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Number of Axons in the Corpus Callosum of the Mature Macaca Nemestrina: Increases Caused by Prenatal Exposure to Ethanol

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ABSTRACT

The effects of prenatal exposure to ethanol on the number of callosal axons were examined. Pregnant *Macaca nemestrina* were treated with ethanol (1.8 g/kg b.wt.) 1 day per week during the first 6 weeks (Et6) or full 24 weeks (Et24) of gestation. Control macaques were intubated with an isocaloric amount of sucrose water (Ct). The mid-sagittal area of the corpus callosum in 4- to 5-year-old offspring was examined in magnetic resonance (MR) images and in fixed brains. The number of callosal axons was determined by using electron microscopy. In both MR images and fixed brains of macaques treated with ethanol, the corpus callosum was 26% larger than in the controls. The rostral portion was particularly affected by ethanol; it was 55% larger in Et6- and Et24-treated macaques. Axonal size and myelin thickness were unaffected by ethanol, but ethanol-treated macaques had more callosal axons (13.7×10^7) than did controls (9.4×10^7 axons). The increase in the rostral corpus callosum suggests that parietal and frontal cortices are particularly susceptible to ethanol. The altered callosal connectivity may be a component of the structural abnormalities that underlie executive processing problems associated with fetal alcohol syndrome. J. Comp. Neurol. 412:123–131, 1999.

Indexing terms: alcohol; axonal pruning; callosal axons; fetal alcohol syndrome; microencephaly; prefrontal cortex

One common manifestation of fetal alcohol syndrome (FAS) and models of FAS is microencephaly (e.g., Jones et al., 1973; Randall et al., 1977; Clarren et al., 1978; Miller, 1988a, 1996a). This reduced size is reflected in the numbers of neurons in the neocortex (Miller and Potempa, 1990; Mooney, 1997). For example, the total numbers of neurons in the primary somatosensory cortices of ethanol-treated rats are 33% fewer than those in control rats. A similar reduction is evident in each cortical layer and in the numbers of glia in each layer. A decreased size of brain is not inevitable in alcohol teratogenesis.

Despite generalized reductions in cell number, the brains of ethanol-treated rats are not miniaturized brains. For example, ethanol exposure affects the mix of neurons in each cortical layer. Ethanol-treated brains commonly have ectopic neurons, i.e., neurons with a specific phenotype that are located at inappropriate sites (e.g., Clarren et al., 1978; Miller, 1986a, 1988a, 1993, 1997; Miller et al., 1990). The connectivity of cortical neurons is also affected by ethanol exposure. The scope of dendritic trees (Shapiro et al., 1984; Pentney et al., 1984; Fabregues et al., 1985; Miller et al., 1990), the morphology of dendritic spines

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TABLE 1. Subjects[1]

Subjects	Controls (n = 6)	Ethanol treatment: 6 weeks (n = 5)	Ethanol treatment: 24 weeks (n = 4)
34/LP*	x		
35/OH*	x		
36/SB*	x		
37/RS*	x		
38/TU	x		
40/DX*	x		
48/KX		x	
49/RG*		x	
50/SA*		x	
51/SC*		x	
52/SR*		x	
55/SH*			x
56/SI			x
58/EY*			x
60/KH*			x

[1]Three principal groups of macaques were examined: those treated with ethanol once per week for 6 weeks or for 24 weeks and controls (treated with sucrose and water once per week). Subjects scanned for magnetic resonance images are noted by asterisks.

(Stoltenburg-Didinger and Spohr, 1983; Miller et al., 1990), the density of cortical efferents (Miller and Al-Rabiai, 1994), and cortical synaptology (Al-Rabiai and Miller, 1989) are altered.

One of the major cortical pathways is the system of interhemispheric connections, i.e., the callosal system. The callosal system is responsible for giving the subject a unified, seamless image of the sensory world. Various reports show that a small number of children with severe FAS exhibit agenesis or dysgenesis of the corpus callosum (e.g., Clarren et al., 1978; Peifer et al., 1979; Pratt and Doshi, 1984; Schaefer et al., 1991; Riley et al., 1995; Swayze et al., 1997; Roebuck et al., 1998). It has been estimated that 6.8% of children with FAS have callosal defects as compared with 0.3% of the general population and 2.3% of those afflicted with developmental disabilities. In one sample, however, as many as 6 of 10 children with FAS exhibited partial or full callosal agenesis (Swayze et al., 1997).

Many data from animal studies support the clinical findings of callosal defects. For example, immature, ethanol-treated rodents have smaller corpora callosa than do controls (Wainwright and Gagnon, 1985; Wainwright and Fritz, 1985; Zimmerberg and Scalzi, 1989). Interestingly, these reductions are absent in mature animals suggesting that the corpus callosum is vulnerable to an ethanol-induced developmental delay. Recent evidence suggest that this delay is followed by an overshoot and that mature rats prenatally exposed to ethanol have more callosal projection neurons than do controls (Miller, 1997). To examine the apparent discrepancy between the human and experimental animal findings, we examined the corpora callosa of non-human primates that were treated with ethanol prenatally.

MATERIALS AND METHODS

Subjects

The subjects were pigtail macaques (*Macaca nemestrina*). These were a subset of the animals used in previous studies (Clarren and Astley, 1992; Clarren et al., 1992; Astley et al., 1995). The macaques that had been exposed for 3 weeks were not included in the present study because only one survived to 4–5 years of age. Brain tissue was obtained from 15 animals and magnetic resonance images were taken from 12 of these macaques (Table 1).

Care and feeding

The primates were clustered into three groups; two groups were exposed to ethanol prenatally and the third group was a control group. Care and handling of the animals was in accordance with institutional guidelines. Details about the care and feeding of the macaques have been described (Clarren and Astley, 1992; Clarren et al., 1992).

The dams of the ethanol-treated groups received a single dose of ethanol (1.8 g/kg b.wt.; Et) once a week for 6 weeks (Et6; i.e., the first quarter of gestation) or for 24 weeks (Et24; i.e., throughout the pregnancy). The Et6-fed animals were fed a sucrose solution (that was isocaloric/isovolumetric to the ethanol dose) once per week for the last 18 weeks of the pregnancy (i.e., from prenatal weeks 7 to 24). The dams of the control-treated macaques were given the sucrose solution (Ct) one day in each week of gestation.

All pregnant macaques were fed chow and water ad libitum. Nonhuman primates were consistently provided their aliquots of the Et or Ct on the same day of the week; for example, on gestational day (G) 3, G10, G17. . . , or on G5, G12, G19. . . In the cadre of nine Et-treated macaques used in the present study, the dosing was initiated on G3, G5, G6, or G7. On the eve of a dosing day (at 5:00 PM), all chow and water was removed. In the morning, the pregnant macaques were weighed and at 8:00 AM the animals were dosed. All pregnant animals were administered with the Et or Ct over a 5-minute period by means of soft nasogastric tubes. Chow and water was reintroduced at 5:00 PM.

Blood ethanol concentrations (BEC) were determined from each mother (Clarren et al., 1992). Samples of blood (1.0 ml) were taken 40–400 minutes post-dosing and BEC was measured with gas chromatography. Based on previous studies (Clarren and Astley, 1992; Clarren et al., 1992), peak BEC was attained 100 minutes after the Et was administered. At this time, the Et6- and Et24-treated macaques had mean BECs of 231 ± 18 mg/dl and 234 ± 14 mg/dl, respectively.

Magnetic resonance imaging

Animals lived in a complex community structure and were routinely subjected to psychometric tests (the outcome of some of these tests have been published elsewhere; Clarren et al., 1992). When the macaques were 3–5 years old, 12 of them (see Table 1) were sedated with an intramuscular injection of ketamine and xylazine (10 mg/kg b.wt.).

The procedures for obtaining the magnetic resonance (MR) images were the same as those described by Astley and colleagues (1995). Briefly, the macaques were placed in a prone position and aligned with the Frankfort horizontal plane perpendicular to the horizontal axis of a General Electric Signa whole body MR Scanner. The heads were placed inside a knee coil to accommodate the small size of a macaque head. A standard cranial series was obtained including T1-weighted images in the mid-sagittal plane (repetition time of 400–500 msec and echo time of 12 msec) were taken. A 16-cm field of view was used with a slice thickness of 3–4 mm. The images were analyzed with a Bioquant Image Analysis System, R & M Biometrics, Nashville, TN) to determine the cross-sectional area of the corpus callosum.

Tissue preparation

At the completion of the original study (Clarren et al., 1992), the 15 surviving macaques (the 12 used in the MR imaging study plus an additional one per treatment group; see Table 1) were euthanized for neuroanatomic evaluation. The macaques were 3.56–5.32 years old. Then, brain samples were prepared for electron microscopy by standard protocols. Briefly, animals were anesthetized with ketamine and xylazine and then killed by a three-stage intracardial perfusion. Each macaque was perfused with 200–300 ml of 0.10 M phosphate buffer (pH 7.4) in saline (PBS), 2–3 liter of 4.0% paraformaldehyde in 0.10 M phosphate buffer, and finally with 1 liter of 10% sucrose in PBS. The brain was removed from the cranium and stored in buffered 30% sucrose.

The brainstem and cerebellum were removed and the forebrain was hemisected. The wet weight of each whole brain was measured. The medial surface of the brain was imaged with the Bioquant System and the cross-sectional area of the corpus callosum was determined.

Electron microscopy

Samples from three sites in the corpus callosum (from the right hemisphere) were removed: the genu, body, and splenium. These areas interconnect the prefrontal cortex, primary and secondary somatosensory cortices, and primary and secondary visual cortices, respectively (LaMantia and Rakic, 1990a). Each block measured 1 mm high by 2 mm wide by 1 mm deep. The blocks were dehydrated, fumigated, and embedded in plastic. A series of ultrathin sections was taken from each block. Every eighth to tenth section was used in the analysis. These sections were cut in a sagittal plane to generate cross-sections of the corpus callosum.

A micrograph was taken of 5 non-overlapping fields (15μm × 15μm) in each of five representative sections per animal. Thus, a total of 25 micrographs was made for each callosal site for each animal. Three measures describing the composition of the corpus callosum in each field were taken by using the Bioquant System. (1) The size of each axon in the field was measured. This constituted the area within the outer aspect of the axolemma. (2) The thickness of the myelin was determined. Three independent measures from each myelin profile were examined and the mean was taken as the value for that axon. Each measurement was made at a site where the laminations were regular and parallel. (3) The number of axons included in each field was counted. Only axonal profiles that were fully within the defined area or intersected two of four sides of the measuring box (10μm x 10μm) were included in the tallies.

Analysis

In all phases of the study, the investigators were blind to the source of the sample. The mean values for the five non-overlapping fields per section were calculated. In turn, the mean (± the standard error of the mean) for the five sections from each callosal segment (i.e., the genu, body, and splenium) was determined. The latter mean was considered representative of the callosal segment that was used in the statistical analyses. Analyses of variance were used to assess differences among locations within the corpus callosum by prenatal treatment group. In cases where significant differences were detected, post-hoc Student-Newman-Keuls tests were performed.

TABLE 2. Effect of the Duration of Ethanol Exposure on Body and Brain Weight[1]

Parameter	Ct (n = 6)	Et6 (n = 5)	Et24 (n = 4)
Age (yr)	4.80 ± 0.18	4.95 ± 0.09	4.31 ± 0.32
Body weight (kg)	5.3 ± 0.8	6.9 ± 0.5	5.2 ± 0.6
Brain weight (g)	85.2 ± 8.7	88.3 ± 4.0	87.6 ± 4.8

[1]The age, body weight, and brain weight at sacrifice are given for macaques in the five treatment groups. The offspring of macaques were fed a sucrose control (Ct), fed ethanol once per week for 6 weeks (Et6), or fed ethanol once a week for 24 weeks (Et24). Each value is a mean (± the standard error of the mean).

RESULTS

Body and brain size

Data on the age, body weight, and brain weight of the macaques at sacrifice are provided in Table 2. Mean body weight was significantly ($P < 0.05$) greater in the Et6-treated animals than in the Ct- and Et24-treated macaques. The brain weight was unaffected by the prenatal exposure to ethanol.

Size of the corpus callosum

Magnetic resonance images of the living brain. The corpus callosum was measured in mid-sagittal MR images of 12 macaques (Fig. 1; Table 1). The mid-sagittal area of the corpus callosum was significantly ($F_{2,11} = 6.78$; $P < 0.019$) larger in the animals prenatally exposed to ethanol (Table 3). Student-Newman-Keuls tests show that the corpora callosa of both Et6- and Et24-treated macaques were significantly ($P < 0.05$) larger than those of the Ct-treated macaques. Differences between the two Et-treated groups were not statistically significant.

Planimetry of the corpus callosum in fixed brains. The corpus callosum was measured on the medial surface of each hemisphere of the 15 fixed brains (Fig. 2). No significant differences between the sizes of the sectioned corpora callosa in each hemisphere (i.e., in comparisons between the right and left sides from the same brain) were detected in any treatment group (Table 3). Although this was expected, the small differences (less than 2.0%) indicate that experimental error was inconsequential. The data from the two hemispheres were pooled and grand means (designated as "total" in Table 3) were generated based on animal units. An analysis of variance of these pooled data showed that the corpus callosum was larger in the Et6- (23.6%) and Et24-treated (22.3%) primates than in the controls ($F_{2,14} = 5.25$; $P < 0.023$).

The ethanol-induced increase was not even throughout the rostrocaudal extent of the corpus callosum. The corpus callosum was divided into two portions; the border between the two portions was at the level of the rostral thalamus where the body of the corpus callosum and the body of the fornix diverged. The size of the caudal segment was similar in Ct-, Et6-, and Et24-treated macaques. On the other hand, the rostral segment was significantly ($P < 0.05$) larger in the Et6- (62.6%) and Et-treated (50.3%) macaques than in the controls.

The corpus callosum in the fixed brains was smaller than it was in the MR images. It is interesting to note, that the difference was consistent among all of the treatment groups; about 8% (i.e., fixation ratios of about 0.92; see Table 3). The implications from these data were (1) that the fixation process caused a small amount of shrinkage independent of ethanol treatment and (2) that prenatal treatment did not affect the fixation ratio.

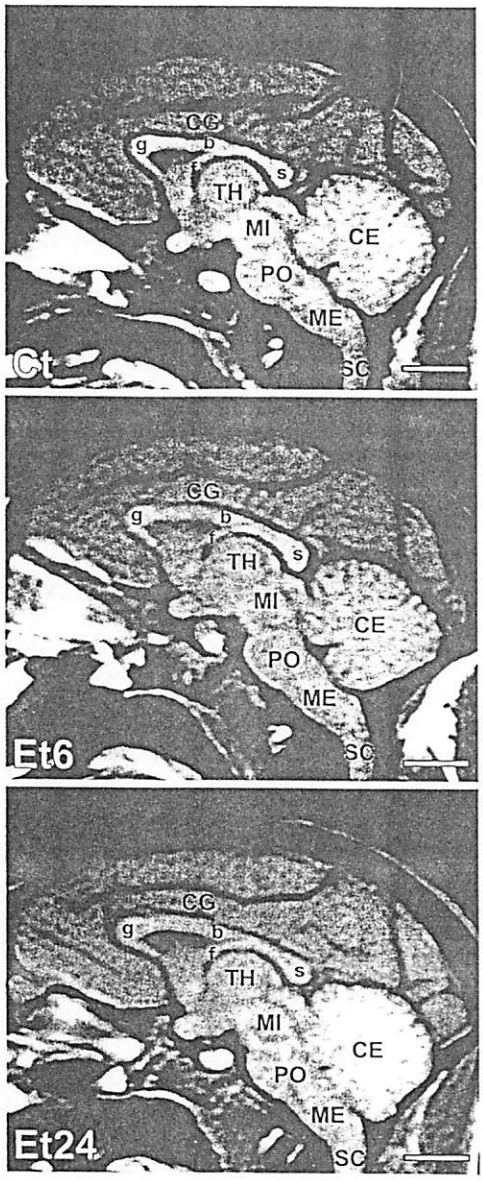


Fig. 1. MR images of ethanol- and control-treated macaques. These MR scans of the mid-sagittal planes of the brains of Ct- (top), Et6- (middle), and Et24-treated (bottom) macaques were taken within 0.5 years of the time of death. CE, cerebellum; CG, cingulate gyrus; b, body of the corpus callosum; f, fornix; g, genu of the corpus callosum; ME, medulla; MI, midbrain; PO, pons; s, splenium of the corpus callosum; SC, spinal cord; TH, thalamus. Scale bars = 1.0 cm.

Composition of the corpus callosum

The cross-sectional area of callosal axons, the thickness of the myelin, and the axonal density were measured in electron micrographs of the corpus callosum (Fig. 3). Each feature was examined at three locations, the genu, body, and splenium of the corpus callosum. Regardless of the treatment group and callosal location, only 10–15% of the callosal axons were non-myelinated.

In the control macaques, axonal size varied among the three locations. Axons in the genu were at best half the size of those at the other two locations (Fig. 4). This difference was statistically significant ($F_{2,4} = 36.2$; $P < 0.001$). A similar pattern was evident in the Et6- and Et24-treated macaques.

TABLE 3. Size of the Corpus Callosum in Magnetic Resonance Images and Fixed Brains[1]

	Ct (n = 6)	Et6 (n = 5)	Et24 (n = 4)
MR images			
Right hemisphere ($\times 10^6$ µm²)	86.2 ± 5.5	112 ± 4*	109 ± 10*
Rostral segment ($\times 10^6$ µm²)	37.4 ± 3.3	60.8 ± 6.3*	56.2 ± 5.2*
Caudal segment ($\times 10^6$ µm²)	48.8 ± 3.9	51.2 ± 4.5	53.0 ± 5.5*
Fixed brains			
Right hemisphere ($\times 10^6$ µm²)	81.1 ± 2.3	102 ± 6*	100 ± 8*
Rostral segment ($\times 10^6$ µm²)	35.2 ± 1.8	54.9 ± 3.6*	52.9 ± 3.2*
Caudal segment ($\times 10^6$ µm²)	45.8 ± 1.1	47.4 ± 2.5	47.0 ± 2.2
Left hemisphere ($\times 10^6$ µm²)	82.3 ± 3.8	100 ± 4*	100 ± 8*
Total ($\times 10^6$ µm²)	81.7 ± 3.5	101 ± 5*	100 ± 8*
Fixation ratio	0.941 ± 0.061	0.911 ± 0.054	0.917 ± 0.059

[1]The rostral and caudal segments were demarcated by the junction/separation of the body of the corpus callosum and the body of the fornix. The total size of the corpus callosum was compiled from the pooled data for the size of the corpus callosum in each hemisphere. The fixation ratio for the corpus callosum in the right hemisphere was calculated as the size of the corpus callosum in the fixed brains to the callosal size in the magnetic resonance images. Ct, sucrose control; Etb, fed ethanol once per week for 6 weeks; Et24, fed ethanol once per week for 24 weeks.
*Asterisks denote statistically significant ($P < 0.05$) differences relative to the size in the Ct-treated macaques.

The mean thickness of the myelin sheaths paralleled the mean size of the axons (Fig. 5). Thus, the axons in the genu had the thinnest myelin and those in the splenium the thickest ($F_{2,4} = 16.1$; $P < 0.001$). At a particular callosal site, no differences among the Ct-, Et6-, and Et24-treated macaques were detected.

The mean axonal density was calculated as the quotient of the mean number of axons counted in a 100 µm² square. An analysis of variance ($F_{2,4} = 64.4$; $P < 0.001$) showed that density was affected by both the treatment group and the location in the corpus callosum. Regardless of the treatment group, the density was greatest in the genu and lowest in the splenium (Fig. 6). The only site where the axonal density was affected by an ethanol treatment was in the body. Here the density was significantly ($P < 0.05$) higher in the Et6- and Et24-treated macaques than in the controls.

An estimate of the total number of axons was calculated as the product of the size of the corpus callosum and the mean axonal density (Fig. 7). The latter value was determined as the mean of the axonal density at the three sites. Accordingly, the Et6- and Et24-treated macaques had significantly more (48.8% and 41.2%, respectively) axons than did the controls ($F_{2,12} = 14.4$; $P < 0.001$). No differences between the two ethanol treatment groups were observed; that is, the duration of the ethanol exposure had no effect.

DISCUSSION

Size and axonal content of the corpus callosum in normal non-human primates

The corpus callosum is the largest commissure in the primate brain. Its cross-sectional area in the *M. nemestrina* is 81.7 mm². This is similar to the size of the corpus callosum in the *M. mulatta* (76.4 mm²d Rakic, 1990a). In both the *M. nemestrina* and the rhesus monkey, axonal density varies with location; the density in the genu is about twice that in the body and the splenium. On the other hand, regardless of location, the present study shows that the density of axons in the corpus callosum of the *M.*

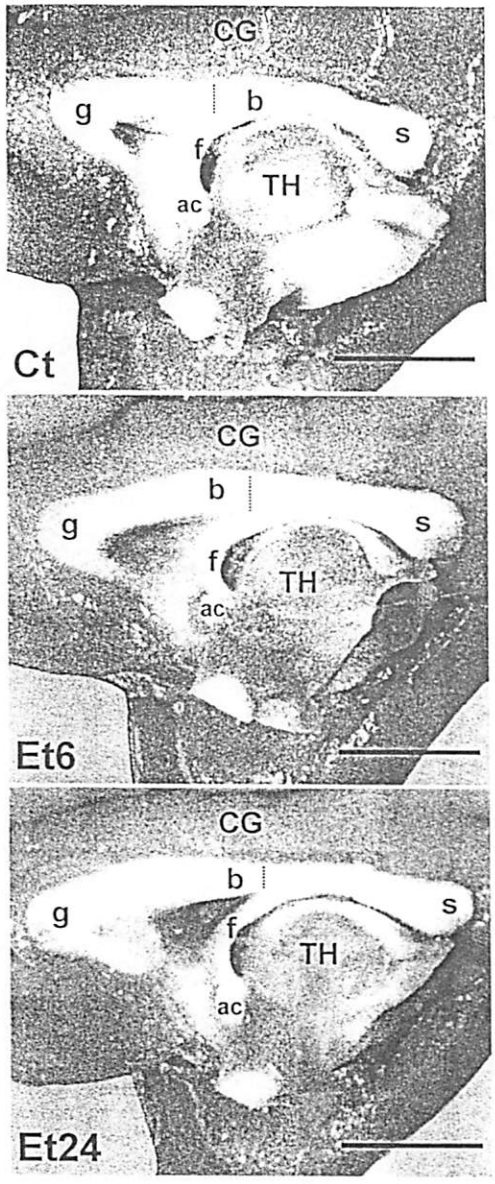


Fig. 2. Corpora callosa of macaques prenatally exposed to ethanol. The medial aspects of the same brains as those depicted in Figure 1 are shown; Ct- (top), Et6- (middle), and Et24-treated (bottom) macaques. The dotted line through the body of the corpus callosum identifies the border between the rostral and caudal segments. ac, anterior commissure; b, body of the corpus callosum; CG, cingulate gyrus; g, genu of the corpus callosum; f, fornix; s, splenium of the corpus callosum; TH, thalamus. Scale bars = 1.0 cm.

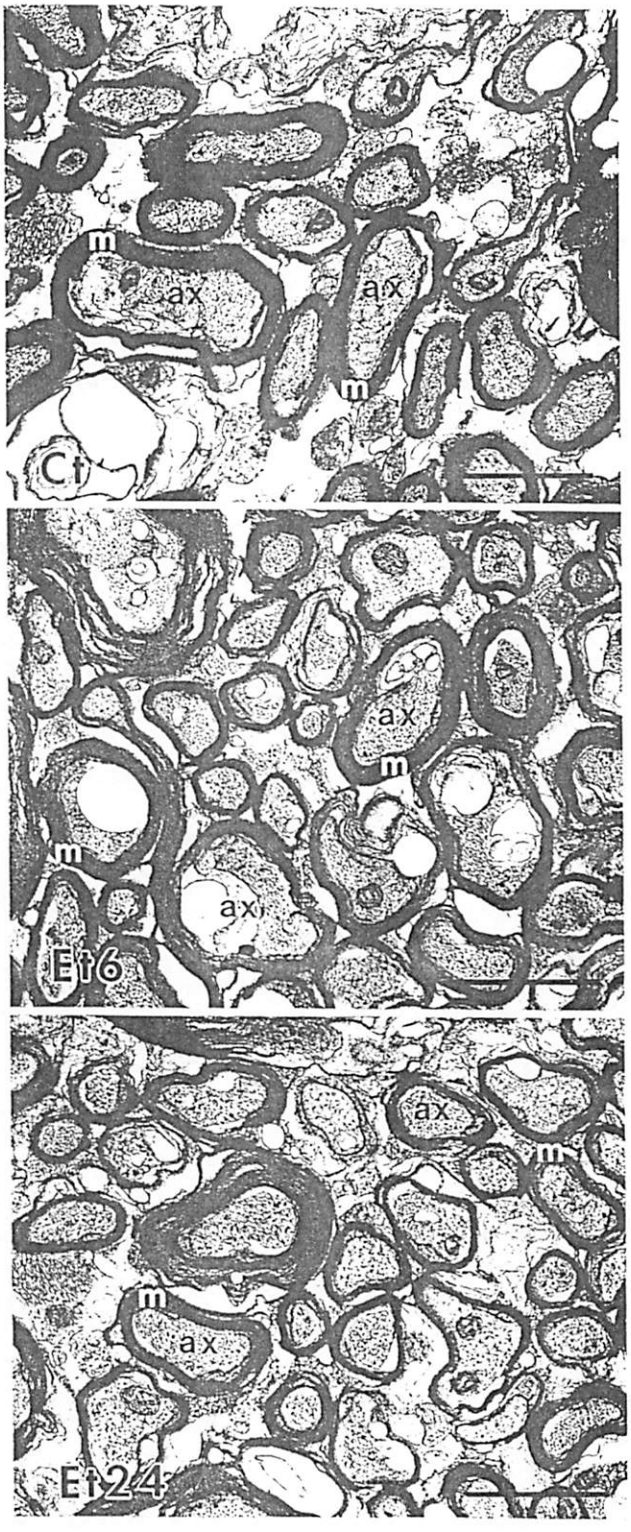


Fig. 3. Ultrastructure of the genu of the corpus callosum. Myelinated axons (ax) are the most common feature of the corpus callosum in Ct- (top), Et6- (middle), and Et24-treated (bottom) macaques. m, myelin. Scale bars = 2.0 μm.

nemestrina (1.18 axons/μm^2 is 40–50% greater than in the rhesus monkey (0.766 axons/μm^2; LaMantia and Rakic, 1990a). The net result is that the estimated number of callosal axons in the *M. nemestrina* (94.2 × 10^6 axons) is 68.2% higher than in the *M. mulatta* (56.0 × 10^6 axons; LaMantia and Rakic, 1990a). It must be kept in mind that the weights of the mature brains of the two macaque species are similar (*cf.* the present results and Holloway and Heilbroner, 1992). The inter-species differences be-

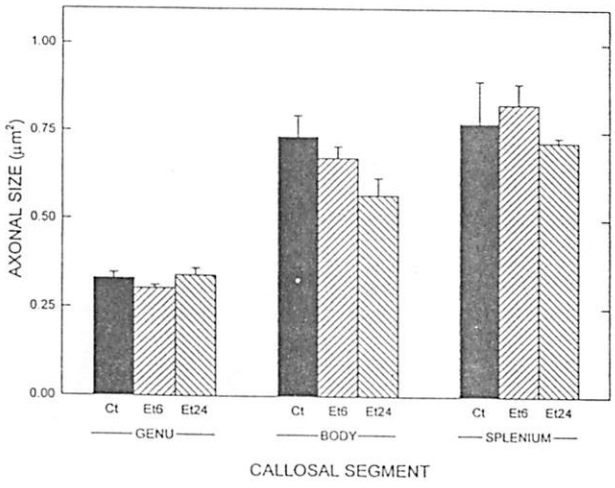


Fig. 4. Axonal size. The mean cross-sectional area of axons in a 100 μm² box (± the standard error of the mean) was plotted for the five treatment groups. A separate mean was calculated for axons in the genu (left), body (middle), and splenium (right) of the corpus callosum. At each callosal site, no differences among treatment groups were detected.

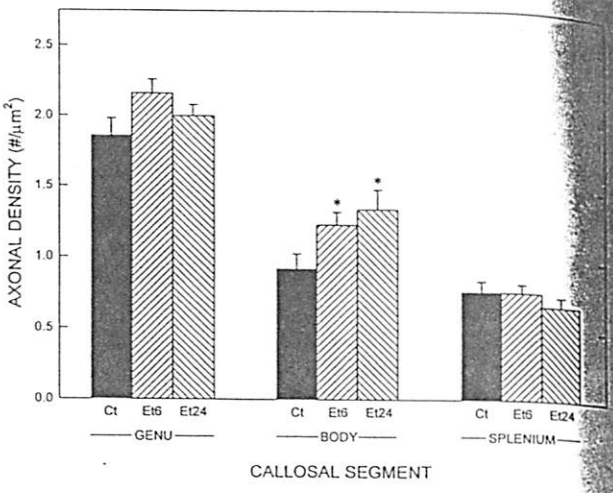


Fig. 6. Axonal density. The packing density of axons varied with the location; the density was greatest rostrally and lowest caudally. Prenatal treatment only had a significant effect ($P < 0.05$; noted by asterisks) on axonal density in the body of the corpus callosum. There, the density of callosal axons was greater in all animals treated with the Et, regardless of the presentation (e.g., Et6 or Et24).

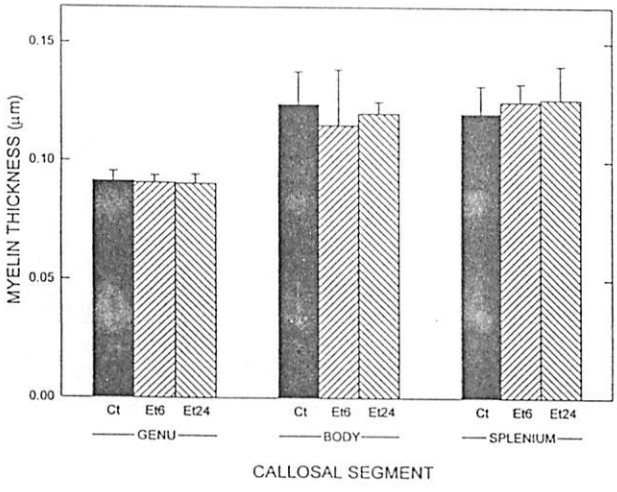


Fig. 5. Myelin thickness. At each callosal site, the mean thickness of the myelin sheath was not significantly different in the Ct-, Et6-, and Et24-treated macaques. T-bars denote the standard errors of the means.

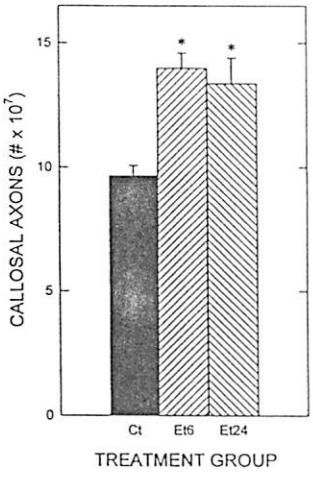


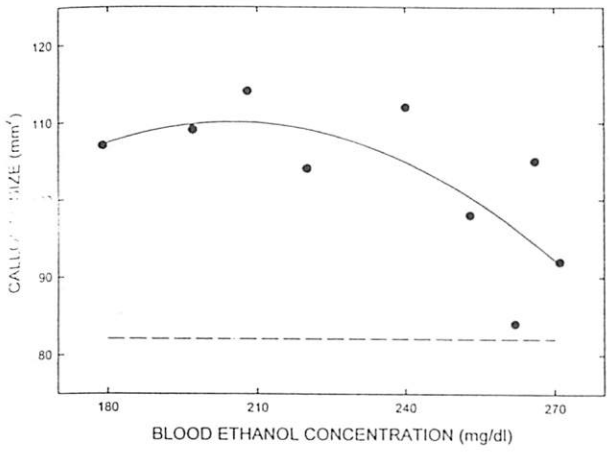
Fig. 7. Total number of axons in the corpus callosum. Regardless of whether the Et was provided over 6 weeks (Et6) or 24 weeks (Et24), the estimated total number of callosal axons was greater in the Et-treated macaques than it was for controls. Statistically significant differences ($P < 0.05$) are noted by asterisks.

tween the *M. nemestrina* and the *M. mulatta* in callosal size are small. After all, despite no change in brain weight, the size of the corpus callosum in the *M. mulatta* is 42.7% larger than cross-sectional area of the corpus callosum in another macaque, the *M. fascicularis* (Holloway and Heilbroner, 1992).

Species differences in ethanol-induced changes in callosal size

Exposure to moderate amounts of ethanol, once per week during gestation for as little as 6 weeks, increases the size of the corpus callosum in the macaque. This ethanol-induced increase in callosal size results from an increase in the number of callosal axons. Furthermore, the increase occurs regardless of how long the fetus is exposed to ethanol (i.e., 6 or 24 weeks).

In addition to the present study, data from rat investigations also show that prenatal exposure to ethanol increases the number of callosal projection neurons (Miller, 1997). Such findings are at variance with the data from various studies of children with severe brain alterations in FAS (e.g., Clarren et al., 1978; Peifer et al., 1979; Pratt and Doshi, 1984; Schaefer et al., 1991; Riley et al., 1995; Swayze et al., 1997). These clinical studies show that such children with severe brain abnormalities have a higher incidence of callosal agenesis or dysgenesis than that for



8. Effects of blood ethanol concentration on callosal size. The ... on the callosal size were plotted against peak blood ethanol concentration for each animal. The solid line depicts the second order curve that best fits the data ($P < 0.05$) and the dashed line describes the mean callosal size in the controls.

the general population. Such clinical data, however, must be interpreted with caution. There is the great risk that by focusing on children with FAS who have the more severe or obvious brain disruptions, the data sample may not be generalizable to all children with fetal alcohol damage. ... clinical studied are biased in that they present data ... severely affected children. The monkeys in the present study evidently are less severely affected because they are not microencephalic. The results of a recent MR imaging study support this contention (Streissguth and Bookstein, personal communication). In this study, children with a wide range in severity of CNS dysfunction in FAS and alcohol-related neurodevelopmental disorder were examined. The range of callosal defects varies widely; in fact, some of the children exhibit enlarged corpora callosa.

One key factor determining the effect of ethanol on ...losal size may be the peak BEC. Ethanol-treated ma...ques and rodents with larger corpora callosa (Miller, 1997; present study) had BECs of <250 mg/dl. In contrast, the mothers of rodents and people exhibiting callosal damage likely had BECs of >250 mg/dl. Interestingly, a curious relationship emerges when the data for the callosal size are plotted against BEC (Fig. 8). Exposure to moderate BEC results in a modest increase in callosal size. The maximal effect is evident with BEC between 205 and 240 mg/dl. At BEC of >250 mg/dl, callosal size falls with BEC. These data should be interpreted with caution, ...wever, because all animals in the present study were ...eated with the same amount of ethanol and peak BEC can vary within an individual animal by 25% or more (Bonthius and West, 1990). Nevertheless, the data are compelling and it is important to note that dose-dependent effects for dopamine neurochemistry in macaques (Astley et al., 1995) and for hippocampal morphology in rats (Miller, 1995) have also been described.

Critical windows of ethanol exposure

The timing of ethanol exposure may have profound ...ratogenic effects. Sulik has shown that mice treated with ...thanol on G7 exhibit the full array of craniofacial malformations that are characteristic of FAS (Sulik, 1984). The brains of these animals also are malformed, particularly midline structures such as the septal nuclei and the corpus callosum (e.g., Sulik and Johnston, 1982; Schambra et al., 1989). The BEC of these mice was extremely high, 500–600 mg/dl. Rats exposed to ethanol through much of the prenatal period (including G8 and G9; the days when gastrulation occurs in the rat) also have callosal damage (Zimmerberg and Scalzi, 1989; Miller, 1997).

It is difficult to draw parallels between the timing of rat and macaque development. If we use the time of gastrulation and the timing of cortical neuronogenesis as bases of comparisons, however, some meaningful conclusions can be drawn. Gastrulation occurs on G8 or G9 in the rat and on G19 or G20 in the macaque. Cortical neurons in macaques are generated between G45 and G102 (Rakic, 1974, 1978) and between G12 and G21 in the rat (Bruckner et al., 1976; Miller, 1988a,b). Thus, based on studies with mice (Sulik, 1984), it can be predicted that macaques exposed to ethanol on G19 or G20, would experience craniofacial and midline brain damage. Data show that the macaques exposed to ethanol on G19 or G20 do, in fact, exhibit craniofacial anomalies (Astley et al., 1999).

The data for the macaque and the rodent are not wholly consistent. The lack of a difference between the Et6- and Et24-treated macaques suggests that brain alterations can result from episodic ethanol exposure before embryonic implantation, but before the period of cortical neuronogenesis. In contrast, cortical damage in the rat may not be caused by such early exposure. For example, the proliferation of cortical progenitors in the rat is unaffected by exposure to ethanol between G6 and G9, whereas exposure between G12 and G15 or between G18 and G21 significantly alters cell proliferation (Miller, 1996b).

Regional specific effects of ethanol

The rostral portion of the macaque corpus callosum is the most affected by prenatal exposure to ethanol. This segment interconnects the frontoparietal lobes in the two hemispheres. In the rat we know that both motor (frontal) and somatosensory (parietal) cortices are profoundly affected by ethanol (e.g., Miller, 1987, 1997; Miller and Potempa, 1990). Although no comparable data are available for the non-human primate, there is reason to suspect that somatosensory cortex in the macaque brain is also targeted by prenatal ethanol treatment.

Evidence implicating the frontal lobe is interesting in light of data from children with FAS. The size of frontal cortex is disproportionately altered in children with FAS (S.N. Mattson, personal communication). By itself, this reduction may be meaningless, but such children also exhibit abnormalities in executive functions (Kodituwakku et al., 1995; Kopera-Frye et al., 1996) and executive functions have been attributed to prefrontal processing (e.g., Eslinger et al., 1992; Bechara et al., 1996, 1998). Thus, the abnormal structure of frontal cortex and likewise the altered numbers of axons in the rostral segment of the corpus callosum may underlie prefrontal dysfunction of executive activities.

Ethanol-induced overgrowth of axons and dendrites

Studies in the rodent show that early exposure to ethanol can promote the growth of axons. Prenatal exposure to ethanol induces an increase (1) in the number of callosal projection neurons in the somatosensory cortex of

the rat (Miller, 1997), (2) in the number of corticospinal projection neurons in somatosensory and motor cortices (Miller, 1987), and (3) in the number of their descending axons in the lower pyramidal tract (Miller and Al-Rabiai, 1994). Recent evidence shows that ethanol treatment promotes axonal growth by cultured hippocampal neurons (Clamp and Lindsley, 1998).

Ethanol-induced overgrowth is not restricted to axonal systems. Prenatal exposure to ethanol also can induce the postnatal hypergrowth of dendrites. Corticospinal projection neurons in somatosensory cortex of Et-treated rats and cerebellar Purkinje neurons have more complex dendritic trees (i.e., more branches) and longer dendrites than do neurons in controls (Pentney et al., 1984; Miller et al., 1990). These data were based on rats that were treated with ethanol from G6 to G21. Similar data were garnered from mice exposed to ethanol only on G10 and G11 (R.F. Mervis, personal communication), that is, early in the period of cortical neuronogenesis (Angevine and Sidman, 1961; Bruckner et al., 1976; Gardette et al., 1982; Miller, 1988a,b).

Callosal (e.g., O'Leary et al., 1981; Ivy and Killackey, 1982; LaMantia and Rakic, 1990b) and corticospinal axons (e.g., Stanfield et al., 1982; O'Leary and Stanfield, 1986) and the dendrites of cortical pyramidal neurons (Miller, 1981, 1986b, 1988b) undergo considerable pruning during early development. We hypothesize that ethanol reduces the pruning process. Various data support this hypothesis. In the developing macaque, the number of callosal axons reaches a maximum of 3.5-fold more than that in the adult (LaMantia and Rakic, 1990b). Et-treated macaques have only 0.45-fold more callosal axons than do controls. This is consistent with the notion that the pruning of callosal axons that normally occurs in all developing macaques is partially blocked by prenatal exposure to ethanol. Further support comes from a study of pyramidal tract axons in developing rats. The number of these axons falls during early postnatal development, but the fall is not as precipitous or profound in Et-treated rats as it is in controls (Miller and Al-Rabiai, unpublished results). The increases in axonal and dendritic fields could be affected (1) by blocking the action of a pruning regulator (e.g., Sato et al., 1994; Frisen et al., 1998) or (2) by facilitating the action of an agent(s) that promotes neuritic growth such as a neurotrophin. Interestingly, ethanol potentiates neurotrophin activity in vitro so that the ethanol-treated cultured neurons elaborate more neurites (Messing et al., 1993; Zou et al., 1993, 1995).

In summary, macaques exposed to moderately high levels of ethanol exhibit increases in the sizes of their corpora callosa and in the numbers of callosal axons. These alterations likely result from interference with substances that regulate normal neuronal growth, e.g., neurotrophins. It is generally assumed that cognitive deficits result from reductions in axonal and/or dendritic fields, however, evidence that ethanol-induced cognitive deficits are caused by an overabundance of axons and dendrites is building.

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