Neuroscience Letters 525 (2012) 12–16

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

In vivo comparison of harmine efficacy against psychostimulants: Preferential inhibition of the cocaine response through a glutamatergic mechanism

Suzan Owaisat[a], Robert B. Raffa[a], Scott M. Rawls[b,c,*]

[a] Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA, USA
[b] Center for Substance Abuse Research, Temple University, Philadelphia, PA, USA
[c] Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA, USA

HIGHLIGHTS

- Harmine displays selective efficacy against different drugs of abuse *in vivo*.
- Harmine preferentially antagonizes cocaine-induced stereotyped responses in planarians.
- Harmine efficacy against cocaine is reduced by a glutamate transporter subtype 1 (GLT-1) inhibitor.
- Harmine displays efficacy against methamphetamine.
- Harmine displays efficacy against the psychoactive bath salt compound mephedrone.

ARTICLE INFO

Article history:
Received 8 April 2012
Received in revised form 19 June 2012
Accepted 22 July 2012

Keywords:
Harmine
β-Carboline
Mephedrone
Cocaine
Glutamate
Planaria

ABSTRACT

Harmine is a β-carboline compound that targets glutamatergic, monoaminergic, and GABAergic pathways underlying drug addiction. We compared the efficacy of harmine against different psychoactive drugs using an invertebrate (planarian) assay designed to quantify 'C-shape' responses. Harmine itself (0.01–10 μM) did not produce C-shapes. However, when applied over the same concentration range, harmine significantly inhibited C-shapes elicited by cocaine, with a concentration of 0.1 μM producing almost 90% inhibition. Consistent with its putative actions, harmine produced a similar, though less efficacious, inhibition of C-shapes elicited by the substituted amphetamines methamphetamine and mephedrone (4-methylmethcathinone) but was much less effective against nicotine. When tested in the presence of the glutamate transporter inhibitor dihydrokainate (DHK) (0.1, 1 μM), harmine (0.1 μM) efficacy against cocaine-induced C-shapes was significantly reduced. Harmine also attenuated C-shapes elicited by N-methyl-D-aspartate (NMDA) and by glutamate itself. The present data suggest that harmine displays preferential efficacy against different addictive substances (cocaine > amphetamines > nicotine) and, at least for cocaine, is dependent on the glutamate system.

1. Introduction

Harmine is a member of the heterocyclic β-carboline family of indole alkaloid compounds (consisting of a pyridine ring fused to an indole skeleton). It produces multiple biological effects, including glutamate transporter subtype 1 (GLT-1) activation [20], monoamine oxidase A inhibition [17], 5-HT_{2A} receptor activation [11], cyclin-dependent kinase inhibition [34], imidazoline site interactions, and inverse agonist actions at the benzodiazepine site of $GABA_A$ receptors [1,8,15,25]. Despite targeting biological systems that underlie drug addiction, harmine has not been extensively tested for its efficacy against different classes of abused drugs. Rat studies indicate that norharman, a related β-carboline compound, reduces cocaine intake in a U-shaped manner and that harmine reduces severity of the naloxone-precipitated morphine withdrawal syndrome and antagonize licking induced by apomorphine [2,5,8]. The present study used a simple invertebrate (planarian) assay to compare the efficacy of harmine against psychostimulants. Planarians possess a simple, yet centralized, nervous system and express mammalian-like neurotransmitter systems, including glutamate, dopamine, 5-HT, acetylcholine, and GABA [7,22–24,26,37]. Furthermore, similar to rats and mice [19], planarians display C-shape responses during exposure to cocaine, amphetamines, nicotine, and glutamate. C-shapes provide a reproducible, quantifiable, and common endpoint for comparing the efficacy of a test compound such as harmine [28,31,35]. Using the

* Corresponding author at: Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, 3450 North Broad Street, Philadelphia, PA 19140, USA. Tel.: +1 215 707 4942; fax: +1 215 707 3678.
E-mail address: scott.rawls@temple.edu (S.M. Rawls).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.07.052

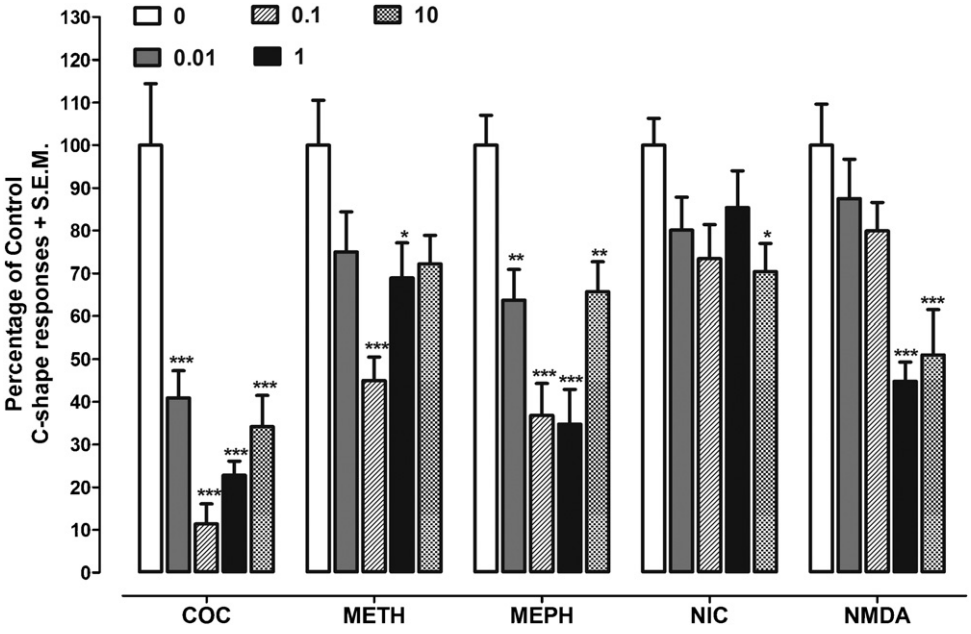

Fig. 1. Harmine (0.01–100 μM) effects on C-shapes elicited by cocaine (COC, 3 mM), methamphetamine (METH, 3 mM), mephedrone (MEPH, 1 mM), glutamate (3 mM), nicotine (NIC, 3 mM), and NMDA (5 mM) during a 5-min exposure. Mean number of C-shapes (±S.E.M.) for the respective control groups (*i.e.*, 0 μM harmine) were: COC (18.6 ± 2.9); METH (22.5 ± 2.5); MEPH (25.5 ± 1.9); NIC (33.4 ± 2.3); and NMDA (36.0 ± 3.7). Data are presented as percentage of control C-shapes + S.E.M. in respective harmine-naïve group. $N = 8$ planarians/group. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ compared to control group (0 μM harmine).

invertebrate assay, we now report that harmine displays particular efficacy (inhibition) against cocaine-induced C-shapes (nearly 90%) through a glutamate-based (dihydrokainate (DHK)-sensitive) mechanism.

2. Materials and methods

Planarians (*Dugesia dorotocephala*) purchased from Carolina Biological Supply (Burlington, NC, USA) were acclimated to room temperature (21 °C) and tested within 3 days of receipt. Cocaine and methamphetamine were provided by the National Institute on Drug Abuse. (*R,S*)-mephedrone (4-methylmethcathinone or 4-MMC) was purchased from Fox Chase Chemical Diversity Center (Doylestown, PA, USA). Dihydrokainate (DHK), N-methyl-D-aspartate (NMDA), (−)-nicotine ditartrate, and L-Glutamic acid (glutamate) was purchased from Tocris Biosciences (St. Louis, MO, USA). Harmine (free base) was purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Stock solutions of harmine (1 mM) were prepared in 0.1% dimethylsulfoxide (DMSO) and then diluted down to desired concentrations with distilled water containing Amquel® water conditioner. To control for any residual effect of the organic solvent, all experimental solutions (including controls that did not contain harmine) contained 0.1% DMSO. Group mean (±S.E.M.) comparisons were evaluated by two-way ANOVA followed by a Dunnett's analysis or one-way ANOVA followed by Tukey's analysis. Values of $p < 0.05$ were considered statistically significant.

A range of harmine concentrations was tested against C-shapes produced by cocaine, methamphetamine, mephedrone, nicotine, and NMDA. The nature of the C-shape responses has been described [26,31]. Approximately equi-effective concentrations of each substance were chosen based on prior work [23,24,28,30,31]. Individual planarians were placed randomly into a petri dish (5.5 cm diameter) containing cocaine (3 mM) or a combination of cocaine (3 mM) and harmine (0.01, 0.1, 1, 10 μM) for 5 min. The number of C-shapes during the 5-min exposure interval was determined. Subsequent testing of harmine against methamphetamine (3 mM), mephedrone (1 mM), nicotine (3 mM), and NMDA (5 mM) used the identical experimental design. Harmine (0.01, 0.1, 1, 10, 100 μM) effects by itself were also investigated. The possibility that harmine acted through a glutamatergic mechanism to inhibit C-shapes elicited by cocaine was investigated by testing the following groups: cocaine (3 mM); harmine (0.1 μM)/cocaine (3 mM); DHK (0.01, 0.1, 1 μM)/harmine (0.1 μM)/cocaine (3 mM); and DHK (1 μM)/cocaine (3 mM).

3. Results

Effects of harmine on C-shapes elicited by different drugs of abuse are presented in Fig. 1. Two-way ANOVA indicated a significant drug effect [$F(4, 35) = 18.8$, $p < 0.0001$], dose effect [$F(4, 175) = 33.9$, $p < 0.0001$], and interaction [$F(16, 175) = 3.28$, $p < 0.0001$]. Harmine (0.01, 0.1, 1, 10 μM) did not produce C-shapes or observable behaviors at the dose range tested here. For combination experiments presented in Fig. 1, C-shapes induced by cocaine were inhibited during co-exposure with harmine (μM harmine concentration, % inhibition): (0.01, 59.1); (0.1, 88.6); (1, 77.2) and (10, 65.8) ($p < 0.001$). C-shapes induced by mephedrone were also attenuated by harmine (μM harmine concentration, % inhibition): (0.01, 36.3) ($p < 0.05$); (0.1, 63.2) ($p < 0.001$); (1, 65.2) ($p < 0.001$); and (10, 34.3) ($p < 0.05$). For experiments with harmine and methamphetamine, C-shapes produced by methamphetamine were reduced by 55% during co-exposure with harmine (0.1 μM) ($p < 0.001$). Harmine was less effecting in reducing C-shapes produced by nicotine as only a concentration of 10 μM produced significant inhibition (29.6%) ($p < 0.05$). For harmine and NMDA experiments, C-shapes induced by NMDA were inhibited during co-exposure with harmine (μM harmine concentration, % inhibition): (1, 55) and (10, 49) ($p < 0.001$).

Harmine (0.1 μM) efficacy against C-shapes elicited by cocaine (3 mM) was investigated in the presence of the glutamate uptake inhibitor DHK (Fig. 2). One-way ANOVA indicated a significant main effect [$F(5, 54) = 6.93$, $p < 0.0001$]. Harmine inhibited C-shapes elicited by cocaine ($p < 0.001$), but its efficacy was significantly

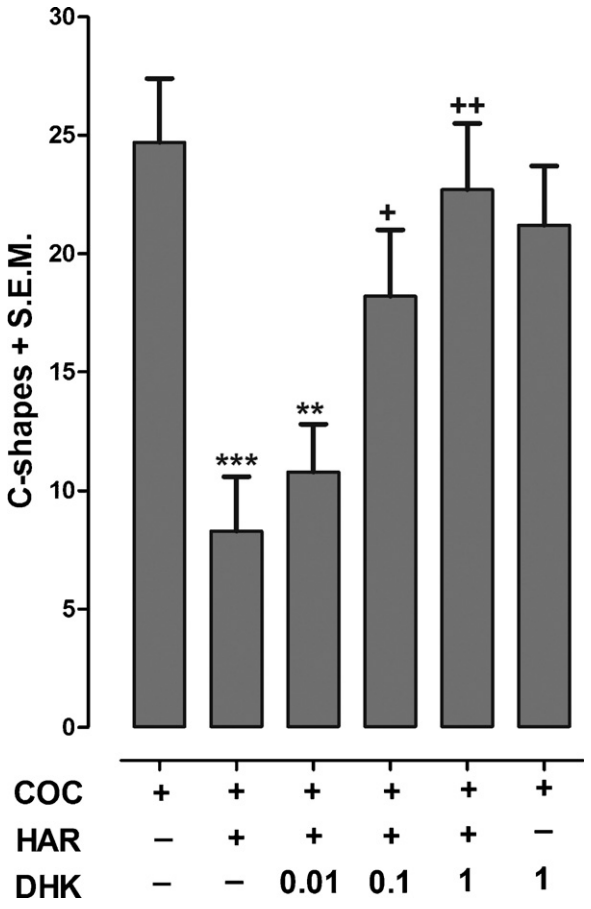

Fig. 2. Dihydrokainate (DHK, 0.01–10 μM) effects on C-shapes elicited by cocaine (COC, 3 mM) in the absence and presence of harmine (HAR, 0.1 μM) during a 5-min exposure. Data are presented as C-shapes + S.E.M. $N = 8$ planarians/group. $^{***}p < 0.001$, $^{**}p < 0.01$ compared to COC group and $^{+}p < 0.05$, $^{++}p < 0.05$ compared to COC + HAR group.

reduced by co-administration with 0.1 μM ($p < 0.05$) and 1 μM ($p < 0.01$) DHK. DHK (1 μM) did not significantly affect C-shapes produced by cocaine ($p > 0.05$) or cause C-shapes by itself (not shown). A role for glutamate in the effects of harmine was further investigated by testing a range of harmine concentrations (0, 0.01, 0.1, 1, 10 μM) against C-shapes induced by glutamate (3 mM) [31]. One-way ANOVA indicated a significant main effect [$F(4, 35) = 16.9$, $p < 0.0001$]. The mean number of C-shapes produced by glutamate (3 mM) was 21.9 ± 2.3. For combination experiments, harmine attenuated C-shapes induced by glutamate (3 mM) (μM harmine concentration, % inhibition): (0.01, 54.9 ± 4.8); (0.1, 42.3 ± 4.9); (1, 36.6 ± 2.7) and (10, 52.0 ± 5.3) ($p < 0.001$).

4. Discussion

Harmine displayed preferential efficacy against different psychoactive compounds. Effects of harmine were most robust against cocaine and substituted amphetamines with lesser efficacy detected against nicotine. Inhibition of cocaine-evoked C-shapes was notably pronounced, with a concentration of 0.1 μM producing about 90% inhibition. The U-shaped concentration curve for harmine against cocaine, methamphetamine, and mephedrone is consistent with the U-shaped inhibition of cocaine intake by norharman in rats and indicative of more than one mechanism of action [1,5,8,15,25]. A recently documented effect of harmine is enhancement of cellular glutamate uptake [20]. *In vivo* studies indicate that harmine displays efficacy in a mouse model of amyotrophic lateral sclerosis and increases both glutamate uptake activity and GLT-1 protein expression [20]. In the present experiments, cocaine responses were most effectively inhibited by harmine concentrations (0.1–10 μM) that also induce GLT-1 gene expression in mouse and human astrocytes (3 μM) and elicit promoter activation in cultured cells (10 μM). Our results also revealed that harmine failed to inhibit cocaine-induced C-shapes in the presence of a glutamate transporter inhibitor (DHK), providing pharmacological evidence that the efficacy of harmine is dependent on enhancement of glutamate uptake. Specific glutamate transporter genes and proteins have not yet been identified in planarians, although elements of a functionally active glutamate system, including endogenous glutamate and ionotropic glutamate receptors, are present [6,37]. A more established glutamate uptake activator, ceftriaxone, displays efficacy against cocaine responses across different species [32]. Vertebrate studies indicate that ceftriaxone reduces reinforcing and drug-seeking properties of cocaine in self-administration assays [18,38]. Planarian studies reveal that ceftriaxone blocks C-shapes produced by acute cocaine exposure and withdrawal responses following discontinuation of cocaine exposure [30,31]. A comparison of ceftriaxone and harmine efficacies against cocaine-induced C-shapes indicates that ceftriaxone was less efficacious (about a 50% inhibition) than harmine, perhaps due to the latter compound acting through at non-glutamate substrates such as 5-HT receptors, imidazoline sites, or cyclin-dependent kinases [15].

The substituted amphetamines, methamphetamine and mephedrone, elicited C-shapes that were also attenuated by harmine. Compared to its effects against cocaine, harmine displayed lesser efficacy against mephedrone and methamphetamine, a finding possibly related to amphetamines acting through a broader range of mechanisms (*i.e.*, monoamine release, monoamine transporter uptake block, monoamine oxidase inhibition, *etc.*). The underlying mechanism of action of harmine is unclear, but GLT-1 transporter activation is again a possibility because ceftriaxone inhibits acute and sensitized amphetamine-induced responses in rats [29]. Harmine inhibits monoamine oxidases [10], but this mechanism is unlikely to have contributed significantly to results presented here because amphetamines produce directionally similar effects on monoamine oxidase activity [12]. Furthermore, monoamine oxidase inhibition prevents dopamine catabolism leading to increased dopamine concentrations, and dopamine and dopamine agonists elicit behavioral responses in planarians [36]. Other possible mechanisms of action for harmine observed here include 5-HT_{2A} receptor activation, cyclin-dependent kinase inhibition, imidazoline site interactions, and inverse agonist actions at the benzodiazepine site of $GABA_A$ receptors [1,8,11,15,25,34].

Harmine effects on mephedrone are especially interesting because mephedrone is a principal ingredient of psychoactive bath salts, a dangerous street drug linked to fatalities and non-fatal overdoses [39]. The American Association of Poison Control reported that mephedrone exposures increased at least 10-fold from 2010 to 2011, and several factors associated with mephedrone, such as its relative purity, ease of synthesis from available precursors, widespread internet marketing, and perceived quality of high, are attractive to end users of the drug and elements of organized crime [40]. Limited preclinical evidence suggests a psychostimulant profile for mephedrone, including enhancement of extracellular monoamine levels in the limbic system, augmentation of locomotor activity, reinforcing properties in self-administration assays, and serotonergic deficits following repeated exposure [3,13,14,16,21]. The psychostimulant-like effects of mephedrone also extend to planarians, where it elicits dopamine-sensitive C-shapes following acute exposure, withdrawal responses during drug absence, and place conditioning effects [28]. The present results suggest

mephedrone displays an intermediate efficacy relative to more established psychostimulants, producing C-shapes that are greater than those produced by methamphetamine but less than those elicited by cocaine. Furthermore, the present findings suggest that mephedrone-induced C-shapes are not only sensitive to dopamine receptor activation [28], but also to the actions of harmine.

Harmine inhibited NMDA-evoked C-shapes by approximately 55%. Prior work indicates that planarians display C-shapes following exposure to NMDA that are antagonized by topiramate and carbamazepine [26,27]. Although NMDA can activate its receptors independent of glutamate release, *in vivo* microdialysis experiments indicate that it does increase extracellular glutamate levels in the rat brain [41]. Thus, NMDA may have produced C-shapes in planarians through overlapping mechanisms that include direct receptor activation and enhanced glutamate release. In this case, it is possible that harmine, by increasing glutamate uptake activity, inhibited the component of NMDA-evoked C-shapes that is dependent on enhanced glutamate release. The interpretation is supported by our finding that harmine also inhibits C-shapes induced by administration of glutamate itself. Future behavioral and neurochemical studies using rats are planned to test this hypothesis.

Although pharmacological evidence presented here suggests a glutamate-based mechanism underlies the efficacy of harmine against cocaine, pharmacokinetic factors and alternative mechanisms of action may have contributed. One consideration regarding planarian assays is that the entire organism is exposed to the drug(s). Thus, in the present study, one could speculate that the efficacy of harmine was related to occlusion of substances across the planarian tegument. However, in the case in which a glutamate transporter inhibitor (DHK) was added to a solution containing cocaine and harmine, the cocaine-induced response was completely restored even in the presence of harmine. This finding indicates that simple occlusion of diffusion across the tegument by harmine could not have been responsible for its efficacy against cocaine. It should also be noted that in order for compounds to display efficacy in planarian assays, higher concentrations are often required relative to those needed for rodent brain studies. The rate of uptake of compounds into planarians has not been determined, but at least two possibilities may account for the concentration differences between planarians and rodents that we and others have reported [26–28,30,31], neither of which alter the conclusions of the present results. The first possibility is that there is not 'free diffusion' in planarians and the diffusion barrier is greater than that in rodents' brain, which seems unlikely but would not affect the results with harmine. A second possibility is that the affinity of compounds for mammalian targets of drugs (receptors, uptake sites, *etc.*), or second messenger transduction efficacy of compounds, is lower in planarians than in higher-order animals, thus requiring higher concentrations to achieve the same level of receptor/uptake site occupancy or signal transduction (level of effect). Finally, the presence of glutamatergic and cholinergic receptors on muscles and the body surface of flatworms raises the possibility that C-shapes elicited by stimulants were muscle contractions [33]. While conducting contraction assays is beyond our capability, any effect on contraction is unlikely to be phasic in nature and simultaneously caused by all six of the substances tested *viz.*, cocaine, methamphetamine, mephedrone, nicotine, NMDA, and glutamate.

In conclusion, harmine displayed selective efficacy against drugs of abuse that was dependent on the class of compound (cocaine > substituted amphetamines > nicotine). The experiments utilized an invertebrate assay that quantifies C-shape responses [23,24,26,27]. With the information obtained in the present study, future studies will investigate more detailed aspects of the mechanisms of action of β-carboline compounds in mammals [20].

Acknowledgments

This study was supported by NIDA grants P30 DA013429 and DA030676. The authors thank Timothy Shickley, Ph.D., for suggesting *Planaria* as an *in vivo* test model.

References

[1] M.S. Allen, A.J. LaLoggia, L.J. Dorn, M.J. Martin, G. Costantino, T.J. Hagen, K.F. Koehler, P. Skolnick, J.M. Cook, Predictive binding of beta-carboline inverse agonists and antagonists via the CoMFA/GOLPE approach, Journal of Medicinal Chemistry 35 (1992) 4001–4010.
[2] F. Aricioglu-Kartal, H. Kayir, I. Tayfun Uzbay, Effects of harman and harmine on naloxone-precipitated withdrawal syndrome in morphine-dependent rats, Life Sciences 73 (2003) 2363–2371.
[3] M.H. Baumann, M.A. Ayestas Jr., J.S. Partilla, J.R. Sink, A.T. Shulgin, P.F. Daley, S.D. Brandt, R.B. Rothman, A.E. Ruoho, N.V. Cozzi, The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue, Neuropsychopharmacology 37 (2012) 1192–1203.
[5] S.L. Cappendijk, D. Fekkes, A. Van Dalen, L. Pepplinkhuizen, The acute effects of norharman on cocaine self-administration and sensorimotor function in male Wistar rats, European Neuropsychopharmacology 11 (2001) 233–239.
[6] F. Cebria, T. Kudome, M. Nakazawa, K. Mineta, K. Ikeo, T. Gojobori, K. Agata, The expression of neural-specific genes reveals the structural and molecular complexity of the planarian central nervous system, Mechanisms of Development 116 (2002) 199–204.
[7] K.S. Eriksson, P. Panula, gamma-Aminobutyric acid in the nervous system of a planarian, Journal of Comparative Neurology 345 (1994) 528–536.
[8] D. Farzin, A. Haghparast, S. Motaman, F. Barya, N. Mansouri, Effects of harmane and other β-carbolines on apomorphine-induced licking behavior in rat, Pharmacology Biochemistry and Behavior 98 (2011) 215–219.
[10] N. Ginovart, J.H. Meyer, A. Boovariwala, D. Hussey, E.A. Rabiner, S. Houle, A.A. Wilson, Positron emission tomography quantification of [11C]-harmine binding to monoamine oxidase-A in the human brain, Journal of Cerebral Blood Flow and Metabolism 26 (2006) 330–344.
[11] R.A. Glennon, M. Dukat, B. Grella, S. Hong, L. Costantino, M. Teitler, C. Smith, C. Egan, K. Davis, M.V. Mattson, Binding of beta-carbolines and related agents at serotonin (5-HT(2) and 5-HT(1A)), dopamine (D(2)) and benzodiazepine receptors, Drug and Alcohol Dependence 60 (2000) 121–132.
[12] A.L. Green, The human brain. Inhibition of rat and mouse brain monamine oxidases by (+)-amphetamine, Biochemical Journal 121 (1971) 37P–38P.
[13] G.C. Hadlock, K.M. Webb, L.M. McFadden, P.W. Chu, J.D. Ellis, S.C. Allen, D.M. Andrenyak, P.L. Vieira-Brock, C.L. German, K.M. Conrad, A.J. Hoonakker, J.W. Gibb, D.G. Wilkins, G.R. Hanson, A.E. Fleckenstein, 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designer stimulant of abuse, Journal of Pharmacology and Experimental Therapeutics 339 (2011) 530–536.
[14] P.K. Huang, S.M. Aarde, D. Angrish, K.L. Houseknecht, T.J. Dickerson, M.A. Taffe, Contrasting effects of D-methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxypyrovalerone, and 4-methylmethcathinone on wheel activity in rats. Drug and Alcohol Dependence, in press, http://dx.doi.org/10.1016/j.drugalcdep.2012.05.011.
[15] S.M. Husbands, R.A. Glennon, S. Gorgerat, R. Gough, R. Tyacke, J. Crosby, D.J. Nutt, J.W. Lewis, A.L. Hudson, Beta-carboline binding to imidazoline receptors, Drug and Alcohol Dependence 64 (2001) 203–208.
[16] J. Kehr, F. Ichinose, S. Yoshitake, M. Goiny, T. Sievertsson, F. Nyberg, T. Yoshitake, Mephedrone, compared to MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and serotonin levels in nucleus accumbens of awake rats, British Journal of Pharmacology 164 (2011) 1949–1958.
[17] H. Kim, S.O. Sablin, R.R. Ramsay, Inhibition of monoamine oxidase A by beta-carboline derivatives, Archives of Biochemistry and Biophysics 337 (1997) 137–142.
[18] L.A. Knackstedt, R.I. Melendez, P.W. Kalivas, Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking, Biological Psychiatry 67 (2010) 81–84.
[19] G.F. Koob, Neural mechanisms of drug reinforcement, Annals of the New York Academy of Sciences 654 (1992) 171–191 (Review).
[20] Y. Li, R. Sattler, E.J. Yang, A. Nunes, Y. Ayukawa, S. Akhtar, G. Ji, P.W. Zhang, J.D. Rothstein, Harmine, a natural beta-carboline alkaloid, upregulates astroglial glutamate transporter expression, Neuropharmacology 60 (2011) 1168–1175.
[21] J. Martinez-Clemente, E. Escubedo, D. Pubill, J. Camarasa, Interaction of mephedrone with dopamine and serotonin targets in rats, European Neuropsychopharmacology 22 (2011) 231–236.
[22] K. Nishimura, Y. Kitamura, T. Taniguchi, K. Agata, Analysis of motor function modulated by cholinergic neurons in planarian *Dugesia japonica*, Neuroscience 168 (2010) 18–30.
[23] O.R. Pagán, A.L. Rowlands, M. Azam, K.R. Urban, A.H. Bidja, D.M. Roy, R.B. Feeney, L.K. Afshari, Reversal of cocaine-induced planarian behavior by parthenolide and related sesquiterpene lactones, Pharmacology Biochemistry and Behavior 89 (2008) 160–170.
[24] O.R. Pagán, A.L. Rowlands, A.L. Fattore, T. Coudron, K.R. Urban, A.H. Bidja, V.A. Eterović, A cembranoid from tobacco prevents the expression of nicotine-induced withdrawal behavior in planarian worms, European Journal of Pharmacology 615 (2009) 118–124.

[25] C.A. Parker, N.J. Anderson, E.S. Robinson, R. Price, R.J. Tyacke, S.M. Husbands, M.P. Dillon, R.M. Eglan, A.L. Hudson, D.J. Nutt, M.P. Crump, J. Crosby, Harmane and harmalan are bioactive components of classical clonidine-displacing substance, Biochemistry 43 (2004) 16385–16392.
[26] R.B. Raffa, S.M. Rawls, A Model for Drug Action and Abuse, Landes Bioscience, Austin, TX, 2010.
[27] L. Ramakrishnan, C. Desaer, Carbamazepine inhibits distinct chemoconvulsant-induced seizure-like activity in *Dugesia tigrina*, Pharmacology Biochemistry and Behavior 99 (2011) 665–670.
[28] L. Ramoz, S. Lodi, P. Bhatt, A.B. Reitz, C. Tallarida, R.J. Tallarida, R.B. Raffa, S.M. Rawls, Mephedrone (bath salt) pharmacology: insights from invertebrates, Neuroscience 208 (2012) 79–84.
[29] B. Rasmussen, E.M. Unterwald, S.M. Rawls, Glutamate transporter subtype 1 (GLT-1) activator ceftriaxone attenuates amphetamine-induced hyperactivity and behavioral sensitization in rats, Drug and Alcohol Dependence 118 (2011) 484–488.
[30] S.M. Rawls, F. Cavallo, A. Capasso, Z. Ding, R.B. Raffa, The beta-lactam antibiotic ceftriaxone inhibits physical dependence and abstinence-induced withdrawal from cocaine, amphetamine, methamphetamine, and clorazepate in planarians, European Journal of Pharmacology 584 (2008) 278–284.
[31] S.M. Rawls, F. Karaca, I. Madhani, V. Bhojani, R.L. Martinez, M. Abou-Gharbia, R.B. Raffa, β-lactamase inhibitors display anti-seizure properties in an invertebrate assay, Neuroscience 169 (2010) 1800–1804.
[32] J.D. Rothstein, S. Patel, M.R. Regan, C. Haenggeli, Y.H. Huang, D.E. Bergles, L. Jin, M. Dykes Hoberg, S. Vidensky, D.S. Chung, S.V. Toan, L.I. Bruijn, Z.Z. Su, P. Gupta, P.B. Fisher, β-Lactam antibiotics offer neuroprotection by increasing glutamate transporter expression, Nature 433 (2005) 73–77.
[33] P. Ribeiro, F. El-Shehabi, N. Patocka, Classical transmitters and their receptors in flatworms, Parasitology 131 (Suppl.) (2005) S19–S40.
[34] Y. Song, D. Kesuma, J. Wang, Y. Deng, J. Duan, J.H. Wang, R.Z. Qi, Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine, Biochemical and Biophysical Research Communications 317 (2004) 128–132.
[35] C. Tallarida, K. Song, R.B. Raffa, S.M. Rawls, Glutamate carboxypeptidase II (GCPII) inhibitor displays anti-glutamate and anti-cocaine effects in an invertebrate assay, Amino Acids 42 (2012) 2521–2524.
[36] G. Venturini, F. Stocchi, V. Margotta, S. Ruggieri, D. Bravi, P. Bellantuono, G. Palladini, A pharmacological study of dopaminergic receptors in planaria, Neuropharmacology 28 (1989) 1377–1382.
[37] C.A. Vyas, S.M. Rawls, R.B. Raffa, J.G. Shackman, Glutamate and aspartate measurements in individual planaria by rapid capillary electrophoresis, Journal of Pharmacological and Toxicological Methods 63 (2010) 119–122.
[38] S.J. Ward, B.A. Rasmussen, G. Corley, C. Henry, J.K. Kim, E.A. Walker, S.M. Rawls, β-lactam antibiotic decreases acquisition of and motivation to respond for cocaine, but not sweet food, in C57Bl/6 mice, Behavioural Pharmacology 22 (2011) 370–373.
[39] A.R. Winstock, L.R. Mitcheson, P. Deluca, Z. Davey, O. Corazza, F. Schifano, Mephedrone, new kid for the chop? Addiction 106 (2011) 154–161.
[40] D.M. Wood, S. Davies, S.L. Greene, J. Button, D.W. Holt, J. Ramsey, P.I. Dargan, Case series of individuals with analytically confirmed acute mephedrone toxicity, Clinical Toxicology 48 (2010) 924–927.
[41] A.M. Young, H.F. Bradford, N-methyl-D-aspartate releases excitatory amino acids in rat corpus striatum in vivo, Journal of Neurochemistry 56 (1991) 1677–1683.