Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats

I. Behavioral results

Anna Y. Klintsova a,*, Rita M. Cowell a, Rodney A. Swain b, Ruth M.A. Napper c, Charles R. Goodlett d, William T. Greenough a,e,f,g,h

a Beckman Institute, University of Illinois, Urbana-Champaign, 405 N. Mathews Ave., Urbana IL 61801, USA
b Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, USA
c Departments of Anatomy and Structural Biology, University of Otago Medical School, Dunedin, New Zealand
d Department of Psychology, Indiana University–Purdue University, Indianapolis, USA
e Department of Psychology, University of Illinois, Urbana-Champaign, USA
f Departments of Cell and Structural Biology, University of Illinois, Urbana-Champaign, USA
g Department of Psychiatry, University of Illinois, Urbana-Champaign, USA
h Neuroscience Program, University of Illinois, Urbana-Champaign, USA

Accepted 28 April 1998

Abstract

The effects of complex motor task learning on subsequent motor performance of adult rats exposed to alcohol on postnatal days 4 through 9 were studied. Male and female Long–Evans rats were assigned to one of three treatments: (1) alcohol exposure (AE) via artificial rearing to 4.5 g kg$^{-1}$ day$^{-1}$ of ethanol in a binge-like manner (two consecutive feedings), (2) gastrostomy control (GC) fed isocaloric milk formula via artificial rearing, and (3) suckling control (SC), where pups remained with lactating dams. After completion of the treatments, the pups were fostered back to lactating dams, and after weaning they were raised in standard cages two–three animals per cage until they were 6 months old. Rats from each of the postnatal treatments then spent 20 days in one of three conditions: (1) inactive condition (IC), (2) motor control condition (MC) running on a flat oval track, or (3) rehabilitation condition (RC) (learning to traverse a set of 10 elevated obstacles). After that all the animals were tested on three tasks, sensitive to balance and coordination deficits (parallel bars, rope climbing and traversing a rotating rod). On parallel bars, both male and female rats demonstrated the same pattern of outcomes: AE-IC rats made significantly more mistakes (slips and falls) than IC rats from both control groups. After 20 days of training in the RC condition, there were no differences between AE and both SC and GC animals in their ability to perform on the parallel bars test. On rope climbing, female animals showed a similar pattern of abilities: AE-IC rats were the worst group; exercising did not significantly improve the AE rats’ ability to climb, whereas the RC groups SC, GC and AE all performed near asymptote and there were no significant differences among three neonatal treatment groups. There was a substantial effect of the male rats’ heavier body weight on climbing ability, and this may have prevented the deficits in AE rats behavior from being detected. Nevertheless, male animals from all three postnatal treatments (SC, GC and AE) were significantly better on this task after RC. Female and male rats from all three postnatal groups demonstrated significantly better performance on the rotarod task after 20 days of ‘rehabilitation’. These results suggest that complex motor skill learning improves some of the motor performance deficits produced by postnatal exposure to alcohol and can potentially serve as a model for rehabilitative intervention.

Keywords: Alcohol; Fetal alcohol effects; Motor learning; Plasticity; Cerebellum

1. Introduction

Children with fetal alcohol syndrome (FAS) or fetal alcohol effects (FAE) exhibit numerous cognitive problems, hyperactivity and motor deficits (e.g. Refs. [14,34,47,71,72,76]). Some of the consequences of this prenatal exposure to alcohol appear to be lifelong while others may dissipate with age [46,69,75,76]. In cases lacking distinct facial abnormalities (sometimes called FAE), a predominant behavioral characteristic that provides a basis for the diagnosis has been cognitive deficits [47] and deficits in motor development and performance [13,14,70].
Not all mothers who consume alcohol during pregnancy produce children with FAS or FAE: the factors that are thought to determine the occurrence of the behavioral and anatomical pathology include the developmental stage(s) when the drinking episode(s) occurred, the pattern of exposure and the peak blood alcohol concentration (BAC) reached during drinking episodes [23,35,68,73,93]. Social drinking during pregnancy or lactation has been reported to cause impaired motor development that lasted through adolescence [43,74].

Animal models of developmental exposure to alcohol exhibit many of the behavioral changes observed in children with FAS and FAE: memory and learning impairments [2,17,26,64,97], developmental impairment of motor skills, poor locomotion and coordination, altered gait [1,20,22,31,39,49,50,56] and hyperactivity [8,9,65,67,82]. Poorly developed motor skills in prenatally and neonatally ethanol-exposed animals prevent them from successfully performing on balance-challenging tasks [20,22,39,49,50,80].

The behavioral deficits appear to reflect underlying structural damage resulting from exposure to ethanol during development: while damage is widespread, impaired motor control appears to be associated primarily with cerebellar damage (e.g., Refs. [4,10,44,59,60,81]).

A few attempts have been made to reverse or mitigate behavioral incompetence resulting from developmental exposure to ethanol. Early behavioral experience (e.g., complex environment rearing, or familiarization with the radial maze) brought about improvement on learning tasks, such as the Morris water maze and the radial arm maze [30,32,57,58,83] and preweaning handling eliminated the deficit in response inhibition in prenatally alcohol-exposed rats [19]. These studies demonstrated that animals exposed to alcohol prenatally can benefit from the effects of an enriched postweaning environment or other behavioral experiences, and that postnatal factors can ameliorate some of the deficits resulting from prior exposure to alcohol.

Rearing rats in an enriched environment after prenatal exposure to alcohol significantly improved behavioral performance, but failed to produce a detectable increase in the density of spines on CA1 pyramidal neurons in hippocampus [5] or an increase in the depth of the occipital cortex [83], although these changes normally occur in control animals (e.g., Refs. [5,33,37,38,66,95]). Berman et al. ’s [5] findings were interpreted to reflect reduced neural plasticity after prenatal exposure to alcohol. This hypothesis was supported by the demonstration that prenatal alcohol exposure reduced reactive axonal sprouting in basal ganglia induced by nigrostriatal lesions [24]. In contrast, an increase of lesion-induced sprouting was reported in hippocampus of prenatally ethanol-exposed rats after entorhinal cortex lesions [15,89]. Hippocampal synaptic plasticity in the form of long-term potentiation exhibits long-lasting deficits after prenatal exposure to alcohol as shown by Swartzwelder et al. [78] and Sutherland et al. [77].

In a previous report [42], we demonstrated that Purkinje neurons, the sole output neurons of the cerebellum, retained a substantial capacity for synaptic plasticity after alcohol exposure on postnatal days 4–9, a model of human maternal binge alcohol consumption during the third trimester of pregnancy. Exposure to a program of complex motor skill training resulted in a significant increase in the number of parallel fiber synapses per Purkinje neuron. Furthermore, performance on the task improved across training such that by the end of 10 days, there were no significant differences between alcohol-exposed animals and controls in terms of time to complete the set of tasks used for the motor training.

Because of the forced nature of the training, however, it was not possible to conclude that there was significant improvement in the specific behavioral performance of the ethanol-impaired animals. In the present study, we report that motor learning, but not simple exercise, produces a true therapeutic effect on balance and coordination impairments resulting from neonatal ethanol exposure.

2. Methods

2.1. Subjects

A total of 130 rats (65 female, 65 male) from 17 litters resulting from timed pregnancies of adult Long–Evans rats (Simonsen Labs, Gilroy, CA) bred in the Indiana University–Purdue University, Indianapolis (IUPUI) vivarium were used in this study. Gestational day 0 was identified by the presence of sperm in a vaginal smear taken the morning after an overnight mating. The day of birth was nearly always gestational day 22 (postnatal day 0), and litters were culled to 10 pups (5 males, 5 females whenever possible) on the day after birth. The breeders, their suckling litters, and the subsequently weaned rats were maintained in the IUPUI vivarium with ad libitum food and water on a 12 h: 12 h light–dark cycle with lights on at 0700 h. Offspring were weaned at 25 days of age and housed 2–4 per cage with same-sex animals.

2.2. Artificial rearing and alcohol exposure

On PD 4, pups were assigned randomly within litter and sex to three groups (Fig. 1): alcohol-exposed (AE)—artificially reared pups given a 10.2% (v/v) ethanol solution in milk formula on two consecutive feedings each day (4.5 g kg⁻¹ day⁻¹) from PD 4–9; gastrostomy control (GC)—artificially reared pups given matched isocaloric maltose/dextrin solutions on PD 4–9; suckle controls (SC)—reared normally by lactating dams. The rats assigned to the artificial rearing groups were surgically implanted with intragastric feeding tubes under methoxyflurane (Pitman–Moore, Mundelein, IL) anesthe-
Fig. 1. Design of the study: pups were evenly distributed across three postnatal treatment groups (suckling control—SC, gastrostomy control—GC, and alcohol-exposed—AE) on postnatal days 4–9, then the tubes were removed and animals were returned to their mothers. When the animals were 6 months old, they spent 20 days in one of three conditions: inactive (IC), motor (MC) or rehabilitation (RC). After completion of this period, they were tested on three specific behavioral tasks.

sia on PD 4 and reared using well-established procedures [10,11,21,22,90].

All intragastric feedings used a customized milk formula [90] delivered every 2 h using programmable Harvard Model 22 infusion pumps. Alcohol (or maltose/dextrin) was provided on the first two feedings after 8:00 h each morning; all other feedings and all feedings on PD 10–12 used milk formula alone. Each day, the rats were fed a volume of formula (in ml) equal to 33% of the mean body weight (in g) of the litter being reared. Seventy minutes after the end of the second alcohol feeding on PD 6, a 20-μl sample of blood was collected in a heparinized capillary tube from a tail-clip of each artificially reared pup. The blood from the alcohol-treated pups was assayed with an enzymatic assay for ethanol content (Sigma kit #332-BT, St. Louis, MO) using a Guilford Response spectrophotometer (absorbency at 340 nm) by comparison to a concurrently derived standard curve of five known alcohol concentrations (0–450 mg/dl).

Artificially reared offspring were fostered back to lactating dams on PD 12; all rats were weaned at 25 days of age, and housed 2–4 per cage with same-sex littermates thereafter. The rats were identified by a paw code (on PD 12) by injection of a small amount of India ink into one or more of the paws for subject number, and by an ear punch code (after PD 60) designating litter number.

Table 1
Female rats body weight at PD 180 and 220

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>N rats</th>
<th>Body weight PD 180</th>
<th>Body weight PD 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>IC</td>
<td>7</td>
<td>294 ± 4</td>
<td>286 ± 4</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>6</td>
<td>290 ± 7</td>
<td>290 ± 7</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>7</td>
<td>303 ± 14</td>
<td>294 ± 10</td>
</tr>
<tr>
<td>GC</td>
<td>IC</td>
<td>6</td>
<td>291 ± 9</td>
<td>292 ± 11</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>6</td>
<td>292 ± 14</td>
<td>277 ± 9</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>7</td>
<td>290 ± 9.5</td>
<td>269 ± 8</td>
</tr>
<tr>
<td>AE</td>
<td>IC</td>
<td>9</td>
<td>292 ± 7.8</td>
<td>278 ± 8</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>8</td>
<td>312 ± 8</td>
<td>301 ± 7</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>9</td>
<td>286 ± 6</td>
<td>285 ± 5</td>
</tr>
</tbody>
</table>
Table 2
Male rats body weight at PD 180 and 200

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>N rats</th>
<th>Body weight PD 180</th>
<th>Body weight PD 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>IC</td>
<td>8</td>
<td>499 ± 12</td>
<td>500 ± 12</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>7</td>
<td>541 ± 16</td>
<td>536 ± 13</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>7</td>
<td>528 ± 15</td>
<td>508 ± 14</td>
</tr>
<tr>
<td>GC</td>
<td>IC</td>
<td>7</td>
<td>491 ± 24</td>
<td>496 ± 24</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>7</td>
<td>515 ± 10</td>
<td>508 ± 11</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>6</td>
<td>482 ± 10</td>
<td>472 ± 11</td>
</tr>
<tr>
<td>AE</td>
<td>IC</td>
<td>9</td>
<td>493 ± 13</td>
<td>509 ± 13</td>
</tr>
<tr>
<td></td>
<td>MC</td>
<td>7</td>
<td>475 ± 14</td>
<td>477 ± 15</td>
</tr>
<tr>
<td></td>
<td>RC</td>
<td>7</td>
<td>500 ± 15</td>
<td>470 ± 13</td>
</tr>
</tbody>
</table>

2.3. Rehabilitative motor skill training procedure

Rats were transferred to the University of Illinois vivarium at age 60–100 days. They were housed in same-sex pairs until they reached age 180 days. At that point, animals from each treatment group (SC, GC and AE) were assigned to one of three training conditions (Fig. 1): an inactive condition (IC), a motor control condition (MC) or a rehabilitative condition (RC). At least 6 animals per group/condition/sex were involved in the study, a total of 65 female and 65 male animals in the nine experimental groups. All animals were coded so that the experimenters were not aware of the early postnatal treatment. Whenever possible, littermates with the same postnatal treatment were assigned to IC, MC and RC; no more than 1 male and 1 female of the same treatment within each litter was assigned to a given training condition. On the first day of training, all rats were housed individually in standard laboratory cages. RC rats were given 5 trials per day on an elevated obstacle course for 20 days as described previously [6,41]. The obstacles included a horizontally placed wooden ladder, narrow rods, a link chain, barriers on narrow beams, a rope ladder, an elastic cord, ascending and descending stairs, and a narrow v-shaped metal bridge. The rats were forced to traverse the obstacle course by gentle prodding of the hindquarters, while the tail was loosely held to prevent falls. The time to complete the entire set of 10 obstacles was recorded on each trial. The mean time to complete 5 trials/day was computed for each animal. Each RC animal was pair matched with an animal in the motor control condition. MC rats were forced to traverse a flat, opaque, oval Plexiglas track equal in length to the acrobatic course. Both animals were placed onto their respective courses at the same time and removed each time that the AC animal had finished each of 5 trials. Animals received the same amount of prodding during training, such that the amount of handling stimulation was comparable for each rat. IC animals were housed individually and received no motor training or extra-cage motor

Fig. 2. Mean daily latencies of female rats on the complex motor skill training. Both AE and GC animals were significantly worse than SC during the first 6 days. Data presented as a mean ± S.E.M.

![Daily times to complete the set of obstacles: Female rats](image-url)
activity but were handled for about 5 min each day. At the completion of the 20 days of rehabilitative motor skill training, the motor ability of each rat was tested on parallel bars, a rotating rod and a vertically suspended rope.

2.4. Parallel bar testing

This task is particularly sensitive to the hindlimb coordination impairment that characterizes alcohol-affected rats (e.g., Ref. [80]). The parallel bar apparatus consisted of two parallel wooden rods (0.48 cm diameter each, 91 cm long) connected to black Plexiglas platforms at each end (15 × 15 cm). The rods were fastened to a wooden block that could be screwed to the platform. Three sets of parallel rods with an inter-rod distance of 2.5 cm, 5 cm or 6.25 cm were used for testing. On the first day, rats were introduced to the 2.5 cm parallel rods. On the second day, the 5 cm bars were used, and on the third day, the 6.25 cm bars were used.

On the first day of testing, the subject was initially placed on the starting platform for 30 s. Approximately half of all rats started to traverse the parallel bars on their own initiative. Those that did not were carefully placed on the rods next to the platform, with both left paws on one bar and both right paws on the other bar. Four successive alternating steps with the hind legs on the rods constituted a successful traversal. The number of times the subject placed two hind paws on one rod, dropped a hind paw below the rod, stepped twice in succession with the same hind paw, fell or swung under the rods was recorded. Subjects were given three consecutive trials on parallel bars of a given distance. The subject was removed from the testing for the day and considered to have ‘failed’ if the number of errors was more than 5 per trial (in which case the subject was assigned 5 errors for that trial and each remaining trial of the day). For each day of testing, the following data were recorded: mean traversal time per trial, number of errors per trial, the type of error.

2.5. Rotating rod testing

The rotating rod apparatus consisted of one of three 2 m long, 15 cm diameter, PVC rods, suspended 1 m above the polyurethane foam-covered bottom of a plywood enclosure. Individual rods either had a roughened surface (by builders’ sand applied to the freshly painted surface of the rod; hurdles 3.3 cm thick, foam strips wrapped and glued to the surface of the rod at about 40 cm intervals); or were smooth. Twenty centimeter wide start and goal platforms were at the ends of the rod. These platforms could be extended by flip-down plywood platforms that covered the entire rod or portions of it. The animal’s task was to traverse the rod lengthwise while it was rotating at a preset RPM. Pretraining consisted of the animal’s traversing the apparatus for food reward with the rod entirely covered by the plywood flaps. On day 1 of testing, the rats traversed the roughened rod at speeds of 0, 6, 15 and 25 RPM. Rats were given 3 trials per speed, and hesitation time on the

![Daily times to complete the set of obstacles: Male rats](image)

Fig. 3. Mean daily latencies of male rats on the complex motor skill training. AE male rats are significantly worse than both SC and GC rats on the first 8 days of training. Data presented as a mean ± S.E.M.
platform and the time to traverse the rod were recorded. The number of slips and ‘falls’ (rats were ‘caught’ by the experimenter such that they did not actually fall) was counted, and the subject was withdrawn from the task after a combination of 5 slips or falls. Days 2 and 3 of testing utilized the smooth rod and the rod with hurdles, respectively, at the speeds reported above. On day 4, the animals were tested on the smooth rod rotating at speeds of 6, 15, 25 and 30 rpm.

2.6. Rope climbing testing

Generally, animals that cannot coordinate their forelimb and hind limb movements fail the rope climbing task, and thus the task is also sensitive to the forelimb–hindlimb coordination impairment that is characteristic of the alcohol-produced damage.

A vertical rope (2.5 cm, 1.9 cm or 1.25 cm in diameter) was suspended from a platform 1 m above the base. During pretraining, the rat was taught by gentle encouragement to climb the upper half of the 2.5 cm thick rope. Following this pretraining session, the rats were tested on the 2.5 cm, 1.9 cm and 1.25 cm thick ropes over the next three consecutive days (1 rope per day, 3 trials per day). Time to climb the entire rope was recorded, and a subjective score was assigned for each trial as follows: 1—excellent performance, no prods or help needed, 2—a small amount of prodding (usually in the beginning of climbing) was required, 3—the rat would climb up with extensive prodding, but would try to go down when the prodding was interrupted, 4—heavy prodding was required along the whole length of the rope, 5—could not climb or even hold the rope, but slid down.

2.7. Data analysis

Although several variables were available for each of the testing procedures, the most straightforward measure of the behavior, i.e., number of failures, was chosen to represent the performance on both the parallel bar and rotating rod tasks. The subjective score was analyzed for the performance on the rope climb. Performance of male and female rats was analyzed separately, because obvious performance differences between the sexes were evident dur-

Fig. 4. Results of female (top) and male (bottom) rats’ performance on parallel bars: both male and female AE rats demonstrated a significantly higher number of slips/falls than their SC and GC littersmates after spending 20 days in IC or MC. Learning the complex motor skill task (RC) resulted in significant improvement of performance (right graphs) in all groups of animals, so that AE rats were no longer different from the two control groups. Data presented as a mean ± S.E.M.
ing behavioral testing. Statistical tests (ANOVA) were run using the SAS general linear model procedure and were conducted at a significance level of alpha = 0.05. Two-way repeated measures ANOVA with GROUP (postnatal treatment: SC, GC or AE) and TRAINING (IC, MC or RC) as between-group factors was used to analyze the results of all three behavioral tests. The Student Newman—Keuls post-hoc (alpha = 0.05) test was used for a posteriori comparisons among individual groups.

3. Results

3.1. Blood alcohol concentration and body weight

The delivery of alcohol in two consecutive feedings resulted in an average peak blood alcohol concentration of 248 ± 10 mg/dl in male animals and 278 ± 14 mg/dl in female animals (blood alcohol concentration was measured in 21 out of 23 male rats and in 20 out of 26 female rats). At 6 months of age, the male rats were about 70% heavier than the females (compare Tables 1 and 2). Body weight did not differ statistically among the animals of the same sex from the three neonatal treatment groups (SC, GC and AE) at PD 180 (Tables 1 and 2), and was not significantly decreased after 20 days of training.

3.2. Rehabilitative motor skill training

During the course of the rehabilitative motor skill learning task employed in this study, it became obvious that animals in all groups significantly improved their motor performance. The performance of male and female AE rats on the obstacle course did not differ statistically from either control group by the end of the 20-day training period (Figs. 2 and 3).

3.3. Parallel bars (Fig. 4)

The mean number of slips on this task was significantly lower overall in female and male rats after complex motor training compared to inactive control and motor control animals. For females, there was a main effect of neonatal treatment (GROUP; $F_{2,140} = 6.65, p < 0.01$) and adult TRAINING ($F_{2,140} = 29.90, p < 0.001$), as well as an interaction (GROUP × TRAINING; $F_{4,139} = 2.93$,

![Rope Climbing Performance](image)

Fig. 5. Results of female (a) and male (b) rats testing on rope climbing task. (a) AE rats (both those that remained in individual cages and those who ran on the track) had the least ability to climb and performed significantly more poorly than the rest of the animals. Rehabilitation training resulted in improvement of motor skills of all SC, GC and AE animals such that the groups no longer differed statistically. (b) Untrained or track-trained male rats from all three SC, GC and AE groups demonstrated poor climbing abilities (due, most probably, to their excessive body weights). Nevertheless, rehabilitation (RC) resulted in significantly better scores in all animals. Data presented as a mean ± S.E.M.
Male rats did not demonstrate a significant interaction between these factors (F(4, 175) = 1.15, n.s.), although there was an effect of GROUP (F(2, 176) = 5.39, p < 0.01) and TRAINING (F(2, 176) = 21.53, p < 0.001). Post-hoc comparisons showed that both female and male IC and MC rats given neonatal ethanol exposure committed significantly more errors on the parallel bar task than rats from either control group (Fig. 4a,b, IC and MC panels). The 20 days of rehabilitative training significantly improved the abilities of the AE male and female rats on Fig. 4a,b, IC and MC panels. The 20 days of rehabilitative training significantly improved the abilities of the AE male and female rats on Fig. 4a,b, IC and MC panels.

Fig. 6. Results of female rats on the rotating rod. AE animals that remained in individual cages had consistently more slips and falls on the test on each day of training (left column, open circles). AE rats that ran on the track (middle column) showed the same pattern of deficits in performance. After 20 days of rehabilitation, the difference in performance disappeared and the skills of all groups were improved (right column). Data presented as a mean ± S.E.M.
this test of hindlimb motor function such that they no longer differed from their SC and GC littermates (Fig. 4a,b, RC panel).

3.4. Rope climbing (Fig. 5)

This task required animals to climb a series of three ropes of diminishing diameter. Performance on this task was scored from 1 to 5, such that the better the performance the lower the score.

Rope climbing performance appeared to be affected by the excessive body weight of the males. For females there was a main effect of neonatal treatment (Fig. 5a) (GROUP; \(F_{2,140} = 11.33, p < 0.001\)) and adult TRAINING (\(F_{2,140} = 22.66, p < 0.001\)), as well as GROUP \(\times\) TRAINING interaction (\(F_{4,139} = 2.69, p < 0.05\)). Male rats did not demonstrate a significant interaction between groups of training (Fig. 5b) (\(F_{4,175} = 0.30, \text{n.s.}\)), nor was there an effect of neonatal treatment (GROUP; \(F_{2,176} = 0.53, \text{n.s.}\)). Only Fig. 7. Male rats’ performance on the rotating rod was not as clear cut as in females: the damage produced by exposure to alcohol was not obvious in animals that remained in individual cages (left column), and was somewhat recognizable in animals after MC (middle column). After 20 days of RC training, the skills were improved in all groups of animals (right column). Data presented as a mean ± S.E.M.
adult TRAINING produced a significant effect on males’ performance \( (F_{2,176} = 41.10, \ p < 0.001) \). Post-hoc comparisons showed that ethanol-exposed IC and MC female rats scored significantly higher (i.e., worse performance) on the rope climbing test than SC and GC counterparts of the same training conditions (Fig. 5a). Female rats from all three postnatal groups given 20 days of ‘rehabilitation’ demonstrated significantly better performance on the rope climbing task (Fig. 5a, filled symbols). Post-hoc comparisons for male rats showed no difference between the performance of IC and MC rats from all three postnatal groups (Fig. 5b), although rehabilitative training resulted in significant improvement of animals from all three groups (Fig. 5, filled symbols). This indicates that rehabilitative intervention did offset the effects of alcohol exposure, but that the intervention had no statistically greater effect in AE rats than it had in controls.

4. Discussion

This study has demonstrated that acquisition of complex motor tasks, but not mere exercise, can rehabilitate the motor deficits occurring as a result of developmental exposure to alcohol. In this animal model of binge-drinking during the period of brain development comparable to that of the third trimester of human pregnancy, substantial loss of cerebellar neurons has been documented in previous studies reviewed in Ref. [23]. The significant improvement in motor performance produced by behavioral rehabilitation was evident despite the permanent loss of cerebellar neurons induced by the neonatal binge alcohol exposure.

Behavioral testing of animals on a set of tasks sensitive to the deficits known to be induced by neonatal alcohol treatment (traversing parallel bars and a rotating rod, and rope climbing) demonstrated a generally consistent pattern of improved motor skills in alcohol-exposed animals after rehabilitative training (AE-RC) to the extent that they were no longer significantly different from the SC and GC rats. Simple physical exercise (running on a track in the motor condition—MC) did not generally improve motor performance of alcohol-exposed animals. The females followed this pattern of changes quite closely across all tasks. One problem with testing the male animals was that, at the older ages used in this study, their excessive body weights interfered with testing across all experimental conditions.

For females, there was a main effect of early postnatal condition (GROUP; \( F_{2,50} = 3.68, \ p < 0.05 \)) and adult TRAINING \( (F_{2,50} = 5.19, \ p < 0.05) \), but no GROUP \( \times \) TRAINING interaction \( (F_{2,50} = 0.73, \) n.s.). There was also a main effect of speed of rotation \( (F_{3,150} = 123.02, \ p < 0.001) \) and a TRAINING \( \times \) SPEED interaction \( (F_{6,150} = 3.49, \ p < 0.01) \). Post-hoc comparisons showed that ethanol-exposed IC and MC female rats made significantly more errors on the rotating rod test than their control counterparts of the same adult training, especially at the higher speeds (Fig. 6, first and second columns of graphs). Female rats from all three postnatal groups demonstrated significantly better performance on the task (Fig. 6, third column of graphs) after 20 days of ‘rehabilitation’. This result also indicates that rehabilitative intervention can offset the effects of alcohol exposure but that the intervention had no statistically greater effect than it had in controls.

For males (Fig. 7), there was a main effect of early postnatal condition (GROUP; \( F_{2,58} = 3.30, \ p < 0.05 \)) and adult TRAINING \( (F_{2,58} = 7.23, \ p < 0.01) \), but the GROUP \( \times \) TRAINING interaction only approached significance \( (F_{4,58} = 2.24, \ p = 0.078) \). There was also an interaction between the effects of neonatal treatment (GROUP), TRAINING and SPEED of rotation \( (F_{12,150} = 2.48, \ p < 0.01) \). Post-hoc comparisons showed that ethanol-exposed IC and MC male rats made significantly more mistakes during all four days of testing than their littermates that underwent the rehabilitative motor learning procedure. A major confound in the testing of the males that apparently contributed to these results was that the excessive body weights of the control males from IC and MC conditions interfered with their performance, making it difficult to detect the effects of early alcohol exposure.

3.5. Rotating rod (Figs. 6 and 7)

The effects of alcohol on the developing CNS are known to depend on the particular period when the drinking episode occurred (so-called ‘windows of vulnerability’), as well as on the dose and BAC reached (see [23]). Animal studies of the behavioral effect of prenatal alcohol exposure have reported increased open-field activity and reduced locomotor habituation [7,8,18,45,55,58,64,65], increased swimming speed in a water maze [82], and impaired performance in the radial arm maze [30,63]. Increased activity after gestational alcohol exposure may result in part from an alteration of the development of the hypothalamo–pituitary–adrenal axis [25,79,84–86], from retarded development of central cholinergic inhibitory systems [64], as well as from damage to certain brain areas (visual and somatosensory cortex, hippocampus) that develop during this gestational period [51–54] and miscommunication and dysregulation between them (e.g., Ref. [88]).
Prior attempts to rehabilitate the effects of prenatal alcohol exposure have included early handling, cross-fostering with normal dams and postweaning exposure to a complex environment [19,32,83,87]. Significant behavioral improvements were established in these studies: Hannigan et al. [32] and Wainwright et al. [83] demonstrated that alcohol-exposed animals learned a Morris water maze task as well as control animals, after the complex environment experience. Gallo and Weinberg [19] reported elimination of the deficit in response inhibition in alcohol-exposed animals after early handling. Weinberg et al. [87] showed that prenatal alcohol-induced deficits in performance on a step-down avoidance test disappeared if the animals were extensively handled during the pre-weaning period. Although successful at the level of behavior, these interventions did not induce detectable morphological plasticity in ethanol-exposed rats [5,83] as they normally do in control animals. For example, Berman et al. [5] did not find increased dendritic plasticity in area CA1 of hippocampus in the animals exposed to enriched environment, and Wainwright et al. [83] reported no change in occipital cortex thickness after complex environment exposure, whereas exposure of normal animals to environmental novelty results in such plasticity [27–29,37,38,66]. These and other [24,77] studies suggest a reduced neural plasticity after prenatal alcohol exposure, though this may depend on brain region and type of measure of neuroplasticity [15,90].

Postnatal exposure of rats to alcohol is a model of drinking during the third trimester of human pregnancy, in terms of comparison based on the timing of developmental events in the brain (e.g., Ref. 16). Fig. 8 represents a comparative chart of time and sequence of events in the cerebellar morphogenesis in humans and rats based on Refs. [3,62,96]. Neonatal exposure results in a significant decrease in the weight of forebrain, cerebellum and brainstem and loss of prenatally generated neurons in some of these areas (cerebellum, hippocampus, olfactory bulb), suggesting that neurons in certain stages of differentiation (not only during neurogenesis) are also vulnerable to the effects of ethanol [11,12,61,80]. Although several brain areas are affected—both structurally and functionally—by exposure to alcohol during the first postnatal days (e.g., Ref. [59]) numerous studies have demonstrated a particular susceptibility of the cerebellum to the neurotoxic effects of alcohol during this period (e.g., [4,36,42,44,91,92,94]). The degree of impairment of cerebellar structure and function is clearly time- and dose-dependent. Alcohol-induced damage to the development of the cerebellum is paralleled by the deficits in motor performance: poor performance on the rotating rod test [20] and decreased parallel bar traversal ability [22,48,49].

To our knowledge, no attempts to rehabilitate the effect(s) of postnatal alcohol exposure had been made prior to Klintsova et al. [42]. There we showed that 10 days of learning a complex motor task results in increased synaptogenesis in the cerebellar cortex of rats that had been exposed to ethanol postnatally.

In this study, the period of rehabilitative motor skill training was increased from 10 to 20 days to prolong the maintenance phase of the task for the AE animals. Several studies [21,40,59] have reported that exposure to ethanol does not completely prevent the animals from learning the tasks; they accomplish the tasks, but at much slower rate than the controls. We made similar observations during the learning period: although AE animals were significantly worse than controls in the beginning of the 20 days’ training, the difference disappeared across the course of training.

Our study supports previous findings of long-term deficits in the motor behavior of alcohol-exposed animals [20,22,48–50,80]: both female and male alcohol-treated

---

**Fig. 8.** Time chart of events in the development of rat (top) and human (bottom) cerebellum: note that in man the PC dendritic tree orientation and synaptogenesis occur during the weeks 26–39 of gestation, whereas in rat this is an exclusively postnatal event.
rats showed significant impairment on parallel bar and rotating rod tests and females also were impaired in the rope climbing test. Most importantly, however, it demonstrates that the ability to learn motor skills after postnatal alcohol treatment can be dramatically improved by a challenging motor skill intervention. A further study is underway to assess morphological plasticity in the cerebellar cortex after 20 days of complex motor task learning.

Acknowledgements

We thank Stephanie Peterson for assistance with artificial rearing, Jennifer Anderson and Brad Weir for assistance in training and testing animals, and Dr. Ed Roy for permission to share his lab space. This work was supported by PHS AA09838.

References


B.L. Marcusson, C.R. Goodlett, J.C. Mahoney, J.R. West, Developing rat Purkinje cells are more vulnerable to alcohol-induced depletion during differentiation than during neurogenesis, Alcohol 11 (1994) 147–156.


M.W. Miller, S. Robertson, Prenatal exposure to ethanol alters the postnatal development and transformation of radial glia to astrocytes in the cortex, J. Comp. Neurol. 337 (1993) 253–266.


[77] R.J. Sutherland, R.J. McDonald, D.D. Savage, Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring, Hippocampus 7 (1997) 232–238.


