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Stereochemistry and neuropharmacology of a 'bath salt' cathinone: *S*-enantiomer of mephedrone reduces cocaine-induced reward and withdrawal in invertebrates

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ABSTRACT

Knowledge about the neuropharmacology of mephedrone (MEPH) applies primarily to the racemate, or street form of the drug, but not to its individual enantiomers. Here, through chemical isolation of MEPH enantiomers and subsequent behavioral characterization in established invertebrate (planarian) assays, we began separating adverse effects of MEPH from potential therapeutic actions. We first compared stereotypical and environmental place conditioning (EPC) effects of racemic MEPH, *S*-MEPH, and *R*-MEPH. Stereotypy was enhanced by acute treatment (100–1000 μM) with each compound; however, *S*-MEPH was less potent and efficacious than racemate and *R*-MEPH. Both *R*-MEPH (10, 100, 250 μM) and racemate (100 μM) produced EPC, but *S*-MEPH was ineffective at all concentrations (10–100 μM). After showing that *S*-MEPH lacked rewarding efficacy, we investigated its ability to alter three of cocaine's behavioral effects (EPC, withdrawal, and stereotypy). Cocaine (1 μM) produced EPC that was abolished when S-MEPH (100 μM) was administered after cocaine conditioning. Spontaneous withdrawal from chronic cocaine exposure caused a reduction in motility that was not evident during acute or continuous cocaine treatment but was attenuated by S-MEPH (100 μM) treatment during the cocaine abstinence interval. Acute stereotypy produced by 1 mM cocaine, nicotine or racemic MEPH was not affected by *S*-MEPH (10–250 μM). The present results obtained using planarian assays suggest that the *R*-enantiomer of MEPH is predominantly responsible for its stimulant and rewarding effects and the *S*-enantiomer is capable of antagonizing cocaine's addictive-like behaviors without producing rewarding effects of its own.

1. Introduction

Mephedrone (4-methylmethcathinone) (MEPH) is a designer cathinone contained in a street drug called "bath salts" (Glennon, 2014; Winstock et al., 2010, 2011). Designer cathinones are β-keto amphetamine compounds, structurally related to the parent compound cathinone, which entered the recreational drug scene as 'legal-high' substitutes for established drugs of abuse including cocaine, methamphetamine, and MDMA (Carroll et al., 2012; Brandt et al., 2010; Brunt et al., 2011; Schifano et al., 2011). The Drug Enforcement Agency (DEA) banned bath salts in 2011 after the American Association of Poison Control Centers reported a 20-fold increase in bath salt exposure in one year. Despite legislation some designer cathinones are gaining a foothold in the illicit drug scene, with MEPH reported as fourth most popular drug of abuse in a survey of UK drug users and methylenedioxypyrovalerone (MDPV) encountered in at least 34 states (Coppola et al., 2012; Prosser and Nelson, 2012).

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Prior knowledge about the neuropharmacology of MEPH applies only to the racemate but not to individual enantiomers. Users of racemic MEPH report both cocaine-like stimulant effects and MDMA-like empathogenic effects (Winstock et al., 2010, 2011; Brunt et al., 2011; Schifano et al., 2011). Racemic MEPH exerts its direct pharmacological actions by acting as a substrate at monoamine transporters to elicit release of dopamine and serotonin (5-HT) that leads to an elevation in extracellular dopamine and 5-HT in the nucleus accumbens of rats (Baumann et al., 2012, 2014; López-Arnau et al., 2012; Eshleman et al., 2013; Kehr et al., 2011). Racemic MEPH also increases locomotor activity following acute exposure, and produces weak but significant behavioral sensitization following repeated treatment (Motbey et al., 2012, 2013; Shortall et al., 2012; Wright et al., 2012; Gatch et al., 2013; Gregg et al., 2013a,b). In addition, racemic MEPH produces conditioned place preference (CPP), facilitates intracranial self-stimulation (ICSS), and is self-administered in rats (Hadlock et al., 2011; Lisek et al., 2012; Robinson et al., 2012; Bonano et al., 2013; Motbey et al., 2013). Taken together, these effects illustrate that MEPH produces behavioral and neurochemical effects consistent with an abuse-liable psychostimulant drug.

MEPH, similar to amphetamine and cathinone, has a chiral center at its α-carbon, and stably exists as a racemic mixture of two enantiomers, *R*-mephedrone (*R*-MEPH) and *S*-mephedrone (*S*-MEPH). Stereospecific effects of amphetamines and cathinone derivatives have been demonstrated. For instance, *R*-Cathinone is three times more potent than *S*-cathinone in causing dopamine release in the CNS, while *S*-MDMA displays 30-fold greater affinity for the dopamine transporter (DAT) than *R*-MDMA (Kalix, 1986; Setola et al., 2003). R- and S-methcathinone both produce neurotoxicity in dopamine neurons but only *S*-methcathinone produces 5-HT neurotoxicity (Sparago et al., 1996). *S*-methcathinone shows a 3-fold greater potency as a discriminative stimulus substituting for cocaine compared to *R*-methcathinone in rats, and both *S*-MDMA and racemic MDMA are more consistent reinforcers of self-administration than *R*-MDMA in rhesus monkeys (Glennon et al., 1995; Wang and Woolverton, 2007).

The purpose of the present study was to begin characterizing behavioral effects of *R*- and *S*-MEPH using established planarian assays. Planarians are aquatic flatworms with a centralized nervous system often considered to be the simplest 'brain' (Raffa and Rawls, 2008; Buttarelli et al., 2008). They also express neurotransmitter systems including glutamate, dopamine, serotonin, acetylcholine, and GABA (Eriksson and Panula, 1994; Vyas et al., 2011; Nishimura et al., 2010) and display mammalian-like behavioral responses (stereotypy, motility changes, abstinence-related withdrawal, behavioral sensitization, and environmental conditioning) following exposure to drug and natural rewards (Palladini et al., 1996; Pagán et al., 2008, 2009, 2013; Rowlands and Pagán, 2008; Rawls et al., 2010, 2011; Ramoz et al., 2012). Following synthesis and isolation of *R*- and *S*-MEPH, we first compared stereotypical and environmental place conditioning (EPC) effects of racemic MEPH, *S*-MEPH, and *R*-MEPH and then investigated effects of *S*-MEPH, which lacked rewarding efficacy of its own, against three hallmark behavioral effects (reward, withdrawal, and stereotypy) of cocaine.

2. Experimental procedures

2.1. Subjects and drugs

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply (Burlington, NC, USA). Upon arrival in the laboratory, planarians were maintained in the aqueous solution provided by Carolina Biological Supply, acclimated to room temperature (21 °C), and tested within 3 days of receipt. (−)-Cocaine hydrochloride was generously provided by the National Institute on Drug Abuse (Bethesda, MD, USA). (−)-Nicotine ditartrate was obtained from Sigma–Aldrich (St. Louis MO, USA).

Racemic MEPH (50:50 ratio of *R*-MEPH and *S*-MEPH), *R*-MEPH and *S*-MEPH were synthesized by Fox Chase Chemical Diversity (Doylestown PA, USA) Racemic MEPH was prepared using the method discussed by Camilleri et al. (2010). *R*-MEPH (d-MEPH/(+)-MEPH) and *S*-MEPH (l-MEPH/(−)-MEPH) were prepared starting from natural amino acids in such a way that stereochemistry is clearly known (Gregg et al., 2014). *R*-MEPH and *S*-MEPH conformations are stable in the solid state, and undergo pH dependent racemization in solution, where higher pH promotes greater deprotonation and racemization. After 1.5 h at 37 °C, a slight decrease in enantiomeric excess (e.e.) was observed for R-MEPH in rat plasma (97.5–87.5% e.e.) and PBS buffer (pH 7.2, 97.5 to 92.5%). After 5 h at 37 °C, e.e. values in rat plasma and PBS buffer were 58.6% and 87.5%, respectively.

Stock solutions of each drug were prepared daily in a vehicle of tap water containing AmQuel® water conditioner. Treatment solutions were diluted with tap water containing AmQuel® water conditioner. Concentrations of cocaine, racemic MEPH, and nicotine were based on prior behavioral work in planarians (Tallarida et al., 2014; Owaisat et al., 2012; Ramoz et al., 2012; Pagán et al., 2008, 2009, 2013; Rawls et al., 2010, 2011).

2.2. Behavioral experiments

2.2.1. Stereotypical activity

Stereotypical activity has been previously defined as the number of C-shape movements across a defined time interval (Palladini et al., 1996; Passarelli et al., 1999; Rawls et al., 2011; Tallarida et al., 2014). Each planarian was removed from its home jar and placed for 5 min into a petri dish (5.5 cm diameter) containing water or drug. C-shaped movements were quantified over the 5-min exposure interval by a trained observer blinded to drug treatment with a stopwatch. The response was recorded each time the planarian made a C-shaped behavior and then relaxed, and this sequence translated to one individual C-shape. The duration of individual C-shapes was not quantified. Quantifying the frequency of a specific *in vivo* response is also common practice in rodent models, such as the quantification of withdrawal signs in physically dependent animals (*e.g.* wet-dog shakes, escape behavior, teeth chattering, eye blinking). Prior work has demonstrated that C-shaped movements displayed by planarians are not caused by changes in the pH or osmolarity of the solution (Raffa and Rawls, 2008). The first set of experiments investigated effects of different concentrations (100, 250, 500, 750, 1000 μM) of racemic MEPH, *S*-MEPH, and *R*-MEPH on stereotypical responses (Fig. 1). A second set of experiments investigated effects of *S*-MEPH (0, 10, 100, 250 μM), on stereotypical activity induced by a fixed concentration (1 mM) of cocaine, racemic MEPH or nicotine (Fig. 5). For combination experiments, drugs were administered concurrently and stereotypical activity was determined for 5 min.

2.2.2. Environmental place conditioning (EPC) experiments

EPC, similar to conditioned place preference (CPP) experiments in rodents, is an assay in which planarians exposed to a distinct environment in the presence of a rewarding substance will later show preference for that same environment when given a choice (Zhang et al., 2013; Ramoz et al., 2012). EPC experiments were divided into 3 different phases; 1) pre-conditioning (pre-test) in water; 2) conditioning in

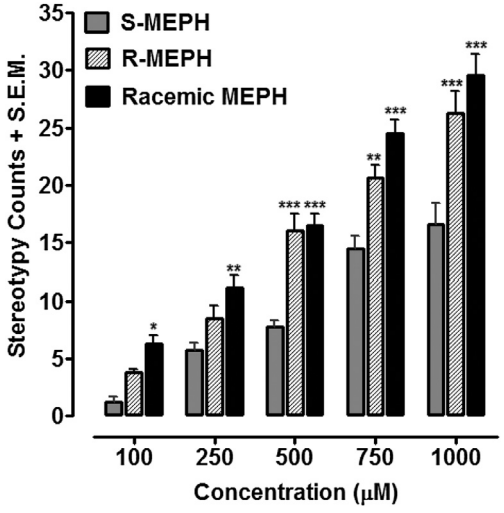

Fig. 1. Effects of S-MEPH, R-MEPH and racemic MEPH on stereotypical movements. Planarians were exposed to different concentrations (100, 250, 500, 750, 1000 μM) of *S*-MEPH, *R*-MEPH, or racemic MEPH and the number of C-shape movements over 5 min were determined and presented as mean stereotypy counts ± S.E.M. $N = 8$ planarians/group. $^{***}P < 0.001$, $^{**}P < 0.01$ or $^{*}P < 0.05$ compared to respective concentration of *S*-MEPH.

which drug (unconditioned stimulus) is paired with a specific environment (conditioned stimulus); and 3) post-conditioning (post-test) in the absence of the unconditioned stimulus. Since planarians naturally avoid a light environment, we used a biased conditioning design to assess effects of drug conditioning (Tallarida et al., 2014; Ramoz et al., 2012). Dark and "ambient" light environments were created by covering half (top and bottom) of a petri dish containing water with black paper. For the pre-test, conducted in the absence of drug, individual planarians were placed at the midline of the dish and given free access to both the light and dark sides of the dish. The side on which a planarian spent the least amount of time (*i.e.*, non-preferred environment) was designated as the drug-paired side. Conditioning then involved 30 min of drug exposure on the non-preferred side followed immediately by 30 min of exposure to water on the opposite side. Controls were also included in which exposure to only water occurred on both sides. Immediately following conditioning, a post-test was conducted in the absence of the hypothesized rewarding substance in which planarians were placed at the midline of a petri dish and allowed free access to the light and dark sides of the dish for 5 min. The time spent in the naturally aversive, drug-paired environment was determined. The difference in time spent in the naturally aversive environment (post-test minus pre-test times) was determined. The experimental design for each EPC experiment is shown in Table 1.

2.2.3. Abstinence-induced withdrawal experiments

Individual planarians were randomly removed from their home jars and placed for 60 min into a petri dish containing water or cocaine (1 μM). Planarians from both groups were then removed and placed into another Petri dish containing water, cocaine (1 μM), or S-MEPH (1, 100 μM) (Table 2). As previously described (Zhang et al., 2013; Raffa et al., 2003), motility counts were quantified as the number of gridlines crossed, or re-crossed, by placing the transparent petri dish over graphing paper with gridlines spaced 0.5 cm apart. Decreased motility following discontinuation of exposure to an addictive substance is a characteristic withdrawal response displayed by planarians (Raffa and Rawls, 2008).

2.3. Data analysis

Comparisons of group means (±S.E.M.) were evaluated by one-way ANOVA, and in cases of significance, followed by Dunnett's *post-hoc* test to identify significant differences between individual groups. For stereotypy experiments comparing racemate, *S*-MEPH, and *R*-MEPH, a two-way ANOVA (drug, concentration) was used to compare group means (±S.E.M.) followed by a Bonferroni's test. In all cases, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of racemic and enantiomeric MEPH on stereotypical activity

Fig. 1 presents effects of *S*-MEPH, *R*-MEPH, and racemic MEPH on stereotypical activity in planarians. In control experiments planarians exposed to water did not display C-shape movements (data not shown). A two-way ANOVA revealed significant drug, concentration and interaction effects (drug [$F(2, 105) = 64.76$, $P < 0.0001$]); concentration [$F(4, 105) = 153.19$, $P < 0.0001$]; interaction [$F(4, 35) = 3.30$, $P < 0.01$]. *Post-hoc* analysis indicated that S-MEPH produced less stereotypy than racemic MEPH (*i.e.,* racemate) at each concentration tested: [100 μM, $P < 0.05$; 250 μM, $P < 0.01$; 500 μM, $P < 0.001$; 750 μM, $P < 0.001$; 1000 μM, $P < 0.001$]. *S*-MEPH also produced less stereotypical activity than *R*-MEPH: [500 μM, $P < 0.001$; 750 μM, $P < 0.01$ 1000 μM, $P < 0.001$]. *Post-hoc* analysis revealed that stereotypical activity produced by *R*-MEPH and racemate did not differ at any of the concentrations tested ($P > 0.05$).

Table 1
Environmental place conditioning (EPC) studies. A) Experimental design for EPC studies comparing effects of S-MEPH, R-MEPH, and racemic MEPH (Racemate) (0, 10, 100, 250 μM) and B) assessing effects of *S*-MEPH (100 μM) on EPC produced by cocaine (1 μM). A).

Group	Pre-test (5 min) in split dish	Conditioning (30 min) on aversive side	Exposure (30 min) to opposite side	Post-test (5 min) in split dish
A				
Water	Water	Water	Water	Water
S-MEPH	Water	*S*-MEPH	Water	Water
R-MEPH	Water	*R*-MEPH	Water	Water
Racemate	Water	Racemate	Water	Water
B				
Water	Water	Water	Water	Water
S-MEPH	Water	Water	Water	*S*-MEPH
Cocaine	Water	Cocaine	Water	Water
Cocaine + *S*-MEPH	Water	Cocaine	Water	*S*-MEPH

Table 2
Withdrawal Studies. Experimental design for studies testing effects of *S*-MEPH (1, 100 μM) on abstinence-induced withdrawal response produced by cocaine (1 μM). Motility counts were determined over the 5-min treatment period.

Group	Pretreatment (60 min)	Treatment (5 min)
Water/water	Water	Water
Water/cocaine	Water	Cocaine (1 μM)
Cocaine/cocaine	Cocaine (1 μM)	Cocaine (1 μM)
Cocaine/water	Cocaine (1 μM)	Water
Cocaine/S-MEPH (1 μM)	Cocaine (1 μM)	S-MEPH (1 μM)
Cocaine/S-MEPH (100 μM)	Cocaine (1 μM)	S-MEPH (100 μM)

3.2. Effects of racemate and MEPH enantiomers on EPC

EPC effects of *S*-MEPH, *R*-MEPH, and racemic MEPH are presented in Fig. 2. Consistent with their natural aversion to light (Inoue et al., 2004), most planarians spent a greater amount of time in the dark environment during the pre-test [*S*-MEPH (30/32 planarians, or 94%); *R*-MEPH (31/32 planarians, or 97%), and racemate (29/32 planarians, or 91%)]. As noted above, the environment in which a planarian spent the least amount of time during the pre-test was designated as the drug-paired side in which conditioning for that planarian was conducted. For the *S*-MEPH data set presented in Fig. 2A, a significant main effect was not detected by one-way ANOVA [$F(3, 28) = 1.526$, $P > 0.05$]. In contrast, for the *R*-MEPH data set presented in Fig. 2B, one-way ANOVA did identify a significant main effect [$F(3, 28) = 14.05$, $P < 0.0001$]. *Post-hoc* analysis indicated that each concentration of *R*-MEPH produced EPC relative to water-exposed controls [10 μM, $P < 0.01$; 100 μM, $P < 0.001$; 250 μM, $P < 0.001$]. For racemic MEPH (Fig. 2C), a significant main effect was detected by one-way ANOVA [$F(3, 28) = 6.910$, $P < 0.01$], and *post-hoc* analysis indicated that a concentration of 100 μM produced significant EPC relative to water-treated controls ($P < 0.05$). The maximal place conditioning effect for each compound, which was expressed as the percentage of their respective water-treated control, was [*S*-MEPH, 199 ± 55%; *R*-MEPH, 530 ± 41%; and racemate, 227 ± 29%], thus suggesting that the rewarding strength of MEPH is predominantly mediated by the *R*-enantiomer.

3.3. Effect of S-MEPH on EPC produced by cocaine

In the next set of experiments, effects of *S*-MEPH against different behavioral actions of cocaine were investigated. In Fig. 3 the effect of *S*-MEPH (100 μM) against EPC induced by a fixed concentration of cocaine (1 μM) is presented. One-way ANOVA indicated a significant main effect [$F(3, 42) = 5.214$, $P < 0.01$]. Planarians conditioned with cocaine displayed significant EPC compared to drug-naïve control planarians ($P < 0.05$). In the case in which planarians were conditioned with cocaine and then tested in S-MEPH after conditioning, the place conditioning effect was significantly reduced compared relative to planarians treated with cocaine and tested in water ($P < 0.001$). *S*-MEPH, relative to water-treated controls, did not produce any significant effects ($P > 0.05$).

3.4. Effect of S-MEPH on cocaine withdrawal response

The effect of *S*-MEPH (1, 100 μM) against abstinence-induced withdrawal caused by a fixed concentration of cocaine (1 μM) is

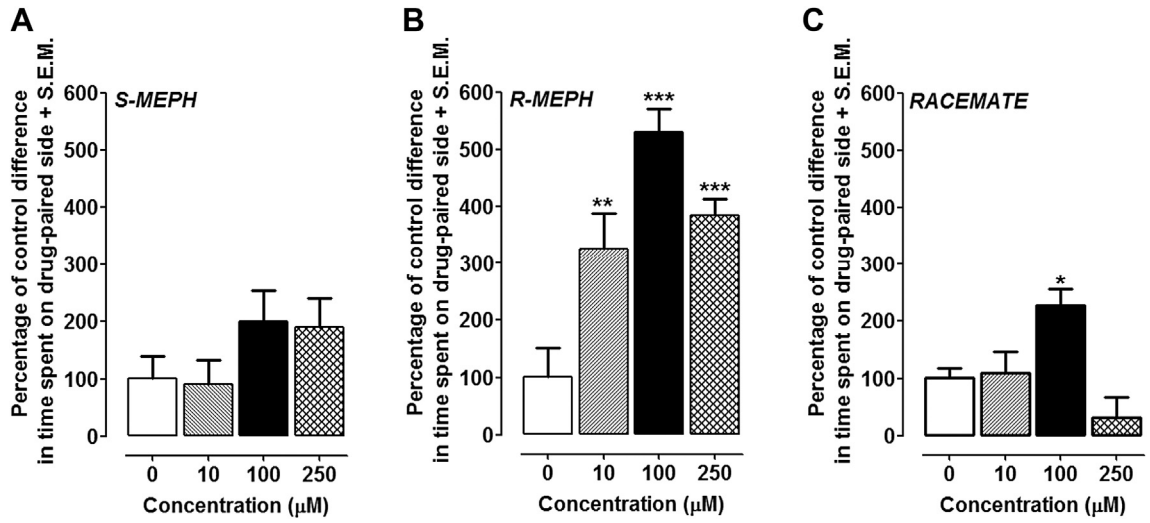

Fig. 2. Effects of S-MEPH, R-MEPH and racemic MEPH on environmental place conditioning (EPC). Planarians were conditioned with different concentrations (0, 10, 100, 250 μM) of S-MEPH (2A), R-MEPH (2B), or racemic MEPH (2C) and EPC was determined as the difference in time spent on the drug-paired side before and after conditioning (post-conditioning time minus pre-conditioning time). Data are presented as the percentage of control difference in time spent on the drug-paired side ±S.E.M. $N = 8$ planarians/group. $^{***}P < 0.001$, $^{**}P < 0.01$ or $^{*}P < 0.05$ compared to respective water control (*i.e.*, 0 μM on the *x*-axis).

presented in Fig. 4. A significant main effect was detected by one-way ANOVA [$F(5, 42) = 24.64$, $P < 0.0001$]. Planarians pretreated with cocaine for 60 min and then withdrawn and tested in water for 5 min displayed decreased motility relative to drug-naïve planarians (W/W) and planarians exposed to acute (W/C) and chronic cocaine (C/C) ($P < 0.001$). In the case in which planarians were pretreated with cocaine and then withdrawn and tested in a solution of *S*-MEPH (100 μM), the decreased motility was less pronounced than in planarians pretreated with cocaine and then withdrawn and tested in water ($P < 0.05$). A 100-fold lower concentration of *S*-MEPH (1 μM) did not affect the decreased motility in cocaine-withdrawn planarians ($P > 0.05$).

3.5. Effects of S-MEPH on stereotypy produced by cocaine, nicotine, or racemic MEPH

Effects of increasing concentrations of *S*-MEPH (10, 100, 250 μM) on stereotypy (*i.e.*, C-shape movements) induced by acute exposure to cocaine (1 mM), nicotine (1 mM), or racemic MEPH (1 mM) are shown in Fig. 5A–C. A one-way ANOVA conducted on each individual data set indicated that *S*-MEPH did not affect stereotypical activity produced by any of the drugs tested (cocaine, [$F(3, 28) = 0.3026$, $P > 0.05$]; nicotine, [$F(3, 28) = 0.3838$, $P > 0.05$]; racemic MEPH, [$F(3, 24) = 0.7966$, $P > 0.05$]).

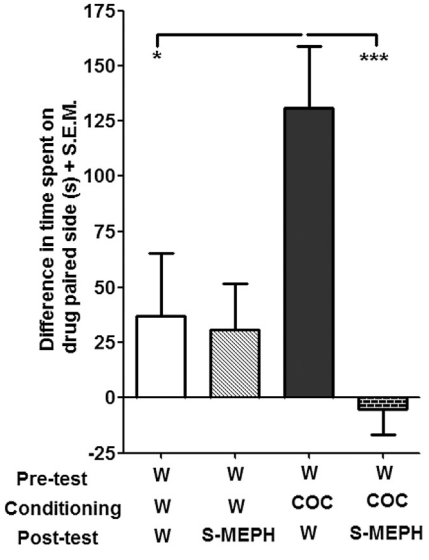

Fig. 3. S-MEPH inhibits environmental place conditioning (EPC) produced by cocaine. All planarians underwent a pre-test in water (W) to determine the naturally less-preferred environment that was designated as the drug-paired side on which conditioning occurred. Planarians were next conditioned with water (W) or cocaine (COC) (1 μM) before undergoing a post-test in either water (W) or *S*-MEPH (100 μM). EPC was determined and presented as the difference in time spent on the drug-paired side before and after conditioning (post-test minus pre-test times). $N = 11$–12 planarians/group. $^{***}P < 0.001$ or $^{*}P < 0.05$ compared to cocaine only group (*i.e.*, W/COC/W).

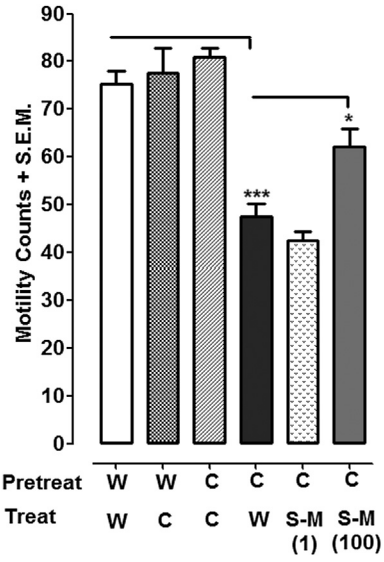

Fig. 4. S-MEPH attenuates withdrawal response produced by cocaine. Planarians were pretreated for 60 min in water (W) or cocaine (C) (1 μM). Water-pretreated planarians were withdrawn and treated for 5 min with water (W) or cocaine (C) (1 μM). Cocaine-pretreated planarians were withdrawn and treated for 5 min with cocaine (C) (1 μM), water (W) or *S*-MEPH (1, 100 μM). Motility was determined over the 5-min treatment interval and presented as motility counts + S.E.M. $N = 8$ planarians/group. $^{***}P < 0.001$ or $^{*}P < 0.05$ compared to cocaine withdrawn group (C/W).

4. Discussion

The present study provides investigation into the stereospecific effects of MEPH enantiomers both in producing psychostimulant-like reward, as well as therapeutic effects. We used established invertebrate assays to probe differences between the individual enantiomers of MEPH (Pagán, 2014). Our results suggest that the *R*-enantiomer of MEPH is primarily responsible for the stereotypical and rewarding effects of the drug in planarians. In addition, our findings suggest that the *S*-enantiomer of MEPH is capable of reducing cocaine reward and abstinence-induced withdrawal in planarians without producing positive rewarding effects of its own.

The behavioral effects of racemic MEPH, the street form of the drug, have been demonstrated in planarians (Ramoz et al., 2012) and are consistent with rewarding and motor effects produced by established psychostimulants (Tallarida et al., 2014; Pagán et al., 2008, 2009, 2013; Rawls et al., 2010, 2011). In the present experiments, all three forms of MEPH (racemate, *S*-MEPH, and *R*-MEPH) produced C-shape movements following acute exposure (Passarelli et al., 1999; Rawls et al., 2011; Tallarida et al., 2014), but the magnitude of the response was influenced by stereochemistry. The *S*-enantiomer displayed less strength and efficacy than both *R*-MEPH and racemate in producing C-shape movements, suggesting that stereotypical effects of MEPH are predominantly mediated by the *R*-enantiomer. The greater potency displayed by *R*-MEPH in planarians is different from results from rat studies in which the enantiomers of amphetamine and methamphetamine produce increases in stereotypy that are not significantly different (Kuczenski et al., 1995; Gregg et al., 2014). The effects of MEPH on motility, which is related to ambulation in rodents, was not quantified here, but an inverse relation between stereotypy and motility was noted (*i.e.*, increasing concentrations of MEPH were associated with enhanced stereotypical activity and reduced motility, and vice versa). For acute psychostimulant exposure, such a concentration-related phenomenon is not unexpected, as a similar correlation has been shown for nicotine in planarians (Rawls et al., 2011) and for cocaine and amphetamines in rats (Gold et al., 1989; White et al., 1998). However, when using a design analogous to the one applied here, the increased motility displayed by rodents following acute stimulant exposure is often not as pronounced in planarians (Rawls et al., 2011;), and this is one reason we chose not to quantify motility. One reason for this difference may be that drug-naïve planarians are already moving at a maximum speed in the aquatic media prior to drug exposure, and that any further increases in motility are masked by the existing 'ceiling effect'.

The most profound stereospecific difference for MEPH was detected in the environmental place conditioning (EPC) assays. Racemic MEPH, as was previously demonstrated in rats and planarians (Ramoz et al., 2012; Lisek et al., 2012), produced modest place conditioning. For the enantiomers, *R*-MEPH produced robust place conditioning whereas *S*-MEPH lacked efficacy across the entire range of concentrations tested. The most parsimonious explanation for this difference is that positive rewarding effects of MEPH, at least in planarians, are predominantly mediated by its *R*-enantiomer. The greatest reward effects for each form of MEPH were detected at a concentration (100 μM) that produced only limited stereotypical activity. In fact, at a concentration of 250 μM, the reward efficacy of both racemate and *R*-MEPH had decreased, suggesting that enhanced stereotypy associated with higher concentrations counteracted any positive rewarding effects. A similar dose-related correlation between rewarding and aversive effects of psychostimulants has been demonstrated in rat assays. For example, in conditioned place preference (CPP) studies, cocaine displays an inverted U-shaped dose response in which doses at the higher end produce weaker rewarding responses due to greater aversive effects such as enhanced stereotypy and eventually seizure (Bardo et al., 1995; Tzschentke, 2007; Zakharova et al., 2009; Estevez et al., 1979; Bhattacharyya and Pradhan, 1979; Zagnoni and Albano, 2002). Our results with MEPH are suggestive of a similar phenomenon in which its positive rewarding effects are strongest at lower concentrations that produce fewer stereotyped movements.

The first part of the study demonstrated that the *S*-enantiomer of MEPH lacks rewarding efficacy in planarians but displays a modest degree of stimulant activity, albeit weaker than *R*-MEPH and racemate. These data indicate that *S*-MEPH may produce some enhancement of monoamine transmission while lacking significant abuse liability, thereby offering a potentially desirable neuropharmacological profile for reducing the withdrawal-induced anxiety and craving in cocaine addicts that often triggers relapse. On the basis of this tenet and the structural similarity between *S*-MEPH and bupropion, which is a cathinone derivative approved for relapse prevention in cigarette smokers and depression, we hypothesized that *S*-MEPH treatment during forced cocaine abstinence could reduce cocaine's rewarding strength and withdrawal response without producing addictive-like effects of its own.

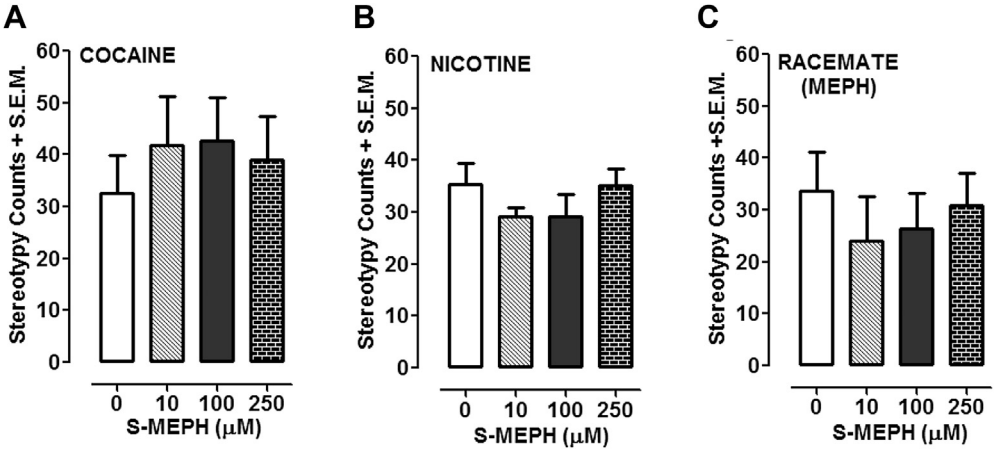

Fig. 5. Effects of S-MEPH on stereotypical movements produced by cocaine, nicotine or racemic MEPH. Planarians were exposed for 5 min to a combination of S-MEPH (0, 10, 100, 250 μM) and a fixed concentration (1 mM) of cocaine, nicotine or racemic MEPH. Motility was determined over the 5-min interval and presented as motility counts + S.E.M. $N = 8$ planarians/group.

Consistent with prior work (Tallarida et al., 2014; Ramoz et al., 2012; Rawls et al., 2011), cocaine produced place conditioning effects suggestive of positive reward. Yet, in the case in which planarians that had already been conditioned with cocaine were treated with *S*-MEPH, the expression of cocaine's rewarding efficacy was prevented.

The cocaine withdrawal response was reduced when *S*-MEPH was administered to planarians during the abstinence interval following discontinuation of chronic cocaine exposure. This withdrawal response to cocaine in planarians manifests as decreased motility following spontaneous discontinuation of cocaine exposure and extends to related drugs of abuse including amphetamines, nicotine, benzodiazepines, and opioids (Zhang et al., 2013; Raffa et al., 2008; Raffa and Rawls, 2008; Sacavage et al., 2008; Pagán et al., 2008, 2009). Prior work indicates that planarian motility is not reduced following discontinuation of exposure to "control" drugs that lack significant abuse liability, such as opioid antagonists, glutamate receptor antagonists, and nitric oxide synthase inhibitors (Rawls et al., 2007; Raffa et al., 2008). Furthermore, in the present experiments, the decrease in motility was reduced only during the condition of cocaine abstinence and not during acute or continuous cocaine exposure. Since a distinguishing feature of physical dependence is the appearance of a somatic sign or response only during drug abstinence, these data suggest that cocaine is capable of producing physical dependence in planarians. Other possibilities are that the decreased planarian motility during cocaine abstinence reflected a "depressive-like state" caused by catecholamine depletion, similar to the immobility that psychostimulant-withdrawn rats display in the forced swim assay (Magalhães et al., 2002), or an "anxiogenic-like state" displayed by cocaine-withdrawn rats in the elevated plus maze (EPM) assay (Rogerio and Takahashi, 1992). Although it is difficult to directly compare withdrawal responses in planarians and rats and unclear if the reduced motility in cocaine-withdrawn planarians reflects physical dependence, depression, or anxiety, it is clear that the response is partially counteracted by administration of *S*-MEPH.

The mechanism by which *S*-MEPH was counteracted cocaine's rewarding and withdrawal effects in planarians is unclear, and it will be important to now conduct related experiments in rats to determine the translational validity of our findings. Racemic MEPH exerts its direct pharmacological actions by acting as a substrate at monoamine transporters to increase dopamine and 5-HT release that leads to an elevation in extracellular dopamine and 5-HT in the nucleus accumbens of rats (Baumann et al., 2012; López-Arnau et al., 2012; Eshleman et al., 2013; Kehr et al., 2011). Neurochemical profiles of the individual MEPH enantiomers have not been characterized, but stereospecific effects of MEPH on dopamine and 5-HT release are predictable and may have contributed to the stereochemical separation of MEPH effects on planarian behavior. Since the rewarding efficacy of MEPH, like other drugs of abuse, is reliant on increased dopamine transmission, the dopamine-releasing effect of MEPH may reside predominantly in its *R*-enantiomer, which displayed greater rewarding and stereotypical effects than *S*-MEPH in our planarian experiments. The weaker rewarding and stereotypical effects of *S*-MEPH relative to *R*-MEPH, as well as its ability to reduce cocaine reward and withdrawal signs in planarians with prior cocaine history, may be due to weaker effects on dopamine release, stronger effects on 5-HT release, or a combination of the two. Significant preclinical and clinical evidence suggests that deficits in both dopamine and 5-HT function in brain reward circuits are associated with withdrawal from cocaine (Rothman et al., 2008; Volkow et al., 2002; Weiss et al., 1996; Martinez et al., 2007) and has led to the proposal of a dual deficit model of cocaine addiction in which drug-induced dopamine and 5-HT dysfunction contributes to withdrawal symptoms, drug craving, and relapse. Thus, at least in planarians, the efficacy of *S*-MEPH efficacy may have been related to its ability to offset some of the dopamine and 5-HT dysfunction during cocaine abstinence. Since the planarian assays are better for screening purposes, as opposed to studying mechanisms of action, it is now important to investigate *S*-MEPH in rodent models of cocaine withdrawal and addiction and to determine its selectivity to promote release of dopamine, 5-HT and norepinephrine (Banks et al., 2011).

In conclusion, we demonstrated that the behavioral profile of MEPH is stereospecific, with the *R*-enantiomer being primarily responsible for its rewarding and stereotypical effects, and that *S*-MEPH reduces cocaine's rewarding strength and withdrawal response without producing rewarding effects of its own. Since enantiomeric specificity of action correlated with neuropharmacology is often a starting point for further structure activity relationship (SAR) development, one future application of these findings may be drug discovery. The isolation of specific enantiomers of a compound, or formulation of specific enantiomeric ratios, can also be used to separate therapeutic actions while minimizing potential side effects. For example, Adderall is a widely prescribed therapeutic for attention deficit disorder that uses a 3:1 ratio of *S*:*R*-amphetamine salts as a way to both optimize the pharmacokinetic profile and lower its abuse liability (Heal et al., 2013; Bidwell et al., 2011). An example of a drug in which one enantiomer is active, while the other enantiomer is inactive is the antihypertensive drug atenolol, in which its ability to block β-adrenoceptors resides in its *S*-form. Finally, considering the dangers of MEPH abuse, future studies are planned using rats to identify neurochemical mechanisms that are responsible for MEPH stereospecificity.

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