

Cocaine in adolescent rats produces residual memory impairments that are reversible with time

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Abstract

Rats received injections (subcutaneous) of either 10 or 20 mg/kg cocaine on postnatal days 26–33, while lab chow-fed and pair-fed controls received saline. Spatial memory in a Morris water maze was assessed on four different occasions commencing 10 days postcocaine and ending ~ 12 months later. To determine whether there existed long-term changes in cholinergic processes, maze performance was evaluated following 1 mg/kg scopolamine challenge 4 months postcocaine. Subjects survived under standard laboratory housing conditions until they died. Results from the first assessment indicated a working memory deficit in the low-dose cocaine group and a long-term memory impairment in the high-dose cocaine group. These decrements neither were permanent nor were exacerbated by age-related processes in that cocaine-treated subjects performed at control levels on subsequent assessments. An exception to this was the results derived from the third assessment indicating that animals previously treated with 20 mg/kg cocaine were impaired when challenged with scopolamine. Examination of mortality rates revealed that cocaine-treated rats died significantly sooner than lab chow-fed control subjects. Taken together, these data indicate that cocaine during adolescence causes residual, but not permanent, deleterious effects on memory that may be mediated by alterations in cholinergic neurochemistry. More provocatively, the results showed that cocaine during adolescence shortened the lifespan of rats. This latter finding suggests that cocaine during adolescence may produce residual physiological effects that last well into adulthood. © 2004 Elsevier Inc. All rights reserved.

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1. Introduction

Cocaine abuse continues to represent a serious biopsychosocial health issue in the United States. Results from national surveys have estimated cocaine to be involved in nearly half of all drug-related deaths and emergency room visits [18]. Disturbingly, the number of young to older adolescents who admit to using cocaine has risen significantly over the past decade [16]. This is especially alarming because cocaine in this age group produces a variety of psychosocial dysfunctions and psychiatric symptoms and is as addictive, or possibility more addictive, than in adults [8]. Given these data, it is critical that basic research studies aimed at identifying cocaine's neurobiobehavioral effects

when administered during adolescence be conducted. In this regard, using animal models is essential given the ability of such approaches to eliminate methodological confounds such as polydrug use, difficulties accessing quality health care, socioeconomic variability, differences in lifestyle, etc., inherent in studying drug use in humans.

Although relatively very small in size, an empirical literature reporting on the results of cocaine administration in adolescent animals is now emerging. One particular issue of paramount importance is determining whether cocaine produces delayed or residual cognitive effects, especially on learning and memory. Impetus for examining this specific question is derived from previous findings indicating the ability of prenatal cocaine to produce deficits of learning [11,13,14], memory [12,26], and attention [10,23], with some impairments lasting well into adulthood [13,14,26].

Using an eight-arm radial maze, Melnick et al. [25] have examined spatial learning and memory in 4-month-old rats

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previously treated with 50 mg/kg cocaine on postnatal days 11–20. These researchers found that their cocaine-treated subjects were impaired on both initial acquisition and relearning of the task when conducted in a different contextual environment. Moreover, consistent with other data indicating an increased sensitivity in female rats to prenatal cocaine's effects on spatial learning [21], Melnick et al. reported that female animals previously treated with cocaine were found to be more impaired than male subjects on a second relearning session administered in the original experimental training room. In contrast, using the Morris water maze as the assessment instrument, Vorhees et al. [34] observed neither acquisition nor memory deficits in 2-month-old rats previously treated with cocaine (15 mg/kg, four times a day) on either postnatal days 1–10 or 11–20. In fact, these investigators reported enhanced performance in the early cocaine-treated group relative to control subjects.

Given the paucity of available data to date and the lack of consistency in the results that have been reported, additional studies are needed to more fully evaluate cocaine's effects on learning and memory when administered during the developmentally critical period of adolescence. In such studies, issues related to dose, age, and length of abstinence extending beyond a few months are among the many variables that need to be examined. The present investigation was an effort at contributing further to the literature by examining the effects of two cocaine doses (10 or 20 mg/kg) administered during early adolescence (postnatal days 26–33; [32]) on spatial learning and memory in rats trained in a Morris water maze at four postadministration time points extending over the course of about 12 months. Moreover, we determined whether differences in mortality rates would emerge over the second year of life after an early trend was observed suggesting that cocaine-treated animals had shorter lifespans. Finally, to evaluate the potential consequences on learning and memory produced by cocaine's anorexic effects, we included a critical control group [pair-fed (PF) control] consisting of animals with food intakes and body weights mirroring that of the 20 mg/kg cocaine dose group.

2. Methods

2.1. Subjects

Forty male Sprague–Dawley adolescent rats (25 days old on arrival) purchased from Taconic Farms (Germantown, NY) were used. Rats were housed individually in standard stainless steel cages in a temperature-controlled (21 ± 1 °C, 60–80% humidity) vivarium and were maintained on a 12-h light/dark schedule (lights on at 7:00 a.m.). Subjects had unlimited access to food and water throughout, except for those animals in the PF control condition. During the cocaine injection period, rats in the PF control group had their access to food limited to the average amount of food

consumed by animals in the 20 mg/kg group on the prior day. Otherwise, subjects in this control condition had free access to food and water. The Manhattanville College Institutional Animal Care and Use Committee approved the care and use of the subjects, and American Psychological Association's ethical standards were followed.

2.2. Apparatus

A Morris water maze was used to test spatial memory. The maze consisted of a black circular fiberglass pool (Fibertech Engineering, El Cajon, CA) filled 24.0 cm high with 27 ± 1 °C water. The pool measured 121.9 cm in diameter and 61.0 cm high and was in an isolated experimental room rich with extramaze cues. Two strips of colored ribbon were suspended from rim to rim over the maze at 90° angles and served as guides for identifying the borders of the pool's four quadrants. Pool water was made opaque by the addition of black nontoxic tempera paint and was replaced daily. A 10.7 × 10.7-cm black ceramic tile mounted on a 23.0-cm-high black plastic submergible stand served as the escape platform. The escape platform was placed in the middle of one of the four quadrants with all four quadrants used equally often. The particular quadrant in which the platform was placed was determined for each animal according to a balanced procedure that ensured an approximately equal number of animals in each condition were trained to each of the four quadrants. One experimenter measured escape latencies (0.1 s) with a hand-held stopwatch, while another experimenter recorded distance swum (cm) data via a computer-assisted digitizing tracking procedure [31].

2.3. Cocaine administration

Cocaine HCl (Sigma, St. Louis, MO) was mixed with 0.9% saline, and suspensions were kept refrigerated in light-tight bottles. All injections (subcutaneous) were administered in a volume of 2 ml/kg. Initial body weights were used as the basis for the random assignment of subjects to ensure that each of the four treatment conditions [lab chow-fed (LC) control $n = 10$, PF control $n = 10$, 10 mg/kg cocaine (10 mg) $n = 10$, and 20 mg/kg cocaine (20 mg) $n = 10$] contained animals of equal average weight [$M_s = 51.8, 50.4, 50.1,$ and 50.3 g, respectively; $F(3,39) < 1.0, P > 0.05$]. All control animals received volumetrically equivalent injections of saline. The injection regimen commenced the day after the animals arrived (postnatal day 26) and continued for 8 consecutive days. A 10-day no injection recovery period followed before animals were assessed behaviorally. Body weights were recorded periodically during the cocaine administration period.

2.4. Morris maze: general procedures

Acquisition training consisted of placing the rat in the pool facing the wall at one of four cardinal start locations

designated N, S, E, and W. The four start locations were balanced with respect to the quadrant in which the platform was placed so that the platform was located either far or near, and either to the left or to the right, of the starting locale an equal number of times on each day. The animal swam until it found and climbed onto the escape platform with all four paws. Once securely on the platform, the animal was allowed to remain there for 10 s. If the animal did not find the platform within 60 s, it was placed on it for 10 s. Trials were separated by an ~ 1-min intertrial interval. Reversal training was identical to acquisition, except that animals were required to swim to the platform placed in the quadrant diagonal from the one used during acquisition. Escape latency (s), distance swum (cm), and swim rate (cm/s) (distance swum \div escape latency) served as the dependent variables. Probe trials, when administered, were conducted with the platform removed from the pool. The total number of times the rat's entire body (minus its tail) entered the quadrant in which the platform was located and/or the total time (s) spent in that quadrant were recorded.

2.5. Assessment sequence in the Morris maze

2.5.1. First assessment

Animals were initially assessed after the end of the 10-day recovery period starting when they were 44 days old. Subjects were trained to find the submerged platform for four trials administered on the same day followed 4 days later by a single probe trial. Four days after the completion of the acquisition probe trial, animals were trained for four trials on reversal followed 4 days later by another probe trial. The start points used on probe trials were the same ones used on the first trial of acquisition/reversal.

2.5.2. Second assessment

Animals were between 142 and 150 days old, and it had been 105–113 days since the end of the cocaine treatment period. Animals were trained on acquisition for four trials followed by a probe trial ~ 4 days later ($M=4.4$ days). Three weeks later ($M=20.3$ days), subjects were trained for four trials on reversal. Three weeks thereafter ($M=18.6$ days), subjects were assessed on a probe trial. A long retention interval was used before assessing animals on the reversal probe trial to determine whether there might be a differential decay of long-term memory. For both probe trials, animals were started from the same start location used on trial 4 during acquisition/reversal training. The sequence of start locations and the quadrant in which the platform was located during acquisition/reversal training were the same for all animals.

2.5.3. Third assessment

Animals were 158–166 days old, and it had been 121–129 days since the end of the cocaine treatment period. One

animal from the 10 mg cocaine condition had died in the interim, thus reducing the number of subjects in this group to 9. The general training procedures used here were identical to that employed in the second assessment's acquisition phase. In addition, each animal received either a 1.0 mg/kg/ml injection (intraperitoneal) of scopolamine HCl (Sigma) or an equivolumetric injection of 0.9% saline (the vehicle) 15 min prior to training (N_s per group = 5, except 10 mg cocaine/saline, $n=4$).

2.5.4. Fourth assessment

Animals were 410–416 days old, and it had been 377–383 days since the end of the cocaine treatment period. Two more animals in the 10 mg cocaine condition and two animals in the 20 mg cocaine condition had died in the interim, thus leaving seven and eight subjects in these two groups, respectively. The working memory procedure generally followed that used in previous assessments, except that animals were trained for four trials per day for 4 days. The location of the platform remained in the same quadrant for the four trials administered on each day but changed to a new location from one day to the next. Because each of the four quadrants was used once over the course of the 4-day training regimen, both working memory and proactive interference from prior learning could be assessed.

2.6. Age at death

Animals were allowed to survive under standard housing conditions until they died. The vivarium was monitored daily for dead animals. Four animals (two in the PF group and one each in the LC and 10 mg groups) that were 694 days old at the time the study was terminated were killed with an overdose of sodium pentobarbital (130 mg/2 ml; Sigma). For these animals, their age at death was recorded as 694 days.

2.7. Statistical analysis

Data were analyzed with the aid of a statistical computer program available commercially [33]. Appropriately designed analyses of variance (ANOVAs) were performed to detect overall effects followed by pairwise Fisher Least Significant Difference Tests when appropriate. Finally, to estimate whether sufficient statistical power ($1 - \beta$) was present to detect differences among the four dose groups, a power analysis for a one-way ANOVA was performed using a fixed effects model, a medium root mean square standardized effect of 0.30,¹ and 10 subjects in each dose group. This analysis yielded a value of $1 - \beta$ of 0.23, much smaller than the typical desired proportion of 0.80.

¹ A root mean square standardized effect of 0.30 is comparable with Cohen's [6] medium effect size (f) of 0.25 [33].

3. Results

3.1. Body weights

Animals in the 10 mg ($M=64.6$ g) and 20 mg ($M=63.6$ g) groups had lower overall lower body weights than subjects in the LC control condition [$M=70.8$ g; $F(3,47)=6.72$, $P<0.001$]. The pair-feeding regimen proved successful in that the body weights of PF control animals ($M=64.3$ g) did not differ from the body weights of subjects serving in the two cocaine dose groups (both $P>0.05$), whereas they did differ from those of LC control subjects ($P<0.01$). By the end of the injection period, the body weights of 10 mg animals ($M=66.5$ g) resembled those of LC rats (72.0 g; $P>0.05$), although the weights of both PF ($M=65.1$ g) and 20 mg ($M=64.9$ g) subjects remained lower (both $P<.05$). Body weights, however, had stabilized by the time animals were evaluated for the third assessment in the Morris maze when they were ~ 5 months of age [$F(3,35)=1.73$, $P>0.05$], with all group averages being comparable (means: LC = 546.5 g, PF = 523.1 g, 10 mg = 535.8 g, and 20 mg = 560.7 g).

3.2. First assessment

3.2.1. Swim rates

A one-way ANOVA for independent samples analyzed the aggregate swim rates of the four groups of subjects during acquisition and reversal training. Results indicated no differences among the groups with averages for LC, PF, 10 mg, and 20 mg animals being 14.3, 13.3, 15.2, and 15.2 cm/s, respectively [$F(3,319)=1.13$, $P>0.05$].

3.2.2. Acquisition and reversal

Because the distance swum and latency to escape measures yielded an identical pattern of results, only the more

traditional latency measure is described here and for all subsequent assessments. The average acquisition and reversal escape latencies for the four groups over the four trials are presented in Figs. 1 and 2, respectively. A 4 (Cocaine) \times 2 (Phase) \times 4 (Trials) mixed factorial ANOVA with repeated measures on the last two variables was performed on these data. Results from this analysis indicated that, overall, animals found the platform in less time during the reversal phase ($M=36.0$ s) relative to acquisition [$M=48.5$ s; $F(1,36)=56.64$, $P<0.0001$] and required less time to find the platform over the four trials [M s: trials 1–4 = 52.5, 44.0, 38.6, and 34.0 s, respectively; $F(3,108)=18.96$, $P<0.0001$]. More importantly was the presence of a significant interaction between Cocaine and Phase [$F(3,36)=4.15$, $P<0.02$]. Subsequent post hoc tests examined this interaction in more detail and revealed several interesting findings. First, 10 mg subjects performed more poorly than PF and LC control subjects during acquisition and reversal, respectively (both $P<0.01$). Second, in comparing trial 1 with trial 4 at both acquisition and reversal, animals in the 20 mg and in both control groups showed savings or shorter latencies on trial 4 (all $P<0.05$). In contrast, the 10 mg group did not reveal evidence of similar savings between these terminal trials ($P>0.05$). Third, in opposition to the observation that memory for the spatial location acquired during acquisition in LC, PF, and 20 mg animals served to interfere proactively with learning to escape to the opposite quadrant during reversal, rats in the low-dose cocaine group were unaffected by their previous maze experience. Relative to trial 4 at acquisition, LC, PF, and 20 mg animals all exhibited longer trial 1 latencies when trained at reversal (M s = 37.3 vs. 44.3, 31.6 vs. 54.1, and 36.4 vs. 54.5 s, respectively), although the difference for LC animals failed to reach statistical significance ($P>0.05$); rats in the 10 mg group, on the other hand, exhibited nominally shorter and statistically comparable

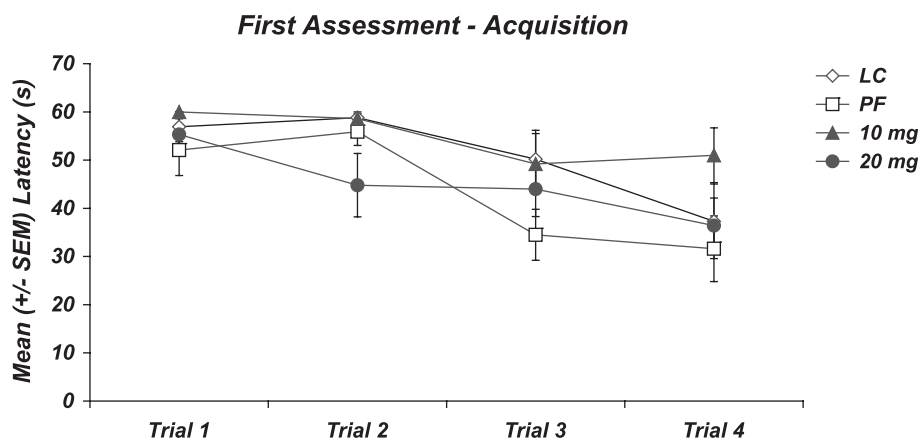


Fig. 1. Mean \pm S.E.M. latencies (s) to find the escape platform for the four groups of subjects during the four acquisition training trials. Impairments of working memory in 10 mg cocaine rats were indicated by the failure of these animals to exhibit significant trial 1 to trial 4 improvement. In contrast, the latencies for each of the other three groups were reduced over the course of training with each group exhibiting shorter latencies on trial 4 relative to trial 1. Finally, the overall performance of 10 mg animals was inferior to that of PF control subjects. (Cocaine was administered on a mg/kg basis on postnatal days 26–33.)

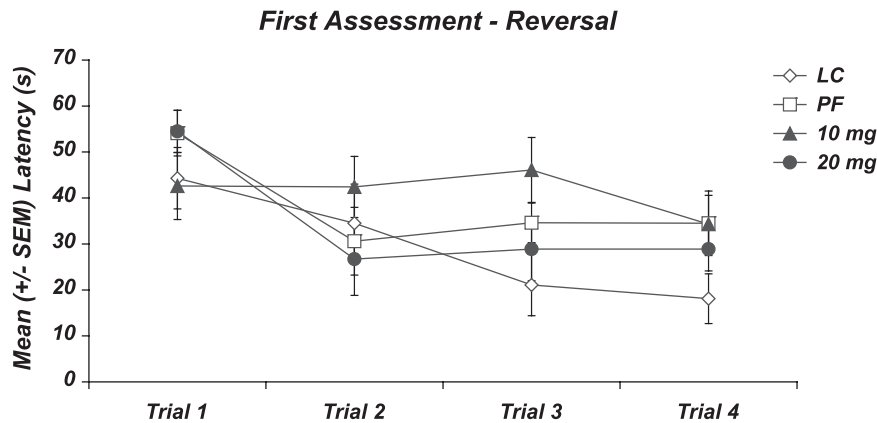


Fig. 2. Mean \pm S.E.M. latencies (s) to find the escape platform for the four groups of subjects during the four reversal training trials. Impairments of working memory in 10 mg cocaine rats were indicated by the failure of these animals to exhibit significant trial 1 to trial 4 improvement. In contrast, the latencies for each of the other three groups were reduced over the course of training with each group exhibiting shorter latencies on trial 4 relative to trial 1. Finally, the overall performance of 10 mg animals was inferior to that of LC control subjects. (Cocaine was administered on a mg/kg basis on postnatal days 26–33.)

latencies on trial 1 at reversal ($M=42.6$ s) relative to trial 4 at acquisition ($M=51.0$ s; $P>0.05$).

3.2.3. Probe trials

Separate 4 (Cocaine) \times 2 (Phase) mixed design ANOVAs were used to analyze the number of entries made into the previously reinforced quadrant and the amount of time spent in the previously reinforced quadrant during probe trials. No significant effects were found when quadrant entries were examined (all $F_s < 1.3$, all $P_s > 0.05$); all animals entered the previously reinforced quadrant with equal frequency on both probe trials (acquisition M_s : LC=3.1, PF=3.7, 10 mg=2.9, and 20 mg=3.3; reversal M_s : LC=4.1, PF=3.1, 10 mg=3.0, and 20 mg=3.3). Significant between-group differences were detected when the time spent in the previously reinforced quadrant was analyzed [see Fig. 3;

$F(3,36)=4.24$, $P<0.02$]. Relative to the PF control group, post hoc tests revealed that animals in both 10 mg and 20 mg conditions spent, on average, less time in the previously reinforced quadrant during the acquisition probe trial (both $P_s \leq 0.05$). However, this outcome for the 10 mg group is tempered by the fact that these animals failed to show proficient trial-to-trial improvement at acquisition. In addition, during the reversal probe, 20 mg subjects spent, on average, less time in the previously reinforced quadrant than did animals in the LC control condition ($P=0.05$).

3.3. Second assessment

3.3.1. Swim rates

A one-way ANOVA for independent samples was used to analyze aggregate swim rates derived from acquisition

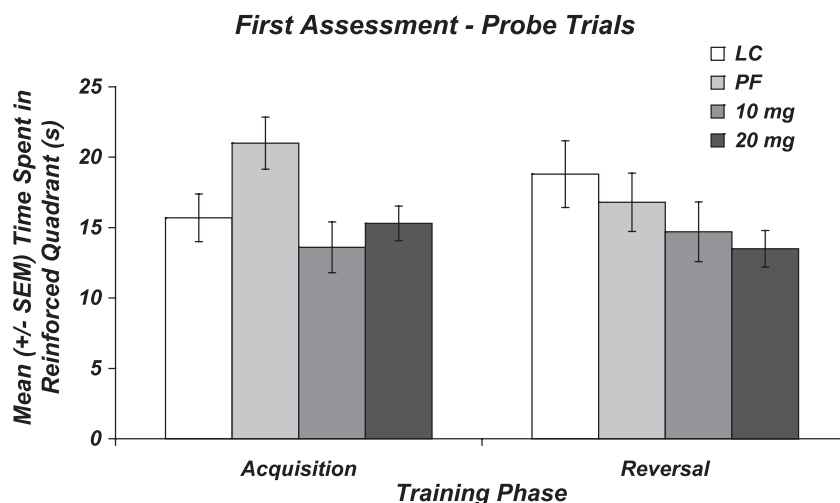


Fig. 3. Mean \pm S.E.M. time (s) spent in the previously reinforced quadrant for the four groups of subjects during acquisition and reversal probe trials. Relative to PF control subjects, animals in both cocaine dose groups spent less time in the previously reinforced quadrant during the acquisition probe trial. Subjects in the 20 mg cocaine group also spent less time in the previously reinforced quadrant during the reversal probe trial relative to the LC control condition. (Cocaine was administered on a mg/kg basis on postnatal days 26–33.)

and reversal training. Results from this analysis revealed no differences among the four groups [$F(3,316) < 1.0$, $P > 0.05$].

3.3.2. Acquisition and reversal

Cocaine did not affect escape latencies [all $F_s < 1.27$, all $P_s > .05$]. Overall, animals improved across trials [$F(3,108) = 15.98$, $P < 0.0001$], with average latencies significantly lower on trials 2–4 relative to trial 1 (all $P_s < .05$). Reversal latencies ($M = 23.5$ s) were generally longer than those at acquisition [$M = 17.8$ s; $F(1,36) = 10.21$, $P < 0.01$] due to the disruption in performance on trial 1 caused by the shift in the platform's location (M_s : trial 4, acquisition vs. trial 1, reversal: LC = 11.1 vs. 32.9, PF = 12.2 vs. 24.2, 10 mg = 10.7 vs. 34.8, and 20 mg = 14.3 vs. 31.8 s (all $P_s < 0.05$, except for the PF group, $P > 0.05$).

3.3.3. Probe trials

No group differences in the amount of time spent in the reinforced quadrant on either acquisition or reversal probe trial were noted [acquisition M_s : LC = 19.9, PF = 19.2, 10 mg = 20.0, and 20 mg = 18.1 s; reversal M_s : LC = 14.6, PF = 11.7, 10 mg = 12.7, and 20 mg = 15.4 s; both $F_s(3,36) < 2.33$, both $P_s > 0.05$].

3.4. Third assessment

3.4.1. Swim rates

A 4 (Cocaine) \times 2 (Scopolamine) factorial ANOVA for independent samples was used to analyze swim rates. Result revealed neither a significant main effect for either Cocaine [$F(3,148) < 1.0$, $P > 0.05$] or Scopolamine [$F(1,148) < 1.42$, $P > 0.05$] nor a significant two-way interaction [M_s : LC =

17.4, PF = 16.3, 10 mg = 16.4, and 20 mg = 16.3 cm/s; $F(3,148) < 1.27$, $P > 0.05$].

3.4.2. Acquisition

An overall 4 (Cocaine) \times 2 (Scopolamine) \times 4 (Trials) mixed design ANOVA with repeated measures on the last variable was used to analyze the latency data. Results from this analysis indicated a significant overall main effect of Trials [$F(3,93) = 2.86$, $P < 0.05$], with latencies generally decreasing over trials (M_s trials 1–4: 23.2, 17.8, 18.0, and 14.0 s, respectively). More importantly was the presence of a significant interaction between Cocaine and Scopolamine [$F(3,31) = 2.97$, $P < 0.05$], indicating that scopolamine differentially affected the performances of animals in the various cocaine conditions. Because there was no significant interaction effect involving Trials, the data were collapsed across this variable to subsequently analyze “daily latency” scores with a 4 (Cocaine) \times 2 (Scopolamine) ANOVA for independent samples (see Fig. 4). Results revealed an expected significant main effect of Cocaine [$F(3,148) = 2.76$, $P < 0.05$] and, more importantly, a significant interaction between Cocaine and Scopolamine [$F(3,148) = 5.90$, $P < 0.001$]. Although no differences among the four groups injected with saline 15 min prior to training were detected (all $P_s > 0.05$), significant pair-wise differences were obtained between the 20 mg cocaine/1 mg scopolamine group ($M = 34.4$ s) and the other three scopolamine-injected groups (M_s : LC/1 mg scopolamine = 11.1, PF/1 mg scopolamine = 12.4, and 10 mg cocaine/1 mg scopolamine = 16.6 s; all $P_s < 0.01$). In contrast, scopolamine failed to disrupt the performances of subjects in the LC, PF, and 10 mg groups in as far as the scores of

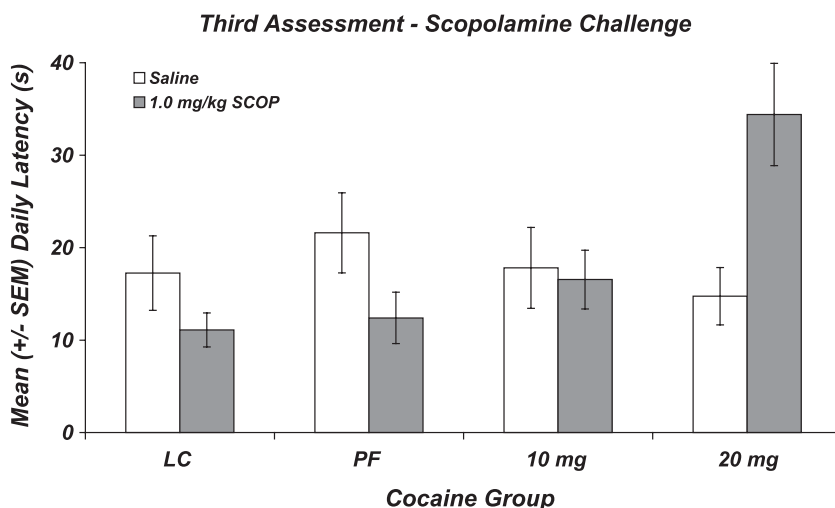


Fig. 4. Mean \pm S.E.M. daily escape latencies (s) for LC, PF, 10 mg cocaine, and 20 mg cocaine subjects that were treated with saline or 1 mg/kg scopolamine 15 min before training in the Morris maze. Subjects in the 20 mg cocaine group injected with scopolamine showed working memory impairments; these subjects had longer latencies than those of their saline-injected counterparts. No other group differences existed. (Cocaine was administered on a mg/kg basis on postnatal days 26–33.)



Fig. 5. Mean \pm S.E.M. age at death (days) for the four groups of subjects in the study. Animals in both 10 and 20 mg cocaine groups died sooner (19.3% and 18.0%, respectively) than LC control subjects. (Cocaine was administered on a mg/kg basis on postnatal days 26–33).

these animals were statistically comparable with those of their saline-injected counterparts (all P s > 0.05).

3.5. Fourth assessment

3.5.1. Swim rates

Results from the one-way ANOVA for independent samples used to analyze aggregate swim rates failed to reveal statistically significant differences among the data from the four groups of subjects [M s: LC = 23.9,

PF = 23.4, 10 mg = 23.7, and 20 mg = 24.5 cm/s; $F(3,136) < 1.00$, $P > 0.05$].

3.5.2. Acquisition

A 4 (Cocaine) \times 4 (Days) \times 4 (Trials) mixed design ANOVA with repeated measures on the last two variables revealed only expected main effects of Days [$F(3,93) = 9.18$, $P < 0.0001$] and Trials [$F(3,93) = 3.05$, $P < 0.05$], with generally improved performance observed over both the 4 days of training (M s days 1–4: 36.2, 27.0, 27.8, and 25.8 s,

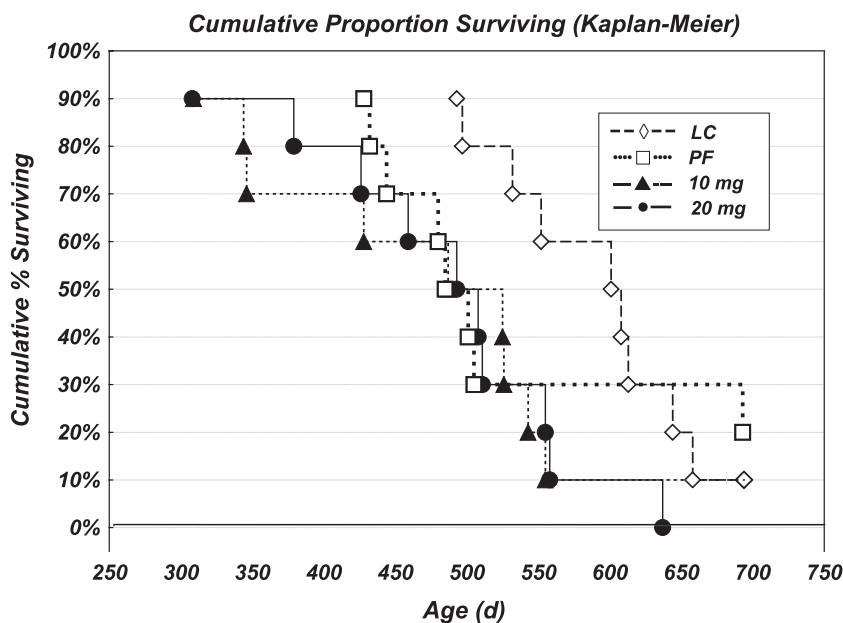


Fig. 6. A Kaplan–Meier cumulative proportion survival curve (expressed in % survival) for the four groups of animals. A survival analysis yielded a marginal effect [$\chi^2(df=3) = 5.50$, $P = .09$]. Because each group originally had 10 animals each, each 10% reduction in cumulative survival represents one death. Four animals were censored at 694 days of age (two in the PF group and one each in the LC and 10 mg/kg cocaine group). (\diamond = LC, \square = PF, \blacktriangle = 10 mg, and \bullet = 20 mg.)

respectively; days 2–4 vs. day 1, all P s < 0.001) and the four daily trials (M s trials 1–4: 33.0, 27.0, 27.6, and 29.2 s, respectively; trials 2 and 3 vs. trial 1, both P s < 0.05).

3.6. Age at death

A one-way ANOVA for independent samples revealed a significant overall main effect [$F(3,36) = 2.74$, $P = .05$] when the age at death data were analyzed (see Fig. 5). Pairwise differences existed when the average age of death associated with each of the two cocaine groups were compared with that of the LC condition (both P s < .05). On average, subjects in the 10 and 20 mg cocaine conditions died 19.3% and 18.0% sooner, respectively, than those animals in the LC group. A similar pattern of finding was detected when the averages associated with cocaine-treated rats were compared with that of PF control subjects (11.2% and 9.7%, respectively), although these differences failed to reach statistical significance (both P s > 0.05). The difference between the two control groups was not statistically significant ($P > 0.05$).

A Kaplan-Meier survival analysis was also performed on these data. The results from this test revealed a marginal difference between the groups [$\chi^2(df = 3) = 6.50$, $P = 0.09$]. The cumulative proportion survival curve from this analysis is presented in Fig. 6.

4. Discussion

Table 1 presents a summary of the study's experimental parameters and relevant findings. The pertinent outcomes can be summarized as follows: (1) Cocaine administered during adolescence produced residual deleterious effects on both working and long-term memory when assessed after a brief (10 days) abstinence period, although the adverse effect of 10 mg cocaine on long-term memory was tempered by the poor acquisition scores exhibited by these animals; (2) These impairments were independent of cocaine's anorexic consequences; (3) Cocaine's effects on memory were dose dependent in that 10 mg/kg affected acquisition (and possibly long-term memory), whereas 20 mg/kg impaired only long-term memory; (4) Memory decrements were not permanent inasmuch as memory recovery was observed

after intermediate (3–4 months) abstinence periods and endured for an additional 12 months (the longest abstinence period studied); (5) However, because scopolamine disrupted working memory in otherwise cognitively intact cocaine animals previously treated with 20 mg/kg, recovery was judged to be incomplete; (6) Motor ability, as assessed with swim rates, was unimpaired; and (7) Cocaine produced premature death.

The present investigation represents the first empirical study evaluating the lifetime consequences of cocaine administered in adolescence. The demonstration of memory decrements observed after a 10-day abstinence is consistent with the results reported by Melnick et al. [25]. In their study, spatial learning and memory impairments in animals treated with 50 mg/kg cocaine on postnatal days 11–20 were observed when an eight-arm radial maze served as the assessment instrument. Taken together, the data support the view that cocaine produces residual cognitive effects in rats when administered during periadolescence. Because this conclusion might be limited to tests of spatial memory [25] and/or to the subcutaneous route of administration [4] or moderately high doses employed vis-à-vis human use [17,18], the extent to which our results generalize needs to be established.

Although our observation of memory impairments after a brief abstinence period is consistent with the findings by Melnick et al. [25], these researchers reported on the existence of impairments well after cocaine cessation. Taken at face value, such a finding appears not to be in accord with the null results derived from the intermediate and long cocaine abstinence periods used in the present investigation. This inconsistency, however, may be attributed to differences in dosing parameters with Melnick et al. using a larger dose of cocaine for a longer period of time. This would suggest that the persistency of cocaine administration on cognition is dependent on the dose and dosing schedule with larger doses administered more frequently being able to affect different developmentally sensitive processes and thus produce longer-lasting deficits. On the other hand, the fact that subjects in our study might have been habituated to stress-related processes as a result of the repeated test procedure might also serve to account for the different pattern of results between the two studies. Alternatively, a more interesting perspective to account for the inconsistency

Table 1
Experimental parameters and summary of results

Assessment	Age of subjects (days since cocaine)	No. of daily trials, no. of training days; probe trial	Working memory deficit	Long-term memory deficit
First	44 days old (10 days)	Four trials, 1 day of acquisition; yes Four trials, 1 day of reversal; yes	10 mg 10 mg	10 (?) and 20 mg 20 mg
Second	142–150 days old (105–113 days)	Four trials, 1 day of acquisition; yes Four trials, 1 day of reversal; yes	None None	None None
Third	158–166 days old (121–129 days)	Four trials, 1 day of acquisition; no	20 mg + scop	–
Fourth	410–416 days old (377–383 days)	Four trials, 4 days of acquisition; no	None	None

scop = 1 mg/kg scopolamine HCl injected 15 min before training; all other doses refer to per kg cocaine. (?) = The fact that animals in the 10 mg cocaine group failed to exhibit proficient acquisition performance obscured detecting reliable long-term memory impairments.

between the two studies is the timing of cocaine administration. Animals in our study were injected at the beginning of adolescence, whereas subjects in the study conducted by Melnick et al. employed preadolescent subjects. This leads to an intriguing prospect that cocaine's ontogenetic effects on cognition are age dependent with preadolescent administration producing more enduring deleterious consequences. Given its differentially pattern of development during these age periods [32], the mesocortical system, especially the prefrontal cortex, may be especially vulnerable to the disruptive effects of preadolescent cocaine administration. More specifically, it is suggested that given its relatively increased rate of development during adolescence [32], the mesocortical-prefrontal system may have the necessary neural resources to respond in a more flexible way to the promote plasticity. With this said, it is equally important to acknowledge the possible role stress had in affecting our results. Our animals were not only housed singly but also were introduced to the experimental manipulation the day after being received into our vivarium; as such, isolation-induced or transportation-induced stress might have played an important mitigating factor in our results [35]. Moreover, there was some variability in terms of the retention interval used prior to probe testing that might have obscured detecting significant differences. All these factors might have, therefore, conspired to produce the unique set of findings reported herein.

Another important implication provided by the present results is the importance of the duration of drug abstinence in determining whether cocaine produced residual deleterious effects on cognition. Memory impairments herein were observed after 10 days of abstinence but recovered after a 4-month drug-free period, an effect that endured for 12 months. These findings are viewed as being consistent with the failure of Vorhees et al. [34] to detect spatial learning and memory impairments in 2-month-old rats treated with 15 mg/kg four times per day on postnatal days 1–10 or 11–20 but contrast with the aforementioned results reported by Melnick et al. [25]. Clearly, additional studies are needed to better define the exact parameters that govern recovery from early cocaine administration.

Although memory deficits in the present study recovered over time, the results from the third assessment during which animals were challenged with scopolamine suggest that the nature of this recovery may not be complete. Subjects previously treated with 20 mg/kg cocaine during adolescence were impaired on working memory when injected with scopolamine prior to working memory training when assessed some 4 months after the drug had been terminated. Interestingly, only subjects in the high-dose cocaine group were sensitive to scopolamine's impairing effects, this time suggesting that long-term perturbations to cholinergic systems involved with learning and memory are produced only when relatively large doses of cocaine are administered in adolescence. Of course, because, in the present study, scopolamine's effect on memory was deter-

mined only once, it is entirely possible that anti-cholinergic-induced impairments may exist in cocaine-treated animals at earlier (or later) time points.

Although we did not explicitly assess the morphological or neurochemical integrity of the cholinergic system, it is entirely possible that, as in prior studies, cocaine administration was neurotoxic to cholinergic cells in our animals producing decreases in muscarinic receptor densities and smaller synaptic pools of the neurotransmitter. For example, residual decreases in muscarinic binding [19,22,39] have been reported in adult rats treated continuously with cocaine, whereas decreased acetylcholine release has been noted following chronic [15] or acute [1] cocaine administration in adult and adolescent rats, respectively, and in adult rats exposed to cocaine during embryonic development [2,3]. Such changes have been accompanied by significant reductions in the number of neurons within the cholinergic basal forebrain as detected with choline acetylcholinesterase immunoreactivity [5]. Interestingly, many of these perturbations have occurred within brain structures intimately linked to learning and memory such as the hippocampus, amygdala [39], nucleus basalis [5], and anterior cingulate cortex [19]. Assuming the existence of similar changes in our subjects, it is reasonable to speculate that scopolamine acted on cholinergic systems that were already beset by previous cocaine treatment, thus making working memory more vulnerable to scopolamine's impairing effects. Given the important role played by acetylcholine in supporting learning and memory processes [30], it is not surprising that scopolamine in cocaine-induced cholinergically compromised animals is able to produce cognitive impairments or to exacerbate existing impairments. These data underscore the subtle nature of drug-induced memory deficits and suggest that a similar phenomenon may possibly exist in recovered addicts who appear to be cognitively intact.

Although their exact mechanisms of action remain unknown, the behavioral deficits observed in this investigation appear not to reflect cocaine's general systemic effects within the central nervous system. This conclusion is based on the fact that at no time decrements in motor performance were observed, at least as judged by swim rates. This is consistent with the metabolic findings of Dow-Edwards et al. demonstrating the failure of adolescent cocaine administration to produce short-term [9] or long-term [7] aberrations in glucose metabolism within motor structures in male rats. Other studies need to be conducted to more fully determine the extent to which cocaine administered during adolescence residually affects specific structures within the nervous system.

The most unexpected finding was the premature deaths of subjects in the two cocaine groups. Animals in the 10 and 20 mg cocaine groups died 19.3% and 18.0% sooner than animals in the LC control group and 11.2% and 9.7% sooner than animals in the PF control group, respectively. These differences would have been even more pronounced had

three of the four surviving animals (age 694 days old) not been from the two control groups at the time the study was terminated. Interestingly, the overall premature death trend started to emerge early with 35% of cocaine-treated subjects (4 in the 10 mg group and 3 in 20 mg group) dying before any one subject in either control group had died. This trend continued with 61% (7 from each dose) of the first 23 deaths and 62% (9 from each dose) of the first 29 deaths coming from the cocaine-treated groups. The fact that body weights recovered when the animals were ~ 5 months of age suggests that cocaine-induced effects on longevity are independent of long-term alterations of metabolic processes and, as such, indicate the potential life-threatening hazard posed by cocaine exposure early in life. Although the precise mechanism(s) accounting for the accelerated death rate in our subjects is unknown at this time, it is worth noting that cocaine is known to produce a variety of cardiotoxic [29] and hepatotoxic [24] effects—coronary thrombosis, myocardial infarction, contraction band necrosis, altered concentrations of P450 enzymes, and hepatocytic necrosis. It is interesting to note that alterations in muscarinic cholinergic receptors have been implicated in mediating cocaine's lethality [20,27,28], especially given the results derived from the third assessment in which pretraining injections of scopolamine produced working memory impairments in animals previously treated with 20 mg/kg cocaine. Finally, the possibility of anorexia partially contributing to cocaine's lethality effect cannot be entirely ruled out. Although no difference was observed between the two control groups in terms of age at death, only nominal, but not statistically significant, differences were noted when cocaine-treated subjects were compared with PF control animals. As an aside, the fact that PF control rats did not survive statistically longer than LC control subjects (or, for that matter, than cocaine-treated subjects) was a bit puzzling given the often-reported longevity effects produced by dietary restriction [38], although such a result can easily be explained by the fact that the extent and duration of food restriction used herein was minimal [36,37]. On the other hand, it must be made clear that statistically significant differences between LC and cocaine-treated animals might have been obscured by the fact the 2 of the 10 animals in the LC control group were censored at 694 days. Perhaps, group differences would have been evidenced if these two control animals had been allowed to survive until they had died from natural causes. Nevertheless, additional studies are needed to identify the exact basis(es) for cocaine's long-term lethality effect and its relationship to food intake.

In conclusion, the present findings indicate that cocaine during early adolescence is able to produce residual effects on working and long-term memory as it has been shown when this drug is administered to young (periadolescent) animals [25]. These cognitive impairments are recoverable as the animal ages into early and middle adulthood, although low statistical power might have obscured detecting

significant group differences at later ages (see Section 2.7). However, what was clear from the results was the prospect that memory recovery in adulthood may not be complete in that subtle perturbations to cholinergic systems involved in learning and memory may persist. Finally, relative to LC control subjects, cocaine produced accelerated death rates in both dose groups, suggesting that this drug's cardiotoxic, hepatotoxic, and/or neurotoxic effects may linger into adulthood and conspire with age-related processes to produce premature death.

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