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Samidorphan, an opioid receptor antagonist, attenuates drug-induced increases in extracellular dopamine concentrations and drug self-administration in male Wistar rats

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ABSTRACT

Opioid receptors modulate neurochemical and behavioral responses to drugs of abuse in nonclinical models. Samidorphan (SAM) is a new molecular entity that binds with high affinity to human mu- (μ), kappa- (κ), and delta- (δ) opioid receptors and functions as a μ-opioid receptor antagonist with partial agonist activity at κ- and δ-opioid receptors. Based on its in vitro profile, we hypothesized that SAM would block key neurobiological effects of drugs of abuse. Therefore, we assessed the effects of SAM on ethanol-, oxycodone-, cocaine-, and amphetamine-induced increases in extracellular dopamine (DA_{ext}) in the nucleus accumbens shell (NAc-sh), and ethanol and cocaine self-administration behavior in rats. In microdialysis studies, administration of SAM alone did not result in measurable changes in NAc-sh DA_{ext} when given across a large range of doses. However, SAM markedly decreased average and maximal increases in NAc-sh DA_{ext} produced by each of the drugs of abuse tested. In behavioral studies, SAM attenuated fixed-ratio ethanol self-administration and progressive ratio cocaine self-administration. These results highlight the potential of SAM to counteract the neurobiological and behavioral effects of several drugs of abuse with differing mechanisms of action.

1. Introduction

The mesolimbic dopamine (DA) pathway, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is involved in the motivational and rewarding effects of drugs of abuse (Koob, 1992; Koob and Volkow, 2010). Many drugs abused by humans increase extracellular concentrations of DA (DA_{ext}) in the shell and core subregions of the NAc in animals (Di Chiara and Imperato, 1988; Di Chiara et al., 1987; McKittrick and Abercrombie, 2007; Volkow and Morales, 2015; Willuhn et al., 2010; Wise and Rompre, 1989). A critical role for increased mesolimbic DA in the rewarding and reinforcing effects of multiple drugs of abuse has also been suggested. Accordingly, lesions of the VTA and NAc that deplete DA (Lyness et al., 1979; Roberts and Koob, 1982; Roberts et al., 1980), as well as systemic and local administration of DA receptor antagonists (Anderson et al., 2003; Bari and Pierce, 2005; Caine and Koob, 1994; Rassnick et al., 1992), decrease drug self-administration and drug-induced conditioned place preference. Conversely, optical activation of VTA dopaminergic neurons facilitates such behaviors (Adamantidis et al., 2011; Tsai et al., 2009; Witten et al., 2011).

Opioid receptors and their endogenous ligands are present throughout the mesolimbic system (Mansour et al., 1987; Mansour et al., 1988; Svingos and Colago, 2002), providing a mechanism whereby exogenous opiates can influence these neurochemical circuits and modulate neurobiological effects of drugs of abuse. Selective and nonselective opioid modulators attenuate increased NAc-sh DA_{ext}

Abbreviations: ANOVA, analysis of variance; C_{max}, mean maximal concentration; DA, dopamine; DA_{ext}, extracellular dopamine; DAT, dopamine transporters; FR, fixed ratio; IP, intraperitoneal; NAc, nucleus accumbens; NAc-sh, nucleus accumbens shell; PO, orally; PR, progressive ratio; SAM, samidorphan; SC, subcutaneously; TO, time out; VTA, ventral tegmental area.

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produced by several drugs including ethanol (Gonzales and Weiss, 1998; Heyser et al., 1999), morphine (Di Chiara and Imperato, 1988), and amphetamine (Hooks et al., 1992; Schad et al., 1995). Similarly, opioid modulators attenuate ethanol (Gonzales and Weiss, 1998), morphine (Glick et al., 1995), and cocaine self-administration (Corrigall et al., 1999; Glick et al., 1995; Ward et al., 2003), and amphetamine reinstatement (Haggkvist et al., 2009) in rodents. These nonclinical studies, among others, support a critical role for opioid receptor modulation in the neurochemical and behavioral effects of drugs of abuse (Le Merrer et al., 2009). Furthermore, these studies provided the rationale for the use of naltrexone and naloxone in the treatment of alcohol and opioid addiction and the reversal of opioid overdose, respectively (SAMSHA, 2009; Volpicelli et al., 1992).

Samidorphan (3-carboxamido-4-hydroxynaltrexone; SAM), a new molecular entity that binds with high affinity to μ-, κ-, and δ-opioid receptors, acts as a μ-opioid receptor antagonist with partial agonist activity at κ- and δ-opioid receptors (Bidlack et al., 2018; Wentland et al., 2009). Notably, when compared with NTX, SAM binds with higher affinity to μ-, κ, and δ-opioid receptors and functions as a more potent μ-opioid receptor antagonist (Bidlack et al., 2018; Raynor et al., 1994). Given its activity at μ-, κ-, and δ-opioid receptors, we hypothesized that SAM would block key neurobiological effects of drugs of abuse. Therefore, the present studies were designed to determine if SAM would inhibit the neurochemical and behavioral effects of commonly abused drugs in nonclinical rat models. The primary aim of these studies was to determine whether administration of SAM would attenuate increased NAc-sh DA_{ext} produced by ethanol, oxycodone, cocaine, and amphetamine (four drugs of abuse with differing mechanisms of action) in microdialysis studies. Following neurochemical assessment, the second aim of these studies was to determine whether administration of SAM would block ethanol and cocaine self-administration.

2. Materials and methods

2.1. Animals

Opioid-naïve male Wistar rats (250–350 g) were used in all experiments. Male rats alone were used due to the well characterized differences in metabolism of morphinans between the sexes in rodents (Baker and Ratka, 2002; South et al., 2009; Doyle and Murphy, 2018). For microdialysis experiments and ethanol self-administration, rats were obtained from Charles River Laboratory (Raleigh, NC) and studies were conducted at Alkermes, Inc. (Waltham, MA). For cocaine self-administration experiments, rats were obtained from Harlan (Livermore, CA) and studies were conducted at Behavioral Pharma, Inc. (La Jolla, CA). Rats were pair-housed except after microdialysis surgery. Rats were acclimated to the animal colony and handled gently for at least 3–4 days before experimentation. Rats were maintained on a 12-h/12-h light-dark cycle (0600:1800 h light) with a room temperature of 22 ± 3 °C and a relative humidity level of 45% ± 10%. Food and water were available ad libitum unless otherwise noted. Animals used for these studies were housed, managed, and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and experiments were approved by the Alkermes or Behavioral Pharma Institutional Animal Care and Use Committees.

2.2. Drugs

For microdialysis studies, Ethanol (40% solution in sterile water; 2.5 g/kg; Pharmco-Aaper, Shelbyville, KY) was prepared fresh and administered orally (PO) via gavage. Oxycodone hydrochloride (3 mg/kg; Spectrum, New Brunswick, NJ), cocaine hydrochloride (5 mg/kg; Mallinckrodt, Hazelwood, MO) and d-amphetamine hemisulfate salt (0.5 mg/kg; Sigma-Aldrich, St. Louis, MO) were prepared fresh in sterile 0.9% saline for injection and administered via intraperitoneal (IP) or subcutaneous (SC) injection. Drug doses for ethanol, cocaine and amphetamine were chosen based on literature reports and preliminary data from our laboratory. To our knowledge, few studies have measured NAc-sh DA_{ext} in response to oxycodone administration in rats. As such, a preliminary dose response (0.03–10 mg/kg) studies were performed to determine the minimal dose of oxycodone that would produce the maximal increase in NAc-sh DA_{ext}. For self-administration studies, ethanol was prepared as described below, and cocaine hydrochloride (Sigma-Aldrich) was administered as a 0.5 mg/kg infusion in sterile saline. SAM was synthesized by Cambridge Major Laboratories (Germantown, WI). SAM (1 mg/kg, calculated as free base) was dissolved in sterile saline and administered subcutaneously (SC) 30 min before drug challenge or test session in all studies. The dose of SAM used in the current studies brackets clinically relevant plasma concentrations of SAM (Turncliff et al., 2015) and was chosen based on previous studies where SAM (0.3–3 mg/kg) dose-dependently attenuated NAc-sh DA_{ext} increases produced by buprenorphine, a μ-partial agonist (Deaver et al., 2013). Importantly in these dose ranges, SAM produces no measurable effect on locomotor activity in the open field (data not shown). In addition, SAM did not alter immobility in the forced swim test (Smith et al., 2019) further demonstrating that SAM does not affect general activity or locomotor behavior at these doses.

2.3. Effects of SAM on drug-induced increases in NAc-sh DA_{ext}

Rats were anesthetized with a ketamine:xylazine solution (80 mg/kg:6 mg/kg, IP), and guide cannula (CMA 12, CMA Microdialysis, Stockholm, Sweden) were stereotaxically implanted above the NAc shell (NAc-sh; final microdialysis probe coordinates relative to the bregma: A/P + 1.70; M/L ± 0.8; D/V −6.0 from dura) (Paxinos and Watson, 1997). Guide cannulas were secured with three 1/8″ jewelers' screws (Small Parts, Seattle, WA) and cranioplastic cement (GC Fuji Plus Capsule; Henry Schein, Melville, NJ). After 3–4 days of recovery, concentric microdialysis probes (CMA 12, CMA Microdialysis) with a 2-mm active membrane were inserted through the guide cannula and rats were individually tethered to a CMA 120 microdialysis system (CMA Microdialysis). Rats were continuously perfused overnight with sterile artificial cerebrospinal fluid (CMA CNS Perfusion Solution, CMA Microdialysis) via a syringe pump at 0.2 μl/min. The following morning, the flow rate of artificial cerebrospinal fluid was increased to 2.0 μl/min and equilibrated for at least 2 h before experimentation. To measure the effects of SAM on basal concentrations of NAc-sh DA_{ext}, perfusates were collected continuously over 30-min intervals using a refrigerated autofraction collector (CMA 470, CMA Microdialysis). Six baseline fractions (−2.5 to 0 h) were collected, followed by administration of SAM, and an additional six fractions (0.5–3 h) were collected. In subsequent experiments measuring the effects of SAM on drug-induced increases NAc-sh DA_{ext}, perfusates were collected continuously over 15-min intervals to capture drug-induced increases in NAc-sh DA_{ext}. Four baseline fractions (−1.25 to −0.5 h) were collected followed by administration of SAM (1 mg/kg) or vehicle. Two subsequent fractions (−0.25 to 0 h) were collected and rats were then administered test drugs. The neurochemical response to each drug of abuse was then measured for 12 fractions (0.25–3 h). To avoid neurotransmitter degradation, fractions were collected in 5 or 10 μl of 0.75 M formic acid. Microdialysis fractions were analyzed via high-performance liquid chromatography with electrochemical detection using an ALEXYS monoamine analyzer (Antec Leyden, Leiden, the Netherlands) using an automated sample handler. An aliquot of each fraction (10 μl) was injected onto a 1-μm reverse-phase C18 column (ALF-105, Antec Leyden) for monoamine separation. DA was eluted using a mobile phase (pH 6.4) consisting of 50 mM phosphoric acid, 8 mM KCL, 0.1 mM EDTA, 10% methanol, and 500 mg/l octane sulfonic acid. DA was detected using a Decade II amperometric detector (Antec Leyden) with a glassy carbon electrode maintained at approximately 0.3 V relative to a Ag/AgCl reference electrode. This method provided a limit of quantification of approximately 0.1 pg/10 μl. Data were recorded and DA concentrations quantitated using Clarity 3.0

software (Data Apex, Prague, Czech Republic) and are reported as on-column DA in pg of DA per 10 μl injected.

To verify probe placement, rats were euthanized with an IP injection of 50% Euthasol (Virbac, AH Inc., Fort Worth, TX) shortly after microdialysis and perfused with Chicago Sky Blue dye (Sigma-Aldrich). Brains were dissected rapidly and frozen on dry ice and stored at −80 °C. Coronal sections (approximately 20 μm) were then sliced on a cryostat at the level of the nucleus accumbens and photographed for archival purposes. Only data from rats with verified probe placements were included in the analysis. A total of 3 rats were excluded due to incorrect probe placement and 1 rat was removed due to sampling error during the microdialysis procedure.

2.4. Effects of SAM on drug self-administration

2.4.1. Ethanol self-administration

Rats were trained to orally self-administer ethanol using a modified operant procedure (Rassnick et al., 1992). Each operant chamber (Coulbourn Instruments, Whitehall, PA) consisted of a single lever with a white cue light, a tone generator (2.9 KHz Sonalert) and a liquid dipper with an 0.1-cc cup. The operant chamber was located in an isolation cubicle with a ventilation fan and internal background white noise. Rats were hand-shaped over a period of 1–3 days to press the lever once under a fixed-ratio (FR1) schedule of reinforcement for a 0.1% saccharine solution following overnight water deprivation. Once lever pressing behavior was established, water was again made freely available in their home cage. A saccharine-fading procedure was then utilized to initiate ethanol drinking. Rats were started on 5% ethanol in 0.1% saccharine, and the ethanol concentration gradually increased to 10% and the saccharine concentration was then decreased to 0.04% over the next 20–40 sessions. Briefly, the start of the session was signaled by the activation of the house light. A cue light above the lever was turned on and the rat was required to press the lever two times (FR2) to receive 3-s access to the ethanol cocktail from a liquid dipper. The presented reinforcer was signaled by an 0.5-s tone and a light located in the dipper receptacle. There was a 5-s inter-trial interval. Programming of the session and data recording was made using Graphic State 3 software (Coulbourn) running on a Windows XP compatible computer. Each daily session (5 days per week) lasted 30 min. Rats that consistently consumed a minimum of 0.6 g/kg/h of ethanol (at least 60 bar presses in 30 min with a 10% ethanol in 0.04% saccharine cocktail) over a 4-week period were used in these studies. Approximately 60% of the rats that began training were able to meet this criterion. For these experiments, rats were placed into test chambers 30 min after SAM or vehicle administration and bar pressing for ethanol was measured for 30 min.

2.4.2. Intravenous catheter insertion and maintenance for cocaine self-administration studies

Rats were anesthetized with an isoflurane-oxygen mixture (1%–3% isoflurane) and silastic jugular catheters were inserted into the external jugular and passed subcutaneously to a polyethylene assembly mounted on the rat's back. The catheter assembly consisted of a 13-cm length of silastic tubing (inside diameter 0.31 mm; outside diameter 0.64 mm) attached to a guide cannula that is bent at a right angle. The cannula was embedded into a dental cement base and anchored with a 2 × 2-cm cm square of durable mesh. The catheter was passed subcutaneously from the rat's back to the jugular vein, where it was inserted and secured with a non-absorbable silk suture. Upon successful completion of surgery, rats were given 3–5 days to recover before self-administration sessions started. During the recovery period, rats remained on ad libitum food access, and catheter lines were flushed daily with 30 units/ml of heparinized saline containing 100 mg/ml of Timentin to prevent blood coagulation and infection in the catheters. During self-administration sessions, catheters were flushed with saline before each test session to ensure catheter patency, and again flushed after the test session with 30 units/ml of heparinized saline, containing 100 mg/ml of Timentin.

2.4.3. Cocaine self-administration

Food training and cocaine self-administration were performed in 12 standard operant chambers (Coulbourn). Each chamber was housed in a sound-attenuating box. Operant chambers were equipped with two levers mounted 2 cm above the floor, and a cue light mounted 2 cm above the lever on the back wall to the right of the food hopper. The right lever was the active lever and the left lever was the inactive lever. For food training, a food hopper was located 2 cm to the left of the active lever, in the middle of the back wall. Intravenous infusions were delivered in a volume of 0.1 ml over 4 s via an infusion pump (Razel Scientific, VT).

Lever pressing was established by the method outlined by Hyytia et al. (1996). Initially, rats were restricted to 15 g of chow daily (to reduce body weight to approximately 85% of their free-feeding weight). After the second day of food restriction, rats were trained to respond for food under a FR1 schedule of reinforcement (1 food pellet for each lever press) with a 1-s time out (TO) after delivery of each reinforcement. Training sessions lasted for 30 min daily, and the TO was gradually increased to 20 s. Once rats obtained steady baseline responding at a FR1–TO–20 s schedule of reinforcement, they were returned to ad libitum food prior to intravenous jugular catheter implant surgery.

2.4.4. Fixed ratio schedule of reinforcement

Rats were trained to self-administer cocaine (0.5 mg/kg infusion) intravenously in 2-h baseline sessions, 5–7 days per week, under a FR1–TO–20 s schedule of reinforcement until stable responding was achieved (<20% variability across three consecutive sessions). Subsequently, vehicle injections were administered to habituate rats to the injections so there was no "injection effect" on cocaine response before drug testing. SAM was administered to rats (1 mg/kg, SC) 30 min before a 2-h test session. Subsequently, a final vehicle injection was given to rats to verify a lack of injection effects.

2.4.5. Progressive-ratio schedule of reinforcement

Rats were tested on a progressive-ratio (PR) schedule of reinforcement during a 6-h session, with each reward resulting in a progressive increase in the number of lever presses required for the subsequent reward. The progression of lever presses was 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95 etc., derived from the formula $(5 \times e0.2n) - 5$ rounded to the nearest integer, where n is the position in the sequence of ratios, where initially the first lever press delivered a cocaine reinforcer. Breakpoint was defined as the last ratio completed with no responses for 30 min. SAM was administered to rats (1 mg/kg, SC) 30 min before testing.

2.5. Statistical analysis

To assess the effects of SAM on basal and drug-induced increases in NAc-sh DA_{ext}, the raw DA (pg/10 μl) values for each rat were converted to A) percentage change from baseline (defined as the average of the baseline samples) and B) the absolute change in DA concentration above baseline (in pg/10-μl sample). All statistics were performed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA) or Sigma Plot 12.5 (Systat Software, San Jose, CA). Means are reported ±SEM. A two-way analysis of variance (ANOVA) with repeated measures was used to analyze average basal DA_{ext} values pre and post SAM administration in the microdialysis experiments. To assess the effects of SAM on NAc-sh DA_{ext}, mean neurotransmitter values before ($t = -2.5$ to 0 h) and after ($t = 0.5$–3.0 h) SAM were calculated and compared using a two-way ANOVA with repeated measures. In addition, the mean maximal concentration (C_{max}) of DA above baseline after drug administration was calculated and analyzed using a one-way ANOVA. To assess the effects of SAM on drug-induced increases in NAc-sh DA_{ext}, percentage change DA data (over the 4.5-h experiment) were analyzed using a two-way repeated measures ANOVA with Geisser-Greenhouse sphericity correction. In addition, the C_{max} of DA above baseline after drug administration was calculated and analyzed using a t-test. Self-administration data

were collected online simultaneously from multiple operant chambers. The results of the operant procedures, reported as mean cumulative bar presses during test sessions, were analyzed using a *t*-test for ethanol and cocaine. For ethanol self-administration, because of the established literature that ethanol drinking behavior in rats is reduced with opioid antagonist treatment (Gonzales and Weiss, 1998), we used a one-tailed *t*-test. Because ratios, or the number of operant responses required for reinforcement, violate the assumption of homogeneity of variance (Richardson and Roberts, 1996) breakpoint data during cocaine self-administration were analyzed with a non-parametric Mann–Whitney *U* test using StatView (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Experiment 1: effects of SAM on basal concentration of NAc-sh DA_{ext}

Average baseline DA_{ext} concentration across treatment groups was 1.67 pg (±0.17) per 10-μl sample and did not differ between groups ($F_{(2,14)} = 0.08$, $p = 0.93$). SAM (0.1, 1, and 10 mg/kg, SC) treatment produced no measurable effect on basal concentration of NAc-sh DA_{ext}, (treatment × time interaction ($F_{(2,28)} = 0.48$, $p = 0.63$), treatment ($F_{(2,28)} = 0.48$, $p = 0.63$), and time ($F_{(1,28)} = 5.68$, $p < 0.05$)), (Fig. 1). In addition, treatment with SAM did not affect C_{max} concentrations of DA_{ext} at any dose tested, ($F_{(2,14)} = 0.44$, $p = 0.65$).

3.2. Experiment 2: effects of SAM on ethanol-induced increases in NAc-sh DA_{ext}

Before vehicle or SAM administration, the average baseline DA_{ext} concentration across treatment groups was 1.77 pg (±0.31) per 10-μl sample and did not differ between groups ($F_{(1,12)} = 0.003$, $p = 0.96$). SAM (1 mg/kg) administered 30 min before ethanol did not affect baseline DA_{ext} concentrations ($F_{(1,12)} = 0.005$, $p = 0.95$). Ethanol administration (2.5 g/kg, PO) produced an approximate 40% increase in NAc-sh DA_{ext} above baseline concentrations (Fig. 2). Treatment with SAM attenuated ethanol-induced increases in NAc-sh DA_{ext} (treatment × time interaction: $F_{(17,198)} = 2.62$, $p < 0.001$; effect of treatment: $F_{(1,12)} = 14.99$, $p < 0.05$; effect of time: $F_{(2.2,25)} = 1.94$, $p < 0.05$). In addition,

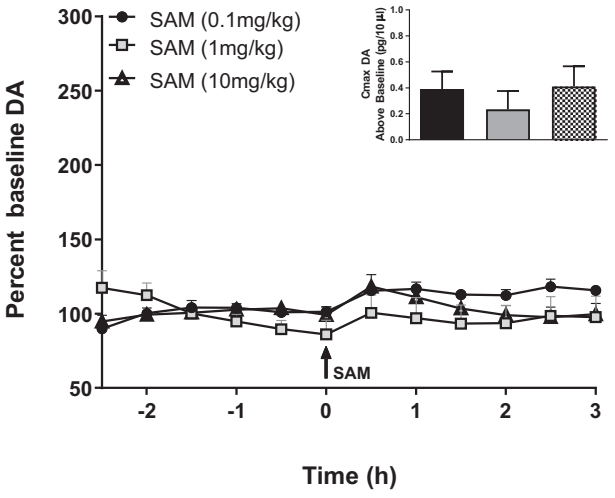

Fig. 1. Effects of SAM on basal extracellular concentrations of DA in the NAc-sh of male Wistar rats. Data are depicted as the percentage change from vehicle ± standard error or the mean (SEM) and average C_{max} concentration above baseline. Subcutaneous administration of SAM (0.1, 1, and 10 mg/kg) produced no measurable effects of extracellular concentrations of DA ($p = 0.63$, drug × time interaction; $p = 0.63$ drug; $p > 0.05$, time) or average C_{max} concentration of DA above baseline ($p = 0.65$) at any dose tested. $n = 5$ (SAM 0.1 mg/kg) and $n = 6$ (SAM 1 mg/kg and 10 mg/kg).

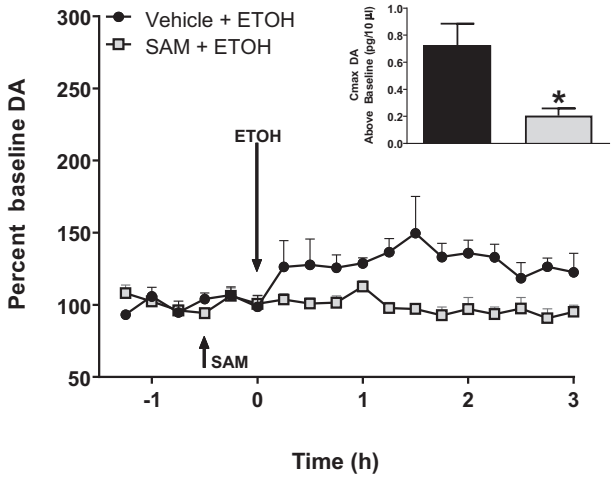

Fig. 2. Effects of SAM on ethanol-induced increases in extracellular concentrations of DA in the NAc-sh of male Wistar rats. Data are depicted as the percentage change from vehicle ± SEM and average C_{max} concentration above baseline. Extracellular concentrations in the NAc-sh increased approximately 40% above baseline following ethanol (2.5 g/kg; PO). Subcutaneous administration of SAM (1 mg/kg) 30 min before ethanol significantly attenuated ethanol-induced increases in DA ($p < 0.001$, drug × time interaction; $p < 0.05$, drug; $p < 0.05$, time) and maximal (C_{max}) concentrations in DA (*$p < 0.01$) above baseline in the NAc-sh. $n = 7$ per group. ETOH denotes ethanol.

treatment with SAM attenuated ethanol-induced increases in C_{max} concentrations of DA_{ext} SAM ($t_{(12)} = 3.12$, $p < 0.01$).

3.3. Experiment 3: effects of SAM on oxycodone-induced increases in NAc-sh DA_{ext}

Before vehicle or SAM administration, the average baseline DA_{ext} concentration across treatment groups was 2.03 pg (±0.35) per 10-μl sample. Average baseline DA_{ext} did not differ between groups ($t_{(9)} = 0.97$, $p = 0.36$). Oxycodone administration (3 mg/kg) produced an approximate 240% increase in NAc-sh DA_{ext} above baseline concentrations (Fig. 3). SAM (1 mg/kg) treatment attenuated oxycodone-induced increases in NAc-sh DA_{ext} (treatment × time interaction: $F_{(17,153)} = 9.14$, $p < 0.001$; effect of treatment: $F_{(1,9)} = 11.32$, $p < 0.01$; effect of time: $F_{(1.5,13.52)} = 11.17$, $p < 0.001$). In addition, treatment with SAM attenuated oxycodone-induced increases in C_{max} concentrations of DA_{ext} by SAM ($t_{(9)} = 2.22$, $p < 0.05$).

3.4. Experiment 4: effects of SAM on cocaine-induced increases in NAc-sh DA_{ext}

Before vehicle or SAM administration, the average baseline DA_{ext} concentration across treatment groups was 1.51 pg (±0.17) per 10-μl sample and did not differ between groups ($F_{(1,9)} = 0.71$, $p = 0.42$). SAM (1 mg/kg) administered 30 min before cocaine did not affect baseline DA_{ext} concentrations ($F_{(1,9)} = 0.48$, $p = 0.51$). Cocaine administration (5 mg/kg, IP) produced an approximate 400% increase in NAc-sh DA_{ext} above baseline concentrations (Fig. 4). Treatment with SAM attenuated cocaine-induced increases in NAc-sh DA_{ext} (treatment × time interaction: $F_{(17,150)} = 16.42$, $p < 0.001$; effect of treatment: $F_{(1,9)} = 17.62$, $p < 0.01$; time: $F_{(3.64,32.1)} = 35.50$, $p < 0.001$). In addition, treatment with SAM attenuated cocaine-induced increases in C_{max} concentrations of DA_{ext} ($t_{(9)} = 3.24$, $p = 0.01$).

3.5. Experiment 5: effects of SAM on amphetamine-induced increases in NAc-sh DA_{ext}

Before vehicle or SAM administration, the average baseline DA_{ext}

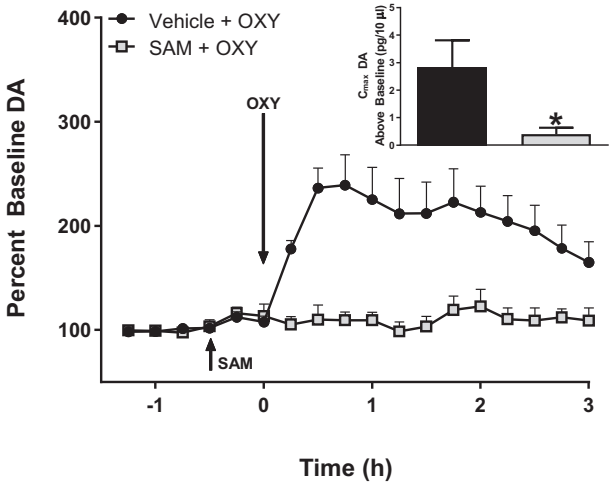

Fig. 3. Effects of SAM on oxycodone-induced increases in extracellular concentrations of DA in the NAc-sh of male Wistar rats. Data are depicted as the percentage change from vehicle ± SEM and average C_{max} concentration above baseline. Extracellular concentrations in the NAc-sh increased approximately 240% above baseline following oxycodone (3 mg/kg; SC). SAM (1 mg/kg) 30 min before oxycodone significantly attenuated oxycodone-induced increases in DA ($p < 0.001$, drug × time interaction; $p < 0.01$, drug; $p < 0.001$, time) and maximal (C_{max}) concentrations in DA (*$p < 0.05$) above baseline in the NAc-sh. n = 6 (Vehicle + OXY) and n = 5 (SAM + OXY). OXY denotes oxycodone.

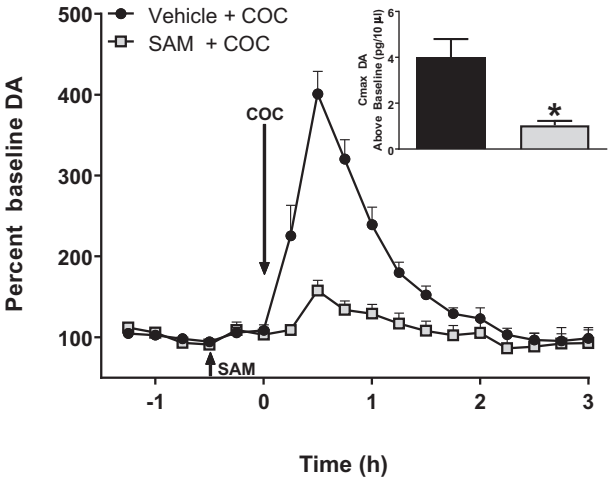

Fig. 4. Effects of SAM on cocaine-induced increases in extracellular concentrations of DA in the NAc-sh of male Wistar rats. Data are depicted as the percentage change from vehicle ± SEM and average C_{max} concentration above baseline. Extracellular concentrations in the NAc-sh increased approximately 400% above baseline following cocaine (5 mg/kg; IP). Subcutaneous administration of SAM (1 mg/kg) 30 min before cocaine significantly attenuated cocaine-induced increases in DA ($p < 0.001$, drug × time interaction; $p < 0.01$, drug; $p < 0.001$, time) and maximal (C_{max}) concentrations in DA (*$p < 0.05$) above baseline in the NAc-sh. n = 6 (vehicle + COC) and n = 5 (SAM + COC) per group. COC denotes cocaine.

concentration across treatment groups was 0.97 pg (±0.09) per 10-µl sample and did not differ between groups ($F_{(1,10)} = 2.08$, $p = 0.18$). SAM (1 mg/kg) administration before amphetamine did not affect baseline DA_{ext} concentrations ($F_{(1,10)} = 0.230$, $p = 0.64$). Amphetamine administration (0.5 mg/kg, IP) produced an approximate 360% increase in NAc-sh DA_{ext} above baseline concentrations (Fig. 5). Treatment with SAM on amphetamine-induced increases in DA_{ext} were time-dependent and less pronounced when compared with other drug treatments

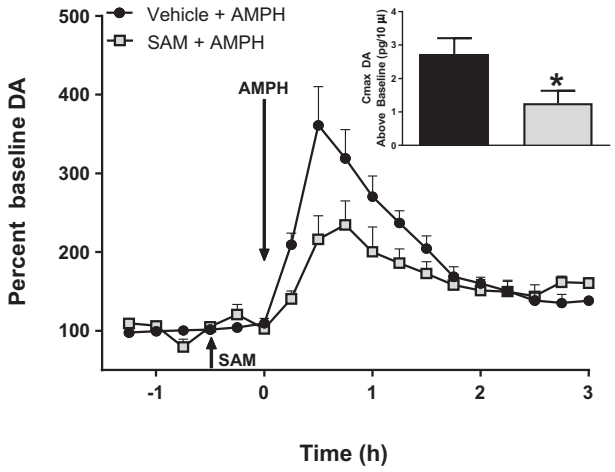

Fig. 5. Effects of SAM on amphetamine-induced increases in extracellular concentrations of DA in the NAc-sh of male Wistar rats. Data are depicted as the percentage change from vehicle ± SEM and average C_{max} concentration above baseline. Extracellular concentrations in the NAc-sh increased approximately 360% above baseline following amphetamine (0.5 mg/kg; IP). Subcutaneous administration of SAM (1 mg/kg) 30 min before amphetamine significantly attenuated amphetamine-induced increases in DA ($p < 0.001$, drug × time interaction; $p = 0.13$, drug; $p < 0.01$, time) and maximal (C_{max}) concentrations in DA (*$p < 0.05$) above baseline in the NAc-sh. n = 6 per group. AMPH denotes amphetamine.

(treatment × time interaction: $F_{(17,158)} = 4.20$, $p < 0.001$; time: $F_{(1.79,16.70)} = 32.69$, $p < 0.001$; and no significant effect of treatment: $F_{(1,10)} = 2.50$, $p = 0.14$). However, amphetamine-induced increases in C_{max} concentrations of DA_{ext} were significantly attenuated by SAM ($t_{(10)} = 2.43$, $p < 0.05$).

3.6. Experiment 6: effects of SAM on ethanol self-administration

Ethanol (10% ethanol in 0.04% saccharine cocktail) maintained reinforced operant responding in vehicle-treated rats (average 100 lever presses during test session – average 50 reinforcers due to the FR2 schedule). SAM, given 30 min before behavioral testing, attenuated the number of rewards (average 20 rewards during test session) when administered under an FR2 schedule of reinforcement ($t_{(7)} = 2.25$, $p < 0.05$, one-tailed; Fig. 6).

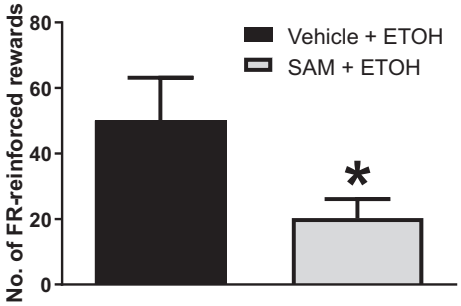

Fig. 6. Effect of SAM on FR2 ethanol self-administration. Rats were trained to orally administer ethanol (10% ethanol in a 0.04% saccharin solution) and tested in a FR2 schedule of reinforcement. Bars represent average (±SEM) number of reinforced rewards per session. SAM administered 30 min before the test session significantly decreased the number of FR-induced reinforced rewards when compared with vehicle (*$p < 0.05$). $n = 4$ (vehicle) and n = 5 (SAM 1 mg/kg).

3.7. Experiment 7: effects of SAM on cocaine self-administration

During FR1–TO–20 s self-administration sessions, cocaine (0.5 mg/kg infusion) maintained reinforced operant responding in vehicle-treated rats. There was a 13% reduction in the number of cocaine rewards during FR responding after SAM treatment, however, this was not statistically significant under this schedule of reinforcement ($t_{(17)} = 1.43$, p = ns; Fig. 6). Under a PR schedule of reinforcement, vehicle-treated rats reached a breakpoint of 62 with an average of 12.4 infusions of cocaine. In contrast, SAM attenuated cocaine self-administration; rats reached a breakpoint of 18 with an average of 6.8 infusions ($U = 4.5$, $p < 0.005$; Fig. 7).

4. Discussion

The current studies characterized the effects of SAM on drug-induced increases in NAc-sh DA_{ext} and drug-seeking behavior. As expected, ethanol, oxycodone, cocaine, and amphetamine increased concentrations of DA_{ext} above basal levels in the NAc-sh. SAM decreased the neurochemical effects of each drug of abuse, though the magnitude of attenuation differed among the drugs. To relate the observed neurochemical effects to drug-seeking behavior, SAM was administered to rats trained to self-administer ethanol and cocaine. SAM significantly decreased self-administration of ethanol, but not cocaine, in FR schedules of reinforcement. Cocaine-seeking behavior was, however, decreased by SAM during a PR schedule of reinforcement. Collectively, these studies indicate that SAM limits the neurochemical and behavioral effects of multiple drugs of abuse.

Opioid receptors and their endogenous ligands are present throughout the mesolimbic DA system (Mansour et al., 1987; Mansour et al., 1988; Svingos and Colago, 2002), providing a mechanism for opioid modulation of these neurocircuits. When compared with other opioid antagonists such as naloxone, naltrexone, and nalmefene, SAM is a more potent μ-antagonist with favorable pharmacokinetic properties (Bidlack et al., 2018; Peng et al., 2007; Raynor et al., 1994; Turncliff et al., 2015; Wentland et al., 2009). Importantly, systemic administration of SAM across a wide range of doses did not result in measurable changes in NAc-sh DA_{ext} relative to baseline values. This indicates that the in vitro partial agonist activity of SAM at κ- and δ-opioid receptors do not correlate with changes in mesolimbic activity associated with opioid receptor agonism in vivo (Di Chiara and Imperato, 1988; Spanagel et al., 1990; Spanagel et al., 1992).

In previous studies, SAM dose-dependently attenuated NAc-sh DA_{ext} increases produced by buprenorphine, a μ-partial agonist (Deaver et al., 2013), an effect attributed to its μ-opioid antagonist activity. Likewise, the ability of SAM to attenuate the elevations in NAc-sh DA_{ext} produced by ethanol, oxycodone, cocaine, and amphetamine is likely driven by its μ-antagonist activity. This is consistent with reports that selective μ-antagonists block elevations of DA_{ext} in the NAc induced by ethanol (Tanda and Di Chiara, 1998; Valenta et al., 2013), morphine (Di Chiara and Imperato, 1988; Valenta et al., 2013), and amphetamine (Schad et al., 1996). In addition, commonly used opioid antagonists such as naltrexone and naloxone also attenuate increases in NAc DA_{ext} induced by morphine (Di Chiara et al., 1987), ethanol (Gonzales and Weiss, 1998), and amphetamine (Hooks et al., 1992; Schad et al., 1995) in rats. To our knowledge, this is the first report that a compound with μ-antagonist activity can block oxycodone-induced increases in NAc DA_{ext}. Furthermore, we are unaware of any studies that report test compound attenuation of cocaine-induced increases in NAc-sh DA_{ext} via a μ-antagonist mechanism of action. For example, Schad et al. (1995) reported no effect (±) of naloxone on cocaine-induced increases in NAc DA_{ext}. Thus, SAM appears to be unique compared with other known opioid modulators in its ability to attenuate increases in NAc-sh DA_{ext} produced by all four drugs of abuse.

One common neurobiological effect of drugs of abuse including ethanol, morphine, cocaine, and amphetamine administration is an increase of the endogenous opioid agonists β-endorphin, enkephalin, and dynorphin within the mesolimbic DA system (Jarjour et al., 2009; Marinelli et al., 2006; Olive et al., 1995; Roth-Deri et al., 2003; You et al., 1996). As μ- and δ-opioid receptor agonists increase NAc DA_{ext} (Di Chiara and Imperato, 1988; Spanagel et al., 1990), and κ-opioid receptor activation decreases NAc DA_{ext} (Di Chiara and Imperato, 1988; Maisonneuve et al., 1994; Spanagel et al., 1992), changes in opioid tone may contribute to observed changes in NAc DA concentrations. Consequently, the neurochemical effects of SAM may be due to modulation of drug of abuse–induced changes in opioid activity within the mesolimbic system. For example, SAM would be expected to counteract the pharmacologic effects of increased β-endorphin or enkephalin via its μ-antagonist effects. Additionally, SAM would be expected to counteract δ-opioid–related effects of increased enkephalin concentrations via its low intrinsic activity at δ-opioid receptors. Thus, the ability of SAM to counteract changes in μ- or δ-opioid tone produced by drugs of abuse could, in part, result in the observed attenuation of drug stimulated increases in NAc DA.

In the presence of increased κ-opioid tone (e.g., increased dynorphin), SAM would also be expected to function as a κ-opioid antagonist. κ-opioid antagonists amplify ethanol-induced (Zapata and Shippenberg, 2006) and heroin-induced (Xi et al., 1998) increases, or have no effect on cocaine-induced increases, in NAc-sh DA_{ext} (Maisonneuve et al., 1994). In contrast, selective κ-opioid receptor agonists attenuate NAc-sh DA_{ext} produced by amphetamine (Gray et al., 1999) and cocaine (Maisonneuve et al., 1994), but the ability of κ-opioid receptor agonists to decrease basal concentrations of DA_{ext} in the NAc limits the interpretation of these data (Carlezon et al., 2006; Di Chiara and Imperato, 1988;

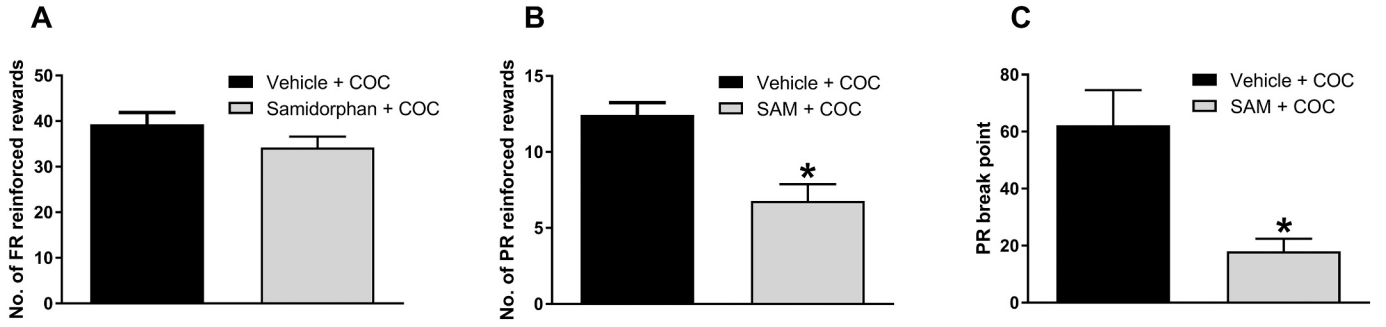

Fig. 7. Effect of SAM on the FR and PR cocaine self-administration. Rats were trained to lever-press for cocaine (0.5 mg/kg infusion; intravenous) and tested on a FR and PR schedule of reinforcement. Bars represent average (±SEM) number of cocaine infusions per session. A) SAM (1 mg/kg, SC) administered 30 min before the test session produced a modest but nonsignificant decrease in the number of rewards during FR1 self-administration ($p = 0.17$); $n = 10$/group. B) SAM significantly attenuated the number of cocaine rewards (*$p < 0.01$) compared with vehicle control and C) breakpoints for cocaine during PR self-administration (*$p < 0.01$); $n = 9$/group.

Maisonneuve et al., 1994; Spanagel et al., 1992). Because SAM itself did not decrease basal NAc-sh DA_{ext} in a similar manner to a κ-opioid receptor agonist, it is unlikely that any κ-opioid receptor activity plays a significant role in the ability of SAM to attenuate acute drug-induced increases in DA_{ext}.

An interesting finding in the current set of studies is that SAM attenuated the neurochemical effects of ethanol and oxycodone to a greater extent than cocaine and amphetamine at doses utilized in these studies. Ethanol and μ-opioid receptor agonists such as morphine increase NAc-sh DA_{ext} through enhanced activity of VTA dopaminergic projection neurons (Gessa et al., 1985; Matthews and German, 1984; Nowycky et al., 1978). This is likely the case for oxycodone however recent studies have shown that other mechanisms may be involved in the rapid increases in NAc-sh DA_{ext} following intravenous administration of oxycodone and morphine (Vander Weele et al., 2014). Cocaine and amphetamine reportedly increase VTA firing (Shi et al., 2000; Steffensen et al., 2008), but also increase DA_{ext} by inhibiting reuptake through DA transporters (DAT) on nerve terminals (Heikkila et al., 1975; Ritz et al., 1990; Seiden et al., 1993). Thus, drug-induced increases in DA_{ext} produced by cocaine and amphetamine are likely the summation of multiple events including 1) increased DA cell firing in the VTA and 2) decreased DA uptake in the NAc. The greater attenuation of ethanol and oxycodone-induced NAc-sh DA_{ext} suggests that SAM is most effective when attenuating DA_{ext} produced by drug-induced VTA cell firing rather than terminal effects within the NAc. Moreover, SAM (at a concentration of 10 μM, CEREP Bioprint Panel; data not shown) did not exhibit appreciable binding to human DAT expressed in CHO cells, and as a result, does not attenuate NAc-sh DA_{ext} by competing with cocaine and amphetamine at transporters. While the discussion above provides interesting hypotheses, it is plausible that the effects are limited by dose selection in the current study. Therefore, future extensive dose response experiments would be required to fully interpret these observations.

Notably, when comparing cocaine and amphetamine, SAM was more effective in attenuating cocaine-induced increases in DA_{ext}. In addition to blocking DAT, amphetamine promotes presynaptic release of cytoplasmic DA and reversal of DAT transporters (Arnold et al., 1977; Sulzer et al., 1993). Therefore, amphetamine, unlike cocaine, does not require cell firing to increase DA_{ext} (Benwell et al., 1993). These differences in synaptic mechanisms may explain why SAM was less effective in attenuating amphetamine-induced DA_{ext}. Importantly, the ability of SAM to attenuate the neurochemical effects of both drugs is in contrast to reports that naloxone attenuates increases in NAc-sh DA_{ext} induced by amphetamine, but not those induced by cocaine (Schad et al., 1995). While the reason for these differences remains unclear, these data distinguish the unique pharmacodynamic effects of SAM compared with other opioid antagonists such as naloxone and naltrexone.

Given the neurochemical effects above, we chose to investigate the effects of SAM on ethanol and cocaine drug-seeking behavior. A critical role for NAc DA in the initiation and maintenance of ethanol and cocaine reinforcement is well established. For example, NAc DA_{ext} concentrations are increased during self-administration of ethanol (Weiss et al., 1993) and cocaine in rats (Hemby et al., 1997; Pettit and Justice Jr., 1989; Wise et al., 1995). Furthermore, dopamine depletion and dopamine antagonists injected into the NAc block cocaine self-administration (Bari and Pierce, 2005; Roberts et al., 1980), but there is notably both a dopamine-dependent and a dopamine-independent action of opioid peptides in the NAc through which both opioid drugs and ethanol act (Nestler, 2005). The dose of SAM that decreased ethanol-induced NAc-sh DA_{ext} also attenuated bar-pressing for ethanol drug reward during FR test sessions. These results are consistent with studies in which naloxone and naltrexone block ethanol self-administration (Froehlich et al., 1990; Gonzales and Weiss, 1998; Samson and Doyle, 1985; Stromberg et al., 2001; Weiss et al., 1990). Notably, the behavioral effects of SAM are likely driven by activity at multiple opioid receptors. For example, selective μ- (Honkanen et al., 1996; Stromberg et al., 1998) and δ-opioid receptor antagonists (Hyytia and Kiianmaa, 2001; Krishnan-Sarin et al., 1995) block ethanol-seeking behavior, while κ-opioid receptor antagonists generally have no effect on oral ethanol self-administration in non-dependent rats (however, see (Mitchell et al., 2005)).

In contrast, SAM produced a nonsignificant decrease in FR cocaine self-administration. Historically, the effects of opioid antagonists on FR cocaine self-administration have been equivocal. While several reports indicate that naltrexone and naloxone attenuate cocaine self-administration in FR paradigms (Corrigall et al., 1999; Ramsey et al., 1999), others report no effect (Ettenberg et al., 1982) or an increase in cocaine-responding after naltrexone pretreatment (Carroll et al., 1986). Similarly, selective μ- and δ-opioid receptor antagonists either decrease (Corrigall et al., 1999; Reid et al., 1995) or have no effect (De Vries et al., 1995; Ward et al., 2003) on FR cocaine-responding. In the current neurochemical studies, cocaine produced an increase of ~150% in DA_{ext} above baseline in the presence of SAM. If these effects translated to the behavioral studies, such elevation of DA_{ext} may have sustained operant responding during the minimally demanding FR1 schedule. Consequently, a higher dose of SAM that might have further blocked NAc-sh DA_{ext} increases may have attenuated cocaine FR self-administration. It remains possible however that a higher dose of SAM may not fully block DA_{ext} because SAM would not directly affect cocaine-induced DA release produced by DAT activity. Given studies suggesting that more demanding schedules of reinforcement are sensitive to NAc-sh DA concentrations (Correa et al., 2002; Salamone et al., 2001), rats were subsequently tested in a PR schedule of reinforcement. Consistent with reports that μ-, δ-, and κ-opioid receptor antagonism attenuates cocaine-responding in a PR schedule of reinforcement (Ward et al., 2003; Ward and Roberts, 2007; Wee et al., 2009), SAM significantly reduced the number of rewards for cocaine-responding during PR test sessions. Furthermore, SAM decreased the breakpoint during PR cocaine administration, a paradigm designed to assess the maximal effort expended for reward and overall drug-reinforcing efficacy (Stafford et al., 1998). These results suggest that SAM may decrease some of the reinforcing efficacy and motivational drive for cocaine. Together, these data support an important role for the opioid system in the behavioral effects of cocaine, which may be dependent on distinct modulation of the different opioid receptors and/or schedule of reinforcement.

In summary, SAM 1) does not produce measurable changes in baseline NAc-sh DA_{ext}, and 2) attenuates the increases in dopamine and the reinforcing effects of multiple drugs of abuse in nonclinical models. Although outside the scope of this work, it is acknowledged that extensive dose response studies for each drug of abuse and SAM would be required to rule out any dose-dependency of these effects. This limitation would need to be addressed in future studies. Nevertheless, the molecular properties of SAM result in its ability to attenuate, to differing degrees, the neurobiological effects of ethanol, oxycodone, cocaine, and amphetamine. Importantly, the nonclinical studies described here are consistent with clinical observations in which SAM attenuated changes in pupillary response and visual analogue scale (VAS) drug-liking produced by the μ-opioid agonists remifentanil and buprenorphine (Ehrich et al., 2015; Shram et al., 2015).

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