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# Cocaine withdrawal in *Planaria*

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**Abstract**

Cocaine-exposed planarians displayed abstinence-induced withdrawal behavior when placed into cocaine-free, but not cocaine-containing, water. The effect, manifested and quantified using a new spontaneous locomotor velocity metric, was dose-dependently related to cocaine exposure ($8 \times 10^{-9}$ to $8 \times 10^{-5}$ M). Ultraviolet light (254 nm = $7.83 \times 10^{-19}$ J), which was previously shown to interfere with drug-receptor interactions in *Planaria*, enhanced the abstinence-induced decreased locomotor velocity. 

*Keywords:* Cocaine; *Planaria*; UV light

Abstinence-induced or precipitated withdrawal behaviors are common experimental paradigms for the assessment of physical dependence or 'craving' in mammals (e.g., Grimm et al., 2001). However, to our knowledge, a quantitative assessment of a withdrawal phenomenon in *Planaria* has not been reported. *Planaria*, a type of flatworm, is of particular interest because it is considered the lowest species having a mammalian-like brain (centralized and clustered nerve cell bodies at the cephalic end) and spinal cord (two ventral nerves that run the length of the body and send out branches from ganglia in a ladder-like design). Planarians respond with characteristic behaviors to dopaminergic agonists and antagonists, including drugs of abuse. For example, amphetamine and cocaine induce 'screw-like hyperkinesias' or 'C-like position' (e.g. Carolei et al., 1975; Venturini et al., 1989; Palladini et al., 1996). That such effects are mediated via dopaminergic pathways has been demonstrated by the increase in cAMP induced by dopamine agonists (Palladini et al., 1996) and the enantiomeric-selective nature of the response to dopamine antagonists (Raffa et al., in press). We recently devised a convenient and sensitive metric to quantify planarian behavioral responses: locomotor velocity (designated *p*LMV) (Raffa et al., 2000; Raffa et al., in press). We now report on the use of this metric to identify an abstinence-induced cocaine withdrawal behavior in *Planaria*.

Brown planarians (*Dugesia gonocephala*, s.l.) were purchased from Carolina Biological Supply (Burlington, NC, USA) and were used within 3 days. Prior to the measurement of locomotor velocity, each planarian was placed into individual 0.5-ml vials containing room temperature (19 °C), treated tap water (AmQuel® water conditioner) (the vehicle) or (−)-cocaine HCl (Sigma, St. Louis, MO, USA) ($8 \times 10^{-9}$ to $8 \times 10^{-5}$ M) for 1 h. To measure *p*LMV, planarians were placed individually into a clear plastic petri dish (14-cm diameter) containing vehicle or cocaine solution ($8 \times 10^{-5}$ M) at room temperature. The petri dish was located over graph paper with gridlines (square pattern) spaced 0.5 cm apart. *p*LMV was quantified by counting the number of gridlines crossed or re-crossed by each planarian per minute over a 5-min observation period and is expressed herein as the mean (±S.E.M.) of the cumulative number of gridlines crossed by each planarian per minute. Some planarians were additionally exposed to long-wave ultraviolet light (UV-L; 366 nm = $5.43 \times 10^{-19}$ J = 3.39 eV) or short-wave ultraviolet light (UV-S; 254 nm = $7.83 \times 10^{-19}$ J = 4.89 eV) (5-in perpendicular above) during the measurement of *p*LMV. All experiments were conducted in a well-lit room between 9:00 a.m. and 6:00 p.m. Each planarian was used only once.

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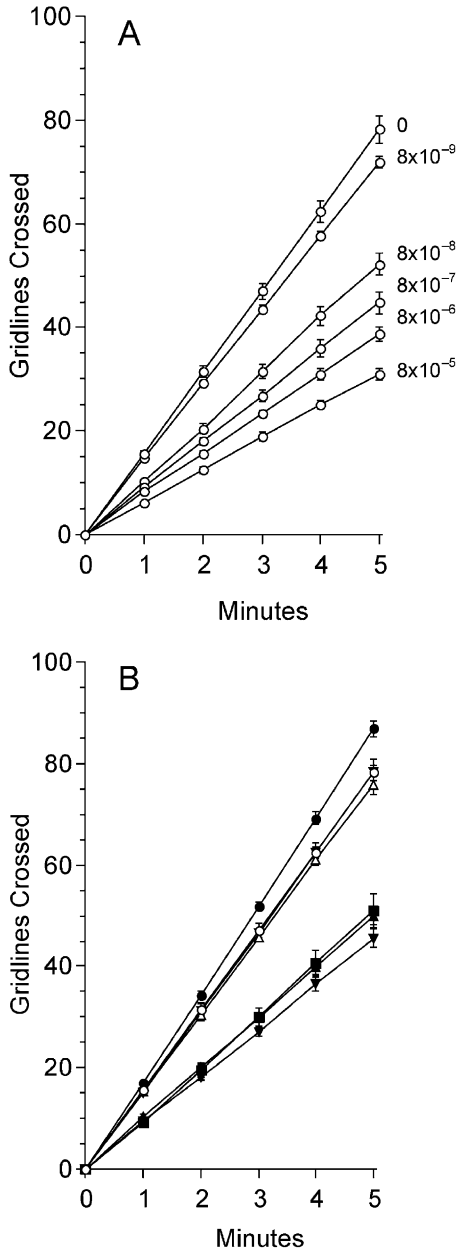


Fig. 1. The locomotor activity of planarians, expressed as the means ± S.E.M. of the cumulative number of gridlines crossed per minute. (A) ($N = 4–5$ each line); cocaine-exposed (molar concentration indicated) tested in cocaine-free water; (B) ($N = 10–14$ each line); (○) cocaine naive in cocaine-free water; (●) cocaine-naïve in water containing cocaine ($8 \times 10^{-5}$ M); (■) cocaine-exposed ($8 \times 10^{-5}$ M) in cocaine-free water; (△) exposed to UV-L (366 nm) only; (▽) exposed to UV-S (254 nm) only; (▲) cocaine-exposed ($8 \times 10^{-5}$ M) + UV-L; (▼) cocaine-exposed ($8 \times 10^{-5}$ M) + UV-S.

The results of all groups are summarized in graphical form in Fig. 1. The data are plotted as the means (± S.E.M.) of 4–14 planarians/group. Consistent with previous results (Raffa et al., 2000; Raffa et al., in press), cocaine-naive planarians displayed a characteristically relatively constant locomotor velocity of about 15–16 gridlines/min (cumulative mean = 78.2 ± 2.5 over 5 min) in cocaine-free water. Neither UV-L nor UV-S alone had any effect on the *p*LMV of untreated planarians. Cocaine-exposed planarians placed in cocaine-containing ($8 \times 10^{-5}$ M) water displayed *p*LMV which was slightly, but significantly ($P < 0.05$), greater than the *p*LMV of cocaine-naive planarians. However, cocaine-exposed planarians placed in cocaine-free water displayed dose-related reduced *p*LMV (ANOVA; $F = 54.3$; $P < 0.05$). Qualitatively, these animals displayed what appeared to be nondirected (indecisive) behaviors. The abstinence-induced decrease of *p*LMA was further enhanced ($P < 0.05$) by UV-S, but not ($P > 0.05$) by UV-L.

The decrease in *p*LMV displayed by cocaine-exposed planarians in cocaine-free water is consistent with dilution of intracellular cocaine levels and abstinence-induced withdrawal, particularly, since there was no diminution of *p*LMV of cocaine-exposed planarians placed in cocaine-containing water. The additional decrease in *p*LMV by UV-S, but not UV-L, is consistent with our previous demonstration that UV-S either stimulates the release of some as yet unidentified substance in a wavelength-dependent manner, or disrupts dopaminergic binding or transduction processes in *Planaria* (Raffa et al., 2000).

In summary, when cocaine-experienced planarians where placed into cocaine-free water, an abstinence-induced cocaine withdrawal was elicited, manifested and quantified using a decrease in a spontaneous locomotor velocity metric. The magnitude of the effect was dose-dependently related to prior cocaine exposure (Fig. 1A) and was not elicited when cocaine-exposed planarians were placed into cocaine-containing water (Fig. 1B). Ultraviolet light, previously shown to interfere with drug-receptor interactions in *Planaria* (Raffa et al., 2000) and without effect of its own, enhanced the abstinence-induced decreased locomotor velocity. We conclude that these phenomena offer novel approaches for studying cocaine or other drug-withdrawal processes in a simple in vivo model.

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