Evaluation of Modafinil in Preclinical Behavioral Assays of Abuse Liability

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EVALUATION OF MODAFINIL IN PRECLINICAL BEHAVIORAL ASSAYS OF
ABUSE LIABILITY

by

Amanda J. Quisenberry

A dissertation submitted to the Graduate College
in partial fulfillment of the requirements
for the degree Doctor of Philosophy
Department of Psychology
Western Michigan University
December 2013

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Modafinil is an FDA-approved drug for the treatment of narcolepsy with efficacy in the treatment of chronic fatigue syndrome, obstructive sleep apnea, and shift-work sleep disorder. Modafinil’s wake-promoting and cognitive-enhancing effects are reportedly similar to those of traditional psychostimulants, but without the side effects typically associated with these substances. Modafinil has also been investigated as an agonist replacement therapy for psychostimulant dependence, although results of clinical trials are equivocal.

Few studies have examined its behavioral effects in combination with psychostimulants and the neuropharmacological actions of modafinil are not well understood. The primary aim of this study was to assess modafinil’s effects in combination with the psychomotor stimulant, d-amphetamine, in four experiments utilizing preclinical behavioral assays of abuse liability. A secondary aim was to investigate modafinil’s neuropharmacological actions utilizing drug discrimination, an in vivo preclinical screening procedure with established predictive validity.

The first experiment utilized a behavioral sensitization assay to determine if repeated d-amphetamine treatment followed by a washout period would produce cross sensitization to modafinil. Experiment 2 utilized a conditioned place
preference (CPP) assay to determine if modafinil would establish a CPP or influence d-amphetamine-induced CPP. Experiment 3 utilized a drug discrimination assay to evaluate generalization with modafinil alone and with d-amphetamine in rats trained to discriminate d-amphetamine. Experiment 4 assessed several dopaminergic compounds for substitution or antagonism in rats trained to discriminate 256 mg/kg modafinil. Experiment 1 results indicated that repeated d-amphetamine treatment does not induce cross sensitization to modafinil. Experiment 2 results demonstrated that modafinil does not readily establish CPP or potentiate d-amphetamine-induced CPP. Modafinil produced dose-dependent d-amphetamine-lever responses and partial substitution for d-amphetamine in Experiment 3. Experiment 4 results represent the first demonstration that modafinil’s actions at the dopamine transporter are important in maintaining its discriminative stimulus effects. Considered together, these findings support previous reports of modafinil’s low abuse potential, but also indicate that it may have additive effects with psychomotor stimulants. In consideration of modafinil as a potential candidate for agonist replacement therapy, further preclinical investigations of modafinil in combination with other stimulants, such as drug self-administration, may be warranted.
ACKNOWLEDGMENTS

This dissertation could not have been completed without the assistance of many individuals to whom I am deeply indebted. I would like to begin by thanking my advisor, mentor, supervisor, and friend, Dr. Lisa Baker, for the guidance and assistance provided during my doctoral career. Not only did she teach me all I know about behavioral pharmacology, she also taught me how to write manuscript style publications and provided me with funding while in the graduate program at WMU. I certainly would not be the scientist I am today without her assistance and I am forever grateful.

I am also thankful to the other members of my dissertation committee who have made me a more proficient scientist in a variety of capacities. I would like to thank Dr. Russ Morgan for providing my first opportunities as a graduate student and experimental researcher and for shaping me into a student capable of completing a Ph.D. program. I would also like to thank Dr. Cindy Pietras for initiating my excitement for behavior analysis at WMU and for giving me the opportunity to teach a class heavily focused in that content area. I also thank Dr. Al Poling for his encouraging words near the completion of my degree, which eased anxiety and made the process more enjoyable.

Finally, I would like to thank all other individuals at WMU who made a lasting impact on my life. My professors, colleagues, students, and friends have provided an overwhelming amount of support and assistance throughout the past
Acknowledgements – continued

four years. Thank you all for everything. I would not be the person I am today without you.

Amanda J. Quisenberry
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CHAPTER I
INTRODUCTION

General Overview

Modafinil is a wake-promoting drug manufactured by Cephalon with FDA approval for the treatment of narcolepsy and is reportedly effective in the treatment of chronic fatigue syndrome (Turkington, Hedwat, Rider, & Young, 2004), obstructive sleep apnoea/hypopnoea syndrome, and shift work sleep disorder (Keating & Raffin, 2005). In addition to being prescribed for these FDA-approved disorders, it has also been investigated to treat fatigue in patients with Parkinson’s disease (Hogl, Saletu, Brandauer, Glatzi, Frauscher, Seppi, Ulmer, Wenning, & Poewe, 2002), amyotrophic lateral sclerosis (Carter, Weiss, Lou, Jensen, Abresch, Martin, Hecht, Han, Weydt, & Kraft, 2005), fibromyalgia (Pachas, 2003), cancer (Blackhell, Petroni, Su, Baum, & Farace, 2009), multiple sclerosis (Volkmer, Heesen, & Liepert, 2009), and dementia (Howcroft & Jones, 2005) in addition to attention deficit hyperactivity disorder (Taylor & Russo, 2000). Modafinil’s wake-promoting (Hermant, Rambert, & Duteil, 1991; Silvestri, Sanford, Ross, Mann, Pavlock, & Morrison, 2002; Webb, Pollock, & Mistlberger, 2006) and cognitive-enhancing effects (Turner, Robbins, Clark, Aron, Dowson, & Sahakian, 2003) are similar to those of traditional psychostimulants, apparently without the side effects (e.g., tolerance, abuse potential, sleep rebound, and increased locomotor activity or hyperactivity) typically associated with these substances (Deroche-Gamonet, Darnaudery, Bruins-Slot, Piat, & Piazza, 2002; Hermant et al., 1991; Lin, Roussel, Akaoka, Fort, Debilly, & Jouvet, 1992). The
following literature review addresses the current state of knowledge regarding the
cognitive effects, abuse liability, and neurochemical actions of modafinil based on
human and nonhuman research investigations.

Cognitive Enhancing Effects of Modafinil

Human Populations

Human clinical investigations of the cognitive and performance-enhancing
properties of modafinil have mainly involved assessments in sleep-deprived
individuals or in populations with neurological disorders. Improved response time
on vigilance tasks (Wesensten, Killgore, & Balking, 2005) and executive function
measures (Walsh, Randazzo, Stone, & Schweizer, 2004; Baranski, Gill,
McLellan, Moroz, Buguet, & Radomski, 2002) were enhanced by modafinil
administration in sleep-deprived individuals. Other studies utilizing sleep-
deprived individuals have found improved performance and alertness in aviators
in a simulated helicopter flight (Caldwell, Caldwell, Smythe, & Hall, 2000). In
fact, modafinil attenuated the decline in simulated flight performance (Dagan &
Doljansky, 2006), and improved attentional focus, response selection, and
reduced impulsivity (Gill, Haerich, Westcott, Godenick, & Tucker, 2006) in this
population.

Studies of individuals with schizophrenia or ADHD also show
enhancement of cognitive abilities following modafinil administration. Modafinil
administration in adults with ADHD led to improved scores on short-term memory
span (the Digit Span Task), visual memory (Pattern Recognition Memory and
Delayed Matching to Sample Tasks), spatial planning (Tower of London Task),
decision-making (Gamble Task), and sustained attention in the Rapid Visual
Information Processing (RVIP) Task (Turner, Clark, Dowson, Robbins, &
Sahakian, 2004a). In patients with schizophrenia, modafinil resulted in cognitive
improvements in the Digit Span Task and an intra-dimensional/extra-dimensional
task (IDED), which is a three-dimensional version of a set-shifting task measuring
selective attention (Turner, Clark, Pomarol-Clotet, McKenna, Robbins, &
Sahakian, 2004b). Few studies have demonstrated modafinil-induced cognitive
benefits in patients with other conditions associated with cognitive decline,
although one study in participants with narcolepsy described a decreased
number of errors from baseline seen on the Wisconsin Card Sorting Task
(Schwartz, Nelson, Schwartz, & Hughes, 2004). In addition, a recent study of
seven methamphetamine-dependent participants seeking treatment documented
significant increases in verbal memory recall and trends toward improvements in
other executive functions (Hester, Lee, Pennay, Nielsen, & Ferris, 2010).

Despite considerable evidence that modafinil produces cognitive benefit
in some neurological populations, results of research involving non sleep-
deprived, healthy individuals are equivocal. Randall and colleagues reported that
modafinil does not act as a cognitive-enhancer in young (n = 30) or middle-aged
individuals who are not sleep-deprived (Randall, Shneerson & File, 2004;
Randall, Shneerson, Plaha & File, 2003). However, a later study by the same
investigators involving more young participants (n = 60) found that modafinil
enhanced the speed of response time in the color naming task of the Stroop test,
and improvements in the Digit Span task (a measure of working memory), the Pattern Recognition Memory (PRM) tests, and the RVIP in the healthy young adult sample (Randall, Viswanath, Bharania, Elsabagh, Hartley, Shneerson, & File, 2005; Turner et al., 2003). Specifically, improvements in verbal recall were found in the Digit Span Task and the modafinil group recognized more patterns in the PRM tests.

In addition to enhancements in working memory and performance on spatial tasks, modafinil appears to have an effect on attention and impulsivity measures. A study in healthy individuals found modafinil decreased the number of omission errors in the RVIP Task after administration of 200 mg/kg modafinil (Randall et al., 2005) and after approximately 300 mg/kg in the Detection of Repeated Numbers (DRN) Task (Baranski, Pigeau, Dinich, & Jacobs, 2004), both measures of sustained attention. Increased accuracy in attentional set-shifting has also been reported (Marchant, Kamel, Echlin, Grice, & Lewis, 2009), but only at the most difficult levels of the task. Reduced impulsivity or increased inhibition has also been demonstrated in humans using the stop-signal task (STOP) (Turner et al., 2003).

In sum, paradigms utilizing working memory tasks have shown decreased errors after modafinil administration that were not dependent on task difficulty, but rather on baseline performance (Muller, Steffenhagen, Regenthal, & Bublak, 2004). Those individuals who had poorer baseline scores were those who benefited the most from modafinil use. Modafinil, therefore, may improve cognitive performance in young, healthy individuals on specific types of cognitive
tasks, such as working memory and spatial tasks. However, evidence for improvements in impulsivity, attentional measures, and decision making tasks are equivocal and may be more specific to task difficulty or those individuals with preexisting cognitive deficits, such as would exist in sleep-deprived or aged individuals, or individuals with ADHD, schizophrenia, or other cognitive impairments.

Nonhuman Animal Populations

A number of animal studies investigating modafinil have also demonstrated cognitive improvements in non sleep-deprived, healthy, young rats. Specifically, improvements have been found in learning and working memory tasks, consistent with the human literature (Randall et al., 2005; Turner et al., 2003). Modafinil-treated rats learned the win-stay rule in a Serial Spatial Discrimination Reversal (SSDR) Task (Beracochea, Celerier, Peres, & Pierard, 2003; Beracochea, Celerier, Borde, Valleau, Peres, & Pierard, 2002) and in a Delayed Nonmatching to Position Swim Task (Ward, Harsh, York, Stewart, & McCoy, 2004) in fewer trials than controls. Acquisition of the Morris water maze task, a measure of spatial memory, was enhanced in a group of mice that received 75 mg/kg modafinil during training, although an acute injection of the same dose did not enhance performance in animals with no drug pretreatment (Shuman, Cai, Sage, & Anagnostaras, 2012). Assessment of modafinil pretreatment on learning in a Pavlovian fear conditioning paradigm revealed significant differences from saline-treated animals in task acquisition and percent of time spent freezing (Shuman, Wood, & Anagnostaras, 2009). In a study
examining the cognitive enhancing effects of modafinil in an animal model of pharmacologically-induced impairment with phencyclidine (PCP), modafinil attenuated effects in the extradimensional set shift task of the preclinical ID-ED task, the cognitive function most impaired by PCP administration (Goetghebeur & Dias, 2009).

Assessments of attention and impulsivity measures have also found promising, though equivocal, performance-enhancing results after modafinil administration in young, non-impaired rats. In a Five-Choice Serial Reaction Time Task (5-CSRTT), modafinil administration decreased the number of omission errors, indicating an increased level of sustained attention, but increased the number of premature responses, suggesting a lack of inhibition or increased impulsivity (Milstein, Dalley, Theobold, & Robbins, 2003). Other research utilizing the same paradigm found discrepant results and reported no improvement in sustained, selective, or divided attention after modafinil administration (Waters, Burnham, O’Connor, Dawson, & Dias, 2005). Furthermore, research utilizing a modified version of the 5-CSRTT with only three operand (3-CSRTT) found no effects on sustained or selective attention in young, healthy adult rats after modafinil administration (Colclasure, Campbell, Quisenberry, Miller, Dopheide, & Morgan, 2006), although another study utilizing this apparatus did find increased premature responses after modafinil (64 mg/kg) administration in young Long-Evans rats (Quisenberry, 2007). It should be noted that the age of the animals was not reported in the Milstein et al. (2003) or the
Waters et al. (2005) studies and age may very well influence the cognitive enhancing effects of modafinil.

Similar to the findings of modafinil-induced enhancement in cognitively-impaired clinical populations, a study of middle-aged animals found evidence of modafinil-induced facilitation of attention (Morgan, Crowley, Smith, LaRoche, & Dopheide, 2007). Specifically, improvements in impulse control were seen, as measured by a reduction in the percentage of premature responses, as well as improved response accuracy and reaction time following oral administration of 64 mg/kg modafinil, but not with lower doses. A similar study validated the results of the attention measures and reported increased sustained attention, measured by an increase in percent accurate trails, but reported no effect on premature responses or impulsivity in aged Long-Evans rats (Quisenberry, 2007).

Cognitive improvements associated with modafinil use may contribute to its potential for abuse in some populations. However, the clinical and preclinical research literature indicate that modafinil’s abuse liability is fairly low. This literature is reviewed in the next section.

Abuse Liability Assessments and Therapeutic Implications for Modafinil in Substance Abuse/Dependence

Basic Human Studies on Subjective Effects

Several double blind, placebo controlled studies have evaluated the subject-rated effects of modafinil in healthy adults with or without substance abuse histories. For example, in a sample of 16 healthy adults without a substance abuse history, the Profile of Mood States, Addiction Research Center
Inventory, and a Visual Analog scale were utilized to compare subject-rated and behavioral effects of a single oral dose of 300 mg modafinil and 15 mg dextroamphetamine at 1, 2, 4 and 8 hour post-dose intervals (Warot, Corruble, Payan, Weil, & Puech, 1993). The authors reported that modafinil ratings were markedly different from those of d-amphetamine and more similar to caffeine ratings. In contrast, in a more recent study evaluating a wider range of doses in 12 healthy adults without a substance abuse history, modafinil and amphetamine were reported to produce qualitatively and quantitatively similar effects (Makris, Rush, Frederich, Taylor, & Kelly, 2007).

Other human laboratory studies of modafinil’s psychoactive effects indicate that oral modafinil administration at clinically effective doses does not appear to have strong reinforcing properties, and may produce different subject-rated effects in participants with and without a history of psychostimulant use (Malcolm, Swayngim, Donovan, DeVane, Elkashef, Chiang, Khan, Mojsiak, Myrick, Hedden, Cochran & Woolson, 2006; Rush, Kelly, Hays, Baker, & Wooten, 2002; Warot et al., 1993). Participants with a history of stimulant use reported different ratings on measures of drug-liking, mood, anxiety, and fatigue between modafinil and amphetamine (Malcolm et al., 2006). On the contrary, humans without a history of psychostimulant use appear to be more likely to characterize the effects of modafinil as amphetamine-like (Mackris et al., 2007; Stoops, Lile, Fillmore, Glaser, & Rush, 2005). Only one study has investigated the abuse liability of modafinil in a human choice self-administration paradigm. The results of this study support results obtained from subject-rated effects in
psychostimulant users. Twelve cocaine abusers not seeking treatment did not choose modafinil more frequently than placebo (Vosburg, Hart, Haney, Rubin & Foltin, 2010).

Findings are generally consistent that participants with a history of psychostimulant abuse can readily distinguish the effects of modafinil from either cocaine or amphetamine (Malcolm et al., 2006; Rush et al., 2002). However, evidence that psychostimulant users can discriminate the effects of modafinil from those of other psychostimulants does not necessarily preclude the possibility that modafinil could be established as a therapeutic agent to assist in recovery from psychostimulant abuse and/or dependence.

Modafinil as a Treatment for Dependence

Modafinil has been evaluated as a potential treatment for amphetamine (Mann & Bitsios, 2008), methamphetamine (Shearer, Darke, Rodgers, Slade, van Beek, Lewis, Brady, McKetin, Mattick, & Wodak, 2009), and cocaine (Dackis, Kampman, Lynch, Pettinati, & O’Brien, 2005) dependence and abuse in clinical populations. Many factors make modafinil a promising candidate for this type of pharmacotherapy. Notably, no major side effects have been reported, no deaths have occurred, and any effect of modafinil overdose was mild (Carstairs, Urquhart, Hoffman, Clark, & Cantrell, 2010). Abuse liability assessments in animals (Deroche-Gamonet et al., 2002) and humans (Vosburg et al., 2010) indicate a low potential for abuse when modafinil is used alone, however there is some evidence to suggest exposure to modafinil in combination with cocaine (Schmitz, Rathnayake, Green, Moeller, Dougherty & Grabowski, 2012) or after
repeated exposure to cocaine (Andersen, Kessler, Murnane, McClung, Tufik & Howell, 2010) may make an individual more susceptible to relapse. Moreover, results of clinical studies investigating modafinil as a potential agonist replacement therapy have yielded mixed results (Hart, Haney, Vosuburg, Rubin, & Foltin, 2008; Anderson et al., 2012).

In a sample of eight participants (one female and seven male African-Americans) with a history of cocaine abuse, modafinil attenuated cocaine self-administration as well as subjective measures of craving in controlled laboratory conditions (Hart et al., 2008). In an earlier study, subjective ratings of cocaine-induced euphoria were attenuated by modafinil administration in cocaine-dependent participants (Dackis, Lynch, Yu, Samaha, & Kampman, 2003). In addition, a trend toward attenuation of the subjective effects of methamphetamine was observed after modafinil administration in 13 methamphetamine-dependent individuals not seeking treatment (De La Garza, Zorick, London, & Newton, 2010). These participants made fewer choices for methamphetamine during self-administration sessions. However, double-blind placebo controlled studies with larger samples have yielded inconsistent findings. One study evaluating modafinil for the treatment of methamphetamine dependence found no significant differences between modafinil and placebo on subject-rated cravings or methamphetamine use (Heinzerling, Swanson, Kim, Cederblom, Moe, Ling, & Shoptaw, 2010). Anderson et al. (2012) evaluated modafinil (200 and 400 mg/kg) as a treatment for cocaine dependence and found no statistically significant differences between placebo and modafinil.
Interestingly, when alcohol-dependent subjects were excluded from this analysis, significant differences were reported on the number of cocaine abstinent days.

Preclinical Studies

Although clinical observations seem to indicate modafinil has a relatively low abuse liability even in people with a history of psychostimulant dependence, an extensive evaluation of its reinforcing effects utilizing standard preclinical drug screening procedures may be warranted before promoting its use in a population with a substance abuse history. To date, only a few preclinical studies have evaluated modafinil in abuse liability screening procedures, such as behavioral sensitization, conditioned place preference (CPP), drug discrimination, and drug self-administration.

Locomotor Activity and Behavioral Sensitization

Locomotor activity is a measure commonly utilized in drug screening that elucidates the motor effects of a drug. More importantly, this screening procedure may elucidate similarities in the mechanism of action between known drugs of abuse and novel compounds (Curzon, Zhang, Radek, & Fox, 2009). Studies investigating the locomotor activating effects of modafinil have found both no effect and increases compared to baseline or control groups. No increases in activity were reported in Syrian hamsters (Webb, Pollock, & Mistelberger, 2006) or in fruit flies (Hendricks, Kirk, Panckeri, Miller, & Pack, 2003). However, modafinil-induced increases in activity were reported in the bungalow test and human threat test in Marmoset monkeys (Van Vilet, Jongsma, Vanwersch, Olivier, & Philippens, 2006) and increases in nighttime activity were reported in
Rhesus monkeys (Andersen et al, 2010). Studies utilizing rats have reported increases in general activity (Zolkowska, Jain, Rothman, Partilla, Roth, Setola, Prisinzano, & Baumann, 2009) and movement in the Morris water maze (Ward et al., 2004), while examination of sensitization in mice has yielded results consistent with the other rodent studies. Specifically, activity and exploratory rearing movements were increased after modafinil administration (Young, Koolstra, & Geyer, 2011). Together these studies indicate modafinil may have motor activating effects in monkeys, mice, and rats although there is no promising evidence utilizing other species.

Behavioral sensitization is a paradigm that incorporates measures of locomotor activity after a period of repeated exposure to a test compound followed by a washout period. A challenge session with a lower dose of the test compound or a different test compound follows the washout period. An increase in activity after the challenge dose that is increased above the levels obtained on the first day of drug administration is indicative of neuroadaptive changes in the mesotelencephalic dopamine pathway (Louk, Vanderschuren, & Kalivas, 2000) and deemed sensitization. The incentive sensitization theory states that these neuroadaptive changes culminate in hypersensitivity to the stimuli associated with the drug and may account for the drug “wanting” effects observed in substance users. When this effect is paired with executive functioning deficits, the major symptoms of addiction are observed (Robinson & Berridge, 2000).

There are two different measures used to assess behavioral sensitization. Induction of sensitization is displayed when the response to the drug increases
with repeated exposure. Expression of sensitization is demonstrated by an increased response on the challenge day, which typically occurs after a washout period, compared to the response on day one of drug exposure. Cross-sensitization often occurs to drugs in the same drug class (Stewart & Bdiani, 1993) although cross-sensitization can occur between drugs of distinct pharmacological classes (Valjent, Bertran, Gonzalez, Aubier, Greengard, Herve, & Girault, 2010). For example, caffeine pre-exposure has been shown to sensitize rats to the locomotor activating effects of cocaine (Schenk, Horger, & Snow, 1990). These authors suggest exposure to other legal or prescription stimulants may prime nervous system responsivity to cocaine.

Studies utilizing the sensitization assay with modafinil have reported results indicative of sensitization or cross-sensitization. One study found no evidence of behavioral sensitization in mice repeatedly exposed to a high or low dose of modafinil when presented with a challenge dose of the same drug (Shuman et al., 2012). In contrast, studies utilizing the same species have demonstrated induction of sensitization with a single dose of modafinil (64 mg/kg, i.p.) (Wuo-Silva et al., 2011) and expression of sensitization to a challenge dose of modafinil (75 mg/kg, i.p.) following repeated treatment with a higher modafinil dose (150 mg/kg) (Paterson, Fedolak, Olivier, Hanania, Ghavami & Caldarone, 2010). Cross-sensitization investigations have found expression of sensitization with a challenge dose of cocaine after only two days of exposure to modafinil (Wuo-Silva et al., 2011). Investigations of modafinil in combination with other psychostimulants demonstrate sensitization with a combination of cocaine and
modafinil although these effects were not significantly different from the effects of cocaine alone (Shuman et al., 2012). Cross-sensitization was produced after a challenge dose of modafinil in cocaine-pretreated mice (Shuman et al., 2012) and a subgroup of methamphetamine pretreated mice (Da Costa Soeiro, Moreira, Abrahao, Quadros, & Oliveira, 2012). Likewise, cross-sensitization to a challenge dose of methamphetamine was reported in a subgroup of mice after repeated exposure to modafinil (Da Costa Soeiro et al, 2012). Additive effects between modafinil and cocaine were also shown to develop robust induction and expression of sensitization to cocaine (Wuo-Silva et al., 2011). It is noteworthy to mention that sensitization to modafinil, unlike that of methamphetamine, was context-dependent and only expressed in the context in which drug administration occurred (Da Costa Soeiro et al., 2012).

Place Conditioning/Conditioned Place Preference

The conditioned place preference paradigm is a preclinical screening assay widely used to assess drug-induced conditioned reward by testing the amount of time an animal spends in a drug-paired chamber after several pairings between the drug and a distinct environmental context. It is well documented that many drugs of abuse readily establish a CPP (Bardo, Rowlett, & Harris, 1995) in a variety of species including amphibians (Presley, Lonergan, & Chu, 2010), crayfish (Alcaro, Panksepp, & Huber, 2011), and rodents (Bardo et al., 1995). This assay utilizes Pavlovian conditioning strategies by pairing a drug with a specific, salient context repeatedly, while pairing the absence of drug with another, salient context. On the test day, the animal is placed in the chamber
and can freely move about both environments. Time spent in both compartments is measured and if the animal spends significantly more time in the drug-paired context, the drug is considered to have rewarding properties (Bardo & Bevins, 2000).

Evaluation of modafinil in place conditioning procedures has yielded somewhat inconsistent findings. For example, Deroche-Gamonet et al. (2002) assessed a range of modafinil doses (32-256 mg/kg) administered via intraperitoneal (i.p.) injection, none of which reliably established CPP in rats. In contrast, it was reported that 64 mg/kg (i.p.) (Wuo-Silva et al., 2011) and 125 mg/kg (i.p.) (Nguyen, Tian, You, Lee, & Jang, 2011) modafinil established CPP in mice. Besides species differences, a number of methodological differences between these studies could account for the discrepant findings, such as number and length of habituation sessions, the cues used in the chambers, and drug pretreatment time.

Drug Discrimination

Drug discrimination is a widely accepted preclinical behavioral assay predictive of pharmacological mechanisms of drug action and is frequently used to examine drug interactions. In this paradigm, animals are trained to discriminate between at least two compounds, typically a drug and vehicle condition, and responses on one of two levers are reinforced, with each lever paired with a particular discriminative stimulus condition. After acquisition of the discrimination, other compounds are tested for stimulus generalization or stimulus antagonism to the training drug. This procedure can be used to assess
many characteristics of a drug that include dose-response curves, rate effects, generalization, and pharmacological mechanism of action (Glennon & Young, 2011).

To date, modafinil has been investigated in five published drug discrimination studies with nonhumans. In the first of these studies, generalization was assessed in six rats trained to discriminate 10 mg/kg cocaine (Gold and Balster, 1996). Modafinil produced dose-dependent increases in cocaine-lever selection, but group data yielded only partial substitution (67%) at doses that significantly suppressed responding. However, it is noteworthy that four of the six rats exhibited complete stimulus generalization to cocaine following administration with 250 mg/kg modafinil in that study. More recently, an investigation utilizing Rhesus monkeys found that modafinil substituted for low (0.18 mg/kg i.m.) and high (0.4 mg/kg i.m.) training doses of cocaine (Newman, Negus, Lozama, Prisinzano, & Mello, 2010). Paterson et al. (2010) reported full substitution with 300 mg/kg modafinil and partial generalization after 100 mg/kg modafinil in rats trained to discriminate 10 mg/kg cocaine. Moreover, Loland, Mereu, Okunola, Cao, Prisinzano, Mazier, Kopajtic, Shi, Katz, Tanda & Newman (2012) found that a much lower dose (56 mg/kg) and a similar dose (100 mg/kg) of modafinil and both modafinil enantiomers fully substituted for cocaine in mice trained to discriminate 10 mg/kg cocaine. In all of these studies, cocaine and modafinil were administered by intraperitoneal injection. Dopheide, Morgan, Rodvelt, Schachtman & Miller (2007) tested modafinil (32, 64, 128 mg/kg) administered by oral gavage at various post-injection times (10 to 240 min) in
three groups of male Sprague-Dawley rats trained to discriminate one of two low doses of cocaine (1.6, 5 mg/kg, i.p.) or a single dose of d-amphetamine (0.3 mg/kg, s.c.). They also tested 32 mg/kg modafinil in combination with a range of cocaine and d-amphetamine doses in all three groups of rats. Partial substitution was seen with 64 mg/kg and 128 mg/kg modafinil in both cocaine training dose groups and the d-amphetamine training group. Although modafinil alone failed to fully substitute for cocaine or d-amphetamine in that study, 32 mg/kg modafinil enhanced the discrimination of low doses of cocaine and d-amphetamine and shifted the dose-effect curves to the left. These findings suggest that modafinil may have additive effects with other stimulants.

Self-Administration

The self-administration paradigm is considered the golden standard paradigm in addiction research for assessing abuse liability and involves utilizing a drug as the reinforcer for lever pressing behavior. This procedure can also be used to investigate whether pretreatment with pharmacological compounds attenuates the rate of drug-taking behavior. This is useful for evaluation of candidates for agonist replacement therapies and has relevance to treatment in the clinical population of drug-dependent individuals (Haney & Spealman, 2008).

The first self-administration study utilizing modafinil was reported by Gold and Balster (1996) and demonstrated that 0.3 mg/kg modafinil substituted for cocaine in three rhesus monkeys that had been previously trained to self-administer cocaine. The number of modafinil infusions was comparable to or greater than the number of cocaine infusions by the same animals, although a
larger dose of modafinil was required to produce effects similar to that of cocaine. In contrast, Deroche-Gamonet et al. (2002) reported that modafinil did not substitute for cocaine self-administration in rats, nor did it induce reinstatement (described below) after cocaine self-administration was extinguished.

Reinstatement of Drug-Seeking

The reinstatement paradigm has been used as an animal model of drug relapse and craving (Andersen et al., 2010) and can be evaluated after extinction of a conditioned place preference or drug maintained responding in the self-administration paradigm. After a response in either of these assays is extinguished, a cue or drug is presented and the response is measured. Reinstatement is said to occur if the response level is similar to the response obtained prior to extinction. Experiments utilizing these paradigms have demonstrated modafinil-induced reinstatement of extinguished cocaine-maintained responding (Andersen et al., 2010) in the self-administration paradigm as well as modafinil-induced reinstatement of an extinguished cocaine place preference (Bernardi, Lewis, Lattal, & Berger, 2009). In the self-administration paradigm, 10 mg/kg modafinil reinstated responding in Rhesus monkeys trained to self-administer 0.1 mg/kg cocaine intravenously (i.v.) (Anderson, Reid, Shou-Hua, Holmes, Shemanski, Slee & Elkashef, 2009), while 32 mg/kg and 56 mg/kg modafinil reinstated behavior maintained by a higher dose of cocaine (0.4 mg/kg, i.m.) in the same species (Newman et al., 2010). Treatment with 0.3 mg/kg modafinil did not significantly increase responding compared to placebo (Anderson et al., 2009).
In methamphetamine-trained rats, modafinil alone injected i.p. (Reichel & See, 2010) or i.v. (Holtz, Lozama, Prisinzano, & Carroll, 2012) did not reinstate self-administration of methamphetamine. Interestingly, when modafinil was administered prior to methamphetamine-primed reinstatement conditions, it dose-dependently attenuated the reinstatement of lever pressing. The highest dose of modafinil attenuated the methamphetamine-primed reinstatement of lever pressing most robustly (Reichel & See, 2010). This phenomenon was replicated in methamphetamine-trained rats, but only one dose of modafinil was tested, which attenuated the methamphetamine paired responding (Holtz et al., 2012). Moreover, chronic modafinil administration during extinction of methamphetamine maintained responding attenuated cue-primed and methamphetamine-primed reinstatement (Reichel & See, 2012). In addition, a challenge test with methamphetamine two weeks after modafinil treatment ceased resulted in lower drug responding compared to control, indicating chronic modafinil treatment has enduring effects (Reichel & See, 2012).

Modafinil's effects in the place conditioning reinstatement paradigm appear to depend on the particular drug utilized to establish CPP. Tahsili-Fahadan, Carr, Harris, and Aston-Jones (2010) reported that 300 mg/kg modafinil completely blocked a morphine-primed reinstatement of morphine place preference. In contrast, following extinction of cocaine-induced place preference, 128 mg/kg modafinil has been reported to reinstate a place preference (Bernardi, et al., 2009). Evidence from this paradigm suggests modafinil may not be a promising agent for therapeutic use in psychostimulant
abusers and dependent individuals, although evidence from many other assays suggest the opposite (Paterson et al., 2010, Dopheide et al., 2007). Research investigating modafinil’s mechanism of action provides further support for the use of modafinil as an agonist replacement therapy.

Modafinil’s Pharmacological Mechanisms of Action

Discerning the neuropharmacological actions of modafinil is essential to fully understanding its behavioral effects and its potential clinical utility. The remainder of the literature review therefore emphasizes research findings based on various assays utilized to discover modafinil’s neurochemical mechanisms of action. Investigations of the neuropharmacological actions of drugs are performed using a variety of different assays and populations. Some of the in vitro techniques that have been utilized to study modafinil’s neurochemical actions include competitive receptor binding assays and measures of spontaneous and electrically-evoked release of neurotransmitters in brain tissue slices. Other procedures include electrophysiology to assess neural firing and in vivo microdialysis to assess localized drug-induced changes in neurotransmitter or metabolite levels in specific brain regions in either anesthetized or awake animals. Drug discrimination offers an alternative in vivo approach to investigating the neuropharmacological actions of drugs. As noted above, this approach involves establishing a drug as a discriminative stimulus and assessing other substances for stimulus generalization or antagonism. One advantage of the drug discrimination procedure over neuropharmacological assays is that it is
conducted in a live behaving animal. Moreover, this assay is pharmacologically specific and has considerable predictive validity. Nevertheless, a potential limitation of the drug discrimination procedure is that neural mechanisms of drug action must be inferred rather than measured directly. Collectively, a wide variety of in vitro and in vivo techniques can be used to provide converging evidence regarding drug mechanisms of action.

Research exploring modafinil’s neuropharmacological actions has implicated several neurotransmitter systems, including serotonin (5-HT), orexin, norepinephrine (NE) and dopamine (DA) (Dopheide, et al., 2007; Minzenberg & Carter, 2008; Wisor, Nishino, Sora, Uhl, Mignot, & Edgar, 2001; Zolkowska et al. 2009). Most evidence of 5-HT involvement results from investigations utilizing microdialysis or receptor and transporter binding assays. In these binding assays, modafinil enhances electrically evoked, but not spontaneous 5-HT release in rat frontal cortical slices (Ferraro, Fuxe, Tanganelli, Fernandex, Rambert, & Antonelli, 2000), while Loland et al. (2012) found no measurable binding of modafinil to the serotonin uptake inhibitor. Microdialysis studies in the awake rat found that modafinil produced dose-dependent increases of 5-HT release in the frontal cortex, without affecting the release of 5-HT from the synaptic vesicles or blocking reuptake in the behaving rat (Ferraro et al., 2000) and increased levels of 5-HT in the frontal cortex, amygdala, and dorsal raphe nuclei in the brainstem (Ferraro, Antonelli, Tanganelli, O’Connor, de la Mora, Mendez-Fanco, Rambert, & Fuxe, 1999). However, modafinil appears to have a very weak effect or no effect on the 5-HT levels in the nucleus accumbens
(Zolkowoska et al., 2009). These regional differences in the serotonergic actions of modafinil could possibly account for its cognitive-enhancing effects due to actions in the frontal cortex as well as explain the lack of reinforcing effects typically attributed to the nucleus accumbens.

Research on the involvement of the orexin and histamine systems in modafinil’s actions was initiated based on knowledge that narcolepsy is associated with a deficiency in these peptide systems (Nishimo, Ripley, Overeem, Lammers, & Mignot, 2000). An increase in Fos-IR neurons, which represents currently active neurons, was seen in the tuberomammillary nucleus following oral administration of modafinil (75 and 100 mg/kg) administration in the rat (Scammell, Estabrooke, McCarthy, Chemelli, Yanagisawa, Miller & Saper, 2000). However, modafinil injections directly into the rat tuberomammillary nucleus produced no change in histamine release (Ishizuka, Sakamoto, Sakurai, & Yamatodani, 2003), indicating the histaminergic system does not directly account for modafinil’s effects.

The noradrenergic system has also been implicated in modafinil's neurochemical effects. Pretreatment with prazosin, an alpha-1 adrenergic antagonist, blocked the locomotor activating effects of modafinil in rhesus monkeys (Hermant et al., 1991), reversed the modafinil-induced increase in brain temperature (Lin et al., 1992), partially attenuated the wake promoting effect of modafinil measured by EEG in the cat brain (Lin et al., 1992), and attenuated the locomotor activating effect of modafinil in a control group of wild type mice (Mitchell et al., 2008). Pretreatment with yohimbine, an alpha-2 receptor
antagonist, on the other hand, enhanced the wake-promoting and temperature increasing effects of modafinil, while β-receptor antagonists moderately decreased these same effects of modafinil (Lin et al., 1992). Although these results suggest the NE system is heavily involved in the neurochemical actions of modafinil, other research has found conflicting results. An in vitro study in the rat brain, examining the effect of prazosin on modafinil-induced firing in the ventral tegmental area found no difference from control when modafinil was presented alone (Korotkova, Klyuch, Ponomarenko, Lin, Haas, & Sergeeva, 2007) and in an investigation of norepinephrine transporter binding, no binding was seen after modafinil administration in human COS-7 cells (Loland et al., 2012). These results point to a potential role of alpha-1 receptors and possibly a role for β-receptors, but imply that the norepinephrine transporter system is not involved in the behavioral effects of modafinil, while alpha-2 receptor antagonism may result in additive effects when combined with modafinil.

Most psychostimulant drugs produce their effects by elevating DA levels in the central nervous system and some authors have suggested that because modafinil lacks the behavioral effects typically seen with traditional psychostimulants, it does not work through the DA system (Engber, Dennis, Hones, Miller, & Contreras, 1998). However, more recent research utilizing genetic modification models, microdialysis and in vitro binding assays has demonstrated that modafinil does, in fact, exert effects on the dopaminergic system (Loland et al., 2012; Murillo-Rodriguez, Haro, Palomero-Rivero, Millan-Aldaco, & Drucker-Colin, 2007; Qu, Huang, Xu, Matsumoto & Urade, 2008;
Volkow et al., 2009). For example, in a study utilizing dopamine-b-hydroxylase -/- (Dbh-/-) mice, which have virtually no NE in the CNS and hypersensitivity to DA, demonstrated increased sensitivity to the behavioral activating effects of modafinil (Mitchell et al., 2008). In addition, a microdialysis study demonstrated increases in extracellular DA in the nucleus accumbens shell after modafinil administration (Murillo-Rodriguez et al., 2007). Another study utilizing in vitro binding assays in human brain tissue reported decreased binding potential for [11C]-raclopride after modafinil administration in the caudate, putamen, and nucleus accumbens, indicative of increased DA binding (Volkow, Fowler, Logan, Alexoff, & Zhu, 2009). Binding assays in other populations have demonstrated that modafinil (31.25, 62.5, 125 mg/kg, i.p.) treatment significantly increases D1 DA receptor binding compared to controls in the mouse caudate putamen, the nucleus accumbens, and the substantia nigra (Nguyen et al., 2011). Increased D2 binding in the mouse caudate putamen, nucleus accumbens (Nguyen et al., 2011) and rat ventral tegmental area, and substantia nigra (Korotkova et al., 2007) following modafinil administration were reported in similar assays.

Another study evaluating the role of D2 receptors in modafinil's actions reported that modafinil does not function as a wake-promoting agent in D2 receptor knockout mice (Qu et al., 2008). In addition, differences after administration of a D2 antagonist, raclopride, at both low and high doses attenuated the modafinil-induced increases in EEG functions (Qu et al., 2008) although the locomotor activating effects were not completely abolished (Qu et al., 2008). However, in an earlier study the D2 dopamine receptor antagonist,
haloperidol, did not weaken the locomotor activating effect of modafinil in mice 
(Simon, Hemet, Ramassamy, & Costentin, 1995). Investigation of modafinil- 
induced D_{1} receptor activation reported decreased wakefulness after 
administration of modafinil and a D_{1} antagonist, SCH23390, at a low dose. (Qu et 
al., 2008). Together, this evidence suggests both D_{1} and D_{2} receptors are 
necessary for the wake-promoting effects of modafinil, although D_{2} receptor 
activation may not be necessary for its locomotor activating effects and the role 
of the D_{1} receptor is uncertain.

Other studies involving in vivo assays indicate that modafinil may indirectly 
affect brain DA systems. Evidence suggests that interference with the DA 
reuptake (i.e., the dopamine transporter) system can prevent the effects on EEG 
readings induced by modafinil in a cat model (Lin et al., 1992). Furthermore, in 
dopamine transporter (DAT) knockout mice, the wake-promoting effects of 
modafinil were not seen, indicating the DAT is necessary for the wakefulness 
effect of modafinil (Wisor et al., 2001). Furthermore, when rats are pretreated 
with the DAT inhibitor, nomifensine, modafinil-induced dopamine overflow in 
striatal brain slices is eliminated (Dopheide et al., 2007). In addition to these 
investigations, Zolkowska et al. (2009) reported that pretreatment with modafinil 
decreased methamphetamine- induced dopamine release in male Sprague- 
Dawley rats indicating that the dopamine transporter is involved in its primary 
mechanism of action.

Investigations utilizing in vitro techniques to assess modafinil-induced DAT 
binding also produce results that strongly suggest DAT involvement in modafinil's
effects. Binding studies have found modafinil administration results in 60 % DAT occupancy in cells from Rhesus monkeys (Anderson et al., 2009) and 64 % occupancy of the DAT in the putamen and 60% in the caudate in human cells (Loland et al., 2012). Evidence demonstrating increased binding to the DAT in the rat prefrontal cortex, caudate putamen, and nucleus accumbens after modafinil administration (Nguyen et al., 2011) and decreased binding potential of \([^{11}C]\)-cocaine after modafinil administration in PET measures of DA efflux in the human brain, which reflects DAT occupancy (Volkow et al., 2009) supports the claim that modafinil exerts its actions through the DAT.

Traditional psychomotor stimulants exert effects by stimulating the monoamine system, in particular DA (Boutrel & Koob, 2004). Cocaine and methamphetamine both function by inhibiting the DAT, while d-amphetamine acts as a vesicle DA releaser (Boutrel & Koob, 2004). Given the similar receptor mechanisms underlying the central nervous system actions of modafinil and psychomotor stimulants, a thorough evaluation of modafinil’s abuse liability is warranted.

Aims

The aim of this study was to evaluate the abuse liability of modafinil alone and combined with d-amphetamine in three preclinical behavioral assays. Four experiments were conducted to this end. The aim of the first experiment was to evaluate the locomotor activating effects of repeated d-amphetamine treatment and cross-sensitization of modafinil in male and female Sprague-Dawley rats.
The second experiment examined a low dose combination of modafinil (64 mg/kg) and d-amphetamine (0.3 mg/kg) in comparison to each drug alone and to a higher dose of d-amphetamine (2.0 mg/kg) using place conditioning procedures in rats in an effort to evaluate the conditioned reward properties of the drug and combination. The third study sought to replicate and expand the findings of Dopheide et al. (2007) by testing modafinil alone and in combination with d-amphetamine in rats trained to discriminate either a low dose (0.3 mg/kg) or moderately high dose (1.0 mg/kg) of d-amphetamine. The aim of the fourth experiment was to evaluate the combined effects of d-amphetamine and modafinil in rats trained to discriminate 256 mg/kg modafinil from vehicle (5% arabic gum) and to elucidate the pharmacological mechanism of action of modafinil utilizing the drug discrimination paradigm.
Overview

Previous research has demonstrated sensitization and cross-sensitization to modafinil after repeated treatment with modafinil (Paterson et al., 2010), cocaine (Wuo-Silva et al., 2011), and methamphetamine (Da Costa Soeiro et al., 2012). To date, no published data has investigated cross-sensitization to modafinil after daily d-amphetamine treatment. This experiment sought to determine if cross-sensitization would occur with a low challenge dose (64 mg/kg) of modafinil after repeated d-amphetamine treatment in male and female individually- or pair-housed drug- naïve Sprague-Dawley rats. The housing conditions were a result of previous experimental conditions and although both male and female rats were utilized in this experiment, sex was not initially a variable of interest.

Methods

Subjects

Eighteen male and 17 female drug naïve Sprague-Dawley rats (bred in Western Michigan University’s animal colony) were utilized in this study. The animals were singly- or pair-housed in polycarbonate cages with corncob bedding where ad libitum access to food and water was available. Animals were housed at Western Michigan University’s animal facilities in a humidity and temperature-controlled room maintained on a 12:12 hour light/dark cycle with
lights on at 7:00 a.m. All procedures were conducted in accordance with the
*Guide for the Care and Use of Laboratory Animals* (National Academy of
Sciences, 2011) and were approved by the Institutional Animal Care and Use
Committee at Western Michigan University.

**Apparatus**

The apparatus consisted of six custom-designed open field chambers
constructed of acrylic (40.5 cm x 40.5 cm x 40.5 cm) and housed within an
Accuscan automated activity monitoring system (Accuscan Instruments, Inc.,
Columbus, OH) equipped with infrared emitters and detectors connected to a
microprocessor. Measures of horizontal activity and vertical activity were
recorded.

**Procedures**

Singly-housed males (n = 10), pair-housed males (n = 8), singly-housed
females (n = 8), and pair-housed females (n = 7) were assigned to one of eight
custom-made chambers. The males were assessed during the two morning 30
min sessions and assigned to session and chamber in a counterbalanced order.
Females were assessed during the afternoon sessions and assigned to chamber
and session in a counterbalanced order. Within each group, the animals were
randomly assigned to receive d-amphetamine or saline treatment. Three 30
minute habituation sessions were conducted to acclimate the animals to the
apparatus and to obtain an accurate measure of baseline activity. For the next
five days, d-amphetamine (3 mg/kg s.c) or saline was administered immediately
prior to placement in the apparatus for 30 minutes. A 10 day washout period
followed the repeated administration of d-amphetamine or saline, where animals were confined to home cages and no experimentation was completed. On day 19, a challenge test was conducted with modafinil (64 mg/kg) in all animals and a vehicle test was conducted in all animals on the following day. For these test sessions, animals were injected immediately prior to placement in the chambers for 90 minutes.

Drugs

Modafinil was synthesized in the laboratory of Dr. Thomas Prisinzano using previously described methods (Prisinzano, Podobinski, Tidgewell, Luo, & Swenson, 2004), prepared on the day of use by suspension in a 5% arabic gum solution (Sigma Aldrich, St. Louis, MO) and administered by oral gavage (i.g.) in a volume of 10 ml/kg. The d-amphetamine-hemisulfate (Sigma Aldrich, St. Louis, MO) was suspended in a 0.9% NaCl solution and administered subcutaneously (s.c) in a volume of 1 ml/kg. Doses were based on the weight of the salts.

Data Analysis

Separate statistical analyses were conducted for each group: singly-housed males, pair-housed males, singly-housed females, and pair-housed females. Horizontal activity and vertical activity measures collected during 30 min d-amphetamine or saline treatment sessions were analyzed utilizing a repeated measures two-factor (drug treatment, treatment session) ANOVA to investigate whether differences in drug treatments varied over repeated dosing. Repeated measures two-factor (pretreatment, test drug) ANOVAs were also conducted on horizontal and vertical activity measures
obtained during the first 30 minutes of the 90 minute test sessions to determine if pretreatment (d-amphetamine or saline) differentially influenced activity during test session (modafinil or saline).

Results

Figure 1 depicts horizontal activity and Figure 2 represents vertical activity during three 30 min habituation and five treatment sessions for all four groups, with data for males in the upper panels and data for females in lower panels. Data from singly-housed animals are depicted in the left panels and data from pair-housed animals are presented in the right panels. As expected, d-amphetamine treatment increased activity compared to habituation sessions and compared to saline treatment. Statistical analyses are reported below for each sex and housing group.

Assessment of Sensitization Induction

Single Males. A two-factor repeated measures ANOVA on horizontal activity across test sessions revealed a main effect of drug that approached significance \[F(1, 8) = 4.50, p = 0.06\], in addition to a significant main effect of test session \[F(4, 32) = 7.52, p < 0.0001\], and a significant drug x test session interaction \[F(4, 32) = 4.47, p < 0.01\], although there were no significant Bonferroni post-hoc tests (see Figure 1). Analysis of vertical activity with a repeated measures ANOVA resulted in a significant main effect of drug \[F(1, 8) = 5.70, p < .05\] and test session \[F(4, 32) = 2.67, p = .05\], although there was not a significant interaction (see Figure 2).
Paired Males. A two-factor repeated measures ANOVA on horizontal activity revealed a significant main effect of drug \([F(1, 6) = 17.89, p < 0.001]\), test session \([F(4, 24) = 2.84, p < 0.05]\), but no significant drug x test session interaction (see Figure 1). Analysis of vertical activity with a repeated measures two factor repeated measures ANOVA found a significant drug effect, \([F(1, 6) = 16.84, p < 0.001]\), but no significant test session main effect or drug x test session interaction (see Figure 2).

Single Females. Analysis of horizontal activity with a two-factor repeated measures ANOVA revealed a significant main effect of drug \([F(1, 7) = 39.55, p < 0.01]\), test session \([F(4, 28) = 4.84, p < 0.001]\) although there was not a significant drug x test session interaction (see Figure 1). A two-factor repeated measures ANOVA on vertical activity also revealed a significant main effect of drug \([F(1, 7) = 28.19, p < 0.001]\), test session \([F(4, 28) = 5.75, p < 0.01]\), but no significant interaction effects (see Figure 2).
Figure 1. Total horizontal activity during three 30 min habituation sessions (H1-H3) and five 30 min treatment sessions (T1-T5) following d-amphetamine or saline injections. The upper panels depict the activity of males and the lower panels depict for the activity of females. Left panels display activity of singly-housed animals and right panels display the activity of pair-housed animals. Data points represent group means (± S.E.M.) Asterisks indicate significant Bonferroni post-hoc tests between d-amphetamine and saline on a given test session (* p < 0.05, ** p < 0.01).
Figure 2. Total vertical activity during three 30 min habituation sessions (H1-H3) and five 30 min treatment sessions (T1-T5) following d-amphetamine or saline injections. The upper panels depict the activity of males and the lower panels depict for the activity of females. Left panels display activity of singly-housed animals and right panels display the activity of pair-housed animals. Data points represent group means (± S.E.M.) Asterisks indicate significant Bonferroni post-hoc tests between d-amphetamine and saline on a given test session (* p < 0.05, ** p < 0.01).
**Paired Females.** Analysis of horizontal activity with a two-factor repeated measures ANOVA revealed a significant main effect of drug \[ F(1, 6) = 74.33, p < 0.001 \], test session \[ F(4, 24) = 10.54, p < 0.010 \], and a significant drug x test session interaction \[ F(4, 24) = 6.52 \ p < 0.001 \]. Bonferroni post-hoc tests revealed a significant difference between the saline and d-amphetamine treated animals on the first \( p < 0.01 \) and second \( p < 0.05 \) day of drug administration (see Figure 1). A two-factor repeated measures ANOVA on vertical activity revealed a significant main effect of drug \[ F(1, 6) = 8.30, p < 0.05 \], test session \[ F(4, 24) = 6.92, p < 0.001 \], and a significant drug x test session interaction \[ F(4, 24) = 3.09 \ p < 0.05 \], although there were no significant Bonferroni post-hoc tests (see Figure 2).

**Evaluation of Cross Sensitization**

**Single Males.** Figure 3 represents horizontal activity measures during the modafinil and vehicle challenge days separated by pretreatment group (d-amphetamine or saline). Figure 4 displays the same information for vertical activity measures. A repeated measures two (modafinil test vs. vehicle test) X two (d-amphetamine vs. saline pretreatment) ANOVA on horizontal activity found a significant effect of test drug, \[ F(1, 8) = 18.37, p < 0.01 \], but no main effect of pretreatment. The same analysis of vertical activity showed similar results. There was no main effect of pretreatment group, but a significant effect of test drug \[ F(1, 8) = 10.53, p < 0.01 \]. There were no significant interaction effects.
Figure 3. Mean (± S.E.M.) total horizontal activity during a 30 minute sampling period following 64 mg/kg modafinil or vehicle for each pretreatment group (d-amphetamine or saline). Asterisks indicate statistically significant differences in activity between modafinil and vehicle tests (* p < 0.05, ** p < 0.01, # p < 0.001).
Figure 4. Mean (± S.E.M.) total vertical activity during a 30 minute sampling period after modafinil and vehicle administration for each pretreatment group (d-amphetamine or saline). Asterisks indicate statistically significant differences in activity between modafinil and vehicle tests (* p < 0.05, ** p < 0.01, # p < 0.001).
Paired Males. A repeated measures two-factor ANOVA on horizontal activity following modafinil treatment found no main effect of pretreatment condition, but did reveal a significant test drug main effect, $[F(1, 6) = 17.55, p < 0.01]$ (see Figure 3). The same analysis with vertical activity revealed an effect of pretreatment that approached significance $[F(1,6) = 4.72, p = 0.07]$ and a significant main effect of test session $[F(1, 6) = 14.86, p < 0.01]$, although there were no significant interaction effects (see Figure 4).

Single Females. A repeated measures two (modafinil test vs. vehicle test) X two (d-amphetamine vs. saline pretreatment) ANOVA on horizontal activity found a significant effect of test drug $[F(1, 7) = 67.24, p < 0.001]$, but no main effect of pretreatment or interaction (see Figure 3). The repeated measures ANOVA on vertical activity found a pretreatment effect that was almost significant, $[F(1, 7) = 5.26, p = 0.055]$ and a significant effect of test drug $[F(1, 7) = 75.82, p < 0.001]$, although there were no significant interaction effects (see Figure 4).

Paired Females. A repeated measures two-factor ANOVA on horizontal activity found no main effect of pretreatment group, but did reveal a significant effect test drug, $[F(1, 5) = 60.57, p < 0.001]$ (see Figure 3). The repeated measures ANOVA on vertical activity showed no effect of pretreatment, but there was an effect for test drug $F(1, 5) = 31.59, p < 0.01$, but there were no significant interaction effects (see Figure 4).
Discussion

This experiment investigated the expression of cross-sensitization to a low dose of modafinil following a brief history of daily repeated d-amphetamine treatment and a 10 day washout period in singly- or pair-housed male or female Sprague-Dawley rats. The results demonstrate that repeated d-amphetamine treatment significantly increases horizontal and vertical activity, but this effect decreases over five daily treatment sessions and thus, no induction of sensitization was displayed. Although no statistically significant differences in pretreatment with d-amphetamine or saline were seen, and thus no expression of cross-sensitization, a modest increase in the locomotor activating effects of modafinil was seen in some animals pretreated with d-amphetamine. Most importantly, an increase in horizontal and vertical activity relative to vehicle was shown in all groups following modafinil administration. Although there was no evidence of induction of sensitization or expression of cross sensitization, this study confirmed previous findings that modafinil administration at low doses can induce an increase in locomotor activity (Zolkowska et al., 2009; Andersen et al, 2010; Van Vilet et al., 2006).

This is the first study to investigate the locomotor effects of modafinil following repeated d-amphetamine treatment in any species, however previous studies utilizing mice have demonstrated cross sensitization to modafinil following treatment with cocaine (Shuman et al., 2012) or methamphetamine (Da Costa Soeiro et al., 2012). Cross sensitization to acute modafinil pretreatment has been also been demonstrated with a challenge dose of cocaine (Wuo-Silva
et al., 2011). The results of the current study are somewhat surprising compared to previous research findings. One assumption of the behavioral sensitization paradigm is that drugs that produce cross-sensitization to each other have similar neurochemical targets in the central nervous system (Stewart & Badiani, 1993). The discrepancy between current results and this theoretical perspective along with previous studies investigating modafinil sensitization could be due to differences in the species utilized, route of administration, or doses of the test compounds. In addition, the small number of subjects per group could account for the lack of statistically significant effects of d-amphetamine pretreatment on cross-sensitization to modafinil in the current study. It is also possible that procedural differences, like treatment regime, between studies are responsible for the discrepant results. This study utilized daily administration of d-amphetamine or vehicle for 5 days, while other studies that have demonstrated expression of cross-sensitization to modafinil utilized intermittent pre-exposure to cocaine (Paterson et al., 2010) or methamphetamine (Shuman et al., 2012). However, modafinil cross-sensitization was reported after 10 daily methamphetamine injections (Da Costa Soerio et al., 2012) in one study indicating that repeated daily treatment could result in cross sensitization. Perhaps, a longer duration of exposure is necessary with daily drug administration.

It is also possible that modafinil does not share a similar of a mechanism of action with d-amphetamine. At least one study has demonstrated that D₁ receptors are critical for d-amphetamine sensitization to occur, but not for
cocaine sensitization to occur (Vanderschuren & Kalivas, 2000), which could explain the discrepancy between findings. The role of DA receptors in modafinil's neurochemical mechanisms of action was not assessed in this experiment, but was examined utilizing a drug discrimination procedure in the experiments described in Chapter 5.

Despite the lack of evidence for cross-sensitization between d-amphetamine and modafinil in this preliminary experiment, additional studies were pursued to explore the behavioral effects of concurrent administration of modafinil and d-amphetamine, in order to determine if these drugs exert additive effects. Experiment 2 utilized a well-established preclinical assay of drug abuse liability, conditioned place preference, and Experiments 3 and 4 utilized drug discrimination procedures to evaluate the combined stimulus effects of these drugs. The aim of Experiment 2 was to determine if combined low dose administration of modafinil and d-amphetamine produced greater CPP than either drug alone. The primary aim of Experiments 3 and 4 was to determine if modafinil potentiated the discrimination of d-amphetamine or vice versa. Evidence for additive effects of modafinil and d-amphetamine in these preclinical assays could be informative regarding similarities in their mechanism of action and have implications for the combined subjective effects of these substances in humans.
Overview

Few studies have examined modafinil in the place preference paradigm. One study reported no evidence of place preference after i.p. administration of a range of modafinil doses in rats (Deroche-Gamonet et al., 2002), whereas two studies that used mice as subjects reported modafinil established CPP at low (Wuo-Silva et al., 2011) and moderate (Nguyen et al., 2011) doses. This experiment sought to systematically replicate the experiment designed by Deroche-Gamonet et al. (2002) with an oral dose of modafinil in addition to evaluating CPP with a combination of modafinil and d-amphetamine.

Methods

Subjects

Forty male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) 50-60 days old at the start of the experiment were acclimated to the animal facilities for at least one week prior to initiation of place conditioning experiments. A separate group of 15 adult male Sprague-Dawley rats were assessed in a supplemental experiment to determine the effects of 64 mg/kg modafinil on locomotor activity. Animals were individually housed in polycarbonate cages with corncob bedding where *ad libitum* access to food and water was available. All animals were housed in Western Michigan University’s animal facilities in a humidity and temperature-controlled room with a 12:12 hour light/dark cycle with
lights on at 7:00 a.m. All procedures were conducted in accordance with the
*Guide for the Care and Use of Laboratory Animals* (National Academy of
Sciences, 2011) and were approved by the Institutional Animal Care and Use
Committee at Western Michigan University.

**Apparatus**

The apparatus utilized for place conditioning and locomotor activity
assessments consisted of eight custom-designed open field chambers
constructed of acrylic and measuring 40.5 cm x 40.5 cm x 40.5 cm. For place
conditioning experiments, the chambers were divided into two-compartments
with an acrylic wall and removable 12.8 cm X 18 cm door. Each compartment
contained distinct visual and tactual cues. One compartment contained walls
covered with alternating vertical black and white stripes and a textured plastic
floor. The other compartment contained walls covered with alternating horizontal
black and white stripes and an aluminum floor with 1.1 cm diameter holes spaced
approximately 0.5 cm apart. Each chamber was housed within an Accuscan
automated activity monitoring system (Accuscan Instruments, Inc., Columbus,
OH) equipped with infrared emitters and detectors connected to a
microprocessor. Locomotor activity and time spent in each side of apparatus
were processed using Versamax software (Accuscan Instruments, Inc.,
Columbus, OH).

**Procedures**

*Place Conditioning Trials.* Thirty-two animals were randomly assigned to
one of the following four treatment groups: 5% arabic gum + 0.9% saline
(VEH+SAL), 5% arabic gum + 0.3 mg/kg d-amphetamine (VEH+AMPH), 64 mg/kg modafinil + saline (MOD+SAL), 64 mg/kg modafinil + 0.3 mg/kg d-amphetamine (MOD+AMPH). These four groups were assessed during the same consecutive 10 day period. Four squads of eight animals were run simultaneously with two animals from each treatment group in each squad. For comparison, a separate squad of eight animals was assessed for place conditioning with 2.0-mg/kg d-amphetamine approximately one month later. Assignments of test chamber and drug-paired compartment were counterbalanced within and between treatment groups.

A single habituation session was conducted 24 hours prior to commencing place conditioning. Animals were habituated to the entire test apparatus with the doors removed for a period of 15 minutes. On the next day, place conditioning commenced for eight days with a single 30-minute trial per day. During conditioning, the removable doors were attached and rats only had access to one compartment. On conditioning days 1, 3, 5, and 7, rats were administered their respective drug treatments (see above) prior to placement into one compartment. On conditioning days 2, 4, 6 and 8, all rats were administered both 5% arabic gum and saline before placement into the opposite side of the chamber. Modafinil or 5% arabic gum was administered 30 minutes before and d-amphetamine or saline was administered 10 min before placement into the chambers. Horizontal activity was recorded during all conditioning trials.

CPP test. The test session was conducted 24 hours after the last conditioning session. Rats were placed in the test apparatus for 15 min with the
doors removed to allow access to both compartments. Horizontal activity and time spent in each compartment was electronically recorded. During all phases of the experiment the floors and walls of the apparatus were wiped down with a 35% isopropyl alcohol solution after each rat was removed.

**Acute Assessment of Locomotor Activity.** A supplemental experiment was conducted with 15 rats to assess the effects of 64 mg/kg modafinil on locomotor activity. The walls and floors used in the place conditioning experiment were not used for this assessment. Animals were administered 64 mg/kg modafinil by oral gavage immediately before placement in the apparatus for a period of 60 minutes. Horizontal activity and vertical activity were determined from infrared beam breaks.

**Drugs**

Modafinil was synthesized in the laboratory of Dr. Thomas Prisinzano using previously described methods (Prisinzano, et al., 2004), prepared fresh each day of use by suspension in a 5% arabic gum solution (Sigma Aldrich, St. Louis, MO) and administered by oral gavage (i.g.) in a volume of 10 ml/kg. The d-amphetamine-hemisulfate (Sigma Aldrich, St. Louis, MO) was suspended in a 0.9% NaCl solution and administered subcutaneously (s.c) in a volume of 1 ml/kg. Doses were based on the weight of the salts.

**Data Analysis**

A repeated measures two-factor ANOVA was conducted on horizontal activity during conditioning sessions with treatment group as a between subjects factor and conditioning trial as a within subjects factor. Bonferroni post-hoc tests
were conducted for significant differences between specific treatment groups. For each treatment group, paired t-tests were conducted on the time spent in the drug-paired compartment and vehicle-paired compartment during the 15 min test session. In the supplemental experiment to assess horizontal and vertical activity over a 60 min period immediately following 64 mg/kg modafinil, a two way repeated measures ANOVA was conducted with time as a within subjects factor and treatment as a between subjects factor.

**Results**

Locomotor activity did not differ significantly among the treatment groups during the 15 min habituation period prior to the onset of conditioning nor was activity different among these groups during the vehicle conditioning trials (data not shown). Figure 5 displays the mean (± S.E.M.) horizontal activity during 30 min drug conditioning trials for each treatment group. Both 0.3 and 2.0 mg/kg d-amphetamine substantially increased activity relative to vehicle, whereas 64 mg/kg modafinil did not. Although the MOD+ 0.3 AMPH combination produced slightly greater activity than either drug alone during the first conditioning trial, this enhancement was not observed on subsequent drug conditioning trials. A two-way repeated measures ANOVA on horizontal activity during drug conditioning trials revealed a significant main effect of treatment group \( F(4, 35) = 33.79, p < 0.001 \), conditioning trial \( F(3, 105) = 4.69, p < 0.01 \), and a significant interaction between treatment group and conditioning trial \( F(12, 105) = 2.41, p < 0.01 \). Significant Bonferroni post-tests comparing treatment groups to vehicle
and to modafinil on each of the four drug conditioning trials are shown with symbols in Figure 5.

Prior to conditioning, there was no consistent preference among animals for either compartment. Following conditioning trials, vehicle and modafinil treatment groups did not show preference for either compartment, whereas both d-amphetamine treatment groups and the MOD+AMPH treatment group showed a preference for the drug-paired compartment. Figure 6 displays the mean (± S.E.M.) time spent in each compartment on the test day for each treatment group. Paired t-tests comparing time spent in the drug-paired compartment with time spent in the vehicle-paired compartment were statistically significant for both the 0.3 mg/kg d-amphetamine group ($t(7)=3.84, p < 0.01$) and the 2.0 mg/kg d-amphetamine group ($t(7)=3.12, p < 0.05$). The animals in the MOD + 0.3 AMPH treatment group also spent more time in the drug-paired compartment following conditioning trials, but the difference in time spent between drug and vehicle compartments was not statistically significant in this group.

A supplemental experiment was conducted to confirm that oral administration of 64 mg/kg modafinil is behaviorally active. Results of this experiment, shown in Figure 7 indicate that modafinil-treated animals exhibited increased horizontal and vertical activity at nearly all post-injection time intervals compared to vehicle treated animals. A two way ANOVA revealed the effect of modafinil on horizontal activity was not quite statistically significant [$F(1,13) = 3.65, p = 0.078$], although the main effect of time was statistically significant [$F(11,143) = 18.99, p <0.0001$]. The main effects of modafinil treatment [$F(1,13) =$
9.28, \( p = 0.01 \) and time \( F(11, 143) = 6.57, p < 0.0001 \) on vertical activity were both statistically significant.
Figure 5. Horizontal activity during 30 min drug conditioning trials. Each data point represents the group mean (± S.E.M.) activity. * (p < 0.05) or ** (p < 0.001) indicates statistically significant compared to the vehicle control group; # (p < 0.05) or ## (p < 0.001) indicates significantly different compared to the modafinil treatment group (n=8 per group).
Figure 6. Mean (± S.E.M.) time spent in drug-paired (dark bars) and vehicle-paired (light bars) compartments during the test phase. (n=8 per group). * (p < 0.05) or ** (p < 0.01) indicates time in drug-paired compartment significantly different from time in vehicle-paired compartment.
Figure 7. Mean (± S.E.M.) horizontal (left) and vertical (right) activity for each 5-minute interval during a 60 min period immediately following 64 mg/kg modafinil (n=8) or vehicle (n=7) administration.
Discussion

Results of the current study indicate that a moderately low oral dose of modafinil (64 mg/kg) does not establish conditioned place preference in adult male Sprague-Dawley rats. These results are consistent with a previous report that modafinil (32-256 mg/kg, i.p.) fails to establish CPP in rats (Deroche-Gamonet et al., 2002); although others have reported 64 mg/kg modafinil to establish CPP in mice (Wuo-Silva et al., 2011). Besides the species difference, several other methodological differences could account for discrepant findings. For example, Wuo-Silva et al. (2011) conducted 10 minute conditioning trials 30 minutes after i.p. modafinil injection and both drug and vehicle trials were conducted on the same day with a six hour intertrial interval. Deroche-Gamonet et al. (2002) conducted 30 min conditioning trials immediately following i.p. modafinil injection and drug and vehicle trials were separated by 24 hours. The methods were modeled after those employed by Deroche-Gamonet et al. (2002) with the exception that animals in the current study were administered i.g. modafinil 30 minutes prior to 30 minute conditioning trials. Nevertheless, the present findings confirm previous suggestions that modafinil has a low abuse liability in drug naïve individuals, in contrast to most psychomotor stimulants (Bardo et al., 1995).

Amphetamine-induced place preference in rodents is a well-established phenomenon at doses ranging from 0.3 mg/kg to 3 mg/kg (Bardo et al., 1995; Deroche-Gamonet et al., 2002) and the current results with d-amphetamine are consistent with these findings. It is somewhat surprising that there was no
evidence for the development of sensitization with repeated d-amphetamine exposure in the current study. However, two important measures of activity that typically display sensitization, vertical activity and stereotypy, were not assessed due to constraints of the place conditioning apparatus in which locomotor activity was assessed.

The lack of a significant difference in locomotor activity between modafinil and vehicle treatment groups during drug conditioning trials suggests the possibility that this low oral dose of modafinil was not behaviorally active. Therefore, a supplemental experiment was conducted with a separate group of rats to determine that i.g. administration of 64 mg/kg modafinil does increase locomotor activity (see Figure 7 and 8). Results of this assessment are supported by results of the preliminary experiment described in Chapter 2, that demonstrated increased activity following modafinil administration in a within subjects design (see Figure 3). The current assessment showed a visually-evident increase in horizontal activity and a statistically significant increase in vertical activity in modafinil-treated rats compared to vehicle-treated rats. As noted above, vertical activity was not assessed during place conditioning trials, but there was no evidence of an increase in horizontal activity after modafinil administration. It is possible that confinement to one compartment during place conditioning trials limited horizontal movement and masked any differences between modafinil and vehicle treatment groups.

The current findings also established that concurrent administration of a low oral dose of modafinil dose does not enhance the hyperlocomotor effects or
CPP established by a low dose of d-amphetamine. There was a visually evident trend toward additive acute locomotor effects of 64 mg/kg modafinil and 0.3 mg/kg d-amphetamine only on the first drug conditioning day. The lack of a statistically significant increase in activity with repeated exposure and the lack of evidence for enhanced CPP with this drug combination indicate these drugs do not have additive effects, at least at low doses. These findings suggest that low oral doses of modafinil will likely not enhance the behavioral or reinforcing effects of psychostimulants. However, in consideration of previous reports that higher modafinil doses significantly increase locomotor activity (Deroche-Gamonet et al., 2002), the next experiment was designed to determine if dose-dependent increases in the additive effects of modafinil and d-amphetamine are different than effects obtained by either compound alone. A preclinical behavioral assay sensitive to the combined effects of compounds, the drug discrimination paradigm, was utilized.
CHAPTER IV

EXPERIMENT 3: COMBINED EFFECTS OF MODAFINIL AND d-AMPHETAMINE IN MALE SPRAGUE-DAWLEY RATS TRAINED TO DISCRIMINATE d-AMPHETAMINE

Overview

The specific aim of Experiment 3 was to evaluate the combined effects of d-amphetamine and modafinil in a drug discrimination assay. This assay has been used to evaluate changes in dose-response functions as a result of pre-treatment with drugs with similar mechanisms of action to the training drug. In this paradigm, increased potency is demonstrated by a curve shift to the left. At the time of this research, only one published study had evaluated modafinil in combination with another substance in this assay. Dopheide et al. (2007) tested modafinil (32, 64, 128 mg/kg) at varied post-injection times (10 to 240 min) in rats trained to discriminate cocaine (1.6, 5 mg/kg, i.p.) or d-amphetamine (0.3 mg/kg, s.c.). Subsequently, they examined the effects of modafinil in combination with cocaine or d-amphetamine. Although modafinil alone only partially substituted for cocaine and d-amphetamine, 32 mg/kg modafinil enhanced the discrimination of low doses of cocaine and d-amphetamine and shifted the dose-effect curves to the left, which suggests modafinil may have additive effects when used in combination with other psychostimulants. The present experiment evaluated the combined effects of modafinil and d-amphetamine in two groups of rats trained to discriminate 0.3 mg/kg or 1.0 mg/kg. In addition, a positive control (PNU-91356A) and two negative control conditions (morphine, ethanol) were tested to ensure reliability of discrimination.
Methods

Subjects

Sixteen male Sprague-Dawley rats (Charles River, Portage, MI) approximately four months old and drug naïve at the beginning of the study were utilized. All animals were housed individually in polycarbonate cages lined with corncob bedding in a colony room with a 12:12 light/dark cycle with lights on from 7:00 a.m. to 7:00 p.m. Water and food were provided *ad libitum* during the acclimation phase. The animals’ weight was then restricted to 80% of their free-feeding weight by restricting the amount of food given each day until the goal weight was reached. All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee and were in accordance with the guidelines of the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, 2011).

Apparatus

Training and testing sessions were conducted in eight standard operant conditioning chambers (ENV-001; MED Associates Inc., Georgia, VT, USA), housed within sound- and light- attenuating shells. Each chamber was equipped with three removable levers located on the front panel, a food pellet dispenser, a 28-V house light, and fan. Forty-five mg food pellets served as the reinforcers (Bioserv; Frenchtown, NJ). Experimental events were programmed and controlled using Version IV Med-PC software (MED Associates Inc., St. Albans, VT, USA).
Drugs

d-Amphetamine-hemisulfate (Sigma-Aldrich, St. Louis, MO) was dissolved in a 0.9% NaCl saline solution and administered s.c. Modafinil was synthesized in the laboratory of Dr. Thomas Prisinzano using previously described methods (Prisinzano, et al., 2004). Suspensions were prepared fresh each day in a 5% arabic gum solution (Sigma Aldrich, St. Louis, MO) and administered by oral gavage (i.g.) in a volume of 10 ml/kg 30 minutes prior to test sessions. Morphine (Sigma-Aldrich, St. Louis, MO) and PNU-91356A (Pharmacia & Upjohn, Inc., Kalamazoo, MI) were dissolved in 0.9% NaCl and administered s.c. 10 minutes prior to test sessions. Ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) was diluted in sterile water and administered i.g. in a volume of 10 ml/kg 10 minutes prior to test sessions.

Procedures

Preliminary Training. Initial training consisted of a single one hour session with no levers present and rats were exposed to a fixed-time 60 second schedule of food delivery to acclimate them to the sound and location of food pellet delivery. All subsequent training sessions lasted 20 minutes and were conducted once per day, five to six days a week between 4:00 and 6:00 p.m. All rats were initially reinforced for responses on the center lever via an autoshaping program under a continuous reinforcement schedule for one (n=9) or two (n=7) 20 min sessions. Only the center lever was present during autoshaping sessions. Once all animals were reliably lever pressing, errorless training commenced with only the left or right lever present.
Drug (D) or vehicle (V) injections were administered subcutaneously 10 minutes prior to errorless training sessions. Drug injections consisted of 0.3 mg/kg d-amphetamine for one group (n=8) and 1.0 mg/kg d-amphetamine for the other group (n=8) and vehicle injections consisted of 0.9% saline for both groups. For half the animals in each group, errorless training sessions with only the right lever present followed drug injections and errorless training sessions with only the left lever present followed saline injections. Conditions were reversed for the remaining animals in each group. All rats were exposed to twelve errorless training sessions in the following order: V, V, D, D, V, D, D, V, V, D.

Responses were initially reinforced under a fixed-ratio 1 (FR 1) schedule and the FR value was gradually incremented within each training session and across the six errorless training sessions with each stimulus condition. Within each session, the FR was programmed to increment by a designated amount (e.g., 1, 2, or 5) after the delivery of five reinforcers at a particular FR value. Across training sessions, the starting FR value was determined for each individual rat by the last FR value obtained in the previous session. All rats were responding on a FR 20 schedule by the last errorless training session with each stimulus condition.

Discrimination Training Procedures. Immediately following the completion of errorless training, discrimination training commenced with both levers present. The first session with both levers present was designated as day one for determining the number of training sessions required for each animal to meet the discrimination criteria. Similar to errorless training sessions, discrimination training under each stimulus condition began on a FR 1 schedule and the FR
was increased gradually until the final FR 20 was reached under each stimulus condition. Once animals were reliably responding on an FR 20 schedule under both stimulus conditions, the FR 20 remained in effect for subsequent training and the remainder of the study.

Drug and saline training sessions were administered in a pseudo-random order, with the limitation that no animal received more than two consecutive drug or two consecutive saline sessions throughout the study. For example, within a six day period, drug (D) and vehicle (V) training sessions occurred in one of the following orders: VVDDVD, DVDVVD, DVVDDV, DDVDVV, or DDVDDV. Levers corresponding to stimulus conditions were held constant for each animal throughout the entire study. Consistent with errorless training conditions, half of the rats in each training group were reinforced for right lever responses following d-amphetamine injections and for left lever responses following saline injections; conditions were reversed for the remaining rats in each group. The chambers and levers were wiped clean with isopropyl alcohol after each session to reduce the influence of olfactory stimuli on lever selection (Extance and Goudie, 1981).

The criteria for stimulus discrimination required animals to emit a minimum of 80% correct lever responses prior to the delivery of the first reinforcer and for the remainder of the training session for at least 8 of 10 consecutive discrimination training sessions.

*Testing Procedures.* Stimulus generalization tests commenced when each subject met the criteria described above. In between test sessions, animals were administered no less than one drug training session and one
vehicle training session and were required to exhibit 80% response accuracy on the first FR as well as during the total session under both conditions to continue testing. If an animal did not meet these criteria, training sessions continued until criterion was met with each stimulus condition on two consecutive days. Generalization tests were conducted no more than two times per week with the following compounds: d-amphetamine (0, 0.03, 0.1, 0.3, and 1.0 mg/kg); modafinil (0, 32, 64, 128, and 256 mg/kg); modafinil (32 mg/kg) in combination with d-amphetamine (0, 0.03, 0.1, 0.3, 1.0 mg/kg); the selective D2 dopamine agonist, PNU-91356A (0.01, 0.03, 0.1, and 0.3 mg/kg); morphine (2.5 and 5.0 mg/kg); ethanol (1.5 mg/kg). These compounds were tested in the order listed above, and individual test doses of each compound were administered in a counterbalanced order among the eight subjects in each training group. At each dose level, half of the animals in each group were tested on a day following a drug training session and the other half were tested on a day following a saline training session. Test sessions were similar to discrimination training sessions with the exception that no reinforcers were delivered and the animal was immediately removed from the chamber following the completion of 20 consecutive responses on either lever or after 20 minutes had elapsed, whichever occurred first. As with training sessions, incorrect responses reset the response counter, but completion of a single FR ended a test session.

Data Analysis

The mean (± S.E.M.) number of sessions to meet the discrimination criteria was calculated for each training group and a t-test was conducted to
determine statistical significance in the sessions to criteria between groups. Stimulus generalization was quantified as the percentage of total responses emitted on the drug-appropriate lever. Complete stimulus generalization was defined as a group mean of 80% or higher on any given dose. A group average of 20 % to 80 % drug-lever selection was considered partial substitution for a particular test dose. Response rate was expressed as responses per second and calculated by dividing the total number of responses on either lever by the number of seconds to complete a test session. Means (± S.E.M.) for these dependent variables were calculated for each test dose and dose-response curves were plotted from these data for each training group. For test compounds producing dose-dependent increases in drug lever selection, separate one-way repeated measures analysis of variance (ANOVA) tests were conducted to assess the main effect of dose on percent drug-lever responses and response rate. For animals that did not emit at least 20 responses during a test session, that animal’s percent drug-lever selection data were excluded from graphs and statistical analyses, but response rate was included. A nonlinear regression was conducted on the d-amphetamine dose-response curves to estimate the median effective dose (ED$_{50}$) with and without the addition of 32 mg/kg modafinil. Statistical analyses were conducted with SPSS (SPSS Statistics, Chicago, IL, USA) and Prism GraphPad (GraphPad, San Diego, CA, USA).
Results

Discrimination Acquisition

Stimulus control was readily established by both AMPH training doses, although acquisition occurred more rapidly in the animals trained with 1.0 mg/kg d-amphetamine (1.0 AMPH) compared to those trained with 0.3 mg/kg d-amphetamine (0.3 AMPH). All eight animals in the 1.0 AMPH group met the discrimination criteria within 16 (± 0 S.E.M.) discrimination training sessions, whereas the 0.3 AMPH group met these criteria within an average of 30 (± 2.8 S.E.M.) sessions (range: 22-46). A t-test on the number of sessions to meet criteria was statistically significant between training groups [t (14) = 4.92, p < 0.01].

Determination of d-amphetamine Dose-response Curves

Dose-response curves for d-amphetamine in both the 0.3 AMPH and 1.0 AMPH training groups are depicted in Figure 8. The top graphs show the mean (± S.E.M.) percentage of d-amphetamine-lever responses and the bottom graphs depict mean (± S.E.M.) response rate. A one-way repeated measures ANOVA on the percentage of drug-lever responses showed significant dose effects in the 1.0 AMPH group [F (4, 28) = 18.06, p < 0.001] and Bonferroni post-hoc tests indicated that both 0.3 and 1.0 mg/kg produced significantly greater AMPH-lever responses than saline in this group (p < 0.001). Response rates following d-amphetamine were fairly stable across doses in the 1.0 AMPH group. A one-way repeated measures ANOVA on response rate was not significant for this group.
A one-way repeated measures ANOVA showed significant dose effects on percent drug-lever selection in the 0.3 AMPH group \(F(4, 24) = 7.63, p < 0.001\). Bonferonni post-hoc tests were statistically significant between saline and 0.3 mg/kg \(p < 0.01\) and between saline and 1.0 mg/kg \(p < 0.05\) d-amphetamine in the 0.3 AMPH training group. A dose-dependent decrease in response rate was observed with d-amphetamine in the 0.3 AMPH group and this effect was statistically significantly \(F(4, 28) = 6.49, p < 0.01\), with significant Bonferroni post-hoc tests between 1.0 mg/kg d-amphetamine and saline \(p < 0.05\).
Figure 8. d-Amphetamine dose-response curves in rats trained to discriminate 0.3 mg/kg (n=7-8) or 1.0 mg/kg (n=8) d-amphetamine. The mean (± S.E.M.) percentage of responses on the d-amphetamine-associated lever is depicted in the top graph and response rate is shown in the bottom graph.
Modafinil Substitution Tests

Figure 9 depicts the results of stimulus generalization tests with modafinil in both the 0.3 AMPH and 1.0 AMPH training groups. In both training groups, modafinil produced a dose-dependent increase in d-amphetamine-lever responses, with slightly higher group means following 32, 64, and 128 mg/kg modafinil in the 0.3 AMPH group. However, a slightly higher percentage of drug-lever selection was observed following 256 mg/kg modafinil in the 1.0 AMPH group (78%) compared to the 0.3 AMPH group (66%). Five of the eight animals in the 0.3 AMPH group and six of the eight animals in the 1.0 AMPH group exhibited complete stimulus generalization following the highest dose of modafinil.

A repeated measures one-way ANOVA revealed a significant effect of modafinil dose on percentage of drug-lever responses in the 1.0 AMPH group \([F (4, 28) = 5.92, p < 0.01]\). Bonferroni post-hoc tests were significant between the 256 mg/kg dose and vehicle \((p < 0.001)\). Modafinil had little effect on response rate in the 1.0 AMPH group. A one-way repeated measures ANOVA on percent drug-lever selection was also significant in 0.3 AMPH group \([F (4, 28) = 3.01, p < 0.05]\). Bonferroni post-hoc tests were significant between the 256 mg/kg dose and vehicle for this group \((p < 0.05)\). A one-way ANOVA on response rate in the 0.3 AMPH group was significant \([F (4, 28) = 2.92, p < 0.05]\), although there were no significant Bonferroni post-hoc tests.
Figure 9. Modafinil dose-response curves in rats trained to discriminate 0.3 mg/kg (n=7-8) or 1.0 mg/kg (n=8) d-amphetamine. The mean (± S.E.M.) percentage of d-amphetamine-lever responses is depicted in top graph and response rate is shown in bottom graph.
Substitution Tests with PNU-91356A, Ethanol, and Morphine

Results of stimulus generalization tests with PNU-91356A, ethanol, and morphine are depicted in Table 1. As demonstration of a positive control, the D₂ dopamine agonist, PNU-91356A fully substituted in both training dose groups. In contrast, ethanol and morphine showed no evidence of substitution in either group, thus serving as valid negative controls. A one-way repeated measures ANOVA on percentage of drug-lever responses following PNU-91356A was significant in the 1.0 AMPH group \([F(4, 16) = 18.61, p < 0.01]\). Bonferroni post-hoc tests showed significant differences between vehicle and the 0.3 mg/kg dose in this group \((p < 0.05)\). Response rate was significantly reduced by PNU-91356A in this group \([F(4, 20) = 23.82, p < 0.001]\), with significant post-hoc tests between vehicle and both 0.1 and 0.3 mg/kg \((p < 0.05)\).

A one-way repeated measures ANOVA on percentage of drug-lever responses following PNU-91356A was also significant in the 0.3 AMPH group \([F(3, 18) = 29.33, p < 0.001]\) with significant Bonferroni post-hoc tests between vehicle and 0.1 mg/kg \((p < 0.001)\). Response suppression was severe in the 0.3 AMPH group following the administration of 0.3 mg/kg PNU-91356A; none of the animals made 20 responses and the majority of them made fewer than five responses following this dose. Furthermore, a one-way repeated measures ANOVA on response rate in this group was statistically significant \([F(4, 28) = 9.22,\)
| Test Drug  | Dose | Mean | SEM | N   | p   | Mean | SEM | N   | p   | Percent  | Rate  | Percent  | Rate  |
|-----------|------|------|-----|-----|-----|------|-----|-----|-----|-----|---------|-------|---------|-------|
| PNU-91356A | 0    | 0.00 | 0.00 | 5   | N.S. | 1.20 | 0.11 | 8   | N.S. | 16.47 | 13.96  | 7     | N.S. | 0.97 | 0.12 | 8     | N.S. |
|           | 0.01 | 2.65 | 1.75 | 5   | N.S. | 0.82 | 0.16 | 6   | N.S. | 14.65 | 14.23  | 7     | N.S. | 1.13 | 0.35 | 8     | N.S. |
|           | 0.03 | 3.00 | 1.25 | 5   | N.S. | 0.44 | 0.11 | 6   | N.S. | 3.40  | 1.95   | 7     | N.S. | 0.30 | 0.09 | 8     | <0.05|
|           | 0.1  | 74.23| 18.71| 5   | N.S. | 0.04 | 0.02 | 8   | <0.05| 95.88 | 2.29   | 7     | <0.001 | 0.07 | 0.02 | 8     | <0.05|
|           | 0.3  | 83.66| 10.20| 5   | <0.05| 0.03 | 0.01 | 6   | <0.05| NC    | NC     | 0     | NC     | 0     | 0     | 8     | <0.05|
| Morphine  | 2.5  | 12.39| 5.66 | 7   | N.S. | 0.32 | 0.10 | 8   | N.S. | 20.06 | 13.33  | 6     | N.S. | 0.20 | 0.08 | 8     | N.S. |
|           | 5.0  | 10.69| 10.69| 5   | N.S. | 0.15 | 0.08 | 7   | N.S. | 0.00  | 0.00   | 1     | N.S. | 0.01 | 0.01 | 6     | N.S. |
| Ethanol   | 1.5  | 7.76 | 5.13 | 8   | N.S. | 0.49 | 0.11 | 8   | N.S. | 11.00 | 6.84   | 8     | N.S. | 0.28 | 0.10 | 8     | N.S. |

N.S. = not statistically significant, NC = not calculated due to lack of responding

Table 1. Percent drug-lever responding and rate of responding in generalization tests conducted with PNU91356-A, ethanol, and morphine in animals trained to discriminate 1.0 mg/kg d-amphetamine and 0.3 mg/kg d-amphetamine
Effects of Modafinil Pretreatment on d-amphetamine Discrimination

Figure 10 illustrates the results of substitution tests with 32 mg/kg modafinil administered in combination with each dose of d-amphetamine (32 MOD+AMPH) in both the 1.0 AMPH training group (left panel) and the 0.3 AMPH training group (right panel). For comparison, these graphs also include the d-amphetamine dose-response curves previously depicted in Figure 8. In animals trained to discriminate 1.0 AMPH, pretreatment with 32 mg/kg modafinil did not alter the d-amphetamine dose-response function. A nonlinear regression using a sigmoidal dose-response function equation indicated a slight reduction of the ED$_{50}$ from 0.71 mg/kg (95% CI: 0.05-10.6) to 0.29 mg/kg (95% CI: 0.05-1.67). A one-way repeated measures ANOVA on percentage of drug-lever responses following the 32 MOD+AMPH dose combinations in the 1.0 AMPH group was significant [$F(4, 28) = 15.44$, $p < 0.001$], with significant Bonferroni post-hoc tests only between 32 MOD+SAL and 32 MOD+1.0 AMPH ($p < 0.01$). Although modafinil pretreatment appeared to reduce the effects of d-amphetamine on response rate, a one-way repeated measures ANOVA indicated no significant rate suppressant effects of 32 MOD+AMPH.

Pretreatment with 32 mg/kg modafinil also shifted the d-amphetamine dose-response curve slightly to the left in the 0.3 AMPH group, with a reduction in the ED$_{50}$ from 0.07 mg/kg (95% CI: 0.01-0.74) to 0.03 mg/kg (95% CI: 0.001-3.7). A one-way repeated measures ANOVA on percentage of drug-lever
responses following the 32 MOD+AMPH dose combinations in the 0.3 AMPH group was statistically significant \(F(4, 20) = 6.90, p < 0.001\) and Bonferroni post-hoc tests were significant between 32 MOD+SAL and both 32 MOD+0.3 AMPH \((p < 0.01)\) and 32 MOD+1.0 AMPH \((p < 0.01)\). Similar to the effects of d-amphetamine alone, 32 MOD+AMPH significantly reduced response rate in the 0.3 AMPH group \(F(4, 28) = 3.35, p < 0.05\) with significant Bonferroni post-hoc tests between 32 MOD+SAL and 32 MOD+1.0 AMPH \((p < 0.05)\).

Discussion

This study evaluated the wake-promoting agent, modafinil, for stimulus generalization to the psychomotor stimulant, d-amphetamine, and assessed the combined effects of these drugs in rats trained to discriminate either 0.3 or 1.0 mg/kg d-amphetamine. Consistent with a previous report by Dopheide et al. (2007), the current findings indicate that modafinil produced only partial substitution for d-amphetamine, while a low modafinil dose appeared to augment the discrimination of low
Figure 10. d-Amphetamine dose-response curves following modafinil (32 mg/kg) pretreatment in rats trained to discriminate 0.3 mg/kg (n=7-8) or 1.0 mg/kg (n=8) d-amphetamine. For comparison, dose-response curves with d-amphetamine alone are also shown.
d-amphetamine doses. Dopheide et al. (2007) reported a four-fold reduction in the d-amphetamine ED$_{50}$ by pretreatment with 32 mg/kg modafinil, whereas the current results showed approximately a two-fold decrease. This discrepancy could be due to various methodological differences between the two studies, such as different vehicles used for the modafinil mixture and different testing procedures. Dopheide et al. (2007) delivered a single reinforcer following completion of the FR requirement on either lever during test sessions, whereas the current study conducted test sessions under extinction. Furthermore, Dopheide et al. (2007) controlled for the pretreatment injection procedure by administering vehicle prior to d-amphetamine test sessions, whereas the current study did not utilize this control. This limitation precludes statistical analyses to compare d-amphetamine dose response tests with and without modafinil pretreatment. Nevertheless, the current findings indicate a visually evident trend in the same direction as that reported by Dopheide et al. (2007), who demonstrated that low doses of modafinil and d-amphetamine have additive effects.

In parallel experiments, Dopheide et al. (2007) reported similar additive effects of modafinil and cocaine, but only in rats trained to discriminate 1.6 mg/kg and not those trained to discriminate 5 mg/kg cocaine. A slightly higher percentage of cocaine-lever responding (60 and 70%) was observed in the group trained to discriminate 1.6 mg/kg cocaine, but similar to the d-amphetamine trained animals, none of the modafinil doses assessed substituted for either dose of cocaine. Results of the current study are consistent with those of Dopheide et
al. (2007) in that 32-128 mg/kg modafinil produced only partial substitution in rats trained to discriminate either 0.3 or 1.0 mg/kg d-amphetamine-hemisulfate (s.c.). Additionally, the present study found a higher modafinil dose (256 mg/kg) was required to produce full substitution in a significant portion of the animals tested. Although the group average did not exceed 80% drug lever selection, it is noteworthy that the majority of animals, five in the 0.3 AMPH training group and six in the 1.0 AMPH training group, exhibited full stimulus generalization to 256 mg/kg modafinil. A higher dose of modafinil may produce full substitution for d-amphetamine. Indeed, Paterson et al. (2010) demonstrated full substitution with 300 mg/kg modafinil (i.p.) in rats trained to discriminate 10 mg/kg cocaine.

A recent comprehensive review by Stolerman, Childs, Ford & Grant (2011) summarizes several key principles derived from research on the role of training dose in drug discrimination. Notably, higher training doses tend to produce enhanced discrimination accuracy, whereas lower training doses tend to produce lower ED50 values with the training drug and enhanced sensitivity in stimulus generalization tests with other test compounds. Previous studies comparing different d-amphetamine training doses generally support these conclusions (Stolerman and D'Mello, 1981; Barrett and Steranka, 1983; Stadler, Caul & Barrett, 2001). Results of the current study are partly consistent with these general principles. The 0.3 AMPH group required nearly twice as many training sessions to attain the criteria for stimulus control and the d-amphetamine ED50 was 10 times lower in this group compared to the 1.0 AMPH group. However, the dose-response curves between the two training groups were not as robust as
expected. Stimulus generalization to modafinil also varied only slightly between the 0.3 AMPH and 1.0 AMPH training dose groups. Lower modafinil doses (32, 64, 128 mg/kg) produced only slightly higher d-amphetamine-lever selection in the 0.3 AMPH group compared to the 1.0 AMPH group, and 256 mg/kg modafinil actually produced a slightly greater mean percentage of drug-appropriate responses in the 1.0 AMPH group. Minor differences in the modafinil dose-response curves between the two training groups fail to support the principle that a lower training dose yields greater sensitivity to other test compounds, it is worth noting that the 0.3 AMPH group was somewhat more sensitive to the combined effects of low modafinil and d-amphetamine doses compared to the 1.0 AMPH group.

Although the drug discrimination paradigm does not directly assess abuse liability, it is a useful model for assessing qualitative responses to drug combinations and may provide a method for investigating the influence of prior drug history on drug sensitivity. Regular use of low dose oral medications for therapeutic purposes and the high dose use by injection or intranasal routes for recreational purposes represent two distinct patterns and levels of psychostimulant use by humans. While modafinil use at low doses for therapeutic purposes presents a low risk for abuse and dependence, more frequent use of high doses may pose a higher risk for dependence. Moreover, the concurrent use of modafinil with other stimulants might pose a greater risk for abuse due to additive effects. The drug discrimination results of Dopheide et al. (2007), and to some extent the current findings indicate modafinil may have additive effects with
other psychostimulants. Thus, an experiment was designed to more directly investigate the combined effects of d-amphetamine and modafinil in the drug discrimination paradigm by training animals to discriminate 256 mg/kg modafinil. A secondary aim of this experiment was to elucidate the pharmacological mechanism of action of modafinil utilizing an *in vivo* assay.
Overview

Given that higher doses of modafinil partially or fully substitute in animals trained to discriminate cocaine (Loland et al., 2012; Newman et al., 2010; Paterson et al., 2010; Gold & Balster, 1996) or d-amphetmaine (Dopheide et al., 2007), it is possible that cocaine or d-amphetamine will produce stimulus generalization in animals trained to discriminate modafinil. No published study to date has investigated modafinil as the trained discriminative stimulus in a drug discrimination paradigm. In order to directly assess modafinil's pharmacological mechanism of action, animals were trained to discriminate 256 mg/kg modafinil and substitution tests were conducted with d-amphetamine, modafinil plus d-amphetamine, and several other dopaminergic agonists or antagonists.

Methods

Subjects

Eight male Sprague-Dawley rats (Charles River, Portage, MI) were singly housed in polycarbonate cages lined with corncob bedding in a colony room at Western Michigan University maintained on a 12:12 light/dark cycle with lights on from 7:00 a.m. to 7:00 p.m. Water was provided ad libitum and food was restricted in order to maintain animals' weights at 85% - 90% of their free-feeding weights. A standard 14 grams of rat chow was received on all training days and
standard 18 grams was received on all test days and days when data was not collected. All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee.

Apparatus

Training and testing sessions were conducted in eight standard operant conditioning chambers (ENV-001; MED Associates Inc., Georgia, VT, USA), housed within sound- and light- attenuating shells. Each chamber was equipped with three removable levers located on the front panel, a food pellet dispenser, a 28-V house light, and a fan. Forty-five mg food pellets served as the reinforcers (Bioserv; Frenchtown, NJ). Experimental events were programmed and controlled using Version IV Med-PC software (MED Associates Inc., St. Albans, VT, USA).

Drugs

Modafinil was synthesized in the laboratory of Dr. Thomas Prisinzano using previously described methods (Prisinzano, et al., 2004). Suspensions were prepared fresh each day in a 5% arabic gum solution (Sigma Aldrich, St. Louis, MO) and administered by oral gavage (i.g.) in a volume of 10 ml/kg 30 minutes prior to test sessions. d-Amphetamine-hemisulfate (Sigma-Aldrich, St. Louis, MO) and PNU-91356A (Pharmacia & Upjohn, Inc., Kalamazoo MI) were dissolved in 0.9% NaCl and administered i.p. 10 minutes prior to test sessions. GBR 12909 bismethanesulfonate monohydrate was prepared in the Chemical Biology Research Branch (National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism) and was dissolved in sterile water.
and administered i.p. 30 minutes prior to test sessions. (-)-Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% NaCl and administered i.p. 15 minutes before testing. Schering 39166 (Shering-Plough Corporation, Bloomfield, NJ) was dissolved in 0.9% NaCl and administered i.p. 10 minute prior to modafinil (256 mg/kg) administration. Haloperidol (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% NaCl and administered i.p. 30 minute prior to modafinil (256 mg/kg) administration.

Procedures

Preliminary Training. Preliminary training consisted of two one-hour sessions with no levers present and food pellets were delivered according to a fixed-time 60 second schedule to acclimate the rats to the sound and location of food pellet delivery. All subsequent training sessions lasted 20 minutes and were conducted once per day, five or six days a week. Lever pressing was initially reinforced on the center lever via an autoshaping program under a continuous reinforcement schedule for one 20 min session. Center lever press training continued for seven additional sessions during which the FR schedule was programmed to increment by a designated amount (e.g., 1, 2, or 5) after the delivery of five reinforcers at a particular FR value. Once all animals were reliably lever pressing at FR 20, errorless training commenced with only the left or right lever present.

Drug (D) or vehicle (V) injections were administered via oral gavage (i.g.) 30 minutes prior to errorless training sessions. Drug injections consisted of 256 mg/kg modafinil and vehicle injections consisted of a 5 % arabic gum solution.
For four rats, errorless training sessions with only the right lever present followed drug injections and errorless training sessions with only the left lever present followed saline injections. Conditions were reversed for the remaining four rats. All rats were exposed to six errorless training sessions in the following order: V, V, D, D, V, D. Responses were initially reinforced under a FR 1 schedule and the FR value was gradually incremented within each training session and across the six errorless training sessions with each stimulus condition. Within each session, the FR was programmed to increment by a designated amount (e.g., 1, 2, or 5) after the delivery of five reinforcers at a particular FR value. Across training sessions, the starting FR value was determined for each individual rat by the last FR value obtained in the previous session. All rats were responding on a FR 20 schedule by the last errorless training session with each stimulus condition.

*Discrimination Training Procedures.* Immediately following the completion of errorless training, discrimination training commenced with both levers present. The first session with both levers present was designated as day one for determining the number of training sessions required for each animal to meet the discrimination criteria. Similar to errorless training sessions, discrimination training under each stimulus condition began on a FR 1 schedule and the FR was increased gradually until the final FR 20 was reached under each stimulus condition. Once animals were reliably responding on an FR 20 schedule under both stimulus conditions, the FR 20 remained in effect for subsequent training and the remainder of the study.
Drug and saline training sessions were administered in a pseudo-random order, with the limitation that no animal received more than two consecutive drug or two consecutive saline sessions throughout the study. Consistent with errorless training conditions, half of the rats were reinforced for right lever responses following modafinil injections and for left lever responses following vehicle injections; conditions were reversed for the remaining rats. The chambers and levers were wiped clean with isopropyl alcohol after each session to reduce the influence of olfactory stimuli on lever selection (Extance and Goudie, 1981). The criteria for stimulus discrimination required animals to emit a minimum of 80% correct lever responses prior to the delivery of the first reinforcer and for the remainder of the training session for at least 8 of 10 consecutive discrimination training sessions.

Testing Procedures. Stimulus generalization tests commenced when each subject met the criteria described above. In between test sessions, animals were administered no less than one drug training session and one vehicle training session and were required to exhibit 80% response accuracy on the first FR as well as during the total session under both conditions to continue testing. If an animal did not meet these criteria, training sessions continued until criterion was met with each stimulus condition on two consecutive days. Generalization tests were conducted no more than two times per week with the following compounds: modafinil (0, 32, 64, 128, 256, and 384 mg/kg); d-amphetamine (0, 0.03, 0.1, 0.3, and 1.0 mg/kg); d-amphetamine (0, 1.0, or 0.1 mg/kg) in combination with modafinil (0, 32, 64, 128, 256, and 384 mg/kg); the selective D2 dopamine
agonist, PNU-91356A (0.01, 0.03, 0.1, and 0.3 mg/kg); the DAT inhibitor, GBR 12909 (0, 5, 10, 20, and 30 mg/kg); nicotine (0, 0.1, 0.2, 0.4, and 0.8 mg/kg); and cocaine (0, 2.5, 5, and 10 mg/kg). Antagonist tests were conducted with the selective D\textsubscript{1} DA antagonist, Schering 39166 (0, 0.03, 0.1, 0.3 mg/kg, i.p.) and the D\textsubscript{2} DA antagonist, haloperidol (0, 0.125, 0.25, 0.5 mg/kg, i.p.). Individual test doses of each compound were administered in a counterbalanced order among the eight subjects. At each dose level, half of the animals were tested on a day following a drug training session and the other half were tested on a day following a vehicle training session. Test sessions were similar to discrimination training sessions with the exception that no reinforcers were delivered and the animal was immediately removed from the chamber following the completion of 20 consecutive responses on either lever or after 20 minutes had elapsed, whichever occurred first. As with training sessions, incorrect responses reset the response counter, but completion of a single FR ended a test session.

Data Analysis

Stimulus generalization was quantified as the percentage of total responses emitted on the drug-appropriate lever. Complete stimulus generalization was defined as a group mean of 80% or higher on any given dose. A group average of 20% to 80% drug-lever selection was considered partial substitution for a particular test dose. Response rate was expressed as responses per second and calculated by dividing the total number of responses on either lever by the number of seconds to complete a test session. Means (± S.E.M.) for these dependent variables were calculated for each test dose and
dose-response curves were plotted from these data for each training group. For test compounds producing dose-dependent increases in drug lever selection, separate one-way repeated measures analysis of variance (ANOVA) tests were conducted to assess the main effect of dose on percent drug-lever responses and response rate. For animals that did not emit at least 20 responses during a test session, that animal’s percent drug-lever selection data were excluded from graphs and statistical analyses, but response rate was included. Statistical analyses were performed using Prism GraphPad (GraphPad, San Diego, CA, USA).

Results

Discrimination Acquisition

Modafinil established stimulus control in all eight animals within an average of 36 (± 2.4) discrimination training sessions (range: 23-43). At session numbers 180 and 195, two animals stopped discriminating during the drug stimulus condition though reliably met criteria during the vehicle stimulus condition. As a result, these animals were returned to errorless training sessions with 256 mg/kg modafinil and vehicle stimulus conditions for 24 sessions. After both levers were presented again, each of these animals still did not meet discrimination criteria during the drug condition. For one animal, the dose was then increased to 384 mg/kg and 10 errorless training sessions were run. This rat met the discrimination criteria after 20 additional discrimination training sessions. The other animal was not put on errorless training when the dose was increased
to 384 mg/kg and failed to reach discrimination criteria after 20 sessions. As a result these two animals are excluded from statistical analyses for all data obtained after the training dose was increased. The data from these animals is only included in the statistical analyses for modafinil and d-amphetamine dose-response curves.

Determination of Modafinil dose-response Curves

Figure 11 displays the agonist substitution tests with PNU-91356A, nicotine, d-amphetamine, GBR 12909, cocaine, and modafinil. Percent drug-lever selection is shown in the upper panel and response rate is displayed in the bottom panel. Modafinil produced a dose-dependent increase in percent modafinil-lever responses with full substitution at the training dose. A repeated measures one-way ANOVA revealed a significant effect of modafinil dose on percentage of drug-lever responses \( F(6, 42) = 5.29, p < 0.001 \). Tukey post-hoc tests were significant between the 256 mg/kg dose and both the vehicle condition \( (p < 0.001) \) and the 16 mg/kg dose \( (p < 0.05) \). There were also significant differences between 384 mg/kg and the vehicle condition \( (p < 0.01) \). A one-way repeated measures ANOVA on response rate after modafinil administration was not statistically significant.

Substitution Tests with d-amphetamine

Half of the animals were severely disrupted when tested with 3.0 mg/kg and did not meet response criteria to be included in the analyses. As such, this dose was eliminated from the statistical analysis but is included in the visual representation of the data (see Figure 11). d-Amphetamine produced dose-
dependent increases in percent modafinil-lever responses with significant partial substitution at the highest dose tested that also markedly decreased response rate. A one-way repeated measures ANOVA excluding the 3 mg/kg dose (due to low N) revealed a significant effect of d-amphetamine dose on percentage of drug-lever responses \( F (4, 28) = 3.76, p < 0.05 \). Tukey post-hoc tests were significant between 1.0 mg/kg and the vehicle control dose \( (p < 0.05) \) and between 0.03 mg/kg and 1.0 mg/kg d-amphetamine \( (p < 0.05) \). A one-way repeated measures ANOVA on response rate for the five doses in the
Figure 11. Results of substitution tests with PNU-91356A, nicotine, d-amphetamine, GBR 12909, cocaine, and modafinil. Percent drug-lever selection is shown on the top and response rate is shown on the bottom.
d-amphetamine generalization gradient was statistically significant \[ F(4, 28) = 4.92, \ p < 0.001 \] with significant Tukey post-hoc tests between 1.0 mg/kg and three conditions: vehicle (\( p < 0.05 \)), 0.03 mg/kg (\( p < 0.05 \)), 0.1 mg/kg (\( p < 0.01 \)).

Substitution Tests with PNU-91356A

Most of the animals were severely disrupted on the highest dose tested (0.3 mg/kg) and didn't emit enough responses to be included in the statistical analysis. As such, this dose was excluded from the statistical analysis of percent modafinil-lever responding, but was included in the statistical analysis of response rate and the graphic depiction of the dose-response curve (see Figure 11). Substantial partial substitution for modafinil was observed with 0.1 mg/kg PNU-91356A while no substitution was observed with the other doses. A one-way repeated measures ANOVA on percent modafinil-lever selection revealed a significant main effect of PNU-91356A dose \[ F(3, 9) = 5.12, \ p < 0.05 \]. Tukey post-hoc tests were significant between the 0.1 mg/kg dose and vehicle (\( p < 0.05 \)). Response rate decreased dose-dependently and the one-way repeated measures ANOVA was significant \[ F(4, 12) = 6.51, \ p < 0.001 \]. Significant Tukey post-hoc tests revealed a difference between the vehicle condition and both the 0.1 mg/kg (\( p < 0.01 \)) and 0.3 mg/kg dose (\( p < 0.01 \)).

Substitution Tests with GBR12909

GBR12909 administration produced dose-dependent increases in drug-appropriate responding, with full substitution at the highest dose (30 mg/kg). The dose response curve for this test compound is depicted in Figure 11. A one-way repeated measures ANOVA on percent modafinil-lever responses revealed a
significant effect of GBR12909 dose \[ F (4, 20) = 3.47, p < 0.05 \]. Tukey post-hoc tests were significant between the highest test dose (30 mg/kg) and the vehicle \((p < 0.05)\) test condition. This compound also produced dose-dependent decreases in response rate. A one-way repeated measures ANOVA on response rate for the GBR12909 generalization tests was statistically significant \[ F (4, 20) = 6.15, p < 0.01 \] with significant Tukey post-hoc tests between the highest dose tested (30 mg/kg) and three conditions: vehicle \((p < 0.001)\), 5 mg/kg \((p < 0.05)\), and 20 mg/kg \((p < 0.05)\).

Substitution Tests with (-)-Nicotine Hydrogen Tartrate

Only partial generalization was produced by nicotine administration even at the highest dose (see Figure 11). A repeated measures one-way ANOVA revealed a significant effect of nicotine dose on percentage of drug-lever responses \[ F (4, 20) = 8.11, p < 0.001 \]. Tukey post-hoc tests were significant between the vehicle condition and both 0.4 mg/kg \((p < 0.01)\) and 0.8 mg/kg \((p < 0.01)\) conditions. There was also a significant difference between 0.2 mg/kg and 0.4 mg/kg \((p < 0.05)\). Visual analysis of response rate data after nicotine administration suggests a dose-dependent decrease in responding, however a one-way repeated measures ANOVA on response rate for the nicotine found no statistically significant differences among doses.

Substitution Tests with Cocaine

The cocaine dose-response curve represents only four animals that have completed all test doses to date. Full substitution for modafinil was observed with 2.5 mg/kg and 5 mg/kg cocaine while partial substitution was observed at 1.25
mg/kg and 10 mg/kg cocaine. Moreover, a one-way repeated measures ANOVA on percent modafinil-lever selection revealed a significant main effect of cocaine dose \([F(4, 12) = 6.78, p < 0.01]\). Tukey post-hoc tests were significant between the all test doses and the vehicle \((p < 0.05)\) condition. Response rate decreased dose-dependently, but the one way repeated measures ANOVA was not significant.

Substitution Tests with Modafinil in Combination with d-amphetamine (0.1 and 1.0 mg/kg)

Figure 12 represents the results of 1.0 mg/kg or 0.1 mg/kg d-amphetamine + modafinil (16-384 mg/kg) compared to modafinil alone with percent drug-lever selection presented on the right and response rate shown on the left. Only five animals completed the dose-response curve for modafinil in combination with 0.1 mg/kg d-amphetamine. A two way repeated measures ANOVA on the difference among the three curves (modafinil alone, modafinil + 1.0 mg/kg d-amphetamine, and 0.1 mg/kg d-amphetamine + modafinil) revealed significant main effects of pretreatment \([F(2, 12) = 10.51, p < 0.01}\) and dose \([F(6, 54) = 4.55, p < 0.001]\), but no significant interaction. A two factor repeated measures ANOVA on response rate found a significant effect of pretreatment \([F(2, 12) = 8.09, p < 0.01]\).

Another two way repeated measures ANOVA utilizing data from seven animals was conducted to compare the results of modafinil substitution tests and the substitution tests with 1.0 mg/kg d-amphetamine + modafinil. There were significant main effects of dose \([F(6, 72) = 5.39, p < 0.001]\) and pretreatment \([F(1, 12) = 40.08, p < 0.001]\) and a significant interaction effect \([F(6, 72) = 3.53, p\)]
< 0.01. Tukey post-hoc tests indicate statistically significant differences in percent drug-lever responses following the 1.0 d-Amphetamine + vehicle \((p < 0.01)\) and the 1.0 d-Amphetamine + 16 mg/kg modafinil \((p < 0.01)\) dose combinations. A two factor repeated measures ANOVA comparing response rate following modafinil and 1.0 mg/kg d-amphetamine + modafinil administration revealed a main effect of pretreatment \([F(1, 12) = 27.97, p < 0.001]\) and no other significant effects.

**Antagonist Tests**

Figure 13 displays the results of Sch 39166 and haloperidol administered in combination with modafinil. Percent modafinil-lever selection is presented in the right panel and response rate is displayed in the left panel. Dose-dependent decreases in percent modafinil-lever selection and response rate are evident for both compounds. A repeated measures one-way ANOVA revealed a significant effect of Sch 39166, dose on percentage of drug-lever responses \([F(3, 15) = 18.29, p < 0.001]\).
Figure 12. Results of stimulus generalization tests with 1.0 mg/kg or 0.1 mg/kg d-amphetamine + modafinil (16-384 mg/kg) compared to modafinil alone. Percent drug-lever selection is shown on the left and response rate is shown on the right.
Tukey post-hoc tests were significant between the vehicle condition and both 0.1 mg/kg ($p < 0.001$) and 0.3 mg/kg ($p < 0.001$) test doses and between the 0.03 mg/kg test dose and both 0.1 mg/kg ($p < 0.001$) and 0.3 mg/kg ($p < 0.01$). Response rate was analyzed with a repeated measures one-way ANOVA and revealed no significant effect of Sch 39166 dose.

A repeated measures one-way ANOVA revealed a significant effect of haloperidol, dose on percentage of drug-lever responses [$F(3, 9) = 4.99, p < 0.05$]. Tukey post-hoc tests were significant between the vehicle condition and the highest dose tested (0.5 mg/kg) ($p < 0.01$). Response rate was analyzed with a one-way repeated measures ANOVA, which revealed a significant main effect of dose [$F(3, 12) = 9.63, p < 0.01$]. Tukey post-hoc tests were significant between the highest dose (0.5 mg/kg) and all other dose conditions ($p < 0.01$).
Figure 13. Results with Sch 39166 and haloperidol administered in combination with 256 mg/kg modafinil. Percent drug-lever selection is shown on the top and response rate is shown on the bottom.
Discussion

This experiment investigated the pharmacological mechanism of action of modafinil using an *in vivo* behavioral assay (drug discrimination) and evaluated the combined effects of d-amphetamine and modafinil for potentiation in animals trained to discriminate 256 mg/kg modafinil. This is the first study to evaluate the pharmacological mechanism of modafinil by utilizing the compound as the training compound in a drug discrimination paradigm. Moreover, it is the first to demonstrate that 256 mg/kg modafinil can function as a discriminative stimulus in rodents. Substitution tests with d-amphetamine produced nearly complete substitution for modafinil at a dose that markedly suppressed responding. This finding is consistent with previous reports (Dopheide et al., 2007) that modafinil partially substitutes in rats trained to discriminate a low dose of d-amphetamine. Results showing partial substitution with the D$_2$ agonist, PNU-91356A, full substitution with the DAT inhibitors, GBR 12909 and cocaine in addition to complete blockade with the D$_1$ antagonist, Sch 39166 and the D$_2$ antagonist, haloperidol suggest dual dopaminergic mechanisms contribute to the discriminative stimulus functions of modafinil. These results confirm those of previous studies using *in vitro* techniques that report inhibition of the DAT and both D$_1$ and D$_2$ DA receptors are involved in modafinil’s neuropharmacological actions (Loland et al., 2012; Anderson et al., 2009; Nguyen et al., 2011; Korotkova et al., 2007) and behavioral effects (Wisor et al., 2001).

The unexpected finding that the cholinergic agonist, nicotine, produced partial substitution for modafinil is of particular interest. Although nicotine is not a
direct DA agonist, it does increase dopamine efflux in the nucleus accumbens and dopamine antagonists have been shown to block its discriminative stimulus effects (Di Chiara, 2000). The results from this study suggest modafinil and nicotine may have similar stimulus properties. Given that it took researchers several years to determine the environmental events necessary to establish nicotine self-administration or CPP in nonhumans (Le Full & Goldberg, 2006), further research may be required to determine whether variables such as age, motivation, drug history, or schedule or reinforcement (Le Full & Goldberg, 2006) could affect abuse liability screening results with modafinil. Further research investigating these procedural variables and extent to which dopaminergic actions contribute to similar motor and discriminative stimulus functions of nicotine and modafinil warrant further investigation.

The results of the combination dose-response curves demonstrated that a dose of d-amphethamine (1.0 mg/kg) that initially produced only partial substitution for modafinil produced complete substitution when combined with a range of modafinil doses. However, for the present analysis there was not a proper control condition to compare the combination curves. A modafinil + d-amphetamine vehicle dose-response curve must be determined for a proper comparison. Generalization tests with this control condition are currently in progress. In addition to this limitation, there were also no negative control tests conducted to verify discrimination to a specific class of drugs versus a simple psychoactive effect. With the exception of nicotine, drugs tested for substitution or blockage were dopaminergic agonists and antagonists, therefore no
conclusions can be made regarding the possible role of other neurotransmitter systems in modafinil’s discriminative stimulus effects. Future investigations should focus on the evaluation of other neurotransmitter systems, in particular the NE and 5-HT systems. Although, cocaine and d-amphetamine are well known to act upon the DAT system, both also produce mechanistic actions involving these neurotransmitter systems (Glennon & Young, 2011). DAT and 5-HT transporter inhibitors readily substitute for cocaine and enhance the discriminative stimulus effects of low doses of cocaine in rats trained to discriminate low dose cocaine (Kleven & Koek, 1998). These authors also argue that interactions with the NE are sufficient, but not necessary in the discriminative stimulus effects of cocaine. Evaluation of the discriminative stimulus effects of d-amphetamine however revealed substitution with NE transporter inhibitors (Kamien & Woolverton, 1988) suggesting this is a primary mechanism involved in maintaining the discriminative stimulus effects of d-amphetamine. Given that the NE system appears to be important for the locomotor activating effects of modafinil (Hermant et al., 1991), further investigation of shared discriminative stimulus function of modafinil and other psychostimulants is warranted.
CHAPTER VI

GENERAL DISCUSSION

These studies evaluated the abuse liability of low to moderate doses of the alertness-promoting drug, modafinil, in three preclinical behavioral assays. Results of the behavioral sensitization and CPP experiments confirm previous reports that modafinil has low abuse liability. Specifically, a brief history of repeated d-amphetamine exposure did not sensitize rats to the locomotor effects of modafinil (Experiment 1), nor did concurrent administration of low doses of modafinil and d-amphetamine exert additive effects on CPP (Experiment 2). However, results of the drug discrimination experiments (Experiments 3 and 4) may be interpreted to suggest the opposite. Evidence of partial substitution with modafinil in animals trained to discriminate either a low or moderately high dose of d-amphetamine in addition to partial or full substitution with dopamine agonists in animals trained to discriminate modafinil indicate dopaminergic mechanisms may be critically important to maintaining modafinil’s discriminative stimulus effects. Many abused substances increase DA levels in the mesolimbic pathway that includes the ventral tegmental area, nucleus accumbens, and other corticolimbic regions. As such, these areas are implicated in the abuse liability of many psychoactive drugs. Psychostimulants, in particular, exert effects through the DA system and increase concentrations in this reward pathway (Spanagel & Weiss, 1999). Modafinil likely influences activities within this system and therefore may have potential for abuse.
In a recent review of preclinical, human laboratory, and clinical research, Herin, Rush, & Grabowski, (2010) summarized the rationale for agonist-like pharmacotherapy for stimulant dependence; medications with properties similar to the abused drug, but exhibiting a lower abuse liability may modify neurochemistry and stabilize behavior, and subsequently reduce drug use. Evidence demonstrated by the experiments in this study seem to support this claim. Furthermore, recent *in vitro* research demonstrating modafinil-induced DAT binding had a longer duration of action and was less efficacious than cocaine supports the use of modafinil as an agonist replacement therapy for cocaine dependence (Loland et al., 2012).

The results of investigations by Dopheide et al. (2007) and Experiment 3 of the current research indicate modafinil can augment the discrimination of low dose psychostimulants. Similarly, the results of Experiment 4 demonstrate that d-amphetamine can augment the discrimination of modafinil. Some authors have argued that drug discrimination in nonhuman models can be used to predict the potential therapeutic effectiveness of medications for the treatment of stimulant dependence (Li, Campbell & Katz, 2006). Specifically, Li et al. (2006) suggest that a drug that produces a leftward shift in a stimulant dose effect curve in drug discrimination might be a viable option for agonist replacement therapy. In consideration of this suggestion, the current experimental findings as well as those reported by Dopheide et al. (2007) support further evaluation of modafinil as an agonist replacement therapy for psychostimulant dependence.
Results of studies utilizing animal models of drug reinstatement following self-administration also seem to support modafinil as a treatment for psychostimulant dependence. Following extinction of methamphetamine self-administration, modafinil did not reinstate methamphetamine seeking and actually blocked methamphetamine-primed and cue-primed reinstatement (Reichel & See, 2010). In a follow up investigation, Reichel and See (2012) reported that chronic modafinil treatment attenuated cue-induced and methamphetamine-primed reinstatement, and even reduced methamphetamine-seeking behaviors following discontinuation of treatment. They also reported only a high dose of modafinil (300 mg/kg) reduced methamphetamine intake during maintenance of self-administration.

Although the results of some preclinical studies provide a rationale for modafinil as a pharmacotherapy for psychostimulant dependence, substantial evidence is currently lacking regarding its clinical efficacy in this regard. Indeed, recent clinical investigations of modafinil as an agonist replacement therapy have yielded mixed results. In a double-blind placebo controlled trial, Shearer et al. (2009) compared modafinil (200 mg/day) to placebo in participants seeking treatment for methamphetamine dependence. Treatment retention and medication adherence were equivalent between groups, though modafinil was reported to reduce methamphetamine use only in participants who received modafinil and remained medication compliant over the 10-week treatment period. Based on these findings, Shearer et al. (2009) promoted the continued assessment of modafinil for the treatment of methamphetamine dependence in
large multi-site clinical trials. Although preliminary clinical trials showed increased
cocaine abstinence in modafinil-treated patients compared to placebo-treated
patients over an eight week period (Dackis et al., 2005), a more recent study
failed to support these findings. In a double-blind, placebo-controlled randomized
clinical trial with cocaine-dependent participants. Schmitz et al. (2012) compared
the therapeutic effectiveness of 400 mg modafinil to 60 mg d-amphetamine and
the combination of 200 mg modafinil and 30 mg d-amphetamine. Retention rates
did not differ among treatment groups and the participants administered the
medication combination actually showed a trend for increased cocaine use over
the course of the 16-week study compared to groups administered only
d-amphetamine or placebo. Based on these preliminary findings, Schmitz et al.
(2012) advised against continued assessment of this dual-agonist medication
combination in a larger population.

Few studies have examined the combined effects of modafinil and other
psychostimulants for additive effects. One possible interpretation of the findings
from Experiment 3 and 4 is that modafinil’s subjective effects may be enhanced
when used in combination with d-amphetamine, which could increase its abuse
liability. The findings of Schmitz et al. (2012) regarding increased relapse to
cocaine use in patients administered combination therapy with modafinil and
amphetamine is consistent with this interpretation. Thus, despite modafinil’s
apparent low abuse liability, the combined behavioral effects of modafinil and
psychostimulants should be evaluated more carefully before continuing clinical
assessments with modafinil for psychostimulant dependence. In particular,
additional preclinical investigations utilizing other methodologies to examine modafinil across a wide range of doses and in combination with other stimulants, such as behavioral sensitization paradigms or drug self-administration may be of interest.
Appendix  
Institutional Use and Care Committee Approval Letters  

Western Michigan University  
Institutional Animal Care and Use Committee  

Date: December 8, 2010
To: Lisa Baker, Principal Investigator
From: Robert Eversole, Chair
Re: IACUC Protocol No. 10-12-01

Your protocol titled “Evaluation of Drug Combinations for Behavioral Sensitization” has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: December 8, 2011
Date: April 10, 2013

To: Lisa Baker, Principal Investigator
    Missy Peet, Student Investigator
    Amanda Quisenberry, Student Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol Number 13-04-03

Your protocol entitled “Conditioned Place Preference Procedures in Rats” received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: April 10, 2014
Date: March 13, 2013
To: Lisa Baker, Principal Investigator
From: Robert Eversole, Chair
Re: IACUC Protocol Number 13-03-03

Your protocol entitled “Drug Discrimination Studies of Psychoactive Drugs in Rats” has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: March 13, 2014
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