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Influence of cocaine self-administration on learning related to prefrontal cortex or hippocampus functioning in rats

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Abstract *Rationale:* Individuals who abuse cocaine have cognitive deficits, particularly in functions associated with the orbitofrontal cortex. It is not clear to what extent the impact of cocaine on cognitive functioning is related to its role as a behavioral reinforcer. A preclinical means to investigate this issue is to use a yoked-triad procedure in which sets of three animals either contingently self-administer cocaine or receive passive administration of cocaine or saline in a noncontingent manner. *Objective:* Using this procedure, we assessed cocaine's effect on learning that requires a functionally intact prefrontal cortex (prelimbic or insular/orbital subregions) or hippocampus. *Methods:* Rats self-administering 1-mg/kg unit doses of cocaine responded under a fixed-ratio 5, time-out 20-s schedule of drug delivery. Testing took place in a radial-arm maze within the first 30 min after 2-hr drug sessions ended, beginning after 2.5 months of cocaine or saline exposure. *Results:* Rats self-administering cocaine earned 14–18 infusions on average throughout different phases of the study. In group-wise comparisons, learning in the visually guided delayed win-shift (prelimbic prefrontal cortex-related) and win-shift (hippocampus-related) tasks was not influenced by con-

tingent or noncontingent cocaine exposure. Session latency, though, was shorter in both cocaine-exposed groups during the win-shift task. During the odor-guided delayed win-shift task (insular/orbital prefrontal cortex-related), learning was disrupted in rats self-administering cocaine, with no influence of noncontingent cocaine exposure. *Conclusions:* Based on these and previous findings, learning related to functioning of the insular/orbital prefrontal cortex and amygdala is the most consistently disrupted in cocaine-intoxicated rats after long-term drug exposure.

Keywords Cocaine · Delayed win-shift · Hippocampus · Insular/orbitofrontal · Learning · Memory systems · Prefrontal cortex · Prelimbic · Self-administration · Win-shift

Introduction

Cocaine abusers with evidence of recent cocaine use just before or 36–48 h preceding testing have deficits in decision making, judgment, and working memory, which are suggestive of prefrontal cortex dysfunction, particularly in the orbitofrontal subregion (Grant et al. 2000; Simon et al. 2002). It is not clear, however, to what extent the effect of cocaine on cognitive functioning is related to its role as a behavioral reinforcer. A preclinical means to investigate this issue is to use a yoked-triad procedure in which sets of three animals either contingently self-administer cocaine or receive passive administration of cocaine or saline in a noncontingent manner (Dworkin and Smith 1989). Although numerous animal studies report a variety of physiological and neurochemical distinctions between contingent and noncontingent cocaine exposure (Wilson et al. 1994; Hemby et al. 1997; Broadbear et al. 1999; Kuzmin and Johansson 1999; Galici et al. 2000; Porrino and Lyons 2000; Crespo et al. 2002; Smith et al. 2003), it is relatively unknown if these physiological and neurochemical differences have functional behavioral consequences. For example, it was reported that the effects of cocaine on complex operant behavior in monkeys did not differ substantially as a func-

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tion of contingent or noncontingent drug administration (Winsauer et al. 2003).

We recently incorporated a yoked-triad procedure in rats (Udo et al. 2004) to examine the influence of contingent and noncontingent cocaine exposure on learning that requires a functionally intact amygdala (conditioned cue preference task) or dorsal striatum (win-stay task), using specific radial-arm maze tasks (McDonald and White 1993; Kantak et al. 2001). In the current study, we extended this prior research to examine the impact of contingent and noncontingent cocaine exposure on learning related to two functionally distinct prefrontal cortex subregions in rats: the prelimbic subregion, which is important for acquisition of rule learning used to guide visuospatial working memory (Eichenbaum et al. 1983), and the insular/orbital subregion, which is important for acquisition of rule learning used to guide odor working memory (Otto and Eichenbaum 1992). We used two complementary radial-arm maze tasks to study prefrontal-cortex-related learning. The first was the visuospatially guided delayed win-shift task that was originally shown to require a functionally intact prelimbic prefrontal cortex and hippocampus for accurate performance once the task was acquired (Floresco et al. 1997). More recently, it has been determined that acquisition of this task requires a functionally intact prelimbic prefrontal cortex, but not insular/orbital prefrontal cortex or hippocampus (Di Pietro et al. 2004; unpublished findings). The second was the odor-guided delayed win-shift task that was newly developed to study more lateral aspects of the rodent prefrontal cortex. Acquisition of this task has been shown to require a functionally intact insular/orbital prefrontal cortex, but not prelimbic prefrontal cortex (Di Pietro et al. 2004). The win-shift task, which lacks a delay and requires a functionally intact hippocampus, but not amygdala, dorsal striatum, or prefrontal cortex, for both acquisition and accurate performance (McDonald and White 1993; Floresco et al. 1997; Black et al. 2004), was evaluated as well to more fully document the effects of long-term cocaine self-administration on multiple memory system functioning.

Materials and methods

Subjects Male Crl(WI)BR rats (Wistar strain, Charles River Laboratories, Portage, MI) were housed in individual hanging plastic cages (24×22×20 cm) within a temperature (21–23°C) and light-controlled (on at 0800 hours; off at 2000 hours) vivarium. The triads used in the current study were the same as those used previously to examine the influence of long-term cocaine self-administration on learning that requires a functionally intact amygdala or dorsal striatum (Udo et al. 2004). Rats had approximately 2.5 months of cocaine or saline exposure before the start of radial-arm maze tasks in the current study, whereas they had approximately 1 month of cocaine or saline exposure before the start of the radial-arm maze tasks in the previous study. The first task in the current study (visually guided delayed win-shift) was conducted 1 week after completion of the last task in the previous study (win-stay). There was no co-

caine-abstinence period between studies, as rats continued with self-administration sessions during this intervening period. Rats continued to be maintained on a restricted feeding schedule by being provided approximately 16 g of food per day after each session and on weekends. All rats fully consumed their daily food ration. Between experimental sessions, the rats were allowed ad libitum access to water in their home cages. Body weights were recorded daily. Details of the apparatus, surgical procedures for intravenous catheterization, and training procedures for drug self-administration can be found in our earlier publication (Udo et al. 2004). The same cocaine dose, reinforcement schedule, and daily session length were used in the current study. A curtain surrounded the maze for controlling exposure to extramaze visual cues. The policies and procedures set forth in the *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health (vol. 25, no. 28, revised 1996) were followed.

Drugs Cocaine hydrochloride (gift from NIDA, Bethesda, MD) was dissolved in a sterile solution containing 0.3 IU of heparin (LymphoMed, Rosemont, IL)/0.1 ml 0.9% saline (American Pharmaceutical Partners, Schaumburg, IL). Throughout self-administration sessions, a 1.0-mg/kg unit dose of cocaine was delivered intravenously by infusing a 4.0-mg/ml solution at a rate of 1.2 ml/min. To attain a dose of 1.0 mg/kg, infusion volume was adjusted for body weight, which resulted in drug delivery times of approximately 4–5 s in individual rats. For rats passively yoked with saline, heparinized saline solution was substituted for cocaine. Catheters were maintained by flushing them daily with 0.1 ml of a 0.9% saline solution containing 3.0 IU heparin and 6.7 mg of an antibiotic mixture comprised of tricarcillin disodium and clavulanate potassium (Timentin, SmithKline Beecham Pharmaceuticals, Philadelphia, PA). In addition, catheters were checked for function weekly or as needed by infusing 1.0 mg/0.1 ml methohexital sodium (Brevital, Eli Lilly and Co., Indianapolis, IN) and noting the presence or absence of sedation. For leaky or nonfunctional catheters, a replacement catheter was implanted into the right femoral vein.

Self-administration sessions Each of six triads consisted of a rat actively self-administering cocaine, a rat passively yoked with cocaine, and a rat passively yoked with saline. Only responses from the rat actively self-administering cocaine controlled infusion delivery by responding under a fixed-ratio (FR) 5, time-out (TO) 20-s schedule. The right lever was assigned as the active lever and the left lever as the inactive lever. Rats passively yoked with cocaine and saline received either a 1.0-mg/kg cocaine infusion or a sterile 0.9% saline infusion, respectively, each time the rat actively self-administering cocaine completed the response requirements for drug delivery. The stimulus light located above the right lever remained illuminated during the infusion and the 20-s TO period for each member of the triad. The TO period was signaled by the offset of the house light. The 2-h drug or saline sessions were conducted 5 days a week (Monday through Friday) during the light

phase, whether or not animals were tested in the radial-arm maze environment. During these sessions, five dependent measures were recorded. For each member of the triad, the number of responses on the right and left levers was recorded. In addition, the number of infusions and the inter-infusion intervals were recorded for the rat actively self-administering cocaine in each triad. Since the half-life of a single intravenous infusion of 1 mg/kg cocaine is approximately 38 min (Lau and Sun 2002), the time between the last infusion and the end of the session was calculated to prospectively establish that cocaine-exposed rats (contingent and noncontingent) were relatively equally under the influence of cocaine while being tested in the eight-arm radial maze tasks.

Radial-arm maze procedures Immediately after 2-h drug or saline sessions, each member of the triad was removed from the operant chamber and placed into its home cage to await testing in the radial-arm maze. Catheters were flushed after testing in the maze rather than after the self-administration sessions to avoid noncontingent delivery of cocaine that remained in the catheter before testing in the maze. Each member of the triad was individually tested in the eight-arm radial maze. Cocaine-exposed rats were tested in the maze in a counterbalanced order. The rat actively self-administering cocaine was tested first and the rat passively yoked with cocaine second in half the triads, and vice versa in the remaining triads. The rat passively yoked with saline was always tested last to maximize cocaine intoxication in the cocaine-exposed groups. All testing in the radial-arm maze for each triad was completed within 30 min after the end of the self-administration sessions. The maze was cleaned before testing each rat. A 1-week interval intervened between the end of one task and the beginning of the next task, but 2-h drug or saline sessions continued during these periods. These intervening periods were designated as Baselines 1, 2, and 3 for purposes of the current study. This study consisted of an additional three phases in which a different radial-arm maze task was examined in the following order.

Delayed win-shift task: visual version For this task, a rat was required to learn, after a delay, to enter arms baited with a reinforcer (a single piece of Froot Loops cereal) that were in different spatial locations than the reinforced arms experienced before the delay to measure visuospatial working memory (Floresco et al. 1997; Di Pietro et al. 2004). Visual cues were placed at 90° intervals on the curtain surrounding the maze and on a circular piece of plywood suspended from the ceiling as previously described in detail (Di Pietro et al. 2004). Daily acquisition sessions consisted of two phases: a training phase and a test phase, with a 5-min delay separating the two. During the training phase, the rat was placed inside the central hub and given access to four randomly selected arms (no more than two adjacent arms), with each arm containing a reinforcer placed at the distal end. The doors to the nonselected arms remained closed. The training phase terminated after all four reinforcers were retrieved and consumed or 5 min had elapsed. For the test

phase after the 5-min delay, all eight arms of the maze were open and only the arms that were previously blocked were baited with a reinforcer. Again, a maximum of 5 min was allowed to retrieve all four reinforcers. If a rat entered an arm that was unbaited or one in which the reinforcer had already been retrieved, an error was recorded. Sessions were conducted Monday through Friday; arms were randomly selected each day before the training phase for each triad. The four arms used in the training phase were cleaned to eliminate residual rat odors before the testing phase. Daily sessions ended after the saline-exposed member of the triad had one or fewer errors for two consecutive daily sessions in both the training and test phases (Floresco et al. 1997). If this acquisition criterion in drug-exposed rats was not achieved within the same time frame as the saline-exposed rats, a maximum of five additional maze sessions were conducted. For rats that did not learn the task with extended training, the maximum number of sessions conducted was assigned as the “sessions to criterion” for statistical purposes.

Win-shift This task measures spatial navigation guided by extramaze visual cues (McDonald and White 1993; Black et al. 2004). A rat was required to enter each of eight baited arms once to receive a reinforcer (a single piece of Froot Loops cereal). Four unique and visually distinct extra-maze cues, as used above, were placed at 90° intervals on the curtain surrounding the maze and on the circular piece of plywood suspended from the ceiling (a different arrangement of cues was used for this task and the visually guided delayed win-shift task). During training, all arms of the eight-arm radial maze were accessible and a reinforcer was placed in the food well at the distal end of each arm. The rat was placed in the central hub of the apparatus with all doors initially open. After a choice was made, doors located at the proximal ends of nonselected arms were closed. Upon the rat's return to the central hub, the previously selected door closed, and a 10-s waiting period was initiated. After the waiting period, all doors were again opened until the next choice was made. The session terminated after either 10 min had elapsed or all eight reinforcers were retrieved. The number of errors in a test session, an indication of performance on the task, was determined by tabulating the number of revisits to previously selected arms within the first eight choices (McDonald and White 1993). Criterion for acquisition of the task was reached when one or fewer errors were made within a session for two consecutive daily sessions in the saline-exposed rat. If this acquisition criterion in drug-exposed rats was not achieved within the same time frame as the saline-exposed rat, a maximum of five additional maze sessions were conducted.

Delayed win-shift: olfactory version For this task, a rat was required to learn, after a 5-min delay, to selectively dig in each of four reinforcer-baited, sand-filled cups that contained odors that were different from the four reinforced odors experienced before the delay to measure odor-guided working memory (Di Pietro et al. 2004). Before the start of the experiment, rats were given ten training trials to learn to

dig in unscented sand for a hidden reinforcer (a single piece of Froot Loops cereal). Daily acquisition sessions consisted of two phases: a training phase and a test phase, with a 5-min delay separating the two. During both phases, four arms were randomly selected and baited with a reinforcer. The reinforcer was located inside a clear plastic cup (6.5 cm in diameter by 6.5 cm in height) containing 125 g of sand mixed with 5 g of an odor cue. The reinforcer was placed approximately 1 cm under the sand to avoid any visual cues. During the training phase, the rat was placed inside the central hub and given access to the four randomly selected arms (no more than two adjacent arms) containing a reinforcer-baited cup scented with allspice, basil, celery seed, or dill weed. The doors to the nonselected arms remained closed. The cups containing the reinforcer were placed 17 cm inside the selected arms. The training phase ended when the rat dug for and retrieved all four reinforcers or after 5 min had elapsed. The four arms and cups used in the training phase were cleaned to eliminate residual rat odors before the testing phase. Four arms were again randomly selected and four different cups scented with paprika, thyme, cinnamon, and marjoram were baited with a reinforcer and placed in these arms. The previously used scented cups were placed in the remaining arms, but did not contain a reinforcer. During the test phase after the 5-min delay, the rat was again placed in the central hub, and this time given access to all eight arms of the maze. The test phase ended when all four reinforcers were recovered or after 5 min had elapsed. If a rat dug in the sand of an unbaited cup or one in which the reinforcer had already been retrieved, an error was recorded. Sessions were conducted Monday through Friday; arms that were reinforced during training and test phases were randomly selected each day for each phase in each triad. Daily sessions ended after the saline-exposed member of the triad had one or fewer errors for two consecutive daily sessions during the training and test phases (Di Pietro et al. 2004). If this acquisition criterion in drug-exposed rats was not achieved within the same time frame as the saline-exposed rat, a maximum of five additional maze sessions were conducted. For rats that did not learn the task with extended training, the maximum number of sessions conducted was assigned as the “sessions to criterion” for statistical purposes.

Data analyses

The body weight and self-administration data were analyzed via one-factor (phase) or two-factor (Group×Phase) ANOVA, with repeated measures for phase. Post hoc Tukey tests were used for follow-up analyses. In individual animals, the five dependent measures in the self-administration sessions described above were averaged over the five sessions preceding the visually guided delayed win-shift task (Baseline 1); all sessions during the visually guided delayed win-shift task (visual delayed win-shift); the five sessions between the visually guided delayed win-shift task and the win-shift task (Baseline 2); all sessions during the win-shift task (win-shift); the five sessions between the win-shift task

and the odor-guided delayed win-shift task (Baseline 3); and all sessions during the odor-guided delayed win-shift task (odor delayed win-shift). It should be noted that one rat from the passive cocaine group died before any testing in the radial-arm maze was completed, leaving an overall group size of five rats for this treatment condition.

Data from the radial-arm maze tasks were analyzed via one-factor (group) ANOVA and post hoc Tukey tests, or in certain cases, unpaired *t*-tests. The dependent measures for both delayed win-shift tasks included number of sessions to acquire the task during training and test phases, latency to complete the sessions at criterion (last two sessions) during training and test phases, and session errors at criterion during the test phase. Total session errors during the test phase for each version of the delayed win-shift task consisted of between-phase errors (initial entry/dig into arms/cups previously baited during the training phase) and within-phase errors (entry/dig into arms/cups already visited during the test phase). For the win-shift task, dependent measures included number of sessions to acquire the task, latency to complete the sessions at criterion, and session errors at criterion.

In addition to the above analyses, other statistical testing was performed to determine if prior experience with any one of the five tasks (present study; Udo et al. 2004) could predict or influence learning in subsequent maze tasks. A partial correlation procedure (SPSS, version 11.0; two-tailed probability testing) was used to associate learning measures while controlling for the effects contributed by level of cocaine intake.

Results

Body weight Body weights before any drug exposure, after approximately 2.5 months of drug exposure, and after approximately 6 months of drug exposure were 323±17, 393±12, and 405±12 g in the passive saline group; 317±9, 365±6, and 362±7 g in the passive cocaine group; and 321±11, 388±7, and 397±10 g in the cocaine self-administration group, respectively. ANOVA analysis revealed significant group differences in body weight at the end of the study [$F(2,14)=4.7$, $p\leq 0.03$], but not at the earlier time points. Post hoc testing indicated that by the end of the study, the body weights in the passive saline and cocaine self-administration groups were significantly greater ($p\leq 0.03$) than the body weights in the passive cocaine group, but were not significantly different from each other. Based on growth charts for Wistar rats provided by Charles River Laboratories, ending body weights were at approximately 78% of ad libitum levels in rats passively exposed to cocaine and at approximately 88 and 86% of ad libitum levels in rats passively exposed to saline and actively self-administering cocaine, respectively.

Self-administration performance For the number of right lever responses (Table 1), there was a significant group difference [$F(2,14)=706$, $p\leq 0.0001$]. Post hoc analysis indicated that there were significantly more right lever responses

Table 1 Mean±SEM lever responses and other measures during the six self-administration phases

Group	Lever	Baseline 1	Visual delayed win-shift	Baseline 2	Win-shift	Baseline 3	Odor delayed win-shift
Cocaine SA	Right	71±6*	80±5*	76±5*	84±5*	87±6*	93±6*
	Left	7±5	2±1	3±1	1±1	1±1	1±1
Passive cocaine	Right	2±1	4±2	8±2	4±1	5±2	12±6
	Left	11±4	10±7	10±5	6±2	24±20†	29±17†
Passive saline	Right	2±1	2±1	2±1	2±1	2±1	1±1
	Left	3±1	4±1	5±1	3±1	5±2	2±1
Other measures							
Infusions		14.2±1.1	15.9±1.0	15.9±0.7	16.4±0.7	16.7±1.1	17.7±1.0
Interinfusion intervals (min)		9.1±1.4	7.4±0.5	7.7±0.2	7.1±0.3	7.0±0.4	6.9±0.5
Time since last infusion of session (min)		6.2±0.5	5.1±0.5	7.7±1.2	5.5±0.9	5.7±0.7	8.0±1.8

* $p=0.05$, significantly different from the right lever responses in the passive cocaine and passive saline groups

† $p=0.05$, significantly different from the left lever responses in the cocaine self-administration and passive saline groups

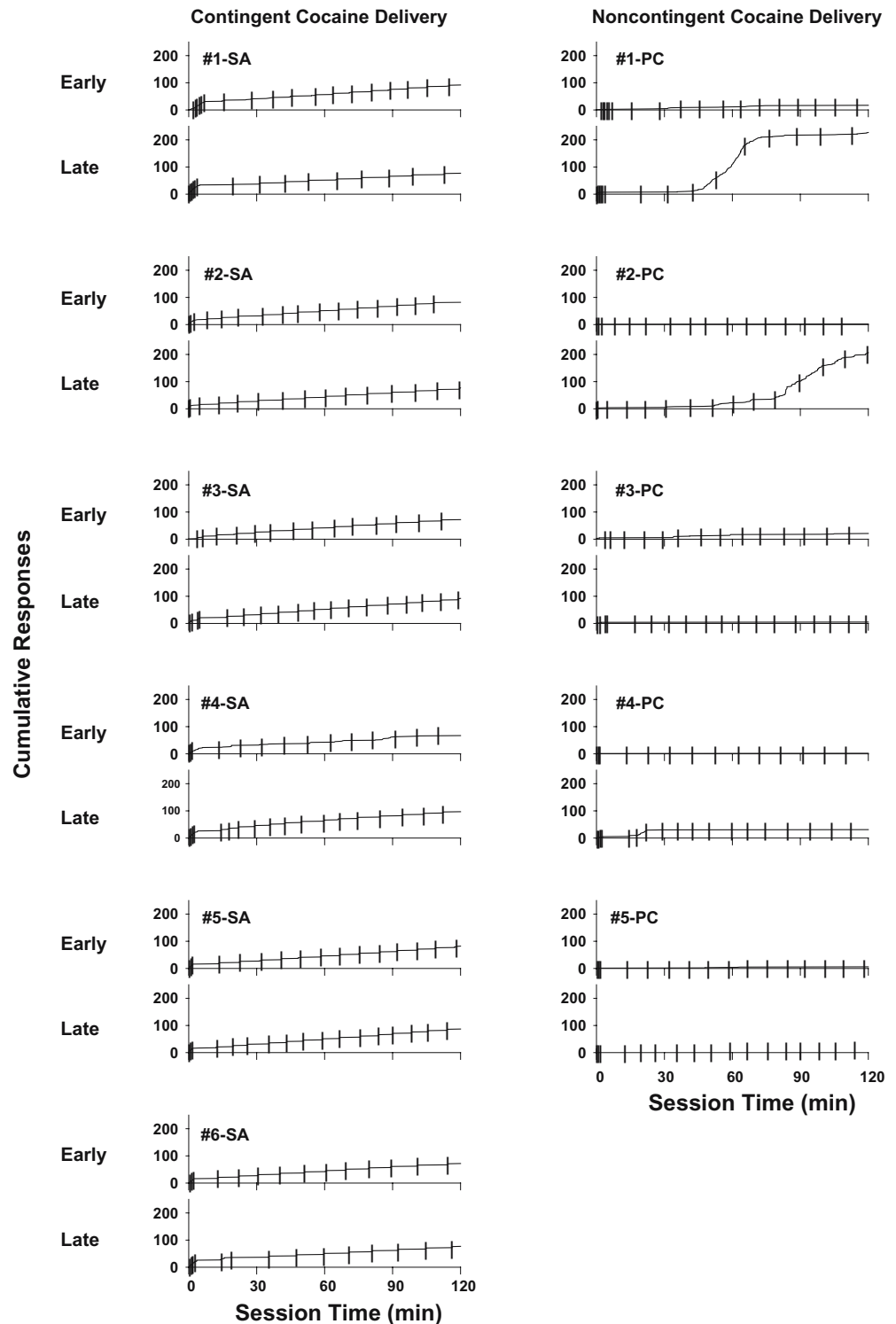
in the cocaine self-administration group compared to either the passive cocaine or passive saline groups ($p \leq 0.05$). The number of right lever responses was low in the passively yoked groups, with no significant differences between them. Responses on the right lever did not significantly vary as a function of phase or Group×Phase, suggesting that right lever responding was relatively stable in each group throughout the study. In terms of left lever responses (Table 1), groups differed as a function of phase [$F(2,14)=8.2$, $p \leq 0.004$]. Post hoc analysis indicated that rats in the passive cocaine group had a significantly greater number of left lever responses during the last two phases of the study ($p \leq 0.05$) compared to rats in the cocaine self-administration and passive saline groups, which did not differ from each other. These group-associated differences were related to high levels of left lever responding in two rats passively exposed to cocaine during the last two phases of the study. Regarding the number of infusions (Table 1), all rats received approximately 14 to 18 infusions throughout the six phases, with no significant differences among phases. The mean interinfusion intervals remained stable as well from phase to phase, with no significant differences among the six phases (Table 1). In addition, the time between the last infusion and the end of the session (Table 1) was consistently between 5 and 8 min, with no significant differences among the six phases.

Figure 1 shows cumulative response records for each rat contingently and noncontingently exposed to cocaine. These data illustrate that the contingent delivery of drug in rats self-administering cocaine resulted in increasing linear rates of lever responding that were associated with regularly spaced drug infusions after the initial short burst of rapidly delivered infusions. This suggests that responding in this group of rats was under the control of the FR 5 schedule and that cocaine was serving as a behavioral reinforcer. In contrast, the noncontingent delivery of cocaine resulted in either low to moderate rates of lever responding or nonlinear high rates of lever responding throughout the 2-h sessions. Lever responding in the noncontingent group was unrelated to the time of drug delivery, even for the two rats who had high levels of left lever responding during the

last two phases of the study (#1 PC and #2 PC). The majority of infusions were delivered at times when rats passively yoked with cocaine were not pressing the lever. Thus, responding in this group of rats was not under the control of drug delivery or the light stimuli that were noncontingently presented at the time of infusion. As responding was not autoshaped to passive drug delivery, cocaine did not serve as a behavioral reinforcer in the noncontingent group of rats.

Delayed win-shift: visual version During the training phase of this task, the three groups of rats took an average of seven to nine sessions to achieve acquisition criterion and completed the sessions at criterion within an average of 63 to 77 s. There were no significant differences among passive saline, passive cocaine, and cocaine self-administration groups during the training phase. During the test phase of this task after the 5-min delay (Fig. 2, top), the three groups of rats took an average of 16 to 19 sessions to achieve acquisition criterion and 78 to 84 s to complete the session. Neither these differences nor the differences in the number of total errors at criterion were significant across the three groups. These measures, however, include data from two rats in both the cocaine self-administration and passive cocaine groups who failed to learn the task, even with extended training. The learning deficits in a small percentage of cocaine-exposed rats produced the high degree of variability in the number of total errors at criterion and caused these error rates to be greater than what is generally accepted for indicating accurate group performance (one or fewer errors on average; Floresco et al. 1997). In the cocaine self-administration group, the number of total errors was significantly greater [$t(4)=2.6$, $p \leq 0.031$] in rats who did not reach the acquisition criterion (3.3 ± 1.2) compared to rats who did (0.5 ± 0). The number of total errors was also significantly greater [$t(3)=2.7$, $p \leq 0.037$] in passively yoked cocaine rats who did not reach the acquisition criterion (3.0 ± 0.8) compared to rats who did (1.0 ± 0). The majority of errors in this task were of the between-phase type in all three groups of rats (Table 2).

Fig. 1 Cumulative response records in individual rats contingently (*SA*) and noncontingently (*PC*) exposed to cocaine. Data were taken from the drug sessions just before conducting the first maze session in the Udo et al. (2004) study (*Early*, after approximately 1 month of exposure) and just before conducting the last maze session in the current study (*Late*, after approximately 6 months of exposure). Plotted are cumulative responses on the active lever for rats contingently self-administering cocaine and cumulative responses on both levers for rats noncontingently exposed to cocaine. Time of infusion (whether contingently or noncontingently



Win-shift During this task (Fig. 2, middle), the three groups of rats took an average of seven to eight sessions to achieve acquisition criterion. There were no significant differences among passive saline, passive cocaine, and cocaine self-administration groups for this measure. However, rats self-administering cocaine and rats passively exposed to cocaine took a significantly ($p=0.05$) shorter amount of time to complete the sessions at criterion compared to rats

passively exposed to saline [$F(2, 14)=12.7, p=0.001$]. The two cocaine-exposed groups were not different from each other for this measure. Each group averaged less than one error at criterion, with no significant differences among the three groups.

Delayed win-shift: olfactory version During the training phase of this task, the three groups of rats took an average

Fig. 2 Mean (\pm SEM) number of sessions to criterion (*left*), session latency in seconds at criterion (*center*) and total errors at criterion (*right*) in passive saline (PS), passive cocaine (PC), and cocaine self-administration (CocSA) groups during the visuospatially guided delayed win-shift (*top*), win-shift (*middle*) and odor-guided delayed win-shift (*bottom*) tasks. *Significantly different ($p \leq 0.05$) from the passive saline group. †Significantly different ($p \leq 0.05$) from the pas-

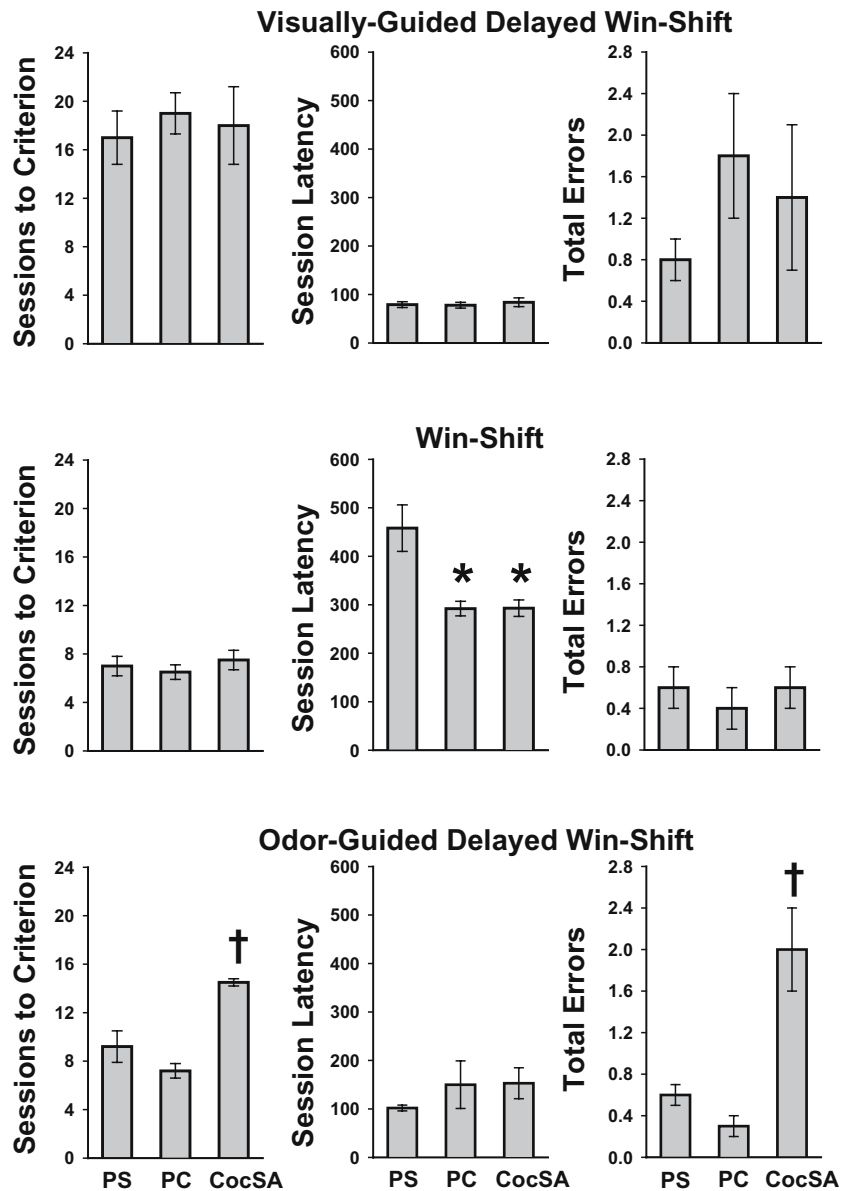


Table 2 Mean \pm SEM between-phase and within-phase errors during the test phase of the visual and olfactory versions of the delayed win-shift task

Task	Group	Between-phase errors	Within-phase errors
Visual delayed win-shift	Passive saline	0.75 \pm 0.21	0 \pm 0
	Passive cocaine	1.50 \pm 0.32	0.30 \pm 0.30
	Cocaine SA	1.00 \pm 0.41	0.43 \pm 0.33
Odor delayed win-shift	Passive saline	0.60 \pm 0.19	0 \pm 0
	Passive cocaine	0.25 \pm 0.14	0 \pm 0
	Cocaine SA	1.67 \pm 0.44*	0.25 \pm 0.17

* $p=0.05$, significantly different from the between-phase errors in the passive cocaine and passive saline groups

of six to nine sessions to achieve acquisition criterion and completed the sessions at criterion within an average of 116 to 158 s. There were no significant differences among

passive saline, passive cocaine, and cocaine self-administration groups during the training phase. During the test phase of this task after the 5-min delay (Fig. 2, bottom), five of six rats self-administering cocaine failed to learn this task, even with extended training. This resulted in conducting significantly ($p=0.05$) more sessions during the test phase in the rats self-administering cocaine compared to rats passively exposed to saline or cocaine, who achieved acquisition criterion [$F(2,14)=11.5$, $p \leq 0.001$]. The passive cocaine and passive saline groups did not significantly differ for this measure. Session latencies did not significantly differ among the three groups. The number of total errors was significantly ($p=0.05$) greater in rats self-administering cocaine compared to passive cocaine or passive saline groups, which did not differ from each other [$F(2,14)=7.5$, $p \leq 0.006$]. The majority of errors in this task were of the between-phase type (Table 2) in all three groups of rats,

with rats self-administering cocaine making a significantly ($p=0.05$) greater number of between-phase errors than rats passively exposed to saline or cocaine [$F(2,14)=6.4$, $p\leq 0.011$]. Within-phase errors did not significantly differ among the three groups.

Partial correlation analyses Among the five radial-arm maze tasks, the only significant correlations were between the number of sessions to learn the win-stay task and session latency ($r=-0.75$, $p<0.003$) and session errors ($r=-0.58$, $p<0.04$) at criterion during the test phase in the visually guided delayed win-shift task. These negative correlations suggest that the more highly proficient rats were in learning to repeat arm selections to obtain reinforcement (“stay”), the more time they needed within a session to accurately use a strategy that now required choosing arms not reinforced before the delay (“shift”). This prior maze experience, however, was not associated with how many sessions it took to learn the visually guided delayed win-shift task.

Discussion

Self-administration performance was sufficiently stable to appropriately compare drug- and saline-exposed rats on multiple memory system functioning. Specifically, cocaine-exposed rats received similar amounts of drug before, during, and after completion of each task (14–18 mg/kg). Furthermore, cocaine-exposed rats were relatively equally under the influence of drug during each task, as latency between time of the last drug infusion and end of the self-administration session did not deviate by more than 3 min on average. More importantly, responding in rats actively self-administering cocaine, but not those passively receiving cocaine, was temporally related to cocaine delivery, making it possible to determine if the behavioral reinforcing effects of cocaine produced any selective effects on memory system functioning.

The results of testing in the radial-arm maze indicate that long-term exposure to daily doses of approximately 14–18 mg/kg cocaine produced differential effects on multiple memory system functioning. The results show that contingent and noncontingent cocaine exposure did not produce significant deficits in the visually guided delayed win-shift task for these groups as a whole, although a small number of rats from each cocaine-exposed group failed to learn the task. If the significant bimodal performance levels are real or spurious trends in cocaine-exposed groups is not clear. That additional subjects were not available to test in this task is a shortcoming of this research. Nonetheless, during the odor-guided delayed win-shift task, the majority of rats self-administering cocaine failed to learn the task, with no influence of noncontingent cocaine exposure. These results are in general agreement with studies in rats showing that noncontingent cocaine injections moderately impaired inhibitory control in a task sensitive to prelimbic prefrontal cortex lesions (Paine and Olmstead 2004), but were ineffective ultimately for impairing impulse control in a

task sensitive to orbitofrontal cortex lesions (Paine et al. 2003).

During the win-shift task, shorter session latencies at criterion were observed after contingent and noncontingent cocaine exposure, which did not alter the speed or accuracy of learning. This suggests that cocaine was not having a direct influence on the functioning of the hippocampus at the daily doses consumed. Given that activity in one memory system can exert a modulatory influence on behavior in tasks mediated by other memory systems (White and McDonald 2002; Poldrack and Packard 2003), shorter session latencies in the win-shift task may have resulted from cocaine affecting a site or sites outside the hippocampus. It has been shown that infusion of amphetamine into the basolateral amygdala shortens the escape latency in the spatial (hippocampal) version of the water-maze task (Packard et al. 1994). Consequently, shorter session latencies in the win-shift radial-arm maze task may have resulted from a pharmacological influence of cocaine within the amygdala, whose functioning is disrupted by cocaine exposure (Udo et al. 2004).

The basis for a selective effect of cocaine self-administration on disrupting odor-guided working memory is unknown. Given that the behavior of rats passively exposed to cocaine was no longer influenced in the radial-arm maze during this last task, one possibility is that this group of rats developed behavioral tolerance after approximately 6 months of cocaine exposure. Before this last task, contingent and noncontingent cocaine exposure had identical effects on radial-arm maze performances (Udo et al. 2004; present findings). Some literature, though, indicates that behavioral tolerance develops within the first few days of intravenous delivery of contingent and noncontingent cocaine and reaches a maximal level by the fourth day of administration (Emmett-Oglesby et al. 1993). The cumulative response records in Fig. 1 confirm that neither tolerance nor sensitization to the behavioral reinforcing effects of cocaine in the contingent group developed over the course of the experiment, as the shape and position of these records were similar for the early and late self-administration time points. Other research on the long-term effects of cocaine has shown that rats passively, but not actively, exposed to cocaine in daily 6-h sessions eventually die, which may be indicative of a progressively sensitized toxic reaction to cocaine (Dworkin et al. 1995).

The inability of rats passively exposed to cocaine to continue gaining weight as efficiently as the other groups of rats near the end of the study may be indicative of a progressively sensitized toxic reaction to cocaine. This slowing in weight gain occurred although all rats fully consumed the daily food ration and ate all cereal reinforcers during testing in the radial-arm maze. Previous studies have demonstrated that behavioral and neurochemical sensitization to cocaine develops to a greater extent in rats maintained at 80 vs 100% ad libitum body weight (Cadoni et al. 2003). If sensitized, this might also explain the high rates of noncontingent lever responding observed in two rats (#1 PC and #2 PC) near the end of the study, which may have resulted from the emergence of motor stereotypies

directed toward the left lever. Supporting this view is the fact that this behavior mainly occurred as a single run of responses once initiated after several noncontingent infusions of cocaine. As rats were not observed in the operant chambers, it is unknown if other types of motor stereotypies may have emerged in rats whose lever responding was still low (#3 PC and #5 PC) to moderate (#4 PC) near the end of the study. Future investigations will need to consider this possibility when designing experiments.

It is not clear if the eventual development of sensitization in rats noncontingently exposed to cocaine relates to the selective effect of cocaine self-administration on learning in the odor-guided working memory task. Other studies in animals have shown that noncontingent cocaine injections, resulting in behavioral sensitization, disrupt learning that is sensitive to lesions of the orbital prefrontal cortex (Jentsch et al. 2002; Schoenbaum et al. 2004). However, these effects were measured several days and weeks after drug withdrawal, raising the possibility that either these are discrepant findings or the influence of noncontingent cocaine exposure on this memory system may be more marked during drug abstinence vs drug intoxication. It is noteworthy that in human cocaine abusers, scores from cognitive tests associated with prefrontal cortex functioning are negatively related to length of cocaine abstinence (Bolla et al. 1998).

In conclusion, these findings indicate that long-term cocaine exposure produces differential effects on multiple memory system functioning in rats. The results of the current and previous (Udo et al. 2004) studies using the same sets of yoked triads indicate that tasks requiring a functionally intact amygdala or insular/orbital prefrontal cortex are the most consistently disrupted by recent cocaine intoxication. This latter finding is consistent with studies in cocaine abusers who show disruptions in tasks requiring intact orbitofrontal cortex functioning after recent use (Grant et al. 2000; Simon et al. 2002). Our findings predict that learning related to amygdala functioning may show disruptions as well in cocaine abusers. Collectively, the methods described herein may provide a preclinical model for investigating the mechanisms by which drug use leads to disrupted learning and memory functioning in humans. Furthermore, these methods may be useful for testing novel pharmacotherapies designed to ameliorate drug-induced cognitive deficits that may be critical for maintaining an addictive state (Volkow and Fowler 2000).

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