7-1-2011

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Accepted version. *Drug and Alcohol Dependence*, Vol. 116, No. 1-3 (July 1, 2011): 72-79. DOI.

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Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

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Abstract: Cocaine addiction is characterized by a persistently heightened susceptibility to drug relapse. For this reason, the identification of medications that prevent drug relapse is a critical goal of drug abuse research. Drug re-exposure, the onset of stressful life events, and exposure to cues previously associated with drug use have been identified as determinants of relapse in humans and have been found to reinstate extinguished cocaine seeking in rats. This study examined the effects of acute oral (gavage) administration of levo-tetrahydropalmatine (*l*-THP), a tetrahydroprotoberberine isoquinoline with a pharmacological profile that includes antagonism of D1, D2 and D3 dopamine receptors, on the reinstatement of extinguished cocaine seeking by a cocaine challenge (10 mg/kg, ip), a stressor (uncontrollable electric footshock [EFS]) or response-contingent exposure to a stimulus (tone and light complex) previously associated with drug delivery in male Sprague-Dawley rats. Extinguished drug seeking was reinstated by ip cocaine, EFS, or response-contingent presentation of drug-associated cues in vehicle-pretreated rats following extinction of iv cocaine self-adminisration. Oral administration of either 3.0 or 10.0 mg/kg *l*-THP one hour prior to reinstatement testing significantly attenuated reinstatement by each of the stimuli. Food-reinforced responding and baseline post-extinction responding were significantly attenuated at the 10.0, but not the 3.0 mg/kg, *l*-THP dose, indicating that the effects of 3 mg/kg *l*-THP on reinstatement were likely

Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

independent of non-specific motor impairment. These findings further suggest that *l*-THP may have utility for the treatment of cocaine addiction.

Keywords: relapse, dopamine, THP, craving, cocaine, reinstatement

1. Introduction

The unpredictable relapse of drug use that occurs even after extended periods of drug abstinence is the primary obstacle to the effective management of cocaine addiction. Although a number of factors likely contribute to relapse, drug re-exposure, the onset of stressful life events, and exposure to cues previously associated with drug self-administration are among the most important determinants of drug use. Relapse precipitated by each of these stimuli can be studied in rats using the self-administration/reinstatement approach (de Wit and Stewart, 1981; Erb et al., 1996; Meil and See, 1996; Ahmed and Koob, 1997).

Although a number of potentially promising medications have been identified for treating drug-dependent individuals (Vocci et al., 2005), a demand exists for new and more effective medicinal approaches, particularly those that target drug craving and relapse (O'Brien, 2005). Despite their potential efficacy, traditional herbal preparations are often not considered to be viable options for treating drug addiction, due in part to the limited number of reliable clinical and preclinical studies examining their utility. However, there has been a recent effort by many to test the effectiveness of such agents and their active constituents using accepted preclinical disease models and well-controlled clinical trials (Ernst, 2005; Lu et al., 2009).

Tetrahydropalmatine (THP) is a tetrahydroprotoberberine (THPB) isoquinoline alkaloid and a primary active constituent of herbal preparations containing plant species of the genera Stephania (Menispermaceae family) and Corydalis (Fumariaceae family), including Bin Ju Huan, Yan Hu Suo, Di Bu Long and Hua Jian Jiu Teng. Two of these species, Corydalis ambigua (yan hu suo) and Stephania tetranda (fang ji) are among the 50 fundamental herbs in Chinese herbology and have been used traditionally for their sedative, neuroleptic and analgesic properties (Ding, 1987). In particular, the levo isomer of THP (*l*-THP) appears to contribute to many of the

therapeutic effects of these preparations, presumably through its interactions with dopamine (DA) receptors (Jin, 1987; Chu et al., 2008).

A large body of data, including our own preliminary analyses, indicates that *l*-THP is an antagonist at DA D1 and D2 receptors (D1R, D2R; Jin, 1987; Guo et al., 1997; Mantsch et al., 2007; Huang and Jin, 1992; Hu and Jin, 1999; Jin et al., 1986; Sun et al., 1992; Wu et al., 1990; Xu et al., 1989; Zhu et al., 1991). Considering the involvement of dopaminergic neurotransmission in drug addiction and relapse (Volkow et al., 2004; Anderson and Pierce, 2005), compounds such as *l*-THP which antagonize DA receptors have long been thought to represent potential medications for the management of cocaine addiction. However, despite its promise, this approach has been largely unsuccessful due to lack of efficacy and/or the occurrence of use limiting side effects, attributable in part to undesirable pharmacological profiles that include high affinity antagonism of D2R (Platt et al., 2002). Compared to many other DA receptor antagonist drugs, *l*-THP has lower affinity for D2R relative to D1R (Jin, 1987). *l*-THP also binds to D3 DA receptors (Mantsch et al., 2007), likely functioning as an antagonist, and has secondary actions at a number of non-DA receptors (Lu et al., 1996; Halbsguth et al., 2003). The unique pharmacological profile of *l*-THP distinguishes it from other DA antagonist drugs and may make it a more suitable medication for treating cocaine addiction.

We and others have recently demonstrated that *l*-THP attenuates cocaine self-administration (SA; Mantsch et al., 2007; Xi et al., 2007), cocaine-evoked reinstatement (Mantsch et al., 2007), cocaine discrimination (Mantsch et al., 2010) and cocaine-induced reductions in intra-cranial self-stimulation (ICSS) thresholds (Xi et al., 2007) in rats at doses that produce little or no impairment of motor function. Along with the findings of a recent preliminary clinical trial demonstrating that *l*-THP attenuates craving and relapse in recovering heroin addicts (Yang et al., 2008), these data suggest that further investigation of the utility of *l*-THP for the treatment of cocaine addiction is warranted.

In the present study we investigated the ability of *l*-THP isolated from the roots of Stephania delavayi Diels (Di Bu Long) acquired from

Yunnan province in China, when administered orally by gavage, to block reinstatement of extinguished drug-seeking behavior by drug re-exposure, stress, and the presentation of drug-associated cues. In an attempt to further dissociate *l*-THP's effects on reinstatement from its sedative/motor effects, we also examined the effects of orally administered *l*-THP on food-reinforced lever pressing in a separate group of rats and on baseline lever pressing following extinction.

2. Methods

2.1. Drugs

Cocaine hydrochloride was obtained through the National Institute on Drug Abuse Drug Supply Program and was dissolved in 0.9% NaCl. *l*-THP, isolated from the crushed and dried tuberous roots of Stephania delavayi Diels (Di Bu Long) grown in Yunnan province in China, was acquired from Guangxi Nanning Baihui Pharmaceutical Group (Nanning, Guangxi, China) via the Beijing Basic Science Research Institute (Beijing, China) and was dissolved in sterile water. The purity of the isolated *l*-THP, according to the manufacturer, was greater than 99% as determined using high performance liquid chromatography.

2.2. Subjects

A total of 44 adult male Sprague-Dawley rats (Harlan Laboratories, St. Louis, MO) weighing 325g – 350g at the start of the study were used. Rats were housed individually in a temperature- and humidity-controlled environment in an AAALAC-accredited animal facility under a 12 h/12 h reversed light/dark cycle (testing during the dark cycle). Availability of food was restricted to 20-25 g each day, a condition that maintains body weight at approximately 80-85% of free-feeding levels, in order to facilitate the acquisition of SA (De Vry et al., 1989). Notably, it has been reported that this level of chronic food restriction does not alter the reinstatement of extinguished cocaine-seeking behavior by cocaine, stress, or cocaine-assocaiated cues (Bongiovanni and See, 2008). Water was available at all times during the study, including in the SA chambers. All procedures were

carried out in accordance with the Guide for the Care and Use of Animals as adopted and promulgated by the NIH.

2.3. Catheterization Surgery

Rats were implanted with indwelling jugular catheters attached to a back-mounted guide cannula under ketamine (100 mg/kg, ip) and xylazine (2 mg/kg, ip) anesthesia and were allowed to recover for five days before SA, during which time they were provided acetaminophen (480 mg/l) in their drinking water. After implantation, rats were injected daily with an antibiotic, cefazolin (15 mg, iv). For SA, a polyurethane delivery line encased in a stainless-steel leash was connected to the cannula to permit infusion of drug from a 30-ml syringe mounted in a motor-driven syringe pump via a leak-proof swivel suspended above the chamber. The swivel-and-leash assembly was counterbalanced to permit relatively unrestrained movement within the chamber. Catheters were filled daily with heparin (83 IU/ml) and capped when the delivery line was disconnected.

2.4. Effects of l-THP on cocaine- and EFS-induced reinstatement

Fifteen total rats were tested for the effects of oral pretreatment with *l*-THP on reinstatement induced by cocaine (10 mg/kg) and a stressor, uncontrollable intermittent electric footshock (EFS).

2.4.1. Cocaine SA

Rats were initially trained to self-administer cocaine (1.0 mg/kg/inf, iv) by pressing a lever under a fixed-ratio one (FR1) schedule during daily 2-h sessions, within which the active (i.e., front) lever was extended into the chamber and the corresponding stimulus light was illuminated. Pressing this lever resulted in an iv infusion of cocaine solution (200µl delivered over 5.0 s) followed by a 25-s time-out period during which the stimulus light was extinguished but the lever remained extended. Responding on the second, inactive (i.e., back) lever was recorded but had no programmed consequences. Once stable and significant SA under the FR1 schedule was observed (i.e., > 10 infusions), the response requirements for SA were gradually

increased to FR4. Once a stable response pattern was observed under the FR4 schedule (total responding < 10% variation from the mean over 3 consecutive sessions), rats were provided daily 6-h access to cocaine for SA under the FR4 schedule for 14 consecutive sessions prior to extinction. We have found that later reinstatement by cocaine or EFS is most pronounced following SA under these conditions (Mantsch et al., 2004; Mantsch et al., 2008).

2.4.2. Extinction

Extinction conditions were identical to those during SA, except that the extinction sessions only lasted for two hours and the cocaine solution was replaced with saline. Rats were tested daily for extinction until they emitted less than 15 responses during an extinction session or until they displayed stable patterns of low-level (<25 responses/session) responding over two consecutive sessions, at which time they were tested for reinstatement.

2.4.3. Cocaine- and EFS-Induced Reinstatement

Each rat was tested twice for reinstatement following ip administration of 10 mg/kg cocaine and twice for reinstatement following EFS (four tests total: once following vehicle pretreatment and once following *l*-THP pretreatment for each stimulus). Separate groups of rats were used to test the effects of 3 mg/kg *l*-THP and 10 mg/kg *l*-THP on reinstatement. We have found that prior testing for EFS-induced reinstatement fails to alter subsequent cocaine-induced reinstatement and vice versa. Nonetheless, we counterbalanced the sequence of cocaine- and EFS-induced reinstatement testing such that half of the subjects received cocaine first and the other half received EFS first. Likewise, the sequence of vehicle and *l*-THP pretreatment was counterbalanced for each reinstating stimulus. Reinstatement sessions were identical to extinction sessions except that they were preceded by an ip cocaine injection or EFS and a pretreatment with *l*-THP or vehicle. Cocaine-induced reinstatement testing consisted of ip administration of 10 mg/kg cocaine immediately prior to placing the rats into the chambers for the 2-h reinstatement session. EFS-induced reinstatement consisted of intermittent uncontrollable shocks through stainless steel grid floors of the SA/extinction/reinstatement test chambers for 10 minutes immediately prior to the 2-h reinstatement

Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

session. Shocks (0.5 mA, 0.5 s duration) were delivered on an average of every 40 s (range 10 – 70) during the 10-min period within which the house light was on, both response levers were retracted and the stimulus lights were extinguished. Rats were treated with *l*-THP or vehicle by oral gavage one hr prior to each reinstatement test session. Reinstatement test sessions were separated by additional extinction training. Rats were required to exhibit responding within 10% of earlier extinction levels before the next reinstatement test.

2.5. Cue-Induced Reinstatement

A total of 20 rats were assigned to the cue-induced reinstatement experiment. For this experiment, rats were tested under SA, extinction, and reinstatement conditions similar to those previously reported (Meil and See, 1996; See et al., 2001) with the exception that cocaine during the SA sessions and response-contingent cues during the reinstatement sessions were delivered under a FR4 schedule of reinforcement.

2.5.1. Cocaine SA

For these rats, cocaine SA training and testing were similar to the conditions described above except that: 1) SA sessions during the test phase only lasted two hours, 2) the stimulus light above the cocaine lever was extinguished at the start of the session and 3) each cocaine infusion self-administered under the FR4 schedule was paired with the concurrent presentation of a 5-s auditory/visual compound stimulus consisting of illumination of the stimulus light above the drug lever and a 78 dB, 2 kHz tone. After the SA training criteria were reached, rats were tested for SA under these conditions for an additional 14 days prior to extinction training.

2.5.2. Extinction

For these rats, extinction sessions were identical to the SA sessions except that lever pressing produced no consequences (i.e., no infusions or compound stimulus presentation). As described in Section 2.4.2., rats were tested daily for extinction until they emitted less than 15 responses during an extinction session or until they displayed stable patterns of low-level (<25 responses/session) responding over

two consecutive sessions at which time they were tested for reinstatement.

2.5.3. Reinstatement

Each rat was tested twice for cue-induced reinstatement (once following vehicle pretreatment and once following *l*-THP pretreatment for each stimulus). Half of the rats were tested first for reinstatement following administration of oral *l*-THP. The remaining rats were tested first for reinstatement following vehicle administration. Separate groups of rats were used to test the effects of 3 mg/kg *l*-THP and 10 mg/kg *l*-THP on reinstatement. Reinstatement conditions were identical to those during extinction, except that responses on the previously active lever resulted in 5-sec presentations of the cocaine-paired light-tone stimulus complex in the absence of cocaine reinforcement. Rats were treated with *l*-THP or vehicle by oral gavage one hr prior to each reinstatement test session. Reinstatement test sessions were separated by additional extinction training as described in section 2.4.3.

2.6. Effects of l-THP on Baseline Post-Extinction Responding

Since we did not design our reinstatement experiments to determine if *l*-THP altered lever pressing in the absence of a reinstating stimulus, nine rats were tested for the effects of oral *l*-THP (3 and 10 mg/kg) and vehicle on baseline responding following extinction. These rats were randomly selected from the rats used to determine the effects of *l*-THP on cocaine- and EFS-induced reinstatement and were tested under the extinction conditions described above (Section 2.4.2.) after reinstatement testing was complete. Each rat was tested for the effects of *l*-THP (3 and 10 mg/kg) or vehicle administered orally one hour prior to the extinction session in counterbalanced sequence. Rats were required to display stable patterns of responding prior to testing (total responding < 10% variation from the mean over 3 consecutive sessions) and were required to exhibit responding within 10% of the earlier 3-day mean before the next test.

2.7. Effects of l-THP on Food-Reinforced Responding

To further examine the potential role of sedation-related motor impairment in the effects of *l*-THP on reinstatement, the effects of oral *l*-THP administration (0, 3, and 10 mg/kg) on food-reinforced lever pressing was examined in a separate group of rats trained to self-administer 45 mg sucrose-sweetened food pellets (Noyes Precision Pellets, Formula F; Research Diets, New Brunswick, NJ) under an FR4 schedule of reinforcement during daily 30-min sessions. Food availability was indicated by illumination of a stimulus light above the lever. Once stable patterns of lever pressing were observed (total responding < 10% variation from the mean over 3 consecutive sessions), rats were tested for the effects of oral *l*-THP (3 or 10 mg/kg) or vehicle, administered one hr before a food session, on responding. Each rat was tested with each *l*-THP dose and vehicle in counterbalanced sequence. Rats were required to display stable response patterns (within 10% of the earlier 3-day mean) prior to testing with the next *l*-THP dose or vehicle.

2.8. Statistical Analyses

All statistical analyses were conducted using PASW (Predictive Analytics SoftWare) statistics software (SPSS, Inc.). The effects *l*-THP on reinstatement by each of the three stimuli (cocaine, stress and cues) were initially examined using 3-way (reinstatement × *l*-THP treatment × *l*-THP dose) ANOVA with reinstatement and *l*-THP treatment as within subjects measures and dose as a between subjects measure. These analyses were followed by 2-way (reinstatement × *l*-THP) repeated measures ANOVA at each *l*-THP dose for each stimulus. Post-hoc testing consisted of Bonferroni-corrected *t*-tests. One-way ANOVA (vehicle, 3 and 10 mg/kg *l*-THP) followed by post-hoc testing using Bonferroni-corrected *t*-tests was used to examine the effects of *l*-THP on baseline post-extinction and food-reinforced responding. For all analyses, significance was defined as $P < 0.05$.

3. Results

3.1 Cocaine SA and Extinction

Five of the 35 total rats used for the SA/reinstatement experiments did not complete the study due to catheter failure, illness or death. The mean numbers of training sessions prior to the acquisition of SA and extinction sessions prior to the first reinstatement test for each group are shown in Table 1.

Table 1
Acquisition and extinction of cocaine self-administration. Data represent the mean number of days of training (±S.E.) before rats in each experimental group reached the criteria for acquisition of SA prior to SA testing or extinction of cocaine seeking prior to reinstatement testing.

	EFS/Coc reinstatement exp.		Cue reinstatement exp.	
	3 mg/kg[a]	10 mg/kg[a]	3 mg/kg[a]	10 mg/kg[a]
Acquisition (Days ± S.E.)	10.14 ± 1.63	10.43 ± 0.48	16.40 ± 1.14	18.57 ± 1.36
Extinction (Days ± S.E.)	9.86 ± 0.60	21.43 ± 4.11	5.00 ± 1.36	6.41 ± 1.52

[a] *l*-THP dose group.

3.2 Cocaine-induced reinstatement

Seven rats were tested for the effects of 3 mg/kg *l*-THP and six rats were tested for the effects of 10 mg/kg *l*-THP on cocaine-induced reinstatement (Figure 1). A 3-way ANOVA was used to examine cocaine-induced reinstatement (cocaine vs. extinction; repeated measure) following pretreatment with vehicle or *l*-THP (repeated measure), at two oral *l*-THP doses (3 mg/kg and 10 mg/kg; between subjects measure). Significant main effects of cocaine administration ($F_{1,11}$=13.611; P<0.01), and *l*-THP treatment ($F_{1,11}$=13.001; P<0.01) but not *l*-THP dose (P=0.932) were observed, as was a significant interaction between *l*-THP treatment and cocaine-induced reinstatement ($F_{1,11}$=;14.007; P<0.01). Significant *l*-THP dose × reinstatement or *l*-THP treatment × dose × reinstatement interactions were not observed.

Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

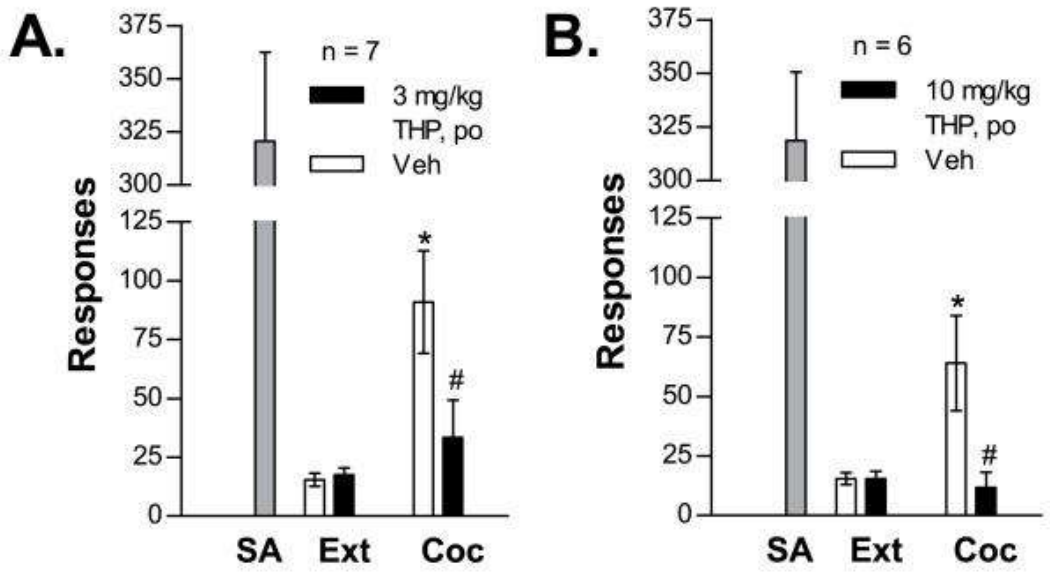

Figure 1 *l*-THP attenuates cocaine-induced reinstatement. Data represent responses on the cocaine lever during the final 6-h cocaine SA session (SA), the 2-h extinction session prior to reinstatement testing (Ext), and the 2-h reinstatement test session prior to which a 10 mg/kg (ip) injection of cocaine (Coc) was administered in rats tested for reinstatement following oral delivery of vehicle or 3 mg/kg (1A; n=7) or 10 mg/kg (1B; n=6) *l*-THP. Cocaine-induced reinstatement was observed following vehicle but not *l*-THP pretreatment (*P<0.05 vs. Ext) and *l*-THP pretreated rats displayed significantly less cocaine-induced reinstatement compared to Veh controls (#P<0.05 vs. Veh).

To further examine the effects of *l*-THP on cocaine-induced reinstatement, 2-way cocaine reinstatement × *l*-THP pretreatment ANOVAs were used for each dose group. In rats pretreated with the 3 mg/kg *l*-THP dose (Figure 1A), significant main effects of both cocaine administration ($F_{1,6}$=6.621; P<0.05) and *l*-THP treatment ($F_{1,6}$=10.354; P<0.05) were observed, as was an *l*-THP × cocaine reinstatement interaction ($F_{1,6}$=6.316; P<0.05). Post-hoc testing revealed that significant cocaine-induced reinstatement was observed in vehicle but not 3 mg/kg *l*-THP treated rats and was significantly reduced in rats pretreated with 3 mg/kg *l*-THP compared to rats pretreated with vehicle (P<0.05). In rats pretreated with the 10 mg/kg *l*-THP dose (Figure 1B), a significant main effect of cocaine administration ($F_{1,5}$=8.188; P<0.05) but not 10 mg/kg *l*-THP treatment (P=0.106) was observed, as was a 10 mg/kg *l*-THP × cocaine reinstatement interaction ($F_{1,5}$=11.443; P<0.05). Once again, post-hoc testing showed that significant cocaine-induced reinstatement was observed in vehicle but not 10 mg/kg *l*-THP treated rats and was

Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

significantly reduced in rats pretreated with 10 mg/kg *l*-THP compared to rats pretreated with vehicle ($P<0.05$).

3.3 Stress-induced reinstatement

Seven rats were tested for the effects of 3 mg/kg *l*-THP and six rats were tested for the effects of 10 mg/kg *l*-THP on stress- (EFS-) induced reinstatement (Figure 2). A 3-way ANOVA was used to examine stress-induced reinstatement (EFS vs. extinction; repeated measure) following pre-treatment with vehicle or *l*-THP (repeated measure), at two *l*-THP doses (3 mg/kg and 10 mg/kg; between subjects measure). Significant overall main effects of stress ($F_{1,11}=11.171$; $P<0.01$), and *l*-THP treatment ($F_{1,11}=9.552$; $P=0.01$) but not *l*-THP dose ($P=0.932$) were observed, as was a significant interaction between *l*-THP treatment and stress-induced reinstatement ($F_{1,11}=11.442$; $P<0.01$). Significant *l*-THP dose × reinstatement or *l*-THP treatment × dose × reinstatement interactions were not observed.

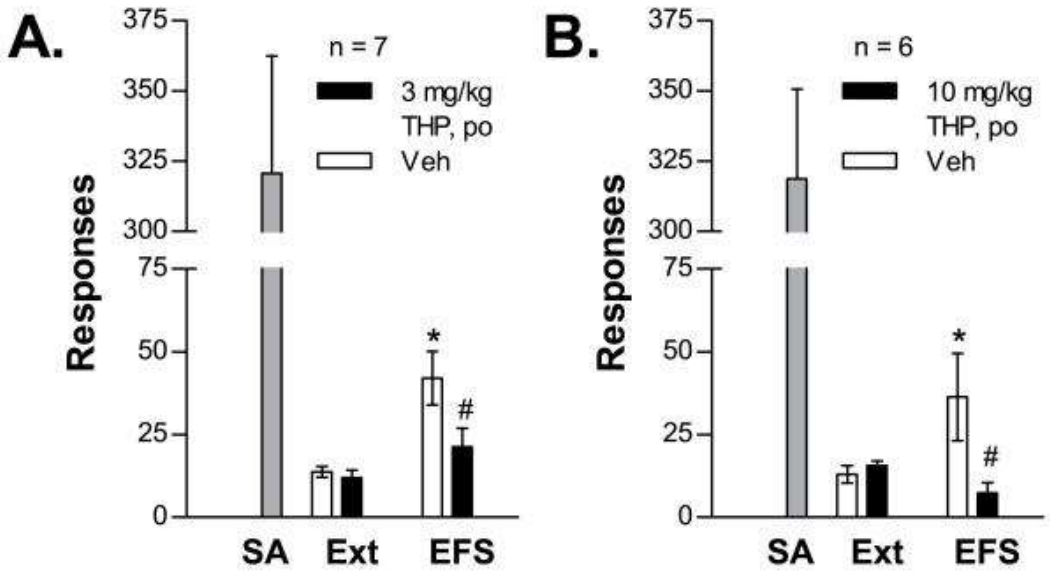

Figure 2 *l*-THP attenuates the reinstatement of extinguished cocaine seeking by a stressor, uncontrollable electric footshock (EFS), delivered intermittently prior to the test session. Data represent responses on the cocaine lever during the final 6-h cocaine SA session (SA), the 2-h extinction session prior to reinstatement testing (Ext), and the 2-h reinstatement test session (EFS) in rats tested for reinstatement following oral administration of vehicle or 3 mg/kg (2A; n=7) or 10 mg/kg (2B; n=6) *l*-THP. EFS reinstated cocaine seeking following vehicle but not *l*-THP pretreatment

(*P<0.05 vs. Ext) and *l*-THP pretreated rats displayed significantly less EFS-induced cocaine seeking compared to Veh controls (#P<0.05 vs. Veh).

To more closely examine the effects of *l*-THP on stress-induced reinstatement, 2-way stress-induced reinstatement × *l*-THP pretreatment ANOVAs were used for each dose group. In rats pretreated with the 3 mg/kg *l*-THP dose (Figure 2A), significant main effects of both stress ($F_{1,6}$=10.338; P<0.05) and 3 mg/kg *l*-THP treatment ($F_{1,6}$=12.946; P<0.05) were observed, as was a 3 mg/kg *l*-THP × stress-induced reinstatement interaction ($F_{1,6}$=7.396; P<0.05). Post-hoc testing revealed that significant EFS-induced reinstatement was observed in vehicle but not 3 mg/kg *l*-THP treated rats and that stress-induced reinstatement was significantly reduced in rats pretreated with 3 mg/kg *l*-THP compared to rats pretreated with vehicle (P<0.05). In rats pretreated with the 10 mg/kg *l*-THP dose (Figure 2B), a significant 10 mg/kg *l*-THP × stress-induced reinstatement interaction ($F_{1,5}$=7.545; P<0.05) but no significant main effect of EFS or 10 mg/kg *l*-THP treatment was observed. Once again, post-hoc testing showed that significant stress-induced reinstatement was observed in vehicle but not 10 mg/kg *l*-THP treated rats and that stress-induced reinstatement was significantly reduced in rats pretreated with 10 mg/kg *l*-THP compared to rats pretreated with vehicle (P<0.05).

3.4 Cue-induced reinstatement

Ten rats were tested for the effects of 3 mg/kg *l*-THP and seven rats were tested for the effects of 10 mg/kg *l*-THP on cue-induced reinstatement (Figure 3). A 3-way ANOVA was used to examine cue-induced reinstatement (cue vs. extinction; repeated measure) following pre-treatment with vehicle or *l*-THP (repeated measure), at two *l*-THP doses (3 mg/kg and 10 mg/kg; between subjects measure). Significant main effects of cue presentation ($F_{1,15}$=11.512; P<0.01), and *l*-THP treatment ($F_{1,15}$=14.983; P<0.01) but not *l*-THP dose (P=0.247) were observed, as was a significant interaction between *l*-THP treatment and cue-induced reinstatement ($F_{1,15}$=20.674;P<0.001). Significant *l*-THP dose × cue reinstatement or *l*-THP treatment × dose × cue reinstatement interactions were not observed.

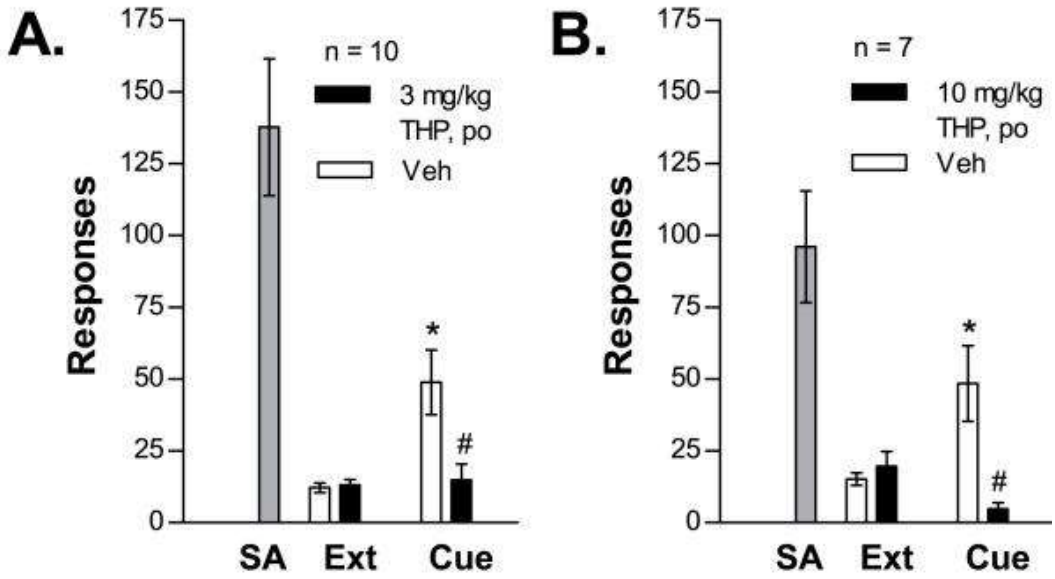

Figure 3 *l*-THP prevents the reinstatement of extinguished cocaine seeking by response contingent presentation of cues (tone-light complex) previously associated with cocaine SA. Data represent responses on the cocaine lever during the final 2-h cocaine SA session (SA), the 2-h extinction session prior to reinstatement testing (Ext), and the 2-h reinstatement test session (Cue) in rats tested for reinstatement following oral administration of vehicle or 3 mg/kg (3A; n=10) or 10 mg/kg (3B; n=7) *l*-THP. Cue presentation reinstated cocaine seeking following vehicle but not *l*-THP pretreatment (*P<0.05 vs. Ext) and *l*-THP pretreated rats displayed significantly less cue-induced cocaine seeking compared to Veh controls (#P<0.05 vs. Veh).

As described above, to further examine the effects of *l*-THP on cue-induced reinstatement, 2-way cue-induced reinstatement × *l*-THP pretreatment ANOVAs were used for each dose group. In rats pretreated with the 3 mg/kg *l*-THP dose (Figure 3A), significant main effects of both cue presentation ($F_{1,9}$=11.144; P<0.01) and *l*-THP treatment ($F_{1,9}$=8.433; P<0.05) were observed, as was an *l*-THP × cue presentation interaction ($F_{1,9}$=10.968; P<0.01). Post-hoc testing revealed that significant cue-induced reinstatement was observed in vehicle but not *l*-THP treated rats and was significantly reduced in rats pretreated with *l*-THP compared to rats pretreated with vehicle (P<0.05). In rats pretreated with the 10 mg/kg *l*-THP dose (Figure 3B), significant main effects of cue presentation ($F_{1,6}$=6.559; P<0.05) but not 10 mg/kg *l*-THP treatment (P=0.156) were observed, as was a 10 mg/kg *l*-THP × cue presentation interaction ($F_{1,6}$=9.176; P<0.05). Post-hoc testing revealed that significant cue-induced reinstatement was observed in vehicle but not 10 mg/kg *l*-THP treated rats and was

Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

significantly reduced in rats pretreated with 10 mg/kg *l*-THP compared to rats pretreated with vehicle (P<0.05).

3.5 Effects on baseline post-extinction responding

Since our original reinstatement design did not include testing for the effects of *l*-THP on baseline extinction responding, an additional nine rats were tested for the effects of 3 and 10 mg/kg *l*-THP on responding during an extinction session in order to determine if *l*-THP induced reductions in reinstatement may have been attributable to non-specific impairment of the ability of the rats to press the lever (Figure 4). Although one-way repeated measures ANOVA failed to show a significant effect of *l*-THP on responding (P=0.058), lever pressing was markedly reduced in rats following administration of the 10 mg/kg *l*-THP dose. Post-hoc testing using Bonferroni-correct *t*-tests revealed that responding after administration of 10 mg/kg *l*-THP was significantly reduced compared to responding following administration of 3 mg/kg *l*-THP (P<0.05) but not vehicle (P=0.173).

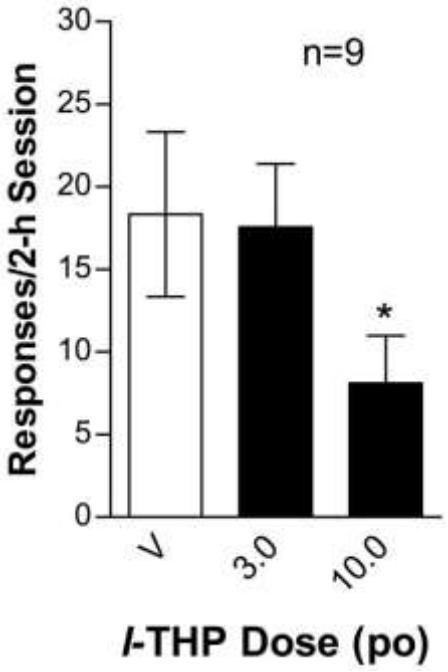

Figure 4 Effects of *l*-THP on baseline extinction responding. Lever pressing during the 2-h session was significantly reduced by oral administration of 10 mg/kg *l*-THP compared to 3 mg/kg *l*-THP but not vehicle (V; *P<0.05). No effects of 3 mg/kg *l*-THP were found.

3.6 Food-reinforced lever pressing

To further test if *l*-THP induced reductions in reinstatement may have been attributable to non-specific motor impairment, the effects of oral *l*-THP on food-reinforced lever pressing were examined in eight rats trained to self-administer sucrose-sweetened food pellets under a FR4 schedule of reinforcement and are shown in Figure 5. One-way repeated measures ANOVA showed a significant effect of *l*-THP on food-reinforced responding ($F_{2,14}$=12.315; P=0.001). Post-hoc testing using a Bonferroni-corrected *t*-test showed that responding was significantly attenuated following oral administration of the 10 mg/kg, but not 3 mg/kg, *l*-THP dose (P<0.05).

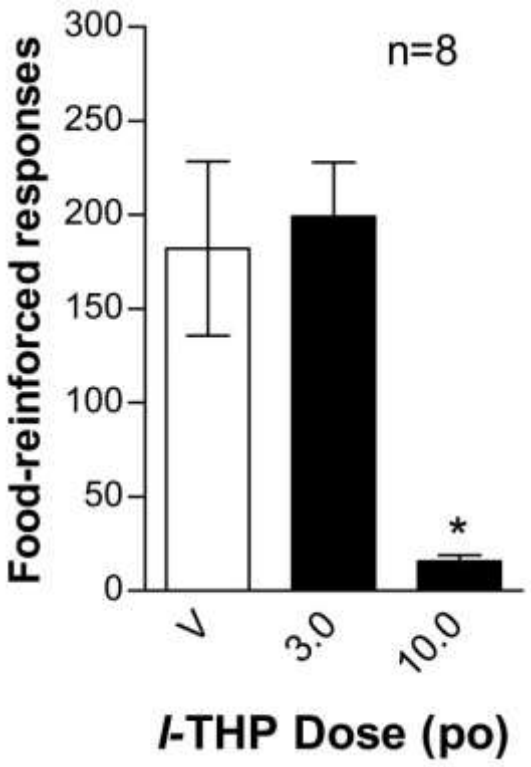

Figure 5 Effects of *l*-THP on food-reinforced lever pressing. Food- (sucrose-sweetened pellet-) reinforced lever pressing under a FR4 schedule during 30 min sessions was significantly reduced by oral administration of 10 mg/kg but not 3 mg/kg *l*-THP (*P<0.001 vs. V).

4. Discussion

4.1 Summary of findings

We and others have previously reported that ip administration of *l*-THP attenuates cocaine SA under both FR and PR (progressive ratio) schedules (Mantsch et al., 2007; Xi et al., 2007; Mantsch et al., 2010), cocaine-induced reductions in ICSS thresholds (Xi et al., 2007), cocaine discrimination (Mantsch et al., 2010) and cocaine-induced reinstatement of extinguished cocaine-seeking behavior (Mantsch et al., 2007). This paper extends these findings by demonstrating that *l*-THP, administered orally, also attenuates cocaine-induced reinstatement, as well as reinstatement by a stressor (uncontrollable EFS) or response-contingent delivery of conditioned cues previous paired with cocaine SA. In each case, *l*-THP decreased reinstatement at a dose (3 mg/kg) that failed to alter food-reinforced lever pressing or baseline post-extinction responding, suggesting that *l*-THP-induced decreases in cocaine seeking were likely not secondary to non-specific motor impairment by *l*-THP.

4.2. l-THP as an anti-craving/anti-relapse agent

The sudden and often unpredictable relapse of drug use after extended periods of abstinence continues to pose a major challenge to the effective treatment of cocaine addiction. For this reason, the identification and development of medications aimed at preventing relapse through the targeting of neurobiological pathways that underlie drug craving continues to be a high priority for addiction researchers (O'Brien, 2005). Although the ability of the reinstatement approach to predict effective anti-craving medications remains a matter of debate (Epstein et al., 2006; Yahyavi-Firouz-Abadi and See, 2009), our findings that *l*-THP, when administered orally, attenuates reinstatement by cocaine re-exposure, presentation of drug-associated cues, or stress, three of the primary precipitating factors of craving in human addicts, while at the same time attenuating the reinforcing effects of cocaine (Mantsch et al., 2007; Xi et al., 2007; Mantsch et al., 2010), suggests that examination of the efficacy of *l*-THP in human populations may be justified. Accordingly, a recent preliminary clinical trial reported that *l*-THP reduces drug craving and relapse in

recovering heroin addicts (Yang et al., 2008). Clinical testing of *l*-THP effectiveness in the US awaits further examination of its safety for use in humans.

4.3. Dopamine receptor antagonist properties of l-THP

It is likely that the effects of *l*-THP on cocaine-seeking behavior are related to its antagonism of DA receptors. A large body of data, including our own preliminary analyses, indicates that *l*-THP is an antagonist at D1R and D2R (Jin, 1987; Guo et al., 1997; Mantsch et al., 2007; Huang and Jin, 1992; Hu and Jin, 1999; Jin et al., 1986; Sun et al., 1992; Wu et al., 1990; Xu et al., 1989; Zhu et al., 1991). Considering the involvement of dopaminergic neurotransmission in cocaine-seeking behavior (see Volkow et al., 2004; Anderson and Pierce, 2005 for reviews), compounds such as *l*-THP that antagonize DA receptors have long been thought to represent potential medications for the management of cocaine addiction. Compared to many other DA receptor antagonist drugs, *l*-THP has lower affinity for D2R relative to D1R (Jin, 1987). *l*-THP also binds to D3 DA receptors (Mantsch et al., 2007) at concentrations reached in the brain following oral administration of the doses tested in this study (Hong et al., 2006), likely functioning as an antagonist; an effect that may contribute to its blockade of cocaine-mediated responses (Mantsch et al., 2010). The blockade of pre-synaptic autoreceptors by *l*-THP results in increased dopamine release (Marcenac et al., 1986) and it has been suggested that lower affinity of *l*-THP for D2R may confer some degree of autoreceptor selectivity (Jin et al., 1986).

4.4. Dopamine receptors and relapse

In addition to establishing *l*-THP as a potentially effective medication for the management of relapse, the present data suggest that DA may be a common mediator of relapse in response to stress, cocaine re-exposure, and exposure to cocaine-associated cues. These findings are consistent with a large body of literature implicating DA receptors in reinstatement (see Volkow et al., 2004; Anderson and Pierce, 2005 for reviews). DA receptor agonists (Khroyan et al., 2003; de Vries et al., 1999) and selective DA transport blockers (Schmidt and Pierce, 2006b; Schenk and Gettings, 2003) reinstate extinguished

Drug and Alcohol Dependence, Vol. 116, No. 1-3 (July 2011): pg. 72-79. DOI.

cocaine seeking, while systemic administration of DA antagonists and low-efficacy agonists at D1, D2, and D3 DA receptors (D1R, D2R, and D3R) have been reported to attenuate drug seeking evoked by cocaine re-exposure (Khroyan et al., 2003: D1R; Khroyan et al., 2000: D1R and D2R; Schenk and Gettings, 2003: D2R; Vorel et al., 2002: D3R; Xi et al., 2005: D3R; Peng et al., 2009: D3R), presentation of cocaine-associated cues (Ciccocioppo et al., 2001: D1R; Alleweireldt et al., 2002: D1R; Feltenstein et al., 2007: D2R; Crombag et al., 2002: D1R and D2R; Cervo et al., 2003: D2/3R; Cervo et al., 2007:D3R; Di Ciano et al., 2003: D3R; Gilbert et al., 2005: D3R), and stress (Xi et al., 2004; D3R).

While DA may be involved in reinstatement in response to each of these stimuli, the neuroanatomical site of DA action appears to vary, depending on the reinstating stimulus. For example, DA activation of D1R in the amygdala, particularly the basolateral amygdala appears to play a critical role in cue-induced reinstatement (See et al., 2001; Alleweireldt et al., 2006), while prefrontal cortical D1R activation appears to be important for stress-induced reinstatement (McFarland et al., 2003; Capriles et al., 2003; Sanchez et al., 2003), and D1R and D2R activation in the nucleus accumbens shell (Anderson et al., 2003; 2006; Bachtell et al., 2005; Schmidt et al., 2006a; Schmidt and Pierce, 2006) as well as D1R activation in prefrontal cortical regions (McFarland et al., 2001; Sun and Rebec, 2005) appears to be required for reinstatement in response to cocaine. Notably, high levels of cocaine intake such as those reached during the 6-h sessions used for the rats tested for cocaine- and stress-induced reinstatement in the present study and often observed in cocaine addicts appears to recruit DA involvement in drug seeking in additional brain regions, such as the NA core (Madayag et al., 2010), further establishing the importance of the DA system as a target for pharmacotherapy.

4.5. Effects of l-THP at non-DA receptors

Our own preliminary binding analysis suggests that *l*-THP, at brain concentrations comparable to those that may be reached following oral *l*-THP delivery (Hong et al., 2006) also displays moderate binding to alpha-1 and alpha-2 adrenergic receptors as well

as strong binding to 5-HT_{1A} receptors (Mantsch et al., 2007 and unpublished findings). *l*-THP is an antagonist at alpha-1 adrenergic receptors (Lu et al., 1996). The effects at alpha-2 adrenergic receptors are unclear; *l*-THP has been reported to both mimic (Wu and Jin, 1995) and block (Liu et al., 1989) the effects of the alpha-2 adrenergic receptor agonist, clonidine. The actions of *l*-THP at 5-HT_{1A} receptors (i.e., full agonist, partial agonist, vs. antagonist) have not been reported. It has also been found that *l*-THP has positive cooperative effects at the $GABA_A$ receptor (Halbsguth et al., 1996), an effect that may contribute to the high-dose behavioral suppressive effects of *l*-THP (Xu et al., 1981). Despite its use as an analgesic, no interaction of *l*-THP with opioid receptors has been reported, and it analgesic effects are naloxone-independent (Hu and Jin, 1999).

These interactions of *l*-THP with non-DA receptors, most notably its likely antagonism of alpha-1 adrenergic receptors and interaction with 5-HT_{1A} receptors, may also contribute to its effects on cocaine seeking behavior and could mitigate some of the undesirable effects associated with DA receptor antagonism, thus further distinguishing *l*-THP from other DA receptor antagonist drugs. Alpha-1 adrenergic (Zhang and Kosten, 2005) and 5-HT_{1A} (Burmeister et al., 2004) receptor antagonists have been previously reported to attenuate cocaine seeking in rats. Secondary actions of *l*-THP at 5-HT_{1A} and alpha adrenergic receptors could also minimize the extrapyramidal effects associated with DA receptor antagonism by *l*-THP (Imaki et al., 2009; Kalkman et al., 1998; Millan et al., 2000; Wadenberg, 1996).

4.6. Sedative effects of l-THP

The effectiveness of DA antagonists for the treatment of cocaine addiction has been limited by problematic side effects, including motor impairment/sedation and anhedonia, attributable in part to undesirable pharmacological profiles (Platt et al., 2002). Additionally, the primary clinical feature of overdose intoxication with *l*-THP-containing preparations (which also typically contain a number of other pharmacologically active constituents) is central nervous system depression (Horowitz et al., 1996). To ensure that motor impairment did not contribute to the observed effects on reinstatement and to demonstrate dose separation between the toxic and therapeutic effects

of *l*-THP, we tested rats for non-specific motor impairment at the same doses used for reinstatement testing. Consistent with previous findings with ip *l*-THP (Mantsch et al., 2007; Xi et al., 2007), we found that, although *l*-THP produced dose-dependent motor impairment, as demonstrated by reductions in baseline extinction and food-reinforced responding, effects on reinstatement were observed at an *l*-THP dose (i.e., 3 mg/kg) that had no effects on lever-pressing during the food or extinction sessions.

Although we assume that the behavioral suppression observed at the higher (i.e., 10 mg/kg) *l*-THP dose was primarily attributable to high-dose sedative effects, it is possible that aversive effects may have also contributed. *l*-THP, at higher doses, has been reported to increase thresholds for ICSS (Xi et al., 2007) and to reduce the breaking point for non-drug (sucrose sweetened) reinforcement under a progressive ratio schedule (Mantsch et al., 2010). However, in both cases, these effects, which potentially reflect anhedonia, emerged at doses higher than those at which effects on responses to cocaine were observed and may have been related to motor impairment. Further, *l*-THP, at doses as high as 18.5 mg/kg (ip), does not produce conditioned place aversion (Liu et al., 2009) and anxiogenic effects of *l*-THP have not been reported. In fact, racemic (dl) THP has been reported to have anxiolytic properties (Leung et al., 2003) and *l*-THP containing preparations are often used for their sedative effects. Thus, we consider it to be unlikely that the general disruption of behavior observed at the higher *l*-THP dose was due to anxiogenic effects.

Although approved for use in China, further preclinical and clinical examination of *l*-THP is needed to determine if it is safe enough for the US market and to distinguish from other DA antagonist drugs.

4.7. Conclusions

To summarize, orally administered *l*-THP was effective in preventing reinstatement by three different stimuli in a rat model of drug relapse at doses that did not produce measurable motor impairment. These findings add to a growing body of evidence suggesting that *l*-THP and other similar compounds derived from natural sources may themselves have medicinal value and/or may provide insight into desirable pharmacological profiles for future

medications. Although additional determination of the efficacy and safety of *l*-THP in human populations is necessary, our findings suggest that *l*-THP or *l*-THP containing preparations may have utility for facilitating relapse prevention and for the treatment of cocaine addiction.

Footnotes

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