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Voluntary exercise influences behavioral development in rats exposed to alcohol during the neonatal brain growth spurt

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Abstract

Children exposed to alcohol prenatally may suffer from severe brain damage, expressed as a variety of behavioral problems, including hyperactivity and learning deficits. There is a critical need to identify effective treatments for fetal alcohol effects. Physical exercise enhances cognitive ability and increases neurogenesis in the hippocampus, a brain area important for learning and memory. Thus, the present study examined whether physical exercise might reduce the severity of alcohol-induced behavioral alterations. Sprague-Dawley rats were intubated with 5.25 g/kg/day ethanol during the third trimester equivalent (postnatal days (PD) 4-9). Intubated sham control and nontreated controls were included. From PD 21-51, half of the subjects were given access to running wheels. On PD 52, subjects were tested on the Morris water maze and on PD 60, open field activity levels were measured. Morris maze performance was significantly impaired among ethanol-exposed subjects; exercise significantly improved performance of all groups. Similarly, ethanol-exposed subjects were overactive in the open field, an effect attenuated with exercise. In sum, these data suggest that exercise may increase neuronal plasticity not only in controls, but also in subjects exposed to alcohol during development.

Keywords

Fetal alcohol syndrome; teratogenic; treatment; behavior; open field; Morris maze; spatial learning; activity level

Introduction

Children exposed prenatally to alcohol suffer from a range of developmental alterations, referred to as fetal alcohol spectrum disorders. On the severe end of the continuum, the child may be diagnosed with fetal alcohol syndrome (FAS), characterized by facial dysmorphology, pre- and postnatal growth deficiencies, and central nervous system (CNS) damage (Hoyme *et al.*, 2005; NIAAA, 2000). Many children exposed to alcohol prenatally may not exhibit all of the diagnostic criteria for FAS, but may still suffer from severe brain damage (Riley & McGee, 2005; Sokol, Delaney-Black, & Nordstrom, 2003). The CNS damage is expressed as a variety of behavioral problems, including hyperactivity, attention deficits, motor dysfunction, language impairments, and learning and social skill difficulties

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(Coles *et al.*, 1997; Kelly, Day, & Streissguth, 2000; Mattson, Schoenfeld, & Riley, 2001; Riley & McGee, 2005; Roebuck, Mattson, & Riley, 1999). Unfortunately, some women continue to drink alcohol during pregnancy; thus, there is a need to understand how prenatal alcohol influences neuronal plasticity and to develop treatments that can attenuate the behavioral alterations experienced by children with FASD.

Animal models suggest that various pharmacological interventions may reduce the severity of fetal alcohol effects (Bonthius, Karacay, Dai, & Pantazis, 2003; Chen, Charness, Wilkemeyer, & Sulik, 2005; Endres *et al.*, 2005; Thomas, Fleming, & Riley, 2001; Wilkemeyer *et al.*, 2003; Wilkemeyer, Menkari, Spong, & Charness, 2002); however, concerns of the safety of these agents may prevent their clinical use and it is less likely that an intervention would be successfully administered during prenatal alcohol exposure. Although clinical data are limited (see Kable, Coles, & Taddeo, 2007), animal studies have demonstrated that experiences, such as environmental enrichment (Hannigan & Berman, 2000; Hannigan, Berman, & Zajac, 1993) or acrobatic motor training (Klintsova, Goodlett, & Greenough, 2000; Klintsova *et al.*, 2002) may reduce the severity of some fetal alcohol effects. These studies indicate that the nervous system continues to be plastic beyond the period of early alcohol exposure and that manipulation of behavior and/or environmental conditions may attenuate ethanol-related behavioral alterations.

Recent animal studies have illustrated that voluntary physical activity and exercise promotes neuronal plasticity, enhancing neurogeneration, neuroadaptation and neuroprotection (Cotman & Berchtold, 2002). Exercise can have general effects on the CNS by increasing vasculature (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; Isaacs, Anderson, Alcantara, Black, & Greenough, 1992), but also can specifically influence hippocampal plasticity. Running increases neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Cotman & Berchtold, 2002; Farmer *et al.*, 2004; Neeper, Gomez-Pinilla, Choi, & Cotman, 1996), increases cell proliferation and neurogenesis in the dentate gyrus (Cotman & Berchtold, 2002; Redila & Christie, 2006; van Praag, Kempermann, & Gage, 1999), stimulates dendritic growth (Redila & Christie, 2006) and enhances long-term potentiation (Farmer *et al.*, 2004; van Praag, Christie, Sejnowski, & Gage, 1999), a process believed to be a mechanism for learning and memory. Consistent with hippocampal changes, voluntary running can enhance cognitive abilities, particularly learning tasks that depend on the functional integrity of the hippocampus (van Praag, Christie *et al.*, 1999). Recent studies further illustrate that exercise enhances cognitive function and increase neurotrophic factors among humans (Winter *et al.*, 2007)

Prenatal alcohol exposure disrupts the development of numerous CNS regions, including the hippocampal formation (Barnes & Walker, 1981; Berman & Hannigan, 2000; Livy, Miller, Maier, & West, 2003; Savage *et al.*, 1992; Sutherland, McDonald, & Savage, 1997; West, Hodges, & Black, 1981). Ethanol-induced hippocampal neuropathology has been linked to impairments in spatial learning and memory (Berman & Hannigan, 2000). Given that developmental alcohol exposure damages the hippocampus and that physical exercise enhances hippocampal function, access to a running wheel may effectively stimulate neuronal plasticity and reduce the severity of some fetal alcohol effects. In fact, recently, Christie and colleagues illustrated that physical exercise in mice exposed to alcohol prenatally may enhance electrophysiological activity in the hippocampus, spatial memory performance (Christie *et al.*, 2005), and cytogenesis (Redila *et al.*, 2006).

The present study examined the effects of exercise in rats exposed to alcohol during the 3rd trimester equivalent brain growth spurt (Dobbing & Sands, 1979), a period of brain development during which the hippocampus is particularly vulnerable to alcohol's teratogenic effects (Bonthius & West, 1991; Greene, Diaz-Granados, & Amsel, 1992; Tran

& Kelly, 2003). All subjects were housed in standard cages with a dam until weaning, after which half of the subjects were housed in cages with running wheels and half in standard cages for a total of thirty days. Performance on a spatial learning task and activity levels were then assessed. It was hypothesized that voluntary exercise would attenuate ethanol's adverse effects on spatial learning abilities and activity levels.

Methods

Subjects

Subjects were ninety-six Sprague-Dawley rats (8 subjects/ treatment /sex), offspring of animals bred at the San Diego State University Animal Care Facility. Multiparous dams were housed with males overnight, and the presence of a seminal plug in the morning indicated mating and constituted gestational day (GD) 0. Pregnant females were then singly housed with food and water *ad libitum* on a 12:12-hr light/dark schedule with lights on at 0600. On postnatal day (PD) 1 (GD 23), litters were culled to 10 pups (5 males and 5 females when possible). All procedures included in this study were approved by the SDSU IACUC and are in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Treatment Design

On PD 4, subjects were randomly assigned to one of 6 treatment groups (3 (ethanol (EtOH), intubation control (SHAM), non-intubated control (NI CONT)) $\times$ 2 (exercise, no exercise)), with no more than one sex pair per litter assigned to each group. Ethanol-treated subjects received a total of 5.25 g/kg/day ethanol via oral intubation (11.9% v/v, twice a day, 2 hours apart) from PD 4-9 (a period of rapid brain development that occurs during the 3rd trimester in humans), followed by two additional oral intubations of milk formula 2 hours apart each day (see Goodlett & Johnson, 1997 for details). This alcohol treatment produces peak blood alcohol levels around 300-350 mg/dl (Thomas, et al. 2007). Intubation controls (SHAM) were orally intubated but did not receive alcohol, whereas non-intubated controls (NI CONT) were removed from the dam during the gavage period but received no intubation. Between intubations, subjects remained with the dam.

All subjects were weighed daily during treatment and periodically thereafter to monitor body growth. On PD 10, the day after the final intubations, all subjects were assigned a numerical paw code through subcutaneous injections of India ink that would allow experimenters to be blind to treatment condition during behavioral testing. All subjects were housed in standard cages with their dam until weaning on PD 21.

On PD 21, half of the subjects from each treatment group (EtOH, SHAM, and NI CONT) were placed in cages with access to running wheels and the remaining subjects were placed in standard cages. Subjects were randomly housed with same-sex pairs. Subjects with access to running wheels had a small wheel inside the cage from PD 21-39, and a larger wheel adjacent to the cage from PD 40-51. The number of wheel rotations on the large wheels was measured every 24-hour period. The average number of revolutions was 10700 ± 1017 , which was approximately 5.9 km/day per rat.

On PD 51, subjects were removed from the cages with running wheels and housed in standard cages during behavioral testing. All behavioral testing was conducted by experimenters blind to the treatment condition.

Behavioral Assessments

Morris Water Maze—On PD 52-57, subjects were tested on a Morris water maze, a task of spatial learning which requires subjects to find a hidden escape platform in a pool of

water. The testing apparatus consisted of a circular white tank (174-cm diameter) filled with 26° C water. An escape platform (10 cm diameter) was submerged 1.25-inches below the surface and powdered milk was added to the water so that the platform was not visible. The room was full of visuospatial cues (e.g. posters, sink, shelves, and video monitors), which remained static throughout the testing trials. The escape platform was located in the center of one of the four quadrants; the position of the platform was pseudorandomly assigned before testing began, with the location counterbalanced across treatment groups. The position of the platform remained constant throughout the testing period for each subject.

Subjects were tested for four trials each day for six consecutive days. During each trial, subjects were placed in the tank and allowed to find the hidden platform. To prevent the use of motor strategies, starting location was varied from trial to trial. Subjects were placed, facing the outer edge of the tank, in one of 12 pre-determined, pseudorandom starting positions. When the subject reached the escape platform, they remained on the platform for 10 sec. If the subject was unable to locate the platform within 60 sec, the experimenter led the subject to the platform where they remained for 10 sec. Path length and latency to find the platform, as well as heading angle (the angle between the initial swimming direction and location of the platform), served as performance measures. On the last day of training, subjects were tested on a probe trial, during which the escape platform was removed and the time spent in the target disc area (3 times the diameter of the platform), as well as passes through the target area, were measured for a 60-sec trial.

Open Field Activity—On PD 60-63, activity levels were measured in an automated open field activity chamber (Hamilton-Kinder, San Diego, CA), consisting of a Plexiglas box (height, 37cm; length, 39cm; width, 43.5cm) equipped with an air circulation fan and an infrared light beam recording system. The chamber was carefully cleaned prior to each test to eliminate any odor cues and white noise was played during the testing to mask any outside noises. Prior to testing, subjects were placed in the testing room and acclimated to the white noise for 30 minutes. Subjects were then individually placed in an activity chamber for 60 minutes during the dark cycle, beginning at 1800 hr each evening, for four consecutive days. The number of interruptions of infrared light beams was recorded and automatically processed by the Hamilton Kinder software. Data from the activity chambers were collected and recorded in twelve 5-minute bins each day.

Data Analyses

All data were analyzed with ANOVAs with treatment group (EtOH, SHAM, NI CONT), exercise (exercise, no exercise) and sex (male, female) as between- subject variables. Day and trial served as repeated within-subject variables for Morris maze testing and day and 5-min bin served as repeated within-subject variables for open field activity. Newman-Keuls post hoc tests were conducted with $p < .05$.

Results

Body Weight

Body weights were analyzed with day as a repeated, within-subject factor from PD 4-15, which included the period of ethanol treatment. All groups increased in body weight over days, producing a significant main effect of day [$F(11,924) = 4341$, $p < .001$]. As seen in Figure 1, panel A, ethanol-exposed subjects lagged in growth compared to both control groups, beginning on PD 5, producing a significant group $\times$ day interaction [$F(22, 924) = 8.7$, $p < .001$] as well as a main effect of group [$F(2,84) = 7.8$, $p < .001$]. At PD 21, ethanol-exposed subjects continued to weigh significantly less than both control groups [$F(2,84) = 5.1$, $p < .01$], but by PD 30, the body weights of ethanol-exposed subjects started catching up,

differing significantly only from SHAM controls [$F(2,84) = 4.1, p < .05$]; there was also a main effect of sex [$F(1,84) = 13.4, p < .001$] as males weighed more than females (data not shown). By PD 40, at the end of the exercise treatment period, there were no longer any effects of treatment; males continued to weigh more than females [$F(1,84) = 124, p < .001$]. Importantly, exercise had no significant effects on body growth.

Morris Water Maze

Ethanol exposure during the neonatal brain growth spurt significantly impaired Morris maze performance and performance was improved among all groups with access to a running wheel. Path length to find the hidden platform is shown in Figure 2; data from one subject was lost due to computer failure. As can be seen, there was a significant effect of treatment [$F(2,83) = 4.8, p < .001$], as well as exercise [$F(1,83) = 11.9, p < .001$]. Follow-up analyses confirmed that ethanol-exposed subjects took longer path lengths to find the hidden platform compared to both control groups who did not differ from one another. Performance improved in all groups, producing a significant effect of day [$F(5,415) = 62.6, p < .001$], trial [$F(3,249) = 40.4, p < .001$] and a day $\times$ trial interaction [$F(15,1245) = 3.3, p < .001$]. In addition, there was a significant interaction of trial $\times$ exercise $\times$ sex [$F(3,249) = 3.0, p < .05$], due to faster improvement in performance within trials among the groups that received exercise, which was more robust among the males than the females (data not shown). A similar pattern was observed for the latency to find the hidden platform (data not shown).

Performance was not affected by treatment-related differences in swimming speed, as there were no effects of treatment or exercise on swimming speed. There were only effects of sex [$F(1,83) = 6.3, p < .05$] due to faster swimming speed in females (males = 0.23 m/sec, females = 0.25 m/sec), day [$F(5,415) = 31.1, p < .001$], trial [$F(3,249) = 17.9, p < .001$] and day $\times$ trial [$F(15,1245) = 2.5, p < .001$], due to reductions in swim speed over training (data not shown).

Heading angle provides a measure of accuracy in the initial swimming direction. As seen in Figure 3, ethanol exposure impaired accuracy, producing a significant main effect of treatment [$F(2,83) = 7.4, p < .001$]. Although exercise appears to have improved performance primarily among ethanol-treated subjects, there was no significant interaction of treatment $\times$ exercise. However, there was a significant effect of exercise [$F(1,83) = 4.7, p < .05$], as exercise improved accuracy when data were collapsed across treatment groups. There were also significant effects of day [$F(5,415) = 13.1, p < .001$], trial [$F(3,249) = 4.0, p < .01$], day $\times$ trial [$F(15,1245) = 2.2, p < .01$] as well as day $\times$ sex [$F(5,415) = 2.4, p < .05$] and trial $\times$ sex [$F(3,249) = 3.3, p < .05$] interactions, as females tended to show more improvement over testing. There were no interactions of sex with any of the treatment conditions.

During the probe trial, there were no significant effects of ethanol treatment; however, exercise improved performance as measured by target passes and time spent in the target area (within an area three times the diameter of the escape platform) (see Figure 4). All groups spent significantly more time in the target area compared to similar areas in other quadrants [main effect of quadrant, $F(3,249) = 198, p < .01$]. There was also a significant interaction of quadrant $\times$ exercise [$F(3,249) = 9.3, p < .01$], due to a main effect of exercise on time spent in target area [$F(1,83) = 10.6, p < .01$] (Figure 4A). It should be noted that the area around the target constitutes about 3% of the total surface area. In addition, there was a significant effect of exercise [$F(1,83) = 11.4, p < .001$] on the number of passes through the target area (Figure 4B).

Open Field Activity

A similar pattern was seen in open field activity. Total distance traveled in the open field chamber is shown in Figure 5. Ethanol-exposed subjects were significantly overactive compared to both control groups, whose activity levels did not differ from one another, producing a main effect of treatment [$F(2,84) = 7.0, p < .01$]. Exercise reduced ambulatory activity in all groups, producing a main effect of exercise [$F(1,84) = 16.2, p < .001$]. There was also a main effect of sex [$F(1,84) = 13.5, p < .001$] as females were more active than males (data not shown), as well as effects of day [$F(3,252) = 88.8, p < .001$], bin [$F(11,924) = 789.5, p < .001$] and day $\times$ bin [$F(33,2772) = 14.6, p < .001$] due to habituation both within and between sessions. There were also significant interactions of bin $\times$ treatment [$F(22,924) = 2.6, p < .001$], bin $\times$ exercise [$F(11,924) = 10.0, p < .001$], bin $\times$ sex [$F(11,924) = 1.9, p < .05$], day $\times$ sex [$F(3,252) = 3.0, p < .05$] and day $\times$ bin $\times$ exercise [$F(33,2772) = 2.4, p < .001$], since differences among treatment, exercise and sex were most obvious during the first half of the testing sessions (Figure 5A). Follow-up analyses confirmed that ethanol-exposed subjects traveled further on bins 2-6 compared to both control groups and exercise improved performance on bins 1-8. Differences were not detected during the latter part of the session, possibly due to a floor effect. Similar effects were observed in distance traveled in the center and periphery of the chamber, movement time, and number of fine movements.

Ethanol exposure also increased the number of entries into the center of the open field, and this effect was mitigated with exercise (see Figure 6A & B). There were main effects of group [$F(2,84) = 8.4, p < .001$], as ethanol exposure significantly increased center entries compared to both control groups, which did not differ significantly from one another. Exercise reduced the number of center entries among all groups, producing a main effect of exercise [$F(1,84) = 15.2, p < .001$]. There was also a significant effect of sex [$F(1,84) = p < .05$], as females entered the center more frequently than males (data not shown). There were effects of day [$F(3,252) = 50.1, p < .001$], bin [$F(11,924) = 555.6, p < .001$] and day $\times$ bin [$F(33, 2772) = 10.9, p < .001$] due to habituation within and between sessions. Finally, there were also interactions of bin $\times$ group [$F(22, 924) = 3.6, p < .001$], bin $\times$ exercise [$F(11,924) = 11.1, p < .001$] bin $\times$ day $\times$ exercise [$F(33, 2772) = 1.9, p < .01$] and bin $\times$ sex [$F(11,924) = 2.2, p < .05$] as treatment and sex effects were more evident early in the session.

A similar result was seen for movement time in the center (data not shown), even though overall time spent in the center was less robustly affected by exercise. As seen in Figure 6C & D, ethanol-exposed subjects spent a significantly greater amount of time in the center of the chamber compared to both control groups, producing a significant main effect of ethanol treatment [$F(2,84) = 12.8, p < .001$]. There were bin $\times$ exercise [$F(11,924) = 11.1, p < .001$] and bin $\times$ day $\times$ exercise [$F(33,2772) = 10.9, p < .001$] interactions, as exercise reduced center time more potently during the early part of the session during the first days of testing; however, the the main effect of exercise failed to reach statistical significance ($p = .07$). As with the other measures, there were significant effects of day [$F(3,252) = 50.1, p < .001$], bin [$F(11,924) = 555.6, p < .001$], day $\times$ bin [$F(33,2772) = 10.9$], as well as interactions of day $\times$ sex [$F(3,252) = 5.3, p < .001$], bin $\times$ sex [$F(11,924) = 2.2, p < .05$], and bin $\times$ group [$F(22,924) = 3.6, p < .001$].

Discussion

The present study illustrates that experience can influence the adverse effects of prenatal alcohol exposure on behavioral development. Consistent with previous findings (Berman & Hannigan, 2000; Goodlett & Johnson, 1997; Kelly, Goodlett, Hulsether, & West, 1988), alcohol exposure during the 3rd trimester equivalent impaired spatial learning performance, increasing path lengths and latencies to find a hidden platform. Voluntary exercise significantly improved performance among both controls and ethanol-exposed subjects,

indicating that exercise can improve behavioral performance among subjects exposed to alcohol during development.

Ethanol exposure significantly impaired performance during the acquisition phase of the Morris maze task, but did not significantly impair performance during the probe trial. This was surprising, both given the magnitude of group effects at the end of acquisition and previous studies that have shown that alcohol exposure during the neonatal brain growth spurt impairs performance both during acquisition and probe trials (Goodlett et al., 1987; Goodlett & Johnson, 1997; Berman & Hannigan, 2000), although at least one other report found ethanol-related acquisition, but not probe, deficits (Marino et al., 2004). Thus, it is possible that other performance variables, rather than spatial learning, were more affected by early alcohol exposure in the present study. Nevertheless, exercise improved performance on both acquisition and probe phases of training, suggesting that exercise does improve spatial memory.

Similarly, exercise attenuated ethanol's effects on open field activity. Ethanol-treated subjects were overactive and less thigmotaxic, behavioral changes that have been commonly reported (Abel, 1984; Bond, 1981; Iqbal, Dringenberg, Brien, & Reynolds, 2004; Kelly, Pierce, & West, 1987; Mattson & Riley, 1998; Thomas, Biane, O'Bryan, O'Neill, & Dominguez, 2007). Exercise altered open field performance, reducing ambulatory and thigmotaxic activity among both controls and ethanol-exposed subjects. Importantly, open field activity levels were examined 10 days after subjects no longer had access to the running wheels, so these results are not simply due to the exercise subjects having an opportunity for more activity in the home cage.

The behavioral effects of exercise were observed several days after subjects were removed from the running wheels. It is currently not known how enduring the behavioral effects of exercise can be, nor is it known how the developmental timing and duration of exercise influences its behavioral effectiveness in ethanol-treated subjects. In the present study, subjects were afforded exercise two weeks after ethanol treatment and for a period of 30 days. This age and duration were chosen to maximize an effect of exercise, but it is possible that shorter durations may also be effective. In fact, behavioral benefits of exercise have been reported following 7 days of voluntary running (Vaynman, Ying, & Gomez-Pinilla, 2004) and BDNF mRNA increases in the hippocampus after only 2 nights of wheel running (Neeper *et al.*, 1996); BDNF enhances brain plasticity and facilitates learning (Tokuyama, Okuno, Hashimoto, Xin Li, & Miyashita, 2000).

It is important to note that exercise was beneficial to ethanol-exposed subjects as well as controls, consistent with the growing literature that exercise enhances cognitive ability not only following brain injury (Will, Galani, Kelche, & Rosenzweig, 2004) but also in control subjects (Fordyce & Wehner, 1993; van Praag, Christie *et al.*, 1999; van Praag, Shubert, Zhao, & Gage, 2005). In the present study, subjects were housed in pairs to eliminate the possibility that isolation-induced stress, which impairs hippocampal plasticity, would interact with exercise (Stranahan, Khalil, & Gould, 2006). Thus, individual running levels are not known. However, there were no significant differences in the number of wheel revolutions between cages that contained ethanol-treated subjects from those that did not (ethanol-exposed subjects, 11482 ± 1337 revolutions/day; non-ethanol-exposed subjects, 9858 ± 1567 revolutions/day). Similarly, we have previously found that although ethanol-exposed subjects have a tendency to run more on wheels as adults, there were no statistically significant effects of neonatal alcohol exposure on overall running (Sakata-Haga *et al.*, 2006). Interestingly, the magnitude of behavioral changes are similar among ethanol-exposed and control subjects on most measures on both the Morris maze and open field.

The mechanisms by which running alters spatial memory and open field behaviors also have yet to be elucidated. Voluntary physical exercise can induce a variety of CNS changes, including increased blood flow (Endres *et al.*, 2003) and angiogenesis (Swain *et al.*, 2003), as well as increases in various neurotrophic levels, such as BDNF (Farmer *et al.*, 2004) and nerve growth factor (NGF) (Pham, Ickes *et al.*, 1999; Pham, Soderstrom, Winblad, & Mohammed, 1999). The hippocampus is one CNS area that is particularly affected by exercise. As noted above, voluntary physical exercise alters hippocampal gene expression (Cotman & Berchtold, 2002; Tong, Shen, Perreau, Balazs, & Cotman, 2001), enhances long-term potentiation (LTP) (van Praag, Christie *et al.*, 1999), stimulates dendritic branching (Redila & Christie, 2006) and promotes neurogenesis (van Praag, Kempermann *et al.*, 1999). In contrast, developmental alcohol exposure damages the hippocampus (Berman & Hannigan, 2000), reduces neurotrophic support (Heaton, Mitchell, Paiva, & Walker, 2000), leads to cell loss (Livy *et al.*, 2003), and impairs LTP (Richardson, Byrnes, Brien, Reynolds, & Dringenberg, 2002), including LTP in the dentate gyrus (Sutherland *et al.*, 1997). It is likely that the behavioral changes observed in the present study may be related, at least in part, to hippocampal function (Riley, Barron, & Hannigan, 1986); thus, some of the beneficial effects of exercise may be related to alterations in hippocampal functioning. In fact, Christie *et al.* (2006) reported that exercise in mice exposed to alcohol during gestation increases hippocampal cytogenesis, although baseline and exercise-induced rates of cytogenesis did not differ between ethanol-exposed and pair-fed controls (Redila *et al.*, 2006). Integration of newly formed cells in the dentate gyrus, as well as potential increases in dendritic branching, alterations of LTP, neurochemical changes, or even increased blood flow could alter the function of hippocampal circuitry.

This leads to the question of how early alcohol exposure influences responsiveness to environmental/behavioral experience. Many studies indicate that experience can influence behavioral development among subjects exposed to alcohol during development, but the findings have been inconsistent (see Hannigan, O'Leary-Moore, & Berman, 2006 for review). For example, environmental enrichment has been shown to mitigate alcohol-related performance deficits on motor tasks (Hannigan *et al.*, 1993) and enhance spatial learning in subjects exposed to alcohol prenatally, although prenatal alcohol exposure failed to impair spatial learning performance in these studies (Hannigan *et al.*, 1993; Wainwright, Levesque, Krempulee, Bulman-Fleming, & McCutcheon, 1993); others have found no benefit of environmental enrichment (i.e. open field activity and avoidance learning) (Osborne, Caul, & Fernandez, 1980). Similarly, handling itself can mitigate ethanol's effects and improve some developmental outcomes (Gallo & Weinberg, 1982; Weinberg & Gallo, 1982), but not all (Gabriel, Johnston, & Weinberg, 2002; Gabriel & Weinberg, 2001; Weinberg, Kim, & Yu, 1995). Recently, Christie *et al.* (2005) (Christie *et al.*, 2005) reported that physical exercise attenuates prenatal ethanol's effects on a working memory version of the Morris maze spatial learning task, consistent with the present study.

The effects of experience on neuronal plasticity are similarly inconsistent. Rema and Ebner (1999) (Rema & Ebner, 1999) reported that prenatal alcohol exposure impairs cortical plasticity, an effect that environmental enrichment attenuated but did not normalize. In contrast, Berman *et al.* (1996) (Berman, Hannigan, Sperry, & Zajac, 1996) reported that environmental enrichment increases the density of hippocampal dendritic spines among controls, but not subjects exposed to alcohol prenatally. Similarly Choi *et al.* (2005) (Choi, Allan, & Cunningham, 2005) reported that exposure to moderate levels of alcohol during gestation prevented the ability of environmental enrichment to enhance hippocampal neurogenesis. Of relevance to the present study, exercise can increase hippocampal cytogenesis in mice exposed to alcohol prenatally, although baseline and exercise-induced rates of cytogenesis did not differ between ethanol-exposed and pair-fed controls in that study (Redila *et al.*, 2006). The present behavioral data suggest that exercise enhances

neuronal plasticity in the hippocampus following ethanol-induced brain damage; further studies will need to examine hippocampal structure and function with the alcohol treatment and physical exercise parameters used in the present study to verify this.

The issue of whether prenatal alcohol exposure compromises plasticity in response to environmental/behavioral manipulations is an important one as it relates to the likelihood of successful treatments for children with FASD. These data indicate that experience, such as physical exercise, may enhance plasticity and recovery of function, influencing the behavioral development of children exposed to alcohol prenatally.

Acknowledgments

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Figure 1.

Body growth from PD 4-15 (panel A) and PD 21-40 (panel B), collapsed across sex. Ethanol-exposed subjects lagged in growth compared to both control groups beginning on PD 5, but body weights did not differ among groups by the time of behavioral testing (PD 40). Exercise had no significant effects on body growth.

MORRIS WATER MAZE

Figure 2.

Ethanol-exposed subjects were significantly impaired on the Morris water maze task, requiring significantly longer path lengths to find the hidden platform. Panel A shows the average path length per trial, collapsed across trials over the training days, whereas Panel B shows the average path length per trial, collapsed across both trials and day. Data are also collapsed across sex. Exercise improved performance among all groups, including the ethanol-exposed groups.

MORRIS WATER MAZE

Figure 3.

Ethanol-exposed subjects were significantly less accurate in their initial swimming direction compared to both control groups. Panel A shows the average heading angle per trial over training days and Panel B shows the mean heading angle collapsed across days. Although largely observed in ethanol-exposed subjects, there was a main effect of exercise, as exercise improved performance with data collapsed across groups.

Figure 4.

Performance on the probe trial was significantly improved following exercise. Panel A shows the amount of time spent in a disc area 3 times the diameter of the escape platform in target and other quadrants and the bottom Panel B shows the number of passes through the target area.

OPEN FIELD AMBULATORY ACTIVITY

Figure 5.

Ethanol exposure during the brain growth spurt significantly increased ambulatory activity in the open field. In contrast, exercise significantly reduced ambulatory activity in all groups. Panel A shows the distance traveled for each 5-min bin, collapsed across day, whereas Panel B shows the average daily distance traveled collapsed across bin, day and sex. It is notable that subjects no longer had access to running wheels during testing, so this was not due to differences in activities within the home cage.

Figure 6.

Ethanol-exposed subjects entered the center of the chamber significantly more frequently (A & B) and spent more time in the center of the chamber (C & D) compared to either control group. Exercise significantly reduced the number of center entries among all groups, but had less robust effects on center time, significantly reducing center time only during the beginning of each session. Panels A and C show average daily data collapsed across day, whereas Panels B and D show data collapsed across day, bin and sex.