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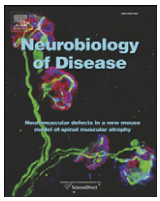

# Alcohol-induced neuroapoptosis in the fetal macaque brain

Nuri B. Farber *, Catherine E. Creeley, John W. Olney

*Department of Psychiatry, Washington University, St. Louis, MO, USA*

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ABSTRACT

The ability of brief exposure to alcohol to cause widespread neuroapoptosis in the developing rodent brain and subsequent long-term neurocognitive deficits has been proposed as a mechanism underlying the neurobehavioral deficits seen in fetal alcohol spectrum disorder (FASD). It is unknown whether brief exposure to alcohol causes apoptosis in the fetal primate brain. Pregnant fascicularis macaques at various stages of gestation (G105 to G155) were exposed to alcohol for 8 h, then the fetuses were delivered by caesarian section and their brains perfused with fixative and evaluated for apoptosis. Compared to saline control brains, the ethanol-exposed brains displayed a pattern of neuroapoptosis that was widespread and similar to that caused by alcohol in infant rodent brain. The observed increase in apoptosis was on the order of 60-fold. We propose that the apoptogenic action of alcohol could explain many of the neuropathological changes and long-term neuropsychiatric disturbances associated with human FASD.



## Introduction

Every year in the U.S., approximately 80,000 women binge drink during pregnancy and it is estimated that one of every 100 newborns is afflicted with fetal alcohol spectrum disorder (FASD) (May and Gossage, 2001). Of the women who drink alcohol during pregnancy, those who binge drink have a higher incidence of FASD in the offspring (Bailey et al., 2004; Sayal et al., 2009). Patients with FASD display a variety of neurobehavioral disturbances, including hyperactivity/attention deficit and learning disorders, mental retardation, affective illness, and psychosis (Famy et al., 1998; Streissguth and O'Malley, 2000; Guerri et al., 2009).

*In vivo* rodent models have utilized chronic exposure models. While these models have demonstrated smaller brains and behavioral deficits after long-term exposure, they have been unsuccessful at capturing neurons in the process of dying and thus the neurodegenerative process by which alcohol produces these changes has remained unknown. Recently it has been shown that brief acute exposure to agents that either block *N*-methyl-D-aspartate (NMDA) glutamate receptors or have $GABA_A$ agonist properties and alcohol, which has both of these properties, causes widespread apoptotic neurodegeneration in the developing brain (Ikonomidou et al., 1999, 2000; Bittigau et al., 2002; Olney et al., 2002a,b; Scallet et al., 2004; Fredriksson et al., 2004, 2007; Ma et al., 2007; Cattano et al., 2008; Johnson et al., 2008). The period of peak vulnerability to this apoptogenic action coincides with the brain growth spurt period (Ikonomidou et al., 1999, 2000), which in mice and rats occurs primarily in the first 2 postnatal weeks, but in humans extends from about mid-gestation to several years after birth (Dobbing and Sands, 1979). In infant rodents, a single high dose of alcohol causes widespread apoptosis of neurons in the forebrain, midbrain, brain stem, cerebellum, spinal cord and retina (Ikonomidou et al., 2000; Olney et al., 2002a,b; Tenkova et al., 2003; Young et al., 2003, 2005; Dikranian et al., 2005), and results in long-term learning/memory deficits that persist through adolescence into adulthood (Wozniak et al., 2004). There is no evidence for replacement of missing neurons by neo-neurogenesis (Olney et al., 2002b; Wozniak et al., 2004), and a minimal, but statistically significant, neuroapoptosis response is elicited in the infant mouse brain by a blood alcohol elevation in the range of 50 mg/dl for 45 min (Young and Olney, 2006). Thus, alcohol's neuroapoptogenic action is a candidate mechanism to explain some of the neuropathological changes and neurobehavioral disturbances that characterize FASD (Ikonomidou et al., 2000; Guerri et al., 2009).

Although multiple exposures of non-human primate (NHP) fetuses to alcohol during specific developmental periods cause a reduced number of Purkinje cells in the cerebellum (Bonthius et al., 1996) and neurons in the frontal lobes (Burke et al., 2009), and long-term neurobehavioral disturbances (Clarren et al., 1992; Schneider et al., 2001a,b), there have been no studies designed to determine

* Corresponding author. Department of Psychiatry, Washington University, Campus Box 8134, 660 S. Euclid Ave., St. Louis, MO 63110-1093, USA.
*E-mail address:* farbern@psychiatry.wustl.edu (N.B. Farber).
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whether exposure of the fetal NHP brain to alcohol on a single occasion causes acute neurodegeneration. Therefore, we undertook the present study in which alcohol was administered to pregnant fascicularis macaques by a dosing regimen that maintained a steady state blood alcohol concentration (BAC) for 8 h in a range that a human fetus might experience during a single episode of maternal binge drinking, and examined the fetal brains for evidence of acute apoptotic neurodegeneration.

## Materials and methods

The NHP subjects for this research were supplied by Alpha Genesis Inc., a non-human primate facility in Yamassee, South Carolina. All experimental procedures were conducted at the Alpha Genesis facility under the supervision of one of the authors (N.B.F.) and the Alpha Genesis veterinary staff, and the brains of the fetuses were transported to the authors' laboratories at Washington University for histological evaluation. All aspects of the research were approved by the Institutional Animal Care and Use Committees of Alpha Genesis Inc. and Washington University School of Medicine, and were conducted in full accordance with the PHS Policy on Humane Care and Use of Laboratory Animals. The NHP fetuses used in these experiments were all of the fascicularis (cynomolgus) macaque specie. In the interests of conserving scarce and precious primate resources, this study was designed to obtain a maximal amount of information from as small a number of NHP fetuses as possible. Thus, nine NHP fetuses were used (four saline controls and five exposed to alcohol) and we studied an age-matched control/experimental pair at gestational age 150–155 days (G150–155), and additional pairs at ages G140–145, G130–135 and G120–125. In addition, we studied one experimental fetus in the age range of G105–G110 for which there was no corresponding age-matched control. The full term gestation period for this macaque specie is 160–165 days (Jewett and Dukelow, 1972; Dukelow et al., 1979; Ferre et al., 1985; Tarantal and Hendrickx, 1988).

Pregnant female NHPs at the desired gestational stage received an intravenous injection of alcohol (2.15 g/kg) administered over a 2 min period (time zero), then every hour for 6 h a maintenance dose (0.2 g/kg) was administered to maintain the BAC in the range of 300–400 mg/dl (Kalhorn et al., 1986). This blood level was chosen because it approximates the BAC that a human fetus would be exposed to during a moderately heavy maternal binge drinking episode. In fact, these blood levels are on the low side compared to the findings of Minion et al. (1989) who reported that 204 patients seen in an adult emergency room for alcohol intoxication had an average BAC = 467 mg/dl, and some of them had BACs > 600 mg/dl. Others (Davis and Lipson, 1986) have reported very similar data, and the alcohol literature, in general, documents that individuals who are dependent on alcohol tolerate and crave much higher BACs than alcohol-naive individuals can tolerate (Adams and Victor, 1989).

One hour after the last dose of alcohol (i.e. 8 h from time zero), the mother was anesthetized and the fetus delivered by caesarian section, then immediately euthanized (while still under anesthesia) by perfusion of fixative (4% paraformaldehyde in phosphate buffer). The fetal brains were additionally preserved in the same fixative for one week, then sections were cut 70 μm thick in the transverse plane and stained with antibodies to activated caspase-3 (AC-3; Cell Signaling Technology, Beverly, MA) by methods previously described (Olney et al., 2002a; Tenkova et al., 2003; Young et al., 2003; Dikranian et al., 2005; Young et al., 2005; Young and Olney, 2006). Caspase-3 is the major effector caspase in the CNS and the immunohistochemical detection of activated caspase-3 immunoreactivity has proven to be a sensitive measure of apoptosis (Kuan et al., 2000; Olney et al., 2002a). To corroborate that AC-3-stained profiles were undergoing cell death, we stained additional sections by the de Olmos cupric silver method (de Olmos and Ingram, 1971) that faithfully marks cellular profiles that are in a relatively advanced stage of cell death.

Histological analysis was conducted in a blinded fashion (J.W.O.) beginning with a qualitative evaluation of each age-matched pair to identify brain regions having an exceptionally high density of apoptotic profiles in at least one member of the pair. A quantitative evaluation was then conducted using 3 brain sections randomly selected at approximately 2 mm intervals from the central portion of each identified brain region. The sampling procedure was computer assisted with StereoInvestigator software (Microbrightfield Inc., Williston, VT) so that the total volume of tissue sampled from each region in a given pair of alcohol and control brains was identical. The number of AC3 positive neurons from each region and the volume of tissue sampled were summed for each brain. Dividing the total volume into the total profile count yielded a density count (profiles per $mm^3$) for each alcohol-exposed and each control brain. This provided a quantitative basis for comparing each age-matched alcohol/control pair and also for comparing the total mean density counts for all alcohol-exposed brains combined with the total mean density counts for all control brains combined. Given the substantial amount of growth in fetal brain size during the period of vulnerability, it was felt that comparing the density of AC3 positive neurons would be a more appropriate measure than total number of AC3 positive neurons since a density measure would correct for increases in brain size.

To determine if differences between the experimental and control brains were statistically significant, an unpaired students $t$ test with Welch correction was performed comparing the total mean (± SEM) density counts for the alcohol brains combined ($n = 5$) versus the total mean (± SEM) density counts for the control brains combined ($n = 4$).

## Results

Consistent with prior findings in rodent brain (Dikranian et al., 2001; Dikranian et al., 2005; Olney et al., 2002a; Olney et al., 2002b; Tenkova et al., 2003; Wozniak et al., 2004; Young et al., 2003; Young et al., 2005), immunohistochemical staining with AC3 antibodies proved to be an excellent method for visualizing neurons undergoing apoptosis in the fetal NHP brain. When neurons die by apoptosis they generate large amounts of AC3, which distributes throughout the cell body and dendritic tree, making the entire cell immunoreactive with AC3 antibody. In the early stages, the intact cell body and its arbor are fully displayed in apparent good health, whereas in later stages as the cell deteriorates, the arbor appears fragmented and the cell body becomes shrunken and condensed. Thus, with the AC3 stain, we were able to not only identify the cellular profiles undergoing apoptosis, but could assess whether they were in an early or late stage of degeneration. Using the DeOlmos cupric silver stain, we were able to corroborate that in those brain regions or cell layers where we found an appreciable display of AC3-positive profiles, adjacent sections stained by the silver method also marked a similar number of neurons in the same regional or cell layer location (Fig. 1).

The initial qualitative neuropathological evaluation revealed no differences in the gross appearance of the alcohol-exposed versus the control brains, but at the microscopic level, the differences were striking. Consistently, the alcohol-exposed brains had a high density of AC3-positive profiles in many brain regions, whereas the control brains had no regions where a high density of stained profiles could be found. The stained profiles in the alcohol brains were distributed unevenly, signifying that the pathological process more severely affected some brain regions and certain neuronal populations within a given region. By contrast, in the control brains, stained profiles were lightly scattered in random distribution, and there were very few neuronal populations showing more than an occasional stained profile.

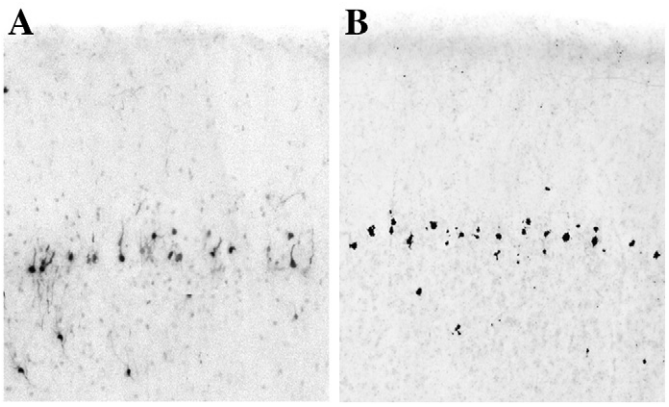


**Fig. 1.** An image from the somatosensory cortex of a G155 NHP fetus exposed *in utero* to alcohol. Panel A was stained with AC3 antibodies and Panel B is an adjacent section stained by the DeOlmos cupric silver method. Note that each stain detects the same population of neurons, which are distributed primarily in a single superficial layer of the neocortex. Other neuronal populations throughout the brain that were selectively AC3-positive were also selectively silver positive. Magnification, 130×.

The alcohol-exposed NHP brains showed an age-related regional distribution of neurodegeneration similar to that we have described in alcohol-exposed infant rodents (Ikonomidou et al., 2000; Olney et al., 2002a; Dikranian et al., 2005). In general, at the younger ages (G105 to G135) certain subcortical structures (caudate/putamen, nucleus accumbens, thalamus, subiculum, entorhinal cortex, inferior colliculus) and the cerebellum were primarily affected, whereas at the older ages (G140 to G155), these subcortical regions and the cerebellum were relatively spared, and various divisions of the cerebral cortex were primarily affected. The cerebrocortical pattern of degeneration that occurred typically at the older ages, tended to have a laminar distribution usually involving superficial cortical layers (Figs. 1 and 2). Some brain regions, such as the anterior cingulate, posterior cingulate, and prefrontal cortices were affected at both the younger and older ages, but the neuronal populations affected within these brain regions at the younger ages were not the same as those affected at the older ages (Fig. 2). The most severe damage observed in this series of brains was in certain regions of the alcohol-exposed younger brains, including the caudate/putamen (Fig. 3), subiculum (Fig. 4), inferior colliculus (Fig. 5), thalamus (Fig. 6) and cerebellum (Fig. 7).

The cerebellum was examined carefully in search for evidence that might help explain the frequently reported loss of Purkinje and granule cells from the developing rodent cerebellum following alcohol exposure. Purkinje cell degeneration was clearly evident following alcohol exposure at G105–110 (Fig. 7A), but not thereafter. The cerebellar cell type most severely affected by alcohol was one of uncertain identity (Fig. 7B) that shifted in location during the developmental period studied. In the youngest brains, this cell type was located in the medullary zone internal to the inner granule layer and in the older brains it was sprinkled throughout the granule cell layer and border zone between this layer and the medullary layer. In a prior study (Dikranian et al., 2005) we observed the same effect of alcohol on the infant rodent cerebellum, and concluded that the highly vulnerable unidentified cell is not a granule cell, but rather is probably a cerebellar cortical interneuron (basket, stellate or Golgi neuron), in that these neurons are known to migrate during this developmental period from the medullary zone through the internal granule cell zone to reach their final destination in the molecular and Purkinje cell zone (Zhang and Goldman, 1996).

Quantitative counts were performed on eight brain regions in each alcohol-exposed brain and on identical regions and identical volumes of tissue in the control brains. Although brain regions affected differed at different ages, the counts were performed on the same brain regions in each age-matched pair of experimental and control brains. The mean (± SEM) density of apoptotic neurons in the alcohol-exposed brains ($5330 \pm 668.40$ profiles per $mm^3$) was 61.8-fold higher than in the control brains ($86.25 \pm 27.75$ profiles per $mm^3$), a difference significant at $p = 0.0014$ (Fig. 8). Although the density count in one alcohol-exposed brain was substantially lower than in the other four, the density count in this brain was, nevertheless, 32.7-fold higher than the mean density count for the control brains.

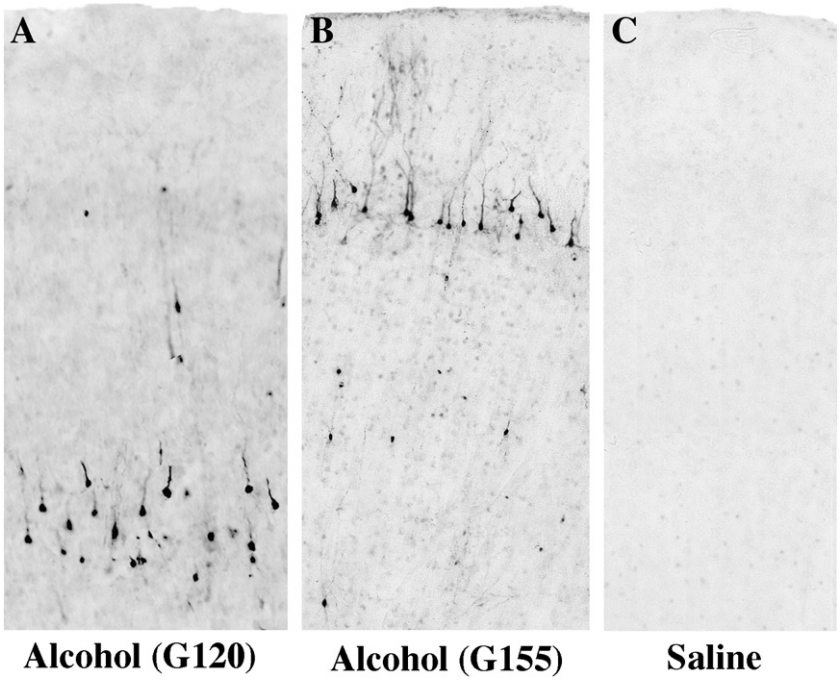


**Fig. 2.** Anterior cingulate cortex of NHP fetuses exposed to alcohol (Panels A and B) or saline (Panel C) at G120 (Panel A) or G155 (Panels B and C) and stained by the de Olmos cupric silver method. Following alcohol exposure at G120 (Panel A), apoptotic neurons are concentrated in deeper layers, whereas after alcohol at G155 (Panel B), apoptotic neurons are concentrated more superficially. Controls exposed to saline at either age had rare or no apoptotic neurons in either layer (Panel C). Magnifications, 150× (Panel A) and 130× (Panels B and C).

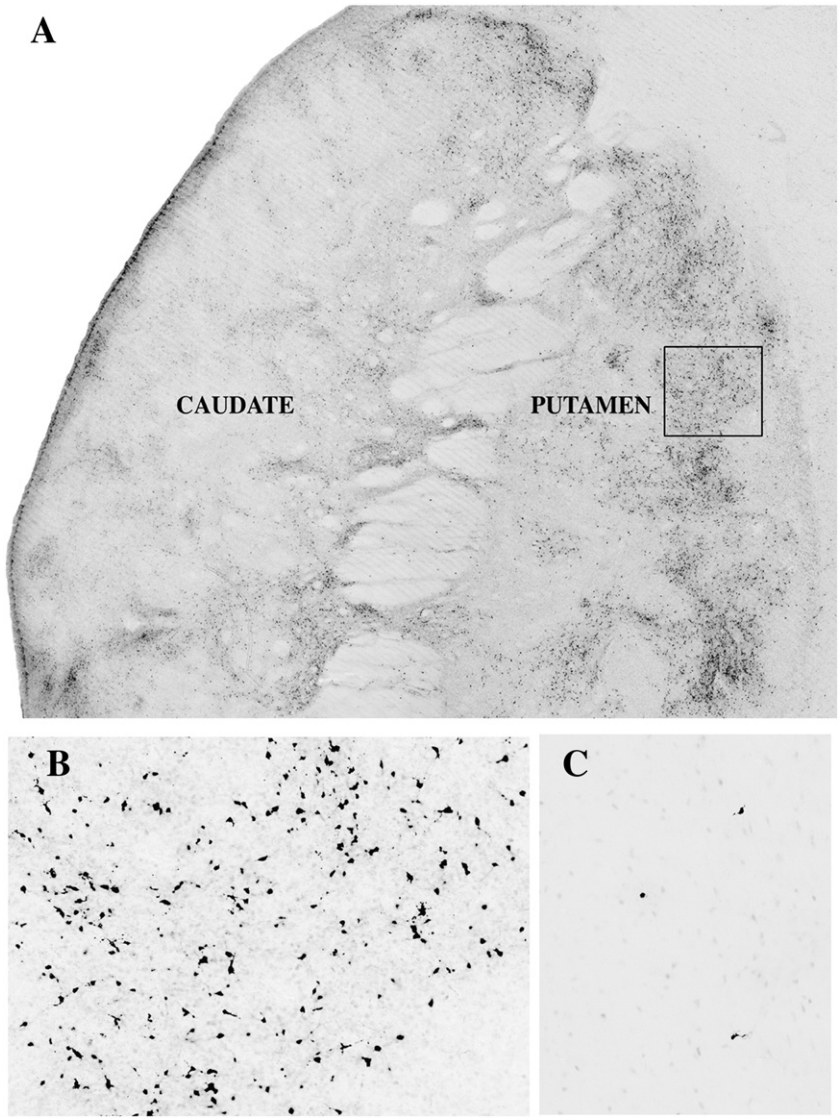


**Fig. 3.** Panel A is a section through the caudate–putamen from a G120 fetal NHP brain following alcohol exposure showing a dense pattern of neuroapoptosis (AC3-positive staining) in both regions, especially putamen. Each tiny dark speck is a degenerating neuron. Panel B is from the boxed region in A showing apoptotic putamen neurons at higher magnification. Panel C is from the putamen of a G120 control brain demonstrating that the physiological cell death rate under normal circumstances is very low. Caudate–putamen neurons respond very early to alcohol and by 8 h the stained cells have a shrunken and irregular shape reflecting deteriorative changes. The pattern of degeneration in the NHP caudate–putamen following alcohol treatment is remarkably similar to that which occurs in the infant mouse caudate–putamen following alcohol treatment (Olney et al., 2002b, Fig. 3). Magnifications, 10× (Panel A) and 55× (Panels B and C).

## Discussion

Here we have demonstrated that during the third trimester of pregnancy a single binge-like episode of maternal alcohol exposure, which should produce an ethanol level in the range of 300–400 mg/dl, triggers widespread neuroapoptosis in the developing NHP fetal brain. Consistent with recent findings in rodents, numerous populations of neurons were affected at each developmental age studied, and the populations affected at the earlier ages were different from the populations affected at later ages. Brain regions preferentially involved at one stage or another in the third trimester fetal NHP brain were very similar to those involved at comparable stages of development in the postnatal rodent brain. Brain regions preferentially affected in both rodent and NHP brain include all divisions of the limbic and neocortex, the striatum, nucleus accumbens, thalamus, subiculum, ventral pallidum, entorhinal cortex, inferior colliculus and cerebellum. Despite age-specific differences in neuronal populations involved, the magnitude of the neuroapoptosis response was similar at each age, and was demonstrated by quantitative counts to be in the range of a 60-fold increase compared to the rate of spontaneous neuroapoptosis in age-matched control brains. Given the rather large increase in apoptosis produced by this moderately high dose of ethanol, it is likely that much lower levels of ethanol would also produce apoptosis. In the rodent alcohol produces apoptosis in a dose responsive fashion and at levels as low as 50 mg/dl (Young and Olney, 2006). While the study can be criticized for not using a large number of animals at several developmental ages and at several different doses of ethanol, we do not feel that such a study would be consistent with the need to conserve and use non-human primates in only a limited manner. Instead such detailed studies should be carried out in rodents.

Exposure of the human fetus to alcohol has been shown in several neuroimaging studies (Mattson et al., 1996; Archibald et al., 2001; Riley and McGee, 2005) to cause a reduced mass of the caudate–putamen, often referred to as the striatum, but when or how this damage occurs is poorly understood. It seems likely that injury to this brain region could be explained by the apoptogenic action of alcohol in that we found the caudate–putamen in the alcohol-exposed NHP


**Fig. 4.** AC3-stained section from the subiculum of the fetal NHP brain in early gestation (G120) following exposure for 8 h to saline or alcohol. In the saline-treated brain there are very few AC3-positive neuronal profiles (none in this scene), whereas in the alcohol-treated brain such profiles are abundant. We judge these cells to be in an early stage of degeneration based on their display of a relatively intact and normal-appearing microanatomy. This pattern of degeneration in the NHP subiculum shows a strong resemblance to that we have demonstrated (Olney et al., 2002a,b, Fig. 3) in the infant mouse subiculum following alcohol treatment. Magnification, 75×.

brain to be one of the regions that is most severely damaged. Our findings indicate that sensitivity of caudate–putamen neurons to the apoptogenic action of alcohol is greatest in the G105 to G130 period, but vulnerability continues until approximately G145.

Following an early report by Bauer-Moffett and Altman (1977) that alcohol exposure of infant rats caused a reduction in the number of Purkinje and granule cells in the cerebellum, this brain region became a major focus of interest among fetal alcohol researchers. In the ensuing two decades, these cell losses were confirmed (Bonthius and West, 1990; Goodlett et al., 1990; Bonthius et al., 1996; Thomas et al., 1998), but no new insights were gained regarding the

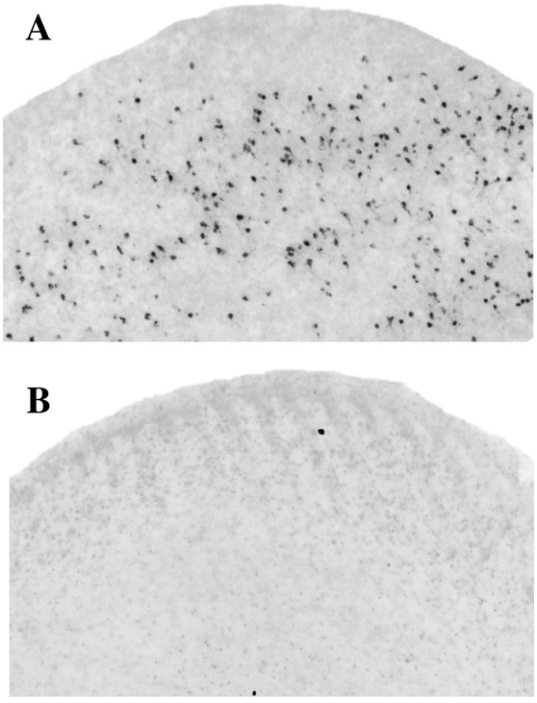


**Fig. 5.** AC3-stained sections from the inferior colliculus of the NHP fetal brain at G130-135 following alcohol (Panel A) or saline (Panel B). Similar to what is seen in the superior colliculus (Tenkova et al., 2003), inferior collicular neurons are relatively more sensitive than most neuronal populations and begin degenerating early. Therefore, by 8 h immunoreactivity is receding and is primarily confined to the cell body. There is an abundance of AC3-positive profiles in the alcohol-exposed inferior colliculus and very few such profiles following saline. Magnification, 100×.

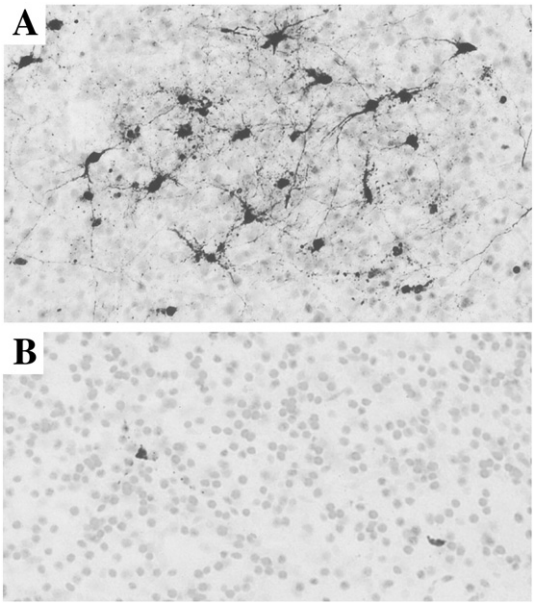


**Fig. 6.** AC3-stained sections from the anterdorsal (AD) nucleus of the thalamus of the fetal NHP brain (gestation age 130–135 days) following alcohol (A) or saline (B). In the section from the saline-treated animal there is one or possibly two faintly positive profile(s), whereas in the section from the alcohol-treated animal there are approximately 35 intensely immunoreactive profiles. The dendritic processes of the degenerating thalamic neurons (Panel A) are intensely AC3-positive, signifying that these neurons are in a relatively early stage of degeneration. The particulate debris in the vicinity of the degenerating dendrites signifies that they are beginning to deteriorate and become fragmented. As we have demonstrated previously (Olney et al., 2002b), Fig. 5), alcohol has a similar predilection for damaging the AD thalamic nucleus in the infant mouse brain. Magnification, 180×.

mechanism of alcohol's deleterious action on cerebellar neurons (West, 1993). It was of interest, therefore, to carefully evaluate the acute effects of alcohol on NHP cerebellar neurons in the present study. We found evidence for apoptosis of Purkinje neurons (Fig. 7), but only following alcohol exposure at G105–110, the most likely interpretation being that alcohol does cause NHP Purkinje neurons to undergo acute apoptosis, but the susceptibility period is prior to G120. This is consistent with our observations (Dikranian et al., 2005) in the infant mouse brain that peak sensitivity of Purkinje neurons to alcohol's apoptogenic action occurs during an early and relatively narrow time window between postnatal days 2 and 5. Similar findings have been reported for the developing rat brain (Hamre and West, 1993; Light et al., 2002). We also found in the infant mouse cerebellum that alcohol's most prominent apoptogenic action was directed toward an unidentified cell type that was located near the inner border of the internal granule cell zone, and seemed to be migrating into or through that zone, but we could not identify the susceptible cell type as a granule cell because immunohistochemical staining revealed that it did not have the same immunoreactive properties as other cells in the internal granule cell zone. In the present NHP study a cerebellar cell type having the same appearance and following a similar migratory path was especially sensitive. Tentatively, we believe these cells are cerebellar cortical interneurons (basket, stellate and Golgi neurons) based on evidence (Zhang and Goldman, 1996) that these neurons in abundant numbers migrate from the white matter through the granule cell zone during this developmental period. Our findings in both rodent and NHP brain are consistent with an interpretation originally advanced by Bauer-Moffett and Altman (1977) that alcohol exerts a direct toxic action against Purkinje cells but not against granule cells. If this interpretation is correct, it calls into question the widely accepted practice of using *in vitro* cultures of

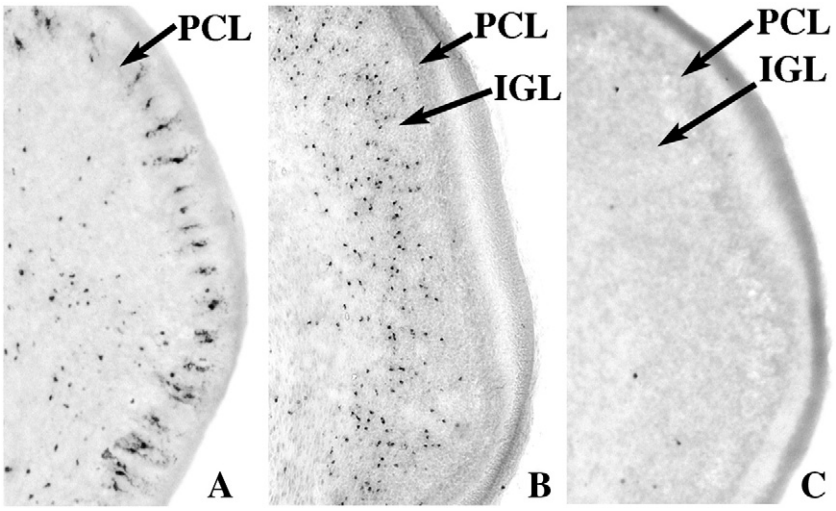


**Fig. 7.** Alcohol-induced neurodegeneration in the fetal NHP cerebellum following alcohol exposure at G105-110 (Panel A) or G120 (Panel B). Panel C is from a control animal. AC3 staining. PCL = Purkinje cell layer; IGL = internal granule layer. Magnification, 40×.

cerebellar granule cells to study the neurotoxic effects of alcohol on the developing brain.

It is interesting to consider to what extent the neuroapoptogenic action of alcohol that we are describing can explain the neuropsychiatric disturbances associated with FASD. Learning and neurocognitive disturbances are often associated with hippocampal damage, and we did not see striking evidence of hippocampal damage in the alcohol-exposed NHP brains. However, in rodents we have observed that the period of peak hippocampal sensitivity to alcohol is very early, at approximately postnatal days 1 and 2, which would be the equivalent of perhaps G100 or earlier for the fetal NHP brain. Therefore, our findings do not rule out a substantial impact on the hippocampus at this earlier age. Alternatively, in both the rodent and NHP brain we have observed that certain brain regions, including the subiculum, retrosplenial/posterior cingulate cortex and anterior thalamic nuclei, are severely damaged by alcohol and these brain regions have been identified as primary components of an extrahippocampal circuit that has been implicated in learning/memory disturbances (Aggleton and Brown, 1999). An additional mechanism that might explain such disturbances is suggested by the findings of Klintsova et al. (2007) that alcohol treatment of infant rodents impairs the neurogenesis process by which new neurons are formed throughout life in the dentate hippocampal gyrus. The present study was not designed to explore this mechanism and, to the best of our knowledge, it has not been explored in any other NHP studies.

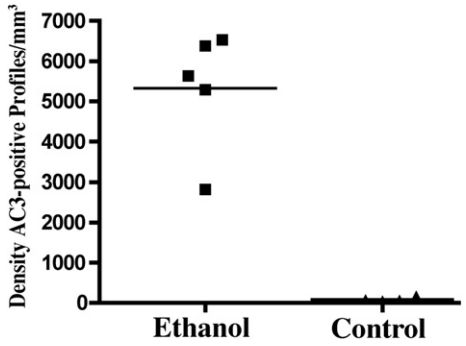


**Fig. 8.** Density of AC3-positive profiles in 5 NHP fetal brains exposed to alcohol compared to 4 NHP control brains. The mean (± SEM) density for the alcohol brains (5330 ± 668.40 profiles per $mm^3$) was 61.8-fold higher than in the control brains (86.25 ± 27.75 profiles per $mm^3$), a difference significant at $p = 0.0014$.

Our finding that alcohol causes widespread neuronal degeneration at any age during the third trimester provides a potential explanation for some of the myriad neurobehavioral disturbances typically seen in FASD patients, especially if multiple binge drinking episodes occurred during the third trimester. Elsewhere (Olney, 2004) we have discussed the likelihood that certain CNS neuronal populations may be sensitive to the apoptogenic action of alcohol as early as the beginning of the second trimester. However, an apoptogenic action of alcohol on post-mitotic fully differentiated neurons cannot explain the finding by Clarren et al. (1992) that weekly alcohol exposure of NHP fetuses during the first trimester of gestation caused neurobehavioral abnormalities. A likely explanation for such early effects is suggested by the findings of Sulik and colleagues (Dunty et al., 2001) that *in utero* exposure of mouse embryos to alcohol as early as embryonic days 7 and 8 can cause apoptosis of CNS progenitor cells, and a reduced size of the forebrain. Similar research in which early NHP embryos are exposed to alcohol, then evaluated for acute apoptotic degeneration of CNS progenitor elements, would help close an important information gap and provide a more complete understanding of FASD neurodevelopmental disabilities.

## Disclosure statement

Dr. Farber was previously on the speaker's bureau of Janssen Pharmaceutica for which he gave talks on antipsychotic medications. This last occurred in 2005. Compensation was not greater than $10,000 per year.

Dr. Farber gave an educational talk to representatives of Astra Zeneca in 2007 about schizophrenia. He received an honorarium for this talk on his university paycheck.

Dr. Farber provided consultation to Neuronetics about Transcranial Magnetic Stimulation in 2008 for which he received an honorarium.

Dr. Farber holds several patents concerning ways of blocking damage in the adult CNS by NMDA glutamate antagonists.

Dr. Creeley has no financial conflicts to declare.

Dr. Olney is named on a pending patent application pertaining to neuroprotection of the developing brain.

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