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Cognitive deficits and neurotoxicity induced by synthetic cathinones: is there a role for neuroinflammation?

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Abstract

Rationale.—The number of synthetic derivatives of cathinone, the primary psychoactive alkaloid found in *Catha edulis* (khat), has risen exponentially in the past decade. Synthetic cathinones (frequently referred to as “bath salts”) produce adverse cognitive and behavioral sequelae, share similar pharmacological mechanisms of action with traditional psychostimulants, and may therefore trigger similar cellular events that give rise to neuroinflammation and neurotoxicity

Objectives.—In this review, we provide a brief overview of synthetic cathinones, followed by a summary of cognitive deficits in animals and humans that have been documented following acute or repeated exposure. We also summarize growing evidence from in vitro and in vivo studies for synthetic cathinone-induced neurotoxicity, and provide a working hypothetical model of potential cellular mechanisms.

Results.—Synthetic cathinones produce varying effects on markers of monoaminergic terminal function, and can increase the formation of reactive oxygen and nitrogen species, induce apoptotic signaling, and cause neurodegeneration and cytotoxicity. We hypothesize that these effects result from biochemical events similar to those induced by traditional psychostimulants. However, empirical evidence for the ability of synthetic cathinones to induce neuroinflammatory processes is currently lacking.

Conclusions.—Like their traditional psychostimulant counterparts, synthetic cathinones appear to induce neurocognitive dysfunction and cytotoxicity, which are dependent on drug type, dose, frequency, and time following exposure. However, additional studies on synthetic cathinone-induced neuroinflammation are clearly needed, as are investigations into the neurochemical and neuroimmune mechanisms underlying their neurotoxic effects. Such endeavors may lead to novel therapeutic avenues to promote recovery in habitual synthetic cathinone users.

Keywords

Psychostimulant; synthetic cathinone; designer drug; abuse; neurotoxicity; neuroinflammation; cognitive deficits

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Conflict of Interest Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Overview of Synthetic Cathinones

Synthetic cathinones are chemical derivatives of cathinone, a naturally occurring β -ketone amphetamine found in the shrub *Catha edulis* (khat) indigenous to eastern Africa and the Arabian Peninsula. Cathinone derivatives, also referred to as substituted cathinones, are considered “designer drugs” since their chemical structures are easily modified in order to circumvent existing legal restrictions. Synthetic cathinones are also referred to under the broader term “new psychoactive substances” which also includes synthetic cannabinoids, opiates, benzodiazepines, phenethylamines, piperazines, or derivatives of ketamine (Baumann and Volkow 2016). Upon their emergence onto drug markets, synthetic cathinones were sold as crystalline mixtures or powders in packaging intentionally labeled to resemble commercially available bath products, hence the name “bath salts”. Although other colloquialisms including “research chemicals”, “plant food”, “glass cleaner”, etc. have been used, the term “bath salts” has remained the most popular jargon term for synthetic cathinones (Banks et al. 2014; Baumann 2014; Simmons et al. 2018).

Regardless of the terminology, synthetic cathinones are psychostimulants designed to mimic the effects of more traditional psychostimulants such as cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA, “Ecstasy”). However, like their traditional counterparts, synthetic cathinones carry a high risk of inducing severe adverse effects including agitated delirium, psychosis, seizures, multiple organ failure, and death (Fass et al. 2012; Karila et al. 2018; Zaami et al. 2018). Initially, the most widely used cathinone derivatives that emerged in the early 2000’s were 4-methylmethcathinone (4-MMC, mephedrone), 3,4-methylenedioxypropylvalerone (MDPV), and 3,4-methylenedioxy-N-methylcathinone (MDMC, bk-MDMA, or methylone), often referred to as “first generation” synthetic cathinones (Baumann 2014). However, a steady stream of additional cathinone derivatives have since emerged, including but certainly not limited to the following:

- α -pyrrolidinopentiophenone (α -PVP)
- α -pyrrolidinopropiophenone (α -PPP)
- α -pyrrolidinobutiophenone (α -PBP);
- α -pyrrolidinononanophenone (α -PNP)
- α -pyrrolidinovalerothiophenone (α -PVT)
- 3,4-methylenedioxy- α -pyrrolidinobutiophenone (MDPBP)
- 3,4-methylenedioxy- α -pyrrolidinopropiophenone (MDPPP)
- β -keto-N-methylbenzodioxolylbutanamine (β k-MBDB, butylone)
- 4-methylethcathinone (4-MEC)
- 4-fluoromethcathinone (4-FMC, flephedrone)
- 3,4-dimethylmethcathinone (3,4-DMMC)
- α -methylamino-butyrophenone (buphedrone) and its N-ethyl, diethyl, and dimethyl derivatives

- 4-methoxymethcathinone (methedrone)
- α -methylamino-valerophenone (pentadone) and its N-ethyl, diethyl, and dimethyl derivatives
- naphthylpyrovalerone (naphyrone)

To date, hundreds of substituted cathinones have been identified, and it has been estimated that up to 250 new cathinone-related chemical entities may emerge each calendar year (Karch 2015; Weinstein et al. 2017).

The known pharmacological mechanisms of action of synthetic cathinones generally fall into one of two categories: blockade of presynaptic reuptake transporters for monoamines (dopamine, DA; norepinephrine, NE; serotonin, 5-HT), or action as a substrate for these transporters as well as the vesicular monoamine transporter 2 (VMAT2), the latter of which results in substantial efflux of monoamines from presynaptic terminals (Simmler and Liechi 2017; Simmons et al. 2018). Cathinone itself was originally characterized as an amphetamine-like monoamine releasing agent (Kalix 1980; 1981; 1982) but additional studies have suggested it has DA reuptake inhibiting properties as well (Wagner et al. 1982).

The myriad of synthetic cathinone analogues that currently exist vary widely with regards to their affinity for VMAT2 and presynaptic DA, NE or 5-HT transporters (DAT, NET, or SERT, respectively), as well as their specific modes of actions. For example, some synthetic cathinones such as MDPV are potent and lasting reuptake inhibitors that are fairly selective for DA and NE, similar to the actions of cocaine (Baumann et al. 2013; Glennon and Young 2016; Simmler et al. 2013). On the other hand, other cathinone derivatives such as mephedrone induce the release of monoamines and block reuptake, similar to the actions of MDMA and methamphetamine (Baumann et al. 2012; Simmler et al. 2013; Simmler et al. 2014). Frequently, transporter affinities of various cathinones are expressed as a ratio of DAT:SERT, with higher values indicating a preferential affinity for DAT and possessing a higher potential for compulsive and abuse-like intake patterns, and lower DAT:SERT ratios indicate a preferential affinity for SERT and generally entactogenic and producing episodic intake patterns similar to those observed with MDMA (Negus and Banks 2017; Simmler and Liechi 2017; Simmons et al. 2018). DAT-preferring synthetic cathinones induce depolarizing inward sodium currents at this transporter (Kolanos et al. 2013), whereas MDMA-like synthetic cathinones induce inward currents but have a much slower rate of dissociation from the transporter, producing persistent leak currents (Dolan et al. 2018).

Only a handful of non-monoaminergic mechanisms of action of synthetic cathinones have been investigated in detail. First, unlike cocaine and amphetamine-type stimulants, synthetic cathinones appear to lack significant affinity for the trace amine receptor 1 (TAAR1) (Simmler et al. 2016). In addition, recent findings indicate that amphetamine acts as a substrate releasing at type 3 organic cation transporters (OCT3), which unlike DAT, NET and SERT are cocaine-insensitive (Mayer et al. 2018b). Given the structural similarities between classical amphetamines and cathinone derivatives, these same investigators also determined in that mephedrone and MDPV are not OCT3 substrates when tested alone in vitro, but when NET is fully occupied by MDPV, mephedrone exerts substrate-releasing effects via OCT3 (Mayer et al. 2018a). These findings suggest novel mechanisms of action

of when reuptake-inhibiting cathinone derivatives are combined with substrate-releasing analogues.

There is some evidence for an ability of MDPV to down-regulate expression of the glutamate transporter GLT-1 (Gregg et al. 2016), which is responsible for clearance of the majority of extracellular glutamate, and as a result this down-regulation of GLT-1 raises extracellular glutamate levels, potentially leading to excitotoxicity. In addition, some of the behavioral effects of MDPV (reward and hyperlocomotion) have been shown to be attenuated by antagonism of CXCR4 chemokine receptors (Oliver et al. 2018). However, as will be detailed later in this review, it is still unclear if any of these non-monoaminergic mechanisms of action contribute to the ability of synthetic cathinones to induce lasting cognitive dysfunction, neurotoxicity and neuroinflammation.

Cognitive deficits associated with synthetic cathinone abuse

Chronic abuse of traditional psychostimulants such as cocaine or amphetamine-type stimulants is associated with varying degrees of cognitive dysfunction in humans. Cognitive domains that have been found to be affected include verbal information processing (learning, fluency, and recall), visuospatial perception, working memory, cognitive flexibility (i.e., set-shifting), executive function (i.e., selective attention, response inhibition, decision-making, impulsivity), psychomotor speed, and social functioning. As a detailed discussion of cocaine or amphetamine-type stimulant induced cognitive dysfunction, as well as controversy over their magnitude, scope, and causality, is beyond the scope of the present review, the reader is referred to several comprehensive reviews (Dean et al. 2013; Frazer et al. 2018; Hart et al. 2012; London et al. 2015; Potvin et al. 2018). However, it should be noted that co-morbid conditions such as attention-deficit hyperactivity disorder, psychosis, depression, anxiety, sleep pattern alterations, and use of other drugs of abuse may contribute significantly to the occurrence of drug-induced cognitive impairments, as can pre-existing cognitive issues that promote vulnerability to an addictive phenotype. Thus, in human studies examining cognitive dysfunction in the context of drug abuse, it is difficult to parse out the precise neurotoxic effects of the drug itself vs. contributions of pre-existing variables or co-morbid disorders (Cadet and Bisagno 2013; Gould 2010).

Habitual khat users exhibit some deficits in learning, memory, psychomotor speed, cognitive set-shifting, and response inhibition. These studies are detailed in Table 1. A handful of studies have also suggested that the deleterious effects of regular khat use may be exacerbated in users who also use tobacco products (Hoffman and Al'absi 2013a; Nakajima et al. 2014). Evidence is now emerging that habitual synthetic cathinone users also may exhibit similar degrees of cognitive dysfunction. As shown in Table 1, three separate studies have shown regular mephedrone users exhibit impaired verbal recall and fluency as well as short-term memory deficits. However, as with many studies assessing cognitive function in human drug users, it is difficult to determine the specific contribution of mephedrone use to these cognitive impairments due to the high level of co-morbidity with other neuropsychiatric disorders, polydrug use, questionable reliability of self-reports of prior drug use, and a lack of baseline measures of cognitive performance. In addition, there are considerable variations in the time elapsed between drug use and cognitive assessment, and the possible contribution

of other psychoactive substances, since many synthetic cathinone products contain psychoactive adulterants (Palamar et al. 2016). It is thus difficult to attribute the use of specific synthetic cathinones with specific measures of cognitive impairment.

Nonetheless, in one of the few controlled laboratory studies conducted in humans, it was shown that acute administration of mephedrone (200 mg) to human polydrug users (including prior mephedrone use) impaired short-term spatial memory while leaving divided attention intact, and actually improved psychomotor reaction time (de Sousa Fernandes Perna et al. 2016). Interestingly, despite frequent co-abuse of alcohol with synthetic cathinones, the observed memory deficits were not counteracted by acute co-administration of alcohol (0.8 g/kg). We are unaware of any other clinical studies utilizing controlled administration of synthetic cathinones in human subjects.

Studies in laboratory animals such as mice, rats, and non-human primates allow for more direct and controlled assessment of the acute or long-term effects of psychoactive drugs on cognition. A summary of such studies published to date on synthetic cathinones, as well as khat extracts containing psychoactive cathinone and cathine, is provided in Table 2. These studies have assessed a wide range of cathinone derivatives, doses, treatment frequency and duration, routes and behavioral contingency of administration, as well as time between end of drug exposure and assessment of cognitive function. Various behavioral tasks have been employed to assess cognitive function in these studies, including the Morris water maze, T- or Y-maze alternations, passive avoidance, novel object recognition, sequential operant responding, and visuospatial information processing.

As can be seen, the results thus far have been mixed, but a clear pattern is beginning to emerge in that repeated exposure to high doses produces impaired cognitive function in the domains of spatial working and recognition memory. These patterns of findings are not unlike those that have been found with traditional psychostimulants such as methamphetamine and MDMA (Easton and Marsden 2006; Moszczynska and Callan 2017). However, several important points should be noted here. First, a few of these studies have actually noted cognition-enhancing effects of synthetic cathinones. For example, den Hollander et al (2013) noted that mice treated with methylone (30 mg/kg i.p., 2x daily for 4 d) displayed increased search time in the target quadrant during probe trial reversal learning in the Morris water maze when tested 5 weeks post-treatment. Also, Wright and colleagues demonstrated that acute administration of mephedrone (0.178 – 0.56 mg/kg i.m.) to rhesus monkeys increased performance in the visuospatial component of a stimulus-associative memory task (Wright et al. 2012), consistent with the psychomotor performance-enhancing effects of amphetamine-type psychostimulants (Wood et al. 2014). On the other hand, mephedrone-induced disruptions in operant conditioning task performance in ovariectomized female rats has been shown to be potentiated by administration of 17- β -estradiol (Weed et al. 2014), suggesting that cognition-impairing effects of synthetic cathinones may be influenced by ovarian hormones. Yet as shown in Table 2, synthetic cathinone-induced deficits in cognition have been observed in animals of both sexes. Clearly, studies examining sex-specific effects of synthetic cathinones on cognitive performance in direct side-by-side comparisons are needed. Finally, it should be noted that cognitive deficits have been observed following administration of synthetic cathinones with

either monoamine releasing (i.e., mephedrone and methylone) and monoamine reuptake blocking (i.e., MDPV) mechanisms of action. It is therefore difficult to predict which modes of action carry a higher potential for inducing cognitive dysfunction. In addition, given that only a handful of cathinone analogues have been assessed thus far, speculations regarding structure-activity relationships relative to the potential for inducing cognitive impairments at this point would be premature.

Another important caveat in interpretation of the results summarized in Table 2, as well as those in the extant literature with regards to classical psychostimulants, is that resulting blood and brain levels of the drug that are produced by high “binge-like” dosing procedures (i.e., bolus doses administered one more times daily for 3–10 days) likely far exceed levels that would be obtained if subjects were to voluntarily self-administer the drug via the intravenous or other route. In addition, such high binge-like dosing procedures via passive drug exposure induce vastly different pharmacodynamic and physiological processes than those invoked by voluntary intake (Jacobs et al. 2003; Stuber et al. 2010). While self-administration procedures are clearly more laborious, challenging, and costly than passive administration approaches, they arguably offer more face validity for modeling human intake patterns. Nonetheless, studies employing passive administration of high doses of synthetic cathinones may offer initial insight into the cognitive and pathophysiological sequelae that occur following prolonged voluntary intake. As an example, Motbey et al. (2012) showed that repeated administration of high doses of mephedrone to rats resulted in deficits in object recognition memory, and similar deficits were observed in a recent study conducted by our laboratory following long-term binge-like MDPV self-administration in multiple 96-hr access sessions over a period of 5 weeks, which resulted in intake levels that exceeded 100 mg/kg per week (Sewalia et al. 2018). Although these studies examined the effects of two different synthetic cathinones (mephedrone and MDPV), similar aspects of cognitive dysfunction was observed. Thus, one key area of future research is to determine which specific cognitive deficits can be accurately and reliably modeled by short-term passive administration vs. those that occur only after voluntary intake. Moreover, it remains to be determined which cognitive domains are susceptible to long-term impairments by synthetic cathinones, which cathinone derivatives carry the highest risk of cognitive impairments, what duration of intake is required to induce such effects, and to what degree can such deficits “recover” after a period of abstinence. The presence of cognitive dysfunction following long term exposure or intake also begs the question – does long-term exposure to synthetic cathinones result in neurotoxicity and/or neuroinflammation?

Cellular toxicity induced by synthetic cathinones

Various distinct yet interactive biological processes are believed to contribute to the neurotoxic effects of classical psychostimulants and associated cognitive impairments, and we hypothesize that similar mechanisms and processes occur following chronic intake of synthetic cathinones (see Figure 1 and conclusory remarks below). These processes include increases in oxidative stress, excitotoxicity, metabolic compromise, induction of apoptosis, neuroinflammation and gliosis, and compromised blood-brain barrier (BBB) function. For detailed reviews on each of these mechanisms as they relate to traditional psychostimulants such as cocaine, methamphetamine and MDMA, the reader is directed to several reviews

elsewhere (Cadet et al. 2014; Cui et al. 2014; Moratalla et al. 2017; Moszczynska and Callan 2017; Soleimani et al. 2016). In addition, for a review on evidence of these phenomenon that may occur in the context of khat use in humans, see (Aleryani et al. 2011). Here, we will provide a brief overview of the proposed biochemical events that are believed to underlie psychostimulant-induced neurotoxicity, with the exception of neuroinflammation, which will be discussed separately below. We will then summarize existing evidence that many of these events are induced by synthetic cathinones.

Through their antagonistic and/or substrate-mimicking effects on monoamine transporters (i.e., DAT, NET, and SERT), psychostimulants including synthetic cathinones induce a substantial and prolonged accumulation of monoamines in the presynaptic terminal or extracellular space (Baumann et al. 2012; Baumann et al. 2013). The resulting overabundance of DA can result in excess DA metabolism that leads to the formation of cell damaging DA-o-quinones as well as reactive oxygen and nitrogen species (ROS and RNS, respectively) such as hydrogen peroxide and peroxynitrite. ROS and RNS production can also result from excessive glutamate release induced by psychostimulants, which results in excessive Ca^{2+} influx and ROS/RNS formation. Cell-damaging superoxides can also be formed by drug-induced compromise of cellular respiration and inhibition of electron transport chain complexes, which results in incomplete reduction of oxygen. The resulting oxidative stress, often paralleled by changes in levels of endogenous antioxidants such as glutathione, induces protein and lipid peroxidation and/or DNA oxidation, which can ultimately lead to cell dysfunction and death (Salim 2017). ROS/RNS can also activate endogenous caspase signaling pathways, promoting autophagy and apoptosis. Finally, psychostimulants can compromise BBB function through mechanisms including induction of hyperthermia, activation of matrix metalloproteinases which disrupt the integrity of the vascular basement membrane via cleavage of extracellular matrix proteins, and neuroinflammatory processes that will be discussed below.

There is now emerging evidence that synthetic cathinones engage some of the aforementioned neurotoxic processes (see also (Angoa-Perez et al. 2017; Pantano et al. 2017)). These in vivo and in vitro studies summarized in Tables 2 and 3, respectively. The in vitro studies have been conducted in numerous cell types, and have shown cytotoxic effects of all cathinone derivatives tested. Of relevance to toxicity to the brain, evidence of oxidative stress have been observed in neuronal cell lines including differentiated SH-SY5Y (dopaminergic neuroblastoma), HT22, SK-N-SH, and TGW cells after exposure to mephedrone, methylone, MDPV, α -PNP, naphyrone, or 3-fluoromethcathione. Evidence for oxidative stress includes observations of increased ROS/RNS production and/or depletion of reduced glutathione and increases in oxidized glutathione levels. As well, these in vitro studies also demonstrated an ability of various synthetic cathinones to deplete intracellular ATP levels, impair mitochondrial function, reduce cell proliferation, and induce autophagy, necrosis and/or apoptosis as measured by chromatin condensation, pyknotic nuclei formation, and caspase activation. den Hollander and colleagues also identified formation of cytotoxic breakdown products such as methylbenzamides in response to mephedrone and 4-methylmethcathinone (den Hollander et al. 2015). Some of the aforementioned effects were reversed by incubation of these cells with antioxidants such as N-acetylcysteine (Matsunaga et al. 2017; Valente et al. 2017a), further underscoring a critical role of oxidative

stress in these cytotoxic effects. Cell damaging effects of MDPV have been observed in endothelial cells, serving as a potential basis for disruption of BBB function. Finally, oxidative stress and cell death has been observed in cultured hepatocytes, which was more prominent under hyperthermic vs. normothermic conditions (Valente et al. 2016). Thus, it is critical to take into account the potential exacerbation of adverse drug effects that are a result of body temperature-elevating effects common to synthetic cathinones.

However, it should be noted that in most (but not all) cases, cytotoxicity was observed following incubation times > 24 hr, and at very high concentrations, often in the high micromolar to low millimolar range. While these studies are informative, such steady state high concentrations of cathinone derivatives do not likely occur in vivo, and it would be of great interest to assess the effects of fluctuating concentrations that are more reflective of cell exposure patterns in human users. In addition, Gaspar and colleagues recently reported an apparent positive relationship between increased cytotoxicity in some cathinone derivatives and the presence of methyl substituents on aryl moieties as well as increased carbon length of acyl chains group, as in mephedrone, DMC and 4-MDMC (Gaspar et al. 2018). These authors speculated that different numbers and positions of methyl groups may lead to changes in hydrophobicity and lipophilicity, and as a result increased oral bioavailability, BBB permeability, and possibly neurotoxicity. While these hypotheses need to be tested and confirmed in vivo, the in vitro findings summarized in Table 3 offer wealth of evidence that synthetic cathinones exert cytotoxic effects, likely via oxidative stress and/or apoptotic mechanisms.

In assessing the potential neurotoxic effects of synthetic cathinones in animal models, many investigators have focused on the primary pharmacological sites of action of these drugs - the monoaminergic presynaptic terminal - quite often in the dorsal or ventral striatum, though other areas have also been studied. Degeneration of striatal monoaminergic terminals has been observed in both animals and humans following long-term exposure to traditional psychostimulants (Moratalla et al. 2017; Moszczynska and Callan 2017; Pereira et al. 2015), and some have speculated that psychostimulant-induced dopamine terminal damage may increase the risk of Parkinson's disease (Kish et al. 2017). Toxicity to monoaminergic terminals is typically assessed by changes in protein levels of monoamine synthesizing enzymes such as tyrosine hydroxylase (TH) or tryptophan hydroxylase 2 (TPH2), presynaptic monoamine transporter (i.e., DAT or SERT) protein levels or radioligand binding, or tissue monoamine and/or metabolite content. With regards to this latter index of monoaminergic dysregulation, studies examining the effects of synthetic cathinones have yielded inconsistent results. As shown in Table 2, passive administration of mephedrone or methylone produces only transient or minimal changes in DA and 5-HT levels as well as turnover ratios in the striatum, hippocampus or prefrontal cortex (also see (Pail et al. 2015; Shortall et al. 2013)). However, it should be noted that most of these studies primarily used once-daily or subchronic dosing procedures, and all of them utilized non-contingent administration.

More consistent depletions or alterations in tissue monoamine content, as well as changes in the expression of monoamine terminal markers (i.e., DAT, SERT, VMAT2), have been observed following administration of synthetic cathinones such as mephedrone or methylone

at higher doses and at increased frequency (i.e., 20 mg/kg, 3 to 4 times per day). Still, other investigators using high and frequent dosing procedures have observed no effects of MDPV or methylone on striatal tissue monoamine content (Anneken et al. 2017; Miner et al. 2017). Counterintuitively, it has been reported that some synthetic cathinones display neurotoxic effects only in the presence of other psychostimulants. For example, methylone (4×30 mg/kg at 2 hr intervals) can enhance putative damage to dopamine nerve endings as measured by decreases in DAT levels or binding more robustly in the presence of methamphetamine, yet oddly the same doses of MDPV appear to exert neuroprotective effects (Anneken et al. 2015). More predictably, additive or synergistic neurotoxic effects have been shown when mephedrone is administered in combination with methamphetamine (Anneken et al. 2018) or ethanol (Ciudad-Roberts et al. 2016). Thus, there are highly complex mechanism of action of synthetic cathinones with regards to their ability to produce neurotoxic or monoamine-altering effects, and it is clear that such effects are dependent on a plethora of variables such, dose and frequency of administration, the presence of other drugs of abuse.

Other than measurements of striatal monoaminergic transmission, only a handful of in vivo studies have assessed other markers of cytotoxicity produced by synthetic cathinones. Kaminska and colleagues showed that repeated administration of a relatively low (5 mg/kg) dose of mephedrone to male adolescent rats daily for 8 days resulted in evidence of oxidative DNA damage in the frontal cortex as assessed by an alkaline comet assay (Kaminska et al. 2018). Additionally, Lopez-Arnau et al. found evidence of lipid peroxidation and increased glutathione peroxidase in the frontal cortex of adolescent rats following acute high dose administration of mephedrone (3×25 mg/kg at 2 hr intervals for 2 days) (Lopez-Arnau et al. 2015). Recent studies have also documented neurotoxic effects of synthetic cathinones following in utero or early life exposure (Adam et al. 2014; Naseri et al. 2018). Finally, in our recent study in which rats were allowed to self-administer MDPV in repeated 96-hr binge-like episodes, we observed evidence of neurodegeneration as measured by FluoroJade C staining in the perirhinal and entorhinal cortices, brain regions which are critical for object recognition memory which was also impaired in these animals (Sewalia et al. 2018). However, contrary to the findings of Kaminska et al. (2018), we observed no evidence of MDPV-induced neurodegeneration in the prefrontal cortex or hippocampus. Future studies are clearly warranted to determine the cellular and molecular mechanisms underlying synthetic cathinone induced neurotoxicity, environmental and hormonal influences on these effects, as well as pharmacological approaches to prevent or attenuate this cytotoxicity.

Do synthetic cathinones induce neuroinflammation?

Psychostimulant-induced terminal degeneration and other manifestations of neurotoxicity are often accompanied, and may even be preceded, by induction of inflammatory processes in the brain (Lacagnina et al. 2017; Loftis and Janowsky 2014; Soleimani et al. 2016). Psychostimulants of various classes (i.e., cocaine or amphetamine-type stimulants) can induce the release of pro-inflammatory cytokines and activation of microglia, which in turn may result in neurotoxicity and/or cognitive dysfunction. As a specific example, cocaine activates microglia within the nucleus accumbens, leading to an increase in the formation

and release of tumor necrosis factor alpha (TNF- α) (Lewitus et al. 2016). This release of TNF- α in turn reduces indices of synaptic plasticity in this region, specifically ratios of excitatory postsynaptic currents mediated by α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to those mediated by N-methyl-D-aspartate (NMDA) receptors within medium spiny neurons (MSNs) (Lewitus et al. 2016). Furthermore, autophagy is induced via the ability of cocaine to activate microglia in vitro and in vivo and invoke endoplasmic reticulum stress-mediated pathways (Guo et al. 2015). MDMA and methamphetamine-induced neurotoxicity of striatal dopaminergic terminals has been associated with activation of microglia (Thomas et al. 2004), and inflammatory processes can either contribute to or even protect against psychostimulant-induced neurotoxicity (Mayado et al. 2011).

Unfortunately, the evidence to date for synthetic cathinone-induced neuroinflammation is scant. An in vitro study revealed that incubation of SH-SY5Y cells with mephedrone, but not methylone, increased the formation of TNF- α (den Hollander et al. 2015). However, in this study, the neuroblastoma cell line was cultured in the absence of astrocytes or microglia, and thus whether such increases occur in a mixed heterogeneous cell environment needs to be determined. In addition, the concentrations of mephedrone at which increases in TNF α were very high (millimolar), and it is uncertain whether or not such concentration would be observed in vivo. Along these lines, various animal studies (see Table 2) have reported that repeated administration of mephedrone or methylone, even with high and frequent dosing procedures, does not induce immunohistochemical evidence of astrogliosis or microgliosis (Angoa-Perez et al. 2012; Angoa-Perez et al. 2014; Lopez-Arnau et al. 2015; Martinez-Clemente et al. 2014; Motbey et al. 2012). On the other hand, some investigators have reported increases in astrocyte or microglia number in regions such as the frontal cortex, striatum or hippocampus following repeated high dose administration of mephedrone, methcathinone, or methylone (Anneken et al. 2018; Lopez-Arnau et al. 2014a; Lopez-Arnau et al. 2014b). Some of these inconsistencies are likely a result not only of the use of different dosing paradigms, but also the amount of time elapsed between the end of drug exposure and the assessment of gliosis, which vary from as few as several days to over a month. Also, in these studies, evidence of gliosis was assessed either by quantification of immunostaining for proteins specific for either astrocytes (i.e., glial fibrillary associated protein, GFAP) or microglia (i.e., ionized binding adapter protein 1, Iba1, and isolectin B4, ILB₄), or radioligand binding to the microglia specific 18 kDa translocator protein (TSPO). It is widely accepted that microgliosis is a highly dynamic process characterized not just by changes in astrocyte or microglial cell number, but also transient release of pro-inflammatory cytokines, morphological changes in cellular structure, cell migration, changes in inflammation-related gene expression, and intracellular indices of oxidative stress such as dense cytoplasmic granules and enlarged lumen of the endoplasmic reticulum (Bisht et al. 2016; Eggen et al. 2013; Ransohoff and Engelhardt 2012). Unfortunately, none of changes are reliably detected with the aforementioned immunostaining or radioligand binding methods. Thus, although evidence of microgliosis may not have been detected in the studies described above, new advancements in methodologies for detecting neuroimmune signaling and activation may unveil evidence for microgliosis induced by synthetic cathinones that were not previously observed.

Currently, we are unaware of any published empirical studies providing evidence that synthetic cathinones induce specific neuroinflammatory processes in vivo. This is not to assert that synthetic cathinones do not induce neuroinflammation; rather, it is a phenomenon that has not yet been investigated, likely a result of the relative infancy of synthetic cathinone research. Indeed, one recent study demonstrated attenuation of MDPV-induced reward and hyperlocomotion by a CXCR4 chemokine receptor antagonist (Oliver et al. 2018), providing one of the first glimpses of evidence for neuroimmune crosstalk in the central actions of synthetic cathinones. Thus, avenues of future research should include detailed analyses of the myriad of quantifiable mechanisms and processes of neuroinflammation that are potentially activated by synthetic cathinones. This is especially important in light of the aforementioned evidence of synthetic cathinone-induced cytotoxicity, which are likely to be accompanied by some form of microgliosis and/or inflammation for clearance of cellular debris following neurodegeneration, as well as synapse and circuit modification via neuroimmune communication.

Conclusions

A considerable amount of work has identified neurotoxic and neuroinflammatory effects of classic psychostimulants, including cocaine, methamphetamine and MDMA. However, the body of literature and studies identifying these effects induced by cathinones and synthetic cathinones remains far from being explored in detail. While the detrimental cognitive effects of synthetic cathinones and some level of neurotoxicity via oxidative stress mechanism are apparent, their ability to induce neuroinflammatory processes needs to be empirically determined. A thorough understanding of the neuroinflammatory and neurotoxic effects induced by synthetic cathinone consumption could provide a basis for novel antioxidant or immunomodulatory therapeutic avenues for habitual abusers of these novel psychoactive substances, as well as traditional psychostimulants.

Based on evidence and existing models for toxicity induced by traditional psychostimulants such as cocaine, amphetamines, and MDMA (Marshall and O'Dell 2012; Moratalla et al. 2017; Moszczynska and Callan 2017), we propose a working hypothetical model of the cytotoxic mechanisms of synthetic cathinones (Figure 1). We focus primarily on potentiated dopaminergic signaling as an initial mechanism, since in vivo and in vitro studies using ligands that either target DAT and/or alter the readily releasable pool of dopamine have for the most part established a critical role of this neurotransmitter in cellular dysfunction induced by synthetic cathinones (Anneken et al. 2017; 2018; Valente et al. 2017b). As inhibitors and/or substrates of monoamine transporters (i.e., DAT and/or VMAT2), synthetic cathinones induce prolonged accumulation of DA in the presynaptic terminal cytoplasm and extracellular space. We hypothesize that the resulting excess DA metabolism by monoamine oxidases and/or catechol-O-methyltransferase results in the formation of cell damaging DA-o-quinones, reactive oxygen and nitrogen species (ROS and RNS, respectively), in parallel with the normal DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (not shown). Since synthetic cathinones such as MDPV also reduce expression of the glutamate transporter GLT-1 (Gregg et al. 2016), primarily localized to astrocytes, we hypothesize such changes may result in excess glutamate accumulation in the extracellular space and potential excitotoxicity via excessive Ca^{2+} influx through ionotropic

glutamate receptors (iGluRs). Oxidative stress resulting from prolonged DA-o-quinone elevations can in turn result in protein and lipid peroxidation and/or DNA oxidation, as well as activation of pro-apoptotic signaling pathways, which ultimately lead to cell death. We hypothesize that toxic effects of synthetic cathinones on endothelial cells may also result in reduced BBB integrity. However, the degree of induction of neuroinflammatory processes, including activation of microglia and the production and release of pro-inflammatory cytokines such as TNF- α and/or chemokines, are currently unknown and need to be explored.

Synthetic cathinones vary widely in their structure- activity relationships with respect to DAT:SERT ratios, affinity for VMAT2, lipophilicity, and BBB permeability, as well as the biological activity of cathinone stereoisomers (Luethi et al. 2018; Negus and Banks 2017; Simmler and Liechti 2017; Valente et al. 2014). While some in vitro evidence has hinted at potential relationships between chemical structure (i.e., acyl chain length and presence of methyl groups) and cytotoxicity and abuse potential (Gaspar et al. 2018), structure-activity relationships related to neurocognitive dysfunction, neuroinflammation and neurotoxicity have not yet evaluated systematically in animals or humans. Thus, it would be premature to predict which cathinone derivatives with defined mechanisms of action or chemical composition are more likely to have deleterious effects on brain function and health.

Finally, we would like to make several points of caution. First, most of the studies reviewed here suggestive of cognitive dysfunction, neurotoxicity, and possible neuroinflammation induced by synthetic cathinones have utilized either steady state incubation with high concentrations in vitro, or in the case of animal studies, have examined effects of behaviorally non-contingent (i.e., experimenter-administered) binge-like dosing procedures. To our knowledge, only one study to date has examined the effects of a voluntarily self-administered synthetic cathinone on neurocognitive dysfunction (Sewalia et al. 2018). Furthermore, varying lengths of time following cessation of drug treatment prior to behavioral or postmortem assessment have been used, which offer only a snapshot of the highly dynamic nature of cognitive impairment, neurotoxicity, and neuroinflammation. It should also be noted that many psychostimulants including synthetic cathinones exert hyperthermic or hypothermic effects to varying degrees (Table 2), which can affect neurocognitive function, inflammation, and toxicity (Kiyatkin 2014; McConnell et al. 2015). Yet only a portion of the studies reviewed here have examined changes in body temperature that accompany any cognitive or neurobiological and changes, and it is important to monitor this potential contributing factor in future studies.

One last point of caution should be made with respect to extrapolation of doses of synthetic cathinone administered to laboratory animals to those used by humans. As mentioned earlier, many synthetic cathinone products contain psychoactive adulterants including other amphetamines, cocaine, and caffeine. Thus, the self-reported doses and purity of any synthetic cathinone used, whether in a recreational or habitual context, may or may not be reliable. Furthermore, doses of psychostimulants administered ingested by humans are not typically based on body weight, as is the case in animal studies, and the use of scaling methods for dose extrapolation between animals and humans is often insufficient. For example, in the study by de Sousa Fernandes Perna and colleagues (2016), mephedrone at

doses of 200 mg were administered acutely to human subjects. When divided by a typical adult body weight of 70 kg, this would equate to a dose of 2.85 mg/kg, which is below doses of 5–30 mg/kg required to produce conditioned place preference in rodents (Karlsson et al. 2014; Lisek et al. 2012). Such scaling based on body mass can often lead to incorrect assumptions about equivalent doses in humans vs. animals, primarily due to differences in route of administration, plasma protein binding, drug physicochemical properties, transport and metabolism, and oral bioavailability. Such extrapolations most likely require more complex allometric or body surface area-based scaling approaches (Kenyon 2012; Sharma and McNeill 2009). Nonetheless, in order to maintain a translational impact on the field, it is important to keep a keen eye on the importance of dose, frequency, duration, behavioral contingency, and assessment of multiple time points in future studies examining neurocognitive dysfunction produced by synthetic cathinones.

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Figure 1.

A working hypothetical model of the cytotoxic mechanisms of synthetic cathinones, adapted from existing models for toxicity induced by traditional psychostimulants such as cocaine, amphetamines, and MDMA. Acting as inhibitors and/or substrates of monoamine transporters (i.e., DAT), synthetic cathinones induce prolonged accumulation of DA in the cytoplasm of the presynaptic terminal and the extracellular space. This results in excess DA metabolism and the formation of cell damaging DA-o-quinones, reactive oxygen and nitrogen species (ROS and RNS, respectively), as well as the normal DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (not shown). Synthetic cathinones such as MDPV can also down-regulate expression of the glutamate transporter GLT-1, primarily localized to astrocytes, resulting in excessive glutamate accumulation in the extracellular space and potential excitotoxicity via excessive Ca^{2+} influx through ionotropic glutamate receptors (iGluRs). The overall oxidative stress induces protein and lipid peroxidation and/or DNA oxidation, as well as activation of pro-apoptotic signaling pathways (not shown), which ultimately lead to cell dysfunction and death. Finally, synthetic cathinones may exert toxic effects on brain endothelial cells, resulting in reduced BBB integrity. Currently unknown are the degrees of activation of microglia, secretion of pro-inflammatory cytokines such as $\text{TNF-}\alpha$ and/or chemokines, and other neuroinflammatory processes. Note that microglia and astrocytes are not drawn to scale relative to the synaptic terminal.

Table 1.

Studies demonstrating evidence of cognitive impairments induced by regular khat use or synthetic cathinones in human subjects.

Drug	Use patterns	Cognitive deficits	Reference
khat	daily, almost daily or occasional use	↓ visuoperceptual memory and fluency, spatial memory, short and long-term memory	(Khattab and Amer 1995)
	1 yr regular use	↓ response inhibition	(Colzato et al. 2010)
	1 yr regular use	↓ cognitive flexibility, working memory	(Colzato et al. 2011)
	1 yr daily use	↓ working memory, n.c. information processing speed	(Hoffman and al' Absi 2013b)
	1x week, >1 yr	↓ serial learning, psychomotor speed, set-shifting	(Ismail et al. 2014)
mephedrone	>1 yr of regular use	↓ verbal recall and fluency	(Freeman et al. 2012)
	<48 hr prior to psychological test	↓ verbal recall and fluency	(Herzig et al. 2013)
	1x month; self-reported use examined over 9 d	↑ self-reported cognitive impairment (unspecified)	(Homman et al. 2018)
	6x prior use;; acute 200 mg dose administered p.o.	↓ short term memory, n.c. divided attention effects not counteracted by alcohol (0.8 g/kg)	(de Sousa Fernandes Perna et al. 2016)

Table 2.

Animal studies demonstrating evidence of cognitive dysfunction, neurotoxicity, or neuroinflammation induced by khat extract or synthetic cathinones.

Species	Sex	Dose	Cognitive deficits	Toxicity	Inflammation	T _B	Reference
mouse	M	khat extract 120–360 mg/kg i.p. 1x daily for 5 d	↑↓ MWM acq., probe trial, and reversal learning (Tx prior to testing)	ND	ND	ND	(Kimani and Nyongesa 2008)
	np	khat extract 40–360 mg/kg i.p. 1x daily for 17 d	↑↓ MWM acq., probe trial, and reversal learning (time relative to Tx not provided)	ND	ND	ND	(Kimani et al. 2016)
	M	khat extract 100–400 mg/kg i.g. 1x daily for 60 d	↓ social interaction, ↓ MWM acq and probe trial (variable time relative to Tx)	ND	ND	ND	(Bogale et al. 2016)
	F	mephedrone 40 mg/kg i.p. 4x @ 2 hr intervals	ND	↓ striatal DAT n.c. striatal GFAP	n.c. striatal ILB ₄	↑	(Angoa-Perez et al. 2012)
	F	mephedrone 40 mg/kg i.p. + METH (2.5 or 5 mg/kg), D-amphetamine (5 mg/kg) or MDMA (20 mg/kg) 4x @ 2 hr intervals	ND	enhanced ↓ in striatal DAT and TH	ND	↑	(Angoa-Perez et al. 2013)
	M	mephedrone 30 mg/kg i.p. 2x daily for 4 d	↓ spontaneous alt. in T-maze (3 wks post-Tx) n.c. MWM (5 wk post-Tx)	ND	ND	↑	(den Hollander et al. 2013)
	F	mephedrone 40 mg/kg i.p. 4x @ 2 hr intervals	ND	n.c. hipp SERT n.c. striatal GFAP	ND	↑	(Angoa-Perez et al. 2014)
	F	mephedrone 40 mg/kg i.p. + METH 5 mg/kg or MDMA 20 mg/kg 4x @ 2 hr intervals	ND	n.c. hipp SERT, TPH2 or GFAP	ND	ND	(Angoa-Perez et al. 2014)
	M	mephedrone 50 mg/kg s.c. 4x @ 2 hr intervals for 1 d	ND	↓ DAT binding in striatum and FC; ↓ SERT binding in hipp and FC	ND	↑	(Martinez-Clemente et al. 2014)
	M	mephedrone 25 mg/kg s.c. 4x @ 2 hr intervals	ND	↓ DAT binding in FC but not striatum; n.c. SERT binding in FC or hipp	ND	↑	(Martinez-Clemente et al. 2014)
	M	mephedrone 25 mg/kg s.c. 3x @ 2 hr intervals for 2 d	ND	↓ DAT binding in FC and striatum; ↓ SERT binding in FC and hipp; ↓ TPH2 levels in hipp; n.c. GFAP in hipp	ND	↑	(Martinez-Clemente et al. 2014)
	F	mephedrone 40 mg/kg i.p. 4x @ 2 hr intervals	ND	↓ striatal DAT	ND	↓↑	(Anneken et al. 2018)
	F	mephedrone 40 mg/kg i.p. + METH 2.5 mg/kg 4x @ 2 hr intervals	ND	↓ striatal DAT, TH ↑ striatal GFAP	↑ striatal Iba1	↑	(Anneken et al. 2018)
	F	mephedrone 40 mg/kg s.c. 4x @ 2 hr intervals	ND	n.c. striatal DAT, TH, or GFAP;	ND	↑	(Anneken et al. 2015)

Species	Sex	Dose	Cognitive deficits	Toxicity	Inflammation	T _B	Reference
	F	mephedrone 40 mg/kg s.c + MDPV 30 mg/kg. 4x @ 2 hr intervals	ND	n.c. striatal DAT, TH, or GFAP;	ND	ND	(Anneken et al. 2015)
	F	mephedrone 40 mg/kg s.c + methylone 30 mg/kg 4x @ 2 hr intervals	ND	n.c. striatal DAT, TH, or GFAP;	ND	ND	(Anneken et al. 2015)
	M	mephedrone 25 mg/kg s.c. + ethanol (2, 1.5, 1.5, 1 g/kg i.p.) 4x @ 2 hr intervals	↓ MWM acq.; potentiation of mephedrone- induced deficits in probe trial performance (1 wk post-Tx)	potentiated mephedrone- induced ↓ DAT binding & ↓ TH levels in FC, ↓ ↓ SERT binding & TPH2 levels in, Hipp, ↑ lipid peroxidation in FC and hipp, ↓ hipp neurogenesis	ND	ND	(Ciudad-Roberts et al. 2016)
	M	mephedrone 2.5 mg/kg i.p. acute	n.c. passive avoidance (15 min post-Tx)	ND	ND	ND	(Budzynska et al. 2017)
	M	mephedrone 2.5 mg/kg i.p. + MDMA 1 mg/kg acute	↓ passive avoidance (15 min post-Tx)	ND	ND	ND	(Budzynska et al. 2017)
	F (pd)	mephedrone 150 mg/kg s.c. 1x daily for 14 d GD 5–18	↓ acq. and reference memory in MWM in 60 d old offspring	↓ cell proliferation and ↑ apoptosis in hipp of offspring	ND	ND	(Naseri et al. 2018)
	F (pd)	mephedrone 50 mg/kg s.c. 3x daily @ 2 h intervals on 6 of GD days 5–18	↓ acq. and reference memory in MWM in 60 d old offspring	↓ cell proliferation and ↑ apoptosis in hipp of offspring	ND	ND	(Naseri et al. 2018)
	?	MDPV 10 mg/kg i.p. acute (PND 7)	ND	↑ apoptosis in cortex, hipp, nucleus accumbens	ND	n.c.	(Adam et al. 2014)
	F	MDPV 30 mg/kg s.c. 4x @ 2 hr intervals	ND	n.c. striatal DAT, TH, or GFAP;	ND	↑	(Anneken et al. 2015)
	F	MDPV 30 mg/kg s.c + methylone 30 mg/kg 4x @ 2 hr intervals	ND	n.c. striatal DAT, TH, or GFAP;	ND	ND	(Anneken et al. 2015)
	F	MDPV 10–30 mg/kg s.c + METH 2.5–10 mg/kg 4x @ 2 hr intervals	ND	protection against METH-induced ↓ in striatal DAT/TH and ↑ in GFAP	ND	↑	(Anneken et al. 2015)
	F	MDPV 10–30 mg/kg s.c + D-amphetamine 5 mg/kg 4x @ 2 hr intervals	ND	protection against METH- induced ↓ in striatal DAT/TH and ↑ in GFAP	ND	ND	(Anneken et al. 2015)
	F	MDPV 1 mg/kg i.p. 4x @ 2 h intervals	ND	n.c. striatal TH or GFAP	ND	n.c.	(Miner et al. 2017)
	F	MDPV 1 mg/kg i.p. + MDMA 15 mg/kg 4x @ 2 h intervals	ND	n.c. striatal TH or GFAP	ND	↑↓	(Miner et al. 2017)
	M	methylone 30 mg/kg i.p.	↑ MWM probe trial performance (5 wks post-Tx)	ND	ND	↑	(den Hollander et al. 2013)
	F	methylone 20 mg/kg i.p. 4x @ 2 h intervals	ND	n.c. striatal TH or GFAP	ND	↑↓	(Miner et al. 2017)
	F	methylone 20 mg/kg i.p. + MDMA 15 mg/kg 4x @ 2 h intervals	ND	n.c. striatal TH or GFAP	ND	↑↓	(Miner et al. 2017)

Species	Sex	Dose	Cognitive deficits	Toxicity	Inflammation	T _B	Reference
Rat	F	methylone 30 mg/kg s.c. 4x @ 2 hr intervals	ND	n.c. striatal DAT, TH, or GFAP;	ND	↑	(Anneken et al. 2015)
	F	methylone 30 mg/kg s.c. + METH 2.5 mg/kg 4x @ 2 hr intervals	ND	enhancement of METH-induced ↓ in striatal DAT/TH and ↑ in GFAP	ND	↑	(Anneken et al. 2015)
	M	methylone 25 mg/kg s.c. 3x @ 3.5 hr intervals	ND	↓ SERT binding in FC and hipp	ND	↑	(Lopez-Arnau et al. 2014a)
	M	methylone 25 mg/kg s.c. 4x @ 3 hr intervals	ND	↓ SERT binding, ↓ TPH2 levels in FC and hipp; ↑ GFAP in hipp	ND	↑	(Lopez-Arnau et al. 2014a)
	F	methcathinone 20–40 mg/kg i.p. 4x @ 2 hr intervals	ND	↓ striatal DAT, TH ↑ striatal GFAP	ND	↑↓	(Anneken et al. 2017)
	F	methcathinone 80 mg/kg i.p. 4x @ 2 hr intervals	ND	↓ striatal DAT, TH ↑ striatal GFAP	↑ Iba1 in striatum	↓	(Anneken et al. 2018)
Rat	F	methcathinone 80 mg/kg i.p. + METH 2.5 mg/kg 4x @ 2 hr intervals	ND	↓ striatal DAT, TH ↑ striatal GFAP	↑ Iba1 in striatum	↓	(Anneken et al. 2018)
	M	mephedrone 10 or 25 mg/kg. i.p. 4x @ 2 hr intervals	ND	↓ hipp 5-HT uptake ↓ striatal DA uptake	ND	ND	(Hadlock et al. 2011)
	M	mephedrone 30 mg/kg i.p. 1x daily for 10 d	↓ novel object exploration (5 wk post-Tx)	↓ 5-HT metab. in hipp, striatum	n.c. TPPO binding in hipp, striatum	ND	(Motbey et al. 2012)
	M	mephedrone 0.3 mg/kg/inf IVSA for 10 d	ND	n.c. in DAT or SERT binding in various regions	non-sig. ↑ TPPO binding in various regions	ND	(Motbey et al. 2013)
	M	mephedrone 30 mg/kg i.p. 2x daily for 4 d	ND	↓ SERT binding in hipp	ND	n.c.	(den Hollander et al. 2013)
	M	mephedrone 25 mg/kg s.c. 3x @ 2 hr intervals for 2 d	n.c. MWM acq. (1 wk post-Tx); ↓ ref. memory in probe trial	↓ DAT binding and TH levels in FC ↓ SERT binding and TPH2 levels, and ↑ antioxidant enzyme levels in FC, striatum, and hipp; ↑ lipid in peroxidation in striatum	ND	↑	(Lopez-Arnau et al. 2015)
	F	mephedrone 10 mg/kg i.p. 2 d/wk for 30 wk	↑ errors in operant response sequence task; further ↑ by 17β-estradiol (Tx during behavioral testing)	ND	ND	ND	(Weed et al. 2014)
	M	mephedrone 5 mg/kg i.p. acute	ND	↑ DNA oxidation in cerebral cortex	ND	ND	(Kaminska et al. 2018)
	M	mephedrone 5 mg/kg i.p. 1x daily for 4 d (repeated 1x after 3 d drug free)	ND	↑ DNA oxidative in cerebral cortex	ND	ND	(Kaminska et al. 2018)
	M	MDPV 0.03 mg/kg/inf i.v. self-administered 96-h sessions for 5 wk	↓ novel object recognition; n.c. object placement memory (3 wks post self-admin)	↑ neurodegeneration in entorhinal and	ND	ND	(Sewalia et al. 2018)
Rat	M	methylone 30 mg/kg i.p. 2x daily for 4 d	ND	↓ SERT binding in FC, hipp	ND	↑	(den Hollander et al. 2013)

Species	Sex	Dose	Cognitive deficits	Toxicity	Inflammation	T _B	Reference
	M	methyline 20 mg/kg s.c. 4x @ 3 hr intervals	n.c. MWM acq. (1 wk post-Tx); ↓ reference memory in probe trial	↓ SERT binding and TPH2 levels in striatum, FC, hipp; ↑ GFAP in FC	ND	↑	(Lopez-Arnau et al. 2014b)
	M/F	methyline 8 mg/kg i.p. 1x daily for 10 d	↓ spatial memory in Y-maze test (40–50 d post-Tx)	ND	ND	ND	(Daniel and Hughes 2016)
Monkey	M	mephedrone 0.32–0.56 mg/kg i.m. acute	↑ visuospatial ND memory; n.c. spatial working memory (Tx immediately prior to testing)	ND	ND	ND	(Wright et al. 2012)

Abbreviations: ↑, increased; ↓, decreased; ↑↓, biphasic effect; 5-HT, 5-hydroxytryptamine (serotonin); acq., acquisition; alt., alternation; DA, dopamine; DAT, dopamine transporter; F, female; FC, frontal cortex; GD, gestational date; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adaptor molecule 1; LDH, lactate dehydrogenase; M, male; MDMA, methylenedioxymethamphetamine; MDPV, methylenedioxypyrovalerone; Morris water maze, MWM; n.c., no change; ND, not determined; pd, pregnant dam; SERT, serotonin transporter; TPH2, tryptophan hydroxylase 2; TSPO, 18 kDa translocator protein; Tx, treatment

Table 3.

In vitro studies demonstrating evidence of neurotoxicity by cathinone derivatives in various cell lines.

Cell type	Drug	Neurotoxicity	Reference
SH-SY5Y (dopaminergic neuron)	mephedrone	↑ LDH release (>500 µM) ↓ MT activity (1 mM) ↑ redox activity	(den Hollander et al. 2014)
	mephedrone	↑ LDH release (1 mM) ↑ ROS production (1 mM) ↑ oxygen consumption rate (1 mM) ↑ p53 levels (1 mM) ↑ TNFα levels (1 mM)	(den Hollander et al. 2015)
	pyrovalerone	↓ cell viability (200 µM) ↓ MT function (200 µM)	(Wojcieszak et al. 2016)
	MDPV	↓ cell viability (100 µM) ↓ MT function (100 µM)	(Wojcieszak et al. 2016)
		↑ LDH release (1 mM) ↓ proliferation (250 µM) ↑ apoptosis (250 µM) ↑ ROS production (10 µM)	(Rosas-Hernandez et al. 2016)
		↑ autophagy (>750 µM) ↑ ROS/RNS production (>1 mM) ↑ apoptosis (>1 mM)	(Valente et al. 2017a)
		↑ cell death (EC ₅₀ =1.5 mM) ↑ LDH release (EC ₅₀ =1.5 mM) ↑ ROS/RNS production (>1.2 mM) ↑ apoptosis (>1.2 mM) ↑ oxidized glutathione (>1.2 mM) ↓ reduced glutathione (>1.2 mM)	(Valente et al. 2017b)
	methylone	↑ LDH release (>1 mM)	(den Hollander et al. 2014; den Hollander et al. 2015)
		↑ autophagy (>1 mM) ↑ ROS/RNS production (>1 mM) ↑ apoptosis (>1 mM)	(Valente et al. 2017a)
		↑ cell death (EC ₅₀ =2.5 mM) ↑ LDH release (EC ₅₀ =3.2 mM) ↑ ROS/RNS production (>1.9 mM) ↑ apoptosis (>1.9 mM)	(Valente et al. 2017b)
cultured cortical neurons	α-PVT	↓ cell viability (25 µM) ↓ MT function (25 µM)	(Wojcieszak et al. 2016)
	α-PV9	↓ cell viability (200 µM) ↓ MT function (200 µM) ↑ LDH release (200 µM)	(Wojcieszak et al. 2016)
	mephedrone	↓ cell viability (>100 µM)	(Martinez-Clemente et al. 2014)
	methylone	↓ cell viability (>300 µM)	(Lopez-Arnau et al. 2014a)
HT22 (neuronal)	3-fluoro-methcathinone	↓ cell viability (1 mM) ↓ cell cycle arrest (1 mM) ↑ ROS production (1 mM) ↑ autophagy (1 mM) ↑ apoptosis (1 mM)	(Siedlecka-Kroplewska et al. 2014) (Siedlecka-Kroplewska et al. 2018)
SK-N-SH (neuronal)	α-PNP	↓ cell viability (10 µM) ↑ ROS production (50 µM) ↑ lipid peroxidation (50 µM) ↑ apoptosis (10 µM) ↑ autophagy (10 µM)	(Matsunaga et al. 2017)
TGW (neuronal)	α-PNP	↓ cell viability (20 µM)	(Matsunaga et al. 2017)
pulmonary	α-PNP	↓ cell viability (10 µM)	(Matsunaga et al. 2017)

Cell type	Drug	Neurotoxicity	Reference
kidney	α -PNP	↓ cell viability (20 μ M)	(Matsunaga et al. 2017)
PC12	Cathinone phthalimide	↑ LDH release (10 μ M) ↓ MT function (1 mM)	(Lantz et al. 2017)
aortic smooth muscle	α -PNP	↓ cell viability (20 μ M)	(Matsunaga et al. 2017)
endothelial cells	pyrovalerone	↓ cell viability (>30 μ M) ↓ MT function (>100 μ M)	(Wojcieszak et al. 2016)
	MDPV	↓ cell viability (>30 μ M) ↓ MT function (>100 μ M) ↑ LDH release (>100 μ M) ↑ LDH release (500 μ M) ↓ proliferation (1 mM) ↑ ROS production (500 μ M)	(Wojcieszak et al. 2016) (Rosas-Hernandez et al. 2016)
	α -PVT	↓ cell viability (25 μ M) ↓ MT function (25 μ M)	(Wojcieszak et al. 2016)
	α -PV9	↓ cell viability (100 μ M) ↓ MT function (100 μ M) ↑ LDH release (100 μ M)	(Wojcieszak et al. 2016)
	α -PNP	↓ cell viability (5 μ M)	(Matsunaga et al. 2017)
intestinal cells	α -PNP	↓ cell viability (20 μ M)	(Matsunaga et al. 2017)
hepatocytes	mephedrone	↑ cell death (2 mM) ↑ ROS production (1 mM) ↓ MT function (1 mM)	(Luethi et al. 2017)
		↓ cell viability (EC ₅₀ =1.3 mM)	(Gaspar et al. 2018)
	pyrovalerone	↓ cell viability (300 μ M) ↓ MT function (300 μ M)	(Wojcieszak et al. 2016)
	MDPV	↓ cell viability (>100 μ M) ↓ MT function (>100 μ M) ↑ LDH release (>100 μ M)	(Wojcieszak et al. 2016)
		↑ cell death (EC ₅₀ =0.7 mM)	(Araujo et al. 2015)
		↑ cell death (2 mM) ↑ ROS production (2 mM) ↓ MT function (1 mM)	(Luethi et al. 2017)
		↓ cell viability (200 μ M) ↑ LDH release (200 μ M) ↑ ROS/RNS production (1.6 mM) * ↑ apoptosis (1.6 mM) *	(Valente et al. 2016)
		↓ cell viability (200 μ M) ↑ LDH release (400 μ M)	(Silva et al. 2016)
	methcathinone	↓ cell viability (EC ₅₀ =5.8 mM)	(Gaspar et al. 2018)
	methylone	↑ cell death (EC ₅₀ =1.2 mM)	(Araujo et al. 2015)
		↑ cell death (2 mM)	(Luethi et al. 2017)
	methedrone	↑ cell death (2 mM)	(Luethi et al. 2017)
	naphyrone	↑ cell death (0.5 mM) ↑ ROS production (200 μ M) ↓ MT function (200 μ M)	(Luethi et al. 2017)
	pentedrone	↑ cell death (EC ₅₀ = 0.7 mM)	(Araujo et al. 2015)
	buphedrone	↓ cell viability (EC ₅₀ =2.9 mM)	(Gaspar et al. 2018)
	4-MEC	↑ cell death (EC ₅₀ =1.3 mM)	(Araujo et al. 2015)
		↓ cell viability (EC ₅₀ =1.5 mM)	(Gaspar et al. 2018)
	4-MDMC	↓ cell viability (EC ₅₀ =0.8 mM)	(Gaspar et al. 2018)
	4-MDEC	↓ cell viability (EC ₅₀ =5.4 mM)	(Gaspar et al. 2018)

Cell type	Drug	Neurotoxicity	Reference
	3,4-DMMC	↓ cell viability (EC ₅₀ =1.2 mM)	(Gaspar et al. 2018)
	DEC	↓ cell viability (EC ₅₀ =6.6 mM)	(Gaspar et al. 2018)
	DEB	↓ cell viability (EC ₅₀ =1.8 mM)	(Gaspar et al. 2018)
	DEP	↓ cell viability (EC ₅₀ =1.5 mM)	(Gaspar et al. 2018)
	DMC	↓ cell viability (EC ₅₀ =7.8 mM)	(Gaspar et al. 2018)
	DMB	↓ cell viability (EC ₅₀ =1.2 mM)	(Gaspar et al. 2018)
	DMP	↓ cell viability (EC ₅₀ =1.1 mM)	(Gaspar et al. 2018)
	NEC	↓ cell viability (EC ₅₀ =2 mM)	(Gaspar et al. 2018)
	NEB	↓ cell viability (EC ₅₀ =1.2 mM)	(Gaspar et al. 2018)
	NEP	↓ cell viability (EC ₅₀ =1.5 mM)	(Gaspar et al. 2018)
	MPPP	↓ cell viability (EC ₅₀ =2.3 mM)	(Gaspar et al. 2018)
	α-PBP	↓ cell viability (EC ₅₀ =4.9 mM)	(Gaspar et al. 2018)
	α-PPP	↓ cell viability (EC ₅₀ =6.2 mM)	(Gaspar et al. 2018)
	α-PVP	↓ cell viability (EC ₅₀ =2 mM)	(Gaspar et al. 2018)
	α-PNP	↓ cell viability (20 μM)	(Matsunaga et al. 2017)
	α-PVT	↓ cell viability (1 μM) ↓ MT function (>1 μM)	(Wojcieszak et al. 2016)
	α-PV9	↓ cell viability (>25 μM) ↓ MT function (>25 μM) ↑ LDH release (200 μM)	(Wojcieszak et al. 2016)

Concentration at which statistically significant effects were observed as compared to untreated cells are provided in parentheses.

* denotes concentrations where effects observed under normothermic conditions that were also observed at lower concentration under hyperthermic conditions. Abbreviations: α-PNP, α-pyrrolidinononanophenone; α-PVT; α-pyrrolidinovalerothiophenone; α-PV9, α-pyrrolidinooctanophenone; α-PBP, α-pyrrolidinobutiophenone; α-PPP, α-pyrrolidinopropiophenone; 3,4-dimethylmethcathinone (3,4-DMMC); 4-MDEC, 4-methyldiethylcathinone; 4-MDMC, 4-methyldimethcathinone; 4-MEC, 4-ethylethcathinone; DEB, diethylbuphedrone; DEP, diethylpentedrone; DMB, dimethylbuphedrone; DMC, dimethylcathinone; DMP, dimethylpentedrone, EC₅₀, effective concentration 50; LDH, lactose dehydrogenase; MPPP, 4-methyl-α-pyrrolidinopropiophenone; MT, mitochondria; ND, not determined; NEB, N-ethylbuphedrone; NEC, N-ethylcathinone; NEP, N-ethylpentedrone; RNS, reactive nitrogen species; ROS, reactive oxygen species.