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Characterization of Cocaine Self-Administration and Pharmacokinetics as a Function of Time of Day in the Rat

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BAIRD, T. J. AND D. V. GAUVIN. *Characterization of cocaine self-administration and pharmacokinetics as a function of time of day in the rat.* PHAMACOL BIOCHEM BEHAV **65**(2) 289–299, 2000—Two experiments examined the influence of time of day on the intravenous self-administration of cocaine and its associated pharmacokinetic profile in male Sprague–Dawley rats. In both experiments, individual rats were randomly assigned to experimental groups (n = 6/group) according to four selected times of day, 0100, 0700, 1300, and 1900 h, during which experimental procedures were conducted. In both experiments, rats were maintained under a 12 L:12 D ambient lighting cycle, with lights on at 0600 h. Training and testing was thus conducted either 1 (0700, 1900) or 7 (1300, 0100 h) hours into the light and dark phases. In Experiment 1, characteristics of cocaine self-administration across a behaviorally active dose range were assessed. Statistically significant differences were observed in the rates and patterns of self-administration across the four experimental groups, most notably characterized by an apparent shift in the dose of cocaine, which engendered peak rates of responding. Specifically, groups tested at 0100 and 1300 h appeared to exhibit enhanced sensitivity to the reinforcing properties of low-dose cocaine relative to groups tested at 0700 and 1900 h. The observed differences in apparent sensitivity of experimental subjects to low-dose cocaine were not related in any simple way to ongoing patterns of general locomotor activity, and were not accompanied by corresponding variance in the pharmacokinetic profiles of cocaine when assessed over 1 h following an intravenous infusion (1.8 mg/kg) at each of the four sampling periods noted above. © 2000 Elsevier Science Inc.

Cocaine Self-administration Chronopharmacology Biological rhythms Pharmacokinetics High-performance liquid chromatography

IT has been repeatedly demonstrated that the pharmacological, physiological, and behavioral effects of drugs are not invariant with respect to the time of the administration over a 24-h cycle (13,20,29,39,47,62,73). Likewise, a wealth of literature currently exists, demonstrating that interactions between drug effects and circadian rhythms appear to be reciprocal (63). Although it has been recognized that the effects of drugs are often dependent upon the time of day at which they are administered, it is also clear that many drugs, potentially acting at different levels within the hierarchy of the circadian system, have the capacity to alter the expression of biobehavioral rhythms (16,50,69). The discipline of chronopharmacology encompasses basic research on the time-dependent activity, toxicity, and kinetics of drugs, as well as the applied practice of circadian phase-adjusted administration of drugs to coincide with peak periods of therapeutic potency and/or efficacy (10,12,62,65).

There are indications that the rate of clearance of a drug from a biological system also displays marked variability according to the time of its administration across the circadian cycle—a finding that may account for circadian fluctuations in the drug's effect (3,10). Chronopharmacokinetics describes the mechanisms mediating time-dependent variation in drug absorption, distribution, metabolism, and excretion (10,37,62, 65). Circadian rhythms in four major types of drug metabolism—oxidation, reduction, hydrolysis, and conjugation—

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have been noted, which at times appear to correspond to time-dependent alterations in the activity of specific enzymes, and can be meaningfully related to gross behavioral or physiological function of an organism following drug administration (3,10,37). For example, there is a significant inverse relation between the circadian profiles of hepatic hexobarbital oxidase activity, and hexobarbital-induced sleep duration in rats (53,54). It has been emphasized, nevertheless, that the acrophase (i.e., time at which the rhythm reaches peak values) of drug potency does not necessarily coincide with the acrophase of the level of drug in biological tissue or fluids (63).

Many psychoactive drugs display differential potency and/or efficacy as a function of the circadian phase at which they are administered. Time-of-day differences in the sensitivity of rodents to amphetamine (75), methylphenidate (26), nicotine (74), diazepam (70), chlordiazepoxide (44), pentobarbital (56,57,74), hexobarbital (49,54), phenobarbital (49) morphine (5,22,48,80) methadone (41), ethanol (1,25,30,80) and toluene (28), have been described. Humans display analogous 24-h patterns of differential sensitivity to some of the same psychoactive substances; for instance, ethanol (64) diazepam (63), and morphine (27,38) engender varying gradations of effect, depending upon the time of administration. Additionally, circadian rhythms in the reinforcing properties of drugs, as demonstrated in self-administration paradigms, and most notably with respect to ethanol, have been described across species, including mice, rats, nonhuman primates, and humans (17,19, 23,25).

Very few studies have addressed the chronopharmacology of cocaine, a fact that is somewhat surprising, given the voluminous extant literature addressing the systemic and behavioral effects of this drug, and especially in light of considerable research demonstrating the importance of circadian factors as partial determinants of drug potency and/or efficacy. Some recent reports have provided tentative indications that the probability and rate of cocaine self-injection may be variable according to the time of day under restricted-access protocols (21,55,67), but no clear and consistent time-of-day differences in the quantitative or qualitative patterns of unrestricted cocaine intake have yet been described. These results are in contrast to unambiguous demonstrations of 24-h variation in the reinforcing properties of ethanol, morphine, and pentobarbital (19). There are two reports of a circadian rhythm in the probability of the initiation of cocaine self-administration in rats under a discrete trials procedure where subjects were limited to fewer than four opportunities per hour to respond on a lever for a cocaine bolus (21,67). However, the limiting conditions in these previous experiments did not afford the opportunity to assess time-of-day differences in the more typical "binge" pattern of cocaine self-administration, where a drug is repeatedly ingested over a period of several hours, which is characteristic of response profiles by human cocaine users, as well as nonhuman primate and rodent models of cocaine abuse (79).

There are a number of reasons to expect that behavioral responses to cocaine, as well as cocaine pharmacokinetics, toxicity, and self-administration may be variable across the 24-h cycle. Several reports indicate that drugs with modes of action somewhat similar to cocaine display altered potency as a function of the time of day of administration. For instance, amphetamine, fencamfamine, and methylphenidate all appear to have greater potency, with respect to various behavioral and neurophysiological dependent measures, when administered in the light phase of the light/dark (L/D) cycle (2,11, 18,26,59,71). These 24-h rhythms in psychomotor stimulant effectiveness are accompanied by corresponding rhythmic changes in functional properties of aminergic systems thought to mediate the central nervous system effects of these drugs, at the level of the neurotransmitter, receptor, and second messenger (4,18,34,35,40,45,72,73,77,81,82). These findings suggest that similar circadian or ultradian variations in sensitivity to cocaine as a reinforcer may well exist.

Several studies suggest that the pharmacokinetic profile of cocaine may not remain constant over a 24-h cycle. Cocaine is rapidly metabolized by esterases in both blood and liver. The two main metabolites produced are ecgonine methyl ester (via cleavage of the benzoyl ester bond), and benzoylecgonine (via hydrolysis of the methyl ester bond), both of which are centrally inactive, and excreted in urine (24,33). Norcocaine is another metabolite of cocaine, formed by the cytochrome P-450 mediated N-demethylation of cocaine (24,36). Researchers have not yet characterized the 24-h profile of cocaine's pharmacokinetics, although several studies have found circadian variation in plasma and brain cholinesterases, which are major catalytic molecules in cocaine metabolism (60,66). Reports indicate that cocaine elimination kinetics are inversely related to levels of plasma cholinesterases, which serve as the major catalysts for cocaine metabolism (9,42,46), and reach peak levels near the end of the light phase of the light/dark (L/D) cycle in humans (66). Accordingly, it might reasonably be expected that cocaine's pharmacokinetic profile would be influenced by circadian fluctuations in these enzymes.

The following two experiments were designed to assess the influence of time of day on quantitative (rates) and qualitative (patterns) parameters of cocaine self-administration in rats under limited-access "binge" conditions, and to furthermore relate these behavioral data to putative time-of-day variations in cocaine pharmacokinetics. In view of the studies describing amphetamine, fencamfamine, and methylphenidate chronopharmacology noted above, it was hypothesized that subjects would display differential sensitivity to the reinforcing properties of cocaine, with groups tested in the light phase demonstrating increased sensitivity relative to groups tested in the dark phase of the L/D cycle. Further, it was predicted that the increased behavioral sensitivity of rats tested in the light phase would be associated with higher overall blood levels as well as attenuated rates of cocaine metabolism.

EXPERIMENT 1: COCAINE SELF-ADMINISTRATION

Time-of-day differences in intravenous cocaine self-administration over a broad dose range were assessed by training and testing independent groups of rats at one of four different time points distributed over the 24-h cycle. Data were analyzed to determine statistically significant differences in both rates and patterns of cocaine self-administration across experimental groups.

Method

Subjects. Twenty-four male Sprague–Dawley rats (Sasco Inc., Omaha, NE) were utilized to characterize qualitative and quantitative aspects of time-dependent alterations in operant cocaine self-administration. Experimental procedures commenced 2 weeks after receipt of the animals to allow time for acclimation to the laboratory environment. Subjects were housed individually in standard stainless steel suspended cages in an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited colony room on a 12 L:12 D cycle (lights on at 0600 h), with temperature and humidity within nominal limits. Rats in the self-administration

experiment were food restricted to 85% of free-feeding weight for 1 week during the course of early operant training to train the lever-press operant response, and maintain food-motivated responding under a FR 5 schedule, but were placed on ad lib access to both food and water for the remainder of the experiment (i.e., after operant responding for cocaine had been established).

Apparatus. Six operant chambers (Lafayette Instruments, Lafayette, IN), equipped with a stimulus lamp, response lever, food dispenser, food cup, and pneumatic syringe apparatus (Ledger Technical Services, Kalamazoo, MI), to which catheterized rats could be connected for the intravenous delivery of cocaine, were used for the self-administration experiments. The operant chambers were interfaced with an IBM compatible computer with input/output controller cards (Med Associates, Inc., Georgia, VT). Training schedules, test contingencies, and data collection were controlled by a Clarion-based software package (American Neuroscience Foundation, Yukon, OK). White noise was provided throughout operant sessions by means of a white noise generator (Model 15800, Lafayette Instruments, Lafayette, IN), connected to an audio speaker located at a fixed position within the room housing the operant chambers.

Behavioral training. After the 1-week acclimation period to the laboratory environment, subjects were randomly assigned to groups and behavioral training commenced. Rats were not allowed access to food for 24 h prior to the first training session. The four independent groups of subjects were trained, in 2-h sessions at each of four times of day (0100, 0700, 1300, and 1900 h), to press the response lever by the method of successive approximations to receive food pellets (45 mg each, P.J. Noyes, Inc., Lancaster, NH). The fixed ratio response requirement to obtain food pellets was successively increased from a fixed-ratio 1 (FR1) until subjects had established stable lever-press performance at an FR 5 schedule. Catheterization surgery was then performed. Food was provided ad lib for the first 3 days of recovery from surgery, but was then restricted for 24 h on the fourth day to ensure that subjects responded when operant sessions commenced. Following surgery and the 4-day recovery period, rats were placed back in the training apparatus and allowed to respond for the simultaneous delivery of food pellets and cocaine on an FR5 schedule. Each food pellet delivery was accompanied by an intravenous bolus of cocaine (0.5 mg/kg). Following this induction procedure for cocaine self-administration 1) the pellet dispenser was made inoperable, 2) rats were allowed ad lib access to food, and 3) rats had the opportunity to respond only for cocaine in operant sessions. A single "priming" injection (approximately 40 μl) was administered to each rat at the beginning of the daily self-administration session to flood the catheter with the drug so that it was immediately available to subjects following the very first response ratio completion. Rats were tested only after exhibiting stable rates of responding, which was defined as ±15% variability in reinforcer deliveries per 2-h session over 3 consecutive days. Self-administration training and test sessions occurred at each of four different times of day. A dose–response curve was generated by substituting a randomly selected cocaine test dose for the training dose in subjects that met criterion for testing, until data were collected for each rat, in all four groups, at each concentration of cocaine.

Surgeries. Indwelling venous catheters were constructed of three different diameters of PE tubing; lengths of PE 90 (6.0 cm), PE 50 (9.0 cm), and PE 10 (6.0 cm) were fused together under a hot air stream. Silastic® silicone tubing (3.85 cm) was joined to the PE 10 by stretching it over the PE tubing so that it overlapped by approximately 5 mm, and sealing the outside joint with heat-shrink tubing. The resulting distance from the heat-shrink tubing junction to the end of the silicone tubing was therefore approximately 3.35 cm. This length allowed positioning of the distal tip of the silicone tubing just outside the right atrium of the heart.

Surgical procedures were performed under anesthesia provided by separate injections of ketamine hydrochloride (110 mg/kg, IP) and sodium xylazine hydrochloride (10 mg/kg, IM), following a 20-min pretreatment with atropine (0.1 ml of 0.54 mg/ml, SC). Anesthesia was considered adequate to commence surgery when each rat demonstrated all three of the following: 1) loss of the righting reflex, 2) no eyeblink to digital palpation around the orbit, and 3) no muscular or vocal response to firm tail pinch. A 1" incision was made through the skin on the dorsal surface, at the midscapular level, and perpendicular to the longitudinal axis of the rat. Another 1" incision was made ventrally, on the area of the neck overlying the right jugular vein, and parallel with the longitudinal axis. After the right external jugular vein had been isolated, the silicone tip of the intravenous catheter was inserted into the vein up to the shrink tubing joint between the silicone and PE tubing. The shrink tubing joint was sutured to the vein, and anchored to the surrounding tissue at two points. The portion of the catheter comprised of PE tubing was threaded around the right foreleg of the rat subcutaneously to the incision point on the dorsal surface, and subsequently attached to a stainless steel (Harvard Scientific, Holliston, MA) or plastic (Kent Scientific, Litchfield, CT) anchoring button. The anchoring button was then sutured to the musculature and secured in place by suturing a 3 × 3-cm piece of sterile Lars Mesh (Meadox Medicals, Oakland, NJ) over the base of the button and to the underlying tissue. Both dorsal and ventral incisions were then closed with suture and cyanoacrylate (Super Glue®).

Following surgery, and for all 4 days of the recovery period, each rat was administered 0.1 ml of saline containing penicillin G sodium (250,000 U/ml), via the catheter, which was then immediately flushed with a solution of heparin (10 U/ml) in saline. When animals resumed training, catheters were flushed with 0.1 ml of heparinized saline (10 U/ml) each day immediately before and after self-administration sessions. Catheter patency was evaluated once each week by a single injection of methohexital sodium (0.1 ml of a 10 mg/ml solution), which caused immediate loss of the righting reflex if the catheter was patent. Additionally, catheter patency was confirmed at the completion of testing for each animal by the same method, and further anatomical examination. Rats were euthanized before necropsy by an overdose of 100 mg/kg sodium pentobarbital (50 mg/ml), delivered through the jugular catheter.

Experimental design. Dose–response curves were generated for each of four selected time points across the 24-h cycle (0100, 0700, 1300, and 1900 h). Six rats were tested at each of six randomly selected doses (saline, 0.018, 0.056, 0.18, 0.56, and 1.8 mg/kg/injection) at each of the four time periods. The selected cocaine doses span a behaviorally active range that has been shown to produce "extinguished" responding at the low end of the dose range, maximal rates-of-responding at intermediate doses, and response-rate suppression at the high end of the dose range.

Drugs. Ketamine hydrochloride (expressed as the salt; Sigma, St. Louis, MO), cocaine hydrochloride (expressed as the sale; National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC), penicillin G so-

dium (Marsam Pharmaceuticals Inc., Cherry Hills, NJ), and methohexital sodium (Eli Lilly & Co., Indianapolis, IN) were mixed in sterile 0.9% saline. Premixed solutions of atropine sulfate, 0.54 mg/ml (Vedco, Inc., St. Joseph, MO) and pentobarbital sodium, 50 mg/ml (Abbott Laboratories, North Chicago, IL) were purchased from the University Hospital pharmacy (Oklahoma City, OK).

Data analysis. Data were analyzed in two ways to assess statistically significant between-group differences in cocaine self-injections in terms of both the response rates and the patterns of responding, or reinforcer deliveries, across each 2-h session. First, to quantify the group-dependent dose–response relationships, a 4 × 6 mixed-factor MANOVA was conducted on total session reinforcer deliveries. Group membership (four times of day) was loaded as the between-subjects factor and the individual cocaine test doses [6] were loaded as the within-subjects (repeated-measures) factor. Both absolute rates of responding and the percentage change from baseline (i.e., the maintenance dose of 0.5 mg/kg/injection) were evaluated in this manner. Baseline responding under the maintenance dose was defined as the average number of cocaine infusions self-administered over the 2 days immediately prior to a test day. Tukey HSD post hoc tests were used for individual group and dose comparisons across the cocaine dose–response curve.

In the second set of analyses, the patterns of responding displayed by each time-of-day group across the 2-h sessions were compared for each individual dose of self-administered cocaine. Individual patterns of responding displayed by the 0100-, 0700-, 1300-, and 1900-h groups over the length of a session were analyzed by breaking each 2-h session down into 12 consecutive 10-min bins. Differential rates of responding across the four time points in each of these bins were analyzed with a 4 (time-of-day group) × 6 (cocaine dose) × 12 (serial time bins) mixed between- and within-subjects MANOVA, with group loaded as the between-subjects factor, and dose and time loaded as within-subject factors. Individual ANOVAs and subsequent Tukey HSD tests were used as simple effects tests to determine the statistical significance of comparisons across time-of-day groups at each individual time bin. All statistical analyses were performed using the CSS: Statistica (Complete Statistical Systems, Tulsa, OK) software package. The criterion for statistical significance was set at $p < 0.05$.

Results and Discussion

A typical inverted U-shaped function characterized the cocaine dose–response curves for self-administration (see Fig. 1). Across all four time-of-day groups, lower rates of lever pressing were observed at the low and high ends of the dose range, while significantly greater rates of response were associated with intermediate doses [main dose effect: $F(5, 100) = 39.96$, $p < 10^{-8}$]. However, the dose–response curves were not identical across all four groups, as there appeared to be a shift in the cocaine dose that engendered peak rates of responding in the 0100- and 1300-h groups, relative to the 0700- and 1900-h groups [main group effect: $F(3, 20) = 3.76$, $p < 0.027$; group × dose interaction: $F(15,100) = 3.57$, $p < 0.0001$]. Rats in the 1300-h group self-administered a significantly greater number of cocaine infusions at the 0.056 dose, relative to rats in the 0700-h ($p < 0.03$) and 1900-h ($p < 0.02$) groups. With respect to the 0100-h group, individual comparisons revealed no statistically significant differences in the number of self-injections at the 0.056 dose between this group and the 0700-, 1300-, and 1900-h groups.

Figures 2–7 depict patterns of responding engendered by each cocaine dose for each time-of-day group. An overall MANOVA conducted across all four time-of-day groups and cocaine doses revealed that rates of responding varied across these sessions according to time-of-day [main group effect: $F(3, 20) = 6.22$, $p < 0.004$], cocaine dose [main dose effect: $F(5, 100) = 49.70$, $p < 10^{-8}$], and individual time bin [main time-bin effect: $F(11,220) = 24.31$, $p < 10^{-8}$]. Figure 4 depicts patterns of responding at the 0.056 mg/kg/inj dose, over the 12 individual time bins comprising the 2h self-administration session, at each of the four different times of day, 0100, 0700, 1300, and 1900-h. Group-dependent variability in responding appeared to be limited to the 0.056 mg/kg/inj dose [main group effect: $F(3, 20) = 10.18$, $p < 0.0003$; main time-bin effect: $F(11, 220) = 2.56$, $p < 0.005$], as patterns of responding within sessions did not vary significantly across experimental groups at any other dose of self-administered cocaine (all ps > 0.05). One-way ANOVAs, used as simple effects tests, revealed significant group-dependent differences in responding for 11 out of the 12 consecutive 10-min time-bins at the 0.056 mg/kg/inj dose (all ps < 0.05, see Fig. 4). The 1300-h, and to a lesser extent, the 0100-h groups responded at a consistently higher rate over the course of the 2-h self-administration session in comparison to the 0700-h group, but not the 1900-h group (Tukey post hoc tests, all ps < 0.05).

The differential pattern of low-dose cocaine self-administration is clearly seen in Fig. 8, which displays event records for individual rats that were judged to be representative of the group averaged patterns of responding engendered at the

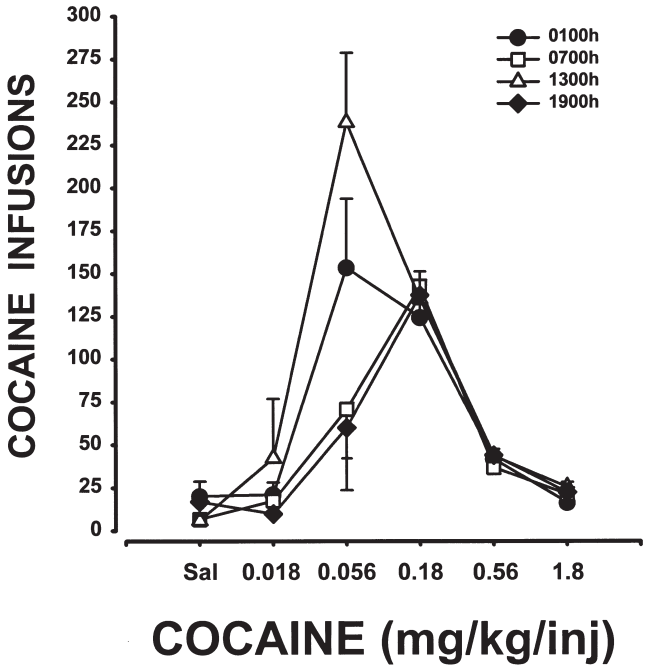

FIG. 1. Cocaine self-administration dose–response curve. Each point represents the mean number of cocaine self-injections administered at a given dose for each time-of-day group, 0100 h (closed circles), 0700 h (open squares), 1300 h (open triangles), and 1900 h (closed diamonds). Error bars indicate standard error of the mean (SEM).

0.056 dose. Self-administration was sustained for the entire 2-h session in the 0100- and 1300-h groups, while a pattern more analogous to an "extinction" of responding, as occurs with saline or nonreinforcing doses of cocaine, was characteristic of the 0700- and 1900-h groups.

EXPERIMENT 2: COCAINE PHARMACOKINETICS

Experiment 2 examined the 24-h profile of cocaine's pharmacokinetics following a single intravenous bolus of the highest dose selected for self-administration in Experiment 1 (1.8 mg/kg/inj). Experimental manipulations were conducted at the same times of day as those assessed in Experiment 1 to determine whether observed differences in the reinforcing properties of cocaine as a function of the time of day were related to corresponding alterations in drug bioavailability.

Method

Subjects. Arterial blood cocaine levels were quantified at the same four time points across the 24-h period as those chosen for the behavioral experiment. Six rats were randomly assigned to 0100-h, 0700-, 1300-, and 1900-h groups (n = 24 rats). These subjects were kept in the same colony room as described previously, under identical ambient conditions, and allowed 2 weeks to acclimate to the laboratory environment before experimental manipulations were initiated.

Surgery. Surgical anesthesia was achieved through separate injections of ketamine hydrochloride (110 mg/kg, IP) and xylazine hydrochloride (10 mg/kg, IM). Anesthesia was deemed adequate to commence surgery according to the same criteria described in Experiment 1. Rats were instrumented with indwelling jugular catheters, using the exact procedure described

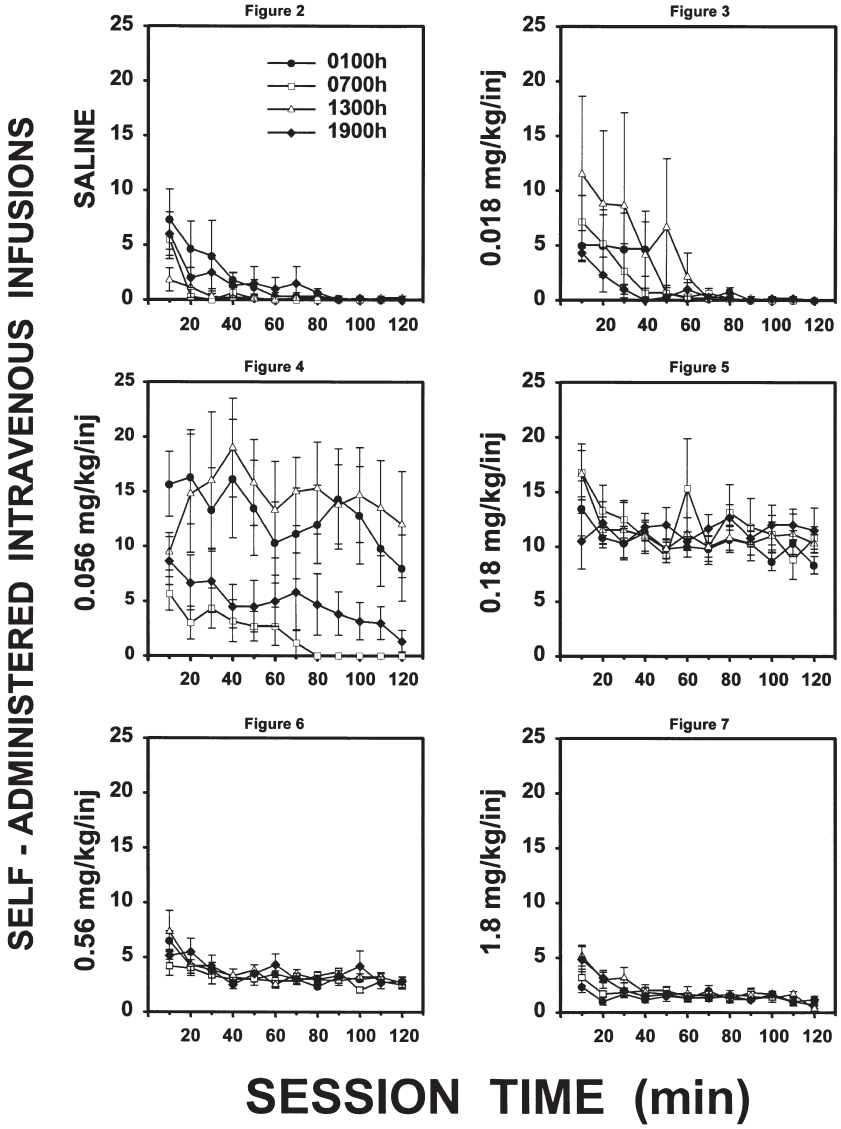

FIG. 2–7. Panels display patterns of responding for intravenous saline infusions, collapsed in 10-minute time bins, across daily 2-h testing sessions conducted at 0100 h (closed circles), 0700 h (open squares), 1300 h (open triangles), and 1900 h (closed diamonds). Each point represents the average (±SEM) of six rats.

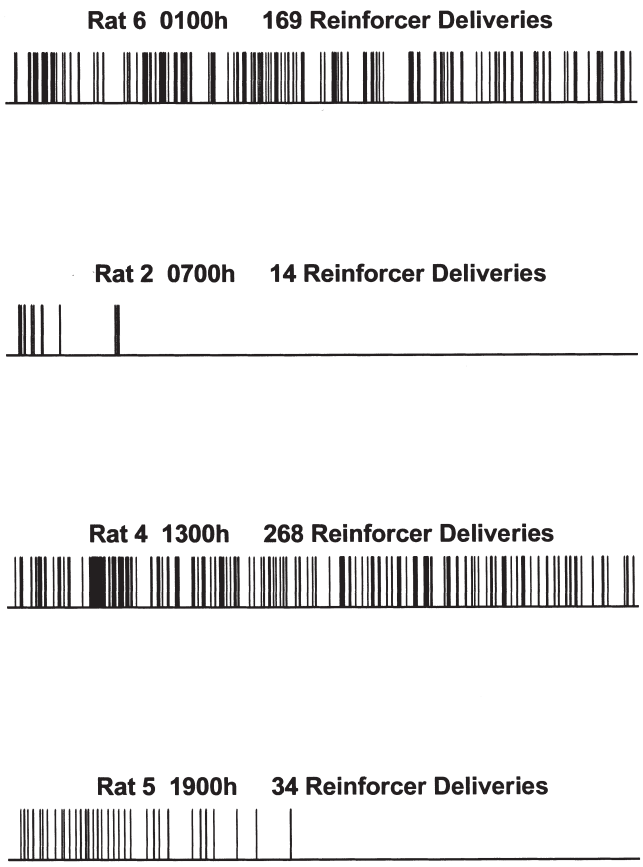

FIG. 8. Individual cocaine self-administration event records of four representative subjects from each experimental group, 0100, 0700, 1300, and 1900 h are displayed. Each vertical tick on the x-axis indicates the time within the 2-h testing session at which a cocaine self-injection was recorded.

in Experiment 1. Subjects were additionally instrumented with a catheter directed into the left internal carotid artery, constructed of lengths of PE50 (3.7 cm) and Tygon® (15 cm) tubing joined with cyanoacrylate (Super Glue®). The end constructed of PE50 was inserted into the internal carotid artery, sutured in place, and the other end tunneled subcutaneously to exit out the back at the midscapular level. The cocaine bolus was delivered through the jugular catheter, while arterial blood samples for high-performance liquid chromatographic (HPLC) analysis were drawn through the internal carotid catheter. Both catheters were sutured to the musculature at the midscapular level, and exited transcutaneously. Custom made rodent vests held the catheters in place and protected them from damage, while allowing freedom of movement within the home cage. Subjects were allowed 2 days to recover from surgery before blood samples were collected. Upon completion of arterial blood sampling, rats were euthanized by an overdose of sodium pentobarbital (100 mg/kg) delivered through the jugular catheter. Proper placement of the catheters was then verified by necropsy.

HPLC measurement of plasma cocaine levels. The HPLC system (Bio Analytic Systems, BAS) used for determination of plasma cocaine levels was comprised of a pump (BAS model PM-60), UV detector (BAS model UV-108), and injector fitted with a 20-μl injection loop (Rheodyne model 7125). The HPLC system was fitted with a BAS 100 × 3.2 mm Phase II ODS 3 micron column, and was interfaced with a Hewlett Packard integrator (model 3390A). The protocol used in the current study is an adaptation of that described by Jatlow and Nadim (31).

The mobile phase consisted of a solution of 500 ml of acetonitrile and 1 l of 50 mM/l phosphate buffer (6.9 g KH_2PO_4 in 1 l of distilled water, adjusted to a pH of 3.0 with orthophosphoric acid) containing 1.88 g/l of the sodium salt of hexanesulfonic acid as an ion pairing reagent. The mobile phase was vacuum filtered (Gelman Sciences 47 mm Nyaflo 0.2 μm nylon membrane) and degassed under vacuum for 20 min. A carbonate buffer (700 mg of a dry mixture of 20 g Na_2CO_3 and 17.5 g $NaHCO_3$, constituted in 10 ml of HPLC grade water) was used to prepare samples. Chromatography was performed with a mobile phase flow rate of 1.0 ml/min, and the effluent was monitored at 235 nm with sensitivity and rise time set at 0.01 AUF and 1.0 s, respectively.

Procedures. Following the 2-day recovery period from catheterization surgery, rats were transferred to a standard plastic shoebox cage for administration of the cocaine infusion and subsequent blood withdrawals. Based on previous work characterizing the time course of cocaine's pharmacokinetic profile (6,8,43), blood samples (400 μl) were extracted for analysis at six individual time points (1, 5, 15, 30, 45, and 60 min) after intravenous (IV) administration of a 1.8-mg/kg cocaine bolus. This dose was chosen because it represented the highest dose of cocaine selected for self-administration in project 1, and also because of the potential for higher doses to initiate seizure activity.

Whole blood samples to be analyzed for cocaine content were deposited into ice-cooled 1.5 ml microcentrifuge tubes containing 10 μl of a saturated sodium fluoride solution (to prevent hydrolysis of cocaine by esterases). Samples were centrifuged at 3100 RCF for 5 min, after which 200 μl of plasma was transferred to a new microcentrifuge tube, frozen, and stored at −80°C until HPLC analysis. Samples and standards were prepared by the addition of 40 μl of the internal standard (lidocaine, 2500 ng/ml in methanol), 50 μl of carbonate buffer, and 1.2 ml of a isoamyl alcohol (20 ml)/hexane (1.0 l) solution. Samples were then extracted on a platform mixer for 20 min and centrifuged at 3100 RCF for 10 min. The upper organic layer was transferred to a new microcentrifuge tube containing 40 μl of 0.1 N HCl reagent, vortex mixed for 2 min, and centrifuged for 5 min. The upper solvent layer was discarded, and the lower aqueous phase injected into the HPLC. Standard cocaine solutions (5000, 2500, 1000, 500, and 250 ng/ml concentrations) were prepared from a stock solution of 1 mg/ml cocaine (calculated as the base) mixed in methanol, and extracted in the same manner as the unknown samples. Additional standards, purchased from Sigma Chemical Co., were also quantified to assess the accuracy of our standards. HPLC-grade reagents and chemicals used in the preparation of the mobile phase, samples, and standards were obtained from Fisher Scientific (Springfield, NJ).

Drugs. Ketamine hydrochloride (expressed as the salt; Sigma, St. Louis, MO) and cocaine hydrochloride (expressed as the salt; National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC) were mixed in sterile 0.9% saline. Xylazine hydrochloride (Mobay Corporation, Animal Health Division) was purchased in a premixed solution.

Data analysis. A mixed-factor, repeated-measures MANOVA with (time-of-day) group loaded as the between-subjects factor, and blood sampling time loaded as the repeated-mea-

sures factor, was used to determine statistical significance of comparisons of absolute cocaine blood levels across groups. Post hoc Tukey HSD tests were used for individual comparisons across groups by the within-subjects factor. Statistical analyses were performed using the CSS: Statistica (Complete Statistical Systems, Tulsa, OK), and Pharmacologic Calculation System (PHARM/PCS; Life Science Associates, Bayport, NY) software packages. PCS was used to calculate cocaine pharmacokinetic parameters (i.e., slopes, Ke values, and tests for parallelism). The criterion for statistical significance was set at $p < 0.05$.

Results and Discussion

Figure 9 displays the mean cocaine concentrations in plasma (ng/ml) for each of the four time-of-day groups across the 1-h sampling period. A significant, time-dependent decline in plasma cocaine concentrations was observed for all groups [main time effect: $F(5,100) = 66.79$, $p < 10^{-8}$]. Plasma cocaine concentrations declined equivalently across all four groups over the six individual time points in the 1-h sampling period [main group effect: $F(3,20) = 1.42$, $p = 0.27$; group × time interaction: $F(15, 100) = 0.72$, $p = 0.76$]. There were no significant differences between the four time-of-day groups in the calculated elimination kinetic constants (Ke) for cocaine [main group effect: $F(3, 20) = 1.06$, $p = 0.39$], and all kinetic functions were parallel (1 vs. 2 $t_{calc}[8df] = 0.144$; 1 vs. 3, $t_{calc}[8df] = 0.443$; 1 vs. 4, $t_{calc}[8df] = 0.551$.

Although no significant differences were observed in plasma concentrations of cocaine or in calculated kinetics functions for the experimental groups, rats tested in the light phase (0700 and 1300 h) exhibited an increased incidence of convulsions immediately following intravenous administration of the 1.8 mg/kg dose of cocaine. Five rats in the 0700-h group, and four rats in the 1300-h group displayed tonic–clonic motions, vocalization, and wild jumping movements within seconds of the cocaine infusion, while none of the rats tested at 1900 and 0100 h exhibited such behaviors. These results, and those of Experiment 1, appear to indicate that time-of-day fluctuations in cocaine-induced, and cocaine-reinforced behaviors, respectively, are not due to corresponding alterations in cocaine's pharmacokinetics. Time-of-day changes in drug bioavailability, therefore, can likely be ruled out as a causal factor in these behavioral effects.

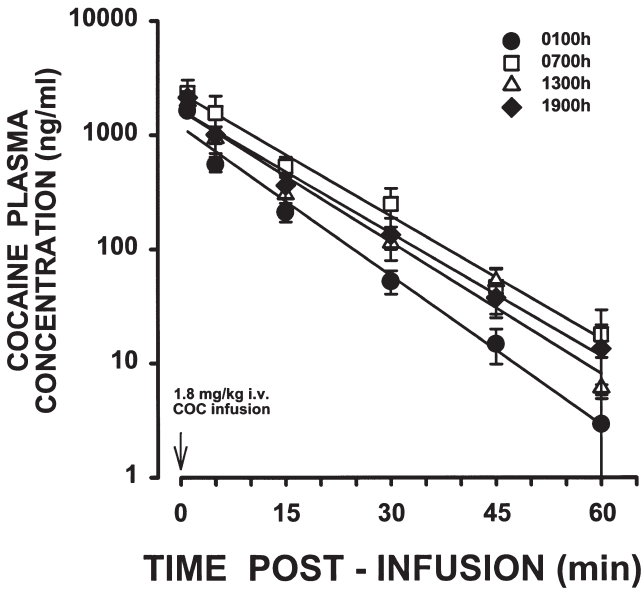

FIG. 9. Concentrations of cocaine recovered from plasma are displayed for each time-of-day group, 0100 h (closed circles), 0700 h (open squares), 1300 h (open triangles), and 1900 h (closed diamonds). An intravenous bolus (1.8 mg/kg) of cocaine was infused at time 0. Each point represents the mean (±SEM) of six rats.

GENERAL DISCUSSION

Early studies in which primates were given continuous access to response-contingent cocaine failed to demonstrate any reliable circadian pattern in cocaine self-administration (19,32), although Deneau et al. (19) did show fairly consistent circadian variations in pentobarbital and morphine intake, and self-administration of caffeine and ethanol, which approximated circadian rhythmicity. In a more recent study by Bozarth and Wise (7), rats displayed an escalating and erratic pattern of cocaine self-administration over days, culminating in death for a significant number of subjects. Although these results were consistent with the previous studies using primates, another point of congruence was that Bozarth and Wise also did not report a circadian rhythm in cocaine self-administration.

Circadian patterns have, however, been recently described in the probability of cocaine self-administration (21,67). In these studies, the experimental protocol was designed to assess the influence of restricted and unrestricted access on cocaine self-administration in rats. Experimental groups were designated in the following manner: a continuous access group had cocaine available for self-injection 24 h a day, while the discrete-trials groups had access to a single intravenous cocaine delivery at one of three frequencies, one, two, or four trials per hour. The authors found that the calculated probability of cocaine self-administration in any given discrete trial did not vary systematically over the 24-h cycle unless subjects were restricted to fewer than four access periods (discrete trials) per hour, or relatively low (0.2 mg/kg/inj) unit doses of cocaine. They interpret this to indicate that binge cocaine self-administration, as might be observed in subjects given unrestricted, continuous access to relatively high individual doses of cocaine, did not display circadian parameters, but that the probability of the initiation of cocaine use under limited access conditions, as observed in the discrete trials procedures, did demonstrate a circadian rhythm.

There are two important differences in the methodologies of the Fitch and Roberts (21) and Roberts and Andrews (67) studies and that of our experiment. First, the experimental parameters under which subjects were given access to cocaine are quite different. In the former studies, rats were given continuous access to cocaine, either unlimited, or limited by the inclusion of timeouts following completion of the required response ratio for drug administration. Subjects in Experiment 1 of the present study were only limited in the amount drug they could self-administer by the time required to complete the response requirements of a fixed-ratio 5 schedule of reinforcement for each cocaine infusion, and by the temporal parameters of the 2-h self-administration session. Second, the earlier studies employed within-subject designs, while the present experiment utilized a between-subjects, independent groups design. This independent groups design was used in an attempt to minimize the influence of continuous cocaine exposure on subsequent rates and patterns of cocaine-maintained responding, and also to simulate the periodic "binge"-

type pattern of cocaine self-administration that is frequently observed in the clinical and experimental literatures.

Temporal variations in the behavioral and toxic effects of many legal and illicit drugs have been described in the chronopharmacology literature. Numerous studies have additionally described time-dependent variability in factors affecting drug absorption, distribution, and pharmacokinetics. There appear to be several factors that may potentially account for a drug having variable effects based solely on the time, within a 24-h cycle, at which it is administered. These include: 1) fluctuation in drug bioavailability related to temporal differences in factors affecting absorption, distribution, metabolism, and elimination, 2) differences in the number, sensitivity, or the efficiency of receptor-effector mechanisms; and 3) differences in the temporal organization of general arousal, learning processes, or behavioral patterns of the organism, and/or their neurobiological substrates (52). Based on the results of Experiment 1, at least one of the above factors, bioavailability, can tentatively be ruled out as a potential explanation for the circadian variability in cocaine's behavioral effects found in the present study. It should be noted, however, that the absence of group-dependent variability in blood levels of cocaine may not necessarily translate into nonsignificant differences in brain cocaine concentrations. Also, the failure to find a statistically significant difference as a function of the time of day of drug administration in the present study may be due to relatively high within-group errors associated with measurements of cocaine plasma levels. Further studies using more sampling periods to provide better temporal resolution should be conducted to conclusively determine whether or not cocaine's pharmacokinetics vary as a function of the time of day. Nevertheless, and in view of these caveats, it may be hypothesized that the observed effects in these experiments relate to circadian variation in either the sensitivity of target systems mediating cocaine's physiological actions, or in the expression of some unique pattern of interaction of cocaine administration with ongoing patterns of biobehavioral activity.

Studies using dopaminergic agonists with actions similar to cocaine have found results that are complementary to those of Experiment 1. Planeta et al. (59), and DeLucia et al. (18) have reported a circadian rhythms in the behavioral and neurophysiological effects of the indirect dopamine agonist, fencamfamine (2-ethylamino-3-phenylnorcamphane). In these studies, fencamfamine administration resulted in a relatively greater increase of general locomotor activity and stereotypic behaviors in rats during the light phase of the L/D cycle. These behavioral effects observed in the light phase were accompanied by relatively greater inhibition of dopamine uptake, and augmented dopamine release in the striatum, as assessed by in vitro experiments (18). The authors interpreted these findings as reflecting a relatively greater susceptibility of dopaminergic presynaptic terminals to the actions of fencamfamine during the light phase. Interestingly, similar circadian rhythms in the neurophysiological effects of amphetamine have been noted; specifically the release of dopamine from striatal synaptosomes is augmented during the light portion of the L/D cycle (2). Although the specific mechanisms mediating differential susceptibility of aminergic neurons to stimulated activity are not known, significant 24-h variability in serotonergic and dopaminergic receptor binding as well as transmitter and metabolite levels has been reported.

In a comprehensive review of the literature on circadian rhythms in mammalian neurotransmitter receptor binding, Wirz-Justice (81) detailed the results of several interesting studies that may hint at potential mechanisms mediating the time-of-day variability in cocaine's behavioral effects observed in the present experiment. Several studies revealed that [^{3}H]spiroperidol and [^{3}H]imipramine binding were maximal, coincident with levels of serotonin (5-HT) and a major metabolite 5-hydroyindoleacetic acid (5-HIAA), in the early portion of the dark phase, from approximately 1900–2200 h, while values of all these markers of serotonergic function were minimal at approximately midway through the light phase (81). Other reports have indicated that dopaminergic receptor binding also shows variation across the L/D cycle, but the pattern has not been characterized as circadian. Rather, the 24-h rhythm is bimodal, with peaks occurring approximately midway through both light and dark phases (39,51). Interestingly, this bimodal pattern of dopamine receptor binding is mirrored by changes in absolute levels of dopamine in various brain regions, including those that have been strongly implicated in the reinforcing properties of cocaine (40,72,73,77).

The circadian rhythms in rat brain dopaminergic receptors (36,53) and dopamine neurotransmitter levels (40,72,73,77), with peaks occurring midway through the light and dark phases of the L/D cycle, appear to correspond fairly well with the patterns of cocaine self-administration observed in Experiment 1. Additionally, the pattern of circadian variation in parameters of serotonergic function relative to that of cocaine self-administration observed in Experiment 1 suggests an inverse relationship between serotonergic activity and the sensitivity of subjects to the reinforcing properties of cocaine. The peak in cocaine self-administration at the 0.056 mg/kg/inj dose occurred at 1300 h, or approximately midway through the light phase of the L/D cycle, which is the same time at which Wirz-Justice (81) and others (45) reported nadirs for multiple indices of serotonergic function previously mentioned. This result is intriguing in light of accumulating evidence that augmentation of serotonergic function results in decreases in rates of cocaine self-administration, and breaking points for cocaine self-administration on progressive ratio schedules, while depletion or antagonism of serotonin function appears to have the opposite effect of augmenting cocaine self-administration on fixed-ratio schedules, and increasing breaking points for cocaine on progressive ratio schedules (14, 15,33,58,68). Although this conceptual scheme is somewhat simplistic, and a more complex picture appears to be emerging [e.g., (78)], these results appear to indicate that the chronopharmacologic effects of cocaine described herein may be significantly related to 24-h variability in the functional characteristics of endogenous aminergic systems.

In light of the previous discussion of circadian and ultradian rhythms in functional properties of monoaminergic systems, it should be noted that interactions between cocaine administration and circadian rhythms may be reciprocal. Just as the sensitivity of an organism to a constant level of drug may vary over a 24-h cycle, drugs also can shift biological rhythms in physiology and behavior. The observed time-of-day group differences in the propensity for low-dose cocaine self-administration, therefore, may be related to systematic variations in drug sensitivity over the 24-h cycle (assuming uniform entrainment of circadian rhythms across experimental groups), or differential entrainment in the functions of underlying biological or behavioral mechanisms.

In conclusion, the current project has demonstrated time-of-day differences in cocaine reinforced behavior, the parameters of which are apparently not the result of coincident variations in cocaine's pharmacokinetic profile. Inasmuch as rodent models of drug self-administration behavior may be

generalized across species, these findings suggest that cocaine's reinforcing and other effects may likewise be heterogeneously expressed across the 24-h cycle in humans. At least one relatively recent report provides some support for this hypothesis. Upon retrospective examination of hospital emergency department records, Raymond et al. (61) found that cases of opiate, ethanol, phencyclidine, benzodiazepine, and cocaine abuse and/or overdose presented with significant ultradian, circadian, and circannual periodicities. Most interesting was the finding that cocaine overdoses presented consistently at 0700 and 1900 h, in a bimodal rhythm opposite to the pattern of sensitivity to cocaine reinforcement observed in the present study, and in previous studies examining functional aspects of the rat dopaminergic system (40,72,73). The possibility that diurnal humans may display essentially a "mirror-image" pattern of sensitivity to cocaine's effects, relative to that of the nocturnal rat is intriguing, and should be further explored, as it would likely prove informative with respect to our understanding of temporal factors mediating the expression of drug consummatory behaviors among different species.

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