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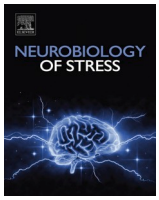

Basal cortisol level modulates stress-induced opioid-seeking behavior

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ABSTRACT

In preclinical studies and our human laboratory, the α_2-noradrenergic autoreceptor antagonist yohimbine was found to promote drug-seeking behavior. This study evaluated effects of dose-combinations of yohimbine and the glucocorticoid receptor agonist hydrocortisone to model intensity-dependent effects of stimulating each neurochemical system, alone and together, on stress-reactivity and opioid-seeking. Twelve regular heroin-using participants diagnosed with opioid use disorder (OUD) were stabilized on sublingual buprenorphine (8-mg/day), then passed a hydromorphone 18-mg vs. placebo intramuscular reinforcement screen. Across 9 experimental conditions (3 × 3 within-subject, randomized crossover, placebo-controlled, double-blind design) during inpatient buprenorphine maintenance, combinations of oral pretreatment doses of yohimbine (0, 27, 54-mg; $t = 0$ min) then hydrocortisone (0, 20, 40-mg; $t = 45$ min) were administered. In each condition, subjective drug and mood effects, cardiovascular responses, and saliva cortisol and α-amylase levels were assessed to evaluate stress-reactivity, and participants completed a 12-trial choice progressive ratio task during which they could earn units of hydromorphone (1.5-mg intramuscular) and/or money ($2.00). Yohimbine dose-dependently increased blood pressure, α-amylase, and anxiety scores, and decreased opioid agonist symptoms; hydrocortisone dose-dependently increased cortisol levels. Yohimbine/hydrocortisone dose-combinations significantly shifted within-session responding from money to opioid-seeking among participants with lower basal cortisol levels. These findings replicate yohimbine effects on stress biomarkers and demonstrate that noradrenergic/glucocorticoid-potentiated opioid-seeking is modulated by basal cortisol level. In persons with OUD stabilized on buprenorphine, basal HPA-axis activity and acute stressors can enhance opioid relative reinforcing efficacy. These factors may limit OUD treatment efficacy and highlight the need for novel interventions that prevent stress-induced opioid-seeking.

1. Introduction

Stressors (stimuli that challenge homeostasis) increase persistence of substance use (Greenwald, 2018; Koob, 2008; Sinha et al., 2011). Preclinical studies demonstrate that stressors including food deprivation (Carroll and Meisch, 1984), social isolation (Alexander et al., 1978; Bozarth et al., 1989), immobilization (Shaham et al., 1992), and intermittent footshock (Shaham and Stewart, 1994; 1996, 2000) enhance opioid-seeking among opioid-exposed animals. Yet, few human studies have experimentally manipulated stressors to determine effects on drug-seeking and self-administration. This is a critical area of inquiry, as stress-reactivity among persons with opioid use disorder (OUD) may predict overdose risk or treatment outcomes such as retention and ongoing opioid use (MacLean et al., 2019). There is an unmet need for improving outcomes related to stress-exposure, no FDA-approved interventions exist for treating stress-related drug use exist (although clonidine is used off-label to manage opioid withdrawal and may have stress-blunting effects), and there have been no systematic studies for this purpose.

Stressors activate multiple neurochemical systems (Joëls and Baram, 2009) including the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis (Banihashemi and Rinaman, 2006; Brown et al., 2009; Greenwald, 2018; Koob, 2008; Lee et al., 2004; Solecki et al., 2019). Within the SNS, noradrenaline has been found to modulate opioid self-administration (Aston-Jones and Harris, 2004; Davis et al., 1975; Jasmin et al., 2006; Olson et al., 2006; Shaham et al.,

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2000; Ventura et al., 2005; Weinshenker and Schroeder, 2007). In contrast, HPA-axis stimulation *alone*, e.g. administering hydrocortisone (or corticosterone in rodents), does not reliably increase drug-seeking (Mantsch et al., 2016; McReynolds et al., 2018; Shaham et al., 1997). An unexplored possibility is whether tonic HPA-axis activity (e.g. cortisol rhythm or troughs) modulates the effect of stressors on drug reinforcement. Interestingly, *coactivation* of noradrenergic and glucocorticoid systems modulates HPA-axis responses (Solecki et al., 2019; Hill et al., 2003; Reuter, 2002), decision-making (Margittai, Nave et al., 2018; Margittai, van Wingerden et al., 2018), and drug-seeking (Brown et al., 2009; Platt et al., 2007; Smith and Aston-Jones, 2008; Zislis et al., 2007).

The α_2-adrenergic autoreceptor antagonist yohimbine is a useful neuropharmacological probe that mimics exogenous stress-induced SNS activation. With effects also at post-synaptic α_1 and α_2 receptors (Doxey et al., 1984; Goldberg and Robertson, 1983), yohimbine-mediated increases in noradrenaline can regulate serotonin and dopamine neurotransmission (Hopwood and Stamford, 2001; Maura et al., 1982; Raiteri et al., 1990; Söderpalm et al., 1995a, 1995b) in a brain region-dependent manner (Millan et al., 2000), as well as HPA axis activity (Banihashemi and Rinaman, 2006; Lee et al., 2004; Smythe et al., 1983). In animal models of stress-induced reinstatement of drug-seeking behavior, yohimbine reliably potentiates drug-seeking during drug cues (Banna et al., 2010; Buffalari and See, 2011; Feltenstein et al., 2012) and in their absence (Feltenstein and See, 2006; Gass and Olive, 2007; Lê et al., 2005; Shepard et al., 2004). For clinical studies of stress-induced drug use, yohimbine affords methodological rigor: (a) its duration of action (4–6 h) enables broad assessment of drug-seeking and psychobiological outcomes; (b) dose-variation (including placebo) corresponds to stressor intensity; (c) dose-blinding can control expectancy effects; (d) participants do not habituate to yohimbine, allowing repeated-exposures with reliable responding; (e) its neurochemical specificity enables assessment of the independent and combined effects of SNS and HPA-axis stimulation; and (f) cross-species comparisons are possible (See and Waters, 2010; Sinha et al., 2011). We previously found that yohimbine 54-mg + hydrocortisone 10-mg increased nicotine-seeking in daily tobacco smokers (Woodcock et al., 2020). In patients with OUD in methadone treatment, intravenous yohimbine increased opioid craving, withdrawal symptoms and anxiety (Stine et al., 2002). Our initial study with heroin-dependent, buprenorphine (8-mg/day)-stabilized, non-treatment volunteers demonstrated that oral yohimbine (0, 16.2 and 32.4-mg) dose-dependently increased opioid-seeking, blood pressure and opioid withdrawal symptoms (from a low baseline level), and decreased positive mood and opioid agonist symptoms (Greenwald et al., 2013).

It is well-established that stressors can increase HPA-axis reactivity and negative affect (Childs et al., 2014). Yet, an understudied issue is whether *basal* HPA-axis activity or negative emotionality (e.g. depression and anxiety symptoms), which are interrelated (Cunningham-Bussel et al., 2009; Doane et al., 2011; Madsen et al., 2012) and commonly present in persons with OUD (Gerra et al., 2014; Kakko et al., 2008; Kroll et al., 2019), could modulate stress-reactivity and drug-seeking. Koob (2008) defined 'hedonic homeostatic dysregulation' as abnormally decreased reward function and increased stress-reactivity, a consequence of repeated lifetime exposures to stressors, drug use and withdrawal. These exposures can occasion allostatic change in hedonic tone and reward tolerance (Koob and Le Moal, 2001), mediated by hyperactivation of the extended amygdala and connected striato-pallidal circuitry implicated in motivation and motor output (Dejean et al., 2013). These neuroadaptations are associated with compulsive drug-use driven by negative reinforcement (Koob, 2009, 2015, 2021). Consistent with this framework, we demonstrated that anhedonia (pleasure deficit) is a common risk factor for increased opioid and benzodiazepine demand among persons with OUD (Greenwald et al., 2023). In animal models, anhedonic- and depressive-like signs are associated with diminished effortful responding for rewards (Salamone, Correa, Ferrigno et al., 2018; Salamone, Correa, Yang et al., 2018), highlighting the importance of assessing reinforcer-specific and global responding. Thus, the present study uses a drug vs. money choice progressive ratio task to help interpret findings with regard to these issues.

To advance understanding of neurobiological mechanisms that underlie stress-induced opioid-seeking, the present study examined the separate and combined effects of SNS (noradrenergic) and HPA-axis (glucocorticoid) activation under randomized, double-blind, placebo-controlled, within-subject, inpatient conditions. We extended our initial work (Greenwald et al., 2013) by administering a wider range of yohimbine doses, combined with placebo and active doses of hydrocortisone, a glucocorticoid receptor agonist, and we measured the HPA-axis biomarker cortisol and indirect SNS biomarker α-amylase (Ditzen et al., 2014; Granger et al., 2007; Nater and Rohleder, 2009). In the present study, we predicted yohimbine and hydrocortisone would modulate stress-biomarkers and opioid-seeking. We further examined whether cortisol levels (reflecting HPA-axis activity function) or allostatic load (α-amylase/cortisol ratio, reflecting SNS/HPA-axis balance [Ali and Pruessner, 2012]) under basal (non-stressed) conditions during buprenorphine stabilization or pre-experimental negative emotionality (depressive or anxious symptoms) modulated acute stress-induced opioid-seeking.

2. Materials and methods

The local IRB approved all procedures. This study was registered on ClinicalTrials.gov (NCT01536925). Participants provided informed consent.

2.1. Participants and selection criteria

Male and female volunteers (18–55 years), self-identifying as regular (at least weekly) heroin users and not currently seeking treatment for their opioid or other substance use disorders, were recruited via advertisements and word-of-mouth referral. Candidates were screened using a Drug Use History Questionnaire (available on request), medical history questionnaire, routine blood and urine chemistry, electrocardiogram, tuberculin test, physical exam, and Semi-Structured Clinical Interview for DSM-IV (SCID [First et al., 1996]), modified for DSM-5, to determine psychiatric and substance use disorders (American Psychiatric Association, 2015).

Volunteers had to meet DSM-5 criteria for current OUD, and were excluded if they: met criteria for a lifetime serious psychiatric disorder (specifically psychosis, bipolar, and major depression that was not substance-induced) or current moderate/severe substance use disorders (except OUD and nicotine); reported neurological, cardiovascular, pulmonary or systemic diseases; were cognitively impaired (IQ $<$ 80; Shipley Institute of Living Scale [Zachary, 1991]); were lactose-intolerant (due to placebo capsules); or, because of the required hydromorphone injections, scored $>$15 on the Injection and Blood Withdrawal Phobia subscale of the Medical Fear Survey [Kleinknecht et al., 1999]. Women who were pregnant (urine HCG), lactating, or heterosexually active but not using medically approved birth control were excluded.

During screening, volunteers provided a urine sample positive for opioids ($>$300-ng/ml) and negative for methadone (due to buprenorphine maintenance in this study), benzodiazepines ($<$300-ng/ml), and barbiturates ($<$200-ng/ml) (the latter have long half-lives and augment opioid respiratory depression). Urine samples positive for cocaine ($>$300-ng/ml) or THC ($>$50-ng/ml) were allowed. Volunteers had to provide an alcohol-free breath sample ($<$0.02%). Notably, this study was conducted prior to widespread adulteration of the heroin supply with synthetic opioids, benzodiazepines, and other substances (e.g. xylazine). Thus, exclusions for urine drug screen results were likely based on intentional use and not a contaminated supply.

2.2. Study design

This experiment had two phases. During phase 1 (opioid reinforcement assessment), each participant was exposed to hydromorphone 0-mg (placebo) and 18-mg IM in double-blinded, counterbalanced order, 4-hr apart (day 1), then had the opportunity to work for 1/12th units of these maximum doses (day 2). Only participants who preferred active hydromorphone over placebo by responding (see below) to earn ≥7 of 12 possible hydromorphone 1.5-mg units continued in the study. In phase 2 (consecutive weekdays), a within-subject, randomized crossover, placebo-controlled, double-blind design was used to test independent and combined effects of yohimbine (0, 27, 54-mg) and hydrocortisone (0, 20, 40-mg) doses on opioid-seeking (hydromorphone 1.5-mg vs. $2 units). Nine sessions were required to evaluate effects of all yohimbine X hydrocortisone dose-combinations.

2.3. Protocol timeline

Participants were stabilized on buprenorphine 8-mg/day for ≥10 outpatient days before a 12-night inpatient stay. Physical dependence is prominent in OUD; buprenorphine stabilization can blunt the physiological drive for compulsive opioid intake (negative reinforcement) which, if uncontrolled, would confound assessment of stress-effects on drug-seeking. Residential living, staff observation and daily urine-drug and breath-alcohol testing ensured abstinence from unsanctioned substance use during test sessions. During non-experimental periods (evenings and weekends), volunteers could engage in recreational activities on the unit. Cigarette smoking or use of nicotine replacement products (provided free by the research team) were allowed *ad libitum* on the residential unit and, on session days, permitted until 10am (prior to the drug/money choice task) and resumed at 2:30pm (after the choice task).

Hydromorphone vs. money choices. Nine experimental sessions were conducted on consecutive weekdays. Hydromorphone (1.5-mg) and money ($2) unit amounts, progressive ratio schedule parameters, and task instructions (Appendix) were similar to Greenwald et al. (2013). Supplementary Fig. 1 illustrates the appearance and response requirements of the drug/money choice progressive ratio task. Participants began the task at 11am. On the computer screen, two colored boxes were labeled Money (green) and Drug (red); adjacent boxes indicated the number of units earned for money and drug; and another box displayed a timer showing residual session time. After the participant completed each choice, a tone signaled they earned the money or drug unit amount. After a 10-sec time-out, the next choice trial began. Across trials within session, response requirements for drug and money options independently increased in an exponential function: 125, 225, 365, 590, 950, 1500, 2300, 3415, 4915, 6875, 9375 and 12,500.

During the 3-hr choice task, participants were not allowed to use their phones, read, smoke cigarettes, eat, or watch television, and had to remain seated (except bathroom breaks) until time expired. Participants could drink water but no other beverages. At 2pm, the computer program quit unless the participant completed all 12 choices early (at which time the program quit, and the participant had to wait until the 3-hr period ended), then the earned hydromorphone dose was injected immediately, and the earned money was added to their study payment.

2.4. Drug administration

Buprenorphine sublingual tablets were consumed under observation during outpatient and inpatient periods (Subutex™; Indivior, Hull, UK; from Research Triangle Institute, NC). During the inpatient study, buprenorphine was ingested at 8pm, i.e. 12.5-hr before each session. The 8-mg/day buprenorphine dose was selected to minimize opioid withdrawal symptoms, but insufficient to completely block the reinforcing effects of hydromorphone (Greenwald et al., 2014, 2024), as confirmed during the hydromorphone reinforcement screen.

Hydromorphone doses (Dilaudid-HP™ in 50 mg/5 ml ampoules) were injected IM into the deltoid muscle. A nurse administered the total earned drug dose (variable dose/volume).

Yohimbine hydrochloride powder USP (Spectrum Chemical Manufacturing, Gardena, CA) was weighed on a Mettler balance and placed with lactose filler inside opaque capsules for oral administration. Placebo capsules contained only lactose. Dose-selection was based on studies of oral yohimbine effects on pharmacokinetics (Grasing et al., 1996; Sturgill et al., 1997), at doses up to 21.6-mg), anxiety ([Mattila et al., 1988] at a dose of 0.8-mg/kg), and opioid-seeking ([Greenwald et al., 2013] at doses of 16.2 and 32.4-mg). Yohimbine was administered at 9:30am (t = 0-hr session timeline).

Hydrocortisone tablets (Cortef™ 20-mg each) were placed with lactose filler inside opaque capsules. Placebo capsules contained only lactose. Dose-selection was based on studies of oral hydrocortisone (±yohimbine) effects on emotion processing (Reuter et al., 2002; van Stegeren et al., 2010), decision-making (Margittai, Nave et al., 2018; Margittai, van Wingerden et al., 2018; Kluen et al., 2017; Putman et al., 2010) and operant behavior (Schwabe et al., 2010, 2012). Hydrocortisone was administered at 10:15am (45-min post-yohimbine) to coordinate peak and sustained effects of both yohimbine and hydrocortisone.

2.5. Measures

Negative emotionality. At screening, volunteers completed the Beck Depression Inventory (BDI-II [Beck et al., 1996]) to measure recent depressive symptoms; and State Trait Anxiety Inventory (STAI [Spielberger et al., 1970]) to measure trait-anxious symptoms. These measures were used to assess negative emotionality (for covariate analysis) and were not exclusionary.

Vital signs and subjective effects. During each choice session, vital signs (blood pressure, heart rate, oxygen saturation, breathing rate, core body temperature) and subjective effects (described below) were measured −0.5, 0.5, 1, 2, 3 and 4-hr relative to yohimbine, with additional post-hydromorphone time points for safety monitoring (excluded from analysis).

Heroin craving was measured with the 34-item Heroin Craving Questionnaire (Schuster et al., 1995); each item is scored 1 to 7, yielding a total score (range, 34 to 238).

Visual analog scale (VAS, 0–100) drug-ratings were obtained: Bad Effect, Good Effect, High, Liking, Sedated and Stimulated.

Opioid agonist symptoms (16 items) and withdrawal symptoms (16 items) were self-rated (Schuster et al., 1995). Each item is scored 0 (not at all) to 4 (extremely), yielding subscale scores ranging from 0 to 64 for each scale.

The Profile of Mood States (POMS [McNair et al., 1971]) was used to assess momentary mood state on 8 subscales: Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, and Elation (items for each scale rated on 0–4 scale); and two composite scales, Arousal and Positive Mood.

Saliva cortisol and α-amylase. In each choice session, 8 saliva samples were collected at −0.5, 0.5, 1, 1.5, 2, 2.5, 3 and 4-hr relative to yohimbine dose. To reduce measurement variability (Rohleder and Nater, 2009), we controlled wake-up time, caffeine intake, cigarette smoking, and food intake (see Appendix). To minimally disrupt choice-task responding during saliva collection, the participant held a cotton salivette in his/her mouth, without chewing, for 3-min. Salivettes were centrifuged and saliva was transferred to cryogenic tubes and frozen at −20 °C until radioimmunoassay for levels of cortisol and α-amylase (Salimetrics, State College, PA).

"Basal cortisol level" for each participant was defined as the last-measured value at 1:30pm during the dual-placebo condition. We computed an 'allostatic load' index (Ali and Pruessner, 2012) for each participant as the area under the curve (AUC) $\log_{10}$ α-amylase ÷ $\log_{10}$ cortisol ratio across session time points in the dual-placebo condition, and we explored α-amylase/cortisol ratio values within each session at each time point to index acute change in SNS/HPA axis balance.

Drug vs. money reinforcement. Measures of drug vs. money reinforcing efficacy included total earned hydromorphone and money choices and breakpoints (highest response requirement completed). We calculated a novel within-session breakpoint difference (drug–money) score, referred to as opioid vs. money relative reinforcing efficacy ('RRE'; see Bickel et al. [2000]). As response requirements on each choice trial differed on the progressive ratio schedule, responding more on one choice option exponentially increased the breakpoint difference score. Positive scores indicate greater opioid RRE, negative scores indicate greater money RRE, whereas zero indicates no preference.

2.6. Data analyses

Analyses were conducted using SPSS v.29. For all analyses, the null hypothesis rejection criterion was $p < 0.05$. Huynh-Feldt adjusted p values were used for sphericity violations.

Subjective effects and vital signs during hydromorphone choice sessions were first analyzed using Yohimbine Dose (0, 27, 54-mg) X Hydrocortisone Dose (0, 20, 40-mg) X Session Time (which varied by outcome) repeated-measures analyses of variance (rmANOVAs). Due to different timing of assessments, area-under-the-curve ($AUC_{0.3–4hr}$) post-yohimbine scores were subjected to Yohimbine Dose X Hydrocortisone Dose rmANOVAs. Opioid-seeking indices (hydromorphone choices and breakpoint, and drug–money breakpoint difference [opioid RRE]) were analyzed using Yohimbine X Hydrocortisone Dose rmANOVAs. Analyses of Covariance (rmANCOVAs) examined covariates of interest: basal cortisol level and allostatic load index in the placebo condition, and recent depression symptoms (BDI-II), and trait anxiety (STAI) scores.

Given the small sample size and limited statistical power of this study, we emphasize effect sizes (partial eta-squared [η_p^2] values), reflecting the proportion of variance explained by the independent variable, relative to total variance remaining, after accounting for all other independent variables in the model. η_p^2 ranges from 0 to 1; small effect sizes are 0.01; moderate effect sizes are 0.06; and large effect sizes are $\geq$0.14.

3. Results

3.1. Participant characteristics

Fig. 1 presents the CONSORT diagram. Four initially eligible participants did not pass the hydromorphone reinforcement screen and were excluded. Table 1 describes characteristics of the 12 participants who completed the study.

3.2. Reinforcement screening sessions

Relative to placebo, hydromorphone 18-mg produced expected physiological, subjective, and behavioral effects for all included participants. For details, see Supplementary Table 1.

3.3. Choice sessions

Physiological and subjective effects. Table 2 summarizes yohimbine

Table 1
Participant characteristics (n = 12).

Measure	Number (%) or Mean (SD)
Demographics	
Sex at birth (self-reported)	10 male (83%), 2 female (17%)
Race (self-reported identity)	5 black (42%), 4 white (33%), 1 multiracial (8%)
Age (years)	41.2 (10.7)
Education (years)	12.9 (1.2)
Body mass index (kg/m^2)	22.1 (1.8), range: 18.7–25.6
Heroin use	
Duration of regular use (years)	14.4 (10.2), range: 3–36
Quit attempts (#)	15.6 (28.0), range: 2–100
Current route of use	7 injection (58%), 5 intranasal (42%)
Daily use amount ($10 bags)	4.5 (1.2), range: 2–6
Other current substance use	
Tobacco (cigarettes/day)	11.8 (7.1), range: 2–21
Alcohol (past-month # days)	0.5 (1.0), range: 0–3
Cannabis (past-month # days)	2.3 (6.3), range: 0–22
Cocaine (past-month # days)	1.8 (4.6), range: 0–15
Negative emotionality scores	
BDI-II depression	8.8 (8.4), range: 1–29
STAI trait anxiety	36.0 (11.7), range: 20–61

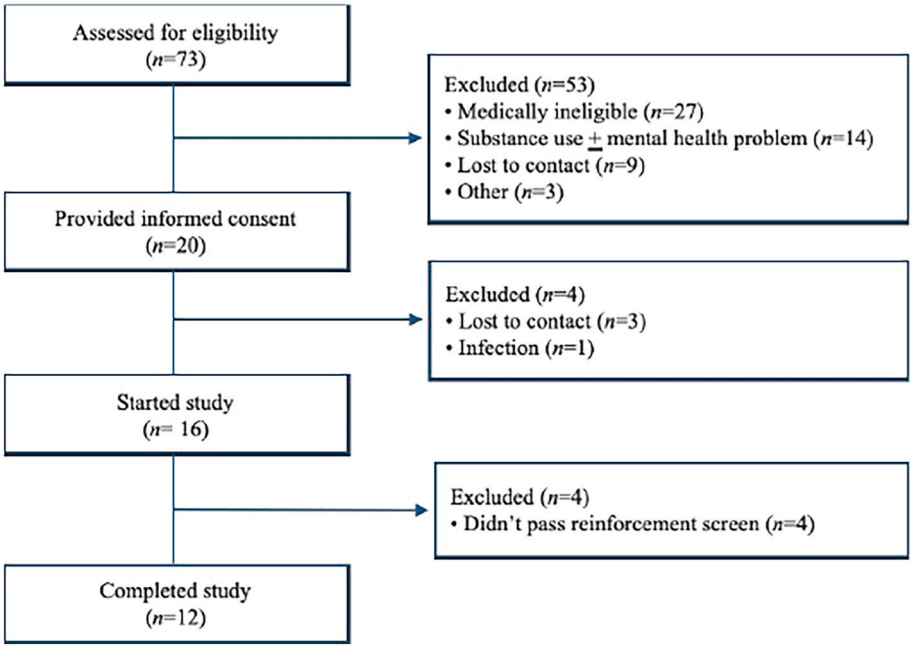

Fig. 1. CONSORT diagram, showing the flow of participants through the study.

Table 2
Yohimbine post-drug (mean ± 1 SEM area under the curve) responses.

	Yohimbine Dose			Dose (AUC)
Measure	0 mg	27 mg	54 mg	$F[2,22]$ (p), η_p^2
Opioid symptom scales (0–64)				
Agonist	**8.69 (1.80)**	**8.56 (1.81)**	**7.54 (1.54)**	**3.64 (0.04), 0.25**
Withdrawal	1.66 (0.39)	2.98 (0.82)	2.81 (0.55)	2.46 (0.11), 0.18
Heroin craving total (34–238)	167.1 (3.8)	166.5 (4.8)	165.7 (4.2)	0.50 (0.61), 0.04
POMS scales				
Anger	0.02 (0.01)	0.06 (0.02)	0.07 (0.03)	1.83 (0.18), 0.14
Anxiety	**0.39 (0.07)**	**0.51 (0.09)**	**0.60 (0.08)**	**3.39 (0.05), 0.24**
Confusion	0.54 (0.03)	0.55 (0.03)	0.57 (0.04)	1.96 (0.17), 0.15
Depression	0.09 (0.04)	0.11 (0.04)	0.11 (0.04)	0.31 (0.74), 0.03
Elation	0.62 (0.21)	0.51 (0.14)	0.48 (0.14)	1.27 (0.30), 0.10
Fatigue	0.16 (0.10)	0.09 (0.03)	0.17 (0.06)	0.68 (0.52), 0.06
Friendliness	1.27 (0.36)	1.24 (0.36)	1.26 (0.36)	0.08 (0.92), 0.01
Vigor	0.67 (0.26)	0.70 (0.24)	0.66 (0.20)	0.12 (0.89), 0.01
Arousal	0.35 (0.27)	0.55 (0.22)	0.52 (0.22)	1.31 (0.29), 0.11
Positive Mood	0.53 (0.22)	0.40 (0.16)	0.37 (0.15)	1.32 (0.29), 0.11
VAS ratings (0–100)				
Bad drug effect	8.5 (3.5)	13.6 (4.7)	14.7 (5.1)	2.28 (0.13), 0.17
Good drug effect	6.6 (2.9)	5.9 (3.2)	6.3 (2.9)	0.20 (0.82), 0.02
High	1.5 (0.5)	2.2 (1.1)	3.3 (1.8)	1.33 (0.28), 0.11
Like Drug	3.2 (1.2)	2.6 (1.2)	3.5 (1.6)	0.42 (0.66), 0.04
Sedated	1.7 (0.5)	1.7 (0.6)	2.6 (1.6)	0.49 (0.62), 0.04
Stimulated	3.5 (1.6)	5.6 (2.0)	5.5 (2.0)	0.82 (0.46), 0.07
Vital signs				
Systolic BP (mm Hg)	**112.4 (3.4)**	**119.2 (3.5)**	**121.4 (3.5)**	**16.74 (<0.001), 0.60**
Diastolic BP (mm Hg)	**70.7 (2.8)**	**73.4 (2.9)**	**74.6 (2.7)**	**11.15 (<0.001), 0.50**
Heart rate (bpm)	68.0 (1.7)	69.8 (2.0)	70.7 (2.3)	1.48 (0.25), 0.12
Core body temp. (°F)	97.3 (0.2)	97.3 (0.2)	97.3 (0.2)	0.11 (0.90), 0.01
Oxygen saturation (%)	98.5 (0.3)	98.6 (0.3)	98.8 (0.2)	1.84 (0.18), 0.14
Breathing rate (bpm)	15.4 (0.7)	15.6 (0.7)	15.2 (0.6)	1.92 (0.17), 0.15
Salivary levels				
Cortisol ($\log_{10}$ µg/dL)	0.25 (0.04)	0.28 (0.03)	0.25 (0.04)	1.89 (0.18), 0.15
α-amylase (Δ $\log_{10}$ U/mL)	0.01 (0.06)	0.06 (0.07)	0.13 (0.04)	2.10 (0.15), 0.16
α-amylase/cortisol	31.25 (4.71)	25.92 (2.63)	29.90 (4.98)	0.58 (0.57), 0.05

Notes: Yohimbine dose means are averaged across hydrocortisone dose conditions, because hydrocortisone affected only cortisol levels (see Fig. 3). For yohimbine dose effects where $p < 0.20$, partial *eta*-squared (η_p^2) values are provided to indicate effect size. Measures with significant dose effects ($p < 0.05$) are bolded.

dose-effect AUC scores for non-choice measures. Hydrocortisone did not affect, or interact with yohimbine on, most non-choice measures; thus, we report yohimbine dose-condition scores (collapsed across hydrocortisone dose) in Table 2.

Fig. 2A–D and Supplementary Table 2 illustrate yohimbine dose- and time-related effects on physiological measures. Yohimbine significantly increased systolic and diastolic blood pressure (larger/more sustained effects for 54-mg), heart rate (slower habituation for 54-mg), and change-from-baseline $\log_{10}$ α-amylase levels (gradually rising for 54-mg), but did not significantly impact $\log_{10}$ cortisol levels, core body temperature, respiration rate or oxygen saturation.

Fig. 2E–H and Supplementary Table 2 illustrate yohimbine dose- and time-related changes on subjective effects. Yohimbine significantly increased POMS Anxiety and decreased Positive Mood scores (peaking at 1–2 h), increased opioid withdrawal symptoms (although these remained at very low levels) and decreased opioid agonist symptoms. Yohimbine and hydrocortisone jointly modulated heroin craving AUC scores (Fig. 3): during placebo hydrocortisone, yohimbine elevated craving, whereas during active hydrocortisone, yohimbine decreased craving. In Dose X Time analyses, yohimbine decreased POMS Elation and Positive Mood, increased POMS Anger and Depression, and increased VAS ratings of 'bad drug effect' and 'sedated', at some time points (not shown).

Fig. 4(A and B) shows that hydrocortisone produced significant dose- and time-dependent increases in cortisol levels and decreases in α-amylase/cortisol ratio scores (lower SNS vs. HPA-axis balance), whereas α-amylase levels were unaffected (not shown; Dose and Dose X Time $ps > 0.25$).

Opioid-seeking. Fig. 5 and Supplementary Table 3 presents rmANOVA results for opioid-seeking indices without covariate adjustment. Yohimbine non-significantly increased the total number of hydromorphone choices ($\eta_p^2 = 0.14$), total hydromorphone dose earned (Fig. 5A; $\eta_p^2 = 0.14$), hydromorphone breakpoint (Fig. 5C; $\eta_p^2 = 0.22$), and the opioid–money breakpoint difference score ($\eta_p^2 = 0.15$); these effect sizes were all 'large'. At the same time, yohimbine non-significantly decreased the total number of money choices ($\eta_p^2 = 0.14$), total money amount earned (Fig. 5B; $\eta_p^2 = 0.14$), and money breakpoint (Fig. 5D; $\eta_p^2 = 0.10$). Hydrocortisone did not influence number of opioid choices ($\eta_p^2 = 0.06$), opioid dose earned ($\eta_p^2 = 0.06$), opioid breakpoint ($\eta_p^2 = 0.002$), or opioid–money breakpoint difference ($\eta_p^2 = 0.06$); nor number of money choices ($\eta_p^2 = 0.05$), total money amount earned ($\eta_p^2 = 0.05$), or money breakpoint ($\eta_p^2 = 0.11$); these effect sizes ranged from 'small' to 'moderate'. Total per-session drug + money choices (mean levels of 11.9–12 [maximum] across all conditions) were not significantly affected by yohimbine or hydrocortisone (η_p^2 values = 0.08). There were no significant Yohimbine × Hydrocortisone interactions on opioid-seeking measures when covariates were excluded from the model (all η_p^2 values ≤ 0.08).

Fig. 4(C and D) illustrates between-subject differences in basal cortisol level, which served as the covariate in adjusted analyses of opioid-seeking behavior. Fig. 6 and Table 3 summarize these covariance analyses. Among participants with lower basal cortisol levels (defined as below the median split), mean RRE was biased toward opioid-seeking and relatively insensitive to the effects of stress (yohimbine + hydrocortisone); in contrast, participants with higher basal cortisol levels (defined as above the median split) preferred money in the absence of stress, and yohimbine and hydrocortisone dose-dependently shifted RRE toward opioid-seeking. Notably, basal cortisol level did not significantly modulate measures of physiological or subjective stress-reactivity.

Basal cortisol level also significantly modulated stress-induced effortful responding. Supplementary Fig. 2 illustrates results of these covariance analyses. For participants with higher basal cortisol levels, total (drug + money) breakpoint decreased when hydrocortisone 40-mg was combined with increasing yohimbine doses, indicating stress-induced reduction in global effort among persons with tonic HPA-axis hyperactivation. In contrast, allostatic load did not significantly affect

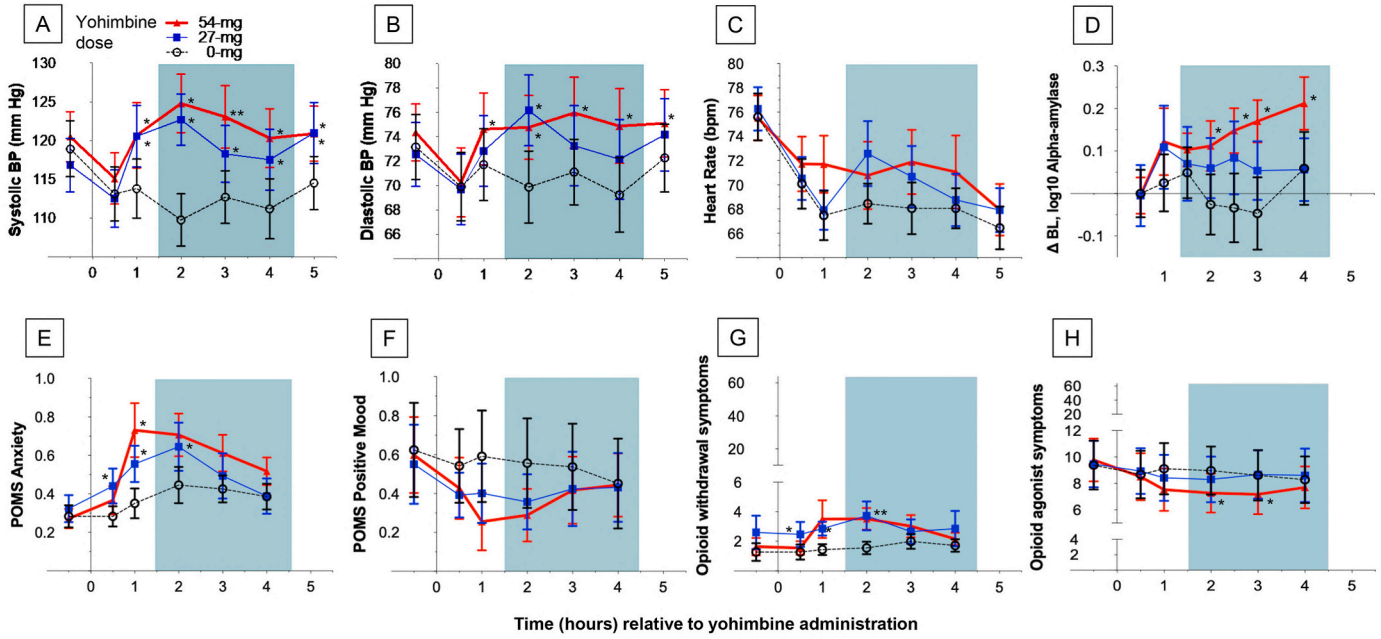

Fig. 2. Effects of yohimbine dose (0, 27 and 54-mg oral, administered at $t = 0$ h) on autonomic nervous system indices (A) systolic blood pressure, (B) diastolic blood pressure, (C) heart rate, (D) saliva α-amylase change-from-session baseline scores; and on self-reported Profile of Mood States (POMS) (E) Anxiety and (F) Positive Mood scales, and (G) opioid withdrawal symptoms and (H) opioid agonist symptom scores. The shaded region in each panel depicts the 3-hr period (1.5–4.5 h post-yohimbine) when the hydromorphone vs. money choice task occurred. These effects were not significantly affected by hydrocortisone dose, therefore, yohimbine means are averaged across hydrocortisone doses. Yohimbine × Time interaction simple effects at each time point (least significant difference test): * significantly different from placebo; ** significantly different from placebo and 27-mg yohimbine. See statistical summary for AUC scores (Table 1) and Dose X Time effects (Supplementary Table 2).

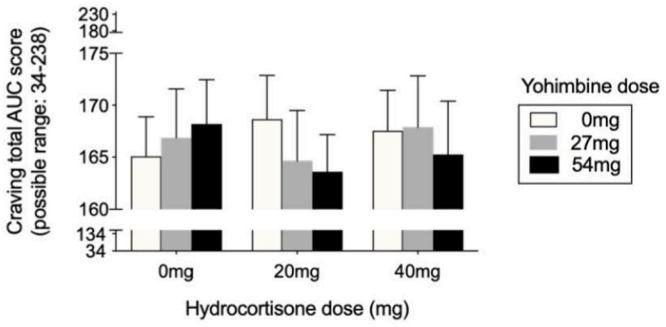

Fig. 3. Average heroin craving AUC_{1-4hr} scores were in the moderate range during buprenorphine 8 mg/day stabilization. On top of this baseline, yohimbine dose ($F[2,20] = 0.50$, $p = 0.61$) and hydrocortisone dose ($F[2,20] = 0.79$, $p = 0.46$) did not significantly affect craving; however, yohimbine and hydrocortisone jointly modulated heroin craving AUC_{1-4hr} scores: during placebo hydrocortisone (left group of bars), yohimbine dose-dependently elevated craving whereas during active hydrocortisone (middle and right groups of bars), craving diminished with increasing yohimbine dose (Yohimbine X Hydrocortisone Dose $F[4,44] = 3.05$, $p = 0.03$, $\eta_p^2 = 0.22$). Basal cortisol level did not significantly modulate heroin craving (unlike measures of opioid seeking).

stress-induced opioid-seeking.

Pre-study negative emotionality. BDI-II depression and STAI trait anxiety scores at screening were highly correlated ($r = 0.86, p < 0.001$). Supplementary Fig. 3 illustrates the effects of depression scores (Supplementary Fig. 3A) and trait anxiety scores (Supplementary Fig. 3B) on stress-induced opioid RRE. Participants with lower depression or anxiety scores were relatively insensitive to the effects of stress, whereas those with numerically higher depression or anxiety scores exhibited dose-linear increases of yohimbine and hydrocortisone on breakpoint difference. Effect sizes were slightly larger for depression than trait anxiety scores, but effect sizes for negative-emotionality modulation of opioid RRE were smaller than for basal cortisol levels.

Correlations between basal cortisol levels, negative emotionality, and other pre-study measures can be found in Supplementary Table 4. Basal cortisol levels significantly correlated with BDI-II depression scores ($r = 0.76, p = 0.004$) but did not correlate with STAI trait anxiety ($r = 0.52, p = 0.085$), age, body mass index, heroin-use characteristics (e.g. duration of use, current use, consequences of use, quit attempts), or tobacco use. Demographic and heroin-use measures were unrelated to opioid-seeking.

4. Discussion

This study examined noradrenergic/glucocorticoid stressor dose-effects on opioid-seeking in people with OUD not currently seeking treatment. There are two primary findings. First, in participants with OUD who were initially stabilized on buprenorphine to suppress opioid withdrawal, yohimbine increased stress-biomarkers, highlighting SNS centrality in vulnerability to persistent opioid use. Second, tonic HPA-axis activation (basal cortisol level in the absence of stress), more robustly than measures of allostatic load and negative emotionality, interacts with yohimbine, hydrocortisone, and their dose-combination, to increase opioid-seeking: participants with higher basal cortisol exhibited greater stress-induced shifts to opioid-seeking.

Yohimbine reliably increased blood pressure, anxiety ratings, and opioid withdrawal symptoms (which remained at sub-clinical levels throughout [scores <10] on this measure [Greenwald, Johanson et al., 2003; Greenwald, Schuh and Stine, 2003]) while decreasing agonist symptoms during buprenorphine maintenance [8-mg/day SL]). These results replicate our prior study (Greenwald et al., 2013) and findings in methadone-maintained patients with OUD that yohimbine increased blood pressure, anxiety, cortisol, opioid craving and withdrawal symptoms (Stine et al., 2002). In dose X time analyses, yohimbine increased heart rate and α-amylase levels, increased 'bad drug effect' and 'sedated' VAS ratings, and altered POMS scores (decreased Elation and Positive

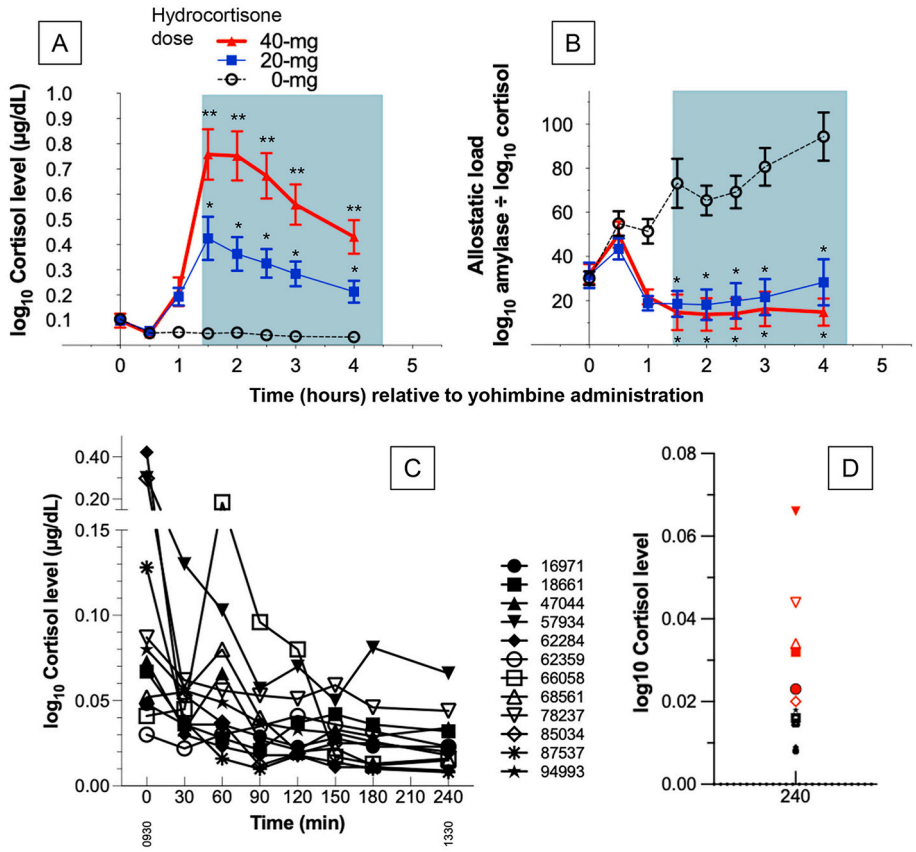

Fig. 4. Effect of hydrocortisone dose (0, 20 and 40-mg oral; administered 45 min post-yohimbine) on (A) salivary cortisol levels and (B) change-from-baseline α-amylase/cortisol ratio scores (SNS vs. HPA-axis balance); the shaded region in each panel indicates the 3-hr period (1.5–4.5 h post-yohimbine) when the opioid vs. money choice task occurred. Hydrocortisone dose-dependently increased cortisol levels overall (AUC: Dose $F[2,44] = 42.84$, $p < 0.001$, $\eta^2_p = 0.80$) and across the session (Dose linear X Time $F[1,11] = 51.94$, $p < 0.001$, $\eta^2_p = 0.83$). Hydrocortisone also reduced α-amylase/cortisol ratio scores overall (AUC: $F[2,44] = 47.22$, $p < 0.001$, $\eta^2_p = 0.81$) and across the session (Dose linear X Time $F[1,11] = 23.33$, $p < 0.001$, $\eta^2_p = 0.68$). These effects were not significantly affected by yohimbine dose; therefore, hydrocortisone means are averaged across yohimbine doses. Hydrocortisone × Time interaction simple effects at each time point (least significant difference test):* significantly different from placebo; ** significantly different from placebo and 20-mg hydrocortisone. (C) Between-subject differences in saliva cortisol levels during the dual-placebo condition across session time (from 9:30 a.m. [$t = 0$ min, yohimbine administration] to 1:30 p.m. [$t = 240$ min]); the terminal value measured at 1:30 p.m. was used as the covariate in adjusted rmANOVAs of opioid-seeking behavior. (D) Distribution of basal cortisol levels at 240-min session time point; participant data shown in red are above the median, and those in black are below the median (median split used for group illustration in Fig. 3). Alternative measures of basal cortisol (e.g. non-linear slope across time) were examined, however, the subject classification and its effect on predicting opioid seeking did not change. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Mood, increased Anger and Depression) at some session time points. Thus, yohimbine produced interoceptive cues consistent with other stressors. The only significant hydrocortisone effect was a dose-dependent increase in saliva cortisol levels. Craving scores showed a more complex pattern: yohimbine alone increased craving, whereas yohimbine + active hydrocortisone decreased craving; these results suggest that increased noradrenaline stimulation might energize craving, whereas increased glucocorticoid stimulation may attenuate craving (Reuter et al., 2002). Interestingly, these effects manifested during moderate-dose buprenorphine stabilization which, like other chronic opioid exposure, tends to suppress HPA-axis function (Wemm and Sinha, 2019). Our results echo findings that 20-mg hydrocortisone reduced craving in low-dose (but not high-dose) heroin users (Walter et al., 2015).

This study examined stress-related opioid-seeking during stabilization on intermediate-dose buprenorphine to suppress withdrawal, reflecting the reality that patients treated with medications for OUD (MOUD) remain susceptible to stress-induced opioid use. Averaged across all conditions, participants chose hydromorphone slightly less often than money (Ms = 5.73 and 6.22, respectively), suggesting that intermediate-dose buprenorphine partly suppressed opioid motivation in this non-treatment sample, and that stress-effects must be interpreted against this background. Although one rodent study found that intermittent footshock increased heroin-seeking both during heroin maintenance and withdrawal (Shaham et al., 1996), most preclinical research has examined the effect of yohimbine or other stressors on opioid reinstatement (analog of return to use) during opioid abstinence, both in contexts where drug-associated cues are present (Banna et al., 2010; Buffalari and See, 2011; Feltenstein et al., 2012) and absent (Feltenstein and See, 2006; Gass and Olive, 2007; Lê et al., 2005; Shepard et al., 2004). To promote translational research, preclinical studies should evaluate the effects of stressors on drug-seeking during maintenance on MOUD, which more closely corresponds to the clinical situation.

In our prior study (Greenwald et al., 2013), yohimbine increased opioid-seeking for 1-mg but not 2-mg hydromorphone unit doses; the lack of effect for 2-mg units may have reflected a ceiling effect on hydromorphone choice. In the present study we tested an intermediate unit dose (1.5-mg) to minimize ceiling effects from stress-exposure and to examine robustness of this experimental model. Without covariate adjustment, yohimbine and hydrocortisone did not significantly affect opioid-seeking at this unit dose, although yohimbine dose-effect size was large ($\eta^2_p = 0.19$ for opioid RRE); this is surprising as the current study was powered to detect large effects at power $\beta = 0.80$. Nonetheless, results align with noradrenaline-potentiated drug motivation

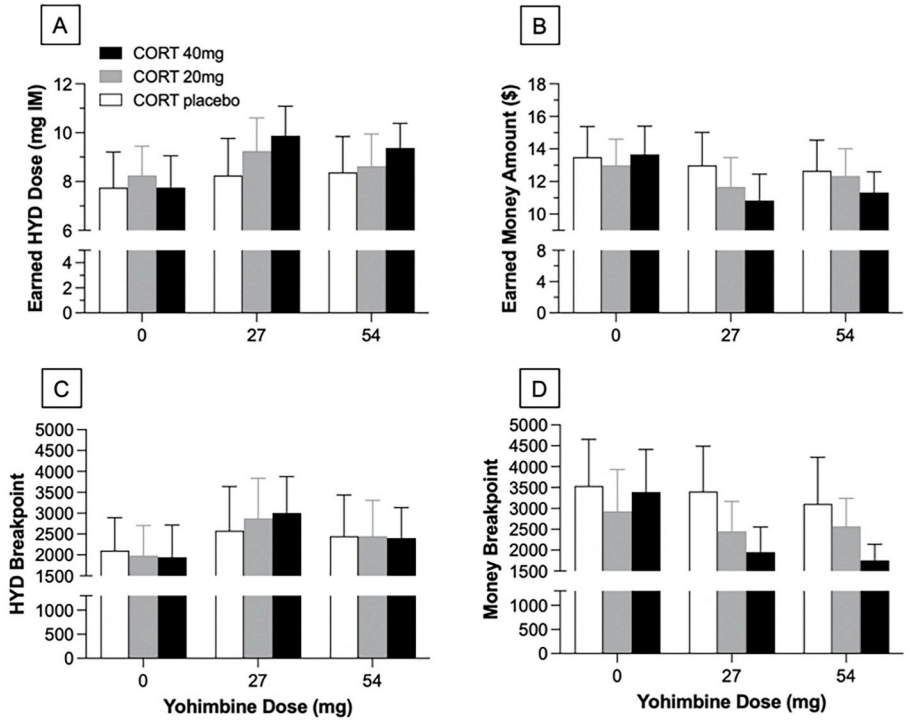

Fig. 5. Effects of yohimbine and hydrocortisone doses on mean ± 1 SEM raw scores for (A) earned hydromorphone dose, (B) earned money amount, (C) hydromorphone breakpoint, and (D) money breakpoint. All effects were non-significant, although effect sizes for yohimbine were large (see text). Yohimbine tended to increase earned hydromorphone dose and breakpoint, while decreasing earned money amount and breakpoint, whereas hydrocortisone effects on these measures was much weaker.

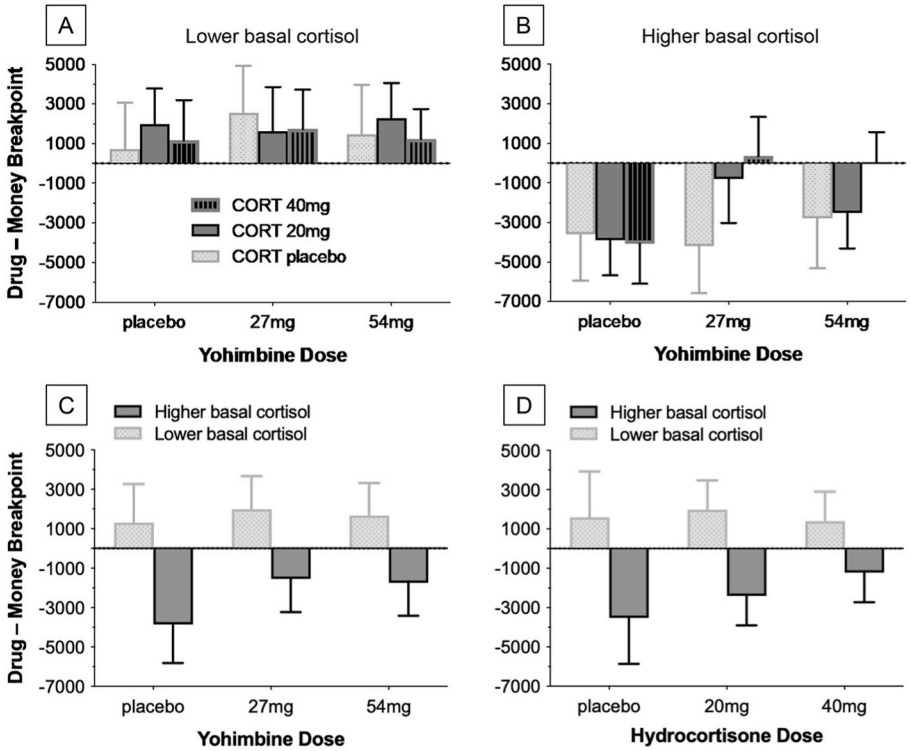

Fig. 6. Effects of yohimbine and hydrocortisone doses on opioid–money breakpoint difference (relative reinforcing efficacy [RRE]) scores. Upper panels illustrate mean ± 1 SEM opioid vs. money RRE across all nine experimental conditions for participants with (A) lower vs. (B) higher basal cortisol levels (three-way interaction). RRE was modulated by the interaction of basal cortisol level with doses of (C) yohimbine and (D) hydrocortisone (two-way interactions); Table 3 presents two-way and three-way interactions from rmANOVAs. Participants (subject numbers shown) with lower basal cortisol levels exhibited mean RRE that was modestly positive (toward the opioid) for all conditions and insensitive to stressor manipulation. In contrast, participants with higher basal cortisol levels exhibited much greater RRE for money during placebo stress, whereas doses of yohimbine and hydrocortisone shifted RRE toward the opioid.

Table 3
Summary of stress effects (covarying for basal cortisol level) on opioid-seeking behavior.

Effect	Opioid choices	Opioid breakpoint	Opioid–money breakpoint difference
Yohimbine dose[a]	**3.56 (0.048), 0.26**	1.14 (0.339), 0.10	**4.14 (0.042), 0.29**
Yohimbine dose X Basal cortisol[a]	**11.24 (<0.001), 0.53**	**6.44 (0.007), 0.39**	**13.08 (<0.001), 0.57**
Hydrocortisone dose[a]	3.14 (0.065), 0.24	2.66 (0.094), 0.21	**3.55 (0.048), 0.26**
Hydrocortisone dose X Basal cortisol[a]	**7.27 (0.004), 0.42**	**4.03 (0.034), 0.29**	**8.06 (0.003), 0.45**
Yohimbine dose X Hydrocortisone dose[b]	2.13 (0.094), 0.18	1.77 (0.154), 0.15	**2.71 (0.044), 0.21**
Yohimbine dose X Hydrocortisone dose X Basal cortisol[b]	**4.10 (0.007), 0.29**	**2.71 (0.043), 0.21**	**5.12 (0.002), 0.34**

[a] $F[2,20]$ (p), η^2_p.
[b] $F[4,40]$ (p), η^2_p.

observed in animal models (Aston-Jones and Harris, 2004; Davis et al., 1975; Jasmin et al., 2006; Olson et al., 2006; Shaham et al., 2000; Ventura et al., 2005; Weinshenker and Schroeder, 2007). In contrast, glucocorticoid alone-mediated opioid motivation was less robust in this study, also consistent with animal models (Mantsch et al., 2016; McReynolds et al., 2018; Shaham et al., 1997).

We found that noradrenergic ± glucocorticoid stimulation shifts RRE (drug–money breakpoint difference), primarily depending on participants' basal cortisol level (tonic HPA-axis activity) and, secondarily, negative emotionality (depression and trait anxiety), but not allostatic load (α-amylase/cortisol ratio) scores. Participants with higher basal cortisol level worked more for money during placebo (i.e. opioid-seeking was suppressed under non-stressed conditions), and yohimbine/hydrocortisone combinations shifted responding toward opioid-seeking, whereas participants with lower basal cortisol generally worked slightly more for hydromorphone than money regardless of yohimbine/hydrocortisone condition. Similar effects were found for opioid choice and breakpoint measures. Cortisol (released from the adrenal cortex or mimicked by exogenous hydrocortisone) inhibits release of corticotropin-releasing hormone (CRH, from hypothalamus) and adrenocorticotropic hormone (ACTH, from pituitary), providing rapid buffering of stress-reactivity. One hypothesis for further exploration is that higher early-afternoon basal cortisol level might reflect an endogenous negative feedback deficit that increases vulnerability to noradrenergic/glucocorticoid stress-induced opioid motivation, whereas lower basal cortisol could indicate more effective negative feedback that, in turn, increases resilience to stress-induced opioid-seeking.

The finding of differential stress-induced opioid motivation in this population on this choice-effort task generates hypotheses for advancing theory and applied research. Our experimental analog demonstrates *higher basal cortisol level (or, secondarily, greater negative emotionality) interacting with stress-exposure shifts response priority from non-drug reinforcers to opioids*. Among participants with higher basal cortisol level (or negative emotionality scores), stress shifted responding from money to the opioid, i.e. a reinforcer-specific effect. Yet, these participants also decreased their global effort (total drug + money breakpoint) by shifting to a balance between opioid and money choices, because working equally for drug and money on this progressive ratio task reduces overall effort. However, in the absence of acute stress, basal cortisol level (or negative emotionality scores) did not impact total number of choices, i.e. decision-making was unaffected. These results indicate that, during stress-exposure, individuals with higher basal cortisol level or negative emotionality maximize reinforcement while minimizing total effort; this suggests a mechanism by which such individuals might compensate for acute stress-induced hedonic deficits (Koob, 2008). The current results echo our recent findings that higher scores for depression and, more so anhedonia, predicted increased opioid demand among patients in treatment for OUD (Greenwald et al., 2023). A potential clinical implication is that patients with OUD presenting with HPA-axis dysregulation (e.g. possibly using take-home saliva collection for cortisol testing) or negative emotionality (using questionnaire assessment) may have elevated risk of stress-induced exacerbation of, or return to, opioid use that would require more intensive prophylactic care. This highlights the importance of developing interventions to recognize and manage underlying SNS and/or neuroendocrine dysregulation and associated psychiatric symptoms, towards improving resilience to stress-related drug use.

Several study limitations have implications for future research. First, yohimbine/hydrocortisone dose-combinations may not fully capture heterogeneous effects of real-world stressors. For example, our hydrocortisone doses likely produced supraphysiological cortisol levels (Jung et al., 2014), possibly leading to a ceiling on opioid-seeking; however, this experimental approach probes two key neurochemical systems underlying stress-reactivity. Second, the study's small sample size reflects the challenge of having persons with OUD complete a complex, within-subject crossover design under highly controlled conditions. The small sample size likely explains why unadjusted analyses of opioid-seeking were non-significant despite large effect sizes; broader conclusions are limited until these outcomes are evaluated in larger-scale studies. Third, our finding that basal cortisol level modulated stress-induced opioid-seeking assumes that early-afternoon levels are a stable phenotype; although we measured cortisol at multiple time points from morning into early afternoon, it was not feasible to conduct full circadian assessment of cortisol rhythm. Fourth, our secondary findings that subclinical depression or anxiety scores moderated stress-induced opioid-seeking could be complicated by other factors, e.g. females experience depression and anxiety symptoms more severely/commonly than males with OUD (Sordo et al., 2012), but current diagnosis of major depressive disorder was exclusionary, and our sample was mostly male and underpowered to examine this issue. Fifth, we did not measure participants' stress-coping ability and its effect on drug responding. Sixth, due to the complex stress manipulation we used a single hydromorphone unit dose, which limits generalizability. Seventh, this study was conducted with participants who were stabilized on buprenorphine; results may not generalize to those maintained on methadone or naltrexone. Finally, this study was conducted with persons not currently seeking treatment for OUD; although each participant reported having tried to quit heroin at least twice, results might not generalize to a treatment-seeking population.

In conclusion, our study findings align with a model of addiction in which chronic opioid use engenders, to varying degrees across individuals, neuroadaptations (e.g. in the HPA-axis) and negative emotionality that can alter susceptibility to stress-potentiated drug use, consistent with the concept of 'hyperkatifeia' (Koob, 2020). Despite having stabilized participants on buprenorphine to suppress withdrawal symptoms, we demonstrated that dose-dependent noradrenergic/glucocorticoid stimulation (stress) can differentially increase opioid-seeking in individuals with elevated basal HPA-axis function. Thus, risks of opioid withdrawal (due to non-optimal dosing with MOUD) and stress-exposure, overlaid on an individual's basal neurophysiology and hedonic tone, might jointly determine ongoing opioid-seeking. Future studies should investigate neurochemical pathways that modulate gain of the stress-response system in drug-appetitive behavior, toward the goal of developing medications or neuromodulation to attenuate stress-potentiated drug use (Greenwald, 2018; Koob, 2021).

CRediT authorship contribution statement

Mark K. Greenwald: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Eric A. Woodcock:** Writing – review & editing, Visualization, Formal analysis, Conceptualization. **Tabitha E.H. Moses:** Writing – review & editing, Conceptualization. **Leslie H. Lundahl:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mark Greenwald reports financial support was provided by National Institute on Drug Abuse. Eric Woodcock reports financial support was provided by National Institute on Drug Abuse. Tabitha Moses reports financial support was provided by National Institute on Drug Abuse. Mark Greenwald reports a relationship with Indivior Inc that includes: consulting or advisory. Note: Although I have received consulting fees from Indivior Inc., that work was unrelated to this project and Indivior did not play a role in the present project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2024.100684.

Data availability

Data will be made available on request.

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