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Yohimbine Increases Opioid-Seeking Behavior in Heroin-Dependent, Buprenorphine-Maintained Individuals

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Abstract

Rationale—In laboratory animals, the biological stressor yohimbine (α_2-noradrenergic autoreceptor antagonist) promotes drug seeking. Human laboratory studies have demonstrated that psychological stressors can increase drug craving but not that stressors alter drug seeking.

Objectives—This clinical study tested whether yohimbine increases opioid seeking behavior.

Methods—Ten heroin-dependent, buprenorphine (8-mg/day) stabilized volunteers, sampled two doses of hydromorphone (12 and 24 mg IM in counterbalanced order, labeled Drug A [session 1] and Drug B [session 2]). During each of six later sessions (within-subject, double blind, randomized crossover design), volunteers could respond on a 12-trial choice progressive ratio task to earn units (1 or 2 mg) of the sampled hydromorphone dose (Drug A or B) vs. money ($2) following different oral yohimbine pretreatment doses (0, 16.2 and 32.4 mg).

Results—Behavioral economic demand intensity and peak responding (O_{max}) were significantly higher for hydromorphone 2-mg than 1-mg. Relative to placebo, yohimbine significantly increased hydromorphone demand inelasticity, more so for hydromorphone 1-mg units (P_{max} = 909, 3647 and 3225 for placebo, 16.2 and 32.4 mg yohimbine doses, respectively) than hydromorphone 2-mg units (P_{max} = 2656, 3193 and 3615, respectively). Yohimbine produced significant but clinically modest dose-dependent increases in blood pressure (systolic ≈15 and diastolic ≈10 mmHg) and opioid withdrawal symptoms, and decreased opioid agonist symptoms and elated mood.

Conclusions—These findings concur with preclinical data by demonstrating that yohimbine increases drug seeking; in this study, these effects occurred without clinically significant subjective distress or elevated craving, and partly depended on opioid unit dose.

Keywords

Stress; Opioid; Drug abuse; Human; Motivation; Noradrenergic; Reinforcement; Self-administration; Behavior

Stressors are stimuli that challenge an organism's capacity to maintain homeostasis and thus can result in psychobiological deviations, including dysregulation of brain reward circuits

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Disclosures/Conflict of Interest

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(e.g., Kreek and Koob 1998; McEwen 2007; Koob 2008). Preclinical animal studies have demonstrated that stressors can increase drug seeking behavior. These observations are relevant to the chronic, relapsing nature of addiction because stressors frequently prevent drug abstinence initiation and can precipitate relapse (Bradley et al. 1989; Stewart 2003). Extant pharmacotherapies for substance use disorders typically employ agonist substitution or blockade approaches that are not specifically designed to mitigate stress-potentiated drug use, and mechanistic understanding of this problem is currently limited.

In laboratory animals, several experimental stressors enhance opioid self-administration, including social isolation (Alexander et al. 1978; Bozarth et al. 1989), food deprivation (Carroll and Meisch 1984), immobilization (Shaham et al. 1992), and intermittent footshock (Shaham et al. 1994, 1995, 1996, 2000). Similarly, the α_2-adrenoceptor antagonist yohimbine reliably potentiates drug seeking behavior under non-cued conditions (Feltenstein and See 2006; Gass and Olive 2007; Lê et al. 2005; Shepard et al. 2004) as well as under conditioned cue-induced conditions (Banna et al. 2010; Buffalari and See 2011; Feltenstein et al. 2012). Yohimbine increases noradrenergic (NA) neurotransmission by blocking inhibitory feedback at the presynaptic autoreceptor (Doxey et al. 1984; Goldberg and Robertson 1983). Yohimbine-induced NA activation also regulates serotonin and dopamine transmission (Millan et al. 2000; Söderpalm et al. 1995), and hypothalamic-pituitary-adrenal axis activity (Banihashemi and Rinaman 2006; Brown et al. 2009; Lee et al. 2004). Yohimbine has anxiogenic effects in animals (Pellow et al. 1987), healthy human volunteers (Cameron et al. 2000; Charney et al. 1983; Mattila et al. 1988) and patients with panic disorder (Albus et al. 1992; Charney et al. 1992). In methadone-maintained patients, yohimbine infusion provoked opioid craving, withdrawal symptoms and anxiety (Stine et al. 2002).

Human laboratory studies have found that acute psychological stressors can increase craving for alcohol (Coffey et al. 2006; Fox et al. 2007) and cocaine (Sinha et al. 1999, 2000). Recent studies using the Trier Social Stress Test found mixed effects on alcohol use, including sex-influenced findings (Nesic and Duka, 2006), equivalent ethanol and water intake (de Wit et al. 2003), and higher proportion of subjects drinking the maximum ethanol amount but no significant differences in amount consumed (Thomas et al. 2011). Childs and de Wit (2010) reported that the Trier Social Stress Test increased cigarette craving but not smoking. However, an earlier study found that social speech (anxiogenic) and monotonous-task (attention-demanding) stressors increased smoking, compared to a relaxation control condition (Rose et al. 1983).

Unfortunately, most psychogenic stressors have non-trivial limitations: (1) they are short-acting, which limits sampling of drug seeking/use (which may explain why self-reported craving has been the focal outcome in prior studies) and differs from sustained stressors; (2) it is difficult to manipulate their intensity, precluding “dose-effect” comparisons; (3) inability to experimentally blind these stimuli (no placebo) may produce expectancy effects, necessitating less-sensitive between-subject designs; and (4) participants may habituate to these challenges, which limits repeated-exposure studies and relevance to chronic stressors (Harris et al. 2005).

Behavioral economic methods provide an alternative and advantageous means to evaluate the effects of stressors on drug-reinforced behaviors. Specifically, stressors may not exert uniform effects on drug reinforcing efficacy; rather, such effects often depend on prevailing environmental conditions including the behavioral cost (unit price) of the drug. At low drug prices, drug demand tends to be “inelastic” or less price-sensitive whereas, at higher drug prices, drug demand becomes “elastic” or more price-sensitive (Bickel et al. 1993). Drug unit price can be experimentally manipulated by increasing response requirement

(progressive ratio schedule) relative to the dose earned. Thus, we predict that stressors may differentially potentiate drug demand at moderate to higher drug unit prices, making demand more inelastic when stressor-induced psychobiological changes overcome normal price restraints on behavior. In other words, stressors should make drug abusing individuals "defend consumption" of their preferred drug despite its higher behavioral cost, which translates into greater demand inelasticity.

In summary, potentiation of drug-maintained responding by stressors is a significant issue, yet human experimental paradigms for investigating this phenomenon have restricted validity. Ideally, clinical studies might employ sustained, neurochemically-defined, placebo-controlled, dose-dependent stressors; in addition, outcomes should focus on drug seeking/use to achieve "reverse translation" with preclinical literature (Sinha et al. 2011), improve our mechanistic understanding, and develop novel therapeutics. To fill this scientific void, the present study aimed to determine yohimbine dose-effects on opioid seeking and biobehavioral indices in heroin-dependent, buprenorphine-stabilized volunteers. In the present approach, yohimbine is operationally defined as the experimental stressor with three dose levels (placebo, lower and higher dose). Using a behavioral economic analytic approach, we hypothesized that yohimbine *vs* placebo pretreatment would increase demand inelasticity and responding for the *mu*-opioid agonist hydromorphone. We also hypothesized that active yohimbine doses would alter biobehavioral markers beyond resting-state or placebo-pretreatment levels, including increases in blood pressure and negative mood.

Materials and Methods

Participants

The Wayne State University IRB approved all procedures. This study was conducted according to Declaration of Helsinki guidelines adopted by the National Institutes of Health. A certificate of confidentiality was obtained. Male and female volunteers from 18–55 yr, self-identifying as regular heroin users and not presently seeking treatment for their problematic opioid use, were recruited using advertisements and word-of-mouth referral. Candidates were screened using medical history, routine blood and urine chemistry, electrocardiogram, tuberculin test, physical exam, and psychiatric interview (SCID-IV; First et al. 1996). Volunteers were excluded who met DSM-IV criteria for current Axis I disorders (except Opioid and Nicotine Dependence), reported chronic health problems or taking prescribed medications, were cognitively impaired (IQ < 80) based on the Shipley Institute of Living Scale (Zachary, 1991), or scored more than 15 on the 10-item Injection and Blood Withdrawal Phobia subscale of the Medical Fear Survey (Kleinknecht et al. 1999).

During screening, volunteers had to provide a urine sample positive for opioids (> 300 ng/ml) and negative for methadone, benzodiazepines (< 300 ng/ml) and barbiturates (200 ng/ml). Urine samples positive for cocaine (> 300 ng/ml) or THC (> 50 ng/ml) were allowed but subjects who met DSM-IV criteria for Cocaine or Cannabis Use Disorders were excluded. Volunteers had to provide an alcohol-free breath sample (< .002%). Volunteers provided separate written informed consent for screening and the study.

Study Design

This experiment had two parts. In part 1 (drug sampling; two sessions), each participant was exposed to hydromorphone 12 mg and 24 mg in counterbalanced order. Individuals who reported greater subjective effects to hydromorphone 24 mg than 12 mg, with no adverse effects, were allowed to continue. In part 2 (drug vs. money choice; six sessions), a within-subject randomized crossover design was used to test effects of Yohimbine Dose (0, 16.2

and 32.4 mg) and Hydromorphone unit dose (1 and 2 mg) on opioid seeking. The six sessions were required to evaluate the effects of the three yohimbine doses (in the presence of the two hydromorphone unit doses) or…under both hydromorphone unit dose conditions.

Protocol Timeline

Participants were initially stabilized on buprenorphine 8-mg/day for ≥10 outpatient days before inpatient admission, and duration of inpatient stay was typically 12 nights. Residential living, staff observation and daily urinalyses ensured abstinence from unsanctioned drug use during study procedures. Testing was conducted on consecutive weekdays. During non-experimental periods, volunteers could engage in recreational activities, e.g., read, listen to music, ride an exercise bicycle, watch television or movies, do arts and crafts, and make telephone calls.

Hydromorphone sampling—Subjects sampled hydromorphone on the first two inpatient weekdays (11:30 am – 3:00 pm). Injections in sessions 1 and 2 were called “Drug A” and “Drug B”, respectively. Participants were asked to attend to effects of each drug and instructed that, during later choice sessions, they could work for $1/12^{th}$ units of these sampled drug doses *vs* money. Whether hydromorphone 12 mg or 24 mg served as Drug A or Drug B was counterbalanced across subjects.

Hydromorphone vs. money choices—Six choice sessions were conducted during the subsequent inpatient weekdays (8:30 AM – 4:30 PM). Hydromorphone (1 and 2 mg) and money ($2) unit amounts, progressive ratio schedule parameters, and task instructions (similar to Greenwald and Hursh 2006) were as follows:

> “Today, you can work *for* Drug ___ (A or B) or money during the session. From 10:00 AM to 1:00 PM, you can work for all or part of Drug __ (A or B), money or neither. There will be 12 trials. On each trial, you will see the words ‘Drug’ and ‘Money’ on the computer screen. Once you complete a single key press on one option, you will be committed to that choice and a box will appear on the screen surrounding whatever option you have chosen for that trial. However, once you complete responding for that trial, you are again free to choose drug or money for the next trial. If you respond for money, you will earn $2 per trial that you complete. If you respond for drug, you will earn $1/12^{th}$ of the total drug (A or B) per trial that you complete. If you choose neither, then you will not earn any money or any drug for that trial. Please be aware that even if you choose all money, you will still receive a placebo (blank) injection.” This last contingency (in addition to excluding individuals with injection phobia) reduced the possibility that participants would choose money simply to avoid an injection.

Participants began the choice progressive ratio task at 10:00 AM. A sign was posted to remind the participant which maximum hydromorphone dose (Drug A or Drug B from sampling) they could work for during each choice session. Across the top of the computer screen, two colored rectangular boxes arranged side-by-side were labeled Money (green) and Drug (red); across the middle of the computer screen, rectangular boxes arranged side-by-side (in corresponding colors) indicated the number of units earned for money and drug; and at the bottom of the computer screen, another box counted down the time remaining (sec) in the session. Immediately after the participant completed each choice, a tone sounded to indicate they had earned the unit amount of money ($2.00) or drug. Next, a different screen appeared for 10 sec (inter-trial interval), during which responding had no consequences and a timer counted down. After this time-out period, the original display re-appeared to begin the next choice opportunity.

During the 3-h session, participants were prohibited from reading, smoking cigarettes, eating, watching television, and remained seated (except for bathroom breaks) until time expired. Participants could drink water but not other beverages. Three hours after the task began (1:00 PM), the computer program quit unless the participant had already completed all 12 choices (at which time the program quit), and the amount of earned hydromorphone was delivered. The participant was told the final number of drug and money choices earned in each session, and signed his/her subject identification code to provide a written record of drug and money earnings.

Drug Administration

Buprenorphine—Participants consumed buprenorphine 8-mg sublingual tablets during initial outpatient and inpatient periods or, during detoxification, multiple 2-mg tablets and matching placebos (Subutex™; Reckitt-Benckiser, Hull, UK; from Research Triangle Institute, Research Triangle Park, NC). During the inpatient study, buprenorphine was always administered at 8:00 PM, i.e., more than 12 h before each experimental session. As in our prior studies, the intermediate buprenorphine maintenance dose (8 mg/day) was intended to help participants feel comfortable (minimal withdrawal symptoms) while living on the inpatient unit without access to heroin, but low enough so that we could surmount buprenorphine's blockade to observe agonist – including reinforcing – effects of hydromorphone.

Hydromorphone—Doses of hydromorphone (Dilaudid-HP™ in 50 mg/5 ml ampoules; purchased from hospital pharmacy) were injected (constant volume = 2.4 ml) into the deltoid muscle under double blind conditions. Doses were 12 mg (1.2 ml hydromorphone and 1.2 ml physiological saline), 24 mg (2.4 ml hydromorphone) or, following the choice task, the response-contingent dose (variable).

Yohimbine—Yohimbine tablets (5.4 mg each; Glenwood Pharmaceuticals, Englewood, NJ) were crushed and placed with lactose filler inside multiple opaque size-0 capsules for oral administration. Placebo capsules contained only lactose. Yohimbine oral doses selected for this study (16.2 and 32.4 mg) were based on prior studies in which yohimbine was administered to healthy, non-dependent human subjects by the oral route to examine pharmacokinetics (Grasing et al. 1996 [21.6 mg]; Sturgill et al. 1997 [up to 21.6 mg]) and anxiogenic effects (Mattila et al. 1998 [0.8 mg/kg]), and by the intravenous route to examine anxiogenesis in healthy subjects (Cameron et al. 2000 [up to 10 mg]) and methadone-maintained subjects (Stine et al. 2002 [0.4 mg/kg]).

Measures

Subjective effects and vital signs—During each hydromorphone-dose sampling session, vital signs (respiration rate, oxygen saturation, heart rate and blood pressure) and subjective drug effects described below were assessed −0.5, 0.5, 1, 1.5, 2, 2.5 and 3 h relative to hydromorphone administration. During each drug/money choice session, yohimbine was administered at 9:30 AM and the choice task ran from 10:00 AM – 1:00 PM. Vital signs were measured −0.5, 0.3, 1, 1.5, 2, 2.5, 3 and 3.7 h relative to yohimbine (the last time point being just before receiving the earned hydromorphone dose). All subjective effects were evaluated at −0.5 h (baseline), 0.3 h (before choice task), 2 h (during choice task) and 3.7 h (after choice task), and opioid agonist and withdrawal symptoms were also assessed 1 and 3 h post-yohimbine.

Heroin craving was measured with a 10-item total score (S.T. Tiffany, personal communication, 11/23/99); this brief version derives from the Heroin Craving Questionnaire, which yielded a 34-item total score and subscales that have been sensitive to

naloxone-precipitated and spontaneous opioid withdrawal states (e.g., Schuster et al. 1995; Greenwald 2002; Greenwald 2005; Greenwald et al. 2003). Seven visual analog scale (VAS, 0–100) drug-ratings were obtained: Any Effect, Good Effect, Bad Effect, High, Liking, Stimulated, and Sedated. Opioid agonist symptoms (16 items) and opioid withdrawal symptoms (16 items) were self-rated (Schuster et al. 1995). Each item was scored from 0 (not at all) to 4 (extremely), yielding total subscale scores ranging from 0 to 64. The Profile of Mood States (POMS; McNair et al. 1971) was used to assess mood on eight subscales: Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, and Elation; and two composite scales, Arousal and Positive Mood. Participants rated each item (0 to 4) based on their momentary mood.

Upon completion of each sampling session, participants made choices between that dose and 44 different money amounts from $0.25 to $25.00 using a modified Multiple Choice Procedure (Griffiths et al. 1993) questionnaire. The amount at which the participant switched from choosing drug to money reflects drug monetary value.

Drug reinforcement—During choice sessions, a 12-trial choice progressive ratio procedure was used. Across trials within session, response requirements for hydromorphone (1 or 2-mg units) and money ($2.00) options increased independently in an exponential function: 125, 225, 365, 590, 950, 1500, 2300, 3415, 4915, 6875, 9375 and 12500. This schedule was used to define the increasing unit prices of each reinforcer (response requirement ÷ unit dose) across 12 independent choice trials within the session. This schedule provides three advantages for measuring drug reinforcement. First, it offers a highly efficient method for evaluating drug reinforcing efficacy as a function of increasing response requirement. Second, by varying the unit dose per completed fixed ratio in combination with different response requirements, 24 unit prices across a 200-fold range (62.5–12500) were investigated. Third, this schedule avoids direct effects of the drug reinforcer by delaying its delivery until the choice session is completed. Measures of drug reinforcing efficacy included total number of drug choices and breakpoint (highest response requirement completed) prior to receipt of drug, i.e., seeking behavior, as the total earned hydromorphone dose was injected as a bolus immediately after the session.

Money reinforcement—Participants could earn $40 nightly for living on the inpatient unit and $2 per money choice (for a maximum of $24 if they worked only for money) in each of the 6 sessions. Payments were disbursed at discharge (all choice earnings plus half of inpatient nights), and the remainder (other half of inpatient nights) was given in two payments during buprenorphine dose tapering.

Data Analyses

All analyses were conducted using SPSS v.20, except for behavioral economic regressions that used GraphPad Prism® (described below). For all analyses, the criterion for null hypothesis rejection was $P < 0.05$. Huynh-Feldt adjusted P values were used for sphericity violations.

Sampling sessions—Subjective effects and vital signs during hydromorphone sampling sessions were analyzed using two-way Hydromorphone Dose (12 and 24 mg) X Session Time repeated measures Analyses of Variance (ANOVAs).

Choice sessions—Subjective effects and vital signs during hydromorphone choice sessions were first analyzed using three-way Yohimbine Dose (0, 16.2 and 32.4 mg) X Hydromorphone Unit Dose (1 and 2 mg) X Session Time (varied, depending on measure) repeated measures ANOVAs. There were no significant pre-yohimbine baseline differences

between conditions. Due to different timing of assessments, area-under-the-curve (AUC) scores were computed from 0.3 until 3.7 h post-yohimbine. Two-way Yohimbine Dose X Hydromorphone Unit Dose repeated measures ANOVAs were conducted on these AUC scores. Number of hydromorphone choices and breakpoints were analyzed using two-way Yohimbine Dose X Hydromorphone Unit Dose ANOVAs.

Behavioral economic analyses—Drug unit prices were defined as progressive ratio schedule response requirements divided by hydromorphone unit dose. As in prior studies (e.g., Greenwald and Hursh 2006), group demand curve analyses were conducted. When binary-coded (0/1) drug choices for each individual are averaged across volunteers at each unit price in each condition, "group-percent choice" across unit prices can be analyzed using behavioral economic assumptions. Within GraphPad Prism® v.4 (San Diego, CA, USA), a demand curve on unit prices was fit to the $\log_{10}$–transformed group-percent choice, using the exponential regression equation: $Y=\log(L) * \exp(-A^{*}X)$

In this equation, parameter Y is group-percent choice; L is level of drug choice at the lowest unit price (demand *intensity*); X is unit price; and A is rate of change in slope (demand *elasticity*). To evaluate the effect of yohimbine dose, the ANOVA function in GraphPad Prism® tested whether L and A parameters of the group-percent hydromorphone choice curves were explained by a single model (accept null hypothesis) or not (reject null hypothesis) based on goodness-of-fit criteria, i.e., sums of squares of the vertical distances of data points from the curve. P_{max} (unit price on the curve where slope equals −1) is a derived measure that equals $0.29 \div A$ (Greenwald and Hursh 2006) and is reported here because it is more readily understood than the slope index.

The response output curve was constructed in a similar manner. At each unit price, sum of group responding was calculated (i.e., number of subjects X response requirement completed). This metric, corresponds to behavior "spent" on hydromorphone at a given unit price by this "market"; the point of peak responding on the curve is called O_{max}. Figure 2 (lower left) illustrates second-order polynomial (bitonic) curves fit to these data, where X is unit price and Y is total group responding (Table 3).

Results

Participants

Ten participants completed the study: 7 males (3 white, 3 African American and 1 Hispanic) and 3 females (2 white and 1 African American). These individuals were (mean ± SD) 43.2 ± 10.5 years old (range, 25–54), had 12.1 ± 1.4 years of education (range, 9–14), and used heroin regularly for 13.3 ± 10.5 years (range, 1–31). Primary route of heroin use was intravenous for 5 volunteers and intranasal for 5 volunteers. Nine of the 10 participants reported daily tobacco use and, these smokers consumed 13.0 ± 4.5 cigarettes/day (range, 7–20).

Sampling Sessions

Table 1 summarizes hydromorphone sampling session data. Multiple Choice Procedure crossover points were significantly higher for the hydromorphone 24 mg than 12 mg sampling dose. Relative to hydromorphone 12 mg, the hydromorphone 24 mg dose significantly increased opioid agonist symptoms, POMS Fatigue and Friendliness scores, and VAS drug ratings of Any Effect, Good Effect, High, Liking, and Stimulated (Table 1). There were no significant hydromorphone dose differences on other subjective effects or any vital signs measures.

Choice Sessions

Subjective and physiological effects—There were no significant Hydromorphone Unit Dose effects on subjective or physiological measures (as expected, because subjects were not exposed to hydromorphone until after the choice task); hence, we focus on Yohimbine Dose effects. Effects of yohimbine are summarized in Table 2 (AUC scores) and selected yohimbine dose- and time-related effects are shown in Figure 1. Pretreatment with yohimbine 16.2 and 32.4 mg *vs* placebo significantly increased opioid withdrawal symptoms (partial *eta*-squared [effect size], $\eta^2 = 0.40$) and decreased opioid agonist symptoms ($\eta^2 = 0.30$), but did not significantly increase heroin craving ($\eta^2 = 0.07$). Yohimbine significantly decreased ratings on the POMS Elation scale ($\eta^2 = 0.30$). Yohimbine marginally (*p*s < .10) increased ratings on the POMS Anxiety scale ($\eta^2 = 0.24$) and VAS Bad Effect ($\eta^2 = 0.28$). Yohimbine significantly increased systolic blood pressure ≈15 mmHg ($\eta^2 = 0.67$) and diastolic blood pressure ≈10 mmHg ($\eta^2 = 0.61$). Yohimbine did not significantly alter other vital signs.

Drug choices and breakpoints—Table 3 summarizes measures of hydromorphone reinforcing efficacy, separately for each unit dose. There was a robust hydromorphone unit-dose effect: Hydromorphone choices were significantly higher for the 2-mg than 1-mg unit dose across yohimbine doses ($F[1,9] = 11.17$, $p = 0.01$, $\eta^2 = 0.55$), and similarly for breakpoints ($F[1,9] = 7.20$, $p = 0.03$, $\eta^2 = 0.44$). Pretreatment with yohimbine increased hydromorphone choices ($F[2,18] = 2.22$, $p = 0.14$, $\eta_2 = 0.20$) and breakpoints ($F[2,18] = 3.03$, $p = 0.08$, $\eta^2 = 0.25$) independent of unit dose, but these overall effects were not statistically significant.

Behavioral economic measures—As expected, group-percent hydromorphone choices exhibited a positively decelerating relationship to unit prices. At unit prices < 900, hydromorphone demand was inelastic across experimental conditions. At unit prices > 900, demand became elastic but this transition depended on the hydromorphone unit dose and yohimbine pretreatment condition.

Yohimbine significantly altered the overall hydromorphone demand curve, i.e., when using both hydromorphone unit doses to define unit prices. Table 3 indicates that active yohimbine (*vs* placebo) pretreatment increased hydromorphone demand inelasticity, as reflected in significantly smaller *A* slope parameter, leading to higher derived P_{max} value. Figure 2 shows that hydromorphone 2-mg curves were shifted upward (increased demand intensity) *vs* hydromorphone 1-mg curves, and attempts to fit the same curves using unit prices from both hydromorphone doses were not optimal. Thus, for clarity, the effect of yohimbine dose on opioid demand was analyzed (Table 3) and plotted (Figure 2) separately for each hydromorphone unit dose. The *L* parameter of the regression equation (peak demand at the lowest unit price) was adjusted for each hydromorphone unit dose, given that group-percent choice reached 100% at the lowest price for the 2-mg unit dose (Figure 2 right panel), but only 70% at the lowest price for the 1-mg unit dose (Figure 2 left panel).

In the absence of yohimbine (placebo pretreatment), indices of hydromorphone demand (*A* slope and P_{max}) were nearly 3-fold higher for the 2-mg than 1-mg unit dose (see Table 3). For the 1-mg unit dose, hydromorphone demand increased nearly 4-fold during exposure to yohimbine 16.2 and 32.4 mg doses *vs* placebo (P_{max} = 3648 and 3255 vs. 909, respectively). In contrast, for the 2-mg unit dose, hydromorphone demand showed a non-significant increase during exposure to yohimbine 16.2 and 32.4 mg doses *vs* placebo (P_{max} = 3194 and 3616 vs. 2656, respectively).

It is also helpful to interpret yohimbine-induced alterations in opioid demand in terms of changes in hydromorphone group-percent choice in proportion to changes in unit price. As

shown in the best-fit demand curves, when unit price escalated 10-fold from 125 to 1250 we observed 71.2% reduction in hydromorphone 1-mg choice in the yohimbine placebo condition indicative of price-elastic demand, but only 25.1% and 48.2% reductions in hydromorphone 1-mg choice in the yohimbine 16.2 and 32.4 mg conditions, respectively (Figure 2 left panel). In short, yohimbine made hydromorphone 1-mg demand more price-resistant, such that participants defended opioid choice against price increases more so than after placebo pretreatment. Across this same 10-fold range of unit prices, hydromorphone 2-mg choice decreased 35.9% in the yohimbine placebo condition, and 29.7%, and 31.6% reductions in the yohimbine 16.2 and 32.4 mg conditions, respectively (Figure 2 right panel). Thus, active yohimbine doses altered price-sensitivity more for hydromorphone 1-mg than for 2-mg unit doses.

As Table 3 and Figure 2 (lower panels) indicate, O_{max} (peak responding) values varied 12-fold from 2300 to 28,000 across experimental conditions, and were much higher for hydromorphone 2-mg than 1-mg. Within each hydromorphone unit dose, O_{max} values were significantly higher following active yohimbine *vs* placebo pretreatment. For the hydromorphone 1-mg unit dose, yohimbine *vs* placebo produced an upward shift in drug seeking at unit prices < 3000 (i.e., more subjects responding for hydromorphone at low response requirements), and increased responding (rightward extension of curve) among subjects who continued to seek hydromorphone at unit prices > 3000.

Discussion

The principal finding of this study is that the α_2-antagonist yohimbine significantly increased behavioral economic demand and responding for the *mu*-opioid hydromorphone in heroin-dependent volunteers. This finding corresponds with substantial preclinical data that yohimbine robustly and reliably potentiates non-cued drug seeking (Feltenstein and See 2006; Gass and Olive 2007; Lê et al. 2005; Shepard et al. 2004), as well as cue-induced drug seeking (Banna et al. 2010; Buffalari and See 2011; Feltenstein et al. 2012). Notably, α_2-agonists (which reduce NA transmission by enhancing pre-synaptic inhibition) block footshock-induced reinstatement of drug seeking in rats (Erb et al. 2000; Shaham et al. 2000b) and can attenuate psychological stressor-reactivity in opioid-detoxified patients (Sinha et al. 2007).

In the present study yohimbine increased opioid seeking behavior in heroin-dependent individuals who were maintained on buprenorphine 8mg/day, a dose that suppressed baseline opioid withdrawal symptoms to negligible levels. It is thus unlikely that these participants sought hydromorphone purely to relieve opioid withdrawal symptoms in the absence of yohimbine. During the 3-h choice task when yohimbine enhanced responding to earn hydromorphone, yohimbine concurrently increased opioid withdrawal and decreased opioid agonist symptoms although withdrawal symptom elevations were not clinically significant (relative to other work from our laboratory using identical measures). These results are consistent with a study of methadone-maintained patients that showed yohimbine (0.4 mg/kg IV) *vs* placebo infusion increased opioid craving, wtihdrawal symptoms, anxiety, blood pressure and cortisol levels (Stine et al. 2002). In preclinical studies, the effects of stressors are typically examined in a reinstatement paradigm, in which responding is not reinforced with drug delivery (i.e., drug seeking behavior); however, one study in rats showed that a footshock stressor could increase heroin seeking during heroin maintenance and 24 h after during spontaneous heroin withdrawal (Shaham et al. 1996). Taken together, it appears the trophic effect of stressors on drug reinforcement may generalize across phases of the addictive cycle (e.g., maintenance, detoxification, abstinence).

One hypothesis that could be examined in future studies with opioid dependent individuals is whether opioid-seeking behavior is directly related to withdrawal-related effects *vs* yohimbine-induced effects that are independent of opioid withdrawal. Buprenorphine is a partial *mu*-opioid agonist, but also an antagonist at *kappa* and *delta* receptors. Given that the *kappa*-antagonist JDTic could attenuate footshock-induced reinstatement of drug seeking (Beardsley et al. 2005) and a *delta*-antagonist could attenuate yohimbine reinstatement of ethanol seeking (Nielsen et al. 2012), buprenorphine's *kappa* or *delta* antagonist actions might have limited yohimbine's effect on opioid seeking in this study. This hypothesis should be evaluated in other drug abusing populations where physical dependence is a lesser issue, as it might offer useful clues for anti-stressor medication development.

Recent preclinical work has demonstrated that the α_2-agonist guanfacine reduced anxiety-like behavior in rats withdrawn from cocaine self-administration and yohimbine-facilitated reinstatement of cocaine seeking (Buffalari et al. 2012). In another study, a high dose of guanfacine attenuated yohimbine-induced reinstatement of alcohol (but not food) seeking whereas the α_1-agonist prazosin blocked yohimbine-induced reinstatement of alcohol and food seeking, as well as footshock-induced reinstatement of alcohol seeking (Lê et al. 2011). Similarly, in clinical studies that all used stressful vs. neutral mental imagery procedures, prazosin attenuated stressor-induced alcohol craving (Fox et al. 2012), and the α_2-agonists clonidine and lofexidine blunted stressor-induced craving for both cocaine (Jobes et al. 2011) and opioids (Sinha et al. 2007). Therefore, noradrenergic medications with pharmacological actions that at least partially oppose those of yohimbine seem to represent one potential approach for blocking the ability of stressors to increase behavior maintained by various drug and non-drug reinforcers.

In the present study, behavioral economic metrics were more sensitive to the effect of yohimbine on opioid reinforcement than choice and breakpoint indices. The behavioral economic approach offers conceptual and analytic advantages over conventional measures of drug reinforcement. First, the influences of several cost and benefit parameters can be parsimoniously integrated within the single independent variable, unit price (Bickel et al. 1993). Second, graphical and analytical data from behavioral economic analyses such as intercept (L), slope (A), P_{max}, and O_{max} offer insights that are not obvious from conventional response data, e.g., downward curve shift indicating fewer subjects responding at low prices, and/or increased elasticity indicating that all subjects are more sensitive to unit price changes. Furthermore, the number of drug choices is neutral to response requirement, and breakpoints depend on characteristics of the progressive ratio schedule (i.e., number of trials and step size) and unit doses/amounts, resulting in findings that cannot readily be compared across studies. For instance, a recent study from this laboratory also found that behavioral economic metrics provided greater sensitivity than choice and breakpoint measures when examining the effect of punishment on hydromorphone reinforcement (Greenwald, 2010).

The present study is the first placebo (expectancy)-controlled, dose (intensity)-dependent clinical evaluation of a longer-acting stressor on drug seeking behavior. To date, human experimental studies in this area are limited because they have examined effects of psychogenic stressors (which cannot be experimentally blinded) that are brief (which may not resemble the natural ecology and are subject to habituation), have uniform intensity (whereas stressor effects likely depend on severity), and have measured only drug craving (which may not correlate with drug seeking). In the model used here, participants could make multiple drug vs. money choices, with each successive choice requiring greater responding, and earned hydromorphone delivery was delayed until the end of the 3-h task. A strength of this procedure (in addition to the strengths noted above) is that responding maintained by the drug option can be interpreted as a pure measure of "drug seeking"

behavior, in contrast to experimental models in which interim drug deliveries could produce satiety or rate-altering effects. This contingency arrangement enables the examination of the effect of yohimbine or other potential stressors on drug *motivation*. On the other hand, it is not possible to directly address whether yohimbine can alter ongoing drug consumption, because participants received their response-contingent hydromorphone dose when the effects of yohimbine were beginning to subside. Thus, studies using this model should attend to potential implications of this methodological difference.

This study also has several limitations, with implications for future work. First, while yohimbine significantly altered biobehavioral markers, effects were clinically modest, e.g., peak blood pressure was not hypertensive for most participants. These data suggest that participants were exposed to a relatively low yohimbine dose, that oral bioavailability of yohimbine might have been restricted and perhaps highly variable across individuals as has been reported (Grasing et al. 1996; Sturgill et al. 1997) or, as noted above, buprenorphine *kappa*/*delta* antagonism played a role. The finding that yohimbine dose-responses were mild but still potentiated drug seeking is consistent with a similar observation using a footshock stressor in rats (Shaham and Stewart 1994). Importantly, yohimbine-induced opioid seeking behavior and subjective craving were dissociated in this study, suggesting that craving may be a less sensitive measure of reactivity to stressors. Interestingly, Stine et al. (2002) observed an increase in craving in methadone-stabilized volunteers following intravenous yohimbine administration; thus, the discrepancy between that study and the present findings could be due to differing measures of craving (e.g., relative to a single-item scale, the 10-item scale used here is a broader index although insensitive items could dilute its value), methadone-maintained treatment seeking patients versus buprenorphine-maintained non-treatment volunteers, or route of yohimbine delivery (e.g., IV administration could potentiate craving more than oral administration) and subjects' drug use histories. Second, yohimbine biobehavioral effects had not peaked when the choice task started, which suggests the impact of yohimbine on hydromorphone-maintained responding could be underestimated. Based on these considerations, our ongoing work is examining effects of higher yohimbine doses and a longer pretreatment time on drug seeking behavior. Third, a significant hydromorphone unit dose effect may have limited the ability of yohimbine to potentiate hydromorphone 2mg-responding. This unit dose effect produced a parallel demand curve (intensity) shift, necessitating separate elasticity analyses for the two unit doses of hydromorphone. A previous study using these same hydromorphone doses in heroin-dependent, buprenorphine-maintained volunteers demonstrated both functional equivalence, i.e., hydromorphone demand was independent of unit dose, as well as more inelastic demand (Greenwald and Hursh 2006). The failure to find functional equivalence in the present study may be due to differences between these two study samples. Fourth, this proof-of-concept study used a small sample size, which may explain why some subjective measures of yohimbine effect (e.g., POMS Anxiety and VAS 'Bad Drug Effect') only exhibited trends; in general, effect sizes ranged from being relatively small to moderate, suggesting that some tests were underpowered and broader conclusions may be limited until these outcomes are evaluated in larger-scale studies. Finally, we did not assess the effects of yohimbine on cortisol response, which could have been useful for measuring HPA axis activation.

In summary, the approach pursued in the present study aligns with a model of addiction in which chronic substance use leads to neurobehavioral counter-adaptations (e.g., allostasis, altered hedonic set point) that dysregulate motivation (Koob 2008). This problem led us to stabilize opioid-dependent individuals on buprenorphine while being studied for stress-responsiveness. Although the brain's system for responding to stressors is more sensitive during drug abstinence (Koob 2010; Smith and Aston-Jones 2008), testing this hypothesis would require parametrically (orthogonally) varying opioid abstinence and stressor levels. In

the first phase of our work, we sought to disentangle opioid withdrawal discomfort (which buprenorphine suppressed to low levels) from biobehavioral responses precipitated by yohimbine. Future studies could address this clinically relevant interaction, and explore neurochemical pathways that modulate gain of the stressor-response system in drug-appetitive behavior, toward the long-term goal of developing medications to attenuate stressor-potentiated drug abuse and relapse.

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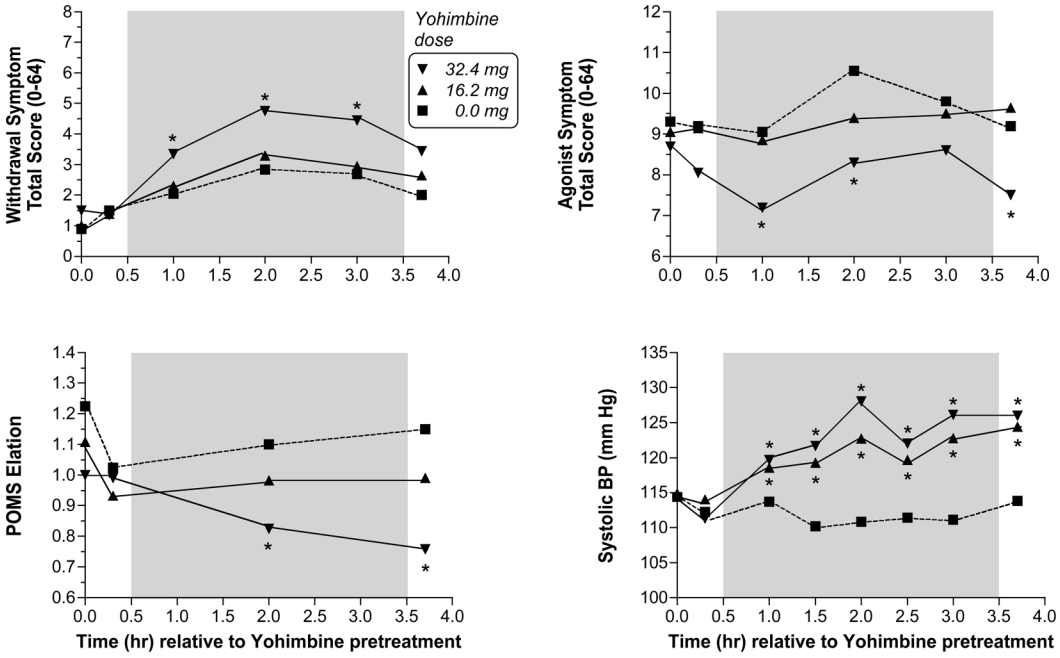

Figure 1.

Statistically significant effects of oral yohimbine dose (0, 16.2 and 32.4 mg) across time on subjective and physiological responses during choice sessions. Gray squares indicate the 3-h period when the drug vs. money choice task occurred. For each measure illustrated, post-yohimbine area under the curve (0.3 – 3.7 h time point) scores showed a dose-related effect (see Table 2). Asterisks indicate the time points at which an active yohimbine dose significantly ($p < .05$) differed from the placebo condition. *Upper left*: Yohimbine increased opioid withdrawal symptom scores. *Upper right*: Yohimbine decreased opioid agonist symptom scores. *Lower left*: Yohimbine decreased Profile of Mood States (POMS) Elation scale scores. *Lower right*: Yohimbine increased systolic blood pressure (as well as diastolic blood pressure; not shown).

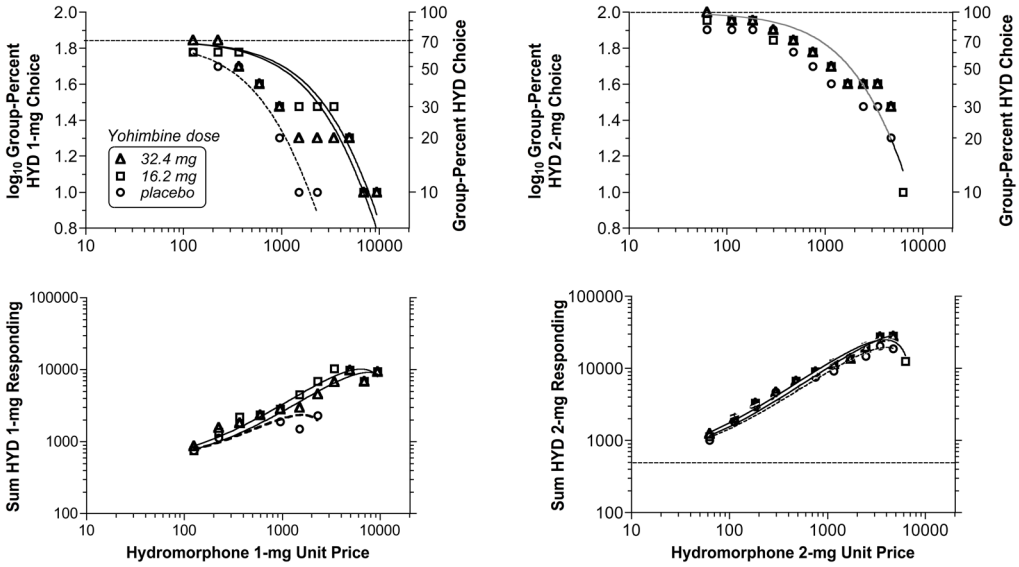

Figure 2.

Upper panels Effects of oral yohimbine pretreatment dose (0, 16.2 and 32.4 mg) on demand for hydromorphone (HYD) 1-mg unit dose (*upper left*) and 2-mg unit dose (*upper right*). Each panel shows the relationship between HYD unit prices (response requirements of the progressive ratio schedule [125, 225, 365, 590, 950, 1500, 2300, 3415, 4915, 6875, 9375 and 12500] divided by the HYD unit dose) and $\log_{10}$ group-percent HYD choice (left ordinate) and corresponding absolute group-percent choice (right ordinate). Yohimbine 16.2 and 32.4 mg doses *vs* placebo significantly increased demand inelasticity with the HYD 1-mg unit dose (3.5 to 4-fold rightward curve shifts in left panel) whereas yohimbine produced a non-significant increase in demand inelasticity with the 2-mg unit dose (hence, a single curve fit is used in the right panel, reflecting the null hypothesis). The horizontal dashed line in each panel represents peak choice at the lowest unit price (parameter L = 70 for the 1-mg unit dose, and L = 100 for the 2-mg unit dose); this regression parameter was incorporated in the separate non-linear curve fits. See Table 3 for elasticity (A parameter), P_{max} and curve fit (R^2) values. *Lower panels*: Effects of oral yohimbine dose on total group responding for (HYD) 1-mg unit dose (*lower left*) and 2-mg unit dose (*lower right*). Curves reflect overall drug seeking, and were fit by the second order polynomial equation: $Y=A+BX+CX^2$, where A = intercept (fixed at 500 here), B and C are slope parameters, X = unit price and Y = total group responding. Actual group responding data are the points fitted by the curves. O_{max} is located at the peak of each bitonic response curve. See Table 3 for O_{max} and curve fit values.

Table 1

Sampling Sessions: Hydromorphone Post-Drug (3-hr Area Under the Curve) Responses

	Hydromorphone Dose		
Measure	**12 mg**	**24 mg**	**Dose *F[1,9]* (*p*)**
MCP crossover ($)	5.70 (1.82)	11.05 (1.87)	**6.66 (.03)**
Opioid scales (0 – 64)			
Agonist	13.3 (1.6)	17.7 (1.9)	**14.19 (.005)**
Antagonist	1.6 (0.6)	2.2 (0.7)	1.90 (.21)
Heroin craving (7 – 70)	25.4 (5.2)	26.9 (4.4)	0.66 (.44)
POMS scales			
Anxiety	0.36 (0.07)	0.39 (0.08)	1.42 (.26)
Depression	0.13 (0.05)	0.16 (0.07)	1.91 (.20)
Anger	0.08 (0.04)	0.11 (0.06)	1.29 (.29)
Vigor	1.53 (0.28)	1.65 (0.26)	1.44 (.26)
Fatigue	0.15 (0.08)	0.26 (0.11)	**7.28 (.03)**
Confusion	0.54 (0.07)	0.55 (0.08)	0.08 (.78)
Friendliness	1.90 (0.24)	2.06 (0.25)	**6.86 (.03)**
Elation	1.39 (0.22)	1.53 (0.23)	3.41 (.10)
Arousal	1.20 (0.30)	1.24 (0.29)	0.14 (.72)
Positive Mood	1.27 (0.22)	1.37 (0.21)	2.46 (.16)
VAS ratings (0 – 100)			
Any drug effect	22.4 (7.1)	38.3 (7.0)	**6.56 (.04)**
Good drug effect	28.0 (8.2)	43.6 (8.0)	**7.02 (.03)**
Bad drug effect	3.3 (1.3)	4.5 (1.9)	2.43 (.15)
High	24.2 (8.3)	40.1 (7.9)	**11.62 (.01)**
Like Drug	30.4 (8.7)	46.6 (9.4)	**13.79 (.01)**
Stimulated	17.1 (6.0)	32.7 (8.6)	**7.23 (.03)**
Sedated	19.9 (6.9)	29.3 (6.6)	3.49 (.10)
Vital signs			
Systolic BP (mm Hg)	116.0 (4.6)	113.5 (3.2)	0.80 (.40)
Diastolic BP (mm Hg)	70.6 (3.2)	70.1 (2.9)	0.14 (.72)
Heart rate (bpm)	64.8 (2.4)	68.0 (3.2)	1.71 (.23)
Respiration rate (bpm)	15.5 (0.6)	14.2 (0.8)	2.98 (.12)
Oxygen saturation (%)	97.6 (0.3)	97.3 (0.2)	0.85 (.38)

Table 2

Choice Sessions: Yohimbine Post-Drug (Mean ± 1 SEM Area Under the Curve) Responses

	Yohimbine Dose			Dose *F[2,18]* (*p*)
Measure	**0 mg**	**16.2 mg**	**32.4 mg**	
Opioid scales				
Agonist	34.4 (5.6)	33.0 (4.6)	28.4 (4.8)	**3.78 (.05)**
Antagonist	8.3 (2.5)	9.4 (2.5)	13.3 (2.5)	**5.98 (.03)**
Heroin craving	85.6 (15.5)	84.9 (12.7)	95.7 (16.3)	0.67 (.49)
POMS scales				
Anxiety	1.39 (0.24)	1.37 (0.19)	1.75 (0.19)	2.81 (.09)
Depression	0.39 (0.12)	0.26 (0.09)	0.30 (0.11)	1.18 (.33)
Anger	0.23 (0.09)	0.23 (0.09)	0.27 (0.10)	0.14 (.83)
Vigor	4.41 (1.07)	4.21 (1.01)	4.09 (0.88)	1.36 (.29)
Fatigue	0.46 (0.18)	0.38 (0.15)	0.35 (0.12)	0.31 (.74)
Confusion	1.70 (0.20)	1.77 (0.19)	1.64 (0.17)	0.32 (.74)
Friendliness	5.71 (0.83)	5.86 (0.70)	5.74 (0.77)	0.10 (.90)
Elation	3.87 (0.78)	3.45 (0.72)	3.04 (0.65)	**3.82 (.05)**
Arousal	3.64 (1.10)	3.43 (1.12)	3.86 (0.80)	0.57 (.56)
Positive Mood	3.48 (0.83)	3.22 (0.69)	2.84 (0.67)	2.37 (.13)
VAS ratings				
Any drug effect	19.6 (8.8)	17.0 (5.5)	21.5 (7.9)	0.12 (.79)
Good drug effect	23.5 (14.7)	14.4 (5.0)	7.8 (3.2)	0.98 (.36)
Bad drug effect	11.3 (4.4)	15.6 (6.7)	32.7 (10.7)	3.54 (.06)
High	7.7 (3.1)	8.5 (3.7)	5.2 (2.4)	0.76 (.45)
Like Drug	26.4 (14.0)	20.2 (9.0)	9.1 (4.6)	1.44 (.27)
Stimulated	16.5 (5.9)	12.4 (3.8)	13.0 (6.5)	0.19 (.74)
Sedated	27.3 (15.6)	18.2 (7.1)	9.3 (3.6)	1.54 (.25)
Vital signs				
Systolic BP (mm Hg)	396.9 (10.9)	426.2 (10.8)	433.0 (9.6)	**18.55 (.0001)**
Diastolic BP (mm Hg)	245.5 (10.3)	259.8 (9.1)	266.4 (9.0)	**13.84 (.0001)**
Heart rate (bpm)	238.8 (10.3)	236.2 (9.2)	240.2 (9.9)	0.27 (.77)
Oxygen saturation (%)	350.2 (0.7)	350.6 (0.7)	350.1 (0.7)	0.52 (.57)

Table 3

Yohimbine Dose Effects on the Reinforcing Efficacy of Hydromorphone (HYD) Unit Doses

	Yohimbine Dose			Yohimbine Dose Effect Test
Measure	**0 mg**	**16.2 mg**	**32.4 mg**	
Choices (Mean ± 1 SEM)				
HYD 1 mg	2.40 (0.81)	3.80 (1.32)	3.60 (1.20)	$F(2,18)= 1.76$, $p<.20$
HYD 2 mg	5.90 (1.30)	6.80 (1.30)	6.90 (1.17)	$F(2,18)= 0.46$, $p=.61$
Breakpoints (Mean ± 1 SEM)				
HYD 1 mg	492 (226)	1903 (989)	1665 (976)	$F(2,18)= 2.15$, $p<.17$
HYD 2 mg	3267 (1210)	4361 (1483)	4083 (1305)	$F(2,18)= 0.69$, $p=.50$
A slope parameter[§]				
Both doses	.0001474	.0000857[∞]	.0000894[∞]	**$F(2,60)= 4.46$, $p<.02$**
HYD 1 mg	.0003189	.0000795[∞]	.0000891[∞]	**$F(2,26)=22.07$, $p<.0001$**
HYD 2 mg	.0001092	.0000908	.0000802	$F(2,31)= 2.01$, $p<.16$
Demand curve fit (R^2)				
Both doses	.343	.806	.707	
HYD 1 mg	.932	.818	.691	
HYD 2 mg	.765	.859	.761	
P_{max}[¶]				
Both doses	1967	3384	3244	
HYD 1 mg	909	3648	3255	
HYD 2 mg	2656	3194	3616	
O_{max} *(actual)* [£]				
HYD 1 mg	2,300	10,245	9,830	
HYD 2 mg	20,625	28,125	28,125	
O_{max} *(polynomial fit)*[£]				
HYD 1 mg	2,350	10,196	9,164	**$F(4,23)= 3.83$, $p<.02$**
HYD 2 mg	19,572	25,001	28,666	**$F(4,28)= 9.24$, $p<.0001$**
Responding curve fit (R^2)				
HYD 1 mg	.182	.859	.902	
HYD 2 mg	.977	.941	.984	

[§]Demand curve regression equation *L* parameter = 70% for hydromorphone 1-mg dose (Figure 2, upper left), *L* = 100% for hydromorphone 2-mg dose (Figure 2, upper right), and *L* = 85% for combined analysis across both hydromorphone unit doses. Unequal denominator degrees of freedom across yohimbine dose-effect tests reflect the differing number of non-zero percent choice data points fit by the demand curve (see text for further description).

[∞]Slope parameter *A* in the yohimbine 16.2 and 32.4 mg dose conditions significantly differed from the placebo condition, but did not differ from each other.

[¶]P_{max} is not statistically tested; it is calculated directly from the non-linear regression *A* parameter ($P_{max} = 0.29 \div A$) and can be interpreted as the hydromorphone unit price at which the demand curve slope = −1 (see Figure 2, upper panels).

[£]For each experimental condition, a response curve was fit by a second-order polynomial equation (see Figure 2, lower panels). Based on visual analysis, the equation *Y*-intercept value (A) was fixed = 500; thus, each equation solved for the B and C values. Degrees of freedom reflect the

number of parameters and non-zero data points fit. O_{max} corresponds to the peak of the bitonic response curve, and can be characterized by the observed data or the crest of the estimated curve; both are provided here.