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Abuse Liability of Novel “Legal High” Designer Stimulants: Evidence From Animal Models

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Abstract

In the last few years, the variety and recreational use of “legal high” designer stimulants has increased to unprecedented levels. Since their rapid emergence in drug markets, numerous adverse physical and psychological effects have been extensively reported. However, less is understood about the potential for compulsive use of and addiction to these drugs. Recently, a small collection of scientific studies assessing abuse liability of these drugs has emerged. This new knowledge has been derived primarily from animal studies using behaviorally-based procedures which include intravenous self-administration, conditioned place preference, intracranial self-stimulation, and drug discrimination. In this review we present a brief history of the recent rise in designer stimulant use followed by a short methodological description of the aforementioned procedures. We then review neurochemical and abuse liability studies on designer stimulants that have been examined to date. Finally, we conclude with a discussion of these collective findings, our current understanding of abuse liability of these drugs in relation to each other and the illicit drugs they are designed to mimic, and recommend future research directions.

Keywords

Animal models; addiction; abuse liability; synthetic cathinones; piperazines; designer stimulants

Introduction

In recent decades, the use of “legal high” designer stimulants has escalated dramatically in the western world (Spiller et al., 2011; Rosenbaum et al., 2012). Concomitant with this escalated use has been an unprecedented increase in the variety of these substances now available in drug markets (Rosenbaum et al., 2012). Furthermore, experts predict that many more designer stimulants (i.e., multiple generations) are likely to emerge as replacements once popular (i.e., older generation) analogues are banned by regulatory agencies, a pattern that has already been reported (Brandt et al., 2010; Baumann et al., 2013b; DEA, 2013).

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Designer stimulants have led to numerous published reports of bizarre and violent behavior, toxicity, and death (for reviews see Spiller et al., 2011; Rosenbaum et al., 2012), forcing regulatory agencies such as the United States (US) Drug Enforcement Administration (DEA) to institute emergency bans citing serious hazards to public health and safety (DEA, 2002; DEA, 2011a). Given these trends, the scientific study of designer stimulant abuse/addiction liability is of the utmost importance for (1) providing evidence-based information to healthcare experts charged with treating abusers of designer stimulants, (2) guiding government agencies responsible for regulating these substances, and (3) informing the public about the potential risks of abuse of and dependence on these drugs. While the scientific evaluation of novel designer stimulant abuse liability is still in its infancy and continues to try to keep pace with the ever-increasing variety of designer stimulants, recently a small body of literature has emerged providing an evidence-based estimation of abuse/addiction liability for these substances in relation to each other and the prototypical illicit stimulants they are designed to mimic. These new data have been obtained largely from behaviorally-based procedures using animal subjects. These include the widely employed paradigms of intravenous self-administration (IVSA), conditioned place preference (CPP), intracranial self-stimulation (ICSS), and drug discrimination. Each of these established procedures measures somewhat distinct drug effects (e.g., reinforcing, appetitive conditioning, or interoceptive effects) and together can provide a solid foundation for inferring abuse liability. Finally, we discuss findings from these studies to (1) estimate the abuse liability of these novel stimulants relative to traditional illicit stimulants and each other, (2) recommend and/or support regulatory status and (3) suggest future research directions.

Historical Overview

The recent waves of designer stimulant use began primarily in the late 1990's to mid-2000's following an increase in government bans on phenylethylamine-based drugs such as methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) (Elliott, 2011). The first major designer stimulants to appear as "legal highs" were piperazine analogues, some of which had been slowly gaining in popularity since they emerged as club drugs in 1970's (Yeap et al., 2010). Marketed primarily for their ability to mimic the subjective effects of amphetamine, MDMA and/or lysergic acid diethylamide (LSD) (Nikolova and Danchev, 2008), these purely synthetic drugs are sold as "party pills", "herbal highs", "legal X", "legal E", or "synthetic stimulants", as well as being sold under multiple brand names (De Boer et al., 2001; Yeap et al., 2010; Elliott, 2011). Desired effects include increases in energy, libido, empathy, euphoria, alertness, and well-being (Nikolova and Danchev, 2008). However, adverse effects can include confusion, agitation, hypertension, tachycardia, hyperthermia, headache, dizziness, anxiety, psychosis, organ toxicity, seizure and death (Nikolova and Danchev, 2008). These drugs are consumed primarily as powders and pills and sold on the internet or in night clubs. Most often these drugs are blends of different piperazines combined with adulterants including caffeine, vitamins, and other binding/cutting agents (Nikolova and Danchev, 2008). These designer piperazines fall under two sub-classes: benzylpiperazines and phenylpiperazines. The two most common *benzyl*piperazines include N-benzylpiperazine (BZP or "A2") and 1-(3,4-methylenedioxybenzyl)piperazine (MDBP),

and the most common *phenyl*piperazines include 1-(3-chlorophenyl)piperazine (mCPP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP or “Molly”), and 1-(4-methoxyphenyl)piperazine (MeOPP) (Arbo et al., 2012; Yeap et al., 2010). In 2004, both BZP and TFMPP were permanently classified as Schedule I controlled substances in the US (DEA, 2012a). However, other piperazine derivatives are still legal to possess and continue to be present in drug markets (Rosenbaum et al., 2012; Yeap et al., 2010).

Starting in the mid-to-late 2000s, another drug class known as synthetic cathinones emerged as stimulants of abuse, largely replacing the banned piperazine derivatives mentioned above. Synthetic cathinones are derivatives of cathinone, the primary naturally occurring amphetamine-like alkaloid found in *Catha edulis* (Khat)(Kalix and Khan, 1984). It was not until 1975 that cathinone was determined to be the main active ingredient of Khat, however the practices of chewing or brewing tea with Khat leaves to extract cathinone dates back centuries in the Horn of Africa and Arabian Peninsula (Manghi et al., 2009). Also, while not a member of the recent synthetic cathinone drug wave, methcathinone (N-methylcathinone, β -keto-methamphetamine, ephedrone) was the first synthetic derivative of cathinone to emerge and was initially developed and used as an antidepressant in Russia in the 1930's. Eventually, methcathinone became a drug of abuse in Russia in the 1970's and in the US in the early 1990's (Kelly, 2011). Both cathinone and methcathinone are Schedule I controlled substances in the US (DEA, 2012a). The most recent waves of synthetic cathinones have been predominantly marketed (and are generally referred to) as “bath salts”, however they are falsely marketed as many other commercial products such as “plant food”, “insect repellent”, “stain removers”, or “research chemicals”. These false products are sold under many different brand names and are usually labeled “not for human consumption” or “for research purposes only” in an attempt to circumvent government controls (Madras, 2012; Spiller et al., 2011). As with the piperazines, synthetic cathinones are intended to be used as “legal high” alternatives to illicit stimulants such as cocaine and amphetamines, the latter of which they share a similar chemical structure (Hillebrand et al., 2010; Vardakou et al., 2011; Prosser and Nelson, 2011; Coppola and Mondola, 2012). Like piperazines, synthetic cathinones are usually found in tablets, capsules, or powders, and can be ingested through swallowing, smoking, injecting, snorting, or rectally administering the drug (Rosenbaum et al., 2012). Desired effects include increases in energy, libido, empathy, euphoria, alertness, openness, and well-being (Prosser and Nelson, 2011). However, adverse effects can include confusion, agitation, sleep deprivation, hypertension, tachycardia, hyperthermia, headache, dizziness, anxiety, appetite suppression, persistent psychosis, aggression, organ toxicity, seizure and death (Spiller et al., 2011; Prosser and Nelson, 2011; Fass et al., 2012; Mas-Morey et al., 2012). Synthetic cathinones have emerged rapidly in drug markets, stemming largely from their widespread sale over the internet and in adult novelty stores, gas stations, tobacco stores, and head shops (NDIC, 2011). In 2011, the US DEA published a call for information for the most common synthetic cathinones found on US drug markets (DEA, 2011b); these included mephedrone (4-methylmethcathinone, 4-MMC), MDPV (3,4-methylenedioxypyrovalerone), methylone (3,4-methylenedioxymethcathinone, MDMC), naphyrone (naphthylpyrovalerone, NRG-1), 4-fluoromethcathinone (4-FMC, flephedrone), 3-fluoromethcathinone (3-FMC), methedrone (4-methoxymethcathinone, bk-PMMA, PMMC), and butylone (bk-MBDB, beta-keto-N-methylbenzodioxolylpropylamine). Of these, MDPV,

mephedrone, and methylone, representing approximately 98% of all synthetic cathinones encountered by law enforcement agencies (DEA, 2011c) and referred to as first-generation “bath salts” (Shanks et al. 2012), were *temporarily* classified as Schedule I controlled substances in 2011 in the US (DEA, 2011a). In 2012, MDPV and mephedrone were *permanently* classified as Schedule I controlled substances in the US (America, 2012), while the temporary Schedule I classification of methylone was extended until April 2013 to allow further information to be gathered (DEA, 2012b). Most recently, the DEA published a new request for information regarding newer “second-generation” synthetic cathinones including 4-methyl-N-ethylcathinone (4-MEC), 4-methyl-pyrrolidinopropiophenone (4-MePPP), alpha-pyrrolidinopentiophenone (α -PVP), 2-(methylamino)-1-phenylpentan-1-one (pentedrone), beta-keto-methylbenzodioxolypentanamine (pentytone), and 4-fluoro-N-methylcathinone (4-FMC, flephedrone) (DEA, 2013).

Before reviewing the behavioural procedures used to assess abuse liability in animals, it is important to briefly discuss scheduling status in the United States. The US DEA designates Schedule I substances as having no accepted medical value and a high potential for abuse or addiction. Schedules II-V on the other hand, designates controlled substances with progressively increased medical value with progressively reduced abuse/addiction risk. The US Department of Health and Human Services utilizes data from animal, human, and epidemiological studies in determining the abuse liability of a particular drug, thus the studies reviewed here should be considered for future regulatory decisions made by the governmental agencies of the US and other countries (O'Connor et al., 2011).

Behavioral Procedures for the Assessment of Abuse Liability

Intravenous Self-Administration

Intravenous drug self-administration (IVSA) is the most widely used behavioral procedure in preclinical addiction research and is arguably the “gold standard” for evaluating the abuse potential of chemical compounds (Panlilio, 2011). The IVSA model has high predictive validity and years of extensive research have shown that in controlled laboratory settings, non-dependent drug-naïve animals will self-administer drugs in patterns similar to those of human drug addicts (Schuster and Thompson, 1969; Yokel and Pickens, 1973; Collins et al., 1983; Gardner, 2010; O'Connor et al., 2011). Generally, drugs that are not typically abused in humans are also not readily self-administered by animals (Griffiths et al., 1979; Collins et al., 1983).

The following overview of IVSA methods is intended to briefly discuss only the most common task parameters used to date to assess abuse liability of the designer stimulants reviewed in this article. Thus, it does not present all the possible variations of the IVSA procedure with regards to apparatus, schedule of reinforcement, subject species, or other task parameters. For more detailed reviews of IVSA procedures, see O'Brien and Gardner, 2005; Sanchis-Segura and Spanagel, 2006; Panlilio and Goldberg, 2007; Howell and Fantegrossi, 2009; Thomsen and Caine, 2011; Panlilio, 2011;. The basic IVSA procedure starts by implanting rats, mice, or non-human primates with indwelling intravenous catheters in either the femoral or jugular veins and attaching the other end to a vascular access port either secured to the head or implanted under the skin on back of the animal (Sanchis-Segura

and Spanagel, 2006; Panlilio, 2011). IVSA sessions are conducted in operant chambers that are equipped with response manipulanda. One common procedure involves an apparatus which contains multiple response manipulanda such that responses on one manipulandum produces drug infusions (i.e., “active” responses) on a pre-determined reinforcement schedule (see below) while responses made on the second manipulandum (i.e., “inactive” responses) produces no programmed consequences. While a multiple manipulanda procedure is common, it is not universal and other procedures such as single manipulanda or chained responses in order to access the active manipulanda have been used (Sanchis-Segura and Spanagel, 2006). Subjects are placed in a testing chamber and connected to drug infusion tubing via a vascular access port. A drug-filled syringe is placed in an infusion pump outside the chamber and is interfaced to a computer which controls drug infusions and stimuli presentations, and records responses. Drug infusions are typically accompanied by discrete cues such as a tone and/or the illumination of a stimulus light. During training subjects can either spontaneously acquire the operant response or it can be acquired using autoshaping, drug priming injections, or food training (Sanchis-Segura and Spanagel, 2006; Carroll and Meisch, 2011).

IVSA studies can be conducted using various schedules of reinforcement; however simple fixed-ratio (FR) and progressive ratio (PR; see below) schedules are the most routinely used (Sanchis-Segura and Spanagel, 2006). The simplest FR schedule is continuous reinforcement (i.e., FR1), where each “active” operant response yields an infusion of the drug while responses on other “inactive” manipulanda, if used, have no programmed consequences. In the IVSA paradigm, in order to assert that a drug functions as a reinforcer, it is necessary and sufficient to establish that its presentation following a response increases the probability of that response in the future (Skinner, 1938). In addition, a number of additional response patterns are also sometimes used to corroborate reinforcing effects of a drug, although their occurrence is not necessary nor sufficient (Meisch, 1987). First, reinforcing effects are inferred if the drug elicits significantly more active responses than inactive responses across multiple experimental sessions. Second, responding maintained by the drug is significantly greater than responding that occurs when saline is substituted as the reinforcer. Third, if a wide range of doses is assessed, drug intake follows a clear dose-dependent pattern that typically (but not always) follows an inverted U-shaped curve (Carter and Griffiths, 2009; Panlilio, 2011). Lastly, if a novel drug substitutes for a known addictive drug such as methamphetamine or cocaine, it is also suggestive of reinforcing effects. Typically, with restricted access (i.e., “short” 1–3 hr daily sessions), stimulant self-administration will reach asymptotic stabilization after which animals will maintain a consistent daily level of drug self-administration (Meisch, 1987).

While most IVSA studies employ restricted access, another increasingly popular manipulation to the standard IVSA is extended access, whereby daily IVSA sessions are extended to 6 hr or more (Ahmed, 2012). Typically, extended access sessions begin after stabilization of responding under restricted access conditions and maintain identical task parameters. The primary dependent measure is escalation of drug-intake across experimental sessions, particularly increased overall drug intake and rate of intake, an effect not typically seen in restricted access sessions. Evidence shows that illicit stimulants that support compulsive use in humans (cocaine, methamphetamine, amphetamine) have all been shown

to lead to escalated intake in extended access, whereas stimulants that typically only support episodic abuse patterns (MDMA) generally do not lead to robust escalated intake (Schenk 2009; Schenk et al., 2012). Evidence also suggests that extended access to some abused drugs can lead to patterns of responding reflective of diagnostic criteria for substance dependence in humans including escalated drug intake, resistance to punishment and negative consequence, impaired inhibitory control over drug intake, preference of the drug over other non-drug reinforcers, and increased motivation to obtain or self-administer the drug (Ahmed and Koob, 1998; Ahmed and Koob, 1999; Koob et al., 2004; Ferrario et al., 2005; Knackstedt and Kalivas, 2007; O'Dell et al., 2007; Ben-Shahar et al., 2008; Gipson and Bardo, 2009; Ahmed, 2010; Ahmed, 2011; Ahmed, 2012).

In addition to basic FR schedules, the other most common schedule used in IVSA paradigms is the PR schedule (Richardson and Roberts, 1996; Stafford et al., 1998) which assesses a drug's "reinforcing efficacy" is determined by the "breakpoint" (Gardner, 2010). While PR schedules can differ between laboratories, the essential feature is an escalating reinforcer cost or behavioral demand; that is, the number of responses required to receive a drug infusion increases following each successive drug infusion. The breakpoint is often the primary dependent measure, and occurs when the animal ceases responding, presumably due to the behavioral demand for obtaining drug exceeding its reinforcing value. PR procedures are typically employed after stable FR responding and can provide additional information beyond basic FR schedules such as the differential hierarchical reinforcing efficacy between different drugs, doses of the same drug, or the effects of other manipulations on motivation (Stafford et al., 1998).

Intracranial Self-Stimulation (ICSS)

Intracranial self-stimulation is a behavioral task in which an organism performs an operant (wheel turn, nose poke, or lever press) in order to receive a short pulse of reinforcing electrical current directly into a specific brain region through a chronically implanted electrode (Olds and Milner, 1954; Olds et al., 1962). Here, rats quickly learn to respond for electrical stimulation directly into brain regions such as the medial forebrain bundle, although other regions such as the ventral tegmental area and lateral septum have also been used (Redgrave et al., 1981; Vlachou and Markou, 2011). In ICSS procedures, the implanted electrode is tethered to a stimulation wire that is connected to an electrical stimulation box and interfaced with a computer that applies stimulation in accordance with the selected ICSS procedure. Across different protocols, stimulation parameters can vary in their intensity of the current delivered (μA), duration of pulses (ms), frequency of pulse as a function of pulse/interpulse interval, and waveform of pulses (Vlachou and Markou, 2011). While various ICSS procedures have been developed (Vlachou and Markou, 2011), the two most extensively used in evaluating abuse liability are the rate-frequency curve-shift procedure (Carlezon and Chartoff, 2007) and the discrete-trial current threshold intensity procedure (Kornetsky and Esposito, 1979; Markou and Koob, 1992).

In the curve-shift procedure, the electrical stimulation frequency (Hz) is varied while the current intensity (μA), pulse width (duration of stimulation), and train duration parameters are kept constant. Once placed in the operant chamber, animals are tested in a series of short

(1 min) trials, with the stimulant intensity varied across trials. During these trials, the animal receives five non-contingent (free) priming stimulations consisting of the stimulation frequency for the current trial. Following stimulation primes, the animal is given 50 – 60 sec to respond for the identical stimulation train. After the responding phase, a time-out period is introduced in which no stimulation is available. During the time-out period, the pulse frequency is lowered, typically by a log unit of 0.05–0.1, for the subsequent short trial. Thus, the short trials are conducted across a series of ascending and descending trials in which pulse frequency is systematically altered across trials (Carlezon and Chartoff, 2007; Vlachou and Markou, 2011). Based upon these trials, responding is averaged to generate a sigmoidal response rate-frequency threshold. This procedure is conducted daily until responding stabilizes to preset conditions. While thresholds can be calculated based upon the frequency, intensity, pulse width, or train duration, the most widely used measures are the “half-maximum” (M50) or the Theta-O (T_0) (Miliaressis et al., 1986). The M50 measure is defined as the minimum stimulation frequency necessary to maintain responding at 50% the maximum rate. In the T_0 measure, thresholds are an estimate of the theoretical frequency at which stimulation becomes rewarding (responding > zero) to the animal (Carlezon and Chartoff, 2007).

In the discrete-trials current threshold procedure (Kornetsky et al., 1979; Markou and Koob, 1992; Lin et al., 1999; Vlachou and Markou, 2011), following basic response acquisition, discrete-trial training begins where trials start with a free stimulation, followed by an inter-trial-interval (ITI) where responses yield no programmed consequences. After the ITI, responding yields stimulation identical to the previously received free stimulation. Once animals learn to inhibit responding during the ITI periods, baseline threshold training begins. Threshold procedures begin at a preset level of current intensity (i.e., 120 μ A). Trials are conducted in blocks (3 to 5 trials) and current intensity remains the same for the entire block. Following a free stimulation and subsequent ITI, animals can respond to receive an identical stimulation train as the free stimulation that began the trial. Responding on the majority of trials lowers the current intensity (e.g., 5 μ A) for the next block of trials whereas not responding on most trials increases the current intensity (e.g., 5 μ A) for the next trial block. This titrating stimulation pattern goes through a series of 2 ascending and 2 descending current intensities. Across the 2 ascending and descending series, thresholds are calculated as the mean of μ A between blocks that support or do not support responding. Following stability, drugs are administered prior to ICSS sessions and relative reductions from baseline thresholds are indicative of hedonic rewarding effects of the drug, whereas elevations are indicative of aversive effects. ICSS procedures have been used since the 1950's and have revealed that drugs which support compulsive abuse in humans, especially stimulants, all lower ICSS thresholds (Vlachou and Markou, 2011).

Conditioned Place Preference (CPP)

Conditioned place preference is a widely used method of assessing conditioned drug effects. Historically the CPP paradigm has been used as a measure of drug “reward” (i.e., positive hedonic value) (Tzschentke, 1998; Bardo and Bevins, 2000; Tzschentke, 2007; Cunningham et al., 2011; Huston et al., 2013). However, Huston et al. (2013) recently challenged this notion and asserted that CPP assesses incentive-driven behavior related to secondary

reinforcers, operant conditioning of behavior prevailing at the conditioning site, conditioned treatment effects, or combinations of these processes. Regardless, it can be generally asserted that CPP is a measure of drug conditioning, and in the context of designer stimulants we assert that it is a measure of the appetitive conditioning effects of these drugs. In the CPP paradigm, a drug (unconditioned stimulus) is repeatedly paired with distinct environmental and contextual stimuli which, through Pavlovian conditioning processes, acquire secondary rewarding (i.e., act as a conditioned stimuli, or CS+) effects (Tzschentke, 1998). CPP procedures generally have three phases; pre-test baseline, conditioning, and test. During the pre-test phase, subjects are allowed to freely roam among the two distinct environmental contexts (compartments) and allowed to habituate to the novel CPP apparatus. Olfactory, visual and tactile stimuli typically differ across the two environmental contexts and preference for either context is measured by the time spent in each context. Most CPP studies use two- or three-compartment apparatuses (Tzschentke, 1998). Two-compartment apparatuses contain equally sized conditioning compartments separated by wall (during conditioning) that is removed during pre-test and test phases to allow movement across compartments. If a third “start-box” compartment is used, it is typically smaller than and situated between the two conditioning chambers. For a discussion on the advantages and disadvantages of two- and three-compartment apparatuses see Tzschentke (1998). When untrained animals show a strong preference (e.g., significantly greater than 50%) for one context during pre-test the apparatus is considered “biased”. Alternatively, an “unbiased” apparatus, which is generally preferred, is one in which no significant group preference for one context over the other exists during pre-test. The biased-unbiased distinction is also used to assign drug-context pairing (Tzschentke, 2007). An unbiased design is one in which subjects are randomly assigned to have the drug paired with one compartment or the other during conditioning without consideration of individual pre-test preferences (Tzschentke, 2007). In a biased design the drug is either paired with the initially preferred or non-preferred compartment during conditioning (Shippenberg and Koob, 2002).

During conditioning, the drug of interest (unconditioned stimulus) is repeatedly paired with one context (CS+) (typically the non-preferred context). Subjects also receive equal exposure to the other context (CS-) without the unconditioned stimulus. In the test phase, subjects are allowed free access to both contexts in the absence of the drug and a CPP occurs when the animal spends more time in the drug-paired context relative to pre-test baseline or saline-paired context. The basic procedure is generally the same across most CPP studies, however, there is substantial variability in a number of methodological variables (Tzschentke, 1998; Tzschentke, 2007; Cunningham et al., 2011).

Drug Discrimination

Drug discrimination procedures provide a useful tool in assessing the abuse liability of novel compounds by assessing the relative similarity of interoceptive cues produced by different substances (Ator and Griffiths, 2003). Drug discrimination procedures typically use the two-lever choice paradigm where subjects are differentially reinforced for responding on one lever (i.e., the left lever) following treatment of a training drug while responses on second lever (i.e., the right lever) are not. During training, drug treatment sessions alternate with vehicle treatment sessions during which responses made to the second (i.e., right) lever are

reinforced, but responses on the other (i.e., drug-paired) lever are not (Ator and Griffiths, 2003). Correct responses made during drug and vehicle sessions are typically reinforced on a fixed ratio schedule, though other schedules have also been used (Colpaert et al., 1976; Young, 2009). Drug discrimination performance relies on the subject's ability to distinguish between the interoceptive cues produced by the training drug versus vehicle (Shippenberg and Koob, 2002; Young, 2009). Thus, interoceptive cues serve as discriminative stimuli that guide reinforced responding. The percent of responding on the drug-paired lever is the primary dependent measure. The number of responses on the drug lever (i.e., drug-appropriate responding) is divided by the total number of responses made on both levers and multiplied by 100 to yield the percent of drug lever responses (Young, 2009). A stability criterion of 80 % drug-lever responding following training drug injections and 20% drug-lever responding following vehicle injections is often used prior to the onset of generalization testing (Young, 2009).

Stimulus generalization tests are conducted to determine if the discriminative stimulus properties of the test drug substitutes for the drug used during training (i.e. the training drug generalizes to the test drug). These test sessions are generally interspersed among training sessions and reinforcement is provided on both levers according to the same schedule used during training. The distribution of responses across both levers is recorded and the percentage of (training) drug-appropriate responses is calculated as an index of the degree of generalization (Shippenberg and Koob, 2002; Young, 2009). Complete stimulus generalization is said to occur when most of the responses (typically 80%) are made on the drug-appropriate lever. Partial generalization is said to have occurred if subjects produce 40–70% drug-appropriate responses. Additional dependent measures obtained from test sessions are the number of animals that predominantly made drug-appropriate responses and the response rate (Young, 2009).

Neurochemical Actions and Abuse Liability of Designer Stimulants

Before reviewing the potential abuse liability literature of novel designer stimulants, it is first important to define a few terms. Throughout the text, the terms “compulsive use” and “addiction” are based on accepted DSM-IV criteria for substance dependence that include the emergence of withdrawal symptoms upon discontinuation of drug use, substance use taken over a longer period than intended, increased amount of time spent in activities necessary to obtain/use/recover from the substance, and social/occupational/recreational activities are given up or reduced because of substance use. We believe that these criteria are more akin to compulsive and chronic use as opposed to “episodic” use. Furthermore, “episodic” use we believe is more characteristic of substance abuse, as defined in the DSM-IV, as opposed to substance dependence.

Both piperazine- and cathinone-based designer stimulants are designed to mimic the effects of traditional illicit stimulants such as cocaine or amphetamines. Because of this, comparison of their neurochemical and behavioral effects provides a logical starting point for evaluating relative abuse potential. Not surprisingly, like traditional illicit stimulants, designer stimulants primarily exert their behavioral and psychological effects by dynamically modulating monoamine transmission in the brain (Cozzi et al., 1999; Baumann

et al., 2002; Baumann et al., 2004; Baumann et al., 2005; Nagai et al., 2007; Baumann et al., 2011; Cozzi et al., 2013; Cameron et al., 2013; Simmler et al., 2013). Generally, the relative abuse liability of amphetamine-type stimulants is highly correlated with the balance between increases in synaptic dopamine (DA) and serotonin (5-HT) induced by the drug (Rothman and Baumann, 2003; Wee et al., 2005; Rothman and Baumann, 2006). That is, 5-HTergic drugs are generally more hallucinogenic and produce episodic patterns of abuse (e.g., MDMA), whereas primarily DAergic drugs support more compulsive abuse patterns (e.g. cocaine, amphetamine, and methamphetamine). Thus, drugs such as methamphetamine, amphetamine, and cocaine are considered highly addictive substances, whereas MDMA-like drugs are generally not considered addictive as consumption patterns are primarily episodic. However, while rare, researchers have shown that MDMA dependence is possible in subsets of users (Jansen, 1999; De La Garza et al., 2007). Thus, MDMA-like drugs appear to possess a relatively lower abuse liability compared to other illicit stimulants, and the relative abuse liability of designer stimulants are likely to fall somewhere along this continuum. Given this understanding, it is important to discuss both the pharmacodynamic and behavioral effects of designer stimulants together for a more complete understanding of abuse liability.

Piperazines

BZP and TFMPP

The two most studied piperazines are BZP and TFMPP. TFMPP acts both presynaptically and as a non-selective 5-HT receptor agonist (Schoeffer and Hoyer, 1989; Nikolova and Danchev, 2008). In vitro data has revealed that TFMPP acts as a substrate releaser at the serotonin transporter (SERT) (Baumann et al., 2004; Baumann et al., 2005). Consistent with this data, previous work in diencephalon brain tissue (Auerbach et al., 1990) showed that TFMPP induced the release of 5-HT only, but was approximately three-fold less potent compared to MDMA and much less efficacious overall as higher doses were unable to match maximum MDMA induced 5-HT release (Baumann et al., 2005). These results extended previous work also revealing TFMPP induced endogenous release of 5-HT from brain tissue (Pettibone and Williams, 1984; Auerbach et al., 1990).

For BZP, in vitro studies have revealed that BZP acts as substrate releaser at the dopamine transporter (DAT) (Baumann et al., 2004; Baumann et al., 2005). However, the in vitro release of DA was less potent compared to methamphetamine. Interestingly, in vivo microdialysis data revealed BZP induced elevations in extracellular DA in the nucleus accumbens, but also 5-HT despite the lack of in vitro 5-HT release. Similar to methamphetamine, effects of BZP on DA were predominant (Baumann et al., 2005).

Because BZP and TFMPP are often co-administered together, typically in a BZP:TFMPP ratio ranging from 1:1 to 3:1 (Nikolova and Danchev, 2008), their combination has also been assessed with microdialysis. At a low dose (3 mg/kg, i.v.), BZP/TFMPP increased extracellular DA and 5-HT in the nucleus accumbens in a qualitatively and quantitatively similar manner to low dose MDMA (1 mg/kg, i.v.). However, at a high-dose (10 mg/kg, i.v.) increased extracellular DA was even greater than the sum total when BZP and TFMPP were assessed alone, suggesting that the combination of these drugs increases dialysate DA in a

synergistic manner (Baumann et al., 2005). These pharmacodynamic results corroborate reports (Nikolova and Danchev, 2008; Lin et al., 2009; Lin et al., 2011) that BZP alone possesses subjective effects more similar to amphetamine whereas the BZP/TFMPP combination has effects more closely resembling MDMA (Baumann et al., 2002; Baumann et al., 2004; Baumann et al., 2005). Furthermore, these pharmacodynamic data would suggest that BZP would possess a relatively higher risk of addiction than TFMPP or a BZP:TFMPP combination.

BZP and TFMPP (doses of 0.01, 0.03, 0.1, and 1 mg/kg/infusion for each drug) were tested for their ability to maintain intravenous self-administration on an FR10 reinforcement schedule in rhesus monkeys trained to self-administer cocaine (0.01 mg/kg/infusion) (Fantegrossi et al., 2005). BZP maintained robust self-administration above vehicle at the three highest doses. Furthermore, responding maintained at the 0.1 and 1 mg/kg doses were similar to or higher than baseline cocaine sessions for all subjects, and response rates remained elevated for several hours when saline was substituted for BZP. In contrast to BZP, TFMPP did not maintain self-administration above vehicle at any of the doses tested. When combining BZP (0.01 or 0.03 mg/kg/infusion) and TFMPP (0.01, 0.03, or 0.1 mg/kg/infusion), self-administration rates decreased in a TFMPP dose-dependent manner and were lower compared to BZP alone, suggesting that TFMPP attenuated the reinforcing effects of BZP. In a separate study using rats (Brennan et al., 2007), separate groups were initially trained to intravenously self-administer either cocaine or BZP in 2-hr daily access sessions and an FR1 schedule. In cocaine trained rats (0.5 mg/kg/infusion), BZP at doses of 0.125, 0.25 and 0.5 mg/kg/infusion maintained and increased self-administration, but responding extinguished when saline was substituted. Furthermore, in drug-naïve animals, rats learned to discriminate active vs. inactive levers by day 10 of BZP self-administration with a starting dose of 0.5 mg/kg/infusion and responding was maintained when doses as low as 0.125 were substituted. Together, the results of these studies reveal that BZP, but not TFMPP, can both maintain self-administration following cocaine self-administration and elicit self-administration without prior training (spontaneous acquisition).

In a counterbalanced, biased design CPP procedure, BZP has also been shown to induce CPP in rats at doses of 1.25, 5, and 20 mg/kg (i.p.) (Meririnne et al., 2006). This effect was reversed by the DA D1 receptor antagonist SCH23390 and 5-HT3 receptor antagonist MDL72222, but not the D2 antagonist raclopride, indicating that BZP reward is partially mediated by dopaminergic and serotonergic mechanisms similar to cocaine, but not amphetamines where CPP can be blocked by both D1 and D2 antagonism (Tzschentke, 1998).

In a drug discrimination study using adult rhesus monkeys (Fantegrossi et al., 2005), BZP doses (1, 3, 10, 17, and 30 mg/kg i.g.) produced dose-dependent increases in responding on a lever previously associated with amphetamine (1 mg/kg). Full substitution for amphetamine was reached for 2 male monkeys at a dose of 17 mg/kg and at a 30 mg/kg BZP dose for 1 female monkey (Fantegrossi et al., 2005). The full substitution of BZP for amphetamine indicated that these two drugs possess similar interoceptive effects, suggesting a similar level of abuse liability. However, in this same study, TFMPP did not elicit amphetamine-like responding at any dose tested (1, 3, 10, 17, and 30 mg/kg i.g.), likely

reflecting its primary effects on serotonin systems. Together, both the IVSA and drug-discrimination data show that BZP possesses stimulant-like effects that resemble amphetamine and reinforcing effects similar to cocaine. However, TFMPP did not substitute for amphetamine, was not readily self-administered, and decreased cocaine and BZP self-administration, suggesting the TFMPP possesses a relatively lower abuse liability compared to BZP, amphetamine, and cocaine.

In a separate drug-discrimination study, mice were initially trained to discriminate either S(+)-MDMA or R(-)-MDMA (1.5 mg/kg, i.p.) from saline (Yarosh et al., 2007). Following stable responding, BZP, TFMPP and mCPP were tested and all dose-dependently and fully substituted for the stimulant-like S(+)-enantiomer of MDMA at doses of 30 mg/kg, 10 mg/kg, and 3 mg/kg, respectively. Interestingly, none of these drugs substituted for the hallucinogenic R(-)-enantiomer of MDMA and total suppression of responding occurred BZP doses of 30 mg/kg, TFMPP doses of 3 mg/kg, and mCPP doses of 3 mg/kg.

mCPP, meOPP, and MDPB

In addition to its actions as a 5-HT receptor agonist, *in vitro* assays have revealed that mCPP blocks 5-HT reuptake (Pettibone and Williams, 1984; Wolf and Kuhn, 1991). *In vivo* microdialysis studies have shown that mCPP increases extracellular 5-HT in the diencephalon and hippocampus of rats via reversal of SERT, as 5-HT uptake blockers such as fluoxetine and citalopram both attenuate these effects (Baumann et al., 1993; Eriksson et al., 1999). mCPP also increases extracellular DA, but to a lesser extent than 5-HT (Eriksson et al., 1999). Thus, mCPP is somewhat similar to MDMA; however it does not induce 5-HT vesicular release like MDMA. Thus, mCPP only increases cytoplasmic 5-HT (Baumann and Ayestas, 2001) and does not lead to long-term 5-HT depletion typically seen with MDMA. Less is known about the pharmacodynamic effects of meOPP, however it has relatively high monoamine reuptake and releasing activity in rat brain synaptosomes (Nagai et al., 2007). It is typically used alone or in a 1:1 ratio with BZP and user reports reveal subjective effects of meOPP alone or when combined with BZP are similar to MDMA (Tancer and Johanson, 2001; Nikolova and Danchev, 2008). Compared to BZP and TFMPP, relatively less pharmacodynamic and behavioral data exists regarding the piperazine derivatives MeOPP, and MDPB. To our knowledge, there have been no published reports that mCPP, meOPP, or MDPB are readily self-administered, elicit CPP, or reduce ICSS thresholds.

Synthetic Cathinones

Mephedrone and methylone

Two of the most studied synthetic cathinones to date are mephedrone and methylone. For both mephedrone and methylone, *in vitro* studies initially showed activity at monoamine transporters. However, because traditional uptake assays are unable to discriminate between blockers and releasers, the precise mechanism was unknown (Cozzi et al., 1999; Hadlock et al., 2011; López-Arnau et al., 2012; Martínez-Clemente et al., 2012; Simmler et al., 2013). Recently it has been shown that mephedrone and methylone, like amphetamines, are monoamine substrate releasers (Baumann et al., 2011; Baumann et al., 2013b). *In vitro* release data revealed preferential affinity for SERT, with the DAT:SERT ratios of

mephedrone (2.41) and methylone (1.82) being more similar to MDMA (0.97) than methamphetamine (152.0). Using human DAT (hDAT) expressed in oocytes, mephedrone produces an inward current similar to methamphetamine, corroborating its effect as a DAT substrate releaser (Cameron et al., 2013). Furthermore, studies have also revealed that both mephedrone and methylone increase extracellular DA and 5-HT in the nucleus accumbens, with preferential effects on 5-HT similar to MDMA (Kehr et al., 2011; Baumann et al., 2011). Both methylone and mephedrone increase extracellular DA and 5-HT in a qualitatively similar manner to MDMA, however, methylone is slightly less potent than MDMA and about half as potent as mephedrone (Baumann et al., 2011). Furthermore, MDMA is approximately twice as potent as mephedrone in terms of its ability to increase 5-HT release (Kehr et al., 2011). Together, these results suggest that methylone and mephedrone are likely to have behavioral effects more similar to MDMA than to amphetamine and methamphetamine.

Mephedrone

To date, two published studies have employed the IVSA paradigm to study the abuse liability of mephedrone (Hadlock et al., 2011; Aarde et al., 2013). In the Hadlock et al. study, mephedrone was self-administered (0.24 mg per 10 μ l infusion) significantly more than saline in rats when placed in daily 4-hour access sessions for eight days. Furthermore, compared to the same dose of methamphetamine, mephedrone elicited greater responding. While this finding appears to suggest that mephedrone is more reinforcing than methamphetamine, comparisons of reinforcing effects are difficult to interpret without a wider dose range. For instance, this dose of methamphetamine is towards the upper end of the inverted U-shaped dose range typically found in IVSA studies. This dose of mephedrone, however, may reside towards the lower end of this curve and thus requires higher response rates for a similar level of reinforcement. While this study was first to establish that mephedrone functioned as a reinforcer, it did not establish clear dose-effect curves in order to determine the relative reinforcing effects of mephedrone compared to other known addictive stimulants. More recently, it has been shown that mephedrone dose-dependently supports IVSA at 0.5 and 1 mg/kg/infusion doses in 1 hour daily access sessions in both Wistar and Sprague-Dawley rats (Aarde et al., 2013). Furthermore, the mephedrone dose of 0.5 mg/kg/infusion produced similar responding to methamphetamine at a 0.05 mg/kg/infusion dose, showing that mephedrone is less potent than methamphetamine.

To our knowledge, only one published study has employed an ICSS model to analyze the rewarding effects mephedrone (Robinson et al., 2012). Using male C57BL/6J mice, mephedrone and cocaine were compared using the curve-shift ICSS method. The results revealed that both mephedrone and cocaine (matched doses of 1, 3, and 10 mg/kg, i.p.) produced similar dose-dependent leftward shifts in rate-frequency curves and were very similar in their effects for M50 (half-maximal responding) and brain stimulation reward thresholds. However, during the initial 15 minutes following injections, only the 10 mg/kg cocaine dose caused a leftward shift in rate-frequency and lowered the rate-frequency supported half-maximal responding. Relative to vehicle, mephedrone (10 mg/kg) produced leftward shift in rate-frequency curves similar to cocaine (10 mg/kg), but only after the first 15 minutes following injections. During the 16–30 minute time frame, mephedrone and

cocaine (at matched doses of 3 and 10 mg/kg) caused similar significant reductions for M50. It is well known that drug properties such as liquid solubility, route of administration, first-pass metabolism, and other factors can affect the rate of drug absorption and bioavailability and that these effects are positively correlated with increased abuse liability (Farre and Cami, 1991). Thus, while these results indicate that mephedrone and cocaine possess a similar capacity to engage reward systems, the delayed effects of mephedrone vs. cocaine likely reflects differential pharmacokinetics and suggests a relatively lower abuse liability compared to cocaine.

Using CPP procedures, researchers have shown that mephedrone possesses appetitive conditioning effects (Lisek et al., 2012). Rats receiving either 3, 10 or 30 mg/kg (i.p.) in 4 conditioning trials doses and mice receiving 30 mg/kg (i.p.) in 6 conditioning trials were tested. Both rats and mice displayed CPP for all doses tested. These effects were similar to previously reported CPP for cocaine, amphetamine, and methamphetamine under similar conditions (Tzschentke, 1998; Leri and Franklin, 2000). However, as the researchers discuss, these doses are much higher than those that elicit CPP for cocaine and amphetamines (Tzschentke, 1998; Cunningham and Noble, 2006; Sanchis-Segura and Spanagel, 2006).

In a recently published drug discrimination study, rats were initially trained to discriminate between mephedrone (3.2 mg/kg, i.p.) and saline on a FR20 schedule to a criterion of 90% drug-appropriate responding (Varner et al., 2013). Following training, test sessions with varying doses of mephedrone, cocaine, MDMA, methamphetamine, fenfluramine, morphine, and PCP were conducted. Of the psychostimulants tested, all produced dose-dependent mephedrone responding. However, only mephedrone and MDMA (both 3.2 mg/kg) produced full substitution for mephedrone. Both cocaine (18 mg/kg) and methamphetamine (1 mg/kg) produced 76% and 73% mephedrone-responding, respectively, but did not count as full substitution (80% mephedrone responding). Of the other non-stimulant drugs, only fenfluramine (1–3.2 mg/kg) produced roughly 50% mephedrone-responding while neither morphine nor PCP produced greater than 50% mephedrone-responding. These results suggest that mephedrone produces interoceptive effects most resembling MDMA, but with some similarity to cocaine and methamphetamine, corroborating user reports of similar subjective effects (Motbey et al., 2012).

Methylone

Thus far, only one study has employed the IVSA paradigm to assess abuse liability of methylone (Watterson et al., 2012). In this study, rats were allowed to spontaneously acquire methylone at doses of 0.05, 0.1, 0.2 and 0.5 mg/kg in 2 hour daily access sessions for 21 days. Methylone functioned as a reinforcer as animals in each of the dose groups, as animals successfully learned to discriminate between active and inactive levers. Responding during PR sessions revealed a positive relationship between methylone dose and breakpoints observed. Furthermore, responding for methylone during PR schedules led to higher breakpoints than those previously reported for MDMA (Schenk et al., 2007), suggesting that methylone has greater reinforcer efficacy than MDMA. However, when these rats were placed in daily extended access procedures (6 hr/day) for 10 days, none of the dose groups displayed escalated drug intake across sessions. As discussed by the authors, this is in

contrast to escalation typically found in illicit stimulants such as cocaine and methamphetamine. Together, this study revealed more robust self-administration compared to published MDMA studies, but the lack of escalation suggests a relatively lower potential for high level use compared to cocaine and methamphetamine (Watterson et al., 2012).

In this same study, using the discrete-trial current threshold ICSS procedure, methylone was unable to significantly lower ICSS thresholds, although a trend ($p < 0.09$) was observed. However, as the authors discuss, the most robust threshold reduction (13% below baseline vehicle) occurred for the 10 mg/kg dose which was smaller than the lowest dose of MDPV (16%) under identical procedures (Watterson et al., 2013). Furthermore, these ICSS threshold reductions were similar, but less robust than those previously reported for MDMA (Hubner et al., 1988; Lin et al., 1997). Thus, while the self-administration data suggests more robust reinforcing effects of methylone, the ability of methylone to affect brain stimulation reward were similar when compared to MDMA.

Other studies have revealed that methylone possesses appetitive conditioned effects in CPP procedures (Miyazawa et al., 2011). Using a counterbalanced, biased design, mice received injections of 1, 2.5, and 5 mg/kg (i.p.) for all conditioning trials. The results revealed dose-dependent CPP effects, with the 2.5 and 5 mg/kg doses producing significantly greater effects relative to vehicle. In comparison, MDMA elicits CPP at doses of 9 – 10 mg/kg in mice (Salzmann et al., 2003; Robledo et al., 2004), and in rats at doses as low as 1.5 mg/kg (Bilsky et al., 1990; Bilsky et al., 1991; Bilsky and Reid, 1991; Schechter, 1991). Together, these data suggest that methylone has more potent conditioning effects than MDMA.

In a drug discrimination study investigating several cathinone analogues (Dal Cason et al., 1997), rats were initially trained to discriminate amphetamine, MDMA, or the serotonergic hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from vehicle. Methylone dose-dependently and fully substituted for amphetamine at the 3 mg/kg (i.p.) dose. In DOM-trained rats (1 mg/kg i.p.) methylone did not elicit DOM-like responding at any dose tested (1, 1.5, or 2 mg/kg, i.p.). However, in MDMA-trained (1.5 mg/kg i.p.) animals, methylone did dose-dependently elicit MDMA-like responding, reaching full substitution at the 2 mg/kg i.p. dose. Together, these effects corroborate the results from other in vitro and in vivo studies showing that methylone possesses effects similar to MDMA and amphetamine.

MDPV

In contrast to mephedrone and methylone, in vitro data has revealed that MDPV is a selective catecholamine transporter inhibitor, similar to its parent drug pyrovalerone and cocaine (Meltzer et al., 2006), and is approximately 50 times more potent than cocaine at DAT and 10 times more potent at NET (Baumann et al., 2013a; Simmler et al., 2013). MDPV has very weak effects at SERT, and when compared to other illicit stimulants such as cocaine, amphetamine, and methamphetamine, is significantly more potent in its inhibition of DAT. Furthermore, in vivo microdialysis data has revealed that MDPV-induced elevations in extracellular DA in the nucleus accumbens are at least 10 times higher than those observed after cocaine. Furthermore, while dialysate DA returned to baseline approximately

40 minutes after cocaine injections, DA remained elevated after MDPV for greater than 60 min (Baumann et al., 2013a). In a separate study using hDAT expressed in oocytes, MDPV induced an outward current similar to, but much more potent and longer-lasting than cocaine, corroborating its effects as a DAT blocker (Cameron et al., 2013). Together these data suggest that behavioral effects of MDPV are likely to be similar to highly addictive stimulants such as cocaine and methamphetamine and similarly have an extremely high potential for abuse/addiction.

To date, two published studies have used the IVSA paradigm to assess MDPV (Watterson et al., 2013; Aarde et al., 2013). In the first, rats were initially allowed to intravenously self-administer MDPV at doses of 0.05, 0.1, and 0.2 mg/kg/infusion in 2-hour daily access sessions (FR1) for 10 days (Watterson et al., 2013). Next, rats were placed into PR tests to assess dose-dependent effects of reinforcing efficacy. Following PR tests, dose-groups were split such that half the animals were placed in 6-hr long-access (LgA) sessions while the other half remained in 2-hr short access (ShA) sessions for 10 additional days. This study also employed a methamphetamine positive control in the PR and LgA tests (0.05 mg/kg/infusion) to assess for abuse potential relative to a known addictive stimulant. The results of this study revealed that rats successfully discriminated between active and inactive levers at each dose by day 5 of IVSA procedures, indicating that MDPV is a reinforcer. Furthermore, a dose-dependent relationship was revealed for both active lever presses and infusions and were very similar to previously published methamphetamine effects under identical conditions (Gass et al., 2008). The PR tests also revealed dose-dependent differences in reinforcer efficacy as revealed by PR breakpoints and at the 0.05 mg/kg/infusion dose, breakpoints were nearly identical to the methamphetamine positive control (0.05 mg/kg/infusion). Furthermore, breakpoints at the 0.1 and 0.2 mg/kg/infusion doses were also similar to previously published results for methamphetamine and d-amphetamine under identical procedures (Richardson and Roberts, 1996; Gass et al., 2008). Finally, under extended access procedures, both the 0.1 and 0.2 mg/kg/infusion doses lead to escalated MDPV intake across experimental sessions, similar to previously published results for methamphetamine and cocaine (Ahmed and Koob, 1998; Ahmed, 2012).

In the IVSA experiments by Aarde et al. (2013), across ten 1 hr IVSA acquisition sessions conducted with an FR5 schedule of reinforcement, male Wistar rats robustly self-administered both MDPV (0.05 mg/kg/infusion) and methamphetamine (0.05 mg/kg/infusion), with MDPV supporting a higher infusion rate compared to methamphetamine. Following this acquisition phase, in order to assess dose-response curves, doses of 0.01, 0.05, 0.1, and 0.5 mg/kg/infusion of MDPV and methamphetamine were then substituted in a block-randomized design with 4 sessions per block with each block assessing one of the four doses. For both MDPV and methamphetamine, orderly dose-response rates were observed and increases in the per-infusion dose corresponded with increases in post-reinforcement pauses and decreases in infusion rate. Following FR5 dose-response testing, rats were also assessed for dose-response testing in PR sessions utilizing the same doses block-randomized design. In PR tests, results revealed that increases in the per-infusion dose lead to increases in the percentage of responses on the drug-paired lever as well as increases in the post-reinforcement pause. Under both PR and FR dose-response tests, responding for MDPV was greater than that for methamphetamine at matched doses, suggesting the MDPV

has greater reinforcing effects than methamphetamine. These findings are in contrast with Watterson et al., (2013) who showed similar responding for MDPV and methamphetamine under PR tests and during extended access sessions on an FR1 schedule. Thus, these results suggest that MDPV possesses greater reinforcing effects and possibly higher abuse liability compared to methamphetamine.

MDPV has also been studied for its threshold-lowering effects in a discrete-trials current threshold ICSS procedure (Watterson et al., 2013). At all doses tested (0.1, 0.5, 1 and 2 mg/kg, i.p.), MDPV produced significant dose-dependent reductions in ICSS thresholds relative to vehicle. While this study did not directly compare these results to other prototypic stimulants, the results match closely with discrete-trials current threshold response patterns previously published for methamphetamine (Vlachou and Markou, 2011). These results indicate that, in addition to possessing reinforcing effects, MDPV engages the mesolimbic reward circuitry and possesses hedonic/rewarding effects similar to known addictive stimulants.

In a recent drug discrimination study (Fantegrossi et al., 2013), mice were initially trained to discriminate MDPV (0.3 mg/kg, i.p.) from saline in daily 30 minute FR10 sessions. Following stable baseline responding, substitution tests revealed that MDPV, methamphetamine, and MDMA all dose-dependently and fully generalized to the MDPV training dose and that the ED₅₀ values for MDPV and methamphetamine were nearly identical. Unexpectedly, the ED₅₀ for MDMA was similar to methamphetamine and MDPV. This finding suggests similar interoceptive effects between these three drugs. This finding is surprising given that both MDPV and methamphetamine, compared to MDMA, possess more robust reinforcing (i.e., more robust self-administration) and hedonic effects (i.e., more robust ICSS threshold reductions) (Watterson et al., 2013). Furthermore, MDPV is a cocaine-like DAT inhibitor with little activity at SERT, whereas MDMA is a potent 5-HT substrate releaser. However, as the authors discuss, methamphetamine (5-HT substrate releaser) and cocaine (DAT inhibitor) have been shown to substitute for one another, as do cocaine and MDMA. Together, the results of this study suggest that MDPV possess similar interoceptive effects as methamphetamine and MDMA in mice and therefore likely has similar subjective effects in humans.

To our knowledge, there are currently no published studies showing that MDPV can elicit a CPP. However, given its robust effects in IVSA, ICSS, and drug-discrimination, it is likely that MDPV would possess appetitive conditioned effects.

Conclusions, Implications for Drug Policy, and Future Directions

While users report many reasons for using designer stimulants, perceived safety of these drugs relative to illicit drugs is often reported (Ramsey et al., 2010; Yeap et al., 2010; Rosenbaum et al., 2012; Arbo et al., 2012). However, in addition to the numerous reports of toxicity and death (Spiller et al., 2011; Rosenbaum et al., 2012), animal abuse liability studies also suggest that designer stimulants can possess a similar potential for abuse and addiction compared to the illicit stimulants they are designed to mimic.

For the piperazines, BZP is readily self-administered by both monkeys and rats, and yields response patterns similar to that of cocaine (Fantegrossi et al., 2005; Brennan et al., 2007). Furthermore, BZP substitutes for amphetamine and (S+)-MDMA in drug-discrimination tests (Yarosh et al., 2007). On the other hand, TFMPP does not support self-administration or substitute for amphetamine (Fantegrossi et al., 2005), but does substitute for (S+)-MDMA (Yarosh et al., 2007). Together, BZP studies indicate stimulant effects similar to cocaine and amphetamines, whereas TFMPP more closely resembles MDMA. Thus, BZP likely poses a significant risk for compulsive use, whereas TFMPP likely does not. Relatively less is known of the other piperazine-based stimulants mCPP, meOPP, MBDB and more abuse liability research is needed before definitive predictions about these drugs can be made. However, the in vitro and in vivo studies would suggest that both mCPP and meOPP would likely resemble MDMA in their behavioral effects (Nagai et al., 2007).

For the synthetic cathinones, MDPV is robustly self-administered, significantly decreases ICSS levels (Watterson et al., 2013; Aarde et al., 2013), and both methamphetamine and MDMA fully substitute for MDPV in drug discrimination tests (Fantegrossi et al., 2013). In vitro and in vivo studies reveal that MDPV acts similarly to cocaine as a DAT blocker, but is approximately 10–50 times more potent and leads to longer lasting elevations in extracellular DA (Baumann et al., 2013a; Cameron et al., 2013). While first glance of these results might suggest a greater abuse liability of MDPV compared to cocaine, evidence also shows that drugs with a relatively faster rate of elimination generally support higher rates of self-administration and lead to the earlier emergence of withdrawal symptoms (Farre and Cami, 1991). In both self-administration and drug discrimination tests, behavioral effects of MDPV are nearly identical to matched doses of methamphetamine, suggesting equipotency of these drugs. Furthermore, like cocaine, amphetamine, and methamphetamine, MDPV leads to escalated drug intake in extended access sessions (Watterson et al., 2013). Thus, these results suggest that MDPV possesses a similar and potentially greater addiction liability than other illicit stimulants such as cocaine and methamphetamine.

Mephedrone and methylone both possess neurochemical properties more similar to MDMA than to amphetamine, methamphetamine, or cocaine (Baumann et al., 2011). However, unlike MDMA which generally only supports weak self-administration and typically only in a subset of animals (Schenk et al., 2003), both methylone and mephedrone are robustly self-administered in rats (Hadlock et al., 2011; Watterson et al., 2012; Aarde et al., 2013). However, while not assessed for with mephedrone, methylone does not lead to escalated drug intake in extended access sessions, suggesting a lower potential for compulsive use compared to cocaine, amphetamine and methamphetamine (Watterson et al., 2012). Like MDMA, both mephedrone (Robinson et al., 2012) and methylone (Watterson et al., 2012) dose-dependently reduce ICSS thresholds and elicit CPP (Miyazawa et al., 2011; Lisek et al., 2012), indicating these drugs do possess hedonic effects similar to MDMA. Thus, of these three synthetic cathinones, MDPV likely possesses the greatest potential for compulsive use. The studies on mephedrone and methylone suggest a slightly greater risk of compulsive abuse than MDMA, but consumption patterns are predicted to be much more episodic than compulsive like other prototypical stimulants.

Given that the piperazines or synthetic cathinones reviewed here appear to possess a relative abuse liability that is similar in nature to the Schedule I illicit drugs they are designed to mimic, and there are no established medical uses of these substances, it is apparent that these drugs and continually emerging analogues should also likely be categorized as Schedule I controlled substances. One exception to this could be TFMPP, which based on the animal studies reviewed here, does not appear to possess a high degree of abuse liability. Despite the lack of evidence for abuse revealed in animal studies, TFMPP is currently classified as a Schedule I substance in the US. However, the US DEA also uses data from human studies in determining classification, and human self-report data reveals that TFMPP does possess subjective effects similar to d-amphetamine and increased ratings of “drug-liking” and “high” (Jan et al., 2010). Thus, the schedule I status of TFMPP is likely due to data gathered from human studies.

While these initial studies represent a significant first step in abuse/addiction liability evaluation, new designer drugs continue to appear on already flooded drug markets. For example, following the bans on mephedrone, methylone and MDPV, the DEA soon afterward reported the emergence of second generation replacements in a published request for new information (DEA, 2013). Thus, while future research should continue assessing newer analogues as they appear, this research is also relatively slow to keep pace with the ever-changing designer drug market. Thus, additional future research should also focus on educating the public about the dangers of “legal highs” to prevent designer stimulant use. In addition, the exponential growth of different analogues also suggest that other methods with higher throughput capability for assessing abuse liability, such as the development of computer models or predictive in vitro systems (such as DAT:5-HT ratio) should be investigated.

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References

- Aarde SM, Angrish D, Barlow DJ, Wright MJ Jr, Vandewater SA, Creehan KM, Houseknecht KL, Dickerson TJ, and Taffe MA (2013). Mephedrone (4-methylmethcathinone) supports intravenous self-administration in Sprague-Dawley and Wistar rats *Addiction Biology*, in press.
- Aarde SM, Huang PK, Creehan KM, Dickerson TJ, Taffe MA (2013) The novel recreational drug 3,4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: Self-administration and locomotor activity in rats. *Neuropharmacology* 71:130–40. [PubMed: 23597511]
- Ahmed SH, and Koob GF. (1998). Transition from moderate to excessive drug intake: change in hedonic set point. *Science* 282:298–300. [PubMed: 9765157]
- Ahmed SH, and Koob GF (1999). Long-lasting increase in the set point for cocaine self-administration after escalation in rats. *Psychopharmacology* 146:303–12. [PubMed: 10541731]
- Ahmed SH (2010). Validation crisis in animal models of drug addiction: beyond non-disordered drug use toward drug addiction. *Neuroscience and Biobehavioral Reviews* 35:172–84. [PubMed: 20417231]
- Ahmed SH (2011). Escalation of drug use In: *Animal Models of Drug Addiction*, Neuromethods 53. Olmstead MC (editor). Humana Press; p. 23–67.
- Ahmed SH (2012). The science of making drug-addicted animals. *Neuroscience* 211:107–25. [PubMed: 21864653]

- One Hundred Twelfth Congress of the United States of America (2012). S. 3187: Food and Drug Administration Safety and Innovation Act.
- Arbo MD, Bastos ML, and Carmo HF (2012). Piperazine compounds as drugs of abuse. *Drug and Alcohol Dependence* 122:174–85. [PubMed: 22071119]
- Ator N, and Griffiths (2003). Principles of drug abuse liability assessment in laboratory animals. *Drug and Alcohol Dependence* 70:S55–S72. [PubMed: 12759197]
- Auerbach S, Kamalakannan N, and Rutter J (1990). TFMPP and RU24969 enhance serotonin release rat hippocampus. *European Journal of Pharmacology* 190:51–57. [PubMed: 2076760]
- Baumann M, and Ayestas M (2001) 1-(m-Chlorophenyl) piperazine (mCPP) dissociates in vivo serotonin release from long-term serotonin depletion in rat brain. *Neuropsychopharmacology* 24:492–501. [PubMed: 11282249]
- Baumann MH, Ayestas M, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al. (2011). The Designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* 35: 192–203
- Baumann MH, Ayestas M a, Sharpe LG, Lewis DB, Rice KC, and Rothman RB (2002). Persistent antagonism of methamphetamine-induced dopamine release in rats pretreated with GBR12909 decanoate. *Journal of Pharmacology and Experimental Therapeutics* 301:1190–7. [PubMed: 12023554]
- Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, and Rothman RB (2004). Effects of “Legal X” piperazine analogs on dopamine and serotonin release in rat brain. *Annals of the New York Academy of Sciences* 1025:189–97. [PubMed: 15542717]
- Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, and Rothman RB (2005). N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, or “Ecstasy”). *Neuropsychopharmacology* 30:550–60. [PubMed: 15496938]
- Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF et al. (2013a). Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive “Bath Salts” products. *Neuropsychopharmacology*, 38:552–562. [PubMed: 23072836]
- Baumann MH, Partilla JS, and Lehner KR (2013b). Psychoactive “bath salts”: Not so soothing. *European Journal of Pharmacology*, 698: 1–5. [PubMed: 23178799]
- Baumann MH, Rutter JJ, and Auerbach SB (1993). Intravenous administration of the serotonin agonist m-chlorophenylpiperazine (mCPP) increases extracellular serotonin in the diencephalon of awake rats. *Neuropharmacology* 32:1381–6. [PubMed: 8152528]
- Ben-Shahar O, Posthuns EJ, Waldroup SA, and Ettenberg A (2008). Heightened drug-seeking motivation following extended daily access to self-administered cocaine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 32(3): 863–869. [PubMed: 18281138]
- Bilsky E, Hubbell C, Delconte J, and Reid L (1991). MDMA produces a conditioned place preference and elicits ejaculation in male rats: a modulatory role for the endogenous opioids. *Pharmacology Biochemistry and Behavior* 40:443–447.
- Bilsky E, and Reid L (1991). MDL72222, a serotonin 5-HT₃ receptor antagonist, blocks MDMA’s ability to establish a conditioned place preference. *Pharmacology Biochemistry and Behavior* 39:509–512.
- Bilsky EJ, Hui YZ, Hubbell CL, and Reid LD (1990). Methylenedioxymethamphetamine’s capacity to establish place preferences and modify intake of an alcoholic beverage. *Pharmacology, Biochemistry, and Behavior* 37:633–8.
- Brandt SD, Sumnall HR, Measham F, and Cole J (2010). Analyses of second-generation “legal highs” in the UK: initial findings. *Drug Testing and Analysis* 2:377–82. [PubMed: 20687197]
- Brennan KA, Lake B, Hely LS, Jones K, Gittings D, Colussi-Mas J, Fitzmaurice PS, Lea RA, and Schenk S (2007). N-benzylpiperazine has characteristics of a drug of abuse. *Behavioural Pharmacology* 18:785–90. [PubMed: 17989516]
- Cameron KN, Kolanos R, Solis E, Glennon R, and De Felice LJ (2013). Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. *British Journal of Pharmacology*, 168:1750–7 [PubMed: 23170765]

- Carlezon WA, and Chartoff EH (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nature Protocols* 2:2987–95. [PubMed: 18007634]
- Carroll ME, and Meisch RA (2011). Acquisition of drug self-administration In: *Animal Models of Drug Addiction*, Neuromethods 53. Olmstead MC (editor). Humana Press; 237–266.
- Carter LP, and Griffiths RR (2009). Principles of laboratory assessment of drug abuse liability and implications for clinical development. *Drug and Alcohol Dependence* 105: s14–s25. [PubMed: 19443137]
- Collins RJ, Weeks JR, Cooper MM, Good PI, and Russell RR (1983). Prediction of abuse liability of drugs using IV self-administration by rats. *Psychopharmacology* 82:6–13.
- Colpaert FC, Niemegeers CJE, and Janssen PAJ (1976). Theoretical and methodological considerations on drug discrimination learning. *Psychopharmacologia* 46:169–177. [PubMed: 951454]
- Coppola M, and Mondola R (2012). 3,4-Methylenedioxypyrovalerone (MDPV): Chemistry, pharmacology and toxicology of a new designer drug of abuse marketed online. *Toxicology Letters* 208:12–5. [PubMed: 22008731]
- Cozzi N, Sievert M, Shulgin A, and Jacob III P (1999). Inhibition of plasma membrane monoamine transporters by beta-ketoamphetamines. *European Journal of Pharmacology* 381:63–69. [PubMed: 10528135]
- Cozzi NV, Brandt SD, Daley PF, Partilla JS, Rothman RB, Tulzer A, Sitte HH, and Baumann MH (2013). Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs. *European Journal of Pharmacology*, 699:180–187. [PubMed: 23178523]
- Cunningham C, Groblewski P, and Voorhees C (2011). Place Conditioning In: *Animal Models of Drug Addiction*, Neuromethods 53. Olmstead MC (editor). Humana Press; p 167–190.
- Cunningham C, and Noble D (2006). Methamphetamine-induced conditioned place preference or aversion depending on dose and presence of drug. *Annals of the New York Academy of Sciences* 654:431–433.
- Dal Cason TA, Young R, and Glennon R (1997). Cathinone: an investigation of several N-alkyl and methylenedioxy-substituted analogs. *Pharmacology Biochemistry and Behavior* 58:1109–16.
- Drug Enforcement Administration, United States Department of Justice (2002). Drug Scheduling Actions - 2002 - Temporary Placement of Benzylpiperazine and Trifluoromethylphenylpiperazine Into Schedule I. Available from: http://www.deadiversion.usdoj.gov/fed_regs/rules/2002/fr09202.htm
- Drug Enforcement Administration, United States Department of Justice (2011a). Chemicals used in “bath salts” Now under federal control and regulation. October 21, 2011 Available from: <http://www.justice.gov/dea/pubs/pressrel/pr102111.html>
- Drug Enforcement Administration, United States Department of Justice (2011b). Synthetic Cathinones - DEA Request for Information.
- Drug Enforcement Administration, United States Department of Justice (2011c). background, data and analysis of synthetic cathinones: Mephedrone (4-MMC), methylone (MDMC) and 3,4-methylenedioxypyrovalerone (mdpv). Available from: http://www.deadiversion.usdoj.gov/fed_regs/rules/2011/HHS%20PDF/background.pdf
- Drug Enforcement Administration, United States Department of Justice (2012a). Scheduling Actions - Alphabetical Order -. 811:1–7. Available From: http://www.deadiversion.usdoj.gov/schedules/orangebook/a_sched_alpha.pdf.
- Drug Enforcement Administration, United States Department of Justice (2012b). Schedules of controlled substances: extension of temporary placement of methylone into schedule I of the Controlled Substances Act. Final order. Available from: http://www.deadiversion.usdoj.gov/fed_regs/rules/2012/fr1018_11.htm
- Drug Enforcement Administration, United States Department of Justice (2013). Synthetic Cannabinoids and Cathinones - DEA Request for Information. Available from: http://www.cpdd.vcu.edu/Pages/Links/Links_PDFs/SYNTHETIC%20CATHINONES.pdf
- De Boer D, Bosman IJ, Hidvégi E, Manzoni C, Benkő AA, Dos Reis LJ, and Maes R (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic Science International* 121:47–56. [PubMed: 11516887]

- De La Garza R, Fabrizio KR, and Gupta A (2007). Relevance of rodent models of intravenous MDMA self-administration to human MDMA consumption patterns. *Psychopharmacology* 189:425–34. [PubMed: 16470404]
- Elliott S (2011). Current awareness of piperazines: pharmacology and toxicology. *Drug Testing and Analysis* 3:430–8. [PubMed: 21744514]
- Eriksson E, Engberg G, Bing O, and Nissbrandt H (1999). Effects of mCPP on the extracellular concentrations of serotonin and dopamine in rat brain. *Neuropsychopharmacology* 20:287–96. [PubMed: 10063489]
- Fantegrossi WE, Winger G, Woods JH, Woolverton WL, and Coop A (2005). Reinforcing and discriminative stimulus effects of 1-benzylpiperazine and trifluoromethylphenylpiperazine in rhesus monkeys. *Drug and Alcohol Dependence* 77:161–8. [PubMed: 15664717]
- Fantegrossi WE, Gannon BM, Zimmerman SM, Rice KC (2013). In vivo effects of abused “bath salt” constituent 3,4-methylenedioxypyrovalerone (MDPV) in mice: drug discrimination, thermoregulation, and locomotor activity. *Neuropsychopharmacology* 38:563–73. [PubMed: 23212455]
- Farre M, and Cami J (1991). Pharmacokinetic consideration in abuse liability evaluation. *British Journal of Addiction* 12: 1601–1606.
- Fass JA, Fass AD, and Garcia AS (2012). Synthetic cathinones (bath salts): legal status and patterns of abuse. *Annals of Pharmacotherapy* 46: 436–41 [PubMed: 22388331]
- Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, and Robinson TE (2005). Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biological Psychiatry* 9: 751–759.
- Gardner EL (2010). What we have learned about addiction from animal models of drug self-administration. *American Journal on Addictions* 9:285–313.
- Gass JT, Osborne MPH, Watson NL, Brown JL, and Olive MF (2008). mGluR5 antagonism attenuates methamphetamine reinforcement and prevents reinstatement of methamphetamine-seeking behavior in rats. *Neuropsychopharmacology* 34:820–833. [PubMed: 18800068]
- Gipson CD, and Bardo MT (2009). Extended access to amphetamine self-administration increases impulsive choice in a delay discounting task in rats. *Psychopharmacology* 3: 391–400.
- Griffiths R, Brady J, and Bradford L (1979). Predicting the abuse liability of drugs with animal drug self-administration procedures: Psychomotor stimulants and hallucinogens In: Thompson T, Dews PB, editors. *Advances in Behavioral Pharmacology*. 2nd ed. New York: Academic Press p 164–200.
- Hadlock GC, Webb KM, McFadden LM, Chu PW, Ellis JD, Allen SC, et al. (2011). 4-Methylmethcathinone(mephedrone): neuropharmacological effects of a designer stimulant of abuse. *Journal of Pharmacology and Experimental Therapeutics* 339:530–536. [PubMed: 21810934]
- Hillebrand J, Olszewski D, and Sedefov R (2010). Legal highs on the Internet. *Substance Use & Misuse* 45:330–40. [PubMed: 20141450]
- Hubner C, Bird M, and Rassnick S (1988). The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. *Psychopharmacology* 95:49–51. [PubMed: 2898790]
- Huston JP, de Souza Silva MA, Topic B, and Muller P (2013). What’s conditioned in conditioned place preference? *Trends in Pharmacological Sciences*, 34(3): 162–166. [PubMed: 23384389]
- Jan RK, Lin JC, Lee H, Sheridan JL, Kydd RR, Kirk IJ, and Russell BR (2010). Determining the subjective effects of TFMPP in human males. *Psychopharmacology* 211:347–353. [PubMed: 20552171]
- Jansen KL (1999). Ecstasy (MDMA) dependence. *Drug and Alcohol Dependence* 53:121–4. [PubMed: 10080038]
- Kalix P, and Khan I (1984). Khat: an amphetamine-like plant material. *Bulletin of the World Health Organization* 62:681. [PubMed: 6334569]
- Kehr J, Ichinose F, Yoshitake S, Gojny M, Sievertsson T, Nyberg F, and Yoshitake T (2011). Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. *British Journal of Pharmacology* 164:1949–58. [PubMed: 21615721]

- Kelly JP (2011). Cathinone derivatives: A review of their chemistry, pharmacology and toxicology. *Drug Testing and Analysis* 3:439–453. [PubMed: 21755607]
- Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, et al. (2004). Neurobiological mechanisms in the transition from drug use to drug dependence. *Neuroscience and Biobehavioral Reviews* 27:739–49. [PubMed: 15019424]
- Knackstedt LA, and Kalivas PW. (2007). Extended access to cocaine self-administration enhances drug-primed reinstatement but not behavioral sensitization. *The Journal of Pharmacology and Experimental Therapeutics* 322: 1103–1109. [PubMed: 17601982]
- Kornetsky C, and Esposito R (1979). Euphorigenic drugs: effects on the reward pathways of the brain. *Federation Proceedings* 38:2473–6. [PubMed: 488370]
- Kornetsky C, Esposito RU, McLean S, and Jacobson JO (1979). Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. *Archives of General Psychiatry* 36:289–292. [PubMed: 420547]
- Leri F, and Franklin KB (2000). Effects of diazepam on conditioned place preference induced by morphine or amphetamine in the rat. *Psychopharmacology* 150:351–60. [PubMed: 10958076]
- Lin D, Koob GF, and Markou A (1999). Differential effects of withdrawal from chronic amphetamine or fluoxetine administration on brain stimulation reward in the rat--interactions between the two drugs. *Psychopharmacology* 145:283–94. [PubMed: 10494577]
- Lin HQ, Jackson DM, Atrens DM, Christie MJ, and McGregor IS (1997). Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. *Brain Research* 744:351–7. [PubMed: 9027397]
- Lin JC, Bangs N, Lee H, Kydd RR, and Russell BR (2009). Determining the subjective and physiological effects of BZP on human females. *Psychopharmacology* 207:439–46. [PubMed: 19777213]
- Lin JC, Jan RK, Lee H, Jensen M-A, Kydd RR, and Russell BR (2011). Determining the subjective and physiological effects of BZP combined with TFMPP in human males. *Psychopharmacology* 214:761–8. [PubMed: 21060995]
- Lisek R, Xu W, Yuvashva E, Chiu Y-T, Reitz AB, Liu-Chen L-Y, and Rawls SM (2012). Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation. *Drug and Alcohol Dependence* 126: 257–262 [PubMed: 22652295]
- López-Arnau R, Martínez-Clemente J, Pubill D, Escubedo E, and Camarasa J (2012). Comparative neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylone. *British Journal of Pharmacology* 167:407–420. [PubMed: 22509960]
- Madras BK (2012). Designer drugs: an escalating public health challenge. *Journal of Global Drug Policy and Practice*:1–57.
- Manghi RA, Broers B, Khan R, Benguettat D, Khazaal Y, and Zullino DF (2009). Khat use: lifestyle or addiction? *Journal of Psychoactive Drugs* 41:1–10. [PubMed: 19455904]
- Markou A, and Koob GF (1992). Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiology & Behavior* 51:111–119. [PubMed: 1741436]
- Martínez-Clemente J, Escubedo E, Pubill D, and Camarasa J (2012). Interaction of mephedrone with dopamine and serotonin targets in rats. *European Neuropsychopharmacology* 22:231–6. [PubMed: 21824752]
- Mas-Morey P, Visser MHM, Winkelmolen L, and Touw DJ (2012). Clinical toxicology and management of intoxications with synthetic cathinones ("bath salts"). *Journal of Pharmacy Practice*, in press
- Meisch RA (1987). Factors controlling drug reinforced behavior. *Pharmacology Biochemistry and Behavior* 27:367–71.
- Meltzer PC, Butler D, Deschamps JR, and Madras BK (2006). 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (Pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. *Journal of Medicinal Chemistry* 49:1420–32. [PubMed: 16480278]
- Meririnne E, Kajos M, Kankaanpää A, and Seppälä T (2006). Rewarding properties of 1-benzylpiperazine, a new drug of abuse, in rats. *Basic & Clinical Pharmacology & Toxicology* 98:346–50. [PubMed: 16623856]

- Miliaressis E, Rompre PP, Laviolette P, Philippe L, and Coulombe D (1986). The curve-shift paradigm in self-stimulation. *Physiology & Behavior* 37:85–91. [PubMed: 3016774]
- Miyazawa M, Kojima T, and Nakaji S (2011). Behavioral and rewarding effects of methylene, an analog of MDMA in mice. *Hiroaki Medical Journal*. 62:56–71.
- Motbey CP, Hunt GE, Bowen MT, Artiss S, and McGregor IS (2012). Mephedrone (4-methylmethcathinone, “meow”): acute behavioural effects and distribution of Fos expression in adolescent rats. *Addiction Biology* 17:409–22. [PubMed: 21995495]
- Nagai F, Nonaka R, and Satoh HK (2007). The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *European Journal of Pharmacology* 559:132–7. [PubMed: 17223101]
- National Drug Intelligence Center, United States Department of Justice (2011). Synthetic cathinones (bath salts): an emerging domestic threat. Available From: <http://www.justice.gov/archive/ndic/pubs44/44571/44571p.pdf>
- Nikolova I, and Danchev N (2008). Piperazine based substances of abuse: A new party pills on Bulgarian drug market. *Biotechnology and Biotechnological Equipment* 22:652–655.
- O’dell LE, Chen SA, Smith RT, Specio SE, Balster RL, Paterson NE, Markou A, Zorrilla EP, and Koob GF (2007). Extended access to nicotine self-administration leads to dependence: Circadian measures, withdrawal measures, and extinction behavior in rats. *The Journal of Pharmacology and Experimental Therapeutics* 320: 180–193. [PubMed: 17050784]
- Olds J, and Milner P (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology* 47:419–427. [PubMed: 13233369]
- Olds J (1962). Hypothalamic substrates of reward. *Physiological Reviews* 42:554–604 [PubMed: 13939938]
- O’Brien CP, and Gardner EL (2005). Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacology & Therapeutics* 108:18–58. [PubMed: 16183393]
- O’Connor EC, Chapman K, Butler P, and Mead AN (2011). The predictive validity of the rat self-administration model for abuse liability. *Neuroscience and Biobehavioral Reviews* 35: 912–938. [PubMed: 21036191]
- Panlilio LV (2011). Stimulant Self-Administration In: *Animal Models of Drug Addiction*, Neuromethods 53. Olmstead MC (editor). Humana Press 3:57–81.
- Howell LL, and Fantegrossi WE (2009). Intravenous drug self-administration in nonhuman primates In: *Methods of Behavioral Analysis in Neuroscience*. 2nd edition Buccafusco JJ(editor). CRC Press Chapter 9.
- Panlilio LV, and Goldberg SR (2007). Self-administration of drugs in animals and humans as a model and investigative tool. *Addiction and Its Sciences* 12: 1863–1870.
- Pettibone DJ, and Williams M. (1984). Serotonin-releasing effects of substituted piperazines in vitro. *Biochemical Pharmacology* 33:1531–5. [PubMed: 6610423]
- Prosser JM, and Nelson LS (2011). The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology* 8: 33–42.
- Ramsey J, Dargan PI, Smyllie M, Davies S, Button J, Holt DW, and Wood DM (2010). Buying “legal” recreational drugs does not mean that you are not breaking the law. *QJM* 103:777–83. [PubMed: 20675395]
- Redgrave P, and Dean P, (1981). Intracranial self-stimulation. *British Medical Bulletin* 37:141–146. [PubMed: 7032647]
- Richardson NR, and Roberts DC (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods* 66:1–11. [PubMed: 8794935]
- Robinson JE, Agoglia AE, Fish EW, Krouse MC, and Malanga CJ (2012). Mephedrone (4-methylmethcathinone) and intracranial self-stimulation in C57BL/6J mice: Comparison to cocaine. *Behavioural Brain Research* 234:76–81. [PubMed: 22728726]

- Robledo P, Balerio G, Berrendero F, and Maldonado R (2004). Study of the behavioural responses related to the potential addictive properties of MDMA in mice. *Naunyn-Schmiedeberg's Archives of pharmacology* 369:338–49.
- Rosenbaum CD, Carreiro SP, and Babu KM (2012). Here today, gone tomorrow...and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), Kratom, *Salvia divinorum*, methoxetamine, and piperazines. *Journal of Medical Toxicology* 8: 15–32. [PubMed: 22271566]
- Rothman RB, and Baumann MH (2003). Monoamine transporters and psychostimulant drugs. *European Journal of Pharmacology* 479:23–40. [PubMed: 14612135]
- Rothman RB, and Baumann MH (2006). Balance between dopamine and serotonin release modulates behavioral effects of amphetamine-type drugs. *Annals of the New York Academy of Sciences* 1074:245–60. [PubMed: 17105921]
- Salzmann J, Marie-Claire C, Le Guen S, Roques BP, and Noble F (2003). Importance of ERK activation in behavioral and biochemical effects induced by MDMA in mice. *British Journal of Pharmacology* 140:831–8. [PubMed: 14517176]
- Sanchis-Segura C, and Spanagel R (2006). Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. *Addiction Biology* 11:2–38. [PubMed: 16759333]
- Schechter MD (1991). Effect of MDMA neurotoxicity upon its conditioned place preference and discrimination. *Pharmacology, Biochemistry, and Behavior* 38:539–44.
- Schenk S (2009). MDMA self-administration in laboratory animals: a summary of the literature and proposal for future research. *Neuropsychobiology* 60: 13–136.
- Schenk S, Gittings D, Johnstone M, and Daniela E (2003). Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rats. *Psychopharmacology* 169:21–7. [PubMed: 12774185]
- Schenk S, Hely L, Lake B, Daniela E, Gittings D, and Mash DC (2007). MDMA self-administration in rats: acquisition, progressive ratio responding and serotonin transporter binding. *European Journal of Neuroscience* 26:3229–36. [PubMed: 18005064]
- Schoeffer P, and Hoyer D (1989). Interaction of arylpiperazines with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors: do discriminatory 5-HT_{1B} receptor ligands exist? *Naunyn-Schmiedeberg's Archives of Pharmacology* 339:675–83.
- Schuster CR, and Thompson T (1969). Self administration of and behavioral dependence on drugs. *Annual Review of Pharmacology* 9:483–502.
- Shanks KG, Dahn T, Behonick G, and Terrell A (2012). Analysis of first and second generation legal highs for synthetic cannabinoids and synthetic stimulants by ultra-performance liquid chromatography and time of flight mass spectrometry. *Journal of Analytical Toxicology* 36:360–371. [PubMed: 22586208]
- Shippenberg TS, and Koob GF (2002). Recent Advances in Animal Models of Drug Addiction In: *Neuropsychopharmacology: The Fifth Generation of Progress*. Lippincott Williams and Wilkins, p 1381–1397.
- Simpler L, Buser T, Donzelli M, Schramm Y, Dieu L-H, Huwyler J, Chaboz S, Hoener M, and Liechti M (2013). Pharmacological characterization of designer cathinones in vitro. *British Journal of Pharmacology* 168:458–70. [PubMed: 22897747]
- Skinner BF (1938). *The Behavior of Organisms*. New York: Appleton-Century-Crofts.
- Spiller HA, Ryan ML, Weston RG, and Jansen J (2011). Clinical experience with and analytical confirmation of “bath salts” and “legal highs” (synthetic cathinones) in the United States. *Clinical Toxicology* 49:499–505. [PubMed: 21824061]
- Stafford D, LeSage MG, and Glowa JR (1998). Progressive-ratio schedules of drug delivery in the analysis of drug self-administration: a review. *Psychopharmacology* 139:169–84. [PubMed: 9784071]
- Tancer ME, and Johanson CE (2001). The subjective effects of MDMA and mCPP in moderate MDMA users. *Drug and Alcohol Dependence* 65:97–101. [PubMed: 11714594]
- Thomsen M, and Caine SB (2007). Intravenous drug self-administration in mice: practical considerations. *Behavior Genetics* 37: 101–118. [PubMed: 17226102]

- Tzschentke TM (1998). Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology* 56:613–72. [PubMed: 9871940]
- Tzschentke TM (2007). Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addiction Biology* 12:227–462. [PubMed: 17678505]
- Vardakou I, Pistos C, and Spiliopoulou C (2011). Drugs for youth via Internet and the example of mephedrone. *Toxicology Letters* 201:191–5. [PubMed: 21187132]
- Varner KJ, Daigle K, Weed PF, Lewis PB, Mahne SE, Sankaranarayanan A, and Winsauer PJ (2013). Comparison of the behavioral and cardiovascular effects of mephedrone with other drugs of abuse in rats. *Psychopharmacology* 225:675–85. [PubMed: 22972412]
- Vlachou S, and Markou A (2011). Intracranial self-stimulation In: *Animal Models of Drug Addiction*, Neuromethods 53. Olmstead MC (editor). Humana Press; p 3–56.
- Watterson LR, Hood LE, Sewalia K, Tomek SE, Yahn S, Johnson CT, et al. (2012). The reinforcing and rewarding effects of methylone, a synthetic cathinone commonly found in “bath salts”. *Journal of Addiction Research and Therapy* S9:1–8.
- Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Grabenauer M, Thomas BF, et al. (2013). Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV). *Addiction Biology*, in press
- Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, and Woolverton WL, (2005). Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. *Journal of Pharmacology and Experimental Therapeutics* 313:848–854. [PubMed: 15677348]
- Wolf W, and Kuhn D (1991). The 5-HT transporter is an additional site of action for the 5-HT agonists RU 24969 and TFMPP. *Neurochemistry International* 19:39–44.
- Yarosh HL, Katz EB, Coop A, and Fantegrossi WE (2007). MDMA-like behavioral effects of N-substituted piperazines in the mouse. *Pharmacology Biochemistry and Behavior* 88:18–27.
- Yeap CW, Bian CK, Fahmi A, and Abdullah L (2010). A review on benzylpiperazine and trifluoromethylphenylpiperazine: origins, effects, prevalence and legal status. *Health and the Environment Journal* 1:38–50.
- Yokel RA, and Pickens R (1973). Self-administration of optical isomers of amphetamine and methylamphetamine by rats. *Journal of Pharmacology and Experimental Therapeutics* 187:27–33. [PubMed: 4795731]
- Young R (2001). *Drug Discrimination In: Methods of Behavior Analysis in Neuroscience*. Buccafusco J(editor). 1st ed Boca Raton, FL: CRC Press86–95.