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Locomotor Stimulant and Discriminative Stimulus Effects of “Bath Salt” Cathinones

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Abstract

A number of psychostimulant-like cathinone compounds are being sold as “legal” alternatives to methamphetamine or cocaine. The purpose of these experiments was to determine whether cathinone compounds stimulate motor activity and have discriminative stimulus effects similar to cocaine and/or methamphetamine. 3,4-Methylenedioxypyrovalerone (MDPV), methylone, mephedrone, naphyrone, flephedrone and butylone were tested for locomotor stimulant effects in mice and subsequently for substitution in rats trained to discriminate cocaine (10 mg/kg, i.p.) or methamphetamine (1 mg/kg, i.p.) from saline. All compounds fully substituted for the discriminative stimulus effects of cocaine and methamphetamine. Several commonly marketed cathinones produce discriminative stimulus effects comparable to those of cocaine and methamphetamine, which suggests that these compounds are likely to have similar abuse liability. MDPV and naphyrone produced locomotor stimulant effects that lasted much longer than cocaine or methamphetamine and therefore may be of particular concern, particularly since MDPV is one of the most commonly found substances associated with emergency room visits due to adverse effects from taking “bath salts”.

Keywords

cathinones; drug discrimination; locomotor activity; abuse liability; mouse; rat

Introduction

Cathinones are a class of compounds derived from the active compound in khat, a mild stimulant widely used in the Middle East. The compounds have chemical structures similar to those of the amphetamines, and so may potentially share the psychostimulant effects of the amphetamines. There has been increasing use of synthetic cathinones quasi-legally marketed as “plant food” or “bath salts” in Europe and the United States (NDIC, 2011). In 2011, the Drug Enforcement Agency identified a group of cathinones of “great concern” for which they requested information, and obtained temporary scheduling for three compounds they identified as being of most concern: methylenedioxypyrovalerone (MDPV), methylone, and mephedrone (DEA, 2011).

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The molecular activity of these compounds is similar to those of other psychostimulants. Mephedrone, butylone, naphyrone and methylone inhibited serotonin and dopamine transporters and reduced uptake of serotonin and dopamine (Cozzi et al., 1999; Meltzer et al., 2006; Hadlock et al., 2011; López-Arnau et al., 2012; Martínez-Clemente et al., 2012). One report indicated that all 3 compounds inhibited the VMAT-2 as well (López-Arnau et al., 2012), but another reported only weak inhibition of VMAT by methylone (Cozzi et al., 1999). Mephedrone and methylone increase the release of dopamine, norepinephrine and serotonin (Nagai et al., 2007; Kehr et al., 2011; Baumann et al., 2012), although repeated dosing produced depletions of serotonin in cortex and striatum similar to that produced by MDMA (Nagai et al., 2007; Hadlock et al., 2011; Baumann et al., 2012). Taken together, these findings suggest that these compounds may produce behavioral effects similar to abused psychostimulants.

Unfortunately, there is a paucity of behavioral studies on many of these molecules. Mephedrone is one that has been more thoroughly studied. Mephedrone increased locomotor activity (Nagai et al., 2007; Kehr et al., 2011; Baumann et al., 2012; Lisek et al., 2012; López-Arnau et al., 2012; Motbey et al., 2012) and wheel running (Huang et al., 2012). Mephedrone also induced CPP (Lisek et al., 2012) and maintained self-administration (Hadlock et al., 2011). These results are quite similar to those of known psychostimulants; however, mephedrone decreased social interaction (Motbey et al., 2012).

Of the other cathinones that have been assessed, methylone and butylone also increased locomotor activity, which was blocked by various dopaminergic antagonists, as was the locomotor stimulant effects of mephedrone (Baumann et al., 2012; Lisek et al., 2012; López-Arnau et al., 2012). MDPV also increased wheel running (Huang et al., 2012). Only methylone has been assessed in drug discrimination. It fully substituted for the discriminative stimulus effects of amphetamine and MDMA, but not of DOM, a serotonergic hallucinogen (Dal Cason et al., 1997).

The purpose of the present study was to characterize the potential abuse liability of several cathinone compounds identified by the DEA as of particular concern (DEA, 2011). Abuse liability testing involves assessing the subjective and reinforcing effects of drugs; that is, do potential drugs of abuse produce discriminative and/or reinforcing stimulus effects similar to those of known drugs of abuse. The drug discrimination assay is a useful animal model for studying the perceptions of drug effects (Balster, 1991). Another approach is to examine other features/aspects of potential drugs of abuse and compare them to known drugs of abuse. For example, abused psychostimulants, such as cocaine and methamphetamine, are so-named because they produce characteristic increases in excitation and locomotor activity. Of course, not all compounds that increase motor activity are abused; however, screening for increased locomotor activity is “permissive” in that all cocaine/amphetamine-like compounds do increase locomotor activity, so a compound which fails to alter locomotor activity has a much reduced chance of being an abused substance.

Initially, to better understand the active time course and dose ranges of these compounds, 8-hour tests for locomotor stimulant effects were conducted using multiple doses. The

discriminative stimulus effects of these cathinones were tested in rats trained to discriminate cocaine or methamphetamine from saline.

Methods

Subjects

Male Swiss–Webster mice were obtained from Harlan (Indianapolis, IN) at approximately 8 weeks of age and tested at approximately 10 weeks of age. Mice were group housed in cages on a 12:12-h light/dark cycle and were allowed free access to food and water. Male Sprague-Dawley rats were obtained from Harlan-Sprague Dawley (Indianapolis, IN). All rats were housed individually and were maintained on a 12:12 light/dark cycle (lights on at 7:00 AM). Body weights were maintained at 320–350 g by limiting food to 15 g/day which included the food received during operant sessions. Water was readily available. All housing and procedures were in accordance with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and were approved by the University of North Texas Health Science Center Animal Care and Use Committee.

Locomotor Activity

The study was conducted using 40 Digiscan (model RXYZCM, Omnitech Electronics, Columbus, OH) locomotor activity testing chambers (40.5 X 40.5 X 30.5 cm) housed within sound-attenuating chambers in sets of two. A panel of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. A 7.5-W incandescent light above each chamber provided dim illumination and fans provided an 80-dB ambient noise level within the chamber.

Separate groups of 8 mice were injected with either vehicle (0.9% saline) or a test compound: methamphetamine (0.5, 1, 2 or 4 mg/kg); cocaine (5, 10, 20 or 40 mg/kg); methylone or butylone (1, 3, 10 or 30 mg/kg); MDPV, mephedrone or flephedrone (0.3, 1, 3, 10 or 30 mg/kg); or naphyrone (3, 10, 30 or 100 mg/kg), immediately prior to locomotor activity testing. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 hours within 10-min periods, beginning at 0800 hrs (1 hr after lights on). Behavioral observations were recorded on each mouse at 30, 120 and 480 minutes following 30 mg/kg (methylone, MDPV, mephedrone, butylone, and flephedrone) or 100 mg/kg (naphyrone).

Discrimination Procedures

Standard behavior-testing chambers (Coulbourn Instruments, Allentown, PA) were connected to IBM-PC compatible computers via LVB interfaces (Med Associates, East Fairfield, VT). The computers were programmed in Med-PC for Windows, version IV (Med Associates, East Fairfield, VT) for the operation of the chambers and collection of data.

Using a two-lever choice methodology, a pool of rats previously trained to discriminate either methamphetamine (1 mg/kg) or cocaine (10 mg/kg) from saline as previously described (Gatch et al., 2011) were tested. Rats received an injection of either saline or drug

and were subsequently placed in the behavior-testing chambers, where food (45 mg food pellets; Bio-Serve, Frenchtown, NJ) was available as a reinforcer for every ten responses on a designated injection-appropriate lever. The pretreatment time was 10 min. Each training session lasted a maximum of 10 min, and the rats could earn up to 20 food pellets. The rats received approximately 60 of these sessions before they were used in tests for substitution of the experimental compounds. Rats were used in testing once they had achieved 9 of 10 sessions at 85% injection-appropriate responding for both the first reinforcer and total session. The training sessions occurred on separate days in a double alternating fashion (drug-drug-saline-saline-drug; etc.) until the training phase was complete, after which substitution tests were introduced into the training schedule such that at least one saline and one drug session occurred between each test (drug-saline-test-saline-drug-test-drug; etc.). The substitution tests occurred only if the rats had achieved 85% injection-appropriate responding on the two prior training sessions.

Test sessions lasted for a maximum of 20 min. In contrast with training sessions, both levers were active, such that 10 consecutive responses on either lever led to reinforcement. Data were collected until the first reinforcer was obtained, or for a maximum of 20 min. Each compound was tested in groups of six rats. A repeated-measures design was used, such that each rat was tested at all doses of a given drug. Intraperitoneal injections (1 ml/kg) of saline, methylone (0.5 – 5 mg/kg), mephedrone (0.5 – 5 mg/kg), MDPV (0.05 – 2.5 mg/kg), naphyrone (0.5 – 5 mg/kg), or butylone (0.5 – 10 mg/kg) occurred 15 min prior to the start of the test session. Flephedrone (0.5 – 10 mg/kg) was administered 60 min prior to the start of the test session.

Drugs

(-)-Cocaine hydrochloride, (+)-methamphetamine hydrochloride, methylone (3,4-methylenedioxy-*N*-methylcathinone hydrochloride), MDPV (3,4-methylenedioxypyrovalerone hydrochloride), mephedrone (4-methylmethcathinone hydrochloride), butylone (β -keto-*N*-methylbenzodioxolylbutanamine hydrochloride), flephedrone (4-fluoromethcathinone hydrochloride) and naphyrone (naphthylpyrovalerone hydrochloride) were provided by the National Institute on Drug Abuse Drug Supply Program. All drugs were dissolved in 0.9% saline and were administered i.p. in a volume of 1 ml/kg. Dose increments were based on 1/3 logs.

Data Analysis

Drug discrimination data are expressed as the mean percentage of drug-appropriate responses occurring in each test period. Rates of responding were expressed as a function of the number of responses made divided by the total session time. Graphs for percent drug-appropriate responding and response rate were plotted as a function of dose of test compound (log scale). Percent drug-appropriate responding was shown only if at least 3 rats completed the first fixed ratio. Full substitution was defined as >80% drug-appropriate responding and not statistically different from the training drug.

The potencies of methylone, mephedrone, MDPV, naphyrone, or butylone were calculated by fitting straight lines to the dose-response data for each compound by means of

TableCurve 2D (Jandel Scientific, San Rafael, CA). Straight lines were fitted to the linear portion of dose-effect curves, including not more than one dose producing <20% of the maximal effect and not more than one dose producing >80% of the maximal effect. Other doses were excluded from the analyses. Differences among ED50 values were tested by one-way ANOVA followed by Tukey's test to compare individual means. Rates of responding were expressed as a function of the number of responses made divided by the total session time. Response rate data was analyzed by one-way repeated-measures analysis of variance. Effects of individual doses were compared to the vehicle control value using a priori contrasts. The criterion for significance was set a priori at $p < 0.05$.

Locomotor activity data were expressed as the mean number of photocell counts in the horizontal plane (ambulation counts) during each 10-min period of testing. A 30-min period, beginning when maximal stimulation of locomotor activity first appeared as a function of dose, was used for analysis of dose-response data and calculation of ED50 values. TableCurve 2D was used to estimate the peak ambulation under each cathinone. The ED50 values were then calculated by estimating the dose producing $\frac{1}{2}$ of the peak ambulation from the ascending linear portion of the dose response curve. A two-way repeated -measures analysis of variance was conducted on horizontal activity counts/10 min interval. A one-way analysis of variance was conducted on horizontal activity counts for the 30-min period of maximal effect, and planned comparisons were conducted for each dose against saline control using single degree-of-freedom F tests.

Correlations between behavioral data and binding/functional data (from Eshleman et al., 2013) were performed by least squares regression analysis using Excel 2007. ED50 values for locomotor activity, substitution for cocaine or methamphetamine, and cocaine/methamphetamine potency ratio from the present study were compared with receptor binding (K_i), IC50 of the inhibition of neurotransmitter uptake, EC50 of release of neurotransmitter, and percent maximum release for butylone, 4-FMC, MDPV, mephedrone, methylone and naphyrone at DAT, SERT, NET and VMAT; and with binding to 5-HT1A, 5-HT2A and 5-HT2C receptors (K_i), EC50 and percent maximum stimulation of 5-HT1A receptor and IC50 and percent maximum inhibition of 5-HT2A and 5-HT2C receptor signaling.

Results

Locomotor Activity

Figures 1 and 2 show average horizontal activity counts/10 min as a function of time (0-8 hr) and dose of each test compound. Figure 3 shows dose-effect curves for each compound at their time of peak effect, and Table 1 shows the ED50 values. Each compound showed increases in locomotor activity as dose increased, up a maximal effect, whereupon higher doses produced sharp decreases in locomotor activity. The 2-way analysis of variance performed on data for each compound yielded a significant main effect of Treatment, as well as a Treatment x Time Period interaction (all $ps < 0.01$)

MDPV—MDPV produced time- and dose-dependent stimulation of locomotor activity in doses from 0.3 to 30 mg/kg (Fig. 1). Stimulant effects of 1 and 3 mg/kg MDPV occurred

within 10 minutes following injection and lasted 190 minutes. Stimulant effects lasted 250 min following 10 mg/kg, whereas following 30 mg/kg, stimulant effects did not occur until 80 min after administration and lasted 300 min. MDPV depressed locomotor activity between 10 to 50 minutes following injection of 30 mg/kg. During the 30-minute time period in which maximal stimulant effects first occurred (10 to 40 minutes following injection), significant stimulant effects (ED_{50} of 1.26 ± 0.08 mg/kg) occurred following 1, 3 and 10 mg/kg (Fig. 3).

Mephedrone—Treatment with 3 and 10 mg/kg mephedrone resulted in stimulant effects that occurred within 10 minutes following injection and lasted 40 to 60 minutes (Fig. 1). Based on the 30-minute time period in which maximal stimulant effects first occurred (0 to 30 minutes following injection) significant effects (ED_{50} of 1.38 ± 1.22 mg/kg) were observed following 3 and 10 mg/kg (Fig. 3).

Flephedrone—Treatment with 10 mg/kg flephedrone caused an initial depression of locomotor activity (between 10 and 30 min), followed by stimulation (Fig. 1). The depressant effect of 10 mg/kg occurred within 20 minutes following injection and lasted 20 minutes. The stimulant effect occurred within 50 minutes following injection and lasted 90 minutes. For the 30-minute time period in which maximal stimulant effects first occurred (40 to 70 minutes following injection), significant stimulant effects (ED_{50} = 2.04 ± 0.35 mg/kg) occurred only following 10 mg/kg (Fig. 3).

Methamphetamine—Treatment with 0.5 and 2 mg/kg methamphetamine resulted in stimulant effects that occurred within 10 minutes following injection and lasted 140 to 210 minutes (Fig. 1). Methamphetamine depressed locomotor activity between 30 to 60 minutes following administration of 4 mg/kg. Stimulant effects did not occur until 90 min after administration of 4 mg/kg and lasted 270 min. For the 30-minute time period in which maximal stimulant effects first occurred (20 to 50 minutes following injection), significant stimulant effects (ED_{50} = 0.30 ± 0.40 mg/kg) occurred following 0.5 and 2 mg/kg (Fig. 3).

Naphyrone—Naphyrone resulted in time- and dose-dependent stimulation of locomotor activity in doses from 10 to 100 mg/kg (Fig. 2). Stimulant effects of 10 and 30 mg/kg occurred within 10 minutes following injection and lasted 110 to 280 minutes. Naphyrone depressed locomotor activity between 10 to 50 minutes following injection of 100 mg/kg. Stimulant effects of 100 mg/kg did not occur until 80 min after administration and lasted close to 8 hr. Based on the 30-minute time period in which maximal stimulant effects occurred (0 to 30 minutes following injection), significant effects (ED_{50} = 8.91 ± 0.09 mg/kg) were observed following 10 and 30 mg/kg (Fig. 3).

Methylone—Treatment with methylone resulted in time- and dose-dependent stimulation of locomotor activity in doses from 3 to 30 mg/kg (Fig. 2). Stimulant effects of 3 and 10 mg/kg occurred within 10 minutes following injection and lasted 60 to 120 minutes. Stimulant effects of 30 mg/kg fluctuated during the first 30 min (but were significantly different from vehicle control) and lasted 180 min. Significant stimulant effects during the time in which maximal stimulant effects first occurred (0 to 30 minutes following injection)

were observed following 3, 10 and 30 mg/kg (Fig. 3) and an ED_{50} of 1.48 ± 0.07 mg/kg was calculated.

Butylone—Butylone produced time- and dose-dependent stimulation of locomotor activity in doses from 10 to 30 mg/kg (Fig. 2). Stimulant effects of 10 mg/kg occurred within 10 minutes following injection and lasted 130 minutes. Stimulant effects of 30 mg/kg fluctuated during the first 30 min (but were not significantly different from vehicle control) and lasted 190 min. Based on the 30-minute time period in which maximal stimulant effects first occurred (0 to 30 minutes following injection), significant effects (ED_{50} of 2.57 ± 0.21 mg/kg) were observed following 10 mg/kg (Fig. 3).

Cocaine—Treatment with cocaine resulted in time- and dose-dependent stimulation of locomotor activity following 10 to 40 mg/kg (Fig. 2). Stimulant effects of 10, 20 and 40 mg/kg occurred within 10 minutes following injection and lasted 120 to 170 minutes. Based on the 30-minute time period in which maximal stimulant effects occurred (0 to 30 minutes following injection), significant effects ($ED_{50} = 7.24 \pm 0.14$ mg/kg) were observed following 10, 20 and 40 mg/kg (Fig. 3).

Discrimination

Cocaine-trained rats—Each of the cathinones fully substituted for the discriminative stimulus effects of cocaine (Fig. 4). ED_{50} values differed across test drugs [$F(5,30) = 97.003$, $p < 0.001$] and are shown in Table 1. Rank order of potency in cocaine-trained rats was MDPV = methylone = mephedrone > naphyrone = flephedrone > butylone. None of the compounds produced a significant overall effect on response rate.

Methamphetamine-trained rats—Each of the cathinones also fully substituted for the discriminative stimulus effects of methamphetamine (Fig. 5). ED_{50} values differed across test drugs [$F(5,30) = 18.823$, $p < 0.001$] and are shown in Table 1. Rank order of potency in methamphetamine-trained rats was MDPV = mephedrone > butylone = methylone = flephedrone = naphyrone. Mephedrone decreased response rate following 2.5 and 5 mg/kg, with the maximum effect (62% of vehicle control) following 5 mg/kg mephedrone [$F(4,20) = 5.31$, $p = .004$]. None of the other compounds produced a significant overall effect on response rate.

Correlations

Potency of these cathinones at inhibiting uptake of norepinephrine at VMAT2 from a recent study (Eshleman et al., 2013) correlated with their potency at stimulating locomotor activity in the present study ($r^2 = 0.72$, $p = 0.033$). Binding or function (uptake inhibition or release) of DAT, NET, and SERT did not correlate with potency in stimulating locomotor activity. Binding or activation of 5-HT1A, 5-HT2A or 5-HT2C receptors also failed to correlate with potency in stimulating locomotor activity.

Differences in potency among the cathinones at substituting for cocaine in the present study were predicted by their potency at activating 5-HT1A signaling in the Eshleman study ($r^2 = 0.7$, $p = 0.038$); similarly, differences in potency of substituting for methamphetamine

were predicted by efficacy of release of norepinephrine at VMAT2 ($r^2=0.76$, $p=0.023$). Further, in the present study, potency of the cathinones at substituting for cocaine did not correlate well with their potency at substituting for methamphetamine ($r^2=0.45$, $p=0.142$). However, the ratio of the potencies for methamphetamine and cocaine substitution by the cathinones correlated with their potency at activating 5-HT_{1A} signaling in the Eshleman study ($r^2=0.66$, $p=0.049$). Binding or function (uptake inhibition or release) of DAT, NET, and SERT did not correlate with potency in substitution for cocaine or methamphetamine or with the cocaine/methamphetamine potency ratio. Binding or activation of 5-HT_{2A} or 5-HT_{2C} receptors also failed to correlate with potency in discriminative stimulus activity.

Discussion

In the present study, a group of synthetic cathinones (methyldone, mephedrone, MDPV, naphyrone, flephedrone and butylone) flagged for study by the DEA all produced locomotor stimulant effects similar to those of methamphetamine, and produced discriminative stimulus effects similar to both cocaine and methamphetamine. These findings agree with earlier findings that methyldone, mephedrone, MDPV and butylone increased locomotor activity (Baumann et al., 2012; Huang et al., 2012; Lisek et al., 2012; López-Arnau et al., 2012), and that methyldone fully substituted for the discriminative stimulus effects of the psychostimulant amphetamine (Dal Cason et al., 1997). Taken together, these findings suggest that these compounds produce behavioral effects similar to cocaine and methamphetamine and that they may share the abuse liability of these two abused psychostimulants.

These findings are in agreement with earlier studies that concluded that the pharmacological activity of these compounds is similar to those of other psychostimulants, such as blockade of monoaminergic transporters and/or increased release of monoamines (e.g., Cozzi et al., 1999; Meltzer et al., 2006; Nagai et al., 2007; Baumann et al. 2012). In addition, a recent study thoroughly characterized the receptor mechanisms of each of the six cathinone compounds tested in the present study (Eshleman et al., 2013). MDPV, butylone and naphyrone were monoamine uptake inhibitors (similar to cocaine), whereas methyldone, mephedrone and flephedrone were monoamine releasers (similar to methamphetamine). The cathinone compounds had no direct DA receptor effects and only weak effects at the vesicular monoamine transporter 2 (VMAT2); but did have some serotonin receptor effects: they were agonists at 5-HT_{1A} and antagonists at 5-HT_{2A} and 2C receptors (Eshleman et al., 2013).

In the present study, differences in potency among the cathinones in producing locomotor stimulant effects or substitution for methamphetamine were predicted by potency of VMAT effects in the Eshleman study. Difference in potency of substitution for cocaine and the cocaine/methamphetamine potency ratio were predicted by potency at activation of 5-HT_{1A} receptors. However, the potency or efficacy of monoamine activity did not predict differences in potency of the cathinones at producing behavioral effects in the present study. It should be noted that these correlations predict differences in potencies of the various cathinones. Changes in monoamine levels via release or uptake at the membrane transporters are likely important mechanisms for the behavioral effects of these cathinones as for other

psychostimulants. It is likely that reaching a threshold level of release or uptake is sufficient to produce the behavioral effects; subtle differences do not seem to be important. In contrast, 5-HT1A receptor and VMAT2 activity may contribute to individual differences in potency of these cathinones. Confirmation of these putative roles will require direct antagonism studies of the behavioral effects, and their generality across cathinones will require further study.

Different species were used for the locomotor activity (mice) and discrimination assays (rats), based on ease of use and the availability of existing literature. Despite the physiological differences between the species, the murine locomotor activity data have been excellent at predicting dose ranges and pretreatment times for rat drug discrimination. In the present study, the locomotor activity assay was a good predictor for dose range in the drug discrimination assay, although naphyrone was less potent at stimulating locomotor activity than at producing cocaine- and methamphetamine-like discriminative stimulus effects. However, magnitude of the locomotor stimulant effect does not seem to be a good predictor of abuse liability, since mephedrone produced relatively modest increases in locomotor activity, yet produced full substitution for both cocaine and methamphetamine, and also induced a conditioned place preference (Lisek et al., 2012) and maintained self-administration (Hadlock et al., 2011).

What may be of more interest in the present data set is the duration of the locomotor stimulant effects. The present study tested extended (8 hr) time courses of a full dose range of these compounds. Acute increases in psychostimulant effects were observed as noted in the earlier studies; but large doses of some compounds had very long acting effects and dose effect functions for all compounds were biphasic—showing a descending limb at high doses. MDPV, similar to methamphetamine, produced long-lasting stimulant effects of 5 to 6 hours. The top dose of naphyrone (100 mg/kg) did not produce stimulant effects for two hours, but those effects lasted until nearly 8 hours after administration. In fact, MDPV and naphyrone both produced depressant effects for nearly an hour after administration. The other compounds produced a similar pattern, although the degree of depression was not so large, and the extension of the time course of stimulation not nearly so large as for MDPV, naphyrone and methamphetamine.

There have been anecdotal reports in the news media of extremely long-lasting and unpleasant effects of some of these compounds. Unfortunately it is impossible to tell exactly what compounds illicit users have taken; however, MDPV is one of the most commonly found compounds in samples of “bath salts” (Spiller et al., 2011). The long time course of MDPV combined with the substantial depressant effects may account for the frequency of emergency room visits by users of MDPV-containing “bath salts”.

The present findings indicate that these six synthetic cathinones produce locomotor stimulant and discriminative stimulus effects similar to abused psychostimulants. Further, the long time courses of MDPV and naphyrone suggest that uncontrolled use of those compounds may be hazardous. Initial conditioned place preference studies conducted in our laboratory suggest that these cathinones can produce reward-like stimuli (data not published), and other studies have reported that mephedrone produced a conditioned place

preference (Lisek et al., 2012) and maintained self-administration (Hadlock et al., 2011). Confirmation that animals will self-administer the remainder of these compounds is essential, but taken together, these data suggest that these compounds have similar abuse liability as cocaine and methamphetamine and should be controlled to a similar degree.

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Figure 1. Time course of locomotor stimulant effects

Average horizontal activity counts/10 min (Ambulation counts) as a function of time and dose for MDPV, mephedrone, flephedrone and methamphetamine. Each panel shows the effects of one dose of compound versus the vehicle. $n=8$ except where noted. Each column of 4 panels shows the data for the 4 doses tested. The gray bar shows the time range of peak effect. $n=8$ except where noted. Data are from independent groups of 8 mice per dose. * indicates stimulant effects ($p < 0.05$) against vehicle control. † indicates depressant effects ($p < 0.05$) against vehicle control.

Figure 2. Time course of locomotor stimulant effects

Average horizontal activity counts/10 min (Ambulation counts) as a function of time and dose for naphyrone, methylone, butylone and cocaine. Each panel shows the effects of one dose of compound versus the vehicle. $n=8$ except where noted. Each column of 5 panels shows the data for the 5 doses tested. The gray bar shows the time range of peak effect. Data are from independent groups of 8 mice per dose. * indicates stimulant effects ($p < 0.05$) against vehicle control. † indicates depressant effects ($p < 0.05$) against vehicle control.

Figure 3. Dose effect of locomotor activity

Average horizontal activity counts/10 min ($\pm$ SE) during the 30 min of peak effect as a function of dose for each of the six cathinones (panels left to right). Data are from independent groups of 8 mice per dose. All of the cathinones increased ambulation (* indicates $p < 0.05$ against vehicle control) and showed an inverted U-shaped dose response († indicates depressant effects $p < 0.05$ against vehicle control).

Figure 4. Substitution for the discriminative stimulus effects of cocaine

Top, Percentage of total responses made on the drug-appropriate lever. Bottom, Rate of responding in responses per second (r/s). All of the cathinones fully substituted for the discriminative stimulus effects of cocaine (>80% drug-appropriate responding). n=6

Figure 5. Substitution for the discriminative stimulus effects of methamphetamine

Top: Percentage of total responses made on the drug-appropriate lever. Bottom: Rate of responding in responses per second (r/s). All of the cathinones fully substituted for the discriminative stimulus effects of methamphetamine (>80% drug-appropriate responding).

n=6

Table 1

ED50 values (mg/kg) for locomotor activity and for discriminative stimulus effects of cathinones in cocaine- and methamphetamine-trained rats. Potency ratio shows the relative potency for cocaine versus methamphetamine (cocaine ED50 / methamphetamine ED50).

Drug	Locomotor Activity	Methamphetamine Discrimination	Cocaine Discrimination	Potency Ratio Cocaine/Methamphetamine
Methylone	1.48±0.35	2.66±0.06	1.47±0.07	0.533
MDPV	1.26±0.08	0.67±0.11	0.68±0.06	1.015
Mephedrone	1.38±1.22	1.27±0.12	1.47±0.07	1.157
Butylone	2.57±0.21	2.52±0.18	4.78±0.07	1.897
Flephedrone	2.04±0.35	2.69±0.06	3.24 ± 0.10	1.204
Naphyrone	8.91±0.09	2.96±0.10	3.01±0.07	1.017
Methamphetamine	0.30±0.40	0.37±0.07	--	--
Cocaine	7.24±0.14	--	3.09±0.09	--