Published in final edited form as:

Neurotoxicol Teratol. 2009 ; 31(1): 40–48. doi:10.1016/j.ntt.2008.08.001.

Insulin-like growth factor-I mitigates motor coordination deficits associated with neonatal alcohol exposure in rats

Nancy N.H. McGough, Jennifer D. Thomas, Hector D. Dominguez, and Edward P. Riley

Center for Behavioral Teratology, Department of Psychology, San Diego State University, 6363 Alvarado Ct., Ste 209, San Diego, CA 92120, USA

Abstract

Prenatal alcohol exposure can affect brain development, leading to behavioral problems, including overactivity, motor dysfunction and learning deficits. Despite warnings about the effects of drinking during pregnancy, rates of fetal alcohol syndrome remain unchanged and thus, there is an urgent need to identify interventions that reduce the severity of alcohol's teratogenic effects. Insulin-like growth factor I (IGF-I) is neuroprotective against ethanol-related toxicity and promotes white matter production following a number of insults. Given that prenatal alcohol leads to cell death and white matter deficits, the present study examined whether IGF-I could reduce the severity of behavioral deficits associated with developmental alcohol exposure. Sprague-Dawley rat pups received ethanol intubations (5.25 g/kg/day) or sham intubations on postnatal days (PD) 4–9, a period of brain development equivalent to the third trimester. On PD 10–13, subjects from each treatment received 0 or 10 µg IGF-I intranasally each day. Subjects were then tested on a series of behavioral tasks including open field activity (PD 18–21), parallel bar motor coordination (PD 30–32) and Morris maze spatial learning (PD 45–52). Ethanol exposure produced overactivity, motor coordination impairments, and spatial learning deficits. IGF-I treatment significantly mitigated ethanol's effects on motor coordination, but not the other two behavioral deficits. These data indicate that IGF-I may be a potential treatment for some of ethanol's damaging effects, a finding that has important implications for children of women who drink alcohol during pregnancy.

Keywords

fetal alcohol; open field activity; cerebellum; motor coordination; spatial learning

1. Introduction

Children exposed to alcohol prenatally exhibit alterations in brain structure and function [68,80]. The consequences of alcohol-related neuropathology include long-lasting behavioral changes, such as overactivity, impairments in attention and memory, altered social behavior, and deficits in balance and motor coordination [31,54,78]. Despite warning labels on alcoholic beverages, heavy alcohol abuse during pregnancy continues to be a problem worldwide and it is estimated that as many as 1 in 100 live births may be affected

Corresponding Authors: Jennifer Thomas, Ph.D., 6363 Alvarado Ct. Ste. 200, San Diego, CA 92120, Ph: 619-594-0681, Fax: 619-594-1895, Thomas3@mail.sdsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

by prenatal alcohol exposure [82]. Thus, there is a need to identify novel treatments to reduce the severity of these fetal alcohol effects.

Prenatal alcohol exposure disrupts CNS development by altering many developmental events such as neurogenesis, migration, differentiation and cell survival [43,55,62], suggesting that alcohol causes neuropathology via a number of mechanisms. Interference with neurotrophic factors may be one of the various mechanisms by which alcohol disrupts brain and behavioral development. For example, the level of nerve growth factor (NGF) [2], the expression of NGF receptors [36] and NGF-mediated cell survival [84] are inhibited by pre- or perinatal ethanol treatment. Similar alcohol-related reductions have been reported for other neurotrophic factors, including brain derived neurotrophic factor (BDNF) [29,50,51,57], basic fibroblast growth factor [41,52,61,64,81], glial derived neurotrophic factor (GDNF) [69,70] and insulin-like growth factor (IGF) [20,40,89]. Conversely, administration of various trophic factors, including NGF, BDNF, GDNF and FGF can protect against some of alcohol's teratogenic effects [9,16,19,63,70,73,93]. In the present study, we investigated the possibility that IGF-I might reduce some of the behavioral alterations associated with developmental alcohol exposure.

Insulin-like growth factors (IGF-I and IGF-II) are peptides that exert significant effects on cell metabolism and growth. These growth factors not only promote the proliferation, differentiation and maturation of neuronal and glial cells, but also reduce apoptotic cell death [37,83]. Illustrating the role of IGF-I in development, humans with defects in the genetic encoding of IGF-I exhibit severe intrauterine growth failure, sensorineural deafness and mild mental retardation [105]. Similarly, whole brain size is significantly reduced in null IGF-I knockout mice, with reductions in all brain regions examined, including the cerebral cortex, cerebellum, hippocampus and diencephalon [12,27,111]. Moreover, myelin staining, expression of myelin-specific protein genes, and oligodendrocyte number are significantly reduced in IGF-I knockout mice [12,111]. Conversely, IGF-I overexpression in mice increases brain weight, number of both neurons and glial cells and both the percentage of myelinated axons and the thickness of myelin sheaths [108].

Ethanol exposure during pregnancy can disrupt the production and/or response to IGF-I. For example, animal model studies have demonstrated that developmental alcohol decreases the level of IGF-I in the fetus [20,40,89]. The expression of genes encoding insulin, IGF-II, and the IGF-I and IGF-II receptors are also significantly reduced in cultured cerebellar granule neurons from ethanol-exposed pups [34]. Ethanol also affects IGF-I signaling. When activated, the IGF-I receptors undergo autophosphorylation, which activates a signaling cascade [14]. Ethanol inhibits the phosphorylation of the IGF-I receptor [76,85,86], as well as the intracellular signaling responses to IGF-I, leading to impaired cell growth and survival [33,48,76,85,86,113]. Thus, interference with IGF-I signaling may contribute to alcohol-related neuroteratogenicity.

The possibility that IGF-I could serve as a potential treatment for fetal alcohol effects is strengthened by evidence that IGF-I can protect against CNS insults [22,107,110], enhancing both neuronal generation and growth as well as white matter production. In fact, IGF-I has been shown to attenuate the neuropathological effects of developmental alcohol exposure. Transgenic mice that overexpress IGF-I are less susceptible to the intoxicating/sedative effects of ethanol than control subjects [75] and ethanol's toxic effect on neuronal survival can be reversed by IGF-I treatment in cultured embryonic cortical neurons [9].

The main effects of IGF-I are mediated by IGF-I receptors which are under critical spatial and temporal control in the developing CNS. The expression of IGF-I mRNA is observed in the CNS in late embryonic days in the cerebellum, striatum, olfactory bulb, thalamus,

hippocampus and retina [6,10,13]. High densities of IGF-I receptors are observed in the olfactory bulb, cortex, hippocampus, septum, nucleus accumbens and the cerebellum on embryonic days 18–20 in rats [10,53]. During postnatal days (PD) 1–14, the density of IGF-I receptors starts to decrease in the olfactory bulb, cortex, and hippocampus, although relatively high densities of IGF-I receptors are still observed in the cortex and hippocampus. Unlike other brain regions, the cerebellum shows a gradual increase in the density of IGF-I binding sites from PD 1–14 and reaches the maximum value at PD14.

No studies, to date, have examined the consequences of administering IGF-I in vivo, nor have they examined the behavioral effects of this treatment, using animal models investigating developmental alcohol exposure. In the present study, subjects were exposed to alcohol during the period IGF-I receptors are expressed in most brain regions, which is also the period during the 3rd trimester equivalent brain growth spurt. The brain growth spurt is a period of rapid brain development during which the developing CNS is particularly vulnerable to alcohol and other insults. This period of brain development occurs during the 3rd trimester in humans, but during the early postnatal period in rats [35]. Alcohol exposure during this period of brain development produces neuropathology in many brain regions, including the hippocampus [60,102], cerebellum [42,66], olfactory bulb [15] and cortex [46]. Alcohol exposure during this period also disrupts white matter development [49,116]. Consistent with the various CNS regions affected, alcohol exposure during the 3rd trimester equivalent alters behavioral development in a number of domains, including learning and memory [44,92,96], activity level [11] and motor function [99]. To be more clinically relevant, IGF-I treatment was initiated after cessation of ethanol treatment, but still within the time period that high IGF-I receptor are expressed in brain regions involved in ethanol-induced behavioral impairment.

2. Materials and Methods

2.1. Subjects

Subjects were 77 (9–11 per sex per group) Sprague-Dawley rats, offspring from the breeding colony at the Center for Behavioral Teratology, San Diego State University. A male and female were housed together overnight and the presence of a sperm plug on the following morning indicated mating and was designated as gestational day (GD 0). Pregnant dams were then single housed in a temperature- and humidity-controlled room with food and water *ad lib*. On the morning following birth, litters were pseudorandomly culled to 8 pups, with 4 males and 4 females (when possible).

2.2. Treatment procedure

To determine the effects of IGF treatment on ethanol-related behavioral alterations, IGF-I was administered after the cessation of ethanol exposure during the 3rd trimester equivalent brain growth spurt. On postnatal day (PD) 4, pups were randomly assigned to one of four treatment groups derived from the factorial combination of EtOH vs. SHAM and IGF vs. vehicle (VEH). To control for potential litter effects, no more than one sex pair per litter was assigned to any treatment group. On PD 4–9, EtOH subjects received 5.25 g/kg/day (11.9% v/v) ethanol in a binge-like manner via intragastric intubation (27.5 ml/kg). Specifically, subjects received 2.625 g/kg ethanol in a milk formula in each of two intubations, separated by two hours. Since alcohol causes reductions in suckling, EtOH subjects received two additional intubations of a nutritionally balanced milk formula at 2-hour intervals following ethanol intubations [44]. During the SHAM intubation, the intubation tube was guided down the pup's esophagus into stomach; however, no formula was administered. All subjects were returned to the dam between intubations. This is a fairly standard procedure and similar to that used in various labs [44,106].

Unlike many neurotrophic factors, IGF-I crosses the blood brain barrier [4,77] and therefore has an effect on the CNS even when administered peripherally [77]. However, to reach the desired therapeutic dose in the CNS, higher IGF-I doses must be injected peripherally. Since IGF-I is a potent anti-apoptotic growth factor, it has been claimed that high doses of systemic IGF-I administration may have adverse side effects, including increased risk of tumorigenesis [101]. An alternate method for administering IGF-I into the CNS without presenting high doses peripherally is intranasal delivery, which has been shown to protect against neurological insults [58,59]. The present study used the intranasal route to deliver IGF-I into the CNS.

On PD 10–13, all subjects receive intranasal treatment under hypothermic anesthesia. More specifically, half of subjects in the EtOH and SHAM groups received 10 µg/10 µl IGF-1 (Peptotech, Rocky Hill, NJ) injections intranasally while the other half received saline vehicle injections. During the intranasal administration, all rat pups were protected with a latex glove and then placed into crushed ice/ice water for 6–8 minutes. After anaesthetized by hypothermia, subjects were placed on their backs and a total of 10 µl solution was administered in nose drops (1 µl/drop) over a 15-minute period, altering drops every 1.5 minute between the right and left nostril. The mouth and the opposite naris were closed during the administration so the drops could be naturally inhaled high into the nasal cavity. A second, third and fourth dose were given to each subjects 24, 48 and 72 hours after the first injection, for a total of four doses of IGF-1 or vehicle per rat.

On PD 6, 20 µl of blood was collected from each pup's tail 1.5 h after the second EtOH feed. Blood samples were centrifuged and supernatant was collected. Samples were analyzed with the Analox Alcohol Analyzer (Model AM1, Lunenburg, MA) for blood ethanol content. On PD 10, subjects were coded via injections of India ink into paws so that each subject could be identified, but testers could remain blind to treatment conditions. Subjects remained with the dam until weaning on PD 21.

2.3. Locomotor Activity Testing

On PD 18–21, activity level was monitored in a Plexiglas open field (40 × 40 × 30.5 cm) contained within an optical beam activity monitor (Hamilton-Kinder, San Diego, CA). The open field was housed in enclosed, ventilated chambers. White noise was present during testing to mask any outside noises. Subjects were placed in the testing room for at least 30 min prior to testing to allow the subjects to acclimate. Prior to testing each subject, each open field was carefully cleaned to eliminate any odor cues. Each subject was then placed in the center of the chamber and infrared beams interruptions were recorded every 5 min during a 60-min test. Number of beam breaks, total distance traveled, time spent in the center of the chamber, rearing and fine movements served as performance measures. Subjects were tested on four consecutive days during the light cycle between 9:30–13:30, since previous reports demonstrated elevation of activity levels resulting from neonatal ethanol treatment during both light and dark cycle [94,95,98].

2.4. Parallel bar motor coordination task

On PD 30–32, subjects were tested on a parallel bar motor coordination task. The parallel bars were two steel rods (0.5-cm diameter each, 91 cm long) held between two platforms (15.3 × 17.8 cm). The rods were fastened onto a rack of 28 grooved slots (0.5 cm apart) on each platform, allowing the distance between the rods to be altered. The platforms stood 63 cm above a floor of wood chip bedding. The subject was initially placed on each platform for 30 sec. Then, the subject was carefully placed on the rods halfway between the platforms, 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.

If the subject placed two hind paws on one rod, fell or swung under the rods, the trial was unsuccessful. The initial distance between the rods was set at 3.5 cm. Subjects were tested for up to five consecutive trials at a given width, with an intertrial interval of 5–10 sec. Once successful at a given width, the distance between the rods was increased by 0.5-cm. If unsuccessful after five consecutive trials, testing for the day was terminated. Subjects were tested a maximum of 15 trials a day, for 3 consecutive days. Each day, the distance between rods was set at the last successful distance for each individual animal. The maximum distance successfully traveled each day, the number of successful to total traversals as well as the number of trials to reach the first successful traversal served as performance measures.

2.5. Morris water maze training

On PD 45–52, subjects were tested on a Morris water maze task. This task utilized a circular water tank filled with water (26° C) made opaque with the addition of powdered milk. The tank was housed in a room filled with spatial cues. An escape platform (10 cm diameter) was hidden below the surface and could not be seen by the subject because of the opacity of the water. Each subject was randomly assigned one of four escape locations and the platform remained in that location throughout the testing period. Platform position was counterbalanced across treatments.

Prior to testing, each subject was marked with a black marker to allow the video system to track the movements of the rat in the white pool. A trial was initiated with the placement of the rat at one of 12 starting positions along the perimeter of the tank. Starting position differed for each trial and followed a pseudorandom sequence, with the condition that the subject was started from each quadrant during each testing day. During each trial, the subject was placed in the tank, facing the rim, and was required to swim to the location of the escape platform. If the subject failed to escape within 60 sec, the subject was manually placed on the platform for 10 sec. Between trials, subjects were kept in a heated environment (31° C) to prevent hypothermia.

Subjects were tested for four trials each day, with an intertrial interval of 3–5 min, for 6 consecutive days. After the last acquisition trial, the platform was removed for a 60-sec probe trial. On PD 51–52, subjects were tested with a visible platform, to determine if any deficits were related to non-spatial performance variables. On these days, white sheets surrounded the pool, removing spatial cues, and the platform was set above water level, made visible with a brightly colored cue. The location of the platform changed on each trial, thus subjects were required to swim to the platform, regardless of spatial location. Subjects were tested for 4 trials on each of 2 days.

All data were collected with the Plus 2020 Tracking system (HVS image, Hampton, UK). Latency to escape, path length, swimming speed, and initial heading angle were measured. On the probe trial, the time spent in each quadrant, time spent near the platform (within 26 cm), and number of passes through the target area were measured.

2.6. Data Analyses

Data were analyzed using SPSS software. All data were analyzed with ANOVAs with a 2 (Ethanol, SHAM) × 2 (IGF, VEH) × 2 (Male, Female) between-subjects design, with the exception of blood alcohol concentration which was analyzed with a 2 (IGF) × 2 (Sex) design. Day served as a repeated within-subject variable for body weight and parallel bar performance. Activity data were measured with day (4) and 5-min bin (12) as repeated, within-subject variables. The Morris water maze data from acquisition and visible platform period were analyzed with day and trial as repeated, within-subject variables. The probe trial

data of Morris water maze data from were analyzed with quadrant as within-subject variables. Post hoc comparisons were conducted with Neuman-Keuls analyses (p 's <0.05).

3. Results

3.1. Body Weights

Body weights during ethanol and IGF treatment (PD 4–13) and during PD 15–45 are shown in Figure 1 and Table 1, respectively. Because variability increases with increasing age, data collected during each of these periods was analyzed separately. During PD 4–13, there was a significant effect of day, due to growth in all groups [$F(9,621) = 1979.3$, $p < 0.05$] and a significant day by ethanol effect [$F(9,621) = 15.3$, $p < 0.05$], as well as a main effect of ethanol [$F(1,69) = 31.3$, $p < 0.05$]. Although there were no significant differences between groups on PD 4, beginning on PD 5, the ethanol-treated subjects lagged in growth compared to controls. During PD 15–45 (see Table 1), besides a significant effect of day [$F(6,414) = 13228$, $p < 0.05$] and a main effect of ethanol [$F(1,69) = 14.3$, $p < 0.05$], a significant sex effect was observed [$F(1,69) = 149.8$, $p < 0.05$], due to heavier weight of males compared with females. A significant day by sex by ethanol interaction was also observed [$F(6,414) = 3.0$, $p < 0.05$]. Subsequent follow-up tests revealed that during this period, body weights of ethanol-exposed females, but not males, caught up with those of controls by PD 35. IGF treatment had no significant effect on body growth.

3.2. Blood alcohol concentration

Blood alcohol concentrations were 343.8 ± 7.3 mg/dl and 348.2 ± 7.2 mg/dl for ethanol-exposed rats receiving intranasal IGF-1 and vehicle injection, respectively. There was no significant difference between groups [$F(1,41) = 0.2$, $p = 0.63$]. Similar blood alcohol levels have been shown in clinical case reports [1,88,103] and in other labs using a similar protocol [17].

3.3. Locomotor Activity Testing

Figure 2 shows the number of total beam breaks in the open field during each 1 hour session. During the 4-day activity testing, activity levels decreased gradually between sessions (day [$F(3,207) = 18.8$, $p < 0.05$]) and within sessions (bin [$F(11,759) = 338.4$, $p < 0.05$]). The ethanol-exposed subjects had higher activity levels compared to sham control subjects, producing a significant effect of ethanol treatment [$F(1,69) = 18.6$, $p < 0.05$]. However, IGF treatment did not significantly affect activity levels in either ethanol-treated or control subjects. A similar pattern was observed for total distance traveled, rearing and fine movements (data not show); on all measures, ethanol exposure during development led to increased activity levels, but IGF had no significant effect.

3.4. Parallel bar motor coordination

In contrast to activity level, IGF treatment reduced the severity of ethanol-related motor deficits. The number of trials to the first successful traversal is shown in Figure 3A. EtOH + VEH rats required more trials to the first success compared to the other three groups, producing a significant interaction of ethanol by IGF [$F(1,68) = 4.1$, $p < 0.05$], as well as main effects of ethanol [$F(1,68) = 14.7$, $p < 0.05$] and IGF [$F(1,68) = 6.1$, $p < 0.05$]. Follow-up comparisons demonstrated that the EtOH + VEH rats performed worse than the EtOH + IGF group, whereas performance of the EtOH + IGF group did not differ significantly from that of the control groups.

A similar pattern is observed on the percent of trials that were successful traversed (Figure 3B). The success percent was significantly reduced in EtOH + VEH rats, producing a significant interaction of ethanol by IGF [$F(1,68) = 5.6$, $p < 0.05$], as well as main effects of

ethanol [$F(1,68) = 21.6, p < 0.05$] and IGF [$F(1,68) = 13.7, p < 0.05$]. Follow-up comparisons demonstrated that the EtOH + VEH rats achieved fewer successes than all other groups, including the EtOH + IGF group; performance of the EtOH + IGF group did not differ significantly from that of the control groups.

The maximum gap successfully traversed on the parallel bars for each testing day is shown in Figure 4. Performance of all subjects showed gradual improvement over days (main effect of day [$F(2,120) = 204.0, p < 0.05$]). There was also a significant day by ethanol by IGF interaction [$F(2,120) = 4.6, p < 0.05$]. Follow-up analyses indicated a significant effect of ethanol [$F(1,68) = 12.8, p < 0.05$] on the first day, as ethanol-exposed subjects were not able to traverse the widths traversed by controls. By the second and third days, there were significant ethanol by IGF interactions [day 2 $F(1,68) = 4.8, p < 0.05$; day 3 $F(1,68) = 4.3, p < 0.05$] and main ethanol effects [day 2 $F(1,68) = 17.2, p < 0.05$; day 3 $F(1,68) = 19.2, p < 0.05$], while a main IGF effect was observed on day 2 [$F(1,68) = 19.2, p < 0.05$] but not on day 3 [$F(1,68) = 2.7, p = 0.1$]. Follow-up comparisons demonstrated that the EtOH + VEH rats successfully traversed significantly smaller widths between bars compared to the other three groups the second and third days of testing. In fact, performance of the EtOH + IGF group did not differ significantly from that of the two control groups. Interestingly, IGF treatment did not significantly influence performance of control subjects.

3.5. Morris water maze training

IGF treatment did not significantly mitigate ethanol-related spatial learning deficits. During the acquisition training, only subtle ethanol-related deficits were evident. For example, the path length from starting position to the escape platform is shown in Figure 5A. There were no significant effects of either ethanol or IGF treatment on path length (Figure 5A), latency, or swimming speed (data not shown). However, there was a significant main effect of ethanol treatment on heading angle [$F(1,69) = 10.1, p < 0.05$]. EtOH subjects exhibited a significantly higher initial heading angle compared to the control subjects (Figure 5B), indicating that they were less accurate in their initial estimate of the target location. There was significant learning among all groups over training, producing effects of day [$F(5,345) = 79.1, p < 0.05$], trial [$F(3,207) = 57.3, p < 0.05$], and day by trial interactions in path length [$F(15,1035) = 2.2, p < 0.05$] and heading angle [day $F(5,345) = 31.4, p < 0.05$; trial $F(3, 207) = 14.9, p < 0.05$; day by trial $F(15,1035) = 1.7, p < 0.05$].

Treatment effects were more evident during the probe trial. Fig. 6A shows the percent of time spent in each quadrant. During the probe trial, although all groups stayed longer in the quadrant where the platform had been located [$F(3,207) = 284.5, p < 0.05$], there was a significant quadrant by ethanol interaction [$F(3,207) = 4.9, p < 0.05$]. Ethanol-exposed subjects spent less time in the target quadrant [$F(1,69) = 7.0, p < 0.05$] and longer time in the adjacent quadrant [$F(1,69) = 7.5, p < 0.05$] compared to the sham-intubated subjects. EtOH-treated subjects also made fewer passes through the platform location compared to controls [$F(1,69) = 5.0, p < 0.05$] (Fig 6B). However, IGF failed to significantly influence the ethanol-related deficits in spatial memory. It is unlikely that any ethanol-related deficits were due to differences in motivation, sensory or motor performance, as there were no significant effects of treatment on the visible platform test (data not shown).

4. Discussion

The present study demonstrates that administration of IGF-I can mitigate the effects of 3rd trimester equivalent ethanol on parallel bar motor performance, but failed to attenuate ethanol's effects on activity level or spatial learning. To our knowledge, this is the first report illustrating that the neurotrophic factor IGF-1 can reduce behavioral alterations resulting from developmental ethanol exposure. Although the beneficial effects were task

specific, these data indicate that IGF-I may be a potential treatment for some of ethanol's damaging effects.

Although the concentration of IGF in the brain was not measured in this study, many studies have shown that peripherally administered IGF does enter the brain [4,77]. Moreover, IGF can enter the brain more easily via intranasal administration [100]. Following intranasal administration of isotope labeled IGF-I, IGF, as well as downstream signals activated by IGF-I, was measured in most CNS areas. In that study, adult rats were injected with 143 μ gram/kg intranasally. Twenty minutes after treatment, blood levels of IGF-I were around 0.6 nM and brain levels ranged from 0.3–0.4 nM for areas including the cerebellum, hippocampus and striatum. These brain levels were 100 times higher than those achieved via i.v. injections which produced similar blood concentrations.

The parallel bar motor coordination task requires both balance and fine motor coordination. Although deficits in these skills may be related to dysfunction of a number of motor areas, they are consistent with dysfunction of the cerebellum, which is highly sensitive to developmental alcohol exposure [45,97,104]. Imaging studies indicate that children with FASD have smaller cerebellar volumes, even disproportionately reduced in relation to overall reductions in brain volume [30,78,79,90]. Animal studies show that ethanol exposure during the 3rd trimester equivalent brain growth spurt significantly reduces both cerebellar Purkinje and granule cell number [18,97] and disrupts motor performance [18,45]. Given the pattern of IGF-I effects on behavior, this study suggests that the cerebellum, or other motor areas, are most sensitive to the beneficial effects of IGF-I, at least at the times of administration used in the present study.

The effects of IGF-I on motor performance, but not cognitive performance, may be related to developmental differences in IGF among various CNS regions. The main effects of IGF-I on cell proliferation, survival and differentiation are mediated by IGF-I receptors, which are under critical spatial and temporal control. Although during PD 10–13, the expression of IGF-I receptors is high in the brain regions involved in neonatal ethanol induced behavioral impairment, there are some developmental differences. In the cerebellum, IGF-I mRNA is first detected on PD 0 and reaches its peak at about PD 12 [56]. Most Purkinje cells don't express IGF-I immunoreactivity until PD 7 and start to lose their IGF-I immunoreactivity at PD 15 [87]. IGF-I receptors are not detectable until PD 4 and reach their maximum level at PD 14 [53]. However, in the hippocampus, the number of IGF-I receptors reaches its peak on embryonic day 18 and exhibits a reduction after birth [53]. IGF-I treatment in the present study occurred from PD 10–13, during the development period when IGF-I receptor expression is peak in the cerebellum. Thus, the timing of IGF treatment in the present study matched the pattern of peak IGF-I and IGF-I receptor expression, which might explain the task specificity of IGF-I's effects on ethanol-treated subjects, although further examination of neuropathology is needed to determine whether IGF is neuroprotective in the cerebellum.

Indeed, beneficial effects of IGF-I on motor impairments related to cerebellum degeneration have been evident in different animal models of cerebellar ataxia. For example, systemic IGF-I injections not only improved motor performance but also rescued the inferior olive neurons, the target of the neurotoxin used to induce cerebellar ataxia [67]. Moreover, overexpression of IGF-1 in genetic cerebellar ataxia mice improved muscle strength and reduced ataxia [23]. Therefore, besides the appropriate period of IGF treatment, the task specificity might also be related to the regional specific beneficial effects of IGF-I on cerebellar cells.

The mechanism(s) by which IGF-I reduces alcohol-related motor deficits are not known, since IGF-I serves many functions in brain development. IGF-I may reverse ethanol's effect

through inhibition of cell loss, as well as an elevation of cell proliferation and/or promotion of neuronal development. First, IGF-I is a potent agent for rescuing neurons from apoptosis [38]. For example, overexpression of IGF-I in the mouse cerebellum leads to cerebellar overgrowth characterized by significant increases in the number of granule cells and Purkinje cells [112] due to IGF-I inhibition of apoptosis [28]. Conversely, IGF-I gene expression is low in the Purkinje cell degeneration (pcd) mice, a mutant mouse line characterized by increased cerebellar apoptosis [114]. IGF-I also protects cerebellar granule neurons from apoptosis induced by serum and potassium removal in the culture media [32,39,115]. In cultured cells, IGF-I treatment three days after ethanol exposure reduced ethanol's toxic effect on neuronal survival [9]. Therefore, the improvement of parallel bar performance resulting from IGF-I may due to its anti-apoptotic effects.

IGF-I may also enhance white matter development. There is increasing evidence that IGF-I plays a role in oligodendrocyte development [111]. Oligodendrocytes possess receptors for IGF and addition of IGF-I to developing brain cells in culture increases the number of oligodendrocytes, affecting proliferation, differentiation, and survival of cells [72,109]. More importantly for the present study, IGF-I promotes the regeneration of oligodendrocytes, increases myelin gene expression, and increases myelin content both in vitro and in vivo following an insult [22]. Indeed, IGF-I reduces pathology in an experimental autoimmune encephalomyelitis, which serves as a model for demyelinating diseases [107]. Similarly, transgenic mice that overexpress IGF-I express greater myelin levels and are relatively protected against a reduction in myelin proteins resulting from undernutrition during the early neonatal period. Alcohol exposure during early postnatal development delays both acquisition and maturation of myelin and the oligodendroglia [74]. Clinical studies also indicated that prenatal ethanol exposure decrease white matter in corpus callosum [3,5,65,71,91]. Therefore, the improvement of parallel bar performance result from ethanol treatment by IGF-I may due to its promotion in myelination.

IGF-I has many potential therapeutic uses because of its varied effects [26]. IGF-I was shown to improve metabolic control among patients with type 1 diabetes whose disease was poorly controlled through conventional means [24,25]. Systemic IGF-I injection also increases the growth velocity in prepubertal children with a growth hormone receptor deficiency and individuals with growth hormone insensitivity [21,26,47]. Common side effects of using recombinant human IGF-I systemically includes pain at the site of the injection, hypoglycaemia and headaches [7,8]. During long-term treatment, although overgrowth of soft tissue has been observed, no substantial clinical side effects were found [8,21]. Therefore, IGF-I is still considered an efficacious and safe treatment in the situation for which other alternatives are not yet available. Instead of a peripheral target, the CNS is the target for mitigating prenatal alcohol effects. The intranasal IGF administration method used in the present study is an alternative delivery strategy that minimizes the undesirable side effects induced by systemic IGF injection while preserving the treatment benefit. This is a realistic method that can be used in clinic drug delivery.

In the present study, we found that IGF-I treatment significantly mitigated ethanol's effects on motor coordination, but did not significantly affect ethanol-induced increases in activity level or spatial learning deficits. Future studies will need to identify the specific neural targets and mechanisms of IGF's beneficial actions. Nevertheless, these data indicate that IGF-I may be a potential treatment for some of ethanol's damaging effects, a finding that has important implications for children of women who drink alcohol during pregnancy.

Acknowledgments

This study was supported by grants from NIAAA (T32AA13525; AA014828; AA006902; AA012446).

References

1. Alcohol consumption among women who are pregnant or who might become pregnant--United States, 2002. MMWR Morb Mortal Wkly Rep. 2004; 53:1178–81. [PubMed: 15614234]
2. Angelucci L, Patacchioli FR, Scaccianoe S, Di Sciullo A, Cardillo A, Maccari S. A model for later-life effects of perinatal drug exposure: maternal hormone mediation. Neurobehav Toxicol Teratol. 1985; 7:511–7. [PubMed: 4080068]
3. Archibald SL, Fennema-Notestine C, Gamst A, Riley EP, Mattson SN, Jernigan TL. Brain dysmorphology in individuals with severe prenatal alcohol exposure. Developmental medicine and child neurology. 2001; 43:148–54. [PubMed: 11263683]
4. Armstrong CS, Waurin L, Ishii DN. Uptake of circulating insulin-like growth factor-I into the cerebrospinal fluid of normal and diabetic rats and normalization of IGF-II mRNA content in diabetic rat brain. J Neurosci Res. 2000; 59:649–660. [PubMed: 10686593]
5. Autti-Ramo I, Fagerlund A, Ervalahti N, Loimu L, Korkman M, Hoyme HE. Fetal alcohol spectrum disorders in Finland: clinical delineation of 77 older children and adolescents. Am J Med Genet A. 2006; 140:137–43. [PubMed: 16353236]
6. Ayer-le Lievre C, Stahlbom PA, Sara VR. Expression of IGF-I and -II mRNA in the brain and craniofacial region of the rat fetus. Development. 1991; 111:105–15. [PubMed: 2015788]
7. Azcona C, Preece MA, Rose SJ, Fraser N, Rappaport R, Ranke MB, Savage MO. Growth response to rhIGF-I 80 microg/kg twice daily in children with growth hormone insensitivity syndrome: relationship to severity of clinical phenotype. Clin Endocrinol (Oxf). 1999; 51:787–92. [PubMed: 10619985]
8. Backeljauw PF, Underwood LE. Therapy for 6.5–7.5 years with recombinant insulin-like growth factor I in children with growth hormone insensitivity syndrome: a clinical research center study. J Clin Endocrinol Metab. 2001; 86:1504–10. [PubMed: 11297575]
9. Barclay DC, Hallbergson AF, Montague JR, Mudd LM. Reversal of ethanol toxicity in embryonic neurons with growth factors and estrogen. Brain Res Bull. 2005; 67:459–65. [PubMed: 16216694]
10. Baron-Van Evercooren A, Olichon-Berthe C, Kowalski A, Visciano G, Van Obberghen E. Expression of IGF-I and insulin receptor genes in the rat central nervous system: a developmental, regional, and cellular analysis. J Neurosci Res. 1991; 28:244–53. [PubMed: 1851850]
11. Barron S, White A, Swartzwelder HS, Bell RL, Rodd ZA, Slawecki CJ, Ehlers CL, Levin ED, Rezvani AH, Spear LP. Adolescent vulnerabilities to chronic alcohol or nicotine exposure: findings from rodent models. Alcohol Clin Exp Res. 2005; 29:1720–5. [PubMed: 16205372]
12. Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F. Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. Neuron. 1995; 14:717–30. [PubMed: 7718235]
13. Bondy CA. Transient IGF-I gene expression during the maturation of functionally related central projection neurons. J Neurosci. 1991; 11:3442–55. [PubMed: 1658250]
14. Bondy CA, Cheng CM. Signaling by insulin-like growth factor I in brain. Eur J Pharmacol. 2004; 490:25–31. [PubMed: 15094071]
15. Bonthius DJ, Bonthius NE, Napper RM, West JR. Early postnatal alcohol exposure acutely and permanently reduces the number of granule cells and mitral cells in the rat olfactory bulb: a stereological study. J Comp Neurol. 1992; 324:557–66. [PubMed: 1430337]
16. Bonthius DJ, Karacay B, Dai D, Pantazis NJ. FGF-2, NGF and IGF-1, but not BDNF, utilize a nitric oxide pathway to signal neurotrophic and neuroprotective effects against alcohol toxicity in cerebellar granule cell cultures. Brain Res Dev Brain Res. 2003; 140:15–28.
17. Bonthius DJ, West JR. Blood alcohol concentration and microencephaly: a dose-response study in the neonatal rat. Teratology. 1988; 37:223–31. [PubMed: 3368876]
18. Bonthius DJ, West JR. Permanent neuronal deficits in rats exposed to alcohol during the brain growth spurt. Teratology. 1991; 44:147–63. [PubMed: 1925974]
19. Bradley DM, Beaman FD, Moore DB, Kidd K, Heaton MB. Neurotrophic factors BDNF and GDNF protect embryonic chick spinal cord motoneurons from ethanol neurotoxicity in vivo. Brain Res Dev Brain Res. 1999; 112:99–106.

20. Breese CR, D'Costa A, Ingram RL, Lenham J, Sonntag WE. Long-term suppression of insulin-like growth factor-1 in rats after in utero ethanol exposure: relationship to somatic growth. *J Pharmacol Exp Ther.* 1993; 264:448–56. [PubMed: 7678650]
21. Bucuvalas JC, Chernausk SD, Alfaro MP, Krug SK, Ritschel W, Wilmott RW. Effect of insulinlike growth factor-1 treatment in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 2001; 33:576–581. [PubMed: 11740232]
22. Cao Y, Gunn AJ, Bennet L, Wu D, George S, Gluckman PD, Shao XM, Guan J. Insulin-like growth factor (IGF)-1 suppresses oligodendrocyte caspase-3 activation and increases glial proliferation after ischemia in near-term fetal sheep. *J Cereb Blood Flow Metab.* 2003; 23:739–47. [PubMed: 12796722]
23. Carrascosa C, Torres-Aleman I, Lopez-Lopez C, Carro E, Espejo L, Torrado S, Torrado JJ. Microspheres containing insulin-like growth factor I for treatment of chronic neurodegeneration. *Biomaterials.* 2004; 25:707–14. [PubMed: 14607509]
24. Carroll PV, Christ ER, Umpleby AM, Gowrie I, Jackson N, Bowes SB, Hovorka R, Croos P, Sonksen PH, Russell-Jones DL. IGF-I treatment in adults with type 1 diabetes: effects on glucose and protein metabolism in the fasting state and during a hyperinsulinemic-euglycemic amino acid clamp. *Diabetes.* 2000; 49:789–96. [PubMed: 10905488]
25. Carroll PV, Umpleby M, Alexander EL, Egel VA, Callison KV, Sonksen PH, Russell-Jones DL. Recombinant human insulin-like growth factor-I (rhIGF-I) therapy in adults with type 1 diabetes mellitus: effects on IGFs, IGF-binding proteins, glucose levels and insulin treatment. *Clin Endocrinol (Oxf).* 1998; 49:739–46. [PubMed: 10209561]
26. Chambers RA, Potenza MN, Hoffman RE, Miranker W. Simulated apoptosis/neurogenesis regulates learning and memory capabilities of adaptive neural networks. *Neuropsychopharmacology.* 2004; 29:747–58. [PubMed: 14702022]
27. Cheng CM, Joncas G, Reinhardt RR, Farrer R, Quarles R, Janssen J, McDonald MP, Crawley JN, Powell-Braxton L, Bondy CA. Biochemical and morphometric analyses show that myelination in the insulin-like growth factor 1 null brain is proportionate to its neuronal composition. *J Neurosci.* 1998; 18:5673–81. [PubMed: 9671658]
28. Chrysis D, Calikoglu AS, Ye P, D'Ercole AJ. Insulin-like growth factor-I overexpression attenuates cerebellar apoptosis by altering the expression of Bcl family proteins in a developmentally specific manner. *J Neurosci.* 2001; 21:1481–9. [PubMed: 11222638]
29. Climent E, Pascual M, Renau-Piqueras J, Guerri C. Ethanol exposure enhances cell death in the developing cerebral cortex: role of brain-derived neurotrophic factor and its signaling pathways. *J Neurosci Res.* 2002; 68:213–25. [PubMed: 11948666]
30. Coffin JM, Baroody S, Schneider K, O'Neill J. Impaired cerebellar learning in children with prenatal alcohol exposure: a comparative study of eyeblink conditioning in children with ADHD and dyslexia. *Cortex.* 2005; 41:389–98. [PubMed: 15871603]
31. Coles CD, Platzman KA, Raskind-Hood CL, Brown RT, Falek A, Smith IE. A comparison of children affected by prenatal alcohol exposure and attention deficit, hyperactivity disorder. *Alcohol Clin Exp Res.* 1997; 21:150–61. [PubMed: 9046388]
32. D'Mello SR, Galli C, Ciotti T, Calissano P. Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. *Proc Natl Acad Sci U S A.* 1993; 90:10989–93. [PubMed: 8248201]
33. de la Monte SM, Ganju N, Banerjee K, Brown NV, Luong T, Wands JR. Partial rescue of ethanol-induced neuronal apoptosis by growth factor activation of phosphoinositol-3-kinase. *Alcohol Clin Exp Res.* 2000; 24:716–26. [PubMed: 10832914]
34. de la Monte SM, Xu XJ, Wands JR. Ethanol inhibits insulin expression and actions in the developing brain. *Cell Mol Life Sci.* 2005; 62:1131–45. [PubMed: 15870954]
35. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev.* 1979; 3:79–83. [PubMed: 118862]
36. Dohrman DP, West JR, Pantazis NJ. Ethanol reduces expression of the nerve growth factor receptor, but not nerve growth factor protein levels in the neonatal rat cerebellum. *Alcohol Clin Exp Res.* 1997; 21:882–93. [PubMed: 9267539]

37. Dore S, Kar S, Zheng WH, Quirion R. Rediscovering good old friend IGF-I in the new millenium: possible usefulness in Alzheimer's disease and stroke. *Pharm Acta Helv.* 2000; 74:273–80. [PubMed: 10812969]
38. Feldman EL, Sullivan KA, Kim B, Russell JW. Insulin-like growth factors regulate neuronal differentiation and survival. *Neurobiol Dis.* 1997; 4:201–14. [PubMed: 9361296]
39. Galli C, Meucci O, Scorziello A, Werge TM, Calissano P, Schettini G. Apoptosis in cerebellar granule cells is blocked by high KCl, forskolin, and IGF-1 through distinct mechanisms of action: the involvement of intracellular calcium and RNA synthesis. *J Neurosci.* 1995; 15:1172–9. [PubMed: 7532699]
40. Gatford KL, Dalitz PA, Cock ML, Harding R, Owens JA. Acute ethanol exposure in pregnancy alters the insulin-like growth factor axis of fetal and maternal sheep. *Am J Physiol Endocrinol Metab.* 2007; 292:E494–500. [PubMed: 17003241]
41. Ghiselli G, Chen J, Kaou M, Hallak H, Rubin R. Ethanol inhibits fibroblast growth factor-induced proliferation of aortic smooth muscle cells, Arteriosclerosis, thrombosis. and vascular biology. 2003; 23:1808–13.
42. Goodlett CR, Eilers AT. Alcohol-induced Purkinje cell loss with a single binge exposure in neonatal rats: a stereological study of temporal windows of vulnerability. *Alcohol Clin Exp Res.* 1997; 21:738–44. [PubMed: 9194933]
43. Goodlett CR, Horn KH. Mechanisms of alcohol-induced damage to the developing nervous system. *Alcohol Res Health.* 2001; 25:175–84. [PubMed: 11810955]
44. Goodlett CR, Johnson TB. Neonatal binge ethanol exposure using intubation: timing and dose effects on place learning. *Neurotoxicol Teratol.* 1997; 19:435–46. [PubMed: 9392779]
45. Goodlett CR, Thomas JD, West JR. Long-term deficits in cerebellar growth and rotarod performance of rats following “binge-like” alcohol exposure during the neonatal brain growth spurt. *Neurotoxicol Teratol.* 1991; 13:69–74. [PubMed: 2046629]
46. Granato A. Altered organization of cortical interneurons in rats exposed to ethanol during neonatal life. *Brain Res.* 2006; 1069:23–30. [PubMed: 16386714]
47. Guevara-Aguirre J, Rosenbloom AL, Vasquez O, Martinez V, Gargosky SE, Allen L, Rosenfeld RG. Two-year treatment of growth hormone (GH) receptor deficiency with recombinant insulin-like growth factor I in 22 children: comparison of two dosage levels and to GH-treated GH deficiency. *J Clin Endocrinol Metab.* 1997; 82:629–33. [PubMed: 9024266]
48. Hallak H, Seiler AE, Green JS, Henderson A, Ross BN, Rubin R. Inhibition of insulin-like growth factor-I signaling by ethanol in neuronal cells. *Alcohol Clin Exp Res.* 2001; 25:1058–64. [PubMed: 11505033]
49. Harris SJ, Wilce P, Bedi KS. Exposure of rats to a high but not low dose of ethanol during early postnatal life increases the rate of loss of optic nerve axons and decreases the rate of myelination. *J Anat.* 2000; 197(Pt 3):477–85. [PubMed: 11117631]
50. Heaton MB, Mitchell JJ, Paiva M, Walker DW. Ethanol-induced alterations in the expression of neurotrophic factors in the developing rat central nervous system. *Brain Res Dev Brain Res.* 2000; 121:97–107.
51. Heaton MB, Paiva M, Madorsky I, Shaw G. Ethanol effects on neonatal rat cortex: comparative analyses of neurotrophic factors, apoptosis-related proteins, and oxidative processes during vulnerable and resistant periods. *Brain Res Dev Brain Res.* 2003; 145:249–62.
52. Heaton MB, Paiva M, Swanson DJ, Walker DW. Alterations in responsiveness to ethanol and neurotrophic substances in fetal septohippocampal neurons following chronic prenatal ethanol exposure. *Brain Res Dev Brain Res.* 1995; 85:1–13.
53. Kar S, Chabot JG, Quirion R. Quantitative autoradiographic localization of [125I]insulin-like growth factor I, [125I]insulin-like growth factor II, and [125I]insulin receptor binding sites in developing and adult rat brain. *The Journal of comparative neurology.* 1993; 333:375–97. [PubMed: 8349849]
54. Kelly SJ, Day N, Streissguth AP. Effects of prenatal alcohol exposure on social behavior in humans and other species. *Neurotoxicol Teratol.* 2000; 22:143–9. [PubMed: 10758343]
55. Kumada T, Jiang Y, Cameron DB, Komuro H. How does alcohol impair neuronal migration? *J Neurosci Res.* 2007; 85:465–70. [PubMed: 17139684]

56. Lee WH, Javedan S, Bondy CA. Coordinate expression of insulin-like growth factor system components by neurons and neuroglia during retinal and cerebellar development. *J Neurosci.* 1992; 12:4737–44. [PubMed: 1281494]
57. Light KE, Brown DP, Newton BW, Belcher SM, Kane CJ. Ethanol-induced alterations of neurotrophin receptor expression on Purkinje cells in the neonatal rat cerebellum. *Brain Res.* 2002; 924:71–81. [PubMed: 11743997]
58. Liu XF, Fawcett JR, Thorne RG, DeFor TA, Frey WH 2nd. Intranasal administration of insulin-like growth factor-I bypasses the blood-brain barrier and protects against focal cerebral ischemic damage. *J Neurol Sci.* 2001; 187:91–7. [PubMed: 11440750]
59. Liu XF, Fawcett JR, Thorne RG, Frey WH 2nd. Non-invasive intranasal insulin-like growth factor-I reduces infarct volume and improves neurologic function in rats following middle cerebral artery occlusion. *Neurosci Lett.* 2001; 308:91–4. [PubMed: 11457567]
60. Livy DJ, Miller EK, Maier SE, West JR. Fetal alcohol exposure and temporal vulnerability: effects of binge-like alcohol exposure on the developing rat hippocampus. *Neurotoxicol Teratol.* 2003; 25:447–58. [PubMed: 12798962]
61. Luo J, Miller MW. Ethanol inhibits basic fibroblast growth factor-mediated proliferation of C6 astrocytoma cells. *Journal of neurochemistry.* 1996; 67:1448–56. [PubMed: 8858926]
62. Luo J, Miller MW. Growth factor-mediated neural proliferation: target of ethanol toxicity. *Brain Res Brain Res Rev.* 1998; 27:157–67. [PubMed: 9622617]
63. Luo J, West JR, Pantazis NJ. Nerve growth factor and basic fibroblast growth factor protect rat cerebellar granule cells in culture against ethanol-induced cell death. *Alcohol Clin Exp Res.* 1997; 21:1108–20. [PubMed: 9309325]
64. Ma W, Li BS, Maric D, Zhao WQ, Lin HJ, Zhang L, Pant HC, Barker JL. Ethanol blocks both basic fibroblast growth factor- and carbachol-mediated neuroepithelial cell expansion with differential effects on carbachol-activated signaling pathways. *Neuroscience.* 2003; 118:37–47. [PubMed: 12676135]
65. Ma X, Coles CD, Lynch ME, Laconte SM, Zurkiya O, Wang D, Hu X. Evaluation of corpus callosum anisotropy in young adults with fetal alcohol syndrome according to diffusion tensor imaging. *Alcohol Clin Exp Res.* 2005; 29:1214–22. [PubMed: 16046877]
66. Marcussen BL, Goodlett CR, Mahoney JC, West JR. Developing rat Purkinje cells are more vulnerable to alcohol-induced depletion during differentiation than during neurogenesis. *Alcohol.* 1994; 11:147–56. [PubMed: 8204201]
67. Martin-Algarra RV, Polache A, Fernandez-Villalba E, Pla Delfina JM, Guerri C. Influence of chronic alcohol intake on intestinal taurine and antipyrine transport in pregnant rats. *Alcohol Clin Exp Res.* 1998; 22:463–7. [PubMed: 9581654]
68. Mattson SN, Schoenfeld AM, Riley EP. Teratogenic effects of alcohol on brain and behavior. *Alcohol Res Health.* 2001; 25:185–91. [PubMed: 11810956]
69. McAlhany RE Jr, Miranda RC, Finnell RH, West JR. Ethanol decreases Glial-Derived Neurotrophic Factor (GDNF) protein release but not mRNA expression and increases GDNF-stimulated Shc phosphorylation in the developing cerebellum. *Alcohol Clin Exp Res.* 1999; 23:1691–7. [PubMed: 10550003]
70. McAlhany RE Jr, West JR, Miranda RC. Glial-derived neurotrophic factor (GDNF) prevents ethanol-induced apoptosis and JUN kinase phosphorylation. *Brain Res Dev Brain Res.* 2000; 119:209–16.
71. McGee CL, Riley EP. Brain imaging and fetal alcohol spectrum disorders. *Ann Ist Super Sanita.* 2006; 42:46–52. [PubMed: 16801725]
72. McMorris FA, Mozell RL, Carson MJ, Yhinar, Meyer RD, Marchetti N. Regulation of oligodendrocyte development and central nervous system myelination by insulin-like growth factors. *Ann N Y Acad Sci.* 1993; 692:321–334. [PubMed: 8215042]
73. Mitchell JJ, Paiva M, Moore DB, Walker DW, Heaton MB. A comparative study of ethanol, hypoglycemia, hypoxia and neurotrophic factor interactions with fetal rat hippocampal neurons: a multi-factor in vitro model developmental ethanol effects. *Brain Res Dev Brain Res.* 1998; 105:241–50.

74. Phillips DE, Kreuger SK. Effects of postnatal ethanol exposure on glial cell development in rat optic nerve. *Exp Neurol*. 1990; 103:90–100. [PubMed: 2912754]
75. Pucilowski O, Ayensu WK, D'Ercole AJ. Insulin-like growth factor I expression alters acute sensitivity and tolerance to ethanol in transgenic mice. *Eur J Pharmacol*. 1996; 305:57–62. [PubMed: 8813532]
76. Resnicoff M, Rubini M, Baserga R, Rubin R. Ethanol inhibits insulin-like growth factor-1-mediated signalling and proliferation of C6 rat glioblastoma cells. *Lab Invest*. 1994; 71:657–62. [PubMed: 7526037]
77. Rienhardt RR, Bondy CA. Insulin-like growth factors cross the blood-brain barrier. *Endocrinology*. 1994; 135:1753–1761. [PubMed: 7525251]
78. Riley EP, McGee CL. Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior. *Exp Biol Med (Maywood)*. 2005; 230:357–65. [PubMed: 15956765]
79. Riley EP, McGee CL, Sowell ER. Teratogenic effects of alcohol: a decade of brain imaging. *Am J Med Genet C Semin Med Genet*. 2004; 127:35–41. [PubMed: 15095470]
80. Roebuck TM, Mattson SN, Riley EP. Behavioral and psychosocial profiles of alcohol-exposed children. *Alcohol Clin Exp Res*. 1999; 23:1070–6. [PubMed: 10397293]
81. Rubert G, Minana R, Pascual M, Guerri C. Ethanol exposure during embryogenesis decreases the radial glial progenitor pool and affects the generation of neurons and astrocytes. *J Neurosci Res*. 2006; 84:483–96. [PubMed: 16770775]
82. Sampson PD, Streissguth AP, Bookstein FL, Little RE, Clarren SK, Dehaene P, Hanson JW, Graham JM Jr. Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. *Teratology*. 1997; 56:317–26. [PubMed: 9451756]
83. Sara VR, Carlsson-Skewirt C. The role of the insulin-like growth factors in the regulation of brain development. *Prog Brain Res*. 1988; 73:87–99. [PubMed: 3047812]
84. Seabold GK, Luo J, Miller MW. Effect of ethanol on neurotrophin-mediated cell survival and receptor expression in cultures of cortical neurons. *Brain Res Dev Brain Res*. 1998; 108:139–45.
85. Seiler AE, Ross BN, Green JS, Rubin R. Differential effects of ethanol on insulin-like growth factor-I receptor signaling. *Alcohol Clin Exp Res*. 2000; 24:140–8. [PubMed: 10698364]
86. Seiler AE, Ross BN, Rubin R. Inhibition of insulin-like growth factor-1 receptor and IRS-2 signaling by ethanol in SH-SY5Y neuroblastoma cells. *J Neurochem*. 2001; 76:573–81. [PubMed: 11208920]
87. Sherrard RM, Richardson NA, Sara VR. Localisation of insulin-like growth factor-I (IGF-I) immunoreactivity in the olivocerebellar system of developing and adult rats. *Brain Res Dev Brain Res*. 1997; 98:102–13.
88. Silva PD, Miller KD, Madden J, Keegan KA Jr. Abnormal fetal heart rate pattern associated with severe intrapartum maternal ethanol intoxication. A case report. *J Reprod Med*. 1987; 32:144–6. [PubMed: 3560078]
89. Singh SP, Ehmann S, Snyder AK. Ethanol-induced changes in insulin-like growth factors and IGF gene expression in the fetal brain. *Proc Soc Exp Biol Med*. 1996; 212:349–54. [PubMed: 8751993]
90. Sowell ER, Jernigan TL, Mattson SN, Riley EP, Sobel DF, Jones KL. Abnormal development of the cerebellar vermis in children prenatally exposed to alcohol: size reduction in lobules I–V. *Alcohol Clin Exp Res*. 1996; 20:31–4. [PubMed: 8651458]
91. Sowell ER, Mattson SN, Kan E, Thompson PM, Riley EP, Toga AW. Abnormal cortical thickness and brain-behavior correlation patterns in individuals with heavy prenatal alcohol exposure. *Cereb Cortex*. 2008; 18:136–44. [PubMed: 17443018]
92. Stanton ME, Goodlett CR. Neonatal ethanol exposure impairs eyeblink conditioning in weanling rats. *Alcohol Clin Exp Res*. 1998; 22:270–5. [PubMed: 9514318]
93. Tateno M, Ukai W, Ozawa H, Yamamoto M, Toki S, Ikeda H, Saito T. Ethanol inhibition of neural stem cell differentiation is reduced by neurotrophic factors. *Alcohol Clin Exp Res*. 2004; 28:134S–138S. [PubMed: 15318100]
94. Thomas JD, Fleming SI, Riley EP. MK-801 can exacerbate or attenuate behavioral alterations associated with neonatal alcohol exposure in the rat, depending on the timing of administration. *Alcohol Clin Exp Res*. 2001; 25:764–73. [PubMed: 11371726]

95. Thomas JD, Garcia GG, Dominguez HD, Riley EP. Administration of eliprodil during ethanol withdrawal in the neonatal rat attenuates ethanol-induced learning deficits. *Psychopharmacology (Berl)*. 2004; 175:189–95. [PubMed: 15064913]
96. Thomas JD, Garrison M, O'Neill TM. Perinatal choline supplementation attenuates behavioral alterations associated with neonatal alcohol exposure in rats. *Neurotoxicol Teratol*. 2004; 26:35–45. [PubMed: 15001212]
97. Thomas JD, Goodlett CR, West JR. Alcohol-induced Purkinje cell loss depends on developmental timing of alcohol exposure and correlates with motor performance. *Brain Res Dev Brain Res*. 1998; 105:159–66.
98. Thomas JD, Leany BD, Riley EP. Differential vulnerability to motor deficits in second replicate HAS and LAS rats following neonatal alcohol exposure. *Pharmacol Biochem Behav*. 2003; 75:17–24. [PubMed: 12759109]
99. Thomas JD, Wasserman EA, West JR, Goodlett CR. Behavioral deficits induced by binge-like exposure to alcohol in neonatal rats: importance of developmental timing and number of episodes. *Dev Psychobiol*. 1996; 29:433–52. [PubMed: 8809494]
100. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience*. 2004; 127:481–96. [PubMed: 15262337]
101. Torres-Aleman I. Serum growth factors and neuroprotective surveillance: focus on IGF-1. *Mol Neurobiol*. 2000; 21:153–60. [PubMed: 11379797]
102. Tran TD, Kelly SJ. Critical periods for ethanol-induced cell loss in the hippocampal formation. *Neurotoxicol Teratol*. 2003; 25:519–28. [PubMed: 12972065]
103. Tsai J, Floyd RL, Green PP, Boyle CA. Patterns and average volume of alcohol use among women of childbearing age. *Matern Child Health J*. 2007; 11:437–45. [PubMed: 17333387]
104. West JR. Acute and long-term changes in the cerebellum following developmental exposure to ethanol. *Alcohol Alcohol Suppl*. 1993; 2:199–202. [PubMed: 7748300]
105. Woods KA, Camacho-Hubner C, Barter D, Clark AJ, Savage MO. Insulin-like growth factor I gene deletion causing intrauterine growth retardation and severe short stature. *Acta Paediatr Suppl*. 1997; 423:39–45. [PubMed: 9401537]
106. Woolfrey KM, Hunt PS, Burk JA. Postnatal ethanol exposure disrupts signal detection in adult rats. *Neurotoxicol Teratol*. 2005; 27:815–23. [PubMed: 16115748]
107. Yao DL, Liu X, Hudson LD, Webster HD. Insulin-like growth factor-I given subcutaneously reduces clinical deficits, decreases lesion severity and upregulates synthesis of myelin proteins in experimental autoimmune encephalomyelitis. *Life Sci*. 1996; 58:1301–6. [PubMed: 8614286]
108. Ye P, Carson J, D'Ercole AJ. In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. *J Neurosci*. 1995; 15:7344–56. [PubMed: 7472488]
109. Ye P, D'Ercole AJ. Insulin-like growth factor I protects oligodendrocytes from tumor necrosis factor-alpha-induced injury. *Endocrinology*. 1999; 140:3063–72. [PubMed: 10385398]
110. Ye P, Lee KH, D'Ercole AJ. Insulin-like growth factor-I (IGF-I) protects myelination from undernutritional insult: studies of transgenic mice overexpressing IGF-I in brain. *J Neurosci Res*. 2000; 62:700–8. [PubMed: 11104508]
111. Ye P, Li L, Richards RG, DiAugustine RP, D'Ercole AJ. Myelination is altered in insulin-like growth factor-I null mutant mice. *J Neurosci*. 2002; 22:6041–51. [PubMed: 12122065]
112. Ye P, Xing Y, Dai Z, D'Ercole AJ. In vivo actions of insulin-like growth factor-I (IGF-I) on cerebellum development in transgenic mice: evidence that IGF-I increases proliferation of granule cell progenitors. *Brain Res Dev Brain Res*. 1996; 95:44–54.
113. Zhang FX, Rubin R, Rooney TA. Ethanol induces apoptosis in cerebellar granule neurons by inhibiting insulin-like growth factor 1 signaling. *J Neurochem*. 1998; 71:196–204. [PubMed: 9648866]
114. Zhang W, Ghetti B, Lee WH. Decreased IGF-I gene expression during the apoptosis of Purkinje cells in pcd mice. *Brain Res Dev Brain Res*. 1997; 98:164–76.

115. Zhong J, Deng J, Huang S, Yang X, Lee WH. High K⁺ and IGF-1 protect cerebellar granule neurons via distinct signaling pathways. *J Neurosci Res.* 2004; 75:794–806. [PubMed: 14994340]
116. Zoeller RT, Butnariu OV, Fletcher DL, Riley EP. Limited postnatal ethanol exposure permanently alters the expression of mRNAs encoding myelin basic protein and myelin-associated glycoprotein in cerebellum. *Alcohol Clin Exp Res.* 1994; 18:909–16. [PubMed: 7526726]

Fig. 1. Effects of neonatal ethanol treatment on body weight (mean $\pm$ SEM) from PD 4–13. Ethanol-exposed subjects lagged in growth compared to control groups beginning on PD 5. IGF had no significant effects on body growth.

Fig. 2. Effects of neonatal ethanol treatment on the number of beam breaks (mean $\pm$ SEM) in open-field locomotor activity testing. Ethanol exposure during development led to significant overactivity in the open field. IGF-1 did not affect ethanol-induced hyperactivity.

Fig. 3.

Effects of neonatal ethanol treatment on motor coordination. Ethanol treatment increased the number of trials required to reach the first success (mean + SEM) (3A) and decreased percent of trials that were successful traversed (mean + SEM) (3B). IGF treatment significantly reduced the severity of ethanol-related motor deficits. In fact, performance of the EtOH + IGF group did not significantly differ from that of controls.

Fig. 4. Effects of neonatal ethanol treatment on the maximum gap traversed (mean $\pm$ SEM) in motor coordination performance. IGF-1 treatment attenuated ethanol-related reductions in maximum gap traversed on the parallel bars.

Fig. 5. Effects of neonatal ethanol treatment on the path length (5A) and heading angle performance (5B) in acquisition period in Morris water maze testing. There were no significant effects of either ethanol exposure or IGF treatment on the path length; however, ethanol exposure during development led to significant increases in heading angle. IGF treatment did not mitigate ethanol-related spatial learning deficits.

Fig. 6. Effects of neonatal ethanol treatment on the time spent in each quadrant (6A) and the number of platform location passes (6B) during the probe trial in Morris water maze testing. Ethanol-exposed subjects spent significantly less time in the target quadrant and made fewer passes through the platform location. IGF treatment did not alter ethanol-related deficits in spatial memory.

Table 1Effects of neonatal ethanol treatment on bodyweight (mean $\pm$ SEM) from PD 15–45

Treatment	N	PD						
		15	20	25	30	35	40	45
Male								
EtOH + VEH	10	33.9 ± 0.9	47.5 ± 1.2	74.9 ± 1.5	108.0 ± 1.8	149.2 ± 2.5	192.8 ± 4.4	238.9 ± 4.5
EtOH +IGF	10	34.2 ± 1.1	47.5 ± 1.4	74.4 ± 1.5	107.6 ± 2.0	147.6 ± 2.7	194.7 ± 2.4	241.3 ± 3.2
SHAM + VEH	9	38.4 ± 2.1	52.9 ± 1.6	83.0 ± 2.3	117.2 ± 3.1	158.8 ± 4.5	205.3 ± 5.6	251.4 ± 6.2
SHAM + IGF	9	36.9 ± 1.2	51.1 ± 1.6	81.3 ± 1.4	115.8 ± 1.8	159.0 ± 2.2	207.2 ± 3.0	252.6 ± 2.8
Female								
EtOH + VEH	10	34.3 ± 1.5	47.8 ± 2.1	72.5 ± 2.2	101.3 ± 2.9	130.4 ± 3.7	160.2 ± 4.8	185.7 ± 5.1
EtOH +IGF	11	33.6 ± 1.0	46.5 ± 1.4	70.1 ± 1.5	98.6 ± 1.6	126.8 ± 1.9	157.7 ± 2.5	184.4 ± 2.6
SHAM + VEH	9	37.5 ± 1.1	50.4 ± 1.2	75.5 ± 1.2	104.1 ± 1.9	132.9 ± 2.6	161.1 ± 3.8	182.1 ± 4.3
SHAM + IGF	9	37.2 ± 1.0	50.7 ± 1.6	74.6 ± 3.6	107.8 ± 2.3	138.9 ± 3.9	170.6 ± 6.2	195.2 ± 8.0