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BRIEF COMMUNICATION

Acute Effects of Caffeine on Several Operant Behaviors in Rhesus Monkeys

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BUFFALO, E. A., M. P. GILLAM, R. R. ALLEN AND M. G. PAULE. *Acute effects of caffeine on several operant behaviors in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **46**(3) 733–737, 1993.—The acute effects of 1,3-trimethylxanthine (caffeine) were assessed using an operant test battery (OTB) of complex food-reinforced tasks that are thought to depend upon relatively specific brain functions, such as motivation to work for food (progressive ratio, PR), learning (incremental repeated acquisition, IRA), color and position discrimination (conditioned position responding, CPR), time estimation (temporal response differentiation, TRD), and short-term memory and attention (delayed matching-to-sample, DMTS). Endpoints included response rates (RR), accuracies (ACC), and percent task completed (PTC). Caffeine sulfate (0.175–20.0 mg/kg, IV), given 15 min pretesting, produced significant dose-dependent decreases in TRD percent task completed and accuracy at doses ≥5.6 mg/kg. Caffeine produced no systematic effects on either DMTS or PR responding, but low doses tended to enhance performance in both IRA and CPR tasks. Thus, in monkeys, performance of an operant task designed to model time estimation is more sensitive to the disruptive effects of caffeine than is performance of the other tasks in the OTB.

Caffeine *Macaca mulatta* Operant behavior Time estimation Incremental repeated acquisition Learning Color and position discrimination Temporal response differentiation Time estimation Delayed matching-to-sample Short-term memory Motivation Attention Food reinforcement

CAFFEINE is widely used as a central nervous system stimulant. It is the active ingredient in many over-the-counter preparations sold to increase 'alertness,' with the usual single oral dose being about 200 mg. Most Americans consume caffeine in colas, coffee, or tea, with the average amount of caffeine present in brewed coffee being about 85–115 mg/cup, in instant coffee, 60 mg/cup, in tea, 40 mg/cup, and in 1 cup of caffeinated soda, 24 mg/cup (14). Caffeine is found naturally in many foods and is also present in headache remedies, analgesics, and stimulants.

In the present study, using a range of doses encompassing those relevant to routine human exposure, we sought to determine caffeine's effects on the brain functions modeled in the operant test battery (OTB). Those functions include: motivation (PR), learning (IRA), color and position discrimination (CPR), time estimation (TRD), and short-term memory and attention (DMTS). We have previously used behavior in the National Center for Toxicological Research (NCTR) OTB for evaluating the neurobehavioral effects of a variety of psychoactive drugs (1,5–7,10–12,15–19). Furthermore, Paule et al. (4,8,9) have used a modified version of this OTB to assess the performance of normal and learning-impaired children and have found human and nonhuman OTB performance to be remarkably similar. Thus, results obtained in the monkey model likely will have relevance for the effects of caffeine in humans.

METHOD

Subjects

Seven male rhesus monkeys (*Macaca mulatta*) between 10 and 11 years of age (approximately 35% of maximal achiev-

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able lifespan) and weighing from 7 to 10 kg at the beginning of the study served as subjects. All animals had previously been trained under the schedules in the OTB for approximately 5 years and had been used in previous studies of acute drug administration (1,5-7,10-12,15-19). Animal housing, feeding, etc., were as described previously (6).

Apparatus

The apparatus has been described in detail elsewhere (15) and consisted of portable primate restraint chairs, sound-attenuated behavioral chambers, operant panels, and computer consoles. The operant panels were equipped with three rear-projection press plates, four retractable levers, six serial position indicator lights, and correct and incorrect response indicator lights. The press plates, levers, and indicator lights were aligned horizontally, with the press plates and serial position indicator lights located above the levers. Symbols and/or colors were projected onto the press plates from the rear and, when pressed, each press plate and lever effected a switch closure. Serial position and correct and incorrect indicator lights were illuminated from behind the panel with various

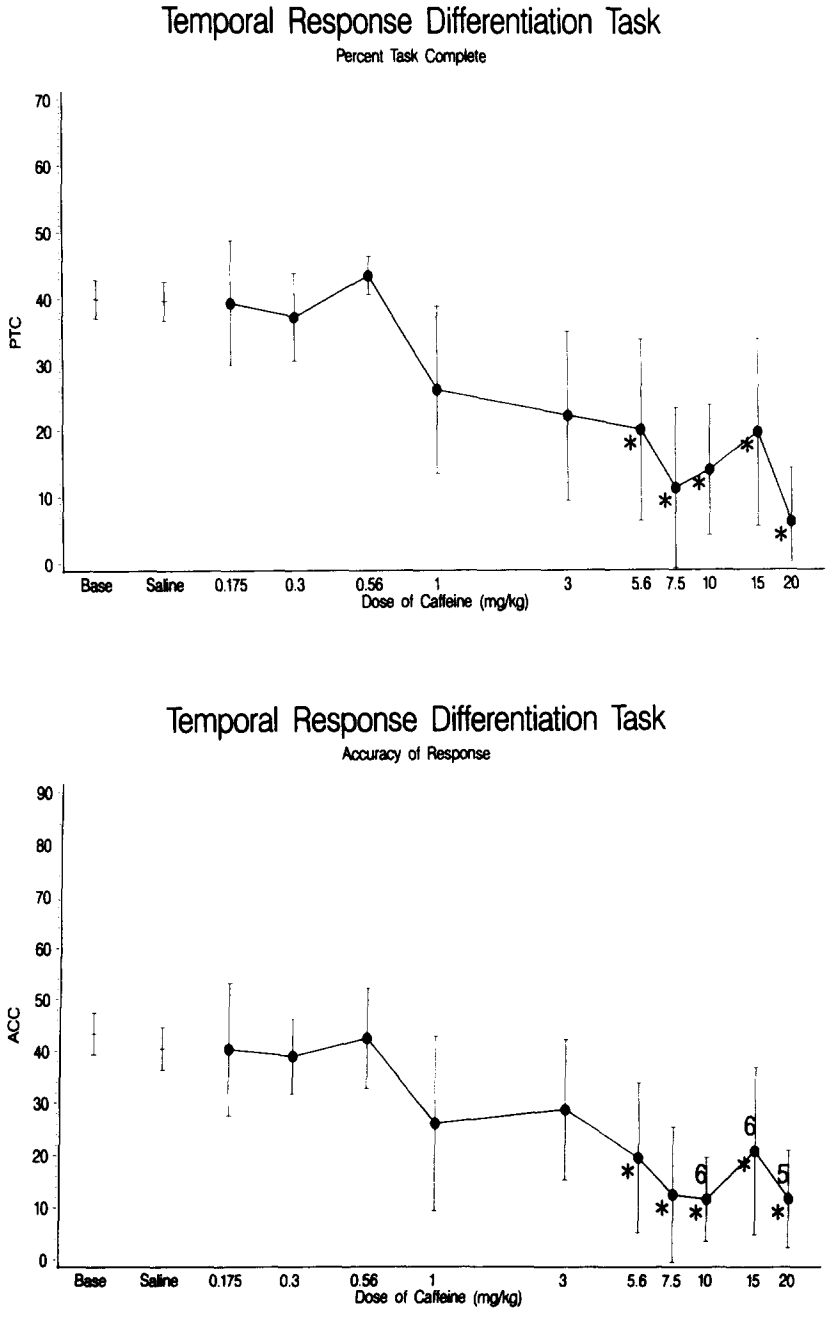

FIG. 1. Effects of caffeine on TRD percent task completed (PTC, top panel) and accuracy (ACC, bottom panel); $n = 7$ unless indicated otherwise. Each point represents the mean ± SE. Data above Base represent preexposure baseline performance (13-14 observations in each of seven animals). Data above Saline represent vehicle control performance (14 observations in each of seven animals). Asterisks indicate significant difference from vehicle controls determined by Bonferroni's t-test ($p < 0.05$).

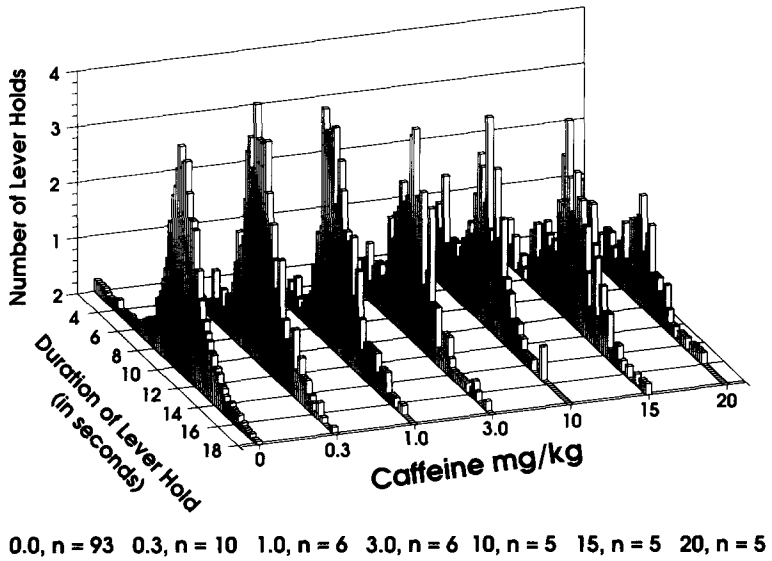

FIG. 2. Effects of caffeine on lever hold duration for the TRD task. Shown are the number of lever holds of specific durations for representative doses of caffeine.

colors. A trough for reinforcer (190 mg banana-flavored food pellet) delivery was centered below the levers.

Operant Schedules

The use and description of the tasks contained in the OTB have been reported in detail and a diagram of the behavioral test panel is published elsewhere (15). A brief description of each task follows.

Progressive ratio (PR). For the PR task, only the far right lever was used. Each monkey was required to increase the number of lever presses required for each subsequent reinforcer. Initially, three to eight lever presses (depending upon the individual monkey but the same for each subject every test session throughout this study) resulted in reinforcer delivery. The number of responses required for the next reinforcer was increased by the initial number of lever presses required for the first reinforcer. Thus, if three lever presses were required for the initial reinforcer, six lever presses were required for the next, then nine, twelve, etc. These initial ratios were chosen such that marked periods of pausing or cessation of responding generally occurred during each baseline or vehicle PR session.

Temporal response differentiation (TRD). For the TRD task, only the far left retractable lever was extended. Subjects were required to hold the lever in the depressed position for a minimum of 10 s but no longer than 14 s. Releasing the lever within this 4-s window resulted in reinforcer delivery. Releasing the lever too early or too late ended the current trial, after which the monkey could immediately start another trial.

Incremental repeated acquisition (IRA). The IRA task used all four response levers, and required subjects to acquire a new sequence of lever presses each test session. IRA began with the presentation of a one-lever response sequence (IRA1). Each response on the correct lever resulted in reinforcer delivery, and after 20 correct responses (criterion performance), a 1-min time-out period was followed by the presentation of an "incremented" two-lever sequence (IRA2), such that a response on a different lever was required before a response on the original lever produced food. After the 20th errorless two-lever sequence (i.e., no errors made between the first and last correct lever presses of the required sequence), the task was incremented to a three-lever sequence and so on, up to six-lever sequences or until the task time ended.

Conditioned position responding (CPR). For the CPR task, only the three press-plates were used. At the start of each trial, the center plate was illuminated with either a solid red, yellow, blue, or green color (side press-plates were dark). Subjects initiated each trial by making an "observing" response (a press) to the center plate, after which it was extinguished and the two side plates were immediately illuminated white. If the center plate color had been either blue or green, a response to the right press-plate resulted in reinforcer delivery. If the center press-plate had been either red or yellow, a response to the left press-plate resulted in reinforcer delivery. Responding to the incorrect position initiated a 10-s time-out period followed by the initiation of a new trial. The sequence of color presentation was random.

Delayed matching-to-sample (DMTS). For the DMTS task, only the three press-plate manipulanda were used. At the start of each trial, one of seven white-on-black geometric symbols (the "sample") was randomly projected onto the center plate (side press-plates were dark). To continue the trial, each monkey was required to make an "observing" response (a press to the "sample" on the center plate). After the observing response was made, the center plate was extinguished for one of six time delays presented pseudorandomly (2, 8, 16, 32, 48, or 64 s). After each time delay, all three plates were illuminated, each with a different geometric symbol, only one of which "matched" the sample. A response to the "match" resulted in reinforcer delivery and initiation of a new trial with another sample stimulus (presented randomly). A nonmatching response was followed by a 10-s time-out period (all press-plates darkened) and then initiation of a new trial.

Behavioral Testing Procedure

Behavioral test sessions were conducted daily (Monday–Friday), and lasted approximately 50 min. Monkeys were rotated through nine identical behavior chambers such that, gen-

erally, no monkey was placed in the same chamber for 2 consecutive test days. Behavioral schedules alternated daily. For example, PR (10 min), IRA (35 min), and CPR (5 min) were presented on 1 test day; TRD (20 min) and DMTS (30 min) were presented the next test day.

Drugs and Dosing Procedure

Caffeine sulfate (Sigma Chemical Company, St. Louis, MO) was dissolved in sterile bacteriostatic (0.9% benzyl alcohol) saline (Elkins-Sinn Inc., Cherry Hill, NJ) such that the final injection volume was 0.1 ml/kg for doses of 0.175, 0.3, 0.56, 1.0, 3.0, 5.6, 7.5, and 10.0 mg/kg, and the volume was 0.2 ml/kg for doses of 15.0 and 20.0 mg/kg. The purity of the caffeine was determined to be 99.5% by in-house gas chromatography. Doses of caffeine were administered IV in a semirandomized order. Caffeine injections were given on Tuesdays and/or Fridays, while vehicle injections were given on Tuesdays, Thursdays, and/or Fridays. Mondays and Wednesdays served as noninjected baseline days. Due to the daily alternation of the presentation of the behavioral tasks, all doses were given at least twice to provide dose–response data for each set. Approximately 15 min following injections, subjects were placed into operant chambers, and behavioral sessions began 1 min later.

Data Analysis

The endpoints measured in each task have been described in detail elsewhere (1,15–17). Two fundamental measures are monitored for each task and include percent task completed and response rate and/or latency. Accuracy data are used in all except the PR task. Percent task completed data are measures of a predetermined criteria of performance (i.e., completing 60 or 120 correct trials would represent 100% task completed for the CPR and the DMTS tasks, respectively) and are functions of both response rates and accuracies. Percent task completed values are calculated by dividing the total number of reinforcers earned by the total number of reinforcers possible for a given task and multiplying this quotient by 100. The percent task completed endpoint is a convenient and comprehensive measure showing intra-animal stability and is useful for comparing drug effects on performance across tasks. For the TRD task, the mean duration and temporal distribution of lever hold durations were also monitored. For the PR task, the breakpoint [the magnitude of the last ratio completed (i.e., number of lever presses made) for the last reinforcer earned] was also measured.

Statistical Analysis

Only those monkeys exhibiting stable performance [standard deviations of percent task completed values after saline (control) injections were less than 15% of mean PTCs] were included in the statistical analyses. For an animal's data to be included in the TRD and CPR accuracy analyses, he must have completed a minimum of three trials. For inclusion in the DMTS and IRA accuracy analyses, a monkey must have completed a minimum of 10 trials. For each behavioral endpoint in each task, the overall effect of drug treatment on performance was determined using a one-way repeated measures analysis of variance (ANOVA). If overall significance was evident ($p \leq 0.05$), then performance at each dose was compared to saline control performance using a Bonferroni correction (3).

RESULTS

Overall Effect of Saline Vehicle

Saline vehicle injections produced no statistically significant effects on any measures when compared to noninjected baseline data.

Progressive Ratio (PR)

Although caffeine tended to decrease percent task completed, breakpoint, and response rate in the PR task, the only significant effect occurred for percent task completed and breakpoint after 10 mg/kg; the effect was not seen at higher doses of 15 or 20 mg/kg (not shown).

Incremental Repeated Acquisition (IRA)

After 0.30 and 0.56 mg/kg caffeine, increases in the IRA percent task completed were noted, although they were not statistically significant (not shown). At 0.30 mg/kg, the total number of errors made early in the acquisition of the three-lever sequence (IRA3) was below the 95% confidence interval for control error levels (not shown). Similar effects were noted for both IRA response rate and accuracy. A statistically significant disruption in percent task completed and response rate occurred at 15.0 mg/kg; however, no effects were noted at the higher dose of 20 mg/kg. There were no significant effects of caffeine on general measures of IRA accuracy at any dose.

Conditioned Position Responding (CPR)

Caffeine, at several doses (0.3, 0.56, 5.6, 7.5, 15.0, and 20.0 mg/kg) tended to increase the percent task completed in this task, an effect that was associated with a trend toward decreases in both observing and choice response latencies. None of these effects were, however, significant by ANOVA. Likewise, no significant effects of caffeine were noted for accuracy (not shown).

Temporal Response Differentiation (TRD)

Caffeine produced dose-dependent decreases in TRD percent task completed, which were statistically significant at doses ≥ 5.6 mg/kg (Fig. 1, top). This effect on percent task completed was not paralleled by the response rate data (not shown) but was evident in accuracy of TRD performance (Fig. 1, bottom). At the highest dose of 20 mg/kg, the mean duration that the lever was held in the depressed position was also significantly decreased (Fig. 2).

Delayed Matching-To-Sample (DMTS)

Caffeine produced no statistically significant effects on any aspect of DMTS behavior.

DISCUSSION

Over the dose range tested, caffeine administration selectively altered performance in only one OTB task, producing a dose-dependent disruption of performance in the time estimation task. Only at the higher doses did it cause unsystematic (not dose-related) disruptions in the motivation and learning tasks; the short-term memory and color and position tasks were not significantly affected. Low doses (i.e., those that would typically be encountered after consumption of a single caffeinated soft drink) gave a hint of increasing learning task performance and decreasing response times in the color and

position discrimination task, but such effects were not statistically significant. These behavioral effects of caffeine are quite different from those obtained for several other psychotropic agents representing a variety of pharmacological classes tested in these same animals performing the same tasks (1,7,8,10–12,15–19).

In behavioral studies with humans, caffeine has been shown to improve vigilance and decrease response times in both children and adults (2,13). In the present study, most doses of caffeine tested tended to decrease response times in the color and position discrimination task. That these effects were not significant likely reflects a 'ceiling' effect for this particular task.

Based on the present observations, one would predict that human consumption of one can of caffeinated soda (about 0.3 mg/kg) will tend to enhance some aspects of brain function. Response or reaction times would likely not be affected or would be expected to decrease over a wider range of doses (1–17 cups of brewed coffee, 1–23 cups of instant coffee, 1–35 cups of tea, or 1–60 cans of soda), and the effects of caffeine to significantly disrupt time estimation behavior would not be anticipated until the user had consumed about 3–4 cups of brewed coffee, 6 cups of instant coffee, 9 cups of tea, or 16 cans of soda.

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REFERENCES

1. Ferguson, S. A.; Paule, M. G. Acute effects of chlorpromazine in a monkey operant behavior test battery. Pharmacol. Biochem. Behav. 42:333–341; 1991.
2. Lieberman, H. R.; Wurtman, R. J.; Emde, G. G.; Coviella, I. L. The effects of caffeine and aspirin on mood and performance. J. Clin. Psychopharmacol. 7:315–320; 1987.
3. Miller, R. G. Simultaneous statistical inference. New York: McGraw Hill; 1966.
4. Paule, M. G.; Cranmer, J. M.; Wilkins, J. D.; Stern, H. P.; Hoffman, E. L. Quantitation of complex brain function in children: Preliminary evaluation using a nonhuman primate behavioral test battery. Neurotoxicology 9(3):367–378; 1988.
5. Paule, M. G.; Schulze, G. E.; Slikker, W., Jr. Complex brain function in monkeys as a baseline for studying the effects of exogenous compounds. Neurotoxicology 9:463–472; 1988.
6. Paule, M. G.; Gillam, M. P. The effects of atropine on operant test battery (OTB) performance in the rhesus monkey. Soc. Neurosci. Abstr. 15(1):735; 1989.
7. Paule, M. G.; Allen, R. R.; Gillam, M. P. The effects of phencyclidine (PCP) on rhesus monkey performance in an operant test battery (OTB). Psychopharmacology (Berlin) 101:42; 1990.
8. Paule, M. G.; Cranmer, J. M. Complex brain function in children as measured in the NCTR monkey operant test battery. In: Johnson, B. L., ed. Advances in neurobehavioral toxicology: Applications in environmental and occupational health. Chelsea: Lewis Publishers; 1990:433–447.
9. Paule, M. G.; Forrester, T. M.; Maher, M. A.; Cranmer, J. A.; Allen, R. R. Monkey versus human performance in the NCTR operant test battery. Neurotoxicol. Teratol. 12:503–507; 1990.
10. Paule, M. G.; Gillam, M. P. The effects of pentobarbital on complex brain function in rhesus monkeys as measured by performance in an operant test battery. Eur. J. Pharmacol. 183(4):1127; 1990.
11. Paule, M. G.; Gillam, M. P. Effects of physostigmine on rhesus monkey performance in an operant test battery (OTB). Toxicologist 11(1):164; 1991.
12. Paule, M. G.; Allen, R. R.; Bailey, J. R.; Scallet, A. C.; Ali, S. F.; Brown, R. M.; Slikker, W., Jr. Chronic marijuana smoke exposure in the rhesus monkey II: Effects on progressive ratio and conditioned position responding. J. Pharmacol. Exp. Ther. 260(1):210–222; 1992.
13. Rapoport, J. L.; Jensvold, M.; Elkins, R.; Buchsbaum, M. S.; Weingartner, H.; Ludlow, C.; Zahn, T. P.; Berg, C. J.; Neims, A. H. Behavioral and cognitive effects of caffeine in boys and adult males. J. Nerv. Ment. Dis. 169:726–732; 1981.
14. Schreiber, G. B.; Maffeo, C. E.; Robins, M.; Masters, M. N.; Bond, A. P. Measurement of coffee and caffeine intake: Implications for epidemiologic research. Prev. Med. 17:280–294; 1988.
15. Schulze, G. E.; McMillan, D. E.; Bailey, J. R.; Scallet, A. C.; Ali, S. F.; Slikker, W., Jr.; Paule, M. G. Acute effects of delta-9-tetrahydrocannabinol (THC) in rhesus monkeys as measured by performance in a battery of complex operant tests. J. Pharmacol. Exp. Ther. 245:178–186; 1988.
16. Schulze, G. E.; McMillan, D. E.; Bailey, J. R.; Scallet, A. C.; Ali, S. F.; Slikker, W., Jr.; Paule, M. G. Acute effects of marijuana smoke in rhesus monkeys as measured by performance in a battery of complex operant tests. Life Sci. 45:465–475; 1989.
17. Schulze, G. E.; Slikker, W., Jr.; Paule, M. G. Multiple behavioral effects of diazepam in rhesus monkeys. Pharmacol. Biochem. Behav. 34:29–35; 1989.
18. Schulze, G. E.; Paule, M. G. Acute effects of *d*-amphetamine in a monkey operant behavioral test battery. Pharmacol. Biochem. Behav. 35:759–765; 1990.
19. Schulze, G. E.; Paule, M. G. Effects of morphine sulfate on operant behavior in rhesus monkeys. Pharmacol. Biochem. Behav. 38:77–83; 1991.