THE EFFECT OF d-GOVADINE ON CONDITIONED PLACE PREFERENCE WITH d-AMPHETAMINE OR FOOD REWARD

by

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Abstract

Pharmacological treatments for drug addiction often relate to the dopamine (DA) system, which is known to play a key role in the development, persistence and relapse to compulsive drug-taking. However, many agents that alter single-receptor DA signaling have been clinically ineffective and there is growing interest in compounds targeting multiple receptors as a more promising therapeutic approach. Tetrahydroprotoberberines (THPB) derived from traditional Chinese herbal medicines have a high affinity for DA D1 and D2 receptors and have potential as novel treatments for drug addiction. This study assessed the effects of the THPB d-govadine on the acquisition, maintenance, expression and reinstatement of amphetamine-induced conditioned place preference (CPP). Furthermore, the effect of d-govadine on the acquisition and maintenance of food CPP was evaluated to gain insight into its action on multiple forms of reward-learning. Amphetamine CPP was established in rats by pairing d-amphetamine (1.5 mg/kg, i.p.) or saline with compartments with distinct contextual cues, and food CPP was induced by pairing Froot loops or no Froot loops with distinct contexts. In separate experiments, rats received d-govadine (0.5, or 1.0 mg/kg, i.p.) or vehicle, a) before each d-amphetamine injection during the conditioning phase, b) before tests for expression of amphetamine-induced CPP, c) before amphetamine-induced reinstatement of CPP, or d) before placement into food-associated compartments during the conditioning phase. CPP was assessed as greater time spent in the amphetamine- as compared to saline-paired contexts. d-govadine did not affect the acquisition of amphetamine CPP. However, groups that were pre-treated with d-govadine dose-dependently extinguished their preference for the amphetamine-associated context at a faster rate compared to vehicle-treated animals. While the expression of amphetamine CPP was not affected by d-govadine administered on the test day, the amphetamine-induced reinstatement of

CPP following an extinction period was blocked by d-govadine (1.0 mg/kg). Finally, the intermediate dose of d-govadine (0.5 mg/kg) blocked the acquisition of food CPP and rats pre-treated with the high dose (1.0 mg/kg) extinguished their preference at a faster rate than vehicle-treated animals. These data suggest that d-govadine affects the acquisition of reward-context associations and the ability of amphetamine to reinstate preference for the amphetamine-associated compartment.

Preface

Data collection and analysis was performed by Maya Nesbit, under the guidance of Dr. Carine Dias. I conducted the statistical analysis of the data with the assistance of Dr. Stan Floresco, Dr. Dias and Dr. Anthony Phillips. The manuscript and figures were created by Maya Nesbit and edited by Dr. Phillips and Dr. Dias. Suggestions from the supervisory committee were also used to edit the manuscript. Jonathan Cunningham and Dr. Dias provided assistance in conducting some tests for conditioned place preference (Figure 3) for which I am most grateful. All experiments were carried out in accordance with the Canadian Council on Animal Care, with approval by the Animal Care Committee at the University of British Columbia under the protocol number A13-0323.

Table of Contents

List of Tables

List of Figures

Acknowledgements

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My experience in graduate school has been especially memorable because of friends who constantly kept my spirits lifted with all of the fun times we've had together. Finally, to my parents and sister, who have endlessly supported me in the hardest of times throughout my studies, I am truly grateful. It is through my family that my curiosity for science was fostered, and their influence has undoubtedly instilled in me a joy of learning that will continue to enrich my life. I would like to especially thank my sister Erina for her unwavering dedication to helping me achieve my goals at every step of the way.

1. Introduction

1.1 The conditioned place preference model of addiction

Drug addiction is characterized by compulsive and escalating drug-seeking behaviour that can recur even after long periods of abstinence (Ahmed and Koob, 1998; Vanderschuren and Everitt, 2004). Current treatments for psychostimulant addiction have limited efficacy (Castells et al., 2010). The rate of relapse to psychostimulant addiction is 50-80% within one year after treatment, which highlights the urgent need to develop effective pharmacotherapies (Hubbard and Marsden 1986; Castells et al., 2010)

Rodent models of drug addiction such as conditioned place preference (CPP) have been developed to study the rewarding effect of drugs of abuse, context-dependent memory of drug reward and relapse to drug-seeking (Carr et al., 1989; Hoffman, 1989; Stolerman, 1992; Bardo and Bevins, 2000). In this paradigm, a distinctive environment is paired with a primary reward while a different environment is associated with the absence of the reward (Phillips and LePaine, 1980; Stolerman, 1992). The protocol can be adapted to model different stages of drug-taking including acquisition, abstinence and relapse. Drugs of abuse such as psychostimulants and opiates reliably produce a conditioned preference for the drug-paired environment and consequently CPP has been widely used to assess the neural mechanisms underlying the rewarding effect of these drugs (Sherman et al., 1980a, 1980b; Spyraki et al., 1982a, 1982b; Hoffman, 1989). To develop effective treatment strategies for drug addiction, it is important for the context-dependent aspect of drug memories to be considered. Contextual cues associated with drug-taking play a significant role in the maintenance and relapse of drug-seeking behaviours (Hyman and Malenka, 2001). The presentation of drug-paired environmental cues can lead to the recollection of the rewarding effects of a drug and elicit drug-seeking behaviour

(O'Brien et al., 1998; Ciccocioppo et al., 2001). This link between memory and addiction-related behaviour is further supported by their distinct but overlapping neural pathways and molecular mechanisms (Nestler, 2002; Robbins et al., 2008). The context-dependent learning processes involved in the CPP model allows for the assessment of these learned associations between environmental cues and drug reward (Bardo and Bevins, 2000).

1.2 The role of dopaminergic systems in psychostimulant-induced conditioned place preference

The development of psychostimulant-induced CPP relies in part on the integrity of the mesocorticolimbic dopamine system, which has long been known to drive reward-related behaviour (Wise, 1978). Drugs of abuse preferentially increase the concentration of dopamine (DA) in the nucleus accumbens (NAc) (Di Chiara and Imperato, 1988), and the intracranial self-stimulation of the mesolimbic pathway is reinforcing (Crow, 1972; Phillips et al., 1975). Dopamine levels are also elevated in the NAc and medial prefrontal cortex (mPFC) when rodents are placed in an environment associated with psychostimulants after the conditioning phase of CPP (Di Ciano et al., 1998; Duvauchelle et al., 2000; Lin et al., 2007) The development, persistence and relapse to psychostimulant-seeking are regulated by both D1-like and D2-like classes of DA receptors (Robledo et al., 1992; Phillips et al., 1994). Indeed, systemic antagonism of DA D1 and D2 receptors blocks the acquisition of psychostimulant CPP (Spyraki et al., 1982a, 1987; Hoffman and Beninger, 1989; Nazarian et al., 2004). A defining feature of psychostimulants is to bind to the DA transporter (DAT), which results in the elevation of DA in the synaptic cleft through two different mechanisms (Kalivas, 2007). Cocaine simply binds DAT to prevent the elimination of DA from the synaptic cleft, whereas amphetamine is transported into the pre-synaptic cell by DAT resulting in its reversal which in turn facilitates the non-

impulse dependent efflux of cytosolic DA into the synaptic cleft (Kalivas, 2007). For this reason, the reinforcing effect of psychostimulants and memory associated with drug intake is thought to be mediated by DA receptors. Furthermore, it has been suggested that adaptations in D1 and D2 DA receptor sensitivity due to repeated drug consumption are associated with behavioural changes that may reflect a transition from a non-addicted to addicted state (Edwards et al., 2007). Preventing these alterations in DA signaling during drug intake or restoring the functional balance between D1 and D2 DA receptors after these changes have occurred may be a potential therapeutic approach for addiction to psychostimulants. However, many agents targeting individual DA receptors have been clinically ineffective because of motor side effects and ability for users to overcome the antagonism by increasing drug intake (Haney et al., 1998; Platt et al., 2002). Therefore, a more promising pharmacotherapy may be to target multiple receptors concurrently.

1.3 Tetrahydroprotoberberines as a treatment for drug addiction

Tetrahydroprotoberberines (THPBs) are a class of alkaloid compounds used in traditional Chinese medicine to treat a variety of neuropsychiatric disorders (Chu et al., 2008). They possess a unique pharmacological profile because they target both DA D1 and D2 receptors, suggesting potential to serve as a novel treatment for drug addiction with low abuse potential and reduced extrapyramidal side effects. l-Stepholidine (l-SPD) is a prototypical THPB isolated from the Chinese herb *Stephania intermedica lo.* It is a DA D1 partial agonist, has DA D2 antagonist properties, and blocks the acquisition and reinstatement of morphine CPP (Guo et al., 1997; Wang et al., 2007). Another THPB that has been recently studied for its therapeutic potential in treating drug addiction is l-Tetrahydropalmatine (l-THP). l-THP acts as a weak antagonist at both D1 and D2 DA receptors and has been shown to attenuate the self-administration of cocaine as

well as block the relapse to cocaine-seeking (Mantsch et al., 2010; Wang and Mantsch, 2012). Furthermore, l-THP has been reported to block psychostimulant-induced conditioned place preference (Ren et al., 2000; Luo et al., 2003; Su et al., 2013).

d,l-Govadine is a synthetic THPB developed at the University of British Columbia and has a similar structure to l-SPD, with a half-life of 6 hrs (Lapish et al., 2012; Phillips et al., unpublished). It targets both DA D1 and D2 receptors in its racemic form and the isolation of its stereoenantiomers revealed that both d- and l-govadine exhibit high affinity for the DA D1 and D2 receptors (Lapish et al., 2012, 2014; Zhai et al., 2012). Both isomers have a higher affinity for DA D1 receptors than D2, a modest affinity for adrenergic receptors and a low affinity for serotonin receptors. Despite these similarities, d- and l-govadine differentially affect the DA system (Lapish et al., 2014). While l-govadine increases DA release in both the prefrontal cortex (PFC) and NAc, d-govadine selectively increases DA release in the PFC and not in the NAc. The mechanism of action giving rise to this unique pharmacological profile remains unclear. Furthermore, l-govadine exhibits DA D2 antagonist properties while d-govadine only acts as a very weak antagonist at both the DA D1 and D2 receptors. Characterization of the behavioural effects of d-govadine showed that it promotes cognitive enhancement on certain memory tasks (Lapish et al., 2014). Interestingly, d-govadine rescues memory impairments associated with suboptimal prefrontal DA levels while disrupting PFC-dependent memory tasks when prefrontal DA is within a presumed optimal range for cognitive functions. Considering the important role that memory plays in addictive behaviours, the effect of d-govadine on learning processes and DA synaptic transmission has implications for the treatment of drug addiction. Moreover, d-govadine does not affect key motor abilities which are disrupted by drugs used traditionally to treat relapse to drug-seeking behaviours (Lapish et al., 2014). In the following experiments, the

effect of d-govadine on the rewarding properties of d-amphetamine, context-dependent memory of amphetamine reward and the reinstatement of drug-seeking was assessed using the CPP model of addiction.

2. Materials and Methods

2.1 Subjects

Male Sprague-Dawley rats (200-210g upon arrival; Charles River, Montreal, Canada) were pair-housed in a colony room (Temperature = 21+/-1 degrees Celsius) under a reverse light cycle (light off during 8:30 – 20:30 h). Rats in the amphetamine-CPP experiment had *ad libitum* access to food and water. Rats in the food-CPP experiment had *ad libitum* access to water and restricted access to food to maintain their weight at 90% of a standard growth curve. All experiments followed the principles of laboratory animal care and were conducted in accordance with the standards of the Canadian Council on Animal Care and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003). All the experiments were approved by the Committee on Animal Care, University of British Columbia.

2.2 Drug preparation

d-govadine was provided by Glenn Sammis (Department of Chemistry, University of British Columbia, Canada). d-govadine was dissolved in 0.1M sulfuric acid, adjusted to pH 5.5 with 0.1M NaOH, and diluted with saline. d-amphetamine (US Pharmacopeia, Rockville, MD) was dissolved in saline.

2.3 Conditioned place preference apparatus

Two larger rectangular compartments (47.2cm x 24.6cm x 31.5 cm) constructed from acrylic panels were connected to a smaller rectangular compartment (21cm x 16cm x 31.5cm) with a smooth Plexiglas floor. The larger compartments could be isolated from the middle passageway by guillotine doors. These compartments were distinguished by different contextual cues with one having black and white striped walls and a metal grid floor while the other had solid grey walls and a floor comprised of smooth Plexiglas bars. Both compartments had a thin

layer of Aspen chip bedding material underneath the floors. The apparatus was illuminated by red light located 41 cm above the smaller middle compartment (40W). A digital video camera placed above the apparatus recorded movement activity of the rats within all three compartments of the CPP apparatus and this digital record was analyzed using tracking software (Ethovision, Noldus) to provide an accurate measure of the amount of time spent in each compartment.

2.4 Amphetamine-induced conditioned place preference

A pre-conditioning test was performed to assess baseline preference for the individual compartments. Animals were placed in the smaller middle compartment and allowed to roam freely between the large compartments for 15 min. Time spent in each compartment was measured and rats were assigned to different treatment groups in a counterbalanced manner. They were trained according to an unbiased protocol, in which subjects are assigned to their prospective conditioning compartment at random. If the time spent in one of the three compartments was not within two standards deviations of the mean, subjects were removed from the experiment.

The CPP paradigm consists of different phases including trials for learning the association between environmental contextual cues and the presence or absence of the rewarding effects of d-amphetamine, and tests for the expression of a preference for the compartment paired with either the drug or vehicle, indicative of context-dependent memory. Specifically, during the conditioning phase, d-amphetamine (1.5 mg/kg, i.p.) was paired with one compartment and saline with the other on alternate days (8 days, 4 pairings each). Following injection with d-amphetamine or saline, guillotine doors were closed, confining animals to one of the two large compartments for 30 min. The expression of CPP was tested 24 and 48 hrs following the last pairing in the conditioning phase. Rats placed in the middle compartment were given access to

all compartments in the CPP apparatus for 15 min. Time spent in each compartment was measured. Three separate experiments assessed the effect of d-govadine on a), the acquisition, b), the expression of amphetamine-induced CPP, while a third study examined its effect on c), drug-priming induced relapse to drug-seeking behavior following extinction of the CPP.

2.4.1 Effect of d-govadine on the acquisition of d-amphetamine conditioned place preference

During the conditioning phase, d-govadine (0, 0.5 or 1.0 mg/kg, i.p.) was administered 5 min prior to each pairing of d-amphetamine with a specific compartment. Following the first test for expression of CPP, rats continued to undergo similar daily test sessions for 7 days to assess the effect of d-govadine administration during the acquisition phase on the maintenance of a CPP for the compartment associated with the effects of d-amphetamine. Extinction was defined as the absence of a significant preference between the amphetamine- and saline-paired compartments for two consecutive days.

2.4.2 Effect of d-govadine on the expression of d-amphetamine conditioned place preference

After the conditioning phase in which d-amphetamine was paired with a specific compartment over 4 sessions, d-govadine (0, 0.5 or 1.0 mg/kg, i.p.) was administered 5 min prior to placement into the CPP apparatus for the expression test.

2.4.3 Effect of d-govadine on drug-induced reinstatement of d-amphetamine conditioned place preference

Following the conditioning phase, rats were tested for the expression of d-amphetamine CPP and subsequently underwent repeated daily test sessions in which the amount of time spent

in each compartment was measured. Twenty four hours after the final extinction session of amphetamine CPP a procedure was followed to examine the possible effect of d-govadine on reinstatement of CPP induced by a priming dose of the psychostimulant drug. To this end, d-amphetamine (1.5 mg/kg, i.p.) was administered immediately prior to placement of the rat into the CPP apparatus. Subjects were given access to all 3 compartments for 15 min to assess whether a CPP for the drug-associated compartment was again displayed. d-govadine (0, 0.5, or 1.0 mg/kg, i.p.) was administered 5 min prior to the priming injection of d-amphetamine.

Several criteria had to be met for rats to be included in the data analysis. Rats were considered to have developed CPP if they spent greater than 60 sec in the amphetamine-associated compartment compared to the saline-associated compartment during the first two expression tests 24 and 48 hrs after completion of the 8 day acquisition phase. (Paolone et al., 2009). Extinction was deemed to have occurred when there was no significant difference between time spent in amphetamine- and saline-paired compartments for two consecutive days. Furthermore, the number of test sessions a rat underwent prior to reinstatement had to fall within two standard deviations of the mean.

2.5 Food-induced conditioned place preference

Similar to the CPP protocol used in the d-amphetamine experiments, a pre-conditioning test was again performed to assess baseline preference for the compartments by placing rats in the middle compartment after which they were free to roam between the large compartments for 15 min. Time spent in each compartment was measured and rats were assigned to different treatment groups in a counterbalanced manner. They were subsequently trained according to an unbiased protocol.

During the 8 day conditioning phase, food-restricted rats were placed into a compartment with either 40 Froot Loops in a metal food cup or with an empty metal food cup, on alternating days (8 days, 4 pairings each). Rats received d-govadine (0, 0.5 or 1.0 mg/kg, i.p.) 5 min prior to placement into the CPP apparatus and were confined to each compartment for 25 min (Dias et al., 2012). Following conditioning, expression of food CPP was tested by measuring time spent in each of the two large compartments during a 15 min test session. The animals then underwent similar daily test trials for 7 days. During each test session, time spent in each compartment was measured and extinction defined as no significant difference between the time spent in the compartments associated with food or the absence of food.

2.6 Statistical analysis

Data from the CPP expression tests were analyzed by a two-way ANOVA with drug-treatment group as the between-subject factor and compartment as the within-subject factor. Experiments with repeated post-acquisition testing were analyzed using a mixed three-way ANOVA for Group × Compartment × Day. All ANOVAs were followed by Fisher LSD post-hoc tests. Analyses specific to each experiment are described in the results section.

3. Results

3.1 Experiment 1

3.1.1 Effect of d-govadine on the acquisition of amphetamine-induced CPP

In this experiment either d-govadine or vehicle was administered prior to each conditioning session with d-amphetamine and the data from a subsequent series of expression tests are presented in Fig. 1. Data from the first expression test used to assess the acquisition of amphetamine-induced CPP were analyzed using an ANOVA for Compartment × Group. This analysis revealed that vehicle-, 0.5 mg/kg d-govadine and 1.0 mg/kg d-govadine-treated groups all showed a preference for the amphetamine-associated compartment during the expression test. Specifically, a significant effect of Compartment was found along with no significant Compartment × Group interaction (compartment; $F(1,28) = 23.04$; $p < 0.001$; compartment × group; $F(2,28) = 1.08$, $p = 0.35$). These results indicate that d-govadine administered at a dose of 0.5 mg/kg or 1.0 mg/kg did not affect the acquisition of amphetamine CPP.

3.1.2 Effect of d-govadine administered during the conditioning trials on the maintenance of amphetamine-induced CPP

In order to determine if d-govadine had an effect on the strength of the conditioned association between the rewarding effects of amphetamine and contextual cues, the same cohort of rats was tested daily for 7 additional sessions to assess the maintenance of the amphetamine CPP. Fig. 1 illustrates the time spent in the amphetamine- and saline-associated compartment by each group in the expression test and subsequent testing over one week. Data from this series of test sessions following the expression test were analyzed by a three-way ANOVA for Compartment × Group × Day. Groups pre-treated with vehicle, 0.5 mg/kg of d-govadine and 1.0 mg/kg of d-govadine differed across days in their preference for the compartment paired with

amphetamine as shown by the significant interaction between compartment and treatment group (compartment × group; $F(2, 28) = 3.40$ $p < 0.05$). Post-hoc analyses revealed that the vehicle-treated group maintained a preference for the amphetamine-associated compartment throughout the 7 days of daily testing, while the group given 0.5 mg/kg of d-govadine extinguished their amphetamine induced CPP by day 6. In contrast, rats pre-treated with 1.0 mg/kg of d-govadine extinguished their preference for the amphetamine-associated compartment by the second post-acquisition expression test.

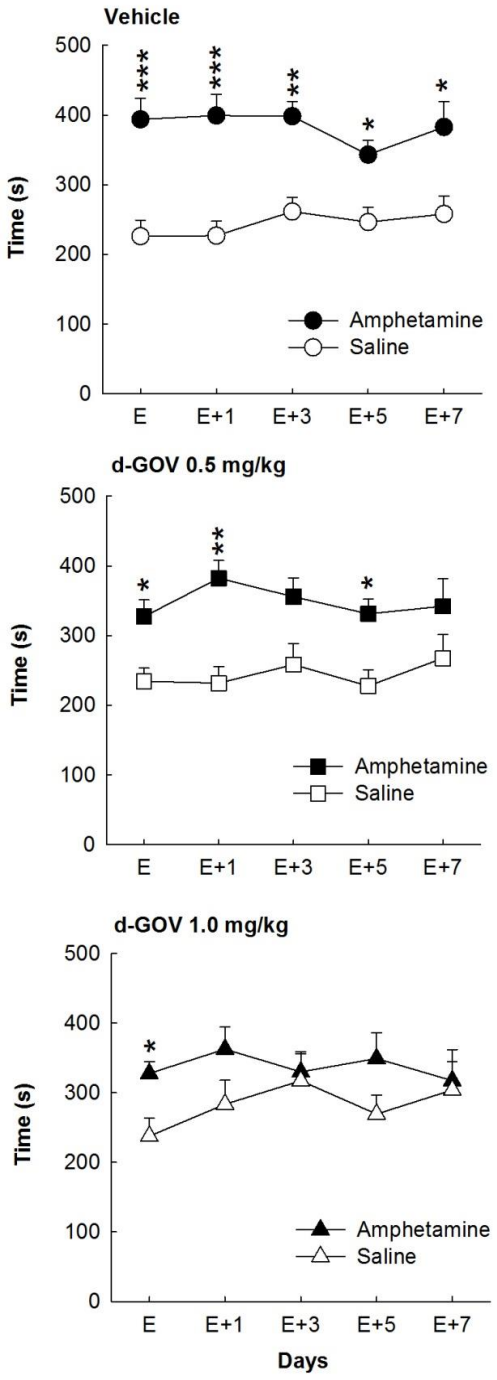

Figure 1. Effect of d-govadine on the acquisition of amphetamine-induced conditioned place preference. The animals received vehicle (n = 10), 0.5 mg/kg (n = 10) or 1.0 mg/kg (n = 11) of d-govadine 5 min before d-amphetamine administration during the conditioning phase. Data represent the mean (±SEM) time spent in the amphetamine- and saline-associated compartments on the 7 post-acquisition expression (E) days during which no drug was administered. *Significant differences between the amphetamine- and saline-associated compartments during the 15 min test and extinction sessions. $^{**}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.2 Experiment 2

3.2.1 The effect of d-govadine on the expression of d-amphetamine-induced CPP

To assess the acute effect of d-govadine on the expression of CPP following conditioning with d-amphetamine, d-govadine or vehicle was administered to rats 5 min prior to placement into the CPP apparatus on the test day. Time spent by each group in the amphetamine or saline compartment is depicted by Fig. 2. ANOVA for Compartment × Group revealed a significant preference for the amphetamine-associated as compared to the saline-associated compartment (compartment; $F(1,24) = 41.25$; $p < 0.001$). A post-hoc test confirmed that each group spent significantly more time in the amphetamine- compared to the saline-associated compartment. Furthermore, the ANOVA did not yield a Group × Compartment interaction indicating that neither dose of d-govadine affected the preference for the amphetamine-associated compartment (compartment × group; $F(2,24) = 0.50$; $p = 0.61$).

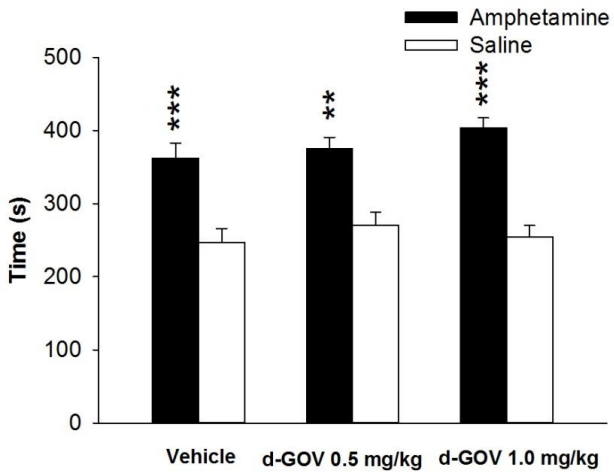

Figure 2. Effect of d-govadine on the expression of amphetamine-induced conditioned place preference. Twenty four hrs after the conditioning phase of amphetamine CPP, animals received vehicle (n = 10), 0.5 mg/kg (n = 8) or 1.0 mg/kg (n = 9) of d-govadine 5 min before the test for CPP. Data represent the mean (±SEM) time spent in the amphetamine- and saline-associated compartments during the 15 min test session. *Significant differences between the amphetamine- and saline-associated compartments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.3 Experiment 3

3.3.1 The effect of d-govadine on the reinstatement of amphetamine-induced CPP

After acquiring and subsequently extinguishing amphetamine CPP, rats received d-govadine or vehicle prior to a priming dose (1.5 mg/kg) of d-amphetamine before being placed into the CPP apparatus. Fig. 3 shows the amount of time spent in the amphetamine- and saline-associated compartments during the first extinction session following the expression test, as well as the final two extinction sessions (averaged), and on the test for reinstatement of amphetamine CPP. A three-way ANOVA for Compartment × Group × Day was conducted and a significant interaction was found between all three factors (compartment × group × day; $F(4,26) = 2.72$, $p < 0.05$) As expected, post-hoc analysis revealed that all groups spent significantly more time in the amphetamine-associated compartment than the saline-associated compartment at the beginning of the extinction phase (Fig. 3A; vehicle; $p < 0.01$, 0.5 mg/kg d-govadine; $p < 0.05$, 1.0mg/kg d-govadine; $p < 0.001$). This preference was extinguished in all groups prior to reinstatement with amphetamine (Fig. 3B). Following administration of the amphetamine prime on reinstatement day, vehicle- and 0.5 mg/kg d-govadine-treated groups spent significantly more time in the amphetamine-associated compartment compared to the saline-associated compartment (Fig. 3C; vehicle; $p < 0.01$, 0.5 mg/kg d-govadine; $p < 0.01$). In contrast, the group treated with 1.0 mg/kg d-govadine prior to the amphetamine prime did not show a significant preference during the test for reinstatement (Fig. 3C).

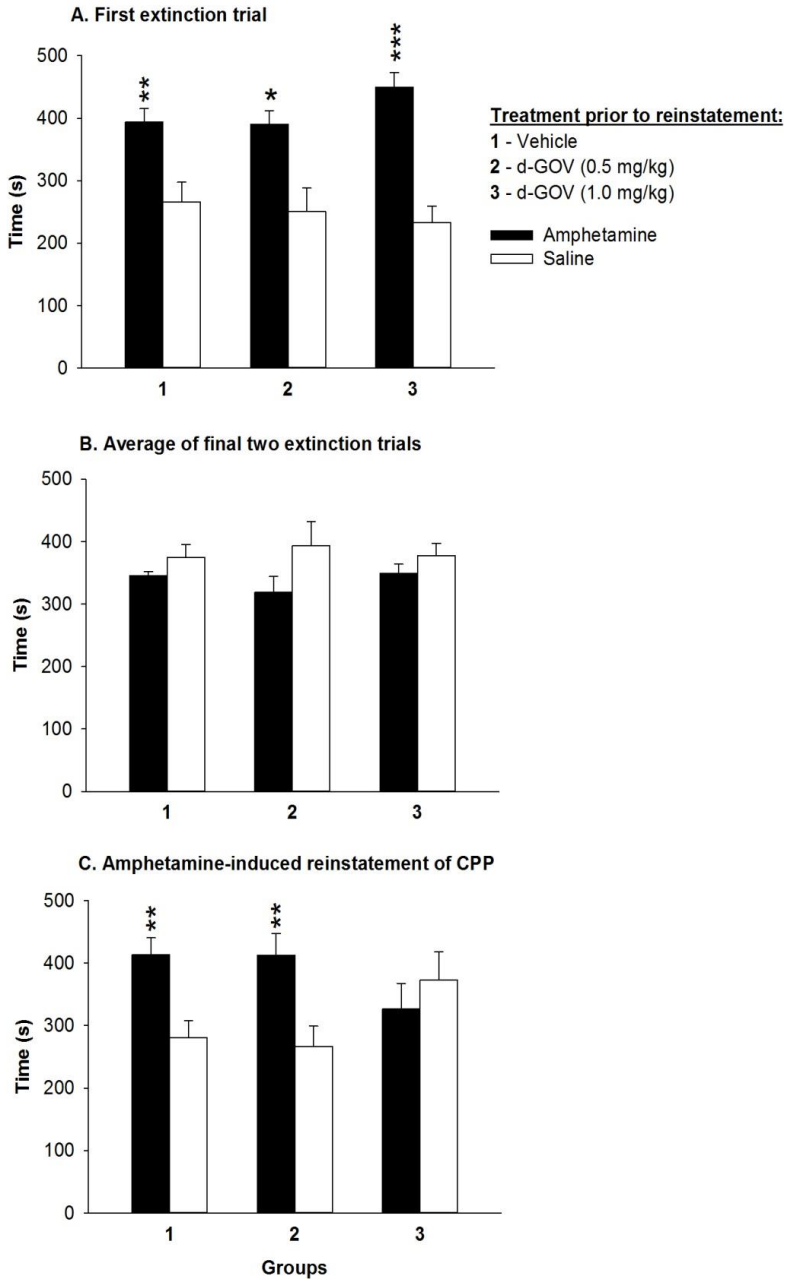

Figure 3. Effect of d-govadine on the reinstatement of amphetamine-induced conditioned place preference. Animals underwent a conditioning phase and were **(panel A)** tested for the expression of amphetamine CPP at the start of the extinction phase. Each group received a daily extinction trial on which they did not receive amphetamine and were tested for extinction **(panel B)**. Twenty four hrs following the final extinction trial, rats received vehicle (n = 11), 0.5 mg/kg (n = 8) or 1.0 mg/kg (n = 10) of d-govadine 5 min prior to a priming injection of 1.5 mg/kg of d-amphetamine and were tested for the expression of amphetamine CPP **(panel C)**. Data represent the mean (±SEM) time spent in the amphetamine- and saline-associated compartments during the 15 min test session. *Significant differences between the amphetamine- and saline-associated compartments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.4 Experiment 4

3.4.1 The effect of d-govadine on the acquisition of food CPP

In order to assess the effect of d-govadine on the rewarding properties of natural reward, this drug (0.5 mg/kg or 1.0 mg/kg) or vehicle was administered during conditioning sessions in which food reward was paired with contextual cues. The acquisition of food CPP was assessed following conditioning in a drug-free state during an expression test (Fig. 4). A 2-way ANOVA for Compartment x Group was conducted on results from the expression test. Overall, there was a significant preference for the food-associated compartment during the expression test and the groups did not differ significantly in their preference (compartment; $F(1,22) = 9.22$ $p < 0.01$, compartment × group; $F(2,22) = 0.50$ $p = 0.61$). A subsequent post-hoc analysis showed that the group treated with 0.5 mg/kg of d-govadine did not have a significant preference for a compartment, suggesting that this group did not acquire a place preference for food. Table 1 shows the number of Froot loops consumed by the groups during each conditioning session, and these data were analyzed by a 2-way ANOVA for Group × Day. As a whole, the number of Froot loops consumed by the animals differed across the four conditioning sessions, but this difference did not vary depending on the treatment group (day; $F(3,20) = 13.1$ $p < 0.01$, group × day; $F(6,42) = 1.19$ $p = 0.32$). Although groups treated with d-govadine appeared to consume less Froot loops than the vehicle-treated group during the first session of conditioning, a one-way ANOVA performed using data from the first conditioning session showed that the groups did not differ in the consumption of Froot loops (group; $F(2,22) = 1.43$ $p=0.261$).

3.4.2 The effect of d-govadine administered during conditioning trials on maintenance of a food CPP

Following the test day for the expression of food CPP, in groups given vehicle or d-govadine during the conditioning phase, 7 additional daily test sessions were conducted to assess the maintenance of food CPP (Fig. 4). A three-way ANOVA for Compartment × Group × Day, revealed that groups given vehicle, or d-govadine (0.5 mg/kg or 1.0 mg/kg) during the initial conditioning phase differed in their preference for the compartments across the 7 test sessions (compartment × group; $F(2,22) = 3.86$, $p < 0.05$). Post-hoc analysis indicated that the control group maintained its preference for the food-associated compartment, whereas the group pre-treated with 0.5 mg/kg d-govadine did not show a preference for the food-associated compartment on 6 of the 7 days. The group pre-treated with 1.0 mg/kg of d-govadine extinguished their preference for the food-associated compartment by the second day of testing.

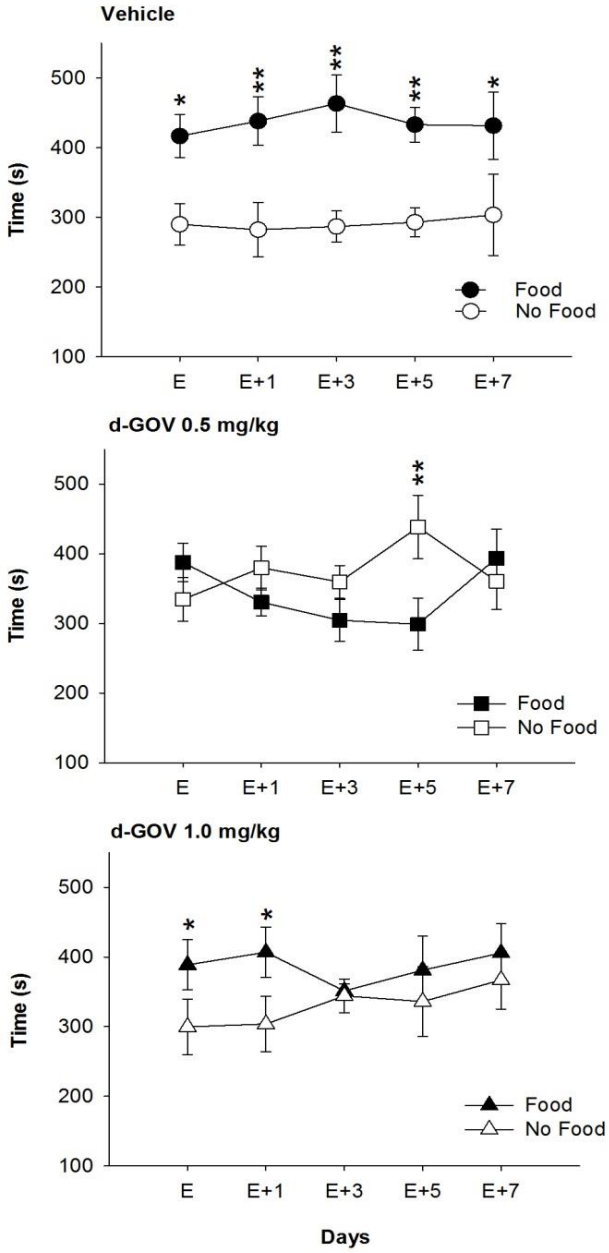

Figure 4. Effects of d-govadine on food conditioned place preference. Animals received vehicle (n = 8), 0.5 mg/kg (n = 8) or 1.0 mg/kg (n = 9) of d-govadine 5 min prior to being placed into a compartment containing Froot loops during the conditioning phase. Data represent the mean (±SEM) time spent in the food- and no food-associated compartments for the 7 days of post-acquisition expression (E) tests for food CPP during which no drug was administered. *Significant differences between the amphetamine- and saline-associated compartments during the 15 min test and extinction sessions. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 1. Effect of d-govadine on the consumption of Froot loops. Animals received vehicle (n = 8), 0.5 mg/kg (n = 8) or 1.0 mg/kg (n = 9) d-govadine 5 min prior to being placed into a compartment containing Froot loops during the conditioning phase. Data represent the mean (±SEM) number of Froot loops consumed over each session of conditioning.

	Number of Froot loops consumed during conditioning			
Treatment	**Session 1**	**Session 2**	**Session 3**	**Session 4**
Vehicle	37±1.3	40	40±0.2	40±0.1
d-govadine (0.5 mg/kg)	31±4.6	40±0.5	40±0.2	40
d-govadine (1.0 mg/kg)	31±2.2	38±1.0	38±1.1	39±1.0

4. Discussion

These experiments were designed to shed light on the possible effects of d-govadine on different aspects of conditioned drug reward. The first important observation was the apparent lack of effect on either the primary reward properties of d-amphetamine or the acquisition of conditioned associations between drug-rewards and the distinctive cues paired with this psychostimulant drug. When d-govadine was administered prior to each pairing of amphetamine and a distinct context, animals were still able to acquire conditioned place preference for the amphetamine-associated context**.** However, when the same cohort of animals were given access to both amphetamine- and saline-associated compartments for several days following the test day, animals treated previously with d-govadine extinguished their amphetamine CPP at a faster rate than control animals in a dose-dependent manner. Furthermore, a similar pattern of behaviour is observed when d-govadine is administered during the acquisition of food-induced CPP. This suggests that d-govadine may affect the learned association between context and reward. In contrast to these significant effects of d-govadine administered repeatedly during conditioning, acute administration of d-govadine directly before the first test for CPP had no effect on the expression of amphetamine CPP thereby indicating that the spontaneous recall of context-dependent drug memory was not affected by the actions of d-govadine. Of greatest clinical relevance were the results of the final experiment showing that d-amphetamine-induced reinstatement of CPP was blocked by d-govadine when acutely administered at a dose of 1.0 mg/kg.

4.1 The effect of d-govadine on the acquisition and maintenance of context-reward associations

4.1.1 Amphetamine-induced conditioned place preference

The ability to express a preference for a specific compartment depends on the association between amphetamine reward and the contextual cues of the compartment (Carr et al., 1989). Systemic administration of psychostimulant drugs including d-amphetamine enhances the efflux of DA in the NAc, thereby initiating neuropsychopharmacological processes that serve as an unconditioned stimulus (US), which is thought to possess incentive-motivational properties (Bindra, 1974; Di Chiara and Imperato, 1988; Robinson and Berridge 1993; Huston et al., 2013). Due to the temporal contiguity of repeated pairings with the US, the contextual cues of the compartment become the conditioned stimulus (CS) associated with the positive affective state induced by amphetamine (Bardo and Bevins, 2000). Consequently, during the expression test the presentation of these contextual stimuli elicits an approach response which is observed as preference for the amphetamine-associated context (i.e. a CPP). In the present study, this association between contextual cues and the affective state induced by amphetamine was evident in the vehicle-treated group as a CPP that was maintained over a 7 days of repeated testing. Administration of d-govadine prior to each conditioning session with amphetamine appears to have altered this association. In animals receiving d-govadine, the association was intact during the first expression test for CPP but was not maintained for the 7 days of testing.

It is also worth noting that d-govadine-treated animals had a weaker preference for amphetamine than vehicle-treated animals during the expression test. The magnitude of CPP during the expression test and the persistence of this preference is positively correlated with the number of drug-context pairings in CPP, as well as the dose of drug administered (Brabant et al.,

2005; Rutten et al., 2011). In one study, a range of drug doses and number of drug-context pairings during the conditioning phase of CPP were tested to assess the effect on the magnitude of CPP expressed on the test day (Brabant et al., 2005). Rodents that were exposed to the fewest number of drug-context pairings and received the lowest dose of drug spent the least amount of time in the drug-conditioned compartment during the expression test, while still showing a preference for this compartment. Furthermore, this relatively weak preference was extinguished at a faster rate than rodents receiving higher doses of drug and a greater number of drug-context pairings (Brabant et al., 2005). Thus, the magnitude of CPP may reflect the strength of conditioning which in turn could be linked directly to the rate of extinction. Alternatively, repeated d-govadine administration prior to the conditioning sessions with amphetamine may have weakened the learned association between the rewarding effects of amphetamine and contextual cues. As a consequence of this weaker drug-context association, extinction of the amphetamine CPP may have occurred more rapidly. This explanation is consistent with the lack of effect of d-govadine on the expression of amphetamine-induced CPP as it suggests that although repeated administration of d-govadine may weaken drug-context associations while being formed, a single dose cannot affect a previously-formed association.

Another interpretation of these findings is that d-govadine facilitated a separate learning process involved in the extinction of amphetamine CPP. Extinction of CPP was achieved by placing rats that had developed a CPP repeatedly into the 3-compartment test apparatus without the amphetamine treatment (Bouton and Moody, 2004). Over days, the contextual cues are no longer able to elicit the approach behaviour previously associated with the amphetamine-induced affective state (Bouton and Moody, 2004). Extinction is an active learning process that is distinct from the learning involved in the acquisition of a conditioned behaviour (Myers and Davis,

2002). Therefore extinction permits the modification of behaviour to reflect changing contingencies in the environment, while maintaining a latent association between reward and contextual cues learned previously (Myers and Davis, 2002). Recent studies show that d-govadine appears to possess pro-cognitive effects that include the enhancement of performance on working memory and Temporal Order Recognition Memory (TORM) (Lapish et al., 2014). This raises the possibility that the facilitated extinction of an amphetamine –induced CPP observed in this thesis may reflect enhanced extinction learning. Against this conjecture one must remember that d-govadine was administered only during the conditioning phase making it unlikely that any pro-cognitive effects it may possess can affect a learning process occurring days later. Therefore, it would appear that the present findings are best explained by the hypothesis that d-govadine weakened the initial context-drug association to a sufficient degree that it had minimal effect on the learning process mediating extinction.

4.1.2 Food-induced conditioned place preference

To gain further insights into the general nature of the effects of d-govadine on CPP, we next examined its effect on the acquisition and maintenance of CPP with food reward. As expected, vehicle-treated rats acquired place preference for the food-associated compartment. However, we observed unanticipated dose effects including the blockade of the acquisition of CPP by 0.5 mg/kg of d-govadine while rats pre-treated with 1.0 mg/kg d-govadine displayed a food-induced CPP on the first test day. Similar to the effects on amphetamine-induced CPP, this preference for the food compartment was no longer observed after the second test session. Vehicle-treated rats maintained a preference for the food compartment for the entire week of testing. Collectively, these observations support the view that d-govadine weakens the drug-context association formed during conditioning rather than facilitating a learning process during extinction. In the animals

pre-treated with the 0.5 mg/kg of d-govadine, the association between food and the contextual cues of the compartment was likely too weak for a preference to be expressed during the test. Since this lower dose of d-govadine had a markedly greater effect on food CPP than amphetamine CPP, d-govadine may exert a stronger effect on food-context associations compared to amphetamine-context associations. Furthermore, the possibility remains that the 0.5 mg/kg dose is the optimal dose for the observed effects of d-govadine on food CPP. On the other hand, 1.0 mg/kg may be close to the optimal dose of d-govadine for exerting effects on amphetamine-context associations. If so, an even higher dose of d-govadine may block the acquisition of amphetamine-induced place preference.

Despite these differences between the effect of d-govadine on the acquisition of food and amphetamine CPP, its overall effects on the extinction of food-induced CPP were quite similar to those observed with amphetamine CPP. These overlapping effects suggest that the actions of d-govadine are not restricted to learning processes associated with drug reward. In fact, the conditioned associations underlying food CPP are distinct from those involved in drug-induced CPP (Spiteri et al., 2000). Drug-induced CPP is characterized by animals spending long periods of time in the compartment associated with drug, whereas animals conditioned with food reward make more frequent but shorter visits to the food-associated compartment (Parker, 1992; Spiteri et al., 2000). This difference may be attributed to the method in which the two types of reward are delivered (Spiteri et al., 2000). Drug reward is generally administered as a single injection prior to presentation of contextual cues, and the resulting positive affect is experienced in a passive manner. In contrast, food reward is presented in the compartment and approach behaviours are engaged to facilitate the location of food. Once consummation begins, the hedonic response is experienced. As noted above, much of the increased time spent in the food-

associated compartment reflects many more visits rather than a longer duration of each visit (Spiteri et al., 2000). Thus, the hedonic response to drug reward is thought to be conditioned in drug-induced CPP and approach behaviours are conditioned in food-induced CPP (Spiteri et al., 2000; Tzschentke, 2007). The fact that d-govadine affects the conditioning of two distinct behaviours in a similar manner suggests that it has a broad effect on learned associations.

4.2 Mechanisms underlying the effect of d-govadine on amphetamine and food CPP

The most notable pharmacological effect of d-govadine observed to-date is its ability to increase DA levels in the mPFC and not the NAc (Lapish et al., 2014). Modulation of DA in the mPFC and the resulting activation of DA receptors have been directly linked to performance on a variety of learning and memory tasks (Phillips et al., 2004; Floresco, 2013). Therefore it is possible that d-govadine-induced modulation of prefrontal DA levels may affect the learning of associations important for CPP.

The activation of mPFC is critical for the induction of cocaine CPP, and the activity of mPFC neurons is involved in the acquisition, expression and extinction of opiate CPP (Isaac et al., 1989; Sun et al., 2011). In fact neural firing in the mPFC increases during conditioning and is highly robust during the recall of the drug-context association during the expression test, but is gradually suppressed over the course of extinction (Sun et al., 2011). Therefore, mPFC activation appears to be correlated with the strength of the association between drug reward and contextual cues (Sun et al., 2011). Interestingly, there appears to be an optimal range of mPFC activation that is associated with the acquisition of drug-induced CPP (Kargari et al., 2012), and electrical stimulation of the mPFC above this range has been shown to block morphine CPP (Kargari et al., 2012).

DA signaling appears to play a key role in regulating mPFC activation. Over-stimulation of DA D1 receptors suppresses mPFC activation, and DA-induced increases in mPFC activity have been shown to be dependent on the activation of DA D4 receptors in the D2 class (Ceci et al., 1999; Seamans et al., 2001). Furthermore, the extent of mPFC DA receptor activation appears to affect performance on working memory tasks in an inverted U-shaped function (Seamans and Yang, 2004; Puig et al., 2014). This optimal range of task performance is primarily observed when altering DA D1 receptor activation in the mPFC, but mPFC DA D2 and D4 receptor stimulation has also been shown to affect learning in a way that is modelled by other functional relationships (Zahrt et al., 1997; Druzin et al., 2000; Floresco and Phillips, 2001; Chudasama and Robbins, 2004; Floresco et al., 2006; Floresco, 2013). In particular, the supra-normal stimulation of both DA D1 and D4 receptors in the mPFC impairs performance on working memory and set-shifting tasks respectively (Zahrt et al., 1997; Floresco et al., 2006). Taken together, these findings suggest that elevation of mPFC DA signaling beyond this optimal range inhibits the activation of the mPFC, which in turn causes deficits in performance on specific learning and memory tasks. This model provides the framework for a possible mechanism through which the potentiation of mPFC DA by d-govadine could impair the learned association between contextual cues and reward. Unfortunately, to-date no published studies have examined the effect of elevating DA or stimulating DA receptors in the mPFC beyond this presumptive optimal range on the acquisition of learned associations in CPP.

Studies assessing the impact of modulating mPFC DA signaling on other forms of associative learning can provide insight into the mechanisms that may underlie the effect of d-govadine on CPP. Using an olfactory fear conditioning experiment, Lauzon et al. (2009) reported that supra-normal stimulation of mPFC DA D4 receptors during conditioning blocked the

acquisition of CS-foot shock associations. Interestingly, stimulation of DA D4 receptors in the mPFC did not affect the expression of associative learning in this task (Lauzon et al., 2009). These data suggest that over-stimulation of DA D4 receptors may prevent the formation of an association between the CS and the foot shock, while having no effect on previously formed CS-foot shock associations (Lauzon et al., 2009). This behavioural profile is reminiscent of the effects of d-govadine on conditioned associations. Since antagonism of mPFC DA D4 signaling has also been shown to block the acquisition of associative fear memories, there may be an optimal range for D4 receptor activation to encode affective associations (Laviolette et al., 2005). In contrast to these findings, over-stimulation of the DA D1 receptor in the mPFC appeared to block the expression but not the acquisition of the associative learning in this task (Lauzon et al., 2009). Furthermore, the supra-normal stimulation of DA D1 receptors blocked the expression of morphine-induced CPP (Lauzon et al., 2013). Overall, these findings point to discrete roles for mPFC DA D1 and D2 receptors in some types of associative learning. These limited studies allow for the speculation that the effects of d-govadine on amphetamine and food CPP may be largely mediated by the excessive stimulation of DA D4 receptors. The elevated DA concentration in the mPFC during conditioning with amphetamine may have led to a level of DA D4 stimulation that allowed for a reward-context association to form but not one strong enough to be maintained. The dose-dependent effects of d-govadine are consistent with this potential mechanism. However, convincing conclusions about the mechanisms of d-govadine at the receptor level cannot be drawn from this small pool of studies because of intrinsic differences in learning mechanisms underlying fear conditioning and conditioned place preference (Raybuck and Lattal, 2014). Nevertheless, there is strong evidence that mPFC DA modulation affects learning and memory and it seems likely that the effect of d-govadine on conditioned associations

between context and reward may be due in part to its selective potentiation of DA in the mPFC. The mechanism underlying the effect of d-govadine on prefrontal DA levels is unclear. However, the fact that d-govadine only has weak antagonistic effects on the DA D1 and D2 receptors suggests that it elevates extracellular DA through pre-synaptic modulation.

The amount of DA release in response to the administration of d-amphetamine is significantly greater than d-govadine-induced elevation of DA in the mPFC (Di Chiara and Imperato, 1988; Lapish et al., 2014). Considering this difference in magnitude of DA release, d-govadine may only slightly elevate DA release in the mPFC above the high DA levels induced by d-amphetamine when they are administered in conjunction. With this in mind, it is hard to conceive of such a small effect on DA release by d-govadine to have exclusively driven the effects it has on multiple aspects of amphetamine-induced CPP. Therefore, the other actions of d-govadine on the DA system likely play a significant role in mediating its effects observed in this thesis.

The weak antagonism of DA D1 and D2 receptors exhibited by d-govadine may contribute to its effects on the conditioned association between context and reward. DA D1 and D2 antagonists are known to block the acquisition of amphetamine CPP in a dose-dependent manner, with the highest dose being the most effective (Hoffman and Beninger, 1989). Specifically, the acquisition of amphetamine CPP appears to be dependent on DA D1 and D2 signaling in the NAc (Liao, 2008). Furthermore, both l-THP and l-SPD which exhibit D1 and/or D2 antagonist properties facilitated the rate of extinction of amphetamine- and morphine-induced CPP respectively (Wang et al., 2007; Su et al., 2013). These properties of d-govadine may have weakened the association between drug reward and contextual cues enough to cause rapid extinction but leave the CPP intact for the test day (Lapish et al., 2014). If this were the case, a

higher dose of d-govadine administered during the conditioning phase may prevent CPP from being expressed on the test day.

4.3 The effect of d-govadine on the reinstatement of context-reward associations

A key finding in the present thesis is the discovery that d-govadine blocked the reinstatement of amphetamine-induced CPP. Following the process of extinction learning in which gradual decrease in place preference is caused by repeated exposure to the contextual cues in the absence of amphetamine reward, the latent association between context and amphetamine can be reinstated by a priming injection of amphetamine (Tzschentke, 2007). The effect of d-govadine on amphetamine-induced reinstatement of CPP can be analyzed from the perspective of incentive motivation (Robinson and Berridge, 1993; Shalev, 2002). During the conditioning phase, amphetamine administration evokes an arousing affective state that can be associated with contextual cues of the compartment. Over time, these contextual cues acquire incentive value in which the amphetamine-induced affective state initiates an approach response toward the drug-paired compartment. Throughout the subsequent extinction process, the approach response is decreased as the amphetamine-associated compartment loses its incentive value. As postulated by Stewart et al. (1984), drug-induced reinstatement may be due to the ability of the drug prime to elicit an incentive motivational state which reactivates the memory underlying approach behaviour towards drug-paired contextual cues. According to this view, the acute administration of 1.0 mg/kg d-govadine may have blocked the ability of a priming dose of amphetamine to restore the incentive salience of a context that was previously paired with amphetamine.

4.4 Mechanisms underlying the effect of d-govadine on amphetamine-induced reinstatement of CPP

Insight into the mechanism underlying the effect of d-govadine on amphetamine-induced reinstatement of CPP is provided by recent behavioural studies involving dopamine-β -hydroxylase (DBH) inhibitors which have a similar pharmacological profile to d-govadine (Devoto et al., 2014a). They selectively increase DA in the mPFC but do not alter DA concentrations in the NAc and have also been shown to potentiate cocaine-induced DA release in the mPFC but not in the NAc (Devoto et al., 2012, 2014b). Most importantly, this acute potentiation of DA release has been shown to prevent cocaine-induced reinstatement of cocaine-seeking behaviour (Schroeder et al., 2010, 2013; Devoto et al., 2014a). Specifically, D1 receptors appear to play a critical role in psychostimulant-induced reinstatement (Devoto et al., 2014a). Antagonism of the D1 receptors in the mPFC has been shown to restore cocaine-induced reinstatement that was blocked by DBH inhibitors, while overstimulation of D1 receptors prevents reinstatement (Devoto et al., 2014a). In contrast, insufficient stimulation of D1 receptors blocks cocaine-induced reinstatement (Sanchez et al., 2003). Together, these findings suggest that an optimal activation of DA D1 receptors in the mPFC may be required for psychostimulant-induced reinstatement of drug-seeking behavior. Furthermore, this behavioural effect of excessive D1 activation may be mediated by increased cAMP/PKA signaling which increases GABAergic interneuron activity, causing feedforward inhibition of pyramidal neurons to suppress the mPFC (Seamans et al., 2001). Indeed, activation of the mPFC appears to be critical for the reinstatement of psychostimulant-seeking behaviour in both the self-administration and CPP paradigms (McFarland and Kalivas, 2001; Capriles et al., 2003; Zavala et al., 2003).

Although the possibility that optimal levels of DA D1 receptor stimulation underlie the ability of a psychostimulant to reinstate drug-seeking has been put forth only recently, this phenomenon has been well-studied with regards to executive functions and cognition as discussed earlier. In particular, mPFC D1 DA receptor activation in a specific range of an inverted U-shaped function is correlated with optimal performance on spatial working memory tasks in both rats and non-human primates (Zahrt et al., 1997; Seamans et al., 1998; Floresco and Phillips, 2001; Vijayraghavan et al., 2007). In a similar pattern, d-govadine has been previously shown to disrupt the expression of TORM, but rescues it when an extended delay is imposed between training and the test that normally impairs memory performance (Lapish et al., 2014). This bidirectional effect of d-govadine on cognition may reflect differences in the modulation of the D1 receptor occupancy. Indeed, it has been shown that D1 stimulation improves working memory under sub-optimal prefrontal DA conditions while disrupting performance when DA is in the normal or supra-optimal range (Floresco and Phillips, 2001). In the case of previously acquired drug-context associations, amphetamine may elevate DA levels within an optimal range for the reinstatement of CPP, and this effect may be mediated by optimal D1 receptor stimulation. In the current study, d-govadine may have enhanced DA efflux to levels beyond the optimal range for amphetamine to induce the reinstatement of CPP. The current literature on the role of DA receptors in drug-induced reinstatement of CPP predominantly focuses on DA D1 receptor activity. However, based on the studies suggesting a role for DA D4 activation in associational learning, it is possible that over-stimulation of DA D4 receptors may contribute to the blocking effect of d-govadine on amphetamine-induced CPP. Future experiments could assess the effect of D1 or D4 agonists on the amphetamine-induced reinstatement of CPP and compare it to the effects of d-govadine. Moreover, administering a D1 or D4 antagonist concurrently with d-

govadine to determine if it reverses the effect of d-govadine on reinstatement would provide a better understanding of the mechanism underlying the present findings.

5. Conclusion

Collectively, the present data show that d-govadine affects multiple aspects of amphetamine-induced place preference but the mechanism through which this is achieved remains unclear. It seems likely that this is in part due to its unique ability to selectively increase DA levels in the PFC. Moreover, the effect of d-govadine on both cognitive function and psychostimulant-induced reinstatement closely resemble that of D1 agonists (Floresco and Phillips 2001; Devoto et al., 2014b). Even so, its high affinity for D1 receptors is not associated with agonist activity. As suggested by Lapish et al. (2014), d-govadine may be modulating DA release pre-synaptically. Interestingly, the effects of d-govadine were only observed when d-govadine was administered immediately prior to amphetamine. d-govadine appears to be altering the acute effects of amphetamine on the DA system to disrupt the maintenance and reinstatement of CPP, but whether it affects the hedonic or motivational properties of amphetamine warrants further investigation. Finally, the observation that the effects of d-govadine extend to learned associations with natural rewards is consistent with previous findings showing that d-govadine affects a variety of cognitive processes.

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Appendix

Appendix A: Effect of d-govadine on body weight

In Experiment 4, d-govadine was administered 5 min prior to placing animals in compartments containing Froot loops during the conditioning phase (4 of the 8 conditioning days; C2, C4, C6, C8). The weight of the animals on the day of the pre-conditioning test, each day of conditioning and each post-conditioning test was recorded and shown on Figure 5. A two-way ANOVA for Group and Day failed to confirm a significant interaction, which suggests that there was no difference in body weight between the groups throughout the conditioning and post-conditioning tests (group × day; $F(30, 14)=0.6$, $p=0.971$). Furthermore, a two-way ANOVA for Group and Day using data from only the conditioning phase also failed to confirm a significant interaction (group × day; $F(14, 30)=0.8$, $p=0.715$). Therefore, the administration of d-govadine during the conditioning phase did not appear to affect body weight throughout the conditioning and the post-conditioning tests.

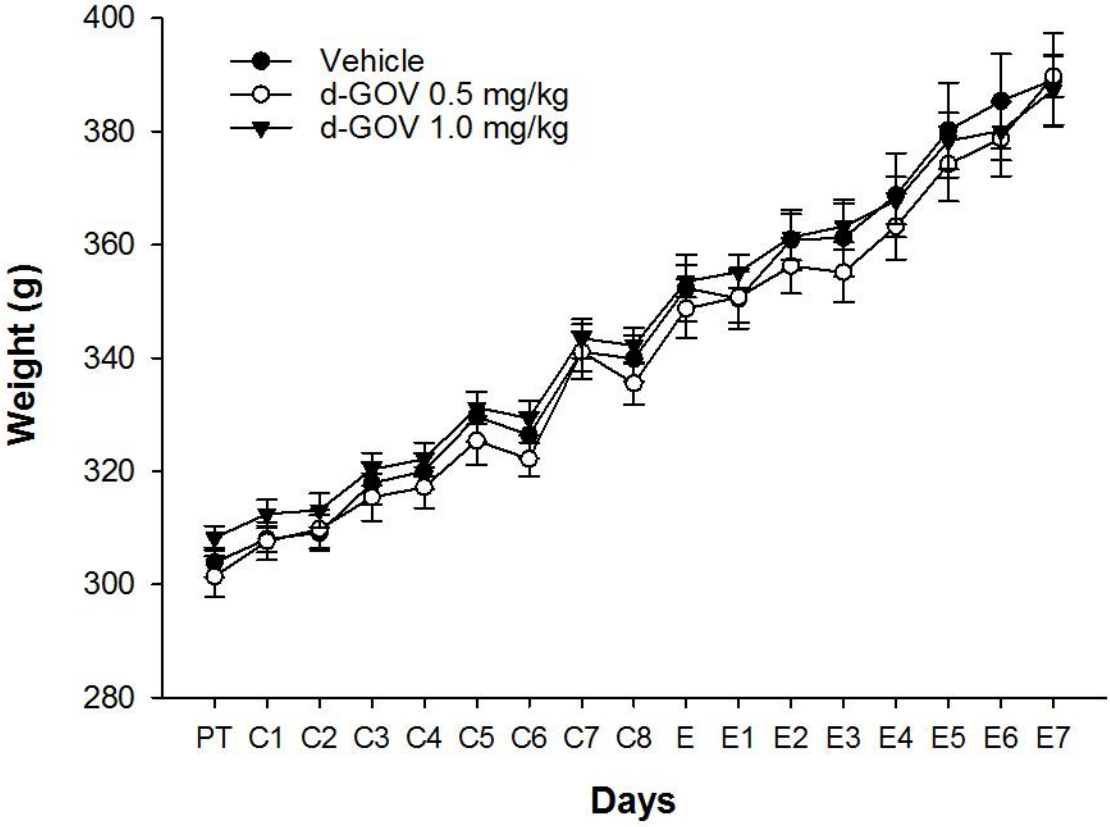

Figure 5. Effect of d-govadine on body weight during the acquisition and maintenance of food-induced conditioned place preference. Animals received vehicle (n = 8), 0.5 mg/kg (n = 8) or 1.0 mg/kg (n = 9) of d-govadine 5 min prior to being placed into a compartment containing Froot loops during the conditioning phase (C2, C4, C6, C8). Data represent the mean (±SEM) body weight on the pre-conditioning test day **(PT)**, 8 days of conditioning **(C1-C8)**, expression test day **(E)**, and 7 days of tests following the expression test **(E1-E7)**.