

# Moderate Alcohol During Pregnancy: Learning and Behavior in Adolescent Rhesus Monkeys

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**Background:** Although high-dose prenatal alcohol exposure is related to cognitive and behavioral impairments in children and adolescents with fetal alcohol syndrome, there is relatively little research on the effects of moderate drinking during pregnancy. We examined learning, memory, and behavior in adolescent rhesus monkeys prenatally exposed to moderate levels of alcohol, psychological stress, or both alcohol and stress.

**Methods:** Forty adolescent rhesus monkey subjects were derived from four groups of female rhesus monkeys that (1) consumed alcohol throughout gestation; (2) experienced prenatal stress; (3) experienced prenatal stress and alcohol consumption; or (4) control group (no alcohol, no stress). The subjects were assessed for number of trials required to reach 90% criterion of correct responses on nonmatching-to-sample task (NMS), followed by trials with delays of 30, 60, or 120 sec. Ratings of behavior during testing were made after each session.

**Results:** Subjects exposed to moderate prenatal alcohol required significantly more trials to reach criterion on the acquisition phase of the NMS task but had no difficulty with delays. Prenatally stressed monkeys showed lower response inhibition or less behavioral restraint, whereas prenatal alcohol plus stress monkeys showed higher activity level and stereotypies compared with controls. High scores on neonatal measures of orientation (attending to novel stimuli) and motor maturity and low scores on irritability, activity, stereotypies, and impulsivity during acquisition were correlated with fewer trials to criterion on acquisition of NMS.

**Conclusions:** NMS trials required to reach criterion and behavior during testing are sensitive to moderate-level prenatal alcohol exposure in monkeys. The most adverse behavioral outcomes (hyperactivity and stereotypies) were associated with prenatal alcohol plus stress, raising concerns that environmental stress might provide the context within which fetal alcohol exposure could promote adverse behavioral outcomes. These effects occurred in the absence of either facial deformities or retarded physical growth.

**Key Words:** Moderate Drinking, Prenatal Stress, Stereotypies, Hyperactivity, Learning.

**A**LTHOUGH MATERNAL ALCOHOL use or abuse during pregnancy is one of the most preventable causes of childhood developmental disabilities, fetal alcohol exposure remains the leading known cause of mental retardation. The most serious adverse consequences found in heavily exposed children with fetal alcohol syndrome (FAS) include growth retardation, craniofacial anomalies, and intellectual and behavioral impairments (Clarren and Smith, 1978; Jones and Smith, 1973). Relatively less is known about the extent to which moderate-level prenatal alcohol exposure contributes to cognitive or behavioral

deficits or how prenatal alcohol exposure may interact with other risk factors to yield developmental deficits. We examined the effects of moderate prenatal alcohol exposure on learning and behavior in rhesus monkeys. We defined moderate dose in accord with Dawson et al. (1995), who classified moderate drinkers as those who consume 4 to 14 drinks per week. The monkeys in this study drank a dose comparable to one or two drinks per day with blood alcohol levels ranging from 20 to 50 mg/dl. In addition, because prenatal stress has also been linked to developmental problems in both humans (Paarlberg et al., 1995; Wadhwa, 1998) and monkeys (Schneider and Moore, 2000), and because stress and alcohol use usually covary, we examined the effects of prenatal alcohol exposure, with and without prenatal stress, on learning and behavior.

Fetal alcohol exposure affects a number of cognitive functions in children. Several studies reported that children without full-blown FAS have lowered IQ scores (Russell et al., 1991; Streissguth et al., 1991). Although less heavily exposed children, without full-blown FAS, do not inevitably show IQ deficits (Fried et al., 1992), they tend to exhibit problems in arithmetic, attention, reading/decoding, spell-

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ing, and premath and reading scores (Coles et al., 1991, 1997; Goldschmidt et al., 1996; Jacobson et al., 1993, 1996; Streissguth et al., 1991).

The relationship between fetal alcohol exposure and attention has been studied since the late 1970s, but results have been inconsistent. For instance, Landesman-Dwyer et al. (1978) observed that fetal-alcohol-exposed infants spent more time with their eyes open but not attending. Streissguth et al. (1983) examined more than 400 infants in a prospective study and found that less habituation (response decrement to repetitive stimulation) and low arousal (alternating between awake and drowsy) were associated with maternal alcohol use, after statistically adjusting for smoking, caffeine use, maternal age, nutrition, and obstetric medication. Coles et al. (1992), however, failed to replicate these neurobehavioral effects. Streissguth et al. (1984, 1986, 1994) also measured attentional behaviors in 512 children at 4, 7, and 14 years of age. Attention was consistently lower across time in the alcohol-exposed children. In contrast, Leech et al. (1999) failed to detect significant effects of prenatal alcohol exposure when they used impulsivity or inattention as measures of attention in a prospective study of 608 children of light-to-moderate urban women who used alcohol, tobacco, and cocaine. Thus, the issue of whether prenatal alcohol exposure adversely affects attention functions remains unresolved.

Others have demonstrated deficits in executive functioning—tasks requiring the individual to plan, analyze a problem, devise a strategy, monitor performance, and modify strategy as performance proceeds—associated with maternal alcohol use (Coles et al., 1997; Kodituwakku et al., 1995; Mattson et al., 1999; Olson et al., 1992). Jacobson et al. (1993) found a relationship between fetal alcohol exposure and reduced speed of information processing in low-socioeconomic-status inner-city infants after statistically controlling for potential confounding factors (prenatal exposure to cocaine, opiates, or smoking). Similarly, Streissguth et al. (1984, 1986) reported slower reaction times on vigilance tasks in older children prenatally exposed to alcohol. Finally, children exposed to alcohol prenatally performed worse on tasks dependent on encoding and retrieval of information from working memory. Once they learned information, however, alcohol-exposed children had little difficulty retaining what they had learned (Mattson et al., 1996a; Olson et al., 1992). Taken together, data from these studies suggest the importance of further research on the components of the cognitive and attention deficits associated with fetal alcohol exposure.

Another issue raised by the human research concerns whether other prenatal variables might exacerbate or ameliorate the effects of alcohol exposure. Abel and Hannigan (1995) have suggested that psychosocial stress might increase susceptibility to fetal alcohol effects (FAE). Because both prenatal stress and prenatal alcohol exposure activate the maternal hypothalamic-pituitary-adrenal axis, it is pos-

sible that they could act synergistically to adversely affect the developing central nervous system of the fetus. Studies of humans are not definitive due to the complex interrelationships of variables such as psychosocial stress, alcohol consumption, and other drug use.

Animal models provide the opportunity to prospectively separate potential teratogenic effects of alcohol from other factors. Animal models also allow for manipulation of timing, exposure level, nutrition, and postnatal environmental factors. Rats exposed to large doses of alcohol (e.g., 6 g/kg) during the period of rapid fetal brain growth demonstrated a range of deficits, including overall physical growth retardation, impaired gait development and righting reflexes, increased motor activity, and learning problems (Barron et al., 1988; Hannigan and Riley, 1989; Nelson et al., 1986; Norton et al., 1988; Riley et al., 1979; Taylor et al., 1986; Weinberg, 1989).

Despite the fact that nonhuman primates provide for a greater generalization to humans than rodents with regard to complex cognitive, behavioral, and social development (Suomi and Higley, 1991), the effects of prenatal alcohol exposure have received limited study in nonhuman primates. Clarren et al. (1992) investigated weekly alcohol exposure by using a binge-drinking model. Pig-tailed macaques were given alcohol during the first 3 or 6 weeks or the entire 24 weeks of pregnancy (1.8 g/kg). Both the 6- and 24-week exposure groups failed to show a preference for novel over familiar objects on a visual recognition memory assessment. Whereas typically developing macaque and human infants show novelty preference, previous studies have shown that infants at risk for cognitive deficits typically have decreased novelty preference; they perform at chance levels (Burbacher et al., 1986). The 24-week-exposed monkeys in the study of Clarren et al. took two or three times longer than controls to discover how to perform learning and memory tasks in the Wisconsin General Test Apparatus (WGTA). Moreover, on the Hamilton Search Task, the 24-week exposed group searched twice as many boxes as controls to locate the food reward (Clarren et al., 1992).

Another issue that has not been adequately addressed concerns whether prenatal stress may render the primate fetus more vulnerable to adverse effects from prenatal alcohol exposure. For instance, Rasco and Hood (1995) reported that restraint stress in mice enhanced the adverse effects of prenatal exposure to all-*trans*-retinoic acid, a potent teratogen to which the early central nervous system is known to be highly sensitive (Snodgrass, 1992). Other studies, however, have reported opposite findings. For example, Ward and Wainwright (1989) reported that prenatal stress actually decreased the adverse effects of prenatal alcohol exposure on sensorimotor development in rats. We recently reported a slight increase in fetal loss associated with exposure to both prenatal stress and prenatal alcohol in rhesus monkeys (Schneider et al., 1997). Thus, additional studies are needed to investigate factors affecting susceptibility to FAE.

In this article, we report the results of cognitive and behavioral testing of 32- to 34-month-old rhesus monkey offspring in our longitudinal study of prenatal alcohol and stress exposure. We examined four groups of monkeys from mothers experiencing one of the following conditions: (1) alcohol exposed (0.6 g/kg/day) throughout gestation (gestation day 0 through birth); (2) prenatally stressed (10-min removal from home cage and exposure to three random loud noises on gestational days 90 through 145); (3) prenatal stress plus alcohol exposed (noise stressor, days 90 through 145, plus alcohol consumed throughout gestation as previously described); or (4) control group (isocaloric control solution throughout gestation). We used a nonmatching-to-sample task (NMS) that required the monkey offspring to learn the rule that the nonmatching object conceals the reward. Learning this task requires the animals to remember whether an object was seen on the immediately preceding trial. This task requires learning a new rule, shifting attention sets, and working memory—functions that are dependent on limbic, basal ganglia, and prefrontal and frontal cortex (Mishkin et al., 1984; Wise et al., 1996), areas known to be sensitive to prenatal alcohol exposure. After the task was learned to criterion, the animals were also tested with 30-, 60-, and 120-sec delays.

Because some studies have found that behavioral abnormalities in children have been associated with fetal alcohol exposure (Coles et al., 1997; Mattson and Riley, 2000; Roebuck et al., 1999; Streissguth et al., 1996; Thomas et al., 1998), we also assessed five behaviors (irritability, activity, stereotypies, inhibition, and impulsivity) during cognitive testing. Finally, we examined the relationship between performance on neurobehavioral tests administered during the neonatal period (Schneider and Suomi, 1992) and performance and behavior in the NMS tasks reported here. It is important to determine whether continuity can be detected between early neurobehavior and later learning deficits. If continuity can be found, it would raise the importance of early intervention, when the brain shows the most plasticity.

Alcohol and stress are manipulated independently, allowing us to test the hypothesis that prenatal stress potentiates the effects of prenatal alcohol exposure on either cognition or behavior or both. Alternately, alcohol and stress may be independent contributors to cognitive and behavioral alterations.

## METHODS

### Maternal Treatment

Healthy adult female rhesus monkeys were identified within the breeding colony that consistently and voluntarily consumed 0.6 g/kg in a 6% (v/v) alcohol solution sweetened with NutraSweet (NutraSweet Co., Chicago, IL) (300 mg/100 ml) when the solution was offered daily over a 2-week period. Before breeding, blood samples obtained 60 min after consumption of alcohol showed blood alcohol concentrations (BACs) of 20 to 50 mg/dl. This dosage is comparable to an average-sized woman consuming approximately one to two drinks daily. Alcohol-consuming females were randomly assigned to one of four groups (control or one of

**Table 1.** Sample Size, Sex Distribution, and Balks

Condition	Females (n)	Males (n)	Balks
Alcohol-exposed	6	3	1
Prenatally stressed	2	6	0
Alcohol + stress	4	4	1
Controls	9	3	1
Total	21	16	3

three experimental groups) in a  $2 \times 2$  factorial design with prenatal alcohol (alcohol present or alcohol absent) and prenatal stress (stress present or stress absent) as the independent variables. All females in the breeding colony were singly housed to minimize variance from different maternal or offspring social experience. One experimental group (alcohol present, stress absent) voluntarily consumed the alcohol solution daily throughout gestation at 1600 hr. Alcohol consumption was begun 5 days before breeding and ended at parturition. Water was available ad libitum, including during the time of day when the alcohol solution was available. A second experimental group (alcohol and stress) consisted of females who voluntarily consumed the alcohol solution daily throughout gestation and were exposed to a mild 10-min psychological stressor during mid to late gestation, days 90 through 145 after conception (see below). A third experimental group (alcohol absent, stress present) consisted of females exposed to a mild 10-min psychological stressor during mid to late gestation, days 90 through 145 after conception. The fourth group consisted of control subjects (alcohol absent, stress absent) that voluntarily consumed a sucrose solution that was approximately equivolemic and equicaloric (8 g/100 ml water) to the alcohol solution. During the remainder of the day, all females were housed under identical conditions, undisturbed except for necessary routine animal husbandry.

### Prenatal Stress

The stress treatment was administered five times per week at approximately 1530 hr for females in the stress and alcohol plus stress conditions. It involved removing the pregnant female from the home cage, placing her in a transport cage, and taking her to a darkened room where three short (less than 1-sec duration) noise bursts were randomly administered over a 10-min period. The noise burst consisted of an alarm horn that produced a 1300-Hz sound of 115-dB intensity at 1 m. Prior studies found higher plasma cortisol levels in stress-exposed females compared with undisturbed controls 30 min after the stress treatment (Schneider et al., 1997).

### Subjects

The subjects in this study were 40 adolescent (32- to 34-month-old) rhesus monkeys (*Macaca mulatta*), 23 females and 17 males, born to the female rhesus monkeys described previously (Table 1). One monkey each from the alcohol, alcohol plus stress, and control group was not testable due to excessive repeated balking (failure to respond for five trials), resulting in a total of 37 monkeys that completed the NMS testing (Table 1).

All monkeys were born between May 1993 and June 1995. They were housed with their mothers in individual cages during the first 6 months of life. All infants were separated from their mothers four times during the first month of life to conduct examinations of early neurobehavioral development and to monitor growth parameters (Schneider et al., 1997). Behavioral observations of mother-infant dyads were made weekly throughout the first 6 months of life. At 6 months, the infants were permanently separated from their mothers and reared in mixed-sex peer groups consisting of five or six monkeys from similar prenatal conditions. Beginning at 24 months of age, they were pair-housed with same-sex peers from similar treatment groups. They were maintained on a diet of Purina Monkey Chow (St. Louis, MO) supplemented three times weekly with fresh fruit. Feeding took place after testing, and water was available ad libitum. All housing conditions were light- (16-hr light/8-hr dark cycle) and temperature- ( $21 \pm 0.5^\circ\text{C}$ ) controlled.



### Cognitive Testing

Cognitive testing was conducted with the WGTA, an apparatus used for assessing learning and memory capabilities in nonhuman primates (Harlow and Bromer, 1938). The WGTA was situated in a dimly lit and sound-shielded room while a masking white noise was generated. Each monkey was placed individually in a cage opposite a human tester, who sat behind the WGTA and manipulated the stimuli and rewards. Testers were blind to the experimental condition of the monkey. Stimulus objects were plastic, three-dimensional, abstractly shaped objects varying in color, size, and texture. A stimulus tray with recessed food wells slid on rollers between the monkey and the tester. Two vertical doors could be moved up and down by the tester; one was on the monkey's side and one was on the tester's side. The tester raised the door on her or his side, arranged the problem on the tray by placing stimulus objects over food wells, and baited the correct object with a food reward. The tester's door was then lowered and the monkey's door was raised, and the tray was rolled forward so that the monkey could respond by reaching through the bars of the cage.

To acclimate the animal to the apparatus, on the first day of testing, the monkey was placed in the WGTA cage and left alone for 30 min with the screen down. On the second day of testing, the monkey was placed in the WGTA cage and left alone for 30 min with the screen up. On day 3, the monkey was placed in the WGTA cage and left alone for 30 min with the screen up and a variety of treats (raisins and Froot Loops; Kellogg Co., Battle Creek, MI) scattered across the board and in the wells. The tester returned after 30 min and noted the treat preference.

### Successive Approximation

The tester placed a treat in the middle well and placed an object behind the treat, leaving the well fully uncovered. Once the animal retrieved the treat, the tester placed another treat in the middle well and covered one fourth of the well with the object. Next, the tester placed a treat in the middle well and covered half of the well with the object, then three fourths of the well, and finally the entire well.

### NMS: Acquisition

In the acquisition phase of the NMS task, first, a sample object was presented over the central, baited food well of a tray containing three wells. The monkey was allowed to obtain the food item by displacing the object. After a 10-sec delay, during which time a screen between the monkey and the experimenter was closed, the sample object (unbaited) and a new object (baited) were presented simultaneously covering the lateral wells of the test tray. The correct strategy was to respond to the new object, displace it, and obtain the food reward. After a 30-sec intertrial interval, a different sample object was presented in the center well, followed 10 sec later by another trial with another novel object. Placement of the sample and novel objects over the left and right wells was determined randomly.

Twenty trials, with 30-sec intervals between trials, were presented 5 days a week until the monkeys reached a learning criterion of 90 correct responses in 100 consecutive trials (90% correct). The outcome measure for the acquisition phase of the NMS task was the number of trials taken by the monkey in reaching this criterion.

### NMS II: Reacquisition

After the monkey reached the criterion of 90% correct performance on NMS I, a 2-week break in testing occurred. After the 2-week break, testing resumed and the same NMS task as discussed previously was administered again to assess memory for the task. The dependent measure was the number of trials to criterion (90% correct, or 90 correct responses in 100 consecutive trials).

**Table 2.** Definitions of Behavioral Rating Scale

Item	Definition	Reliability
Irritability	general fussiness (0 = no fussiness; 1 = fussy 25% of time; 2 = fussy 50% of time; 3 = fussy 90% of time)	0.92
Activity	amount of gross body movement (0 = stays in one place; 1 = active 25%; 2 = active 75%; 3 = continual movement)	0.93
Stereotypies	repetitive three or more rhythmic motor movements (0 = none; 1 = 1–2 times; 2 = 50% of time; 3 = continuous)	0.95
Inhibition	fearful, restrained behavior (0 = bold, not inhibited; 1 = fearful, inhibited 25%; 2 = fearful, inhibited 50%; 3 = extremely fearful, inhibited 90%)	1.00
Impulsivity	grabs hastily (0 = does not grab hastily; 1 = grabs 1–2 times; 2 = grabs hastily 50%; 3 = grabs hastily 90% of time)	0.83

### NMS III: Extended Delay

This task was the same as NMS I and II, except the delay between the first object and the presentation of the two objects was varied. After presentation of the first object, the screen was lowered and stayed down for 30, 60, or 120 sec. On days 1 and 2, a 30-sec delay was used; on days 3 and 4, a 60-sec delay was used; and on days 5 and 6, a 120-sec delay was used. The outcome measure was percentage of correct responses at each delay.

### Behavioral Ratings

Behaviors exhibited during testing that might affect performance of the cognitive task were also rated. A rating scale developed by the first author and adapted from the Bayley Scales of Infant Development (Bayley, 1969) was completed by the tester for each monkey immediately after each testing session (Table 2). Ratings were averaged across testing sessions for each task. Reliability was calculated by the method in Winer (1971, p 283), except for response inhibition, which had 100% agreement.

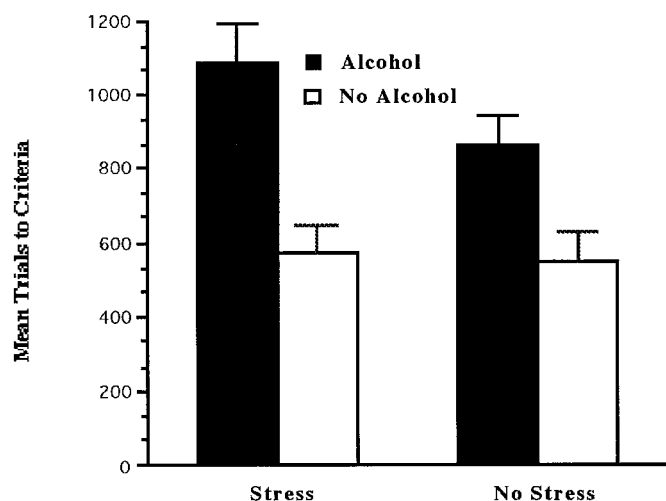
### Data Analysis

Group differences in trials to criterion for NMS I and II and percentage correct for NMS III (30, 60, and 120 sec) were evaluated by ANOVAs to test the overall effects of alcohol exposure (present or absent), stress (present or absent), and delays (30, 60, and 120 sec). Scores for the behavioral measures (irritability, activity, stereotypies, inhibition, and impulsivity) were analyzed by multivariate ANOVA (MANOVA) to test whether the groups differed as a function of alcohol exposure (present or absent) and stress (present or absent). Univariate analyses were also conducted and reported. The significance of associations between trials to criterion and behavioral measures for NMS I and II, and the relationships between neonatal neurobehavioral measures of orientation and motor maturity (Schneider and Suomi, 1992) and cognitive performance, were analyzed by Spearman rank correlational analysis.

## RESULTS

### NMS I: Acquisition Phase

The mean number of trials to criterion for NMS I (acquisition phase) for each group are shown in Fig. 1. The number of trials to criterion was affected by prenatal alcohol [ $F(1,33) = 22.29, p < 0.0001$ ]. There was no significant main effect for prenatal stress [ $F(1,33) = 2.12, p = 0.15$ ], nor was there an interaction effect of alcohol and prenatal stress [ $F(1,33) = 1.22, p = 0.27$ ]. Compared with control animals, which took an average of [mean (SE)] 544 (85)



**Fig. 1.** Performance on the nonmatching-to-sample task of the study is shown. Data are plotted as group mean  $\pm$  SE for trials to criterion, collapsed across sex. The trials to criterion did not differ significantly across prenatal stress groups ( $p = 0.15$ ). However, the fetal-alcohol-exposed group took significantly longer to reach criterion (acquisition phase;  $p < 0.0001$ ).

trials to criterion, prenatally stressed monkeys took 575 (67) trials, alcohol-exposed monkeys took 862 (83) trials, and alcohol plus stress-exposed monkeys took 1086 (102) trials to reach criterion.

Maternal prepregnancy BACs were available for mothers of 16 alcohol or alcohol plus stress infants. Correlations between maternal BACs and trials to criterion on NMS acquisition were positive but not significant ( $n = 16$ ,  $r = 0.412$ ,  $p = 0.11$ ). However, when we analyzed the alcohol-alone group, a significant correlation was found between maternal BAC and offspring trials to criterion ( $n = 9$ ,  $r = 0.707$ ,  $p = 0.033$ ). The correlation for the alcohol plus stress group was not significant ( $n = 7$ ,  $r = 0.055$ ,  $p > 0.50$ ).

#### NMS I: Behavioral Data

There was no significant multivariate effect for alcohol in the MANOVA of irritability, activity, stereotypies, inhibition, and impulsivity [ $F(5,29) = 1.96$ ,  $p = 0.11$ ]. There was, however, a significant multivariate main effect for prenatal stress [ $F(5,29) = 6.62$ ,  $p = 0.0003$ ] and a significant multivariate alcohol  $\times$  prenatal stress interaction [ $F(5,29) = 4.42$ ,  $p = 0.004$ ]. Univariate analyses indicated a significant alcohol  $\times$  prenatal stress interaction for both stereotypies and activity, showing that the monkeys exposed to both alcohol and prenatal stress had higher levels of stereotypies [mean (SE)]—1.55 (0.35) for alcohol plus prenatal stress compared with 0.37 (0.08) for alcohol, 0.14 (0.07) for prenatal stress, and 0.73 (0.19) for controls [ $F(1,33) = 18.23$ ,  $p = 0.0001$ ; Table 3]. Alcohol plus prenatal stress monkeys were more active [1.99 (0.24)] compared with alcohol [1.25 (0.20)], prenatal stress [0.97 (0.28)], and control monkeys [1.25 (0.21),  $F(1,33) = 4.61$ ,  $p = 0.04$ ]. There was a significant main effect of prenatal stress for response inhibition, showing lower response inhibition (less restraint) in the

prenatal stress subjects [0.16 (0.04)] compared with 0.31 (0.13) for alcohol plus stress [0.68 (0.14) for alcohol and 0.93 (0.17) for control monkeys;  $F(1,33) = 15.68$ ,  $p = 0.0004$ ].

#### Correlations Among Measures

To investigate potential patterns of individual differences, behavioral scores were correlated by using Spearman rank correlations. Table 4 shows the correlations between neonatal measures of orientation and motor maturity, trials to criterion on NMS I, and behavioral ratings of irritability, activity, stereotypies, inhibition, and impulsivity during testing. Significant associations between trials to criterion on all five behavioral categories—irritability, activity, stereotypies, inhibition, and impulsivity—were detected. More trials to criterion (slower acquisition learning) were associated with more behavioral irritability, more activity, more stereotypies, and more impulsivity. This pattern of correlations portrays an animal that has difficulty learning and is impulsive, as well as irritable and active, and that shows the abnormal behavior of stereotypies.

#### Developmental Continuity

The correlations in Table 4 also show some continuity in developmental deficits from the neonatal period to adolescence. Higher scores on the neonatal neurobehavioral measures of orientation (reflecting attention) and motor maturity were associated with fewer trials to reach criterion on NMS I.

#### NMS II: Memory Task

After the monkey reached the criterion of 90% correct performance on NMS I, a 2-week break occurred, and the NMS I task was administered again until the monkey again re-achieved 90% correct performance (NMS II). There were no significant effects on trials to criterion for prenatal alcohol or prenatal stress [ $F(1,33) = 0.96$ ,  $p = 0.33$  and  $F(1,33) = 0.032$ ,  $p = 0.86$ , respectively]. There was a trend for an alcohol  $\times$  prenatal stress interaction, with alcohol plus prenatal stress-exposed monkeys tending to take longer to reach criterion [ $F(1,33) = 2.89$ ,  $p = 0.099$ ].

#### NMS II: Behavioral Observations

There were no multivariate significant effects for alcohol or stress exposure for irritability, activity, stereotypies, inhibition, and impulsivity [ $F(5,29) = 0.843$ ,  $p = 0.53$  and  $F(5,29) = 1.83$ ,  $p = 0.14$ , respectively]. There was, however, a significant multivariate alcohol  $\times$  prenatal stress interaction [ $F(5,29) = 3.18$ ,  $p = 0.02$ ]. Univariate analyses indicated a significant alcohol  $\times$  prenatal stress interaction for both stereotypies [ $F(1,33) = 14.68$ ,  $p = 0.0005$ ] and activity [ $F(1,33) = 6.7$ ,  $p = 0.02$ ]. As in NMS I, these interactions were due to higher stereotypies and activity in the alcohol

**Table 3.** Ratings During Learning Task Performance Groups

Tasks	Alcohol + stress	Alcohol	Stress	Controls
NMS I				
Activity	1.99 (0.24)	1.25 (0.20)	.975 (0.28)	1.25 (0.21)
Stereotypies	1.55 (0.35)	0.37 (0.08)	0.14 (0.07)	.73 (0.19)
Inhibition	0.31 (0.13)	0.68 (0.14)	0.16 (.04)	0.93 (0.17)
NMS II				
Activity	2.11 (0.22)	1.2 (0.26)	0.98 (0.19)	1.34 (0.23)
Stereotypies	1.55 (0.38)	0.12 (0.09)	0.21 (0.11)	0.73 (0.24)
Inhibition	0.21 (0.09)	0.53 (0.20)	0.03 (0.02)	0.66 (0.18)
NMS 60-sec delay				
Irritability	1.5 (0.44)	1.56 (0.36)	0.75 (0.40)	0.66 (0.21)
Stereotypies	1.45 (0.34)	0.11 (0.11)	0 (0)	0.79 (0.32)
NMS 120-sec delay				
Irritability	1.5 (0.26)	1.64 (0.29)	0.78 (0.37)	0.98 (0.22)
Inhibition	0.16 (0.10)	0.58 (0.25)	0.09 (0.07)	0.59 (0.13)
Activity	1.75 (0.25)	1.11 (0.29)	0.69 (0.22)	1.18 (0.23)
Stereotypies	1.06 (0.09)	0.33 (0.22)	0.12 (0.08)	0.93 (0.34)

Numbers in parentheses are SEs.

**Table 4.** Spearman Rank Correlations Among Neonatal Scores, NMS I Performance, and Behavioral Ratings

Item	Orientation	Motor maturity	Trials	Irritability	Activity	Stereotypies	Inhibition
Orientation							
Motor maturity	0.44**						
Trials to criterion	-0.34*	-0.38*					
Irritability	-0.29	-0.09	0.40*				
Activity	-0.02	-0.08	0.38*	0.12			
Stereotypies	0.13	0.01	0.42**	0.09	0.84**		
Inhibition	0.20	0.10	-0.19	0.04	-0.33*	-0.06	
Impulsivity	0.04	0.14	0.33*	0.40*	0.49**	0.45**	-0.24

\*  $p < 0.05$ ; \*\*  $p < 0.01$ .

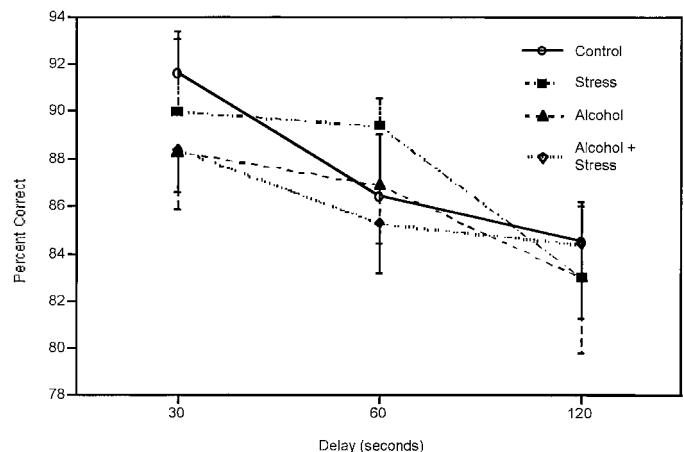
plus prenatal stress monkeys compared with the others. Also, there was a univariate effect of stress for behavioral inhibition that showed that subjects from the prenatal stress condition were lower on behavioral inhibition than subjects from the no prenatal stress condition [ $F(1,33) = 7.1, p = 0.02$ ].

*NMS III: 30-, 60-, and 120-sec Delays*

The mean percentage of correct responses made per delay (30-, 60-, and 120-sec delays) are portrayed in Fig. 2. There were no significant main effects for prenatal alcohol or prenatal stress, and there was no alcohol  $\times$  prenatal stress interaction at any of the delays (all  $p > 0.30$ ). As expected, the percentage of correct responses decreased as a function of the length of delay [90% at 30 sec, 87% at 60 sec, and 83% at 120 sec;  $F(2,64) = 7.7, p = 0.001$ ].

*Behavioral Observations on NMS III: 30-, 60-, and 120-sec Delays*

There were no significant multivariate effects for alcohol, prenatal stress, or alcohol  $\times$  prenatal stress interactions for behaviors during testing at 30 sec. At the 60-sec delay, there was a significant multivariate alcohol  $\times$  prenatal stress interaction [ $F(5,28) = 2.85, p = 0.04$ ] for the behavioral measures. Univariate analyses indicated that, as in NMS I and II, the monkeys from the alcohol plus stress prenatal condition exhibited more stereotypies in NMS III [ $F(1,32) = 13.81, p = 0.0008$ ]. Univariate analyses also showed that alcohol-exposed



**Fig. 2.** Performance on the delayed nonmatching-to-sample task of the study is shown. Data are plotted as the percentage of correct responses at each delay, collapsed across days. There were no differences across groups for any of the delays ( $p > 0.30$ ). The percentage of correct responses decreased as a function of length of delay ( $p < 0.001$ ). Bars represent SEs.

monkeys were more irritable [ $F(1,32) = 4.79, p = 0.04$ ] and that stress-exposed monkeys were less inhibited [ $F(1,32) = 6.8, p = 0.02$ ; Table 3]. There were no multivariate effects for alcohol, prenatal stress, or alcohol  $\times$  prenatal stress at the 120-sec delay; however, univariate analyses at the 120-sec delay showed main effects such that alcohol-exposed monkeys were more irritable [ $F(1,32) = 4.8, p = 0.04$ ] and stress-exposed monkeys were less inhibited [ $F(1,32) = 7.5, p = 0.01$ ]. There were significant alcohol  $\times$  prenatal stress interactions

such that alcohol plus stress-exposed monkeys exhibited more stereotypies [ $F(1,32) = 8.7, p = 0.006$ ] and were more active [ $F(1,32) = 6.8, p = 0.02$ ].

## DISCUSSION

This study adds to the growing body of evidence that suggests that prenatal alcohol exposure, even at moderate levels, is associated with poorer offspring cognitive task acquisition and behavioral disturbances. Moreover, the finding that poorer task acquisition on certain cognitive measures was correlated with a number of abnormal behaviors raises the possibility that fetal alcohol exposure, especially within the context of prenatal stress, may produce a behavioral disturbance that could interfere with the acquisition of certain types of problem-solving tasks.

The principal findings of this study were the following. (1) Prenatal alcohol-exposed monkeys, in comparison with non-alcohol-exposed monkeys, exhibited impairments in the acquisition of an NMS task. They were also rated more irritable during the NMS 60-sec and 120-sec delay tasks than non-alcohol-exposed monkeys. (2) The combination of prenatal alcohol plus stress yielded monkeys that were rated high on stereotypies and activity level for four of five tasks compared with the other three groups. (3) Prenatally stressed monkeys, compared with nonstressed monkeys, did not show decrements on acquisition or memory tests of NMS. They were rated lower on response inhibition (less restrained) on four of five tasks. (4) Higher scores on irritability, activity, stereotypies, and impulsivity were correlated with poorer performance (more trials to criterion) on NMS task acquisition. Finally, (5) lower scores on neonatal orientation (reflecting poor attention) and motor maturity were correlated with poorer performance (more trials to criterion) on NMS task acquisition during adolescence.

One interpretation of the results is that the differences in trials to criterion are due to differences in attention produced by the prenatal alcohol treatment. The overall picture, however, is that poor NMS performance is related to overall poor behavioral regulation, not just attention. The correlations suggest that higher irritability and poor control of activity level or disordered behaviors (stereotypies) all may be playing a role in interfering with performance on the NMS task.

Because there were acquisition deficits in the prenatal alcohol-exposed monkeys, but not deficits in overall performance or relearning of the task (NMS-II) or performing under longer delays after learning to criterion (NMS-III), the deficit may be in the ability to initially acquire tasks with the nonmatching/reward principle (Mishkin et al., 1984). The acquisition deficit does not seem to result from a straightforward impairment in holding the sample objects in memory, because once the principle was acquired, performance was essentially equivalent at all delays (30, 60, and 120 sec) in NMS-III. A similar pattern of deficits in

learning, but not in retention, was reported in children exposed to alcohol in utero and in adults with FAS (Kerns et al., 1997; Mattson et al., 1996a). Other research with humans supports the suggestion that difficulties in encoding or learning of information are affected by prenatal alcohol exposure. Coles et al. (1997) compared children who had FAS or FAE with children with attention-deficit/hyperactivity disorder and found that children with FAS or FAE had difficulty encoding the information they attended to and had difficulty using new information in a meaningful way to develop a rule to solve problems.

Our data suggest that prenatal alcohol exposure affects either how individuals approach problem solution (learning of rules) or their ability to attend or regulate behavior, rather than how they perform once the general problem and probable solutions are understood. A similar effect was reported by Kraemer and Bachevalier (1998) in comparing acquisition of NMS in maternally reared versus maternally deprived monkeys. In this study, groups of monkeys with different early rearing conditions differed in their rate of acquisition and eventual performance of NMS. They did not differ on performance reduction on NMS at 10-, 30-, 60-, and 120-sec delays. It had been demonstrated earlier in this study, however, that monkeys with different early rearing conditions did not differ on acquisition of a task requiring learning of a list of object discriminations or later spatial learning. This suggested that the acquisition differences observed on the NMS task might be peculiar to NMS and not to attentional or behavior differences that also distinguished these groups.

The overall conclusion is that an "attentional" problem most likely contributes to impaired acquisition of the NMS rule in prenatally alcohol-exposed monkeys. Nonetheless, there are examples where, due to an earlier exposure to stress or different rearing conditions, there may be a deficit in NMS acquisition but not on other WGTA tasks also requiring attention (e.g., spatial and list-learning tasks). Therefore, the idea that prenatal alcohol exposure might have an effect on brain mechanisms recruited by NMS problems cannot be ruled out either.

The exact identification of neural dysfunction underlying the NMS acquisition deficit we observed has yet to be determined. A candidate mechanism for the neuropathological basis of FAE, extrapolated from primate localization studies on NMS performance, includes the cortico-limbo-diencephalic circuit. This is based on the notion that to perform NMS, the monkey must discriminate the more difficult cue of the object's familiarity (or novelty) from the more obvious cue of the object's physical characteristics (Bachevalier, 1990). It is interesting to note that Clarren et al. (1992) found that infant monkeys exposed to alcohol prenatally failed to show the typical preference for novelty on tests of visual recognition. Such a difference could account for initial differences in acquisition because a lack of preference for novelty would compromise initial correct choices in solving the NMS problem.



Other researchers have focused on the requirement that to use the nonmatching rule, the subject must have the ability to shift the choice of objects after the first presentation. Shifting choice is a function that requires behavioral flexibility and is specific to the prefrontal cortex (Mirsky, 1996). Petrides (1994) emphasized the role of the frontal cortex and the basal ganglia in tasks that require shifting responses or cognitive flexibility. Reduced basal ganglia functioning may be a candidate mechanism, on the basis of findings by Mattson et al. (1996b), of significant reductions in the volume of basal ganglia of children with FAS, after controlling for brain size. Animal studies have found that caudate lesions (the caudate nucleus is part of the basal ganglia) produced perseveration and difficulty shifting responses (Cote and Crutcher, 1991). Thus it is possible that altered function of basal ganglia could relate to some of the behavioral disturbances and related acquisition impairments on NMS seen in the fetal-alcohol-exposed monkeys. Moreover, the findings of persistent increased abnormal behaviors, such as stereotypies and hyperactivity in the prenatal-alcohol- plus stress-exposed monkeys, suggest that the combination of alcohol and stress exposure might be more damaging in terms of deviant behavior than either prenatal alcohol exposure or prenatal stress alone.

Still other researchers have suggested that prenatal exposure to alcohol can lead to mental health problems in humans (Roebuck et al., 1999; Streissguth et al., 1996). Rodier (1998) made a strong argument that neuroteratologists should study human clinical syndromes, such as autism, bipolar disorder, and obsessive-compulsive disorder. Although generalizations from monkey studies to human studies must be made with caution, it is interesting to note that the closest similarity to the behavioral abnormalities noted in the prenatal stress plus alcohol monkeys reported here fall into the domain of the autistic spectrum disorders. Such disorders are associated with restricted, repetitive, and stereotyped patterns of behavior, including stereotyped repetitive motor mannerisms (DSM-IV; American Psychiatric Association, 1994). It is also interesting to note that Aronson et al. (1997) followed 24 children born to mothers who had abused alcohol throughout pregnancy and reported that 10 had attention-deficit/hyperactivity disorder, 2 had Asperger syndrome, and 1 had an autistic-like condition that failed to meet the criteria for Asperger syndrome. Moreover, Steinhausen and Spohr (1995) found a high occurrence of psychiatric symptoms in children and adolescents with FAS, including abnormal habits and stereotypies.

Our data suggest that reduced attention early in life may be associated with later deficits in acquisition of a simple learning task and rule—"choose the object that is different from that seen before." The performance of the monkeys on early measures of orientation (during the first month of life) correlated with the number of trials to criterion on NMS. These findings provide experimental confirmation of reports in fetal-alcohol-exposed children of decrements in

attention as early as the first day of life (Streissguth et al., 1983) and also confirm that the deficits in attention persist through 10 years of life (Streissguth et al., 1995). If confirmed by further research, our findings suggest that early measures of attention might be useful for identifying fetal-alcohol-exposed children at risk for later cognitive deficits. This finding is promising, because early intervention services could be used from birth, taking advantage of early brain plasticity and the potential role of the environment in modifying brain development (Hannigan et al., 1993; Klintsova et al., 1998; Weinberg et al., 1995).

In summary, our results suggest that prenatal alcohol exposure, even at moderate levels, is associated with acquisition decrements on an NMS task and increased behavioral disturbances, such as increased irritability, stereotypies, and activity, in monkeys. Future studies on these animals, with noninvasive neuroimaging studies, will provide critical information on whether these task acquisition and behavioral deficits are associated with underlying neural function changes, such as alterations in neurotransmitter synthesis, receptor binding availability, or volume of different brain structures. This information could suggest potential pharmacological treatments for individuals with deficits as a consequence of fetal alcohol exposure.

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