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Abstract

Aims: Prenatal exposure to alcohol can have adverse effects on the developing fetus. Two of the hallmarks of children exposed to alcohol prenatally are attention deficits and hyperactivity. While hyperactivity has been observed in rats following prenatal ethanol exposure, few studies have examined these effects in mice. The present study investigated the effects of prenatal ethanol exposure on activity in mice from three inbred strains: C57BL/6 (B6), Inbred Long Sleep (ILS) and Inbred Short Sleep (ISS). **Methods:** On days 7 through 18 of gestation, mice were intragastrically intubated twice daily with either 3.0 g/kg Ethanol (E) or an isocaloric amount of Maltose-Dextrin (MD); Non-Intubated Control (NIC) litters were also generated. Offspring activity was monitored at 30, 60, 90 and 150 days of age. **Results:** While results showed no effects of prenatal ethanol exposure on any measures of activity, we did observe differences in baseline activity among the strains. ISS mice were more active than B6 and ILS for all activity measures except stereotypy; B6 mice had higher measures of stereotypy than ILS and ISS. Younger mice were more active than older mice. The only sex effects were on measures of stereotypy, where males had higher scores. **Conclusions:** Mice are an excellent organism to study genetic influences on many phenotypes. However, our study and others have shown few effects of prenatal ethanol exposure on behavior in mice. It appears as if the prenatal period in mice, corresponding to organogenesis, is not a sensitive period for producing behavioral deficits following ethanol exposure. It is likely that the first two weeks postnatally, corresponding to the brain growth spurt, is more sensitive for producing behavioral effects.

Introduction

Women who consume alcohol (ethanol) during pregnancy place their offspring at risk for a number of teratogenic outcomes. The most severe cases are diagnosed as Fetal Alcohol Syndrome (FAS), a disorder defined by prenatal and/or postnatal growth retardation, a characteristic pattern of craniofacial abnormalities, and central nervous system dysfunction (Jones and Smith, 1973; Jones et al., 1973; Sokol et al., 2003). Because not all offspring exposed to alcohol prenatally display the full spectrum of FAS symptoms (particularly the facial dysmorphology), the term Fetal Alcohol Spectrum Disorders (FASD; Koren et al., 2003; Sokol et al., 2003) has been coined to describe varying degrees of ethanol teratogenesis. The estimated incidence of FASD in the United States is 1% (May and Gossage 2001; Sampson et al. 1997). Neurodevelopmental and behavioral deficits associated with FAS/FASD include developmental delay, attention deficits, hyperactivity, learning and memory impairments and diminished impulse control (Coles, 2001; Kelly et al., 1987; Kvigne et al., 2004; Sampson et al., 1997; Sokol et al., 2003).

Hyperactivity and attention deficits are hallmarks of children exposed to alcohol prenatally (Mattson and Riley, 1998). Most studies have relied on naturalistic observations and have reported children (from a few hours after birth through high school) exposed to ethanol prenatally as hyperactive, tremulous, fidgety, restless, always on the go, unable to sit still and irritable (Hanson et al., 1976; Kvigne et al., 2004; Landesman-Dwyer et al., 1981; Nanson and Hiscock, 1990; Shaywitz et al., 1980; Steinhausen et al., 1993; Steinhausen and Spohr, 1998; Streissguth et al., 1978). This hyperactivity can occur in the absence of intellectual impairment,

at relatively moderate levels of maternal ethanol consumption and persist throughout childhood. How to best model these behaviors in a rodent model is unclear. What behaviors in rats and mice best model "fidgety" or "restless" described in the human condition?

Results of prenatal ethanol exposure on activity in rats have been mixed. While most studies have demonstrated increased activity (Martin et al., 1978; Osborne et al., 1979; Ulug and Riley, 1983; Vorhees and Fernandez, 1986; for reviews see Bond, 1981; Meyer and Riley, 1986), others have not (Carneiro et al., 2005; Vorhees, 1989; Westergren et al., 1996; Wigal and Amsel, 1990). Results depend upon the pattern of ethanol administration, age at which offspring are tested and apparatus in which activity is measured. Most studies have used activity monitors (automated or observer scored) and reported total distance traveled, number of photocell beam interruptions, or number of squares entered/crossed.

Somewhat surprisingly, few studies have examined hyperactivity in mice exposed to ethanol prenatally. Randall and colleagues have investigated the effects of *in utero* ethanol exposure on activity in C57BL/6J (B6) inbred mice. Their paradigms involved giving pregnant dams a liquid diet containing various ethanol concentrations on days 5/6 through 17/18 of gestation. They have reported greater activity in male and female offspring exposed to ethanol prenatally at 23 days of age (Randall et al., 1986), no effect of prenatal ethanol exposure on activity (male and female offspring) at 12, 20, or 36 days (Middaugh et al., 1988) and greater activity in female offspring exposed to ethanol prenatally when tested at 30 days of age (Becker and Randall, 1989). Mothes et al. (1996) reported increased activity (36-42 days of age) in the home cage, but not in an activity monitor, in B6 mice exposed to ethanol on days 14-18 of gestation. Allan et al. (2003) kept B6SJL/F_1 dams on a liquid ethanol diet before and during pregnancy and reported no effects on offspring activity at 60-100 days. Finally, Gilliam and colleagues (Gilliam et al., 1987;

Gilliam 1990) showed either no effect of prenatal ethanol exposure on activity in Long-Sleep (LS) or Short-Sleep (SS) mice (21-25 days of age), or an increase in activity in SS mice and mice derived from reciprocal crosses between SS and LS (150 days of age) exposed to ethanol prenatally.

Studies have shown that, in humans, genetic factors can influence susceptibility and resistance to ethanol teratogenesis. Several case study reports indicate that monozygotic twins are more similarly affected than dizygotic twins (Chasnoff, 1985; Christoffel and Salafsky, 1975; Palmer et al., 1974; Riikonen, 1994). A more comprehensive study examined ethanol exposure *in utero* in both monozygotic and dizygotic twins. The rate of concordance for diagnosis was 5/5 for monozygotic twins and 7/11 for dizygotic twins and the authors concluded that genes had a modulating influence on expression of the teratogenic effects of alcohol (Streissguth and Dehaene, 1993). More recently, several studies have shown that different alleles of the alcohol dehydrogenase gene (*ADH*), an enzyme involved in ethanol metabolism, can influence the severity of teratogenesis in different ethnic populations (Das et al., 2004; McCarver et al., 1997; Stoler et al., 2002; Viljoen et al., 2001). Therefore, characterizing mice for hyperactivity following prenatal ethanol exposure (or any prenatal alcohol trait) is important because mice are a much more tractable species than rat in which to examine genetics. Many more inbred, recombinant inbred, congenic and selectively bred lines of mice exist than rat. In addition, the mouse genome is much better annotated and many more strains of mice have been sequenced. Finally, it is currently much easier to manipulate the mouse genome (i.e. targeted mutagenesis) than the rat genome.

In the present study, we examined activity following *in utero* ethanol exposure in mice from three inbred strains: Inbred Long-Sleep (ILS), Inbred Short-Sleep (ISS) and B6. We chose these

strains because they are among the few mouse strains that have been characterized for any prenatal alcohol phenotypes. The ILS and ISS mice were derived from LS and SS mice, selectively bred for differential sensitivity to a hypnotic dose of alcohol (McClearn and Kakihana, 1981). Previous research has shown that LS mice are more susceptible than SS mice to several measures of teratogenesis, including activity as noted above (Gilliam 1990; Gilliam and Kotch, 1990; Gilliam and Kotch, 1996; Gilliam et al., 1989a; Gilliam et al, 1989b). This suggests that one or more genes that mediate differential sensitivity to the hypnotic effects of ethanol in SS and LS may also mediate differential sensitivity to ethanol teratogenesis. The ILS and ISS mice have not been characterized for any prenatal alcohol phenotypes.

B6 mice are relatively susceptible to fetal weight deficits and kidney, limb and skeletal malformations following prenatal ethanol exposure (Boehm et al., 1997; Downing and Gilliam, 1999; Gilliam and Irtenkauf, 1990; Gilliam et al., 1997; Webster et al., 1980). As discussed above, results have been mixed when B6 mice have been examined for hyperactivity following prenatal ethanol exposure.

Methods

Animals

Male and female ILS, ISS and B6 mice were obtained from and housed in the specific pathogen-free (SPF) facility at the Institute for Behavioral Genetics, Boulder, CO. Males were individually housed while females were housed three to five per cage; mice were maintained on a 12-hr light/dark cycle (lights on at 7:00 am) and were given food and water ad libitum. The temperature was kept at a constant 22º C. All procedures were approved by the University of Colorado Institutional Animal Care and Use Committee, in accordance with National Institute of Health guidelines.

Mating and Dosing

Females weighed a minimum of 18 grams at mating. Two females were placed in each male's cage overnight and examined for a seminal plug in the morning as evidence of mating. Plugged females were weighed, randomly assigned to a treatment condition (Ethanol, E; Maltose-Dextrin, MD or Nonintubated Control, NIC) and single-housed. The day of plug detection was designated as Day 0 of pregnancy (Gestational Day 0: GD 0). On GD 7, females were weighed to ascertain a 1.5 gram minimum weight gain. Pregnant dams were then intubated twice daily (9:00 am and 3:00 pm, GD 7 to GD 18) with either 3.0 g/kg ethanol (20% w:v) or an isocaloric amount of MD. Mice in the NIC group were weighed daily; this group served as a control to assess the effects of repeated handling and intubations in the E and MD groups.

Dams were checked for births twice daily starting on GD 19. Once born (Postnatal Day 0; PND 0), litters were weighed and sexed. Litters were weighed again on PND 3 and offspring sex confirmed. Litters were then culled to four offspring, two males and two females when possible. Offspring were again weighed on PNDs 5, 10, 20 and subsequent days when activity testing took place. Pups were weaned on PND 28 and housed with same-sex mice, 4-5 per cage. Mothers who died during the intubation process or failed to deliver by GD 20 were sacrificed and the number of implantation sites was counted.

Offspring Testing

Offspring were tested for activity in two-day intervals on PNDs 30 and 31, 60 and 61, 90 and 91, and 150 and 151. We chose the 30 and 60 day time-points because they represent a reasonable approximation of the adolescent and young adult stages in mice. We also tested offspring at 90 and 150 days to see if effects persisted into adulthood; Gilliam (1990) found effects of prenatal ethanol exposure on activity in offspring tested at 150 days of age. Testing

took place between 9:00 am and 11:00 am. Mice were placed in an automated activity monitor for 15 minutes (3 successive 5-minute bins.). The activity monitoring system (Digiscan: Accuscan Instruments Inc., Columbus, Ohio) consists of a 16 beam photocell apparatus interfaced with a PC; the monitors consist of a 40 cm^2 chamber with a 30.5 cm ceiling. Software interfacing with the monitors records the total photocell beam breaks, both vertical and horizontal. Computer software recorded a number of other activity measures, as described below.

Maternal Blood Ethanol Concentration

A separate group of pregnant females were examined for blood ethanol concentration (BEC). Dams were intubated twice daily with 3.0 g/kg ethanol from GD 7 to GD 18, as described above. Blood was drawn from the retro-orbital sinus 30, 60, 120 and 180 minutes after the second intubation (3:00 pm) on GD 18. Ten µl of retro-orbital blood was added to 200 µl of perchloric acid on ice to precipitate blood solids. Blood samples were vortexed and centrifuged at 4500 RPM for 10 minutes. The plasma or supernatant was then removed from the pellet and an equal volume of KOH was added to the supernatant to neutralize the perchloric acid. The sample was then vortexed and stored in the freezer until analysis (once per week). BEC was determined by spectrophotometric analysis of an enzyme assay as described by Smolen et al. (1986).

Statistical Analyses

Data were examined using Analysis of Variance (ANOVA) with strain (ILS, ISS and B6), treatment (E, MD, NIC) and sex as grouping factors. For maternal data, percent weight gain during pregnancy, litter size and postnatal mortality were examined. For offspring data, in order to control for litter effects and inflated sample size, litter means were the unit of analyses (Wainwright, 1998; Zorilla, 1997). Offspring weight at birth was examined using ANOVA. Body weight and activity measures from PND 3-151 were examined with repeated measures

ANOVA (RM-ANOVA), with strain, treatment and sex as between-group factors and age as the within-group factor.

The following activity variables were analyzed: horizontal activity, total distance traveled (cm), horizontal movement number, movement time, rest time, vertical movement number, vertical time, stereotypy number and stereotypy time. As noted earlier, most previous studies have simply looked at total distance traveled and number of beam breaks. Examining these additional activity variables should provide insights into what measures in mice may best model "hyperactivity" in children exposed to ethanol *in utero*.

Activity data was analyzed in several ways. First, we analyzed data within each session. For almost every day and measure, we saw habituation; animals were less active in the last 5 minute bin compared to the first 5 minute bin. There were no effects of genotype, treatment or sex on habituation. We also analyzed 15 minute totals and found very few differences compared to individual 5 minute bins; therefore, we present data from 15 minute totals only. Data from the first day of each two-day session was analyzed separately (30, 60, 90 and 150), from the second day of each two-day session (31, 61, 91, 151), and all days (30, 31, 60, 61, 90, 91, 150, 151). While activity measures were generally lower when the second day was analyzed separately (likely habituation), the pattern of results was remarkably consistent. Thus, we present activity analyses (RM-ANOVA) from all days. Figures include means for two day averages (30 and 31, 60 and 61, etc.).

Results

Maternal Data

Resorptions. One out of 11 ISS MD and 1 out of 14 ISS E dams lost their litters (didn't give birth and had resorptions when sacrificed on GD 20). While no ILS dams lost their litters, 3 of 11 E dams and 1 of 9 MD dams died following intubation. No B6 females died during intubations or lost their litters.

Weight Gain, Litter Size and Postnatal Mortality. ANOVA showed significant main effects of strain ($F(2,78) = 42.45$, $p < .001$) and treatment ($F(2,78) = 8.36$, $p < .01$) and a significant strain by treatment interaction ($F(4,78) = 4.16$, $p < .01$) for percent maternal weight gain (Table 1). Post hoc analyses showed that ISS dams put on less weight than ILS and B6 (p's < .01). As expected, NIC dams gained more weight than E and MD treatment groups (p's < .01). Decomposition of the strain by treatment interaction showed that E-treated B6 females put on significantly less weight than MD and NIC controls (p's < .01). In addition, E- and MD-treated ILS females put on less weight than their NIC controls (p's < .03 and .01, respectively).

While there was a main effect of strain ($F(2,80) = 32.83$, $p < .001$), there was no effect of treatment and no strain by treatment interaction on litter size. Post hoc analysis showed that B6 and ILS dams had larger litters than ISS dams (p's < .001; Table 1). It should be noted that litter size is likely an underestimate. On GD 19, we examined dams for litters twice, in the morning and afternoon. In several cases, when litters were found, the dams had partially eaten one or more pups; these pups were included in our "litter size" variable and accounted for some of the postnatal mortality. It seems likely that there were a few dams that had completely eaten pups by the time litters were found, so litter size is likely a bit underestimated.

Postnatal mortality was calculated as: (number of pups on PND 0 – number of pups on PND 3 before culling)/ number of pups on PND 0. There were no main effects of strain or treatment on postnatal mortality.

Blood Ethanol Concentration. Five pregnant dams per genotype were intubated with 3.0 g/kg ethanol, twice daily, from GD 7 to GD 18. Thirty, 60, 120 and 180 minutes following the last intubation on GD 18 (3:00 pm), blood was obtained and assayed for ethanol concentration. Blood ethanol levels averaged 317 mg/dl 30 minutes after the last injection and declined to 56 – 117 mg/dl at 180 minutes (Figure 1). Data was analyzed using RM-ANOVA, with time after intubation (30, 60, 120, 180 minutes) as the within-group variable and strain as the between-group variable. Results showed a significant main effect of time ($p < .01$) but no significant main effect of strain and no significant strain by time interaction. Within all strains, BECs declined across time.

Offspring Data

Birth Weight. Birth weight and offspring weight from PND 3-151 were analyzed using litter means for each sex as the unit of analysis. We found significant main effects of strain ($F(2,119) = 47.49$, $p < .001$), treatment ($F(2,119) = 4.87$, $p < .01$) and sex ($F(1,119) = 6.82$, $p < .02$) for offspring weight at birth, but no significant interactions among the variables. Post hoc analyses showed that ISS offspring weighed more than ILS and B6 (p's $< .001$) and ILS offspring weighed more than B6 ($p < .01$); MD-treated litters weighed more than E-treated litters ($p < .01$) and NIC litters ($p < .02$); and males weighed more than females ($p < .02$; Table 1).

Weight Gain. Offspring weight from PND 3-151 was analyzed using RM-ANOVA, with age as a within-subjects variable and strain, treatment and sex as between-subjects variables. ANOVAs involving repeated measures used the Greenhouse-Geisser adjustment factor to assess

the significance of the observed F ratio. We found significant main effects of age (F(3.79,378) = 12,680, $p < .001$), strain (F(2,100) = 23.32, $p < .001$) and sex (F(1,100 = 189.05, $p < .001$) on weight gain from PND 3-151, but no main effect of treatment. Not surprisingly, age accounted for 98% of the variance in weight gain (partial eta^2). Age also interacted with strain (F(7.57, 378) = 23.67, $p < .001$) and sex (F(3.78, 378) = 186.08, $p < .001$) and there was a significant age by strain by sex interaction (F(7.57, 378 = 2.43, $p < .02$). Simple effects analysis (within strain) at each age showed that in general, ISS offspring gained more weight than the other three strains from PND 3-20, while ILS offspring gained less weight than the other 3 strains from PND 3-60; males gained more weight than females from PND 20-151 (p's < .001).

Measures of Activity. Total distance traveled (TDT) and horizontal activity (HACT) are the two most commonly used measures of behavioral activation in rodents. TDT indicates, in centimeters, the distance traveled during a given sample period, while HACT is the total number of beam breaks that occur in the horizontal plane during a given sample period. For TDT, RM-ANOVA showed significant main effects of age (F(3.84, 421) = 12.55, $p < .001$) and strain (F(2,110) = 34.54, $p < .001$). There was no effect of treatment. Post-hoc analyses (Bonferoni corrected t-tests) showed that ISS offspring had greater TDT (Figure 2) compared to ILS and B6 (p's < .01); younger mice had a higher TDT than older mice (p's < .01). RM-ANOVA also showed a significant age by strain interaction for TDT (F(7.67, 421) = 7.45, $p < .001$). ISS mice had higher TDT (Figure 2) than B6 and ILS at 30, 60 and 90 days of age (p's < .05). We found identical results (with slightly different F-values; data not shown) for HACT. In general, all strains had a decrease in activity across days. This could reflect habituation or perhaps mice simply are not as active when they get older. Interestingly, the exception to this characterization is ILS mice, which had a large increase in activity at 150 days.

Movement number (MOVNO) is the number of separate horizontal movements an animal makes in a given sample period. Movement time (MOVTIM) is the amount of time an animal ambulates (horizontally) in a given sample period while rest time (RSTIM) is the amount of time an animal does not ambulate. For all three variables, RM-ANOVA showed significant main effects of age and strain, and a significant age by strain interaction (all p's < .001). There was no effect of treatment. As can be seen in Figure 3, ISS made more movements and spent more time ambulating than B6 and ILS (p's < .01). Because RSTIM is simply the inverse of MVTIM, we present data from MVTIM only.

We examined two measures of vertical activity. Vertical movement number (VMVNO) is the number of movements an animal makes in the vertical plane (rearing), while vertical time (VTIM) is the amount of time an animal spends rearing. For VMVNO, ANOVA showed significant main effects of age ($F(5.47, 601) = 22.38$, $p < .01$) and strain ($F(2, 110) = 24.09$, $p < .01$) and a significant age by strain interaction ($F(10.94, 601) = 13.22$, $p < .01$). Post hoc analyses showed that ILS offspring made fewer vertical movements than B6 and ISS (p's < .01; Figure 4). All three strains had a significant increase in vertical movements at the 90 day timepoint compared to the other timepoints (p's < .01; Figure 4). For VTIM, ANOVA again showed significant main effects of age ($F(5.14, 565) = 69.15$, $p < .01$) and strain ($F(2, 110) = 42.98$, $p < .01$) and a significant age by strain interaction ($F(10.28, 565) = 16.46$, $p < .01$). Post hoc analyses showed that ISS offspring had significantly greater VTIM compared to B6 and ILS (p's < .01; Figure 4). There were no treatment effects on either variable.

If a mouse repeatedly breaks the same beam or set of beams, the mouse is exhibiting stereotypic behavior. We analyzed two measures of stereotypic behavior: Stereotypy number (STNO) is the number of times the monitor observed stereotypic behavior in the mouse; a break

in stereotypy of one second or more is required to separate one stereotypic episode from the next. Stereotypy time (STIM) is the total amount of time that stereotypic behavior is exhibited. For STNO, RM-ANOVA showed significant main effects of age ($F(5.27, 579) = 25.49$, $p < .01$), strain ($F(2,110) = 40.28$, $p < .01$) and sex ($F(1,100) = 6.91$, $p < .01$) and significant age by strain ($F(10.53, 579) = 6.30$, $p < .01$) and strain by sex ($F(2,110) = 3.97$, $p < .03$) interactions. Post-hoc analyses showed that B6 offspring had significantly greater STNO than ILS and ISS, while ILS offspring had significantly less STNO than B6 and ISS (all *p*'s < .01). In addition, males had significantly more STNO than females at 60, 90 and 150 days of age (*p*'s < .05). As can be seen in Figure 5, the effect of sex was strain and age dependent. Similarly, for STIM, ANOVA showed significant main effects of age ($F(5.01, 551) = 34.63$, $p < .01$), strain ($F(2, 110) = 46.08$, $p < .01$) and sex ($F(1,110) = 5.97$, $p < .02$). Age also interacted with strain ($F(10.02, 551) = 10.25$, $p < .01$) and sex ($F(5.01, 551) = 2.32$, $p < .05$). Post-hoc analyses showed that, similar to STNO, B6 had significantly greater STIM than ILS and ISS while ILS had significantly less STIM than B6 and ISS; males had significantly higher STIM than females (all *p*'s < .01). There was no effect of treatment on STNO or STIM.

Discussion

Attention deficits and hyperactivity are hallmarks of children exposed to ethanol *in utero*. While the effects of prenatal ethanol exposure on attention and activity have been fairly well characterized in rats, few studies have examined these effects in mice. For activity, while two studies have reported increased activity in B6 mice exposed to ethanol prenatally (Becker and Randall, 1989; Randall et al., 1986), two others have not (Middaugh et al., 1988; Mothes et al., 1996). Allan et al. (2004) reported no effects of prenatal ethanol exposure on activity in B6 x

SJL F_1 mice. Results from our study showed no effects of prenatal ethanol exposure on any measures of activity in B6 offspring.

LS and SS mice were selectively bred for sensitivity (LS) and resistance (SS) to the soporific effects of ethanol, as measured by loss of the righting reflex (LORE: Loss Of Righting due to Ethanol; McClearn and Kakihana1981). ILS and ISS were derived by subsequent inbreeding of LS and SS. In addition to the hypnotic effects of ethanol, LS, SS, ILS and ISS differ on many other behavioral and physiological traits. Compared to LS and ILS, SS and ISS mice have higher baseline activity and also show greater activation following a low dose of ethanol (Dudek and Abbott 1984; Owens et al. 2002; Phillips and Dudek 1991). In addition, these lines of mice differ in measures of functional tolerance following ethanol exposure (Bennett et al. 2007; Deitrich et al. 2000; Gill and Deitrich 1998) and in their responses to other sedative hypnotics (Christensen et al. 1996; Simpson et al. 1998).

LORE is a measure of initial sensitivity to alcohol. Previous research has suggested that individual sensitivity to alcohol may influence susceptibility and resistance to some of the detrimental effects of prenatal alcohol exposure. Ethanol-sensitive LS mice are susceptible to fetal weight deficits, postnatal growth deficits, increased postnatal mortality, fetal brain weight reductions and skeletal malformations following prenatal alcohol exposure, while ethanol-insensitive SS mice are relatively resistant (Gilliam and Irtenkauf 1990; Gilliam and Kotch 1990, 1996; Gilliam et al. 1989a, 1989b; Goodlett et al. 1989). In addition, LS mice exposed to ethanol prenatally took significantly more trials to reach a passive avoidance criterion than their controls, while SS mice did not. Two studies have looked at hyperactivity in LS and SS mice following *in utero* ethanol exposure. Gilliam et al. (1987) reported no effects of prenatal ethanol exposure on activity in LS and SS mice. In contrast, Gilliam (1990) reported an increase in activity at 150

days of age in SS, but not LS, mice exposed to alcohol prenatally. This increase in activity at 150 days was not confirmed in ISS mice in the present study. It is interesting to note that Purkinje cells of the cerebellum of LS mice are much more sensitive to the depressant effects of ethanol than Purkinje cells of SS mice, as determined by electrophysiological measures (Basile A. et al. 1983; Sorensen et al. 1980). Levels of aldehyde dehydrogenase in Purkinje cells of the cerebellum are higher in SS mice compared to LS mice (Zimatkin and Deitrich 1995). Purkinje cells of the cerebellum are particularly vulnerable to neonatal (third trimester equivalent) ethanol exposure in rats (Goodlett et al. 1998; Light et al. 2002; Thomas et al. 1998a).

ISS showed greater baseline activity than ILS on all measures. The differences in TDT and HACT verify results from previous studies (see above) and shows that these differences exist at younger ages than previously reported. This suggests that in addition to LORE, differences in baseline activity were also selected for in LS and SS, and were captured during the inbreeding process. B6 were intermediate on most measures of activity except for our two measures of stereotypy, where B6 were higher than both ISS and ILS. The only sex differences in baseline activity were also for stereotypy, where males displayed higher rates than females.

Few studies have demonstrated effects on behavior in mice following prenatal alcohol exposure. This is likely due, at least in part, to behavior being correlated with brain development/functioning. This is one area where humans and mice differ. In mammals, a period of rapid central nervous system growth and proliferation (the “brain growth spurt”) occurs during the third trimester. While the third trimester of pregnancy occurs *in utero* in humans, it occurs during approximately the first two weeks postnatally in rodents (Dobbing and Sands, 1979). Thus, in order to mimic third trimester ethanol exposure in rodents, one must administer ethanol to neonatal pups. Only a handful of studies have examined behavior in mice following early

postnatal ethanol exposure (Ciociola and Gautieri 1988; Pal and Alkana 1997; Pick et al., 1993; Wozniak et al., 2004; Yanai 1983; Yanai and Ginsburg 1977, 1979). When exposed to ethanol neonatally, rats reliably show changes in behavior (Goodlett and Johnson 1997; Goodlett et al. 1987; Kelly et al. 1987; Melcer et al. 1994; Pauli et al. 1995; Thomas et al. 1998b, 2003). Therefore, researchers using mice for FASD research should begin to look at the early postnatal period for ethanol exposure when examining behavior.

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Figure Legends

Figure 1. Mean (SEM) blood ethanol concentration for pregnant C57BL/6J (B6), Inbred Long-Sleep (ILS) and Inbred Short-Sleep (ISS) mice. Blood was drawn 30, 60, 120 and 180 minutes following the last intubation on GD 18. RM-ANOVA showed only a main effect of timepoint ($p < .01$).

Figure 2. Mean (SEM) total distance traveled (TDT) for B6, ILS and ISS mice. Bars represent the average of two days of testing. For example, day 30 equals the average TDT on days 30 and 31. For figures 2-5, sample sizes (litters): B6 E = 13, B6 MD = 9, B6 NIC = 11; ILS E = 7, ILS MD = 7, ILS NIC = 8; ISS E = 10, ISS MD = 9, ISS NIC = 9. * indicates ISS mice were more active than ILS and B6 on days 30, 60 and 90, *p*'s < .05. ** indicates that ILS were more active than B6 at 150 days, $p < .01$.

Figure 3. Mean (SEM) movement number (MVNO) and movement time (MVTIM) in B6, ILS and ISS mice. Each timepoint represents a two-day average. * Indicates that ISS mice had greater MVNO than ILS at 30, 60 and 90 days of age (*p*'s < .001); ISS had greater MVNO than B6 at 30 and 90 days (*p*'s < .001). ** indicates ISS also had greater MVTIM than ILS at 30, 60 and 90 days (*p*'s < .001); they also had greater MVTIM than B6 on all days tested (*p*'s < .001).

Figure 4. Mean (SEM) vertical movement number and vertical movement time in B6, ILS and ISS mice. Each timepoint represents a two-day average. * indicates ILS had less VMOV than ISS and B6 at 30 and 60 days (*p*'s < .01); ** indicates that ILS had less VTIM than ISS and B6 at 30 and 60 (*p*'s < .01); *** indicates that ISS had greater VTIM than B6 at 60 ($p < .05$), 90 and 150 days (*p*'s < .01)

Figure 5. Mean (SEM) stereotypy number (STNO) in B6 (a), ILS (b) and ISS (c) mice. Each timepoint represents a two-day average. * indicates males had significantly greater STNO than females, $p < .01$. ** indicates males had significantly greater STNO than females, $p < .05$.

Table 1. Mean (± SEM) percent maternal weight gain, litter size, prenatal mortality and pup weight at birth.

	C57BL/6			ILS			ISS		
	E	MD	NIC	E	MD	NIC	E	MD	NIC
	(15)	(9)	(12)	(7)	(7)	(8)	(11)	(9)	(9)
% Wt Gain[a]	45[b] (4)	67 (3)	69 (5)	49 (3)	44 (5)	65[c] (2)	30 (2)	30 (5)	33 (4)
Lit Size[d]	6.20 (.75)	7.78 (.72)	7.60 (.43)	5.29 (.42)	5.57 (.90)	6.88 (.40)	3.18 (.40)	3.38 (.32)	3.67 (.37)
% PM[e]	56 (12)	22 (12)	14 (7)	4 (4)	32 (18)	8 (4)	22 (12)	15 (7)	11 (11)
Pup Wt. ♀	1.23 (.05)	1.37 (.04)	1.34 (.04)	1.34 (.05)	1.49 (.06)	1.36 (.03)	1.53 (.03)	1.60 (.05)	1.59 (.04)
Pup Wt. ♂	1.33 (.05)	1.33 (.03)	1.33 (.06)	1.51 (.08)	1.47 (.05)	1.46 (.03)	1.53 (.04)	1.86 (.13)	1.67 (.06)

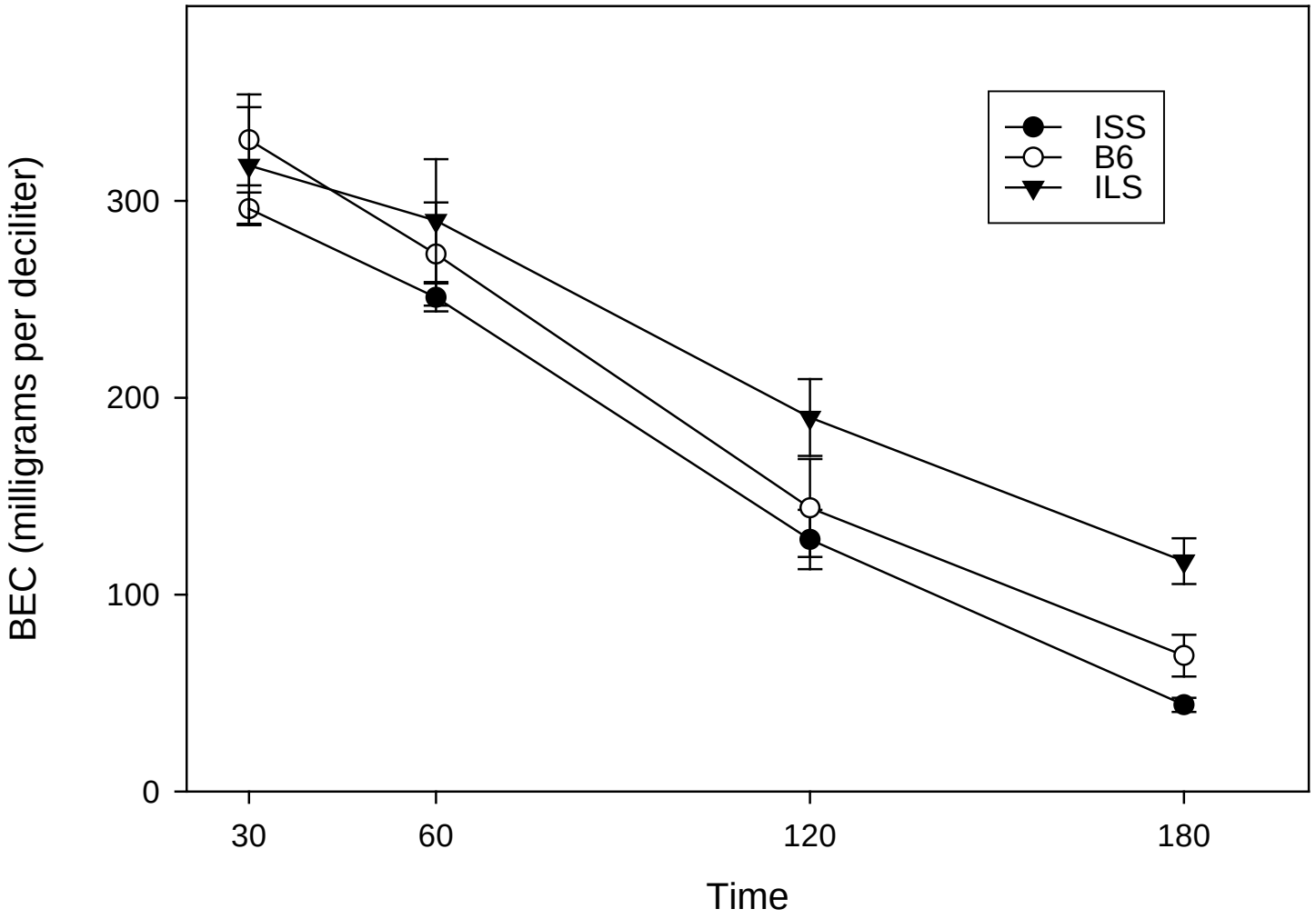
[a] Percent maternal weight gain calculated as: weight on GD 18 – weight on GD 7/weight on GD 7.

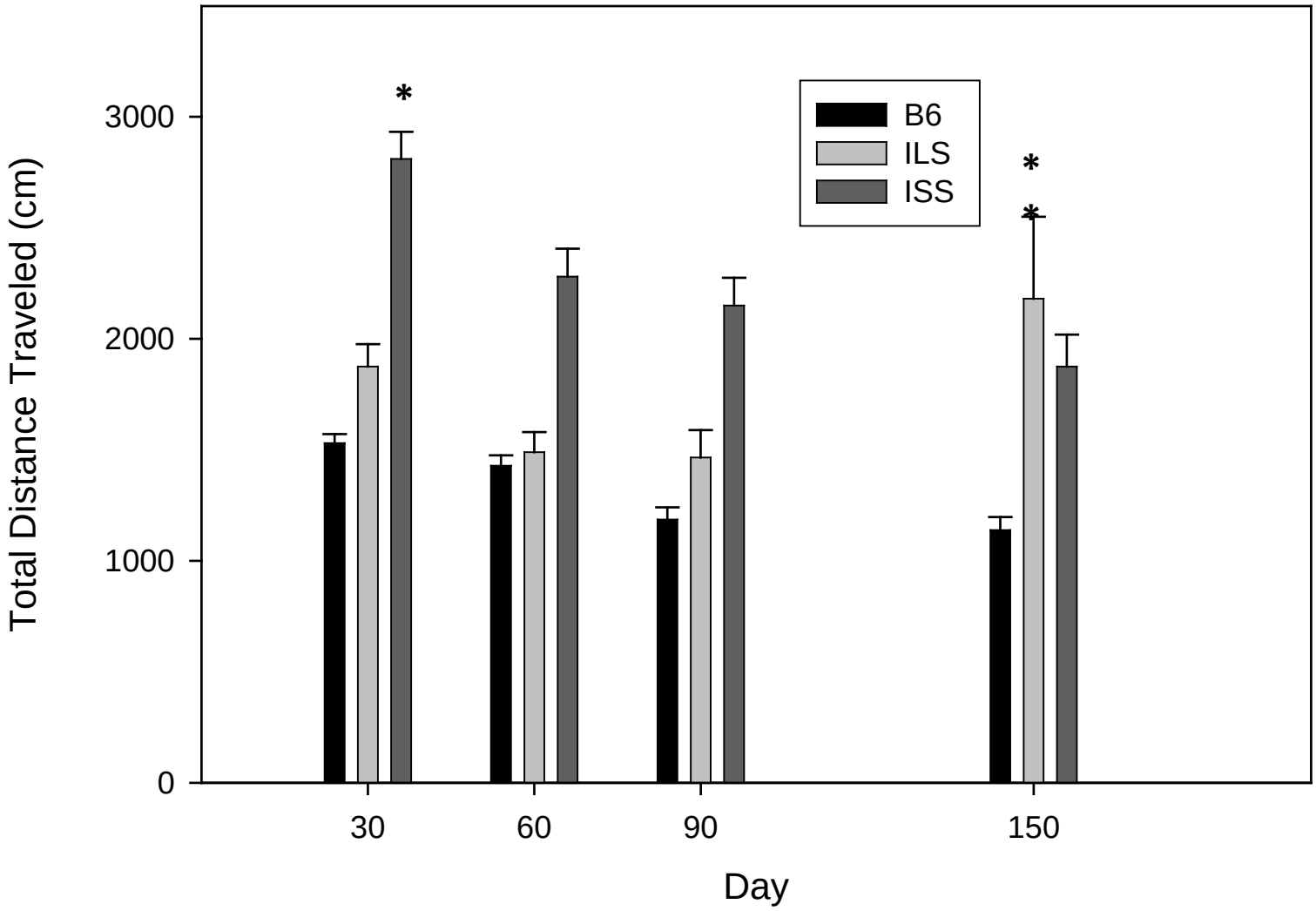
[b] Ethanol-treated B6 dams put on less weight than MD and NIC controls, p's < .01.

[c] Ethanol- and Maltose-treated ILS dams put on less weight than NIC controls, p's < .03 and .01, respectively.

[d] B6 and ILS litters were larger than ISS litters, p's < .001.

[e] Postnatal mortality calculated as: # of pups on PND 0 - # of pups on PND 3 before culling/# of pups on PND 0.


ISS
B6
ILS
BEC (milligrams per deciliter)
0
100
200
300
30
60
120
180
Time


Total Distance Traveled (cm)
3000
2000
1000
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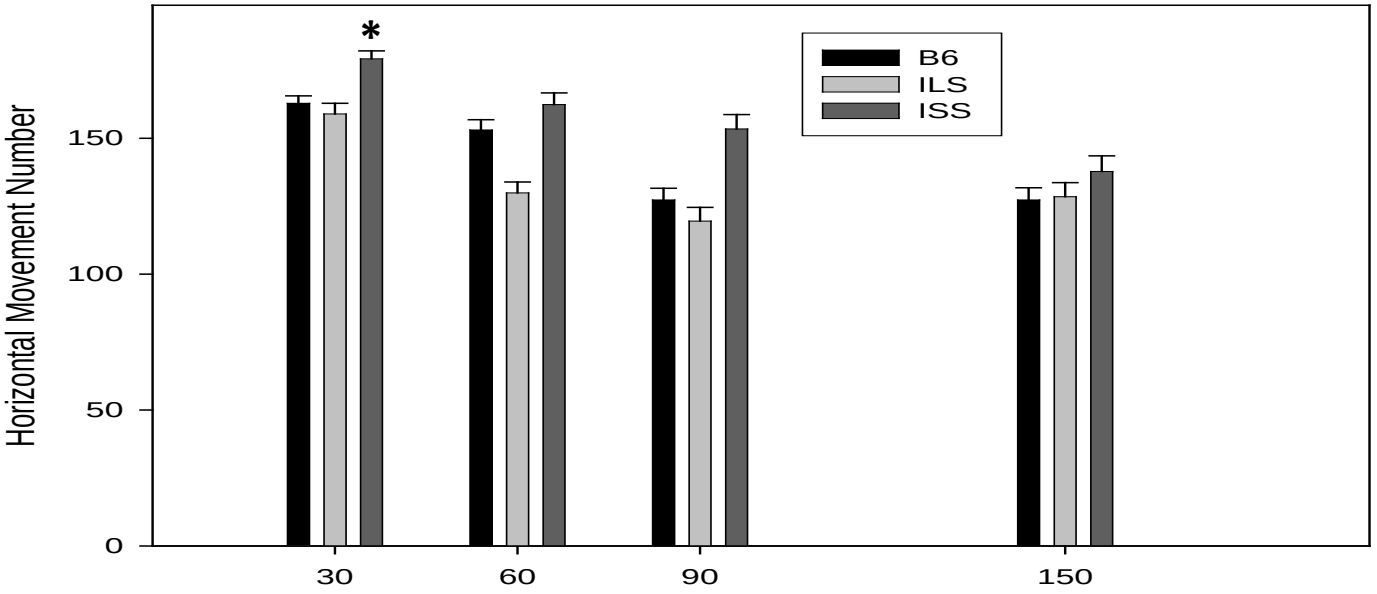
B6
ILS
ISS
*
*
*
30
60
90
150
Day


Horizontal Movement Number
B6
ILS
ISS
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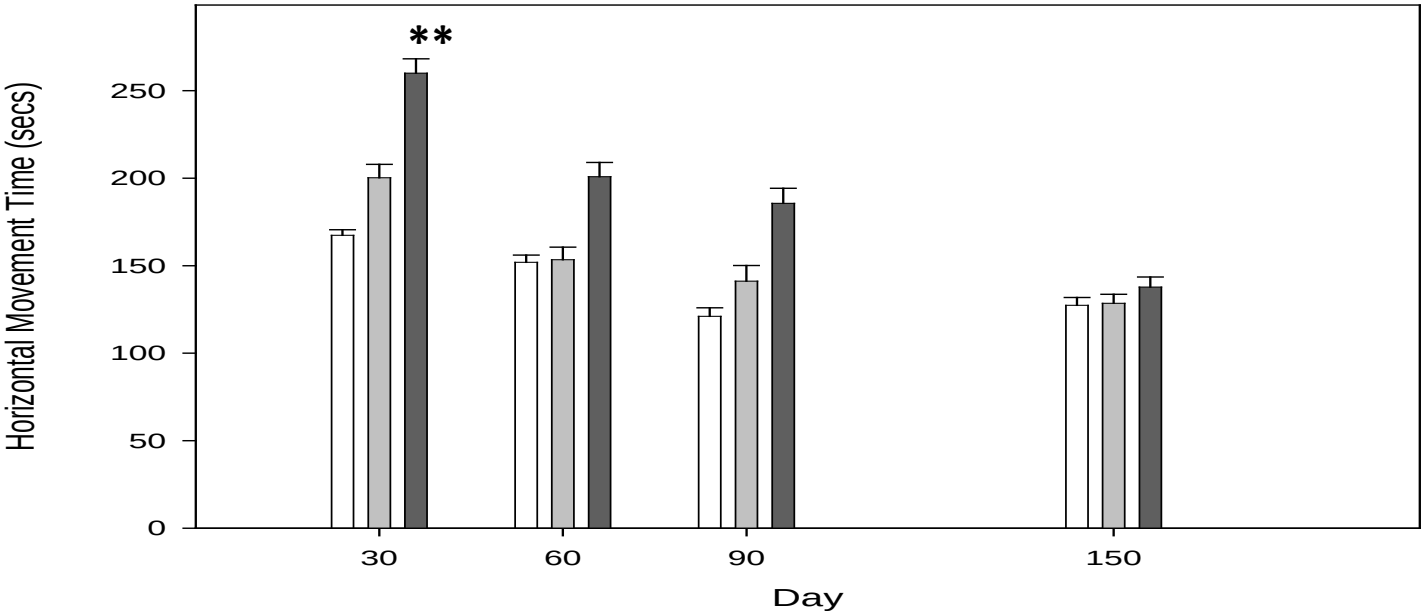
Horizontal Movement Time (secs)
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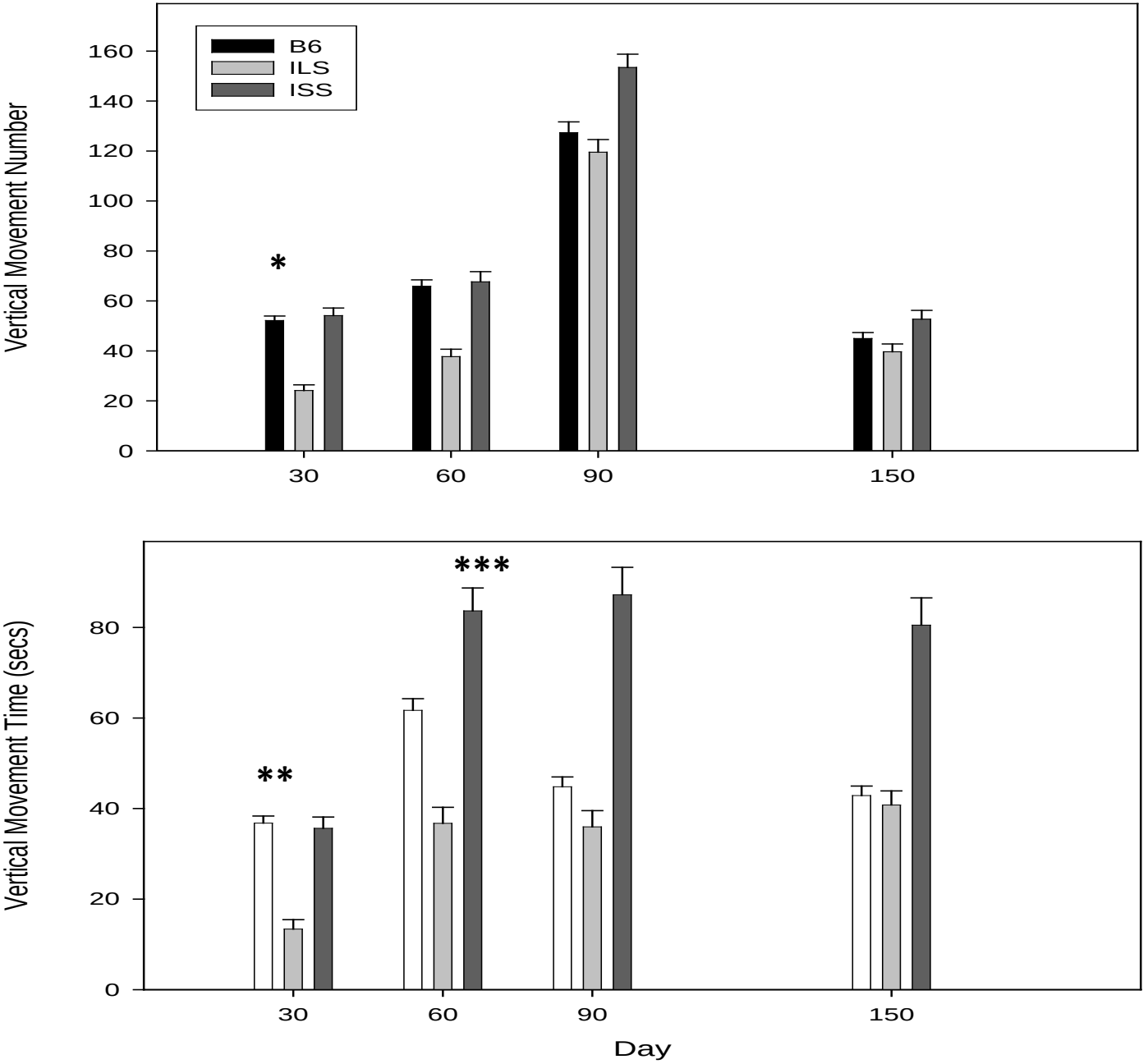
250
30
60
90
150
Day


B6
ILS
ISS
Vertical Movement Number
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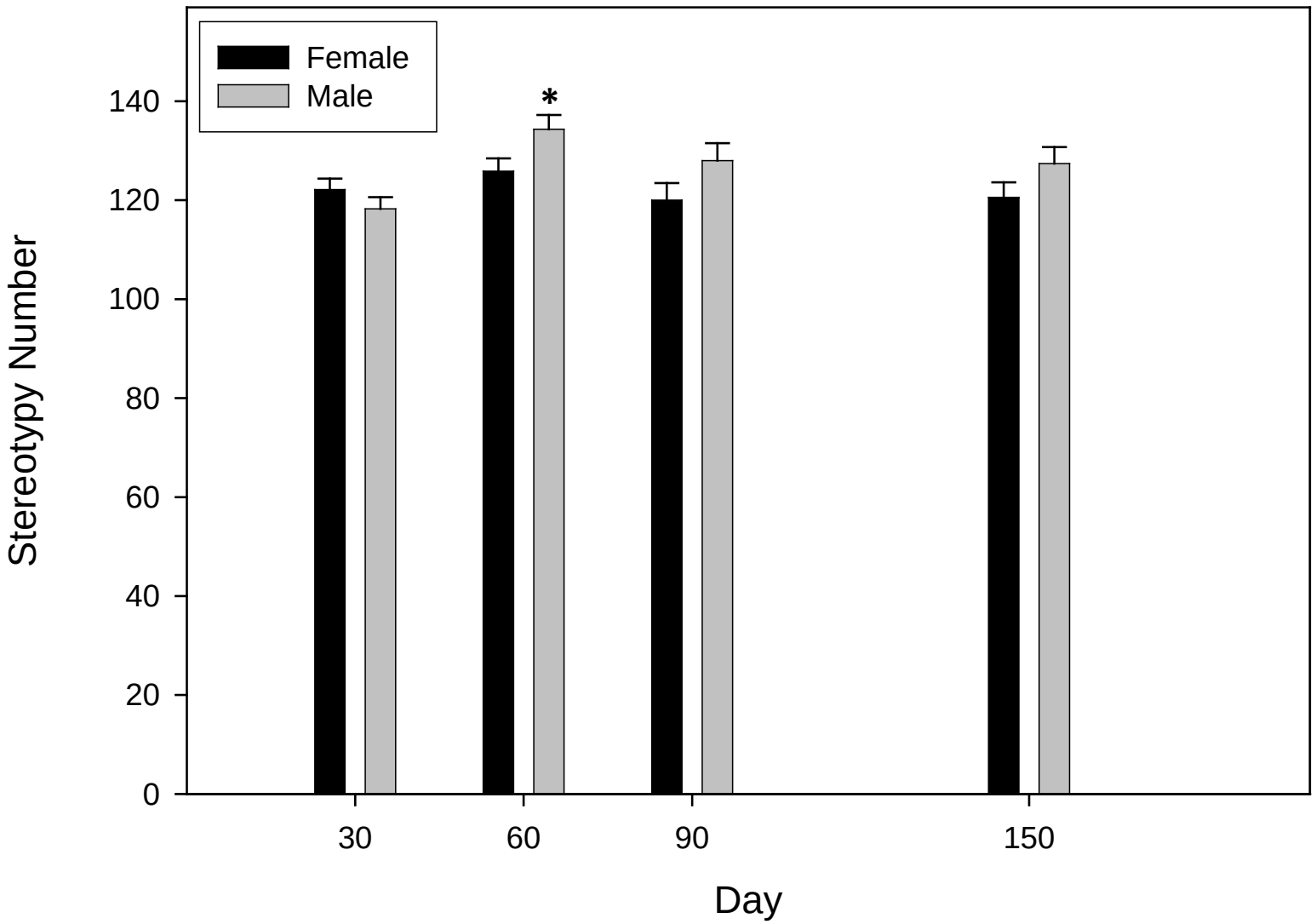
Day


Female
Male
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Stereotypy Number
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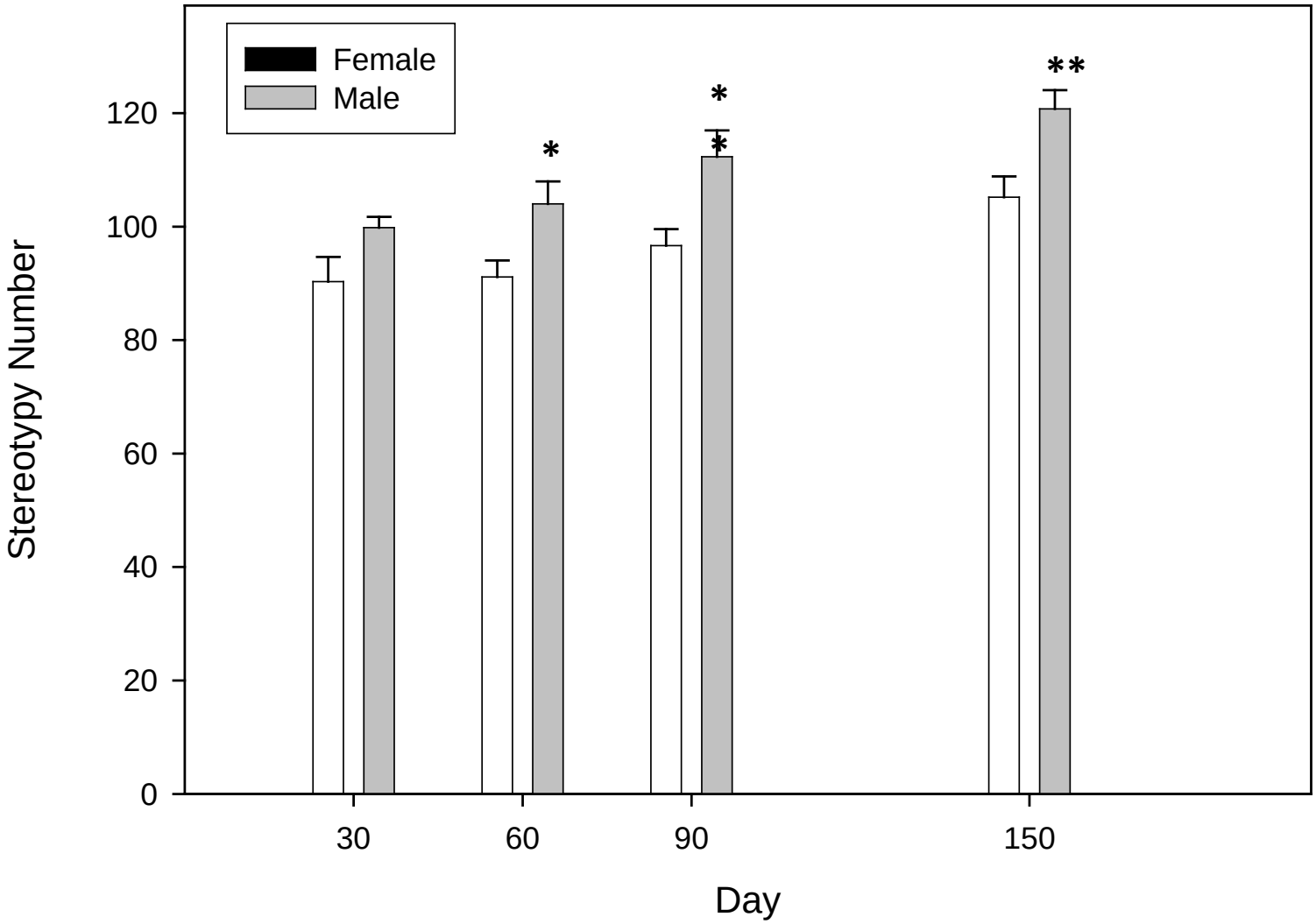
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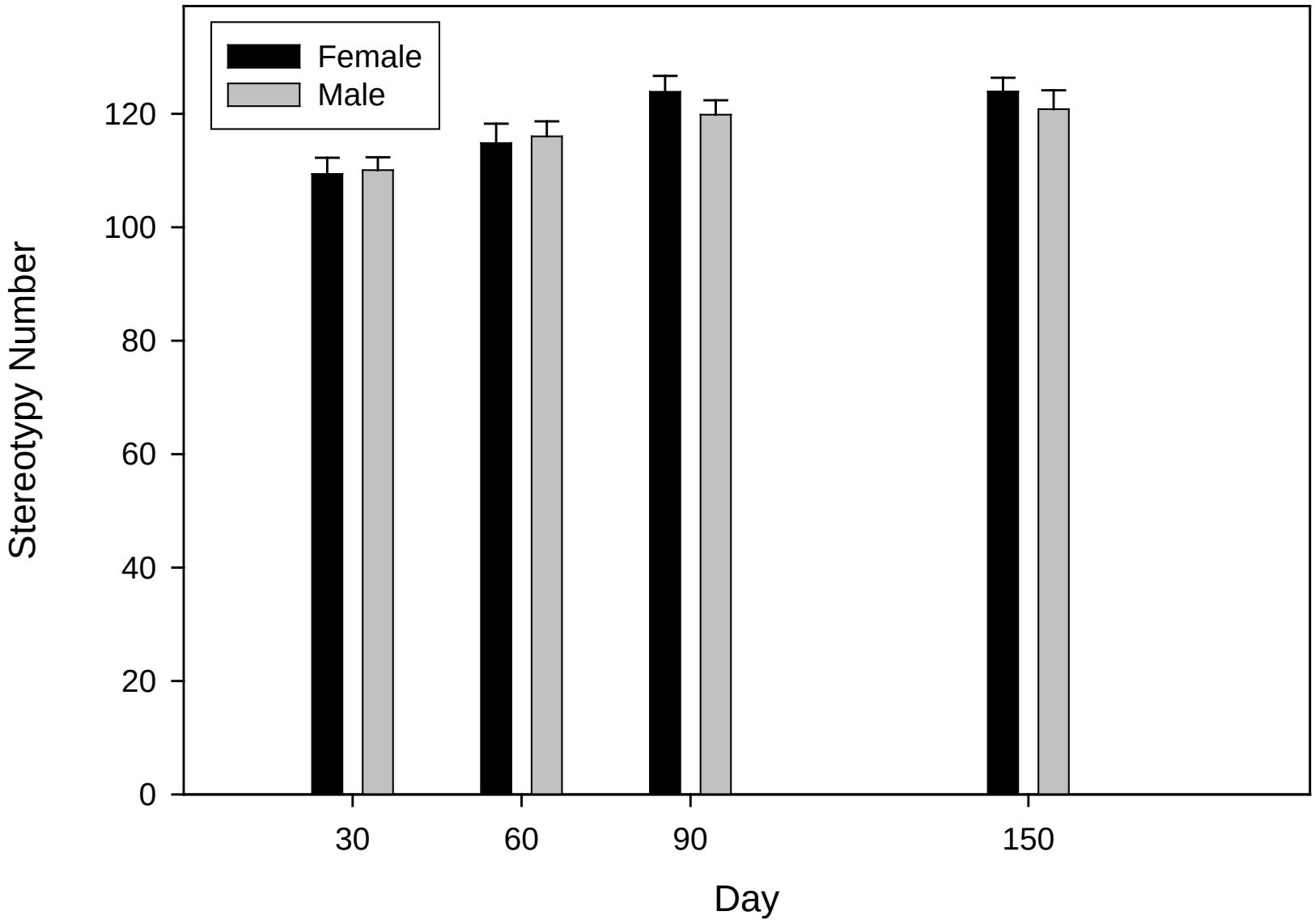
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