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Differential Effects of Injection Regimen on Behavioral Responses to Cocaine

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TERRY, P. *Differential effects of injection regimen on behavioral responses to cocaine.* PHARMACOL BIOCHEM BEHAV 41(2) 365-369, 1992.—Locomotor behavior was measured in mice receiving IP cocaine at 5, 10, 20, or 40 mg/kg. Mice in the *cumulatively dosed* treatment were injected first with saline and then with 5, 5, 10, and 20 mg/kg at 10-min intervals. In the *single-dose* treatment, mice received a single dose of cocaine at a time corresponding to the equivalent cumulative dose, with saline injections at other times. The *single-injection* treatment was similar, but saline injections were omitted. Locomotor activity was measured across each 10-min interval. Mice were retested 6 weeks later and 1 day after that. Dose-response curves were similar for all three treatments on the first test, but diverged markedly on subsequent tests. Significant locomotor sensitization occurred at the higher doses on the second test, particularly in the treatments receiving single cocaine injections. On the third test, convulsions occurred at 40 mg/kg, but only in the singly dosed treatments. The results demonstrate that injection parameters can modify both the behavioral and toxic effects of cocaine.

Cumulative dosing Cocaine Locomotor activity Sensitization Seizure Mouse

CUMULATIVE dosing is a procedure that allows rapid evaluation of drug effects: Complete dose-response curves can be obtained within a single test session. This provides potential advantages not only in time and cost but also in methodology since it allows a rapid assessment of the effects of a range of doses within a fully repeated-measures design.

The technique was initially restricted to in vitro pharmacology, but it has recently become more widespread in behavioral pharmacology. Despite this, there have been few systematic attempts to compare full dose-response profiles obtained cumulatively and discretely; limited comparisons have been provided both in operant procedures [e.g., (2,4,19)] and in locomotor activity tests [e.g., (11)].

The present study was initiated to determine whether cumulative dosing of the psychomotor stimulant cocaine might reliably reproduce the effects of discrete injections on locomotor activity, thereby permitting rapid antagonist studies later. Not only were complete dose-response functions compared across different injection regimens, but tests were also repeated after a long and a short intertest interval and variability of response was examined both within and between subjects. These additional analyses were primarily to determine whether intrasubject variability is a problem in cumulatively dosed subjects with repeated testing, as has been reported (11). A secondary concern was to assess whether the sensitization to the stimulatory effects of cocaine differs according to injection regimen. Repeated administration of cocaine can result in elevated locomotor stimulation and seizure susceptibility (3,13): This process of sensitization is affected by a number of parameters [e.g., see (12,14)], and injection regimen is likely to be an important such influence.

METHOD

Subjects, Apparatus, and Drugs

Male Swiss-Webster mice (Charles River, Wilmington, MA) weighing 25-35 g were housed five or six per cage with free access to standard Purina chow and tapwater. The housing room was environment-controlled, with room lights on between 0600 and 1800 h. Testing was between 1300 and 1600 h in open-field Plexiglas enclosures (39.5 × 39.5 × 31 cm high) inside Digiscan activity monitors (Columbus Instruments, Columbus, OH). Interruption of two consecutive photobeams incremented the ambulatory activity score. Cocaine HCl was from Mallinkrodt/Nuclear (Orlando, FL); all injections were IP at 0.5 ml/100 g.

Injection Treatments and Test Procedure

Mice were tested in separate arenas in squads of four. They were first allowed 40 min to habituate to the apparatus before testing. There were three injection treatments, as follows.

Cumulatively dosed (n = 10). After habituation, mice were removed from the test arenas and injected with physiological saline. They were returned to the test arenas after 2 min (time to inject full squad, injection order balanced) and behavior was monitored for 10 min. They were then removed again, injected with 5 mg/kg cocaine HCl, and returned to the arenas for a further 10 min. This procedure was repeated three more times, injecting 5, 10, and 20 mg/kg cocaine consecutively. This provided total cumulative doses of 0, 5, 10,

20, and 40 mg/kg over the course of the session, with 10 min of activity monitoring at each dose. Activity scores were recorded every 2 min within each 10-min block.

Single dose (n = 40). All mice received a total of five injections, as in the cumulatively dosed group, but only one of these was cocaine (the other injections being saline). A single dose of either 5, 10, 20, or 40 mg/kg cocaine was given as the second, third, fourth, or fifth injection, respectively, with saline injections at other times. A saline control group was given five saline injections only. There were eight mice in each dose group.

Single injection (n = 32). Subjects were injected only once, receiving either 5, 10, 20, or 40 mg/kg cocaine at the time at which those doses were given in the other treatments. No saline injections were administered. There were eight mice in each dose group.

Behavioral Observations

A behavioral checklist was used to examine qualitative changes in behavior, from simple exploratory behaviors to stereotypies [the rating procedure was modified from that used with rats by Kalivas et al. (6)]. Each mouse was observed for 10 s every 2 min and a single category from a 10-category list recorded for that interval. Because all except one of these categories failed to discriminate between treatments, only this exception will be described: occurrence of clonic seizures. A clonic seizure was defined in accordance with Marley et al. (8): either a loss of body posture with convulsive movements in the extremities or a bout of uninhibited running and bouncing clonus.

Repeat Testing

All mice were retested 42–50 days after initial testing (the interval balanced between groups) exactly as before; they were tested again 24 h later. Comparisons between the cumulatively dosed group and the single-dose or single-injection groups *over* tests are only strictly possible at the highest dose (40 mg/kg), the only one for which all injection groups received the same cumulative drug quantity over tests.

Data Analysis

Because seizures were observed during the third test at 40 mg/kg (see below), scores were adjusted to mean counts per 2-min interval, eliminating scores from intervals where seizures were observed (and all subsequent 2-min intervals within a 10-min block).

Statistical comparisons between the cumulatively dosed group and the two singly dosed groups are complicated by the fact that, of necessity, the cumulative-dosing regimen is a repeated measure whereas the single-dosing regimens use independent groups. For this reason, separate comparisons were made at each dose level where necessary. Standard bioassay methods were used to determine lines of regression and to compare cumulative dose-effect functions with the others on each test (18). One score at 5 mg/kg for the cumulatively dosed group on test two was not obtained; to allow full crossover analyses on the bioassays, this score was interpolated as the group mean. Comparisons of frequencies (seizures) were made using Fisher's test of exact probability (17).

RESULTS

Dose-Response Functions

Dose-response relationships for each drug group, and the saline controls, are plotted for each of the three tests in Fig. 1. All drug treatments produced a significant dose-related increase in locomotor activity [cumulative: $F(4,36) = 4.83$; single-dose: $F(4,35) = 3.48$; single-injection: $F(4,35) = 6.95$; all p's < 0.05]. Dose-response curves for the three drug groups were similar at the first test (first panel, Fig. 1): Comparisons between drug groups at each level of dose failed to reveal any reliable differences, even at 40 mg/kg, $F(2,23) = 1.68$, $p > 0.05$.

Differences between injection treatments emerged on subsequent tests (second and third panels, Fig. 1). On test 2, injection treatments differed at 5, 10, and 40 mg/kg, $F(2,23) = 5.20$, $F(2,23) = 7.02$, $F(2,23) = 4.19$, respectively, all p's < 0.05. Posthoc comparisons between treatments (Tukey's) revealed that the greater increase in ambulatory activity observed in the single-injection group compared to the single-dose group was significant at 5 and 10 mg/kg (p's < 0.05). In addition, the increase in ambulatory activity in the single-dose group was significantly greater than that obtained in the

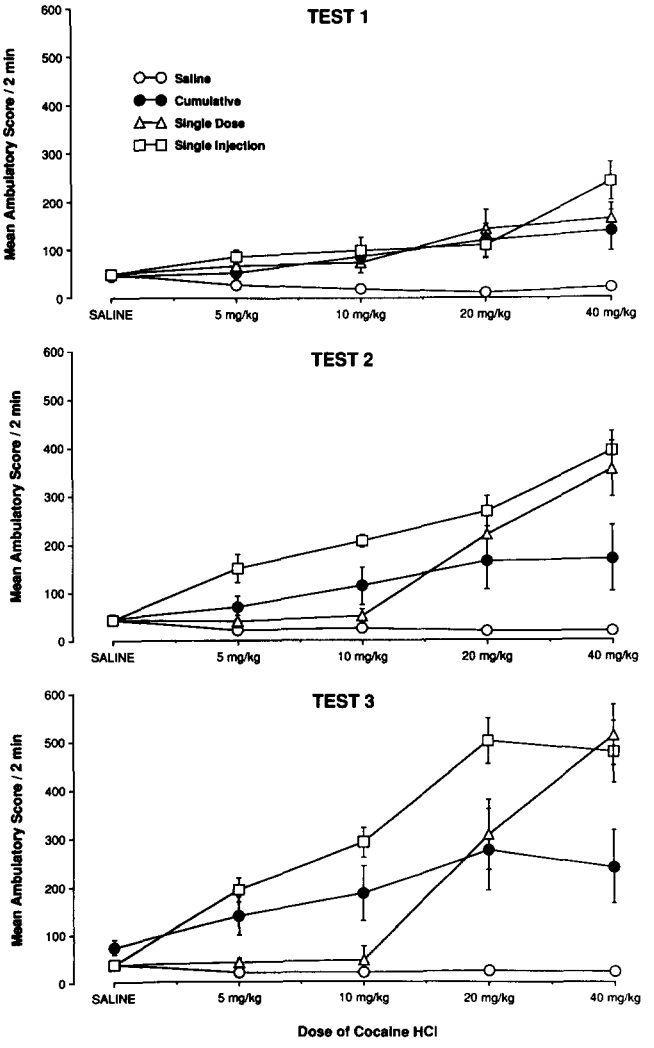

FIG. 1. Dose-response functions for ambulatory activity against dose of cocaine, presenting each of the three injection groups over each of the three tests. Upper panel, Test 1; middle panel, Test 2 (42–50 days later); lower panel, Test 3 (24 h after Test 2). Scores are mean ambulatory counts per 2-min interval.

cumulative-dose group at 40 mg/kg (p's < 0.05). The same pattern occurred on test 3, with treatment differences at 5, 10, and 40 mg/kg, $F(2,23) = 5.64$, $F(2,23) = 6.71$, $F(2,23) = 4.82$, respectively, all p's < 0.05, and posthoc differences between the single-dose and single-injection groups at 5 and 10 mg/kg, and between the single-dose and cumulatively dosed groups at 40 mg/kg (all p's < 0.05). There were no differences at 20 mg/kg.

Because of quantitative and qualitative differences in dose-response functions, ED values were calculated to obtain estimated doses required to produce 100 activity counts. These are presented, with 95% confidence intervals, in Table 1. All linear regressions were significant. The trend is toward increasing sensitization over tests; ED_{100} values decreased across repeated tests. The least sensitization occurred in the single-dose condition. The pattern of differences that emerged from separate analyses of variance (ANOVA's) was confirmed by parallel line bioassay. First, there were no differences between the three injection treatments on test 1, but significant differences in maximal effects between cumulative-dose and single-injection conditions on both tests 2 and 3, $F(1,64) = 15.55$, $F(1,64) = 13.41$, respectively, p's < 0.01. Comparisons between the cumulatively dosed and single-dose conditions were obviated by significant deviations from parallelism on tests 2 and 3, $F(1,64) = 6.4$, $F(1,64) = 10.5$, respectively, p's < 0.05, probably reflecting the limited response at 5 and 10 mg/kg in the single-dose group (Fig. 1, middle and lower panels).

Response to 40 mg/kg Cocaine

Activity counts. Figure 2 plots scores at 40 mg/kg for each drug condition over successive tests. All groups showed sensitization across both intertest intervals, but the rate of increase (and overall amount) of locomotor activity was much reduced in the cumulatively dosed subjects. These patterns were confirmed by comparing single-dose and cumulatively dosed groups (same number of injections, same total drug intake): There was a significant treatment difference, $F(1,16) = 5.03$, $p < 0.05$, a significant difference between tests, $F(2,32) = 14.29$, $p < 0.05$, and a significant interaction of treatment with test, $F(2,32) = 4.54$, $p < 0.05$. None of these effects were significant when comparing the single-dose and single-injection groups.

Seizures. Figure 3 presents percentages of each injection group seizing during third test at 40 mg/kg. No seizures were observed on any other test or at any other dose. Seizures were clearly most prevalent in the single-dose group. The disparities between the single-dose group and the other two groups were both significant (comparison with cumulatively dosed, $p = 0.0015$; with single injection, $p = 0.023$).

Individual differences. To determine whether there is greater intra- or intersubject variability in the cumulatively dosed group compared with the other groups, individual profiles were examined within and between tests. The variation in drug response among cumulatively dosed mice was considerable, with some subjects showing no clear response at any dose. Even so, the degree of variation at each dose is comparable among injection groups (compare standard errors, Fig. 1).

To examine reliability over successive tests in the cumulatively dosed group, area under the curve for individual dose-response functions was correlated between each successive test. There was a high degree of intrasubject consistency: Between tests 1 and 2, $r = 0.87$; between tests 2 and 3, $r = 0.90$; and between tests 1 and 3, $r = 0.81$ (all p's < 0.01). A

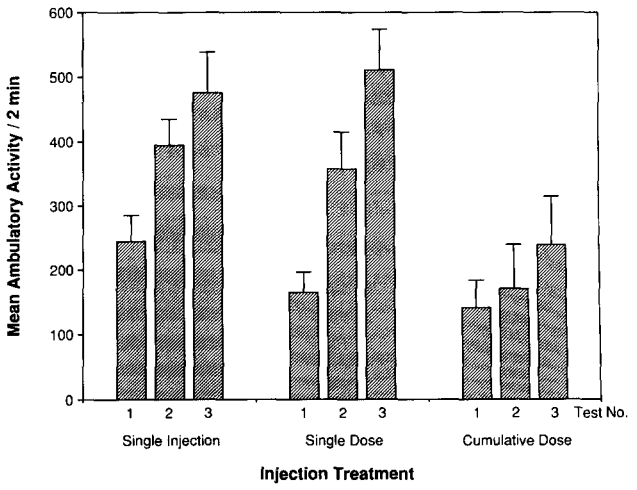

FIG. 2. Locomotor activity (ambulation) at 40 mg/kg for each injection group on each test. Scores and intertest intervals are as in Fig. 1.

TABLE 1

ESTIMATED DOSES OF COCAINE (mg/kg) REQUIRED TO EVOKE 100 AMBULATION COUNTS FOR EACH OF THE THREE INJECTION TREATMENTS ON EACH OF THE THREE DIFFERENT TESTS

Injection Treatment	Test 1	Test 2	Test 3
Cumulative dose (mg/kg)	14.0	7.9	2.1
	(8.9-21.9)	(3.9-16.0)	(0.5-8.8)
Single dose (mg/kg)	11.2	9.3	8.4
	(6.0-20.9)	(6.9-12.6)	(6.4-11.0)
Single injection (mg/kg)	8.5	3.7	2.5
	(4.9-14.8)	(2.0-6.6)	(1.2-5.4)

Values in parentheses are lower and upper 95% confidence limits.

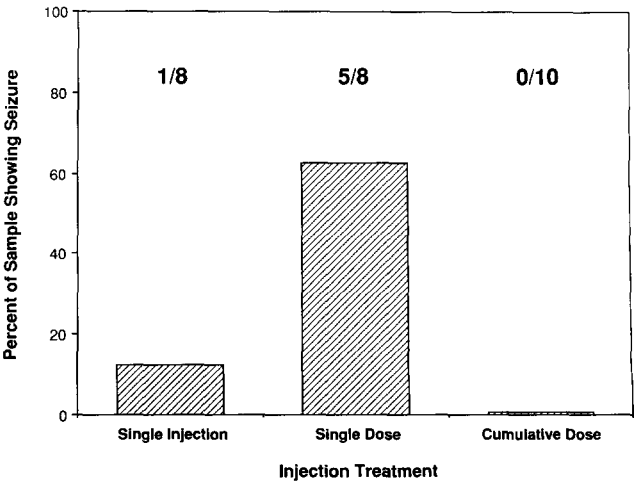

FIG. 3. Percentage of group sample seizing during third test at 40 mg/kg. No seizures were observed on any other test or at any other dose.

similar comparison for the other groups is difficult since the only scores available for correlation are at each individual dose and these did not produce significant correlations even in the cumulatively dosed group (for example, correlating between tests 1 and 2 at 40 mg/kg, single-dose and cumulatively dosed groups, respectively, $r = 0.67$, $r = 0.60$, p's > 0.05).

Behavioral observations. There were no qualitative differences in activity patterns between different drug groups, except for incidence of clonic seizures (see above). The general trend over doses was for infrequent stereotypies to develop at 20 and 40 mg/kg on second and third tests; ataxia was only recorded in the single-dose and single-injection treatments and only on the third test.

DISCUSSION

The psychomotor stimulation produced by cumulative dosing of cocaine presents a similar dose-response profile to individual doses (with or without control for numbers of injections) over a single test session. Although variation in response to drug is considerable in the cumulatively dosed group, with some subjects showing no psychomotor stimulation at any dose, the between-subject variance at each dose is similar to that in the other injection treatments. In addition, there is a high degree of intrasubject consistency in response to drug over tests, at least in the cumulatively dosed group using area under the curve as the measure.

However, despite these apparent similarities in dose-response relations, differences between injection treatments emerge with repeated testing. These differences, in terms of sensitization and seizure susceptibility, suggest that the different injection treatments may not be functionally equivalent; moreover, the fact that differences were identifiable at the second test suggests that there may be a lack of functional equivalence even at the first test. The significant preparations effect between cumulatively dosed and single-injection groups on Tests 2 and 3 demonstrates increased efficacy with the latter treatment; the deviation from parallelism between cumulatively dosed and single-dose conditions on the same tests might indicate qualitatively different modes of action for the two treatments.

More generally, the degree of sensitization over the first intertest interval is surprising given the length of the interval (approximately 6 weeks) and the single dosage. Single injections of cocaine have been demonstrated to induce sensitization measurable after at least 1 week (7), and as few as four daily injections of cocaine can sensitize responding for as long as 2 months after treatment (16). Prolonged or continuous infusions of psychostimulants are less effective in producing sensitization than intermittent challenges (12), and this may account for the differences obtained here between cumulatively dosed mice and those receiving single shots. However, the nature of the interactions between interinjection interval and other critical variables in the promotion of sensitization remains to be quantified (12).

The generalizability of these results to other compounds, procedures, and species (or strains of mice) remains to be determined. In a recent study of schedule-controlled behavior in rats cumulatively dosed with naltrexone (15), repeated weekly tests markedly enhanced behavioral sensitivity, apparently because the regular association between lower and higher doses resulted in conditioned suppression to the lower doses. This enhanced behavioral sensitivity with cumulative dosing is apparently not the case in the present experiment, where behavioral sensitivity is reduced in comparison with discrete dosing, but the number of injection–drug pairings would probably be too low (and the intertest interval too long) for the effect to develop.

It is also to be noted that the single-dose and single-injection groups differed in a number of respects. In particular, they differed markedly at lower doses (5 and 10 mg/kg) on second and third tests. This is partly explicable according to a conditioned sensitization effect [e.g., see (5,10)] since mice in the single-injection group always received cocaine at injection, whereas mice in the single-dose group received four saline injections in addition to one injection of cocaine on each test. The repeated pairing of injection with no behavioral consequences might then impair the response to drug in comparison with the group that always received cocaine at injection [in addition, it has been shown that saline injections can actively suppress locomotion (9,16)]. However, conditioning might then be expected to exaggerate differences between groups at all doses rather than only at the lower doses. This suggests that responses to the higher doses might be independent of such conditioning effects, although control for number of preceding injections would be required. Clearly, the situation requires more direct experimental study.

A second important difference between injection treatments concerns the incidence of seizures. The single-dose group is exceptional in its high rates of seizure at 40 mg/kg, even though levels of locomotor activity are comparable between single-dose and single-injection groups at this dose. It is well established that repeated stressors, such as handling and injection, can act to sensitize responses to psychostimulants [e.g., (1)]. The possibility of an interaction between handling- and cocaine-induced behaviors is increased by the short time interval available for measurement (10 min). However, if the four saline injections preceding the 40 mg/kg cocaine serve as stressors to sensitize behavior, then this process must act specifically on one category of response (seizure and not locomotion). Moreover, acute effects of stress on behavioral responses to dopaminergic agents occur, as well as effects with repeated testing (21); no such acute effect was apparent here (i.e., no differences between curves were identified on Test 1). Finally, an account of stress-enhanced susceptibility to seizures would also necessarily underline the difference between the cumulatively dosed group and the single-dose group since at 40 mg/kg the former group received the same number of injections and the same dose but showed no seizures. It seems that only a constellation of interacting factors can explain the pattern of results obtained.

In conclusion, the results suggest some caution in the use of cumulative-dosing procedures with psychostimulants, at least for the IP route of administration. Although the reduced effect size with chronic testing might be useful in minimizing the risks of toxic reactions to cocaine in chronic studies, the possibility remains that cumulative dosing leads to different effects on central mechanisms or alters cocaine pharmacokinetics (20). These effects may later become apparent by modifying responsivity to coadministered compounds (if compared with singly dosed subjects).

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