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Alteration of the Effects of Caffeine by Prenatal Stress

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POHORECKY, L. A., P. ROBERTS, S. COTLER AND J. J. CARBONE. *Alteration of the effects of caffeine by prenatal stress.* PHARMACOL BIOCHEM BEHAV **33**(1) 55–62, 1989.—We examined the effect of prenatal stress exposure on sensitivity to caffeine using behavioral and physiological measures. Pregnant rats were handled 5 minutes daily from the 14th to 21st day of gestation. Male offspring were tested when 60 days of age in a modified open field apparatus 30 and 90 minutes after injection with caffeine (0, 10, 30 mg/kg). Caffeine increased crossover frequency and duration at the 10 mg/kg dose. Rearing frequency and duration were increased by the 10 mg/kg dose while the 30 mg/kg dose was ineffective. Gnawing was increased by caffeine, especially 90 minutes postinjection. Headpoke activity was decreased by caffeine treatment. Caffeine had no effect on defecation and urination. Gnawing activity was increased by caffeine in prenatally nonstressed animals, but was depressed in prenatally stressed animals. Prenatal stress increased sensitivity to caffeine on corner activity and rearing. The other measures were not affected differentially by prenatal stress exposure. Rectal temperature was depressed 0.75°C in both prenatally stressed and nonstressed animals, by the 30 mg/kg dose of caffeine. Thus, our results indicate that prenatal stress affects sensitivity to caffeine in the adult offspring. However, the long-term effects of prenatal stress exposure are dependent on the measures employed.

Prenatal stress Caffeine Open field activity Corticosterone Rectal temperature

DISTURBANCES of homeostasis during fetal development may have long-term, possibly permanent, effects on the offspring. Exposure to prenatal stress has been shown to alter responsiveness to stressors in the adult offspring (1,17). Prenatal exposure to a pharmacological stressor such as ethanol also has long-term consequences on the offspring. These effects range from neurochemical and behavioral effects to, in its most severe form, the fetal alcohol syndrome (13). Besides ethanol, a variety of other drugs given to pregnant female rodents have been shown to have long-term effects on the offspring (10, 15, 25).

The effect of stress on the response to drugs has not been examined to any great extent. However, since the early 1960's there has been some evidence indicating that stress may modify the response to drugs. For example, stress of hind leg ligation decreased the duration of sleep time for hexobarbital, pentobarbital and meprobamate (23). The stress of aggregation increased the toxicity but not the convulsions produced in mice by cocaine (11,20). Stress of aggregation also increased the toxicity of amphetamine (8). However, the effect of prenatal stress exposure on drug sensitivity has not been examined. Recently we found that prenatal stress can alter sensitivity to ethanol (5). This finding has been confirmed in another laboratory (28). We have also found that prenatally stressed animals were less sensitive on a behavioral measure (open field crossover activity) and endocrine response (plasma testosterone concentration) to diazepam [(22) Pohorecky, Roberts, Cotler and Carbone, submitted]. Presently we have extended the generality of the interaction of prenatal stress to include a widely used stimulant drug, caffeine.

METHOD

Subjects

Male Sprague-Dawley rats were purchased from Hilltop Laboratories (Scottsdale, PA). Animals were housed individually in stainless steel cages in a vivarium which was temperature (21°C) and light (on 9:30 p.m. to 9:30 a.m.) controlled. Food (Purina Rat Chow) and water were available ad lib.

Animals were mated one week after their arrival at our animal facility. Paper cage liners were checked daily and presence of a vaginal plug was designated as day 1 of gestation. From days 14 to 21 of gestation, animals were handled for a period of 5 minutes daily by making them walk repeatedly through an open-ended wire mesh cylinder 20 cm long and 7 cm wide. After birth all pups were crossfostered to nonstressed females giving birth within plus or minus one day. Litters were culled to 10 pups, but were otherwise left intact. Pups were not handled until weaning at the age of 21 days, when they were individually caged.

Male offspring were tested in the open field when they were 60 days of age, at 30 and 90 minutes after injection, after random assignment to the treatment groups. Treatment groups consisted of 15 to 18 subjects.

Caffeine was dissolved in saline and was administered at a dose of either 10 or 30 mg/kg by intraperitoneal injection (IP). Control animals were injected with an equivalent volume of saline.

Open Field Testing

The modified open field apparatus was 40.0 cm long by 31.5 cm wide and 46.0 cm high. Its floor was divided into four equal

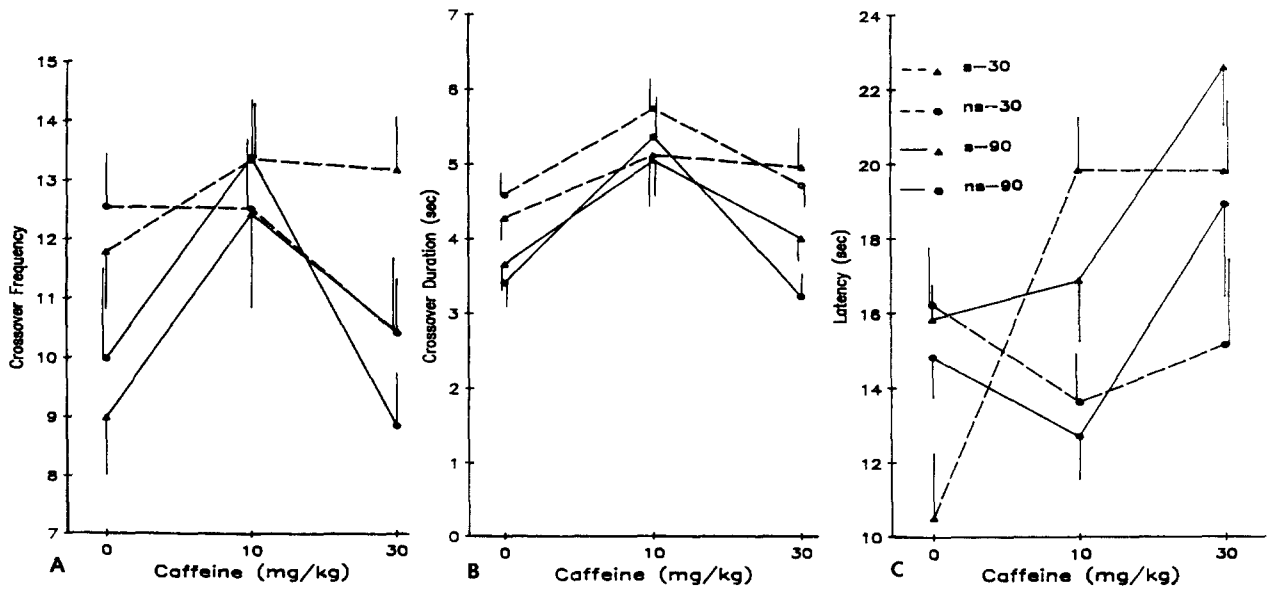

FIG. 1. Effect of caffeine on crossover activity. Prenatally stressed and nonstressed animals (n = 15–17/group) were tested when 60 days of age at 30 and 90 minutes after injection with 0, 10 or 30 mg/kg of caffeine. Crossover frequency (A), duration (B) and latency (C) were recorded as described in the Method section. Results are presented as the mean ± SEM.

squares, each containing four equidistant holes 3.0 cm in diameter. The removable floor of the open field consisted of a plastic tray which was elevated 7.0 cm above the ground. Under the floor was a small lit bulb. There was no overhead illumination. Testing was carried out between 10:00 a.m. and 1:00 p.m. Behavioral activities (headpokes, rearing, crossing and corner activity) were recorded using an automated system. A headpoke was counted when the animal dipped his head into one of the holes past the level of the eyes. Rearing was counted when the animal assumed an upward posture standing on only his back legs. Crossover frequency was counted when the animal crossed from one square to another with all 4 legs. Duration of crossover activity was measured by timing from the moment the rat began crossing a square to the time it stopped moving. For example, in a 10-second period, an animal may have crossed one or more squares. Corner activity consisted of the animal poking or nosing one of the corners. The automated system consisted of an IBM-XT personal computer equipped with a digital interface. The interface contained 4 buttons, each assigned to a particular behavior (crossing, rearing, headpoke, corner). A program was written to monitor the latency to the first press for each key as well as the duration and frequency each key was pressed for during the test period. In addition, the program performed a time block analysis so that the development and maintenance of each behavior could be assessed. Animals were placed in the apparatus for a period of 15 minutes during which time their activity was recorded for a one-minute period every 5 minutes. At the end of the open field test, the floor tray of the apparatus was carefully removed and the presence of urine and fecal boli was recorded.

To obtain an overall indication of the effectiveness of drug treatment, we also calculated the total area under the curve representing the response over time for each behavioral measure.

Plasma Corticosterone Determination

Plasma corticosterone was determined using the radioimmunoassay procedure originally described by Gwosdow-Cohen *et al.* (12). The antibodies for corticosterone were obtained from Dr. G. D. Niswender from the State University of Colorado, Fort Collins, CO.

Plasma Caffeine Determination

Plasma caffeine was measured using a modified method of Blanchard *et al.* (2). Plasma (0.1 ml) was extracted with 0.1 ml of acetonitrile, and 20 μl of the latter was injected directly into the chromatograph. The chromatograph was equipped with a 250 × 4.6 cm i.d. column containing Spherisorb ODS (10 μm) preceded by a Ca:Pell ODS pre-column (Whatman Inc., Clifton, NJ 07014). The mobile phase consisted of a 80/20 mixture by volume of acetate buffer (pH 4.0) and acetonitrile. The flow rate of the mobile phase was 1.0 ml/min. Column effluent was monitored at 273 nm.

Rectal Temperature Measurement

Temperature was measured with a Data-Precision Model 134 multimeter equipped with a Yellow Springs Instruments 400 series rectal probe. Body temperature was measured by inserting the lubricated rectal probe 4.5 cm into the rectum. Temperature was recorded after 1 minute of stabilization.

Data Analysis

Results are presented as the mean ± standard error of the mean. Data were analyzed using an appropriate analysis of variance (stress treatment × drug dose × time) using a computerized package. Differences between groups were considered statistically significant when $p \leq 0.05$.

RESULTS

Crossover Activity

Frequency of crossover activity was affected by the time of

TABLE 1

TOTAL AREA UNDER RESPONSE CURVE FOR CAFFEINE

Behavior	Dose (mg/kg)	Frequency S	Frequency NS	Duration S	Duration NS	Latencies S	Latencies NS
Crossover	0	624.51	677.62	238.78	240.56	804.17	922.77
	10	776.07	780.03	306.30	334.79	1007.51	889.67
	30	710.92	579.25	269.37	238.76	1051.66	1249.39
Rearing	0	402.83	392.61	792.47	831.91	1429.73	1489.99
	10	485.15	457.21	1202.64	1074.32	1448.60	1348.42
	30	359.35	359.65	686.53	713.82	1688.73	1527.78
Headpoke	0	314.98	369.05	581.52	610.57	1145.97	830.98
	10	317.08	242.15	500.76	148.35	1081.88	1236.53
	30	289.19	287.37	557.12	410.06	991.03	1352.91
Corner	0	238.66	209.66	363.73	283.53	1519.29	1306.46
	10	292.41	230.04	372.20	282.83	1475.70	1513.19
	30	233.42	230.04	279.62	306.22	1617.05	1324.25
Gnawing	0	52.21	17.40	116.74	33.00	35645.05	44433.71
	10	16.20	26.11	15.60	60.30	43968.83	42032.22
	30	43.78	65.43	89.39	197.19	34863.74	32018.16

testing, $F(2,92) = 10.804$, $p = 0.0020$ (Fig. 1A). Crossover activity was generally lower at the 90-minute test period, except in animals treated with the 10 mg/kg dose of caffeine. Analysis of variance indicated a significant main effect of drug treatment, $F(2,92) = 3.788$, $p = 0.02$. Neither the effect of prenatal stress nor the interaction of prenatal stress and drug treatment were statistically significant. However, it is apparent for Fig. 1 that in prenatally nonstressed animals caffeine had a biphasic effect on crossover frequency. Crossover frequency increased only with the 10 mg/kg dose of caffeine. The total response indicates that only the 10 mg/kg dose of caffeine had a significant ($p<0.02$) stimulant effect on the frequency of crossover activity (Table 1).

Duration of crossover activity was significantly affected by the time of testing, $F(1,96) = 11.453$, $p<0.013$, with duration being generally less at the 90-minute test period (Fig. 1B). Duration of crossover activity was also affected by caffeine treatment, $F(2,96) = 7.94$, $p<0.001$. Examination of the data reveals that crossover duration was increased by the 10 mg/kg dose of caffeine, while the 30 mg/kg dose of the drug was ineffective. The effect of the 10 mg/kg dose of caffeine on the duration of crossover activity was largest at the 90-minute test time (+38%). There was no significant effect of prenatal stress treatment nor in the interaction with the drug treatment. As indicated by the total area under the curve, 10 mg/kg of caffeine significantly ($p<0.02$) increased the duration of crossover activity in both control and prenatally stressed animals (Table 1).

Latency for crossover activity was 14.0 ± 2.7 seconds for both control and prenatally stressed animals; it did not vary with the time of testing (Fig. 1C). Caffeine treatment had no significant effect on latency of crossover activity in control animals, which is also evident from the integrated data in Table 1.

Rearing Activity

Frequency of rearing was significantly affected by the time of testing, $F(1,97) = 8.056$, $p = 0.005$ (Fig. 2A). Except for animals tested with 10 mg/kg of caffeine, the frequency of rearing was lower on the second test. Frequency of rearing activity was significantly affected by caffeine treatment, $F(2,97) = 3.464$, $p = 0.033$. The interaction of drug treatment and time of testing was also significant, $F(1,97) = 6.98$, $p = 0.002$. In animals injected with the 10 mg/kg dose of caffeine frequency of rearing activity was elevated by approximately 60% ($p = 0.005$), at the 90-minute test in both prenatally nonstressed and in prenatally stressed animals, but had no effect at the 30-minute test. The 30 mg/kg dose of caffeine had no effect at both test times. With respect to the total response, caffeine treatment had a small, significant ($p<0.05$) stimulant effect on rearing activity at the 10 mg/kg dose in nonstressed and stressed animals, respectively. The larger dose was ineffective (Table 1).

Caffeine treatment also had a significant main effect on rearing duration, $F(2,97) = 6.043$, $p = 0.004$ (Fig. 2B). Neither dose of caffeine was effective at the 30-minute test. In prenatally stressed animals the 10 mg/kg dose of caffeine increased duration of rearing activity by 120% while the 30 mg/kg dose decreased this measure by 30% at the 90-minute postinjection. Caffeine treatment had no effect on duration of rearing in control animals. The total response indicated an increase in rearing duration by the 10 mg/kg dose of caffeine in both control (+29%) ($p<0.05$) and prenatally stressed (+52%) ($p<0.01$) animals. The larger dose of caffeine on the other hand, had no effect on rearing duration (Table 1).

Latency for rearing activity was 24.3 ± 3.7 seconds over all treatment groups and time periods. In neither the control nor the stressed animals did latency vary with the time of testing, nor was it affected by the treatment with caffeine (Fig. 2C).

Headpoke Activity

The frequency of headpoke activity was not affected by either caffeine or prenatal stress treatments (Fig. 3A and Table 1).

Duration of headpoke activity was affected by the time of testing, $F(2,97) = 7.950$, $p = 0.006$ (Fig. 3B). Compared to the 30-minute test, headpoke activity was longer during the 90-minute test period (Fig. 3B). Neither drug nor prenatal stress had a significant effect on this measure. The overall duration of headpoke activity was significantly depressed by both the 10 mg/kg (−76%, $p<0.01$) and 30 mg/kg dose of caffeine (−33%), but

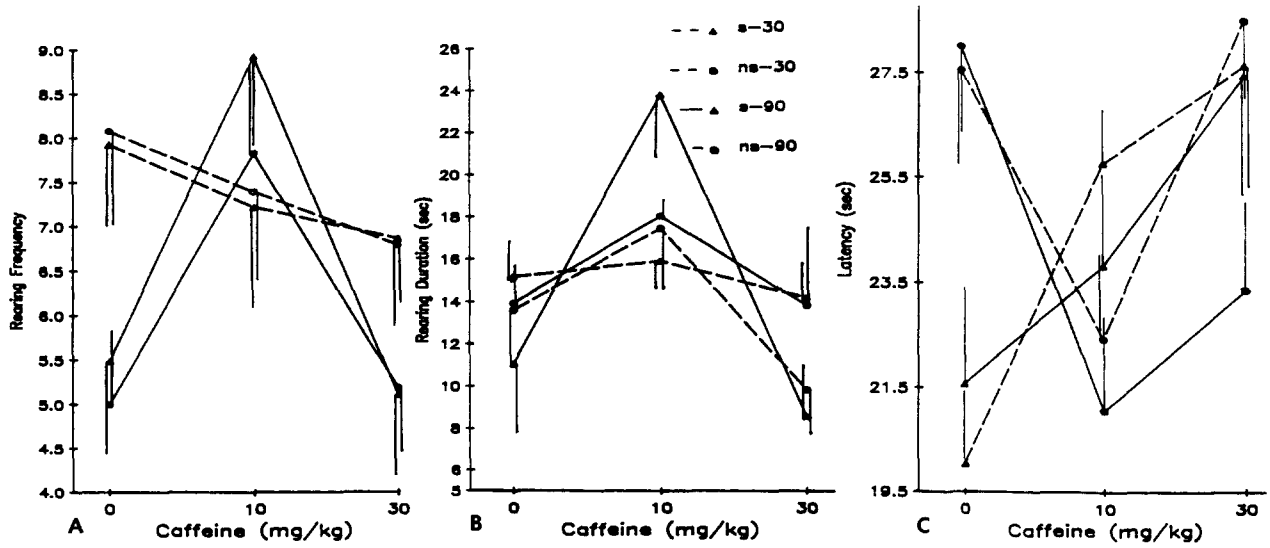

FIG. 2. Effect of caffeine on rearing activity in prenatally stressed and nonstressed animals. Prenatally stressed and nonstressed animals (n = 15–17/group) were tested at 30 and 90 minutes after injection with 0, 10 or 30 mg/kg of caffeine. Rearing frequency (A), duration (B) and latency (C) were recorded as described in the Method section. Results are presented as the mean ± SEM.

only in the nonstressed animals (Table 1).

Latency of headpoke activity was 16.5 ± 3.0 seconds, and did not vary with the time of testing (Fig. 3C). The effect of either caffeine or prenatal stress alone on latency did not reach statistical significance. Integrated measures of latency indicate that caffeine significantly ($p<0.05$) lengthened the onset time for headpoke activity in a dose-dependent manner (+49% and 63% at 10 and 30 mg/kg respectively) in control animals (Table 1).

Corner Activity

As with the other measures, frequency of corner activity was dependent on the time of testing, $F(1,84) = 29.752$, $p<0.001$ (Fig. 4A). The frequency of corner activity was, in general, significantly less at the 90- compared to the 30-minute test period. Caffeine treatment or prenatal stress had no significant effect on frequency of corner activity. However, the triple interaction (stress × drug × time) was significant, $F(2,84) = 4.05$, $p \leq 0.02$. This indicates that the effect of caffeine treatment was affected by both the time of testing as well as by the prenatal exposure to stress. Overall, prenatally stressed animals had higher frequency of corner activity compared to nonstressed animals. Furthermore, in stressed animals caffeine was more effective in increasing corner activity at the 10 mg/kg dose at 30 minutes while at 90

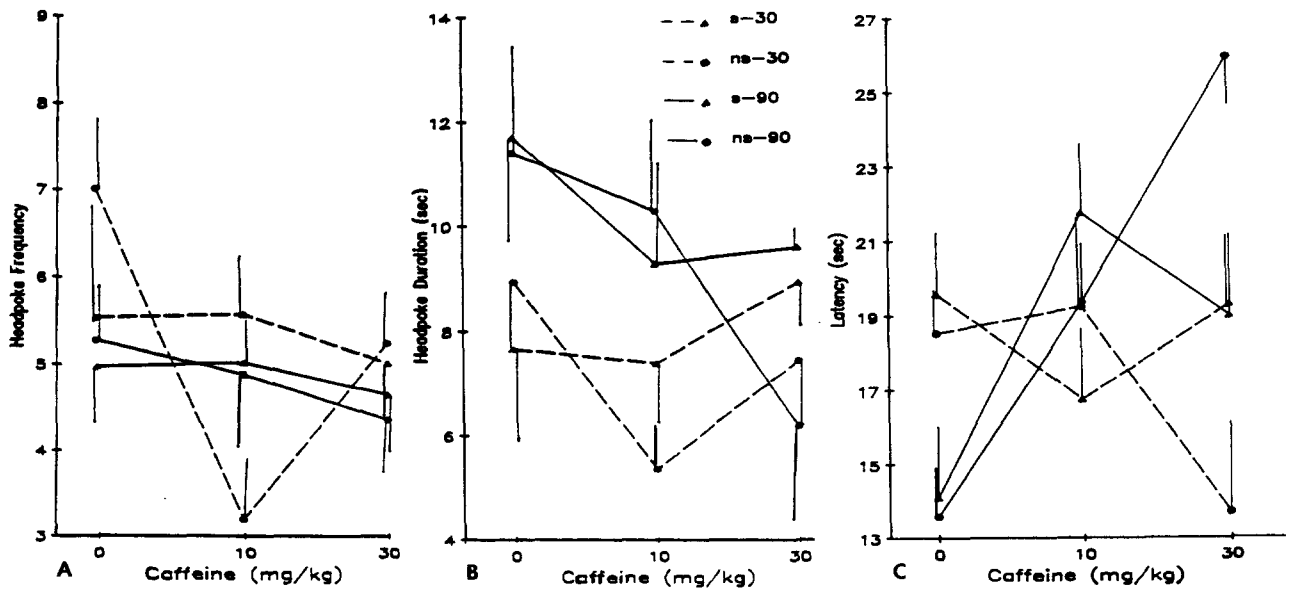

FIG. 3. Effect of caffeine on headpoke activity in prenatally stressed and nonstressed animals. Prenatally stressed and nonstressed animals (n = 15–17/group) were tested at 30 and 90 minutes after injection with 0, 10 or 30 mg/kg of caffeine. Headpoke frequency (A), duration (B) and latency (C) were recorded as described in the Method section. Results are presented as the mean ± SEM.

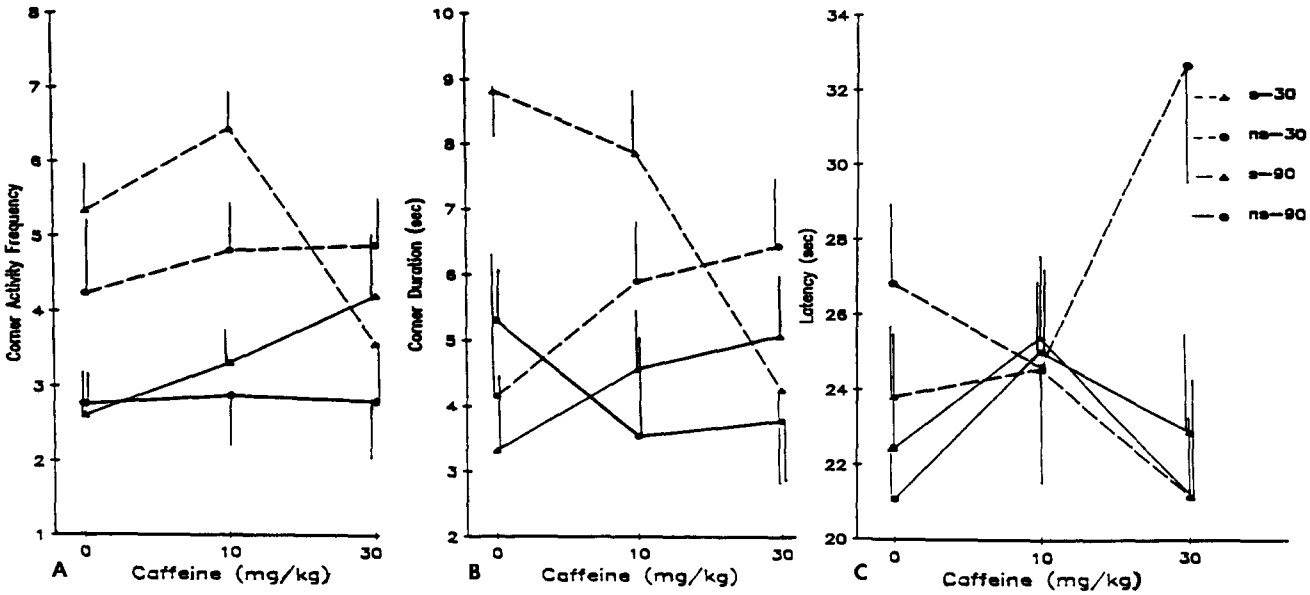

FIG. 4. Effect of caffeine on corner activity in prenatally stressed and nonstressed animals. Prenatally stressed and nonstressed animals (n = 15–17/group) were tested at 30 and 90 minutes after injection with 0, 10 or 30 mg/kg of caffeine. Corner activity frequency (A), duration (B) and latency (C) were recorded as described in the Method section. Results are presented as the mean ± SEM.

minutes only the higher dose of caffeine raised corner activity, compared to nonstressed animals. Data for the total response corroborate the lack of effect of caffeine on corner activity (Table 1).

Duration of corner activity was also dependent on the time of testing, $F(1,84) = 10.631$, $p = 0.002$ (Fig. 4B). Overall duration of corner activity was less at the 90-minute test time. In saline-injected animals the duration of corner activity at the 30-minute test was 175% longer in prenatally stressed animals compared to prenatally nonstressed animals. As with the frequency of corner activity, prenatal stress and caffeine treatment had no significant effect on their own, yet the triple interaction (stress × drug × time) was significant, $F(2,84) = 5.89$, $p = 0.004$. Corner activity of prenatally stressed animals 30 minutes postinjection was 210% higher than that of nonstressed animals. Such difference was not evident in animals treated with the 30 mg/kg dose of caffeine. Caffeine treatment had no effect as far as the total response on either duration or frequency of corner activity in control animals (Table 1).

Latency for corner activity was approximately 13.5 seconds

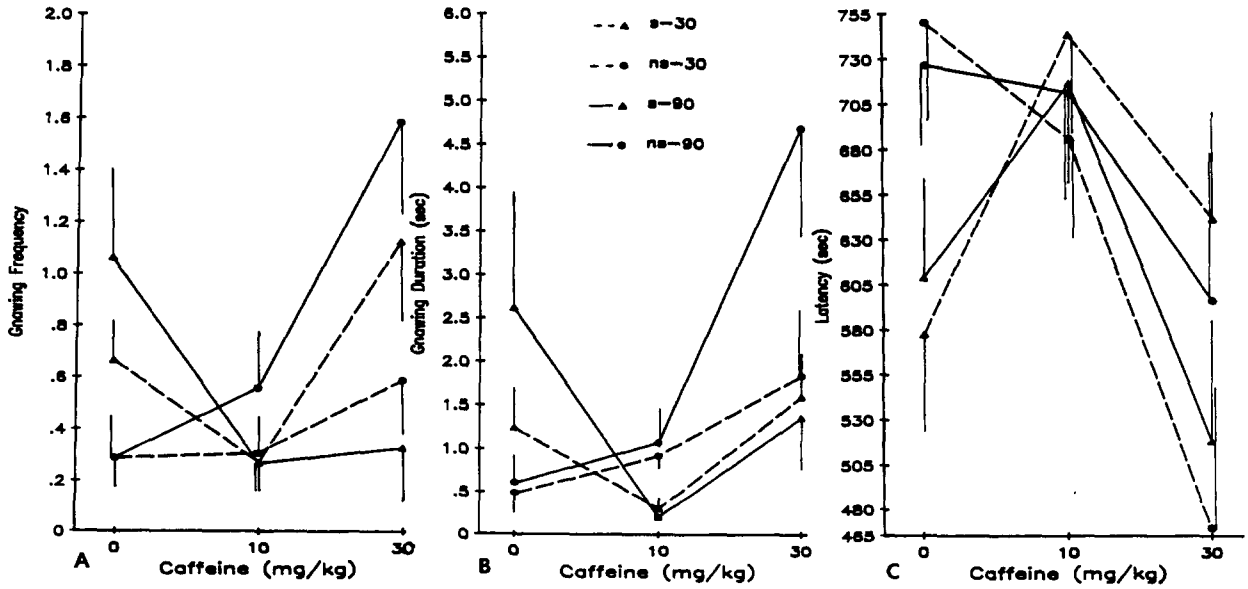

FIG. 5. Effect of caffeine on gnawing activity in prenatally stressed and nonstressed animals. Prenatally stressed and nonstressed animals (n = 15–17/group) were tested 30 and 90 minutes after injection with 0, 10 or 30 mg/kg of caffeine. Gnawing frequency (A), duration (B) and latency (C) were recorded as described in the Method section. Results are presented as the mean ± SEM.

TABLE 2

CAFFEINE PLASMA CONCENTRATIONS IN PRENATALLY STRESSED AND NONSTRESSED RATS

Treatment	Dose (mg/kg)	Caffeine (ng/ml)
Stress	0	ND
	10	2346.2 ± 167.1
	30	7164.7 ± 666.4
Nonstress	0	ND
	10	2649.5 ± 499.9
	30	6262.9 ± 267.2

Pregnant females were stressed as described in the Method section. Adult male offspring were injected with 0, 10 or 30 mg/kg of caffeine and killed 1 hour later. Plasma was analyzed as described in the Method section. Results are given as the mean ± SEM for groups of 8 animals.
ND = nondetectable.

over both treatment groups and time of testing. Neither caffeine treatment nor prenatal stress exposure had any significant effect on the latencies for this measure (Table 1, Fig. 4C). However, latency to corner activity was longer in the nonstressed group given 30 mg/kg of caffeine.

Gnawing Activity

In contrast with the other behaviors, gnawing activity was not influenced by the time of testing. Frequency of gnawing activity was affected by caffeine treatment, $F(2,89) = 3.777$, $p = 0.025$ (Fig. 5A). With the exception of the 90-minute period in prenatally stressed animals, the 30 mg/kg dose of caffeine increased the frequency of gnawing activity in both control and prenatally stressed animals. The effect of caffeine was time dependent, $F(1,89) = 4.652$, $p = 0.030$. The triple interaction (stress × drug × time) was also statistically significant, $F(2,89) = 6.569$, $p = 0.002$, indicating that the effect of caffeine treatment was not only dependent on the time of testing but also on the prenatal treatment. The stimulant effect of caffeine was evident primarily in the prenatally nonstressed animals. These animals showed a 70% and 448% increase in frequency, at the 30- and 90-minute test periods respectively, compared to a 69% increase and a 169% decrease in frequency, at 30- and 90-minute test periods, respectively, in prenatally stressed animals. The total response after treatment with caffeine clearly demonstrates the general stimulant effect of this drug in animals that were not stressed prenatally (Table 1). Caffeine's effect was dose-dependent, with the larger dose increasing frequency by 276% ($p<0.01$). In stressed animals, by contrast, caffeine depressed gnawing activity, with the depression being largest (69%) with the 10 mg/kg dose ($p<0.001$).

Duration of gnawing activity was significantly affected by caffeine, $F(2,89) = 5.38$, $p = 0.006$. Furthermore, the interaction of prenatal stress with caffeine treatment was significant, $F(2,89) = 4.53$, $p = 0.015$ (Fig. 5B). Caffeine treatment dose-dependently increased duration of gnawing activity but only in the prenatally nonstressed animals. In prenatally stressed animals the 10 mg/kg dose of caffeine decreased gnawing activity while the 30 mg/kg dose had no effect. The overall stimulation of caffeine on duration of gnawing was dose-dependent with a 196% ($p<0.001$) increase at the 30 mg/kg dose in control animals (Table 1). Again in stressed animals, caffeine depressed duration of gnawing with peak effect (−23%) ($p<0.001$) at the 10 mg/kg dose.

Onset for gnawing activity was longer than that for all the other measures (Fig. 5C). Although caffeine treatment decreased the time of onset for gnawing of animals that were not stressed prenatally by up to 38% at the 90-minute test, this effect was not statistically significant.

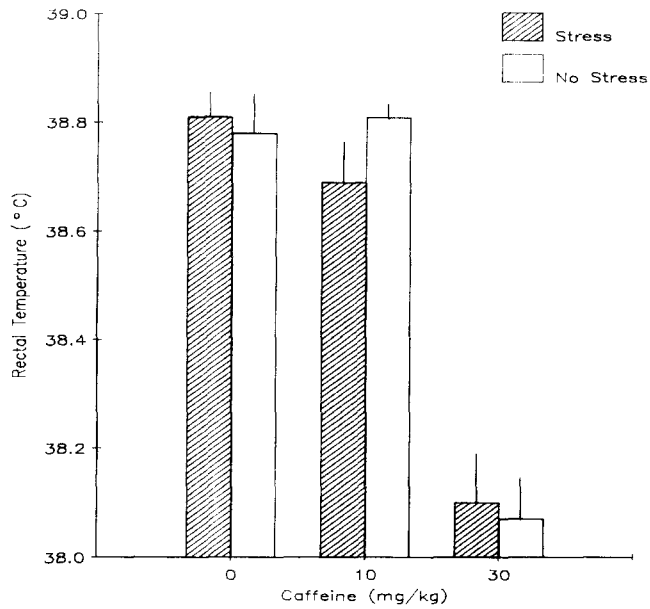

FIG. 6. Effect of caffeine on rectal temperature. Groups of 18 prenatally stressed and nonstressed animals were injected with 0, 10 or 30 mg/kg caffeine. Rectal temperature was recorded prior to and at 60 minutes after the drug treatment. Results are presented as the mean ± SEM.

Defecation and Urination

Caffeine treatment had no statistically significant effect on defecation or urination in the open field apparatus. However, there was a trend for a decrease in the mean number of boli as well as for less urination in animals treated with the highest dose of caffeine.

Rectal Temperature

Treatment with caffeine had a significant effect on rectal temperature, $F(2,99) = 56.34$, $p<0.001$ (Fig. 6). Caffeine produced hypothermia, but only at the 30 mg/kg dose. The hypothermic effect of caffeine was not influenced by prenatal stress exposure.

Plasma Corticosterone

Although plasma corticosterone levels were 12% higher in animals stressed prenatally compared to nonstressed animals, the difference was not statistically significant. The effect of caffeine was also not statistically significant.

Plasma Caffeine

Plasma concentrations of caffeine after the 10 mg/kg dose of the drug were equivalent in both prenatally stressed and nonstressed animals (Table 2). Caffeine plasma concentration was 14% higher in prenatally stressed versus nonstressed animals injected with the 30 mg/kg dose.

DISCUSSION

The open field test has been one of the most widely used

behavioral tests because of its simplicity, ease of quantitation and sensitivity (18). A number of dependent variables can be measured in the open field, possibly representing various underlying constructs. However, proof of construct validity is not always available. Locomotion, rearing and defecation have been generally taken as measures of emotionality (5). Tachibana (27) investigated the correlation and stability of several of the measures taken in an open field test. Correlation between measures was found to increase on repeated testing. Our behavioral test also incorporated headpoke activity, a widely accepted measure of exploration (7). By evaluating a number of behavioral measures, we expected to increase the overall sensitivity of the testing procedure.

Our results indicate that caffeine treatment affected various measures of open field activity. Crossover duration, and the frequency and duration of both rearing and gnawing activities were increased by caffeine treatment. Duration and frequency of crossover were increased by the 10 mg/kg dose in control animals. Overall, caffeine treatment had little effect on rearing activity in control animals. However, the 10 mg/kg dose increased rearing activity by 29% in prenatally stressed animals. In an earlier study, Hughes and Greig (14) reported that 10 mg/kg of caffeine increased ambulation in an open field. The present finding of an increase in rearing activity is in contrast to the report of Hughes and Greig (14) who found no effect of caffeine on rearing activity. As for gnawing behavior, caffeine increased this behavior in control animals dose-dependently (+50% and +83% in frequency and duration after 10 mg/kg, and +276% and +497% in frequency and duration, after 30 mg/kg). The effect of caffeine was monotonically dose-dependent in some cases, but in others it was biphasic. Thus the increase in the frequency and duration of rearing and gnawing activities was evident only at the 10 mg/kg dose of caffeine, while headpoke activity was depressed by this dose of caffeine in control animals. Caffeine showed no effect on a related measure, corner activity.

Caffeine has been shown to stimulate a number of different behaviors (8, 9, 14, 19–21, 28). The reported effects of caffeine were generally not monotonically dose-dependent. For instance, ambulation was increased by a 10 mg/kg dose of caffeine but was unaffected by a 40 mg/kg dose (29). Also responding on a FI300 schedule for food reinforcement increased with both 6 and 12 mg/kg of caffeine, but was depressed by a 24 mg/kg dose (19). A U-shaped effect of caffeine on ambulation has been previously reported in rodents (14). A U-shaped response curve was also obtained in squirrel monkeys maintained on a fixed-interval schedule with either food or electric-shock presentation (16). Our data indicate that low doses of caffeine increased some behavioral measures evidenced in the open field. Both horizontal and vertical motility were affected. Furthermore, activities susceptible to stereotypy, i.e., gnawing and headpoke, were differentially affected. Gnawing activity was increased by caffeine while headpoke activity was depressed.

Our finding that caffeine had no effect on plasma corticosterone levels at doses up to 30 mg/kg dose confirms the earlier report by Spindel *et al.* (26). These investigators found that caffeine at doses greater than 30 mg/kg raised plasma corticosterone dose-dependently. In our hands only the 30 mg/kg caffeine affected body temperature, it produced hypothermia. A previous study reported a biphasic effect of caffeine on body temperature. Schlosberg (24) found hyperthermia with 12.5 and 25 mg/kg and hypothermia with 50 and 100 mg/kg doses of caffeine. The reason for the discrepancy at the 10 mg/kg dose level is not apparent. The decrease in rectal temperature was not related to the behavioral effects of caffeine since it occurred only at a higher dose.

As for the effect of the prenatal stress treatment, these animals were less sensitive to caffeine with respect to gnawing activity (−69% in frequency and +87% in duration). Prenatal stress treatment had no effect, no did it alter the sensitivity to caffeine on the other measures. There was, however, a tendency for greater stimulation of crossover activity after treatment with both doses of caffeine. Thus, prenatal stress exposure altered sensitivity to caffeine on a specific behavior in the open field. It is unclear what the mechanism of the interaction of prenatal stress and caffeine treatments is. Methylxanthines are believed to produce their effects by interacting with adenosine receptors in brain (4). Stress has been shown to affect adenosine receptors (3), therefore one possibility is that prenatal stress may have affected adenosine receptors. That is, neurochemical and/or neuroendocrine effects produced by prenatal stress may interact with the direct acute effects produced by caffeine to either increase or decreases some of the observed effects. In this respect these results are similar to those we observed previously with animals prenatally stressed and tested when adult with an acute dose of ethanol (6). Some acute effects of ethanol were increased (swimming behavior) while others were decreased (i.e., hypothermia).

In the case of other drugs such as barbiturates and amphetamine the effects of stress may have been partially mediated by changes in their metabolism (8,23). In the present study, however, the effect of prenatal stress cannot be ascribed to differences in plasma levels of caffeine. Similarly, prenatally stressed animals did not differ from control animals in plasma levels of diazepam (22).

In summary, caffeine treatment increased gnawing, rearing and crossover activities in control animals, which indicates an overall stimulant effect of this drug. Prenatal stress exposure decreased the effect of caffeine on gnawing behavior in the open field.

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