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The Effects of Postnatal Caffeine Exposure on Growth, Activity and Learning in Rats

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ZIMMERBERG, B., K. L. CARR, A. SCOTT, H. H. LEE AND J. M. WEIDER. *The effects of postnatal caffeine exposure on growth, activity and learning in rats.* PHARMACOL BIOCHEM BEHAV **39**(4) 883–888, 1991.—Caffeine is both ingested by pregnant women in their third trimesters and administered therapeutically to premature infants to stimulate respiration. This experiment attempted to delineate any persistent effects of low dose caffeine exposure during the first week of life in rats, since this time period provides an animal model equivalent to the human third trimester or premature infant exposure. Rat pups who had received either 1 or 9 mg/kg of caffeine during the first week of life grew more slowly, were hypoactive at two weeks of age, and were impaired on an operant spatial learning task as adults. Adding visual cues to the operant task did not improve their performance. The timing of the appearance of developmental landmarks, adult body weight and adult brain weight, however, were not affected by postnatal caffeine exposure. The persistent behavioral deficits noted after postnatal caffeine exposure were all opposite in direction to the acute effects of caffeine, and similar to the effects of adenosine. Thus the behavioral deficits reported here may reflect an upregulation of developing adenosine receptors that persists into adulthood subsequent to early chronic postnatal caffeine exposure.

Caffeine Postnatal Adenosine Growth Learning Activity Rats Development

THE ingestion of caffeine, a methylxanthine derivative found in coffee, tea, cocoa, chocolate, carbonated beverages, and some over-the-counter cold remedies, is so common in our society among all age groups that it is considered more of a dietary component than an abused drug (3). Neurophysiological and neurochemical studies have established that caffeine's behavioral effects are primarily due to its antagonism at adenosine receptors (11, 29, 33, 39). Adenosine analogs have been reported to depress synaptic transmission, spontaneous motor activity, food intake and lipolysis (9, 13, 35, 38, 39). Conversely, caffeine is a well-known stimulant of spontaneous motor activity and food intake, at least at low doses (4, 12, 32). High doses of caffeine are associated with insomnia, restlessness, nervousness, and delirium (12). In contrast to the acute effects of caffeine, chronic caffeine treatment has been found to increase the number of adenosine receptors in the rat cerebral cortex (5, 17, 21, 36), and increase the percentage of agonist receptors in the high affinity state (42); these effects of chronic exposure can be considered evidence of a compensatory "upregulation" of adenosine pathways.

Women who are heavy caffeine consumers during pregnancy have been reported to have higher incidences of prematurity and low birth weight in their infants (8, 26, 43). Prenatal administration of caffeine in animal models has resulted in a variety of deficits in the offspring, although studies have produced contradictory results (6). Skeletal anomalies have been found in the offspring of rat dams who were given a single high dose of 112.5 or 150 mg/kg on gestation day 11 (27). However, rat dams given caffeine throughout gestation (up to 16 mg/day) produced offspring with decreased fetal weight but no malformations (19). Tanaka and Nakazawa found that maternal caffeine ingestion led to lower fetal cerebral weight and an increased concentration of free tyrosine in the fetal cerebrum, but no significant differences in body or liver weights in prenatally exposed pups (44). Prenatal caffeine exposure has also been reported to delay the onset of auditory startle (45) and swimming development (7). Studies of activity levels in the preweaning period of caffeine-exposed offspring have found both decreased (10,45) and increased levels of activity (7). When tested on passive-avoidance tasks, prenatally caffeine-exposed rats have shown either no treatment-related changes (7,40) or deficits in male but not female offspring (34) as compared to controls. Dose may also be an important factor: West and co-workers reported learning deficits in male offspring exposed to 5 and 25 mg/kg caffeine in utero but not to higher doses (45).

There have been few experiments directly testing the effects of postnatally administered caffeine; most studies did not distinguish between prenatal and postnatal treatment or administered the caffeine in the dams' diet during lactation. Gullberg and coworkers (22) administered caffeine postnatally through dam's milk, and found that pups exposed to about 30 mg of caffeine per day weighed less than control pups or pups exposed to about 7.5 mg per day. They reported no significant effects of caffeine on activity in an open-field test given on day 18; however, they did find that high-dose rats showed a significantly earlier onset of auditory startle. Rats exposed to both doses displayed the

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righting reflex earlier than control rats, with low-dose rats showing the earliest onset (22). Postweaning hyperactivity and deficits in left-right discrimination were found to be associated with combined in utero and lactational caffeine exposure (40). In contrast, Concannon and colleagues (10) found that postnatal exposure to caffeine through dam's milk resulted in hypoactivity on postnatal days 15 and 20, but no difference in activity at postnatal day 25. When caffeine (40 and 80 mg/kg) was administered acutely to rat pups at either one or ten days of age, pups had significant deficits in suckling attachment and gained less weight than controls (23). Chronic caffeine administration (20 mg/kg) for nine days after birth also resulted in lower weight gain (23). Single subcutaneous injections of caffeine at one, ten or 15 days of age caused significant hyperactivity; again, the doses used in this study were high (20–120 mg/kg) (24). Daily subcutaneous injections of caffeine during the first postnatal week caused hyperactivity during drug administration (days 1 and 7) but hypoactivity when offspring were tested a month later (15).

Because caffeine stimulates respiratory function, it is routinely administered to premature infants to combat sleep apnea (1,18), despite a paucity of studies on long-term behavioral consequences. Indeed, caffeine is often preferred over theophylline due to caffeine's wider therapeutic range and easier administration (18). Rat pups in the first week of life provide an excellent model for investigating behavioral sequela of drug administration in the third trimester of human pregnancy or in premature infants (37). During the first week of life, adenosine receptors are rapidly developing in the forebrain and cerebellum in rats (31). Since there are few data available on the long-term effects of low dose caffeine exposure during the last trimester, this study examined the effects of caffeine administered to neonatal rats in the first six postnatal days, using doses corresponding to a normal range of caffeine intake by humans. Outcome measures included growth, development and activity levels of preweanling rat pups, and learning an operant task in adult offspring. This operant spatial alternation task has been used previously in this laboratory to detect persistent effects of prenatal alcohol exposure in adult offspring (47).

METHOD

Subjects

The subjects were 64 pups born to eight female Long-Evans rats (Charles River Laboratories, Wilmington, MA) fed a regular laboratory diet and water ad lib. On the day after a birth was noted (postnatal day 1), pups were sexed, weighed, and numbered by toe-clip. One male and one female pup from each litter was assigned to one of four groups: a noninjected control group, a low-dose group (1 mg/kg caffeine in distilled water), a high-dose group (9 mg/kg) and a vehicle control group injected with an equivalent volume of distilled water. The injection volume was 0.07 ± 0.02 ml. These 2 doses are roughly equivalent to ⅓ cup and 3 cups of coffee, respectively. Dams were housed in an isolated temperature- and humidity-controlled nursery, with a 12-hour LD cycle starting at 0700 h.

Apparatus

Open-field activity was monitored by videotape using a 50 cm^2 Plexiglas field divided into 25 squares. The spatial alternation testing was conducted in four operant chambers encased in research chests (Coulbourn Instruments, Lehigh Valley, PA). The chambers were configured with a center lever placed 5 cm above a recessed water trough during initial training, and then with two levers 3.5 cm to the right or to the left of the water trough during the test sessions. The side levers were each 2.5 cm from the floor and 1.5 cm inside from the cell walls. A yellow cue light was situated 4 cm above each lever. The water trough consisted of a plastic fluid reservoir into which a 0.4 cc stainless steel cup was dipped. When the correct lever was pressed, the dipper cup was raised and the feeder light was activated for a duration of five seconds. A 28-V houselight situated above the water trough was activated for the duration of each session. The boxes were interfaced with a microprocessor which recorded responses and errors and controlled the delivery of reinforcements (LabLinc, Coulbourn Instruments, Lehigh Valley, PA).

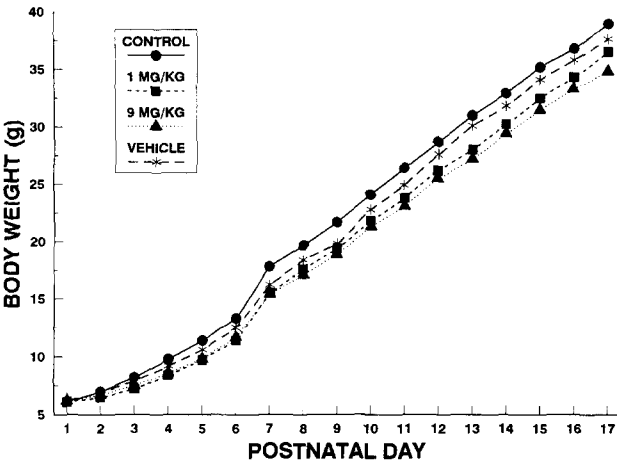

FIG. 1. Body weight (g) for the first 17 days after birth in rat pups administered either 1 mg/kg or 9 mg/kg caffeine or vehicle (distilled water) for their first six postnatal days.

Procedure

At 0800 h, starting on postnatal day (PN)1, all pups were removed from the nest and maintained at nest temperature (34°C) with a heating pad. Pups in injection groups were injected intragastrically with caffeine or vehicle solutions daily on PN days 1 thru 6. Pups were weighed daily on PN days 1 thru 17, and checked daily for eye-opening. Pups were tested for righting reflex starting on day 10. Pups were held ventral side up 30 cm above a padded surface. Landing right-side up on all four paws constituted a correct righting response. The criteria for attaining righting was three righting reflexes out of three drops. Activity was measured on days 13–17. Rat pups were placed individually in the center square of the activity box and videotaped for two minutes. Tapes were scored for the number of squares crossed by the rat's entire body.

Pups were weaned into hanging cages with a same sex sibling at 25 days of age and remained undisturbed until 69 days of age. Subjects were then deprived of water for 24 h, and trained to bar-press for water in daily 1-h sessions for 5 days in the operant chambers with one center bar. Eight subjects (four high-dose, 2 control and 2 vehicle) who failed to learn to bar-press at a consistently high rate were eliminated from the study at this point. The operant chambers were then reconfigured with two bars, one to the left and one to the right of the center. Cue lights were not available during this learning phase. Subjects were required to alternate left and right responses to receive re-

ward. Any responses after the first correct response were errors and only the next response on the other bar was rewarded. Subjects were tested in 30-min sessions daily for two weeks, five days a week. On the following week, testing continued for four days, but cue lights were used to signal the correct bar. All testing took place between 1000 and 1400 h. At the end of the week, the subjects were weighed and their brains removed. After weighing the whole brain, the cerebellum and cerebrum were separated from the brainstem and weighed separately.

RESULTS

One rat from each of the four conditions died—a female low-dose, a female vehicle-dose, a male high-dose and a male control. Body weights for the first 17 postnatal days are shown in Fig. 1. A repeated measures analysis of variance (ANOVA) of these weights indicated a significant interaction between Postnatal Treatment and Day, $F(48,832)=1.67$, $p<0.005$ (Greenhouse-Geiser corrections used for all repeated measures analyses). Control subjects gained weight more rapidly than all other groups; both caffeine-exposed groups gained weight less rapidly than either the vehicle-injected or control groups, but did not differ from each other (trend analysis, p's<0.05). There were no differences between groups in either day of eye-opening or day of achieving righting reflex.

Activity on days 13 through 17, measured by number of squares crossed, is shown in Fig. 2. A repeated measures ANOVA indicated an interaction between Postnatal Treatment and Day, $F(12,208)=2.43$, $p<0.006$, as well as a main effect of Postnatal Treatment, $F(3,52)=3.88$, $p<0.01$. Subsequent post hoc comparisons (Tukey's tests, $p=0.05$) showed that the noninjected and the vehicle-injected control groups differed from both caffeine groups at Days 15 through 17, but the two doses of caffeine did not differ from each other, and the vehicle group did not differ from the control group on Day 16. There was no significant effect of Sex, nor did Sex interact with Postnatal Treatment or Day.

The percentage of correct responses compared to total responses for the alternation test for these subjects tested as adults are shown in Fig. 3. An ANOVA of the results of the learning phase (no cue lights) indicated a significant interaction between

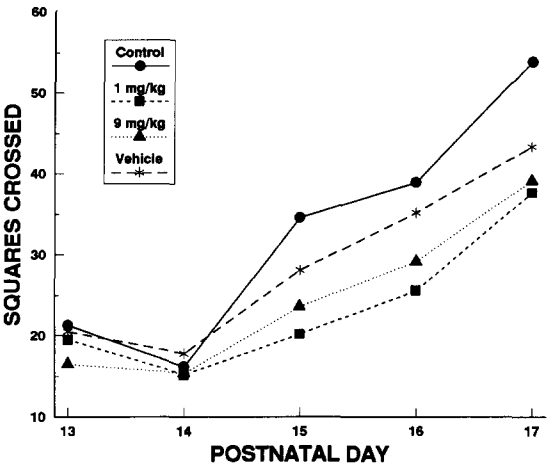

FIG. 2. Locomotor activity (number of squares crossed) in 13–17-day-old rat pups administered either 1 mg/kg or 9 mg/kg caffeine or vehicle (distilled water) for their first six postnatal days.

Postnatal Treatment and Day, $F(27,414)=1.94$, $p<0.01$, and a significant main effect of Postnatal Treatment, $F(3,46)=4.13$, $p<0.01$. Trend analysis (p's<0.05) revealed that the noninjected and vehicle-injected control groups learned at the same rate (e.g., the linear components of the slopes did not differ), while the two caffeine-injected groups learned at a slower rate than the two control groups, but did not differ from each other. ANOVA of the results from the four days with cue lights also revealed a significant interaction between Postnatal Treatment and Day, $F(9,138)=4.84$, $p<0.001$. Both caffeine-injected groups differed significantly from both control groups (p's<0.01). The two control groups did not differ from each other, nor did the two caffeine dose groups differ from each other. Again, no effects of Sex were noted. There were also no differences among the groups or sexes in the total number of responses per session (mean of 240 responses).

Table 1 shows the body weights and weights for whole brain, cerebrum and cerebellum for these subjects after testing was completed. There were significant effects of Sex on all of these

TABLE 1

MEAN BODY AND BRAIN WEIGHTS IN ADULT RATS AFTER EARLY POSTNATAL CAFFEINE ADMINISTRATION

Experimental Condition	Weight (grams) Body (Mean ± SE)	Whole Brain (Mean ± SE)	Cerebrum (Mean ± SE)	Cerebellum (Mean ± SE)
		Males		
Control	467.2 ± 12.4	1.99 ± 0.05	1.45 ± 0.04	0.55 ± 0.01
Caffeine (1 mg/kg)	460.1 ± 11.9	1.97 ± 0.03	1.44 ± 0.02	0.53 ± 0.01
Caffeine (9 mg/kg)	452.6 ± 10.9	1.97 ± 0.06	1.42 ± 0.04	0.55 ± 0.02
Vehicle	451.2 ± 27.0	1.82 ± 0.13	1.40 ± 0.02	0.52 ± 0.01
		Females		
Control	281.1 ± 11.0	1.84 ± 0.03	1.36 ± 0.03	0.48 ± 0.01
Caffeine (1 mg/kg)	289.6 ± 11.1	1.83 ± 0.05	1.34 ± 0.04	0.49 ± 0.02
Caffeine (9 mg/kg)	291.6 ± 13.5	1.80 ± 0.04	1.31 ± 0.03	0.49 ± 0.01
Vehicle	298.8 ± 14.6	1.78 ± 0.02	1.31 ± 0.01	0.47 ± 0.01

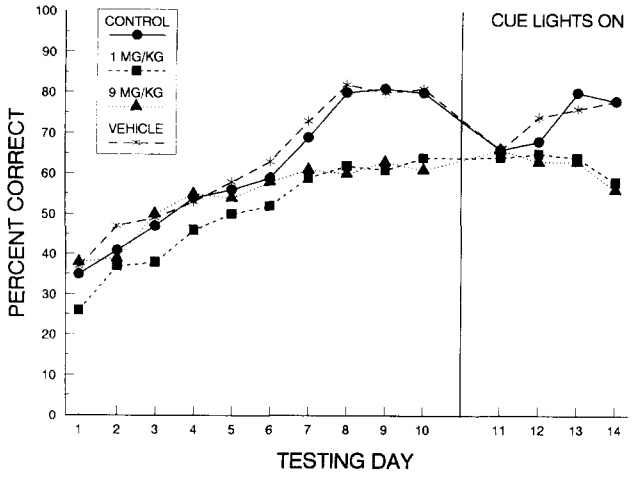

FIG. 3. The percentage of correct responses on a spatial alternation operant task (uncued and cued) in adult rats who had received either 1 mg/kg or 9 mg/kg caffeine or vehicle distilled water) for their first six postnatal days.

measures, F's(1,45) = 287.17, 10.71, 17.35 and 31.57, respectively, p's<0.01, but no effects of Postnatal Treatment.

DISCUSSION

Postnatal administration of caffeine had several deleterious effects on developing rat pups even at these low doses. The caffeine-treated pups gained less weight, were hypoactive as juveniles and showed impaired spatial learning ability as adults. Although one previous study (34) found a sex difference in the effects of prenatal caffeine exposure (only male offspring were affected), we found no evidence of any sex differences in any outcome measure.

Although all of the pups weighed the same at birth, the caffeine-treated rats of both low and high doses gained weight more slowly than vehicle-injected rats, who gained weight more slowly than controls. Slower growth during the caffeine administration period (days 1 through 6) might be attributable to suckling deficits (23). However, the sustained weight difference after cessation of injections suggests that this limited caffeine exposure may have produced a more permanent change in developing adenosine neurons. These results are supported by a previous study in which which found that at weaning, after a 21-day period of caffeine administration in dams' milk, caffeine-treated rat pups weighed less than control pups (22). Hunter and co-workers (25) also found small decreases in body weight in rat pups treated with caffeine on days 4–27. It is possible that the caffeine-treated rats reduced their food intake by emitting fewer behaviors leading to ingestion, since they were also hypoactive in the open-field test. Alternatively, since adenosine has been shown to inhibit lipolysis and reduce food intake (9, 13, 28), perhaps the effects shown here were caused by a caffeine-induced upregulation of adenosine receptors. Interestingly, the vehicle-injected rats also gained weight more slowly than controls, although they were heavier than caffeinated pups. Possibly this reflects an effect of the stress of the injection. None of the developmental measures used in this experiment, namely eye opening or righting, showed any significant effects. Significant effects in these measures are not consistently detected in prenatal caffeine exposure experiments (6). Perhaps higher doses of caffeine might have revealed an effect on these developmental landmarks.

In the open-field activity test, juvenile rats who had been exposed to either dose of caffeine in the first week of life showed significantly less activity than controls or vehicles. The fairly sudden difference in activity beginning on the third day of testing could have been due to the fact that the rats opened their eyes somewhere between days 13 and 15. Therefore, with the sudden presence of visual cues, control rats responded with increased activity, while drug-treated rats remained hypoactive and possibly less attentive. The results of this experiment are supported by studies which found that prenatal (10,44) or early postnatal (15) exposure led to decreased levels of activity. The fact that hypoactivity occurred after this chronic caffeine treatment suggests that caffeine, which normally increases spontaneous motor activity, might have upregulated adenosine receptors so that there was now increased adenosine inhibition of dopaminergic pathways subserving activity (20).

In addition, an effect of vehicle injection alone was also detected, similar to the vehicle-related weight deficit effect. Since all of the pups were removed from the nest for the same period of time, these effects cannot be attributed to any "handling" stress effects (the "handling" paradigm actually refers to a maternal separation procedure, and not to any physical stimulation). However, it is possible that the injection itself is so stressful that there are persistent effects on growth and activity, and that caffeine is potentiating the pups' responses to the stress either through a central adenosine pathway, peripheral adenosine receptors, or via some nonadenosine response. In adult rats, high doses of caffeine cause a "stress-like" endocrine response, including decreased thyrotropin and growth hormones, increased corticosterone and beta-endorphin levels, and increased adrenal weights after chronic administration; however, these responses do not appear to be under direct control of central adenosine receptors (41). Further research on the effects of postnatal caffeine on stress during development may help to determine the site of action.

As adults, the rats who had been caffeine-exposed were significantly impaired in their spatial alternation learning. Control rats learned the task more quickly and had a higher asymptotic performance level. The largest differences between controls and caffeine-exposed rats were seen on the first day and on the last two days of testing. Early caffeine exposure impaired both initial learning and later performance abilities. Studies of the acute effects of caffeine and adenosine on learning in humans and in animals have produced mixed results. In humans, caffeine's effects are dependent on the level of difficulty of the task, the sex of the subject, and dose. For example, caffeine has been found to enhance performance in simple memory and hand-eye coordination tasks, while impairing performance on more difficult tasks placing high demands on working memory or requiring vigilance, sustained cognitive effort, excellent hand-eye coordination, or the ability to give complex responses (14,16). Caffeine did not affect recall in males but impaired females at one level of response rate in a recall task (14). Arnold and co-workers (2) found that caffeine facilitated word recall in females at low (2 mg/kg) and high (4 mg/kg) doses but impaired recall in males only at the low dose level. Animal studies concerned with the role of adenosine in learning have been limited. Winsky and Harvey (46) found that the adenosine analog L-PIA retarded associative learning in a simple classical eyeblink conditioning task in rabbits.

Addition of cue lights, which had ameliorated deficits in prenatal alcohol-exposed offspring (47), did not reverse the performance deficits in these caffeine-exposed subjects. Since the cue lights did not ameliorate the performance deficits in the caffeine-exposed offspring, it cannot be concluded that there was a specific deficit in spatial learning as with the prenatal alcohol-exposed subjects. Interestingly, the cue lights caused a short-

term disruption of the control groups' performance, which then returned to the same asymptotic level seen before the addition of cue lights. The apparent lack of attentiveness to the addition of the lights was striking; perhaps it reflects a behavioral deficit similar to the failure of caffeine-exposed pups to markedly increase activity after their eyes opened, as mentioned above. In this postnatal caffeine paradigm, there may be a persistent deficit in visual discrimination, or a more global learning or attention impairment. It would be important to look for postnatal caffeine-related deficits in learning using additional tasks.

In summary, short-term exposure to low doses of caffeine in a third trimester animal model produced persistent deficits in body growth, juvenile activity and adult learning and performance of a spatial operant task. Since all of these effects are either opposite to those caused by acute caffeine or similar to those caused by adenosine, these results suggest that caffeine upregulates adenosine receptors when administered for six days at this developmental stage. This interpretation is supported by the finding that chronic caffeine administered in the dams' diets increased adenosine receptor binding sites in the cerebellum and brainstem in 16-day-old mice (30). Although there is increasing awareness of the adverse effects of drugs like alcohol and cocaine on the fetus, there is less concern about a more widely used drug, caffeine. In addition, the use of caffeine to stimulate respiration in premature infants may have short-term benefits but long-term consequences that need further elucidation.

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