OXFORD

International Journal of Neuropsychopharmacology (2020) 23(6): 401–405

doi:10.1093/ijnp/pyz070
Advance Access Publication: 12 June 2020
Brief Report

BRIEF REPORT

The Molecular-Container Calabadion-2 Prevents Methamphetamine-Induced Reinstatement in Rats: A Potential Approach to Relapse Prevention?

Michael Z. Leonard*, Paul Rostin*, Kevin P. Hill, Stephanie D. Grabitz, Matthias Eikermann, Klaus A. Miczek

Department of Psychology, Tufts University, Medford, MA (Mr Leonard and Dr Miczek); Department of Anesthesia, Critical Care, and Pain Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA (Mr Rostin, Drs Grabitz, and Eikermann); Division of Addiction Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA (Dr Hill); Department of Psychiatry, Harvard Medical School, Boston, MA (Dr Hill); Department of Anesthesiology and Intensive Care Medicine, University Duisburg-Essen, Essen, Germany (Dr Eikermann); Departments of Neuroscience and Pharmacology, Tufts University, Boston, MA (Dr Miczek).

Correspondence: Klaus Miczek, PhD, Department of Psychology, Tufts University Medford, 530 Boston Ave, 02155, MA (klaus.miczek@tufts.edu) and Matthias Eikermann, MD, PhD, Department of Anaesthesia, Critical Care, and Pain Medicine, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, 330 Brookline Avenue, MA 02215 (meikerma@bidmc.harvard.edu).

*Authors contributed equally.

Abstract

Background: Reexposure to methamphetamine with a single "priming dose" can trigger intense cravings and precipitate relapse in methamphetamine-dependent individuals. The acyclic cucurbit[n]uril "molecular container" calabadion-2 shows a high affinity to bind and sequester methamphetamine in vitro and attenuates its locomotor-stimulating effect in rats. The present study investigates whether pretreatment with calabadion-2 is sufficient to prevent the reinstatement of drug seeking by a priming dose of methamphetamine in rats.

Methods: Male Long-Evans rats were trained to self-administer i.v. methamphetamine (0.06 mg/kg/infusion). Following 10 days of stable self-administration, rats underwent extinction training and were subsequently tested on a multi-phase reinstatement procedure. Drug-primed reinstatement sessions (0.3 mg/kg methamphetamine, i.v.) were preceded by either saline or calabadion-2 (130 mg/kg). Additional reinstatement tests were conducted after administration of yohimbine (1.0 mg/kg, i.v.) to define the pharmacological specificity of calabadion-2.

Results: Pretreatment with calabadion-2 significantly attenuated methamphetamine-induced reinstatement of responding. Cal2 did not affect drug-seeking behavior stimulated by the pharmacological stressor yohimbine, indicating a mechanism of action specific to methamphetamine.

Conclusions: These results demonstrate the effectiveness of calabadion-2 in a preclinical model relapse-like behavior. With further structural optimization, molecular containers may provide a novel and efficacious pharmacokinetic approach to relapse prevention for methamphetamine-dependent individuals.

Keywords: methamphetamine, reinstatement, relapse, self-administration

Received: September 24, 2019; **Revised:** December 9, 2019; **Accepted:** January 2, 2020

Introduction

Methamphetamine (METH) remains among the most widely abused substances worldwide, and its use represents a substantial public health burden (SAMHSA, 2017). Although cognitive and behavioral treatment strategies have been shown to produce positive clinical outcomes in the short term (Lee and Rawson, 2008), maintaining prolonged periods of abstinence is particularly challenging for METH-dependent individuals; an estimated 60% to 80% relapse within a year of treatment (Kay-Lambkin, 2008; Brecht and Herbeck, 2014). Both clinical and laboratory findings have demonstrated that administration of a single, low dose of the previously abused drug (i.e., priming dose) can trigger intense cravings, which dramatically increases the vulnerability to relapse (Jaffe et al., 1989; de Wit, 1996; Bossert et al., 2013). Therefore, therapeutic interventions that can mitigate the impact of drug reexposure might prove useful to clinicians treating patients with stimulant use disorders.

Recent drug development efforts for the treatment of stimulant abuse (i.e., cocaine, METH) have largely focused on small-molecule pharmacotherapies aimed at various CNS neurotransmitter systems. These pharmacodynamic approaches target specific receptors or transporters to either attenuate the positive reinforcing and rewarding effects of the drug or to dampen the negative reinforcing effects brought on by withdrawal from it. Until now, however, this strategy is yet to produce a clinically viable target. The limited efficacy of pharmacodynamic drug candidates can be attributed, in part, to off-target effects and compensatory plasticity associated with chronic engagement of a target signaling system. Intended drug effects may be further complicated by an abundance of genetic polymorphisms known to influence drug metabolism, absorption, and excretion, which likely contribute to the high variability in pharmacological treatment outcomes (Brensilver et al., 2013). Consequently, there are currently no consensus treatments or FDA-approved pharmacotherapies for stimulant use disorders, including METH use disorder, leaving clinicians treating patients addicted to METH with few clinical tools (Morley et al., 2017). Alternatively, agents that alter the pharmacokinetics of METH may offer a favorable therapeutic profile without disturbing endogenous neural dynamics (Owens et al., 2011; Gorelick, 2012). However, studies of the enzymes, antibodies, and vaccines primarily utilized for this manner of treatment have revealed significant limitations of all such strategies due to lack of specificity (Watterson et al., 2013), variation in antibody titer (Yang et al., 2018), or high cost of production (Owens et al., 2011).

Initially developed to accelerate recovery from anesthetic neuromuscular blockade, the calabadion family of acyclic cucurbit[n]uril "molecular containers" facilitate drug clearance through rapid recognition and encapsulation of target molecules (Hoffmann et al., 2013; Diaz-Gil et al., 2016). Host-guest complexes are subsequently excreted in urine (Haerter et al., 2015). In an initial screening of several molecular container compounds with a high affinity toward hydrophobic cations, calabadion-2 (Cal2) was identified as having high affinity and specificity for (+) METH in vitro. Subsequent experiments to clarify its in vivo efficacy demonstrated that i.v. administration of Cal2 was sufficient to prevent and reverse the motor stimulant effects of METH in rats (Ganapati et al., 2017). Since molecular containers possess favorable aqueous solubility, display high biocompatibility (Ma et al., 2012), and can be synthesized on a large scale (Zhang and Isaacs, 2014), these preliminary findings position the calabadion family of compounds as an intriguing candidate therapeutic for pharmacokinetic modulation of METH activity in vivo.

Since doses of stimulant drugs necessary to enhance locomotor activity may exceed those capable of exerting stimulus-effects underlying subjective craving, the present studies sought to empirically examine the effects of calabadion-2 on a drug-primed reinstatement procedure: a preclinical model with both face and predictive validity for therapeutics that decrease drug relapse in humans (Bossert et al., 2013). In these experiments, male rats trained to self-administer i.v. METH were tested in a multi-phase extinction-reinstatement protocol. After rats had undergone extinction training, they were administered a priming dose of METH that was preceded by either saline or Cal2 pretreatment to establish the capacity for Cal2 to prevent METH-induced drug-seeking behavior. Finally, the pharmacological specificity of Cal2 was examined in a series of reinstatement tests preceded by yohimbine (Yoh) injection.

Methods

Subjects

Male Long-Evans rats (n = 12; Charles River Laboratories, Wilmington, MA) weighing 225 to 250 g on arrival were individually housed in clear polycarbonate cages (29 × 25 × 29 cm). After 1 week of habituation to the vivarium, rats were implanted with chronic, indwelling catheters into the jugular vein as previously described (Leonard et al., 2017). Animals were given 1 week to recover before being transferred to operant conditioning chambers, where they remained for the duration of the study. Throughout the experiment rats were maintained on a reverse light/dark cycle (lights on: 8:00 PM–8:00 AM) within a temperature-controlled environment and provided food and water ad libitum. Experimental procedures were approved by the Tufts University Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

METH Self-Administration, Extinction, and Reinstatement

Self-administration sessions were carried out in the rats' home cages, which were custom-built clear acrylic chambers (30 × 30 × 24.5 cm) outfitted with a removable panel containing 2 retractable levers and 2 stimulus lights (Med Associates Inc.). Chambers were housed within sound- and light-attenuating enclosures prepared with a ventilation fan and house light. During self-administration sessions, a green stimulus light was illuminated to signal drug availability, and responses on the active lever were reinforced with 0.06 mg/kg METH infusion and concurrent illumination of a red stimulus light above the lever. Stimulus lights remained illuminated throughout the drug infusion and were extinguished during a subsequent post-infusion timeout period (30 seconds). Responses on the inactive lever or during the timeout were recorded but had no programmed consequence. Training sessions lasted for 5 hours or 30 infusions, whichever came first. After 2 consecutive days in which rats obtained at least 20 drug infusions under a simple fixed ratio (FR) schedule of 1 (each lever press was followed by an infusion), the schedule of reinforcement was gradually increased to a terminal schedule of FR3. In the event of >5% loss of body weight between sessions, rats received 1 day off (no drug) in order to prevent excessive weight loss during initial training.

METH self-administration was maintained under an FR3 schedule of reinforcement for 10 days prior to extinction training. During extinction, responses on the active lever no longer produced any programmed consequences (i.e., no stimulus light presentation and no drug infusion). Each session lasted for 2 hours and was conducted twice daily, separated by 3 hours. Extinction criterion was met when active lever responses were reduced to <20% of the responses emitted during the first extinction session, for 2 consecutive sessions.

For reinstatement tests, drug treatments (130 mg/kg calabadion-2 or saline [Sal]) were delivered i.v. 5 minutes before a priming injection of either METH (0.3 mg/kg, i.v.) or Yoh (1.0 mg/kg, i.v.) at the onset of the session. A higher dose of METH (3.0 mg/kg, i.v.) was further examined in a subset of rats (n = 4).

Reinstatement sessions were identical to the extinction procedure, where lever responses were recorded but had no programmed consequence. Drug test conditions were assigned in a counterbalanced manner to account for potential order effects and were as follows: Sal+METH, Cal2+METH, Sal+Yoh, Cal2+Yoh, Sal+Sal, and Cal2+Sal. All drug tests were conducted in the morning and were followed by a single session of METH self-administration (FR3; 2 hours). Between tests, extinction training was carried out such that the extinction criterion was met (<20% of the responses recorded during the first extinction session) before each successive determination.

Drugs

d-Methamphetamine HCl (METH) was provided by the National Institute of Drug Abuse (Research Triangle Institute, Research Triangle Park, NC) and was dissolved in sterile 0.9% Sal for i.v. administration. Yoh was obtained from Sigma-Aldrich (Saint Louis, MO) and dissolved in sterile distilled water. Calabadion-2 was synthesized according to the previously published protocol (Ma et al., 2012) in the laboratory of Dr Lyle Isaacs, at the University of Maryland (College Park, MD). Cal2 was suspended in sterile distilled water to a concentration of 130 mg/mL immediately prior to use. A dose of 130 mg/kg produced maximal effects without toxicity in previous behavioral studies in rats (Ganapati et al., 2017) and was therefore selected for the present behavioral experiments.

Statistical Analysis

All statistical analyses were conducted using Prism version 7.0 (Graphpad Software Inc.). Active and inactive responses during extinction were assessed via paired *t* test, and behavior during reinstatement sessions was analyzed using factorial ANOVAs. All significant effects ($P < .05$) were followed by post hoc analyses with Bonferroni corrections for multiple comparisons.

Results

Maintenance and Extinction

Rats acquired METH self-administration under an FR3 schedule of reinforcement (i.e., ≥20 infusions per session) in 9.67 ± 1.50 days (mean ± SEM), and responding remained stable across 10 additional maintenance sessions (Figure 1B). Extinction training significantly reduced responding on the drug-paired lever [$t_{(11)} = 5.233$, $P < .001$], and all rats met extinction criteria after 12 sessions (Figure 1C–D). Extinction, however, had no effect on inactive lever responding.

Reinstatement

A priming infusion of METH reinstated responding on the previously extinguished drug lever without altering responding on the inactive lever (Figure 2A). The METH-induced drug-seeking was significantly attenuated by pretreatment with Cal2. Accordingly, main effects were found for both pretreatment ($F_{1,22} = 53.00$, $P < .001$) and drug exposure ($F_{1,22} = 36.94$, $P < .001$) condition, with a significant interaction between the two ($F_{2,22} = 59.72$, $P < .001$). On the other hand, while Yoh produced a similar enhancement of drug-lever responding after extinction ($F_{1,12} = 14.52$, $P < .01$), pretreatment with Cal2 was insufficient to prevent these effects ($P = .773$).

Discussion

The present studies are the first to evaluate an acyclic cucurbit[n]uril molecular container, calabadion-2, as a potential pharmacokinetic intervention for METH relapse-like behavior. Under these conditions, noncontingent administration of METH provoked a prominent burst of responding on the lever previously reinforced with METH. However, when administered prior to drug reexposure, Cal2 is sufficient to prevent METH-triggered drug seeking in rats. Although only examined in a subset of rats (data not shown), a 10-fold higher dose of METH (3.0 mg/kg), which was behaviorally disruptive alone, was sufficient to surmount Cal2 blockade to stimulate reinstatement responding. These findings are collectively consistent with the proposed mechanism that Cal2 can rapidly bind and sequester METH in the periphery to reduce its access to the brain, in turn preventing the stimulus properties of the drug (Ganapati et al., 2017). Furthermore, the vigorous response output exhibited by rats that received Yoh after Cal2 pretreatment undermines the possibility of Cal2 effects being related to general motor impairment or that Cal2 might interact with other behavioral substrates of reinstatement such as associative, motivational, or inhibitory processes.

In addition to drug reexposure, it is important to consider that relapse can also be triggered by nondrug stimuli. Notably, both stress and exposure to drug-associated contexts and cues can stimulate drug craving humans and serve to reinstate METH seeking in animal models of relapse (Sinha, 2001; Shepard et al., 2004; Bossert et al., 2013). The finding that Cal2 failed to prevent the stress-related reinstatement of drug-seeking by Yoh administration, an alpha-2 adrenoceptor antagonist commonly used to induce stress-like behaviors (Bremner et al., 1996), therefore underlines the pharmacological specificity of Cal2 to selectively bind METH molecules. Although Cal2 cannot protect against environmental factors that might influence relapse vulnerability, it may, however, mitigate the intensity or duration of drug taking when a patient relapses back into METH use. Cal2 could provide a critical buffer against the reinforcing effects of METH in the event of resumed drug consumption.

Limitations

The current studies provide a valuable proof of concept for calabadion-like molecular containers as a functional inhibitor of METH in a translationally relevant context. However, there are some practical limitations that constrain the efficacy of Cal2 in vivo. The excretion rate of calabadion-host complexes in urine indicates a functional half-life on the order of hours in rats (Diaz-Gil et al., 2016). The somewhat transient functionality of Cal2 is further complicated by the i.v. route of administration. Both the duration of drug action and route of

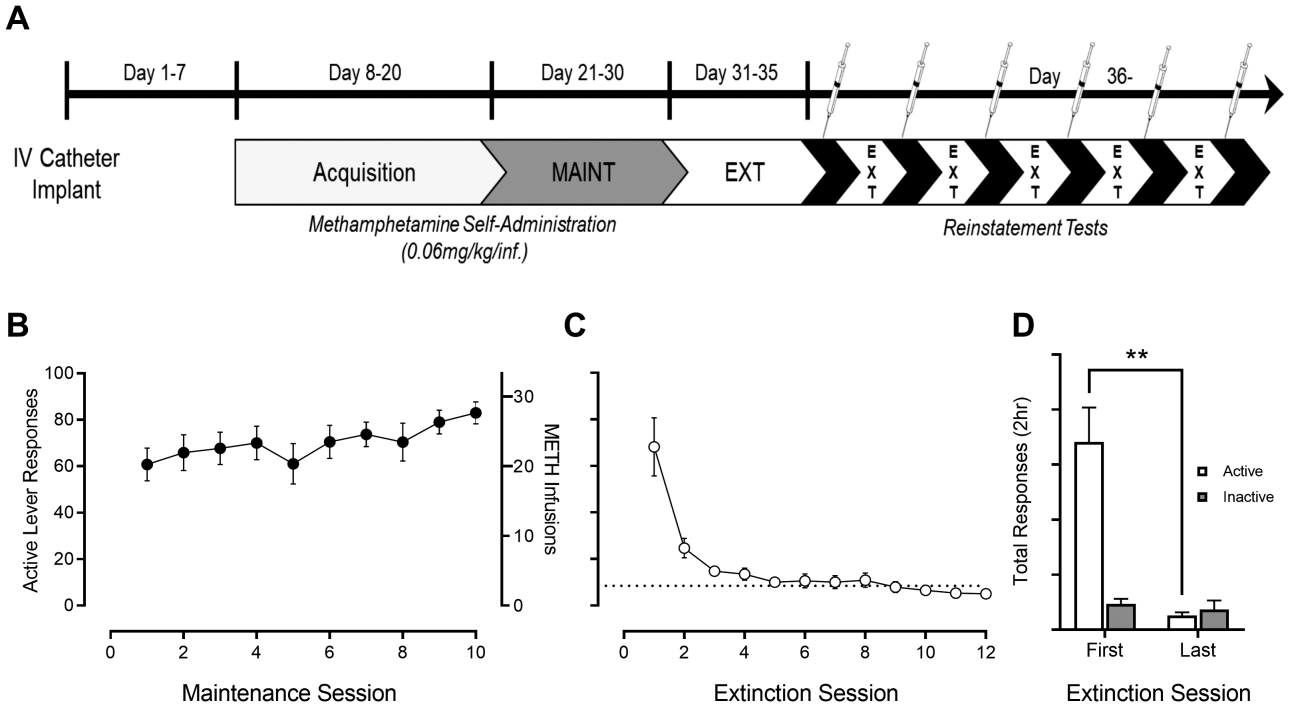

Figure 1. Acquisition and extinction of methamphetamine (METH) self-administration. (A) Experimental timeline. (B) Mean (±SEM) number of active lever responses during maintenance of METH self-administration (0.06 mg/kg/infusion) and (C) extinction training. (D) Total responses recorded on the active and inactive levers during the first and last extinction session. *P < .05; **P < .01.

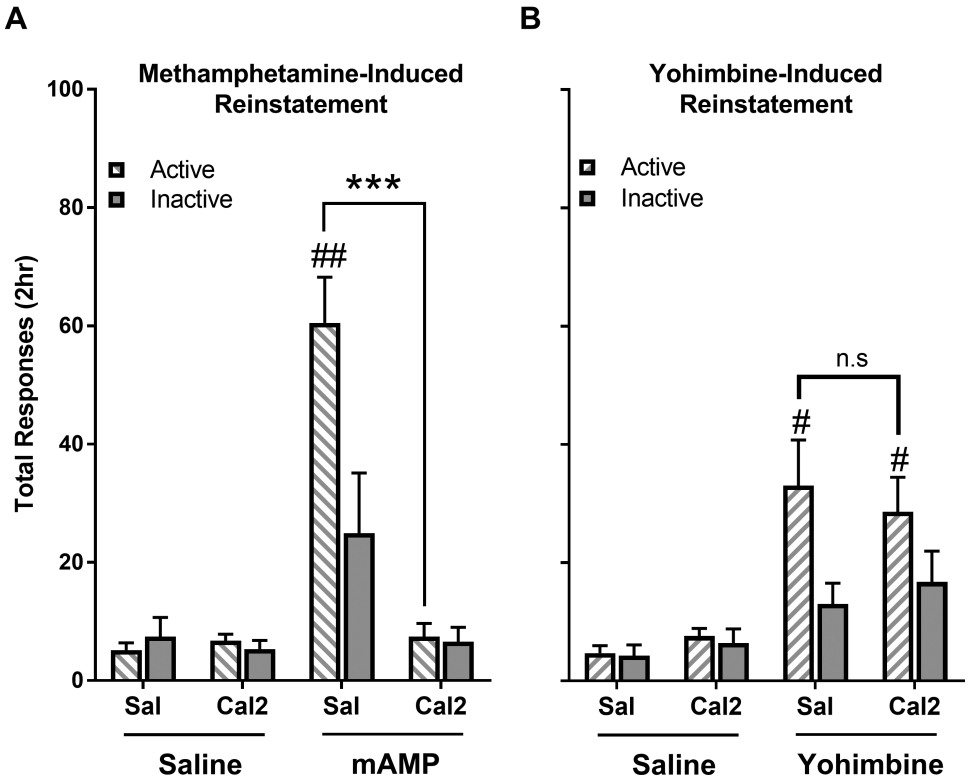

Figure 2. Calabadion-2 specifically prevents methamphetamine (METH)-induced reinstatement. (A) Total responses on the previously active lever or inactive lever after a priming injection of saline (Sal), METH (0.3 mg/kg, i.v.; n = 12), or (B) yohimbine (Yoh; 1.0 mg/kg, i.v.; n = 8). Data are presented as mean (±SEM) of total responses during reinstatement tests. #Difference from Sal+Sal control. #P < .05, ##P < .01, ###P < .001; *P < .05; **P < .01, ***P < .001.

administration are key determinants of compliance across a range of pharmacological therapeutics, where patients are increasingly likely to forego medication that requires frequent or impractical dosing regimens (Burnier, 2019). Importantly, the unpredictability of factors that might drive drug cravings or provoke relapse (i.e., environmental stress) demands consistent therapeutic compliance to be effective.

Further chemical optimization of Cal2 with regards to its binding affinity, selectivity, and half-life may overcome some of these practical limitations. Structural optimization that increases binding affinity includes the incorporation of a covalent capping group as a secondary binding site on one face of the molecule, a variation of the length of the glycoluril oligomer backbone to provide a larger hydrophobic driving force, and an increase in the ion-ion interactions between ammonium ion and solubilizing groups leading to improved electrostatic binding characteristics of the molecule.

In summary, we have demonstrated that Cal2 can selectively and effectively reduce relapse-like METH-seeking behavior in a preclinical model. Further research is required to determine whether molecular containers like Cal2 can provide a feasible pharmacokinetic approach to relapse prevention for METH-dependent individuals.

Acknowledgments

The authors thank Dr Lyle Isaacs and Sandra Zebaze for synthesizing the Cal2 used in the present experiments.
This work was supported by the National Institute on Drug Abuse at the National Institutes of Health, R01DA031734 (K.A.M.).

Statement of Interest

M.E. holds a stake in Calabash Bioscience, Inc. (College Park, MD), which develops Calabadions for biomedical applications. All other authors declare no competing interests.

References

Bossert JM, Marchant NJ, Calu DJ, Shaham Y (2013) The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl) 229:453–476.

Brecht ML, Herbeck D (2014) Time to relapse following treatment for methamphetamine use: a long-term perspective on patterns and predictors. Drug Alcohol Depend 139:18–25.

Bremner JD, Krystal JH, Southwick SM, Charney DS (1996) Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. Synapse 23:28–38.

Brensilver M, Heinzerling KG, Shoptaw S (2013) Pharmacotherapy of amphetamine-type stimulant dependence: an update. Drug Alcohol Rev 32:449–460.

Burnier M (2018) Is there a threshold for medication adherence? Lessons learnt from electronic monitoring of drug adherence. Front Pharmacol 9:1540.

de Wit H (1996) Priming effects with drugs and other reinforcers. Exp Clin Psychopharmacol 4:5–10.

Diaz-Gil D, Haerter F, Falcinelli S, Ganapati S, Hettiarachchi GK, Simons JC, Zhang B, Grabitz SD, Moreno Duarte I, Cotten JF, Eikermann-Haerter K, Deng H, Chamberlin NL, Isaacs L, Briken V, Eikermann M (2016) A novel strategy to reverse general anesthesia by scavenging with the acyclic Cucurbit[n]uril-type molecular container calabadion 2. Anesthesiology 125:333–345.

Ganapati S, Grabitz SD, Murkli S, Scheffenbichler F, Rudolph MI, Zavalij PY, Eikermann M, Isaacs L (2017) Molecular containers bind drugs of abuse in vitro and reverse the hyperlocomotive effect of methamphetamine in rats. Chembiochem 18:1583–1588.

Gorelick DA (2012) Pharmacokinetic strategies for treatment of drug overdose and addiction. Future Med Chem 4:227–243.

Haerter F, Simons JC, Foerster U, Moreno Duarte I, Diaz-Gil D, Ganapati S, Eikermann-Haerter K, Ayata C, Zhang B, Blobner M, Isaacs L, Eikermann M (2015) Comparative effectiveness of calabadion and sugammadex to reverse non-depolarizing neuromuscular-blocking agents. Anesthesiology 123:1337–1349.

Hoffmann U, Grosse-Sundrup M, Eikermann-Haerter K, Zaremba S, Ayata C, Zhang B, Ma D, Isaacs L, Eikermann M (2013) Calabadion: a new agent to reverse the effects of benzylisoquinoline and steroidal neuromuscular-blocking agents. Anesthesiology 119:317–325.

Jaffe JH, Cascella NG, Kumor KM, Sherer MA (1989) Cocaine-induced cocaine craving. Psychopharmacology (Berl) 97:59–64.

Kay-Lambkin FJ (2008) Technology and innovation in the psychosocial treatment of methamphetamine use, risk and dependence. Drug Alcohol Rev 27:318–325.

Lee NK, Rawson RA (2008) A systematic review of cognitive and behavioural therapies for methamphetamine dependence. Drug Alcohol Rev 27:309–317.

Leonard MZ, DeBold JF, Miczek KA (2017) Escalated cocaine "binges" in rats: enduring effects of social defeat stress or intra-VTA CRF. Psychopharmacology (Berl) 234:2823–2836.

Ma D, Hettiarachchi G, Nguyen D, Zhang B, Wittenberg JB, Zavalij PY, Briken V, Isaacs L (2012) Acyclic cucurbit[n]uril molecular containers enhance the solubility and bioactivity of poorly soluble pharmaceuticals. Nat Chem 4:503–510.

Morley KC, Cornish JL, Faingold A, Wood K, Haber PS (2017) Pharmacotherapeutic agents in the treatment of methamphetamine dependence. Expert Opin Investig Drugs 26:563–578.

National Research Council (2011) Guide for the care and use of laboratory animals (8th ed). Washington DC: National Academies Press.

Owens SM, Atchley WT, Hambuchen MD, Peterson EC, Gentry WB (2011) Monoclonal antibodies as pharmacokinetic antagonists for the treatment of (+)-methamphetamine addiction. CNS Neurol Disord Drug Targets 10:892–898.

SAMHSA (2017) 2016 National Survey on Drug Use and Health: detailed Tables. Rockville, MD: Center for Behavioral Health Statistics and Quality. Available at: https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2016/NSDUH-DetTabs-2016.pdf. Accessed January 2020.

Shepard JD, Bossert JM, Liu SY, Shaham Y (2004) The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. Biol Psychiatry 55:1082–1089.

Sinha R (2001) How does stress increase risk of drug abuse and relapse? Psychopharmacology (Berl) 158:343–359.

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Hood LE, Olive MF (2013) Attenuation of reinstatement of methamphetamine-, sucrose-, and food-seeking behavior in rats by fenobam, a metabotropic glutamate receptor 5 negative allosteric modulator. Psychopharmacology (Berl) 225:151–159.

Yang X, Wang Y, Li Q, Zhong Y, Chen L, Du Y, He J, Liao L, Xiong K, Yi CX, Yan J (2018) The main molecular mechanisms underlying methamphetamine- induced neurotoxicity and implications for pharmacological treatment. Front Mol Neurosci 11:186.

Zhang B, Isaacs L (2014) Acyclic cucurbit[n]uril-type molecular containers: influence of aromatic walls on their function as solubilizing excipients for insoluble drugs. J Med Chem 57:9554–9563.