



Short communication

Self-administration of heroin, cocaine and their combination under a discrete trial schedule of reinforcement in rats

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Abstract

Several self-administration models have been used to study the interactions between cocaine and heroin, and the schedule of reinforcement used is an important consideration for these studies. Behavior maintained by cocaine, heroin or their combination was studied using a discrete trial schedule that has been described for cocaine self-administration previously. This schedule permits 24 h access to drug without mortality associated with unlimited access to cocaine, and provides unique measures related to the circadian pattern of drug self-administration. Cocaine and heroin combined maintained higher rates of responding compared to either drug alone when a maximum of three infusions were available each hour (DT3), and decreased food intake compared to cocaine alone. There were significantly greater numbers of hours in both the light and dark cycles during which animals self-administered heroin or the combination of cocaine/heroin compared to cocaine alone. When the FR was increased to 4 under the DT3 access conditions, responding maintained by cocaine or heroin extinguished to levels not different than those maintained by saline while food reinforcement remained intact. The combination of cocaine and heroin maintained robust responding under these conditions. This schedule of reinforcement appears to elucidate behavioral interactions between cocaine and heroin that are more complex than rate of drug consumption and may provide a procedure to address some of the issues related to co-abuse of these drugs.

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1. Introduction

The combined abuse of cocaine and heroin has been documented in humans and studied in laboratory animal models. The results of combining cocaine and heroin on self-administration in rodents and primates appear to be dependent on a number of variables, including the dosing regimen and schedule of reinforcement. Isobolographic analysis of dose–effect curves for self-administration of cocaine, heroin and their combination under FR schedules revealed an additive interaction in rats (Smith et al., 2006) and an additive or sub-additive interaction in primates (Negus, 2005). Some investigators report an enhancement by heroin of the ability of cocaine to maintain responding under progressive ratio schedules while others report no effect of heroin (Ranaldi and Munn, 1998; Ward et al., 2005). Using choice procedures, others have found the combination of cocaine and either heroin or methadone to be more potent than cocaine

alone (Ward et al., 2005; Wang et al., 2001). As no single reinforcement schedule can elucidate all aspects of drug-motivated behavior, there is a need to investigate the behavioral interaction of cocaine and heroin using additional self-administration paradigms.

A discrete trial self-administration procedure has been described that allows several variables to be assessed that are relevant for self-administration and may be relevant to human drug consumption patterns (Lynch and Roberts, 2004; Roberts et al., 2002). A complex interaction between cocaine dose and access conditions has been described with respect to circadian patterns of drug intake as well as the effects on concurrent food reinforcement. The ability of cocaine to produce distinct patterns of drug intake when subjects are given 24 h access can be manipulated by either altering the dose of cocaine available for each infusion or the number of infusions allowed per hour of access. A significant advantage of this procedure is that 24 h access to cocaine self-administration can be studied without lethality. Drug dependence is associated with an alteration of circadian rhythms in humans and circadian rhythms have been shown to be an important variable to consider in the treatment

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of both opioid and cocaine dependence (Sattar et al., 2004; Lin et al., 1997; Oyefeso et al., 1997). Therefore, a discrete trial schedule is potentially useful for evaluating the behavioral interaction between cocaine and heroin self-administration, as this schedule provides a means of assessing diurnal patterns of drug self-administration without the mortality associated with continuous access to psychostimulant self-administration in rodents. This study was undertaken to determine the applicability of the discrete trial schedule described previously (Roberts et al., 2002) in examining the similarities and differences between responding maintained by cocaine, heroin or their combination with respect to diurnal patterns of self-administration and effects on concurrent food reinforcement.

2. Methods

2.1. Subjects

Subjects consisted of 67 male, Fisher 344 rats (Charles Rivers Laboratories, Raleigh, NC) weighing 250–300 g at the beginning of the experiments. Rats were kept on a reversed light:dark cycle (dark 05:00–17:00) in a temperature and humidity controlled environment. Water was available ad libitum at all times during the experiments. All rats were implanted with chronic indwelling jugular catheters and maintained as reported previously (Smith et al., 2006). All procedures were approved by the Animal Care and Use Committee of Wake Forest University School of Medicine and adhered to the guidelines in the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

2.2. Drug self-administration

The procedures were similar to those reported previously (Roberts et al., 2002). For initial training, lever presses were reinforced by delivery of a maximum of 40 infusions/day of either cocaine (0.5 mg/inf, $N=17$), heroin (0.0135 mg/inf, $N=17$) or their combination ($N=N=17$) under an FR1 schedule of reinforcement with continuous access to drug infusions. Infusions were given in 0.2 ml of 0.9% (w/v) NaCl with 1.7 U/ml of heparin sodium (Baxter Healthcare, Deerfield, IL) over 6.2 s and were signaled by operation of a tone. Drug availability was signaled by illumination of a stimulus light above the lever. If the maximum infusion limit was obtained, the drug-reinforced lever was retracted from the chamber and the stimulus light was turned off until 5:00 a.m. the next day. The doses were chosen based on previous work using the DT3 schedule for cocaine and based upon the ratio of cocaine/heroin that has been shown to maintain comparable rates of responding on the descending limb of the dose–effect curve for self-administration under an FR schedule (Smith et al., 2006; Roberts et al., 2002). Once responding on the drug-reinforced lever was stable (defined as 5 successive days during which the number of infusions delivered did not vary by more than 10% of the mean), the schedule of reinforcement was changed to discrete trial (DT3) access during which drug self-administration was available during discrete trials of 20 min as described previously (Roberts et al., 2002).

Each trial was initiated by extension of the drug-reinforced lever into the chamber and illumination of a stimulus light above the lever. The lever was retracted and the stimulus light was turned off following a lever press or after 9 min had elapsed since the beginning of the trial, whichever event occurred first, until the end of the 20 min trial. Separate groups of rats were treated similarly, except that the ratio on the drug lever was gradually increased to 4 during the initial training phase (40 infusion limit). For these rats, drug self-administration was available under a DT3-FR4 schedule, under which four lever presses were required to obtain an infusion (cocaine, $N=6$; heroin, $N=5$, combination, $N=5$).

2.3. Food reinforcement

Lever presses on a second lever were reinforced by delivery of a standard 45 mg rat chow pellet (Research Diets, New Brunswick, NJ). For rats given DT3 access to drug self-administration under FR1, food reinforcement was under FR1 as well. For rats given DT3 access to drug self-administration under FR4, food reinforcement was under FR4. For these animals, the ratio on the food lever was gradually increased across three to four sessions during the initial drug self-administration training sessions (40 infusion limit). Food reinforcement was available on a continuous, unlimited basis for all animals.

2.4. Drugs and chemicals

Cocaine hydrochloride and heroin hydrochloride were obtained from the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD).

2.5. Data analysis

Data were analyzed by ANOVA, with cocaine, heroin or cocaine/heroin dose serving as the independent variables and total daily infusions administered, total daily food pellets delivered, infusions or food pellets delivered in the light or dark phase, hours during which no infusions were administered, hours during which no food pellets were obtained, or the hours during which no infusions or food pellets were obtained in the light or dark phase serving as the dependent measures. Post hoc comparisons were made using Fisher's LSD *t*-test. For the DT3-FR4 schedule, the number of infusions or food pellets delivered was analyzed using ANOVA with the number of days of access serving as the independent variable and post hoc analyses were performed using the Bonferroni–Dunn *t*-test with day 1 of DT3-FR4 access serving as the control.

3. Results

3.1. DT3-FR1

Combining cocaine and heroin resulted in different total drug intake than either drug alone (Fig. 1A). The total number of infusions delivered daily of 0.5/0.0135 mg of cocaine/heroin was

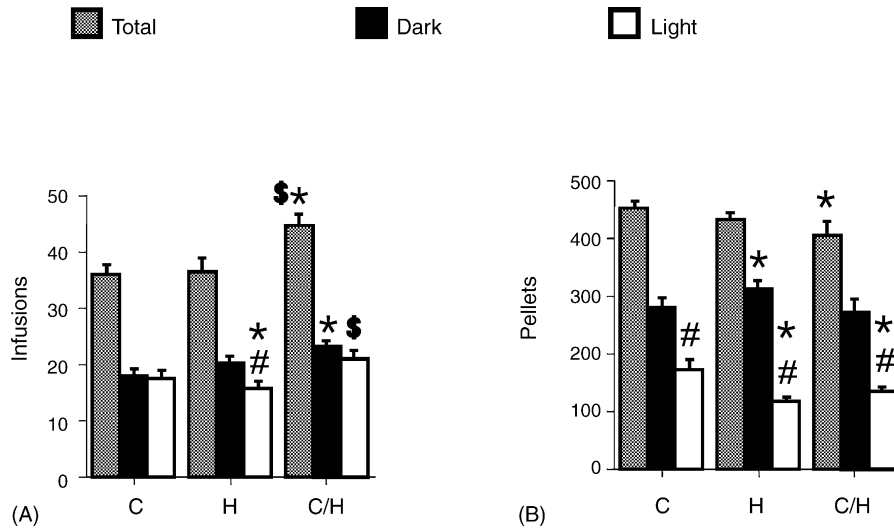


Fig. 1. Self-administration of cocaine, heroin and their combination during light and dark cycles under DT3 access. Number of drug infusions and food pellets (mean \pm S.E.M. for last 5 days of access) obtained during the entire day or in the light or dark cycles are shown for cocaine (C), heroin (H) or their combination (C/H) under DT3-FR1 access (panels A, B) (mean \pm S.E.M.). Significantly different from cocaine alone, * p <0.05. Significantly different from heroin alone, $^{\$}$ p <0.05. Significantly different from dark cycle, $^{\#}$ p <0.05.

greater than either drug alone [$F(2,49) = 5.4, p = 0.008$]. This was not due to a simple increase in self-administration of the combination across all hours compared to each drug separately. Rather, the number of drug infusions of the combination was significantly higher than cocaine alone during the dark phase and significantly higher than heroin alone during the light phase [dark: $F(2,49) = 5.4, p = 0.008$; light: $F(2,49) = 3.8, p = 0.03$].

The number of hours during which rats self-administered drug infusions also differed between cocaine, heroin or the combination. Rats engaged in self-administration of either heroin or cocaine/heroin combinations in more hours during both the light and dark phases of the cycle relative to cocaine alone [$F(2,49) = 15.4, p < 0.0001$] (data not shown). Although cocaine intake was not different between the light and dark cycles (Fig. 1A), the number of hours spent in self-administration was greater in the dark cycle relative to the light cycle. The number of hours during which heroin or cocaine/heroin maintained self-administration were not different between the light and dark cycles and were greater than that found with cocaine alone for each cycle (data not shown).

The effects of the combination of cocaine and heroin self-administration on food reinforcement were minimal (Fig. 1B). Food maintained responding in a highly circadian manner compared to drug infusions and the total daily food intake in rats self-administering cocaine was similar to that reported previously using a similar dose (Lynch and Roberts, 2004). Total daily food reinforcement was less for rats self-administering the combination of cocaine and heroin relative to those with cocaine alone due to a decrease primarily during the light cycle (Fig. 1B). Rats self-administering heroin spent more hours obtaining food pellets during the dark phase than rats self-administering infusions of either cocaine or cocaine/heroin combinations (data not shown) and food intake was higher during the dark cycle and lower during the light cycle relative to the cocaine group (Fig. 1B).

3.2. DT3-FR4

Under DT3-FR4 access conditions, neither 0.5 mg of cocaine nor 0.0135 mg of heroin maintained stable responding (Fig. 2). Both drugs readily engendered responding and animals attained stable baseline responding under FR4 with continuous access to drug. However, when the DT3 schedule was instated, responding extinguished over 4–5 days for all animals self-administering infusions of either 0.5 mg of cocaine or 0.0135 mg of heroin (Fig. 2). The number of infusions administered of 0.5 mg of cocaine was dependent upon the number of days of access [$F(10,49) = 4.9, p = 0.0002$] with the number of infusions being lower than the first day after day 4 of access. Food intake increased as cocaine self-administration extinguished [$F(10,49) = 2.8, p = 0.02$], with food intake being significantly higher on days 8–11 of DT3-FR4 access than on day 1. Self-administration of 0.0135 mg of heroin under DT3-FR4 access was similar to that of cocaine in that lever pressing decreased over 4 days of access [$F(10,49) = 6.7, p < 0.0001$]. Food reinforcement was not affected over this time period however [$F(10,49) = 0.2, p = 0.8$]. Animals reacquired self-administration when allowed free access to a maximum of 40 infusions/day under FR4, however again extinguished when the DT3 schedule was reinstated (data not shown). In contrast, the combination of 0.5 mg of cocaine with 0.0135 mg of heroin maintained stable self-administration under the DT3-FR4 schedule, and the number of infusions did not vary over days of access [$F(8,44) = 1.0, p = 0.4$] (Fig. 2). Food intake increased over the first 3 days of DT3-FR4 access however [$F(8,44) = 4.1, p = 0.001$]. The number of infusions of the combination of cocaine and heroin administered daily was similar between the groups of rats for which self-administration was maintained under the DT3-FR4 schedule compared to the number administered under the DT3-FR1 schedule (Figs. 1A and 2). Food reinforcement was also not significantly different between

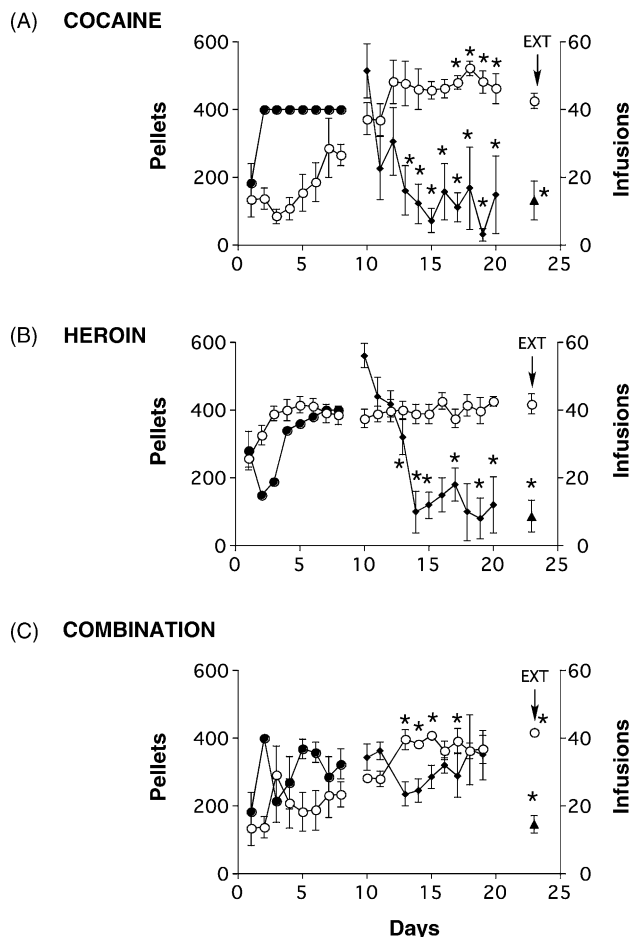


Fig. 2. (A–C) Effect of increasing the FR during DT access on drug and food self-administration. Data are shown from continuous access conditions with 40 infusion/day limit (closed circles) under FR4 schedule and following instatement of DT3 access under FR4 (closed diamonds). Data from sessions in which saline was substituted for drug are denoted by EXT (closed triangles). Concurrent food self-administration under FR4, free access conditions are denoted with open circles. *, Significantly different from first day of DT3-FR4 access.

these groups. Substitution of saline for either cocaine, heroin or cocaine/heroin combinations resulted in low rates of lever pressing that were not significantly different between these groups of animals (Fig. 2).

4. Discussion

These data indicate that a discrete trial procedure is useful for examining multiple behavioral outcomes that are influenced by combining cocaine and heroin in a self-administration paradigm. This paradigm permits multiple measurements that describe several aspects of drug seeking behavior that short session procedures do not provide. Most importantly, the combination of cocaine and heroin was found to maintain self-administration with characteristics similar to that of cocaine for some variables (e.g., equivalent drug intake in the dark and light cycles) and similar to that of heroin for others (e.g., hours spent self-administering drug in the dark and light cycles). Food reinforcement was similar between groups, and generally displayed a more circadian pattern than drug self-administration and was

similar to previous findings under DT3 access conditions of a similar dose of cocaine (Lynch and Roberts, 2004) and in rats not given access to drug self-administration (Martin et al., 2005). The DT3-FR4 schedule appears to indicate that the combination of cocaine and heroin is of greater reinforcing efficacy than either drug dose alone, as doses of cocaine and heroin that would not maintain stable responding alone produced robust and stable self-administration in combination. The present data do not permit an analysis of the interaction between cocaine and heroin with respect to additivity or supra-additivity, however recent studies would suggest that this interaction is additive (Smith et al., 2006). Although previous work has demonstrated an increase in the rate of self-administration of the combination of cocaine and heroin relative to either drug alone (Smith et al., 2006), this occurs at very low doses of each drug that are difficult to study pharmacologically or neurochemically, as the behavior maintained by these doses of cocaine and heroin alone is not robust and varies significantly between subjects. However, the combination of cocaine and heroin maintained higher rates of intake than either drug alone at doses that provide robust behavioral measures for each drug under the DT3 schedule, which should make pharmacological and neurochemical studies more feasible than procedures that utilize small doses on the ascending limb of the dose-effect function. The generalities of these findings need to be determined in future studies using broader dose ranges, as well as other ratios of cocaine and heroin in combination. The ability to study the development and potential negative consequences of drug dependence, such as decreased food intake and loss of circadian behavior, while allowing 24 h access to drug infusions in the absence of lethality could be useful for evaluating prospective pharmacotherapies for co-abuse of these drugs.

Recent studies have shown both similarities and differences in drug intake patterns maintained by cocaine, heroin or their combination. Both cocaine and heroin maintain a circadian pattern of self-administration in rhesus monkeys under a second-order fixed-ratio/variable-ratio schedule, with intake being higher in the afternoon compared to early morning and evening (Negus et al., 1995). In contrast to the present data, the combination of cocaine and heroin under this schedule was found to maintain behavior in a manner similar to each drug alone with respect to total daily drug intake (Mello et al., 1995). Studies using progressive ratio schedules have proved to be equivocal in both rodents and primates with respect to the effect of combining cocaine and heroin on self-administration. Some studies report increases in final ratios obtained or a shift in the dose-effect curve for cocaine by heroin (Rowlett et al., 2005; Duvauchelle et al., 1998; Ranaldi and Munn, 1998; Rowlett and Woolverton, 1997), whereas others report no effect (Ward et al., 2005). Using a drug-food choice procedure, the interaction between cocaine and heroin appears to be additive or sub-additive in primates (Negus, 2005). Using a drug-drug choice procedure, combining a low dose of heroin with cocaine shifted the cocaine dose-effect curve to the left when paired against a moderate cocaine dose in rats, even though heroin was not chosen alone over cocaine (Ward et al., 2005). These latter data suggest that combining cocaine with heroin produces greater relative reinforcing efficacy than

cocaine alone. However, these studies have not addressed the relevance of diurnal rhythms on the combination of cocaine and heroin self-administration, which has been suggested to be an important variable to consider for pharmacological treatment of drug dependence (Sattar et al., 2004; Lin et al., 1997; Oyefeso et al., 1997).

The ability to assess circadian patterns of both drug and food reinforcement could be an important feature of the present paradigm, in addition to examining total drug and food intake. The neurobiology of circadian behavior in rodents has been studied, and the suprachiasmatic nucleus (SCN) of the medial hypothalamus is a key region (Moore and Leak, 2000; Wurts and Edgar, 2000). The SCN sends output to the ventrolateral and median preoptic nuclei to control circadian rhythms and three brain regions appear to be involved in the relay of SCN output to these areas: the medial preoptic area (MPA), subparaventricular zone (SPZ) and the dorsomedial hypothalamus (DMH) (Durveilhaer and Semba, 2003; Chou et al., 2002; Durveilhaer et al., 2002; Leak and Moore, 2001). The ventral tegmental area (VTA) receives input from the MPA and DMH and the locus coeruleus (LC) receives afferents from the DMH (Durveilhaer and Semba, 2005). Since neuronal activity in the VTA and LC are influenced by both stimulants and opioids, input from structures that influence circadian sleep–wake cycles may alter the circadian patterns of drug reinforcement. Serotonergic systems in the brainstem, principally within the raphe nuclei have a prominent role in sleep–wake cycles as well, and there is a vast literature on the anatomical topography and regulation of these regions (review, Abrams et al., 2004). Both opiates and psychostimulants influence serotonergic activity within raphe nuclei and the relevance of the neurochemical effects of cocaine, heroin or their combination on these systems has not been documented in a self-administration paradigm. The investigation of the influence of drugs of abuse on these systems could add significant new information about the development of drug dependence, since disruption of circadian rhythms during this process is likely an important variable to consider for treatment modalities. The DT schedule described in the present study appears to be an appropriate procedure for such studies using cocaine, heroin or their combination.

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