



Withdrawal-like behavior in planarians is dependent on drug exposure duration

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ABSTRACT

Planarians display a concentration-related reduction in locomotor activity following their spontaneous withdrawal from opioids, cannabinoids, stimulants and benzodiazepines. This suggests that planarians display a withdrawal-like behavior that can be quantified as a reduction in locomotor activity. Because withdrawal-like behavior in previous studies has been quantified only following the cessation of a 60-min drug exposure, it is unclear whether the withdrawal response varies with drug exposure duration. Therefore, the goal of this study is to determine if the duration of drug exposure (0, 5, 15, 30, 45, 60 min and 24 h) to three different drugs – methamphetamine, cocaine and caffeine – affects the magnitude of withdrawal-like behavior (i.e., reduced locomotor activity) in planarians. Experiments revealed that methamphetamine (10 μ M) produced significant withdrawal-like behavior regardless of the exposure time ($P < 0.05$). An exposure time of only 5 min resulted in a significant reduction in locomotor activity. The peak effect, although occurring following a 24-h exposure, was only slightly greater than that caused by a 30-min exposure. For cocaine (10 μ M), a longer exposure time (15 min) was required for the manifestation of significant withdrawal-like behavior. The peak cocaine effect was observed following a 24-h exposure. Caffeine (10 μ M) did not produce significant changes in locomotor activity during withdrawal or alter locomotor activity during acute exposure. The present results suggest that the magnitude of withdrawal-like behavior in planarians is dependent on both the duration and type of drug exposure, and that planarians do not display withdrawal to caffeine.

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Abstinence-induced withdrawal phenomena are behavioral manifestations of the physical dependence that develops to drugs of abuse and a common experimental paradigm used to assess and study dependence in mammals. A type of withdrawal phenomenon (hyperresponsiveness to vibration) has also been demonstrated in crickets, and tolerance and behavioral responses, like addiction, have been reported in mollusks and land snails [6,17,18,47]. Our laboratory has previously developed and reported the use of a new metric to identify and quantify an abstinence-induced withdrawal-like behavior in planarians [27]. Planarians are free-living, fresh-water flatworms that are considered to be the most primitive extant animals having bilateral symmetrical nerve processes consisting of cephalic ganglia and peripheral nerve cords. Planarians provide a useful and convenient model for the study of nervous

system function and drug-induced effects because they utilize mammalian-like neurotransmitters; express catecholamines and monoamine-containing neurons; display biochemical changes following exposure to direct- and indirect-acting sympathomimetics; increase cyclic AMP formation following exposure to dopamine agonists; and respond with characteristic behaviors to selective ligands [1–3,7,8,10,11,20,21,24–25,35,40,41,39,44]. The withdrawal-like behavior in planarians is quantified as a reduction in locomotor activity following the cessation of drug exposure [27]. Planarians display withdrawal-like behavior to a number of addictive drugs, including opioids, cannabinoids, benzodiazepines, cocaine and amphetamines, and the effect is not due to factors such as pH and osmolarity [31,32,34,36,38].

One unresolved question is whether withdrawal-like behavior in planarians is dependent on drug exposure duration. It is documented in mammals that the intensity of a withdrawal syndrome, as well as the specific behaviors which accompany withdrawal, vary with the type of drug administered, duration of drug exposure and frequency of drug administration [5,26]. For example, in morphine-dependent rats, a characteristic behavior following the

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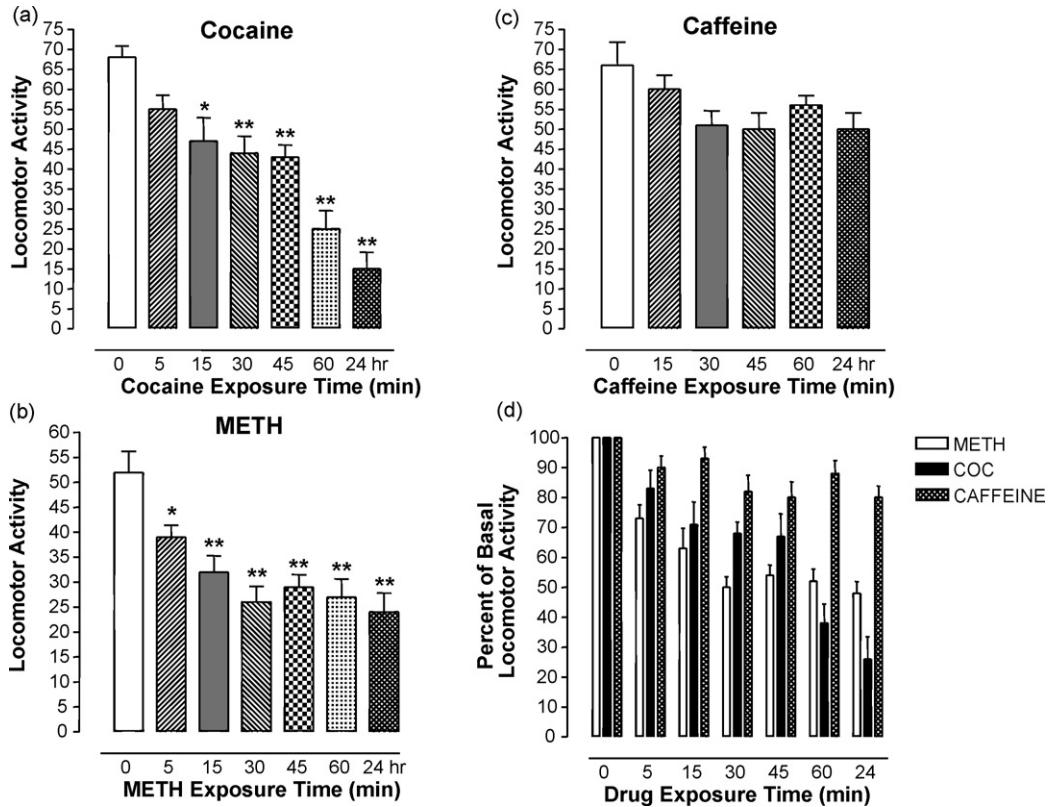


Fig. 1. Effects of drug exposure duration on the degree of withdrawal-like behavior (reduction in locomotor activity) in planarians. (a–c) Planarians were exposed to methamphetamine (10 μ M), cocaine (10 μ M) or caffeine (10 μ M) for 0, 5, 15, 30, 45 and 60 min, and for 24 h, and then tested individually for locomotor activity in water for 5 min. Locomotor activity was quantified as the number of gridlines crossed or re-crossed over a 5-min interval and is expressed as the mean \pm S.E.M. of the cumulative locomotor activity. $N = 10$ –12 planarians per group. * $P < 0.05$ or ** $P < 0.01$ compared to the respective drug exposure time of 0 min. (d) The raw data in 1a–c was expressed as a percentage of basal activity (i.e., activity in groups not exposed to a drug) for each drug at each time point. A two-way ANOVA revealed a significant drug effect [$F_{(2,32)} = 52.15$, $P < 0.0001$] and time effect [$F_{(6,192)} = 30.24$, $P < 0.0001$].

precipitation of withdrawal with naloxone is wet-dog shaking, but the intensity of the shaking is highly sensitive to the duration of morphine exposure and dose of naloxone administered [4]. On the other hand, the frequency of wet-dog shaking following the precipitation of withdrawal in nicotine-dependent rats is much less than the shaking observed during morphine withdrawal [16,19,45]. It is the goal of the present study to determine the effect that duration of drug exposure, and drug type, has on the expression of withdrawal-like behavior in planarians.

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply Company (Burlington, NC). Planarians were acclimated to temperature-controlled room temperature (21 $^{\circ}$ C) and tested within 72 h of arrival. Cocaine hydrochloride was generously supplied by the National Institute on Drug Abuse, and methamphetamine hydrochloride and caffeine were purchased from Sigma–Aldrich (St Louis, MO, USA). Cocaine and methamphetamine solutions (10 μ M) were prepared directly in room-temperature (21 $^{\circ}$ C) tap water containing AmQuel[®] water conditioner. A stock solution of 1 mM caffeine was prepared in a 0.4% methylcellulose/water solution, and then diluted to volume with tap water containing AmQuel[®] water conditioner [15].

Planarian locomotor activity was quantified by placing individual planarians into a clear plastic petri dish containing room-temperature (21 $^{\circ}$ C) tap water containing AmQuel[®] water conditioner [27,28]. The dish was placed over paper with gridlines spaced 0.5 cm apart. Locomotor activity was quantified as the number of gridlines crossed or re-crossed over a 5-min observation period and expressed as the mean (\pm S.E.M.) of the cumulative

number of gridlines crossed by each planarian in 5 min. Prior to behavioral observations, each planarian was placed into individual 0.5 ml vials containing a test drug for a variable amount of time. Planarians were exposed to methamphetamine (10 μ M) for 0, 5, 15, 30, 45 and 60 min, and for 24 h. Following drug pre-treatment, planarians were removed from the drug exposure tubes and placed directly into petri dishes where they were tested for locomotor activity in water for 5 min. Each planarian was used only once. These experiments were repeated with cocaine (10 μ M) and caffeine (10 μ M). To determine the effect of acute caffeine administration on planarian locomotor activity, planarians were placed into water or caffeine (1, 10 or 100 μ M) and tested individually for locomotor activity for 5 min. Comparisons of the cumulative group means were evaluated by a one-way ANOVA, followed by a Dunnett's *post hoc* analysis, or a two-way ANOVA. Values of $P < 0.05$ were considered to be statistically significant.

The effect of cocaine (10 μ M) exposure time on withdrawal-like behavior is presented in Fig. 1a. Locomotor activity of cocaine-exposed planarians tested in water was significantly less than the locomotor activity of cocaine-naïve planarians tested in water for all exposure times except 5 min ($P < 0.05$) (Fig. 1a). Significantly reduced locomotor activity (47 ± 5.9) was first apparent following a 15-min exposure to cocaine, and the peak reduction (15 ± 4.1) occurred following a 24-h exposure to cocaine ($P < 0.05$). Because of the pronounced decline in locomotor activity following the withdrawal of planarians from a 24-h cocaine exposure, a follow-up experiment was conducted to determine if the reduction in locomotor activity has stabilized. Results from that experiment revealed that a 48-h exposure did not further reduce locomotor activity, thus

indicating that the effect had reached its maximum following a 24-h exposure ($P > 0.05$) (data not shown).

Locomotor activity (i.e., withdrawal-like behavior) in methamphetamine-exposed planarians tested in water was significantly less, for all exposure times, than the locomotor activity of methamphetamine-naïve planarians tested in water ($P < 0.05$) (Fig. 1b). In fact, the locomotor activity (39 ± 2.4) of planarians tested in water following only a 5-min methamphetamine exposure was significantly less than the locomotor activity of methamphetamine-naïve planarians (52 ± 4.2) ($P < 0.05$). The peak reduction (24 ± 3.8) in locomotor activity occurred when planarians were tested in water following a maximal methamphetamine exposure time of 24 h, but the effect was only slightly greater than that observed following a 30-min exposure (26 ± 3.2). A comment is warranted on the time course of the locomotor activity in drug-naïve planarians and in planarians withdrawn from either cocaine or methamphetamine. The locomotor activity of drug-naïve planarians (i.e., planarians not exposed to any drug) was consistently greater in the first 2 min after their placement into the test dish, and their locomotor activity declined gradually over the remaining 3 min of the observation interval. The most significant difference between the locomotor activity of cocaine- or methamphetamine-treated planarians and the locomotor activity of drug-naïve planarians was in the first 2 min following withdrawal (i.e., placement into the test dish), with the difference between the drug-treated and drug-naïve planarians becoming less apparent thereafter. In fact, at 60 min after testing, drug-treated planarians displayed locomotor activity that was not significantly different ($P > 0.05$) from drug-naïve planarians (data not shown). At 24 h after testing, drug-treated planarians displayed locomotor activity no different from untreated controls (data not shown).

The effect of caffeine (10 μM) withdrawal on planarian locomotor activity is presented in Fig. 1c. Experiments demonstrated that the locomotor activity of caffeine-exposed planarians tested in water was not significantly different from the locomotor activity of caffeine-naïve planarians tested in water ($P > 0.05$) (Fig. 1c). Although planarians withdrawn from a 30–60 min, or 24 h, exposure to caffeine did display a slight reduction in locomotor activity compared to caffeine-naïve planarians, the effect did not reach statistical significance ($P > 0.05$). Because the effect of caffeine on locomotor activity in planarians has not been tested previously, an additional experiment determined if planarian locomotor activity is altered by an acute caffeine (10 μM) exposure. We found that planarians tested in caffeine (10 μM) for 5 min displayed locomotor activity that was not significantly different from planarians tested in water for 5 min ($P > 0.05$) (Fig. 2).

Planarians display a concentration-related, withdrawal-like behavior following their removal from a 60-min exposure to cocaine, opioids, benzodiazepines, cannabinoids or amphetamines [27–36,38]. The present study used three different drugs, methamphetamine, cocaine and caffeine, to determine whether withdrawal-like behavior varies with drug exposure duration. We found that: [1] methamphetamine and cocaine produced significant withdrawal-like behavior [2] methamphetamine required less exposure time (5 min) than cocaine (15 min) to produce a significant response; [3] methamphetamine and cocaine produced maximal withdrawal-like behavior following a 24-h exposure, but the magnitude of the cocaine effect was greater; and [4] caffeine did not affect locomotor activity following its acute administration or produce withdrawal-like behavior.

Cocaine and methamphetamine both produced maximal withdrawal-like behavior following a 24-h exposure, but there were two differences in their effects. First, methamphetamine required a shorter exposure time than cocaine to produce signifi-

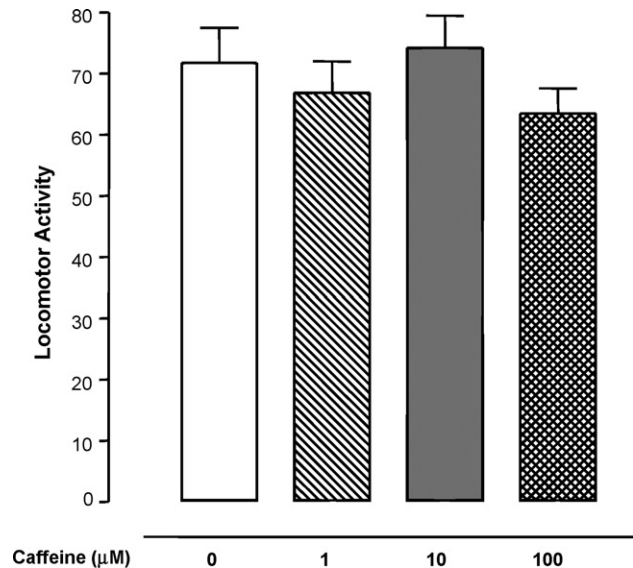


Fig. 2. Effects of acute caffeine exposure on locomotor activity in planarians. Planarians were tested for locomotor activity in water or caffeine (1, 10 or 100 μM) for 5 min. Locomotor activity was quantified as the number of gridlines crossed or re-crossed over a 5-min interval and is expressed as the mean \pm S.E.M. of the cumulative locomotor activity. $N = 12$ –15 planarians per group. A one-way ANOVA of the group means did not reveal a significant drug effect [$F_{(3,51)} = 0.9504$, $P = 0.4233$].

cant withdrawal-like behavior. A significant response was observed following only a 5-min exposure to methamphetamine whereas a 15-min exposure time was necessary for cocaine to produce significant withdrawal-like behavior. Second, the magnitude of the response to cocaine was greater. For example, locomotor activity was $48 \pm 4\%$ of baseline activity during withdrawal from a 24-h methamphetamine exposure whereas locomotor activity was only $27 \pm 7\%$ of baseline activity during withdrawal from a 24-h cocaine exposure. Following the withdrawal of planarians from a 48-h cocaine exposure, a further reduction in locomotor activity was not observed, thus suggesting that the peak withdrawal response to cocaine had been attained. The relevance of our findings to the mammalian withdrawal syndrome is unclear, but it is apparent that the sensitivity to withdrawal is different in planarians and mammals. Planarians display a marked withdrawal response – decrease in locomotor activity – following the spontaneous discontinuation of methamphetamine and cocaine exposure. In contrast, the abrupt withdrawal of these agents does not produce quantifiable withdrawal signs in rats, and only causes minimal signs in humans [14,46].

It is not known why methamphetamine and cocaine produced effects that were not entirely similar. A pharmacodynamic- or dopamine-related reason is possible. Planarians express dopaminergic systems, and selective dopamine agonists produce locomotor effects consistent with those behaviors reported in mammals [23,41]. Methamphetamine and cocaine both increase extracellular dopamine by similar mechanisms of action, but there are differences, with cocaine blocking the cellular reuptake of dopamine and methamphetamine blocking dopamine reuptake and stimulating dopamine release [12,23]. The nonspecific actions of methamphetamine on dopaminergic systems may have contributed to the differences in the behavioral effects of the two drugs. Pharmacokinetics related to the absorption, distribution, metabolism, and excretion of cocaine and methamphetamine may have also played a role. Brain uptake in mammals is faster for cocaine than for methamphetamine (3.5 min vs. 7 min, respectively) and clearance rates are also much faster for cocaine than for methamphetamine,

a result which is consistent with differences in the self-reported temporal course of a “high” between these two drugs [13,22,43].

Caffeine, regardless of exposure duration, did not cause withdrawal-like behavior. This finding is different from the effects of methamphetamine, cocaine, amphetamine, cannabinoid agonists, opioid agonists and benzodiazepines, all of which produce withdrawal-like behavior in planarians [27–36]. Given that caffeine is the world’s most widely consumed psychoactive substance, surprisingly little attention has been paid its withdrawal syndrome. The central nervous system does not seem to develop a great tolerance to the effects of caffeine although dependence and withdrawal symptoms are reported in humans. The acute administration of caffeine did not alter planarian locomotor activity. The ineffectiveness of caffeine is different from the response demonstrated in mammals, where hyperactivity is observed following a single caffeine injection [37]. This is not an unexpected result given that only one drug – the cold channel agonist icilin – has been shown to increase planarian locomotor activity under our conditions [33]. We do not know the reason for this, but speculate that planarians are moving at a maximum speed in our procedure. This would explain why decreased locomotor activity is commonly observed following pharmacological manipulation.

We conclude that the magnitude of withdrawal-like behavior in planarians varies with the duration and type of drug exposure, as well as drug concentration [29]. Our results are consistent with evidence that stimulant withdrawal produces a wide range of dysphoric symptoms in humans that vary with the dosage, pattern and duration of use [42]. Given the degree of conservation between planarian and mammalian genes [9] and evidence suggesting some congruence in the mechanisms involved in the expression of withdrawal in planarians [32], the investigation of physical dependence and withdrawal in planarians should contribute to a better understanding of these processes in humans.

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