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DARK Classics in Chemical Neuroscience: Cathinone-Derived Psychostimulants

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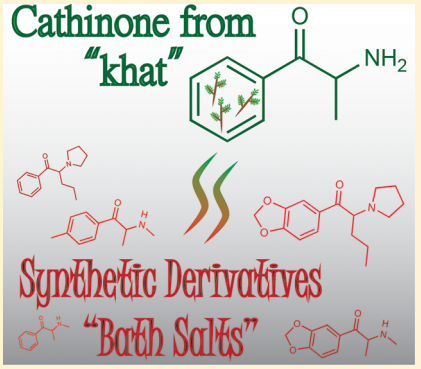
ABSTRACT: Cathinone is a plant alkaloid found in khat leaves of perennial shrubs grown in East Africa. Similar to cocaine, cathinone elicits psychostimulant effects which are in part attributed to its amphetamine-like structure. Around 2010, home laboratories began altering the parent structure of cathinone to synthesize derivatives with mechanisms of action, potencies, and pharmacokinetics permitting high abuse potential and toxicity. These "synthetic cathinones" include 4-methylmethcathinone (mephedrone), 3,4-methylenedioxypyrovalerone (MDPV), and the empathogenic agent 3,4-methylenedioxymethcathinone (methylone) which collectively gained international popularity following aggressive online marketing as well as availability in various retail outlets. Case reports made clear the health risks associated with these agents and, in 2012, the Drug Enforcement Agency of the United States placed a series of synthetic cathinones on Schedule I under emergency order. Mechanistically, cathinone and synthetic derivatives work by augmenting monoamine transmission through release facilitation and/or presynaptic transport inhibition. Animal studies confirm the rewarding and reinforcing properties of synthetic cathinones by utilizing self-administration, place conditioning, and intracranial self-stimulation assays and additionally show persistent neuropathological features which demonstrate a clear need to better understand this class of drugs. This Review will thus detail (i) historical context of cathinone use and the rise of "dark" synthetic derivatives, (ii) structural features and mechanisms of synthetic cathinones, (iii) behavioral effects observed clinically and in animals under controlled laboratory conditions, and (iv) neurotransmitters and circuits that may be targeted to manage synthetic cathinone abuse in humans.



KEYWORDS: *Addiction, cathinone, designer drugs, dopamine, novel psychoactive substance(s), reward, synthetic cathinone(s)*

CATHINONE AND THE RISE OF SYNTHETIC DERIVATIVES

Cathinone is a phenylalkylamine derived from leaves of the khat perennial shrub (*Catha edulis*) that produces psychostimulant effects. Khat leaves are chewed by locals residing in East Africa and the Arabian Peninsula for ability to allay fatigue similarly to how cocaine-containing coca leaves of the *C. erythroxylaceae* shrub are chewed along trails of the Andes Mountains; leaf chewing in this manner is culturally normative and seen as relatively nonproblematic. Khat leaves appear to have a written history of being chewed by natives beginning in the 14th century, and the custom remains commonplace to date.[1] Based on self-reports, khat leaves are chewed for subjective improvements in concentration and libido.[2] In an analysis of 8900 responses from secondary school and college-aged students in Saudi Arabia, over one-fifth reported having chewed khat leaves with the majority attributed to male respondents (37.7%) compared to females (3.8%).[3] Although (−)-cathinone is self-administered by rodents,[4] purified forms of cathinone do not appear to be widely abused in humans, likely as a result of its moderate potency and rapid degradation to cathine during storage.[5] These unfavorable characteristics may have played a role in the development of the first synthetic cathinone analogue methcathinone (ephedrone), which was originally synthesized by Hyde and colleagues[6] and subsequently marketed as an antidepressant in the Soviet Union and a CNS stimulant in the United States[7] Reports of methcathinone abuse surfaced in the United States and elsewhere in the early 1990s,[8,9] leading to its placement (along with its parent compound cathinone) into Schedule I controlled substance status in the United States.

Structurally, cathinone possesses striking resemblance to the classically abused agent phenylisopropylamine (amphetamine) and the empathogenic amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA). Capitalizing on comparable structures, clandestine laboratories altered motifs of cathinone to produce synthetic agents which have been classified under the umbrella of "novel psychoactive substances" (NPS). Many

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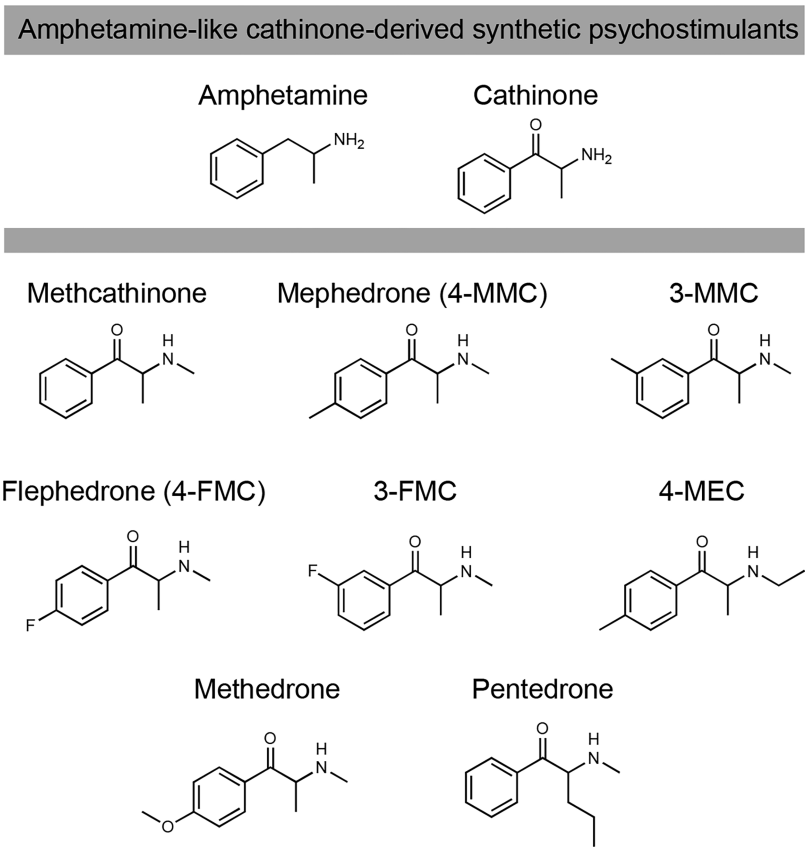

Figure 1. Amphetamine-like cathinone-derived synthetic psychostimulants including methcathinone, "first-generation" mephedrone and its positional isomer 3-MMC, flephedrone and 3-FMC, 4-MEC, methedrone, and pentedrone. Typically, synthetic cathinones in this class possess substitutions along cathinone's benzene ring.

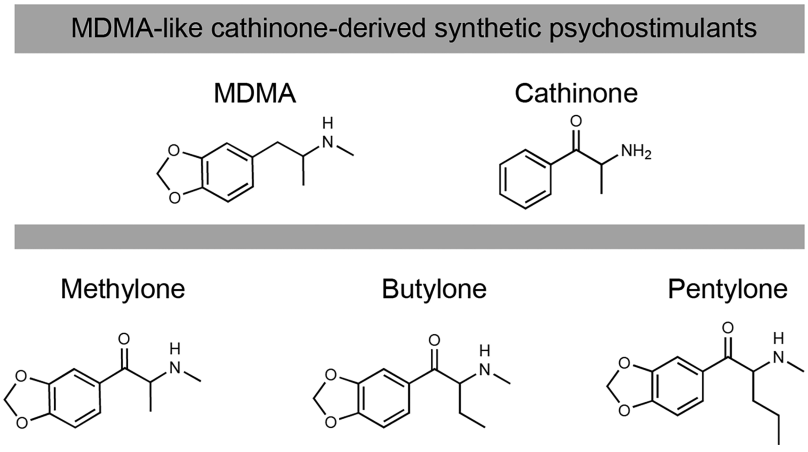

Figure 2. MDMA-like cathinone-derived synthetic psychostimulants including methylone, butylone, and pentylone. Typically, synthetic cathinones in this class contain a methylenedioxy-substitution adjacent to cathinone's benzene ring.

synthetic cathinones are highly abused with evident toxicity conferring their "dark" nature. Common modifications of cathinone's parent structure include alkyl or halogen substitutions at points along the aromatic ring (e.g., 4-methylmethcathinone [4-MMC; mephedrone], 4-fluoromethcathinone [flephedrone]; Figure 1) as well as methylenedioxy substitutions (e.g., 3,4-methylenedioxy-*N*-methylcathinone [methylone]; Figure 2) and pyrrolidinyl substitutions (e.g., α-pyrrolidinovalerophenone [α-PVP]) or a combination therein (e.g., 3,4-methylenedioxypyrovalerone [MDPV]; Figure 3). The United Nations' Convention on Psychotropic Substances recognized cathinone and its methylated analogue (methcathinone, the first synthetic cathinone) as potentially harmful and abuse-prone, leading to Schedule I placement in 1971.

The rise in synthetic cathinone production and aggressive marketing arrived on an international scale beginning around 2010. Around then, synthetic cathinones were advertised and sold online and as fictitious products such as "plant food" and "bath salts", and marked "not for human consumption" to bypass regulations imposed by the Food and Drug Administration. By 2013, over 600 "dark web" sites had been identified in Europe that allowed traffickers and users to

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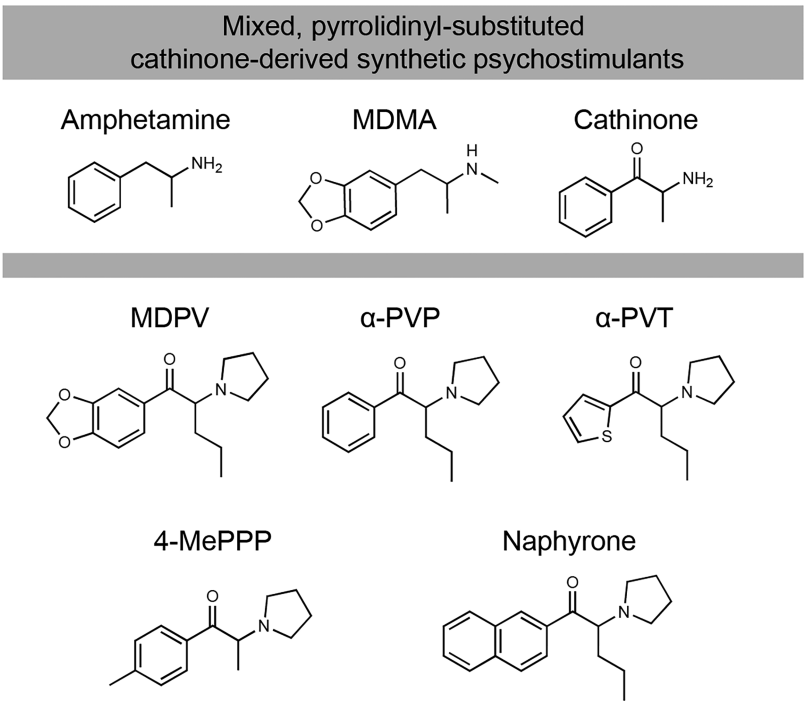

Figure 3. Mixed amphetamine- and MDMA-like cathinone-derived synthetic psychostimulants including MDPV, α-PVP and -PVT, 4-MePPP, and naphyrone. Synthetic cathinones in this class share a benzene ring substitution (methylenedioxy or other) as well as a pyrrolidinyl group along cathinone's tertiary carbon atom.

purchase these drugs anonymously with the use of untraceable digital currencies.[10] In addition, online drug forums, social media plaftorms, and even drug retailers themselves provided users and customers with detailed information on specific product names, availability, slang terminology, packaging imagery, drug effects, efficacy, dosages, and legal status.[11,12] In 2011 alone, 6138 calls were made to the Poison Control Centers from emergency departments related to synthetic cathinone ("bath salt") products.[13] As specific chemical structures became scheduled, new compounds tended to rise in popularity to avoid risk of judicial prosecution. Specifically, whereas the National Forensic Laboratory Information System reported 21 analytically confirmed synthetic cathinone drugs in 2011, 42 different synthetic cathinone drugs were found in 2014.[14] A Poland-based report documented that, while methyl- and methylenedioxy-substituted cathinones (e.g., mephedrone and methylone) were most abundant in seized samples from 2010, "simple cathinone" packages including α-methylamino-valerophenone (pentedrone) as well as positional isomers of former agents (e.g., 3-methylmethcathinone [3-MMC]) were analytically detected in greatest abundance from 2014 to 2015.[15] Like cathinone itself, nearly all synthetic cathinones produce behavioral effects by augmenting monoamine transmission in central nervous system sites of action (i.e., dopamine [DA], noradrenaline [NA], serotonin [5-HT]) through amphetamine-like release facilitation and cocaine-like reuptake inhibition mechanisms. Euphoria and hallucination are met with adverse sympathomimetic effects which collectively make many synthetic cathinone drugs dangerously rewarding and reinforcing "dark" agents.

BIOCHEMICAL STRUCTURES AND NEUROPHYSIOLOGY OF SYNTHETIC CATHINONES

Structural Features. Cathinone's striking resemblance to amphetamine diverges with the addition of a ketone body (carbon−oxygen double bond) at the β-carbon atom. The carbon atom adjacent to amphetamine's (and cathinone's) primary amine is stereogenic thus giving rise to two enantiomers with unique methyl group positional conformations along the *z*-plane. As such, many synthetic cathinones including mephedrone exist as pharmacologically distinct enantiomers, although racemic mixtures predominate illicit formulations. The backbone of amphetamine can be modified by replacing its hydrogen atoms, methyl groups, or amine groups with substituents=cathinone, in this way, is considered a substituted amphetamine (β-ketone-amphetamine). Also included in the domain of substituted amphetamines are methamphetamine and ephedrine as well as the psychedelics MDMA and 2,5-dimethoxy-4-methylamphetamine (DOM).[16] Structural features of synthetic cathinones include substitutions with methyl, ethyl, butyl, isopropyl, methylenedioxy, pyrrolidinyl, fluoro, and benzyl groups against the cathinone backbone (for reviews, see refs 7 and 17). Many synthetic cathinones readily permeate the blood-brain barrier as determined in vitro with alarming high permeability noted for mephedrone and MDPV.[18]

Effects of Cathinone and Synthetic Derivatives on Brain Monoamine Transmission. Consistent with structural resemblance to amphetamine, cathinone and synthetic derivatives exert psychosomatic effects through augmentation of brain monoamine transmission. Cathinone was first shown to augment extracellular DA content by Peter Kalix in the early 1980s.[19,20] Thereafter, cathinone was found to block synaptosomal DA uptake sites (i.e., dopamine transporters [DAT]) at comparable efficacy compared to *d*-amphetamine

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(Adderall) while decreasing DAT content following chronic exposure.[21] When examining enantiomeric differences, (−)-cathinone was found to possess approximately 3-fold greater potency at blocking central DATs compared to (+)-cathinone whereas both enantiomers significantly enhanced extracellular NA in peripheral tissues.[22,23] Pehek and colleagues[24] were the first to use in vivo microdialysis to confirm that cathinone produced amphetamine-like elevations in extracellular DA content within the nucleus accumbens (NAcc) of ventral striatum—a central site of action for psychostimulant-associated reward and reinforcement implicated in the development of drug dependence. Cathinone appreciably elevates striatal DA and NA in an enantiomer-sensitive manner which likely drives, at least in part, the pervasiveness of khat leaf chewing in/around East Africa and the Arabian Peninsula.

Synthetic cathinones share similar mechanistic features as the parent compound. Cathinone's methylated analogue, methcathinone, potently augments extracellular DA content at rates comparable to methamphetamine.[25] Other work finds that systemic mephedrone significantly decreases synaptic uptake of striatal DA and, with greater persistence, hippocampal 5-HT content.[26] In vivo, both mephedrone and the MDMA-like agent methylone significantly elevate ventral striatal DA and 5-HT.[27] Similarly, intravenous MDPV was observed to significantly elevate DA and NA in vivo with approximately 10-fold greater potency compared to intravenous cocaine.[28] Electrophysiological study additionally finds that bath-applied MDPV inhibits clearance of DA within mouse striatal slices after delivery of 5−25 Hz electrical pulses. MDPV also enhances DAT currents in both magnitude and duration.[29] The "second generation" synthetic cathinone α-PVP also elicits elevations in striatal DA in vivo.[30] Many first- and second-generation synthetic cathinones, like the parent compound itself, augment central DA content albeit at varying potencies which likely contributes to differential rates of abuse.

The MDMA-like agents methylone, β-keto-*N*-methylbenzodioxolylbutanamine (butylone), and β-keto-*N*-methylbenzodioxolylpentanamine (pentylone) induce inward currents at serotonin transporters (SERTs) and dissociate slowly.[31] MDMA-like synthetic cathinones are suspected to produce persistent leak currents at SERT comparable to effects of amphetamine on DATs.[32] Described later in this review, MDMA-like agents typically show relatively weak reinforcing effects compared to amphetamine-like comparator agents which is likely due to DAT vs SERT selectivity.

It has been proposed that synthetic cathinones including mephedrone activate DATs and in turn induce depolarizing currents that trigger vesicle fusion and DA release similarly to mechanisms ascribed to amphetamine and cocaine (for review, see ref 9). This "DAT hypothesis" suggests that mephedrone, like amphetamine, binds directly to DAT to activate the catecholamine transport pore and, in turn, allows the bound agent (and sodium) to enter the presynaptic terminal to facilitate vesicular fusion and DA release. However, because studies measuring extracellular DA content show that mephedrone-elicited elevations are lesser than those compared to amphetamine, we suggest that mephedrone may have a relatively weaker affinity for DAT although to our knowledge these protein−protein interactions (i.e., mephedrone/amphetamine-DAT) have not been directly compared.

BEHAVIORAL EFFECTS OF SYNTHETIC CATHINONES DETERMINED FROM CASE REPORTS AND CONTROLLED LABORATORY EXPERIMENTS

As indicated above, cathinone and synthetic derivatives structurally and mechanistically resemble psychostimulants amphetamine and cocaine as well as the empathogen MDMA. Accordingly, euphorigenic effects are typically produced following synthetic cathinone use but are experienced alongside agitation, anxiety, and aggression typified by psychostimulant abuse. The following subsections will detail behavioral effects of cathinone and synthetic derivatives as have been captured in case reports. Thereafter, descriptions of behavioral assays used in animal studies to probe cathinone-related reward and reinforcement under controlled laboratory conditions, as well as effects captured in those studies, will be provided.

Clinical Features and Case Studies. Khat leaf chewing often occurs in social settings which tends to buffer otherwise negative effects including anxiety, irritability, and aggressiveness. Somatically, khat leaf chewing can lead to tachycardia, tachypnea, mydriasis, flushing, and headache as a result of cathinone's sympathomimetic properties. Of perceived benefit, khat leaf chewing reportedly produces euphoria, elation, allayed fatigue, and increased alertness and is generally believed to be a mood elevator. In three vignettes provided by Granek and colleagues,[33] clinically diagnosed hypnagogic hallucinations from khat leaf chewers of 25+ years were described, consistent with earlier anecdotal links between khat leaf chewing and schizophreniform psychosis (e.g., ref 34). The authors reason that, while acute effects of khat leaf chewing are relatively innocuous, chronic use permits greater risk of psychotic episodes warranting clinical attention. Separately, stimulant-like effects of khat leaf chewing can be followed by debilitating rebound effects including lethargy and sleepiness. One case report described the danger of utilizing prescription sedatives (diazepam, in this case) to combat insomnia following episodes of khat leaf chewing in chronic users.[35] In this report, a 35-year-old man was admitted to an emergency department following a midafternoon comatose state attributed to the combination of excessive khat leaf chewing and diazepam which, in turn, was suspected to lead to aspiration pneumonia followed by acute respiratory distress syndrome (ARDS). Khat leaf chewing is not without risk, but these risks are far less severe relative to those associated with synthetic cathinone use.

Administering synthetic cathinones can produce adverse risks ranging from local tissue injury to death following multiorgan failure. Injection-related soft tissue complications are summarized by Dorairaj and colleagues[36] and include cellulitis, vein clotting, abscess formation, and muscle necrosis which can be compounded by poor hygiene, nonsterile injection practices, and lack of injection site asepsis. Injection-related skin infections can often be managed by antibiotic treatment; in extreme cases, surgical debridement may be needed. Administration of synthetic cathinones can produce subjective effects including delusional thoughts, hallucinations, agitation, and aggression as well as, at high doses, severe somatic symptoms resembling cocaine and MDMA overdose including sympathomimetic toxicity and serotonin syndrome. In a recent online survey of self-reported synthetic cathinone users within the United States, subjective effects of greatest frequency included feelings of stimulation/energy, euphoria, improved focus, suppressed appetite, and enhanced sex drive.[37] Additionally,

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self-reported undesired effects included elevated heartrate, excess sweating, nervousness/anxiety, and paranoia.

In one of the first case reports related to synthetic cathinone use, Belhadj-Tahar and Sadeg[38] describe the misfortune of a 29-year-old woman who was admitted to the emergency department following a toxicologically verified methcathinone-potentiated coma presented with mydriasis and hyperpnea. The first "pure" medical case report of mephedrone toxicity was published in 2010 and described the emergence of palpitations, sweating, chest pressure, blurred vision, and sympthaomimetic toxicity following intramuscular injection of 3.8 g of mephedrone in a 22-year-old man.[39] Consumption of just 100 mg of naphthylpyrovalerone (naphyrone) led to clinical diagnosis of sympathomimetic toxidrome in a 31-year-old patient who acutely suffered from insomnia, hallucinations, and "mortal fear".[40] Methylone was linked directly to sudden cardiac death in a 19-year-old user[41] consistent with prior MDMA-related fatality from serotonin syndrome, disseminated intravascular coagulopathy, and anoxic encephalopathy.

Abuse of MDPV was reported in a case report documenting a 25-year-old who was "markedly combative and foaming at the mouth".[42] The patient was hyperthermic, hypertensive and mydriatic upon arrival to the emergency department and subsequently developed renal failure and rhabdomyolysis indicative of multi organ failure although eventually recovered after a prolonged hospital stay. In addition to violent behaviors, MDPV has also been reported to lead to paranoid delusions and hallucinatory delirium which present management obstacles as methods to control erratic behaviors including physical restraints, tasers, and antipsychotics can worsen somatic toxicity.[43] Many users who begin using synthetic cathinones following a history of cocaine and methamphetamine use tend to experience intensified neurological/psychiatric conditions.[44] Indeed, a 39-year-old man with a history of drug and alcohol use was taken to the emergency department after MDPV use in a hyperthermic (peak fever recorded at 107.1 °F) and tachycardic state; after cooling efforts, he became severely bradycardic and died ∼12 h after admission.[45] Collectively, these studies illustrate lethal risk of synthetic cathinone use in human users. To more precisely characterize which domains of behavior synthetic cathinones influence, we turn our attention to animal studies conducted under controlled laboratory conditions.

Behavioral Effects of Synthetic Cathinones in Animal Subjects. Numerous behavioral assays are utilized by preclinical investigators to probe the rewarding and reinforcing effects of drugs that are abused by humans. These behavioral tools in nonhuman animals, including measurement of locomotor activity, place conditioning, drug discrimination, intracranial self-stimulation (ICSS), and intravenous self-administration, help to comprehensively model substance use disorders as observed in human users. Equipped with these tools, preclinical investigators can evaluate neurobiological features that underlie the development to drug dependence. Additionally, intervention strategies including pharmacotherapy can be tested in effort to gauge therapeutic efficacy. This section will briefly describe aforementioned behavioral tools leading into detailed accounts of effects found from experimenter- and self-administered synthetic cathinones.

Locomotor Stimulation and Sensitization. Typical of sympathomimetic drugs, cathinone and synthetic derivatives elevate ambulatory/exploratory behavior as well as "stereotypy" which is often measured as vertical photobeam breaks in a testing arena. Repeated movements, whereby the test subject shuttles between two horizontal locations in a back-and-forth pattern, can additionally be measured. These measures provide simple, targetable behaviors to evaluate the ability of novel psychoactive agents to act in a psychostimulant-like manner. Work in cocaine-injected rats demonstrates a clear, positive relationship between cocaine-stimulated locomotor activity and elevations in ventral striatal DA content;[46] follow-up work revealed that direct injection of cocaine within ventral striatum was sufficient to elevate locomotor activity.[47] Early work also shows that khat plant extract and purified cathinone acutely elevate locomotor activity in laboratory animals, and that (−)-cathinone is the more potent locomotor-activating enantiomer.[19,48] Cathinone-elicited hyperlocomotion is mediated in part by effects on central nervous system sites of action as intracerebroventricular injection of cathinone also significantly elevated ambulation.[49]

In testing a series of synthetic cathinones, Gatch and colleagues[50] showed hyperlocomotor effects in mice after injection of mephedrone, MDPV, and methylone as well as "newer" agents flephedrone, naphyrone, and butylone: robust and persistent effects were noted across the 8 h testing session following injections of MDPV and naphyrone. Gregg and colleagues[51] reported that *R*-mephedrone more potently elevated locomotor activity compared to its racemate *S*-mephedrone. Significant elevations in locomotor activity were additionally found following injection of 3-fluoromethcathinone (3-FMC) and 4-methoxymethcathinone (methedrone) with greater potency ascribed to the former psychoactive agent.[52] Fantegrossi and colleagues[53] reported significant locomotor-stimulating effects of systemic MDPV in mice but only under relatively warm ambient conditions (∼28 °C). In a different study, systemic injection of MDPV (1.0 mg/kg, IP) elicited comparable elevations in locomotor activity compared to methamphetamine-injected rats but failed to appreciably elevate core body temperature.[54] Other synthetic cathinones including α-PVP and pentedrone were shown to significantly elevate locomotor activity.[30,55] Synthetic cathinones appear thus to invariably elevate motor activity in animal subjects albeit with different potencies.

Repeated injections of a psychostimulant often lead to the development of a sensitized behavioral response, typically measured by locomotor activity (ambulation), after many injections compared to initial responses. Sensitization to locomotor-stimulating effects of cathinone and mephedrone were reported from rats receiving a multiday repeated injection regimen.[56,57] Mephedrone was shown to produce sensitization of repetitive movements (i.e., consecutive photobeam breaks) using a 7 day variable-dose injection regimen, and greater potency in inducing behavioral sensitization was attributed to mephedrone's *R*-enantiomer.[51,58] MDPV also produces sensitization to repetitive movements using the variable-dose injection regimen.[59] Other studies find that mephedrone- or MDPV-injected animal subjects ambulate at appreciably greater rates when challenged subsequently with either cocaine or methamphetamine suggesting psychostimulant cross-sensitization.[60,61] The development of locomotor sensitization is characteristic of cocaine and amphetamine, and the above-mentioned experiments additionally support that synthetic cathinones produce this behavioral signature.

Reward: Effects on Affect/Mood. Psychostimulants "prime" brain reward function during assessment of intracranial self-stimulation (ICSS) in animal subjects. Briefly, ICSS utilizes an

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intracranially implanted stimulating electrode lowered to a structure or fiber bundle known to support operant reinforcement. Stimulation current required to support self-stimulation is often taken as a metric of hedonic state ("reward threshold") whereas other teams quantify the rate of responding at a fixed current. Early evidence finds that systemic mephedrone (10 mg/kg, IP) promotes ICSS in mice at lower current intensities relative to cocaine-elicited responding at 15−30 min post-injection.[62] This study supports that mephedrone is capable of priming brain reward function but may take a relatively longer time to reach central sites of action relative to cocaine. Not long thereafter, Watterson and colleagues[63] demonstrated significant facilitation of ICSS following MDPV injection (0.1 to 2.0 mg/kg, IP) in rats. Indeed, numerous other synthetic cathinones including methcathinone and methylone were shown to facilitate self-stimulation upon acute injection [ref 64, but see ref 65]. Interestingly, high-dose methylone and mephedrone (10 mg/kg, IP) were shown to reduce response rates below vehicle-injected control levels suggesting a suppression of brain reward function. Taken together, synthetic cathinones generally promote electrical self-stimulation behavior in rodents supporting their ability to facilitate brain reward function.

Place conditioning can also be used to probe conditioned reward processes attributed to an experimental treatment. Typically, a two-chamber shuttle apparatus is used whereby each chamber is distinguished by a constellation of tactile, visual and olfactory cues. An experimental treatment, such as synthetic cathinone injection, is paired with one chamber and a control treatment such as saline is paired with the other chamber. A preference score is then determined by measuring the amount of time tested subjects spend on each of the two chambers when subjects are provided a choice between the two chambers in a drug-free state. Only a few reports describe the ability of cathinone and synthetic derivatives to induce place preference in animal subjects. The first study was provided by Schechter[66] and showed that systemic injections of (−)-cathinone every other day across an 8 day injection regimen induced significant place preference in rats compared to the saline-paired alternative context. Mephedrone induces place preference which appears to be attributed to the *R*-enantiomer as rats did not show preference toward a context paired with *S*-mephedrone.[51] MDPV (2 and 5 mg/kg, IP) produced place preference across a 4-day injection schedule.[59] Other reports find that pentedrone (3.0 and 10.0 mg/kg, IP) elicits significant place preference at rates comparable to methamphetamine (1 mg/kg, IP). Significance place conditioning was additionally observed for the phenyl/thiophene substitute of α-PVP, α-pyrrolidinopentiothiophenone (α-PVT).[67]

In an alternative test design, drug discrimination is used to compare the interoceptive stimulus effects of injected drugs by measuring the extent to which a new drug is responded for relative to a comparator agent (often cocaine, amphetamine, or MDMA). Drug discrimination can further apply to stereoisomers of a given agent; for example, *l*-amphetamine discriminates for *d*-amphetamine at high doses whereas nicotine, mescaline, and fenfluramine fail to produce stimulus discrimination effects.[68] Kalix and Glennon[69] were among the first to show that (−)-cathinone fully substitutes for systemic amphetamine unlike other tested aminophenones. Methcathinone fully and potently substitutes against racemic amphetamine; in a follow-up study examining enantiomeric differences, *S*-methcathinone was revealed as the potent enantiomer producing amphetamine-like stimulus discrimination.[25,70] Kohut and colleagues[71] observed that methcathinone possesses cocaine-like potency in rhesus monkeys trained in drug discrimination. Methylone failed to fully substitute for amphetamine but retained MDMA-like discriminative stimulus effects.[72] Comparing across drug types, Fantegrossi and colleagues[53] observed that MDPV fully substituted for both methamphetamine and MDMA with 50% efficacy (ED_{50}) achieved ∼0.03 mg/kg across both comparator drugs. "Second-generation" synthetic cathinones α-PVP, α-PVT and 4-methyl-α-pyrrolidinopropiophenone (4-MePPP) fully substituted for methamphetamine whereas 4-methyl-*N*-ethylcathinone (4-MEC) failed to discriminate suggesting a unique interoceptive experience relative to methamphetamine.[67,73] A second report revealed substitution of 4-MEC for methamphetamine but at appreciably higher doses (50.0 mg/kg, IP) compared to those used by Naylor and colleagues (2015; 1.0−8.0 mg/kg, IP).[74] Collectively, drug discrimination has aided our understanding as to the degrees to which novel synthetic psychostimulants resemble the subjective experience attributed to classically abused agents such as cocaine, amphetamine and MDMA.

In effort to capture additional measures noninvasively in existing behavioral experiments, ultrasonic vocalizations (USVs) have proven of great utility in psychostimulant research (for review, see ref 75). USVs are high-pitched vocal emissions produced by constriction and stabilization of the larynx. Rats typically elicit USVs as an intraspecies communication tool which signal "avoid-approach" behaviors. Notably, USVs are often dichotomized based on frequency of emission: 22 kHz USVs are often long, monotonous and are emitted under experimental conditions associated with affective distress including presentation of a predator, electrical foot-shock, and withdrawal from abused drugs including cocaine.[76−78] Conversely, 50 kHz USVs are often shorter, acoustically varied and are emitted under appetitive experimental conditions including electrical brain stimulation, mating opportunity, and acute administration of psychostimulants including cocaine [e.g., refs 79−81]. In an initial demonstration, our team found that systemic MDPV elicits comparable yet more persistent rates of 50 kHz USVs compared to cocaine at one-tenth the dose.[82] As is mentioned below, intravenous self-administration of MDPV additionally elicits 50-kHz USVs at robust rates including from exposure to the MDPV-paired context in the absence of drug receipt.

Finally, several teams have interrogated effects of synthetic cathinones on anxiety-like behavior in rodents. den Hollander and colleagues[83] failed to observe appreciable effects of binge-like mephedrone or methylone on metrics interpreted as reflecting anxiety-like behavior (i.e., % time in open arms of an elevated plus- or zero-maze). Repeated injections of MDPV, however, produced an anxiogenic effect in rats tested 72 h following the last injection.[84] Thus, the negative emotional states emerging following synthetic cathinone use may be mediated by dynamic fluctuations in DA/NA levels which MDPV most robustly influences. Indeed, the lasting negative affective states produced by psychostimulants are commonly attributed to alterations in mesolimbic DA activity and effects therein on reward processing [e.g., ref 85].

Reinforcement As Assessed by Intravenous Self-Administration. Intravenous drug self-administration remains a model with face and construct validity that measures abuse potential and captures the volitional aspect of drug-taking. Nearly all drugs that are abused in humans are reinforcing and self-administered intravenously in laboratory subjects including

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nonhuman primates and rats. In an early comparison study, Johanson and Schuster[86] observed greater reinforcing effects of *l*-cathinone against both the racemic mixture and *d*-amphetamine in intravenously self-administering monkeys. Both forms of cathinone showed broader inverted U-shaped dose−response curves and greater peak responding compared to *d*-amphetamine in tested monkeys. Similar work performed by Woolverton and Johanson[87] supports the ability of intravenous cathinone to function as a positive reinforcer even when an intravenous cocaine option is presented. Gosnell and colleagues[4] provided the first evidence that cathinone is actively self-administered in rats, and that the inverted U-shaped dose−response function was approximately 2-fold leftward shifted relative to intravenous cocaine, suggesting greater potency. Qualitative analysis on patterns of drug-taking revealed that, while intravenous cocaine was self-administered at relatively evenly distributed interinfusion intervals throughout sessions in well-trained rats, intravenous cathinone tended to be rapidly responded for early in sessions suggesting a pattern of use characterized by rapid "load-up" injections followed by even interinfusion intervals to titrate drug levels.

Effects of synthetic cathinones on operational reinforcement were investigated beginning in the early 1990s. Methcathinone was the first cathinone-derived synthetic agent shown to be intravenously self-administered at doses ranging from 0.1 to 1.0 mg/kg/inf.[88] In this study, rates of responding were comparable to those performed for self-administered equipotent cocaine. Hadlock and colleagues[26] showed that rats actively self-administer mephedrone (∼0.80 mg/kg/inf) commensurate with elevations in core body temperature and with significantly greater infusions earned across days 5−8 of daily 4 h self-administration sessions compared to saline infusions. Thereafter, Aarde and colleagues[54] determined the half-life of mephedrone after ∼1.00 mg/kg/inf injection (IV) from plasma to be ∼1-h, and other work from our team observed greater reinforcement associated with *R*-mephedrone compared to its racemate.[89]

The MDMA-like agent methylone is self-administered by rats across a range of doses (0.05−0.50 mg/kg/inf), yet, unlike with intravenous cocaine, rats self-administer appreciably greater infusions of high-dose methylone compared to lower doses under low-effort, fixed-ratio 1 (FR-1) access conditions whereby one operant response performed during the availability period is rewarded with an intravenous drug injection.[65] Watterson and colleagues[63] additionally observed potently reinforcing effects of self-administered MDPV in rats at doses as low as 0.05 mg/kg/inf. Also captured in this study is a remarkably high motivational drive for intravenous MDPV as evidenced by rats responding 100+ times for single injections of MDPV under progressive-ratio (PR) access conditions, a schedule of reinforcement in which exponentially greater operant responses are needed to yield intravenous drug receipt. Compared to self-administered cocaine, MDPV demonstrated comparable reinforcing efficacy at one-tenth the dose, and the latency to begin MDPV self-administration was appreciably shorter compared to cocaine.[82] Methylone possesses relatively weak reinforcing efficacy during intravenous self-administration compared to MDPV likely due to concomitant elevations in ventral striatal DA and 5-HT. In one of the only studies to demonstrate synthetic cathinone reinforcement in female test subjects, self-administered methylone was shown to have significantly lower reinforcing value compared to response rates for intravenous mephedrone.[90] A second report by the same team[91] additionally found that mephedrone is a more effective reinforcer compared to MDMA and methylone in self-administering male rats.

The "second-generation" α-alkyl derived synthetic cathinone α-PVP showed similar reinforcing efficacy during intravenous self-administration compared to MDPV.[92] Moreover, α-PVP was shown to have greater reinforcing efficacy compared to pentedrone and pentylone although all tested agents were more reinforcing than methylone.[93] In a different study, only one of four tested doses of pentedrone (0.3 mg/kg/inf) was appreciably self-administered by rats,[55] suggesting a narrow dose−response function. 4-MePPP, a different pyrrolidinyl substituent synthetic cathinone, showed comparable reinforcing efficacy as α-PVP although with less potency.[94] In this study, both α-PVP and 4-MePPP were more reinforcing than 4-MEC yet only α-PVP was revealed as more potent than methamphetamine. α-PVT showed cocaine-like reinforcing efficacy, reinforcing doses ranging from 0.1 to 1.0 mg/kg/inf in an inverted-U dose−response manner, in self-administering mice.[67] Self-administration remains an excellent tool to capture the volitional aspect of drug-taking as is observed in humans and provides insight on potency and reinforcing efficacy of new synthetic drugs.

Cognitive Effects Associated with Synthetic Cathinone Use. Chronic use of psychostimulants such as cocaine, methamphetamine, or MDMA is associated with cognitive dysfunction. Similar dysfunction appears to manifest following use of cathinone and synthetic derivatives although results tend to vary by drug type, dosing, and duration of use. Studies on habitual khat users demonstrate deficits in several aspects of learning and cognitive set-shifting.[95,96] Chronic users of the synthetic cathinone mephedrone show impaired prose recall, verbal learning and fluency, working memory, and cognitive flexibility,[97,98] although readers should note a high incidence of polydrug use in these cited studies. Recent controlled laboratory studies in humans have confirmed that acute administration of mephedrone (200 mg) impairs short-term spatial memory, although divided attention appears unaffected.[99] Taken together, these studies support that cathinone and synthetic derivatives are capable of contributing to deficits in cognitive functions.

Corroborating with work performed in humans, acute mephedrone (0.56−10 mg/kg) was shown to produce dose-dependent rate-decreasing and error-increasing effects in an operant-based response test in rodent subjects.[100] In nonhuman primates, interestingly, acute mephedrone injection produced improvements in visuospatial attention[101] perhaps due to enhancements in vigilance and concentration commonly reported in human users. A single injection of the potent cocaine-like synthetic cathinone MDPV can induce widespread reductions in functional connectivity between frontal and subcortical structures.[102] Moreover, cognitive deficits associated with acute synthetic cathinone additionally appear sex-dependent and mediated by circulating ovarian hormones.[103] Acute synthetic cathinone use thus appears to produce mixed effects in assessments of cognitive functioning; however, drug abusers (including those who abuse synthetic cathinones) infrequently administer a single, isolated dose of drug.

Several teams have sought to explore how chronic synthetic cathinone use, as is observed in many human users, impacts short- and long-term cognitive functioning. Motbey and colleagues[104] observed object recognition deficits when subjects were tested 35 days after a chronic mephedrone dosing regimen

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(30 mg/kg/day, 10 days). A separate study compared long-term effects of chronic mephedrone to those of the empathogenic agent methylone. Specifically, den Hollander and colleagues[83] found that mice treated with mephedrone (30 mg/kg/day, 2×/day, 4 days) showed deficient alternating T-maze task performance when assessed 3 weeks after drug injections whereas methylone-injected mice remained unimpaired. In a follow-up assessment, mephedrone-injected mice returned to vehicle-injected control levels and, curiously, methylone-injected mice showed improvements in reversal learning test performance. In contrast, other reports detail significant deficits in reference memory as assessed by either Morris water maze or a land-based Y-maze following repeated methylone injections.[105,106] Collectively, long-term synthetic cathinone use generally associates with deficient cognitive functioning which may relate to neurobiological changes in response to repeated drug use.

It should be noted that the aforementioned studies invariably utilized noncontingent "bingelike" passive exposure of subjects to the cathinone-related drug of interest which may produce pharmacokinetic and pharmacodynamic effects that are not necessarily the same as those occurring following voluntary intake. To achieve improved validity in rodent models, we recently demonstrated that long-term access to self-administered MDPV (96 h access periods alternating with 72 h periods of abstinence for a total of 5 weeks) resulted in significant deficits in novel object recognition memory but not object placement memory when tested 3 weeks following the last drug access session.[107] The lack of effect on object placement suggests some sparing of spatial memory function in opposition to findings summarized previously with repeated noncontingent administration. It is therefore of great interest to determine which cognitive deficits are observed following prolonged voluntary access, their dose- and sex-dependency, and the duration of impairment.

NEURAL STRUCTURES AND TRANSMITTER SYSTEMS PROMOTING PHARMACOTHERAPEUTIC INTERVENTION STRATEGIES TO MANAGE SYNTHETIC CATHINONE ABUSE

To date, no pharmacotherapeutic medications are approved for treating stimulant use disorders including to cocaine, amphetamine, and synthetic cathinones. Within this section, we describe new advances from preclinical research teams on our developing understanding of the circuits and transmitters that underlie reward associated with synthetic cathinone use. Exciting progress has supported the targeting of monoaminergic transmission systems as well as glutamatergic, neuropeptide, and central chemokine transmission systems. New "preventative medicine" avenues include CNS-permeable drug-conjugate vaccinations to suppress synthetic cathinone abuse.[108]

Regulation of Monoamine Transmission. In effort to suppress artificially augmented surges in DA content, several studies have incorporated the use of agents acting on D_1- and D_2-like receptors. For example, Young and Glennon[70] observed that the discriminative stimulus effects of *S*-methcathinone against an amphetamine-paired lever are effectively shifted (made less effective) by pretreatment with haloperidol, a D_2-like receptor antagonist. Seminal work additionally reveals that haloperidol can reduce cathinone-elicited hyperthermia in rabbits[19] supporting that cathinone-elicited somatic effects are, at least in part, DA-mediated. Systemic injections of either the D_1-like receptor antagonist SCH23990 or the D_2-like receptor antagonist sulpiride significantly decreases hyperlocomotor activity following α-PVP injection.[30] Other work, however, finds that pretreatment with SCH23390 increases self-administered cathinone infusions relative to vehicle pretreatment levels:[4] the increase in self-administered infusions may reflect effort to reach the subjectively positive experience occurring in sessions prior to DA receptor blockade.

Reinforcing efficacy of abused drugs is largely attributed to augmented DA levels; however, pharmacological agents that regulate 5-HT content show some preclinical efficacy. Gannon and colleagues[109] recently reported on the ability of clinically available lorcaserin, an obesity medication working in part via 5-HT_{2A} activation, to reduce motivated responding for both cocaine and MDPV in self-administering rats. Targeting 5-HT_2 receptors was additionally highlighted by López-Arnau and colleagues[110] who found that ketanserin, a nonselective 5-HT_2 antagonist, decreased mephedrone- and methylone-elicited elevations in locomotor activity which corroborates prior work linking 5-HT to MDMA-elicited locomotion. It thus appears that stimulation of 5-HT_{2A} receptors produces therapeutically favorable effects against the reinforcing effects of psychostimulants, but that other 5-HT receptor subtypes may contribute to locomotor-stimulating effects following synthetic cathinone use.

Regulation of Glutamate Transmission. *Receptor-Based Targeting Strategy.* Targeting presynaptic metabotropic glutamate 2 and 3 receptors (mGluR2/3s) also appears as a promising pharmacotherapeutic strategy to manage psychostimulant addiction (for review, see[111]). Animal studies show enduring neuroadaptations in mGluR2/3 receptors following repeated psychostimulant exposure, which contributes to synaptic plasticity in glutamate and DA levels that drives drug-taking and drug-seeking behavior.[112−116] Accordingly, systemic administration of mGluR2/3 agonists can attenuate cocaine and methamphetamine self-administration as well as reinstatement behaviors.[117−121] Moreover, the glutamate carboxypeptidase II (GCPII) inhibitor 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), which is an indirect mGluR2/3 agonist, attenuates cocaine place preference,[122] cocaine self-administration, and cocaine relapse.[123,124]

Recently, we showed that mGluR2/3 receptors may also possess pharmacotherapeutic potential for the treatment of synthetic cathinone abuse.[125] In our study, we found that 2-PMPA and *N*-acetylaspartylglutamate (NAAG) dose-dependently reduced the expression of MDPV place preference. We confirmed that these effects were mediated via mGluR2/3 receptors, as the inhibitory effects of NAAG were blocked by pretreatment with the mGluR2/3 antagonist LY341495. Currently, we are investigating whether the effects of NAAG and 2-PMPA extend to MDPV self-administration and reinstatement of MDPV-seeking behavior.

Targeting Glutamate Transporters and Clearance Efficiency. A strategy of targeting glutamate transport may offer possible advantages over conventional receptor-based approaches in managing psychostimulant abuse. Since multiple glutamate receptor subtypes contribute to psychostimulant addiction, a putative medication that blocks (e.g., NMDA) or activates (e.g., mGluR2/3) only a single receptor is unlikely to produce lasting efficacy. The glutamate transport system offers a viable target because chronic cocaine regimens disrupt its ability to maintain glutamate homeostasis, and this action leads to glutamate dysregulation that contributes to cocaine

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reinforcement and seeking.[126−128] Activation of the glutamate transport system shapes activity at multiple glutamate receptor subtypes. For example, activation of cystine-glutamate exchange (system Xc) increases glutamatergic tone onto mGluR2/3 receptors and decreases synaptic glutamate release probability.[129] Activation of system Xc leads to activation of presynaptic mGluR2/3 receptors that reduces reinstatement of cocaine seeking by normalizing the reduction in extrasynaptic glutamate following chronic cocaine administration and preventing the increased synaptic glutamate release that drives drug seeking.[130] Activation of glutamate transporter subtype 1 (GLT-1) by the β-lactam antibiotic ceftriaxone reduces reinforcing effects of cocaine in mice and the behavioral-sensitizing effects of amphetamine, counteracts deficits in GLT-1 expression and myelin-related proteins in the NAcc of cocaine-withdrawn mice, and inhibits relapse to cocaine seeking in rat self-administration models of reinstatement.[131−133] Notably, chronic MDPV administration downregulates NAcc GLT-1 expression, and pretreatment with ceftriaxone normalizes GLT-1 levels and suppresses MDPV-associated reward.[59]

Neuropeptides: Hypocretin/Orexin and Dynorphin. Neurotransmitters and peptides that influence mesolimbic DA transmission may additionally be targeted to modulate reward and reinforcement associated with synthetic cathinone abuse. As an example, chronic mephedrone was shown to upregulate neuropeptide dynorphin content in rat striatum;[134] dynorphin is the endogenous ligand of the kappa opioid receptor (KOR) which associates with subjective states of negative affect and negatively regulates DA-producing cellular physiology [e.g., refs 135 and 136]. While KOR blockade failed to appreciably alter cocaine self-administration in nonhuman primates,[137] KORs were observed to regulate cocaine-seeking behavior stimulated by activation of hypocretin/orexin (hcrt/ox) receptors,[138] which is discussed in detail in the following section. It reasons, then, that elevated dynorphin levels may contribute to negative affect associated with cessation of many synthetic cathinone drugs including mephedrone, and that the reinforcing effects of psychostimulants may be mediated by synergistic interactions between KORs and other neuropeptide receptors at the cellular level.

Converging lines of anatomical and behavioral studies support that the hypothalamic peptide hypocretin/orexin (hcrt/ox), which transmits via two excitatory postsynaptic G-protein coupled receptors (GPCRs) (OX_1R and OX_2R), densely innervates DA-producing cellular populations and regulates psychostimulant-associated reinforcement [refs 139 and 140; for review, see, ref 141]. Notably, OX_1R blockade decreases operant responses for intravenous cocaine (as well as for sucrose pellets) but fails to appreciably alter responding for normal food chow suggesting a selective role for hcrt/ox transmission via OX_1Rs in the seeking/retrieval of palatable reinforcers.[142] Muschamp and colleagues[136] soon revealed VTA OX_1Rs as direct contributors to motivated cocaine-taking. Complementary work finds that hcrt/ox transmission to VTA mediates the reinforcing effects of self-administered cocaine and positively regulates mesolimbic DA transmission.[143,144]

In a recent study from our team, we found that suvorexant, a clinically available hcrt/ox receptor antagonist, reduces motivated cocaine-taking without suppressing motor activity of tested rats.[145] We later observed that suvorexant significantly suppresses 50 kHz USVs associated with anticipation and self-administration of MDPV in rats.[146] However, we did not observe significant reductions in the number of self-administered MDPV infusions which was likely due to the employment of a low-effort schedule of reinforcement and a selective role of hcrt/ox in motivational action. Thus, targeting hcrt/ox transmission may favorably normalize pathological motivation including behaviorally activated states associated with the procurement of synthetic cathinone drugs of abuse.

***S*-Mephedrone: An Enantiomer with Antiaddictive Potential.** We have unexpectedly found that the stereochemical effects of racemic mephedrone are dramatic. In preclinical assays, *S*-mephedrone blocks anxiogenic and depressant effects during cocaine withdrawal and displays efficacy against neuropathic pain.[84] Importantly, *S*-mephedrone does not cause extensive amphetamine-like rewarding, reinforcing, motivational, and locomotor-enhancing effects in rats, as does racemic mephedrone and the *R*-enantiomer.[51] Mechanistically, *S*-mephedrone is a "triple monoamine transporter substrate" that enhances release of 5-HT, DA, and NA[27,28,147] but with 50-fold greater potency in releasing 5-HT versus DA.[51] The use of candidate medications for drug addictions that target the same transporters or receptors as the primary drug of abuse is a proven strategy for treating substance abuse disorders, as exemplified by efficacious, approved treatments for cigarette smoking (e.g., nicotine patch) and opioid dependence (e.g., methadone, buprenorphine).

There is strong scientific premise for studying triple 5-HT/DA/NA transporter substrates with preferential 5-HT-releasing effects for managing psychostimulant addiction. Cocaine withdrawal produces a dual deficit of synaptic DA and 5-HT in the brain of rats and humans, indicating the advantage of developing medications that normalize dysregulation of both neurotransmitter systems during cocaine abstinence. Specific evidence indicates that (i) withdrawal from abused stimulants produces a 5-HT deficit that resembles major depressive disorder in humans, (ii) administration of DA and 5-HT-releasing agents alone or together decreases drug-seeking in animals, (iii) increases in extracellular 5-HT in brain antagonize psychomotor activation by DA releasers, and (iv) a single molecule that releases both DA and 5-HT could suppress cocaine self-administration while having low abuse liability [e.g., refs 148 and 149]. A limitation of using amphetamines and preferential DA releasers as medications is a high abuse potential due to increased mesolimbic DA output. Since increases in synaptic 5-HT counteract stimulant and reinforcing effects caused by increased DA transmission, monoamine transporter substrates that release both DA and 5-HT, such as the compound PAL287,[150] are hypothesized to have lower abuse potential while maintaining an ability to antagonize withdrawal symptomatology and relapse. Although *S*-mephedrone does interact with $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, and $5\text{-}HT_{2C}$ receptors, it lacks agonist activity at any of them.[84] As such, potential advantages of *S*-mephedrone are reduced risks of hallucinogenic effects associated with $5\text{-}HT_{2A}$ activation,[18] reduced cardiovascular effects related to $5\text{-}HT_{2B}$ activation,[151] and reduced sensitivity of $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors which may otherwise promote relapse.[152]

Since *S*-mephedrone suffers a slow but characterized racemization to mephedrone, *S*-mephedrone itself is unsuited to be considered as a promising therapeutic agent for treating psychostimulant abuse. The in vivo metabolism of mephedrone is nearly exclusively Cyp2D6-mediated oxidation of the tolyl methyl to hydroxymethyl 7 and oxidative demethylation to normephedrone 8.[153,154] In summary, preclinical evidence with *S*-mephedrone suggests that future work be directed toward

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identification of stereochemically and metabolically stable *S*-mephedrone analogs as potential treatments for psychostimulant abuse and perhaps neuropathic pain due to its dual enhancement of both serotonergic and adrenergic transmission.

Chemokine Modulation of Reward and Reinforcement. A separate body of work has positioned the chemokine receptor−ligand pair, CXCR4-CXCL12, as a modulator of mesolimbic DA activity and as a contributor to the behavioral effects of psychostimulants. CXCL12, for example, is acutely elevated in humans and rodents following cocaine use.[155] The receptor for CXCL12, CXCR4, is a constitutively expressed GPCR localized to adult neurons and glia in reward- and emotion-governing structures including prefrontal cortex, VTA and NAcc.[156,157] While CXCR4 is coupled to $G\alpha_i$, calcium influx elicited by CXCL12 tends to favor neuroexcitatory effect associated with the ligand binding [e.g., ref 158]. In the midbrain, CXCR4 activation by intranigral CXCL12 injection depolarizes ipsilateral neurons residing in substantia nigra leading to DA elevations in dorsal striatal targets commensurate with increased contralateral turning behavior.[159] Moreover, bilateral intra-VTA CXCL12 potentiates cocaine-induced hyperlocomotion, an effect that is blocked by systemic CXCR4 antagonism with the clinically available bone marrow stimulant agent AMD3100 (Plerixafor).[157] In vitro, CXCL12 increases putative DA-producing cellular firing and extracellular DA content in a CXCR4-activation-dependent manner.[158] In rodents, repeated administration of cocaine increases CXCL12 mRNA in the VTA.[160] Notably, CXCR4 inhibition with AMD3100 has proven efficacious in reducing locomotor-stimulating and rewarding effects of MDPV in rats. More recently, we demonstrated that AMD3100 pretreatment blocks place conditioning for an MDPV-paired context and normalizes MDPV-elicited 50 kHz USVs.[161] Together, these findings implicate CXCR4 inhibition with AMD3100 as an effective therapeutic strategy to normalize MDPV-associated reward as well as to suppress protracted anxiogenic effects following chronic MDPV use.

Neurotoxic and Neuroinflammatory Effects: A Role for Anti-Inflammatory Agents? Given their primary pharmacological sites of action, presynaptic monoaminergic terminals in striatum and other forebrain regions are frequently investigated as potential substrates of possible neurotoxic effects following psychostimulant use [e.g., refs 162 and 163]. It is thus surmised that synthetic cathinones, through comparable central sites of action, may exert similar neurotoxic effects on monoaminergic neuron function.[164] However, studies examining the effects of synthetic cathinones on monoamine transmission in the cortex, striatum, and hippocampus have yielded mixed results [for reviews, see refs 165 and 166]. Specifically, some reports indicate that repeated administration of mephedrone or methylone produces transient or minimal changes in central monoamine content (DA and 5-HT) as well as turnover ratios.[57,83,104,167−171] Robust and persistent depletions in tissue monoamine content and/or reduced monoamine terminal markers have been reported following administration of higher doses of these synthetic cathinones at increased frequency (i.e., 20−25 mg/kg, 3−4×/day).[105,170,172,173] Still, other investigators have failed to observe any effects of mephedrone, MDPV, or methylone on tissue monoamine content [e.g., refs 174 and 175].

Psychostimulants promote inflammatory processes such as the release of proinflammatory cytokines and activation of microglia, which in turn may result in neurotoxicity and/or cognitive dysfunction.[163,176,177] There is relatively unconvincing evidence, however, that repeated injections of mephedrone or methylone produce microgliosis despite high doses and frequent injection.[104,105,172] It should be noted that some of these reports have observed increases in glial fibrillary acidic protein (GFAP) immunoreactivity following synthetic cathinone injections.[105,170] A separate body of work has begun uncovering cytotoxic effects of mephedrone, MDPV, and methylone largely derived from in vitro preparations (cultured neurons, endothelial cells, DA-producing SH-SY5Y cells, hepatocytes, and upper airway epithelial cells).[170,178−181]

Recent evidence of neurotoxic effects of long-term MDPV self-administration in object recognition circuitry (e.g., entorhinal and perirhinal cortices) was provided by our laboratory,[107] although no evidence of neurodegeneration was observed in monoamine-populated structures including prefrontal cortex, striatum and hippocampus. However, it was recently observed that repeated administration of high doses of mephedrone (50 mg/kg/day fpr 14 days or 3× daily for 7 days) to pregnant female mice during gestational days 5−18 produced offspring with reduced body weight and increased rate of stillborn birth.[182] In addition, mice exposed to mephedrone in utero displayed significant evidence apoptosis and reduced cell proliferation in the hippocampus. Thus, some synthetic cathinone may induce hippocampal neurotoxic and teratological effects. Consequently, we surmise that additional research is needed to examine multiple forms of toxicity in the central nervous system following prolonged synthetic cathinone exposure or use. Specifically, toxicity-associated measures should include necrosis, apoptosis, necroptosis, autophagy, oxidative stress, excitotoxicity, and activation of inflammatory and cell death signaling pathways. Elucidation of these pathways and mechanisms is needed to understand and potentially mitigate inflammatory and/or cytotoxic effects of synthetic cathinones which may underlie their ability to induce cognitive dysfunction and abuse propagation.

CONCLUDING REMARKS ON THE "DARK" NATURE OF CATHINONE-DERIVED SYNTHETIC PSYCHOSTIMULANTS

An appreciable body of work has corroborated to make clear the lethal risk of single-motif structural alterations to cathinone's parent structure through case studies, self-reports, and controlled laboratory experiments detailing effects of synthetic cathinone abuse. For a detailed account corroborating preclinical and clinical reports related to synthetic cathinone abuse, readers are referred to ref 183. Legislation has adapted to prosecute those possessing and manufacturing certain synthetic drugs, but novel formulations often, to societal detriment, avoid legal risk. Our understanding of how synthetic cathinones affect transmitters and circuits within the CNS has afforded multiple avenues for pharmacotherapeutic intervention testing—notably, some preclinical efforts reveal effective therapeutics from clinically available medications. Investigating the neurotoxic effects of synthetic cathinones, acutely and protractedly, promotes the investigation of anti-inflammatory agents in efforts to normalize long-term impairments in cognitive and emotional health. The rise of synthetic cathinone abuse occurs alongside the emergences of synthetic cannabinoids and opiates. Collectively, these designer drugs create a public health epidemic unlike those the United States has experienced for "pure" agents such as cocaine and heroin. Our teams and others, however, remain optimistic in working to comprehen-

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sively understand the behavioral features and mechanisms of cathinone-derived psychostimulants in hopes to send one or more therapeutic agents to clinics in ultimate efforts to manage their pervasive abuse.

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ABBREVIATIONS

DOM, 2,5-dimethoxy-4-methylamphetamine; two PMPA, 2-(phosphonomethyl)-pentanedioic acid; 3-FMC, 3-fluoromethcathinone; 3-MMC, 3-methylmethcathinone; methylone, 3,4-methylenedioxy-*N*-methylcathinone; MDMA, 3,4-methylenedioxymethamphetamine; MDPV, 3,4-methylenedioxypyrovalerone; 4-FMC (flephedrone), 4-fluoromethcathinone; methedrone, 4-methoxymethcathinone; 4-MePPP, 4-methyl-α-pyrrolidinopropiophenone; 4-MEC, 4-methyl-*N*-ethylcathinone; 4-MMC (mephedrone), 4-methylmethcathinone; pentedrone, α-methylaminovalerophenone; α-PVT, α-pyrrolidinopentiothiophenone; α-PVP, α-pyrrolidinovalerophenone; butylone, β-keto-*N*-methylbenzodioxolylbutanamine; pentylone, β-keto-*N*-methylbenzodioxolylpentanamine; ARDS, acute respiratory distress syndrome; DA, dopamine; DAT, dopamine transporter; GPCR, G-protein coupled receptor; GFAP, glial fibrillary acidic protein; GCPII, glutamate carboxypeptidase II; hcrt/ox, hyporcretin/orexin; OX_1R, hypocretin/orexin receptor subtype-1; OX_2R, hypocretin/orexin receptor subtype-2; ICSS, intracranial self-stimulation; KOR, kappa opioid receptor; LHb, lateral habenula; mGluR, metabotropic glutamate receptor; MAO, monoamine oxidase; NAAG, *N*-acetylaspartylglutamate; naphyrone, naphthylpyrovalerone; NA, noradrenaline; NAT, noradrenaline transporter; NPS, novel psychoactive substance; NAcc, nucleus accumbens; amphetamine, phenylisopropylamine; 5-HT, serotonin; SERT, serotonin transporter; USV, ultrasonic vocalization; VTA, ventral tegmental area

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