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Intravenous cocaine priming reinstates cocaine-induced conditioned place preference

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Separate groups of rats underwent an unbiased conditioned place preference (CPP) procedure involving alternate pairings of distinct environments with intravenous (IV) injections of cocaine (.75 mg/kg) or saline immediately or 15 min after injection. A subsequent extinction phase consisted of exposure to both conditioning environments preceded by saline injection (Ext). Finally, an IV priming injection of cocaine was administered to reinstate the extinguished place preference (Rein). Time spent in the cocaine-paired chamber significantly increased from Pre-test to CPP, decreased from CPP to Ext and increased from Ext to Rein. The temporal delay had no significant effect on performance across all phases of the study. This study confirms previous studies of reinstatement and extends the route of administration under which reinstatement can be demonstrated by replicating the phenomenon with IV drug administration.

Relapse to compulsive drug taking after long periods of abstinence is a major problem in the treatment of drug addiction in humans (Sofuoglu & Kosten, 2004). In order to study the factors that underlie relapse to drug seeking, a variety of animal models have been developed. Reinstatement of drug seeking has been primarily applied to instrumental conditioning of self-administration, where animals are trained to lever-press to obtain intravenous (IV) drug infusions. After this drug-reinforced behavior is extinguished, presentation of a non-contingent priming injection of the drug reinstates lever-pressing (e.g., Shaham, Shalev, Lu, De Wit & Stewart, 2003).

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Conditioned place preference (CPP; Tzschentke, 1998) is another method that has been employed to research relapse in laboratory animals (e.g., Mueller & Stewart, 2000). Here, animals are exposed to two distinctive chambers; one of them is paired with drug, and the other with saline. After multiple pairings, when the animal has access to both chambers, the drug-paired chamber is preferred over the saline-paired chamber or animals spend significantly more time in the drug chamber compared to pre-conditioning levels. After the place conditioning effect is extinguished, a priming injection of the drug reinstates the preference for the drug-paired chamber.

In cocaine-induced CPP, reinstatement has been limited mainly to intraperitoneal (IP) injections (Busse & Riley, 2004; Mueller & Stewart, 2000). In order to explore the generality of reinstatement within the CPP preparation, the present study investigated whether the administration of cocaine via the IV route is also effective in generating reinstatement of drug seeking. Given that human drug abusers often employ IV administration (Anthony, Vlahov, Nelson, Cohn, Astemborski & Solomon, 1991), results from this assessment may provide a useful animal model of drug relapse that more accurately approximates the dynamics of drug administration utilized by human cocaine abusers.

It is also well known that the IV route is a method of drug administration that produces effects of relatively short duration (Ambre, Belknap, Nelson, Rho, Shin & Atkinson, 1988). Previous research suggests that this method may be especially sensitive to temporal manipulations, such as variations in the interval between drug injection and exposure to the drug-paired stimuli (Ettenberg et al., 1999). Thus, a second goal of this study was to evaluate the effect of delaying the placement of the subjects in the conditioning context after an IV cocaine injection on the conditioning, extinction and reinstatement of place preference. This was accomplished by comparing the place preferences acquired by two groups whose pairings of the conditioning chamber with IV cocaine injections occurred immediately (0-min group) or 15 min (15-min group) after drug infusion. If during this 15-min period the positive reinforcing effects of cocaine remain, then one could expect to see equivalent preferences between the two groups. However, if the positive affective properties of cocaine are diminished 15-min after its administration, then the expression of CPP should be attenuated relative to the 0-min group. Finally, according to the opponent-process theory of motivation (Solomon & Corbit, 1974), the initial appetitive state produced by the IV cocaine injection should decline with time and should be followed by an opposite emotional state, that is, an aversive state. As such, the 15-min group should develop a place *aversion*,

instead of a place preference (cf. Ettenberg, Raven, Danluck & Necessary, 1999).

METHOD

Subjects. Adult male Sprague-Dawley rats served as subjects (mean $\pm$ SD weight of 376 $\pm$ 14 g before surgery). All animals were individually housed in plastic bins (18 x 20 x 20 cm; with 2 cm of wood shavings) and were maintained on a 12:12 light/dark schedule (lights on 0800h) at an ambient temperature of 23°C. Rat chow and water were available *ad libitum* in the home bin. All rats were surgically prepared with chronic indwelling jugular vein catheters as described by Kearns and colleagues (Kearns, Weiss, Schindler & Panlilio, 2005). All injections given to the animals were manually administered. All procedures were in compliance with US National Institutes of Health and National Research Council guidelines and were approved by the Institutional Animal Care and Use Committee at American University.

Place Conditioning Regimen

Phase I: Pre-test. A 3-chambered black-and-white place conditioning apparatus was used for all place conditioning procedures (described in detail by Roma & Riley, 2005). Two days prior to conditioning, animals were given access to the entire apparatus for a 15-min pre-test to assess baseline preferences (Pre-test). Total time spent in both main conditioning chambers was determined, and individuals that spent $\geq$ 75% of that time in one chamber were excluded from further study. Animals that met criteria were then ranked in order of preference for the white chamber (seconds in white chamber – seconds in black chamber) and alternately assigned to the 0- and 15-min delay groups. Neither group displayed a significant within-group preference for a conditioning chamber and there were no between-groups differences in time spent in either chamber. Drug-paired chamber assignment was counterbalanced, whereby half of the subjects in each group experienced cocaine's effects in the black chamber while the other half did so in the white chamber. The place conditioning strategy employed was thus fully unbiased, with counterbalanced drug-paired stimulus assignment within an unbiased apparatus (Cunningham, Feree & Howard, 2003; Roma & Riley, 2005).

Phase II: Conditioning. On Day 1 of conditioning, animals from the 0-min group were injected with .75 mg/kg cocaine (generously supplied by the US National Institute on Drug Abuse; 1 mg/ml with saline) over a 4-sec

interval and immediately placed into their respective drug-paired chambers where they were confined for 5 min. Animals from the 15-min group were injected with cocaine, immediately placed back into their individual transport bins where they were held for 15 min and then placed into their respective drug-paired chambers for 5 min. On Day 2, animals from each group were treated identically to Day 1, but received saline injections (equivolume to cocaine) and were confined to the opposite conditioning chamber. This 2-day sequence constituted one conditioning cycle, and Phase II consisted of four conditioning cycles over the course of 8 days. On Day 9, animals received no injections and were given access to the entire apparatus for a 15-min test session (CPP).

Phase III: Extinction. The Extinction phase began the day after the place conditioning test and was conducted identically to the Conditioning phase, except that all animals had saline paired with both chambers. On Day 9 of the Extinction phase, subjects received no injections and were given access to the entire apparatus for a 15-min test session (Ext).

Phase IV: Reinstatement. The day after the extinction test, catheter patency was assessed via anesthetic injection and animals with compromised patency underwent vein-switch surgery. Seven days after the extinction test, the Reinstatement test was conducted. The procedures for each group were similar to conditioning in that each animal from the 0-min Group was injected with .75 mg/kg IV cocaine and immediately placed in the apparatus while the 15-min Group also received .75 mg/kg IV cocaine and was placed in the apparatus after a 15-min holding period. However, instead of being confined to one conditioning chamber, each subject was given access to the entire apparatus for a 15-min test session (Rein).

RESULTS

A number of animals died of respiratory illness over the course of the study; their data were not included, thereby reducing the total number of subjects to 12 (n = 6 per group). The deaths were distributed evenly between the groups and do not reflect the effects of any specific manipulation. For all statistical tests, a significance level of 0.05 was used.

A 2 (Group: 0-min and 15-min) x 4 (Phase: Pre-test, CPP, Extinction and Reinstatement) mixed Analysis of Variance (ANOVA) was performed on the time (sec) spent in the cocaine-paired chamber. This analysis revealed a significant main effect of Phase ($F_{3,30} = 3.465$, $p = .028$), but no main or interaction effects involving Group (Fs < 1.56, ps > .220). Since the temporal delay between injection and placement in the conditioning

chambers had no effect, all subjects were collapsed into a single group for subsequent analyses (n = 12).

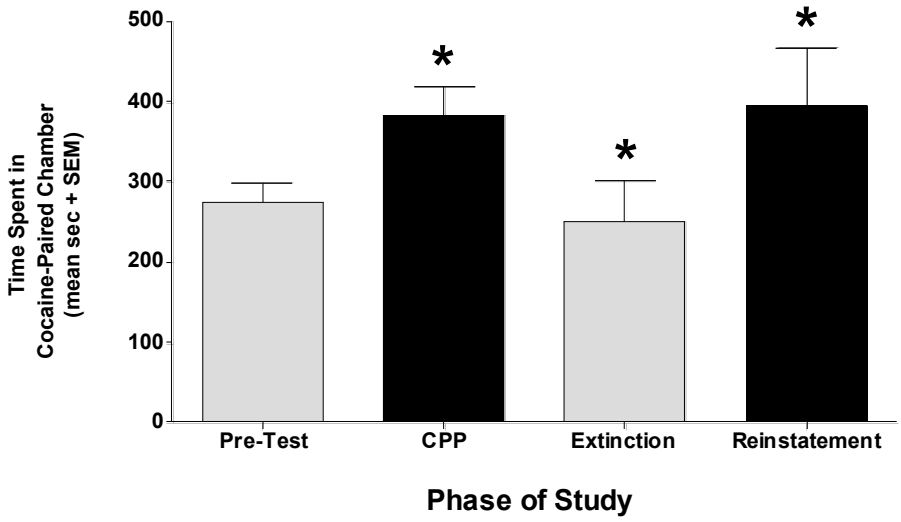

Figure 1. Time spent in the cocaine-paired chamber across all four phases of the experiment (mean sec + SEM, n = 12). Asterisks indicate a significant difference in means compared to the preceding phase (ps ≤ .030); no other pair-wise comparisons were significant (ps ≥ .691).

Paired-samples t-tests revealed a significant increase in time spent in the cocaine-paired chamber from Pre-test to CPP (t_{11} = -2.871, p = .015), a significant decrease from CPP to Extinction (t_{11} = 3.754, p = .003) and a significant increase from Extinction to Reinstatement (t_{11} = -2.495, p = .030). In addition, time spent in the cocaine-paired chamber during CPP and Reinstatement did not differ (t_{11} = -.170, p = .868), further supporting the reinstatement effect.

DISCUSSION

The finding that IV injections of cocaine were capable of generating CPP is in accordance with previous findings (Nomikos & Spyraki, 1988; O'Dell, Khroyan & Neisewander, 1996). There were no differences in CPP, extinction or reinstatement between the 0- and 15-min groups. This is an interesting contrast to the results of Ettenberg et al. (1999) who reported

that animals placed into a distinctive environment 15 min after IV injections of 0.75 mg/kg cocaine developed a place aversion to this environment instead of a place preference. Although the design employed in the present experiment was similar to that used by Ettenberg et al., there are some procedural differences that could potentially have affected the different outcomes. First, Ettenberg et al. administered IV injections via a motorized infusion pump, which delivered the drug at a constant rate. In the present study, the drug was administered to the animals manually. The lack of an avoidance of the cocaine-paired side in the 15-min group may be due to a difference in infusion speed between the two studies, a factor that has been reported to affect the positive reinforcing effects of cocaine (Nelson et al., 2006). In addition, the chambers in the current design were distinguished by color and texture only, while Ettenberg et al. also used an odor cue (acetic acid) to distinguish the chambers. It is possible that the odor cue preferentially contributed to an association between cocaine's aversive properties and the conditioning environment (Garcia & Koelling, 1966). However, given that the drug-paired side was counterbalanced between the odor and non-odor chambers in the Ettenberg et al. design, it is unlikely that the odor cue contributed systematically to the development of place aversions. The discrepancy in results between the present study and Ettenberg et al. is intriguing, but is a phenomenon that warrants further exploration before any confident between-study interpretations can be made.

The results of the present study, which used the IV route of administration, parallel those observed in studies employing IP administration (e.g., Busse & Riley, 2004; Mueller & Stewart, 2000), wherein pairings of a distinct environment with cocaine generated a preference for this environment and, once this conditioned preference was extinguished, a non-contingent injection of cocaine reinstated the preference. This study validates previous work with reinstatement and extends the route of administration under which reinstatement can be demonstrated. This may provide a more accurate animal model of drug reward and relapse within cocaine CPP, since human abusers rarely use IP injections, favoring routes of administration with more rapid onset of drug effects such as those produced by IV infusions (Verebey & Gold, 1988).

RESUMEN

Un priming intravenoso de cocaína reinstaura la preferencia al lugar condicionada inducida por cocaína. Grupos independientes de ratas fueron sometidos a un procedimiento de preferencia al lugar condicionada (PLC) involucrando emparejamientos alternados de ambientes distintivos con inyecciones intravenosas (IV) de cocaína (.75 mg/kg) o de una solución salina, inmediatamente o 15 min después de la inyección. La siguiente fase de extinción consistió en una exposición a ambos ambientes precedidos por una inyección de solución salina (Ext). Finalmente, una inyección priming IV de cocaína fue administrada para reinstaurar la preferencia al lugar extinguida (Rein). El paso del tiempo en el compartimento emparejado con la cocaína aumentó significativamente del Pre-test al PLC, se redujo del PLC al Ext y aumentó del Ext a Rein. El intervalo temporal no tuvo un efecto significativo en la ejecución en ninguna de las fases del estudio. Este estudio confirma estudios previos de reinstauración y extiende la ruta de administración bajo la cual se puede demostrar reinstauración al replicar el fenómeno con una administración IV de droga.

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