Research report

Therapeutic effects of complex motor training on motor performance deficits induced by neonatal binge-like alcohol exposure in rats: II. A quantitative stereological study of synaptic plasticity in female rat cerebellum

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Accepted 31 January 2002

Abstract

Twenty days of complex motor skill training in adult rats was previously demonstrated to rehabilitate motor performance deficits induced by binge alcohol exposure in neonatal rats. This follow-up study evaluated morphological plasticity in the paramedian lobule of the cerebellum (PML) using the same treatment and training regimens. On postnatal days (PD) 4–9, female Long–Evans rats were given either alcohol (Alcohol Exposure — AE, 4.5 g/kg/day via artificial rearing), exposure to gastrostomy control (GC) artificial rearing procedures, or reared normally as suckle controls (SC). After weaning, all rats were housed two to three per cage. At 180 days old, rats were randomly assigned either to a rehabilitation condition (RC: given 20 days of complex motor skill training), or to an inactive condition (IC: remained in their home cage). The AE rats were delayed in acquiring the training, but there were no group differences in performance over the last 2 weeks of training. Unbiased stereological techniques were used to evaluate PML volume, Purkinje cell and parallel fiber synapse density. Although total volume of PML was significantly reduced in the AE rats, complex motor skill training resulted in a significant increase in the PML molecular layer in all three postnatal treatment groups. The RC animals from the SC and AE groups had more parallel fiber synapses per Purkinje cell than corresponding IC animals. These data support the hypothesis that ‘rehabilitative’ motor training stimulates synaptogenesis in the PML, and that Purkinje neurons that survive the early postnatal alcohol insult are capable of substantial experience-induced plasticity. © 2002 Elsevier Science B.V. All rights reserved.

Theme: Development and regeneration

Topic: Motor systems

Keywords: Ethanol; Cerebellum; Purkinje neuron; Synapse; Plasticity; Motor learning

1. Introduction

Alcohol abuse during pregnancy can damage the developing brain and result in developmental impairments in cognitive function, motor performance, and regulation of social and emotional behavior [9,21,39,40,52,61,63,65,68]. These effects have been described in children diagnosed with fetal alcohol syndrome (FAS), and in children with alcohol-related neurodevelopmental disorder (ARND) who have a known history of prenatal alcohol exposure but who do not show the facial malformations required for diagnosis of FAS [22,40,60]. The combined prevalence of FAS
and ARND may be as high as 1 per 100 live births [55]. Faced with the life-long disabilities in the thousands of alcohol-affected children born each year [61], it is crucial that the health community devise rehabilitation programs to improve outcomes in these brain damaged children. However, interventions appropriate for fetal alcohol disorders have neither been tested nor validated scientifically in randomized studies.

Basic research using animal models can inform and guide efforts to develop rehabilitation programs for FAS. Several recent studies have evaluated postnatal behavioral interventions in rat models of early alcohol exposure, including early ‘handling’ experience [70], postweaning environmental enrichment [19,51] or complex motor skill training in adulthood [30]. These interventions resulted in amelioration of some of the functional deficits induced by early exposure to alcohol. In some cases, the interventions stimulated physiological [51] or structural [23,28] neuroplasticity, suggesting that there may be significant potential for therapeutic treatment to stimulate and improve brain function to ameliorate alcohol-induced damage to the developing brain.

This laboratory has focused on complex motor learning as a potential rehabilitation treatment for brain damage induced in a rat model of binge alcohol exposure during the early postnatal brain growth spurt. Brain damage and behavioral dysfunction have been relatively well characterized in this model [12,16,72], in which alcohol exposure occurs during the period of neonatal rat brain development roughly comparable to that of the human 3rd trimester [5,11,71]. Daily episodes of binge-like alcohol exposure induce permanent damage to the developing brain, including cell loss in the cerebellum, brain stem, hippocampus, and olfactory bulb [12,72]. Damage to the cerebellum increases with the peak blood alcohol concentration (BAC) attained, and the threshold for significant Purkinje cell loss is around 150–200 mg/dl. Long-lasting deficits in behaviors that are known to depend on cerebellar function have been documented, including tests of gait, balance and co-ordinated motor performance [13,15,41,66,67] and eyelink classical conditioning [17,59]. There is compelling evidence that binge alcohol exposure during the brain growth spurt in neonatal rats induces severe structural damage to the cerebellum accompanied by long-lasting deficits in cerebellar-dependent behavior. These deficits model effects linked to cerebellar damage in FAS/ARND children [58], including deficits in motor performance, gait and balance [4,20,32,53,54,64].

In normal adult rats complex motor skill training (learning to traverse an obstacle course) increased the number of synapses in specific regions in the cerebellum and motor cortex [1,6,25,26]. Given that motor skill learning stimulates synaptogenesis in the motor areas of the brain, a series of studies in this laboratory have investigated whether complex motor training can potentially provide therapeutic rehabilitation for neonatal alcohol-induced brain damage. Our initial preliminary neuroanatomical report [31] found that 10 days of complex motor skill training (a typical duration used in the normal adult studies) significantly increased the number of parallel fiber synapses per Purkinje cell in the paramedian lobule (PML) of the cerebellum in normal control rats and in alcohol-exposed rats. However, the alcohol-exposed rats and the gastrostomy control rats (both groups artificially reared) took longer to acquire the training than the normal controls, and the gastrostomy control rats failed to show statistically significant training-induced increases in synapse number. For subsequent studies, it was decided to extend the duration of complex motor training to 20 days, to increase the potential rehabilitative effects on brain structure and function.

The present study is the second of a series of complementary studies evaluating rehabilitation induced by 20 days of complex motor skill training in 6-month-old adult Long–Evans rats that were exposed to alcohol on postnatal days (PD) 4–9. The first study, reported previously in this journal [29], focused on behavioral rehabilitation, and demonstrated that the motor skill training resulted in significant amelioration of the deficits in neonatal alcohol-induced deficits in co-ordinated motor performance. Importantly, the improved motor performance was demonstrated on tasks that were not specifically included in the complex motor skill training.

The purpose of the current study was to obtain quantitative estimates of the extent of morphological plasticity in the PML of the cerebellum, using the same 20-day regimen of complex motor training that the first study found to be effective in rehabilitating motor performance deficits [29]. Separate groups of rats were used for the present study of morphological neuroplasticity to avoid potentially confounding effects of changes in synaptic number induced by the post-training behavioral testing. The present study tested the hypothesis that the 20-day complex motor training stimulates synaptogenesis in the PML of the cerebellum of alcohol-exposed rats, supporting the proposal that PML synaptogenesis is one structural correlate of the training-induced behavioral rehabilitation in the alcohol-exposed rats in the previous report. This study used modern stereological methods based on 3-dimensional probes to provide measures of training-related changes of parallel fiber synapses in the PML and is the first to evaluate training-related changes in the volume of the PML and its layers.

2. Materials and methods

2.1. Subjects

Litters from timed pregnancies were obtained by breeding adult Long–Evans rats (Simonsen Labs, Gilroy, CA) in the Indiana University-Purdue University, Indianapolis.
Artificial rearing and alcohol exposure

At PD 4 pups were assigned randomly within litter and sex to three groups: alcohol-exposed (AE)—artificially reared pups given 4.5 g/kg of alcohol each day on PD 4–9, delivered as a 10.2% (v/v) ethanol solution in milk formula on two consecutive feedings, 2 h apart; gastronomy 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) anesthesia on PD 4 and reared using well-established procedures as described in detail previously [13, 29]. All intragastric feedings used a customized milk formula [73] delivered every 2 h using programmable Harvard Model 22 infusion pumps. Formula containing alcohol (or isocaloric maltose/dextrin) was provided on the first two feedings after 8.00 a.m. each morning; the other 10 feedings on PD 4–9 and all feedings on PD 10–12 used milk formula alone. Each day, the rats were fed a total 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 spec trophotometer (absorbance 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 two to four per cage with same-sex littersmates 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.

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 two conditions: either an inactive condition (IC) or a rehabilitative condition (RC). Special attention was paid not to place two littermates of the same sex and postnatal treatment in the same condition. (Note: although animals of both sexes have been included in our ongoing studies, we report here only the data from female rats). At least five animals per group/condition were involved in the study. The volume estimate data were obtained from 44 animals, and the quantitative electron microscopic study was performed on a total of 35 animals in the six groups (some tissue was lost or excluded from the electron microscopy (EM) study based on unsuccessful perfusion/fixation/embedding; one rat in SC–IC, one in AE–IC, four in SC–RC and three in GC–RC). All animals were coded so that the experimenters conducting and scoring the training were not aware of their early postnatal treatment. Whenever possible, littermates with the same postnatal treatment were assigned to IC and RC, to minimize pre-experimental variance. Rats from IC were paired in cages with RC rats and were handled daily. RC rats were trained to traverse an obstacle course on 20 consecutive days, five trials per day, as described previously [29]. The obstacles included narrow rods, ropes, a link chain, barriers on narrow beams, a rope ladder, etc., which rats were forced to traverse by gentle prodding, while the tail was loosely held to prevent falls. The time to complete the entire set of 10 obstacles was recorded on each trial, as well as the number of errors/slips/mistakes during each run on the course. The mean time to complete five trials/day and the mean number of errors per trial were computed for each animal.

Tissue preparation and analysis

On the day of the completion of training the animals were anesthetized with pentobarbital (100 mg/kg) and transcardially perfused with 0.14 M sodium cacodylate buffer (pH 7.3) followed by a mixture of 2% paraformaldehyde and 2% glutaraldehyde in the same buffer. The brains were postfixed overnight in the fixative (4 °C). Sagittal 150 μm serial Vibratome sections were taken through the right hemisphere of the cerebellum and collected in a sequence in cacodylate buffer. A systematic subset of every third section (starting with a randomly-selected section within the first three sections containing PML) was chosen and the PML was dissected (usually four to five sections per animal) and used for transmission electron microscopy (TEM). The remaining two-thirds of the sections were mounted on chrom-alum gelatin-coated slides maintaining the order and were used for quantitative
stereological evaluation of the PML, the volume of the cortical layers and the total number of Purkinje cells (PCs) in the PML.

2.4.1. Total number of PCs (estimation using light microscopy)

To evaluate the volume of PML, its boundaries were defined on the parasagittal sections as follows: the PML appears on lateral parasagittal sections at the level of flocculus/paraflocculus and extends medially above copula pyramidis. PML disappears at the beginning of vermal lobulae VIII and IX [48]. The Cavalieri principle was used to determine the volume of the PML and individual layers within the PML using the StereoInvestigator software package (MicroBrightField, Colchester, VT). Details of the approach are described explicitly in West et al. (1991) [74]. Briefly, a random systematic set of every third section was selected from the entire set of sections containing PML. In each of the selected sections, PCs were counted at the random systematic sampling points (grid size 200×200 μm) within the section (the position of the sampling points was determined in a random systematic manner by the software) (Fig. 1). At each (x,y) position of the grid within the section, only a certain fraction of the tissue was included within the unbiased sampling frame (80×80 μm) of the optical disector. PCs were counted throughout a depth of 30 μm within the section. A guard zone of at least 5 μm was set from the section surface to the top of the optical disector. The total number of PCs in the PML was obtained from the number of PCs in the sampled volume of PML (∑Q⁻), multiplied by the inverse fraction from each of the three sampling levels (section, area, depth):

\[ f_1 = \frac{1}{3} \]

\[ f_2 = \frac{\text{area (counting frame)}}{\text{area (grid)}} = \frac{6400 \, \mu m^2}{40,000 \, \mu m^2} \]

The fractionator method was used to determine the total number of PCs in the PML. The neurons were directly counted using the optical disector method, in a known fraction of the PML using the StereoInvestigator software package (MicroBrightField, Colchester, VT). Details of the approach are described explicitly in West et al. (1991) [74]. Briefly, a random systematic set of every third section was selected from the entire set of sections containing PML. In each of the selected sections, PCs were counted at the random systematic sampling points (grid size 200×200 μm) within the section (the position of the sampling points was determined in a random systematic manner by the software) (Fig. 1). At each (x,y) position of the grid within the section, only a certain fraction of the tissue was included within the unbiased sampling frame (80×80 μm) of the optical disector. PCs were counted throughout a depth of 30 μm within the section. A guard zone of at least 5 μm was set from the section surface to the top of the optical disector. The total number of PCs in the PML was obtained from the number of PCs in the sampled volume of PML (∑Q⁻), multiplied by the inverse fraction from each of the three sampling levels (section, area, depth):

\[ f_1 = \frac{1}{3} \]

\[ f_2 = \frac{\text{area (counting frame)}}{\text{area (grid)}} = \frac{6400 \, \mu m^2}{40,000 \, \mu m^2} \]
\[ f_s = \text{sampling fraction of section thickness (t)} \]
\[ = \text{disector height/average t of sections} \]
\[ = 30 \, \mu m/65.6 \, \mu m \]

Total PC count = \( Q^\sim \times (1/f_1) \times (1/f_2) \times (1/f_3) \)

2.4.2. Electron microscopy

For TEM analysis the PML samples were postfixed in 2% osmium tetroxide in 0.14 M sodium cacodylate buffer for 1 h, stained en block with aqueous 0.5% uranyl acetate, dehydrated and flat embedded in LX112 resin.

All blocks and slides were coded so that the experimenter performing the quantitative morphology was blind to both the neonatal treatment and to the adult training condition. PML was dissected from each cerebellar slab designated for EM and embedded in resin (pre-embedding thickness of 150 \( \mu m \)); two PML blocks per animal were selected at random. First, 60 serial, 1-\( \mu m \) sections were collected from the whole PML (for PC density counts) using a diamond histo-knife (Diatome) on a Reichert ultramicrotome. A set of every other section was mounted on a chrom-alum gelatin-coated slide and stained with Toluidine Blue, and used for determination of PC density \( N_{v}(PC) \). Then a small pyramid was cut out in such a way that its surface extended through the whole depth of the molecular layer and PC layer. A series of 20 consecutive 60-nm sections were then collected from the pyramidal block on the Formvar-coated slot grids for electron microscopy. PC density was determined with the physical disector method using a computer-assisted microscope and a locally written stereology software package (Phokus on Stereology). Briefly, an unbiased counting frame of a known area \( A_{frame} \) was superimposed on the images of the PC layer and the molecular layer on two serial sections of the PML, the first of which was considered the ‘reference’ section and the second, the ‘look-up’ section. The frame was positioned such that the PC layer was at the bottom of the frame that had constant width and variable height equal the depth of the molecular layer. Within the frame the number of PC nucleoli which were present in the ‘reference’ section and absent in the ‘look-up’ section, termed ‘\( Q^\sim \)’, was counted. The nucleolus of the PC is small and densely stained and as each PC has only one nucleolus it is an ideal particle for counting these cells [44]. The disector volume of tissue within which the cells were counted \( V_{dis} \) was estimated as \( V_{dis} = A_{frame} \times t \times n \), where \( t \) is section thickness and \( n \) is the number of sections through which the counting was done. The density of PCs was calculated from:

\[ N_{v}(PC) = Q^\sim /V_{dis} \]

Following the 1 \( \mu m \) sectioning, a pyramid was trimmed from the same block through the full depth of the molecular layer of the PML. From the pyramid, 20 silver/grey serial sections (60 nm) were collected on a Formvar-coated slotted grid (care was taken to obtain sections of uniform color/thickness). Section thickness was measured regularly using the ‘small fold’ method [8,10]. Sections were stained with 10% uranyl acetate solution in methanol followed by 0.25% lead citrate solution in water. Pictures were taken systematically from corresponding areas at the level of the outer two-thirds of the molecular layer of each of 15–20 serial sections. Two or three areas of PML were selected and photographed for each animal. Negatives were printed at a final magnification of \( \times 26,400 \). Parallel fiber to PC dendritic spine synapses show distinct morphological characteristics. An axon varicosity containing small round vesicles, often collected at one site of the fiber, abuts a spine profile containing sparse tubules of smooth endoplasmic reticulum in a fine matrix [46]. Synapses, identified by the presence of a postsynaptic density and at least three vesicles in the presynaptic element, were counted using the physical disector method on adjacent photographs within the series (Fig. 2). The number of parallel fiber synapses per PC \( N_{synapses} / PC \) was then obtained by dividing the density of synapses per cubic millimeter of molecular layer \( (N_{v}(syn)) \) (from the EM serial sections) by the density of PCs per cubic millimeter of the same layer \( (N_{v}(PC)) \) (from the 1-\( \mu m \)-thick sections):

\[ N_{synapses} / PC = N_{v}(syn)/N_{v}(PC) \]

2.4.3. Statistical analysis

Data were analyzed using SPSS software. For the motor training performance measures, a mixed-model, two-way ANOVA was used, with GROUP (SC, GC, AE) as the between-subjects factor and DAY as the repeated factor. The morphological data (PML volume, PC number and synapses/neuron data) were analyzed with a two-way between-groups ANOVA, with GROUP (SC, GC and AE) and TRAINING (IC versus RC) as factors.

3. Results

The delivery of alcohol in two consecutive feedings resulted in an average peak blood alcohol concentration of 225±8 mg/dl. Body weight did not differ statistically across groups at postnatal day 180 and 200 (see Table 1) and was comparable with the weights reported in the earlier study of this series [29].

The average time to complete the task in each session decreased over days for all groups [31] (Fig. 3), but there was a significant main effect of postnatal treatment \( (F_{2,619} = 12.67, P<0.0001) \) on the daily completion times. The SC animals were the fastest to learn, GC animals were intermediate, and AE animals were the slowest. There was no significant GROUP X DAY interaction. However, with a post-hoc division of the training period into four blocks (5 days each), a two-way ANOVA yielded a significant
Fig. 2. EM pictures of disector used to evaluate the density of parallel fiber synapses in the PML molecular layer. In physical disector, parallel fiber synapses (arrows) on PC spines are counted when they are present in one section, the ‘reference’ section (left, arrowhead), but not in the adjacent, ‘look-up’ section (right). Scale bar = 0.5 μm.

GROUP×BLOCK interaction \((F_{6,619} = 2.240, P < 0.05)\). This confirms the slower acquisition of training by the AE group during the first 5 days, the typical period of rapid acquisition of motor skills observed in previous studies [25].

A two-way ANOVA revealed a significant effect of postnatal treatment on the ‘total volume’ of the PML \((F_{2,43} = 8.024, P < 0.01)\). Subsequent multiple comparisons (Student’s Newman–Keuls test; \(P < 0.05\)) showed that the total PML volume in the AE group was significantly lower than the SC and GC groups. For the ‘molecular layer volume’ of the PML, there were significant neonatal treatment effects \((F_{2,43} = 15.68, P < 0.0001)\), along with significant effects of training \((F_{1,43} = 6.61, P < 0.01)\); the GROUP×TRAINING effect was not significant. Post-hoc comparisons (Student’s Newman–Keuls test; \(P < 0.05\)) showed that the molecular layer volume was significantly lower in AE groups than in their control counterparts. The 20-day motor training increased the molecular layer volume in all RC groups when compared with IC groups (within each neonatal treatment condition) (Fig. 4A).

For ‘total number of PCs’ in the PML, the ANOVA yielded a significant effect only of early postnatal treatment \((F_{2,43} = 43.32, P < 0.0001)\). Subsequent multiple comparisons (Student’s Newman–Keuls test; \(P < 0.05\)) showed that early postnatal alcohol exposure significantly reduced the number of PML PCs relative to the SC and GC groups. The PC number in the AE group was reduced by about 40% in comparison with SC and GC animals (Fig. 4B).

For the ‘number of synapses per Purkinje neuron’, ANOVA revealed a significant main effect of training \((F_{1,34} = 6.432, P < 0.01)\) but there was no GROUP×TRAINING interaction \((F_{2,34} = 0.307, P = 0.74)\). Multiple comparisons (Student’s Newman–Keuls test; \(P < 0.05\)) showed that animals trained on the complex motor task (RC) from the SC and AE groups had significantly more parallel fiber synapses per Purkinje neuron than IC animals from the same neonatal treatment groups (Fig. 5). For the GC group, the increase in the number of synapses per PC in the RC condition compared to the IC condition was not statistically significant.

4. Discussion

The primary finding of this study was that complex motor skill training induced morphological synaptic plasticity in surviving adult cerebellar neurons following neonatal alcohol exposure, as shown by significant addition of parallel fiber synapses per PC. The training-induced synaptic plasticity parallels the rehabilitation of motor performance of AE rats by the complex motor skill training, demonstrated in the first article in this series [29]. These data also support previous findings, using normal animals, that complex motor skill learning results in synaptogenesis in areas of the brain involved in co-ordi-
nated motor performance [6,25–27]. In addition, this study is the first to document that the volume of the PML molecular layer was significantly increased by the complex motor skill training, and this effect was evident in all three postnatal treatment groups. The observed large increases in PML molecular layer volume (by 0.41–0.55 mm$^3$) following complex motor skill training likely cannot be accounted for only by the increases in the number of parallel fiber synapses. Because changes in astrocyte volume have been reported with this training [2], it is reasonable to speculate that motor learning stimulated plastic changes in other neuropil elements, in parallel to the addition of synapses.

Although permanent loss of neurons after exposure to alcohol during development is quite well documented [35–37,42,49], the mechanisms of alcohol-induced cell loss are not well understood. The specific brain areas where such loss occurs depends on the developmental timing of the exposure to ethanol and on the peak BAC attained [36,44,47,72]. Cerebellar PCs are particularly vulnerable to alcohol-induced damage during the neonatal period (reviewed in [12,35,72]). Dose- (and BAC-) dependent loss of the total number of PCs occurs regardless of whether it is administered by artificial rearing procedures [13] (as used in this study) or by intragastric intubation [14,50]. Significant PC loss can be detected when peak BACs approach 200 mg/dl, and the extent of loss can exceed 40% when peak BACs are above 300 mg/dl. In this study in which peak BACs reached 225±8 mg/dl, significant loss of PCs (40%) was found within the PML, consistent with effects found in previous studies measuring total PC number. However, alcohol-induced reduction of the total PML volume is unlikely to be solely due to the loss of PCs, but rather may reflect a combination of loss of neurons and glial cells [7,44,56], and diminished soma size and alterations in the dendritic organization of surviving neurons [3,57,69].

Synaptic changes in the brain structures that lose significant numbers of neurons as a result of developmental exposure to alcohol remain relatively unexplored. It is noteworthy that in this stereological study and in our previous preliminary report [31], the number of parallel fiber synapses (per PC) in the IC groups did not differ across neonatal treatment groups. This may indicate that the number of synapses per PC in adulthood was not altered by the significant neonatal alcohol-induced PC loss. In contrast, following prenatal ethanol exposure, others have shown decreases in synaptic density in the molecular layer of vermis [34] and of lobule VI [33]. However, those studies used older methods of estimation of synaptic density per unit area that fail to protect against biased counts and made no correction for the changes in the overall volume. Pre/postnatal alcohol administration also resulted in delayed synapse maturation in folium VIII of the vermis [69] and in lobules II/III [43].

It is possible that dramatic changes in synaptic organization may occur in the cerebellum after developmental alcohol exposure. In the neonatal binge exposure model,
PCs (the sole efferent source from the cerebellar cortex), granule cells (source of parallel fibers, one of two excitatory inputs on PCs), neurons in the cerebellar deep nuclei (target of PC axons), and inferior olive neurons (source of another excitatory input on PCs—climbing fibers) all undergo substantial loss [13,18,38,44,45,47,49,50]. However, as noted above, neither this study nor our previous preliminary report detected any alcohol-related difference in the number of parallel fiber synapses per Purkinje neuron in the IC animals. This suggests that developmental regulation of the formation and maintenance of these synapses in the cerebellar molecular layer was not permanently altered by postnatal alcohol exposure. Since only one synapse type in cerebellar PML was studied here, possible changes in climbing fiber or interneuron synapses cannot be excluded.

Learning of the complex motor task results in the addition of synapses in the molecular layer of PML in adult animals [1,6,26]. At least two types of synapses undergo synaptogenesis in this experimental condition: parallel fiber synapses on PC dendritic spines and climbing fiber synapses [1,26,27]. Our data confirm the significant addition of parallel fiber synapses on PCs after complex motor skill learning in the control animals, and further strengthen the preliminary report [31] that there is significant residual capacity for synaptic plasticity in cerebellum after neonatal binge alcohol exposure. The extent of climbing fiber and interneuron synapse plasticity in AE animals remains to be determined.

Increases in the total volume of PML molecular layer resulting from learning the complex motor task suggests that plastic changes are not limited to the addition of new parallel fiber synapses. One possibility is that molecular layer volume increases may include dendritic growth

Fig. 4. Effect of postnatal exposure and adult training on the morphology of the PML of the cerebellum. (A) Volume of PML molecular layer, 20 days of the complex motor skill learning in the RC resulted in the significant increase of the PML molecular layer volume when compared with the IC group from the same postnatal treatment condition (SC, GC, AE). (B) Total number of PCs in the PML. The total number of PCs in the PML was significantly reduced after exposure to alcohol on PD 4–9. Data presented as mean ± S.E.M.
changes. We have preliminary evidence that learning of the complex motor task is accompanied by an increase in apical dendrite material in motor cortex of both control and AE rats [28]. Plasticity of non-neuronal elements may occur as well. Previous reports from this laboratory have shown that rats given acrobatic motor training have a greater volume of molecular layer (per PC) than the IC or exercise groups [6], and the thickness of the molecular layer is significantly increased in these animals [26]. Glial hypertrophy (glial volume per PC reference volume) was also reported to be associated with synaptogenesis (but not with exercise-related angiogenesis) in the rats given complex motor training [2]. Glial changes such as hypertrophy of glial processes need to be studied directly to determine whether AE rats also retain the capacity for plasticity in non-neuronal elements.

Consistent with cerebellar structural damage and with previous findings of deficits in co-ordinated motor performance, rats in the current study exhibited a delay in learning to traverse the obstacle course, but this difference disappeared by the eighth day of training. In the first publication in this series [29], AE rats not given the acrobatic motor training were significantly impaired in their ability to perform parallel bar, rope climbing and rotarod tasks in post-training behavioral tests consistent with deficits reported in earlier studies [13,15,24]. Thomas and colleagues (1998) recently demonstrated a significant correlation between the loss of PCs in cerebellum and successful parallel bar traversal [66].

Considering the above effects (in the absence of complex motor skill training), motor performance deficits are a consistent consequence of the alcohol-induced structural damage, and are correlated with cell loss in the underlying cerebellar–brain stem circuits. However, complex motor skill training appears sufficient to stimulate synaptogenesis in the PML cerebellar cortex and to improve motor performance deficits. Cerebellar plasticity (i.e. synaptogenesis) may provide a key component of the biological substrate for motor performance rehabilitation following alcohol-induced brain damage. The addition of more parallel fiber synapses on PCs likely serves to increase the strength of this excitatory input onto the cerebellar output neurons, thereby providing enhancement of the cerebellar function underlying improvement of motor performance.

Finally, it should be noted that these findings bode well for systematic investigations of similar interventions in the human clinical conditions associated with third trimester fetal alcohol exposure. There is evidence that characteristics of the rearing environment can influence the outcome in fetal-alcohol disorders in humans [62] that similarly point to the possibility of ameliorative effects of intervention. Clearly, controlled studies of interventions are merited given the outcomes of this animal research.

Acknowledgements

We are grateful to Stephanie Peterson for assistance with the artificial rearing and blood alcohol determination. This work was supported by PHS AA09838.

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