Cocaine effect on light/dark choice in *Planaria*: withdrawal

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Running head: Cocaine effect on choice in *Planaria*

Abstract

Objective: We have previously demonstrated that cocaine dose-dependently reversed planarians' choice behavior of dark over light, from about 80% of a 10-min observation period for naïve animals to about 27% of the period when tested in 8 x 10^{-5} M cocaine (acute exposure). In the present study, we examined: (1) the effect on choice behavior produced by longer exposure to cocaine, and (2) whether cocaine-experienced planarians would display abstinence-induced withdrawal from cocaine.

Methods: Brown planarians were immersed in either water or 8 x 10^{-5} M cocaine for 1 h; then tested in either water or 8 x 10^{-5} M cocaine. Choice was quantified by measuring the time spent in light *vs*. dark during a 10-min observation period.

Results: The acute effect of cocaine on reversal of light/dark choice was magnified by chronic exposure to cocaine. Withdrawal from chronic cocaine exposure was manifested as an increase in the time spent in dark compared to naïve animals.

Conclusion: These results suggest that cocaine physical dependence (manifested as withdrawal) develops in this endpoint in *Planaria*.

Keywords: cocaine; *Planaria*; physical dependence; tolerance; withdrawal

INTRODUCTION

Planaria, a type of flatworm, possesses a simple yet mammalian-relevant nervous system and neurotransmitters such as dopamine, 5-HT (5-Hydroxytryptamine; serotonin), amino acids, and endogenous opioids, and is capable of relatively complex behavioral adaptation, including learning and memory (1,2). Planarians respond to standard dopamine pharmacologic ligands, such as dopamine agonists, antagonists, or neuronal reuptake inhibitors – such as cocaine – with characteristic behaviors or changes in locomotor activity (motility) (*e.g.*, 3–6).

We recently reported (7) that acute exposure to cocaine reversed the strong preference that freely moving planarians display for the dark in a simple choice paradigm. That is, when cocaine-naïve planarians were allowed to choose, they spent about 80% of a 10-min test period in the dark. However, when tested in cocaine (8×10^{-5} M), they reversed their choice and spent about 73% of the 10-min test period in visible light (source maintained at a constant distance, 12.5 cm, above and perpendicular to the test apparatus). Other factors (*e.g.*, ambient light conditions, pH, directional preference, local differences in test apparatus, *etc*.) were carefully controlled or randomized. The effect was neither simply secondary to an increase in locomotor activity, since cocaine only minimally increases planarian locomotor activity at the highest dose tested (8), nor merely a disruption of sensory systems, since the behavior did not revert to a random 50/50 split between light and dark. The effect was likewise not secondary to small variations in water temperature, since planarian activity is not affected by such changes (9). Hence, the effect appeared to be directly related to cocaine.

In our previous work, the planarians were exposed to cocaine acutely, *i.e.*, only during the test period. In the present study, we examined the effect of a longer exposure (one hour) to

cocaine on their subsequent choice behavior and, further, tested whether cocaine-experienced planarians would display withdrawal when cocaine was abruptly removed.

MATERIALS AND METHODS

Animals & cocaine

Brown planarians (*Dugesia gonocephala, s.l.*) were purchased from Carolina Biological Supply Co. (Burlington, NC) and were acclimated to temperature-controlled (21^{o}C) laboratory conditions prior to use. They were tested within 48 h of arrival. Each planarian was used only once. (–)Cocaine hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO).

Testing

The light/dark choice test apparatus was similar to that previously described (7). It consisted of a plastic channel (11.5 x 1.5 x 0.5 cm) that contained room-temperature water. One-half of the channel was covered by an opaque material (**Figure 1**). At the beginning of each run, a planarian was placed at exactly the mid-point of the channel. The planarian was observed for 10 minutes and the amount of time that it spent in the light and dark portions of the channel was recorded. Between runs, the orientation of the tray was randomly varied and the half covered by the opaque material was randomly alternated. The light source was maintained at a constant distance of 12.5 cm above, and perpendicular to, the test apparatus. A total of four conditions of exposure (1 h) and testing (10 min) were examined: (i) baseline test in water; (ii) exposure to water and test in cocaine (8×10^{-5} M); (iii) exposure to cocaine (8×10^{-5} M) and test in cocaine (8×10^{-5} M), and (iv) exposure to cocaine (8×10^{-5} M) and test in water.

Statistics

Comparisons of group means were made by one-way ANOVA followed by Bonferroni post-hoc test (significance criterion $P < 0.05$).

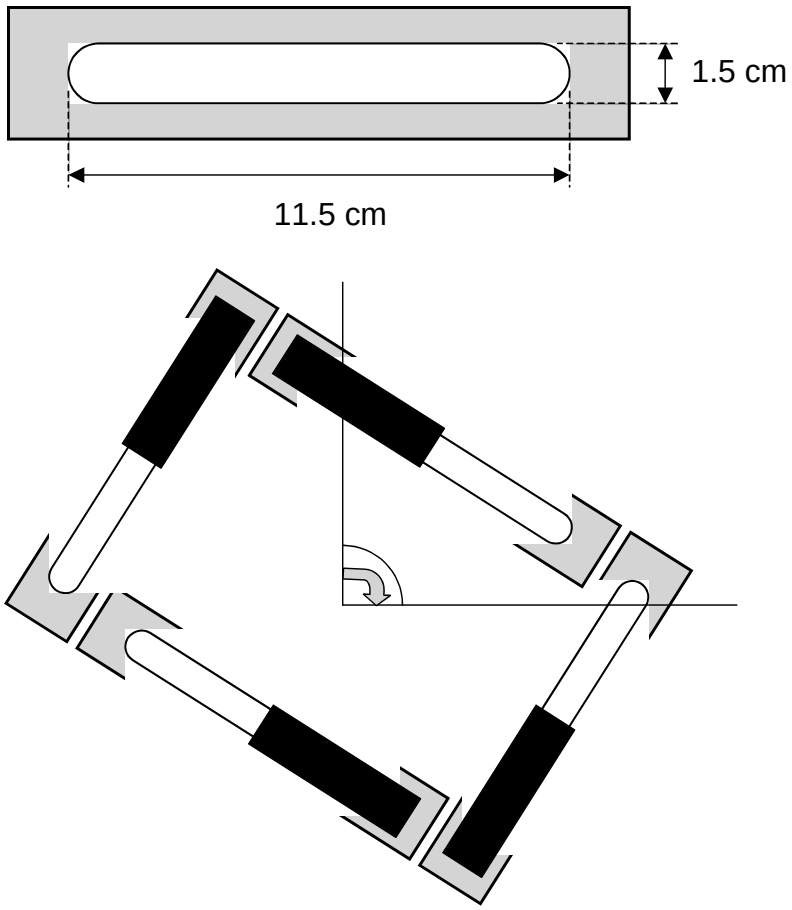

Figure 1 Apparatus used to assess planarian choice behavior. Following pretreatment, planarians were placed individually at the junction of the light-dark portion of the test trough, which was filled with water or cocaine. Both the direction and placement of the opaque cover were randomly changed prior to each run. Choice was measured as the times that each planarian stayed in the light and dark portions of the trough during the 10-min observation period.

RESULTS

Acute and prolonged exposure to cocaine

The results of either acute or prolonged (1 h) exposure on planarian light/dark choice are shown in **Figure 2**. The data are plotted as the means (± S.E.M.) of the time (s) that planarians (N = 5 – 10 per group) spent in the light during the 10-min observation period. Planarians tested in water displayed a characteristic preference for dark, spending only 116.5 ± 20.6 s (19% of the 10-min observation period) in light. Planarians that were exposed to water for 1 h, then tested in cocaine (8×10^{-5} M) displayed a significant increase ($P < 0.001$; $F = 48.67$, $df = 4$) in the amount of time that they spent in the light (416.0 ± 18 s) 69% of the 10-min observation period). No abnormal behaviors that disrupt motility, such as 'screw-like hyperkinesias' (SLH) or 'C-like' position (CLP) (*e.g.*, 4,5,10,11), were observed.

Withdrawal

In order to test for withdrawal, planarians were first exposed to cocaine (8×10^{-5} M) for 1 h, and then were tested for light/dark preference in water (abstinence). As shown in **Figure 2**, cocaine-experienced planarians spent significantly less time ($P < 0.05$) in the light (45.4 ± 13.6 s) (8% of the 10-min observation period) than did cocaine-naïve planarians (116.5 ± 20.6 s) (19% of the observation period), indicative of abstinence-induced withdrawal.

DISCUSSION

Based on previous experiments, we reported (7) that acute exposure to cocaine reverses the preference that freely-moving planarians display for the dark. That is, when planarians exposed to cocaine (8×10^{-5} M) were allowed to choose, they spent over 70% of the test period

in the light portion of the test apparatus, compared to cocaine-naïve planarians, which spent less than 20% of the 10-min test period in the dark portion of the apparatus.

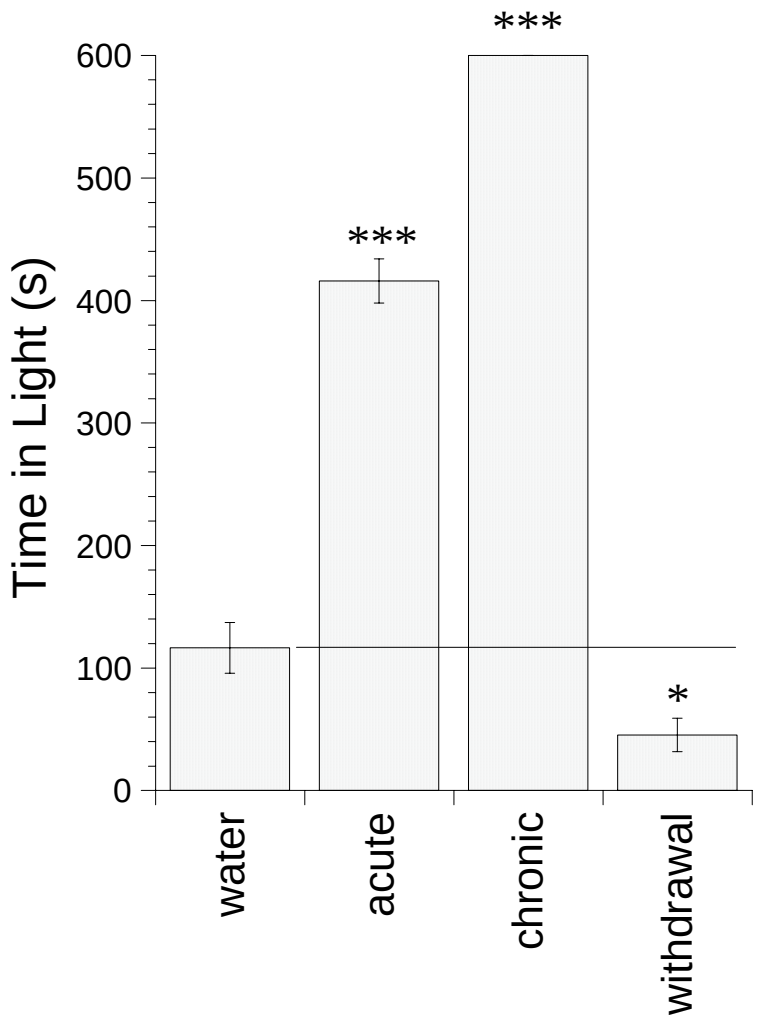

Figure 2 The effect of acute or chronic exposure to cocaine (8×10^{-5} M) on planarian preference for the dark (first three bars) and abstinence-induced withdrawal (fourth bar). Data are the means ± S.E.M. of 5 - 10 planarians per group. Asterisks indicate significant difference from vehicle (water): * = $P < 0.05$; *** = $P < 0.001$.

The present work was undertaken to expand the prior work by investigating: (1) the effect of more prolonged exposure to cocaine on choice behavior, and (2) whether cocaine-experienced planarians would display withdrawal when the cocaine was removed (abstinence). Planarians are a valuable model in which to address questions related to the effect of drugs on behavior. *Planaria* contain several mammalian-like neurotransmitters (*e.g.*, dopamine, 5-HT, opioids, and amino acids) and respond with characteristic alterations in activity to neurotransmitter-selective agonists, antagonists, or neuronal reuptake inhibitors (*e.g.*, 3–5,10,12) and the enantiomer-specific (*S*(–)- *vs* *R*(+)-sulpiride) effect on locomotor activity (6) and changes in 2nd messenger levels (such as in cAMP) (3,5) implicate a receptor- or a carrier-mediated action.

We have previously shown an abstinence-induced withdrawal phenomenon, manifested as an exposure-dependent decrease in spontaneous locomotor activity, in planarians exposed to cocaine (8) or to the opioid κ-receptor agonist U-50,488H (*trans*-(±)-3,4-Dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide) (13). The present work expands these findings to the effect of cocaine on light/dark choice behavior.

Consistent with our previous report (7), planarians exposed acutely to cocaine switched from a strong preference to dark (about 80%) to a strong preference to light (about 70%). Planarians that were exposed longer (1 h) to the same dose of cocaine displayed a remarkably even stronger preference for the light (100%). The mechanism by which cocaine reverses light/dark preference for dark is not presently known. However, the effect is not simply secondary to an increase in locomotor activity, since amphetamine, which increases planarian locomotor activity (4), has no effect on cocaine-induced light/dark preference (7) and it is not merely a disruption of sensory systems, as shown by the fact that the behavior does not revert to

a random 50/50 split. The effect is likewise not due to possible difference in water temperature, since planarian locomotor activity is not affected by small variations in temperature (9). Hence, the effect appears to be related to cocaine action.

The results also demonstrate for the first time a withdrawal phenomenon to a cocaine-induced choice behavior in planarians. Planarians that were exposed to cocaine for one hour and then tested in water displayed an abstinence-induced significant increase in the amount of time that they opted to stay in the dark, from 77% to 91% of the observation period. That this did not represent a deleterious effect on locomotor activity or disruption of directionality is shown by: (1) the fact that the planarians were placed at the mid-point of the choice apparatus and had to move to either the dark or light portion, and (2) planarians still display significant locomotor activity in this situation (8).

In summary, acute exposure to cocaine reverses planarians' normal selection of dark over light and the effect is magnified by longer (one-hour) exposure to cocaine. Furthermore, abstinence-induced withdrawal produced behavioral changes opposite to the drug-induced effect (*i.e.*, an increase in choice of dark compared to cocaine-naïve planarians). Hence, withdrawal – indicative of physical dependence – was displayed to the direct effect of cocaine.

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