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# Levamisole and cocaine synergism: A prevalent adulterant enhances cocaine's action *in vivo*

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ABSTRACT

Levamisole is estimated by the Drug Enforcement Agency (DEA) to be present in about 80% of cocaine seized in the United States and linked to debilitating, and sometimes fatal, immunologic effects in cocaine abusers. One explanation for the addition of levamisole to cocaine is that it increases the amount of product and enhances profits. An alternative possibility, and one investigated here, is that levamisole alters cocaine's action *in vivo*. We specifically investigated effects of levamisole on cocaine's stereotypical and place-conditioning effects in an established invertebrate (planarian) assay. Acute exposure to levamisole or cocaine produced concentration-dependent increases in stereotyped movements. For combined administration of the two agents, isobolographic analysis revealed that the observed stereotypical response was enhanced relative to the predicted effect, indicating synergism for the interaction. In conditioned place preference (CPP) experiments, cocaine produced a significant preference shift; in contrast, levamisole was ineffective at all concentrations tested. For combination experiments, a submaximal concentration of cocaine produced CPP that was enhanced by inactive concentrations of levamisole, indicating synergism. The present results provide the first experimental evidence that levamisole enhances cocaine's action *in vivo*. Most important is the identification of synergism for the levamisole/cocaine interaction, which now requires further study in mammals.



## 1. Introduction

An old drug called levamisole (Ergamisol) that was once used to treat parasitic worm infections in humans is exacerbating health risks for an estimated 2 million cocaine users in the United States (Auffenberg et al., 2013). The news media (*e.g.* Time Magazine), scientific publications, and government agencies are alerting the general public, health officials, and physicians about potentially life-threatening effects of cocaine laced with levamisole (Zhu et al., 2009; Chang et al., 2010; Ullrich et al., 2011). An example of such a warning is the public alert issued in September of 2009 by the U.S. Department of Health and Human Services Substance Abuse and Mental Health Services Administration warning that "a dangerous substance, levamisole, is showing up with increasing frequency in illicit cocaine powder and crack cocaine and can lead to a severe reduction in the number of white blood cells, a problem that is called agranulocytosis". The Drug Enforcement Agency (DEA) estimates that about 80% of the cocaine seized in the US is laced with levamisole (Wolford et al., 2012). DEA data from 2009 also noted an average concentration of approximately 10% levamisole detected in cocaine, and Buchanan et al. (2010) demonstrated the presence of levamisole (as high as 10%) in a patient's crack cocaine pipe, thus confirming levamisole as a cocaine adulterant. Speculation about the addition of LVM to cocaine centers on two hypotheses. One is that LVM increases the amount of 'product' which increases profits. LVM is cheap, has similar physicochemical properties to cocaine, and is easily accessible as a veterinary pharmaceutical in regions in which the laced cocaine originates.

A second hypothesis is that levamisole is added to cocaine to modify the pharmacological properties of cocaine. To probe the latter possibility, we used an established planarian assay to determine if levamisole affects cocaine's action *in vivo*. Planarians are aquatic flatworms with a centralized nervous system often

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considered to be the simplest 'brain' (Raffa and Rawls, 2008; Buttarelli et al., 2008). Planarians contain neurotransmitter systems, including glutamate, dopamine, serotonin, acetylcholine, and GABA (Eriksson and Panula, 1994; Vyas et al., 2011; Nishimura et al., 2010), and to a limited extent display mammalian-equivalent behavioral responses (stereotypical activity, abstinence-related withdrawal, behavioral sensitization, cross-sensitization, and place conditioning) following exposure to addictive substances (Palladini et al., 1996; Pagán et al., 2008, 2009, 2013; Rowlands and Pagán, 2008; Rawls et al., 2010, 2011; Ramoz et al., 2012). The present experiments characterized levamisole and cocaine interactions using two behavioral endpoints. One was stereotypical activity, defined as the number of C-shape movements across a defined time interval (Passarelli et al., 1999; Rawls et al., 2011). The other was conditioned place preference (CPP), an assay in which planarians exposed to a distinct environment in the presence of a positively reinforcing substance will later show preference for that same environment when given a choice (Zhang et al., 2013; Ramoz et al., 2012). Drug combination analysis employing isobolographic theory was used to quantify levamisole–cocaine interactions (Tallarida, 2011, 2012). The isobolographic method is derived from the principle of dose equivalence and is the standard pharmacological approach to analyze observed combination dose effects for comparison with expected, or additive, effects. Its aim is to assess synergistic and/or additive interactions between compounds administered simultaneously (Tallarida, 2011, 2012).

## 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). Levamisole hydrochloride was purchased from Sigma–Aldrich (St. Louis, MO, USA). 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 were based on prior behavioral outcomes in planarians (Owaisat et al., 2012; Pagán et al., 2013; Rawls et al., 2010), and levamisole concentrations were determined empirically using previously reported Ki values as a guide (Anagnostou et al., 1996).

### 2.2. Behavioral experiments

#### 2.2.1. Stereotypical activity

Individual planarians were placed randomly into a transparent petri dish (5.5 cm diameter) containing a solution of cocaine (0, 0.1, 1, 3, 5 mM) or levamisole (0, 0.1, 0.3, 0.75, 1 mM) for 5 min and stereotyped movements were quantified (Ramoz et al., 2012). The concentration-effect data for each individual drug was used to determine the constant–potency ratio of the two drugs at a specified effect level (*i.e.* equi-effective doses of each drug). From this value the isobole, which indicates additivity for the predicted effect of the combination, was constructed and used to determine if the combination was additive, sub-additive or synergistic (super-additive) (Tallarida, 2011, 2012). Combination doses used in actual experiments were determined based on individual drug potencies.

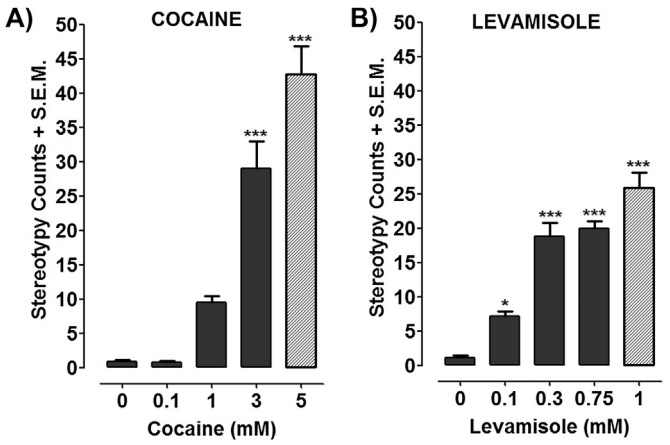


**Fig. 1.** Cocaine and levamisole produce stereotyped movements in planarians. Planarians were exposed to different concentrations of cocaine (1A) or levamisole (1B) for 5 min. The number of C-shape movements over the 5-min exposure interval were determined and presented as mean stereotypy counts ±S.E.M. $N = 8$ planarians/group. $^{***}p < 0.001$ or $^{*}p < 0.05$ compared to the respective water control in cocaine (1A) or levamisole (1B) experiments.

#### 2.2.2. Conditioned place preference (CPP)

CPP) experiments were divided into 3 different phases: 1) pre-conditioning (pre-test); 2) conditioning; and 3) post-conditioning (post-test). Because planarians display a natural preference for a dark environment (Raffa et al., 2003), we used a biased, counterbalanced conditioning design to assess cocaine and levamisole preference (Ramoz et al., 2012). In a biased design, the preference of each individual animal for a particular environment is determined prior to conditioning by placing the animal in the apparatus, and then by assessing the amount of time the animal spends in each compartment. The least-preferred compartment for each animal is then assigned to be the drug-paired compartment. For the pre-conditioning phase, dark and "ambient" light environments were created by covering half (both the top and bottom) of a petri dish containing water with black construction paper. An individual planarian was then placed at the midline of the dish and given free access to roam both the light and dark environments of the dish. The time spent in the least-preferred setting over a 5-min interval was then determined. This value is called the pre-test time. The least-preferred environment, as determined during pre-conditioning, is designated as the environment in which drug conditioning occurs and is therefore called the 'drug-paired' environment. For conditioning, planarians were exposed to either cocaine (0, 0.001, 0.01, 0.1, 1, 100 μM) or levamisole (0, 0.01, 0.1, 1 μM) for 30 min in the least-preferred (drug-paired) environment. For the situation in which the 'drug-paired' environment is ambient light, the petri dish is uncovered during the conditioning phase to allow exposure to the light. For the opposite situation in which the drug-paired environment is the dark, the entire petri dish is covered with black construction paper to enable exposure to a dark environment. Immediately following conditioning, the post-conditioning phase was performed in a manner identical to that described for pre-conditioning. Planarians were placed at the midline of a petri dish containing water and allowed free access to the light and dark sides of the dish for 5 min. Time spent in the drug-paired side (the original least-preferred environment) was determined (post-test), and a preference score was calculated as the difference between the post-test and pre-test times. A similar protocol has been used by our laboratory to demonstrate that planarians display CPP to different addictive substances including designer cathinones, nicotine, and sugar (Rawls et al., 2011; Ramoz