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Research article

# Persistent conditioned place preference to cocaine and withdrawal hypo-locomotion to mephedrone in the flatworm planaria

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## HIGHLIGHTS

- We show conditioned place preference in planaria to cocaine.
- We find long-lasting hypo-motility to mephedrone.
- We have used clinically relevant concentrations.

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## ABSTRACT

The purpose of the present study was to determine the effects of exposure to cocaine and mephedrone on conditioned place preference (CPP) and locomotion in the flatworm planaria. Planaria were treated with either cocaine or mephedrone at 1 or 10 μM. Planaria were exposed to 15 min of drug in their non-preferred place (either a rough- or smooth-floored petri dish) on alternate days, and were exposed to normal water in their preferred place on the following day. There were 5 days of conditioning to drug. Planaria were then tested for CPP on day 2, 6 and 13 after withdrawal. We found that animals exhibited CPP to cocaine at both 1 and 10 μM, but not to mephedrone. When examining locomotor activity we found that neither cocaine nor mephedrone treatment showed any evidence of evoking increased motility or locomotor sensitisation. Hypo-motility was seen on the first day of conditioning at concentrations of 10 μM for both cocaine and mephedrone, but had disappeared by the last day of conditioning. Examining chronic withdrawal, only 10 μM mephedrone had a significant effect on motility, decreasing locomotion on day 2 of withdrawal. Taken together we have shown that cocaine evoked CPP in planaria. We have also shown withdrawal depressing effects of mephedrone on motility.



## 1. Introduction

There is an increasing pressure on researchers to justify their use of vertebrate animals in pre-clinical studies. Over the last decade, the principles of the 3Rs (reduction, refinement and replacement) have gained more prominence and researchers are actively looking for non-vertebrate species in which to undertake their research. One such invertebrate species that has become more popular in pre-clinical research is the flatworm planaria. There are two main advantages of undertaking research in planaria; first its entire genome is known [1] and second it is cheap and easy to maintain. In the field of neuroscience, further advantages to using planaria include its ability to regenerate its nervous system and the belief that it is the simplest organism to have a 'brain' [2].

In the field of neuropharmacology, the planaria may be of use because it possesses most of the main neurotransmitters that are found in rodents and humans [3]. It has been used recently in studying drugs of abuse and has been found to show withdrawal signs, most notably reduced locomotion, after being taken out of various drugs of abuse including cocaine, opioids, methamphetamine, cannabinoids and mephedrone [4–8]. Hypomotility is a core symptom of stimulant withdrawal in humans and rodents. In addition, behaviours such as head-nodding and head-swinging, squirming, clinging, tail-twising and corkscrew spiral motions along the long-axis have been seen [4]. More recently conditioned place preference (CPP) has been shown in planaria for cocaine, mephedrone and MDMA [8,9] and we have shown more subtle responses such as blocking [10] suggesting that planaria show complex information

*Abbreviations:* CPP, conditioned place preference; NPS, novel psychoactive substance; ANOVA, analysis of variance.

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processing. It has also been shown [11] that planaria exhibit environmental familiarisation that endures for up to 14 days. This raises the interesting possibility that drug-induced CPP effects in planaria might be long-lasting, in a manner similar to the effects observed in rodent models [12] where rodents, after conditioning with mephedrone for a number of days, demonstrated significant CPP that lasted for at least 3 weeks. If similar effects were evident in planaria, their utility modeling drug-related changes in mammalian behaviour would be extremely promising.

One possible limitation of previous studies concerns whether the concentrations tested have clinical relevance, where studies use drug concentrations of up to 1000 μM. In the present study we aimed to further show the validity of using planaria in drug abuse research by using drugs at lower, perhaps more clinically relevant, concentrations and by examining the persistence of the conditioning effect. Having a simple model organism that allows high throughput screening of drugs of abuse would be particularly useful in the testing of legal highs (novel psychoactive substances, NPS) where around 100 NPS are found each year, with virtually nothing known of their pharmacology.

## 2. Methods

**Planaria**. Brown planaria (*Dugesia Tigrina*) were purchased from Blades Biological Ltd (UK) and were fed raw chicken every 2 days for 1 h. Their water was changed after feeding. We used AquaSafe© (Tetra, Germany) to treat normal tap water. Planaria were kept about 20 worms to 500 ml treated water in Tupperware boxes. They were kept in a dimly lit room with a dark cycle from 6PM to 9AM.

**Drugs and conditioning**. We examined cocaine, one of the most addictive drugs, and mephedrone for which much less is known about its addictive liability. Cocaine is primarily a DAT inhibitor whereas we have previously shown that mephedrone causes reverse transport of dopamine (Opacka-Juffry et al., 2014). In a pre-test, planaria were allowed to move freely in a plastic petri dish (9 cm diameter) which had one half smooth (white shiny card) and one half rough (brown, wet–dry sandpaper, grade 100 grit). The floor coverings were fitted up the sides of the dishes to avoid planaria getting trapped under paper and also so that if they swam around the edge, they were still in contact with a smooth or rough surface. The dish was filled with 3 ml of treated tap water. This meant that the fluid level was just above the floor of the dish, forcing the worms to contact the different surfaces. The time spent in each half of the dish was measured after 15 min. During conditioning the worms were placed in a dish, which only contained a floor of their non-preferred side (sandpaper or shiny white card) in either 1 or 10 μM of cocaine or mephedrone, made up in treated tap water, for 15 min. The following day worms were placed in a petri dish with their preferred floor, again for 15 min. This was repeated for 10 consecutive days (5 days drug, 5 days water), as is standard in CPP experiments [16]. Planaria were then tested on days 2, 6 and 13 after withdrawal in the original petri dishes, which were ½ sandpaper and ½ shiny white card. The amount of time spent in each half was measured after video analysis. We were also able to measure locomotor activity using video analysis with a suitable sized grid placed in the video monitor, allowing us to estimate the number gridlines crossed. If more than 50% of the planaria's body crossed a line we counted this as a crossing. See Fig. 1 for an overview of the testing and drug administration schedule.

Worms were transferred from dish to dish by gently picking them up with a fine artists brush, occasionally a worm would be damaged during this process and data from these worms were excluded from analysis, (i.e. planaria that did not move). Cocaine was purchased from Sigma–Aldrich, mephedrone was a gift from John Ramsay (TICTAC Communications Ltd., UK).

## 3. Results

In our pre-conditioning test of side preference we found no statistically significant difference in the amount of time spent in each side in all our planaria combined. In the 15 min (900 s) test, planaria spent 411.1 ± 31 s in the rough side and 488.9 ± 31 s in the smooth side, $t(42) = 1.25$, $p = 0.22$. Thus, our CPP design was non-biased.

**Conditioned place preference.** The main finding was that CPP was seen with cocaine at both 1 and 10 μM, but no effect was seen with mephedrone. Fig. 1A shows CPP findings for planaria conditioned with 1 μM cocaine ($n = 9$), 1 μM mephedrone ($n = 13$) and a control group ($n = 7$) conditioned in treated water. Fig 1B shows CPP findings for planaria conditioned with 10 μM cocaine ($n = 6$) or 10 μM mephedrone ($n = 9$). The control group is also

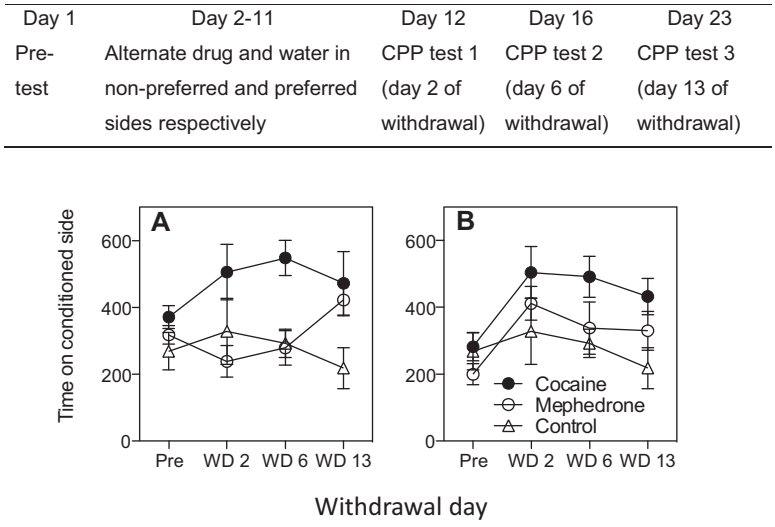


**Fig. 1.** Overview of testing and drug administration schedule and conditioned place preference after cocaine. Top panel: we always started the planaria with drug on day 1 of conditioning, on the non-preferred side. This meant that the last day of conditioning was with water on the preferred side. Thus the CPP tests were done on days 2, 6 and 13 of drug withdrawal (WD). Time spent on the non-preferred side before conditioning (pre) and 2, 6, and 13 days after withdrawal. CPP was seen with cocaine at both 1 and 10 μM, but not with mephedrone, see text for details. A. 1 μM cocaine (closed circles) and 1 μM mephedrone (open circles) and for the control group (open triangles). B. 10 μM cocaine (closed circles) and 10 μM mephedrone (open circles). Control group data (open triangles) are replotted from A. Values are means ± 1 SEM. $N = 6$–13.