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Voluntary exercise rescues deficits in spatial memory and long-term potentiation in prenatal ethanol-exposed male rats

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Abstract

Prenatal ethanol exposure can lead to long-lasting impairments in the ability to process spatial information in rats, as well as produce long-lasting deficits in the ability of animals to exhibit long-term potentiation, a biological model of learning and memory processing. Conversely, we have recently shown that both spatial memory and long-term potentiation can be enhanced in animals that are given access to a running wheel in their home cage. In the present study, Sprague–Dawley rat dams were given one of three diets throughout gestation: (i) a liquid diet containing ethanol (35.5% ethanol-derived calories); (ii) a liquid diet, isocaloric to the ethanol diet, but with maltose-dextrin substituting for the ethanol derived calories and (iii) an *ad libitum* diet of standard rat chow. At weaning (28 days) animals were housed individually in either a standard rat cage, or a cage that contained a running wheel. Adult offspring were tested on a two trial version of the Morris water maze beginning at postnatal day 60, for five consecutive days. Following this, the capacity of the perforant path to dentate gyrus pathway to sustain long-term potentiation was examined in these animals using theta-patterned conditioning stimuli. Our results demonstrate that prenatal ethanol exposure can produce pronounced deficits in both spatial memory and long-term potentiation, but that allowing animal's access to voluntary exercise can attenuate these deficits to the point that those exposed to ethanol prenatally can no longer be differentiated from control animals. These findings indicate that voluntary exercise may have therapeutic benefits for individuals that have undergone prenatal ethanol exposure.

Introduction

Prenatal alcohol exposure can result in a set of characteristic facial anomalies, growth retardation, and neurodevelopmental abnormalities that together are termed 'fetal alcohol syndrome' (FAS; Jones *et al*., 1973). Less severe forms of the disorder, that lack the facial dysmorphology but still exhibit the cognitive deficits, have been termed alcohol-related neurodevelopmental disorders (ARNDs), and along with FAS, have been grouped under the umbrella of fetal alcohol spectrum disorder (FASD; Sokol *et al*., 2003). Prenatal alcohol exposure, even at social drinking levels, is linked to learning and memory problems, among other psychosocial deficits, which can affect academic and social functioning throughout life (Sokol *et al*., 2003). Exposure to alcohol *in utero* has been implicated as the most common cause of mental retardation, and in the United States is the leading preventable cause of birth defects (Stratton & Battaglia 1996), with estimates of FASD incidence approaching one in 100 births (Sampson *et al*., 1997).

Animal models of FAS have been vital to our understanding of the neural structures and behaviours affected by prenatal ethanol exposure (PNEE; Berman & Hannigan, 2000). Among other deficits, PNEE induces impaired spatial learning and memory in the offspring (Blanchard *et al*., 1987; Iqbal *et al*., 2004), with deficits similar to those observed following bilateral lesions of the hippocampus (Morris *et al*., 1982). Although most studies showing Morris water maze deficits in rodents following PNEE have employed the conventional place test (reviewed in Berman & Hannigan, 2000), working memory deficits have also been shown in matching-to-place versions of the Morris water maze (Girard *et al*., 2000; Savage *et al*., 2002). Moreover, similar deficits have been shown in adolescent male humans diagnosed with FAS, using a computerized 'virtual' version of the Morris water maze place task (Hamilton *et al*., 2003). Following PNEE, rodents display a variety of structural and functional deficits, including cell loss, altered dendritic branching, reduced long-term potentiation (LTP) and reduced aspartate release (Sutherland *et al*., 1997; Berman & Hannigan, 2000; Savage *et al*., 2002).

The constellation of structural and functional deficits seen in animal models of FAS is striking in that they are virtually the diametric opposite of the benefits that are seen following voluntary exercise. For example, exercise has been shown to enhance hippocampal cholinergic functioning (Fordyce & Farrar, 1991), neurotrophic factor expression (Farmer *et al*., 2004), performance in the Morris water maze (van Praag *et al*., 1999), performance in the radial arm maze (Anderson *et al*., 2000), long-term potentiation (van Praag *et al*., 1999; Farmer *et al*., 2004), and neurogenesis (van Praag *et al*., 1999; Farmer *et al*., 2004) in rodents. In addition, there is also a plethora of research showing that exercise is beneficial for brain health and function in

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humans, resulting in decreased risk of cognitive impairment, Alzheimer's disease, and dementia in general (Laurin *et al*., 2001; Cotman & Berchtold, 2002). In this study, we examined the capacity of voluntary exercise to rescue the behavioural and electrophysiological deficits normally observed in rats following PNEE.

Methods

Generation of PNEE animals

Twenty-three adult Sprague–Dawley, virgin, female rats (250–275 g; Charles River, Quebec, Canada) were bred with age-matched male rats in suspended stainless steel mesh cages (63 × 24 × 18 cm). During breeding, all rats were given *ad libitum* access to standard rat chow (#5012, Jamiesons Pet Food Distributors, Delta, BC, Canada) and water. The breeding colony room was kept at a constant temperature of 21 °C, and lights were on from 07:00–19:00 h. Animals were checked twice daily for the presence of a vaginal plug. Once a vaginal plug was found, the female rats were singly housed in clear polycarbonate cages (46 × 24 × 20 cm) lined with Carefresh contact bedding (Absorption Corp., Bellingham, WA, USA) and were assigned to one of three groups: (i) prenatal ethanol exposure (PNEE) *ad libitum* access to a liquid ethanol diet (35.5% ethanol-derived calories); (ii) pair-fed (PF) a liquid control diet isocaloric to the ethanol diet (with maltose-dextrin substituting for the ethanol-derived calories), with each PF female offered the amount consumed by an PNEE female on the equivalent day of gestation (in g per kg bodyweight); (iii) *ad libitum* (AL) access to standard rat chow. The PNEE and PF diets were developed by Dr Joanne Weinberg (Department of Anatomy, University of British Columbia) and Dyets, Inc. (Bethlehem, PA, USA) to provide adequate nutrition to pregnant female rats regardless of ethanol intake, and are sold as Modified Lieber-DeCarli High Protein Ethanol Liquid Diet (Dyets # 710324) and Modified Lieber-DeCarli High Protein Control Liquid Diet (Dyets # 710109), respectively. All females were given *ad libitum* access to water throughout gestation. In the late afternoon, just prior to the dark cycle, the PNEE and PF bottles from the previous day were removed and weighed to determine the amount consumed, and appropriate amounts of fresh diet were given. On day 22 of gestation, the PNEE and PF mothers were taken off their respective liquid diets and given *ad libitum* access to rat chow. Birth usually occurred with 24 h.

The pregnant females were weighed on days 1, 7, 14, and 21 of gestation. Forty-eight hours after birth, on postnatal day 2 (PN2), the pups and the mother were weighed and the litters were culled down to ten (five females and five males, when possible), to control for any confounding effects of litter size or sex ratio. The pups were weaned on PN22, after which the offspring were group housed according to litter and sex in clear polycarbonate cages (46 × 24 × 20 cm), with *ad libitum* access to standard rat chow and water.

Experimental subjects

Male offspring were randomly selected at PN54 from all litters to be used as subjects. Two subjects were used from each litter, with one subject being singly housed in a polycarbonate cage (46 × 24 × 20 cm) with a running wheel (Mini Mitter, Bend, OR, USA), while the other was singly housed in the same type of cage without a running wheel. The cages were lined with bedding, and rats had *ad libitum* access to water and rat chow. The light cycle and temperature were kept constant as before. The subjects now formed six experimental groups [the controls – nonrunners, PNEE ($n = 12$); PF ($n = 12$); AL ($n = 10$); and the runners, PNEE-Run ($n = 11$), PF-Run ($n = 12$); AL-Run ($n = 8$)]. Testing on the Morris water maze took place on days PN60–PN64 for each rat, and electrophysiology was carried out for the next 2 weeks thereafter.

Behavioural apparatus

A white circular pool (180 cm diameter × 60 cm high) was filled to a 21-cm level with room-temperature water made opaque with non-toxic white tempera paint powder (Reeves and Poole Group, Toronto, ON). A circular platform (12 cm diameter × 18 cm high) was placed within the pool, submerged approximately 3 cm below the surface of the water. The platform was placed in the centre of a predetermined quadrant (North-east, North-west, South-east, or South-west) for each trial. The experimenters remained concealed behind a large screen, so as not to become visual cues, and observed the rat via a video tracker mounted above the pool. Each rat's swimming pattern was traced and recorded by the computer software HVS Water (HVS Image) with input from the video tracker. The overhead lighting in the room was turned off and lights mounted on the walls of the testing room provided dim lighting. The position of extra-maze cues in the testing room remained constant throughout testing.

Morris water maze procedures

The methods used in the present experiments are similar to those previously reported by others (Girard *et al*., 2000; Savage *et al*., 2002). Four to six rats were brought into the testing room at a time, in temporary holding bins (46 × 24 × 20 cm). The bins were placed on a counter behind the large screen with the experimenters during testing. Each day before a rat's testing, it had a patch of fur between its ears coloured black with a non-toxic marker (Staedtler permanent marker, Lumocolor), to create a colour contrast against the white pool that the video tracker could detect. Rats were tested in pseudorandom order, and experimenters were blind to the treatment groups. All testing was carried out during the light cycle, between 08:00 h and 14:00 h.

The rats performed two trials on each of five consecutive days. Each trial began with the rat being placed in the pool and released facing the side wall at one of four positions (the boundaries of the four quadrants, labelled N, S, E and W). Release positions were randomly predetermined, but the same for all rats on all trials for a given day of testing. The platform was placed in the centre of a different quadrant each day (pseudo-randomly predetermined) but remained in the same quadrant for both trials on a given day. On each trial, the rat was allowed to swim until it found and remained on the platform for 10 s, or until 300 s had passed and it had not found the platform, at which point it was guided to the platform by the experimenter and allowed to stay on the platform for 10 s (while the experimenter returned behind the large screen). Then the rat was removed from the pool, dried, and placed back in its holding bin for a period of 5 min, after which the second trial was conducted.

Swimming speed across trials for all groups was analysed using repeated-measures ANOVA, to ensure that the dependent variable measure, latency to the platform, would not be influenced by extraneous variables such as fitness. Analysis of latency to the platform was performed using the original six treatment groups. All analyses were conducted using Statistica software (Statsoft, Tulsa, OK).

Electrophysiology

Animals were anaesthetized with Somnotol (65 mg/kg, i.p.) and placed into a stereotaxic apparatus (Kopf Instruments). Supplemental

doses of Somnotol were administered as required to maintain a surgical plane of anaesthesia. Rectal temperature was maintained at 37 ± 1.0 °C with a grounded homeothermic temperature control unit (Harvard Instruments, MA, USA). Using stereotaxic coordinates determined previously (Christie & Abraham, 1992a,b; Farmer *et al*., 2004), a 125-μm stainless-steel recording electrode (A–M Systems, Inc., WA, USA) was directed through a trephine hole into the DG (3.5 mm posterior, 2.0 mm lateral to bregma). Similarly, a 125-μm monopolar stimulating electrode (A–M Systems, Inc.) was directed through a trephine hole to activate the medial perforant path input to the DG (7.4 mm posterior, 3.0 mm lateral to bregma). The final depth of the stimulation and recording electrodes was determined by adjusting both electrodes to yield the maximal fEPSP using a minimal degree of stimulation. Once a response was obtained, paired-pulse stimuli (25, 50, 100 ms interpulse intervals) were administered to ensure that medial perforant path responses exhibited paired-pulse depression (McNaughton & Barnes, 1977; Christie & Abraham, 1992a,b). Responses were evoked using single pulse stimuli, of a fixed amplitude and duration (120 μs), and were delivered at 15-s intervals. Baseline responses were standardized to 30% of maximal response size, and recordings were required to exhibit a stable baseline for a minimum of 30 min to be included in the data set. Theta-patterned stimuli (ten bursts of five pulses at 100 Hz with a 200 ms interburst interval) were administered to induce LTP. The administration of the protocol was repeated five times at 2-min intervals. Signals from the dentate hilus were amplified and filtered (1 Hz and 3 kHz) using a differential amplifier (Getting Instruments, San Diego, CA, USA) and then digitized at 5 kHz before being stored on a PC using custom-written software (Lee Campbell; Getting Instruments) and National Instruments data acquisition hardware. The slope of the rising phase of the field EPSP was used to determine alterations in the level of synaptic efficacy (Christie & Abraham, 1992a,b). Following the administration of the conditioning stimuli, single pulse stimuli were again administered for a minimum period of 1 h. All EPSP slope data are presented as the mean per cent change from the pre-conditioning baseline ± SEM. Analysis of variance and unpaired *t*-tests were conducted on electrophysiological data as appropriate using Statistica software (Statsoft, Tulsa, OK).

Results

Pregnancy outcome

Animals administered the liquid diet containing ethanol continued to gain body weight throughout the gestation period, and consumed an average of 10.51 ± 0.001 g of ethanol per kilogram of bodyweight per day. Unfortunately, a technical error with the blood samples from these animals prevented an accurate blood alcohol concentration analysis. Thus, in five separate pregnant female animals that consumed almost identical amount of the diet (10.47 ± 0.001 g/kg ethanol per day), we took additional blood samples and determined that these animals had an average blood alcohol concentration of 184 ± 50 mg/dL. This level of consumption and the blood alcohol levels are similar to those found in other studies using identical procedures (Keiver & Weinberg, 2003, 2004) and are also similar to levels recorded in other animals in separate studies performed in our laboratory (unpublished observations).

The effects of chronic prenatal ethanol exposure on the average litter size, birth weight, and the number of male or female offspring are presented in Table 1. On average, the AL group gave birth 1.55 days earlier than PNEE animals, and 0.68 days earlier than PF animals. This undoubtedly reflects the fact that PNEE and PF animals need to be re-introduced to an AL diet on gestational day 22 to help induce birth (Keiver & Weinberg, 2003, 2004). The AL animals typically produced more male offspring (61% male) than the PNEE animals (41% male); however, we culled litter sizes to ten animals (five males and five females) to minimize any effects differences in the ratio of male to female pups might have had on our study. The differences in size between the AL and PNEE animals disappeared after the first week of life, and, as is shown in Table 1, these animals gained weight normally over the next three weeks and are indistinguishable from AL and PF animals on this measure at weaning. These data are similar to those generated by other groups that have employed similar methods (Keiver & Weinberg, 2003, 2004). Maternal behaviour was also monitored prior to weaning, and no differences were observed in licking, grooming or other indices of maternal behaviour between the different groups.

TABLE 1. The effects of chronic prenatal ethanol exposure on the average litter size, birth weight, and the number of male or female offspring

	Treatment		
Pregnancy outcome variable	PNEE	Pair-fed	*Ad libitum*
Number of pregnant dams	8	8	7
Maternal death/illness/ abnormality	0	0	2
Spontaneous abortion	0	0	0
Perinatal death	8*	0	0
Length of gestation (days)	$23.75 \pm 0.16^{\dagger}$	22.88 ± 0.13	22.20 ± 0.20
Litter size	14.13 ± 1.13	12.75 ± 1.31	15.80 ± 0.58
Offspring weight PN2 (g)	$7.44 \pm 0.33^{\dagger,+}$	8.28 ± 0.58	8.64 ± 0.36
Offspring weight PN8 (g)	$22.7 \pm 0.7^{+}$	24.8 ± 1.0	25.3 ± 0.9
Offspring weight PN15 (g)	38.8 ± 2.3	41.4 ± 1.9	40.7 ± 1.0
Offspring weight PN22 (g)	62.5 ± 2.5	62.3 ± 2.8	61.3 ± 2.1
Male offspring (%)	$41.39 \pm 4.17^{+}$	$40.64 \pm 4.11^{+}$	61.21 ± 6.81
Female offspring (%)	$58.61 \pm 4.17^{+}$	$59.36 \pm 4.11^{+}$	38.79 ± 6.81

The data for maternal death, spontaneous abortion, and perinatal death are reported as the number of occurrences. The other data are reported as group means ± SEM. *Four animals lacking milkbands were euthanized and four animals were lost to maternal cannibalism. $^{\dagger}P < 0.05$ compared with pair-fed group. $^{+}P < 0.05$ compared with *ad libitum* group.

Prenatal ethanol exposure produces impairments in reference and working memory that can be attenuated with voluntary exercise

All animals were tested with the experimenters blind as to the identity of the animals being tested. Following the completion of the task by all animals, analysis of variance revealed that water maze performance of the PNEE, AL control, and PF control groups did not differ significantly on any of the measures taken (latency, path length, swim speed, $P > 0.05$), indicating that nutritional differences did not affect behavioural performance in the control animals. A repeated measures ANOVA revealed that animals in the PNEE non-runner (PNEE-NR) group performed significantly worse than PF-NR and Al-NR animals on Trial 1 of the task over the five day testing period ($F_{5,25} = 43.7$, $P < 0.01$). The Trial 2 performance of PNEE animals was also impaired when compared to animals in the control group ($F_{5,25} = 22.8$, $P < 0.01$), however, this effect is largely due to the large impairment in performance seen on day 1 (Fig. 1B). Thereafter, these did not differ significantly from either PF or AL animals in their ability to find the platform (Table 2).

Surprisingly, when allowed access to voluntary exercise, no differences were observed between animals in the PNEE, AL and

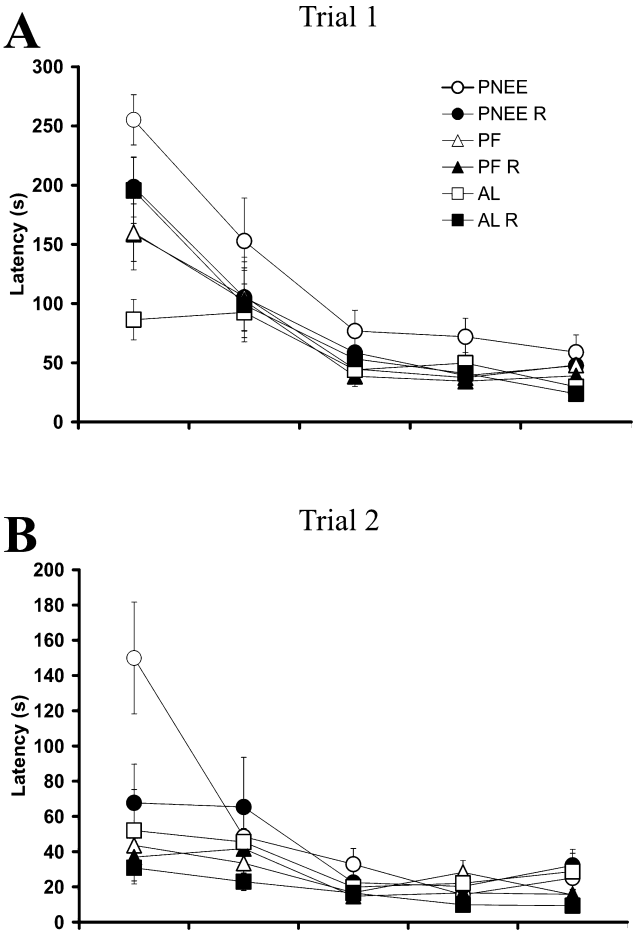

FIG. 1. Effects of PNEE on reference and working memory. (A) PNEE animals that did not exercise (PNEE-NR, open circles) were significantly slower in finding the platform on Trial 1 than either group of non-exercising control animals (AL-NR or PF-NR). (B) When animals were required to find the same platform position five minutes later in Trial 2, they were again initially slower than control animals in performing the task. In contrast PNEE animals that exercised (PNEE-R; filled circles) performed as well as control animals that exercised (AL-R, PF-R) and better than PNEE animals that did not exercise on both Trial 1 (A) and Trial 2 (B).

TABLE 2. Average time to reach platform for each group across days

	Time to reach platform (s)					
	PNEE ($n = 11$)	PF ($n = 12$)	AL ($n = 10$)	PNEE-Run ($n = 10$)	PF-Run ($n = 12$)	AL-Run ($n = 8$)
Trial 1 data						
Day 1	255 ± 21	159 ± 30	86 ± 17	198 ± 25	160 ± 24	195 ± 28
Day 2	153 ± 36	106 ± 29	93 ± 16	105 ± 34	103 ± 25	99 ± 31
Day 3	77 ± 18	45 ± 8	44 ± 8	59 ± 13	39 ± 9	53 ± 11
Day 4	72 ± 16	37 ± 8	50 ± 9	39 ± 8	35 ± 5	41 ± 10
Day 5	59 ± 15	48 ± 11	30 ± 7	48 ± 11	39 ± 8	24 ± 6
Trial 2 data						
Day 1	150 ± 32	44 ± 9	52 ± 23	68 ± 22	37 ± 13	31 ± 9
Day 2	49 ± 16	33 ± 7	45 ± 7	65 ± 28	42 ± 11	23 ± 5
Day 3	33 ± 9	17 ± 3	19 ± 5	22 ± 7	15 ± 4	16 ± 5
Day 4	16 ± 3	28 ± 7	22 ± 4	20 ± 5	17 ± 4	10 ± 2
Day 5	25 ± 7	15 ± 3	28 ± 10	32 ± 9	16 ± 3	9 ± 1

Data are presented as means ± SEM.

PF groups ($P > 0.05$; repeated measures ANOVA). In fact, PNEE animals that exercised (PNEE-Run) performed significantly better than animals that had been exposed to ethanol prenatally but did not exercise ($F_{5,15} = 48.2$, $P < 0.01$). This improvement is reflected in the reduced time it took these animals to find the platform on Trial 1 across days 1–4, and the reduced score for Trial 2 of day 1 seen in the PNEE animals that exercised (Table 2; Fig. 1C and D). Neither exercise nor prenatal diet had any significant effect on swimming speed ($P > 0.05$), indicating that physical fitness was not a confounding measure in this data set. In fact, analysis of variance indicated that all animals in all groups (both runner and non-runner) showed equivalent swim speeds in both Trial 1 and Trial 2 across all days tested.

Voluntary exercise leads to enhanced LTP induction in the dentate gyrus of animals following prenatal ethanol exposure

Electrophysiological recordings were conducted on 45 animals in total, with a minimum of seven animals being tested from each group (AL, PF, PNEE, AL-Run, PF-Run, PNEE-Run). The optimal recording location for each animal was determined as the point at which the largest response could be obtained with a minimal amount of current being used. This procedure is quite robust for determining the optimal recording and stimulating coordinates, and analysis of variance verified that the different groups did not differ in the baseline amplitude of their initial evoked responses (Fig. 2A; $F_{5,37} = 0.11$, $P > 0.05$). Thus, neither prenatal ethanol exposure nor reduced caloric intake (PF animals) produced long-lasting effects on electrophysiological signalling at medial perforant path – dentate gyrus synapses. Furthermore, our analysis indicated that transmitter release at these synapses was equivalent between groups as the degree of paired-pulse depression at these synapses did not vary significantly (Fig. 2B). These analyses indicate that the response profiles in adult animals, following prenatal ethanol exposure, are not significantly different from those of control animals.

Next we sought to determine if the different *in utero* conditions might produce a persistent change in the capacity of medial perforant path, dentate gyrus synapses, to exhibit LTP. For these experiments we use conditioning stimuli that were patterned after the theta activity that is normally observed in the hippocampal EEG of freely moving animals during behavioural testing. With these stimuli, we found that all groups tested were capable of exhibiting a significant degree of LTP, as measured 60 min following the application of the conditioning stimuli; however, analysis of variance revealed there were significant differences between the groups ($F_{1,5} = 10.94$, $P < 0.001$). The rising phase of the EPSP slope was increased in animals from the AL group 30 ± 6% ($t_5 = 4.83$; $P < 0.05$) at this time point, while animals from the PF group showed a 28 ± 5% LTP ($t_7 = 5.85$, $P < 0.05$). In contrast, although showing significant LTP (14 ± 4%; $t_8 = 3.31$, $P < 0.05$), animals in the PNEE group showed significantly less LTP than both PF ($t_{15} = -2.15$; $P < 0.05$) and the AL ($t_{13} = -2.21$; $P < 0.05$) animals (Fig. 3A).

We have previously shown that allowing animals exposure to voluntary exercise significantly enhances LTP induction in both normal mice (van Praag *et al.*, 1999) and rats (Farmer *et al.*, 2004). Similarly, in the present study, animals in the AL-Run group also showed significantly more LTP than control AL animals (57 ± 7%; $t_{11} = 2.84$; $P < 0.05$). Enhanced LTP was also observed in the PF group (60 ± 6%; $t_{12} = 2.50$; $P < 0.05$), indicating that the reduced nutritional intake during pregnancy did not produce long-lasting reductions in the ability of these animals to exhibit 'enhanced' LTP.

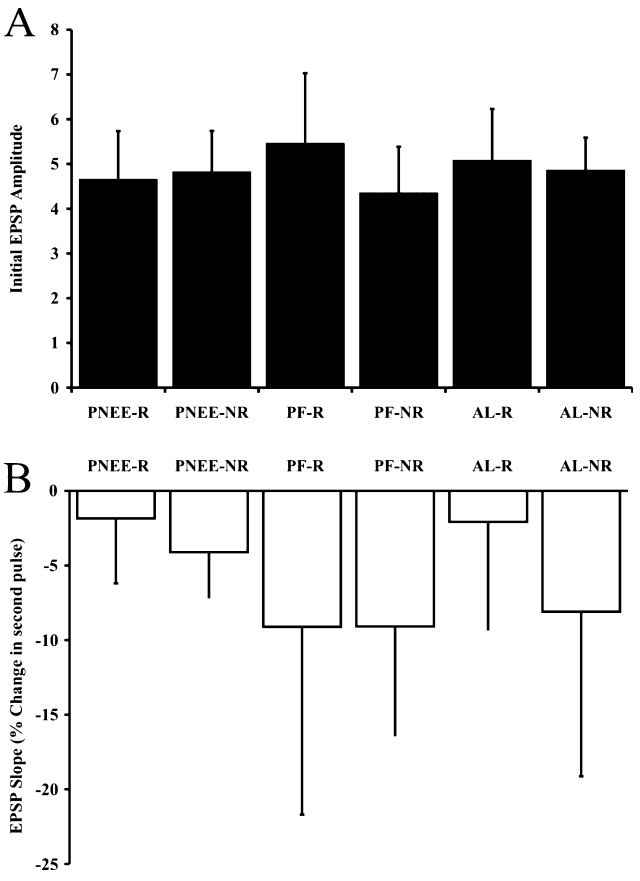

FIG. 2. Prenatal ethanol exposure does not markedly alter evoked responses in the dentate gyrus. (A) Maximal response amplitudes, for medial perforant path evoked responses in the dentate gyrus, did not vary significantly between groups. (B) Paired-pulse responses also did not vary significantly across groups, indicating that transmitter release did not vary significantly across groups.

More strikingly, animals in the PNEE group also benefited from exposure to exercise, showing significantly more LTP than their non-running littermates (34 ± 5%; $t_{14} = 3.19$; $P < 0.05$). While exercise raised the degree of LTP in PNEE animals to a level not significantly different from that seen in the AL and PF control groups ($P > 0.05$), these animals still exhibited significantly less LTP than PF runners ($t_{11} = -3.33$; $P < 0.05$) and AL runners ($t_{12} = -2.75$; $P < 0.05$). Thus, while exercise increases the overall amount of LTP seen following prenatal ethanol exposure, the induction of LTP in PNEE animals is still impaired when compared to runners from the other control groups (Fig. 3B).

Discussion

The present results indicate that prenatal ethanol exposure has deleterious effects on both synaptic plasticity and the initial performance of adult animals in a matching-to-place version of the Morris water maze. The behavioural effects manifested themselves in two types of performance deficits. First, the PNEE animals did not learn as easily that they were required to find a new platform location on the first trial of each day, performing significantly worse than control animals in the PF and AL groups. Second, animals from the PNEE group also did not remember the platform location as accurately when they were re-introduced to the same task 5 min later. This deficit was especially pronounced on day 1. The induction of LTP, with conditioning stimuli patterned after the naturally occurring theta activity in the hippocampal formation (Christie & Abraham, 1992a,b), was also significantly impaired in PNEE animals. These findings are similar to those obtained previously by other researchers using different conditioning stimuli, and different methods for exposing animals to ethanol prenatally (Sutherland *et al*., 1997; Richardson *et al*., 2002). Most striking was the fact that both LTP induction and behavioural performance on the water maze could be improved to levels similar to control subjects, simply by allowing animals in the PNEE group access to voluntary exercise.

Our behavioural data also support those of Savage *et al*. (2002) who found learning deficits in animals that received either a 3% or 5% ethanol diet prenatally. In this study, all of the animals performed equally well on Trial 1; however, there was little improvement on Trial 2 for the last 3 days of testing in animals that were exposed to ethanol prenatally. This indicates that the learning deficits associated with prenatal ethanol administration in their study were more pronounced on working memory and qualitatively different than our results. We saw deficits across the initial days of Trial 1, indicating that reference memory was impaired in our animals. The Trial 2 deficits we observed mainly occurred on the day 1, after which PNEE animals were able to perform at a similar level to both PF and AL control animals. Girard *et al*. (2000) also used the matching-to-place version of the Morris water maze task to evaluate the effects of perinatal binge alcohol exposure on spatial working memory in rats. Rats were given a task in which the platform location was constant over sets of four trials (compared to two trials in our study). In Phase 1 of testing, beginning at PN35 and using an intertrial interval (ITI) of either zero or 60 s, the ethanol-exposed rats performed poorer than controls on Trials 2, 3, and 4. In Phase 2, beginning at 15 weeks postnatal and using the same ITIs, the same ethanol-exposed rats performed poorer on Trials 2 and 3 but not 4. In Phase 3, during the sixth postnatal month, using an ITI of either 60 s or 2 h, ethanol-exposed rats were no longer impaired following the 60-s ITI but were impaired following the 2-h ITI. A practice effect was apparent, as the ethanol-exposed animals 'caught up' with controls in terms of working memory performance on the Morris water maze. The fact that the binge pattern of ethanol exposure (compared to the steady ethanol exposure in our study) caused animals to require more practice to perform as well as controls (Girard *et al*., 2000), is consistent with evidence from a large-scale, population-based study in humans showing that maternal binge drinking is the strongest alcohol-related predictor of adolescent attention/memory deficits (Streissguth *et al*., 1994).

Although prenatal alcohol exposure is often accompanied by numerous cognitive deficits, it has been found that the difference in IQ levels are not sufficient to explain the extent to which subjects with FAS are affected by distractions and requirements for rapid information processing (Kerns *et al*., 1997). One interesting observation from our studies was that the PNEE animals always performed markedly worse on the initial day for both trials. Although we examined the search strategies employed by the animals, no consistent pattern was observed. Instead, the animals adopted a wide variety of (incorrect) search patterns. Because of the magnitude of the effect on day 1, it is possible that their performance on this task is actually indicative of an inappropriate reaction to the 'stress' of the testing situation. Prenatal ethanol exposure, using a similar ethanol-containing diet, has been shown to alter the adrenocortical response to both predictable and unpredictable stressors, causing deficits in the ability of these animals to use or respond to external cues (Weinberg, 1992). This is important, as animals in the matching-to-place version of the water maze rely on

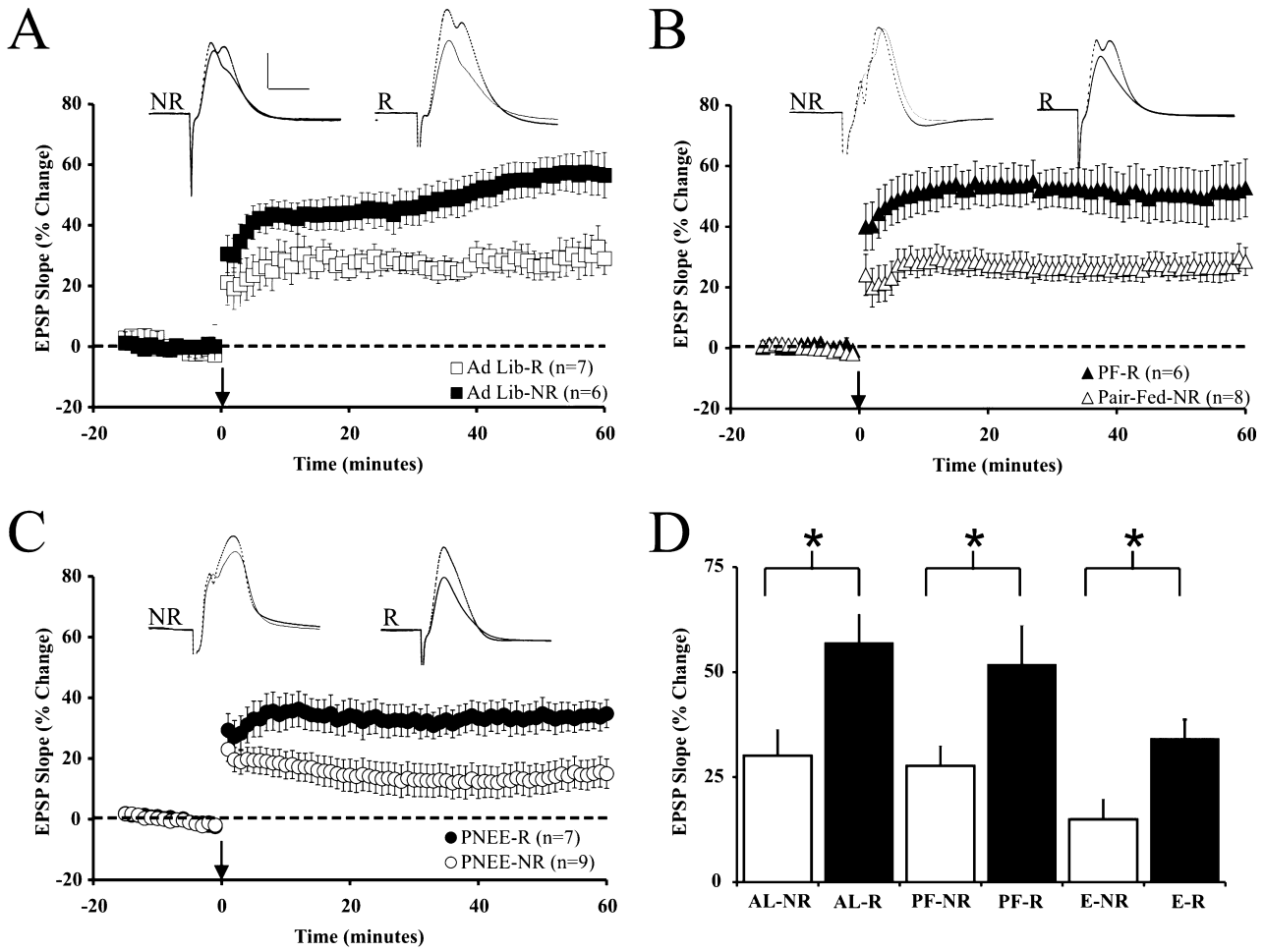

FIG. 3. Exercise attenuates PNEE-induced LTP deficits. Following the administration of theta-patterned conditioning stimuli (arrow), animals that engage in voluntary exercise show enhanced LTP when compared to control animals whether they are derived from animals fed the *ad libitum* diet (A), the pair-fed diet (B), or the ethanol containing diet (C) during gestation. Insets show representative waveforms prior to (solid) and following (dashed) the application of the theta patterned conditioning stimulation in control (left) and runner (right) animals. (D) No matter the diet, LTP was much more reliably and robustly induced in animals that exercised. *$P < 0.05$. Scale bar, 2 mV, 10 ms.

visual-spatial cues as a navigational aid. Thus, it is possible that the initial deficits in learning we observed are the result of an altered stress response in the PNEE animals, which in turn produces impaired learning. Further support for this conjecture comes from the fact that exercise has also been shown to decrease levels of ACTH and corticosterone in behaving rats (Watanabe *et al*., 1991), and in the present study, PNEE animals that exercised did not show these deficits in learning when compared to control animals. Further testing is needed to elucidate whether an abnormal stress response is involved though, as recently it has been reported that animals that exercise only show a reduced stress response when they are exposed to a novel environment that contains a running wheel (Droste *et al*., 2003). In other situations, their response to stress is similar to that of control animals.

Exercise has also been shown to regulate levels of brain-derived neurotrophic factor (BDNF) in the hippocampus (Berchtold *et al*., 2001) and increased hippocampal BDNF following voluntary exercise is one putative link between exercise and improved spatial learning (Neeper *et al*., 1996; Cotman & Berchtold, 2002). BDNF levels, along with the levels of other neurotrophic factors, are affected by *in utero* alcohol exposure in rodents, although the effects are not straightforward and seem to depend on the period of ethanol exposure, the age of the animal when killed, and the specific brain structure being examined (Heaton *et al*., 2000). In rats exposed prenatally to ethanol and killed at gestation day 21 or PN10, there was a significant decrease in BDNF mRNA levels (Maier & West, 2001). In another study using prenatal ethanol exposure, and subsequent killing of the animals at birth, no changes in BDNF were observed in the hippocampus, septum, cortex/striatum or cerebellum (Heaton *et al*., 2000). Following early postnatal ethanol exposure, and killing at PN10, BDNF was elevated in the hippocampus and cortex/striatum (Heaton *et al*., 2000). Heaton and colleagues speculate that while such an increase in BDNF could play a protective role, increases in neurotrophin levels during critical developmental periods could also be detrimental, and potentially even contribute to some of the CNS abnormalities that have been observed following developmental ethanol exposure.

The relationship between altered BDNF levels and prenatal ethanol exposure may be important for explaining the reduction in LTP observed in both the present study and that by Sutherland *et al*. (1997). First, PNEE has been shown to reduce BDNF levels in the brain (Maier & West, 2001). Second, deletions in the coding sequence for BDNF significantly reduce BDNF levels, and this in turn reduces the magnitude of hippocampal LTP (Korte *et al*., 1996). Thus, the

reduction in hippocampal LTP we observed may be due to some mechanism affected by a reduction in BDNF levels. While the relationship between prenatal ethanol exposure and BDNF levels across the life of the animal is likely far more complicated than that presented here, what is interesting is that others have shown that BDNF levels can be restored using a number of procedures. For instance, the effects of gene deletions can be reversed with either viral-mediated gene transfers (Korte *et al*., 1996) or direct BDNF infusions (Patterson *et al*., 1996). Furthermore, brief applications of BDNF, but not other neurotrophic factors, can enhance the ability of both hippocampal and cortical slices to exhibit LTP (Akaneya *et al*., 1997; Huber *et al*., 1998; Kovalchuk *et al*., 2002), and low concentrations of BDNF improve the ability of theta-patterned conditioning stimuli to induce LTP by acting to increase SK2 serine phosphorylation (Kramar *et al*., 2004). Similarly, voluntary exercise has been shown to increase both BDNF levels (Adlard *et al*., 2004; Farmer *et al*., 2004) and LTP induction (Farmer *et al*., 2004). Together this suggests that the enhanced LTP we observed in all groups that engaged in exercise in the present study may be mediated by a BDNF-associated mechanism. Support for this notion can be taken from other models of neuronal injury, where voluntary exercise has been shown to enhance both recovery and function following traumatic brain injury (Griesbach *et al*., 2004) and to reverse nutritionally mediated reductions in learning and LTP (Molteni *et al*., 2004) via a BDNF mediated mechanism(s). Clearly further testing is needed to determine what role, if any, BDNF can play in the recovery of function in FAS.

In conclusion, we report here that prenatal ethanol exposure can produce impairments in both reference and working memory that are apparent well into adulthood, and that these deficits are accompanied by a reduced capacity in the ability of the hippocampal dentate gyrus to sustain long-lasting LTP. Allowing these animals access to voluntary exercise can significantly ameliorate these deficits and dramatically improve both behavioural learning and the capacity for LTP induction in animals following prenatal ethanol exposure, indicating that exercise may have a therapeutic value in helping to improve learning and memory performance in individuals with FAS.

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Abbreviations

AL, *ad libitum*; BDNF, brain-derived neurotrophic factor; FAS, fetal alcohol syndrome; ITI, intertrial interval; LTP, long-term potentiation; PF, pair-fed; PN, postnatal day; PNEE, prenatal ethanol exposure.

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