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Ethanol Self-Administration and Motor Deficits in Adult C57BL/6J Mice Exposed Prenatally to Cocaine

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KELLEY, B. M. AND L. D. MIDDAUGH. *Ethanol self-administration and motor deficits in adult C57BL/6J mice exposed prenatally to cocaine.* PHARMACOL BIOCHEM BEHAV **55**(4) 575–584, 1996.— Daily injections of 10 mg/kg cocaine on gestation days 12–18 did not alter maternal weight gain or offspring birth weight, viability, growth, or adult weight compared to saline controls. Adult male and female offspring were food deprived and trained to lever press for ethanol. Responding on an FR2 schedule and ethanol intake (g/kg) were recorded as measures of the reinforcing effects of ethanol. Lever press duration was used to assess motor performance. Results demonstrate that C57 mice will work for and consume large quantities of ethanol and that prenatal cocaine exposure increased the amounts ingested by both male and female mice. Prenatal-cocaine-exposed males also exhibited motor deficits as indicated by longer response duration times compared to controls. The consumption of large amounts of ethanol exacerbated the motor impairment in prenatal-cocaine males and revealed such deficits in cocaine females. The present results demonstrate that maternal cocaine exposure, at doses having no observable effect upon pregnancy, birth, or offspring growth, can increase the consumption of ethanol and enhance its motor impairing effects on fully mature offspring. **Copyright © 1996 Elsevier Science Inc.**

C57BL/6J Mice Prenatal cocaine Ethanol self-administration Ethanol effects

COCAINE use in general has declined during the last 10 years; however, it is estimated that nearly 50 million Americans (that is one in four) have used cocaine and more than 6 million use cocaine on a regular basis (14). Surveys from around the United States suggest that 5% to 17% of pregnant women use cocaine (10,15,27). The extent of the problem is suggested by a survey of New York City hospitals which indicates that during the early 1980's about 3,000 infants per year were born dependent on "crack" at a perinatal health cost of $48 million (40). While a number of the complications initially associated with prenatal cocaine exposure in humans have not been replicated (e.g., SIDS, 37), low birth weights, small head circumference, and genitourinary malformations have held up under the rigors of more tightly controlled studies (4,15,26,27). Follow-up studies of children exposed prenatally to cocaine beyond early development are few, and the presence of multiple confounding variables (i.e., perinatal, genetic, biological, and environmental factors) has generally prohibited definitive conclusions regarding the degree to which cocaine acts as a developmental toxicant. Research with animals avoids many of the confounds of human studies and in general supports the abnormalities observed for humans during gestation, birth, and the early neonatal period. Furthermore, animal models have allowed investigation of the long-term consequences of prenatal cocaine exposure as well as the potential mechanisms for the behavioral effects (For review see 18,43).

Several studies indicate that prenatal cocaine exposure can alter neurotransmitter systems which are important in the effects of several drugs of abuse. Behavioral pharmacological and neurochemical studies indicate that prenatal cocaine exposure can result in suboptimal functioning of the dopaminergic (DA) system of mice (6), rats (30,31,41,47,52), and rabbits (54) as well as the opioid systems of rats (13,29) and mice (39). Since these systems are important in many of the effects of psychoactive drugs, it is reasonable to hypothesize that prenatal cocaine exposure will produce long-term alterations in the effects of these drugs, including their reinforcing effects. The latter might in turn influence the degree to which these drugs are self-administered.

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The possibility that prenatal cocaine exposure might alter the self-administration of various abused drugs in adulthood has received little attention in either the clinical or the animal literature; however, two studies provide indirect evidence that such exposure might alter the self-administration of cocaine in adulthood. One of the studies indicated that male rats prenatally exposed to cocaine were less sensitive to its discriminative stimulus effects, an effect which was unrelated to the transport of the drug to the brain or to its locomotor stimulant effects (33). The second study indicated that prenatal-cocaine-exposed adult offspring did not develop a conditioned place preference to cocaine associated stimuli under conditions favorable for the control groups (32). The results of either of these studies suggest the possibility that prenatal cocaine exposure could alter the self-administration of the drug in adult animals.

While study of the effects of prenatal cocaine exposure on the sensitivity to cocaine during adulthood is important, from a population perspective the effect of prenatal cocaine exposure on ethanol sensitivity in adulthood might be more important since it is one of the most widely used drugs in the United States. The overuse of ethanol is indicated by the fact that alcoholism has a 15% lifetime prevalence rate in the United States with the disease impacting approximately 15 million people at an annual national cost of about $100 billion (1).

Because prenatal cocaine exposure appears to alter the dopaminergic and opioid systems, and because these systems are important in mediating the rewarding effects of ethanol (34–36,53), as well as its effects on motor systems (5,45,48), it is important to determine if such prenatal exposure might alter the reinforcing and motoric effects of ethanol. The reinforcing or rewarding effects of psychoactive drugs are commonly assessed in laboratory animals using operant procedures in which the animal is required to perform some behavioral response to obtain the drug.

In the present experiment, we examined the effect prenatal cocaine exposure had on responding for access to ethanol, on ethanol consumption, and on fine motor performance in the absence and presence of ethanol in adult male and female C57BL/6J (C57) mice. This particular strain of mice was used because, in contrast to many rodents, C57 mice have a strong preference for ethanol. An operant procedure with ethanol as a reward was used because the procedure allowed an oral route of administration, precise control of environmental factors, and the possibility of maintaining significant levels of ethanol intake. In addition, this procedure is uniquely suited for simultaneously measuring response duration which is an indicant of fine motor performance. The particular procedure used in our study was adapted from previous reports utilizing C57 mice (20,28) and essentially uses food-induced drinking (prandial drinking). The procedure results in the consumption of pharmacologically relevant amounts of ethanol thus insures that the mice are exposed to its interoceptive effects.

The purpose of this study was to examine the reinforcing efficacy of ethanol in C57 mice prenatally exposed to cocaine and was the first investigation into the potential effects of prenatal cocaine exposure on ethanol self-administration and its effects on motor behavior.

METHODS AND PROCEDURES

Subjects

Thirty-three adult C57 male and female mice (Jackson Laboratory, Bar Harbor, MN) weighing 25-28 g and approximately 10–11 weeks old at the beginning of the experiment served as subjects. They were offspring of dams (no more than 1 male and female per litter) injected subcutaneously daily with either 10 mg/kg cocaine (C10, 9 females and 7 males) or saline (SAL, 9 females and 8 males) during gestation days 12 - 18, the period of prenatal development of DA systems (42,46). Cocaine HCl was obtained from NIDA Drug Supply Batch #7890-1022-131B, Research Triangle Institute, Research Triangle Park, NC. No pair-fed saline-injected group was used in this experiment because previous experiments (See 44) indicated that a 20 mg/kg dose of cocaine reduced food consumption only on the first day of exposure, had no effect on the dam's weight gain, and had no effect on the pups birth weight. The offspring were fostered to untreated dams on postnatal day one and remained with them until 35 days of age. At weaning, the mice were separated by sex and were maintained 4 per cage until the onset of this study when they were housed individually. The colony room was temperature controlled (22 ± 1°C) with a 12 h light-dark cycle (0700–1900 light on) and subjects were given free access to laboratory chow and water prior to initiation of the experiment.

Apparatus

Three operant-conditioning chambers were used for this study. These boxes were both sound attenuated and light controlled. The chambers (16 × 16 × 11.4 cm) were constructed of gray opaque Plexiglas with a transparent door and a stainless steel grid floor. A fountain, lights, and a lever were all located on the left wall. Located 4.1 cm to the left of the lever was a 2 × 3 cm opening to an enclosure which housed a 1.1 mm diameter spout that was used to deliver a small droplet of liquid in response to a lick. Electronic circuitry sensed a small current (<5 mAMP) traveling from the brass spout, through the subject's body to the grounded cage floor. As the tongue contacted the spout tip, a solenoid valve opened briefly to deliver a droplet of liquid from reservoirs mounted at equal heights on the outside of each operant chamber. A white house light located 8 cm above the floor and 4 cm to the left of the opening remained on when no reinforcer was available. Termination of the house light and illumination of a red stimulus light mounted outside the operant chamber just above the opening to the fountain indicated that the reinforcer was available. The number of lever-presses, volume consumed, and the lever-press duration (total time the lever was depressed/total number of responses) were recorded during each session.

Procedure

The procedure was divided into the five phases described in detail below. In general, Phase I (4 weeks) involved acclimating the mice to the testing procedure and training them to lever press for liquid reinforcement. During Phase II (6 weeks), the animals were trained to respond for various ethanol concentrations, which insured that they experienced the drug's interoceptive effects. Ethyl alcohol was obtained from the MUSC hospital pharmacy. Phase III (1 week) was to examine the stability of the dependent vari ables across days of the week. Finally Phases IV and V (6 days each) allowed for examination of the reinforcing effects and motor effects of ethanol under different motivational states by testing the mice after their daily food allotment as in previous phases (Phase IV) or prior to feeding (Phase V).

Phase I: Familiarization and weight reduction. At about

9–10 weeks of age, the mice were individually housed, and were allowed a one week period to acclimate to their new cage during which time food and water were available ad lib. At the end of that week, the mice were weighed to establish their ad lib body weight. Food was restricted over the course of a week reducing body weights to 80% of their ad lib levels. Operant training began while the subjects' body weights were being reduced. On Day 1 and Day 2, the mice were placed in the operant chamber for five minutes with the house-lights illuminated without the response levers present. During the next three sessions, each subject was placed in the operant chamber for 30 min. During this time, termination of the house-light and illumination of the red stimulus light signaled that each lick at the spout would produce a droplet of water. To facilitate drinking on Day 3, mice were deprived of water for 23 hours prior to their training session and their daily food allotment was given 1 h before the 30-min session. For the remainder of this week, the mice had water available except during their daily food allotment 60 min prior to the beginning of the training session. Beginning the next week, mice had access to the response lever and all subjects were run on a Fixed Ratio (FR) 1 schedule such that each lever press activated the fountain for ten seconds. For the following week the fixed ratio was increased from FR1 to FR2, and all other variables remained the same.

Phase II: Ethanol concentration assessment. During the next phase of operant training, the subjects responded on a FR2 schedule for various ethanol concentrations to determine the concentration which best functioned as a reinforcer. Ethanol was introduced at 0% during the first week and increased to 3%, 6%, 9%, 12%, and 15%, respectively during the next five weeks (ethanol concentrations reflect a v/v relationship). In order to facilitate ethanol consumption and to overcome the possible aversive taste qualities of ethanol, mice were water deprived for 22.5 hours prior to the first test session for each new concentration of ethanol. During the next four days, the animals were given water ad lib for 22.5 hours a day and their food was provided 1 h prior to the start of the daily operant session.

Phase III: Stability of responding for 12% ethanol. After the weekly sequence of operant testing under increasing ethanol concentrations, mice were retested with 12% ethanol for five consecutive days under the conditions noted in Phase II except that they were not water deprived for 23 hours prior to the Monday test. Thus, each daily session was identical. The purpose of this phase was to establish the stability of lever responding, ethanol intake, and response duration across daily test sessions.

Phases IV and V: Pre- and post-test feeding. During the next two weeks mice were tested daily with ascending concentrations (0, 3, 6, 9, 12, and 15%) of ethanol (i.e., 0% on Monday and 15% on Saturday). During the first week (Phase IV; Pre-Test Feeding), the mice were fed 60 min prior to the operant session without water available. For the second week (Phase V; Post-Test Feeding), the mice were fed their daily food allotment after the operant session and water was available ad lib.

Data analysis. Each data point in this experiment represents only one litter; however, because only some of the litters were represented by offspring of both sexes, the sex factor was mixed with regard to between vs within group variance. In order to maintain the litter as the unit of analysis, data from the two sexes were analyzed separately. Thus, the effects of prenatal cocaine were statistically evaluated within each gender; however, statistical evaluation of the possible effects of the sex factor or its interaction with prenatal condition was not possible. 2(Prenatal Condition) × 5 or 6(Ethanol Concentration) analyses of variance (ANOVA) were performed for each variable. Data with significant F values for the main factors were followed by Tukey's post hoc tests. Since the Ns for the study were relatively small and the power of interaction terms in ANOVA's is low (17), *F*- values for interaction terms with probabilities of $p < 0.10$ were accepted as significant. Data with significant interaction of the main factors were subjected to analyses of the simple main effects. Data for response duration were subject to a natural log transformation prior to data analysis to increase the homogeneity of variance between groups.

RESULTS

Maternal and Litter Data

The characteristics of the breeding group from which the subjects were obtained were unaffected by cocaine exposure. Maternal and litter outcome measures included percent weight gain during pregnancy for the dams, litter mortality (deaths per litter), and pup weight at birth (litter average). Student's t-tests comparing the SAL and C10 groups on each outcome measure revealed no significant differences for maternal weight gain ($\bar{x} \pm$ SEM %: SAL = 41.8 ± 0.9%; C10 = 42.2 ± 1.3%, $t(24) = 0.25$, $p > 0.100$); litter mortality ($\bar{x} \pm$ SEM dead pups/litter: SAL = 0.6 ± 0.2; C10 = 0.8 ± 0.3, $t(31) = 0.99$, $p > 0.100$); or pup weight ($\bar{x} \pm$ SEM g: SAL = 1.32 ± 0.02; C10 = 1.28 ± 0.02, $t(30) = 1.13$, $p > 0.100$). Additional comparisons of body weights prior to the beginning of operant training revealed no significant difference for either males ($\bar{x} \pm$ SEM g: SAL = 24.1 ± 0.4, C10 = 23.6 ± 0.4, $t(13) = 0.39$ $p > 0.100$) or females ($\bar{x} \pm$ SEM g: SAL = 18.5 ± 0.3, C10 = 18.9 ± 0.3, $t(16) = 0.35$ $p > 0.100$).

Ethanol concentration assessment at weekly intervals (Phase II data). Data for response frequency, ethanol intake, and response duration for this phase of the experiment are summarized respectively in the upper, middle, and lower panels of Fig. 1. To avoid the influence of the 23-hr water deprivation which preceded the first day of each new ethanol concentration, data for this phase of the experiment were restricted to the last three days for each concentration. Data collected during these three days were averaged and subjected to 2(Prenatal Condition) × 5 or 6(Ethanol Concentration) ANOVAs for each measure. The number of responses per session (a) tended to increase with increasing ethanol concentration and approached statistical significance for male mice [$F(5, 70) = 1.86$, $p = < 0.110$]; however, prenatal cocaine exposure did not alter responding across ethanol concentrations for either sex.

Ethanol intake (g/kg, Fig. 1b) varied across concentrations for both male [$F(4, 56) = 32.78$, $p < 0.001$] and female mice [$F(4, 64) = 6.72$, $p < 0.001$]. In addition, the change in ethanol intake by male mice across concentration interacted with prenatal condition [$F(4, 56) = 3.88$, $p < 0.008$]. Post hoc analyses on these data revealed a significant increase in intake for the prenatal cocaine but not saline mice when the ethanol concentration was increased from 3% to 6%. Peak ethanol consumption at the 9% concentration was significantly greater than at the 3% concentration for both groups. Ethanol intake of the male-saline group declined when the concentration was increased from 9% to 15%, while intake by the male-cocaine group did not change across these three concentrations. Finally, ethanol intake by male mice at the 15% concentration was significantly higher for the prenatal-cocaine-exposed mice

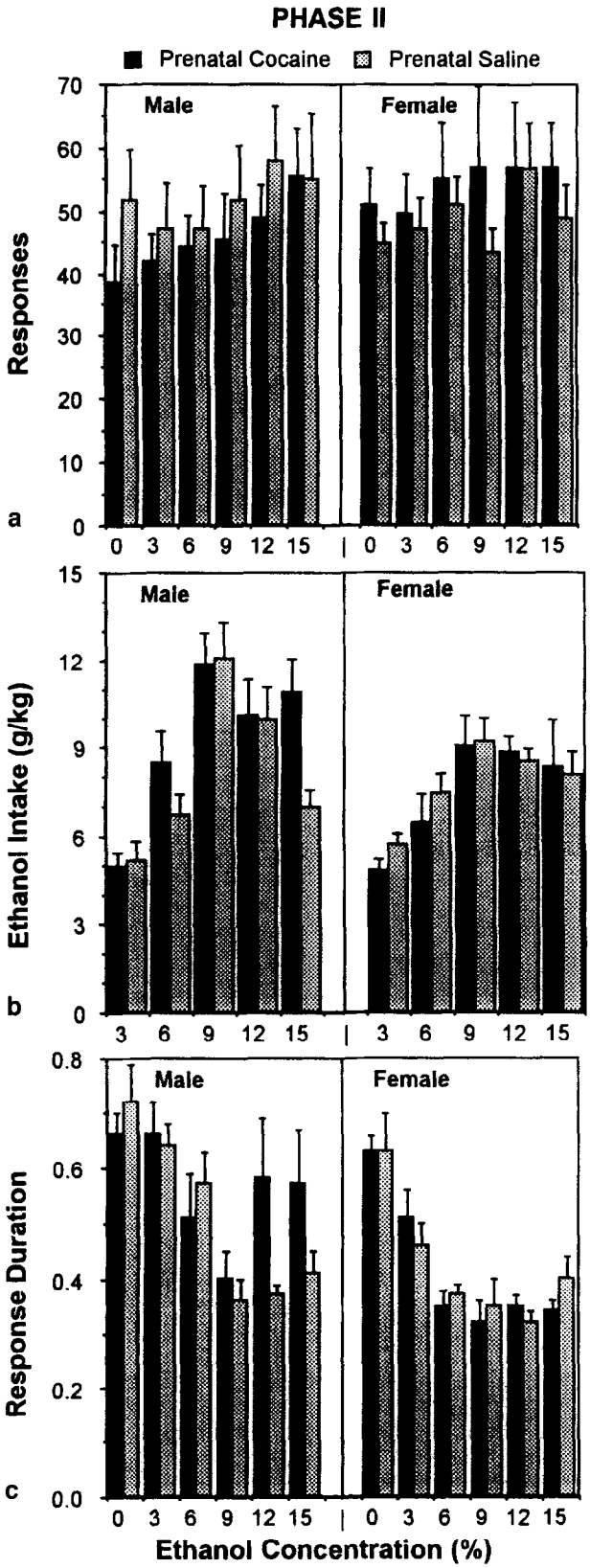

FIG. 1. Phase II: Total responding (a), ethanol intake [g/kg, (b)], and response duration [sec, (c)] during weekly tests with different ethanol concentrations as reward. Mice were fed without access to water for 60 min prior to the start of the operant session. Data represent 3-day averages for each ethanol concentration tested and are summarized as $\bar{x}$ SEM for each group.

than for the saline group. The observed increase in the amount of ethanol ingested by prenatal-cocaine-exposed mice compared to controls was not merely a reflection of group differences in fluid consumption since water consumption ($\bar{x}$ ± SEM μl/g) by prenatal-cocaine-exposed and control mice did not differ for either males (SAL: 214 ± 14.33, C10: 190 ± 10.1; $t(13) = 1.31, p > 0.100$) or females (SAL: 211.75 ± 11.65, C10: 205 ± 13.56; $t(16) = .33, p > 0.100$).

The ANOVAs on the response duration data (log transformed) indicated that duration varied as a function of ethanol concentration for both male [$F(5, 70) = 18.40, p < 0.001$] and female [$F(5, 80) = 25.81, p < 0.001$] mice (Fig. 1c). As noted for ethanol intake, the change across ethanol concentration interacted with prenatal condition [$F(5, 70) = 2.58, p < 0.034$]. Post hoc analysis confirmed the response duration reduction for males of both groups across the first four concentrations of ethanol. In contrast to the stable response duration for the saline males as ethanol concentration was increased from 9% to 15%, the cocaine males exhibited an increase in response duration on the 12% and 15% compared to the 9% ethanol concentration. In addition, at the 12% and 15% ethanol concentrations response duration was significantly higher for the prenatal cocaine males than for their controls.

Response stabilization at 12% ethanol (Phase III data). The ANOVAs revealed no significant differences in total responses, ethanol intake, or response duration across days of the week for either prenatal group of males or females. In addition, there were no significant interactions of Prenatal Condition with Days indicating that responding, ethanol intake, and response duration remained stable across days.

Daily ethanol concentration assessment: pre-test feeding (Phase IV data). Data for response frequency, ethanol intake, and response duration, during this phase of the experiment are summarized respectively in the upper, middle, and lower panels of Fig. 2. Data are daily values across the various ethanol concentrations and were analyzed with 2(Prenatal Conditions) × 5 or 6(Ethanol Concentrations) ANOVAs. For the male mice, response frequency was influenced by the interaction of ethanol concentration and prenatal condition [$F(5, 70) = 1.82, p < 0.100$]. Post hoc comparison supported the apparent reduction in responding for the prenatal cocaine group in comparison to the saline group at the 3% concentration of ethanol. The number of responses by females across the different ethanol concentrations was highly variable and the ANOVA of these data revealed no significant effects of Prenatal Condition, Ethanol Concentration, or their interaction.

Ethanol intake (b) varied as a function of ethanol concentration for males [$F(4, 56) = 62.74, p < 0.001$] and females [$F(4, 64) = 27.21, p < 0.001$] generally increasing with increasing concentration. Although the Prenatal Condition × Ethanol Concentration interaction did not reach significance for the male mice [$F(4, 56) = 1.70, p = 0.160$], mean ethanol consumption tended to be higher for prenatal-cocaine-exposed mice than for their saline controls at the low concentrations. For the females, prenatal-cocaine-exposed mice consumed more ethanol than the saline group [$F(1, 16) = 6.49, p < 0.022$]. As noted above for Phase II, water consumption (μl/g) was not influenced by prenatal cocaine for either males (SAL: 109 ±

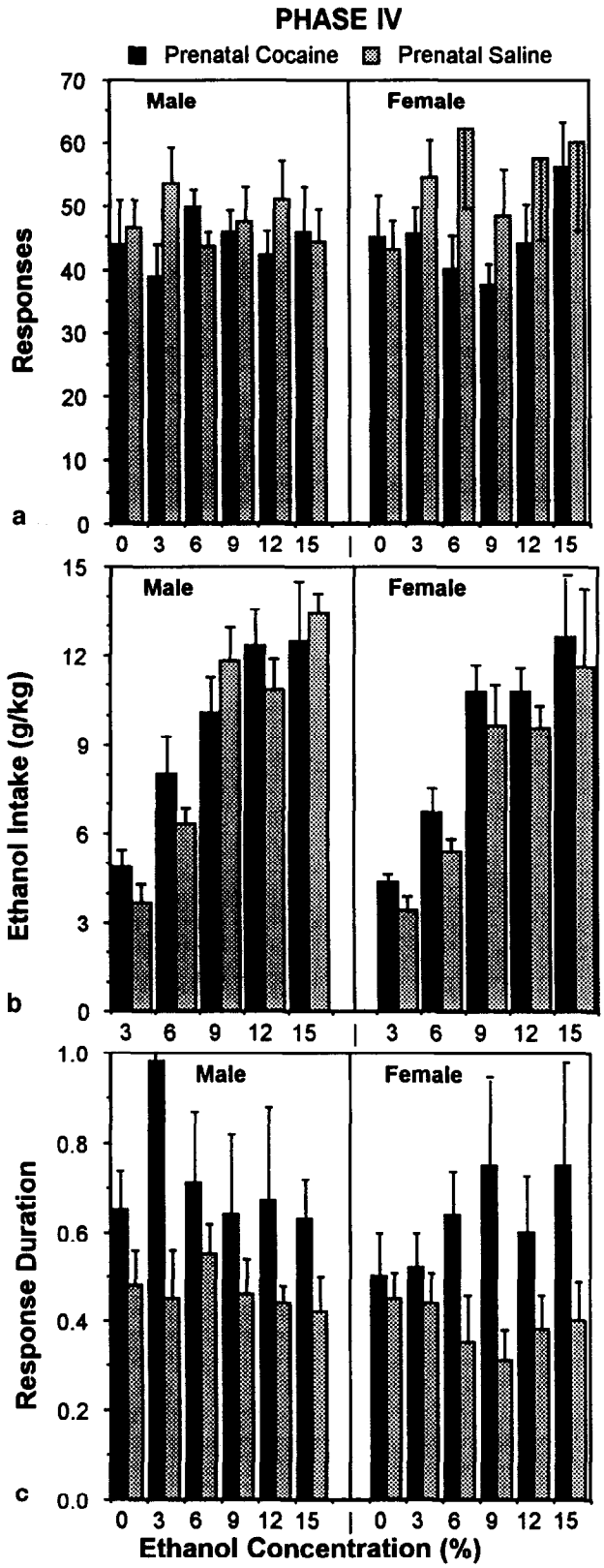

FIG. 2. Phase IV: Total responding (a), ethanol intake [g/kg, (b)], and response duration [(sec, (c)] during daily tests with different ethanol concentrations as reward under the conditions described for Fig. 1. Data represent daily values and are summarized as $\bar{x}$ SEM per group.

13.6, C10: 129 ± 19.8; $t(13) = .80, p > 0.100$) or females (SAL: 99 ± 15.23, C10: 107 ± 9.4; $t(16) = .42, p > 0.100$).

The response duration measure (c) during this phase of the experiment was influenced by prenatal condition and its interaction with ethanol concentration. For the males, response duration was greater for the prenatal cocaine group than for the saline group [$F(1, 14) = 5.37, p < 0.050$] and the elevation interacted with ethanol concentration [$F(5, 70) = 2.33, p < 0.052$]. This interaction was due to a reduction in response duration for cocaine animals from the abnormally high value at 3% ethanol as the concentration increased while the duration for the saline control groups remained stable across concentration. The ANOVA of the female duration data supported the higher duration values for prenatal cocaine exposed compared to controls suggested in Fig. 2 [$F(1, 16) = 4.58, p < 0.048$].

To further assess the influence of prenatal cocaine exposure and ethanol intake on motor systems, the pattern of response duration across the 30 min sessions with either water or 15% ethanol as the reinforcer was evaluated. These data are summarized in Fig. 3 and were subjected to 2(Prenatal Condition) × 6(5-Min Blocks) ANOVAs, one for each sex/ethanol concentration combination. While responding for water (a), response duration varied across the 5-min time blocks for both males [$F(5, 70) = 3.006, p < 0.015$] and females [$F(5, 80) = 4.76, p < 0.001$]. The apparent interaction of the time factor with prenatal cocaine approached significance for the males [$F(5, 70) = 1.68, p < 0.150$], with prenatal cocaine males exhibiting higher duration values. While responding for 15% ethanol (b), response duration also varied across the session for the males [$F(5, 70) = 2.61, p < 0.032$], and approached significance for the females [$F(5, 80) = 1.96, p < 0.094$]. The primary prenatal cocaine effect was an increase in response duration for the males compared with controls [$F(5, 70) = 6.24, p = < 0.025$].

The influence of prenatal cocaine exposure on the changes in response duration during ethanol vs water reinforcement was also assessed. This was accomplished by adding the 0% response duration value at each time point to the corresponding 15% ethanol value and then dividing the ethanol values by the totals (i.e., [15%/(0%+15%) x 100] at 5, 10, 15, 20, 25 and 30 min.). The resultant values reflect the contribution that the 15% ethanol concentration had on response duration across the session. If ethanol consumption contributed little to this measure, then the effect due to ethanol would be around 50% (i.e., 50% of the total duration value was accounted for by ethanol). On the other hand, if a greater percentage of the total response duration value was accounted for by ethanol, then it can be argued that ethanol influenced response duration. These analyses provide additional information about when and to what degree ethanol influenced response duration. The percent of the total response duration value accounted for by ethanol depended on the interaction of prenatal condition and time factors for both male [$F(5, 65) = 2.85, p < 0.022$] and female [$F(5, 85) = 1.90, p < 0.100$] offspring. The greater effects of ethanol on prenatal-cocaine-exposed mice was noted early in the session for the male mice and in the middle of the session for the female mice.

Daily ethanol assessment: post-test feeding (Phase V data). Figure 4 summarizes the data collected during the daily ethanol concentration tests when the animals were fed after rather than before the operant session. Data for this phase of the study were analyzed as described for Phase IV above. Visual inspection of Figs. 2 and 4 suggests that the animals generally responded more but consumed less ethanol when fed after

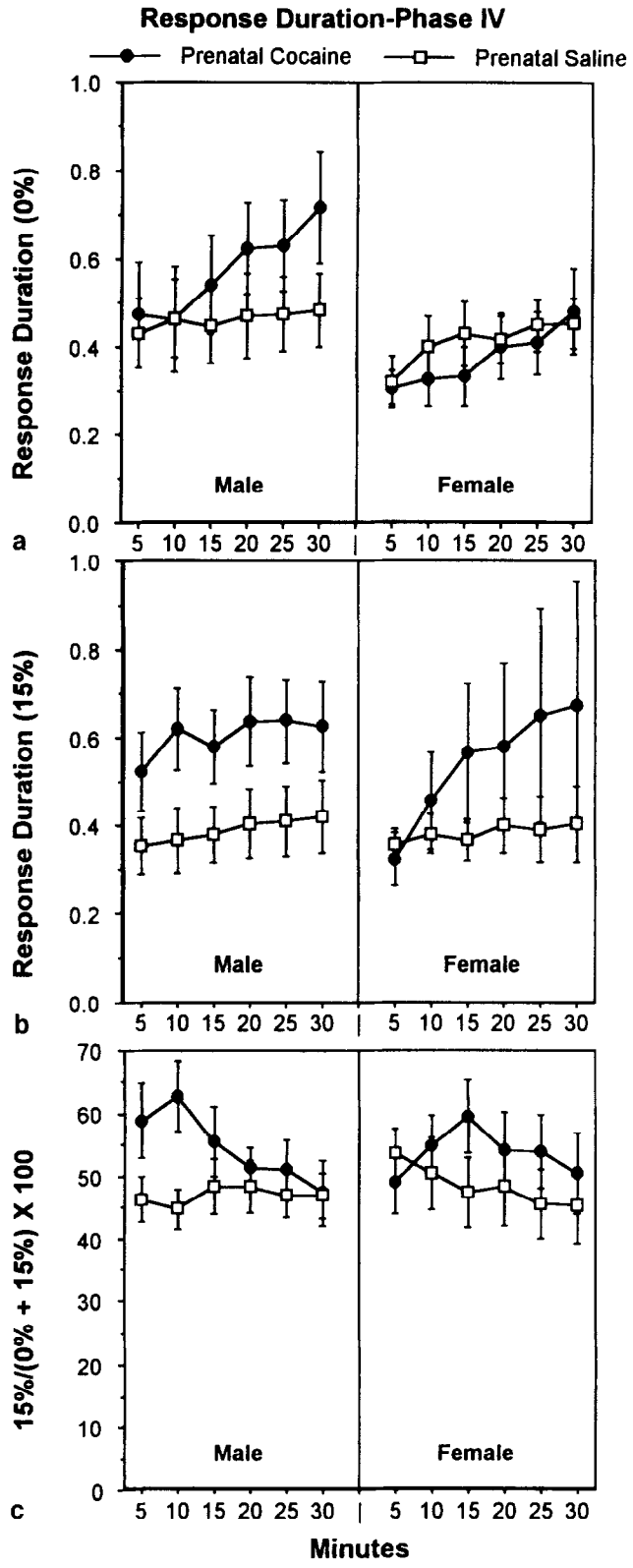

FIG. 3. Response duration (sec) at intervals across the 30 min sessions noted in Fig. 2 with water [0%, (a)] and 15% ethanol (b) as the reward. A comparison of response duration across the session under 0% and 15% ethanol reinforcement [15%/(0%+15%) × 100] is provided in (c).

(Phase V) rather than before (Phase IV) the operant tests. Although responding appeared to change across ethanol concentrations for male mice during this phase, this was not supported statistically [$F(5, 70) = 1.72$, $p < 0.120$]. For the females however responding declined with increasing ethanol concentration [$F(5, 80) = 3.09$, $p < 0.014$]. Prenatal cocaine had no significant effect on this measure for either male or female offspring.

Ethanol intake (b) during the post-session feeding tests varied across ethanol concentrations, generally increasing with increasing concentration for both male [$F(4, 48) = 14.28$, $p < 0.001$] and female mice [$F(4, 64) = 12.53$, $p < 0.001$]. As noted for responding, prenatal cocaine exposure did not influence ethanol ingestion, nor did it influence water intake for males (SAL: 36 ± 10.83; C10: 28 ± 4.67; $t(13) = .66$, $p > 0.100$) or females (SAL: 65 ± 17.52; C10: 59 ± 6.32, $t(16) .35$, $p > 0.100$).

Perhaps the most interesting finding during this phase of the experiment was that ethanol intake influenced response duration across test sessions for both males [$F(5, 70) = 6.75$, $p < 0.001$] and females [$F(5, 80) = 4.04$, $p < 0.003$].

As described for Phase IV, response duration data across the 30-min operant session were examined when responding was maintained by water and the 15% ethanol concentration (Fig. 5). When reinforced with water, response duration across the session depended on the interaction of time and prenatal condition for both male [$F(5, 60) = 2.99$, $p < 0.018$] and female mice [$F(5, 107) = 2.35$, $p < 0.050$]. Response duration was greater for the prenatal-cocaine-exposed mice than the control groups at all but the first time point for males and on the last two time points for females. When reinforced with 15% ethanol, response duration tended to decline across the session for both male [$F(5, 80) = 1.98$, $p < 0.090$] and female [$F(5, 80) = 2.92$, $p < 0.018$] mice; however, the measure was not influenced by prenatal cocaine.

The influence of ethanol on response duration was evaluated in terms of percent of the total response duration value (0%+15%) accounted for by ethanol as described in Phase IV. As noted in Phase IV, this response duration measure was influenced by ethanol as indicated by a reduction in the percent of the total response duration value for both male [$F(5, 60) = 2.97$, $p < 0.019$] and female [$F(5, 80) = 2.28$, $p < 0.054$] mice (Fig. 5). Prenatal cocaine exposure, however, did not influence the effects of ethanol on response duration under this feeding condition.

DISCUSSION

This study indicates that maternal cocaine exposure during fetal development of DA reward and motor systems can increase the consumption of ethanol and disrupt fine motor performance of fully mature offspring, the latter effect being exacerbated by ethanol consumption. These effects were observed to varying degrees in both male and female offspring and were produced by a cocaine dose (10 mg/kg) which had no readily observable effect on pregnancy, birth, or pup growth.

Prenatal cocaine effects on responding for ethanol reward. Both male and female mice responded for ethanol, and the number of responses during a session tended to vary with different ethanol concentrations. This measure, however, did not provide a systematic index of the reinforcing value of ethanol and was not influenced by prenatal cocaine exposure. Although not supported statistically, both male and female mice responded more for some concentrations of ethanol than for water. For example, male mice tended to respond more for 12% ethanol than for water during Phase II (Fig. 1a) and

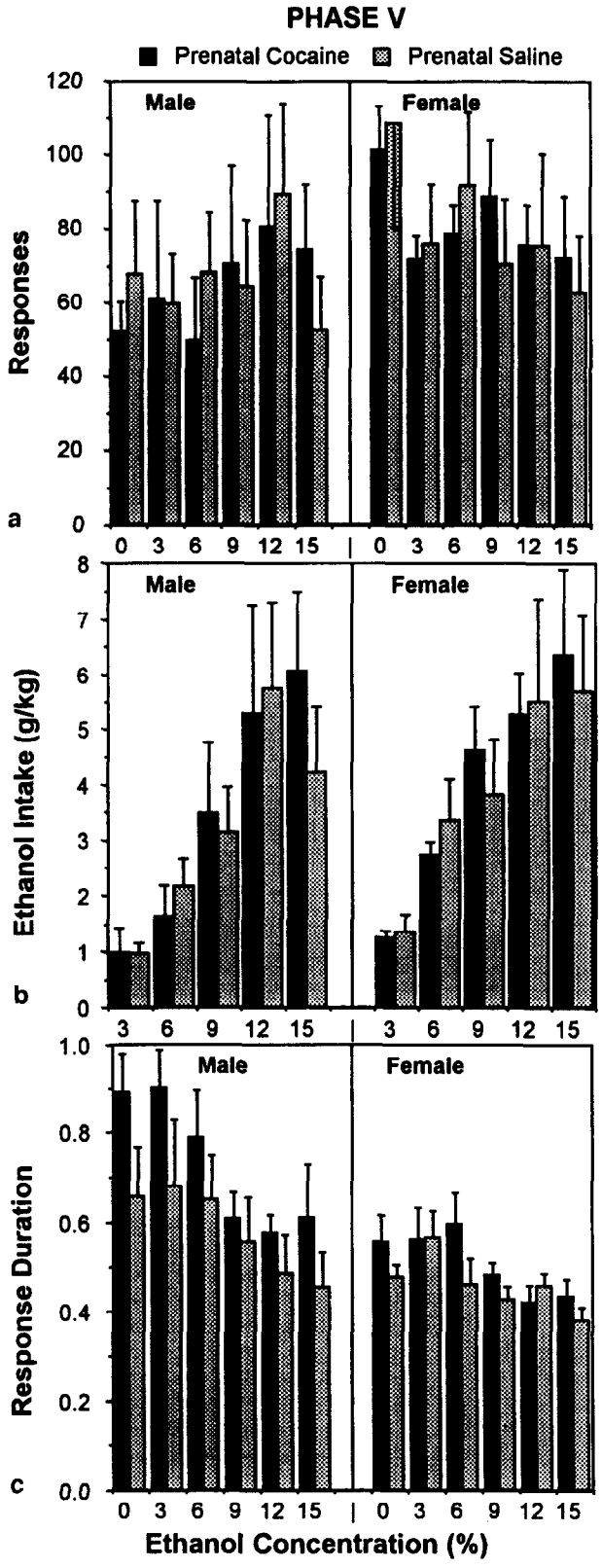

FIG. 4. Phase V: Total responding (a), ethanol intake [g/kg, (b)], and response duration [sec, (c)] during daily tests with different ethanol concentrations as reward. Mice were fed after termination of the operant session. Data summaries are as described for Fig. 2.

during Phase V (Fig. 5a). Females tended to respond more for 12% ethanol than for water when fed prior to the tests (Phases II and IV); however they responded less for ethanol than for water when fed after the tests (Phase V).

Two characteristics of the procedures used in our experiment might have contributed to the relative insensitivity of the response frequency measure to changes in ethanol concentration. First, the access to ethanol after completion of the fixed-ratio may have been too long, thus allowing the mice to consume a desired amount of ethanol with minimal lever-pressing. Second, the FR2 schedule may not have been demanding enough to reflect small changes in reinforcement efficacy. In spite of the marginal sensitivity of the response frequency measure, the fact that males increased their response output for relatively high concentrations of ethanol, and that ethanol maintained responding by females indicates that it can act as a positive reinforcer for either sex. These results confirm previous reports in which male C57 mice lever pressed for various concentrations of ethanol under similar operant conditions (21,22) and suggest that the performance of female mice for ethanol reward might differ from that of males. Additional studies in which the reinforcement schedule and the time allowed for access to ethanol are manipulated will be needed to establish its optimal reinforcing conditions for both sexes. Such studies will also be necessary to fully evaluate whether prenatal cocaine exposure alters ethanol's reinforcing efficacy.

Prenatal cocaine effects on the consumption of ethanol. The amount of ethanol consumed (g/kg) during a session provides another method for assessing its rewarding effects and this measure was influenced by prenatal cocaine exposure. When statistically significant differences in ethanol consumption were observed during the course of the experiment, prenatal-cocaine-exposed mice of either sex consumed more ethanol than their controls. The strongest evidence for increased ethanol consumption by prenatal-cocaine-exposed mice was noted in Phase II for males and Phase IV for females. During Phase II, male prenatal-cocaine-exposed mice increased their consumption of ethanol when its concentration was increased from 3% to 6%, a phenomenon not observed for control mice. In addition, they also consumed significantly more ethanol than controls at the highest concentration tested (15%). During Phase IV prenatal-cocaine-exposed female mice consumed more ethanol across the different concentrations than did their controls. Although the prenatal-cocaine-exposed mice did not consume more ethanol than controls under all of the ethanol conditions tested, under none of these conditions did they consume significantly less than saline control mice. Furthermore, their increased ethanol intake was observed across nine weeks of testing suggesting that the effect is long-lasting, perhaps even permanent.

Several possible mechanisms, either alone or in combination, could conceivably account for the increased ethanol consumption by prenatal-cocaine-exposed mice. In addition to our hypothesis that maternal cocaine exposure altered the development of fetal brain areas involved with reward, thus altering the rewarding value of ethanol, possible changes in food or water regulation, or in taste sensation might also be involved.

Possibly, prenatal-cocaine-exposed mice consumed more ethanol than their controls in order to compensate for a reduction in its reward value. Such an interpretation would be consistent with the previous reports that adult rats prenatally exposed to cocaine exhibited an attenuation of the interoceptive and the reinforcing properties of cocaine (32,33) and

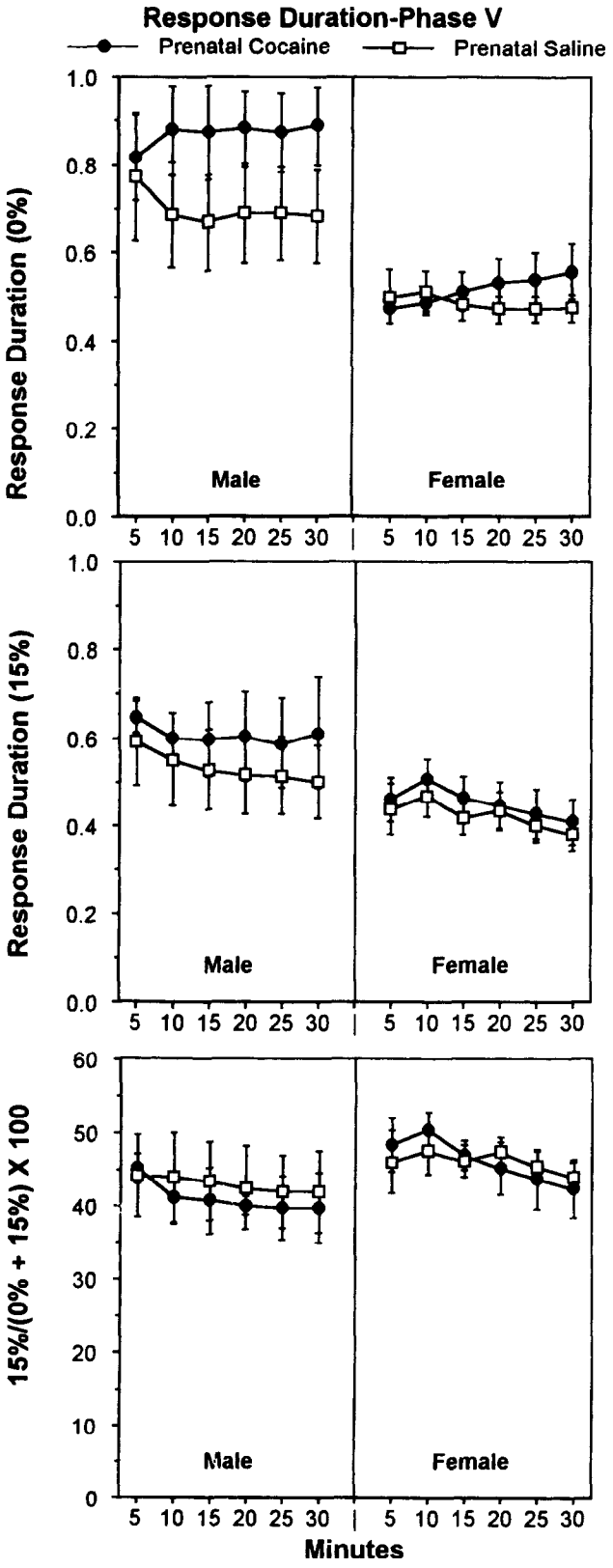

FIG. 5. Response duration (sec) at intervals across the 30 min sessions noted in Fig. 4 with water (0%, a) and 15% ethanol (b) as the reward. A comparison of response duration across the session under0% and 15% ethanol reinforcement [15%/(0%+15%) × 100] is provided in (c).

other reports that prenatal cocaine can alter DA (54) or opioid (29,39) systems which are involved with mediating reward (38).

It is unlikely that ethanol's caloric value alone could have accounted for the differences in ethanol intake observed between the two prenatal treatment groups. It is important to note that the prenatal-cocaine-exposed mice consumed more ethanol than controls only when the animals were tested after eating their daily food allotment, a time of low food motivation but high water motivation. Testing the mice prior to feeding, a condition of relatively high food but low water motivation, resulted in a reduction in the amount of ethanol consumed and eliminated the differences between prenatal-cocaine-exposed and control mice. The reduced consumption of ethanol when food motivation should be higher (i.e., tests prior to the daily food allotment) suggests that caloric need was an unlikely motivating factor. For a similar reason, altered caloric need is not a likely explanation for the increased ethanol consumption by prenatal-cocaine-exposed mice. Although the consumption of ethanol was enhanced by food deprivation, it should be noted that deprivation also enhances the reinforcing effects of several drug classes such as psychomotor stimulants, CNS depressants, opioids, and dissociative anesthetics (7–9,16). Recent literature also suggests that the caloric content of ethanol probably has less influence than other factors on its consumption and on its efficacy as a reinforcer. For example, food deprived rats will stop consuming ethanol (and its associated calories) when given an opportunity to consume a more palatable saccharine solution (no calories) and thereby suffer even further weight loss (49). Further, BALB/cJ mice, a strain known to avoid ethanol, did not lever press for an ethanol reward regardless of food deprivation level (23).

Although the consumption of ethanol in our experiment was higher when the mice were thirsty (prandial drinking following food), an increased need for fluids is an unlikely mechanism for the increased ethanol consumption by prenatal-cocaine-exposed mice for two reasons. First, prenatal-cocaine-exposed mice did not consume more than controls when water was the reinforcer; second they consumed more of only some concentrations of ethanol rather than all concentrations.

It remains a possibility that prenatal cocaine exposure attenuated the taste sensation of ethanol since the elevated consumption by prenatal-cocaine-exposed mice was at the higher ethanol concentrations when taste should have an increasingly dominant role. Since taste appears to be relevant to ethanol ingestion by humans, taste factors are of legitimate concern in animal models. A recent report indicated that C57BL/6ByJ (ethanol preferring) mice had greater preferences for ethanol, sucrose, and citric acid but had similar preferences for capsaicin and quinine compared to 129/J mice (ethanol non-preferring). These findings suggest that ethanol intake by C57 mice depends at least in part on the higher hedonic attractiveness of its sweet taste component (2).

Although the mechanism remains unclear, the present study indicates that prenatal cocaine exposure increased ethanol consumption of fully mature male and female mice at several concentrations, and that altered food and water regulation are not likely mechanisms for the increase.

Prenatal cocaine effects on motor control and the influence of ethanol consumption. The effects of prenatal cocaine exposure and ethanol intake on response duration is of importance because of its sensitivity to changes in fine motor performance. Increases in response duration are reflective of motor impairment to the extent that the subject is requiring more time to complete an operant response, thereby reducing the potential for earning reinforcers. When ethanol intake was high (Phase

II), the prenatal-cocaine-exposed mice exhibited greater deficits (i.e. increased duration) on this measure than controls. Response duration declined for the saline control animals during the early weeks of this phase (0-9% ethanol) and then stabilized during the following two weeks when ethanol concentrations were increased from 9% to 15%. The initial decline during the first 3 weeks was similar for all groups and most likely reflects improved lever performance due to practice and/or learning. For the control mice, response duration values remained relatively constant for the remainder of the experiment which supports the idea that learning/practice may have contributed to this initial decline. Although exhibiting a decline in response duration similar to controls during the early stages of testing when the ethanol concentrations were low (0–9%), male prenatal-cocaine-exposed mice had considerably higher response durations than controls during the 12% and 15% concentrations. Thus, when ethanol intake (g/kg) was high, significant motor impairments were observed. The motor impairment exhibited by the male and to a lesser degree the female prenatal-cocaine-exposed mice during Phase II of the experiment was confirmed in Phase IV when their response duration values were greater than those of controls at almost every ethanol concentration.

Performance within the 0% and 15% daily test sessions also suggests that motor function might be impaired by prenatal cocaine exposure, ethanol intake, or the interaction of these two factors. The increase in response duration across the 30-min session exhibited by prenatal-cocaine-exposed male mice in comparison to controls when responding for access to water indicates that deficits in fine motor performance occurred toward the end of the session. This increase in response duration associated with prenatal cocaine exposure was exacerbated in male mice and revealed in the female mice when 15% ethanol was the reinforcer. When compared to control mice under the 15% ethanol reinforcer, the male prenatal-cocaine-exposed mice had elevated response duration values which occurred very early and continued throughout the session. Female prenatal-cocaine-exposed mice had higher response duration values than controls later in the session.

The increases in response duration relative to control values noted in prenatal-cocaine-exposed mice when ethanol intake was high (Phase II and IV) suggests that they are more sensitive to ethanols motor disruptive effects. The prenatal-cocaine-exposed mice also appeared more sensitive than controls to ethanol's effect on fine motor performance in Phase V. During this phase, however, the consumption of ethanol was much lower for all mice than in the previous stages and its influence on response duration was a decrease rather than an increase. The decrease in response duration, perhaps reflecting the drug's stimulatory effects, appears to be greater in prenatal-cocaine-exposed than control mice of both sexes. Perhaps these observations suggest that prenatal cocaine exposure increases a subjects sensitivity to ethanol, such that when intake is high, greater motor disruptive effects are observed, yet when intake is low, greater motor stimulatory effects are observed. In either case, it is important to note that the observed differences in response duration for the prenatal-cocaine-exposed mice, relative to their control group, occurred without differences in the total number of responses made during a session. Thus, increases in response duration were most likely due to ethanol consumption rather than fatigue or motivation. Although we believe that the observed changes in response duration during these two stages reflect differences in ethanol intake, other factors could have contributed to these changes. For example, in addition to the changes in ethanol consumption under the two test conditions, the change in food presentation schedules might also influence response duration thus confound our conclusions. Additional studies examining fine motor performance with different injected ethanol doses will be necessary to more completely address this issue.

The effects of prenatal cocaine exposure on response duration observed in this study are consistent with other studies that have examined motor ability of prenatal-cocaine-exposed subjects. These reports suggest that prenatal cocaine exposure impairs the motor ability of infants and young children, but that the effects are probably transient (11,12,9, 50,51). In addition, neonatal rat pups exposed to cocaine (20 mg to 60 mg/kg–PND 4–11) showed deficits in measures of balance and coordination at 19–21 and 38–48 days of age (3).

In conclusion this study confirms previous studies indicating that ethanol can serve as a reinforcer for C57 mice (20, 21) and provides the first evidence that maternal cocaine exposure at doses having no observable effect on perinatal measures can alter the consumption of ethanol and its effects on fine motor performance. The altered sensitivity to ethanol occurred after full maturity in both male and female offspring.

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