

5-HT_{1A}-like receptor activation inhibits abstinence-induced methamphetamine withdrawal in planarians

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ABSTRACT

No pharmacological therapy is approved to treat methamphetamine physical dependence, but it has been hypothesized that serotonin (5-HT)-enhancing drugs might limit the severity of withdrawal symptoms. To test this hypothesis, we used a planarian model of physical dependence that quantifies withdrawal as a reduction in planarian movement. Planarians exposed to methamphetamine (10 μM) for 60 min, and then placed (tested) into drug-free water for 5 min, displayed less movement (*i.e.*, withdrawal) than either methamphetamine-naïve planarians tested in water or methamphetamine-exposed planarians tested in methamphetamine. A concentration-related inhibition of withdrawal was observed when methamphetamine-exposed planarians were placed into a solution containing either methamphetamine and 5-HT (0.1–100 μM) or methamphetamine and the 5-HT_{1A} receptor agonist 8-hydroxy-*N,N*-dipropyl-2-aminotetralin (8-OH-DPAT) (10, 20 μM). Planarians with prior methamphetamine exposure displayed enhanced withdrawal when tested in a solution of the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide (WAY 100635) (1 μM). Methamphetamine-induced withdrawal was not affected by the 5-HT_{2B/2C} receptor agonist meta-chlorophenylpiperazine (m-CPZ) (0.1–20 μM). These results provide pharmacological evidence that serotonin-enhancing drugs inhibit expression of methamphetamine physical dependence in an invertebrate model of withdrawal, possibly through a 5-HT_{1A}-like receptor-dependent mechanism.

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A barrier to identification of agents to treat methamphetamine physical dependence is the lack of a sensitive withdrawal measure in mammals. Human trials rely on diverse endpoints, including dysphoria, depression, anxiety, and psychomotor disturbances, that are difficult to quantify [7,36]. Rodents develop methamphetamine physical dependence, but the associated withdrawal symptoms are more difficult to quantify than opiate-induced withdrawal phenomena [6]. A simpler species that displays a quantifiable abstinence-induced withdrawal response to psychostimulants is planarians, aquatic flatworms that utilize mammalian-relevant neurotransmitters, such as serotonin (5-HT), dopamine, and glutamate, that mediate addiction [4,15,17,25–31,39]. Methamphetamine produces alterations in 5-HT function in mammals which underlie its addictive properties [8,12,14,38]. Midbrain 5-HT neurons innervate, and dampen the activity of, mesocorticolimbic dopamine neurons that are activated by methamphetamine [18,19,21,22]. Amphetamine-induced hyperactivity and behavioral

sensitization is attenuated by 5-HT_{1A} receptor agonists, 5-HT_{1B} receptor antagonists, and 5-HT_{2A} receptor antagonists [1,13,23,24]. These collective data suggest that 5-HT-enhancing drugs are capable of managing addiction-related behaviors caused by methamphetamine abuse. We hypothesized that planarians would be an ideal organism to investigate this hypothesis because they contain well-defined 5-HT system and display sensitive responses to methamphetamine, including spontaneous withdrawal and conditioned place preference [10,30,31]. We specifically determined if methamphetamine withdrawal is affected by 5-HT, a 5-HT_{1A} receptor agonist (8-OH-DPAT), a 5-HT_{1A} receptor agonist (WAY 100635), or a 5-HT_{2B/2C} receptor agonist (m-CPZ).

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply and tested within 72 h. Methamphetamine was obtained from the National Institute on Drug Abuse. Serotonergic compounds (5-HT, 8-OH-DPAT, WAY 100635, and m-CPZ) were purchased from Tocris Bioscience. To quantify planarian movement [25–31], individual planarians were placed into a clear plastic petri dish (14-cm diameter) containing room-temperature (21 °C) tap water treated with AmQuel® water conditioner. The dish was located over paper with gridlines spaced 0.5 cm apart. Planarian movement was quantified as the number of gridlines crossed or re-crossed over a 5-min observation period. The

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Table 1

Methamphetamine (10 μ M) produces abstinence-induced withdrawal. Planarians pretreated with water (W) or methamphetamine (M) for 60 min were then treated with W or M for 5 min. Planarian movement (\pm SEM) was quantified over the 5-min treatment interval.

Pretreatment/treatment	Planarian movement \pm SEM	N
W/W	81 \pm 3.2	10
M/W	41 \pm 1.7**	12
M/M	84 \pm 3.8	10
W/M	90 \pm 2.5	10

** $P < 0.01$ compared to W/W.

planarian movement consists of constant-velocity, horizontal, and forward-directed movement, with periodic turns, but rarely any stops. The speed of movement is essentially constant for at least 10 min [25–31,33]. Prior to measurement of planarian movement, each organism was placed into individual 0.5 ml vials containing water or methamphetamine for 60 min. Planarians were then placed for 5 min into one of the following: 5-HT (0.001–100 μ M); 8-OH-DPAT (0.1–20 μ M); WAY 100635 (0.001–1 μ M); or m-CPZ (0.1–20 μ M). The concentration of methamphetamine used to induce abstinence-induced withdrawal, as well as concentrations of 5-HT and 5-HT agonists/antagonists that do not affect planarian movement when given alone, were selected on the basis of prior studies [5,30,31]. Data were expressed as the mean (\pm SEM) of the total number of gridlines crossed by each planarian in the 5-min test interval. Comparisons of group means were determined by a one-way ANOVA followed by a Bonferroni *post hoc* test. Values of $P < 0.05$ were considered statistically significant.

The withdrawal response described here is quantified as a reduction in planarian movement. Drug-naïve planarians normally travel at a constant, apparently near-maximal speed, so the only change is slowing of the speed (movement). Although physiological stresses can alter planarian movement, we have demonstrated that the reduction in planarian movement is due to withdrawal from a specific drug and not some other factor, such as pH or osmolarity [25–31,33]. Equally important, we only describe withdrawal when acute or continued exposure to the same concentration of drug does not decrease planarian movement.

Effects of both acute methamphetamine (10 μ M) exposure and methamphetamine withdrawal on planarian movement are presented in Table 1. One-way ANOVA revealed a significant main effect [$F(3, 38) = 68.09, P < 0.0001$]. Planarians pretreated with methamphetamine and tested in water displayed movement that was significantly less compared to methamphetamine-naïve planarians tested in water ($P < 0.01$) but not significantly different from methamphetamine-pretreated planarians tested in methamphetamine ($P > 0.05$). Planarians that were pretreated with water and tested in water or methamphetamine did not display significantly different movement ($P > 0.05$).

Effects of increasing 5-HT concentrations (0.001, 0.01, 0.1, 1, 10, and 100 μ M) on abstinence-induced methamphetamine (10 μ M) withdrawal are presented in Fig. 1. One-way ANOVA revealed a significant main effect [$F(7, 56) = 5.590, P < 0.001$]. Methamphetamine-treated planarians that were withdrawn and tested in water displayed significantly less movement than methamphetamine-naïve planarians tested in water ($P < 0.001$). Movement displayed by planarians treated with methamphetamine and then tested in water was significantly lower than movement displayed by methamphetamine-exposed planarians tested in 5-HT (10 μ M, $P < 0.05$ and 100 μ M, $P < 0.01$). Additionally, in methamphetamine-exposed planarians, movement was not significantly different when planarians were tested in water or 5-HT (10, 100 μ M) ($P > 0.05$).

Effects of progressively increasing concentrations (0.1, 1, 10, and 20 μ M) of the 5-HT_{1A} receptor agonist 8-OH-DPAT on abstinence-

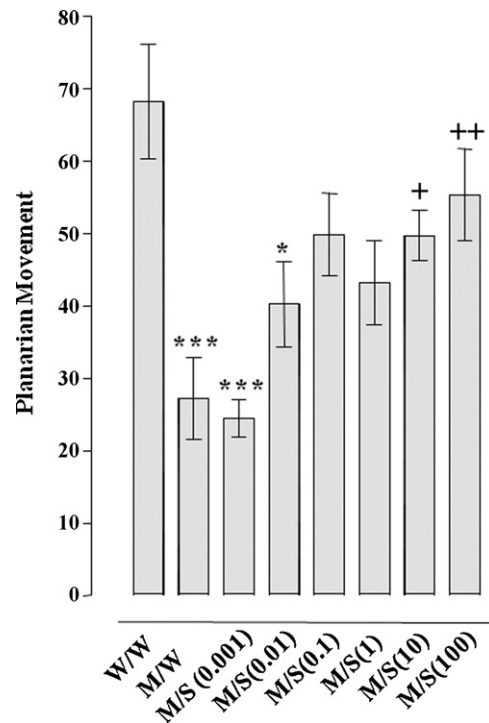


Fig. 1. Serotonin (S) inhibits abstinence-induced methamphetamine withdrawal. Planarians exposed to 10 μ M methamphetamine (M) or water (W) for 60 min were tested in W or serotonin (S) (0.001–100 μ M) for 5 min ($N = 7–9$ planarians/group). * $P < 0.05$, *** $P < 0.001$ compared to W/W and + $P < 0.05$, ** $P < 0.01$ compared to M/W.

induced methamphetamine (10 μ M) withdrawal are presented in Fig. 2. One-way ANOVA revealed a significant main effect [$F(5, 42) = 7.576, P < 0.001$]. Methamphetamine-treated planarians that were withdrawn and tested in water again displayed significantly less movement than methamphetamine-naïve planarians tested in water ($P < 0.001$). Movement displayed by planarians treated with methamphetamine and then tested in water was significantly lower than movement displayed by methamphetamine-exposed planarians tested in 8-OH-DPAT (10 μ M, $P < 0.01$ and 20 μ M, $P < 0.001$). Additionally, in methamphetamine-exposed planarians, movement was not significantly different when planarians were tested in water or 8-OH-DPAT (10, 20 μ M) ($P > 0.05$).

Effects of progressively increasing concentrations (0.001, 0.01, 0.1, and 1 μ M) of the 5-HT_{1A} receptor antagonist WAY 100635 on abstinence-induced methamphetamine (10 μ M) withdrawal are presented in Fig. 3. One-way ANOVA revealed a significant main effect [$F(5, 45) = 23.94, P < 0.001$]. Methamphetamine-treated planarians that were withdrawn and tested in either water or WAY 100635 (0.001, 0.01, 0.1, and 1 μ M) displayed significantly less movement than methamphetamine-naïve planarians tested in water ($P < 0.001$). However, in the case in which methamphetamine-exposed planarians were withdrawn and tested in the highest concentration (1 μ M) of WAY 100635, movement was significantly less than the movement displayed by methamphetamine-exposed planarians that were withdrawn and tested in water ($P < 0.05$). None of the concentrations of WAY 100635 (0.001, 0.01, 0.1, and 1 μ M) significantly affected planarian movement compared to water ($P > 0.05$) (Fig. 3, inset).

Effects of progressively increasing concentrations (0.1, 1, 10, and 20 μ M) of the 5-HT_{2B/2C} receptor agonist m-CPZ on abstinence-induced methamphetamine (10 μ M) withdrawal are presented in Fig. 4. A one-way ANOVA indicated a significant main effect [$F(5, 43) = 15.46, P < 0.001$]. Methamphetamine-treated planarians that were tested in either water or m-CPZ (0.1, 1, 10, and 20 μ M) dis-

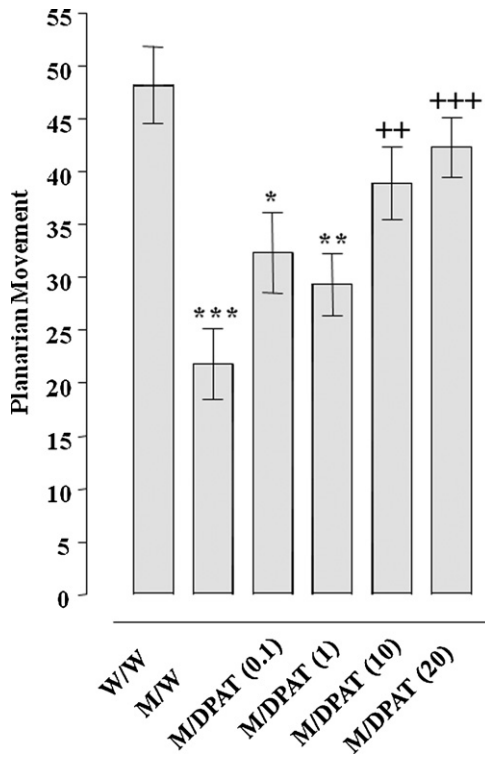


Fig. 2. 8-OH-DPAT (5-HT_{1A} agonist) inhibits abstinence-induced methamphetamine withdrawal. Planarians exposed to 10 μ M methamphetamine (M) or water (W) for 60 min were tested in W or 8-OH-DPAT (DPAT) (0.1–20 μ M) for 5 min ($N=7-10$ planarians/group). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to W/W and ** $P<0.01$, *** $P<0.001$ compared to M/W.

played significantly less movement than did methamphetamine-naïve planarians tested in water ($P<0.001$). Movement displayed by planarians treated with methamphetamine and then tested in water was not significantly different than movement displayed by methamphetamine-exposed planarians tested in any of the concentrations of m-CPZ (0.1, 1, 10, and 20 μ M) ($P>0.05$), although there was a trend toward significance displayed by the higher m-CPZ concentrations (1, 10, and 20 μ M).

We provide evidence that abstinence-induced methamphetamine withdrawal in an invertebrate is inhibited by 5-HT-enhancing agents. Expression of methamphetamine physical dependence was inhibited by administration of 5-HT or by a 5-HT_{1A} agonist (8-OH-DPAT) and slightly, but significantly, enhanced by administration of a 5-HT_{1A} antagonist (WAY 100635). Methamphetamine-induced withdrawal was not affected by a 5-HT_{2B/2C} agonist (m-CPZ), suggesting that the abstinence response was selective for 5-HT_{1A} compounds. A major role for 5-HT_{1A}-like receptors in planarian methamphetamine physical dependence is supported by evidence that planarians display characteristic behavioral responses following acute 5-HT exposure and express 5-HT_{1A}-like receptors that show partial homology to mammalian 5-HT_{1A} receptors which contribute to the psychostimulant-induced 5-HT response [3,32,34,36,37,39]. Four sequences of 5-HT receptors in planarians, designated 5-HTpla1 to 5-HTpla4, have been identified in molecular biology studies, all of which are similar to the human 5-HT_{1A} receptor. The 5-HTpla1 receptor displays partial homology to the human 5-HT_{1A}, human 5-HT₇ receptor, *Xenopus* 5-HT₇, and *Drosophila* 5-HTdro1 receptor. Of the four planarian 5-HT receptors, the 5-HTpla4 receptor is the most abundantly expressed and displays partial homology to the human 5-HT_{1A}, human 5-HT₇ receptor, *Xenopus* 5-HT₇, and *Drosophila* tyramine receptor. Deficits in 5-HT_{1A} receptor function have been linked to addiction-related adverse effects of methamphetamine, including a clinical

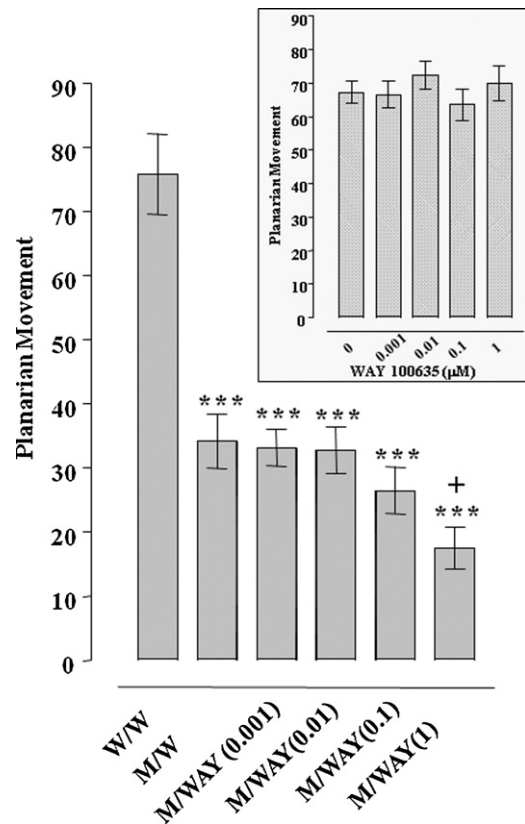


Fig. 3. WAY 100635 (5-HT_{1A} antagonist) enhances abstinence-induced methamphetamine withdrawal. Planarians exposed to 10 μ M methamphetamine (M) or water (W) for 60 min were tested in W or WAY 100635 (WAY) (0.001–1 μ M) for 5 min ($N=7-9$ planarians/group). *** $P<0.001$ compared to W/W and * $P<0.05$ compared to M/W. (Inset) WAY planarians were exposed to WAY (0.01–10 μ M) for 5 min.

trial demonstrating that 5-HT_{1A} receptor dysfunction is a causative factor in methamphetamine-induced psychosis [9]. Neurochemical data indicate 5-HT_{1A} receptor activation inhibits hyperactivity, and prefrontal 5-HT release, produced by acute methamphetamine administration and inhibits behavioral sensitization induced by repeated methamphetamine exposure [1,23]. Furthermore, activation of 5-HT_{1A} receptor-expressing neurons originating in the midbrain diminishes the activity of mesolimbic dopamine neurons activated by methamphetamine exposure [18,19,21,22].

Augmentation of methamphetamine withdrawal by a 5-HT_{1A} receptor antagonist suggests expression of methamphetamine physical dependence is opposed by endogenous tone at 5-HT_{1A}-like receptors. The components required for such a compensatory response are present in planarians as they synthesize 5-HT and display at least four subtypes of 5-HT_{1A}-like receptors [34,39]. In mammalian studies, acute methamphetamine exposure increases extracellular 5-HT in terminal substrates of the brain reward circuit (e.g., nucleus accumbens, prefrontal cortex) but the outcome reverses during psychostimulant withdrawal, where a reduction in limbic 5-HT activity is observed [19,35]. Thus, one explanation for our results with WAY 100635 is that planarians withdrawn from methamphetamine display decreased 5-HT activity that embellishes the withdrawal response. In the case in which methamphetamine-withdrawn planarians are treated with a 5-HT_{1A} antagonist, blockade of 5-HT_{1A}-like receptor activity further inhibits 5-HT activity, thereby producing an exaggeration of the withdrawal response. In the reverse case, in which methamphetamine-withdrawn planarians are treated with a 5-HT_{1A} agonist (8-OH-DPAT), 5-HT activity is either elevated or

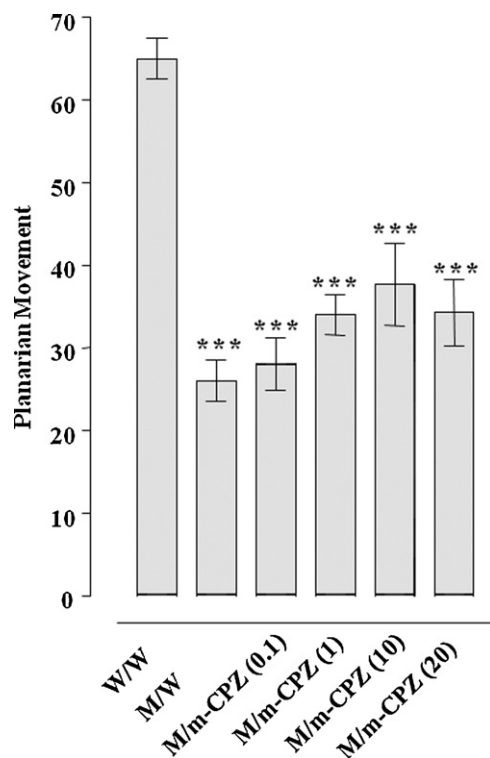


Fig. 4. m-CPZ (5-HT_{2B/2C} agonist) does not affect abstinence-induced methamphetamine withdrawal. Planarians exposed to 10 μ M methamphetamine (M) or water (W) for 60 min were tested in W or m-CPZ (0.1–20 μ M) for 5 min ($N=7$ –10 planarians/group). *** $P < 0.001$ compared to W/W.

restored, thereby reducing the severity of withdrawal. Future studies will attempt to determine the effect of methamphetamine withdrawal on 5-HT activity in planarians, akin to *in vivo* microdialysis experiments conducted in rats.

Insight into the relative contributions of pre- and post-synaptic 5-HT_{1A} receptors to methamphetamine dependence is not provided by the present results. Effects of pre- and post-synaptic 5-HT_{1A} receptors on methamphetamine addiction can be dissociated in rats, with 5-HT_{1A} autoreceptor activation enhancing addiction and post-synaptic 5-HT_{1A} receptor activation opposing addiction [14]. Methamphetamine-induced addiction-related behaviors are believed to be shaped more by the ratio of limbic 5-HT to dopamine levels rather than absolute levels of either neurotransmitter, with a greater 5-HT to dopamine ratio opposing addiction, and vice versa [2,14]. An enhanced 5-HT to dopamine ratio is achieved by activation of 5-HT_{1A} receptors located on limbic dopamine terminals, which causes a direct reduction in extracellular dopamine, whereas 5-HT_{1A} autoreceptor activation, by reducing the level of extracellular 5-HT in limbic areas through inhibition of midbrain 5-HT neuronal activity, shifts the ratio toward greater dopamine levels and thus enhances addiction-related behaviors following methamphetamine administration [1,23,24]. As more information becomes available regarding the planarian genome, it will be important to determine if a single, or multiple, 5-HT_{1A}-like receptor subtypes are involved in psychostimulant physical dependence and withdrawal in this species.

One promising application of the planarian withdrawal assay is *in vivo* screening of potential abuse-deterrent compounds. The translational capacity of the methodology needs to be further delineated, but the 'direction' of the psychostimulant withdrawal response in planarians and rats is similar. Planarians withdrawn from methamphetamine display reduced movement, and rats withdrawn from continuous administration of amphetamines dis-

play hypolocomotion [11,20,26,27,30,31,33]. The planarian assay may also complement existing mammalian assays, such as the elevated maze that determines the anxiety level of rats withdrawn from repeated psychostimulant administration [16]. Advantages of the planarian assay are: (a) amenability to screening therapeutic candidates against different abused drugs, and combinations of abused drugs, which are administered in exposure patterns that mimic recreational and addictive drug use; (b) ability to cost-effectively screen small amounts of synthetic compounds of limited supply; (c) reduction of interpretive complications caused by mammalian pharmacokinetics; and (d) less susceptibility to higher-order confounders such as handling stress, testing-environment familiarity, procedural stresses, and anticipation of drug administration. One limitation of the model is that stimulants do not significantly increase planarian movement or locomotion following acute administration, which is different from the well-documented behavioral activation that stimulants produce in rats and mice. We believe this is because drug-naïve planarians are essentially moving at maximum speed, and that their acute exposure to a stimulant is unable to further increase movement. Another limitation of the planarian model is the lack of knowledge about molecular targets, especially as it relates to the degree of homology between addiction-related substrates in planarians, rodents, and humans.

In conclusion, these results demonstrate that methamphetamine physical dependence in planarians is inhibited by a 5-HT_{1A} agonist, but not by a 5-HT_{2B/2C} agonist. Methamphetamine withdrawal was enhanced by a 5-HT_{1A} antagonist, suggesting that 5-HT_{1A}-like receptor activity tonically opposes the withdrawal response. These combined data provide a functional correlate to previous molecular biology experiments and suggest that 5-HT_{1A}-like receptors regulate methamphetamine physical dependence in planarians.

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