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Benzodiazepine inhibits anxiogenic-like response in cocaine or ethanol withdrawn planarians

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Abstract

Planarians spend less time in light versus dark environments. We hypothesized that planarians withdrawn from cocaine or ethanol would spend even less time in the light than drug-naïve planarians and that a benzodiazepine would inhibit this response. Planarians pre-treated in cocaine or ethanol were placed at the midline of a petri dish containing spring water that was split evenly into dark and light compartments. Planarians withdrawn from cocaine (1, 10, 100 μ M) or ethanol (0.01 %) spent less time in the light compartment than water controls; however, this withdrawal response to cocaine (100 μ M) or ethanol (0.01 %) was abolished by clorazepate (0–100 μ M). These data suggest that planarians, similar to rodents, display benzodiazepine-sensitive, anxiogenic-like responses during cocaine or alcohol withdrawal.

Keywords

planarians; anxiety; cocaine; alcohol; benzodiazepine; withdrawal; addiction; clorazepate; invertebrate

Introduction

Planarians are the simplest living animals having bilateral symmetry and a CNS with cephalization (Pagan, 2014). These flatworms utilize neurotransmitter systems, including glutamate, dopamine, serotonin, acetylcholine, and GABA (Nishimura et al., 2010) and display mammalian-like responses to drugs of abuse including abstinence-related withdrawal, behavioral sensitization, and enhanced stereotypical activity (Pagán et al., 2009; Kusayama and Watanabe, 2000; Palladini et al., 1996). A tendency to spend less time in light versus dark environments is a behavioral phenotype of planarians (Zhang et al., 2013). Using a model based on the rodent conditioned place preference assay, we have shown that the tendency of planarians to spend less time in the light is reduced after a conditioning phase in which the worm is exposed to an addictive substance (*e.g.* cocaine, ethanol,

nicotine, cathinones, sugar) in the light compartment (Tallarida et al., 2014). We hypothesized that planarians, like rodents, would spend even less time in the light following discontinuation of alcohol or cocaine exposure and that a benzodiazepine would inhibit the response.

Methods

Subjects and drugs

Planarians (*Dugesia dorocephala*) were purchased from Carolina Biological Supply (Burlington, NC, USA). (–)-Cocaine hydrochloride was provided by NIDA (Bethesda, MD, USA). Clorazepate dipotassium salt was purchased from Sigma-Aldrich (St. Louis MO, USA). Drugs were dissolved in spring water. Concentrations were based on prior work (Tallarida et al., 2014).

Light/dark experiments

Each planarian was removed from its home jar and placed into a secondary jar (identical to their home jar) containing cocaine (1, 10, 100 μ M) or spring water. Following a 30-min pretreatment, planarians were placed at the midline of petri dish (5.5 cm diameter) containing spring water. A sleeve of black construction paper covered one half of the dish on the top, bottom and vertical sides to create a dark and ‘ambient’ light environment. Each planarian was given free access to roam the ‘ambient’ and dark sides of the dish, and time spent in each compartment was recorded over 10 min. Effects of ethanol (0.0001, 0.001, 0.01 %) were tested similarly except for a 60-min pretreatment time. Separate experiments tested the effect of clorazepate on cocaine- or alcohol-withdrawal responses, with planarians being pre-treated with cocaine (100 μ M) or ethanol (0.01 %) and then tested in clorazepate (1, 10, 50, 100 μ M).

Data analysis

Comparisons of group means ($\pm$ S.E.M.) were evaluated by one-way ANOVA followed by Dunnett’s *post-hoc* test to identify group differences. $P < 0.05$ was considered significant.

Results

Withdrawal from cocaine or ethanol decreases time spent in the light (Fig. 1)

For cocaine experiments (Fig. 1A), a one-way ANOVA revealed a significant drug effect ($[F(3, 58) = 5.011, P < 0.01]$). Planarians withdrawn from cocaine spent less time in the light compared to cocaine-naïve planarians (1 μ M, $P < 0.01$; 10 μ M, $P < 0.01$; 100 μ M, $P < 0.01$). For ethanol experiments (Fig. 1B), a one-way ANOVA revealed a significant drug effect (drug $[F(3, 60) = 5.182, P < 0.01]$). Planarians withdrawn from ethanol (0.01 %) spent less time in the light compared to ethanol-naïve planarians ($P < 0.01$).

Clorazepate attenuates cocaine or ethanol withdrawal response (Fig. 2)

For cocaine experiments (Fig. 2A), a one-way ANOVA revealed a significant drug effect (drug $[F(4, 72) = 7.188, P < 0.0001]$). Planarians withdrawn from 100 μ M cocaine spent 27% (± 11) % as much time in the light as drug-naïve (water) controls (276 ± 51 s compared to

76 ± 26 s). This environmental shift observed in cocaine-withdrawn planarians was reduced by clorazepate (50 µM, $P < 0.01$; 100 µM, $P < 0.001$). For ethanol experiments (Fig. 2B), a one-way ANOVA revealed a significant drug effect (drug [$F(4, 75) = 4.818$, $P < 0.01$]). Planarians withdrawn from 0.01 % ethanol spent 61% (± 11) % as much time in the light as drug-naïve (water) controls (225 ± 26 s compared to 138 ± 25 s). This environmental shift observed in ethanol-withdrawn planarians was reduced by clorazepate (1 µM, $P < 0.05$; 10 µM, $P < 0.001$; 100 µM, $P < 0.01$).

In control experiments, planarians exposed to clorazepate (1–100 µM) during the 10-min test interval did not display motor deficits or spend more time in the light compared to drug-naïve controls ($P > 0.05$, data not shown). In additional experiments, planarians withdrawn from caffeine or table sugar (sucrose) exposure spent less time in the light compared to water controls ($P < 0.01$, data not shown).

Discussion

Planarians, like rodents, tend to spend less time in light versus dark environments. We demonstrated here that planarians spend even less time in the light following abrupt discontinuation of cocaine or alcohol exposure. The withdrawal response was inhibited by clorazepate, suggesting that planarians withdrawn from cocaine or alcohol experience an anxiogenic-like state that manifests as less time spent in the light.

Similar phenomena have been shown in humans and rodents. Drug abusers who discontinue chronic consumption of abused drugs display a negative motivational state that increases vulnerability to relapse (Koob and Kreek, 2007). Rats or mice withdrawn from chronic regimens of an addictive substance spend more time in dark (*e.g.* light/dark box) or closed c (*e.g.* EPM assay) compartments (Bourin and Hascoët, 2003). Classical benzodiazepine anxiolytics are active in these mammalian models where they reduce the anxiogenic-type response during alcohol or cocaine withdrawal (Walf and Frye, 2007; Chaki et al., 2003; Paine et al., 2002) and are the front-line therapy for treating anxiety due to acute alcohol withdrawal syndrome (Perry et al., 2014). In summary, our results suggest that anxiogenic-type effects associated with drug withdrawal may be conserved across species and we propose the planarian light/dark assay as a pre-mammalian, high-throughput screen for identifying anxiolytic and anxiogenic activity.

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Fig. 1. Cocaine or ethanol withdrawn planarians spend less time in the light. Planarians exposed to different concentrations of cocaine (1A) or ethanol (1B) were then placed into spring water and time spent in the light was recorded for 10 min. Data are expressed as percentage of time spent in the light (+ SEM) following discontinuation of cocaine or ethanol exposure; n=15–16 planarians/group. ** $P < 0.01$ or * $P < 0.05$ compared to water control (0 μM or 0 %).

Fig. 2.

Clorazepate inhibits environmental shift in cocaine- or ethanol-withdrawn planarians.

Planarians pretreated with 100 μM cocaine (2A) or 0.01 % ethanol (2B) were then removed and placed into clorazepate (0, 1, 10, 50, 100 μM) and time spent in the dark was recorded for 10 min. In control experiments, planarians pretreated with spring water were placed into a split dish containing spring water and time spent in the dark was recorded. Data are expressed as percentage of the water control time spent in the light (+ SEM); n=14–16 planarians/group. *** $P < 0.001$, ** $P < 0.01$ or * $P < 0.05$ compared to cocaine or ethanol alone group.