Early Alcohol Exposure Induces Persistent Alteration of Cortical Columnar Organization and Reduced Orientation Selectivity in the Visual Cortex

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Medina, Alexandre E., Thomas E. Krahe, and Ary S. Ramoa. Early alcohol exposure induces persistent alteration of cortical columnar organization and reduced orientation selectivity in the visual cortex. J Neurophysiol 93: 1317–1325, 2005. First published October 13, 2004; doi:10.1152/jn.00714.2004. Fetal alcohol syndrome (FAS) is a major cause of learning and sensory deficits in children. The visual system in particular is markedly affected, with an elevated prevalence of poor visual perceptual skills. Developmental problems involving the neocortex are likely to make a major contribution to some of these abnormalities. Neuronal selectivity to stimulus orientation, a functional property thought to be crucial for normal vision, may be especially vulnerable to alcohol exposure because it starts developing even before eye opening. To address this issue, we examined the effects of early alcohol exposure on development of cortical neuron orientation selectivity and organization of cortical orientation columns. Ferrets were exposed to ethanol starting at postnatal day (P) 10, when the functional properties and connectivity of neocortical neurons start to develop. Alcohol exposure ended at P30, just before eye opening at P32. Following a prolonged alcohol-free period (15–35 days), long-term effects of early alcohol exposure on cortical orientation selectivity were examined at P48–P65, when orientation selectivity in normal ferret cortex has reached a mature state. Optical imaging of intrinsic signals revealed decreased contrast in this group of animals ended at P30, just before eye opening at P32. This timing of alcohol treatment is approximately equivalent to the second half of human gestation and the time when the neocortex starts developing and major changes occur in its anatomical and functional properties (Guerrini 1998). Neuronal selectivity to stimulus orientation, a functional property believed to be crucial for normal vision, may be especially affected since it starts developing during this period and continues developing after eye opening (Chapman and Stryker 1993). Moreover, maturation of orientation selectivity is thought to be regulated by neuronal activity (Weliky and Katz 1997) and to require NMDA receptor function (Ramoa et al. 2001).

To examine whether early alcohol exposure disrupts development of neuronal orientation selectivity and orientation columns in the visual cortex, ferrets were injected with alcohol on alternate days starting at postnatal day (P) 10. Alcohol exposure in this group of animals ended at P30, just before eye opening at P32. This timing of alcohol treatment is approximately equivalent to the second half of human gestation and was selected to coincide with the period when several important developmental processes occur in the neocortex, including the formation of the first synapses between thalamic axons and cortical layer IV neurons (Herrmann et al. 1994), neuronal differentiation, and remodeling of neural connections (Guerrini 1998). To test whether effects of alcohol exposure on orientation selectivity are specific to an early developmental age, we also examined a second group of animals treated for the same duration as the P10 group but exposed to alcohol later in life, between P20 and P40. Exposure of alcohol in this group of animals lasted well beyond the time of eye opening. After a prolonged alcohol-free period, long-term effects of early alcohol exposure on cortical orientation selectivity were determined for this age group.

Activity-dependent neuronal plasticity is thought to play a critical role in the development of the mammalian brain and the visual cortex in particular (Constantine-Paton et al. 1990; Katz and Shatz 1996). Some of the key factors involved in neocortical plasticity include N-methyl-D-aspartate (NMDA) receptors (Bear et al. 1990; Roberts et al. 1998), GABA_A receptors (Hensch et al. 1998), and the transcription factor CREB (Mower et al. 2002). Importantly, alcohol exposure may induce substantial and long-lasting functional alterations of each of these factors (Costa et al. 2000; Hsiao et al. 1999; Llovinger et al. 1989; Pandey et al. 1999, 2001; Rema and Ebner 1999; Yang et al. 1998). Therefore early alcohol exposure may disrupt cortical development by interfering with activity-dependent mechanisms of neuronal plasticity. Alcohol exposure during the second half equivalent to human gestation may have severe effects on central processing of information since this is the time when the neocortex starts developing and major changes occur in its anatomical and functional properties (Guerrini 1998). Neuronal selectivity to stimulus orientation, a functional property believed to be crucial for normal vision, may be especially affected since it starts developing during this period and continues developing after eye opening (Chapman and Stryker 1993). Moreover, maturation of orientation selectivity is thought to be regulated by neuronal activity (Weliky and Katz 1997) and to require NMDA receptor function (Ramoa et al. 2001).

To examine whether early alcohol exposure disrupts development of neuronal orientation selectivity and orientation columns in the visual cortex, ferrets were injected with alcohol on alternate days starting at postnatal day (P) 10. Alcohol exposure in this group of animals ended at P30, just before eye opening at P32. This timing of alcohol treatment is approximately equivalent to the second half of human gestation and was selected to coincide with the period when several important developmental processes occur in the neocortex, including the formation of the first synapses between thalamic axons and cortical layer IV neurons (Herrmann et al. 1994), neuronal differentiation, and remodeling of neural connections (Guerrini 1998). To test whether effects of alcohol exposure on orientation selectivity are specific to an early developmental age, we also examined a second group of animals treated for the same duration as the P10 group but exposed to alcohol later in life, between P20 and P40. Exposure of alcohol in this group of animals lasted well beyond the time of eye opening. After a prolonged alcohol-free period, long-term effects of early alcohol exposure on cortical orientation selectivity were determined for this age group.
mimicking third-trimester ethanol exposure in humans (Bellinger et al. 1996). Additional advantages of the ferret model include its immaturity at birth and its relatively slow period of postnatal development. The protracted early postnatal development and the relatively large size of the ferret kit will permit future molecular and pharmacological manipulations targeting a particular area of the neocortex and discrete developmental events at very early stages of development. Moreover, we recently showed that early alcohol exposure disrupts visual cortical plasticity in ferrets studied at a later age (Medina et al. 2003). Exposure of ferrets to alcohol during a relatively brief period of development lasting from P10 to P30 is shown to induce a long-lasting disruption of cortical receptive field properties and orientation columns while preserving robust responses to sensory stimulation.

METHODS

Male and female ferrets in litters of five to six animals were injected with alcohol (3.5 mg/kg, 25% in saline, ip) every other day from P10 to P30, ending the treatment just before eye opening at P32. Littermates were injected with vehicle saline solution from P10 to P30 or did not receive any treatment (see Table 1 for number of animals used in this study). Some of the developmental events observed in ferrets during this period occur in rats from approximately P4 to P10 (Clancy et al. 2001); alcohol exposure during this period has been proposed to mimic third-trimester ethanol exposure in humans (Bellinger et al. 1999; Hsiao and Frye 2003; Maier et al. 1999; Thomas et al. 2003, 2004). However, some of the same developmental events occur in humans from as early as 135 to ≈188 days after conception (Clancy et al. 2001), suggesting correspondence with an earlier period of human development. Additional complexity is added to this comparison by considering the timing of exposure to patterned visual stimulation, an important developmental landmark that plays a major role in the maturation of the visual system (Katz and Shatz 1996). Since the onset of patterned visual stimulation in humans is only after birth, the timing of alcohol treatment in our ferret model of FAS may be approximately equivalent to the second half of human gestation. We also examined a second group of animals treated for the same duration as the P10 group but exposed to alcohol later in life, between P20 and P40. Exposure of alcohol in this group of animals lasted well beyond the time of eye opening.

An important reason for our selection of a relatively late timing of alcohol treatment is to avoid ocular malformations, which result from alcohol intoxication during early embryonic development (Cook et al. 1987; Sulik and Johnston 1983). To eliminate animals that may have developed optical problems, we used an opthalmoscope to examine the optics before each experiment. In every animal used in these studies, we observed clear images of the optic disc and retinal blood vessels before the physiology experiments started. No cases of microphthalmia, hypoplasia of the optic disc, large refractive errors, decreased optic media transparency, or abnormalities of retinal blood vessels were seen in alcohol-treated and control ferrets used in this study.

Blood samples were obtained from the tail tip at different time-points (1, 3, 5, and 3 h) after an injection of animals aged approximately P20. Each subject was bled once. Blood analyzed using a commercial kit (333-A diagnostics kit, Sigma, St. Louis, MO) revealed 254 ± 13 mg/dl alcohol concentration 1–5 h after the injection and undetectable levels 34 h after the injection. This blood alcohol level is higher than required for alcohol intoxication (100 mg/dl) but lower than required to induce a comatose state (400–500 mg/dl) in humans (McLain et al. 1985). To avoid the effect of stress on the physiology studies, animals used for blood alcohol sampling were not included in the experimental studies. Concerning weight of the animals, one-way ANOVA shows an effect of treatment (F = 5.16; P = 0.01). However, our results could not be explained by nutritional deficiencies since ethanol- [275.4 ± 17.8 (SE) g] and saline- (301.7 ± 23.16 g) treated animals presented similar weights (Bonferroni; P > 0.05).

Quantitative single-unit in vivo electrophysiology or optical imaging of intrinsic signals were performed at P48–P65, weeks after the end of treatment. All procedures described in this paper were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

In vivo electrophysiology

Animals were premedicated by subcutaneous injection of a tranquilizer (acepromazine, 1 mg/kg), a muscarinic antagonist (methyl atropine bromide, 0.2 mg/kg) to reduce bronchial secretion, and dexamethasone sodium phosphate (0.5 mg/kg) to reduce inflammation. Animals were anesthetized using sodium pentobarbital (35 mg/kg, Abbott Laboratories, North Chicago, IL) and placed in a stereotaxic frame. No procedures started until the animal was sufficiently anesthetized, as ascertained by the loss of withdrawal and corneal blink reflexes. A tracheal cannulation was performed, and the animal was placed on a ventilator and paralyzed using pancuronium bromide (0.2 mg/kg, ip). To comply with National Institutes of Health guidelines for use of paralytic agents and to certify that the animals were maintained at an appropriate level of anesthesia, use of muscle relaxants was omitted in some experiments, and withdrawal reflexes were monitored in these animals. Similar procedures have been previously described and shown to be appropriate for visual physiology studies conducted in ferrets (Medina et al. 2003). Heart rate, expired CO2, and arterial blood oxygen saturation (SpO2) were monitored continuously and maintained at ≈270 bpm, 4.0%, and >90%, respectively. Body temperature was maintained at 38°C using a homeostatic blanket. Supplemental doses of pentobarbital (12 mg/kg) were given every hour throughout the experiment when heart rate or expired CO2 increased, a procedure previously shown to preserve visual responses over time (Medina et al. 2003). Nictitating membranes were retracted using phenylephrine hydrochloride (2.5%), the pupils were dilated with atropine sulfate (1%), and contact lenses were placed on the corneas. Subcutaneous injections of 10% dextrose and 0.9% saline were given to prevent dehydration.

A craniotomy (3–4 mm diam) was performed to expose the binocular region of the left primary visual cortex (Law et al. 1988) where recordings were performed. Single-unit recordings were conducted using a glass-coated tungsten microelectrode with a 5-μm exposed tip lowered into the primary visual cortex at ≈20° to the vertical. To minimize sampling bias, single units used in this study were separated by ≥100 μm along the electrode track. After the isolation of a single

<table>
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<tr>
<th>Experimental groups</th>
<th>Number of Animals (Number of Cells)</th>
<th>Optical imaging</th>
<th>Single-unit recordings</th>
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<tr>
<td>Untreated</td>
<td>3</td>
<td>6 (120)</td>
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<tr>
<td>Saline</td>
<td>6</td>
<td>3 (66)</td>
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<tr>
<td>Early ethanol (P10–P30)</td>
<td>8</td>
<td>7 (115)</td>
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<td>Late ethanol (P20–P40)</td>
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unit, its receptive field was mapped, and the optimal stimulus orientation, direction, and velocity were determined qualitatively using a moving bar of light projected onto a tangent screen. Ocular dominance, spontaneous activity, and number of spikes per stimulus were quantitatively determined for each cell by presenting a computer-controlled bar of light to each eye. Each stimulus presentation consisted of the bar of light moving across the receptive field at the optimal orientation in one direction and back across in the opposite direction. To assess orientation selectivity, the moving bar of light was presented to each eye separately at four orientations centered around the optimal (0, 45, 90, and 135°). Spikes were collected during the 10 stimulus presentations by a computer using Spike 2 software (Cambridge Electronics Design, Cambridge, UK), and peristimulus histograms were generated. Spontaneous activity was determined by recording activity in the absence of stimulation. At the conclusion of the electrophysiology experiment, ferrets were killed with Euthasol (0.6 ml/kg, ip; Delmarva Laboratories, Midlothian, VA), a solution containing pentobarbital sodium (390 mg/ml) and phenytoin sodium (50 mg/ml).

To quantify the results, an orientation selectivity index (OSI) was obtained for each cell by dividing the response 90° from the optimal by the response at the optimal orientation and subtracting the result from 1. Indices of 1.0 indicate a high degree of selectivity, and indices of 0.0 indicate lack of selectivity. Figure 1 shows peristimulus histograms from three cortical neurons displaying different degrees of selectivity: 1) a highly selective cell with an OSI of 1 responding to stimuli at the optimal orientation but not at orientations 90 or 45° away from the optimal (Fig. 1A), 2) a weakly selective cell with an OSI of 0.6 displaying responses to every orientation tested but showing the strongest response at the optimal orientation (Fig. 1B), and 3) an unselective cortical neuron with an OSI of 0.05 displaying robust responses to every orientation tested (Fig. 1C).

**Optical imaging of intrinsic signals**

Optical imaging of intrinsic signals was performed with Imager 2001 VSD+ (Optical Imaging System, Germantown, NY) using imaging methods slightly modified from those described elsewhere (Medina et al. 2003; White et al. 1999). Briefly, animals were premedicated, anesthetized, and ventilated with similar procedures described for single-unit recordings. Temperature, heart rate, expired CO2, and SpO2 were monitored continuously. A craniotomy was made over the left hemisphere to expose the dorsal area of the occipital cortex. The dura was reflected, and the opening filled with agar (2.5% in saline) and covered with a glass coverslip. An image of the vascular pattern was obtained by illuminating the cortical surface with a green filter (~550 nm) using a tungsten-halogen light source. Next, images of intrinsic signals were obtained using a red filter (~700 nm). Visual stimulation consisting of high-contrast rectangular wave gratings (8.75° dark phase/1.25° light phase) generated on a 21-in monitor (Sony Trinitron) using SGT+ graphics board and STIM software (generously provided by Kaare Christian). Gratings were presented to both eyes at angles of 0, 45, 90, or 135° and drifted (22.5°/s) in both directions along the axis orthogonal to the orientation of the gratings. A single trial consisted of these four gratings and a blank screen presented to each eye for 9 s in a pseudorandom sequence, with data acquisition during the last 8 s. A total of 20 trials was performed for each eye, and the summed images were used to obtain single condition maps by subtracting responses to each angle (0, 45, 90, and 135°) from responses to a blank screen. In these images, dark areas correspond to regions responsive to a specific angle. In addition, differential maps (cardinal and oblique) were obtained by subtracting the images to a specific angle (0° for cardinal and 45° for oblique) from the images to its orthogonal (90° for cardinal and 135° for oblique). In these images, dark areas correspond to best to stimulation by 0 (in cardinal maps) or 45° (in oblique maps) gratings, and white areas respond best to stimulation by 90 (in cardinal maps) or 135° (in oblique maps).

**RESULTS**

We examined the effects of alcohol exposure at P10–P30 and P20–40 on development of cortical orientation selectivity. Selectivity to stimulus orientation was assessed by conducting optical imaging of intrinsic signals and single-unit recordings in ferret visual cortex. As shown in Table 1, results observed in alcohol-treated animals were compared with findings in saline-treated animals and untreated controls. All experimental and control groups were treated in the same age ranges.

**Optical imaging of intrinsic signals**

To examine the effects of early alcohol exposure on development of orientation selectivity columns, we conducted intrinsic signal-imaging experiments in alcohol-treated, salinetreated, and untreated animals. Figure 2 shows the finding that untreated and saline-treated control animals had well-organized, high-contrast differential maps with normal spacing and pattern of domains at both cardinal (Fig. 2, A and B) and oblique (Fig. 2, D and E) orientations. In contrast, orientation maps in ethanol-treated animals had markedly reduced contrast, so that the orientation domains were barely noticeable at both cardinal (Fig. 2C) and oblique (Fig. 2F) orientations.

Single condition maps indicated that reduction in contrast did not result from poor response to visual stimulation. Figure 3 shows the finding that single condition maps in alcohol-treated (Fig. 3, C, F, I, and L) animals showed strong cortical signal in response to visual stimulation. These maps show that
the entire dorsal surface of V1/V2 appeared uniformly darker than the background in response to stimulation at a single orientation. In contrast, control saline-treated (Fig. 3, B, E, H, and K) and untreated animals (Fig. 3, A, D, G, and J) showed clear alternation of responding (dark) and nonresponding (gray areas). Importantly, robust responses were observed in each alcohol-treated animal at every orientation tested, as shown in Fig. 3. This finding rules out the possibility that optical problems, especially astigmatism, may have prevented stimulation at some orientations.

To quantify the effects of early ethanol treatment on maturation of orientation columns, we computed signal intensity observed in the single condition maps, as shown in Fig. 4. We obtained an estimate of signal intensity across the designated rectangular area by computing the position of each pixel along a grayscale containing 100 levels of gray. In this scale, the lightest pixel corresponds to an arbitrary value of 0, which represents lack of response to visual stimulation. The darkest pixel corresponds to an arbitrary value of 100, which represents the strongest possible response to visual stimulation. Signal intensity as a function of position along the main axis of the rectangular region is shown beside each single condition image. Signal intensity was found to vary with position in the case of a saline-treated animal (Fig. 4A), reflecting alternation of regions with high signal intensity located within the orientation columns and regions with low signal intensity located outside of the columns. Signal intensity variations with position were reduced (Fig. 4C) or even absent (Fig. 4B) in the alcohol-treated animals, reflecting reduced contrast of their orientation maps. However, response to visual stimulation in these animals was not reduced, as indicated by the elevated mean pixel intensity.

To quantify these spatial variations in signal intensity, we calculated the SD of the signal observed along the main axis of each rectangular area. A high SD value represents elevated contrast of orientation columns, whereas a low value represents reduced contrast of orientation columns. The SD values were lower in the alcohol-treated animals (5.65 and 9.99) than in the saline-treated animal (13.1) shown in Fig. 4 (right), even though mean signal intensity was elevated in every case. SD values were calculated at every orientation tested in ethanol-treated, saline-treated, and untreated controls. Figure 5 shows striking differences in SD values of alcohol-treated animals relative to both groups of control animals (1-way ANOVA, $F = 28.5; P < 0.001$). Contrast of orientation maps was substantially lower in alcohol-treated animals than in saline-treated (Bonferroni; $P < 0.001$) or untreated animals ($P < 0.001$). In contrast, SD values in saline-treated and untreated animals were statistically indistinguishable ($P = 1$).

Figure 6 shows that mean signal intensity was not reduced in alcohol-treated animals relative to control groups. Rather, a weak but statistically significant enhancement of responses was

**FIG. 2.** Orientation selectivity maps in untreated, saline-, and ethanol-treated animals, as revealed by optical imaging of intrinsic signals. A–C: cardinal differential maps, in which dark areas respond best to 0° gratings (horizontal) and white areas respond best to 90° (vertical) gratings. D–F: oblique differential maps, in which dark areas respond best to 45° gratings and white areas respond best to 135° gratings. Note the poor contrast of cardinal and oblique orientation maps in ethanol-treated animals relative to untreated and saline-treated animals. Ferrets were treated with alcohol from P10 to P30 and examined following a prolonged alcohol-free period. Calibration bar = 3 mm.

**FIG. 3.** Single condition orientation maps in untreated, saline-, and ethanol-treated animals. In these maps, dark areas represent regions responsive to a single angle. Single condition maps in the ethanol-treated animals show strong signals but less clear modular organization than observed in control animals. Stimulus orientations used were 0 (A–C), 45 (D–F), 90 (G–H), and 135° (I–L). Note that robust responses were present at every orientation tested. Calibration bar = 3 mm.
observed in animals treated with ethanol relative to the saline-treated group (1-way ANOVA, $F = 6.0; P = 0.004$; Bonferroni, $P = 0.003$). However, no statistically significant differences were observed between the alcohol-treated and untreated groups ($P = 0.168$) and between saline-treated and untreated animals ($P = 0.675$). These findings show that the effects of alcohol on orientation selectivity did not result from a reduced response to visual stimulation.

**Single-unit responses**

To assess further the effects of early alcohol exposure on cortical orientation selectivity, we conducted single-unit recordings in the binocular region of the primary visual cortex. In some cases, single-unit recordings and optical imaging were conducted in the same animals ($n = 2$ alcohol-treated and 2 saline-treated ferrets). Figure 7A shows the cumulative number of cells (in percent) plotted as a function of the OSI in animals treated with alcohol or saline from P10 to P30 and studied at...
P48–P65. Results from untreated animals are also included. The cumulative curve for the alcohol-treated animal was significantly shifted to the left of the control curves, reflecting weaker neuronal orientation selectivity in ethanol-treated animals (1-way ANOVA, \( F = 9.368; P < 0.001 \)) than in saline-treated (Bonferroni, \( P < 0.001 \)) and untreated animals (\( P < 0.01 \)). In contrast, OSIs in saline-treated and untreated animals were statistically indistinguishable (\( P = 0.379 \)).

Critical timing of treatment

Next, we examined whether the disruptive effects of early alcohol exposure on maturation of orientation selectivity are specific to an early developmental stage. Figure 7B shows that similar cumulative plots were obtained from untreated animals and from animals exposed to alcohol from P20 to P40. The average OSI values for untreated controls (0.74 ± 0.02) and alcohol-treated animals (0.72 ± 0.04) were indistinguishable (Bonferroni; \( P = 0.983 \)). This result contrasts with the finding of decreased orientation selectivity in animals treated at an earlier age, indicating that disruption of orientation selectivity depends on the timing of early alcohol exposure.

We asked whether depression of visually driven activity may underlie the weakened orientation selectivity found in alcohol-treated animals. As shown in Fig. 8, similar mean maximal responses to stimulation at the optimal orientation were observed in animals treated with alcohol or saline from P10 to P30, in animals treated with alcohol from P20 to P40, and in untreated animals (1-way ANOVA, \( F = 0.46, P > 0.05 \)).

**FIG. 7.** Reduced neuronal orientation selectivity in animals exposed to alcohol from P10 to P30 (A) but not P20 to P40 (B). Orientation selectivity index (OSI) was substantially lower in animals treated with alcohol from P10 to P30 (\( n = 115 \) cells from 7 ferrets) than in saline-treated (\( n = 66 \) cells from 3 ferrets; \( P < 0.001 \)) or untreated animals (\( n = 120 \) cells from 6 ferrets; \( P < 0.01 \)). In contrast, orientation selectivity was not affected in animals exposed to alcohol late during development (\( n = 29 \) cells from 3 animals). OSIs in these animals were similar to OSIs in untreated animals (\( P > 0.05 \)).

**FIG. 8.** Mean maximal responses of striate cortical cells to a moving bar of light at the optimal orientation. Similar mean maximal response (in spikes per run) of cortical neurons to stimulation at the optimal orientation observed in early (P10–P30) ethanol-treated, late (P20–P40) ethanol-treated, saline-treated, and untreated animals (\( P > 0.05 \) in each case). These findings indicate that early alcohol exposure preserved robust responses to visual stimulation. Bars indicate SE.

**DISCUSSION**

We have shown that early ethanol exposure significantly affects development of orientation columns and neuronal orientation selectivity in the visual cortex. Optical imaging of intrinsic signals revealed that early alcohol exposure decreased signal contrast of orientation maps while preserving robust signal strength for every orientation tested. Consistent with the optical imaging results, single-unit recordings revealed that early alcohol exposure decreased neuronal selectivity to stimulus orientation while preserving normal strength of visual responses. These effects resemble the findings in ferrets raised under extreme disruption of sensory activity (Weliky and Katz 1997). Collectively, these findings indicate that early alcohol exposure disrupts cortical processing of sensory information at a later age.

Assessment of cortical orientation selectivity was conducted at P48, when orientation selectivity in ferret visual cortex is mature (Chapman and Stryker 1993), and up to P65. Since recordings were conducted following a prolonged alcohol-free period, the findings suggest that effects of ethanol are long-lasting. An earlier, pioneering study also indicated that alcohol administration throughout gestation has a lingering impact on neuronal activity in the rodent barrel cortex (Rema and Ebner 1999). However, both spontaneous and evoked activities were substantially suppressed, precluding an investigation of cortical neuron receptive field properties. In contrast, our procedure of alcohol exposure limited to a shorter period of development corresponding to approximately the second half of gestation is advantageous in preserving visual cortical responsiveness. Another advantage of this relatively late timing of alcohol exposure is that ocular malformations, which result from alcohol intoxication during embryonic development (Chan et al. 1991; Cook et al. 1987), can be avoided. Therefore these findings show for the first time that alcohol intoxication during a relatively brief period of development results in alterations of neuronal receptive field properties and functional architecture in the neocortex.
Relevance to understanding visual deficits in FAS

The abnormalities described here are consistent with reports indicating that deficits in visual processing are relatively common in children with FAS (Mattson et al. 1996; Uecker and Nadel 1996). Prenatal alcohol exposure leads to an elevated prevalence of amblyopia (Stromland and Pinazo-Duran 2002) and poor performance in neuropsychological tests that assess visual perceptual skills (Mattson and Riley 1998; Olson et al. 1998). Children with FAS show especially poor performance in reproducing geometric designs, even after correction for optical problems that may occur after alcohol exposure during embryonic life (Stromland and Pinazo-Duran 2002). Interestingly, the most common types of mistakes are shape distortions and inappropriate reproduction of corners and borders (Mattson et al. 1996; Uecker and Nadel 1996). The perception of geometrical forms may be dependent on the integrity of the orientation tuning of visual cortical neurons, which our findings indicate to be weakened in the ferret model of FAS. Therefore these findings of reduced contrast of cortical orientation columns and abnormal neuronal receptive fields may provide a neurobiological substrate for some types of visual deficits in children with FAS.

Effects of alcohol exposure on activity-dependent mechanisms of development

Ferrets were exposed to alcohol from P10 to P30, when lateral geniculate neurons first form synapses with layer IV neurons (Herrmann et al. 1994), ocular dominance columns are first seen (Crowley and Katz 2000), and orientation selectivity of cortical neurons starts developing (Chapman and Stryker 1993; Chapman et al. 1996; Krug et al. 2001). However, orientation selectivity matures further after the end of alcohol exposure until the adult state is reached ~2 wk later. The question arises of how early alcohol exposure disrupts the development of receptive field properties that only reach a mature state following the end of treatment.

Alcohol may disrupt activity-dependent mechanisms of development by acutely suppressing NMDA receptor function (Lovinger et al. 1989; Rema and Ebner 1999; Savage et al. 1992) while enhancing GABA receptor function (Hsiao et al. 2002) and affecting CREB phosphorylation (Constatinescu et al. 1999; Yang et al. 1998). Following the end of chronic ethanol exposure, neocortical development and plasticity may be further disrupted as a result of substantial and long-lasting alterations of CREB activity (Pandey et al. 1999; Yang et al. 1998), NMDA receptor composition (Costa et al. 2000; Rema and Ebner 1999), and GABAergic inhibition (Durand and Carlen 1984; Hsiao et al. 1999). Significantly, NMDA receptors have been reported to be downregulated for at least several weeks and into adulthood following fetal alcohol exposure (Rema and Ebner 1999; Savage et al. 1992; Valles et al. 1995). NMDA receptors and CREB are involved in visual cortical plasticity (Bear et al. 1990; Hensch et al. 1998; Mower et al. 2002; Roberts et al. 1998), and NMDA receptor function is required for development of orientation selectivity (Ramoa et al. 2001). Moreover, reduction of GABAergic inhibition after eye opening has been shown to perturb the development of cortical columnar architecture (Hensch and Stryker 2004). Therefore long-term disruption of NMDA receptor, GABA receptor, and CREB function at a time when receptive fields properties are developing and ocular dominance plasticity is at a peak (Issa et al. 1999) may result in abnormal development of the neural circuits required for orientation selectivity. Clear effects were observed in animals injected from P10 to P30 but not from P20 to P40. These findings suggest that compensatory effects following the end of chronic alcohol exposure at P30, rather than the acute effects of alcohol intoxication, are responsible for the disruption of orientation selectivity.

The possibility should be considered that malformation of neural circuits in the lateral geniculate nucleus may also contribute to alterations of neocortical responses in animals exposed to alcohol. During the period when alcohol was administered in this study, retinal ganglion cell axons segregate into sublaminae that retain input from ON and OFF-center retinal axons (Hahn et al. 1991). Segregation of ON-OFF pathways has been suggested to play a critical role in the development of cortical orientation selectivity (Miller 1994). Interestingly, ON-OFF segregation requires NMDA receptor function (Hahn et al. 1991), which is acutely inhibited by alcohol (Lovinger et al. 1989; Mirshahi and Woodward 1995). This raises the possibility that NMDA receptor blockade during alcohol exposure prevents the segregation of ON-OFF responses. It should be interesting to examine in future studies what effects early alcohol exposure may have on the development of ON-OFF pathways in the lateral geniculate nucleus and visual cortex. The possibility should also be considered that ethanol’s effects on development of orientation selectivity are even more striking than reported here. Pentobarbital anesthesia, which was used in this study, is known to potentiate GABAergic inhibition (Harris 1990). Since GABAergic inhibition may contribute to sculpt cortical orientation selectivity (Shapley et al. 2003), it is conceivable that this stimulus selectivity may be even weaker in awake alcohol-treated animals than our results indicate.

Neurodegeneration of cortical neurons, another major effect of early alcohol exposure (Ikonomidou et al. 2000), may also contribute to the development of abnormal cortical circuits. Preferential loss of cortical inhibitory neurons during chronic ethanol exposure (Bailey et al. 2004) could permanently disrupt processing of sensory information.

Peripheral effects of alcohol exposure

In alcohol intoxication throughout gestation, the sensory deficits are closely associated with peripheral malformations. Examples of peripheral malformations that may underlie sensory deficits in people with FAS include microphthalmia and optic nerve hypoplasia (Stromland and Pinazo-Duran 2002) and abnormal curvature of the eyes (Garber 1982; Miller et al. 1981). However, it is very unlikely that peripheral problems, especially optical problems, underlie the abnormalities described here. First, ethanol has been shown to result in a spectrum of ocular malformations only when administered to gastrulating embryos (Cook et al. 1987; Sulik and Johnston 1983), during a period that corresponds to the third week after fertilization in humans (Cook et al. 1987). To avoid this problem, we administered alcohol during the second half equivalent of gestation. Second, we administered alcohol at a blood concentration of 250 mg/dl, inferior to the elevated blood alcohol concentration (430 mg/dl) required to elicit loss...
of optic nerve axons and decrease myelination (Harris et al. 2000; Stromland and Pinazo-Durán 2002). Third, we used an ophthalmoscope to examine the ocular media and the retina before each experiment and found no differences between normal, saline-, and alcohol-treated ferrets. Fourth, in every animal, we found visual responses at all orientations tested, precluding astigmatism as an explanation for the abnormalities of cortical response. Fifth, robust visual responses were preserved in alcohol-treated animals, as indicated by imaging of intrinsic signals and single-unit recordings.

Advantages of the ferret visual cortex model of FAS

The visual cortex was used as a model to study the effects of early alcohol exposure on cortical processing of information because it has been the most studied area of the neocortex and because deficits in visual processing are relatively common in children with FAS (Mattson and Riley 1998). However, the studies on this system should assess the effects of alcohol on cortical development in general, and the results should not be restricted to the visual cortex. The ferret visual system in particular was selected because it is characterized by the presence of ocular dominance and orientation columns (Chapman and Stryker 1993; White et al. 1999, 2001), which are present in humans (Goodyear et al. 2002), and a large proportion of neurons showing robust selectivity to stimulus orientation (Chapman et al. 1996). In contrast, rodents lack ocular dominance and orientation columns and have visual cortical neurons that are characterized by weak selectivity to stimulus orientation (Drager 1975). Additional reasons the ferret is an excellent model to study the effects of alcohol on cortical development include its relatively short gestational length of 41 days and the fact that most of the second half equivalent of human gestation occurs postnatally (Clancy et al. 2001). Therefore several developmental events that occur prenatally in cats, primates, and humans occur postnatally in the ferret. In this species, LGN axons reach the subplate at gestational day 37 and invade the cortical plate to form synapses with layer IV neurons during the second week of postnatal life (Herrmann et al. 1994). Alcohol exposure used in our study started at P10 and extended until P30, just before eye opening at P32. This period is approximately equivalent to late second and third trimester equivalent of human gestation and P4–P10 in rats (Clancy et al. 2001). We have focused our study on orientation selectivity because this functional property arises in striate cortex and is believed to be crucial for normal visual processing (Livingstone and Hubel 1998). Moreover, the neuronal circuits underlying orientation selectivity have been previously examined in great detail and are likely to be present in other areas of the neocortex (Shapley et al. 2003; Somers et al. 1995), suggesting that the changes in neocortical organization reported here may impair information processing along different sensory modalities. Future studies using the ferret model of FAS will be instrumental in elucidating how early alcohol exposure disrupts neocortical development and plasticity.

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REFERENCES


