

Choice Between Heroin and Food in Non-Dependent and Heroin-Dependent Rhesus Monkeys: Effects of Naloxone, Buprenorphine and Methadone

S. Stevens Negus

McLean Hospital-Harvard Medical School

Running Title: Heroin vs. food choice in rhesus monkeys

Corresponding Author:

S. Stevens Negus

Alcohol and Drug Abuse Research Center

Harvard Medical School; McLean Hospital

115 Mill St.

Belmont, MA 02178-9106

Phone: (617)855-3324

FAX: (617)855-2519

email: negus@mclean.harvard.edu

Number of Text Pages:

Number of Tables: 2

Number of Figures: 9

Number of References: 37

Number of Words in Abstract: 250

Number of Words in Introduction: 734 (excluding references)

Number of Words in Discussion: 1500 (excluding references)

Abbreviations: FR (fixed ratio), Fixed Interval (FI)

Recommended Section Assignment: Behavioral Pharmacology

ABSTRACT

Several medications are approved for treatment of opiate abuse, but determinants of their clinical effectiveness are not completely understood. States of opiate dependence or withdrawal may constitute one important set of determinants. To test this hypothesis, the effects of naloxone, buprenorphine and methadone were assessed on choice between heroin and food in non-dependent rhesus monkeys and in heroin-dependent monkeys undergoing withdrawal. A choice procedure was used to permit dissociation of medication effects on the relative reinforcing properties of heroin from non-selective effects on response rates. In non-dependent monkeys, increasing unit doses of heroin (0-0.1 mg/kg/injection) maintained dose-dependent increases in heroin choice. Chronic 5-day treatment with naloxone (0.01-0.32 mg/kg/hr) or buprenorphine (0.01-0.1 mg/kg/day) produced dose-dependent rightward shifts in heroin choice dose-effect curves, whereas chronic methadone (0.1-0.56 mg/kg/hr) had little effect on heroin choice up to doses that suppressed responding. In heroin-dependent monkeys, opiate withdrawal produced overt abstinence signs as well as increases in heroin choice, manifested as leftward shifts in heroin choice dose-effect curves. The withdrawal-associated increases in heroin choice suggest that opiate withdrawal increased the relative reinforcing efficacy of heroin in comparison to food—an effect that may be related to relapse in humans. Methadone prevented withdrawal-associated increases in heroin choice, whereas buprenorphine was less effective. These findings suggest that agonist medications such as methadone may derive their clinical utility from their ability to attenuate withdrawal-associated increases in opiate reinforcement. Moreover, this procedure may be useful for exploring mechanisms underlying withdrawal-associated increases in opiate reinforcement and for testing candidate medications.

INTRODUCTION

Opiates, including heroin and many prescription analgesics, constitute one major class of abused drugs, and medications approved for the treatment of opiate abuse and dependence include the high-efficacy mu opioid receptor agonist methadone, the intermediate-efficacy mu agonist buprenorphine, and the mu antagonist naltrexone (Greenstein et al., 1997; Lowinson et al., 1997; Gonzalez et al., 2004). Each of these medications has proven effective under at least some conditions; however, the environmental, pharmacological and biological determinants of their clinical effectiveness are not fully understood. Research on this topic contributes not only to the refinement of treatments for opiate abuse, but also to the evolution of conceptual frameworks that guide strategies to treat other types of addictive behaviors. For example, methadone is considered to be a prototype “agonist” medication, and experience with methadone in the treatment of opiate abuse has helped guide the development of other “agonist” medications, including nicotine formulations for the treatment of nicotine dependence or amphetamine for the treatment of stimulant abuse (Garrett et al., 2001; Grabowski et al., 2004).

One common approach to the preclinical assessment of drug abuse medications has been to train subjects to self-administer a target drug of abuse (e.g. heroin), and then to evaluate the ability of candidate medications to alter rates of drug self-administration (Mello and Negus, 1996). Acute or chronic treatment with opiate antagonists or with buprenorphine has consistently been found to produce rightward and/or downward shifts in opiate self-administration dose-effect curves in non-dependent subjects at doses that produce little evidence of toxicity (Harrigan and Downs, 1978; Harrigan and Downs, 1981; Mello et al., 1983; Winger et al., 1992; Negus et al., 1993; Winger and Woods, 1996; Mello and Negus, 1998). These results are consistent with the interpretation that these and related medications alter opiate self-administration by binding to mu opioid receptors and antagonizing the reinforcing effects of self-administered opiate agonists. Moreover, these findings are consistent with the apparent clinical effectiveness of naltrexone or buprenorphine under conditions in which compliance with the medications can be maintained (Greenstein et al., 1997; Gonzalez et al., 2004). However, there

is less concordance between the preclinical and clinical effects of methadone. Clinical evidence indicates that methadone maintenance can be extremely effective in reducing illicit opiate use by opiate abusers (Lowinson et al., 1997; Gonzalez et al., 2004). However, in preclinical studies, methadone either failed to reduce opiate self-administration (Mello et al., 1983) or reduced rates of opiate self-administration only transiently and/or only at doses that also reduced responding maintained by other reinforcers and produced other evidence of toxicity (Jones and Prada, 1977; Harrigan and Downs, 1981). This discrepancy suggests that standard preclinical approaches to medications evaluation may be insensitive to important attributes of methadone that contribute to its clinical utility.

One factor that may be important is the state of opiate dependence and/or withdrawal. In the preclinical studies cited above, methadone was tested in subjects that had limited daily access to opiate self-administration, and these subjects were unlikely to be in states of significant opiate dependence. However, the existence of opiate dependence, verified by spontaneous or antagonist-precipitated withdrawal, is typically a prerequisite for the clinical use of agonist medications such as methadone (Lowinson et al., 1997). Consequently, preclinical assessment of agonist medications might benefit from inclusion of studies conducted in opiate-dependent subjects. With regard to this possibility, several preclinical studies have found that opiate dependence, and in particular opiate withdrawal, may be associated with increased rates of opiate-maintained responding under a chain schedule (Thompson and Schuster, 1964), increased breakpoints maintained by opiate delivery under progressive-ratio schedules (Yanagita, 1978; Carrera et al., 1999), or increased opiate vs. food choice under concurrent-choice schedules (Spragg, 1940; Griffiths et al., 1975). Moreover, morphine and/or methadone reduced withdrawal-associated increases in opiate-maintained responding (Spragg, 1940; Thompson and Schuster, 1964; Griffiths et al., 1975; Griffiths et al., 1981). Taken together, these findings are consistent with the hypotheses that (a) opiate withdrawal increases the reinforcing effects of opiate agonists, and (b) the clinical utility of agonist medications may be predicted not by their effects on basal opiate self-administration in non-dependent subjects, but rather by their ability to

reduce withdrawal-associated increases in opiate-maintained responding in opiate-dependent subjects.

The purpose of the present study was to further evaluate these hypotheses. Specifically, the effects of naloxone, buprenorphine and methadone on heroin self-administration were evaluated in non-dependent rhesus monkeys, and in heroin-dependent rhesus monkeys undergoing opiate withdrawal. Studies were conducted using a concurrent-choice procedure in which rhesus monkeys could respond for either heroin or food. A choice procedure was used because the primary dependent variable is response allocation rather than response rate, and it has been argued that such choice-based measures permit a dissociation between medication effects on the relative reinforcing efficacy of the self-administered drug and medication effects that may compromise the subject's ability to respond (e.g. sedation) (Griffiths et al., 1975; Johanson, 1975; Woolverton and Balster, 1981; Negus, 2003). Moreover, previous studies using choice procedures have provided the most compelling evidence to date to suggest that opiate agonists may reduce opiate self-administration in a manner consistent with the clinical utility of agonist medications (Spragg, 1940; Griffiths et al., 1975; Wurster et al., 1977; Griffiths et al., 1981). The results of the present study provide additional evidence to suggest that opiate withdrawal increases the relative reinforcing effects of opiate agonists, and that agonist medications such as methadone attenuate withdrawal-associated increases in opiate reinforcement.

METHODS

Animals

Studies were conducted in 3 adult male rhesus monkeys (*Macaca mulatta*) that had been surgically implanted with double lumen catheters using aseptic procedures as described previously (Negus and Mello, 2004). All monkeys had prior exposure to heroin and to the behavioral procedures, but none of the monkeys had received sufficient doses of heroin to produce signs of dependence. Monkeys weighed 6.7-7.5 kg and were maintained on a diet of multiple vitamins, fresh fruit and food biscuits (Lab Diet Jumbo Monkey Biscuits, PMI Feeds, Inc., St. Louis, MO). Biscuits and vitamins were provided in the afternoon between 1 and 2 pm (i.e. within 1 hr after each daily session), and fruit was provided daily between 4 and 5 p.m. In addition, monkeys received up to 50 1 gm banana flavored pellets (Precision Primate Pellets Formula L/I Banana Flavor, P. J. Noyes Co., Lancaster, NH) during daily operant sessions (see below). Water was continuously available. A 12 hr light-dark cycle was in effect (lights on from 7 a.m. to 7 p.m.).

Animal maintenance and research were conducted in accordance with the guidelines provided by the NIH Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was periodically monitored by consulting veterinarians. Monkeys had visual, auditory and olfactory contact with other monkeys throughout the study. Operant procedures and foraging toys provided opportunities for environmental manipulation and enrichment. Music or nature video tapes were also played daily in animal housing rooms to provide additional environmental enrichment.

Apparatus and Catheter Maintenance

Experimental sessions were conducted in each monkey's home cage. The front wall was equipped with an operant response panel (28 x 28 cm²) that included three circular response keys (5.1 cm in diameter) arranged 2.5 cm apart horizontally. Each key could be illuminated by red,

green or yellow stimulus lights. Each housing chamber was also equipped with a pellet dispenser (Gerbrands, Model G5210, Arlington, MA) and two syringe pumps (Model BSP-IE, Braintree Scientific, Braintree, MA; or Model PHM-100, Med Associates Inc., St. Albans, VT), one for each lumen of the double lumen catheter. One syringe pump was used to deliver self-administered heroin injections through one lumen of the double-lumen catheter (the “drug self-administration lumen”). The second syringe pump was used to deliver saline or treatment drugs through the second lumen of the catheter (the “treatment lumen”). This second pump was programmed to deliver 0.1 ml infusions every 20 min from 10:00 a.m. each day until 9:00 a.m. the next morning. Operation of the operant response panels and data collection were accomplished with microprocessors and software purchased from Med Associates Inc. (St. Albans, VT). The intravenous catheter was protected by a tether system consisting of a custom-fitted nylon vest connected to a flexible stainless steel cable and fluid swivel (Lomir Biomedical, Malone, NY). This flexible tether system permitted monkeys to move freely in the cage. Catheter patency was periodically evaluated by i.v. administration of ketamine (5 mg/kg) or the short-acting barbiturate methohexital (3 mg/kg) through the catheter lumen. The catheter was considered to be patent if i.v. administration of ketamine or methohexital produced a loss of muscle tone within 10 sec.

Behavioral Procedures

Training Procedures: Behavioral sessions were conducted 7 days a week from 11 a.m.-1 p.m. as described previously (Negus, 2005; Stevenson et al., 2005). Following initial shaping of key press responding for food reinforcement (1 gm food pellets) and drug injections (0.1 mg/kg/injection heroin), choice training was initiated. The terminal choice schedule consisted of five 20-min response periods separated by 5 min timeout periods (total session duration of 120 min). During each response period, the left, food-associated key was illuminated with red stimulus lights, and completion of the fixed-ratio (FR) requirement resulted in the delivery of a food pellet. The right, heroin-associated key was illuminated with yellow stimulus lights, and

completion of the FR requirement on this key resulted in the delivery of a heroin dose. A different heroin dose was available during each of the five successive response periods (0, 0.0032, 0.01, 0.032 and 0.1 mg/kg/injection during response periods 1-5, respectively), and dose was varied by varying the duration of pump activation and the resulting volume of each injection. Stimulus conditions on the drug-associated key were also varied by flashing the stimulus lights on and off in 3 sec cycles. (Response Period 1: 0 sec on, 3 sec off; Response Period 2: 0.1 sec on, 2.9 sec off; Response Period 3: 0.3 sec on, 2.7 sec off; Response Period 4: 1 sec on, 2 sec off; Response Period 5: 3 sec on, 0 sec off). Thus, longer flashes (and shorter inter-flash intervals) were associated with higher available drug doses. The response requirements were set at FR 100 on the food-associated key and FR 10 on the heroin-associated key for all monkeys, because our previous studies indicated that under these response requirements, monkeys usually switched from the food-associated key to the drug-associated key during the third response period, when an intermediate unit dose of 0.032 mg/kg/injection heroin was available (Negus, 2005). Consequently, it was possible to observe both leftward and rightward shifts in the heroin choice dose-effect curves that might result from manipulation of experimental variables.

During each response period, monkeys could complete up to 10 total ratio requirements on the food- and heroin-associated keys. Responding on either key reset the ratio requirement on the other key. Completion of each ratio requirement initiated a 30 sec timeout, during which all stimulus lights were turned off, and responding had no scheduled consequences. During response periods when the drug-associated key was not illuminated and a “0” dose of drug was available, responses on this key were still recorded, they still reset the FR requirement on the food-associated key, and completion of the FR requirement still counted as one of the 10 allotted ratios and initiated a 30 sec timeout. If all 10 ratio requirements were completed before the 20 min response period had elapsed, then all stimulus lights were extinguished and responding had no scheduled consequences for the remainder of that 20-min response period. Choice training

was considered to be complete when the ED₅₀ value of the dose-effect curve for heroin choice (see Data Analysis) varied by less than 2-fold for three consecutive days.

Testing Procedures: Once training was completed, testing began. Testing was conducted in two phases. In the first phase, two monkeys were tested under conditions in which heroin injections were available only during the daily choice session as described above. Under these conditions, monkeys self-administered approximately 0.6 mg/kg/day heroin, and withdrawal signs were not evident either between daily sessions or after unintended interruption of heroin access (e.g. due to problems with equipment or catheters). Accordingly, this condition will be referred to as the “non-dependent” condition. (It should also be noted that the term “non-dependent” is meant to describe the state of the subjects at the beginning of treatment with medications. As will be discussed below, high doses of buprenorphine and methadone did produce signs of dependence after 5 days of treatment). Choice between heroin and food was evaluated during treatment with naloxone (0.01-0.32 mg/kg/hr), buprenorphine (0.01-0.1 mg/kg/day), and methadone (0.1-0.56 mg/kg/hr). Methadone was tested first, followed by naloxone and buprenorphine. Doses of each drug were tested in a mixed order, and in general, studies with one drug were completed before studies with another drug were begun. The effects of each dose of each treatment drug were evaluated for a period of 5 consecutive sessions. Naloxone and methadone were substituted for saline treatment at 2 pm on the first day of treatment (i.e. 1 hr after the most recent choice session and 21 hr before the next choice session), and 0.1 ml infusions of the test drug were automatically delivered by the syringe pump every 20 min for the next 5 days (except from 9-10am each morning, when data were downloaded and syringes and equipment were checked). The long-acting mu opioid receptor partial agonist buprenorphine was delivered once daily by bolus intravenous infusions. The first infusion was delivered at approximately 2 pm on the first day of treatment, and subsequent injections were delivered each morning at 9am. Doses for each drug were based on preliminary results and on previously published studies (Mello and Negus, 1998; Negus and Mello, 2004). The 5-day duration of each treatment was also based on preliminary studies and on previous studies, which

found that shifts in drug vs. food choice produced by pharmacological or environmental manipulations typically emerge over a period of 2-3 days and stabilize after approximately 3 days (Negus, 2003; Negus, 2005; Stevenson et al., 2005).

At the conclusion of each 5-day treatment period, baseline conditions of saline treatment were reinstated for a period of at least 4 days and until the choice dose-effect curve recovered to its baseline position. The mean (range) of intertest intervals was 11.8 (4-26) days for one monkey and 10.2 (4-28) days for the second monkey.

During these initial studies, it became apparent that chronic treatment with high doses of methadone, and to a lesser extent buprenorphine, produced dependence as indicated by the emergence of opioid withdrawal signs upon termination of treatment (e.g. lying on bottom of cage, emesis, vocalization). Moreover, these withdrawal signs were associated with transient but robust increases in heroin vs. food choice. Both withdrawal signs and withdrawal-associated increases in heroin choice could be attenuated by resuming methadone treatment. To investigate these phenomena further, the second phase of the study was initiated, and a third monkey was added to the group. During the second phase of the study, heroin was available both during the daily choice session, and during a supplemental session that began daily at 1 pm (i.e. immediately after the choice session) and concluded the next morning at 10 am. During this 21-hr supplemental session, the heroin-associated key was illuminated with yellow lights, and heroin (0.1 mg/kg/injection) was available under a FR 10/time out 15 min schedule. Thus, in addition to heroin available during the daily choice sessions, monkeys could also self-administer a maximum of 84 injections for a maximum intake of 8.4 mg/kg heroin during the supplemental sessions.

After at least seven consecutive days, access to supplemental heroin was terminated, and both choice behavior and overt withdrawal signs were examined for seven days. Eight withdrawal signs were counted as present or absent each day, and the total number of withdrawal signs expressed each day were counted to yield a Withdrawal Score (i.e. the maximum Withdrawal Score was "8"). The eight signs were lying on bottom of cage, unusually aggressive

or lethargic response to investigator, increased vocalization, retching/emesis, diarrhea, penile erection/masturbation, tremor/convulsion, and a category for other unusual behaviors.

Termination of access to supplemental heroin produced both increases in withdrawal scores and withdrawal-associated increases in heroin choice (see Results). Accordingly, this condition will be referred to as the “dependent” condition.

Follow-up tests were then conducted to assess the ability of the highest doses of methadone (0.56 mg/kg/hr) and buprenorphine (0.1 mg/kg/day) to attenuate withdrawal signs and withdrawal-associated increases in heroin choice in heroin-dependent monkeys. For these follow-up tests, the supplemental heroin sessions were reinstated for at least 7 days. Subsequently, the supplemental heroin session was omitted for one day, and non-contingent treatment with methadone or buprenorphine was introduced for that day. Access to supplemental heroin was then reinstated for at least 3 days. Methadone was tested first, and it was administered by i.v. injections every 20 min, with treatment beginning at 1 pm and continuing for 24 hr (with an interruption from 9-10 am as described above). Buprenorphine was tested next, 3-14 days after the methadone test. Buprenorphine was administered by bolus i.v. injections at 2 pm and 9 am. At the conclusion of the test period, supplemental heroin sessions were resumed. Buprenorphine (0.1 mg/kg/day) did not prevent withdrawal, so a higher buprenorphine dose (0.32 mg/kg/day) was also tested. This second test occurred 1 month after the first test in one monkey, and three months after the first test in the other two monkeys. Throughout this period of access to supplemental heroin, variations in heroin choice were modest and without trends, withdrawal (either spontaneous or precipitated by an antagonist) reliably produced increases in heroin choice, and treatment with opioid agonists (e.g. methadone,) reliably eliminated withdrawal. Because of the intensity of withdrawal signs observed during buprenorphine treatment, the effects of naloxone in dependent monkeys were not examined. The effects of methadone were replicated at the conclusion of the study (data not shown).

Data Analysis

The primary dependent variables for each response period were (1) percent heroin choice, defined as (number ratios completed on the heroin-associated key ÷ total number of ratios completed)*100, and (2) response rate, defined as the total number of responses ÷ total time responses had scheduled consequences. These variables were then plotted as a function of heroin dose. The ED50 value of the heroin choice dose-effect curve was defined as the dose of heroin that produced 50% heroin choice. ED50 values were calculated by interpolation when only two data points were available (one below and one above 50% heroin choice) or by linear regression when at least three data points were available on the linear portion of the dose-effect curve. In some cases, ED50 values could not be determined because percent heroin choice was above or below 50% for all heroin doses, and these instances are specified in text and tables.

In the first phase of the study, log ED50 values were calculated for individual monkeys during the last three days of each five-day test period, and these individual data were averaged to yield mean log ED50 values (\pm 95% confidence limits). In the second phase of the study, log ED50 values were calculated for each monkey during the last three days of access to supplemental heroin, and during each day after termination of access to supplemental heroin. Log ED50 values were considered to be significantly different if 95% confidence limits did not overlap and log ED50 values and confidence limits were converted to linear values for presentation in tables.

Additional dependent variables collected during each session included total food choices, total heroin choices, total choices, total heroin intake, and (during the second phase of the study) withdrawal score. Values were compared by one-factor ANOVA, with treatment condition as a within-subjects factor, and a significant ANOVA was followed by the Duncan *post hoc* test to compare test conditions with control conditions. The criterion for significance was set at $p < 0.05$.

Drugs

Heroin HCl, buprenorphine HCl and (dl) methadone HCl (National Institute on Drug Abuse, Bethesda, MD) and naloxone HCl (Sigma Chemical Co., St. Louis, MO) were dissolved in sterile water and filter-sterilized using a 0.22 micron Millipore filter. Doses were calculated using the salt forms of the drugs given above.

RESULTS

Baseline choice between heroin and food in non-dependent monkeys. Figures 1-3 (open symbols and open bars) show baseline patterns of choice between heroin and food under the non-dependent condition in two monkeys. When low heroin doses were available (0 or 0.0032 mg/kg/inj), monkeys responded almost exclusively on the food-associated key at rates of approximately 3 responses/sec. Similar high rates of responding were observed during availability of an intermediate dose of 0.01 mg/kg/inj heroin. At this heroin dose, responding on both the food- and heroin-associated keys was observed, although on any given day in a given monkey, responding was usually allocated quantally to either the food- or heroin-associated key. When high heroin doses were available (0.032-0.1 mg/kg/inj) responding was allocated almost exclusively to the heroin-associated key. Choice of the highest dose of heroin (0.1 mg/kg/inj) was also associated with a decrease in response rates to less than 1 response/sec. The baseline ED50 of the heroin choice dose-effect curve is shown in Table 1. ED50 values for heroin choice in non-dependent animals ranged from 0.006-0.018 mg/kg/inj both within and between cycles and across monkeys.

Monkeys completed the response requirements for an average (\pm SEM) of 42.3 (\pm 1.9) total choices out of a maximum of 50 choices. This reflected completion of response requirements for all 10 available choices during availability of 0, 0.0032, and 0.01 mg/kg/inj heroin, 9.2 ± 0.7 choices during availability of 0.032 mg/kg/inj heroin, and 3.1 ± 1.2 choices during availability of 0.1 mg/kg/inj heroin. Thus, choice of the highest heroin dose was associated with a decrease in both response rates and total reinforcers delivered. Overall, monkeys earned 25.4 ± 2.5 food pellets and 16.8 ± 4.4 heroin injections per day, and total heroin intake averaged 0.64 ± 0.16 mg/kg/day.

Effects of naloxone, buprenorphine and methadone on heroin choice in non-dependent monkeys. Figure 1 and Table 1 show the effects of chronic 5-day naloxone treatment on heroin choice in non-dependent monkeys. Naloxone produced dose-dependent rightward shifts in the

heroin choice dose-effect curve and significantly increased the heroin choice ED50 value. In addition, there was a naloxone dose-dependent increase in response rates during availability of the highest dose of 0.1 mg/kg/inj heroin. Naloxone significantly increased total choices and food choices. Naloxone also decreased the number of heroin choices in both monkeys (from 21.2 and 12.4 to 12.0 and 4.2, respectively), but this effect did not achieve statistical significance. Finally, intermediate doses of naloxone (0.01-0.1 mg/kg/hr) increased heroin intake in both monkeys (from 0.81 and 0.48 mg/kg/day to highs of 1.2 and 1.0 mg/kg/day, respectively), although again, this effect did not achieve statistical significance. Note that naloxone simultaneously decreased the total number of heroin injections but increased heroin intake. This occurred because intermediate doses of naloxone decreased the number of injections of lower heroin doses (0.01-0.032 mg/kg/inj; which contributed relatively less to total intake), but increased the number of injections of the highest heroin dose (0.1 mg/kg/inj; which contributed relatively more to heroin intake). For example, 0.1 mg/kg/hr naloxone decreased intake of 0.032 mg/kg/inj heroin from a mean of 9.2 injections (mean intake=0.29 mg/kg) to 4.3 injections (mean intake=0.14 mg/kg). However, the same dose of naloxone increased intake of 0.1 mg/kg/inj heroin from a mean of 3.1 injections (mean intake=0.31 mg/kg) to 8.3 injections (mean intake=0.83 mg/kg).

Figure 2 and Table 1 show the effects of buprenorphine treatment. As with naloxone, buprenorphine produced dose-dependent rightward shifts in the heroin choice dose-effect curve, increased the heroin choice ED50 value, and increased response rates during availability of the highest dose of 0.1 mg/kg/inj heroin. Also like naloxone, buprenorphine increased total choices and food choices while tending to decrease heroin choices, and an intermediate dose of buprenorphine (0.032 mg/kg/day) significantly increased heroin intake.

Figure 3 and Table 1 show the effects of methadone treatment. Methadone doses up to 0.56 mg/kg/hr had little or no effect on the heroin choice dose-effect curve, the heroin choice ED50 value, the number of heroin choices or total heroin intake. There was a trend for the higher doses of methadone to decrease rates of responding and total reinforcers, and 0.32

mg/kg/hr methadone significantly decreased the number of food choices per session. Higher doses of methadone were not tested because (a) higher doses produced severe sedation in our previous studies (Negus and Mello, 2004), and (b) termination of treatment with 0.56 mg/kg/hr methadone produced withdrawal signs (see below).

Although withdrawal signs were not formally monitored or recorded during this phase of the study, monkeys were observed multiple times daily by veterinary and technical staff for deviations in normal behavior or appearance. Notably, these observations did not identify the presence of opiate withdrawal signs between daily sessions, after unintended interruption of heroin access, or during treatment with naloxone or buprenorphine. This contrasts with the unambiguous appearance of withdrawal signs after termination of treatment with high doses of methadone (see below).

Effects of termination of naloxone, buprenorphine and methadone treatment on heroin choice in non-dependent monkeys. Figure 4 and Table 1 show parameters of heroin choice after termination of treatment with the highest doses of naloxone, buprenorphine and methadone. Termination of treatment with 0.32 mg/kg/hr naloxone resulted in a gradual recovery of the heroin choice dose-effect curve over a period of 7 days, and data from days 1, 3 and 7 after naloxone are shown to illustrate this effect. The number of total choices, food choices and heroin choices also recovered to baseline levels in 7 days.

A much different pattern emerged after termination of 0.1 mg/kg/day buprenorphine treatment. On day 1 after buprenorphine treatment, there was a slight recovery toward baseline of the heroin choice dose-effect curve and of the numbers of total choices, food choices and heroin choices. However, over a period of approximately 3-7 days after termination of buprenorphine treatment, there was a large leftward shift in the heroin choice dose-effect curve, and a significant decrease in the heroin ED₅₀ value relative to baseline. Moreover, both monkeys completed the response requirement on the heroin-associated key even during the first cycle of the choice session, when the heroin-associated key was not illuminated, and no heroin

was available. Data from day 5 after termination of buprenorphine treatment are shown to illustrate these effects. The reallocation of responding was also reflected by a significant decrease in the number of food choices and a significant increase in the number of heroin choices. The heroin choice dose-effect curve recovered to its baseline position after 10 days. Heroin intake remained relatively constant after termination of buprenorphine treatment at 0.6-0.7 mg/kg (data not shown), because the greatest changes in heroin choice occurred during availability of low heroin doses that contributed little to overall intake.

A similar pattern of changes in heroin choice occurred with a different time course after termination of treatment with 0.56 mg/kg/hr methadone, and data from days 1, 3 and 7 after methadone treatment are shown. One day after termination of methadone treatment, the heroin choice dose-effect curve shifted approximately one-log unit to the left, and there was a significant decrease in the heroin choice ED₅₀ value. One monkey completed response requirements on the heroin-associated key during the first cycle of the choice session, when the heroin-associated key was not illuminated, and no heroin was available. The other monkey did this on day 2 after methadone (data not shown). There was also a significant reduction in food choices and a significant increase in heroin choices. The heroin choice dose-effect curve gradually recovered to its baseline position after 7 days, as did the numbers of food and heroin choices. Heroin intake remained relatively constant after termination of methadone treatment at 0.7-0.8 mg/kg (data not shown), because the greatest changes in heroin choice occurred during availability of low heroin doses that contributed little to overall intake.

Increases in heroin choice observed after termination of high-dose treatment with methadone, and to a lesser extent buprenorphine, were also associated with signs of opiate withdrawal (e.g. lying on bottom of cage, emesis). At the time these studies were conducted, signs of opiate withdrawal were not formally monitored and scored, and as a result, the magnitude of withdrawal scores cannot be compared. However, opiate withdrawal signs were formally scored in the next phase of the study.

Induction of heroin dependence and effects of heroin withdrawal. Heroin dependence was induced by introducing a supplemental daily session of heroin self-administration in addition to the heroin vs. food choice session. During supplemental heroin access, heroin injections (0.1 mg/kg/inj) were available under a FR 10/TO 15 min schedule from 1pm each day until 10am the next morning. Figure 5 shows patterns of heroin self-administration during access to the supplemental heroin self-administration. For the purposes of data presentation, the 21 hr session was divided into quintiles. Monkeys responded for heroin throughout the supplemental session, with generally lower rates of self-administration during the 3rd and 4th quintiles (i.e. from approximately 9:30 pm until 6:00 am). Figure 5 also shows total daily heroin intake during access to supplemental heroin. On average, the three monkeys in this phase of the study self-administered a total of 5.0 ± 0.3 mg/kg/day heroin (1.1 ± 0.1 mg/kg/day during the choice session and 3.9 ± 0.3 mg/kg/day during the supplemental session).

Figure 6 and Table 2 show parameters of choice between heroin and food before access to supplemental heroin, during access to supplemental heroin, and during the first day after termination of access to supplemental heroin. Prior to access to supplemental heroin, the heroin choice dose-effect curve and other parameters of heroin vs. food choice were similar to those described above. Introduction of access to supplemental heroin decreased response rates but had little effect on the heroin choice dose-effect curve and did not significantly alter the heroin choice ED50 value. Supplemental heroin also did not significantly alter total choices, food choices, heroin choices or heroin intake. However, total choices and food choices decreased in two of three monkeys, and it is notable that heroin intake increased in all three monkeys during supplemental heroin. In the last cycle of the heroin choice sessions, when the highest dose of heroin was available, the number of self-administered heroin injections increased significantly from 3.2 ± 1.1 per session before supplemental heroin to 8.8 ± 0.5 per session during access to supplemental heroin ($p < 0.05$).

On the first day after termination of access to supplemental heroin, there was a leftward shift in the heroin choice dose-effect curve and a significant decrease in the heroin ED50 value.

One monkey responded exclusively on the heroin-associated key even during the first cycle, when the heroin-associated key was not illuminated, and no heroin was available (the other two monkeys did this on the second day after termination of supplemental heroin). The number of food choices decreased in all three monkeys, and although this effect did not achieve statistical significance, two monkeys did not respond for any food pellets on the first day after termination of supplemental heroin. Figure 7 shows a time course for low-dose (0.0032 mg/kg/inj) heroin choice and overt withdrawal signs during the 7 days after termination of access to supplemental heroin. Prior to and during access to supplemental heroin, monkeys never chose 0.0032 mg/kg/inj heroin over food, and no withdrawal signs were observed. For the first three days after termination of access to supplemental heroin, mean choice for 0.0032 mg/kg/inj heroin ranged from 67-75%, and mean withdrawal scores ranged from 2.7-4.3 (out of a maximum of 8). The most commonly observed withdrawal signs were lying on bottom of cage, vocalizations, heightened aggression, and emesis. Both choice of 0.0032 mg/kg/inj heroin and withdrawal scores gradually declined over seven days.

Effects of methadone and buprenorphine on heroin withdrawal. Figure 8 and Table 2 show the effects of methadone (0.56 mg/kg/hr) on parameters of heroin choice after termination of access to supplemental heroin. Methadone completely prevented the emergence of withdrawal signs (mean withdrawal score \pm SEM = 0.3 ± 0.3) and the withdrawal-associated increase in heroin choice. Moreover, response rates that had been suppressed during access to supplemental heroin recovered to rates similar to those observed prior to introduction of supplemental heroin. Methadone did not significantly alter total choices, food choices or heroin choices relative to values observed during access to supplemental heroin. However, as noted above, supplemental heroin decreased total choices and food choices in two of three monkeys, and termination of supplemental heroin coupled with methadone treatment resulted in a recovery

of both total choices and food choices in these monkeys. Heroin intake during the choice session decreased significantly during methadone treatment.

In contrast to the effects of methadone, buprenorphine (0.1 and 0.32 mg/kg/day) did not completely prevent the emergence of withdrawal signs or withdrawal-associated increases in heroin choice. Withdrawal scores \pm SEM were 3.7 ± 0.9 during treatment with 0.1 mg/kg/day buprenorphine and 2.7 ± 0.3 during treatment with 0.32 mg/kg/day buprenorphine. Figure 9 and Table 2 show parameters of heroin choice during buprenorphine treatment. Left shifts in the heroin dose-effect curve and decreases in the heroin choice ED₅₀ values were observed in two of the three monkeys during treatment with each buprenorphine dose. Termination of supplemental heroin sessions in combination with buprenorphine treatment did not significantly alter total choices, food choices, heroin choices or heroin intake; however, one or both doses of buprenorphine decreased food choices and increased heroin choices and heroin intake in every monkey. Higher buprenorphine doses were not tested due to the low response rates observed after treatment with 0.32 mg/kg/day buprenorphine.

DISCUSSION

The present study compared the effects of naloxone, buprenorphine and methadone on choice between heroin and food in non-dependent rhesus monkeys and in heroin-dependent monkeys undergoing withdrawal. In non-dependent monkeys, naloxone and buprenorphine produced rightward shifts in heroin choice dose-effect curves, whereas methadone had no effect. In dependent monkeys undergoing spontaneous opiate withdrawal, methadone prevented the emergence of both overt withdrawal signs and withdrawal-associated leftward shifts in the heroin choice dose-effect curves, whereas buprenorphine was less effective and naloxone was not tested due to concerns about the severity of precipitated withdrawal. These results support the hypothesis that opiate withdrawal may increase the reinforcing effects of opiate agonists, and that agonist medications such as methadone may derive their clinical utility from their ability to attenuate withdrawal-associated increases in the reinforcing effects of opiate agonists. In addition, these results demonstrate that choice procedures may be especially useful as a tool to investigate mechanisms underlying withdrawal-associated increases in opiate reinforcement.

Drug effects on heroin choice in initially non-dependent monkeys. The baseline dose-effect curve for heroin choice in non-dependent monkeys was similar to that described previously for monkeys responding under this procedure (Negus, 2005; Stevenson et al., 2005). Chronic 5-day treatment with naloxone or buprenorphine produced dose-dependent rightward/downward shifts in the heroin choice dose-effect curves in non-dependent monkeys. These results agree with many previous studies that opiate antagonists and buprenorphine produce rightward and/or downward shifts in mu opiate agonist self-administration dose-effect curves under other schedules of reinforcement (Harrigan and Downs, 1978; Harrigan and Downs, 1981; Winger et al., 1992; Negus et al., 1993; Winger and Woods, 1996; Mello and

Negus, 1998). These data are consistent with the conclusion that naloxone and buprenorphine antagonize the reinforcing effects of heroin. These results are also consistent with the finding that maintenance on the opiate antagonist naltrexone or on buprenorphine decreases heroin vs. money choice in detoxified heroin-dependent humans (Mello et al., 1981; Mello et al., 1982; Comer et al., 2005).

The heroin choice dose-effect curve was not affected by chronic 5-day treatment with methadone or by the 8-fold increase in daily heroin intake that occurred after introduction of supplemental heroin self-administration sessions. Methadone treatment also failed to alter heroin intake, although heroin intake increased during access to supplemental heroin. These findings agree with previous reports that chronic methadone up to toxic doses had little effect on opiate agonist self-administration (Harrigan and Downs, 1981; Mello et al., 1983). Similarly, chronic morphine had little effect on fixed-ratio responding maintained either by heroin in rats (Carrera et al., 1999) or by the high efficacy mu agonist alfentanil in rhesus monkeys (Winger and Woods, 2001). However, the latter study did find that chronic morphine produced small rightward shifts in the self-administration dose-effect curves for heroin and morphine and larger rightward shifts in self-administration dose-effect curves for the low efficacy mu agonists nalbuphine and buprenorphine (Winger and Woods, 2001). These results were interpreted to suggest that chronic morphine administration did produce tolerance to the reinforcing effects of mu agonists, and the expression of tolerance was inversely related to the efficacy of the test drug (Winger and Woods, 2001). Moreover, it has also been argued that expression of opiate tolerance is dependent not only on the efficacy of the acutely administered test drug, but also on the efficacy of the chronically administered drug, with lower efficacy agonists occupying more receptors and producing greater tolerance than initially equieffective doses of higher efficacy

agonists (e.g. (Sosnowski and Yaksh, 1990). With regard to the present study, methadone is a high efficacy mu agonist (Selley et al., 1998), and the present results are consistent with the conclusion that chronic treatment with this high efficacy agonist did not produce sufficient tolerance to alter the reinforcing effects of the moderately high efficacy agonist heroin. Chronic access to supplemental heroin also failed to produce tolerance to the reinforcing effects of heroin as measured by drug choice in the present study; however, access to supplemental heroin did produce evidence of tolerance to the rate-decreasing effects of heroin in that monkeys self-administered more injections of high dose heroin (0.1 mg/kg/inj) during access to supplemental heroin than before access to supplemental heroin.

Two other points regarding effects of opiate agonists on heroin choice warrant mention. First, rightward shifts in the heroin choice dose-effect curve produced by buprenorphine may reflect tolerance to the low efficacy agonist effects of buprenorphine rather than receptor antagonism, and these two possibilities are often difficult to dissociate (Winger et al., 1992; Winger and Woods, 1996). Second, drug choice in this procedure provides a measure of the *relative* reinforcing efficacy of heroin in comparison to food rather than the *absolute* reinforcing efficacy of heroin. Consequently, tolerance to the reinforcing effects of heroin could be masked and choice behavior unaffected if agonist treatment decreased the reinforcing efficacies of both heroin and food to similar extents.

Effects of withdrawal on heroin choice. Although chronic methadone treatment and chronic access to supplemental heroin self-administration produced little evidence of tolerance to the reinforcing effects of heroin, these treatments did produce evidence of physical dependence. Specifically, termination of either high-dose methadone treatment or access to supplemental heroin resulted in the emergence of characteristic signs of opiate withdrawal and significant

increases in heroin choice. Withdrawal from buprenorphine treatment also increased heroin choice, albeit at a delayed interval consistent with its slow dissociation from mu receptors (e.g. (Negus and Woods, 1995). Taken together, these results support the hypothesis that opiate withdrawal increased the relative reinforcing efficacy of heroin in comparison to food. This agrees with the finding that opiate dependence and withdrawal increased response rates during the fixed-interval component in rhesus monkeys responding for morphine under a FI-FR chain schedule (Thompson and Schuster, 1964) and increased breakpoints maintained by morphine in rhesus monkeys or heroin in rats responding under progressive ratio schedules (Yanagita, 1978; Carrera et al., 1999).

The present findings in heroin-dependent rhesus monkeys also parallel results obtained in earlier studies of heroin vs. food choice in baboons (Griffiths et al., 1975; Griffiths et al., 1981). In those studies, short-term (1-day) treatment with naloxone produced overt withdrawal signs, indicating that the extent of heroin self-administration was sufficient to produce dependence. Moreover, heroin vs. food choice was increased after short-term naloxone administration, reduction or elimination of the available unit dose of heroin, or termination of chronic 11-day treatment with methadone (8.3 mg/kg/day). Similarly, spontaneous withdrawal in morphine-dependent chimpanzees produced both overt withdrawal signs and increased preference for morphine vs. food (Spragg, 1940). Taken together with the results of the present study, these findings suggest that choice between an opiate agonist and food in opiate-dependent non-human primates can be increased either by antagonist-precipitated withdrawal or by spontaneous withdrawal from opiate agonists.

As a final caveat, it should be noted again that these choice procedures measure the *relative* reinforcing efficacy of heroin in comparison to food, and not the *absolute* reinforcing

efficacy of heroin. Opiate withdrawal is well-known to decrease rates of food-maintained responding (e.g. (Thompson and Schuster, 1964), and withdrawal-associated increases in heroin choice could reflect decreases in the reinforcing efficacy of the food alternative rather than, or in addition to, changes in the reinforcing efficacy of heroin. Either way, though, opiate withdrawal had the net effect of increasing the reinforcing efficacy of opiate agonists in comparison to an alternative reinforcer, and this increase in the *relative* reinforcing efficacy of opiate agonists may also be associated with relapse in human drug users. For example, the time course of withdrawal signs and withdrawal-associated increases in heroin choice described in this study parallel the time course of “nonpurposive symptoms” (e.g. abdominal cramps, goose flesh) and “purposive symptoms” (e.g. efforts to obtain drug) described during opiate withdrawal in humans (Jaffe, 1970). Similarly, withdrawal from methadone maintenance increased overt withdrawal signs and measures of craving, and the expression of these signs and symptoms was associated with relapse to illicit opiate use (Greenwald, 2002).

Drug effects on withdrawal-associated increases in heroin choice. The emergence of withdrawal signs and withdrawal-associated increases in heroin choice could be prevented by treatment with methadone or by continued access to supplemental heroin. Similarly, heroin vs. food choice in opiate-dependent baboons or morphine vs. food choice in opiate-dependent chimpanzees could be reduced by non-contingent treatment with methadone or morphine, or by availability of a higher unit dose of heroin (Spragg, 1940; Griffiths et al., 1975; Griffiths et al., 1981). Accordingly, these results support the hypothesis that opiate agonists may prevent withdrawal-associated increases in the relative reinforcing efficacy of mu agonists, and this effect may contribute to the clinical utility of agonist medications such as methadone in

preventing relapse to opiate use. The lower efficacy mu agonist buprenorphine did not consistently prevent withdrawal-associated increases in heroin choice. This is consistent with the finding that buprenorphine produces only a partial suppression of withdrawal in maximally withdrawn monkeys (Aceto, 1984) and may also be less effective as a maintenance medication than methadone in highly dependent opiate users (Gonzalez et al., 2004).

Conclusions. The main findings of the present study were that withdrawal increased the relative reinforcing efficacy of heroin in comparison to food, and that treatment with opiate agonists prevented withdrawal-associated increases in heroin choice. There are two related implications of the present study. First, optimal strategies for assessment of candidate medications for opiate dependence may include evaluation of medication effects on withdrawal-associated increases in the relative reinforcing efficacy of opiate agonists. Second, the mechanisms that underlie withdrawal-associated increases in reinforcing efficacy are unknown. It has been suggested that chronic opiate administration may activate “anti-reward” processes, and that withdrawal-associated increases in reinforcing efficacy may reflect the ability of opiate agonists to oppose these anti-reward processes (Koob et al., 2004). The present procedure provides one experimental approach that could be used to test this hypothesis, in that non-opiate drugs that might target hypothetical anti-reward systems could be evaluated for their ability to prevent withdrawal-associated increases in drug choice.

ACKNOWLEDGMENTS

The author would like to thank Peter Fivel, Sam McWilliams and Melissa Timm for outstanding technical support and Kevin Costa for writing the computer programs used in this study. The author would also like to thank Andrew C. Barrett and Nancy K. Mello for comments on an earlier version of this manuscript.

REFERENCES

- Aceto MD (1984) Characterization of prototypical opioid antagonists, agonist-antagonists, and agonists in the morphine-dependent rhesus monkey. *Neuropeptides* **5**:15-18.
- Carrera MR, Schulteis G and Koob GF (1999) Heroin self-administration in dependent Wistar rats: increased sensitivity to naloxone. *Psychopharmacology (Berl)* **144**:111-120.
- Comer SD, Walker EA and Collins ED (2005) Buprenorphine/naloxone reduces the reinforcing and subjective effects of heroin in heroin-dependent volunteers. *Psychopharmacology (Berl)*:1-12.
- Garrett BE, Rose CA and Henningfield JE (2001) Tobacco addiction and pharmacological interventions. *Expert Opin Pharmacother* **2**:1545-1555.
- Gonzalez G, Oliveto A and Kosten TR (2004) Combating opiate dependence: a comparison among the available pharmacological options. *Expert Opin Pharmacother* **5**:713-725.
- Grabowski J, Shearer J, Merrill J and Negus SS (2004) Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. *Addict Behav* **29**:1439-1464.
- Greenstein RA, Fudala PJ and O'Brien CP (1997) Alternative pharmacotherapies for opiate addiction., in *Substance Abuse: A Comprehensive Textbook* (Lowinson JH, Ruiz P, Millman RB and Langrod JG eds) pp 415-425, Williams and Wilkins, Baltimore.
- Greenwald MK (2002) Heroin craving and drug use in opioid-maintained volunteers: effects of methadone dose variations. *Exp Clin Psychopharmacol* **10**:39-46.
- Griffiths RR, Wurster RM and Brady JV (1975) Discrete-trial choice procedure: effects of naloxone and methadone on choice between food and heroin. *Pharmacol Rev* **27**:357-365.

- Griffiths RR, Wurster RM and Brady JV (1981) Choice between food and heroin: effects of morphine, naloxone, and secobarbital. *J Exp Anal Behav* **35**:335-351.
- Harrigan SE and Downs DA (1978) Continuous intravenous naltrexone effects on morphine self-administration in rhesus monkeys. *J Pharmacol Exp Ther* **204**:481-486.
- Harrigan SE and Downs DA (1981) Pharmacological evaluation of narcotic antagonist delivery systems in rhesus monkeys. *NIDA Res Monogr* **28**:77-92.
- Jaffe JH (1970) Drug addiction and drug abuse, in *The Pharmacological Basis of Therapeutics* (Goodman LS and Gilman A eds) pp 276-313, Collier-MacMillan, London.
- Johanson CE (1975) Pharmacological and environmental variables affecting drug preference in rhesus monkeys. *Pharmacol Rev* **27**:343-355.
- Jones BE and Prada JA (1977) Effects of methadone and morphine maintenance on drug-seeking behavior in the dog. *Psychopharmacology (Berl)* **54**:109-112.
- Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, O'Dell LE, Parsons LH and Sanna PP (2004) Neurobiological mechanisms in the transition from drug use to drug dependence. *Neurosci Biobehav Rev* **27**:739-749.
- Lowinson JH, Payte JT, Salsitz E, Joseph H, Marion IJ and Dole VP (1997) Methadone maintenance., in *Substance Abuse: A Comprehensive Textbook* (Lowinson JH, Ruiz P, Millman RB and Langrod JG eds) pp 405-415, Williams and Wilkins, Baltimore.
- Mello NK, Bree MP and Mendelson JH (1983) Comparison of buprenorphine and methadone effects on opiate self-administration in primates. *J. Pharmacol. Exp. Ther.* **225**:378-386.
- Mello NK, Mendelson JH and Kuehnle JC (1982) Buprenorphine effects on human heroin self-administration: an operant analysis. *J Pharmacol Exp Ther* **223**:30-39.

Mello NK, Mendelson JH, Kuehnle JC and Sellers MS (1981) Operant analysis of human heroin self-administration and the effects of naltrexone. *J Pharmacol Exp Ther* **216**:45-54.

Mello NK and Negus SS (1996) Preclinical evaluation of pharmacotherapies for treatment of cocaine and opioid abuse using drug self-administration procedures. *Neuropsychopharmacology* **14**:375-424.

Mello NK and Negus SS (1998) The effects of buprenorphine on self-administration of cocaine and heroin "speedball" combinations and heroin alone by rhesus monkeys. *J Pharmacol Exp Ther* **285**:444-456.

Negus SS (2003) Rapid assessment of choice between cocaine and food in rhesus monkeys: effects of environmental manipulations and treatment with d-amphetamine and flupenthixol. *Neuropsychopharmacology* **28**:919-931.

Negus SS (2005) Interactions between the reinforcing effects of cocaine and heroin in a drug-vs-food choice procedure in rhesus monkeys: a dose-addition analysis. *Psychopharmacology (Berl)* **180**:115-124.

Negus SS, Henriksen SJ, Mattox A, Pasternak GW, Portoghese PS, Takemori AE, Weinger MG and Koob GF (1993) Effect of antagonists selective for mu, delta and kappa opioid receptors on the reinforcing effects of heroin in rats. *J. Pharmacol. Exp. Ther.* **265**:1245-1252.

Negus SS and Mello NK (2004) Effects of chronic methadone treatment on cocaine- and food-maintained responding under second-order, progressive-ratio and concurrent-choice schedules in rhesus monkeys. *Drug Alcohol Depend* **74**:297-309.

Negus SS and Woods JH (1995) Reinforcing effects, discriminative stimulus effects, and physical dependence liability of buprenorphine, in *Buprenorphine: Combatting drug*

- abuse with a unique opioid* (Cowan A and Lewis JW eds) pp 71-101, Wiley-Liss, New York.
- Selley DE, Liu Q and Childers (1998) Signal transduction correlates of mu opioid agonist intrinsic efficacy: receptor-stimulated [³⁵S]GPTgammaS binding in mMOR-CHO cells and rat thalamus. *J Pharmacol Exp Ther* **285**:496-505.
- Sosnowski M and Yaksh TL (1990) Differential cross-tolerance between intrathecal morphine and sufentanil in the rat. *Anesthesiology* **73**:1141-1147.
- Spragg SDS (1940) Morphine addiction in chimpanzees. *Comp. Psychol. Mono.* **15**:5-132.
- Stevenson GW, Folk JE, Rice KC and Negus SS (2005) Interactions between delta and mu opioid agonists in assays of schedule-controlled responding, thermal nociception, drug self-administration, and drug versus food choice in rhesus monkeys: studies with SNC80 [(+)-4-[(α)- α -(2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-N,N-diethylbenzamide] and heroin. *J Pharmacol Exp Ther* **314**:221-231.
- Thompson T and Schuster CR (1964) Morphine self-administration, food-reinforced, and avoidance behaviors in rhesus monkeys. *Psychopharmacologia* **5**:87-94.
- Winger G, Skjoldager P and Woods JH (1992) Effects of buprenorphine and other opioid agonists and antagonists on alfentanil- and cocaine-reinforced responding in rhesus monkeys. *J. Pharmacol. Exp. Ther.* **261**:311-317.
- Winger G and Woods JH (1996) Effects of buprenorphine on behaviour maintained by heroin and alfentanil in rhesus monkeys. *Behav Pharmacol* **7**:155-159.
- Winger G and Woods JH (2001) The effects of chronic morphine on behavior reinforced by several opioids or by cocaine in rhesus monkeys. *Drug Alcohol Depend* **62**:181-189.

Woolverton WL and Balster RL (1981) Effects of antipsychotic compounds in rhesus monkeys given a choice between cocaine and food. *Drug Alcohol Depend* **8**:69-78.

Wurster RM, Griffiths RR, Findley JD and Brady JV (1977) Reduction of heroin self-administration in baboons by manipulation of behavioral and pharmacological conditions. *Pharmacol Biochem Behav* **7**:519-528.

Yanagita T (1978) Drug dependence studies in laboratory animals. *NIDA Res Monogr* **19**:179-190.

FOOTNOTES

This work was supported by grants RO1-DA02519 and P01-DA14528 from NIDA, NIH.

FIGURE LEGENDS

Figure 1. Effects of chronic 5-day treatment with naloxone (0.01-0.32 mg/kg/hr) on choice between heroin and food. The top panels show the percent heroin choice (top left panel) or the overall response rates in response/second (top right panel) during treatment with saline (heroin alone) or increasing doses of naloxone. Abscissae: Unit dose heroin in mg/kg injection. The bottom panel shows the total number of choices, total food choices and total heroin choices, as well as the total heroin intake, obtained during treatment with saline or each dose of naloxone. All points and bars show mean data \pm SEM from 2 monkeys obtained during the last 3 days of each 5-day treatment. Asterisks indicate a significant difference from “heroin alone”, $p < 0.05$.

Figure 2. Effects of chronic 5-day treatment with buprenorphine (0.01-0.1 mg/kg/day) on choice between heroin and food. Other details as in Figure 1.

Figure 3. Effects of chronic 5-day treatment with methadone (0.1-0.56 mg/kg/hr) on choice between heroin and food. Other details as in Figure 1.

Figure 4. Choice between heroin and food after termination of chronic 5-day treatment with naloxone, buprenorphine and methadone. Top panels show percent heroin choice as a function of unit dose heroin (mg/kg/inj) during saline treatment (heroin alone), during treatment with the highest dose of each medication, and at selected times after termination of treatment with the highest dose of each medication. Bottom panels show the numbers of total choices, food choices and heroin choices during each phase of treatment and withdrawal. All points and bars show mean data \pm SEM from 2 monkeys. Asterisks indicate a significant difference from “heroin alone”, $p < 0.05$.

Figure 5. Patterns of heroin self-administration during access to supplemental heroin self-administration sessions. The left panel shows the number of 0.1 mg/kg/injection heroin

injections self-administered during each quintile of the daily supplemental heroin self-administration sessions. Times for each quintile were 1:00-5:12pm (quintile 1), 5:12-9:24pm (quintile 2), 9:24pm-1:36am (quintile 3), 1:36am-5:48pm (quintile 4) and 5:48pm-10am (quintile 5). The right panel shows heroin intake in mg/kg self-administered during the choice session, the supplemental heroin self-administration session, and total daily intake (choice session intake + supplemental session intake). All points and bars show mean data \pm SEM from 3 monkeys during the last 3 days of access to supplemental heroin prior to termination of access to supplemental heroin.

Figure 6. Effects of access and termination of access to supplemental heroin self-administration sessions on choice between heroin and food. The top panels show the percent heroin choice (top left panel) or the overall response rates in response/second (top right panel) prior to, during, and on the first day after exposure to supplemental heroin self-administration sessions. Abscissae: Unit dose heroin in mg/kg injection. The bottom panel shows the total number of choices, total food choices and total heroin choices, as well as the total heroin intake during each phase of access to supplemental heroin self-administration. All points and bars show mean data \pm SEM from 3 monkeys. Data collected prior to and during exposure to supplemental heroin were collected from the last three days of each phase. Data collected after termination of access to supplemental heroin show results obtained on the first day of withdrawal.

Figure 7. Time course of overt withdrawal signs (right ordinate, open circles) and withdrawal-associated increases in choice for 0.0032 mg/kg/injection heroin (left ordinate, filled squares) after termination of access to supplemental heroin self-administration sessions. Abscissa: Days after termination. Data above “C” (control) and “SH” (supplemental heroin) show values for each variable prior to and during access to supplemental heroin self-administration sessions, respectively. All points show mean data \pm SEM from three monkeys.

Figure 8. Effects of methadone treatment (0.56 mg/kg/hr) on withdrawal-associated increases in heroin choice. The top panels show the percent heroin choice (top left panel) or the overall response rates in response/second (top right panel) during the last 3 days of exposure to supplemental heroin self-administration sessions and one day after termination of access to supplemental heroin coupled with methadone treatment. Abscissae: Unit dose heroin in mg/kg injection. The bottom panel shows the total number of choices, total food choices and total heroin choices, as well as the total heroin intake. All points and bars show mean data \pm SEM from 3 monkeys. Data collected during exposure to supplemental heroin were collected from the last three days of each phase. Asterisks indicate a significant difference from “heroin alone”, $p < 0.05$.

Figure 9. Effects of buprenorphine treatment (0.1-0.32 mg/kg/day) on withdrawal-associated increases in heroin choice. Other details as in Figure 8.

Table 1. Mean ED50 values (95%CL) for heroin choice in mg/kg/inj in two monkeys during treatment with saline or with increasing doses of naloxone, buprenorphine and methadone. ED50 values observed at various times after treatment with the highest doses of naloxone, buprenorphine, and methadone are also shown.

<u>Treatment</u>	<u>ED50 (95% CL)</u>
Saline Treatment	0.011 (0.006-0.018)
0.01/hr Naloxone	0.019 (0.008-0.043)
0.1/hr Naloxone	0.034 (0.012-0.095)
0.32/hr Naloxone	>0.053*¥
Post 0.32 Nalox Day 1	0.025 (0.002-0.37)
Post 0.32 Nalox Day 3	0.018 (0.002-0.17)
Post 0.32 Nalox Day 7	0.010 (0.004-0.030)
0.01/day Bup	0.015 (0.009-0.025)
0.032/day Bup	0.022 (0.014-0.034)
0.1/day Bup	0.069 (0.034-0.14)*
Post 0.1 Bup Day 1	0.038 (0.014-0.1)
Post 0.1 Bup Day 5	<0.0032*†
Post 0.1 Bup Day 10	0.018
0.1/hr Methadone	0.013 (0.007-0.024)
0.32/hr Methadone	0.010 (0.003-0.030)
0.56/hr Methadone	0.012 (0.010-0.015)
Post 0.56 Meth Day 1	<0.0032*†
Post 0.56 Meth Day 3	<0.0039*†
Post 0.56 Meth Day 7	0.011 (0.003-0.040)

* 95% confidence limits do not overlap with “Saline Treatment”

¥ ED50 could not be determined for at least one monkey because heroin choice was less than 50% across all heroin doses up and including 0.1 mg/kg/inj heroin.

† ED50 could not be determined for at least one monkey because heroin choice was greater than 50% across all heroin doses down to and including 0.0032 mg/kg/inj heroin.

Table 2. Mean ED50 values (95%CL) for heroin choice in mg/kg/inj in three monkeys. Data are shown before access to supplemental heroin sessions, during access to supplemental heroin sessions, or during withdrawal from supplemental heroin. During withdrawal, monkeys were treated with saline, methadone (0.056 mg/kg/hr) or buprenorphine (0.1 or 0.32 mg/kg/day). The number of monkeys in which withdrawal from supplemental heroin produced a decrease in the heroin choice ED50 value is also shown.

<u>Treatment</u>	<u>ED50 (95% CL)</u>	<u># Monkeys with Decreased ED50</u>
Pre-Supplemental Heroin	0.0091 (0.0068-0.012)	
+ Supplemental Heroin	0.016 (0.0051-0.053)	
24 hr after Termination of Supplemental Heroin		
+ Saline	<0.0032 †	3/3
+ 0.056/hr Meth	0.021 (0.017-0.026)	0/3
+ 0.1/day Bup	<0.0046 †	2/3
+ 0.32/day Bup	<0.0038 †	2/3

† ED50 could not be determined for at least one monkey because heroin choice was greater than 50% across all heroin doses down to and including 0.0032 mg/kg/inj heroin.

Figure 1

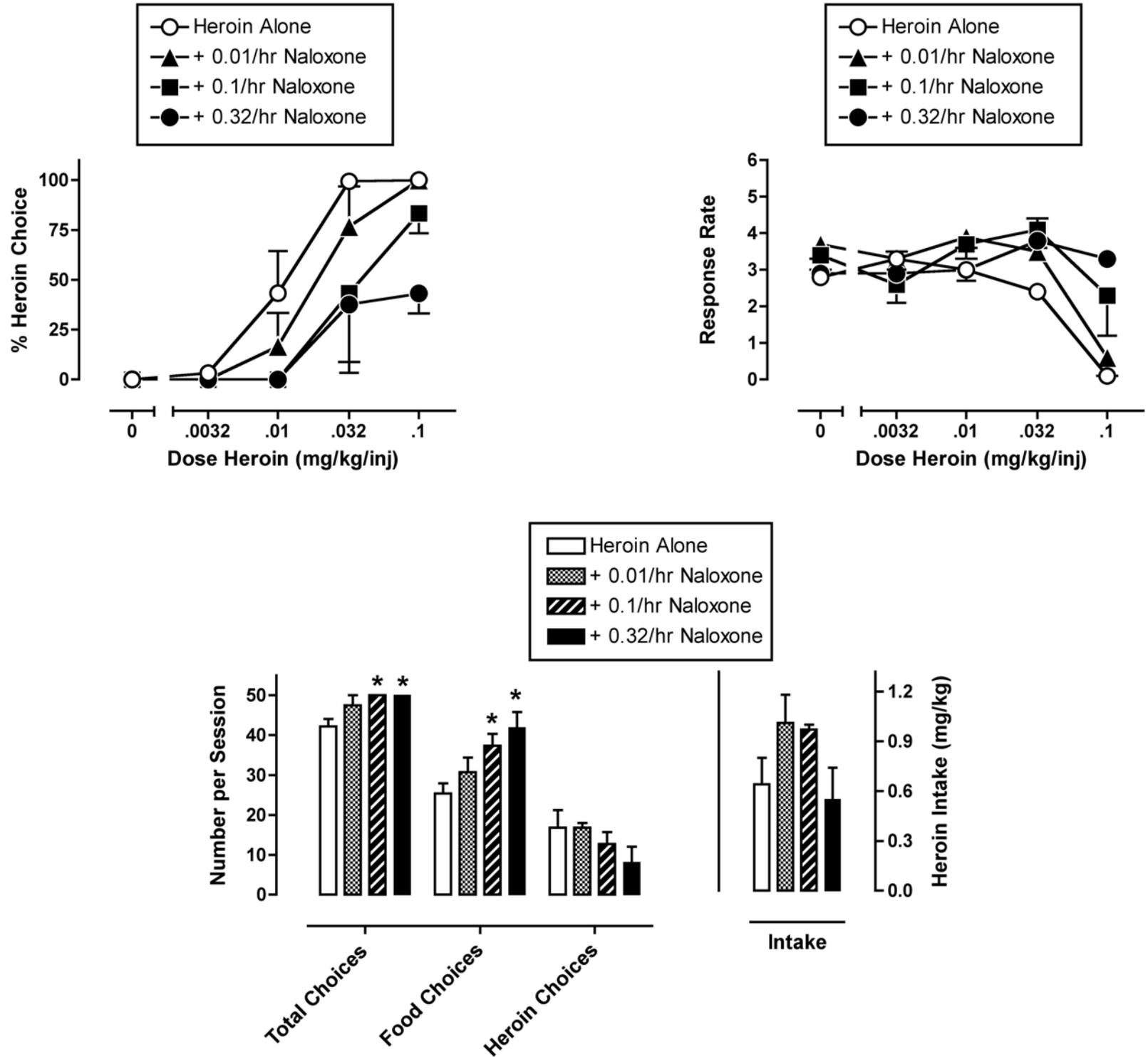


Figure 2

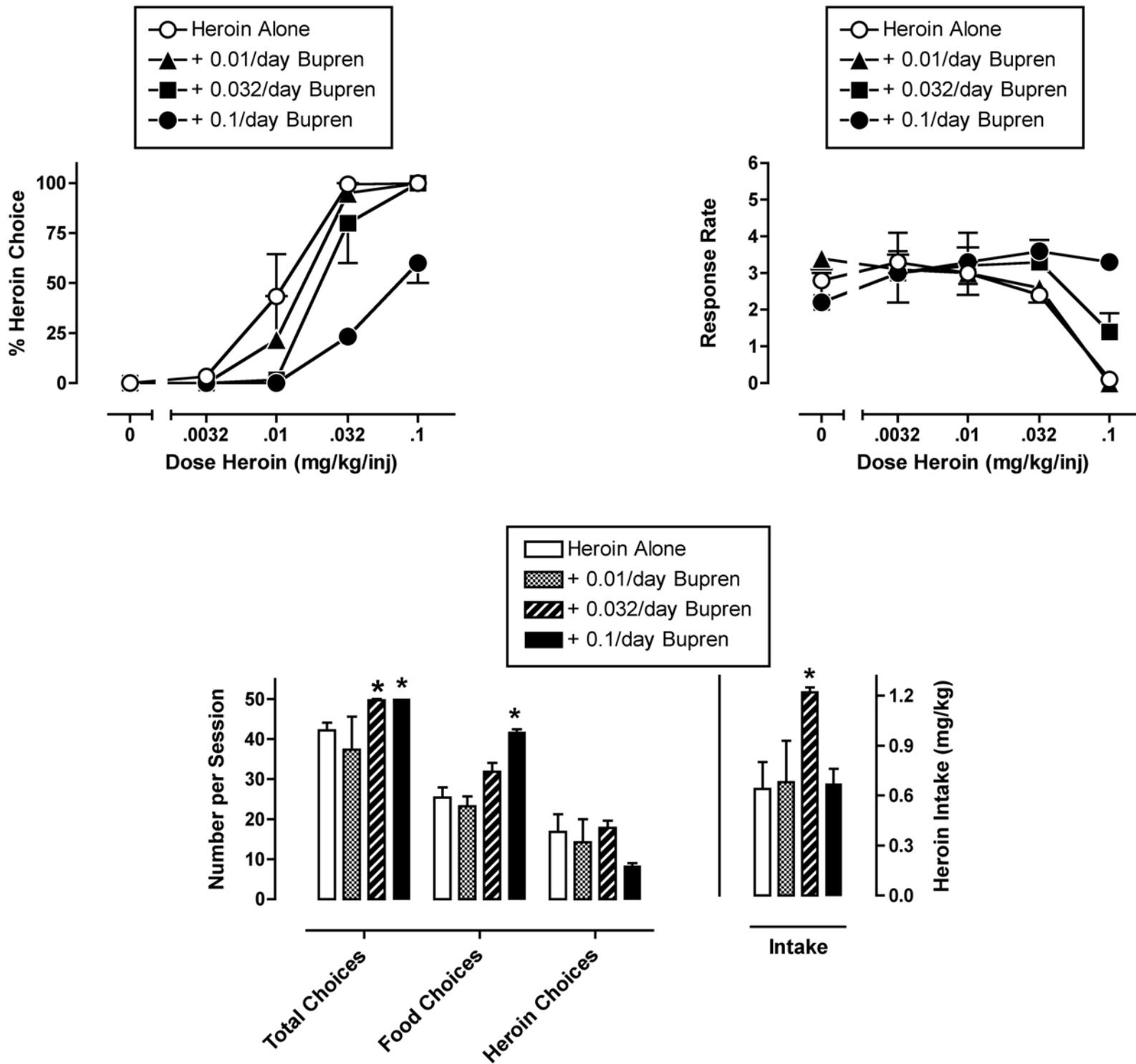


Figure 3

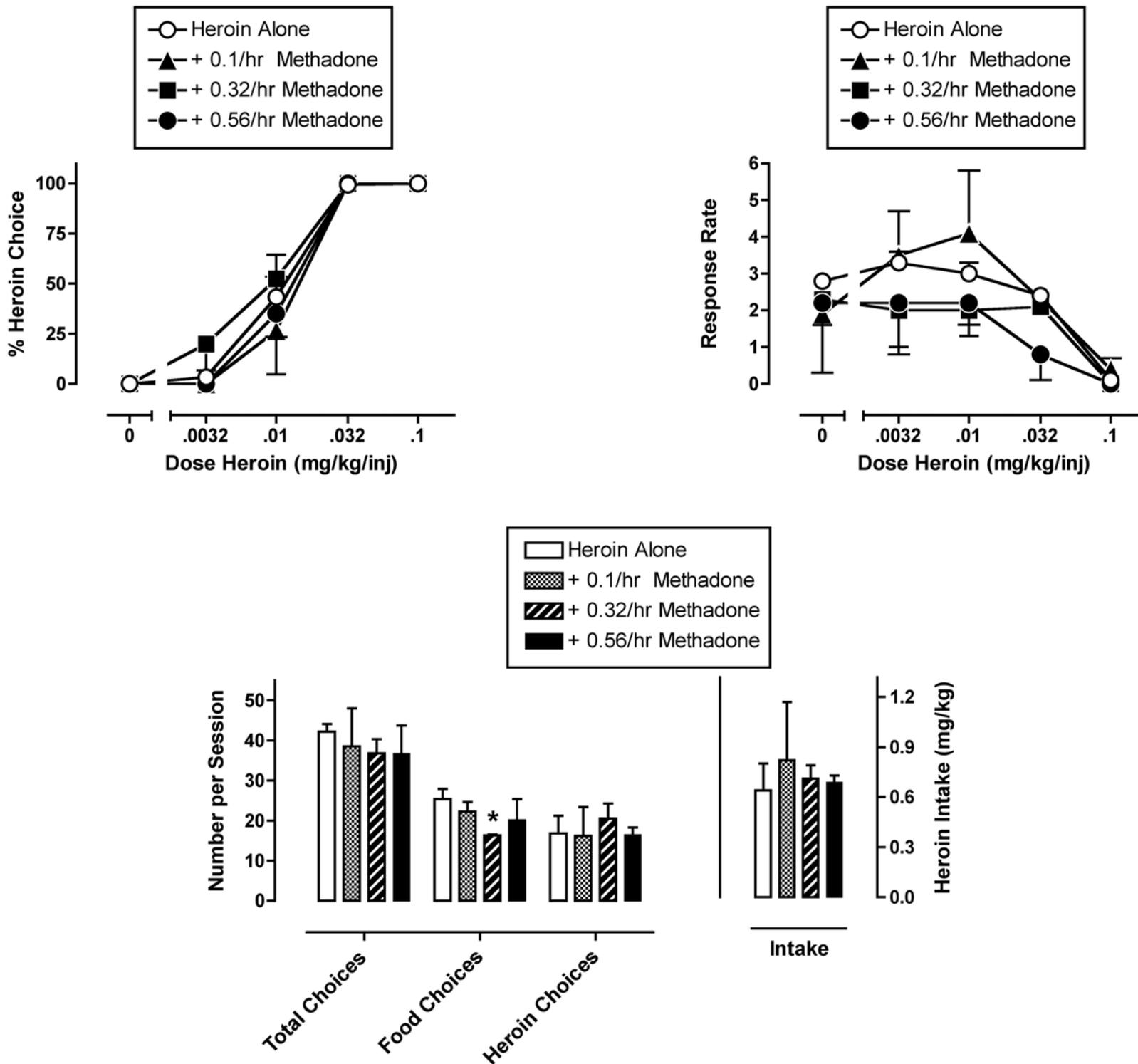


Figure 4

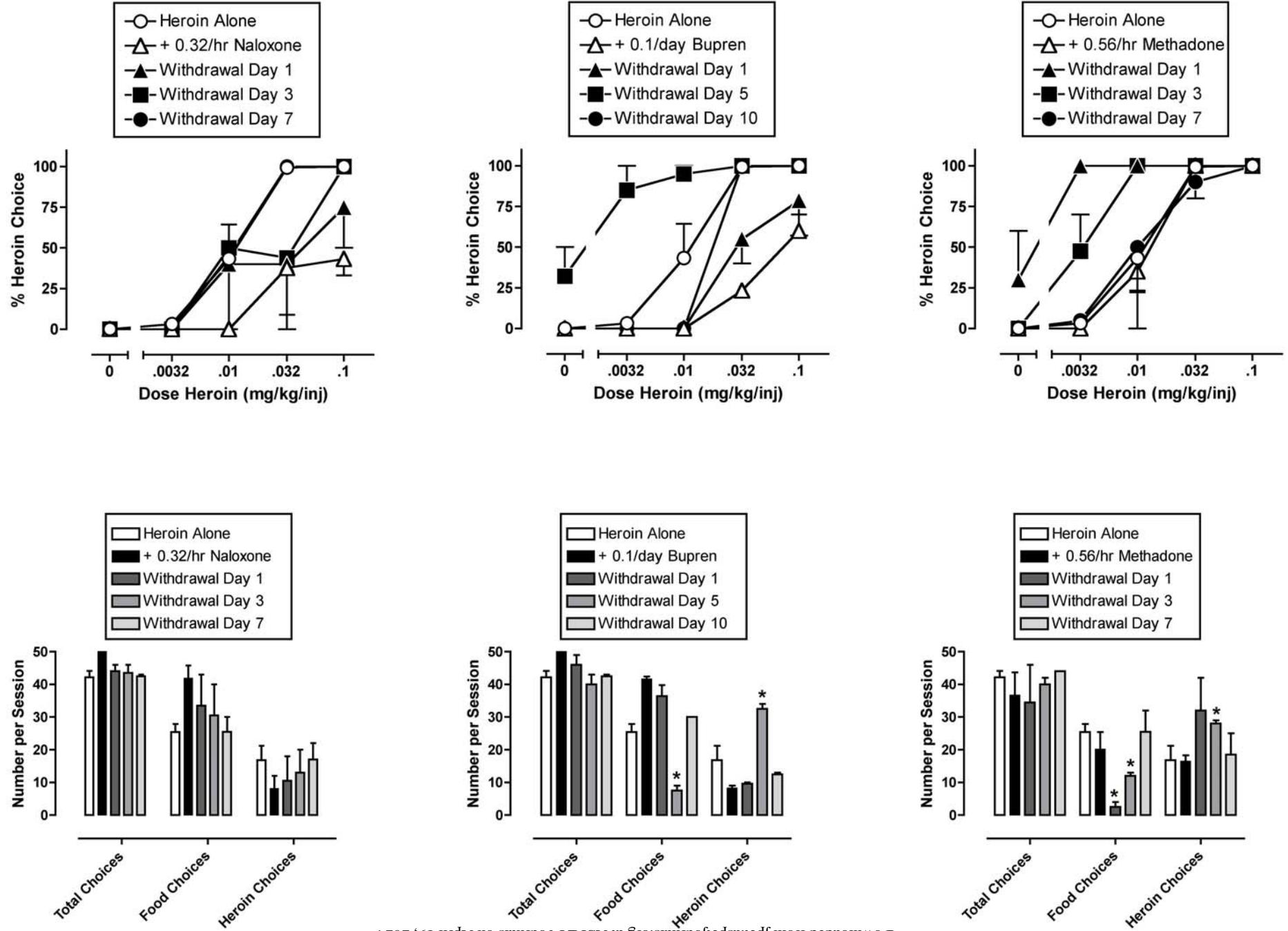


Figure 5

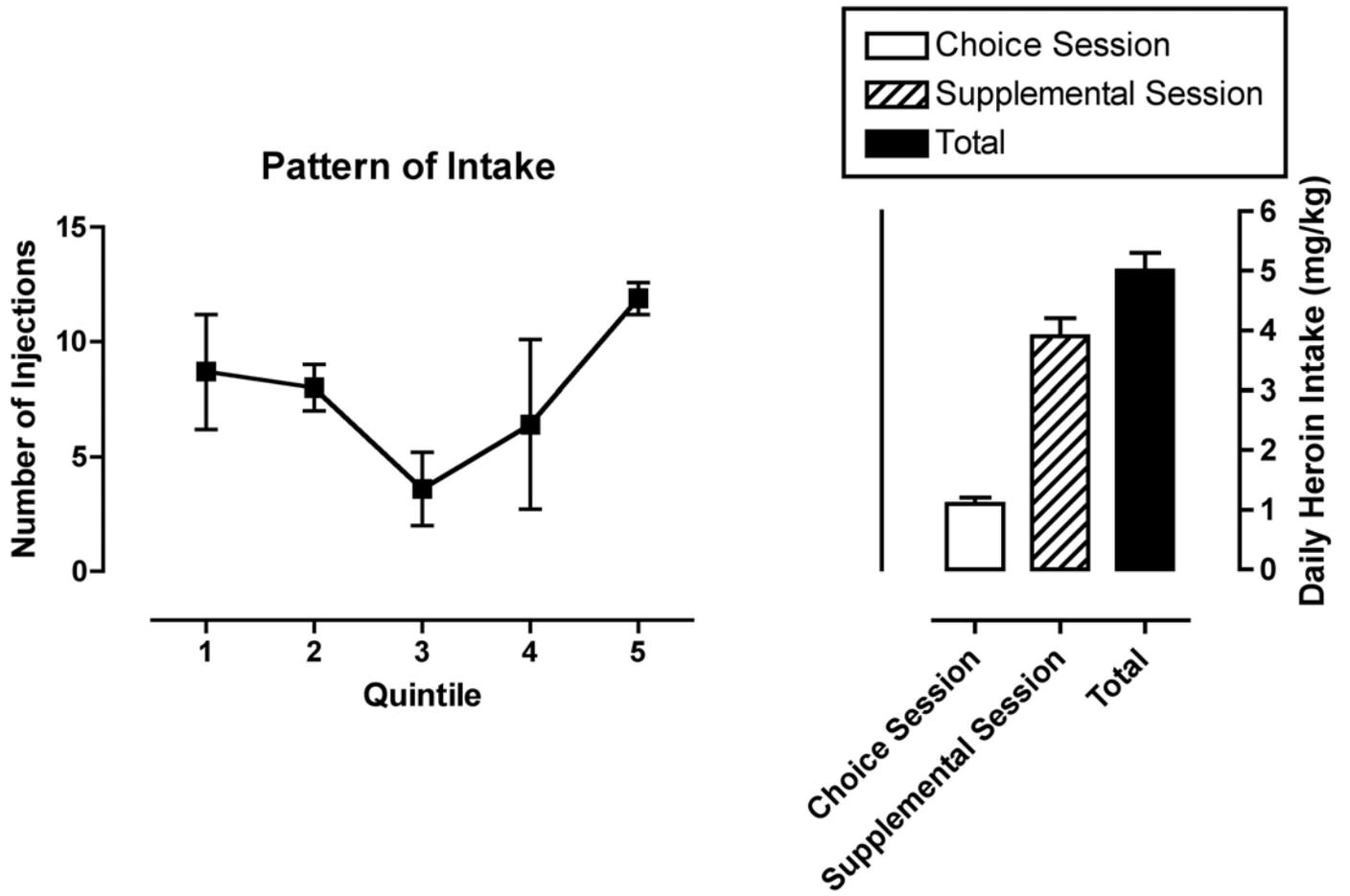


Figure 6

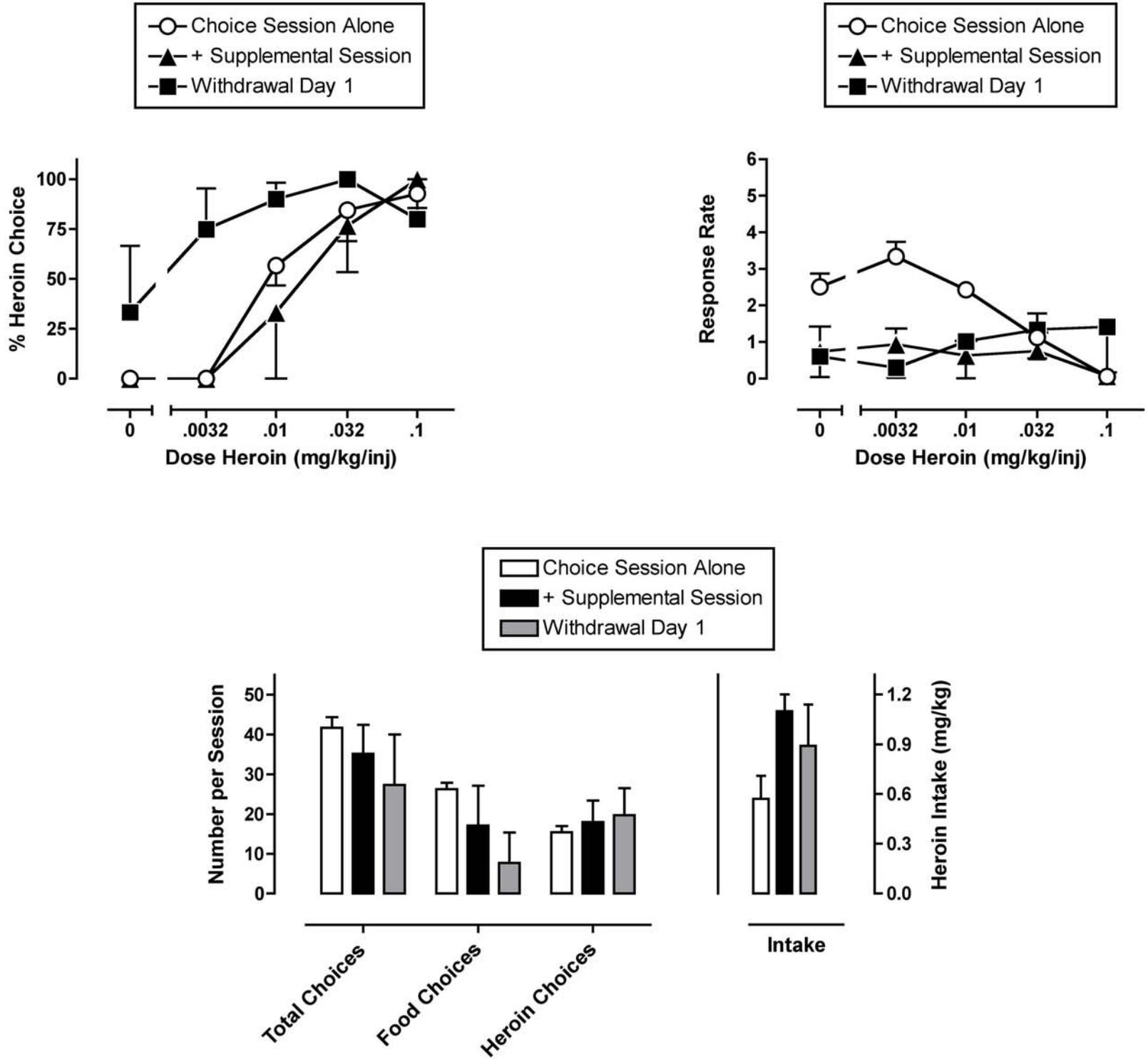


Figure 7

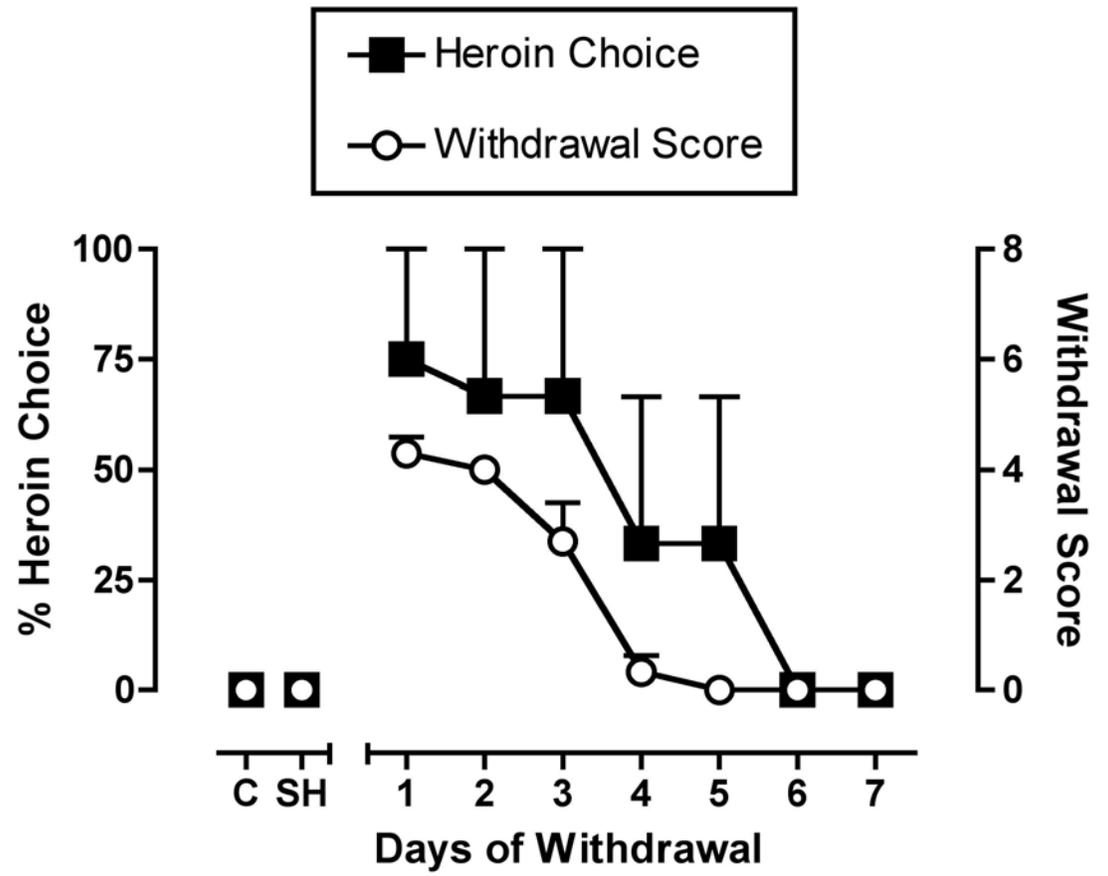


Figure 8

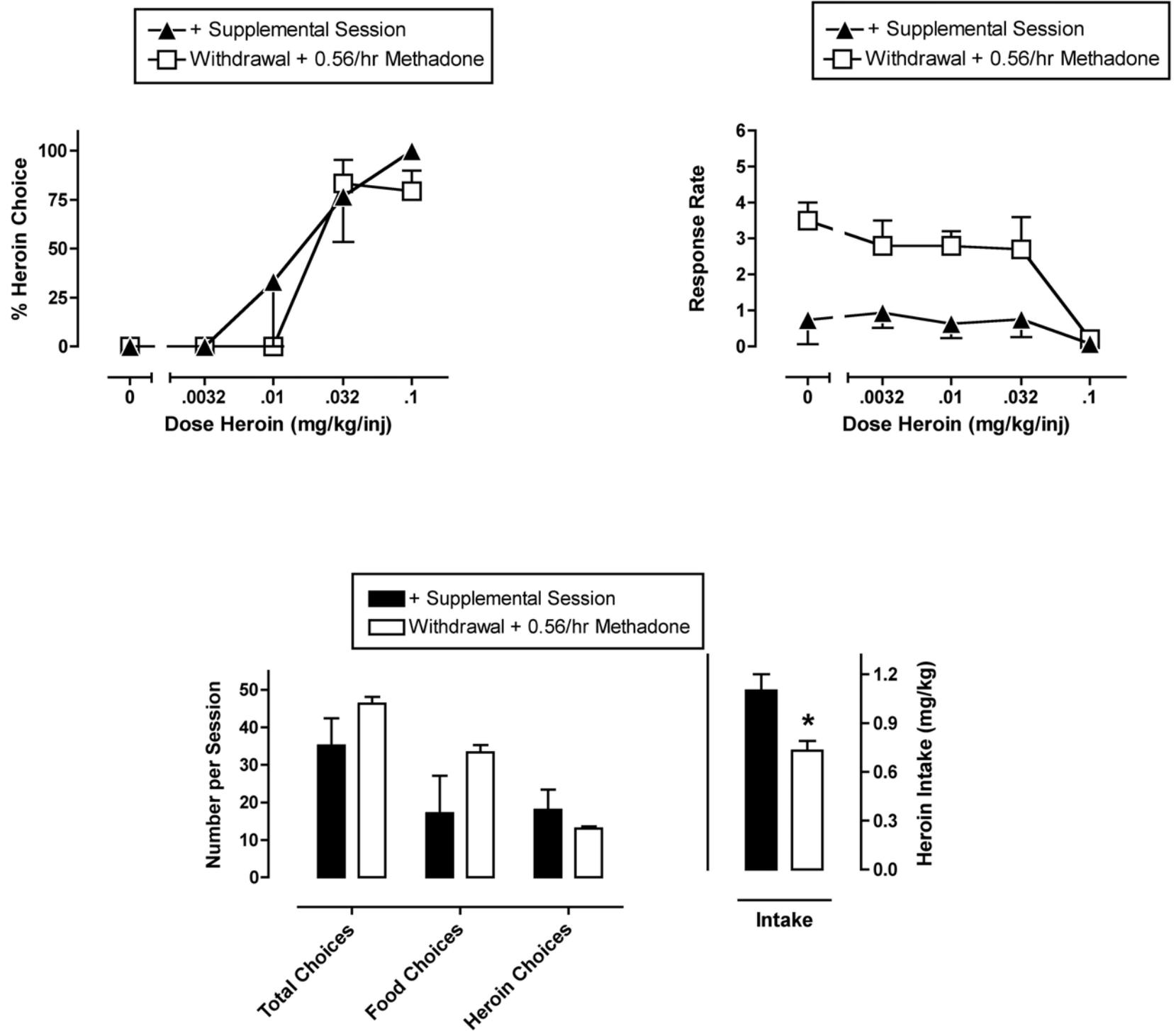


Figure 9

