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Amphetamine Conditioned Place Preference in Planarians

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ABSTRACT

Meth- and other amphetamines currently present major drug-abuse concerns. However, the demonstration and study of abuse-related behaviors expressed in animal models is expensive and time-consuming. We previously reported a novel model of conditioned place preference (CPP), which is a standard tool in abuse research, in invertebrates (planarians). In the present study, planarians were tested for light/dark preference, then exposed for 5 min to either *d*-amphetamine or vehicle (water) in light and then re-tested for place preference (light vs dark). The planarians' natural strong preference for dark (15 of 16) was significantly altered by amphetamine experience, such that 12 of 16 preferred the unnatural, but amphetamine-associated, light side. These results extend the demonstration of CPP to this invertebrate species and provide further evidence in support of this model to testing/screening amphetamine-like and possibly other drugs of abuse.

Keywords: Amphetamine; Conditioned Place Preference; Drug Abuse; *Planaria*

1. Introduction

As a class, amphetamine psychostimulants have value in a number of therapeutic applications (*e.g*., for narcolepsy or ADHD (attention-deficit hyperactivity disorder) [1-3]. But they also have potential for abuse [4], with negative health consequences [5]. An extensive body of evidence suggests that the major rewarding/reinforcing effects of psychostimulants such as the amphetamines is related to their ability to inhibit the neuronal reuptake of dopamine, particularly in the mesocorticolimbic pathway [6-9], and other neurotransmitters such as norepinephrine [10] and the excitatory (glutamate) [6-9] and inhibitory (GABA, γ-aminobutyric acid) [11] amino acids. In addition, there is broad evidence of a functional interaction between the dopamine and endogenous opioid systems, which might play an important role in amphetamine abuse [12-25].

The conditioned place preference (CPP) paradigm is a measure of incentive learning and an indicator of abuse potential [26-30]. Amphetamine-induced CPP has been demonstrated to occur in humans [31]. In animal models, microinjections of amphetamine into nucleus accumbens establishes a CPP, and the effect is attenuated by lesions produced by 6-OHDA (6-hydroxydopamine) [29], or by microinjections into the n. accumbens of α-flupenthixol or reserpine [32], which suggests an involvement of the mesolimbic dopamine pathway in this phenomenon. The acquisition of amphetamine CPP is also attenuated by the selective antagonism of the dopamine D1 [33-34] and the D2 [29,33] receptor or dual antagonism of both subtypes [35]. This plus additional evidence suggests that both of dopamine receptor types are involved when dopamine is released by amphetamine during establishment of CPP [36]. For example, null-mutant orphan G protein-coupled receptor 37 (which colocalizes with the dopamine transporter DAT) knockout mice (GPR37-KO) do not respond to the incentive properties in CPP tests [37].

Other neurotransmitters have been suggested to play a role in CPP in addition to dopamine. For example: the selective serotonin (5-HT, 5-hydroxytryptamine) 5-HT_{2C} receptor antagonist 6-Chloro-5-ethoxy-*N*-(pyridin-2-yl) indline-1-carboxamide hydrochloride (CEPC) potentiates low-dose amphetamine CPP [38]; amphetamine-induced CPP is attenuated by selective antagonism of the growth hormone secretagogue receptor 1A (GHS-R1A), which suggests an involvement of the central ghrelin signaling system [39]; the selective non-competitive antagonist of the NMDA NR2B (*N*-methyl-D-aspartate 2B subunit) receptor, rhynchophylline, reverses the expression of amphetamine-induced CPP [40] and additional evidence suggests that an activation of the NMDA receptor and of CaMKII (calcium/calmodulin-dependent protein kinase II) activity are essential for amphetamine-induced CPP [41]; intracerebroventricular administration of oxytocin inhibits the acquisition and facilitates the extinction of methamphetamine-induced CPP [42]; estradiol-treated female rats have enhanced amphetamine-induced CPP

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compared to vehicle-treated ovariectomized rats [43] (an effect possibly related to the known estrogen enhancement of dopamine-mediated behaviors; amphetamine-induced CPP is blocked by an intra-hippocampal (CA3 region) infusion of an inhibitor of Trk (tyrosine kinase) receptor [44]; intra-accumbens injection of a protein kinase C inhibitor blocks amphetamine-induced CPP in rats [45]; and both pre- and co-injections of diazepam block the formation of amphetamine-induced CPP [46]. There is additional evidence, but it is beyond the scope of the short overview presented here and the present report.

Planarians have the requisite endogenous neurotransmitter systems relevant to a study amphetamine (ab) use (for review see [47]), including dopamine, acetylcholine, and opioids among others [48-55]. Planarians develop a physical dependence to, and display abstinence-induced and antagonist-precipitated withdrawal from, a diverse list of drugs of abuse [56-61]. We recently reported the development of nicotine- [62] and mephedrone- ("bath sat") induced [63] CPP in planarians.

2. Materials and Methods

2.1. Animals and Drugs

The planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply (Burlington, NC). They were acclimated to the laboratory temperature (21˚C) and were tested within two days of receipt. *d*-Amphetamine was obtained from the National Institute of Drug Abuse.

2.2. Behavioral Testing

The methodology was similar to that previously reported by us [62,63]. Briefly, dark and light (ambient) sides were created by covering half of the top, bottom, and sides of a 60 mm diameter petri dish with black paper or tape. Individual planarians were placed at the midline of the dish. The time that the planarian spent in the non-preferred side (light) over a 5-min interval was determined (the pre-pairing response). Planarians were then conditioned with exposure to *d*-amphetamine (0.001, 0.1, or 1 mM) for 30 min in the opposite non-preferred (light) side. Immediately following conditioning, the planarian was placed again at the midline of the petri dish (now half light and half dark) containing vehicle and the amount of time that the planarians spent in the non-preferred side during the 5-min interval was measured.

3. Results

Untreated (drug-naïve) planarians spent 89% of the time in the dark half of the test chamber (petri dish). The effect of 30-minute exposure to *d*-amphetamine on individual planarian light/dark choice is shown in **Figure 1**. The 0.001 mM dose reduced the preference to only about

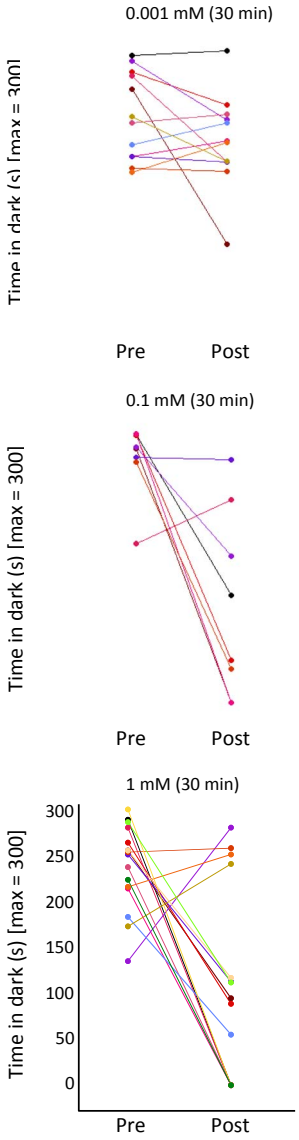

Figure 1. Change in predominant preference for the dark of drug-naïve planarians (Pre) to the dark after 30-min exposure to *d*-amphetamine (Post) (0.001 mM, top graph; 0.1 mM, middle graph; 1 mM, bottom graph).

1/2. The doses of 0.1 and 1 mM reversed the natural preference for dark. Most of the planarians displayed a CPP for the side in which they exposed to *d*-amphetamine, *i.e.*, they spent more time in the light.

The effect of *d*-amphetamine exposure on reversal of planarian light/dark preference choice was dose related. *d*-Amphetamine (0.001, 0.1, and 1 mM) exposure during conditioning produced dose-related increase in the time planarians spent in the light (**Figure 2**). The data are plotted as the mean ± s.e.m. of the percent of time that planarians spent in the light during the 5-min observation period.

The effect of *d*-amphetamine exposure time on reversal of planarian light/dark preference is shown in **Figure 3**.

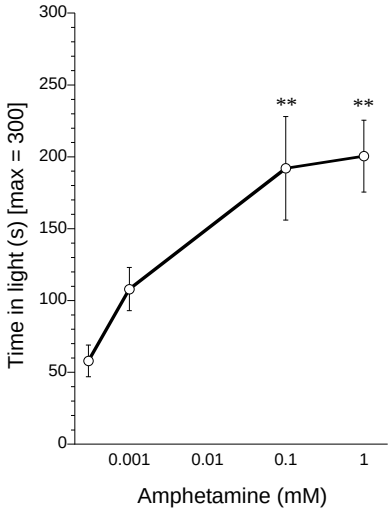

Figure 2. Dose-related change in predominant preference for the dark of planarians. N = 8 - 16 planarians per group. **P < 0.01 (ANOVA df = 51, F = 11.2).

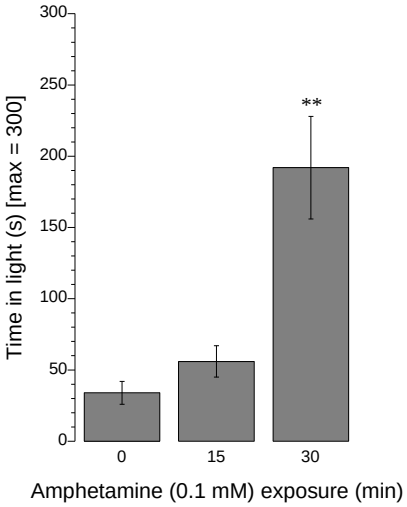

Figure 3. Change in the predominant preference for dark after 30-min exposure to *d*-amphetamine (0.1 mM). N = 8 - 16 planarians per group. **P < 0.01 (ANOVA df = 51, F = 11.2).

4. Discussion

Freely-moving planarians display a strong natural choice for the dark, as in the present study of ~90% of the time. We previously reported [64] that exposure of planarians to cocaine reverses the strong preference that 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, similar to the present study. However, when exposed to cocaine (8×10^{-5} M), they reversed their preference 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, such as the ambient light conditions, test pH, directional preference, local differences in test apparatus, etc. were carefully controlled or randomized. The effect was not simply secondary to an increase in spontaneous or drug-induced locomotor activity, since cocaine only minimally increases planarian locomotor activity at the highest dose tested [58]. It was also not due merely to a disruption of sensory systems, since the behavior did not revert to a random 50/50 split between light and dark. Hence, the effect appeared to be directly related to cocaine.

Similarly, in the present study drug-naïve planarians displayed a clear preference for light (about 90%), which is consistent with our previous findings. Exposure of the planarians to *d*-amphetamine (0.1 mM) for 15 minutes reduced the dark-preference to only ~50% and exposure to amphetamine for 30 minutes inverted the preference to light—demonstrative of a conditioned place preference. This is to our knowledge the first report of the development of CPP to amphetamine in planarians. The demonstration of this phenomenon in planarians is important, because planarians have proven to be a valuable model system for studying drug action and abuse [47]. These results can now form the basis for investigation of other drugs of abuse and a more detailed investigation of the biochemical processes involved.

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