25 and were housed under a 12:12 light/dark cycle in a temperature- and humidity-controlled animal facility.

2.3. Blood alcohol level

On PD 6, 1.5 h after the start of the last alcohol feed, 20 μl of blood were drawn from a tail clip from each artificially reared subject to determine peak blood alcohol level. Blood samples were analyzed using the Analox Alcohol Analyzer (Model AM1).

2.4. Behavioral testing

2.4.1. Activity level

On PD 18 to PD 21, activity level was measured in an automated open field [16 in. (W) × 18 in. (L) × 15 in. (H)]. The Plexiglas open field was contained in a sound-attenuated chamber with a fan, which provided a masking noise and ventilation. The open field contained a grid of infrared beams (Digiscan model RXYCM; Omnitech Electronics) that tracked each subject's movement.

Subjects were placed in the testing room 30 min prior to testing to allow for acclimation. Each subject was then placed in the center of the activity chamber and activity was recorded. Chambers were cleaned prior to testing of each subject to eliminate odor cues. Activity was recorded every 5 min for a period of 0.5 h/day for four consecutive days during the subjects' light cycle. Total distance traveled served as the performance measure.

2.4.2. Parallel bars

On PD 30–32, subjects were tested on a parallel bar motor coordination task. The parallel bar apparatus consisted of two steel rods (0.5 cm diameter, 91 cm long) suspended between two platforms (15.3 × 17.8 cm). The rods and platforms stood at a height of 63 cm above a thick layer of wood chip bedding. Subjects were allowed to acclimate on the platform for 30 s before being balanced on the parallel bars halfway between the platforms, with right paws on one rod and left paws on the other. The subject was then required to traverse toward the platform. A successful trial consisted of four consecutive, alternating steps with the hind-limbs. If the subject dragged its hindlimbs or slipped, the trial was unsuccessful.

At the initiation of training, the width between the rods was 3 cm. Subjects were given up to five consecutive trials to successfully traverse with an intertrial interval of 5–10 s. If unsuccessful after five trials, testing for that day was complete. If successful, the gap between rods was increased in 0.5-cm increments. A maximum of 15 trials were given per day, for three consecutive days. At the beginning of each day, the subject was tested at the maximum successful gap achieved the previous day. Performance was measured by the ratio of successful to total trials, the maximum width successfully traversed, and the number of trials to the first success.

2.4.3. Data analyses

Data were analyzed with Genotype, Treatment, and Sex as between-subject factors, using SPSS software. Day and Bin served as within-subject repeated measures when appropriate. Newman–Keuls post-hoc tests were conducted with $\alpha = .05$.

3. Results

3.1. Body weight

Body weights from PD 4 to PD 14 were analyzed with Day as a repeated measure. There were main effects of Day [$F(10,1930) = 437$, $P < .001$] due to growth over days, Sex [$F(1,193) = 6.2$, $P < .05$], with males being heavier than females; and Genotype [$F(2,193) = 30.3$, $P < .001$] due to the larger weights of S–D rats compared with HAS and LAS rats. In addition, there was a main effect of Treatment [$F(2,193) = 119.4$, $P < .001$], as well as significant interactions of Day × Genotype [$F(20,1930) = 17.2$, $P < .001$], Day × Treatment [$F(20,1930) = 205$, $P < .001$], and Day × Genotype × Treatment [$F(40,1930) = 2.5$, $P < .001$]. The artificially reared groups lagged in growth compared to the suckle controls within each line, and SC subjects weighed more than the EtOH and GC subjects from PD 7 to PD 14 (see Fig. 1). The weight lag was slightly less in the LAS subjects, creating the significant interactions. Importantly, at no time were there significant differences in body

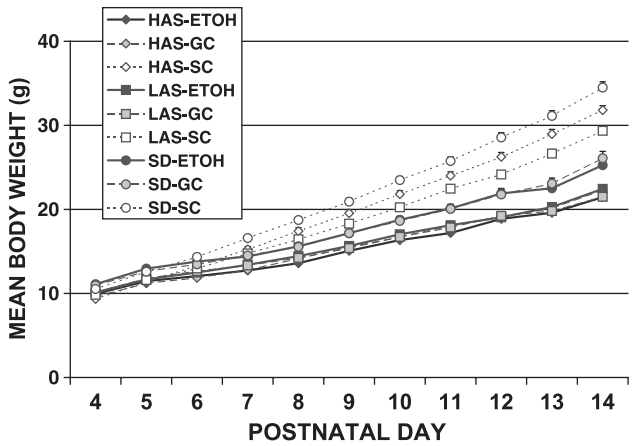

Fig. 1. Mean (±S.E.M.) body weights for all groups. All artificially reared groups lagged in growth compared with the suckle controls. However, there were no significant differences in body weight between EtOH and GC subjects within any genotype.

weight between EtOH and GC subjects within each genetic group.

Artificially reared subjects continued to weigh less than suckle controls during behavioral testing (see Table 1). At PD 18, during activity testing, there was a significant effect of Genotype [$F(2,193)=20.1$, $P<.001$], Treatment [$F(2,193)=175$, $P<.001$], and an interaction of Genotype × Treatment [$F(4,193)=3.2$, $P<.05$]. Suckle controls weighed more than EtOH and GC subjects, although the effect was slightly less pronounced in the LAS rats. Similarly, at PD 30, during parallel bar testing, there was a main effect of Genotype [$F(2,193)=56.5$, $P<.001$] due to the heavier weight of S–D subjects, and Treatment [$F(2,193)=60.7$, $P<.001$] due to the heavier weight of SC subjects. Males weighed more than females at both PD 18 (Sex [$F(1,193)=6.4$, $P<.05$]) and PD 30 (Sex [$F(1,193)=19.6$, $P<.001$]).

3.2. Blood alcohol level

Mean (±S.E.M.) blood alcohol levels were 228±9, 222±13, and 229±22 mg/dl for the HAS, LAS, and S–D groups, respectively. There were no significant differences among lines in blood alcohol level.

3.3. Activity level

Total distance traveled in the activity chamber is shown in Fig. 2. Due to equipment failure, data from 20 subjects were excluded from analyses (one female HAS EtOH, three male HAS GC, one female HAS GC, four male HAS SC, four female HAS SC, three female LAS EtOH, one male LAS GC, two male LAS SC, and one female LAS SC). As seen in Fig. 2, alcohol exposure produced hyperactivity in all genetic groups. However, the effect was differential across

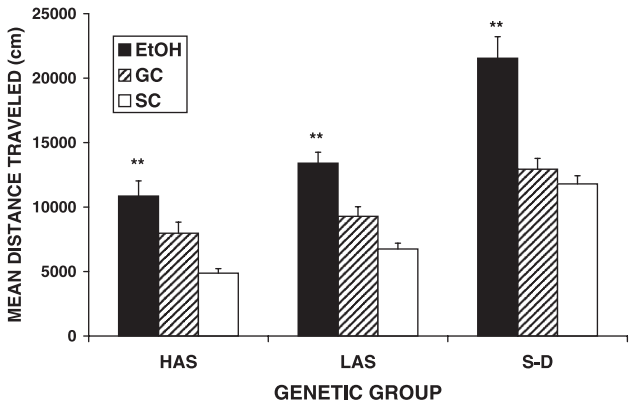

Fig. 2. Mean (±S.E.M.) total distance traveled in the activity chamber over 4 days of testing. Alcohol exposure during the third trimester equivalent produced hyperactivity in all genotypes. There were no significant differences in severity of hyperactivity between the HAS and LAS lines. Alcohol-induced overactivity in the S–D subjects was greater than that observed in HAS and LAS lines when measuring absolute activity ($P<.05$), but not when measuring percent difference from controls. * * Significantly different from GC and SC controls within genetic group.

genotype, producing a significant Genotype × Treatment interaction [$F(4,175)=3.0$, $P<.05$], in addition to significant effects of Treatment [$F(2,175)=59.2$, $P<.001$] and Genotype [$F(2,175)=63.9$, $P<.001$]. Follow-up analyses of the difference in activity level between alcohol-exposed and controls within each genotype indicated that alcohol exposure produced more severe hyperactivity in the S–D subjects compared to HAS and LAS subjects [mean (±S.E.M.) S–D=9288±1506 more centimeters traveled, HAS=4544±1374 cm, LAS=5524±1191 cm; $F(2,52)=3.8$, $P<.05$]. However, given differences in activity levels among controls, percent differences between alcohol-treated and controls within each line were also generated. There were no effects of line on alcohol-induced increases in activity as measured by percent change (HAS=72%, LAS=70%, and S–D=76%; $F<1$). Importantly, there were no differences in the severity of alcohol-induced hyperactivity between the HAS and LAS lines, differing from our finding with the first replicate.

In addition, there were significant effects of Day [$F(3,525)=140.2$, $P<.001$], Day × Genotype [$F(6,525)=4.3$, $P<.001$], Day × Treatment [$F(6,525)=10.4$, $P<.001$], Day × Sex [$F(3,525)=3.1$, $P<.05$], Bin [$F(5,875)=351.9$, $P<.001$], Bin × Genotype [$F(10,875)=5.3$, $P<.001$], Day × Bin [$F(15,2625)=2.0$, $P<.05$], Day × Bin × Genotype [$F(30,2625)=2.4$, $P<.001$], and Day × Bin × Genotype × Treatment [$F(60,2625)=1.4$, $P<.05$]. These interactions were due largely to differences in habituation within and between sessions in the S–D subjects compared to HAS and LAS rats (data not shown). There were no significant differences in habituation between the HAS and LAS lines.

3.4. Parallel bar motor performance

Alcohol exposure during the third trimester equivalent also produced motor deficits, although there was differential vulnerability to ethanol's effects among the genetic groups. Fig. 3 shows the ratio of successful to total traversals for each treatment group. One subject (HAS, SC) died prior to testing and 13 S–D subjects were inadvertently not tested on this task. There was a significant Genotype × Treatment interaction [$F(4,179)=3.8$, $P<.01$], as well as significant effects of Treatment [$F(2,179)=31.6$, $P<.001$] and Genotype [$F(2,179)=6.5$, $P<.01$]. Follow-up comparisons within each genotype illustrated that ethanol-exposed subjects in the HAS and S–D groups were more severely impaired in this task compared with the LAS line. Alcohol-exposed HAS and S–D subjects had significantly lower success ratios compared to their respective control groups (P's $<.05$). In contrast, alcohol-exposed subjects in the LAS line were only mildly affected. Motor performance of ethanol-exposed LAS subjects was significantly impaired compared to LAS SC controls, but did not differ significantly from LAS GC controls. There were no significant effects of sex on this or other motor measures.

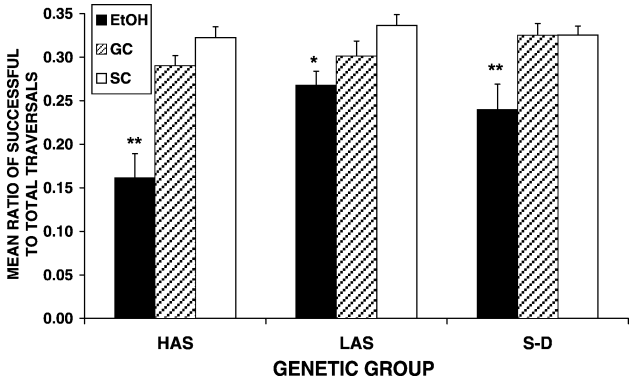

Fig. 3. Mean (±S.E.M.) ratio of successful to total traversals on the parallel bar motor coordination task as a function of treatment group. Ethanol exposure produced severe motor deficits in the HAS and S–D subjects, but only mild deficits in the LAS subjects, resulting in a significant interaction of Genotype × Treatment ($P<.01$). Follow-up analyses confirmed that the severity of ethanol-induced motor deficits in the HAS subjects was significantly greater compared to the LAS subjects. * Significantly different from SC controls within genetic group. * * Significantly different from GC and SC controls within genetic group.

To better illustrate differential vulnerability among genetic groups, difference scores between the success ratios of ethanol-exposed subjects and the mean success ratio of control subjects within genotype were generated. Difference scores were as follows: HAS 0.15 ± 0.03, LAS 0.05 ± 0.02, and S–D 0.08 ± 0.03. The difference in success ratio between EtOH and control groups in the HAS line was significantly larger compared to the LAS line, producing a significant effect of Genotype [$F(2,50)=4.5$, $P<.01$]. The severity of ethanol-related deficits in S–D subjects was intermediate, not differing significantly from either the HAS or LAS line.

Differential vulnerability among genetic groups was also evident in the number of trials to the first successful traversal, as shown in Fig. 4. Significant effects of Genotype [$F(2,179)=9.0$, $P<.001$], Treatment [$F(2,179)=22.6$, $P<.001$], and a Genotype × Treatment interaction [$F(4,179)=3.3$, $P<.05$] were found. Ethanol-treated subjects in each genetic group required more trials to reach success compared to GC and SC control subjects. However, the ethanol-related effects were more severe in the HAS rats compared with the LAS rats. Difference scores between ethanol-treated subjects and controls were generated for each genetic group: ethanol-exposed HAS subjects required 4.6 ± 1.2 more trials to reach success compared to HAS controls, ethanol-exposed LAS subjects required 1.8 ± 0.7 more trials to reach success compared to LAS controls, and ethanol-exposed S–D subjects required 2.0 ± 1.1 more trials to reach success compared to S–D controls. The difference score of HAS subjects was significantly greater than LAS subjects ($P<.05$); however, the difference score of the S–D subjects failed to differ significantly from the HAS line.

Maximum width between rods traversed was analyzed with Day as a repeated measure. There was a significant effect of Day [$F(2,358)=463.8$, $P<.001$] due to an increase in width traversed over days, Treatment [$F(2,179)=27.5$, $P<.001$] due to impaired performance of the ethanol-exposed subjects, and Genotype [$F(2,1790)=6.3$, $P<.01$]. In addition, there were significant interactions of Day × Treatment [$F(4,358)=7.4$, $P<.001$] due to larger improvements in controls compared to ethanol-exposed subjects over days, and a Day × Genotype interaction [$F(4,358)=3.8$, $P<.01$] (data not shown).

Fig. 5 shows the maximum width traversed on the last day of testing. Ethanol-exposed subjects in each genetic group were significantly impaired compared to their respective control groups, producing a main effect of Treatment [$F(2,179)=25.1$, $P<.001$]. In addition, there was a main effect of Genotype [$F(2,179)=6.5$, $P<.001$] due to smaller widths traversed in the HAS group, which was largely

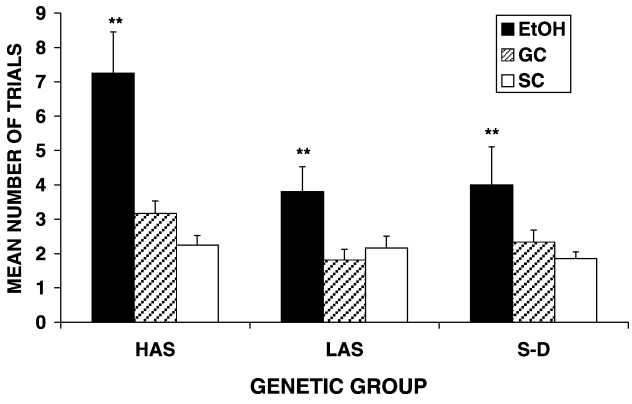

Fig. 4. Mean (±S.E.M.) number of trials to the first successful traversal on the parallel bar motor coordination task. Ethanol-exposed subjects took more trials to reach success compared to controls, but the ethanol effects were more severe in the HAS subjects compared with LAS subjects, producing a significant Genotype × Treatment interaction ($P<.05$). * * Significantly different from GC and SC controls within genetic group.

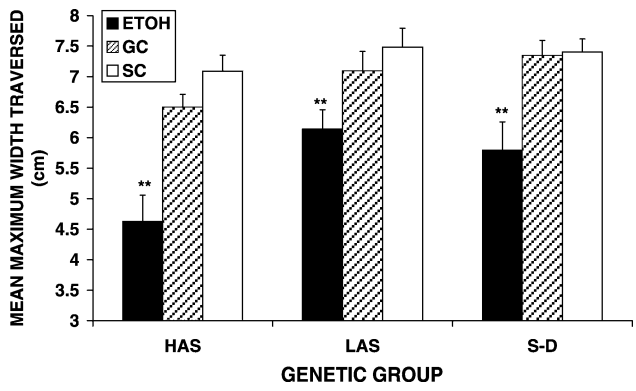

Fig. 5. Mean (±S.E.M.) maximum width successfully traversed on the parallel bar task. Ethanol-exposed subjects traversed significantly smaller widths compared to controls. Although there was a trend for the HAS subjects to be less successful than LAS subjects, the interaction was not statistically significant. * * Significantly different from GC and SC controls within genetic group.

driven by the HAS ethanol-exposed subjects. As seen in Fig. 5, ethanol-exposed HAS subjects showed little improvement, reaching an average maximum width of 4.5 cm (starting width was 3.0 cm). However, the HAS control subjects also tended to be less successful. Thus, although the ethanol-exposed HAS subjects performed more poorly than ethanol-exposed LAS and S–D subjects, the severity of deficit compared to control groups within genotype failed to reach statistical significance. Differences between ethanol-treated and control subjects within genetic group were as follows: HAS EtOH subjects successfully traversed 2.2 ± 0.4 cm less than HAS controls, LAS EtOH subjects traversed 1.2 ± 0.3 cm less than LAS controls, and S–D EtOH subjects traversed 1.6 ± 0.5 cm less than S–D controls.

4. Discussion

The present study demonstrates that HAS rats are more vulnerable to motor deficits induced by developmental alcohol exposure than LAS rats. Consistent with our findings in the first replicate (Thomas et al., 2000), ethanol exposure during the third trimester brain growth spurt produced more severe deficits on the parallel bar motor coordination task in the HAS rats compared with the LAS rats. Thus, HAS rats are more vulnerable, whereas LAS rats are relatively protected against ethanol's damaging effects on brain systems necessary for motor coordination.

In contrast, there were no significant differences among genetic groups in ethanol-induced alterations in activity level. Ethanol treatment during the third trimester equivalent produced hyperactivity in HAS, LAS, and S–D subjects. This differs from our findings with the first replicate, where we reported that HAS subjects were also more vulnerable to hyperactivity following neonatal alcohol exposure compared with LAS subjects (Thomas et al., 1998b). The failure to find similar findings in both replicates suggests that vulnerability of neuronal systems underlying activity changes is not consistently related to the trait for which these rat lines were selected, and that differential vulnerability to alcohol's teratogenic effects in the HAS and LAS lines is task-specific.

The parallel bar motor task requires both balance and fine motor coordination. Although deficits in these skills may be related to dysfunction of a number of motor areas, they are consistent with damage to the cerebellum. Indeed, the cerebellum is vulnerable to ethanol-induced neuropathology during this period of development (Bonthius and West, 1991; Goodlett et al., 1991, 1998; Hamre and West, 1993) and the severity of ethanol-induced motor deficits on the parallel bar task is correlated with cerebellar Purkinje cell loss (Thomas et al., 1998a). Moreover, cerebellar differences between the HAS and LAS lines may underlie the extremes in sensitivity to alcohol's hypnotic effects (Palmer et al., 1992). Cerebellar Purkinje neurons in HAS rats are more sensitive to the depressant effects of ethanol, possibly related to differences in ethanol potentiation of GABA receptor activation (Allan et al., 1991), and show less rapid acute tolerance to alcohol-induced suppression compared with LAS rats (Pearson et al., 1997). The present data suggest that the cerebella of HAS and LAS rats may be differentially vulnerable to alcohol-induced neuropathology, and we are currently evaluating this possibility.

We and others have not found differences in blood alcohol levels between HAS and LAS lines (Deitrich, 1993; Dahchour et al., 2000; Thomas et al., 2000), suggesting that the differential vulnerability is not due to general metabolic differences, but rather to neuronal sensitivity. Others have also found genetically mediated differences in vulnerability to fetal alcohol effects that are not related to the rate of alcohol metabolism (Goodlett et al., 1989; Boehm et al., 1997). This does not preclude the possibility that differences in brain ethanol metabolism may play a role. Although there are no differences in peripheral ethanol metabolism or whole brain aldehyde dehydrogenase (ALDH) activity, HAS rats exhibit higher brain acetylaldehyde levels following acute ethanol exposure compared with LAS rats (Zimatkin et al., 2001). In addition, HAS rats express lower levels of cerebellar ALDH, the enzyme that metabolizes alcohol's metabolite acetylaldehyde (Zimatkin and Deitrich, 1995). Acetylaldehyde can be damaging to the developing brain (Sreenathan et al., 1982); however, it is not known whether differences in levels of brain acetylaldehyde reported are sufficient to account for differential vulnerability to alcohol's teratogenic effects.

Understanding how genetic factors contribute to alcohol's adverse effects on both brain and behavioral development may help to elucidate alcohol's mechanisms of damage. Unfortunately, with few exceptions (Draski et al., 2001), there have been no studies examining the brain and behavioral development of HAS and LAS rats. Thus, at the present time, it is not known whether differential vulnerability is due to genotype-dependent differences in neuronal development, sensitivity to damage, or ability to recover from injury. Again, further investigation into the neuropathology induced by early alcohol treatment in these genetic lines will help to better clarify ethanol's mechanisms of damage.

Convergence of findings from other rat and mouse lines may also be helpful. For example, long-sleep (LS) mice, which are more sensitive to alcohol's hypnotic effects, are more vulnerable to prenatal alcohol-induced mortality, growth retardation, microcephaly, and dysmorphology (Goodlett et al., 1989; Gilliam and Kotch, 1990, 1996) compared to short-sleep (SS) mice. In addition, LS mice exhibit more severe passive avoidance deficits following prenatal alcohol exposure compared to SS mice (Gilliam et al., 1987). Together with the present data, these findings provide further evidence that sensitivity to alcohol plays a role in vulnerability to alcohol's teratogenic effects. In

contrast, preferring (P) rats, who were selected for alcohol preference but also have less sensitivity to alcohol, are more vulnerable to hyperactivity induced by neonatal alcohol exposure compared to nonpreferring (NP) rats (Riley et al., 1993). Melcer et al. (1995) also failed to find differences in the severity of alcohol-induced motor coordination deficits between P and NP rats, although NP rats performed so poorly on the parallel bar motor task that conclusions are limited. It was speculated that the differences in the P and NP rats were related to the differential rate of development of the serotonin system, with the P rats exposed to alcohol at a period when the serotonin system was more vulnerable. It is clear that genetically mediated responses to alcohol may be associated with vulnerability to alcohol's teratogenic effects; however, it depends on the outcome measure and selected genetic trait.

Finally, identification of factors that are associated with increased risk or protection against fetal alcohol effects can help us identify women whose fetuses are at high risk. For example, Viljoen et al. (2001) report that the ADH2*2 allele may serve as a marker for protective effects against FAS. Animal studies have also shown that genetic differences in alcohol metabolism, which leads to higher blood alcohol levels, can also increase vulnerability (Chernoff, 1977). The present data suggest that differences in neuronal sensitivity and behavioral responses to alcohol may also help us to identify at-risk populations. These data suggest that the fetuses of women who are more sensitive to the initial hypnotic effects of alcohol, independent of metabolic differences, may be at high risk for some fetal alcohol effects.

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