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STIMULANTS AS SPECIFIC INDUCERS OF DOPAMINE-INDEPENDENT SIGMA AGONIST SELF-ADMINISTRATION IN RATS

Takato Hiranita, Paul L. Soto, Gianluigi Tanda, Theresa A. Kopajtic and Jonathan L. Katz

Psychobiology Section, Molecular Targets and Medications Discovery Branch, Intramural Research Program, Department of Health and Human Services, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224 (TH, GT, TAK, JLK)

Behavioral Biology Research Center, 5510 Nathan Shock Drive, Suite 3000, Johns Hopkins University Medical School, Baltimore, MD 21224-6823 (PLS)

Running Titles

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b) Correspondence: Jonathan L. Katz, Psychobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, 251 Bayview Blvd, Suite 200, Baltimore, MD 21224, USA

E-mail: jkatz@intra.nida.nih.gov; Phone: 443-740-2582; FAX: 443-740-2829

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d) A list of nonstandard abbreviations

σR: Sigma receptor;

ANOVA, analysis of variance

BD 1008, N-[2-(3,4-Dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine dihydrobromide

BD 1063, 1-[2-(3,4-Dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride

DTG, 1,3-di-*o*-tolylguanidine

EXT, extinction

inj, injection

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LED, light-emitting diode

(+)-MK 801: (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (dizocilpine)

NIDA, National Institute on Drug Abuse

NMDA, *N*-methyl-D-aspartate

OWW, original wet weight

PRE-084, 2-(4-Morpholinethyl) 1-phenylcyclohexanecarboxylate hydrochloride

TO, time-out;

e) Recommended section: Behavioral Pharmacology

ABSTRACT

A previous study showed that cocaine self-administration induced dopamine-independent reinforcing effects of sigma agonists mediated by their selective actions at sigma$_1$ receptors (σ_1Rs), which are intracellularly-mobile chaperone proteins implicated in abuse-related effects of stimulants. The present study assessed whether the induction was specific to self-administration of cocaine. Rats were trained to self-administer the dopamine releaser, *d*-methamphetamine (0.01-0.32 mg/kg/injection), the mu-opioid receptor agonist, heroin (0.001-0.032 mg/kg/injection), and the non-competitive *N*-methyl-D-aspartate (NMDA) receptor/channel antagonist ketamine (0.032-1.0 mg/kg/injection). As with cocaine, self-administration of *d*-methamphetamine induced reinforcing effects of the selective σ_1R agonists, PRE-084 and (+)-pentazocine (0.032-1.0 mg/kg/injection, each). In contrast, neither self-administration of heroin nor ketamine induced PRE-084 or (+)-pentazocine (0.032-10 mg/kg/injection, each) self-administration. Though the σ_1R agonists did not maintain responding in subjects with histories of heroin or ketamine self-administration, substitution for those drugs was obtained with appropriate agonists (e.g. remifentanil, 0.1-3.2 μg/kg/injection, for heroin and (+)-MK 801, 0.32-10.0 μg/kg/injection, for ketamine). The σR antagonist BD 1008 (1.0-10 mg/kg) dose-dependently blocked PRE-084 self-administration but was inactive against *d*-methamphetamine, heroin, and ketamine. In contrast, PRE-084 self-administration was affected neither by the dopamine receptor antagonist, (+)-butaclamol (10-100 μg/kg), nor the opioid antagonist, (-)-naltrexone (1.0-10 mg/kg), whereas these antagonists were active against *d*-methamphetamine and heroin self-administration, respectively. The results indicate that experience specifically with indirect-acting dopamine agonists induces reinforcing effects of previously inactive σ_1R agonists. It is further suggested that induced σ_1R reinforcing mechanisms may play an essential

role in treatment-resistant stimulant abuse, suggesting new approaches for the development of effective medications for its treatment.

INTRODUCTION

Sigma$_1$ receptors (σ_1Rs) are intracellular chaperone proteins that are expressed widely in the central and peripheral nervous systems (Hayashi and Su, 2007; Maurice and Su, 2009). With agonist actions or cellular stress, σ_1Rs translocate from their primary endoplasmic reticulum localization to different subcellular compartments and can regulate ion channels and G-protein-coupled-receptor signaling (Aydar et al., 2002; Cormaci et al., 2007; Hayashi and Su, 2007). The wide-spread distribution of σ_1Rs and their capacity to influence intracellular signaling suggest a substantial role in various physiological functions. Drugs acting at these receptors have been studied for therapeutic effects in cancer, HIV-infection, psychiatric disorders, and substance abuse (Maurice and Su, 2009; Katz et al., 2011).

σ_1Rs also are expressed widely in dopaminergic brain regions (Hayashi et al., 2010), and drugs acting at these receptors have been shown to regulate dopaminergic function (e.g. Nuwayhid and Werling, 2003). Further, σ_1R antagonists have been shown to block several *in vivo* effects that are related to abuse and excessive intake of cocaine (McCracken et al., 1999; Romieu et al., 2000; 2001; 2002; Matsumoto, 2009; Katz et al., 2011). However, several studies have demonstrated a lack of effects of a wide range of the selective σ_1R antagonists on cocaine self-administration (Martin-Fardon et al., 2007; Hiranita et al., 2010; 2011a; 2013), though selective σ_1R antagonists did block cocaine self-administration when combined with standard dopamine uptake inhibitors (Hiranita et al., 2011a). These results suggest a complex involvement of σ_1Rs in cocaine self-administration.

Consistent with that potential complex involvement, a recent study demonstrated that experience with cocaine self-administration induced reinforcing effects of σ_1R agonists that were absent in drug-naïve subjects or subjects with a history of food reinforcement (Hiranita et al.,

2010; 2013). Further, the induced reinforcing effects of the selective σ_1R agonist, PRE-084, were insensitive to pretreatments with dopamine-receptor antagonists (Hiranita et al., 2013). Additionally, another study found little, if any, cocaine-like discriminative-stimulus effects of PRE-084 in rats (Hiranita et al., 2011b). Though PRE-084 administration did dose-dependently stimulate dopamine levels in the nucleus accumbens shell of rats (Garcés-Ramírez et al., 2011), a brain region involved in the reinforcing effects of cocaine (Pontieri et al., 1995; 1996; Tanda et al., 1997), its potency was roughly 30-fold lower than that of cocaine and stimulation of dopamine was absent at self-administered doses (Garcés-Ramírez et al., 2011).

The present study was designed to address the pharmacological specificity of the experience with cocaine self-administration as an inducer of the reinforcing effects of σ_1R agonists. The study by Hiranita et al. (2013) determined that experience with food reinforcement was ineffective as an inducer of σ_1R agonist self-administration, despite the experimental conditions of the exposure being similar to those for cocaine. The present study assessed whether the induction of the selective σ_1R agonists was obtained with the indirect dopamine agonist *d*-methamphetamine, the mu-opioid receptor agonist heroin and the non-competitive *N*-methyl-D-aspartate (NMDA) receptor/channel antagonist ketamine in rats, each representing different classes of abused drugs.

MATERIALS AND METHODS

Drugs. The drugs used in the present study were as follows: *d*-methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, MO), (-)-heroin hydrochloride (RTI International, Research Triangle Park, NC), (±)-ketamine hydrochloride (Ft. Dodge, FT Dodge, Iowa / Bioniche Pharma, Lake Forest, IL,), PRE-084 (Tocris, Ballwin, MO), (+)-pentazocine succinate (National Institute on Drug Abuse), *d*-amphetamine sulfate (Sigma-Aldrich), remifentanil hydrochloride (brand name Ultiva, Hospira, Inc. Lake Forest, IL), (+)-MK 801 hydrogen maleate (Sigma-Aldrich), BD 1008 (Tocris), (+)-butaclamol hydrochloride (Sigma-Aldrich), (-)-naltrexone hydrochloride (Sigma-Aldrich) and haloperidol (Sigma-Aldrich, used only for the σ_2R binding assay). Saline (0.9% sodium chloride, USP, Hospira Inc., Lake Forest, IL) was used as vehicle for all compounds. Drugs used were administered intravenously (*d*-methamphetamine, (-)-heroin, (±)-ketamine, PRE-084, and (+)-pentazocine, remifentanil, (+)-MK 801, BD 1008, (+)-butaclamol, and (-)-naltrexone) or intraperitoneally (BD 1008, (+)-butaclamol, and (-)-naltrexone). BD 1008 and (-)-naltrexone were administered 5 min before sessions. (+)-Butaclamol was administered at 30 min before sessions.

σR Binding. Frozen whole guinea-pig brains (minus cerebellum) were thawed on ice, weighed and homogenized (with a glass and Teflon homogenizer) in 10 mM Tris-HCl with 0.32 M sucrose, pH 7.4 (10 ml/g tissue). Guinea-pig brain was used because of the relatively higher density of those receptors in that tissue compared to rat (Tam, 1983; Nguyen et al., 1996). The homogenate was centrifuged at 1000 x g for 10 min at 4° C. The supernatant was collected into a clean centrifuge tube and the remaining pellet was re-suspended by vortex in 10 ml buffer (tissue) and centrifuged again at 50,000 x g for 15 min at 4° C. The resulting pellet was resuspended in experimental buffer to 80 mg/ml original wet weight (OWW).

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Ligand binding experiments were conducted in polypropylene assay tubes containing 0.5 ml of 50 mM Tris-HCl buffer, pH 8.0. For σ_1R binding, each tube contained 3 nM [^{3}H](+)-pentazocine (PerkinElmer Life Sciences, Waltham, MA) and 8.0 mg tissue, OWW. Nonspecific binding was determined using 10 μM haloperidol. For σ_2R binding, each tube contained 3 nM [^{3}H]DTG (Perkin Elmer Life Science), 200 nM (+)-pentazocine, and 8.0 mg tissue, OWW. Nonspecific binding was determined using 100 μM haloperidol. The reaction was started with the addition of tissue and the tubes were incubated for 120 min at room temperature.

Incubations for all binding assays were terminated by rapid filtration through Whatman GF/B filters, presoaked in polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, Maryland). The filters were washed twice with 5 ml ice cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 ml) was added and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, California) at 50% efficiency. Assays were typically conducted in at least three independent experiments, each performed in triplicate.

For the displacement of radioligand binding, IC_{50} values were computed using a nonlinear, least-squares regression analysis (GraphPad Prism, GraphPad Software Inc., San Diego, CA). Affinities (K_i values) were calculated using the concentration of radioligand used in the assay.

Self-Administration Procedures. Twenty-four male Sprague-Dawley rats (Taconic Farms, Germantown, New York) weighing approximately 300 g at the start of the study, served as subjects. Subjects were acclimated to a temperature- and humidity-controlled vivarium for at least one week with food (Scored Bacon Lover Treats, BIOSERV, Frenchtown, NJ) and tap water unrestrictedly available in their home cages under a 12:12-h light:dark cycle with lights on

at 07:00 hours. After acclimation, rats were assigned to treatment conditions in a non-systematic manner and thereafter maintained at approximately 320 g by adjusting their daily food ration. Subjects were surgically implanted under anesthesia (ketamine 60.0 mg/kg, i.p. and xylazine 12.0 mg/kg, i.p.) in the right external jugular vein with a chronic indwelling catheter that exited at the mid-scapular region of the animal's back. Catheters were infused daily with 0.1 ml of a sterile saline solution containing heparin (30.0 IU/ml), penicillin G potassium (250,000 IU/ml) to minimize the likelihood of infection, and the formation of clots or fibroids. All animals were allowed to recover from surgery for approximately seven days before drug self-administration studies were initiated. Care of the subjects was in accordance with the guidelines of the National Institutes of Health and the National Institute on Drug Abuse Intramural Research Program Animal Care and Use Program, which is fully accredited by AAALAC International.

Experimental sessions were conducted daily with animals placed in operant-conditioning chambers (modified ENV-203, Med Associates, St. Albans, VT) that measured 25.5 x 32.1 x 25.0 cm, and were enclosed within sound-attenuating cubicles equipped with a fan for ventilation and white noise to mask extraneous sounds. On the front wall of each chamber were two response levers, 5.0 cm from the midline and 4.0 cm above the grid floor. A downward displacement of a lever with a force approximating 20 g defined a response, and always activated a relay mounted behind the front wall of the chamber producing an audible "feedback" click. Three light-emitting diodes (LEDs) were located in a row above each lever. A receptacle for the delivery of 45-mg food pellets via a pellet dispenser (Med Associates, Model ENV-203-20) was mounted on the midline of the front wall between the two levers and 2.0 cm above the floor, but was unused in this study. A syringe infusion pump (Model 22, Harvard Apparatus, Holliston, MA) placed above each chamber delivered injections of specified volumes from a 10 ml syringe.

The syringe was connected by Tygon tubing to a single-channel fluid swivel (375 Series Single Channel Swivels, Plymouth Meeting, PA) which was mounted on a balance arm above the chamber. Tygon tubing from the swivel to the subject's catheter was protected by a surrounding metal spring and completed the connection to the subject.

Experimental sessions started with the illumination of the LEDs above each lever, and initially lasted for 120 min during which saline (n=6), *d*-methamphetamine (0.1 mg/kg/injection, n=6), (-)-heroin (0.01 mg/kg/injection, n=6) or (±)-ketamine (0.32 mg/kg/injection, n=6) were delivered following responses. Each response on the right lever turned off the LEDs, produced an audible click, and activated the infusion pump for 10 sec (fixed-ratio or FR one schedule) followed by a 20-sec time-out (TO) period during which LEDs were off and responding had no scheduled consequences. After the TO, the LEDs were illuminated and responding again had the scheduled consequences. Responses on the left lever were recorded but had no scheduled consequences. This condition remained in effect over all the sessions. One group was studied with self-administration of saline injections only, for 14 sessions. For the *d*-methamphetamine, (-)-heroin or (±)-ketamine self-administration groups, subjects were given an opportunity to respond for injections of the respective drugs for 14 sessions to assess reinforcing effects. On the 15th session, PRE-084 (0.32 mg/kg/injection) was substituted for the initially self-administered drug for ten sessions. Subsequently saline was substituted for PRE-084 for seven sessions. Subjects were returned to their home cages in the vivarium after each session.

The self-administration procedure was then modified for assessments of a range of self-administered doses to ensure that the outcomes of the single-dose assessments were generalizable over a range of doses, and to more fully characterize the pharmacological mechanisms of the substitution with PRE-084. Subjects were trained to self-administer *d*-

methamphetamine (0.1 mg/kg/injection), (-)-heroin (0.01 mg/kg/injection) or (±)-ketamine (0.32 mg/kg/injection) under an FR 5 schedule of reinforcement until self-administration was consistent from one session to the next. The session was then divided into five 20-min components, each preceded by a 2-min TO period. This arrangement allowed the assessment of a range of self-administered doses in a single session (Hiranita et al., 2009). By adjusting infusion volumes and durations, the drug dose per injection was incremented in the five sequential components in an ascending dose-order as follows: no injection (also referred to as extinction, or EXT, because responses had no scheduled consequences), 0.01, 0.03, 0.10, and 0.32 mg/kg/injection for *d*-methamphetamine, no injection, 0.001, 0.003, 0.01, and 0.032 mg/kg/injection for (-)-heroin, and no injection, 0.03, 0.10, 0.32, or 1.0 mg/kg/injection for (±)-ketamine. Infusion volumes (and durations) were respectively 0 µl (0 sec), 5.6 µl (0.32 sec), 18.0 µl (1.0 sec), 56.0 µl (3.2 sec), and 180 µl (10.0 sec) based on a body weight of 0.32 kg. A sample injection of the drug at the corresponding dose occurred independently of responding at the end of the TO period that preceded each component except the first.

Training continued until response rates across three consecutive sessions differed by less than 20%. With these stable performances various compounds were substituted for each of the drugs to assess their self-administration. The compounds substituted for each of the self-administered drugs were: PRE-084 and (+)-pentazocine, each across a low (0.032, 0.1, 0.32, and 1.0 mg/kg/injection) and high (0.32, 1.0, 3.2, and 10 mg/kg/injection) range of doses, and the antagonists, BD 1008 (0.03, 0.10, 0.32, or 1.0 mg/kg/injection), (+)-butaclamol (0.0001, 0.00032, 0.001, or 0.0032 mg/kg/injection) (-)-naltrexone (0.03, 0.10, 0.32, or 1.0 mg/kg/injection) and saline. In addition, the indirect-acting dopamine agonist *d*-amphetamine (0.01, 0.03, 0.1, and 0.32 mg/kg/injection) was substituted for *d*-methamphetamine, the mu-opioid receptor agonist

remifentanil (0.0001, 0.00032, 0.001, or 0.0032 mg/kg/injection) was substituted for (-)-heroin, and the non-competitive NMDA receptor/channel antagonist (+)-MK 801 (0.00032, 0.001, 0.0032, or 0.01 mg/kg/injection) was substituted for (±)-ketamine. Due to high rates of remifentanil self-administration (Panlilio and Schindler, 2000; Hutchinson et al., 2012), infusion durations of remifentanil were reduced to 0, 0.24, 0.75, 2.4, 7.5 sec to avoid excessive fluid intake and emptying of the syringe.

Presession injections (i.p.) of the non-selective σR antagonist, BD 1008, the non-selective dopamine receptor antagonist, (+)-butaclamol, or the non-selective opioid-receptor antagonist, (-)-naltrexone, were assessed. These antagonists also were examined in the rats trained with *d*-methamphetamine when PRE-084 (0.03, 0.10, 0.32, and 1.0 mg/kg/injection) was substituted under otherwise identical conditions. The effects of pre-session treatments on respective drug self-administration were separated by a minimum of 72 hours. The antagonists were studied with a mixed order of drugs and doses.

Response rates were determined by dividing responses by elapsed time, excluding all TO periods. The significance of effects on response rates was assessed by ANOVA, with post-hoc Bonferroni t-tests. A two-way repeated-measures ANOVA was used to assess the effects of successive response rates during drug self-administration (factors were session number and lever: right or left). A one-way repeated-measures ANOVA was used to assess the effects of drug substitution on successive response rates during drug self-administration (for data shown in Figure 2). A two-way repeated-measures ANOVA was also used to assess effects of pre-session treatment with antagonists on self-administration with drug-dose and component, no injection or drug pretreatment dose as factors (for data shown in Figures 3 and 4). Presentation of the entire outcomes of the statistical analyses of results shown in Figures 2-4 and their corresponding post-

hoc tests was judged to be cumbrous and impractical. Therefore the present text only indicates for those experiments which outcomes were significant at $p<0.05$. Complete statistical analyses are available in Supplemental Tables 1 and 2.

The ED_{50} values for the effects of antagonist pretreatments were calculated by linear regression (Snedecor and Cochran, 1967) of the response rates on dose of the antagonist, using rates from the dose of the self-administered drug that maintained maximal response rates after a saline pretreatment. From this analysis, ED_{50} values and their 95% confidence limits were derived.

RESULTS

Response rates on the active (right) lever remained infrequent over a series of 14 daily 2-hr sessions in drug-naïve rats (Figure 1a) when each response on the active lever produced an injection of saline (an FR one-response schedule of reinforcement). Additionally, responses on the alternate (left) lever which had no scheduled consequences remained infrequent. A two-way repeated measures ANOVA (lever x sessions) indicated a non-significant effect of session number ($F_{13,65}$=1.38; p=0.193), lever position ($F_{1,65}$=0.112; p=0.752), and their interaction ($F_{13,65}$=0.746; p=0.711).

Responses on the active lever which produced *d*-methamphetamine injections (0.1 mg/kg/injection) increased in frequency (Figure 1b) over the 14 sessions. In contrast, responses on the alternate (left) lever remained infrequent. Starting on session 15, when PRE-084 (0.32 mg/kg/injection) was substituted for *d*-methamphetamine, response rates immediately increased above those maintained by *d*-methamphetamine and further increased in frequency over 10 daily sessions (Figure 1b). When saline was substituted for PRE-084 (for the next seven sessions), response rates decreased to low levels (Figure 1b). A two-way repeated measures ANOVA (lever x sessions) indicated a significant effect of session number ($F_{30,150}$=14.3; p<0.001), lever ($F_{1,150}$=50.5; p<0.001), and their interaction ($F_{30,150}$=15.1; p<0.001).

As with *d*-methamphetamine, responses on the active lever which produced heroin injections (0.01 mg/kg/injection) increased in frequency over the first 14 sessions and those on the alternate (left) lever, which had no scheduled consequences, remained infrequent (Figure 1c). In contrast to results with *d*-methamphetamine, response rates decreased to low levels when PRE-084 was substituted for heroin (Figure 1c). Those levels were similar to levels obtained when saline was subsequently substituted for PRE-084 (Figure 1c). A two-way repeated

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measures ANOVA (lever x sessions) indicated a significant effect of session number ($F_{30,150}$=6.84; p<0.001), lever ($F_{1,150}$=72.5; p<0.001), and their interaction ($F_{30,150}$=7.47; p<0.001).

Responses on the active lever which produced ketamine injections (0.32 mg/kg/injection) dramatically increased in frequency over the 14 sessions of its initial availability (Figure 1d). In contrast, responses on the alternate (left) lever which had no scheduled consequences remained infrequent. As with heroin, response rates decreased to low levels when PRE-084 was substituted for ketamine (Figure 1d), and remained infrequent when saline was substituted for PRE-084 (Figure 1d). A two-way repeated measures ANOVA (lever x sessions) indicated a significant effect of session number ($F_{30,150}$=17.2; p<0.001), lever ($F_{1,150}$=22.8; p=0.005), and their interaction ($F_{30,150}$=15.2; p<0.001).

The dose-related effects of the compounds, substitutions of other compounds, and antagonist pretreatments were subsequently assessed after the schedule was modified to the FR5 schedule with separate components for evaluating a range of doses of the self-administered drugs. As expected, low to intermediate doses of *d*-methamphetamine (0.01-0.1 mg/kg/injection), heroin (0.001-0.01 mg/kg/injection) and ketamine (0.032-0.32 mg/kg/injection) produced dose-related increases in rates of responding (Figure 2a-c, filled symbols). Maximal self-administration of *d*-methamphetamine, heroin, and ketamine was obtained at doses of 0.1, 0.01, and 0.32 mg/kg/injection, respectively, with responding at those doses significantly greater than that obtained with saline injections.

For the subjects trained with *d*-methamphetamine, PRE-084 maintained self-administration across a range of doses, with maximal rates at the 0.32 mg/kg/injection dose greater than those maintained by saline and *d*-methamphetamine (Figure 2a triangles up). Additionally, the selective σ_1R agonist, (+)-pentazocine, maintained response rates statistically

greater than those with saline and greater than those maintained by *d*-methamphetamine across a similar range of doses (Figure 2a triangles down). The indirect-acting dopamine agonist *d*-amphetamine substituted at rates greater than saline and with a potency similar to that of *d*-methamphetamine but with greater maximal effects (Figure 2a open diamonds). In contrast to the other compounds, responding occurred infrequently when saline was available for injection and rates of responding with the antagonists, BD 1008, (+)-butaclamol, or naltrexone, were typically not different from those with saline (Figure 2a). The exception was 0.32 mg/kg/injection of BD 1008 at which response rates were slightly, but significantly, lower than those maintained by saline.

For the subjects trained with heroin, neither PRE-084 nor (+)-pentazocine maintained self-administration at levels appreciably greater than those obtained with saline (Figure 2b), though a small (0.005 response/sec) statistical increase above saline response rates was obtained at 3.2 mg/kg/injection of PRE-084 that was well below rates of responding maintained by remifentanil or heroin (Figure 2b). With (+)-pentazocine small (≤ 0.009 responses/sec) statistical increases above saline response rates were obtained at 0.1 and 1.0 mg/kg/injection that were also substantially less than those obtained with remifentanil and heroin. As expected, remifentanil was approximately 10-fold more potent than heroin and maintained the highest rates of responding among all of the compounds tested (Figure 2b, compare filled symbols with open diamonds). As with subjects trained with *d*-methamphetamine, occasional small statistical increases (≤0.006 responses/sec) in responding were obtained with a few doses of the antagonists, but these drugs did not maintain rates of responding comparable to those maintained by heroin or remifentanil (Figure 2b).

Neither PRE-084 nor (+)-pentazocine maintained self-administration at levels comparable to those maintained with ketamine (Figure 2c), though small statistical increases were obtained with 0.32 and 1.0 mg/kg/injection. In contrast, a bi-phasic dose-related and robust substitution for ketamine was obtained with (+)-MK 801 that was statistically greater than that obtained with saline (Figure 2c). (+)-MK 801 was approximately 100-fold more potent than ketamine though the maximal rates of responding maintained by (+)-MK 801 were substantially lower than those maintained by ketamine (Figure 2c). As with the other training drugs, the specific antagonists, BD 1008, (+)-butaclamol, and naltrexone, produced occasional small statistical increases (≤0.006 responses/sec) in responding compared to those with saline, but response rates were uniformly low and approximated those obtained with saline (Figure 2c).

It is currently unclear how much experience (in cumulative intake or time) is necessary or sufficient for the induction of reinforcing effects of selective σ_1R agonists, and consequently the above described negative outcomes might be altered by extended exposure to the primary self-administered drug. Therefore, the effects of PRE-084 substitutions were assessed again after the experiments shown in Figure 3. For the subjects with a *d*-methamphetamine history, the dose-effect curve for the initially effective PRE-084 was shifted upward on its second assessment (after a total intake of about 165 mg/kg of *d*-methamphetamine) with maximal rates of responding maintained by PRE-084 increasing by a factor of 2.10 (Table 1). During the same period of time there was no appreciable change in the maximal self-administration of *d*-methamphetamine (Table 1). Further exposure to *d*-methamphetamine, however, did not produce further change in the self-administration of PRE-084 (Table 1). In contrast, a greater than six-fold increase in heroin or approximate 4.5-fold increase in ketamine intake did not induce self-administration of PRE-084 (Table 1).

A previous study indicated that the self-administration of PRE-084 was blocked by σR antagonists, while insensitive to dopamine receptor antagonists (Hiranita et al., 2013). Thus the performances maintained by the various drugs were further compared with regard to their sensitivity to pharmacological antagonism. As expected, *d*-methamphetamine self-administration was dose-dependently blocked by the dopamine antagonist, (+)-butaclamol (Figure 3a), with an ED_{50} value of 4.38 μg/kg (95% CLs: 3.98-5.17). (+)-Butaclamol was used because it has low affinity for σRs (Table 2) compared to literature values of other DA antagonists (Andersen, 1988; Bristow et al., 1998). In contrast, neither the σR antagonist, BD 1008, nor the opioid antagonist, naltrexone, altered the self-administration of *d*-methamphetamine (Figure 3b-c).

Compared to *d*-methamphetamine, the self-administration of PRE-084 was not appreciably altered (Figure 3d) at doses of (+)-butaclamol that antagonized *d*-methamphetamine self-administration. Small statistical changes in rates of responding maintained by PRE-084 were obtained at a few doses of (+)-butaclamol that decreased *d*-methamphetamine self-administration. In contrast, PRE-084 self-administration was dose-dependently blocked by BD 1008 (Figure 3e) with an ED_{50} value of 3.45 mg/kg (95% CLs: 2.68-4.52). BD 1008 was used because it is a σR antagonist with comparable affinity for both subtypes of σRs (Table 2). Neither (+)-butaclamol nor BD 1008 produced any appreciable shifts in the self-administration dose-effect curves for heroin or ketamine (Figure 4a-e), though small statistical decreases in rates compared to vehicle treatment were produced by 3.2 μg/kg (+)-butaclamol against 10.0 μg/kg/inj of heroin and 32.0 and 100.0 μg/kg (+)-butaclamol against 0.32 mg/kg/inj of ketamine compared to vehicle treatment. As expected, self-administration of heroin, but none of the other self-administered drugs, was blocked by naltrexone pretreatment (Figures 3c, f, 4c, f) with an ED_{50}

value of 0.514 mg/kg (95% CLs: 0.416-0.669).

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DISCUSSION

The results of the present study indicate that experience with *d*-methamphetamine self-administration induces σ_1R-mediated reinforcing effects. Further, comparable histories of either heroin or ketamine self-administration were insufficient as inducers of σ_1R-mediated reinforcing effects. A previous study found a similar effect with cocaine self-administration (Hiranita et al., 2010; Hiranita et al., 2013). Together these results suggest that the induction of reinforcing effects of σ_1R agonists is due to actions mediated by dopaminergic mechanisms and that this finding is consistent with previous studies indicating that qualitative and profound changes in the behavioral effects of drugs can be obtained due to experiential factors (e.g. Collins and Woods, 2007; 2009; Barrett, 2013).

Several previous findings may be related to the present induction of reinforcing effects of σ_1R agonists. For example passive exposure to cocaine increases levels of σ_1R mRNA and protein in brain regions implicated in drug reinforcement, an effect sensitive to the preferential σ_1R antagonist, BD 1063 (Liu et al., 2005; Liu and Matsumoto, 2008). Moreover, the up-regulation of σ_1Rs by cocaine administration *in vivo* does not occur in mice with a genetic deletion of dopamine D_1 receptors (Zhang et al., 2005). In rats actively self-administering *d*-methamphetamine, increases in midbrain levels of σ_1R mRNA and protein have been found in the hippocampus and olfactory bulb, and were greater than those obtained in rats passively receiving the drug at the same doses and frequencies, i.e. "yoked" controls (Stefanski et al., 2004; Hayashi et al., 2010). Further, the σ_1Rs in the olfactory bulb were found to be co-localized with dopamine D_1 receptors (Hayashi et al., 2010), and a linkage between D_1 and σ_1 receptors is further supported by studies suggesting that these proteins can form heterodimers (Navarro et al., 2010).

Dopamine D_2 receptors may also be involved in the actions of σ_1Rs. Stefanski et al. (1999) have shown that rats with *d*-methamphetamine self-administration histories showed a decrease in levels of dopamine D_2 autoreceptors in the ventral tegmental area and the substantia nigra zona compacta as well as a down-regulation of dopamine D_1 receptors in the shell of the nucleus accumbens – effects not obtained in rats passively receiving *d*-methamphetamine or saline. More recently, Navarro et al. (2013) provided evidence for heteromers of σ_1 and D_2, but not D_3 or D_4, receptors in mouse striatum and that cocaine can inhibit signaling that is not obtained in tissue from mutant mice with the deletion of σ_1Rs. Thus, current evidence points to an activation of dopaminergic effects, involving D_1 and D_2 dopamine receptors, as critical for triggering an up-regulation of σ_1Rs, which in turn may be involved in the induction of σ_1R-agonist reinforcing effects. Further studies assessing the induction of these reinforcing effects with particular molecular targets and anatomic loci will further elucidate these potential pathways.

It remains possible that exposure to heroin or ketamine under other conditions (for example longer exposures or greater cumulative intake) might more effectively induce σ_1R agonist self-administration. However after the initial assessment, further increases in the intake of heroin that were greater than six-fold or ketamine that were approximately 4.5-fold did not induce self-administration of PRE-084. At present it appears that experiences with the dopamine indirect agonists, *d*-methamphetamine (present study) or cocaine (Hiranita et al., 2010; Hiranita et al., 2013) are sufficient, whereas comparable experiences with other abused drugs are insufficient, to induce σ_1R agonist self-administration. Both *d*-methamphetamine and cocaine exert their dopaminergic effects through actions at the dopamine transporter. At present it appears that an indirect action mediated by the dopamine transporter is critical in the induction of

reinforcing effects of σ_1R agonists, however the potential of direct-acting dopamine receptor agonists to induce σ_1R agonist self-administration has not yet been assessed.

Once induced, the reinforcing effects of the selective σ_1R agonists were independent of dopaminergic mechanisms in the present and a previous (Hiranita et al., 2013) study. The lack of substantive dopaminergic mediation of the effects of σR agonists is not surprising in light of behavioral studies showing a failure of the σR agonists, PRE-084 or DTG, to substitute for cocaine in rats trained to discriminate cocaine from saline injections (Hiranita et al., 2011b), a procedure in which a number of indirect dopaminergic agonists fully substitute for cocaine (Witkin et al., 1991; Li et al., 2006). Further, administration of i.v. doses of PRE-084 that maintained self-administration behavior did not significantly stimulate dopamine levels in the nucleus accumbens shell in rats that had self-administered cocaine and PRE-084, or in rats that were not previously exposed to cocaine or PRE-084 (Hiranita et al., 2010; Garcés-Ramírez et al., 2011; Hiranita et al., 2013). Doses of PRE-084 that were 18-30 times greater than the self-administered doses did increase nucleus accumbens dopamine, but the effect was less than that produced by cocaine and was not antagonized by the σR antagonist, BD1063 (Garcés-Ramírez et al., 2011).

The present and similar past *in vivo* results are consistent with an independence from dopaminergic systems once σ_1R-mediated reinforcing mechanisms are induced, and as such they present a picture different from that painted by the findings of Navarro et al. (2013) of cocaine inducing effects mediated through σ_1-D_2 heteromers. In contrast to the present findings, no particular behavioral history was necessary for the interactions with either D_2 or D_1 dopamine receptors obtained by Navarro et al. (Navarro et al., 2010; 2013). Thus it is currently unclear whether or how those *in vitro* outcomes are related to the present *in vivo* findings. Additionally,

a previous study reported comparable stimulation of locomotor activity by methamphetamine in σ_1R knockout mice and their wild-type controls (Fontanilla et al., 2009) suggesting that at least the acute stimulation produced by methamphetamine does not involve dopamine and σ_1 receptor heteromeric complexes. The present results together with these published findings, suggest initially distinct *in vivo* pharmacologically mechanisms of stimulant drugs and σ_1R agonists that interact during experience with stimulant self-administration, followed by minimal if any involvement of dopamine systems after the reinforcing effects of the σ_1R agonists are triggered.

There was a difference between the antagonism of σR agonist self-administration and that of heroin or methamphetamine in the present study. The antagonism of *d*-methamphetamine self-administration by (+)-butaclamol, as well as that of heroin by naltrexone, was mainly surmountable, with decreases in maximal effects only at the highest antagonist doses (see also Harrigan and Downs, 1978; Bertalmio and Woods, 1989; Bergman et al., 1990; Winger et al., 1992; Hiranita et al., 2013). In contrast, the antagonism of PRE-084 self-administration by BD 1008 appeared insurmountable, with prominent dose-related decreases in maximal effect (see also Winger et al., 1992; Hiranita et al., 2010; 2011a; 2013). The bitonic function for self-administration may reflect two different dose-related effects that are differentially sensitive to antagonism. For example, doses on the ascending limb of the dose-effect curve may be competitively antagonized by the σR antagonist with doses on the descending limb insensitive to antagonism, resulting in what appears to be a decrease in maximal effect. Differential mechanisms for ascending and descending limbs of bitonic dose-effect curves have been described in other systems (Collins et al., 2005). Additionally, uniform direct effects on response rates by the antagonist may limit the degree to which it shifts the descending limb of the self-administration dose-effect curve. In that regard, it is interesting to note that the

antagonism of cocaine and PRE-084 self-administration by haloperidol, which has well-known dopamine-antagonist effects but also is an antagonist at σRs (Tam, 1983; Iwamoto, 1989), shows respectively, surmountable or insurmountable antagonism, suggesting that at least for haloperidol the insurmountable antagonism is not due to its own direct effects which would be expected to decrease responding maintained by either cocaine or PRE-084. As such, studies of the nature of the antagonism of σR agonist self-administration might most profitably focus on the sensitivity of doses on the descending limb to antagonism rather than features of the antagonists.

Recently it has been reported that inhibition of the dopamine transporter coupled with antagonism of σRs selectively decreases cocaine self-administration (Hiranita et al., 2011a), a preclinical indication of potential efficacy as a cocaine-abuse treatment (Mello and Negus, 1996). Those effects appear to involve mechanisms similar to those responsible for the present findings. The present results may shed light on the etiology of that efficacy. If cocaine self-administration experience recruits σRs into reinforcement mechanisms such dual targeting may be a preferred approach to the discovery of effective stimulant-abuse treatments and may at least partly explain the intractability of stimulant abuse to various attempts at treatment (Gorelick et al., 2004; Vocci et al., 2005; Vocci and Elkashef, 2005), particularly medical treatments singly targeting dopamine systems.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Hiranita, Kopajtic and Katz.

Conducted experiments: Hiranita, and Kopajtic.

Contributed new reagents or analytic tools: N/A.

Performed data analysis: Hiranita, Kopajtic, and Katz.

Wrote or contributed to the writing of the manuscript: Hiranita, Soto, Tanda, Kopajtic, and Katz.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1: A specific induction of the reinforcing effects of the selective σ_1R agonist PRE-084 in rats with a history of *d*-methamphetamine self-administration. Ordinates: responses per second; abscissae: sessions. Each point represents the mean ±SEM of six subjects. Significant differences between response rates on the active vs. inactive lever are shown as follows: *p<0.05, **p<0.01, ***p<0.001, compared with responding on the inactive lever. Panel a: Lack of self administration with saline injections. Panel b: Substitution of PRE-084 (0.32 mg/kg/injection) after self-administration of *d*-methamphetamine (0.1 mg/kg/injection) and absence of self-administration of saline injections. Panel c: Lack of substitution of PRE-084 (0.32 mg/kg/injection) after self-administration of heroin (0.01 mg/kg/injection) and absence of self-administration of saline injections. Panel d: Lack of substitution of PRE-084 (0.32 mg/kg/injection) after self-administration of ketamine (0.32 mg/kg/injection) and absence of self-administration of saline injections.

Figure 2: Substitution of various compounds in rats trained to self-administer either *d*-methamphetamine, heroin or ketamine. Ordinates: responses per second, log scale; abscissae: drug dose (mg/kg/injection), log scale, or sequential component of the session (for saline). Each point represents the mean ±SEM of six subjects. Panel a: Dose-effects of various drugs in subjects trained to self administer *d*-methamphetamine (●). Each dose-effect curve was determined only once except *d*-methamphetamine (shown as an average of 28 assessments) and PRE-084 (shown as an average of three assessments). Consecutive components with saline injections (○) did not maintain rates of responding greater than those obtained in the first component when response did not produce injections (EXT). Panel b: Dose-effects of various

drugs in subjects trained to self administer heroin (▲). Each dose-effect curve was determined only once except heroin (an average of 22 assessments). Consecutive components with saline injections (○) did not maintain rates of responding greater than those obtained in the first component when response did not produce injections (EXT). For PRE-084 and (+)-pentazocine, their dose-effects were assessed across two overlapping ranges of doses (0.032-1.0 and 0.32-10 mg/kg/injection) and their dose-effect curves are shown as a combination of the two determinations with an average of rates of responding maintained by no injections (EXT), or 0.32 or 1.0 mg/kg/injection of each drug. Panel c: Dose-effects of various drugs in subjects trained to self administer ketamine (■). Consecutive components with saline injections (○) did not maintain rates of responding greater than those obtained in the first component when response did not produce injections (EXT). Each dose-effect curve was determined only once except ketamine (shown as an average of 27 assessments). For PRE-084 and (+)-pentazocine, dose-effects were assessed with two overlapping but different ranges of doses (0.032-1.0 and 0.32-10 mg/kg/injection) and the dose-effect curves for these drugs are shown as described above. Complete statistical analyses are available in Supplemental Tables 1 and 2.

Figure 3: Effects of pre-session treatments with selective antagonists on self-administration of *d*-methamphetamine or PRE-084. Ordinates: responses per second; abscissae: drug dose (mg/kg/injection), log scale. Each point represents the mean ±SEM of response rates on the active lever in six subjects. Injections of (+)-butaclamol were administered i.p. 30 min before sessions. BD 1008 and naltrexone were administered i.p. five-min before sessions. Points labeled 0 mg/kg of the antagonist indicate vehicle pretreatments. Panels a-c: Effects of the antagonists on *d*-methamphetamine self-administration. Panels d-f: Effects of the antagonists

on PRE-084 self-administration. Complete statistical analyses are available in Supplemental Tables 1 and 2.

Figure 4: Effects of pre-session treatments with selective antagonists on self-administration of heroin or ketamine. Ordinates: responses per second; abscissae: drug dose (μg/kg/injection or mg/kg/injection, respectively), log scale. Each point represents the mean ±SEM of response rates on the active lever in six subjects. Injections of (+)-butaclamol were administered i.p. 30 min before sessions. BD 1008 and naltrexone were administered i.p. five-min before sessions. Points labeled 0 mg/kg of the antagonist indicate vehicle pretreatments. Panels a-c: Effects of the antagonists on heroin self-administration. Panels d-f: Effects of the antagonists on ketamine self-administration. Complete statistical analyses are available in Supplemental Tables 1 and 2.

TABLES

Table 1: Effects of extended history of drug self-administration on the induction of reinforcing effects of the σ_1R agonist, PRE-084.

Training Drug	Cumulative Intake of the Training Drug (mg/kg)	Maximal Response Rate Maintained by PRE-084 (responses/sec)	Maximal Response Rate Maintained by the Training Drug (responses/sec)
d-Methamphetamine	64.7 ± 6.75	0.184 ± 0.056 at 0.32 mg/kg/inj	0.086 ± 0.015 at 0.10 mg/kg/inj
	163 ± 21.3	0.388 ± 0.177 at 0.32 mg/kg/inj	0.068 ± 0.011 at 0.10 mg/kg/inj
	243 ± 34.0	0.339 ± 0.145 at 0.32 mg/kg/inj	0.068 ± 0.011 at 0.10 mg/kg/inj
Heroin	3.73 ± 0.292	0.021 ± 0.004 at 1.0 mg/kg/inj	0.064 ± 0.012 at 0.01 mg/kg/inj
	23.7 ± 3.45	0.018 ± 0.004 at 3.2 mg/kg/inj	0.070 ± 0.012 at 0.01 mg/kg/inj
Ketamine	367 ± 30.7	0.028 ± 0.007 at 0.32 mg/kg/inj	0.376 ± 0.100 at 0.32 mg/kg/inj
	1,630 ± 141	0.023 ± 0.004 at 0.32 mg/kg/inj	0.427 ± 0.120 at 0.32 mg/kg/inj

Table 2: Inhibition of the binding of radioligands labeling σ_1 and σ_2 receptors. Values in parentheses are 95% confidence limits.

Compound	**σ_1R K_i Value (nM) determined with [^{3}H]Pentazocine**	**σ_2R K_i Value (nM) determined with [^{3}H]DTG**
PRE-084	48.5 (39.8 – 59.2)	12,700 (9,290 – 17,300)
(+)-Pentazocine	4.59 (4.26 – 4.97)	224 (195 – 257)
d-Methamphetamine	4,390 (3,740 – 5,160)	15,900 (11,700 – 21,500)
Heroin	N.D.	68,600 (27,100 – 173,000)
Ketamine	227,000 (53,400 – 963,000)	36,900 (20,100 – 67,900)
(+)-Butaclamol	4,700 (4,270 – 5,170)	2,100 (1,770 – 2,498)
Naltrexone	N.D.	< 50% displacement at 10 mM

N.D. – No displacement at concentrations up to 10 mM

Figure 1

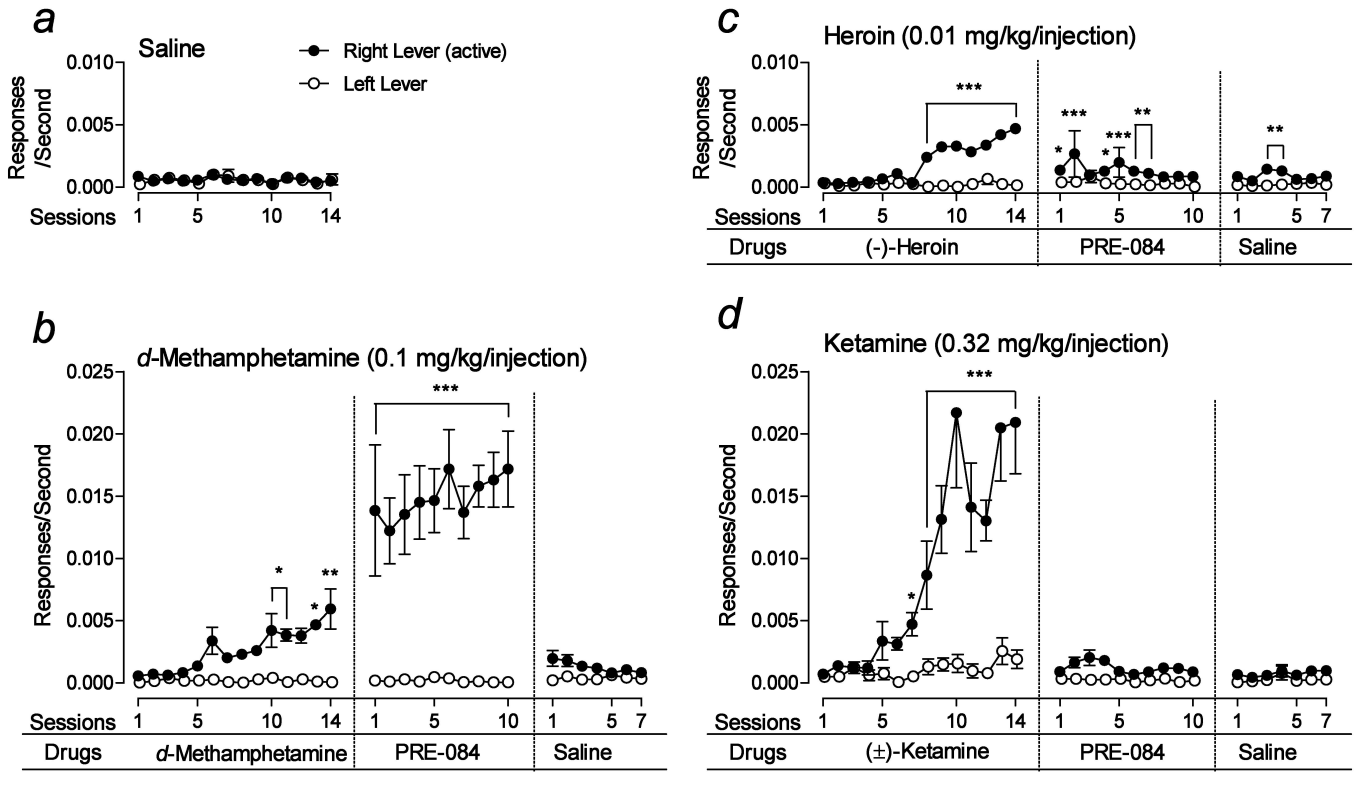

a
Saline
Right Lever (active)
Left Lever
Responses /Second
0.010
0.005
0.000
Sessions 1 5 10 14
b
d-Methamphetamine (0.1 mg/kg/injection)
Responses/Second
0.025
0.020
0.015
0.010
0.005
0.000
***
*
**
*
**
Sessions 1 5 10 14 1 5 10 1 5 7
Drugs d-Methamphetamine PRE-084 Saline
c
Heroin (0.01 mg/kg/injection)
Responses /Second
0.010
0.005
0.000
***
*
***
*
***
**
**
Sessions 1 5 10 14 1 5 10 1 5 7
Drugs (-)-Heroin PRE-084 Saline
d
Ketamine (0.32 mg/kg/injection)
Responses/Second
0.025
0.020
0.015
0.010
0.005
0.000
***
*
Sessions 1 5 10 14 1 5 10 1 5 7
Drugs (±)-Ketamine PRE-084 Saline

Figure 2

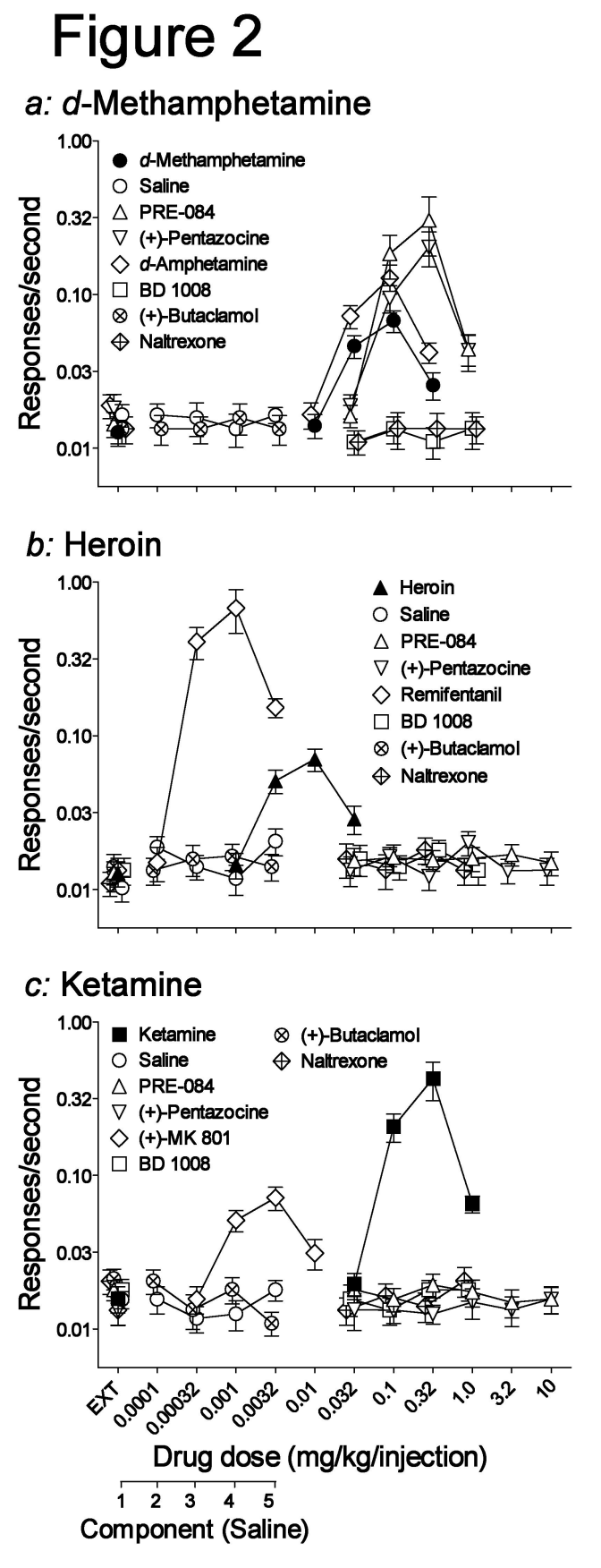

a: d-Methamphetamine
1.00
0.32
0.10
0.03
0.01
Responses/second
d-Methamphetamine
Saline
PRE-084
(+)-Pentazocine
d-Amphetamine
BD 1008
(+)-Butaclamol
Naltrexone
b: Heroin
1.00
0.32
0.10
0.03
0.01
Responses/second
Heroin
Saline
PRE-084
(+)-Pentazocine
Remifentanil
BD 1008
(+)-Butaclamol
Naltrexone
c: Ketamine
1.00
0.32
0.10
0.03
0.01
Responses/second
Ketamine
Saline
PRE-084
(+)-Pentazocine
(+)-MK 801
BD 1008
(+)-Butaclamol
Naltrexone
EXT
0.0001
0.00032
0.001
0.0032
0.01
0.032
0.1
0.32
1.0
3.2
10
Drug dose (mg/kg/injection)
1 2 3 4 5
Component (Saline)

Figure 3

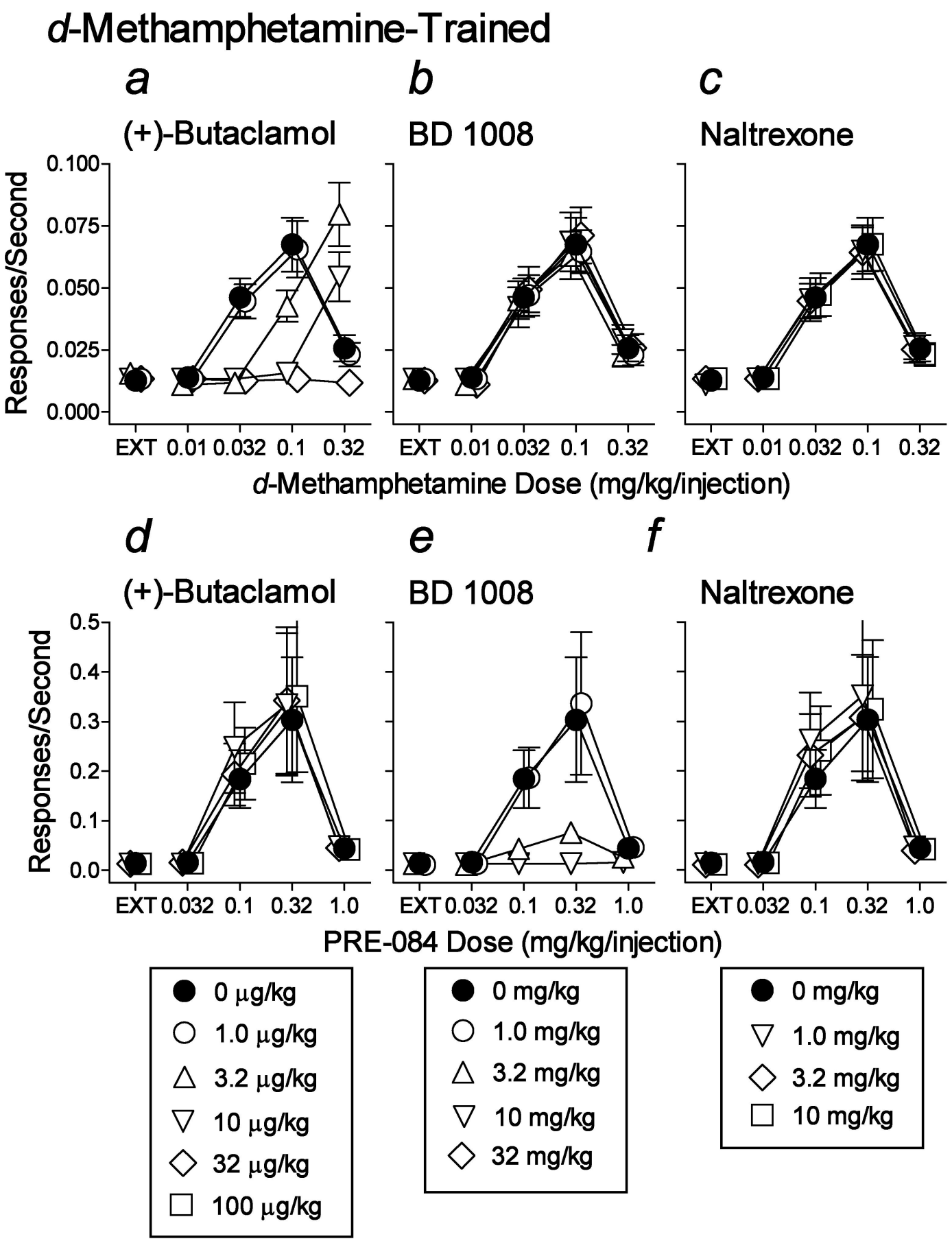
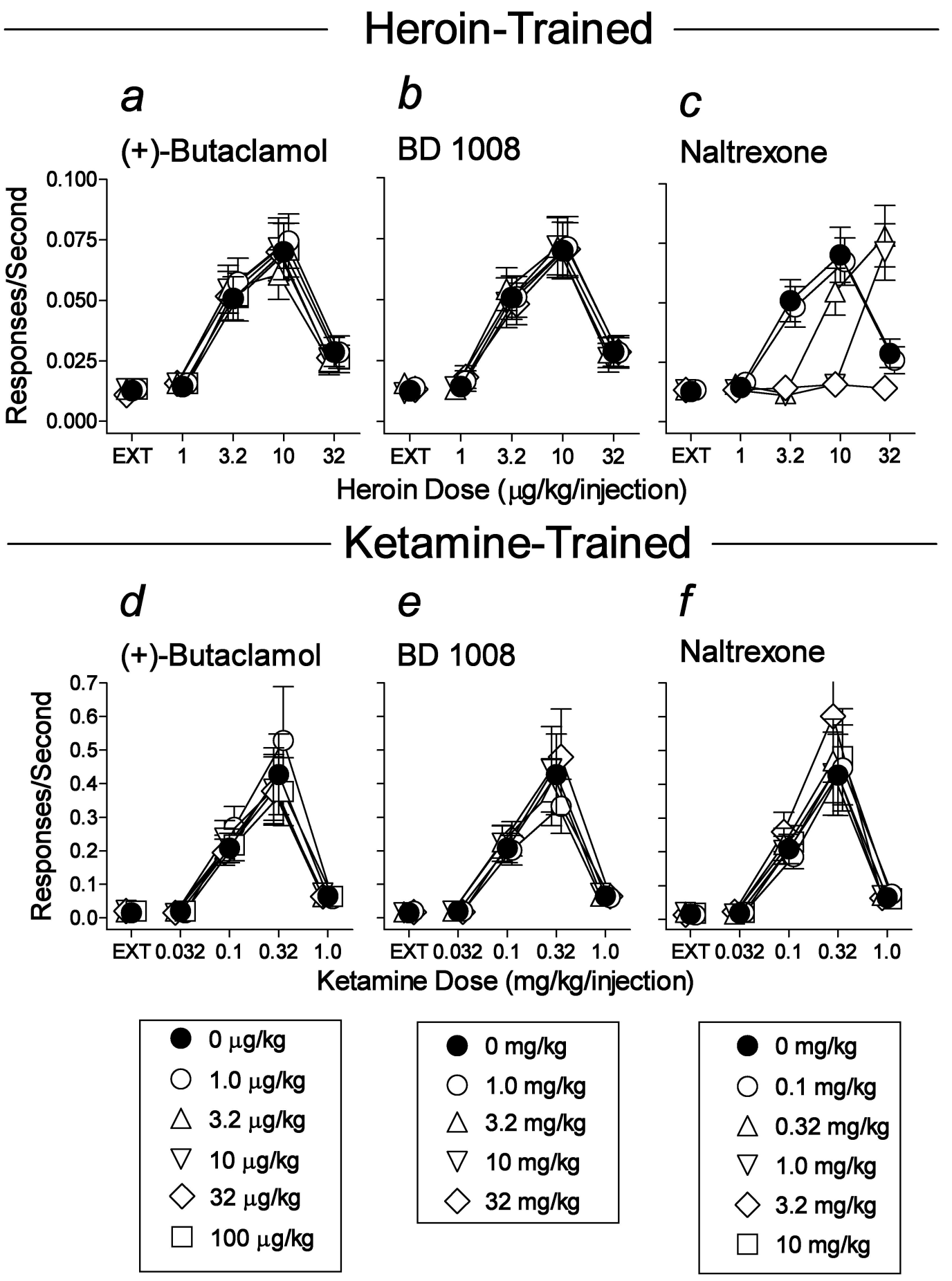

d-Methamphetamine-Trained
a
(+)-Butaclamol
b
BD 1008
c
Naltrexone
Responses/Second
0.100
0.075
0.050
0.025
0.000
EXT 0.01 0.032 0.1 0.32
d-Methamphetamine Dose (mg/kg/injection)
d
(+)-Butaclamol
e
BD 1008
f
Naltrexone
0.5
0.4
0.3
0.2
0.1
0.0
EXT 0.032 0.1 0.32 1.0
PRE-084 Dose (mg/kg/injection)
0 µg/kg
1.0 µg/kg
3.2 µg/kg
10 µg/kg
32 µg/kg
100 µg/kg
0 mg/kg
1.0 mg/kg
3.2 mg/kg
10 mg/kg
32 mg/kg
0 mg/kg
1.0 mg/kg
3.2 mg/kg
10 mg/kg

Figure 4


Heroin-Trained
a
(+)-Butaclamol
b
BD 1008
c
Naltrexone
Responses/Second
0.100
0.075
0.050
0.025
0.000
EXT 1 3.2 10 32
Heroin Dose (μg/kg/injection)
Ketamine-Trained
d
(+)-Butaclamol
e
BD 1008
f
Naltrexone
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
EXT 0.032 0.1 0.32 1.0
Ketamine Dose (mg/kg/injection)
0 μg/kg
1.0 μg/kg
3.2 μg/kg
10 μg/kg
32 μg/kg
100 μg/kg
0 mg/kg
1.0 mg/kg
3.2 mg/kg
10 mg/kg
32 mg/kg
0 mg/kg
0.1 mg/kg
0.32 mg/kg
1.0 mg/kg
3.2 mg/kg
10 mg/kg